# Pharmaceutical Science: New Insights and Developments

## Vol. 8

*Edited by Prof. Wagih Mommtaz Ghannam*

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**Prof. Wagih Mommtaz Ghannam**
Mansoura University, Egypt.

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## *PREFACE*

*This book covers key areas of pharmaceutical science. The contributions by the authors include herbal cream, Biophytum sensitivum, antibacterial activity, Chronotherapy, chrono-pharmaceutics, pharmacodynamics, equivalent dose modelling, dose justification, liver enzymes, sarkaraikolli, glibenclamide, gestational diabetes mellitus, Nyaope, substance abuse, recreational drug, liver damage, drug utilisation review, emergency department, Mimosa pudica, phytotherapeutic, intestinal infections, medicinal plants, breath analysis, volatile organic compounds, fourier transform infrared spectroscopy, non-volatile compounds. This book contains various materials suitable for students, researchers, and academicians in the fields of pharmaceutical science.*

***Chapter 1***
Print ISBN: 978-93-88417-78-5, eBook ISBN: 978-93-88417-68-6

# Formulation and Antibacterial Assessment of a Herbal Cream Containing *Biophytum sensitivum* D.C.

**Haripriya G [a*], Krithika R [a], LisMaria Joseph [a] and Suresh J [a]**

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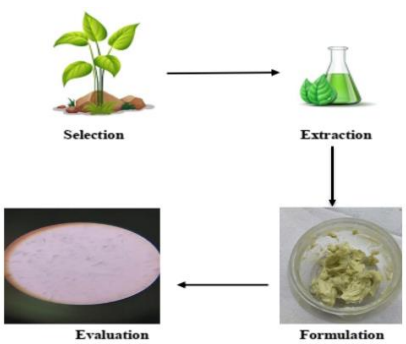
## ABSTRACT

**Aim:** *Biophytum sensitivum DC Oxalidaceae* is a traditional medicinal plant for treating bacterial infections. The goal of the current study was to create and assess an antibacterial cream of hydroalcoholic extract from *Biophytum sensitivium DC.*
**Materials and Methods:** This plant was extracted using the hydroalcoholic method and further evaluated for antibacterial activity against *Escherichia coli (E. coli)* by agar well diffusion method.
**Results:** The presence of phenols and flavonoids in the extract of *Biophytum sensitivum DC Oxalidaceae* exhibited significant activity on *E. coli* organism and activity which is compatible with standard antibiotics.
**Conclusion:** The chemical constituents were tested to identify phenols and flavonoids. The use of hydroalcoholic extract resulted in antibacterial activity, and an antibacterial cream was further formulated.

## GRAPHICAL ABSTRACT

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*Keywords: Biophytum sensitivum; hydroalcoholic extract; antibacterial activity; diffusion method; Escherichia coli; phenols; flavonoids.*

## 1. INTRODUCTION

Presently, life-threatening infections caused by pathogenic microorganisms, lead to the highest morbidity and mortality rate in the world population. The burden of skin disease on the global health system is enormous. One of the most prevalent human illnesses is skin disease. Between 30% and 70% of people are affected by it, and even higher rates are seen in at-risk subpopulations. The antibacterial qualities of a molecule are only associated with compounds that either eliminate or severely restrict the growth of bacteria and viruses. Topical antimicrobials can be used either as a stand-alone therapy or as an addition to systemic therapy. Every age group and every culture are impacted by it. Mupirocin (Bactroban, GlaxoSmithKline) is frequently advised even though several topical antibiotic treatments, including bacitracin, triple antibiotic ointment (polymyxin B, neomycin, and bacitracin), or gentamicin, can be utilised. The increasing emergence of multiresistant organisms, the cost, and potential side effects make systemic antibiotic or antifungal therapy less than ideal. Synthetic drug side effects are Paranoia, quite anxious, hallucinations, seizures, aggression, murderous or suicidal actions, heart attack or chest discomfort. To overcome these effects, natural remedies are prepared (Natarajan et al., 2010, Peterson, J. W., 1996).

## 2. BACTERIAL INFECTION

A bacterial infection is any illness or condition caused by bacteria or toxins growing in the body. Hazardous microorganisms from the surroundings, an infected person or animal, a bug bite, contaminated food, water, or surfaces can all cause infections. Most bacteria do not cause harm. As a matter of fact, our skin and internal organs are teeming with bacteria. Our intestines' (gut's) bacteria aid in food digestion. However, certain bacteria can lead to illnesses. Numerous body parts can be impacted by bacterial infections, including the throat, Lungs, Skin, and Bowel.

**Causes of Spreading Bacterial Infections:** The spreading or transfer of germs leads to bacterial illnesses. Contact with an infected person, touching surfaces with bacteria on them, consuming tainted food or drinking tainted water are all ways that you can get exposed to germs. Common transmission routes are listed below;

**Airborne:** Microscopic airborne respiratory droplets are the primary means of transmission for bacterial infections like tuberculosis. The virus releases these droplets when an infected person laughs, coughs, sneezes, or exhales. The bacteria can land on surfaces, move with air currents, or remain in the air. When the germs encounter mucous membranes or are inhaled by another person, they can cause them to become ill.

**Contaminated food or water:** Consuming raw food or water that has been polluted might transmit bacteria. *Salmonella,* among the bacteria that can cause

food-borne illnesses, is *Clostridium botulinum, Campylobacter, and Escherichia coli.*

**Contaminated objects:** Bacterial infections transmitted through contaminated objects can range from mild to severe, depending on the bacteria involved and the individual's susceptibility. For example, Staphylococcus aureus, which is commonly found on skin and in the environment, can cause infections that range from minor skin infections to life-threatening conditions like pneumonia and bloodstream infections.

**Insect bites:** Insect bites can transmit bacterial illnesses Leprosy (Trench fever), rickettsiosis (typhus), and borreliosis (relapsing fever) are just a few of the infections that lice can transmit.

**Sexual transmission:** The bacterial infections chlamydia, gonorrhea, and syphilis are sexually transmitted. Sexually transmitted diseases (STIs) caused by bacterial infections are a major public health concern worldwide. These infections are typically contracted through unprotected sexual contact with an infected person. The bacterium Chlamydia trachomatis is responsible for one of the most common bacterial STDs. Chlamydia frequently presents with no symptoms, particularly in its early stages, resulting in cases that go undiagnosed and untreated.

**Bacterial Skin Infection:** Skin infections caused by bacteria frequently start as little, red pimples that gradually become larger. While certain bacterial infections can be managed effectively with topical antibiotics, others necessitate the use of oral antibiotics. Various bacterial skin diseases come in the following forms: Cellulitis, Impetigo, Boils, and Hansen's disease (leprosy).

*Biophytum sensitivum* (L.) DC (family: *Oxalidaceae*) has been used in the Indian indigenous system of medicine, Ayurveda, for the treatment of various health ailments (Sakthivel & Guruvayoorappan, 2012). Medicinal plants have always remained an important source of drugs. *B. sensitivum* (L.) DC (family: *Oxalidaceae*), commonly known as "life plant," is a mesophytic under-shrub growing in slightly moist places (Chandra Kala et al., 2014). The plant has been extensively studied for its various biological activities and therapeutic potentials such as analgesic, anti-pyretic, anti-inflammatory, immunomodulatory, antitumor, antidiabetic, antioxidant, antibacterial, antihypertensive, chemoprotective, radioprotective, antifertility, etc (Pawar & Vyawahare, 2015; Jasim et al., 2024; Nair & Mallya, 2025). The aim of the study is to formulate an anti-bacterial cream incorporating *Biophytum sensitivum* D.C. extract and evaluate its physicochemical properties, antimicrobial efficacy, and overall stability.

## 3. MATERIALS AND METHODS

### 3.1 Materials

#### 3.1.1 Plant material collection

The plant *Biorhythm sensitivum* was collected from Kothanallur of Kottayam district, Kerala, India. This plant was authenticated by prof V. Biligiriranga, MSc,

Ph.D. Chairperson PG Department of Botany JSS College Ooty Road, Mysore. The plants were dried in sunlight for 2-3 weeks and powdered.

## 3.2 Plant Profile

### 3.2.1 Taxonomy (scientific classification)

*Biophytum sensitivum* is a fascinating plant species belonging to the Kingdom Plantae. Classified within the Phylum Tracheophyta and the Division Magnoliophyta, it falls under the Class Magnoliopsida and the Order Oxalidales. Within the plant taxonomy, it is further identified in the Family Oxalidaceae. The genus *Biophytum* encompasses various species, with *B. sensitivum (Abhirama, B. R., & ShanmugaSundaram, R. J. (2018), Chatterjee, T.K. et al (2008) and Chitravel, R & Kaliyaperumal, S et.al.,(2018)).*

**Fig. 1*. Biophytum sensitivum***

### 3.2.2 Morphological description of the plant

It is a tiny plant that rarely grows taller than 2.5-20 cm and has an unbranched, upright stem. The plant can be recognised by a pure yellow flower which is a bit smaller than the heterostyled plants.

## 3.3 Chemical Constituents

Whole plant extracts of *Biophytum sensitivum* contained pectin, essential oil, polysaccharides, phenolic and polyphenolic compounds, saponins, and other compounds. From the aerial parts of Biophytum sensitivum, two biflavones (cupressuflavone and amentoflavone), three flavonoids (luteolin 7-methyl ether, isoorientin, and 3'-methoxyluteolin 7-O-glucoside), two acids (4-caffeoylquinic

acid and 5-caffeoylquinic acid), and four flavonoids were isolated (Pawar & Vyawahare, 2014).

**Table 1. Description of the morphological characteristics of the plant**

| Plant parts | Description |
|---|---|
| Leaves | The terminal pair of leaves abruptly, opposing 6-12 pairs, up to 1.5 cm long leaflets, is the largest. Lateral nerves are fine, numerous, and oblique, and the peduncles are slender, shorter, and heavily pubescent. |
| Flowers | Flowers in umbels of 4-8, calyx 12mm across, pedicles three times as long as the sepals, and bracts subulate. The approximation length of the striate, subulate-lanceolate sepals is 7 mm. |
| Capsules | Capsules that are as long as sepals. |
| Fruits | In actuality, fruits are shorter than calyxes, ellipsoid capsules. |
| Seeds | Tuberculate seeds have been discovered. |

## 3.4 Methodology

### 3.4.1 Preparation of extracts

Using aqueous, alcohol and hydroalcoholic solutions in the ratio of 90:10 extraction of aerial parts of *Biophytum sensitivum* has been carried out. A hydroalcoholic solution of about 1500ml was added to the powdered aerial parts. The extraction was carried out by the cold maceration method for 4 days. It was filtered using filter cloth and filter paper using a vacuum filter. 1000ml of filtrate was obtained. Using a rotavapor for distillation, the solvent was then evaporated. The temperature was chosen for cooling, 175 mmHg of pressure, and a boiling point of 60 °C. We have collected a sample of about 100ml. When a thin membranous layer had formed, the sample was scraped off and kept in an airtight container on a petri plate over a boiling water bath (Abhirama, B. R., Rajagopal, S. S., & Nanjan, M., 2017).

### 3.4.2 Microscopic evaluation

A thin portion of the plant was cut with a sharp blade, stained with phloroglucinol, dilute hydrochloric acid and after 5 minutes thin section was transferred to a glass slide.

### 3.4.3 Powder microscopy

Dried powdered aerial parts were treated with phloroglucinol, hydrochloric acid and glycerine and observed under a microscope.

### 3.4.4 Phytochemical investigation

The present study revealed that the various alcoholic and aqueous extracts of the whole part of *the Biophytum sensitivum* plant contain alkaloids, cardiac

glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids. However, phenols, flavonoids and alkaloids were detected in the highest number of yields in the hydroalcoholic extract compared to the aqueous extract. The water-insoluble portions for aqueous extract are less, and the percentage yield is also comparatively less than hydroalcoholic extract (Suresh, K. (2017).

## 3.5 Estimation of Total Flavanoid Content

### 3.5.1 Requirements

Chemicals: Rutin, 5% sodium nitrate, 1M NaOH, 10% $AlCl_3$, Millipore water.

### 3.5.2 Procedure

*3.5.2.1 Preparation of standard*

In this study, 25mg of rutin was dissolved in 25 ml of alcohol (1mg/ml) and considered as solution A. Then, a 25 ml volumetric flask is filled with Millipore water after 2.5 ml of the solution has been transferred to it and is considered as solution B. From solution B, pipette out 1,2,3,4 and 5 ml of solution into test tubes and add 2ml Millipore water and 5% sodium nitrate solution. Keep it aside for 5 minutes. After 5 minutes, add $AlCl_3$ 10% solution 0.3 ml to all the test tubes. After $6^{th}$ minute, add 1M NaOH to all the test tubes and make up the volume using Millipore water. Absorbance is measured at 510nm.

## 3.6 Preparation of Sample

The given plant extract, 10mg, is dissolved in 10ml Millipore water (1mg/ml). From the above solution, pipette out 100µl and 200µl solution using a micropipette and transfer into a test tube. Add 2ml Millipore water and 5% sodium nitrate solution 0.3ml and keep it aside for 5 minutes. After 5 minutes, add $AlCl_3$ 10% solution 0.3 ml to the test tube. After $6^{th}$ minute, add 1M NaOH to the test tubes and make up the volume to 10ml using Millipore water. Absorbance is then measured at 510 nm.

## 3.7 Estimation of Total Phenol Content

Requirements: Gallic acid, $F_C$ reagent, 8% $Na_2Co_3$ solution.

## 3.8 Preparation of Standard

In this investigation, 25mg of gallic acid is dissolved in 25ml Millipore water (1mg / mL) and is considered as solution A. From solution A, 10ml of solution is transferred into a 100ml volumetric flask and filled up to the volume using Millipore water and is considered as solution B. From solution B 10, 20, 40, 60,80,100µg/ml is transferred to test tubes and 1ml Fc reagent is added and kept aside for 3 minutes. After 3 minutes, add 1ml sodium carbonate solution to each

of the test tubes and make up the volume using Millipore water it is then kept for 90 minutes in a dark place, and after that absorbance is measured at 765 nm.

## 3.9 Preparation of Sample

The given plant extract 10mg is dissolved in 10 ml of Millipore water. From the above solution pipette out 100 µl and 200µl using a micropipette and transfer into a test tube. Fc reagent is added to the test tube and kept it aside for 3 minutes. After 3 minutes, add 1ml sodium carbonate solution to the test tube and the volume is made up to 10ml using Millipore water. It is then kept aside for 90 minutes in a dark place. Absorbance is measured at 765nm (Fattahi S et.al., 2014), Kalita, P et al., (2013)

## 3.10 TLC of Plant Extract

A slurry is created by combining water and silica gel. This slurry is uniformly mounted onto a glass plate. This plate is dried in an oven at 110 degrees Celsius. The plate was removed from the oven after one hour. Over 1 cm from the bottom edge, a baseline is drawn. On the glass plate, a plant extract and alcohol mixture has been applied. Ethyl acetate: formic acid: Glacial acetic acid: water in the ratio of 100:11:11:27 was taken as the mobile phase. Separation was viewed under UV long light, UV short light and the fluorescence in the UV cabinet.

## 3.11 Antimicrobial Assay

### 3.11.1 Pathogenic culture and growth conditions

**Bacterial pathogens**: The pathogenic culture *Escherichia coli* Microbial Type Culture Collection and Gene Bank 118 was grown in BHI (Brain Heart Infusion) media for 24 h at 37°C. Sample concentration used: 2, 4, 6 mg/ml (Brantner, A et al., (1994).

**Well diffusion assay:** Poured into sterile petri dishes, BHI media (1.5% w/v agar) was pre-inoculated with bacterial pathogen (1% v/v) and allowed to harden. Utilising a sterile corn borer, 4 mm wells were created and inoculated with the specified sample concentration. For proper diffusion, the plates were maintained at 4C for 30 minutes. Plates were then incubated at 37°C for 24 hours while being watched for an inhibitory zone (mm in diameter). As a positive control, vancomycin (1 mg/ml) was utilised (Kumar, P.T et al., 2013), Varghese, L. S., et al., 2019)

## 3.12 Analysis Using Liquid Chromatography- Mass Spectroscopy

The plant extracts were diluted in 20 liters of methanol prior to the LC-MS/MS (MRM mode) investigations, which were conducted with an LC system connected to the same mass spectrometer, model QTRAP® 5500 (AB Scitex). (Ultimate 3000, Dionex). Nitrogen was utilised as the curtain gas (set to 15), and

Quick polarity switching (50 ms) was used to complete analyses in both the positive (PC) and negative (PE, PS, PA, PI, and PG) modes. Analyses in the negative (PE, PS, PA, PI, PG) and positive (PC) modes were finished with rapid polarity switching (50 ms). With nitrogen set to 15, 20, and 0 for the curtain gas, gas1 and gas2 were used. The delustering potential was either fixed at +40 V or 4,500 or 5,500 V without needle heating. or varied between 180 and 85 V. Additionally, nitrogen served as the collision gas, and Based on the compound, the collision energy varied between 48 and 62 eV and +47 eV. 3 ms was chosen as the dwell time. 21 positive and 127 negative MRM images were used for MS/MS studies. At room temperature (22 °C), HILIC separations were performed on a 1502 mm Luna NH2 100 pore size columns with 5 µm particles from Phenomenex in Le Pecq, France. Eluent A was composed of water plus formic acid and NH4OH, while eluent B comprised acetonitrile. The following was the gradient elution schedule: 0 min., 95% B; 15 min., 50% B. 3-L sample volumes were injected at 200 litres per minute of flow. Standard phase separations were carried out at 22 °C on a 150 mm by 2 mm Luna 5 Silica (2) column containing 5 µm particles and 100 pore size (Phenomenex). CHCl3, CH3OH, H2O, and EtNH2 made up eluent A, and CHCl3, CH3CN, CH3OH, H2O, and EtNH2 made up eluent B. The following was the gradient elution program: 0% B for 0 minutes, 40% B for 8 minutes, and 60% B for 32 minutes. A flow rate of 200 l/min was employed, and 3 L of sample were injected. On a 150 mm x 1 mm Synergic Fusion-RP column with 80 pore size, 4 m particles (Phenomenex), Phase separations in reverse were carried out at 40 °C. Eluent A contained isopropanol, CH3OH, and water, while eluent B contained isopropanol, NH4OH and formic acid. 0 minutes, 30% B, 5 minutes, 50% B, 30 minutes, 80% B, 31–41 minutes, 95% B, 42–47 minutes, 5% B, and 52–62 minutes, 30% B comprised the gradient elution schedule. Three-litre sample volumes were injected at a 40 L/min flow rate (Bure C et al., 2013), Jirovetz, L., et al., (2004) Rajeswari, P et al., 2015).

## 4. RESULTS AND DISCUSSION

The outcomes of our project-related work are as follows;

### 4.1 Preliminary Phytochemical Investigation

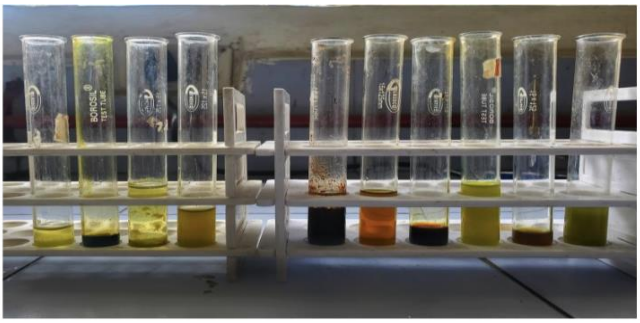

**Table 2. Data of preliminary examination**

| Experiments | Observations | Aqueous Extract | Hydroalcoholic Extract |
|---|---|---|---|
| Test for flavonoids<br>i. Alkaline reagent test<br>ii. Lead acetate test | A yellow tint developed, which was eliminated upon the addition of diluted acid. | Flavonoids are present | Flavonoids are present |
| | Formation of yellow colour precipitate. | Flavonoids are present | Flavonoids are present |
| Test for phenolic compounds | | | |
| i. Ferric chloride test | Formation of bluish - black colour. | Phenols are present | Phenols are present |
| ii. Lead acetate test | Formation of milky white precipitate. | Phenols are present | Phenols are present |
| Test for alkaloids<br>Wagner's test | Formation of red precipitate | Alkaloids are present | Alkaloids are present |

## 4.2 Estimation of Total Flavonoid Content

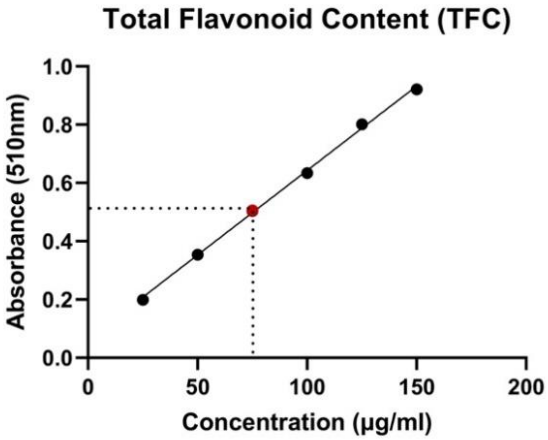


**Fig. 2. Graphical representation of concentration vs absorbance**

The total flavonoid content for the plant extract of *B.Sensitivium* was found to be 0.606.

## 4.3 Estimation of Total Phenolic Content

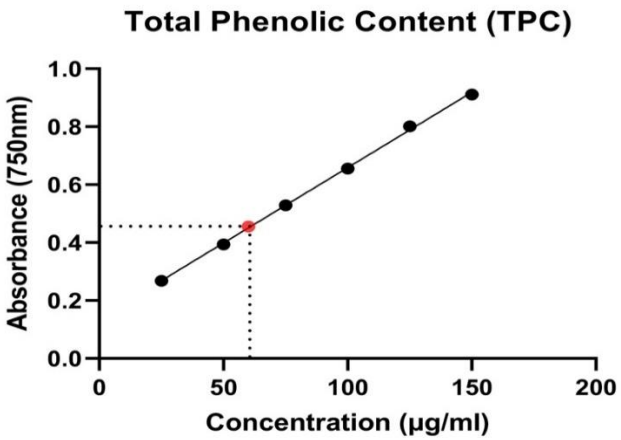


**Fig. 3. Graphical representation of concentration versus absorbance**

The total phenolic content for the plant extract of *B. Sensitivium* was found to be 0.556.

## 4.4 Analysis Using Liquid Chromatography- mass Spectroscopy

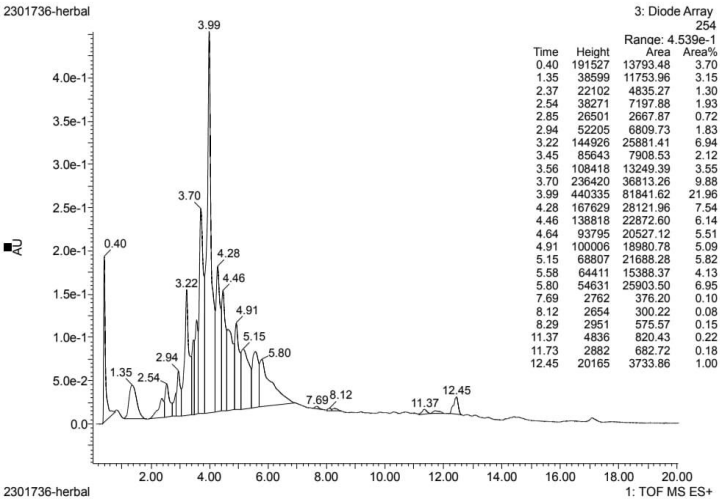


**Fig. 4. Graphical representation of molecular weight of highest peak concentration**

The highest peak time is observed at 3.99, height is 440335, area 81841.62, percentage area is 21.96. The molecular weight of the highest peak was found to be 432.137.

The compound which having a molecular weight of 432.137 is *vitexin.* Vitexin, also known as apigenin flavone glycoside, is a naturally occurring FLAVONOID family molecule (Lima et al., 2018). The antibacterial properties of vitexin have been used for a long time (Babaei et al., 2020).

## 4.5 Antibacterial Assay

**The antibiotic used is vancomycin.** When compared with the standard drug, the 50mg/ml concentration exhibits significant activity. Although 25mg/ml concentration shows comparatively lower efficacy, it still demonstrates the ability to inhibit bacterial growth. The lot of microorganisms resistant to vancomycin it may indicate that this plant can be an alternative choice for vancomycin.

## 4.6 Evaluation of Cold Cream

The formulation and evaluation techniques of herbal cream as per Babu, Raja, et al. (2022), Bharati, A. C., & Sahu, A. N. (2012)

**pH of the cream:** The pH of the cream was found to be 6.2. The herbal formulation had shown a pH closer to the skin.

**Appearance:**

Colour: Pale- Green
Odour: Characteristic
Texture: Smooth

**Viscosity: The** Viscosity of the cold cream was found to be 166.

**Acid and Saponification value:**

Saponification value: 23.4
Acid value :5.9

**Irritancy test:** The cream which has been formulated did not cause any irritation on application to the skin.

**Homogeneity test:** The visual appearance and texture were proven to be satisfactory.

**Spreadability test:** The spreadability test showed that the cream had good spreading ability.

**Dye test:**

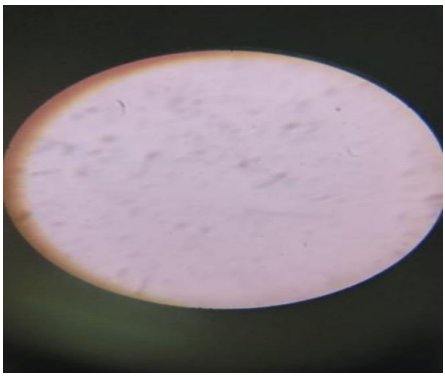

**Fig. 5. Showing under the microscope, the dispersed molecule was found to be colourless**

The dispersed molecule was found to be colourless on a red coloured background. So, it is confirmed that the formulated herbal cream is a w/o type of emulsion.

## 5. CONCLUSION

In this study, an antibacterial cream was formulated using the hydro alcoholic extract of the plant *B. Sensitivum*. The cold cream base used in the formulation of this cream hydrates the skin and leaves it feeling non-greasy after application. After it was discovered that a cream worked best for the intended purpose, these were chosen for additional assessment based on their physical characteristics, in vitro antibacterial activity, and safety with regard to skin irritation and allergic sensitisation. A major finding of this study is that hydro hydroalcoholic extract of the plant *B. Sensitivum* has a synergistic antibacterial activity when compared to the standard drug vancomycin.

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The Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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# Dose Justification for Nanoparticle Formulation

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## ABSTRACT

Dose justification refers to the scientific rationale behind selecting a specific dose of a drug for human use. With the emergence of nanotechnology in pharmaceuticals, particularly nanoparticle formulations, this concept has evolved significantly. This study aims to shed light on the different approaches to justifying the dose of nanoparticles, using ibrutinib nanoparticles as an example. It also examines the relation between the dose and bioavailability, alongside considering relevant pharmacokinetic (PK) and pharmacodynamic (PD) parameters. The biopharmaceutical factors like Cmax, Tmax, and AUC are also considered for dose justification. The comparison of the radiation dose and nanoparticle dose taken internally or externally, and their relation, is highlighted. The NOAEL (No Observed Adverse Effect Level) and its limitations are discussed. The human equivalent dose and inhumane studies were listed along with the equivalent dose of nanoparticles. The Equivalent Dose Model, used in nanotoxicology, is presented as it calculates dose based on nanoparticle surface area rather than mass alone. Comprehensive data support the approval of the 50 mg nanoparticle dose, providing patients with a more effective and safer treatment option. Project Optimus by the FDA, which aims to refine and simplify oncology dose selection, is also considered. Key nanoparticle-specific factors like surface area and zeta potential are explained with the regulatory approvals. Nano drug formulations offer significant improvements in solubility and bioavailability. Incorporating PK/PD data, toxicity thresholds, nanoparticle-specific models, and regulatory guidelines ensures that the new dose maintains safety and efficacy. Finally, the integration of in silico modelling, Quantitative

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Structure-Activity Relationship model, and machine learning is proposed to enhance dose predictions, with standardised weighting factors suggested to improve risk assessment.

*Keywords: Nanoparticle; drug delivery; pharmacokinetics; pharmacodynamics; dose justification; equivalent dose modelling.*

## 1. INTRODUCTION

Before going for dose justification, a brief overview of the drug and its therapeutic use is necessary, along with the potency and half-life of the drug. For a new formulation, special population, or novel route of drug delivery, dose justification is needed. Along with the Drug Profile, the Mechanism of Action, Indications, and Pharmacokinetics (ADME).

Dose justification refers to the scientific rationale behind selecting a specific dose of a drug for human use. With the emergence of nanotechnology in pharmaceuticals, particularly nanoparticle formulations, this concept has evolved significantly. Nanoparticles enhance drug solubility, bioavailability, and therapeutic outcomes. However, due to changes in pharmacokinetics (PK) and pharmacodynamics (PD), dose optimisation is essential to balance efficacy and safety.

The poor solubility and bioavailability limit the drug's efficacy. Nanoparticles in solution form increase the surface area available for dissolution, significantly enhancing solubility and, consequently, bioavailability. In this, a lower dose of the nanoparticle formulation may achieve the same or better therapeutic effect as a higher dose of the conventional formulation due to improved absorption.

The justified dose release of the drug depends on patient-related factors like age and disease condition.

Factors influencing drug dosing include its solubility (affects dissolution and absorption), absorption window (limits the time for uptake), half-life (determines dosing frequency), therapeutic window (defines safe and effective range), mechanism of action (influences dose–response), disease condition (alters drug needs), patient compliance (affects regimen simplicity), site of action (impacts distribution), stability in pH/enzymes (affects bioavailability), and resistance or tolerance (may require dose adjustment).

There are different methods to measure TI (Chinedu et al., 2013; Ramakrishnan & Dhanavelu, 2018).

- Karber's method.
- Fixed dose method.
- Reed-Muench method.
- Miller & Tainter method.

- Lorke method.
- Up & down method.

The Therapeutic Index (TI) is used to compare the therapeutically effective dose to the Toxic dose of a pharmaceutical agent. The TI is a statement of the relative safety of a drug. It is the ratio of the dose that produces toxicity to the dose needed to produce the desired therapeutic response. The common method used to derive the TJ is to use the 50% dose-response points, including TDSO (toxic dose) and ED (effective dose) 50.

$$\mathbf{TI} = \frac{\textit{Toxic dose}}{\textit{Dose for Therapeutic response}} = \frac{\mathrm{TD50}}{\mathrm{ED50}}$$

A clinician would consider a drug safer if it had a TI of 10 than if it had a TI of 3. However, the use of the ED50 and TD50 doses to derive the TI may be misleading about a drug's safety, depending on the slope of the dose-response curves for therapeutic and toxic effects. To overcome this deficiency, toxicologists often use another term to denote the Margin of Safety. The Margin of Safety (MOS) is usually calculated as the ratio of the toxic dose to 1% of the population (TDOI) to the dose that is 99% effective to the population (ED99) ToxTutor. (n.d.).

Along with the TI lethal dose is very important. $LD_{50}$ stands for "lethal dose, 50%": the amount of a substance required to kill 50% of test subjects, typically determined using animal studies (e.g., rats, mice) and expressed in mg/kg of body weight. $LD_{50}$ serves as a comparative measure for acute toxicity. However, translating animal-based $LD_{50}$ data to humans is complex due to interspecies differences. Opioids (Analgesics) like Fentanyl (synthetic opioid): Even < 2 mg can be fatal; 42% of tested counterfeit pills contained ≥ 2 mg, estimated to be a lethal dose. Stimulants like Cocaine have Fatal doses that range from 30 mg to 1.2 g, significantly influenced by user-specific factors. For dose justification, therapeutic window data of drugs should also be considered.

## 2. DOSE CALCULATION FOR SUSTAINED RELEASE (ROBINSON & ERIKSEN, 1966)

Famotidine gastric floating matrix tablets (GFMT) were designed for stomach and upper small intestine absorption using the Robinson and Eriksen PK calculation, with a total dose of 50 mg (8.57 mg initial, 3.68 mg/hour maintenance) for zero-order release over 12 hours. Effervescent GFMTs (EGFMT) using WSR 303, WSR N-12K, and polyethene oxide sustained release for 12–23 hours with zero-order kinetics and non-Fickian diffusion. Non-effervescent GFMTs (NEGFMT) with glyceryl behenate maintained buoyancy for over 24 hours and provided stronger release control, especially with melt granules. Both systems achieved a rapid onset within 15 minutes and prolonged pharmacological action. The optimised formulations showed about a two-fold increase in bioavailability with good IVIVC, ensuring predictable drug performance (Kaur et al., 2025).

**Pharmacodynamic Studies:** To assess the pharmacodynamic (PD) effects of the nanoparticle formulation, we have to measure the therapeutic effect and onset of action in animal models and clinical trials. After that Therapeutic outcomes of nanoparticle and conventional formulations at different doses are compared. As a result, PD profiles are enhanced with a quicker relief of symptoms or enhanced therapeutic effects, and can support dose reduction or confirmation of an effective lower dose. Nanoparticle formulation does not increase toxicity. The toxicology studies are needed to compare the safety profile of the nanoparticle and conventional formulations. Potential increase in adverse effects due to the increased bioavailability. If there is no increase in toxicity despite enhanced bioavailability, then we can justify the chosen dose (Enna & Bylund, 2014).

IVIVC models predict how the nanoparticle formulation behaves in the body. Strong IVIVC can provide predictive justification for dose adjustments. To meet regulatory requirements for dose justification, comprehensive data from preclinical and clinical studies to regulatory agencies must be provided. Using this data, we can justify the proposed dose. Nanoparticles of the Drug increase its solubility by 10-fold. Pharmacokinetic Improvement: PK studies show that a 50 mg dose of the nanoparticle formulation achieves the same Cmax and AUC as a 200 mg dose of the conventional formulation. PD studies demonstrate quicker symptom relief and better overall efficacy at the 50 mg nanoparticle dose. Toxicology studies indicate no increase in adverse effects at the 50 mg nanoparticle dose compared to the 200 mg conventional dose. Phase III trials confirm that the 50 mg nanoparticle dose is as effective as the 200 mg conventional dose in treating the target condition (Rui et al., 2025; Nayak et al., 2025; Hossain et al., 2025).

Comprehensive data support the approval of the 50 mg nanoparticle dose, providing patients with a more effective and safer treatment option. The Oncology field has evolved rapidly over the last few decades, significantly improving clinical outcomes and quality of life for patients with cancer. Project Optimus, introduced by the U.S. Project Optimus by FDA, aims to modify and improve oncology dose selection. Many workshops, like the Oncology Dose Optimisation IQ Working, provided tool kits and ideas to justify the dose. Traditional MTD-based dosing is outdated for targeted therapies and new modalities. There is a need for individualised, case-specific dose optimisation strategies. The surveys considered evidence-based approaches and diseases, population, mechanisms, and therapeutic index (Samineni et al., 2024).

**Drugs and Different Formulations:** Ibrutinib has low oral bioavailability (only 2.9%) because it has poor solubility and undergoes extensive first-pass metabolism. Patients need high daily doses (420–560 mg) to achieve therapeutic effects, which increases the risk of side effects. The bioavailability of ibrutinib also changes with food, leading to variable drug exposure. To overcome these problems, the researchers developed nanosponges using hydroxypropyl-β-cyclodextrin (HPβCD). These nanosponges increase solubility, improve absorption, and release the drug slowly, which helps in maintaining steady drug

levels. In animal studies, the new formulation showed 6.45 times higher maximum drug concentration (Cmax) and 14.96 times more total exposure (AUC) than plain ibrutinib. Therefore, this new nanosponge system allows lower doses to be used while still achieving better effects, reducing side effects, and improving treatment (Sampathi et al., 2025).

The Human Equivalent Dose (HED) is calculated from animal data. A safety factor (commonly 10) is applied for interspecies differences. The goal is to achieve exposure (AUC, Cmax) similar to that observed in effective preclinical models. Due to higher systemic exposure, the conventional 560 mg dose could be reduced to 100–200 mg in the nano form to maintain equivalent therapeutic levels. Clinical and animal bridging studies show Similar BTK inhibition, Comparable AUC, and Faster onset of action.

**MRSD (Maximum Recommended Starting Dose):** Calculated using NOAEL-derived HED and applying appropriate safety factors. There are several Theoretical Dose Adjustment Models based on Biowaiver justification, *ivivc,* etc. For example, Ibrutinib nanoparticles improve solubility, dissolution, and bioavailability, enabling faster and more consistent absorption. They can achieve equivalent therapeutic exposure (AUC) at ~25–40% of the conventional dose, reducing toxicity risk from high Cmax. PK/PD data and animal studies show sustained BTK inhibition at lower doses (100–200 mg/day). Switching formulations may require dose titration, TDM, and consideration of patient-specific factors. Dose justification should rely on exposure–response data, PK modelling, and clinical validation (FDA, 2005).

To calculate the dose for nanoparticles (NP), dose metrics and A proper dose concept are crucial. A general dose calculation model for nanomaterials is not available. Here, we propose how to develop a dose assessment model for NP in analogy to the radiation protection dose calculation, introducing the so-called “deposited and equivalent dose”.

As a dose metric, we propose the total deposited NP surface area (SA), which has been shown frequently to determine toxicological responses, e.g., of lung tissue.

The deposited NP dose is proportional to the total surface area of deposited NP per tissue mass, and takes into account primary and agglomerated NP and physico-chemical properties of the NP that influence the biological responses. These weighting factors consider the specific surface area, the surface textures, the zeta-potential as a measure for surface charge, the particle morphology, such as the shape and the length-to-diameter ratio (aspect ratio), the band gap energy levels of metal and metal oxide NP, and the particle dissolution rate and weighting factors on the equivalent dose of the deposited NP.

There is a need for high-throughput techniques to assess the potential toxicity of nanomaterials. Despite the lack of complete toxicological data, regulatory

agencies must still make safety decisions. Therefore, suitable dose models must be identified to support reliable dose assessment for nanoparticles (NPs).

Surface area, rather than mass alone, is proposed as a better metric for toxicity prediction. A new dose model, therefore, considers the total surface area of deposited NPs per unit tissue mass.

Nanoparticles primarily enter the body via the respiratory, digestive, and skin pathways. This paper focuses on inhalation exposure, excluding intravenous routes used in nanomedicine. Inhalation studies have shown that over 90% of NPs deposited in the lungs' alveolar region are retained, gradually translocate into the bloodstream, and accumulate in various organs. Gastrointestinal uptake has been less studied, though NP ingestion (e.g., in food) is increasing. However, typical oral exposure levels are not considered toxic. Skin absorption of NPs is minimal and thus excluded.

Mathematical models like Quantitative Structure Activity Relationship (QSAR) help predict NP toxicity based on their physico-chemical properties (Balraadjsing et al., 2024). QSAR models also extrapolate findings from tested compounds to untested ones. Nano-QSAR use remains limited due to nanomaterial complexity and unknown behaviours in biological systems.

Among various toxicity mechanisms, oxidative stress is widely recognised as a key mode of NP-induced damage. Excess reactive oxygen species (ROS), generated through immune responses, can lead to lipid peroxidation, protein and DNA damage, enzyme inactivation, and inflammation. Under normal conditions, antioxidant systems balance ROS levels. However, NP-induced oxidative stress disrupts this balance, contributing to cytotoxicity, inflammation, and potential disease development. Thus, this model focuses on oxidative stress as the primary toxicological endpoint.

The concept for dose assessment of nanoparticles uses the total surface area of the NP as a dose metric. This concept is influenced to a large degree by the dose concept developed within the field of radiation protection over the last six decades, and it also has the potential to complement nano-QSAR models.

**Deposited dose:** This is the total surface area of nanoparticles (NPs) that gets deposited in a specific amount of tissue (measured as $m^2$ of NP surface per kg or $m^3$ of tissue). This surface area is important because it can trigger biological effects in the body. This dose includes both individual NPs and those that are clumped together (agglomerated).

**Equivalent dose:** This goes a step further. It adjusts the deposited dose by also considering other physical and chemical properties of the NPs that can influence how harmful they are. The factors considered as Deposited Dose Matters More Than Mass, Agglomeration Is Considered, Equivalent Dose Is a More Advanced Metric, and Physico-Chemical Properties Drive Toxicity.

## 3. ABSORBED DOSE OF IONIZING RADIATION

The absorbed dose is a key concept in radiation safety. It measures the energy from radiation absorbed by a small amount of matter and is expressed in grays (Gy). When considering internal exposure, like when radioactive material enters the body, the absorbed dose for a specific organ over time (usually 50 years for adults) is calculated using the dose rate at each moment.

### 3.1 Dose Comparison: Radiation vs. Nanoparticles (NPs)

Radiation dose is based on energy deposited per kilogram of tissue, while for nanoparticles, dose is measured by the surface area of NPs deposited per tissue mass. Other dose types—like dose rate, committed dose, equivalent dose, and effective dose—have parallels in nanotoxicology. For NPs, these doses are adjusted using factors that reflect their unique properties (like surface area or chemical behaviour), just as radiation doses are adjusted for radiation type. To estimate how much radiation reaches specific organs, scientists use detailed models. These consider how long the radioactive material stays in the body, how it moves through organs, and what kind of radiation it gives off. Established models, like those by ICRP, are used for the respiratory and digestive tracts to simulate this process.

### 3.2 Equivalent and Effective Dose of Radiation

Not all types of radiation cause the same damage, so scientists use "weighting factors" to adjust doses based on how harmful each type is. The effective dose accounts for the fact that different organs are more or less sensitive to radiation. For example, bone marrow and lungs are more sensitive than skin or the brain, so their doses are weighted more heavily.

The Nanoparticles (NPs) can enter the body by inhalation, ingestion, skin contact, or medical injection, with workplace inhalation being the most studied. NP dose is often measured by the total surface area deposited in the body, changing over time as particles degrade or move to other organs. Weighting factors based on NP properties (size, shape, surface charge, etc.) adjust the deposited dose to better estimate biological impact. Factors like specific surface area, texture, zeta potential, shape, band gap energy, and dissolution rate influence NP reactivity and potential harm. NPs have a higher specific surface area, making them more chemically reactive and potentially more toxic.

### 3.3 A New Way to Measure Nanoparticle (NP) Risk, like we do with radiation

Scientists are trying to find better ways to compare how harmful different nanoparticles (NPs) are. Just like in radiation science, where we use concepts like absorbed dose, equivalent dose, and effective dose, they propose a similar system for NPs.

## 3.4 Equivalent Dose (HNP, T)

To calculate how dangerous a nanoparticle is, multiply the deposited dose (how much lands in the organ or tissue) by the product of all weighting factors. This gives the equivalent dose, which shows how damaging the NP really is, taking its unique properties into account. Right now, there's not enough data to say how different tissues or organs respond to NPs. In the future, researchers hope to add tissue-specific weighting factors, like we do for radiation. It is a Useful Starting Point. This new dose model for nanoparticles could help scientists compare and rank the risks of different NPs more accurately. While it needs more data and refinement, especially for the effective dose, it's a step toward safer nanotechnology and better regulation.

The Equivalent Dose Model is used in nanotoxicology; this model calculates dose based on nanoparticle surface area rather than mass alone.

## 3.5 Deposited and Equivalent Dose

Deposited Dose (DD): Total NP surface area per tissue volume ($m^2/kg$ or $m^2/m^3$)

Equivalent Dose (ED) = DD × ∑ weighting factors for NP characteristics.

Clinical Trial Validation in all the phases is required, and adjustments may be needed for Hepatic/Renal Impairment, the Elderly, Pediatric population.

## 3.6 Human Trials of Monoclonal Antibodies (Mabs)

In human trials of monoclonal antibodies (mAbs), it was found that due to low toxicity, the recommended phase II dose (RP2D) was often uncertain or based on limited data. They searched scientific databases for clinical trials (other than the first human trials) involving mAbs. They analysed how doses were chosen and compared those to the initial trials and doses used in trials for FDA-approved mAbs. In 37 follow-up trials, dose guidance (RP2D) was still unclear. Some phase II/III trials tested only the original FIHT dose, while others used different doses without explaining why. FDA registration trials often used doses much lower than was tested in early trials. Introduction to FIHT and RP2D First-in-human trials (FIHTs) help find the maximum safe dose (MTD), which usually guides future dosing (RP2D). But for targeted therapies like mAbs, the RP2D often doesn't match the MTD, making dose selection harder.

## 3.7 Specific mAb Challenges (Viala et al., 2018)

Monoclonal antibodies usually have fewer side effects because they don't target healthy cells. That makes it hard to find the MTD. The RP2D is often chosen based on blood levels or biological effects, but these are not always reliable predictors of effectiveness. The study aimed to understand how mAb doses are selected after FIHTs by reviewing more advanced trials and comparing them to early data.

They searched medical journals for trials on specific mAbs and collected data on disease type, dose, schedule, and how the dose was selected. They analysed how later doses matched or differed from the original trials. The General Trial Data reviewed 144 follow-up trials of 42 mAbs. Most were for cancer and used intravenous dosing. Some used fixed doses instead of adjusting by weight, as in the earlier trials. But only a few trials found a true MTD, and the reasoning behind RP2D was often unclear. In 103 later-stage trials, the dose was rarely the same as the one recommended in FIHTs. Often, doses were picked without clear reasons or based on early effectiveness or blood levels or safety data.

They looked at 60 trials of 27 FDA-approved mAbs. The doses used in these trials were usually lower than those tested in FIHTs, and the severe side effects in later trials were often not predicted in early trials. There is a lot of uncertainty when choosing doses for mAbs in later trials. FIHT results often don't guide dose selection. Preclinical data or blood concentration levels are commonly used instead, but may not reflect actual tumour activity.

Severe side effects often don't appear until late in development. Early trials may miss them because they are short and don't use full dosing. Using doses that are too high or too low can lead to problems. Better dose selection might be achieved by studying how the drug affects biological markers. But these approaches require more data, like repeated biopsies or advanced blood testing. This short window may miss important side effects or drug behaviour. Longer observation or multiple doses may give better safety and dose information. Current early trial methods may not be suitable for mAbs. Standard endpoints like MTD (*Maximum Tolerated Dose)* and RP2D (*Recommended Phase 2 Dose)* often don't help in later stages. New trial designs focusing on biological markers and dose comparison are needed.

## 3.8 Trastuzumab Formulation (Tamura et al., 2019)

Dose justification in oncology. Investigators analysed data from a Phase I (J101) and early Phase II (DESTINY-Breast01) trials of fam-Trastuzumab Deruxtecan (DS-8201a) in HER2-positive breast cancer. DS-8201a is a next-generation HER2-targeted antibody–drug conjugate (ADC) with a high drug-to-antibody ratio and a cleavable linker to deliver a topoisomerase I inhibitor payload. Dose levels between 0.8 and 8.0 mg/kg were assessed in J101, with 5.4 and 6.4 mg/kg selected for expansion due to favourable safety and efficacy profiles. A population pharmacokinetic (PPK) model was developed to calculate individual exposure metrics ($C_{min}$, $C_{max}$, AUC), which were then linked to efficacy and safety endpoints through exposure–response (ER) analyses using logistic regression and Cox modelling. At J101 cutoff (~April 18, 2018), HER2+ BC patients treated at 5.4 and 6.4 mg/kg had confirmed ORRs of 52.6% and 55.7%, respectively. Grade ≥3 adverse events occurred in 35.6% (5.4 mg/kg) vs. 50% (6.4 mg/kg). ER analysis found a significant correlation between higher intact $C_{min}$ and increased ORR (P = 0.035), with a trend toward longer progression-free survival at higher exposure (P = 0.238). Safety-related exposures were significantly associated with neutropenia, anaemia, thrombocytopenia, dose

reductions or discontinuations due to adverse events, and risk of interstitial lung disease (ILD) (P-values ranging from 0.003 to <0.001).

Quantitative method to determine nanoparticle dose, the techniques like Transmission Electron Microscopy (TEM) for accurate nanoparticle counts per vesicle, Flow Cytometry for large-scale population analysis of fluorescence-labelled nanoparticles (quantum dots), and Statistical modelling to link microscopy-derived particle distributions to cytometry-based dose predictions. Nanoparticle-loaded vesicles are inherited during cell division, showing that while vesicle inheritance is nearly symmetric, particle dose to daughter cells is highly asymmetric due to variable vesicle loading.

The internalised dose of nanoparticles is quantified per cell and used to understand how these doses are inherited during cell division. U-2 OS cells were exposed to quantum dots (QDs). The results are that, on average, ~2.4 million QD particles are internalised per cell. There is significant heterogeneity in vesicle number per cell and QD load per vesicle. Dose dilution during cell division does not result in equal nanoparticle distribution due to unevenly loaded vesicles. A calibration model was developed to connect EM-based particle counts with cytometry-derived fluorescence signals. The study enhances nanotoxicology and nanopharmacology by enabling accurate dose quantification and prediction of therapeutic/toxic effects. The method helps improve dose–response modelling for nanomedicine, particularly in rapidly dividing cells like cancer (Summers et al., 2013).

## 4. CONCLUSION

Nano drug formulations offer significant improvements in solubility and bioavailability. However, these enhancements necessitate rigorous dose justification. Incorporating PK/PD data, toxicity thresholds, nanoparticle-specific models, and regulatory guidelines ensures that the new dose maintains safety and efficacy. With continued innovation in modelling and clinical strategy, dose justification for nanomedicines will become increasingly precise and patient-centred. Integration of in silico modelling, QSAR, and machine learning will enhance dose predictions. Standardised weighting factors could improve risk prediction. Personalised dose regimens based on individual response and PK profiling, ICH, FDA, and EMA expect a clear dose selection rationale. Biowaiver is generally not granted unless *ivivc* and safety are established. The dose justification of drugs about nanoparticles involves a thorough evaluation of enhanced solubility, improved pharmacokinetic and pharmacodynamic profiles, safety considerations, clinical efficacy, and regulatory compliance. This multi-faceted approach ensures that the optimised dose is both effective and safe for patient use.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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***Chapter 3***
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# A Novel Polyherbal Formulation-Sarkaraikolli Hastens Wound Healing Activity with Antidiabetic Potential

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## ABSTRACT

Diabetes is a chronic group of metabolic diseases characterised by the constantly elevated blood glucose levels, which leads to the glycation of body proteins, which may further cause serious complications. In recent years, the use of complementary medicine has seen significant growth, particularly in dietary interventions and traditional plant-based therapies derived from systems such as Ayurveda. No systematic studies have been reported for its wound healing and anti-diabetic properties of Sarkaraikolli. An effort has been made to establish the wound healing and anti-diabetic properties of the polyherbal formulation Sarkaraikolli. In this model, animals were administered with test and standard samples by the oral route. Frequently blood samples were taken and examined for blood glucose levels, which were assessed using a glucometer. The animals were sacrificed 2 hours on the 21$^{st}$ day after the treatment by cervical dislocation, and biochemical studies were performed. The blood samples were collected, and serum was separated by centrifugation at 3000 rpm for 15 minutes after a retro-orbital puncture. Different procedures were performed for the determination of liver enzyme tests. In the model of anti-diabetic animals were evenly divided into 5 groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4, and 5 were the treatment groups which were simultaneously treated with standard, 250 and 500 mg/kg extract respectively after glucose loading. Sarkaraikolli possesses significant anti-diabetic and wound healing activity when compared to the conventional medicine like Glibenclamide.

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Significant anti-diabetic action is visible due to lower blood glucose levels and maintenance of steady body weights. Sarkaraikolli wound healing activity is demonstrated by higher wound healing activity as compared to the standard medication. The biochemical parameters like SGOT, SGPT and ALP were determined by using the analytical kits. The results shown by the treatment of Sarkaraikolli were significant and are closer to the control group. This research work proved that Sarkaraikolli possesses significant anti-diabetic and wound-healing activity. When compared to conventional medicine, Glibenclamide, a significant anti-diabetic action is visible due to lower blood glucose levels and maintenance of steady body weights. Sarkaraikolli's wound healing activity is demonstrated by higher wound healing activity when compared to the standard drug (Nitrofurazone).

*Keywords: Sarkaraikolli; blood glucose; wound healing; liver enzymes; diabetes.*

## 1. INTRODUCTION

Plants are extremely beneficial to humans. Many of them are primarily utilized for medical purposes. Historical reports and centuries-old cultural traditions show that some plants can be an alternative to standard pharmacotherapy or, at least, help with treatment or have a preventative effect. Modern science is very eager to verify these properties by analysing the so-called medicinal plants for the presence of valuable bioactive compounds, including antioxidants, and the resulting interesting potential health properties (Przeor, 2022; Kifle et al., 2022). Diabetes is a chronic group of metabolic diseases characterised byconstantly elevated blood glucose levels, which leads to the glycation of body proteins, which may further cause serious complications (Susmitha *et al*., 2024). There are three types of diabetes. Type 1 diabetes (also known as juvenile diabetes or insulin-dependent diabetes) is a long-term disease in which the pancreas produces little or no insulin on its own. Second and most commonly encountered in adults is type 2 diabetes, which arises when the body becomes resistant to insulin or does not produce enough insulin for any reason connected directly or indirectly (Susmitha et al., 2024). Type II diabetes is the common, accounting for 90-95% of all cases. Insulin resistance occurs when the body does not respond effectively to insulin in Type II diabetes (Goldfine, 2001). It is linked to multiple complications such as retinopathy, neuropathy, kidney disease, and cardiovascular disease, all of which increase the risk of mortality and negatively impact their quality of life (Tonga et al., 2025).

The third kind is GDM (Gestational Diabetes Mellitus), which is linked to abnormal glucose intolerance that develops or is identified during pregnancy (Susmitha et al., 2024). Blood glucose testing times and testing frequency should be planned to suit the glucose-lowering medicine regimen and the clinical situation (Ganjal, 2021). Mothers who have GDM are at risk of developing gestational hypertension and preeclampsia, at risk of suffering from caesarean section, and at risk of inducing subsequent type 2 diabetes mellitus and cardiovascular diseases (Zhang et al., 2021). Diabetes mellitus has developed into the third most “killer”, chasing after cardio-/cerebro-vascular diseases and cancer. It is predictable that 5% of every fatality in the world is caused by

diabetes, a figure which will be elevated by 50% in the next 10 years (Kumbhare et al., 2021). It is characterised by abnormalities in carbohydrate, lipid and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis (Dongare et al., 2010; Susmitha et al., 2024).

In recent years, the use of complementary medicine has seen significant growth, particularly in dietary interventions and traditional plant-based therapies derived from systems such as Ayurveda (Kumar et al., 2025).

Wounds are visible results of cell death can be classified by size, site, depth and causation –accident, surgery, or circulatory failure. Wound healing is a process which is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other extracellular macromolecules which are latter remoulded to form Scars and collagen provides strength and integrity to the dermis (Charde et al., 2010; Devi et al., 2009).

A combination of many wound healing models would improve the reliability and validity of the data and also provide a better knowledge of the mechanisms involved in tissue repair (Abdel Halim et al., 2024).

Many indigenous tribes around the world have long suspected that this ubiquitous, annual, herbaceous plant might have medicinal wound healing properties (Jain et al., 2009). The scientific assessment of medicinal plants may result in the discovery of novel, reasonably applicable medicinal plants to treat wounds. In India; there has been interest in the potential of natural products obtained from plants and animals for development of drugs with wound healing properties (Emmanuel et al., 2011). Sarkaraikolli is a polyherbal formulation which consists of 13 vital herbal ingredients. India has more than 50.8 million people with diabetes and projected to increase to 87 million by year 2030 (Chitra Devi & Ramesh, 2018). No systemic studies have been reported for its wound healing and anti-diabetic property of sarkaraikolli. Hence, an effort has been made to establish the wound healing and anti-diabetic property of poly herbal formulation, sarkaraikolli.

**Insulin Resistance:** Insulin resistance occurs when the body is unable to utilize insulin owing to an insulin receptor deficiency. Insulin resistance precedes hyperglycemia, and hyperglycemia eventually leads to Type II diabetes (Tomas et al., 2002).

**Activity for Wound Healing:** Wounds are an unavoidable part of living. Physical, chemical, or microbial causes can all cause wounds to occur. Wound healing is a complicated network of interactions involving a wide range of cell types, cytokine mediators, and the extracellular matrix (Porth, 2014). Natural wound healing includes the stages of hemostasis, inflammation, proliferation, and remodeling. Despite the fact that the wound healing process is ongoing, with each phase overlapping the last, each phase is unique. Because effective wound

healing necessitates adequate blood and nutrition delivery to the area of wounded tissue. It involves various stage

**Inflammatory Stage:** The inflammatory stage begins shortly following the injury and can persist anywhere from 24 to 48 hours, with some cases lasting up to two weeks. The inflammatory phase activates haemostatic mechanisms, which reduce blood loss at the wound site quickly (Heinemann et al., 2012).

**Fibroblastic Stage:** Wound healing's fibroblastic phase follows the inflammatory phase and can last anywhere from 2 days to 3 weeks. This phase is divided into three stages: granulation, contraction, and epithelialization.

**Epithelialization Stage:** One of the most important aspects of wound healing is epithelial cell migration. Epithelialstem cells must travel into the wound after separating from the wound's edges. Hair follicles that have been transected also contribute to the quantity of migratory epithelial cells (Stadelmalmann et al., 1998).

**Stage of Proliferation:** Granulation stage: Fibroblasts lay a bed of collagen to cover the deficiency and create new capillaries during the phase of proliferation (Heinemann et al., 2012) (2 to 3 weeks).

**Stage of Contraction:** To eliminate shortage, wound edges draw closer.

**Stage of Epithelialization:** Crosses the wet surface cell migrate around 3 cm in all directions from the place of genesis (Ioni et al., 2009).

**Phase of Remodelling:** This stage might last anywhere from three weeks to two years. During this stage, new collagen is produced. Collagen intermolecular cross-linking via vitamin-C-dependent hydroxylation promotes tissue tensile strength. The scar flattens and the scar tissues regain 80% of their previous strength (Tamara, 2008).

## 2. MATERIALS AND METHODS

### 2.1 Materials

**Extract Preparation:** The Sarkaraikolli powder was purchased from Rajapalayam, Tamil Nadu: Aravindh Herbals. The aqueous extract was prepared by using a Soxhlet apparatus.

**Chemicals:** Alloxanmonohydrate, Dimethylsulfoxide, Carbinol, Diclofenac (Hindustan chemicals and pharmaceuticals), Glibenclamide (Medico remedies Limited), nitrofurazone, diazepam and ketamine.

All these drugs were dissolved in dimethyl sulphoxide and were freshly prepared just before the administration. Drugs are taken by the gavage route of administration through an oral feeding needle.

**Equipments:** Plethysmometer, Vernier callipers, Glucometer, Glucose measuring strips, Gavageneedle, Petridishes, Autoclave, Laminar air flow chamber, Autoanalyser.

**Analytical Kits:** Serum glutamate oxidase transaminase, Serum glutamic pyruvic transaminase and alanine transaminase were determined using the analytical kits manufactured by Tulip Diagnostics.

**Consumables:** The consumables used for this work are sterile cotton buds, sterile paper discs with 0.5 mm thickness (autoclave), sterile cotton, cotton plugs, and Aluminium foil.

## 2.2 Methodology

### 2.2.1 Analysis of anti-diabetic activity

**Alloxan induced diabetes:** Alloxan is a uride and pyrimidine of mesoxalic acid. Alloxan causes selective necrosis of the pancreatic islets of Langerhans beta cells when induced in rats.

**Animal grouping:** Each group consists of six animals of either sex and is divided into five groups. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC Reg. No. (VIPW/IAEC/1581/PO/Re/S/11/CPCSEA/MPh/002/2021-22).

**Group 1:** The Control group.
**Group 2:** The diseased group was induced with diabetes with alloxan (90 mg/kg IP)
**Group 3:** The standard group was administered with alloxan (90 mg/kg IP & Glibenclamide 10 mg/kg po).
**Group 4:** Test-1 Group administered with alloxan (90 mg/kg IP & Sarkaraikolli extract 250 mg/kg po).
**Group 5:** Test-2 Group administered with alloxan (90 mg/kg IP & Sarkaraikolli extract (500 mg/kg po).

Animals were administered with test and standard samples by oral route. The animals were sacrificed by 2 hours on the 21st day after the treatment by cervical dislocation and biochemical studies were performed and the blood samples were collected and serum was separated by centrifugation at 3000 rpm for 15 minutes after a retro-orbital puncture. Different procedures were performed for the

### 2.2.2 Determination of liver enzyme tests are as follows

*2.2.2.1 Serum glutamate oxidase transaminase (SGOT) determination*

**Procedure:**

Wavelength: 340 nm

Temperature: 370 C Light path: 1 cm

For the test, 0.8 ml of enzyme reagent (L1) and 0.1 ml of extracts were put into a clean and labelled test tube. After roughly 1 minute at 370 C, add a starter of 0.2 ml reagent, stir, and then measure the absorbance at 340 nm.

*2.2.2.2 Serum glutamic pyruvic transaminase (SGPT) determination*

**Procedure:**

Wavelength: 340 nm

Temperature: 370 C Light path: 1 cm

For the test, 0.8 ml of enzyme reagent (L1) and 0.1 ml of extracts were put to a clean and labeled test tube. After roughly 1 minute at 370 C, add a starter of 0.2 ml reagent, stir, and then measure the absorbance at 340 nm.

**2.2.3 Alkaline phosphatase (PNPP method)**

**Procedure:**

Wavelength: 405 nm

Temperature: 37oC Light path: 1 cm

Into a clean and labelled test tube, for test, 1ml of working reagent was added. The tube was incubated for approximately 1 minute at 370 C.0.02 ml of serum sample was added, mixed and absorbance was read at 405nm.

**2.2.4 Assessment of wound healing activity**

**Group 1:** The Control group.
**Group 2:** The diseased group was induced with diabetes with alloxan (90 mg/kg IP).
**Group 3:** The standard group was administered with alloxan (90 mg/kg IP & Nitrofurazone 10 mg/kg po).
**Group 4:** Test-1 group was administered with Alloxan (90mg/kg IP & sarkaraikolli extract 250 mg/kg po)
**Group 5:** Test-2 group was administered with Alloxan (90mg/kg IP & sarkaraikolli extract 500 mg/kg po).

**Incision wound model (Clark, 1993):** The percentage of wound closure, scar size, and shape were all measured on the fourth, eighth, twelfth, sixteenth, and twentieth post-wounding days (Vijayabhaskar et al., 2011). The rat models were also used to conduct wound healing activity. In this activity, the animals were anaesthetized for 18 days using diazepam and ketamine at a dose of 1 mg/kg, and the formula was used to calculate wound contraction.

%Wound closure = (wound on the first day - wound area on day (n) / wound area on first day x 100)

Where n is the number of days ($2^{nd}$,$4^{th}$, etc.).

**Excision Wound Model:** On an anesthetized rat, a circular seal 2.5cm in diameter was used to make an impression on the dorsal thoracic area 1cm away from the vertebral column and5cm away from the ear. The skin of the impressed area was excised to its full thickness, resulting in a wound with a diameter of roughly 500 $mm^2$. Blotting the wound with a cotton swab dipped n normal saline resulted in hemostasis (Lee, 1968). Contractions, which aid in wound closure in the first two weeks, were initially investigated by tracing the wound on transparent paper. The wound area was measured on days 0, 7, 14, 16 and 21 (Werner et al., 1994). The scar area after complete epithelization was then evaluated, as well as the duration for complete epithelization in days, to calculate the degree of wound healing on a millimetre scale (Bura, 2018).

## 3. RESULTS AND DISCUSSION

### 3.1 Assessment of Anti-Diabetic Activity

Anti-diabetic activity was measured by inducing diabetes with alloxan monohydrate (90 mg/kg) intraperitoneally. Then blood glucose levels were measured initially after the induction of diabetes and measured in consecutive days for 21 days. The test and standard drug samples were given continuously for 21 days, and blood glucose levels of the animals were measured by collecting the blood sample using the glucometer. The results were significant and showed a drastic change in the glucose levels. The results were compared with the control. The values were tabulated in the following Table 1, and a graphical representation was shown in Fig. 1.

**Table 1. Anti-Diabetic activity – Blood glucose levels**

| Groups | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **Control** | 109.3±1.014 | 106.7±1.542 | 107.6±0.988 | 105.7±0.802 |
| **Diseased** | 257.5±3.041 | 268.7±6.233 | 268.4±1.118 | 271.3±1.815 |
| **Standard** | 226.5±3.374 | 238.3±1.014 | 188.8±1.851 | 143.6±1.500 |
| **Test-1** | 230.3±9.887 | 236.0±3.568** | 187.5±1.366** | 147.3±2.076** |
| **Test-2** | 235.1±7.587 | 234.6±3.921** | 185.5±1.648** | 138.4±1.249*** |

#### 3.1.1 Effect of extracts on body weight

The body weights of the animals first decreased in alloxan induced diabetic rats by the administration of standard and test samples for initial and on $7^{th}$ day. But the weights gotincreased by the administration of drugs significantly and almost equal to the control group. The values of body weights were tabulated in the following Table 2 and a graphical representation in Fig. 2.

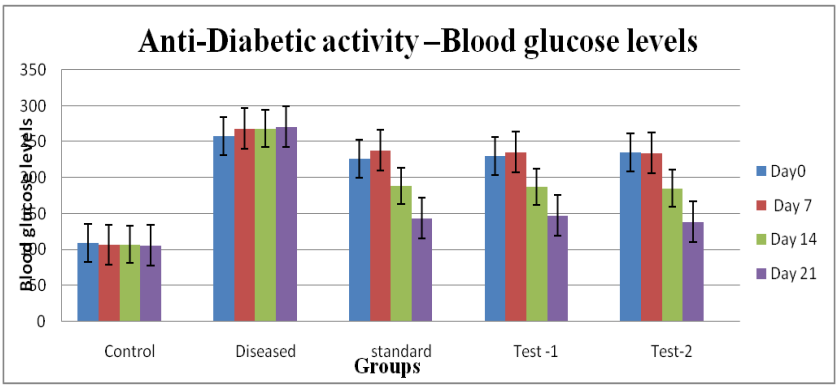


**Fig. 1. Antidiabetic activity of Sarkaraikolli**

**Table 2. Anti-Diabetic activity – Body Weights**

| Groups | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **Control** | 215.19±23.5 | 224.41±4.22 | 226.41±3.62 | 227.47±3.17 |
| **Diseased** | 224.32±2.70 | 220.22±2.42 | 213.25±1.52 | 206.13±2.48 |
| **Standard** | 222.24±2.71 | 222.22±2.25 | 224.53±2.59 | 222.31±2.43 |
| **Test-1** | 225.34±2.32** | 224.34±2.20*** | 225.43±3.42*** | 227.24±2.35*** |
| **Test-2** | 224.24±2.23*** | 225.35±2.32*** | 225.26±1.35** | 226.22±1.43** |

### 3.1.2 Determination of biochemical parameters of liver enzymes

The biochemical parameters like SGOT, SGPT and ALP were determined by using the analytical kits. The results shown by the treatment of Sarkaraikolli were significant and are closer to the control group. The values were tabulated in the following Table 3 and graphical representation was shown in Fig. 3.

**Table 3. Determination of Biochemical Parameters of Liver Enzymes**

| Groups | BGL (mg/dl) | SGOT(µl) | SGPT(µl) | ALP(µl) |
|---|---|---|---|---|
| **Control** | 89±5.7 | 115±1.7 | 95±2.3 | 156±7.5 |
| **Diseased** | 281±1.4 | 199±4.8 | 208±6.1 | 210±10 |
| **Standard** | 189±9.2 | 110±1.2 | 109±7.3 | 181±11 |
| **Test-1** | 200±4.1** | 170±3.1** | 152±7.6*** | 235±21*** |
| **Test-2** | 181±8.9*** | 121±6.6*** | 124±8.3*** | 192±26*** |

## 3.2 Assessment of Wound Healing Activity

The effect of the extract of polyherbal formulation sarkaraikolli on wound healing activity, excision, incision and burned wounds was inflected upon four groups of six rats each. Group 1 was assigned as the control (ointment base). Group 2 was treated with standard nitrofurazone Group 3 was given aqueous extract of 250mg/kg, and Group 4 was given aqueous extract of 500mg/kg. The parameters observed were the wound healing activity of the poly herbal formulation in $mm^2$.

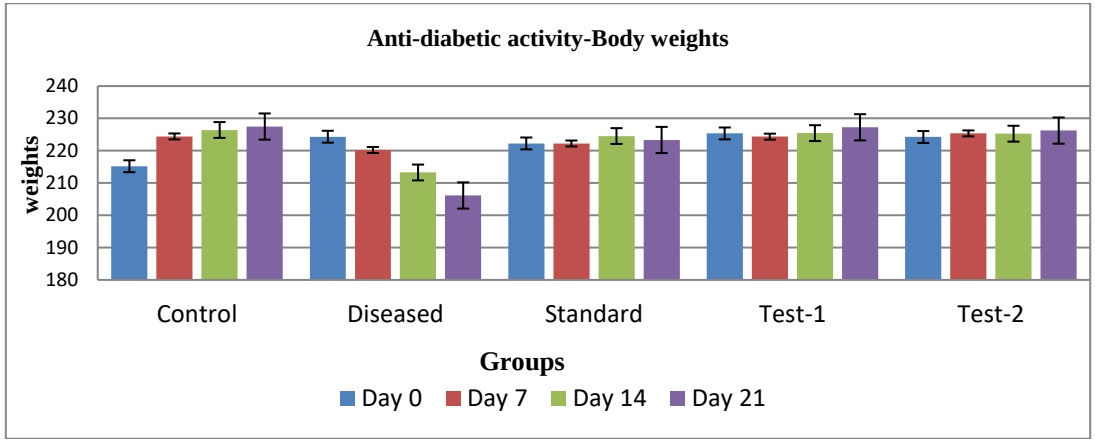


**Fig. 2. Anti-diabetic activity –Body weight**

**Table 4. Wound Healing Activity of Poly herbal formulation in mm²-Sarkaraikolli**

| Days | Control | Std | SK1 | SK2 |
|---|---|---|---|---|
| 0 | 29.8±81 | 30.1±21 | 29.8±0.69** | 30.1±0.21*** |
| 2 | 29.8±81 | 25.9±81 | 26.8±0.87*** | 25.9±0.19** |
| 4 | 29.8±81 | 20.5±81 | 21.3±0.09*** | 20.1±0.22*** |
| 6 | 29.8±81 | 14.4±31 | 15.0±0.86** | 12.6±0.91** |
| 8 | 29.8±81 | 25.4±21 | 9.2 ±0.03*** | 16.7±0.38*** |
| 10 | 29.8±81 | 23.7±10 | 5.2±0.04** | 12.7±0.74** |
| 12 | 29.8±81 | 19.6±13 | 2.8±0.09*** | 17.2±0.07*** |

| Days | Control | Std | SK1 | SK2 |
|---|---|---|---|---|
| 14 | 29.8±81 | 15.6±15 | 1.1±0.12** | 1.2±0.07** |
| 16 | 29.8±81 | 0.66 | 2.9±0.02*** | 0.00 |
| 18 | 29.8±81 | 0.00 | 0.0 | 0.0 |

*Std – Nitrofurazone, SK1 – Sarkaraikolli (250 mg/kg), SK 2 – Sarkaraikolli (500 mg/kg)*

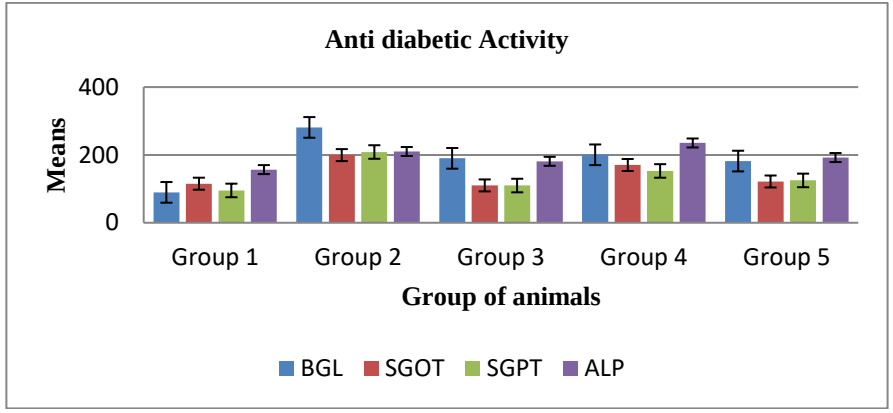


**Fig. 3. Biochemical Parameters of Liver Enzymes**

The influence of the extract of polyherbal formulation Sarkaraikolli on wound healing activity, four sets of six rats were subjected to an incision wound model.

**Group 1:** The control group (ointment base)
**Group 2:** The standard group was given a normal nitrofurazone treatment.
**Group 3:** Test-1 was given an aqueous extract of 250 mg/kg
**Group 4:** Test-2 was given aqueous extract of 500 mg/kg.

The parameters measured were wound contraction.

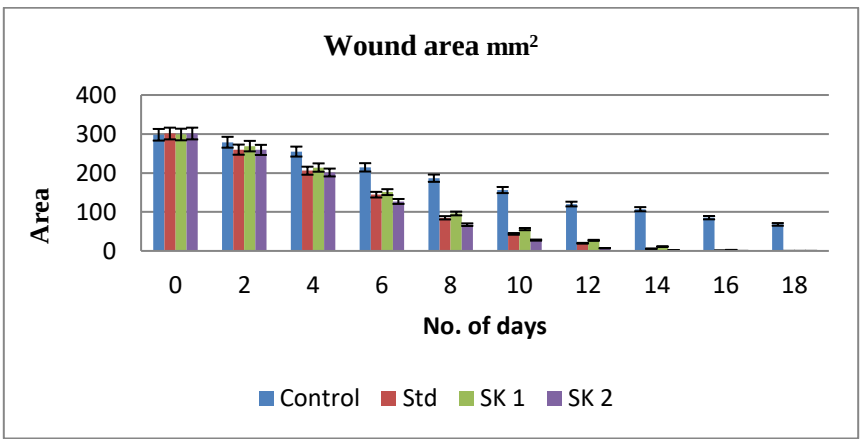


**Fig. 4. Wound Healing Activity of Sarkaraikolli**

## 4. CONCLUSION

This research work proved that Sarkaraikolli possesses significant anti-diabetic and wound-healing activity. When compared to conventional medicine, Glibenclamide, a significant anti-diabetic action is visible due to lower blood glucose levels and maintenance of steady body weights. Sarkaraikolli's wound healing activity is demonstrated by higher wound healing activity when compared to the standard drug (Nitrofurazone).

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It is not applicable.

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It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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# Chronotherapy: Unleashing the Precision of Time for Enhanced Treatment Strategies

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## ABSTRACT

Chronotherapy is an emerging discipline in medicine that emphasises aligning drug administration with the body's inherent biological rhythms, particularly the circadian clock. Recognising that critical physiological functions such as hormone secretion, enzymatic activity, and metabolism fluctuate throughout the day, chronotherapy aims to enhance therapeutic efficacy and reduce adverse effects by synchronising treatment timing with these natural cycles. Recent progress in this field includes the development of time-specific drug formulations and advanced delivery systems, as well as computational tools and algorithms designed to tailor treatment regimens to individual patients. Although still evolving, chronotherapy holds significant promise for optimising clinical outcomes and advancing personalised medicine. The present study focuses on different aspects of Chronotherapy. This study explored the recent advances in chronotherapy, highlighting the potential it holds for revolutionising healthcare. Further research is required to fully understand the intricate mechanisms underlying chronotherapy and to explore its application across various medical conditions.

*Keywords: Chronotherapy; chrono-pharmacology; chrono-pharmaceutics; biological rhythms; suprachiasmatic nuclei.*

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## 1. INTRODUCTION

As residents of a planet where the main source of light and warmth—the sun—is not consistently available, life on Earth has developed intricate biological systems to predict and adjust to these regular environmental changes. These systems, referred to collectively as circadian clocks (derived from the Latin circa diem, which translates to 'about a day'), allow organisms to synchronise their physiological activities with the 24-hour day-night cycle. In mammals, circadian clocks play a crucial role in regulating nearly all significant organ systems, coordinating functions such as hormone secretion, metabolism, immune responses, and cellular repair. Notably, this temporal regulation has significant consequences for human health, as numerous disease processes and their intensities also exhibit variations that depend on the time of day (Dallmann et al. 2014). A disrupted circadian clock is a risk factor for various disorders, including neurodegeneration, metabolic syndrome, diabetes, and cancer. Chronotherapy is a type of therapy provided at specific time intervals based on an individual's circadian rhythm. It considers how circadian clock-driven rhythms influence pharmacokinetic (absorption, distribution, metabolism, and elimination) and pharmacodynamic processes to optimise beneficial effects and/or minimise adverse effects.

"Chrono pharmacology is the science concerned with the variations in the pharmacological actions of various drugs over a period of time" (Shetty, A., & Selvam, 2016). For over three decades, research has demonstrated that the processes of drug absorption and distribution exhibit daily fluctuations in both humans and animal models. Numerous drugs have been shown to undergo significant 24-hour variations in bioavailability. For instance, medications such as acetaminophen (Kamali et al. 1987) and theophylline (Watanabe et al. 1984) display distinct pharmacokinetic behaviours depending on whether they are administered in the morning or evening. Given the extensive influence of circadian rhythms on physiological and pathological processes, it is reasonable to infer that the pharmacokinetics and pharmacodynamics (PK/PD) of many therapeutic agents are also rhythm-dependent. Consequently, both the effectiveness and safety of various drugs may differ based on the time of day they are administered (Dallmann et al. 2014).

"Advancements in chrono-pharmacology have highlighted the critical role of biological rhythms in optimising drug therapy, leading to innovative strategies in the design of oral drug delivery systems" (Shetty, A., & Selvam, 2016).

"Chronopharmacology is a part of pharmacology intended to plan and assess the movement of drugs delivery systems that discharge a bioactive agent at a rate that in a perfect world matches the biological need for a given disorder's treatment or prevention continuously" (Kapadia et al. 2020). "In the treatment of many diseases, chrono therapeutics drug delivery offers a new approach in the pharmacologic interventions for effective treatment of the different types of diseases" (Shetty, A., & Selvam, 2016). Therefore, the newer drug delivery systems designed using a chrono-pharmacological approach offer significant

potential for enhancing patient care. By aligning drug release with the body's natural biological rhythms, these systems can improve therapeutic efficacy, reduce adverse effects, and increase drug tolerance—ultimately leading to safer and more personalised treatment outcomes (Kapadia et al. 2020).

## 2. BIOLOGICAL RHYTHMS

Biological rhythms refer to the natural cycles of change in the chemicals or functions of our body. They function like an internal master "clock" that synchronises the various clocks within your body. This "clock" is situated in the brain, just above the point where the optic nerves intersect. It consists of thousands of nerve cells that assist in coordinating your body's functions and activities. There are four types of biological rhythms:

1. Circadian rhythms: the 24-hour cycle encompassing physiological and behavioural rhythms such as sleeping.
2. Diurnal rhythms: the circadian rhythm that aligns with the cycle of day and night.
3. Ultradian rhythms: biological rhythms characterised by a shorter duration and higher frequency than circadian rhythms.
4. Infradian rhythms: biological rhythms that extend beyond 24 hours, exemplified by the menstrual cycle.

"The circadian clock has a significant role in physical, mental, and behavioural responses to light and darkness. In mammals, processes as varied as fluctuations in temperature and blood pressure, sleep-wake cycles, and the metabolism of glucose and lipids are all regulated by these rhythmic patterns". (Fig. 1) (Smolensky, M., & Lamberg, L., 2001).

## 3. CIRCADIAN RHYTHMS

"The circadian clock system is the totality of all oscillators in organisms coupled to various physiological processes. This system generally consists of three parts in mammals, including the input pathway, the core circadian clock, and the output pathway. The input pathway senses external timing signals, for example, light/dark, and sends information to the core circadian clock. The core circadian clock forms endogenous Circadian Rhythm (CR) according to external time cues to allow for adaptation to the environment. Based on changes in the core circadian clock, the output pathway adjusts the physiological activities in various tissues and organs through neuro-humoral regulation" (Dibner et al. 2010).

"In mammals, the core pacemaker of the CR system exists in the Suprachiasmatic Nucleus (SCN), which exhibits endogenous rhythmic oscillations both at the tissue and cell levels, plays a vital role in maintenance and alterations of CR, and provides outputs to peripheral tissues after synchronisation by external time cues" (Dibner et al. 2010).


**Fig. 1. Schematic representation of key physiological changes over the circadian day. Representation of circadian changes in human physiology**

## 4. MOLECULAR BASIS AND MECHANISM OF CIRCADIAN RHYTHM

“At the molecular level, circadian rhythms are generated by a negative transcriptional feedback loop, which involves transcription factors that drive their own repressors. These repressors are modified throughout the day by various means (such as phosphorylation) and eventually degraded, thereby starting a new cycle” (Tataroglu, O., & Emery, P., 2014).

“Our knowledge of the basis of circadian rhythm generation and its entrainment by environmental cycles has been profoundly influenced by research using Drosophila. The roots of this influence can be traced back to Colin Pittendrigh, one of the founding fathers of chronobiology, who used various Drosophila species to study fundamental aspects of circadian clocks, such as entrainment and temperature compensation” (Pittendrigh, C. S. 1954, Zimmerman et al. 1968, Pittendrigh, C. S. 1993, Pittendrigh, C. S. 1967). Further critical influence came from the work of Seymour Benzer and Ronald Konopka and their initial forward mutagenesis screen using Drosophila, in which they identified the first circadian gene: period (PER) (13 Konopka et al. 1971). “Their work and that of many others following these seminal studies, as well as the powerful techniques

developed by other Drosophila scientists, made fruit flies especially suited to investigate circadian rhythms" (Tataroglu, O., & Emery, P. 2014).

Having a deep understanding of the Drosophila circadian pacemaker (Fig. 2) (Zhang, Y., & Emery, P. 2012, Allada et al. 2010). "The circadian transcription factors CLOCK (CLK) and CYCLE (CYC) form a heterodimeric complex and promote period (per) and timeless (TIM) transcription" (Allada et al. 1998, Rutila et al. 1998, Darlington et al. 1998). PER (period) and TIM (Timeless) proteins accumulate during the night and form a heterodimer as well (Zeng et al. 1996, Gekakis et al. 1995). "The PER/TIM complex enters the nucleus and promotes the phosphorylation of CLK/CYC, which inhibits its activity and reduces its affinity for DNA" (Menet et al. 2010, Yu et al. 2009). "However, PER and TIM are also gradually modified by phosphorylation during the day" (Zeng et al. 1996, Edery et. Al, 1994). This eventually results in their degradation and releases CLK/CYC from repression to start a new cycle (Tataroglu, O., & Emery, P. 2014).

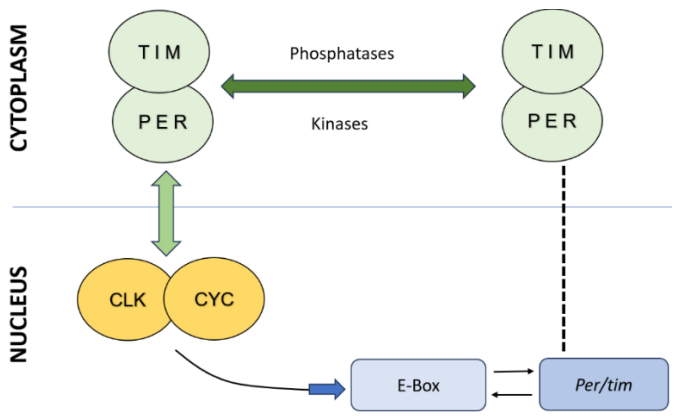


**Fig. 2. The transcriptional feedback loop of the Drosophila circadian clock CLK/CYC drive expression of their own repressors PER and TIM. PER/TIM go through various modifications during the day, until they are eventually turned over to release CLK/CYC from repression, starting the next cycle**

"In mammals, the logic appears to be similar, i.e. transcription factors positively induce 'clock genes' which then negatively feed back, progressively inhibiting transcription, thus forming a 'transcription-translation feedback loop' (TTFL)" (Vansteensel et. al 2008, Brown et al. 2012). "The principal activators within this system are the CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle Arnt-like protein-1) proteins and their homologs, which dimerise and bind to cis-acting E-box promoter elements (with the simple consensus DNA sequence CAANTG) to activate the transcription of a large number of circadian genes. Among these genes are loci encoding the PERIOD and CRYPTOCHROME families of repressor proteins (PER1–3 and CRY1–2)" (Dallmann et al. 2014). "PER and CRY proteins in turn form complexes in the cytoplasm, and at a certain threshold, the PER/CRY complex migrates to the

nucleus, where it inhibits the action of BMAL and CLOCK, and thus PER and CRY transcription" (Horst et al. 1999, Yu et al. 2002, Zheng et al. 2001). The inhibitory PER/CRY complexes are subsequently degraded in the proteasome following phosphorylation by casein kinase Ie (CKIe) (Eide et al. 2005), and then ubiquitination (Siepka et al. 2007), which removes the inhibition on CLOCK and BMAL, allowing the feedback loop to restart again in a 24-hour loop (Robinson et al. 2014).

"The system is further fine-tuned by complex interactions with numerous other intertwined feedback loops. Critically, the retinoic acid receptor-related orphan receptors, REV-ERBa/b and RORa bind to enhancer elements on the Bmal1 promoter to inhibit or promote transcription, respectively" (Preitner et al. 2002). "Oscillations in transcription of REV-ERBa and RORa drive the rhythmic expression of BMAL1, and the BMAL1/CLOCK complex acts directly on the REV-ERB a gene, driving an 'accessory' loop" (Robinson et al. 2014) (Fig. 3).

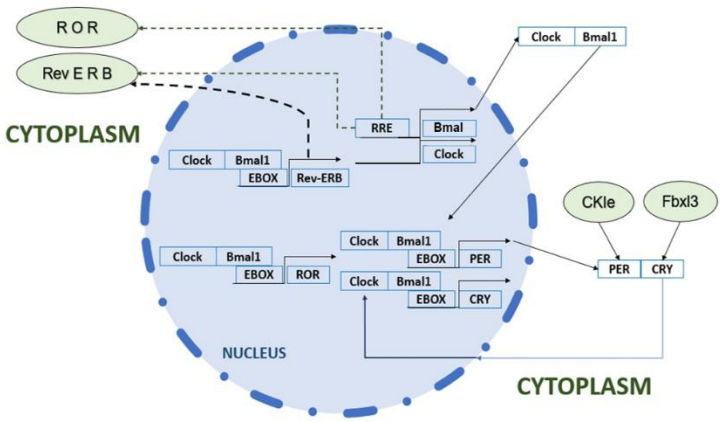


**Fig. 3. Schematic representation of the core circadian clock. The core circadian clock mechanism involves the transcription factors BMAL1 and CLOCK, which form a heterodimer in the cytoplasm and then move into the nucleus. Once inside, they bind to E-box elements in DNA, activating the transcription of target genes including Period (PER), Cryptochrome (CRY), REV-ERB, and ROR. The PER and CRY proteins are synthesised and transported back into the cytoplasm, where they form complexes. These complexes eventually re-enter the nucleus to inhibit the activity of the BMAL1/CLOCK complex, thereby suppressing their own gene expression. PER and CRY proteins are later degraded through interactions with casein kinase I epsilon (CKIε) and the ubiquitin ligase Fbxl3, respectively. Meanwhile, REV-ERB negatively regulates Bmal1 transcription, whereas ROR acts as a positive regulator, helping to maintain the rhythmic expression of BMAL 1**

## 5. HIERARCHICAL ORGANIZATION OF CLOCKS

In mammals, the circadian clock is arranged in a hierarchical manner with a central clock in the brain maintaining the daily rhythm in accordance with the external environment. This timing information is then outputted to the periphery to synchronise the body's tissue clocks (Robinson et al. 2014). A "master clock" tissue within the suprachiasmatic nuclei (SCN) of the hypothalamus receives light input via the retina and communicates timing signals to peripheral "slave" oscillators of similar molecular mechanisms in cells from other tissues. Multiple redundant signals have been described. These include direct signals such as innervation by the autonomous nervous system and hormones such as glucocorticoids and indirect signals emanating from SCN-controlled rhythmic behaviour, such as timing of food intake and small rhythmic changes in body temperature from activity (Dibner et al. 2010).

The SCN gets reset every day by light on the retina, and dedicated monosynaptic projections terminate in the SCN, resulting in activation of various signalling pathways, including transcription of clock genes and chromatin remodelling (Golombek et al. 2010).

Metabolic activity in the SCN is high during the daytime and low at night. However, at night, the metabolic activity can be increased by acute exposure to light. Light is the major Zeitgeber (time giver) in humans and is capable of altering the circadian phase by up to an hour (Skene et al. 2006). Having periods of light and dark is important for normal physiology. Preterm infants exposed to a light/dark cycle in intensive care units gain weight faster than those under traditional continuous light conditions, which may be a result of decreased metabolic demand (Vásquez-Ruiz et al. 2014). While the SCN ensure that peripheral clocks are appropriately synchronised, peripheral tissues oscillate independently of the SCN.

This hierarchical clock structure has two important implications for chronopharmacology. First, if clocks in different tissues govern different aspects of drug activity and metabolism. Second, increasing evidence suggests that chronic circadian dephasing itself has significant negative consequences for health, both for rodents subjected to laboratory conditions of chronic jetlag or shift work and for humans subjected to similar stresses (Golombek et al. 2013, Castanon et al. 2010, Barclay et al. 2012).

## 6. CHRONO PHARMACOKINETICS

Chrono pharmacokinetics examines how drug plasma levels fluctuate based on the time of day and the underlying mechanisms that cause these time-dependent variations (Chawla et al. 2012). Pharmacokinetic parameters, including peak drug concentration ($C_{max}$), time to $C_{max}$ ($t_{max}$), volume of distribution, area under the curve, bioavailability, plasma protein binding, and elimination half-life, which are typically regarded as constant over time, are affected by various physiological functions that exhibit a circadian rhythm. Changes in circadian

gastric acid secretion, gastrointestinal motility, gastrointestinal blood flow, drug protein binding, liver enzyme activity, renal blood flow, and urinary pH can contribute to the time-dependent variation in drug plasma concentrations (Shetty, A., & Selvam, 2016).

## 6.1 Absorption

Oral administration is the most common administration route due to its simplicity and convenience (Bardal et al. 2011). “It is altered by circadian changes in gastric emptying time, gastrointestinal blood flow, gastric acid secretion, and pH. Most lipophilic drugs seem to be absorbed faster when the drug is taken in the morning compared with the evening (Ex: absorption of valproic acid is larger in the morning than in the evening). Drug transporters are expressed in many organs, including the intestine, liver, brain, placenta, testis, and kidney, where they regulate molecular traffic by carrying drugs into or out of cells. In particular, efflux transporters exert a protective role against xenobiotics by either opposing their passage or facilitating their elimination” (Levi et al. 2007)

## 6.2 Distribution

“Drug distribution is influenced by several factors, including cardiac output, blood flow, and drug binding to both tissues and plasma proteins” (Bardal et al. 2011). Peak plasma concentration of plasma proteins like albumin occurs early in the afternoon, while troughs are found during the night, For example, maximum binding of neoplastic drugs like cisplatin to plasma proteins is in the afternoon and minimum in the morning.

## 6.3 Metabolism

“During metabolism, lipophilic drugs are often converted to more hydrophilic polar metabolites through functionalization in phase I reactions, such as oxidation, mainly mediated by cytochrome P450 (CYP) enzymes, and phase II conjugation reactions. Afterwards, in phase III, ABC transporters facilitate the excretion of metabolites into the bile, milk, sweat, bronchial exudate, urine, and/or faeces” (Bardal et al. 2011).

For drugs with low extraction ratio depends on liver enzyme activity. For drugs with high extraction ratio depends on hepatic blood flow.

## 6.4 Excretion

Circadian oscillations of kidney function are well known; the most relevant renal processes that undergo rhythmic variations and affect drug excretion are the GFR, tubular reabsorption, active tubular secretion, renal blood flow, and urinary pH (Boulamery et al. 2007, Firsov et al. 2018). In rodents, renal blood flow and GFR are higher during the activity phase and lower during rest (Okyar et al. 2012).

## 7. CHRONO PHARMACODYNAMIC

Chronopharmacodynamics mainly deals with the biochemical and physiological effects of drugs on the body, the mechanisms of drug action, and the relationship between drug concentration and effect in relation to the circadian clock.

Biological rhythms at the cellular and subcellular levels can provoke significant dosing-time differences in the pharmacodynamics of medications that are unrelated to their pharmacokinetics. This phenomenon is called “chronesthesy” (Ohdo, S. 2007, Reinberg, A. E. 1992, Ohdo, S. 2021, Ohdo, S. 2003, Smolensky et al. 2007, Ohdo et al. 2010, Ohdo et al. 2000).

Chronopharmacodynamics basically endeavours with mechanisms of time-related variation in effects and metabolism of drugs in healthy organisms. However, the term “chronesthesy” can be used instead of chrono-pharmacodynamics, which is a long and difficult word. Rhythms in receptor number or conformation, secondary messengers, metabolic pathways, and/or free-to-bound fraction of medications help explain this phenomenon (Huang et al. 2011). Below, we discuss a few drugs to highlight how timing affects their pharmacodynamic properties.

### 7.1 Anticancer Drugs

For anti-cancer drugs used in infusion form, in order to define the drug regimen, dose administered per unit (usually body surface area or body weight), duration of infusion and frequency of administration are the parameters commonly used (Laposky et al. 2008). If the cancer drug will be infused for a short time, chronotherapy studies can be performed with a timed bolus or a short infusion where the timing of administration (start, peak and stop times) is stipulated (Erkekoglu et al. 2012, Innominato et al. 2010). On the other hand, a constant-rate infusion over 24 hours, or integral multiples of this span, does not consider chronopharmacodynamic properties. Therefore, this procedure should be used as a control administration schedule for studies of cancer chronotherapy for drugs whose pharmacologic properties enable long-term infusion (Erkekoglu et al. 2012).

Here we can give the examples for the change in the pharmacodynamics of some anti-cancer drugs: DNA synthesis in the main target tissues of 5-fluorouracil (5-FU)-induced toxicity (e.g., bone marrow, skin, and oral and rectal mucosa) is lowest during the night and highest during daytime (Erkekoglu et al. 2012, Lévi t al. 2010, Hrushesky et al. 2004, Longley et al. 2003, Bjarnason, G. A., & Jordan, R. 2002, Wood et al. 2006, Smaaland et al. 2002, Lincoln et al. 2000, GA, B. 2001). Therefore, at night, when the whole-body clearance of 5-FU is increased, the proportion of healthy cells potentially damaged by 5-FU is decreased. Whole-body pharmacodynamics of 5-FU, therefore, displays variation along the circadian time scale, with a synchronous phase between different target tissues. The anabolic enzymes (orotate phosphor-ribosyltransferase, uridine phosphorylase, and deoxythymidine kinase) that produce cytotoxic forms

of 5-FU have their highest activity during the dark span of rats or mice, when 5-FU is most toxic to healthy tissues (Etienne-Grimaldi et al. 2008). The activity of the "thymidylate synthetase", which is the target enzyme of 5-FU, has also been studied at the cellular level in the oral mucosa cells of 6 healthy volunteers. The activity of this particular enzyme showed a circadian rhythm with a trough between midnight and 4 A.M. (Hrushesky et al. 2004). Therefore, the molecular target of 5-FU is less active at night. This results in a cellular chronopharmacodynamic pattern of this drug consistent with its lower cytotoxicity to the oral mucosa during the night. The circadian profiles of whole-body and cellular chrono pharmacokinetics and chrono pharmacodynamics in humans would therefore predict a better tolerability of healthy tissues for a nightly administration of 5-FU. This hypothesis has been approved by several clinical studies. On the other hand, the anti-tumour effects of interferon-β (IFN-β) in mice are more efficient during the early rest phase than during the early active phase. The dosing schedule-dependent effect of IFN-β is also closely related to that of IFN receptors and interferon-stimulated gene factor (ISGF) expression in tumour cells or lymphocytes (Ohdo et al. 2000, Lévi, F. 2006).

## 7.2 Cardiovascular Drugs

There is evidence in literature that the circadian clock has an impact on the pharmacodynamics of several cardiovascular drugs (Table 1) (Takane et al. 2000, Nakagawa et al. 2006, Ohdo et al. 2011, Bode-Böger, S. M., & Kojda, G. 2005, Aschoff, J. 1969, Stanton, A. V. 1998). Beta-receptor blocking drugs (β-blockers) consist of an important group of cardioactive drugs. These medications still have great therapeutic value in the treatment of cardiovascular disorders like coronary heart disease, hypertension and arrhythmias. Different β-blockers vary not only in their specific effects, like receptor affinity, receptor selectivity, intrinsic sympathomimetic activity, and in their nonspecific effects related to the lipophilicity of the drug, but also in their biotransformation. Lipophilic β-blockers are usually subject to hepatic biotransformation, whereas hydrophilic ones are mainly eliminated by renal excretion. Various β-blockers have been studied in several animal experiments and in volunteers for their chronopharmacodynamic properties. The peak concentration of propranolol was achieved in the application between 8 A.M. and 2 P.M. However, when applied at 2 A.M., the heart rate can be slightly changed in the following 6 hours. Therefore, it can be stated that sympathetic tonus, which is demonstrated by the rhythm in plasma noradrenaline and cAMP levels, affects the pharmacodynamics of the particular drug, and it can be concluded that propranolol should be applied in hours when sympathetic tonus is high" (Takane et al. 2000).

"Anti-anginal drugs such as nitrates favorably shift the ratio of myocardial oxygen demand and supply by relieving chest pain and reducing the duration and frequency of acute ischemic events" (Nakagawa et al. 2006). "Isosorbide-dinitrate, an important nitrate derivative, shows its highest therapeutic effect around 2 A.M. when it highly induces a decrease in blood pressure and an increase in heart rate. In contrast, at 8 A.M., isosorbide-dinitrate did not significantly increase reflex-induced tachycardia during orthostatic" (Takane et al.

2000). These findings are also in concert with the data that a maximum orthostatic liability is observed around 3 A.M. (Ohdo et al. 2011).

**Table 1. List of cardiovascular drugs that follow the circadian rhythm**

| **Beta blockers** | **Calcium channel blockers** |
|---|---|
| Acebutolol | Amlodipine |
| Atenolol | Nifedipine |
| Bevantolol | Nisoldipine |
| Bopindolol | Nitrendipine |
| Labetalol | Verapamil |
| Mepindolol | **Angiotensin Converting Enzyme** |
| Metoprolol | Captopril |
| Nadolol | Enalapril |
| Oxprenolol | **Nitrates** |
| Pindolol | Glyceryl-trinitrate |
| Propranolol | Isosorbide-dinitrate |
| Sotalol | Isosorbide-5-mononitrate |
| **Diuretics** | **Others** |
| Hydrochlorothiazide | Clonidine |
| Indapamide | Prazosin |
| Piretanide | Potassium Chloride |
| Xipamide | |

“Calcium channel blockers are used in the treatment of coronary heart disease, myocardial infarction, cerebrovascular diseases and hypertension for several years” (S. M., & Kojda, G. 2005). “Verapamil and Diltiazem have a more prominent cardiac effect, whereas dihydropyridine type blockers such as nifedipine have a more dominant vasodilator effect. Several chronopharmacology studies were performed on various calcium channel blockers, and in general, their blood pressure-lowering effect was found to be higher in daytime than nighttime time and the circadian clock-dependent effects of these drugs show a similar pattern as β-blockers” (Aschoff, J. 1969, Stanton, A. V. 1998).

### 7.3 Analgesics and Non-steroidal Anti-inflammatory Drugs

NSAIDs include a wide range of medications, utilised in an assortment of illnesses. These medications ought to be regulated in the morning when the pain is at its most noteworthy, particularly in rheumatoid arthritis. Morning time organisation typically gives a day-long decrease in torment and an increase in life quality.

## 8. EVALUATION OF DISEASES BASED ON THE CIRCADIAN RHYTHM

### 8.1 Diabetes

In type 1 diabetes, the circadian rhythms of insulin and its activity are of physiological interest and clinical significance (White et al. 2002). Thus, insulin is

delivered in a pulsatile style; however, here and there it is unpredictable. Insulin can show its cyclic rhythmicity of 8-30 min, which can show the ideal activity. The insulin release from basal mode acts on β cells in both stimulatory and inhibitory states, and target cell's sensitivity to insulin activity and hyperglycemia is hindered by stress chemicals, cortisol, epinephrine, and development chemical. The characteristic rhythmicity and parchedness have drawn out the insulin withdrawal prompt auxiliary criticism on insulin delivery can assist with raising the blood glucose levels. The modulators of insulin delivery and activity are discharged in a circadian pattern and influence the method of insulin discharge. So, the distinction between the most extreme and least plasma insulin fixation has transient rhythmicity and the complex optional circadian beat is variable early-morning and late-evening insulin obstruction (Bakris et al. 2002).

## 9. Glucose Metabolism and Circadian Rhythm

Notwithstanding the significant taking care of contributions, there are 24-hour exceptionally musical changes in blood glucose levels instigated by changes in insulin sensitivity and insulin secretory patterns (Waldhäusl, W. 1989). In creature models, insulin discharge is musically managed by fringe pancreatic β-cell clocks, even in the separated perfused condition (Singh et al. 2010). The 24-hour pulsatile insulin discharge is seen in people, and has been discovered to be set at a more elevated level in obese subjects and in type 2 diabetes patients and their first-degree family members without diabetes (Polonsky et al. 1998). In a late trial, creature contemplates, worldwide clock freak mice (Clockδ19) created age-dependent hyperglycemia and weight, however showed an improperly low centralisation of insulin with improved insulin sensitivity (Robinson et al. 2014). Twofold Cry1 and Cry2 knockout mice likewise showed impaired insulin secretion, in spite of the fact that their body weight was to that degree not exactly in control mice, and increased sympathetic tone was noticed (Kurose et al. 2014). As of late, hyperglycemia-instigated by removal of twofold Cry quality articulation was demonstrated to be intervened, at least to a limited extent, by hepatic gluconeogenesis because of hepatic over-articulation of Cry by bringing down blood glucose levels and improving insulin sensitivity in insulin-resistant mice (Turek et al. 2005). Moreover, islet-explicit BMAL1 freak mice showed typical weight, ordinary movement, and ordinary care, however, critical hyperglycemia with the debilitated release of insulin because of the inhibition of exocytosis (Ikeda et al. 2007). Furthermore, in diabetes-inclined rodents, disturbance of circadian rhythms speeds up the advancement of diabetes through pancreatic β-cell misfortune and degeneration (Zhang et al. 2010). Insulin sensitivity likewise shows circadian changes, and clock quality interruption incites an absence of rhythmicity in insulin activity and action designs (Marcheva et al. 2010). Utilising a hyperinsulinemic-euglycemic clamp, the creators discovered circadian rhythmicity in insulin activity, which was nullified in BMAL1-knockout mice. The majority of the counter-administrative chemicals that have an impact like an anti-insulin, including glucagon, catecholamines, and cortisol, likewise show the circadian mood. Developmental chemical shows a rest-related flood that incites early morning insulin obstruction (Polonsky et al. 1998). Notwithstanding these systems, autonomic neural guidance from the

hypothalamic SCN applies consequences for the liver (Gale et al. 2011). In this way, insulin sensitivity is under close guideline of circadian control (Qian et al. 2013).

## 10. CIRCADIAN RHYTHMS AND TYPE 2 DIABETES

The significant issue with respect to the role of the circadian clock in diabetes is whether interruption of CLOCK or BMAL1 directly prompts metabolic imperfections or whether origin is indirectly related to clock function (Kalsbeek et al. 2008, Kurose et al. 2014). In the event that interruption of the clock mechanism is straightforwardly associated with the improvement of diabetes, current way of life itself neurotically affects the progression of the disease. Notwithstanding uncommon examples of rest, strange eating behaviour, for example, skipping breakfast and late evening eating, related with shift work or other way of life interruptions of the day-night cycle may obsessively affect the development of diabetes mellitus (Polonsky et al. 1998).

## 11. PEPTIC ULCER DISEASE

Histamine H2 antagonists were administered at a regular interval based on basis of pharmacokinetic properties and circadian rhythm because maximal acid secretion, duodenal ulcer, pain, and peptic ulcer disease are more common at night. Administration of these drugs at bedtime is more effective. Night-time administration of the peptic ulcer drugs doesn't just lessen the corrosive discharge more effectively yet in addition advances the ulcer healing and decreases ulcer recurrence (Kurose et al. 2011). It is grounded that patients with peptic ulcer illness frequently encounter the best level of pain in the close to time that the patient hits the sack, as the rate of stomach corrosive discharge is highest at night (Huang et al. 2011). The circumstance of administration of ulcer medications has a critical outcome on their therapeutic impact and shows the best chronotherapeutic drug delivery in the joint inflammation treatment, which shows the correct treatment as per the rhythms and natural time structure (Khasawneh et al. 1992).

## 12. ALLERGIC RHINITIS

Nasal congestion, sneezing, running nose are the symptoms of allergic rhinitis, which are typically more severe in the early morning hours (Evans, R. M., & Marain, C., 1996). Morning dose of antihistamine is more viable than placebo, but not as viable as a similar dose given in the evening. On the off chance that the administration of the medication can be coordinated with the biological time structure which has the pinnacle pharmacologic action are coordinating with the hour of the most prominent inconvenience, ideal alleviation might be given when it is required for the vast majority of the patients (Bakris et al. 2002).

## 13. CHRONO PHARMACEUTICS

Most recently, the idea of chrono-pharmacokinetics is acquiring the consideration of pharmaceutical researchers. Chrono-pharmacology may be considered as a

bridge to fill up the gap between the existing concepts of chronobiology, chrono-pharmacology, chronotherapeutic and chronotoxicology (Smolensky et al. 1995).

Chronopharmacology is a part of pharmaceutics intended to plan and assess the movement of drugs movement structure which releases a bioactive agent at a time that in perfect world matches the organic need for a given disorder treatment (Reinberg et al. 1985). Chronopharmacology is a part of pharmaceutics that involves the planning and assessment of medication delivery system that delivers a bioactive agent at a rhythm that preferably coordinates with the biological requirements of a given disease treatment.

Ideal Characteristics of Chronotherapeutic Drug Delivery Systems (Sundeep et al. 2011)]:

1. Should be non-toxic within approved limits of use.
2. It should have a real-time and specific triggering biomarker for a given disease state.
3. Should have a feedback control system (example: self-regulated and adaptive capability to circadian rhythm and individual patient to differentiate between awake – sleep status).
4. It should be biocompatible and biodegradable, especially for parenteral administration,
5. Should be simple to administer to patients in order to improve adherence to the dose regimen.
6. It should be simple and cost-effective.

## 14. CHRONO PHARMACEUTICAL TECHNOLOGY

Many technologies have been developed to deliver the drugs to the body according to the biological rhythm of the disease. The formulations that have been approved by the USFDA for chronotherapy of the diseases and the technologies used are given in (Table 2) (Smolensky et al. 1995).

Techniques such as CONTIN® technology, CHRONOTOPIC® technology, OROS® technology, CODAS® technology, CEFORM® technology, DIFFUCAPS® technology, Chrono modulating infusion pumps, TIMERx® technology, PORT® technology, Controlled-release microchip are developed to deliver the drugs to the body according to the biological rhythm.

These technologies were created to treat disease as indicated by the endogenous biologic rhythms which moderate xenobiotic digestion and cell drug reaction. As a result, many mechanisms of diseases and drug effects are controlled by the circadian timing system.

### 14.1 Contin® Technology

It is an innovation(technique) wherein molecular coordination complexes are formed between a cellulose polymer and nonpolar solid aliphatic alcohol alternatively substituted with an aliphatic group by solvating the polymer with a

volatile polar solvent. Reacting the solvated cellulose directly with aliphatic alcohol is preferable as a melt. No advancement of sustained-release tablet forms like aminophylline, theophylline, morphine, and other drugs by this innovation (Devdhawala et al. 2010). It is more effective in controlling of diseases and reduces unwanted side effects.

## 14.2 Oros® Technology

Generally, OROS means osmotic controlled release oral delivery system. It is an osmosis-based system. It is also known as controlled onset extended released. Chronoset® is a proprietary OROS® (developed by Alza Corporation) delivery system that reproducibly delivers a bolus drug dose in a time or site-specific manner to the gastrointestinal tract (Devdhawala et al. 2010). Two compartments are in there, one is a drug vessel and another is an osmotic engine cap. when the system is contacted with an aqueous medium, the water permeates into the osmotic engine cap through a rate-controlling membrane. Due to hydration, expansion of the osmotic engine occurs, which exerts a driving force against the ridge of the drug vessel. Two compartments are separated from each other. After detachment, the open mouth of the drug vessel is exposed to a fluid environment. Chronoset® can deliver the entire dose and minimise the drug residue in the drug vessel after the operation. The vessel is made up of impermeable ethylene co-vinyl acetate polymer while the cap is made up of water-permeable blends of polycaprolactone and flux enhancers (Kotkar et al. 2009).

## 14.3 CODAS® Technology

CODAS technology refers to a chronotherapeutic oral drug absorption system. it was developed by Elan Corporation, USA. it was introduced by the non-enteric release controlling polymer (a combination of water-soluble and water-insoluble polymer) applied to drug-loaded beads. When this drug-loaded bead is contacted with water from git, the water-soluble polymer slowly dissolves and the drug diffuses through the resulting pores in the containing and the water-insoluble polymer continues to act as a barrier, maintaining the controlled release of the drug (Rewara et al. 2014).

## 14.4 CEFORM® Technology

This innovation comprises microspheres uniform in size and shape arranged by the melt spinning method. Obtaining microspheres are spherical with a diameter of around 150-180 micrometres can be utilised in a wide assortment of dosage forms like tablets, capsules and sachets (Shidhaye et al. 2012).

## 14.5 DIFFUCAPS® Technology

This is a capsule-based system that conveys/delivers the medication into the body in a circadian release design, and it contains one additional medication molecule like beads, pellets, granules, and so on. Each bead shows a pre-programmed quick or sustained delivery profile with or without a predetermined lag time (Silas et al. 2017).

**Table 2. Chrono-pharmaceutical systems approved by US-FDA**

| Date of FDA approval | API | Proprietary name; dosage form | Chrono pharmaceutical technology | Indication/rationale for chronotherapy |
|---|---|---|---|---|
| Sept 01,1982 | Theophylline | Uniphyl®; extended-release tablet | CONTIN | Asthma/increased bronchoconstriction in morning. |
| Oct 15,1986 | Famotidine | Pepcid®tablets | Physico-chemical modification of API | Ulcer/increased gastric acid secretion in evening. |
| Dec 23,1991 | Simvastatin | Zocor®tablest | Phsyico-chemical modification of API | Hypercholesterolemia/increased cholestrol synthesis overnight. |
| Feb 26,1996 | Verapamil HCL | Covera HS; extended release tablets | OROS | Hypertension increased BP in early morning. |
| Nov 25,1998 | Verapamil HCL | Verelan®PM;extendedrelase capsules | CODAS | Hypertension. |
| AUG 1,2000 | Methyphenidate HCL | Concerta®tablet. | OROS | Anti-pshycotic. |
| Feb 06,2003 | Diltiazem HCL Verapamil HCL | Cardizem LA; Extended-release tablets | CEFORM | Hypertension. |
| Mar 12,2003 | Propranolol HCL Verapamil HCL | Innopran XL; extended-release capsules | DIFFUCAPS | Hypertension. |
| Dec 19,2006 | Palipenidone | Invega | OROS | Schizophrenia. |

## 14.6 TIMERx® Technology

It is a hydrogel-based controlled delivery device that can give from zero-order kinetics to chronotherapeutic delivery and furthermore give different release kinetics by controlling molecular interactions (Uhumwangho et al. 2011). It consolidates primarily xanthan and locust bean gums blended in with dextrose. The physical interaction between these components works to form a strong binding gel in the presence of water. Medication release is controlled by the rate of the fluid penetration from the GIT into the TIMERx™ gum matrix, which extends to form a gel and subsequently releases the active medication substance (Silas et al. 2017).

## 15. VARIOUS APPROACHES TO DESIGN CHRONOTROPIC SYSTEMS TO ACHIEVE PULSATILE DRUG RELEASE

Generally, we know a pulsatile drug delivery system is the rapid and transits release of a certain amount of drug molecules within a short time period immediately after a predetermined off-release period (lag time).

The principal point of this system is to deliver drugs on a programmed design i.e. at an appropriate time and suitable site of action. It is a chrono pharmacotherapy design according to circadian rhythm of body. A few techniques have been created and intended for pulsatile drug release. These methods include (Mullaicharam 2013).

1. Time-controlled chronotropic systems.
2. Stimuli-induced pulsatile drug delivery systems.
3. Externally regulated pulsatile drug delivery systems.

## 15.1 Time Controlled Chronotropic System

This system is mostly developed in capsule form. The lag time is constrained by a plug, which gets pushed away by swelling or disintegration, and the medication is released as a "pulse" from the insoluble capsule body, also known as Single unit system.

### 15.1.1 Capsular-based system

These dosages consist of an insoluble capsule body that contains medication along with swellable degradable plugs made from hydrophilic polymers and lipids. The lag time can be regulated by adjusting the size and placement of the plug. The polymers utilised in the design of the hydrogel plug include Polymethacrylate (an insoluble yet swellable and permeable polymer), Hydroxypropyl methyl cellulose, Polyvinyl alcohol (an erodible compressed polymer), and pectin (an enzymatically controlled erodible polymer). When these capsules come into contact with the dissolution liquid, they swell, and after a certain lag time, the plug ejects itself from the capsule, resulting in a rapid release of the medication (Dubal et al. 2011).

### 15.1.2 Osmosis-based system

It consists of a capsule coated semipermeable membrane. This system contains medication and a water-absorptive osmotic agent that is put in compartments isolated by a movable partition. The semipermeable film of the capsule permitted the passage of water because of contact between the capsule and the dissolution liquid. This causes the development of osmotic pressure and an insoluble plug expelled after a lag time (Rewar et al. 2014).

## 15.2 Stimuli-Induced Pulsatile Drug Delivery Systems

This system is broadly classified into two systems such as:

I. Temperature-induced system
II. Chemically induced System

### 15.2.1 Temperature-induced system

For pulsatile release thermos responsive hydrogel have been developed. In these systems, the polymer goes through an expanding or deswelling stage in response to the temperature which modulates the drug release in the swollen state. developed an indomethacin pulsatile release design having temperature ranges somewhere in the range of 200°C and 300°C by utilizing reversible expanding properties of copolymers of N-isopropyl acrylamide and butyryl acrylamide (Shetty et al. 2016).

### 15.2.2 Chemically induced system

In these systems, the polymer goes through swelling or deswelling phase in response to chemical response with film, change in pH and inflammation induce release the medications from the polymer by swelling of the polymer. The release mechanism of drugs due to chemically induced system are mentioned in the below (Table 3) (Tajane et al. 2012).

**Table 3. Effect of different chemical stimuli on the release of drug from smart hydrogels**

| Stimulus | Hydrogel | Type of release mechanism |
|---|---|---|
| pH | Acid or basic hydrogel | Change in pH causes swelling which in turn release the drug |
| Ionic strength | Ionic hydrogel | Change in ionic strength cause change in concentration of ions |
| Chemical species | Hydrogel containing electron accepting group | Electron donating compounds causes formation of charge transfer thereby causing swelling and release of drug |

## 15.3 Externally Regulated Pulsatile Drug Delivery Systems

Magnetism, ultrasound, irradiation, and electrical impact are the sorts of external stimuli by which medications are released (Dalvadi et al. 2010). In a magnetically

regulated system, the system contains magnetic beads in the implant. On application of magnetic-field the drug releases. In case of ultrasonic regulated system ultrasonic waves cause the erosion of polymer matrix by which the drug release (Singh et al. 2011).

## 16. ADVANTAGES OF CHRONOTHERAPY

It is more effective when a person sleeps for several hours. While Chronotherapy patients often fall asleep this improves their condition and confidence as well. It is different from other treatments because it got the beginning, middle, and an end. So, one can predict easily the point at which it will work. It gives a new schedule like getting up and sleeping early which will be quite unusual for some days but it will give a period to adjust psychologically (Chandani et al. 2012).

## 17. CONCLUSION

Chronotherapy has emerged as a captivating field in modern medicine, harnessing the power of timing to optimize treatment outcomes. This article explored the recent advances in chronotherapy, highlighting the potential it holds for revolutionizing healthcare. The development of new drug formulations and delivery methods, coupled with the use of personalized treatment schedules, has paved the way for enhanced therapeutic efficacy and reduced side effects. By aligning drug administration with the body's natural circadian rhythms, chronotherapy offers a promising avenue for maximizing treatment benefits. However, further research is still needed to fully understand the intricate mechanisms underlying chronotherapy and to explore its application across various medical conditions. As the field continues to evolve, it is evident that chronotherapy holds great potential to transform the landscape of healthcare, providing patients with targeted and time-driven healing.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

## CONSENT AND ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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***Chapter 5***
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# Hepatotoxicological Evaluation of the Illicit Street Drug Nyaope: Chemical Profiling and Liver Damage in a Rodent Model

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## ABSTRACT

**Introduction:** The liver is one of the most important organs of the body that plays important roles in several functions. The drugs are metabolised in the liver. The most important causes of acute liver disease in the world include drug toxicity. In South Africa, there is a drug called nyaope, which is mainly used for recreational purposes. The administration of nyaope may cause severe health outcomes in the human body system. Nyaope is a cocktail using different household chemical compounds, only known by the dealer.
**Aim:** The aim of the study will be to investigate the effect of nyaope on the morphology of the liver.
**Methods:** The nyaope samples were supplied by the SAPS with the permission of DoH. The Nyaope powder sample was mixed in three different solvents, then analysed using a GC-MS machine. A total of 24 Wistar rats were divided into the control and experimental groups. The nyaope was dissolved in 90% saline water and administered at 0.1mg/ml in the 12 experimental rats for 30 days. After 30 days, the rodents were sacrificed and the livers were harvested and collected, and the blood samples were analyzed for histology analysis and biomarkers analysis, respectively. The Graph Pad was used to analyse the biomarkers. The Kruskal-Wallis test was carried out at $p< 0.05$.
**Results:** The nyaope samples dissolved in the methanol showed more ingredients compared to other solvents. Heroin and its derivatives were identified in all the solvents. The morphology of the liver after histology showed that the central vein was highly affected after administration of the nyaope solution. All liver biomarkers in the experiment were significantly higher in the experimental rodents compared to the control, with a p-value $<0.05$.

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**Discussion:** Heroin was the most common ingredient found in the nyaope samples. In the current study, it was very clear that nyaope can modify the morphology of the liver cells. The morphological change may lead to the presence of biomarkers in the blood samples. Biochemical analysis and histopathological observation showed that heroin-addicted rats had liver injury. It can cause hepatic injury, which might affect the physiological functioning of the liver in the experimental group.
**Conclusion:** The effect of the other compound that is present in the nyaope needs further investigation. There must be an investigation to search for the strategy of controlling the use of nyaope amongst the youth community.

*Keywords: Nyaope; illicit drug; adulterated drug; heroin; substance abuse; street dealers; recreational drug and black community.*

## 1. INTRODUCTION

The use of street adulterated illicit drugs is a worldwide health problem (WHO, 2023). These drugs usually consist of a base drug (cocaine, heroin, amphetamine) mixed with substances that apparently serve to enhance the effects of the base drug (Carroll & Dickinson, 2024; Hegazy et al., 2024; Ahmed et al., 2021; Mthembi et al., 2019). The additional substances are typically household chemicals and over-the-counter medications that are common and easily accessible. Adulterated illicit drugs do not have any scientific nomenclature and are subsequently known by their local name. Nyaope is a relatively new, prohibited, psychostimulatory, adulterated illicit drug used for recreational purposes in South Africa. Its consumers are mainly males, aged between 15 and 35, and the drug is preferably smoked in combination with cannabis (Daniels et al., 2024; Morgan et al., 2019; Fernandes & Mokwena, 2016; Khine et al., 2015). There are several methods used for the administration of nyaope. Some users prefer to smoke cannabis mixed with nyaope. While under, add a small quantity of water to the nyaope powder and flame the mixture to dissolve. This method is preferred by the users that choose to administer the nyaope in the form of infusion through an injection. The users have the skill of identifying the most profound vein or artery in the different points of their body for the administration of nyaope infusion (Akuzawa et al., 2023; Hoeberg et al., 2022). Some users will choose to snort the well-refined powder through their noses. While others prefer to share blood from the primary to the secondary user, which is called “Bluetooth”. Where the primary user will administer the nyaope in any of the above-mentioned methods. After the reaction has taken place and the effect of nyaope has kicked in, then withdraw blood from any point in there that will be transferred to the secondary users. The secondary user will also feel the effect of the nyaope similarly to the primary user. The life span of the nyaope in the blood lasts for approximately 45 minutes in the body system before the user administers the next “hit”. The intake of nyaope has been associated with significant disruptions in the social, mental, and physical lives of young people (Daniels et al., 2024; Morgan et al., 2019; Fernandes & Mokwena, 2016; Khine et al., 2015). Despite these alarming observations, research into nyaope and its toxic effects is limited.

The main constituent has been found to be heroin, but traces of other ingredients (antiretroviral drugs, rat poison, detergents) have also been reported

(Daniels et al., 2024; Mthembi et al., 2019; Khine et al., 2015). One of the aims of the present study was therefore to determine the chemical composition of nyaope samples obtained from the South African Police Services Forensic Laboratory. The toxicity of heroin can be assessed at the whole body, organ system, or cellular level. The behavioural effects of heroin highlight the impact on the function of the central nervous system (Baldo, 2025; Hill et al., 2022; Richardson, 2021; Tolomeo et al., 2021). However, heroin, as an opioid, may also affect other organ systems, including the respiratory (Baldo, 2025; van der Schrier et al., 2022) and gastrointestinal system (Sekhotha & Sekhotha, 2024)(M. S. Milella et al., 2023). The current study focused on the effect of heroin on the liver since a recent study by several authors demonstrated the presence of heroin metabolites (6-monoacetyl-morphine, morphine, 6-acetylcodeine, and codeine) in post-mortem liver tissues of heroin-related fatalities (Owolabi et al., 2021; Ruggiero et al., 2022). Also, a very interesting study by Zhao et al., (2025) and Bardhi et al., (2022) showed that cannabis products inhibit the hydrolysis of heroin in the liver. Decreased hydroxylation of heroin in the liver is of particular importance in the South African context, where nyaope (heroin) is commonly used in conjunction with cannabis and this may explain the persistence of high levels of the drug in the body of nyaope users (Chen et al., 2024; Lee, 2003). While heroin has not yet been implicated in clinically relevant hepatic injury, the prevalence of infectious diseases, such as hepatitis C, in substance users has been documented (Hileman et al., 2023; Farnsworth et al., 2021). The second aim of our study was therefore to determine whether nyaope has direct toxic effects on the liver.

## 2. MATERIALS AND METHODS

Nyaope is an adulterated drug used mainly by youth in South Africa for recreational purposes (Fernandes & Mokwena, 2016). To characterize the contents of nyaope, samples were collected from the South African Police Forensic Services Laboratory (Pretoria, South Africa; SAPS 2019/05). The project obtained permission to have nyaope for experimental purposes from the National Department of Health (NDoH), South Africa (POS 140/2018/2019; POS 385/2019/2020). Samples were documented and stored under strict access control. Witwatersrand University ethics committee awarded me permission to conduct this study.

### 2.1 Chemical Analysis of Nyaope

This section of the study was conducted in conjunction with the Department of Chemical Pathology in the Faculty of Health Sciences at the Witwatersrand University. Street samples were ground into a fine powder using a mortar and pestle. Separate aliquots of the homogenized street sample ranging from 10 mg to 16 mg were weighed into a 20 mL vial and mixed with 1 mL of each of the tertiary butyl alcohol, dichloromethane and isopropanol internal standard solution. This was done to see whether the composition of nyaope was solvent-dependent. Tertiary butyl alcohol has previously been shown to be the solvent of choice for presenting nyaope extracts to the GC–MS (Daniels et al., 2024; Diekhans & Lurie, 2022; Mthembi et al., 2018). Gas chromatography-mass spectrometry was used

to analyse the samples, as this is the method of choice to separate (gas chromatography) and identify (mass spectrometry) small amounts of volatile compounds. The National Institute of Standards and Technology (NIST) database was used to identify compounds according to their Chemical Abstracts Service (CAS) registry number. The CAS registry number is a unique numerical identifier assigned by the Chemical Abstracts Service to every chemical substance described in the open scientific literature. The obtained identifications were verified against another database known as the Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants developed (Brueckner et al., 2025; Maurer, 2021).

## 2.2 Animal Husbandry

A total of 24 male and female Wistar rats were used in this project. There is adequate evidence that shows that different genders react differently to the reaction of administered adulterated drugs (Gallant et al., 2025; Weng et al., 2022). The rats were obtained from and housed in the Wits Research Animal Facility at the University of the Witwatersrand. The six to eight-week-old rats were housed in groups of three in standard Perspex cages with wood chip bedding and *ad libitum* access to water and food mainly the pellets (90% of the diet), The temperature in the housing area was monitored using a HOBO data logger with U10 software (Wantit All (Pty) Ltd, Linbro Park, Frankenwald Gauteng, South Africa) and maintained at 22.5$^{o}$C. The light intensity of the housing room and behavioural test area was similarly kept at about 325 lux. Humidity ranged from 60-70% and the lights were connected to a timer set at a 12-hour cycle, with lights on between 06h00 – 18h00. All animals were weighed daily to accustom them to being handled and to monitor their overall well-being.

## 2.3 Animal Treatment

Rats were grouped into two genders of the age ranging from 8 to 10 weeks old. In subsequent experiments, rats were treated with either saline (1 ml, intraperitoneal, for five consecutive days) to serve as controls, or nyaope (1 mg/kg, intraperitoneal, for five consecutive days as indicated by the pilot study). The nyaope was dissolved in 90% saline, filtered using Grade 4 filter paper, and the supernatant was collected in an Erlenmeyer flask for further use, while the precipitate was discarded. The new, fresh nyaope solution was prepared on a daily basis. The animals were treated for 30 days. The animals were weighed daily to monitor the effect of nyaope on the body mass of the animals and to adjust the volumes of solutions to ensure correct dosing.

## 2.4 Experimental Design and Protocol

A total of 24 Wistar rats were used for this project. The rodents were then divided into two groups. The control groups consist of 12 Wistar rats (i.e., 6 females and 6 males). The experimental group consists of 12 rats (i.e., 12 females and 12 males). The experimental procedure lasted 30 days. All animals were decapitated the day after the end of the experimental protocol (Day 30). While treatments and assessments were staggered, the schedule was designed in such a manner that

representatives of each group were decapitated on one day. Following decapitation, trunk blood was collected and centrifuged and the plasma was kept for biochemical analysis at a later stage. Liver tissue was harvested into saline-buffered 10% formalin for histological investigation.

## 2.5 Blood and Tissue Sampling

Twenty-four hours after the last behavioural test was performed, the animals were anaesthetized (ten seconds of exposure to halothane-saturated air in an anesthetic chamber) before being decapitated. Trunk blood was collected and centrifuged at 1500 rpm for 10 minutes and the plasma was aliquoted into Eppendorf tubes for subsequent biochemical analyses and stored in a -20 $^{o}$C refrigerator. Liver tissue was sampled and placed in a 10% phosphate-buffered formalin solution for subsequent histological analysis.

## 2.6 Assessment of the Effect of Nyaope on the Liver

### 2.6.1 Tissue processing (Embedding)

The 10% formalin-fixed liver tissue samples were cut and mounted into a cassette for paraffin embedding. The cassette was inserted into a tissue processing machine (ATP 300 Close Tissue Processor, LABOTEC, SA) for approximately 24 hours, where the liver tissue was dehydrated and infiltrated with wax, forming a wax block housing the tissue sample. After placing the wax block on ice for 20 minutes, it was positioned in a microtome (Leica RM2125 RTS, LABOTEC, South Africa) for sectioning at 5 µm thickness. The sections were initially placed in a water bath set at 25 $^{o}$C for five minutes before being mounted onto a glass slide. Three sections per animal were placed on one slide. Slides were dried in an incubator at 30 $^{o}$C for four hours before being stained.

### 2.6.2 Staining procedure

Two staining techniques were used. Some slides were subjected to Mayer's hematoxylin and eosin staining to gain insights into the overall cellular structure and nuclear integrity of the tissues, while other slides were stained with Masson's trichrome stain to evaluate the supporting structures within the tissues, for example, connective tissues and collagen fibers. The procedure entailed deparaffinizing and dewaxing the sections with xylene for ten minutes, followed by a series of immersions in 100%, 95% and 70% ethanol for rehydration. The sections were then washed with distilled water for one minute.

For Mayer's hematoxylin staining, the slides were exposed to hematoxylin solution for ten minutes and washed under slow-running tap water for five minutes. The sections were subsequently differentiated by dipping the slides twice in 1% acid alcohol. The sections were checked under the microscope to verify the appropriateness of the stain. The slide was washed under slow-running tap water for five minutes, stained with Scott's tap water (alkaline medium) for five minutes and washed with running tap water for another five minutes. Sections were then counterstained with a 1% eosin solution for ten minutes.

### 2.6.3 Dehydration and mounting

After the sections were washed under tap water for another five minutes, the slides were dehydrated by immersing them in 70%, 95% and 100% ethanol solutions sequentially. The sections were then cleared by immersing them twice in xylene for ten minutes. The sections were finally mounted in Entillin for evaluation under light microscopy (Olympus CX23 Microscope, Olympus, Auckland Park, South Africa). Masson's trichrome staining was done according to the method by Suvarna et al., (2012). After the sections were dewaxed, rehydrated and washed under tap water, they were exposed to a celestine blue solution for five minutes. The slides were rinsed in distilled water and stained with alum hematoxylin for five minutes and washed with tap water for about ten minutes until a noticeable blue color was obtained.

## 2.7 The Effect of Nyaope on Liver Enzymes

The plasma levels of several enzymes were determined using veterinary rapid in-clinic assays, which serve as indicators of liver function. These enzymes were alanine transferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). Additionally, the plasma concentration of albumin was also measured. Ten microliters of plasma were pipetted into a cassette preloaded with reagents for the specific tests and placed in a biochemical analyzer (Catalyst Dx Chemistry Analyzer, IDEXX Laboratories, Midrand, South Africa). The results of this automated technology were subsequently captured on a computer connected to the equipment.

## 2.8 Statistical Analysis

All statistical analyses were done using GraphPad Prism (Version 9.02) software. Descriptive statistics are provided that include minimum and maximum values, median, 25 and 75% percentiles, arithmetic mean, standard deviation, standard error, and lower and upper 95% confidence intervals, where appropriate. Normality of tests was performed using the Shapiro-Wilk test. For parametric data, analyses of variance (ANOVAs) or repeated ANOVAs were used, followed by t-tests. For non-parametric data, Holm-Sidak tests or Kruskal-Wallis tests were used, depending on whether the data were repeated or not. This was followed by Dunn's multiple comparison test. Where group differences (saline vs nyaope; pre- and post-treatment) were obtained, they were shown to be significantly different, and additional analyses were performed to identify sex differences. If no significant differences were found, no further analyses were conducted.

# 3. RESULTS

## 3.1 Composition of Nyaope Using Mass Gas Chromatography

Nyaope samples were obtained from the forensic department of the South African Police Services (SAPS). The nyaope samples were in a powder form that weighed on average 0.07mg (Fig. 1). Three different solvents (dichloromethane, ethanol,

and methanol) were used in the preparation of nyaope for gas chromatography-mass spectrometry (GC/MS) analysis. When nyaope samples were extracted using dichloromethane, significant peaks were identified for caffeine, codeine, heroin, and morphine (Table 1). A similar pattern was observed when nyaope samples were extracted with ethanol (Table 2). Extraction of nyaope using methanol yielded a more comprehensive list of compounds (Table 3). In the current study, significant peaks were identified as 1(3H)-isobenzofuranone, 1H-purine-2,6-dione, 3, 7dihydro-1, 3, 7-trimethyl-acetamide, acetylcodein, 6-MAM and diacetylmorphine (Tables 1, 2 & 3). Despite slight differences, all three solvents showed high concentrations of heroin and heroin-related products.

**Fig. 1. Nyaope packaged for distribution**

## 3.2 Histological Analysis of the Liver Tissue of Rats Treated with Saline or Nyaope

Fig. 2 shows the morphology of the liver of a saline-treated rat. The photomicrographs show the normal structure at 10X (A) and 40X (B) magnification. The central vein, sinusoids and sheets of healthy-looking hepatocytes can easily be identified. Rats treated with nyaope displayed a different morphology. There was collagenous thickening around the central vein and a reduction in sinusoidal space/sizes compared to the saline-treated animals (Fig. 3A). The Masson trichrome stain indicated the presence of connective tissue and fibrosis around the portal tract and central vein of nyaope-treated animals, as well as intense staining of collagen (Figs. 3 B and C).

## 3.3 Analysis of Plasma Enzyme Levels of Rats Treated with Saline and Nyaope

Plasma levels of albumin, alanine transaminase and lactate dehydrogenase were assessed to investigate the effects of nyaope treatment on liver function. Animals treated with nyaope (n=12) had significantly lower plasma albumin levels compared to saline-treated animals ($p<0.0001$, unpaired t-test, n=12, Fig. 4).

**Table 1. Compound identified in nyaope samples using dichloromethane extraction**

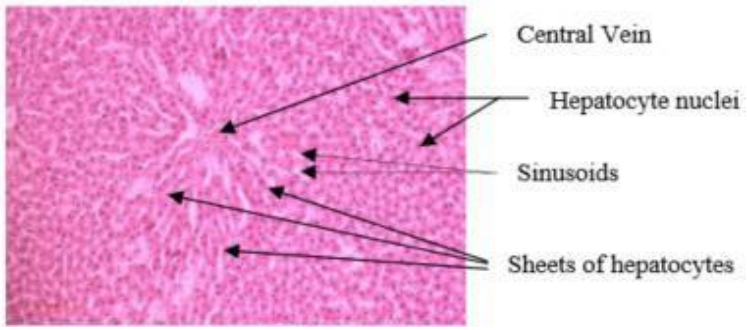
| Retention time (min) | Library / ID | Reference no | CAS # | Quality (%) |
|---|---|---|---|---|
| 8.303 | Caffeine | 191 | 000058-08-2 | 95 |
| 12.556 | Codeine | 224 | 006703-27-1 | 93 |
| 12.685 | Heroin-M (6-acetyl- morphine) | 525 | 059833-14-6 | 95 |
| 13.276 | Morphine 2AC | 225 | 000561-27-3 | 96 |

**Table 2. Compounds identified in samples extracted with ethanol**

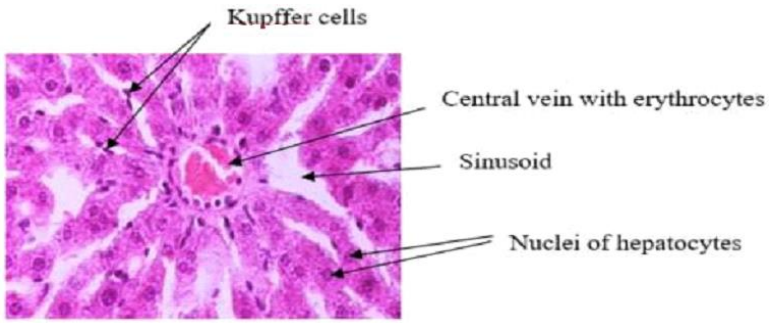
| Retention time (min) | Library / ID | Reference no | CAS # | Quality (%) |
|---|---|---|---|---|
| 8.299 | Caffeine | 191 | 000058-b08-2 | 94 |
| 12.558 | Codeine | 224 | 006703- 27-1 | 93 |
| 12.700 | Heroin-M (6-acetyl morphine) | 525 | 059833- 14-6 | 95 |
| 13.279 | Morphine 2AC | 225 | 000561-27-3 | 95 |

**Table 3. Compounds identified in samples extracted with methanol**

| Retention time (min) | Library / ID | Reference no | CAS # | Quality (%) |
|---|---|---|---|---|
| 13.734 | 1(3H)- isobenzofuranone | 52288 | 000569-31-3 | 99 |
| 16.371 | 1H-Purine-2,6-dione 3,7dihydro-1,3,7- trimethyl- acetamide | 53168 | 000058-080-2 | 97 |
| 16.457 (Same as 16.371) | 1H-Purine-2,6-dione 3,7dihydro-1,3,7- trimethyl acetamide | 53168 | 000058-080-2 | 97 |
| 16.553 (Same as 16.371) | 1H-purine-2,6-dione 3,7dihydro-1,3,7- Trimethyl Acetamide | 53170 | 000058-080-2 | 97 |
| 32.479 | Acetylcodein | 148310 | 1000153-00-2 | 98 |
| 32.537 | 6- Monoacetylmorphine | 140705 | 002784-73-8 | 99 |
| 33.033 (Same as 32.537) | 6 -Monoacetylmorphine | 140705 | 002784-73-8 | 99 |
| 33.575 | Diacetylmorphine | 160913 | 000561-27-3 | 99 |
| 33.627 (Same as 33.575) | Diacetylmorphine | 160913 | 000561-27-3 | 99 |

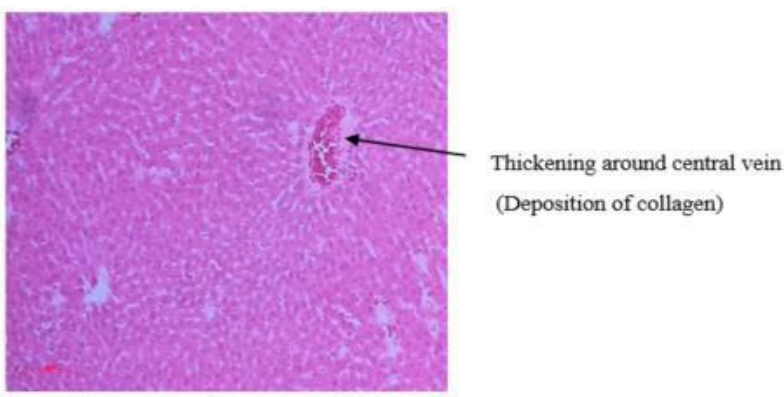



A

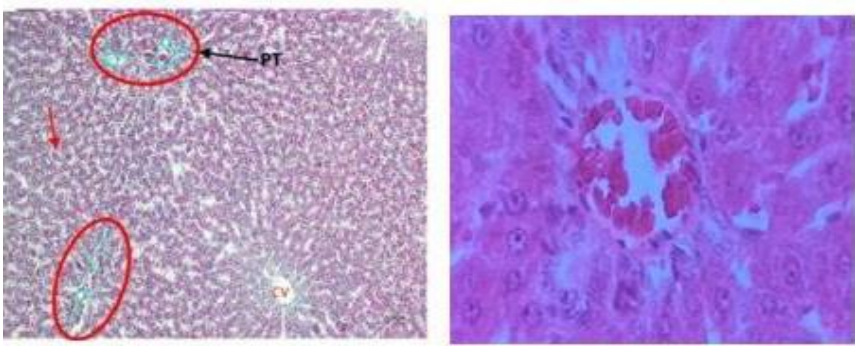



B

**Fig. 2. A representative photomicrograph of the liver tissue of a saline-treated rat at 10X (A) and 40 X (B) magnification**




**A**




**B** **C**

**Fig. 3. Representative photomicrographs of haemotoxylin and eosin stained (A) and Masson trichrome stained (B & C) tissues of the liver of a nyaope-treated rat at 10X (A & B) and 40X (C) magnification (PT- Portal tract; CV- central vein**

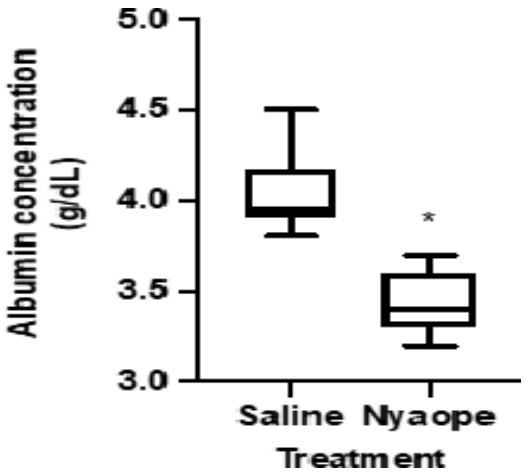



**Fig. 4. Plasma albumin levels of saline (n=12) and nyaope (n=12) treated animals**

*Data are presented as mean ± SD. *p<0.0001, Unpaired t-test*

Fig. 4 shows the plasma albumin levels of male and female rats treated either with saline or nyaope. A significant decrease in plasma albumin levels was observed in both male ($p<0.001$) and female ($p<0.0001$) animals (n=6 per group). The comparison between the saline and nyaope treated animals showed a significant difference with p-value <0.005.

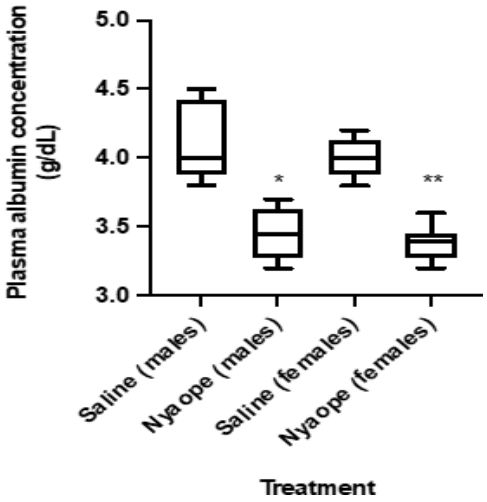



**Fig. 5. Plasma albumin levels of saline and nyaope-treated male and female rats**

**p<0.001, Nyaope (males) vs Saline (males); **p<0.0001, Nyaope (females) vs Saline (females)*

There were 6 animals in each group. Data are presented as mean ± SD. Analysis by one-way ANOVA followed by the Bonferroni post-hoc test (Fig. 5). There was a significant difference between nyaope and saline male rats (P-value <0.05). Furthermore, there was a significant difference between saline and nyaope-treated females with a P-value <0.05.

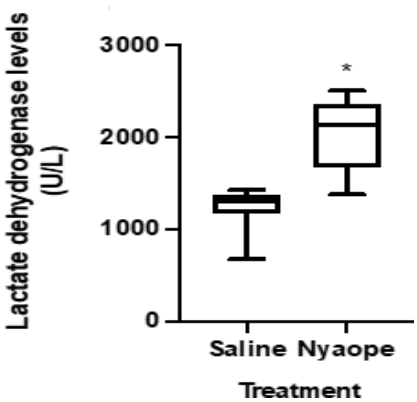



**Fig. 6. The lactate dehydrogenase level of saline (n=12) and nyaope (n=12) treated rats (*p<0.0001, Nyaope vs Saline)**

There was a significant difference between rats treated with saline and nyaope, with a p-value <0.05 (Fig. 6).

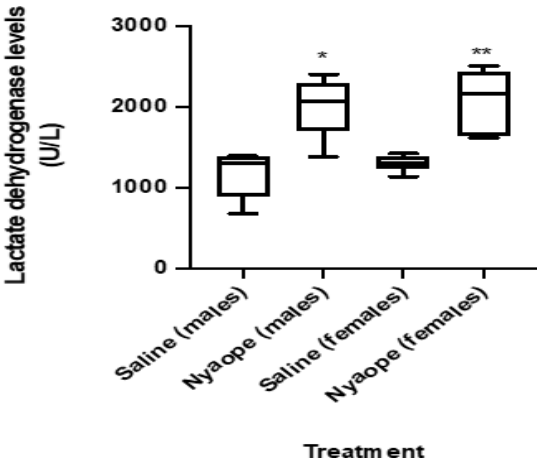



**Fig. 7. Lactate dehydrogenase (LDH) levels of males (n= 6) and females (n=6) treated with saline or nyaope with *p<0.002, Nyaope (males) vs Saline (males); **p<0.001, Nyaope (females) vs Saline (females)**

There was a significant difference based on gender between saline and nyaope-treated animals, with a p-value <0.05 (Fig. 7).

## 4. DISCUSSION

### 4.1 Characteristics of Nyaope Using Mass Gas Chromatography

Characterization or profiling of street drugs is important to gain insights into the active ingredients that mediate their effects. Therefore, drug profiling provides the chemical basis that underpins the pathophysiological impact of the drug. The profiling exercise simultaneously identifies additional substances that are added during the preparation or production of the drug by the dealer. Identifying the composition of a street drug is therefore vital to understanding its mechanism of action and to informing remedial strategies to address harmful effects. Nyaope has previously been described as low-grade heroin to which various other compounds have been added as bulking agents (Daniels et al., 2024; Di Trana et al., 2022; Mthembi et al., 2019; Khine et al., 2015). In the current study, nyaope samples were subjected to three different extraction solvents, namely dichloromethane (Fig. 2), ethanol (Fig. 3), and methanol (Fig. 3). The three methodologies provided comparable data that unequivocally showed that the nyaope samples contained significantly high levels of 6-acetyl-morphine (6-AM) and morphine (Tables 1, 2 &3). In contrast to previous reports, we found nyaope to consist mainly of heroin and heroin intermediates of exceptionally high quality and purity. The data of this project do not support the description of nyaope as a low-grade version of heroin.

Indeed, my study highlights this misconception and encourages scientists and health care professionals to refer to nyaope in the same vein as heroin.

Heroin, or diamorphine, is a diacetyl derivative of morphine. In the body, it is rapidly converted to 6-AM or monoacetylmorphine (MAM). In my analysis of nyaope, this major heroin derivative appeared in significant amounts together with 3-acetyl morphine (3-AM) (Tables 1, 2 & 3). 3-AM is a less active metabolite of heroin compared to the more active 6-AM. A study by several authors showed that 6-AM displays effects like those of pure heroin (Abdoli et al., 2024; Avvisati et al., 2019; Mthembi et al., 2019). For instance, it exhibits robust reinforcing properties in a rat self-administration setting akin to heroin and evokes drug-seeking behaviour equal to heroin. 6-AM is further metabolised to morphine (morphine 2AC) and hence the presence of high concentrations of this compound was not surprising. The study by Avvisati et al. (2019) showed that the heroin metabolite 6-AM resembles heroin in the ability to sustain self-administration behaviour and to precipitate relapse into drug seeking after a period of abstinence. As a result, 6-AM has reinforcing effects and may contribute to addictive behaviour (Tables 1, 2 & 3).

Besides heroin, notable amounts of codeine and caffeine were also identified in the nyaope samples, yet in much smaller quantities (Tables 1, 2 & 3). Since these compounds by themselves have addictive properties (Abdoli et al., 2024; Frigerio et al., 2021), their presence adds to the potential of nyaope to induce SUD. Codeine, a “weak” opioid (Cangadis-Douglass et al., 2025; Maher et al., 2024; Yang et al., 2022), has been suggested to be a gateway substance to other opiates, including morphine and heroin. It is well known that the administration of codeine relieves pain and, when ingested in higher doses, may lead to pleasurable, euphoric sensations. The physiological mechanisms of codeine-induced euphoria are well documented. Codeine binds to μ-opioid receptors to elicit its effects on the central nervous system (Hadland et al., 2024; Rosen et al., 2022; Rieder & Jong, 2021). Codeine is normally O-demethylated to form morphine via the activity of the cytochrome P450 enzyme CYP2D6 (Guo et al., 2021). However, studies have shown that codeine can also be converted to codeine-6-glucuronide and that this metabolite can generate opioid effects (Castellote-Caballero et al., 2025). The combined action of morphine and codeine-6-glucuronide may therefore lead to an escalated nyaope-mediated pleasurable experience.

The effects of caffeine (Table 2) on the central nervous system are varied. Clinical studies have shown an association between chronic caffeine ingestion and negative mood states such as anxiety, restlessness, insomnia, and tachycardia (Nouri-Majd et al., 2022; Rieder & Jong, 2021) and impaired cognitive function ( Yelanchezian et al., 2022; Fiani et al., 2021). On the other hand, preclinical experiments reported caffeine administration to stimulate motor activity in rodents (Lalonde & Strazielle, 2024). The effects of caffeine are proposed to be mediated via activation of adenosine A1/A2 receptors that influence DAergic neurotransmission and subsequent protein kinase A (PKA)-dependent mechanisms (Faraco & Gaspar, 2025; Gurram et al., 2023) Caffeine, in conjunction with heroin, may have synergistic effects that may result in a greater

deleterious impact on the wellbeing of nyaope consumers. For instance, caffeine, which is present in many products, modulates neurotransmitter systems in mesocorticolimbic brain regions. Studies have found that caffeine induces positive effects in animal models of certain neurological diseases, in part by modulating dopaminergic signaling (Ruggiero et al., 2022). It is also worth noting that caffeine, at high doses, induces undesirable health responses in other systems, including cardiovascular, skeletal, and muscular systems (Alasmari, 2020).

Unlike those of others, the extractions showed no significant traces of bulking agents (Nelson & Ramirez, 2022). A reason for this discrepancy could be differences in methodology. Khine and co-workers used two independent methods to characterize their nyaope samples that included both gas chromatography-mass spectrometry (GC-MS) as well as time-of-flight direct sample analysis mass spectrometry (TOF DSA MS). Khine & Mokwena (2016) reported nyaope to contain antiretroviral drugs, antidepressants, and benzodiazepines, in addition to morphine, caffeine, codeine and amphetamine. Despite variations in experimental procedures, the data collected show that nyaope has a strong opiate base and, therefore, is an extremely unsafe substance to consume. Alternatively, variations in the composition of nyaope could also be attributed to differences in additive ingredients used in the production of the drug in the various provinces (Monteiro et al., 2023; Di Trana et al., 2022; Fernandes & Mokwena, 2016). Nevertheless, all studies show that nyaope consists mainly of heroin and its metabolites.

## 4.2 The Effect of Nyaope Treatment on Liver Structure and Function

As a major site of metabolism, the liver has been central to many studies investigating the toxic potential of xenobiotics (Lee, 2003). As such, liver structure and function have been extensively studied in substance abusers (Figs. 2A & B). For instance, a test center in Singapore reported the prevalence of hepatic dysfunction in approximately 75% of 1500 parenteral substance users (Zacharia & Jacob, 2024; Monteiro et al., 2023; Li et al., 2021). Similarly, the intake of illicit substances accounted for approximately 20-40% of all instances of severe and sudden-onset hepatic failure admitted in a drug rehabilitation center in New York ( Robinson et al., 2024; Li et al., 2021; Montazerifar et al., 2021). Epidemiological statistics such as these clearly show the vulnerability of the liver to exogenous drugs.

Morphological changes of the liver associated with extended exposure to substances of abuse included the development of steatosis and inflammation (Montana et al., 2024; Wu et al., 2023; Scendoni et al., 2021). Montana et al. (2024), after completing a clinical study in Italy, reported that in a cohort of 851 patients suffering from substance dependence, steatosis was observed in about 70% and portal inflammation in 93% of cases. At the ultrastructural level, it seems as if mitochondria (Allard et al., 2021; Mihajlovic & Vinken, 2022), hyperplasia and hypertrophy of the smooth endoplasmic reticulum (Milella et al., 2023; Yue et al., 2023), can occur with chronic drug intake as evidenced in heroin addicts. Morphological changes to the sinusoid system of the liver include thickening of the sinusoidal wall and fibrosis of the perisinusoidal space (or space of Disse) - a

location in the liver between a hepatocyte and a sinusoid (Liu et al., 2024; Pinazo-Bandera et al., 2022). In the current study, organelle modification was observed in the liver of nyaope-treated animals (Figs. 3 A, B & C). The walls of the sinusoids are lined with phagocytic cells, called Kupffer cells, that digest old red blood cells and clear the bloodstream of toxins or hazardous substances. The results obtained in the current study agree with the histological findings described in the paragraphs above. The rats treated with nyaope displayed diminished sinusoidal spaces compared to saline-treated rats. In addition, collagen deposits appeared around the central vein and there was a presence of connective tissue and fibrosis in the proximity of the portal tract in the livers of nyaope-treated animals. These structural abnormalities are in line with the observations of comparable clinical and preclinical studies and confirm opioid-mediated increases in liver collagen fibers and the development of liver fibrosis (Maher et al., 2024; Yang et al., 2022).

The morphological abnormalities invoked by substances of abuse are often associated with physiological function disturbances. According to several authors (Charuni, 2024; Nath et al., 2022; Okibor & Mormah, 2022), drug users frequently exhibit hepatocyte damage resulting in impairments in liver protein and enzyme synthesis, location, and activity. Injury to hepatocytes, therefore, is commonly reflected in the release of liver enzymes into the bloodstream (D'Ottavio et al., 2023; Milella et al., 2023). Long-term use of opioids has acute effects on the homeostasis of the body. It is for this reason that the present study also compared the plasma concentrations of albumin, alanine transaminase and lactate dehydrogenase between nyaope-treated animals and their saline-treated controls. The data showed that the plasma albumin levels were significantly lower in nyaope-treated rats compared to saline-treated animals for both males and females (Fig. 7). Hence, in other studies, nearly 56% out of a total of 25 patients, 25-45 years, who were heroin addicts, showed a level of ALT higher than the normal level (D'Ottavio et al., 2023; Montazerifar et al., 2021). While plasma alanine transaminase levels were not significantly different between the two groups of animals, the plasma concentration of lactate dehydrogenase was significantly higher in rats that were treated with nyaope compared to saline-treated rats (Fig. 7).

Together, these biochemical results support the morphological findings, showing nyaope to markedly affect liver structure and function. The fact that the plasma levels of alanine transaminase did not change significantly suggests that the nyaope-induced effects were moderate and not severe enough to cause extensive liver damage (Fig. 7). Such an explanation is plausible and underpins the modest effects of nyaope on the behaviour of the animal. Nevertheless, the nyaope-induced changes observed in the current study are consistent with existing literature reporting opioid dependence to be associated with elevated plasma levels of liver enzymes such as lactate dehydrogenase (Asatiani et al., 2025; Baum et al., 2021; Hesaruiyeh et al., 2022).

## 5. CONCLUSION

Nyaope (also known as "whoonga" or "wunga") is a highly addictive, dangerous, and destructive street drug, unique to the South. Whereby a single "hit" can cost

as little as R30 ($2.12) (1.15 GBP) for a parcel or straw. It is mainly used in highly concentrated impoverished townships in nine provinces in the South. Whoonga is said to be used as a recreational drug. Reports indicate that a whoonga addict needs several doses a day; however, users are typically too poor to afford the drug out of their legal income. It is mainly smoked by heating ingredients and inhaling the fumes. The drug is usually smoked with cannabis in the form of a joint, but methods of preparation for injection do exist. It initially makes users feel euphoric or, when using heavier doses, a wonderful sense of relaxation, but the effect soon wears off and another hit is required. Further investigation needs to be conducted to assess the physiological effect of the method used on the body system. Some less pleasant side effects may include a painful stomach, muscle cramps and generally feeling ill, but when these ease up, they use it again. Whereby the raw ingredients of nyaope are only known by the dealer. But low-grade heroin is mainly used and mixed with other compounds. While it is not always clear what else nyaope consists of, the ingredients vary from place to place. Some of the alleged ingredients are a cocktail of anti-retroviral drugs, milk powder, rat poison, bicarbonate of soda and pool cleaner. But in the sample used for this project, there were no traces of anti-retroviral drugs. There is adequate evidence that dealers are known to add "all sorts of stuff" to a drug to bulk it out for commercial purposes.

Administration of nyaope may lead to serious health conditions such as heart attacks, hepatitis B or C and lung-related diseases such as TB. Substances used to administer the nyaope by injecting the drug are at a higher risk of heart-related illnesses than those who smoke it, who, in turn, are more at risk of lung disease. People who inject the drug often suffer valve failure, which means their heart function is dramatically reduced. Other substances used during the synthesis of the drug affect the brain, leading to impaired reasoning, memory loss, and other mental health disorders. The drug, which is highly addictive, can also trigger mental illness among patients who have a genetic history of mental illness. The presence of the Nyaope in society is one of the biggest concerns that we are faced with in South Africa. Consequently, further research is needed to provide deeper insights into its effects and to inform effective prevention and intervention strategies.

## 6. LIMITATION

This study was carried out for a short period. Also, the dose of the nyaope solution was administered once a day.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Matome M Sekhotha hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## DISCLAIMER

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## ETHICAL APPROVAL

Ethical approval was obtained from the university's Animal Ethics Screening Committee (AESC) (Clearance certificate 2018/09/40/C). All procedures followed the standards approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Witwatersrand University Animal Care and Facilities Committee.

## CONSENT

It is not applicable.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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***Chapter 6***
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# Drug Utilization Pattern at the Emergency Department of a Tertiary Health Care Hospital in India

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## ABSTRACT

Drug utilization studies ensure patient safety by assessing the safe and effective use of drugs, optimizing treatment strategies for better outcomes and contributing to overall quality improvement in emergency care. The aim of the study is to understand drug utilization patterns at the emergency department of a tertiary health care hospital in India. The study was designed as a prospective observational study. The patients' data were collected and distributed on the basis of age, gender, comorbidities, disease and drug class in which the patients were observed for 6 months, and the conclusion was obtained with a sample size of 100 patients. Where the result was obtained according to the gender, males (64%) were more prone to disease than females (36%). The age group 51-60 years was more prone to disease, i.e., 28% than the 61-70 years (24%), and the least age group that visited the Emergency department was those 91-100 years (3%). The most common comorbidities that were observed during the study were hypertension (56 Patients), diabetes mellitus (45 Patients), hypothyroidism (10 Patients), and coronary artery disease (15 Patients), while 10 patients had no comorbidities. The common drugs used in ER are Pantoprazole, Ondansetron, Optineuron and Acetaminophen. In this study, the findings highlight the need for promoting rational drug therapy by encouraging the prescription of essential drugs using their generic names to improve health care outcomes and also in optimising drug therapy in the Emergency department.

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*Keywords: Drug utilization; comorbidities; emergency department; triage; drugs class.*

## 1. INTRODUCTION

Drug utilisation review (DUR) is an authorised, systematic, continuous assessment of the prescription, dispensing, and use of medication. According to WHO (1985 and 1993), DUR is defined as —the marketing, distribution, prescription and use of drugs in society, with special emphasis on the resulting medical, social and economic consequences (Dar et al., 2022; Gangwar et al., 2023). Drug utilization mainly focuses on the various medical, social, and economic aspects of drug use (Steinke, 2019). Drug utilization studies aim to increase our understanding of how medicines are being used in society, ensuring that medicines are used in an appropriate or rational manner (Gisev & Sluggett, 2019). DUR includes a review of drugs based on pre-established criteria, and if these are not satisfied, modifications to medication therapy are implemented. In order to guarantee effective pharmaceutical decision-making and favourable patient outcomes, Trostle (1996), it entails a thorough evaluation of patients' prescriptions and drug data before, during, and after distribution. DUR programs include corrective action, prescriber feedback, and additional evaluations as a quality assurance measure (Carver et al., 2023).

### 1.1 Importance of DUR

The prescription, administration, and use of pharmaceuticals can be better understood, interpreted, evaluated, and improved with the use of DUR programs, which are essential to manage health care systems. Because of their knowledge in the field of pharmaceutical therapy management, chemists are essential to this procedure (Babatabar-Darzi et al., 2020). The managed care pharmacist can use DUR to find patterns in patient prescribing, whether it is based on drug-specific criteria or disease-state criteria like high blood pressure, diabetes, or asthma. Subsequently, chemists could work with prescribers and other members of the healthcare team to improve drug therapy (Kim et al., 2018).

It gives the doctors insightful input regarding the prescription rationality by analysing the results of various intervention types offered to enhance rationality in drug use. It also evaluates the intervention's influence on drug use in the population. Drug use research can be conducted using a variety of techniques that are qualitative or quantitative. (Zachariasse et al., 2017). The comprehension of numerous facets, diverse design and the criteria for carrying out drug use research is highlighted in this study (Goedken et al., 2018).

**Steps involved in conducting Drug Evaluation-** (Thim et al., 2012)

- Identify/determine optimal use
- Measure actual use
- Evaluate
- Intervene

- Evaluate the DUR program
- Report the DUR found

Problems related to drug prescriptions are common worldwide, with medication errors and adverse drug events being the primary causes. In the Emergency department, patients are admitted without any prior appointment by themselves own/with an ambulance for the examination of urgent or emergent conditions requiring after-hours medical attention. Doctors in the ED deal with serious, Bazyar et al., (2019). urgent cases that require prompt, effective care. This makes it difficult for doctors to start and choose the right medications for their patients. Because of that, patients suffering from a wide range of diseases in a variety of acute or high drug use, the ED is an important place for conducting drug utilization studies (Barot et al., 2013). It assesses the initial stages of the disease and diagnoses it. These mostly consist of Adverse drug events and medication errors. Medication errors accounted for 5.7% of all drug administration episodes, according to a meta-analysis of 35 studies published between 1990 and 2005, Cheekavolu et al*.,* (2011). whereas adverse drug events impacted 6.1 patients out of every 100 hospitalised patients. Prescription errors are influenced by many factors, including polypharmacy, lack of adequate pharmacological knowledge, errors in patient records or nursing documentation, inadequate pharmacy staffing, female gender, age > 65 years, renal excretion of drugs, drugs with a narrow therapeutic index, and usage of Anticoagulants and diuretics. Moreover, adverse medication events ranging from 3% to 12% have been routinely recorded in several studies. Based on this research, the emergency department (ED) sees 1.5–3% of all adverse medication occurrences. On the other hand, the EDs had the greatest percentage of avoidable error prevalence (70–82%). FitzGerald et al*.,* (2012).

**Triage Process:** Triage is a system used to prioritise patient treatment according to illness/injury, severity, prognosis, and resource availability. Identifying patients in need of emergency resuscitation, placing them in a designated patient care area to prioritise their care, (Iserson, & Moskop, 2007). and starting diagnostic or therapeutic interventions are the goals of triage (Al Balushi et al., 2013).

The process of triage, which typically involves taking vital signs and assigning a "chief complaint" (e.g., chest pain, abdominal pain, difficulty breathing, etc.), is the first step a patient goes through. The majority of emergency rooms have a specific space set aside for this procedure, and they may employ personnel whose sole responsibility is triaging patients. Most departments assign a triage nurse to this position, Jimmy et al., (2023). Although other medical personnel, including paramedics and physicians, may also be tasked with triage sorting, depending on training standards in the nation and region (World Health Organization, 1993, Malhotra, and Rana, n.d).

**Triage Categories (Christian, 2019; Peta et al., 2023):**

**Red – Emergency**

A life-threatening medical condition. Expect to receive Immediate attention.

**Orange - Very Urgent**

A serious medical condition. Expect attention after red patients have been stabilized.

**Yellow - Urgent**

Expect attention after red and orange patients have been stabilised.

**Green – Routine**

You can function without immediate care and will be attended to as soon as Possible.

## 2. MATERIALS AND METHODS

**Participants:** A Minimum of 100 patients were included in this study

**1. Inclusion criteria:**

- All patients, irrespective of diagnosis, admitted to the emergency department. Kaur et al., (2014).
- Patients above 18 years.
- Patients, irrespective of gender.

**2. Exclusion criteria**

- Incomplete and illegible data were excluded.
- The patients who are from the non-emergency department.
- The drugs that were already being taken by the patient due to their concomitant
- Illnesses were excluded. Kosuge, et al., (2013).
- Pregnant/lactating females and individuals aged less than 18 years.
- Patient who refuses to give consent.

**Study Type and Duration:** The study was designed as a prospective observational study and was conducted over a duration of 6 months.

**Study Procedure:** The study duration:

- Identifying the need for the study.
- Designing of the study proforma.
- After receiving approval from the Institutional Ethical Committee. The study was conducted in the Emergency department.
- This study was observational and prospective in the Emergency Department.
- All case sheets were reviewed, and the cases that met the inclusion and exclusion criteria were selected. Lindner, & Woitok, et al., (2021).

- A proforma was designed in which the demographic details like age, gender, past medical history, present history, final diagnosis, and medication chart are included. In which Patient data will be collected during the study period.
- After collecting the data, a proforma patient treatment chart will be studied.
- Then, we will observe the commonly reported cases and drugs being prescribed for the particular disease in the ER.
- All the prescribed drugs will be noted along with all the data being given in a proforma.

**Statistical Analysis:** Data was entered in Microsoft Excel 201,6, and also data was presented by using descriptive statistics, i.e., Count and percentage. Patel et al., (2013). Data was visually represented by the pie diagram and bar graph. Data analysis was carried out by SPSS software version 22.

## 3. RESULTS

It was a prospective observational study. This study includes 100 patients in hospital.

**Table 1. Distribution of the Patients according to Gender (N=100)**

| Gender | No. of Patients | Percentage |
|---|---|---|
| Male | 64 | 64% |
| Female | 36 | 36% |

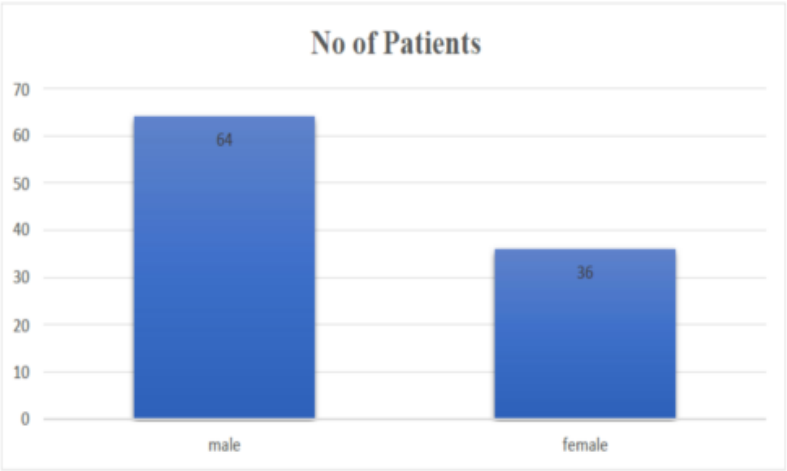


**Fig. 1. Distribution of patients according to gender**

Among the 100 patients, 64% are male and 36% are female. It was observed that the male population is higher than the female population in the emergency department.

**Table 2. Distribution of patients according to age**

| Age | No. of Patients | Percentage |
|---|---|---|
| 20-30 | 5 | 2% |
| 31-40 | 4 | 4% |
| 41-50 | 8 | 8% |
| 51-60 | 28 | 28% |
| 61-70 | 24 | 24% |
| 71-80 | 15 | 15% |
| 81-90 | 13 | 13% |
| 91-100 | 3 | 3% |

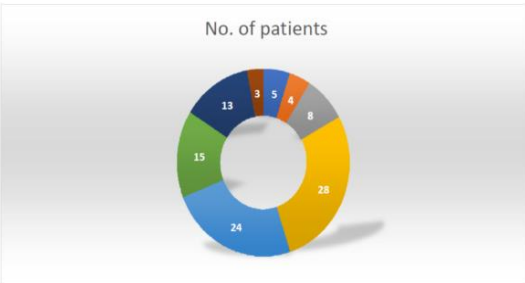


**Fig. 2. Distribution of patients according to age**

Fig. 2 indicates that in 100 patients 05 patients are under 20-30 age group, 04 patients are under 31-40 age group, 08 patients are under 41-50 age group, 28 patients are under 51-60 age group, 24 patients are under 61-70 age group, 15 patients are under 71-80 age group, 13 patients are under 81-90 age group, 03 patients are under 91-100 age group. In our present study, it was found that patients between 50 years and 90 years are more likely to be reported to the ER Ojeniran et al*.,* (2010).

**Table 3. Distribution according to comorbidities**

| Comorbidities | No of Patients |
|---|---|
| HTN | 56 |
| DM1 | 1 |
| DM-2 | 45 |
| LA cervix stage 4 Hemorrhagic | 1 |
| Hypothyroidism | 10 |
| CAD | 15 |
| Seizures | 2 |
| Post-stroke epilepsy | 4 |

| Comorbidities | No of Patients |
|---|---|
| ADHF | 7 |
| Pyelonephritis | 1 |
| Asthma | 4 |
| CVA | 10 |
| Cardioembolic stroke | 1 |
| Psychiatric disorder | 2 |
| Epilepsy | 3 |
| Parkinsonism | 7 |
| Schizophrenia | 1 |
| Aspiration pneumonia | 1 |
| Type 2 respiratory failure | 1 |
| hypoglycemia | 1 |
| Bradycardia | 2 |
| Ischemic stroke | 4 |
| Old Kochs disorder | 1 |
| Cirrhosis of the liver | 1 |
| Tuberculosis | 1 |
| Vertigo | 1 |
| CKD | 7 |
| Splenectomy | 1 |
| Pancreatitis | 2 |
| Hepatic jejunum | 1 |
| PAD | 1 |
| Tibial occlusion | 1 |
| Thrombolysis | 1 |
| Ileostomy | 1 |
| Ppb | 1 |
| Acute Myocarditis | 1 |
| SNHL | 1 |
| PTCA with stunt | 3 |
| Anemia | 1 |
| ACS NSTEMI | 3 |
| Psoriasis | 1 |
| MCTD | 1 |
| ICD | 1 |
| PCOD | 1 |
| Hyperthyroidism | 2 |
| Hyponatremia | 1 |
| Old PTB | 1 |
| Circulation Stroke | 1 |
| Tracheostomy | 1 |
| Hemiparesis | 3 |
| DVT | 2 |
| Acute Cardiogenic Pulmonary Oedema | 2 |
| Moderate ARDS | 2 |
| Moderate LV Dysfunction | 2 |

| Comorbidities | No of Patients |
|---|---|
| ACS TVD | 1 |
| Dry Gangrene | 1 |
| Grade 2 Prostomegaly | 1 |
| CNS lymphoma | 1 |
| Hypertriglyceridemia | 1 |
| Dementia | 2 |
| UTI | 1 |
| Portal HTN | 1 |
| Intracranial SDL | 1 |
| Ild | 2 |
| Nil | 10 |

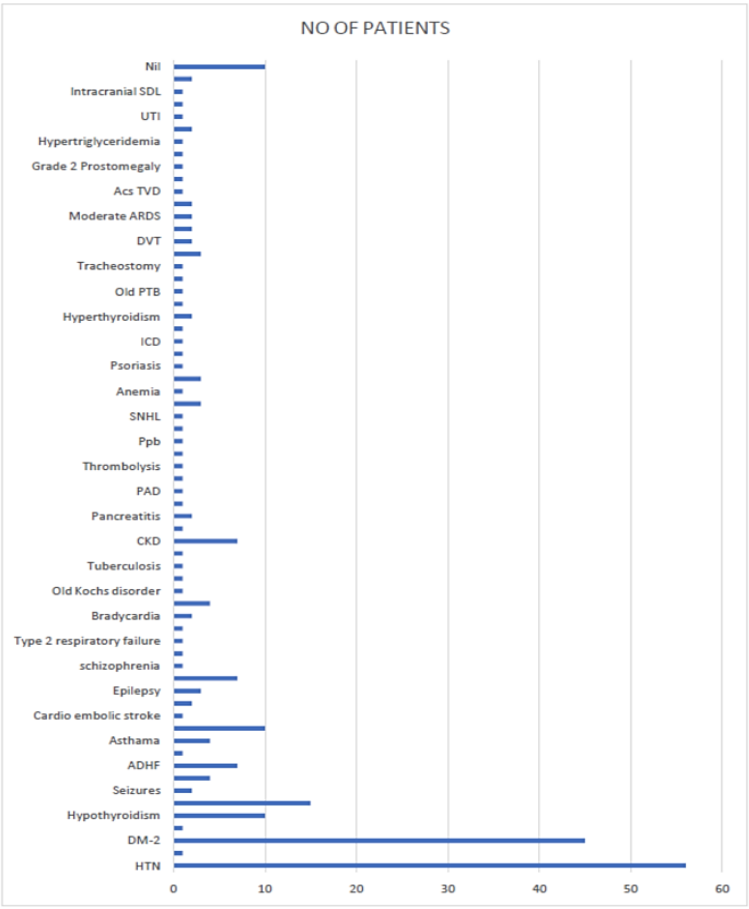


**Fig. 3. Distribution of patients according to comorbidities**

Fig. 3 indicates that out of 100 patients, 56 patients consisted Hypertension, 45 consisted diabetes mellitus, 10 Hypothyroidism, 15 CAD, 7 ADHF, 10 CVA, 7 Parkinson, 7CKD, 4 Post stroke epilepsy, 4 Asthma, 4 Ischemic stroke, 3 Epilepsy, 3 PTC and STUNT, 3 ACS NSTEMI, 3 Hemiparesis, 2 Acute Cardiogenic Pulmonary Edema, 2 Moderate ARDS, 2 Moderate LV Dysfunction, 2 Dementia, 2 Ild, 2 Seizure, 2 Psychiatric disorder, 2 Bradycardia, 2 Pancreatitis, 2 Hyperthyroidism, 2 DVT, 2 Acute cardiogenic and others like ACS TVD, Schizophrenia, DM1, Hypertriglyceridemia, vertigo, old Kochs disorder Type 2 respiratory failure, Hypoglycemia etc. Pendhari et al., (2013), and also, there are 10 patients who do not have any past medical history.

**Table 4. Distribution of patients according to disease**

| Diseases | No of Patients |
|---|---|
| Pneumonia bronchitis | 8 |
| Acute ischemic stroke | 14 |
| Hemorrhagic stroke | 4 |
| Traumatic brain injury | 2 |
| Seizures | 14 |
| CVA | 8 |
| Encephalopathy | 11 |
| LRTI, Type III | 6 |
| Septic shock | 2 |
| COPD | 1 |
| CAD | 7 |
| CKD | 10 |
| AKI | 6 |
| Hemiparalysis | 2 |
| Acute gastric shock | 1 |
| DKA | 2 |
| Fractures | 4 |
| Neurogenic shock | 1 |
| Febrile illness | 4 |
| Cholelithiasis | 1 |
| Parkinsonism | 8 |
| Vertigoz | 1 |
| ADHF | 9 |
| Gallstones in the bladder | 1 |
| Altered mental state | 1 |
| UTI | 4 |
| Asthma | 1 |
| Electric burns | 1 |
| Hernia | 1 |
| Anaemia | 3 |
| AWMI | 1 |
| Myasthenia gravis | 2 |
| Arnold chiarinflammation | 1 |
| Hypoglycaemia | 1 |

| Diseases | No of Patients |
|---|---|
| Hyponatremia | 1 |
| Cirrhosis | 1 |
| Cerebral edema | 1 |
| Old SAH with IVH | 1 |
| IC bleed | 2 |

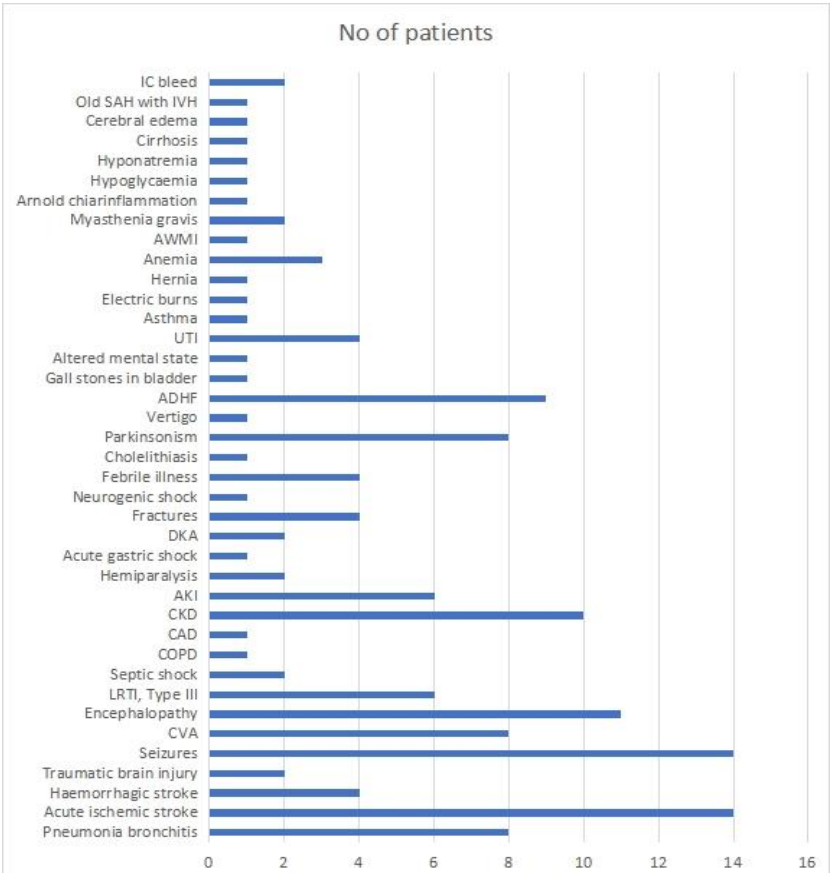


**Fig. 4. Distribution of patients according to disease**

From the Fig. 4, we conclude that out of 100 patient 14 were affected with Acute ischemic stroke, 14 Traumatic brain injury, 11 encephalopathy, 10 CKD, 9 ADHF, 8 Parkinson's, 8 Pneumonia bronchitis, 8 CVA, 6 LRTI type-3, 6 AKI, 4 hemorrhagic strokes, 4 fracture, 4 febrile illness, 4 UTI, 3 Anemia and other diseases like septic shock, COPD, neurogenic shock, electric burn, asthma, hypoglycemia, hernia, altered mental state, hyponatremia, cirrhosis, cerebral edema, old SAH with IVH and IC bleed. Quick et al., (2002).

**Table 5. Distribution of patients according to drug**

| Drugs | No of Patients |
|---|---|
| Sodium Chloride | 13 |
| Furosemide | 10 |
| Pantoprazole | 90 |
| Ondansetron | 46 |
| Mannitol | 4 |
| Dexamethasone | 1 |
| Optineuron | 45 |
| Clopidogrel | 5 |
| Aspirin | 10 |
| Acetaminophen | 28 |
| Duolin | 7 |
| Budesonide | 12 |
| Calcium gluconate | 11 |
| Dextrose | 4 |
| Etomidate | 2 |
| Phytonadione | 7 |
| Tetanus toxoid | 2 |
| Cefuroxime | 1 |
| Levetiracetam | 16 |
| Levothyroxine | 2 |
| Magnesium sulphate | 4 |
| Hydrocortisone | 9 |
| Salbutamol | 11 |
| Ceftriaxone | 2 |
| Ursodeoxycholic Acid | 1 |
| Heptagon | 1 |
| Nor adrenaline | 5 |
| Syndopa | 2 |
| Vasopressin | 1 |
| Insulin | 3 |
| Metoprolol Succinate | 1 |
| Nico Malone | 1 |
| Torsemide | 1 |
| Tramadol | 5 |
| Nicorandil | 2 |
| Telmisartan | 1 |
| Ranolazine | 1 |
| Nitro-glycerin | 1 |
| Carvedilol | 1 |
| Lactulose | 1 |
| Magnex forte | 10 |
| Sodium bicarbonate | 1 |
| Dobutamine | 1 |
| Enoxaparin | 3 |
| Meropenem | 4 |

| Drugs | No of Patients |
|---|---|
| Silver sulfadiazine | 1 |
| piperacillin | 4 |
| Atorvastatin | 7 |
| Alteplase | 1 |
| Ipratropium bromide | 1 |
| Escitalopram | 1 |
| Clonazepam | 1 |
| Clindamycin | 7 |
| Apixaban | 1 |
| Neurobionforte | 1 |
| Metoprolol | 1 |
| kcl+mgso4 | 1 |
| Hyoscine butyl bromide | 1 |
| Fenofibrate | 1 |
| Doxycycline | 1 |
| Erythropoietin. | 1 |
| Atrovent | 1 |
| Tranexamic Acid | 1 |
| Rosuvastatin | 1 |
| Tenecteplase | 1 |
| Amiodaron | 1 |
| Lysergic acid Diethylamide | 1 |
| levocarnitine | 1 |

Out of 100 patients, we observed that 90 patients were given with Pantoprazole, 46 with Ondansetron, 45 with Optineuron, 28 with Acetaminophen, 16 with Levetiracetam, 13 with Sodium Chloride, 12 with Budesonide, 11 Calcium gluconates, 11 with Salbutamol, 10 with Aspirin, 10 with Magnex forte, 10 with Furosemide, 9 with Hydrocortisone, 7 with Phytonadone,7 with Atorvastatin, 7 with Clindamycin, 7 with Duolin, 5 with Tramadol, 5 with Noradrenaline, 5 with Clopidogrel, 4 with mannitol, 4 with Dextrose, 4 with Magnesium sulphate, 4 with Meropenem, 4 with Piperacillin, 3 with Insulin, 3 with Enoxaparin and others like Dexamethasone, Tetanus toxoid, Cefuroxime, Levothyroxine, Ceftriaxone, levocarnitine, Rosuvastatin, Doxycycline, Metoprolol, Clonazepam, Alteplase, Nitro-glycerine, Telmisartan, Ipratropium bromide etc (Fig. 5). Robertson-Steel, (2006).

From Fig. 6, we conclude that out of 100 patients, 90 patients were using the class of proton pump inhibitor, Siddiqi, et al*.,* (2002). 47 multivitamins, 46 antiemetic, 29 antibiotics, 28 analgesics, 16 anticonvulsants, 15 antiplatelet, 13 electrolytes, 12 corticosteroids, 11 bronchodilator, 11 antidiuretics, 11 calcium supplements, 10 NSAIDs, 8 HMG CO-A reductase inhibitor, 7 vitamin supplements, 6 diuretics, 5 Adreno-receptor agonists,5 opioid analgesic, 4 glucose elevating agent, 3 antidiuretics, 3 antidiabetic, 3 beta blockers, 3 anticoagulant, 2 anesthetic, 2 thrombolytic, 2 CNS depressants, 2 dopamine agonist, 2 potassium channel activator, 2 anticholinergic agents, 2 antifibrinolytics and other classes like hepatoprotective agents, nutritional supplement arbs,

vasodilator, antilipemic, laxative agent, metabolic modulator, erythropoiesis stimulating agent, antiarrhythmic agent, psychedelics agents, factor X-A inhibitor, thyroid drugs, immune booster, etc. Suresh, R., & Selva, P. et al*.,* (2020).

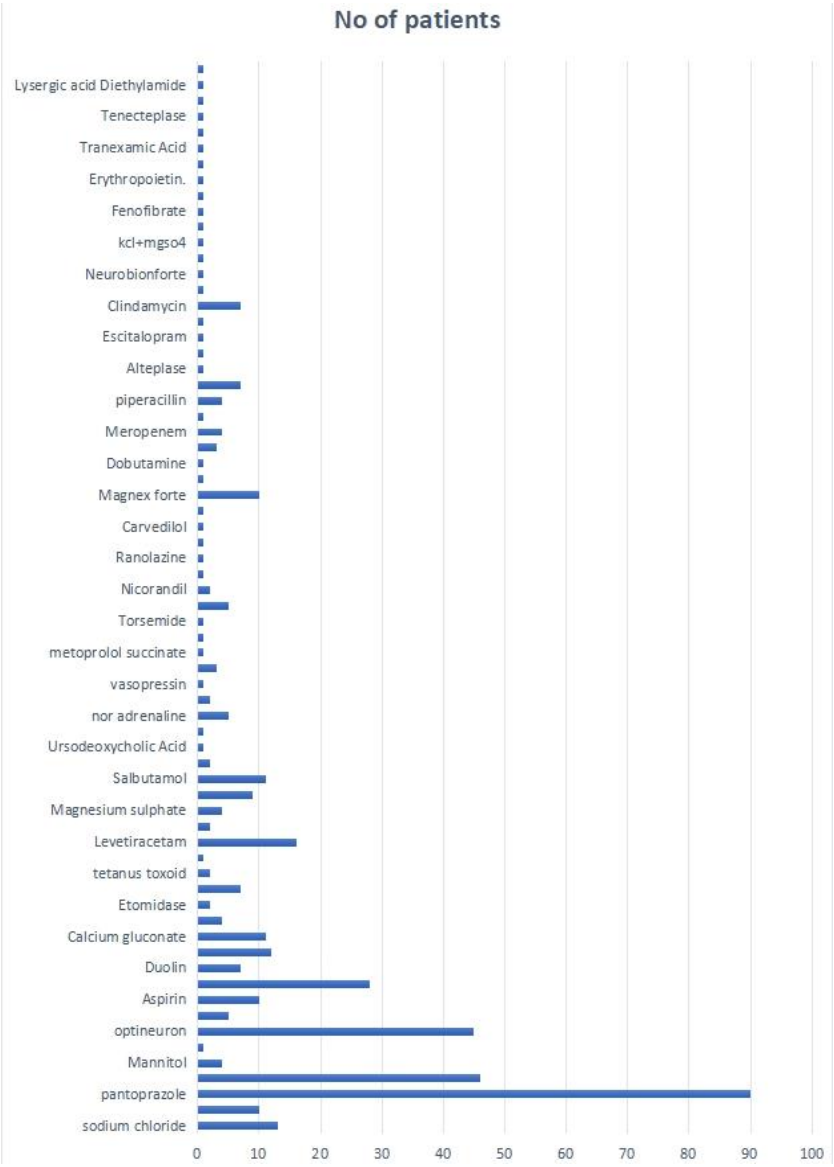


**Fig. 5. Distribution of patients according to drug**

**Table 6. Distribution of drugs according to class**

| Drugs Class | No of Patients |
|---|---|
| Anticonvulsant | 16 |
| Corticosteroid | 12 |
| Bronchodilator | 11 |
| Hepato Protective Agent | 1 |
| Nutritional Supplement | 1 |
| Adrenoceptor Agonists | 5 |
| Dopamine Agonist | 2 |
| Anti Diuretic | 11 |
| Anti Diabetic | 3 |
| Beta Blocker | 3 |
| Anticoagulant | 3 |
| Loop Diuretic | 2 |
| Opioid Analgesic | 5 |
| Potassium Channel Activator | 2 |
| Arbs | 1 |
| Metabolic Modulator | 1 |
| Vasodilator | 1 |
| Antilipemic | 1 |
| Laxative Agent | 1 |
| Tetracyclines | 1 |
| Antibiotics | 29 |
| Anticholinergic Agents | 2 |
| Erythropoiesis Stimulating Agents | 1 |
| Antifibrinolytics | 2 |
| Hmgco-A REDUCTASE INHIBITORS | 8 |
| Antiarrhythmic Agents | 1 |
| Psychedelics | 1 |
| Electrolytes | 13 |
| Anta Acids | 90 |
| Antiemetics | 46 |
| Diuretics | 4 |
| Multivitamins | 47 |
| Antiplatelet | 15 |
| NSAIDS | 10 |
| Analgesics | 28 |
| Calcium Supplements | 11 |
| Glucose-Elevating Agent | 4 |
| Anaesthetic Agent | 2 |
| Immune Booster | 1 |
| Antithyroid Drugs | 1 |
| Alkalizing Agent | 1 |
| Sulphonyl Ureas | 1 |
| Thrombolytics | 2 |
| SSRIS | 1 |
| Factor-Xa Inhibitors | 1 |

| Drugs Class | No of Patients |
|---|---|
| CNS Depressents | 2 |
| Tissue Plasminogen Activator | 1 |
| Ltras | 1 |
| Supplements | 7 |

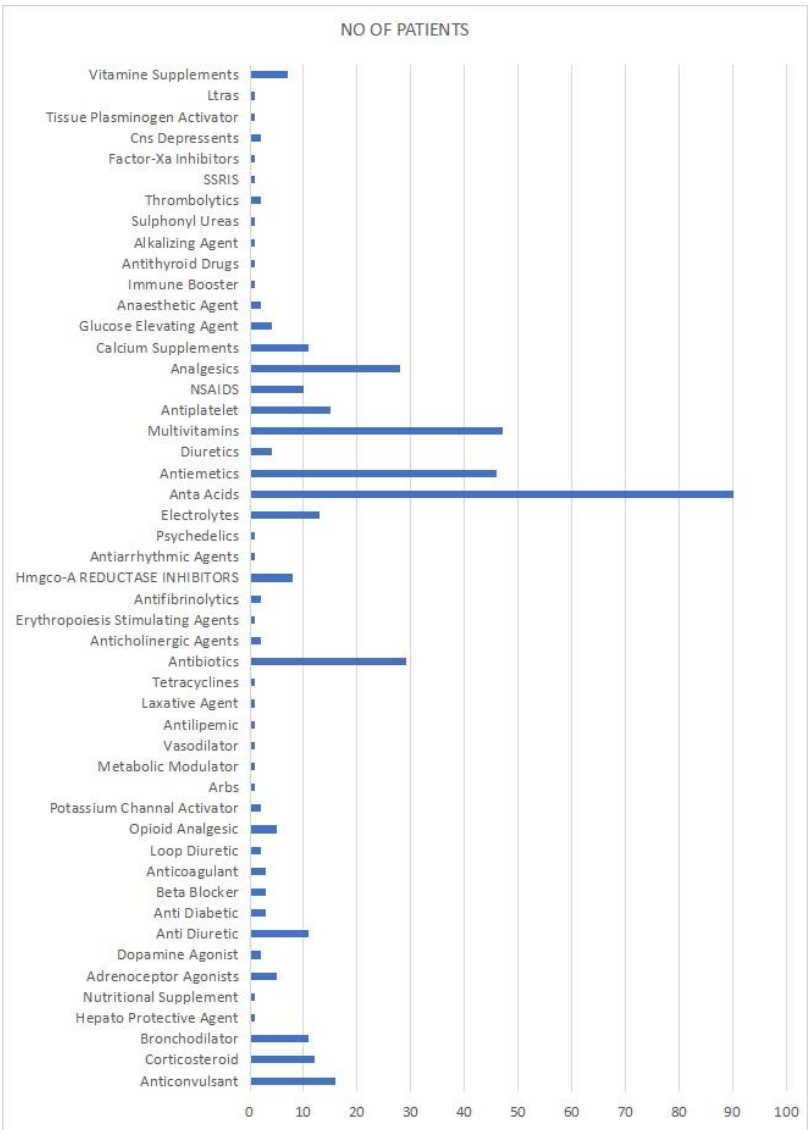


**Fig. 6. Distribution of drugs according to class**

## 4. DISCUSSION

Analysing the drug utilisation pattern in a care hospital involved a thorough examination of prescribing practices, therapeutic classes, and adherence to formulary guidelines. By assessing factors such as generic versus brand usage and the duration of therapy, healthcare professionals can gain insights into the appropriateness and efficiency of medication regimens. Thim et al*.,* (2010).

Our study is a prospective observational study in which 100 patients were examined for around 6 months throughout the study period,100 medication charts in total were examined and the patients' chief complaints regarding the symptoms were observed, and a stabilising treatment was provided in the ER according to a particular diagnosis. In this study, the male population is more recruited for this study compared to females in the emergency department. About 64% were male and 36% were female. Also, as per the data, patients aged 51-90 are more prone to disease; approximately 80% of the patients are under this age group. The most common diseases that were observed were Acute ischemic stroke, Seizures, Encephalopathy, CAD, CKD, ADHF, Parkinson's, hypoglycemia, and Anaemia. As per this study, some common comorbidities associated with the diagnosis are- HTN, DM 2, Hypothyroidism, CAD, ADHF, CVA, Parkinsonism, CKD, Post stroke epilepsy, Asthma, Ischemic stroke, Epilepsy, PTC and STUNT, ACS NSTEMI, Hemiparesis. Also, we observed the common class of drugs that were commonly used in ER to stabilise the patients were proton pump inhibitors, multivitamins, antiemetics, antibiotics, analgesics, anticonvulsants, antiplatelet, electrolytes, corticosteroids, bronchodilators, antidiuretics, calcium supplements, NSAIDs, HMG-CoA-A reductase inhibitors, or diuretics. Thim et al., (2012).

## 5. CONCLUSION

Antibiotics, antacids, proton inhibitors, analgesics, antiplatelets, NSAIDS and multivitamins were the most frequent classes of drugs administered to patients. As per our study, the highest number of drugs was prescribed for diseases like Seizures, Acute ischemic stroke, Encephalopathy, CAD, ADHF, Pneumonia, bronchitis and followed by Parkinsonism and CKD.

There is a need for promotion of rationalised therapy in terms of increasing prescription of drugs from the essential drug list by generic name.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

As per international standards or university standards, written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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***Chapter 7***
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# Pharmacological Potentials of *Mimosa pudica*: Antiparasitic, Antibiofilm and Mucolytic Activities

**Pinheiro, Elizabeth** [a,b++,#,†,‡*]

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## ABSTRACT

*Mimosa pudica* L., traditionally known as the sensitive plant or touch-me-not, is a species with a wide pantropical distribution and a history of ethnomedical use in treating gastrointestinal disorders, infections, and inflammatory processes. The plant has a diverse phytochemical profile, including L-mimosine, flavonoids, saponins, tannins, triterpenes, and a mucilage rich in glucuronoxylans. This metabolic arrangement confers multifunctional pharmacological activities, highlighting three main axes: (i) antiparasitic, through the cytostatic action of mimosine and synergistic effects of flavonoids and saponins on helminths and protozoa; (ii) antibiofilm, with the ability to interfere in critical stages of microbial community formation, reducing adhesion, biomass, and virulence of pathogens such as *Candida albicans* and *Streptococcus mutans*; and (iii) anti-mucoid, attributed to the seed mucilage, which exhibits bioadhesive and mechanical properties capable of promoting the removal of luminal residues and muco-biofilm structures associated with intestinal dysbiosis. From a toxicological point of view, studies indicate low acute toxicity in animal models, but prolonged exposure requires caution due to the iron-chelating effects of mimosine, reinforcing the need for chemical standardization and monitoring of safe doses. Translational perspectives include the standardization of extracts, randomized clinical trials, and integrative applications with probiotics, prebiotics, and conventional antihelminthics, as well as potential use in nanotechnological platforms. It is concluded that *Mimosa pudica* gathers sufficient ethnomedical,

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pre-clinical, and pharmacological evidence to be considered a multifunctional phytotherapeutic for intestinal health. Clinical validation and phytochemical standardization are essential steps to consolidate its role as an integrative and innovative therapeutic resource.

*Keywords: Mimosa pudica; antiparasitic; antibiofilm; mucilage; phytotherapeutic; standardization.*

## 1. INTRODUCTION

Intestinal infections caused by protozoa and helminths remain among the most persistent challenges in global public health, particularly in low- and middle-income countries where environmental and socioeconomic determinants—poor sanitation, contaminated water, social inequality, and limited access to health and education—sustain long-lasting transmission cycles and high reinfection rates (Keiser & Utzinger, 2010). Current estimates indicate that billions are exposed annually to soil-transmitted helminths, with cumulative impacts on anemia, malnutrition, growth retardation, and cognitive impairment in childhood, as well as long-term effects on productivity and well-being in adulthood (WHO, 2023; Pullan et al., 2014; Chen et al., 2024). In parallel, growing concerns about suboptimal cure rates and emerging drug resistance—e.g., with benzimidazoles for helminths and nitroimidazoles for protozoa—narrow the therapeutic arsenal and underscore the urgency for innovative, low-cost, and safe approaches suitable for vulnerable settings (Keiser & Utzinger, 2010; Ashok et al., 2022).

Over recent decades, a paradigm shift has consolidated in the understanding of gastrointestinal pathogenesis: microbial communities structured as biofilms, embedded in a polymeric matrix of exopolysaccharides, proteins, lipids, and extracellular DNA, contribute to chronicity, antimicrobial tolerance, and recurrence (Costerton et al., 1999; Donlan & Costerton, 2002). Endoscopic and molecular observations of adherent biofilm-like communities on intestinal mucosa highlight therapeutic targets across the biofilm life cycle—from initial adhesion and quorum-sensing–regulated maturation to matrix integrity—suggesting that anti-adhesive, anti-quorum, and matrix-disrupting strategies could enhance treatment and reduce relapses (Costerton et al., 1999; Donlan & Costerton, 2002). Although the term “mucoid plaque” is controversial in conventional gastroenterology, reports of viscous mucus/biofilm aggregates in dysbiotic states are conceptually coherent with contemporary biofilm biology and merit careful translational investigation.

Against this backdrop, medicinal plants re-emerge as reservoirs of bioactive molecules with modes of action complementary or alternative to existing drugs (Mandal et al., 2022; Muhammad et al., 2016). Among them, Mimosa pudica L.—the “sensitive plant” or “touch-me-not,” noted for its thigmonastic leaf folding—has attracted interdisciplinary interest. Originally South American and now pantropical, *M. pudica* thrives in ruderal and disturbed environments throughout tropical and subtropical regions, favoring broad ethnomedical availability (Ahmad et al., 2012). Its rapid leaf movement, mediated by ion and water fluxes in pulvini,

has motivated decades of botanical and physiological research; moreover, as a Fabaceae member capable of symbiosis with rhizobia, its rhizospheric ecology may influence phytochemical plasticity and nutrient economy (Ahmad et al., 2012; Rizwan et al., 2022). Historically, *M. pudica* holds a prominent place in traditional medical systems—from Ayurveda in South Asia to Indigenous and Afro-diasporic practices in the Americas and Africa—where it is used for dysentery, chronic diarrhea, amebiasis, hemorrhoids, wound healing, jaundice, pain, and inflammation (Ahmad et al., 2012; Joseph et al., 2017; Hassan et al., 2019). Convergence of ethnomedical records on gastrointestinal complaints suggests a multifaceted pharmacological portfolio triggered by preparations of aerial parts, roots, or seeds, often as decoctions or macerates (Joseph et al., 2017; Hassan et al., 2019).

From a phytochemical standpoint, *M. pudica* displays a complex architecture of secondary metabolites: the non-protein amino acid L-mimosine, flavonoids (notably quercetin and kaempferol), saponins, tannins, phenolic acids, coumarins, triterpenes, and a mucilaginous fraction rich in glucuronoxylans (Azmi et al., 2011; Bukhari et al., 2022; Wu et al., 2022; Noor et al., 2024; Rizwan et al., 2022). Composition varies with plant part, seasonality, geographic origin, and solvent polarity, which alter total phenolic content, glycosylation patterns, and even trace metal profiles—variables with implications for biological activity and batch-to-batch reproducibility (Azmi et al., 2011; Bukhari et al., 2022; Gandhi et al., 2023). Mechanistically, this dense chemical matrix favors synergy: metal chelators like L-mimosine can collapse iron-dependent pathways; saponins increase membrane permeability; polyphenols impose redox stress and interfere with microbial signaling; and the mucilage modulates local rheology and residence time on the mucosa (Panigrahi et al., 2019; Ashok et al., 2022; Rathnamali et al., 2018; Noor et al., 2024; Bukhari et al., 2022).

Among these constituents, L-mimosine stands out for inhibiting critical iron-dependent enzymes (e.g., ribonucleotide reductase), depleting deoxyribonucleotide pools, and blocking DNA replication at the G1/S transition—a cytostatic effect particularly relevant for rapidly dividing organisms, including protozoa (Panigrahi et al., 2019). Flavonoids add orthogonal pressure by disrupting membranes, dissipating proton-motive force, inhibiting energy enzymes, modulating efflux, and exerting anti-inflammatory/antioxidant actions supportive of mucosal healing (Rathnamali et al., 2018; Singh et al., 2014; Barua et al., 2017). This triad—chelation, membrane damage, and redox/signaling interference—supports a multi-target potential against parasites, biofilm microorganisms, and host-side inflammatory pathways.

The experimental literature corroborates a consistent anthelmintic signal. In classic assays with Pheretima posthuma, ethanolic leaf extracts induced paralysis and death in a dose-dependent manner with latencies comparable to albendazole (Bendgude et al., 2012). In vivo evidence in gastrointestinal helminth models further suggests reduced parasite load and viability consistent with luminal action, while at the population level, ongoing reliance on benzimidazoles is challenged by suboptimal cure rates for species such as Trichuris trichiura and

concerns about resistance under mass drug administration (Keiser & Utzinger, 2010). Recent combination strategies—e.g., albendazole plus ivermectin—have outperformed monotherapy in several settings, reinforcing the search for adjuvants that increase efficacy and mitigate resistance (Keiser & Utzinger, 2010).

Beyond direct antiparasitic effects, *M. pudica* exhibits antibiofilm activity against clinically relevant pathogens, including Candida albicans and Streptococcus mutans, reducing adhesion, biomass, and hyphal morphogenesis—key virulence traits underpinning persistence and recurrence (Desrini et al., 2023; Lobo et al., 2021). Because mucosal biofilms share organizational principles with oral and device-associated biofilms (adhesion, quorum sensing, matrix integrity), interventions that destabilize the matrix or interfere with microbial communication can sensitize pathogens to antimicrobials and host defenses (Costerton et al., 1999; Donlan & Costerton, 2002).

An additional differentiator is the abundant seed mucilage, composed primarily of glucuronoxylans with high viscosity, gelling capacity, and pronounced mucoadhesion at near-neutral pH—conditions typical of the distal ileum and proximal colon (Bukhari et al., 2022; Wu et al., 2022; Noor et al., 2024). These properties support roles as bioadhesive carrier, disintegrant, and particle adsorbent, with the mechanistic hypothesis that mucilage could facilitate aggregation and removal of viscous luminal residues (including muco-biofilm structures), provided mucosal safety and biofilm-science principles are respected (Bukhari et al., 2022; Noor et al., 2024). Regardless of terminology, the mucilage–biofilm axis offers a plausible translational pathway for barrier modulation and reduction of luminal microbial/particulate load.

On phytochemistry and safety, recent studies broaden the landscape: phytochemical screening consolidates the metabolite portfolio and suggests additional targets (Rizwan et al., 2022); repeated-dose oral toxicity in rodents has not shown clinically relevant adverse signs within customary exposure ranges (Jacob et al., 2025); and green nanotechnology approaches using *M. pudica* to mediate metallic nanoparticles indicate potential for delivery and anti-inflammatory activity (Abdulmumeen et al., 2024). Concurrently, geographic and seasonal variation in phenolics and trace metals reinforce the need for chemical standardization and quality control, prerequisites for reliable clinical translation (Gandhi et al., 2023; Noor et al., 2024; Rizwan et al., 2022).

Given the confluence of factors—persistent transmission, gaps in preventive chemotherapy coverage, resistance risk, the centrality of biofilms in chronicity, and the presence in *M. pudica* of orthogonal pharmacology (iron-dependent cytostasis by L-mimosine; antimicrobial/antibiofilm actions of flavonoids and saponins) plus a bioadhesive mucilage with favorable physicochemical properties—an integrative hypothesis emerges: standardized *M. pudica* extracts could serve as rational adjuvants in combined strategies for infection control, barrier support, and biofilm modulation in the intestine. This framework motivates a clear translational agenda: defining chemical markers and extract

specifications; characterizing luminal kinetics and local exposure; testing combinations with reference anthelmintics; and mapping effects on microbiota composition and mucosal inflammation in phase I/II clinical studies (Keiser & Utzinger, 2010; Desrini et al., 2023; Bukhari et al., 2022; Noor et al., 2024).

Finally, this chapter proposes a critical synthesis that interweaves the epidemiological and therapeutic landscape of intestinal parasitoses, contemporary foundations of gastrointestinal biofilm biology, the botany and mechanosensitive physiology of *M. pudica*, its phytochemical architecture and the pharmacotechnical properties of its mucilage, culminating in a balanced appraisal of translational evidence, knowledge gaps, and priorities for developing microbiota-conscious, standardized extracts. *M. pudica* is not presented as a panacea, but as a plausible multi-axis candidate for integration into evidence-based intestinal health programs.

## 2. THEORETICAL FRAMEWORK

### 2.1 Ethnomedical History of *Mimosa pudica* L.

*Mimosa pudica L.,* popularly known as the sensitive plant or touch-me-not, is a pantropical Fabaceae whose ethnomedical relevance spans centuries and diverse cultural matrices. Beyond its iconic thigmonastic leaf-folding—rapid laminar closure in response to tactile stimuli—the species has been valued for preparations employing aerial parts, roots, and seeds, credited with effects on gastrointestinal and inflammatory conditions in multiple traditional systems (Ahmad, Mishra, & Gupta, 2012; Joseph, George, & Mohan, 2017; Hassan, Zainal, & Ismail, 2019). The wide ecological availability of *M. pudica* in disturbed tropical landscapes likely facilitated its diffusion and persistence in local pharmacopoeias, enabling therapeutic continuity despite socioeconomic constraints (Ahmad et al., 2012).

In South Asian traditions—especially Ayurveda, where it is referred to as Lajjalu—records describe the use of infusions/decoctions and pastes for diarrhea and dysentery, hemorrhoids, wound healing, jaundice, and pain/inflammation, often empirically and sometimes in combination with other herbal remedies (Ahmad et al., 2012; Joseph et al., 2017). The convergence of gastrointestinal indications is consistent with the traditional clinical experience of “astringency” and “demulcency” attributed to the plant, properties that reflect chemical classes such as tannins (astringent effect) and mucilages (protective/soothing effect), although such attributions require modern standardization for clinical translation (Joseph et al., 2017; Hassan et al., 2019).

In Afro-diasporic and Indigenous contexts in the Americas and parts of Africa, *M. pudica* is used for chronic digestive complaints, "intestinal cleansing," and as an adjuvant against helminthiasis, usually as decoctions or macerations of the leaves and/or aerial parts. These practices reinforce an ethnomedical core focused on enteral disorders and mucosal recovery, with an emphasis on accessibility and low cost (Hassan et al., 2019; Joseph et al., 2017; Rizwan et al.,

2022). Although local terminology varies, the pattern of use suggests a multi-target empirical rationale—antiparasitic, biofilm modulator, and barrier support—that dialogues with contemporary pharmacological hypotheses (Rizwan et al., 2022).

The ethnopharmacology of *M. pudica* also shows specificity by plant parts. Leaves and aerial parts, rich in flavonoids and tannins, are associated with astringent, antimicrobial and anti-inflammatory effects; roots appear in vulnerary formulations; seeds, in turn, provide a glucuronoxylan mucilage, historically used for demulcent purposes to relieve luminal irritation (Ahmad et al., 2012; Kokane et al., 2009; Bukhari et al., 2022; Noor, Muhammad, & Hanif, 2024). Modern animal model trials support some of these practices: leaf extracts exhibit anti-inflammatory and pro-inflammatory mediator modulating activity, consistent with their traditional use in pain and inflammation (Singh, Singh, & Nath, 2014; Barua, Bora, & Bhagabati, 2017), while the mucilage exhibits high gelling and bioadhesion properties, consistent with reported demulcent use (Bukhari et al., 2022; Noor et al., 2024).

At the same time, the ethnomedical literature documents heterogeneity in preparations (infusion, decoction, maceration, poultice) and usage regimens, reflecting local ecological and cultural contexts. This variability is potentially amplified by seasonal and geographic factors that modulate phytochemical content (phenolics, flavonoids, oligosaccharides), impacting perceived potency and reproducibility—a critical point for translation and comparison between studies (Ahmad et al., 2012; Rizwan et al., 2022). Thus, although the cross-cultural recurrence of gastrointestinal and vulnerary indications for *M. pudica* is notable, contemporary interpretation requires standardized collection and extraction protocols, as well as chemical markers that anchor the continuity between traditional knowledge and scientific validation.

In summary, the ethnomedical history of *M. pudica* outlines a broad-spectrum medicinal resource, with a persistent emphasis on gastrointestinal tract conditions and inflammatory/healing processes. This trajectory provides a rational platform for modern research on three axes of particular clinical relevance—antiparasitic, antibiofilm, and antimucoid—while underscoring the need for phytochemical standardization and controlled clinical studies to ensure efficacy and safety (Joseph et al., 2017; Hassan et al., 2019; Rizwan et al., 2022).

## 2.2 Ecological Distribution

*Mimosa pudica* L., belonging to the Fabaceae family, is a perennial herbaceous plant with a wide pantropical distribution, whose ecological and physiological adaptation has contributed to its success as an ethnomedical and pharmacological resource. Originally native to South America, the species was disseminated, by anthropic and natural action, to tropical and subtropical regions of Asia, Africa, and Oceania, establishing itself with ease in disturbed soils, ruderal areas, roadsides, degraded pastures, and poorly managed urban

environments (Ahmad, Mishra, & Gupta, 2012; Rizwan et al., 2022). This capacity for rapid and resilient colonization ensures continuous availability for collection, sustaining the traditional and contemporary use of the plant in different communities, including populations with limited access to conventional medicines. The geographical expansion of the species also facilitates genetic and phytochemical diversity, contributing to local variations in the content of flavonoids, saponins, tannins, and L-mimosine, which can directly impact the effectiveness of ethnomedical preparations (Bukhari et al., 2022).

Botanically, *M. pudica* has morphological characteristics that make it easily identifiable and functionally adapted to adverse environments. The leaves are compound, bipinnate, and consist of small leaflets, capable of performing thigmonastic closure in response to mechanical, light, or thermal stimuli. This rapid movement, mediated by turgor changes in the pulvini and the activation of mechanosensitive ion channels, is not only an impressive visual feature but also a complex physiological manifestation that reflects the plant's ability to respond to environmental stresses (Ahmad, Mishra, & Gupta, 2012). The leaf morphology reduces water loss and protects the plant against herbivory, conferring adaptive advantages that may have been empirically perceived by traditional communities, reinforcing the choice of the species for medicinal purposes. The flowers, globose and pink or lilac in color, are grouped in terminal inflorescences and represent a prominent feature in the identification of the species, while the seeds are encapsulated in short, densely hairy pods, favoring dispersal by gravity, animals, and water flow (Rizwan et al., 2022).

From a physiological point of view, *M. pudica* shows remarkable adaptability to nutrient-poor soils and stress conditions, a characteristic shared by several legumes. The species forms symbiotic associations with β-rhizobia, promoting biological nitrogen fixation and increasing the availability of essential nutrients. This rhizospheric interaction not only sustains growth in degraded soils but may also influence the synthesis of secondary metabolites, such as flavonoids and L-mimosine, modulating the phytochemical profile and, consequently, the plant's pharmacological effects (Rizwan et al., 2022; Bukhari et al., 2022). Furthermore, the ability to colonize different types of soil and microhabitats, including ruderal and urban areas, demonstrates ecological and functional plasticity, allowing the plant to be a reliable resource for human communities, regardless of local environmental conditions.

The seasonal and geographical variability of the species plays a crucial role in its phytochemical composition. Factors such as luminosity, temperature, water availability, soil type, and inter-specific competition affect the concentration of active metabolites, including flavonoids, saponins, and tannins. Studies show that plants collected in regions with greater environmental stress tend to have higher levels of phenolic compounds, possibly as an adaptive defense mechanism, suggesting that the effectiveness of traditional preparations may vary according to geographical origin and collection time (Rizwan et al., 2022). This variation reinforces the importance of standardizing extracts for

pharmacological purposes, as well as offering an explanation for the empirical perception of different therapeutic potencies observed by traditional communities.

The botanical characteristics are also closely linked to ethnomedical practices. The rapid closure of the leaves may have reinforced the perception of the plant's "activity" or "vitality," possibly influencing the choice of specimens for medicinal use. The hairiness of the seeds and the facilitated dispersal by animals may have contributed to the selection of populations with a higher density of plants, ensuring a stable supply for human populations (Ahmad, Mishra, & Gupta, 2012; Joseph, George, & Mohan, 2017). This interaction between ecology, morphology, and cultural perception illustrates how environmental and biological factors can directly influence ethnopharmacology, promoting the continuity of the traditional use of the species over generations.

The ecological and botanical distribution of *M. pudica* is not only relevant for the preservation of the species but also for the planning of pharmacological studies. Knowledge of environmental and physiological variability allows for the selection of representative samples for chemical and biological assays, increasing the robustness of data on efficacy and safety. In addition, understanding its natural habitats and the conditions that affect the production of secondary metabolites provides support for strategies of cultivation, sustainable collection, and development of standardized products, capable of reproducing the therapeutic effects observed traditionally (Rizwan et al., 2022; Bukhari et al., 2022).

In summary, the wide geographical distribution, ecological adaptability, and unique botanical characteristics of *Mimosa pudica* L. are fundamental factors that explain its historical and contemporary relevance in ethnomedicine. The accumulated knowledge about morphology, physiology, and ecology provides a solid basis for modern pharmacological research, integrating empirical observations and scientific evidence. The association between physical characteristics, phytochemical composition, and environmental plasticity highlights the plant's potential as a reliable therapeutic resource, justifying detailed studies on mechanisms of action, standardization of extracts, and innovative applications in intestinal health (Rizwan et al., 2022; Bukhari et al., 2022).

## 2.3 Phytochemical Composition and Bioactivity of the Metabolites of *Mimosa pudica* L.

*Mimosa pudica* L. has a complex phytochemical architecture that supports the multiplicity of therapeutic effects observed ethnomedically and corroborated by recent pre-clinical studies. The plant synthesizes a wide variety of secondary metabolites, including flavonoids, saponins, tannins, triterpenes, coumarins, and the non-protein amino acid L-mimosine, as well as phenolic and antioxidant compounds. This chemical diversity not only provides multiple therapeutic targets but also allows for functional synergies, where different classes of metabolites

act in a complementary manner on parasitic organisms, microbial biofilms, and host tissues (Rizwan et al., 2022; Mandal et al., 2022; Bukhari et al., 2022).

Among the most studied components, L-mimosine deserves special attention for its cytostatic and antiparasitic effects. The molecule acts as an iron chelator, inhibiting crucial iron-dependent enzymes, such as ribonucleotide reductase, blocking DNA synthesis and inducing cell cycle arrest in the G1/S phase. These effects are particularly relevant against rapidly dividing organisms, including protozoa and helminths, providing a mechanistic basis for the antiparasitic activity observed in assays with *Pheretima posthuma* and murine models of helminthiasis (Hueza et al., 2023; Bendgude et al., 2012). The action of mimosine is microenvironment-dependent: its effectiveness increases in conditions of low iron availability, while high concentrations of iron can antagonize its effects, an important aspect to be considered in formulations and experimental design.

The flavonoids present in *M. pudica*, including quercetin, kaempferol, and their glycosylated derivatives, contribute to multiple pharmacological activities. In vitro, these compounds exhibit antimicrobial activity, interfering with cell membrane integrity, altering ion gradients, and promoting intracellular oxidative stress in bacteria and yeasts, including species relevant to gastrointestinal biofilms (Górniak et al., 2019; Singh et al., 2014; Desrini et al., 2023). In addition, flavonoids exert antioxidant and anti-inflammatory effects, modulating signaling pathways such as NF-κB and COX, reducing the production of pro-inflammatory mediators, and protecting the intestinal mucosa against damage resulting from infection or oxidative stress (Barua et al., 2017).

The saponins present in the plant play a complementary role, destabilizing sterol-rich membranes, increasing cell permeability, and inducing osmotic lysis in parasites and microorganisms, while also enhancing the penetration of other active compounds (Ashok et al., 2022). Tannins and triterpenes, in turn, exert additional protective functions, forming complexes with surface proteins of parasites and biofilms, decreasing motility, metabolism, and cell adhesion, thus reinforcing the combined efficacy of the plant on multiple therapeutic axes.

Another relevant component is the mucilage extracted from the seeds, consisting predominantly of glucuronoxylans, with high viscosity, gelling capacity, and adhesion to the mucosa. This polysaccharide fraction not only acts as a pharmacotechnical vehicle, increasing local residence time and the concentration of active metabolites in the intestinal lumen, but may also interact mechanically with biofilms and luminal residues, facilitating the removal of debris and contributing to the maintenance of mucosal integrity (Bukhari et al., 2022; Noor et al., 2024).

The phytochemical profile of *M. pudica* is highly dependent on environmental and seasonal factors. Studies show that plants collected in regions with greater environmental stress, low nutrient availability, or high sun exposure tend to have higher concentrations of flavonoids and phenolic compounds, possibly as an adaptive response to oxidative stress and herbivores (Rizwan et al., 2022). This variation also applies to L-mimosine, whose levels can differ according to soil, climate, and plant age, directly impacting the pharmacological activity of extracts used ethnomedically or in experimental assays.

In summary, the phytochemical composition of *Mimosa pudica* provides a solid basis for its therapeutic multifunctionality, distributing mechanisms of action among antiparasitic, antimicrobial, antibiofilm, anti-inflammatory, and antioxidant activities. This biochemical diversity explains the ethnomedical perception of efficacy in different cultural contexts and allows the plant to be rationally explored in integrative intestinal health strategies, from traditional preparations to standardized pharmaceutical formulations. A detailed understanding of each metabolite, its interactions, and environmental variations is crucial to guide future research, optimize extracts, and develop safe and effective clinical applications (Rizwan et al., 2022; Mandal et al., 2022; Bukhari et al., 2022; Hueza et al., 2023).

## 2.4 Standardization and Phytochemical Variability of *Mimosa pudica* L.

Phytochemical variability is one of the main challenges in the development of phytotherapeutics from *Mimosa pudica* L., as the composition and concentration of its bioactive metabolites are strongly influenced by biological, environmental, and technological factors. This variability can compromise the reproducibility of experimental and clinical results, hindering the translation of ethnopharmacology into evidence-based practices. For this reason, the standardization of extracts becomes an essential step on the path to scientific validation and the incorporation of the species into formal therapeutic protocols (Rizwan et al., 2022; Mandal et al., 2022).

A first critical aspect relates to the part of the plant used. Studies show that leaves, roots, seeds, and aerial parts have distinct chemical profiles. Leaves tend to concentrate flavonoids and tannins, conferring greater antioxidant and antimicrobial activity, while roots are richer in triterpenes and alkaloids associated with healing and anti-inflammatory effects (Kokane et al., 2009). The seeds, in turn, are a source of mucilage rich in glucuronoxylans, with interesting bioadhesive and pharmacotechnical properties, in addition to varying concentrations of L-mimosine, responsible for cytostatic and antiparasitic activities (Bukhari et al., 2022). This heterogeneity imposes the need for a clear definition of which plant part should be prioritized for different therapeutic purposes, avoiding inconsistent results.

The geographical region of collection also has a great influence on the phytochemistry of *M. pudica*. Edaphic (pH, fertility, soil texture), climatic (luminosity, temperature, rainfall regime), and biotic factors (presence of herbivores, interspecies competition) modulate the synthesis of secondary metabolites. Plants grown in poor soils or subject to environmental stresses tend to accumulate higher levels of phenolics and flavonoids, probably as an antioxidant defense strategy, while more stable environments may favor a greater balance between primary and secondary metabolites (Rizwan et al., 2022). This phytochemical plasticity means that two samples of *M. pudica* collected in different regions can have radically different profiles, implying variation in pharmacological efficacy.

Seasonality is another determining factor. Seasonal changes in water availability, luminosity, and temperature modulate the expression of key enzymes in the biosynthesis of phenolic compounds, flavonoids, and alkaloids. Studies with species of the genus *Mimosa* report significant differences in the concentration of mimosine and flavonoids between dry and rainy periods, with peaks generally observed under conditions of greater environmental stress (Rizwan et al., 2022). For the production of consistent extracts, it is essential to establish ideal harvesting periods, standardizing the time of year and cultivation conditions to maximize the concentration of target metabolites.

The extraction method is perhaps the most critical variable in terms of reproducibility. Solvents of different polarities extract distinct chemical classes: ethanol and methanol favor the solubilization of flavonoids and saponins; ethyl acetate concentrates lipophilic aglycones and phenolic compounds; while water predominantly extracts polysaccharides and mucilage (Mandal et al., 2022). In addition, parameters such as time, temperature, plant/solvent ratio, and applied techniques (infusion, decoction, maceration, ultrasound- or microwave-assisted extraction) profoundly affect the yield and profile of the extracts. This explains why traditional ethnomedical preparations often show divergent results compared to laboratory extracts, reinforcing the importance of establishing reproducible and optimized protocols for each pharmacological purpose.

In the context of translational research, phytochemical standardization plays a central role. Clinical trials require chemical consistency so that pharmacological effects can be robustly correlated with the composition of the tested extract. The absence of standardization can lead to inconclusive or contradictory results, compromising the plant's credibility as a therapeutic resource. Defining chemical markers (such as L-mimosine, quercetin, or glucuronoxylan mucilage) and acceptable concentration ranges is an essential requirement for the development of phytopharmaceuticals derived from *M. pudica*. Furthermore, modern strategies such as high-performance liquid chromatography (HPLC), mass spectrometry (LC-MS/MS), and metabolomic fingerprinting techniques can be applied to establish characteristic chemical profiles and detect inter-lot variations. These resources not only ensure greater quality control but also allow for the correlation

of specific compound concentrations with observed biological effects, bringing basic research closer to clinical practice.

Finally, the standardization and control of phytochemical variability are not limited to laboratory research: they are equally fundamental for the industrial production of consistent and safe extracts. Products intended for the pharmaceutical and nutraceutical market need to meet strict regulatory requirements for efficacy, safety, and reproducibility. In this sense, the establishment of good practices for cultivation, harvesting, processing, and formulation represents an indispensable link between ancestral ethnomedical knowledge and the contemporary scientific validation of *Mimosa pudica* as a reliable therapeutic resource (Rizwan et al., 2022; Bukhari et al., 2022; Mandal et al., 2022).

## 2.5 Safety and Toxicology of *Mimosa pudica* L.

Table 2 presents a detailed summary of the safety and toxicology of *Mimosa pudica* L., including data on acute and chronic toxicity, target organs, limitations, and practical recommendations. It is observed that the plant has low short-term toxicity, but the presence of L-mimosine requires caution in prolonged use, reinforcing the need for phytochemical standardization and clinical validation.

The study of the safety and toxicology of *Mimosa pudica* L. is essential to understand the limits of use of its extracts and for the transition from ethnomedical knowledge to evidence-based clinical practice. Although traditional use in various medical systems—such as Ayurveda and Latin American folk medicine—suggests relative safety, the presence of metabolites like L-mimosine, flavonoids, saponins, and tannins calls for caution, especially in prolonged regimens. Available data indicate that the plant has low acute toxicity in animal models, but chronic exposure, especially at high doses, can trigger subclinical effects on target organs and metabolic parameters (Ahmad, Mishra, & Gupta, 2012; Rizwan et al., 2022; Mandal et al., 2022).

Acute toxicity studies show that aqueous and hydroalcoholic extracts of *M. pudica* do not produce relevant clinical signs or mortality in rodents exposed to doses higher than those traditionally used. In mice and rats, no marked behavioral changes were observed, nor significant deviations in basic hematological parameters, reinforcing short-term safety. On the other hand, histopathological analyses in animals subjected to very high doses indicated minor liver and kidney changes, which, although discreet, signal potential risks under conditions of abusive or uncontrolled use (Mandal et al., 2022; Jacob et al., 2025).

**Table 1. Phytochemical variability of *Mimosa pudica* L. and therapeutic implications**

| Plant part | Main compounds | Influence of environmental factors | Ideal extraction method | Associated therapeutic applications | References |
|---|---|---|---|---|---|
| Leaves | Flavonoids (quercetin, kaempferol), tannins, alkaloids | Levels increase in sunny and dry environments; marked seasonal variation | Ethanol, methanol, ethyl acetate | Antiparasitic, antimicrobial, antioxidant, anti-inflammatory | Rizwan et al., (2022); Mandal et al., (2022) |
| Roots | Triterpenes, alkaloids, saponins | Variability according to soil type and rhizobia present | Ethanol, methanol, prolonged maceration | Healing, anti-inflammatory, mucosal regulator | Kokane et al., (2009); Rizwan et al., (2022) |
| Seeds | Mucilage (glucuronoxylans), L-mimosine | Yield and viscosity influenced by humidity and temperature | Aqueous extraction, alcoholic precipitation | Bioadhesive, biofilm modulator, mechanical support for residue removal | Bukhari et al., (2022); Noor et al., (2024) |
| Aerial parts | Mixture of flavonoids, tannins, saponins | Great geographical variation; plants from poor soils tend to accumulate more phenolics | Infusion, decoction, 70% ethanol | Ethnomedicine: general antiparasitic and 'intestinal cleanser' | Ahmad et al., (2012); Joseph et al., (2017) |

**Table 2. Summary of the safety and toxicology findings of *Mimosa pudica* L.**

| Plant part/ Type of extract | Findings in acute toxicity | Findings in chronic toxicity | Target organs / observed effects | Use limits and recommendations | References |
|---|---|---|---|---|---|
| Leaves – aqueous and ethanolic extracts | High doses (>2000 mg/kg, oral) in rodents did not cause mortality or relevant behavioral changes | Limited data; at doses >3000 mg/kg/day, minor hepatic histological changes were observed | Liver and kidneys: discreet hepatocellular vacuolization and renal congestion at high doses | Safe in the short term; standardization of flavonoids and restricted use at traditional doses are recommended | Mandal et al., (2022); Rizwan et al., (2022) |

| **Plant part/ Type of extract** | **Findings in acute toxicity** | **Findings in chronic toxicity** | **Target organs / observed effects** | **Use limits and recommendations** | **References** |
|---|---|---|---|---|---|
| Roots – hydroalcoholic extract | No signs of acute toxicity up to 2000 mg/kg in rats; absence of mortality | Absence of systematic chronic studies; theoretical risk of alkaloid bioaccumulation | Insufficient data; potential cumulative action in the liver and gastrointestinal tract | Traditionally used as healing agents; lack of a defined maximum safe dose; need for subchronic studies | Kokane et al., (2009); Rizwan et al., (2022) |
| Seeds – aqueous extract (mucilage) | Mucilage extracts showed no acute toxicity in rats up to 5000 mg/kg; absence of lethality | Scarce chronic data; risk associated with the variable presence of L-mimosine | Possible metabolic changes in serum iron and hematological parameters with prolonged doses | Considered safe as a functional fiber; it is recommended to monitor mimosine content and standardize viscosity | Bukhari et al., (2022); Noor et al., (2024) |
| Aerial parts – traditional decoctions | Ethnomedical reports indicate safe use in infusions and decoctions; no reports of serious adverse effects | Limited laboratory data; phytochemical variation may alter toxicity | Potential gastrointestinal risk in concentrated tannin extracts | Traditional use considered safe in moderate doses; controlled clinical validation recommended | Ahmad et al., (2012); Joseph et al., (2017) |
| Mimosine (chemical marker) | Low acute toxicity in rodents; cytostatic effect against protozoa and helminths | In prolonged use, it may induce hypoferritinemia, inhibit DNA synthesis, and affect rapidly dividing cells | Bone marrow, intestinal mucosa, liver: risk of cytostasis in host cells | Contraindicated in iron deficiency anemia; HPLC standardization necessary to establish safe limits | Hueza et al., (2023); Jacob et al., (2025) |

Chronic toxicity remains less explored, but reports indicate that prolonged consumption can interfere with iron metabolism and cell replication. L-mimosine, a non-protein amino acid abundant in the plant, acts as an iron chelator and an inhibitor of ribonucleotide reductase, blocking DNA synthesis. This mechanism, although useful for compromising the viability of protozoa and helminths, can also affect host cells with a high proliferative rate, such as enterocytes and bone marrow cells, especially in individuals with iron deficiency. This characteristic highlights the need to limit the dose and consider the patient's nutritional status when using extracts of the plant (Hueza et al., 2023; Bendgude et al., 2012).

Besides mimosine, other secondary constituents contribute to potential adverse effects. Saponins, in high concentrations, are known for their detergent action, which can lead to gastrointestinal irritation and lysis of cell membranes. Tannins can reduce the bioavailability of proteins and minerals by forming insoluble complexes, while excess flavonoids can generate paradoxical oxidative stress in tissues under specific pH and metabolic conditions. Although these effects have not been reported in direct clinical trials with *M. pudica*, they are described in toxicological studies of other Fabaceae rich in these compounds, suggesting the importance of monitoring dose and preparation method (Rizwan et al., 2022; Ashok et al., 2022).

Phytochemical variability is another critical factor for safety. Different parts of the plant concentrate specific metabolic profiles: seeds have a higher content of mucilage and mimosine; leaves are rich in flavonoids and tannins; roots concentrate alkaloids and triterpenes. In addition, factors such as geography, season of collection, and extraction method influence the final levels of active compounds. For example, aqueous extracts tend to be safer as they concentrate mucilage and hydrophilic polysaccharides, while ethanolic or ethyl acetate extracts can concentrate lipophilic compounds with greater potential toxicity (Bukhari et al., 2022; Noor, Muhammad, & Hanif, 2024). This heterogeneity justifies discrepancies in experimental results and reinforces the importance of standardization.

Phytochemical standardization and the definition of chemical markers are indispensable conditions to ensure clinical safety and efficacy. The quantification of L-mimosine by chromatographic methods (HPLC, LC-MS) should be adopted as a quality control criterion, establishing maximum acceptable limits. In parallel, flavonoids and mucilage can be used as secondary markers of efficacy and consistency. Furthermore, phase I clinical trials should monitor biomarkers of hepatic, renal, and hematological toxicity, as well as possible drug interactions, since constituents of *M. pudica* can interact with cytochrome P450 systems and influence the pharmacokinetics of commonly used drugs (Rizwan et al., 2022; Bukhari et al., 2022; Jacob et al., 2025).

In summary, the safety of *Mimosa pudica* L. in acute use is corroborated by experimental studies, while chronic use still requires detailed investigation to elucidate cumulative risks, especially those related to mimosine and iron metabolism. Although data indicate low toxicity at traditional doses,

phytochemical variability and the absence of controlled clinical trials impose the need for rigorous standardization and long-term safety assessment. This approach will allow a widely disseminated ethnomedical tradition to be transformed into a modern, safe, and reliable phytotherapeutic resource (Hueza et al., 2023; Rizwan et al., 2022; Jacob et al., 2025).

## 2.6 Translational Perspectives and Integrative Applications of *Mimosa pudica* L

The transition of *Mimosa pudica* L. from the ethnomedical sphere to evidence-based clinical practice requires a translational approach that considers the plant's phytochemical complexity, its multiple axes of action, and the need for rigorous standardization. Pre-clinical studies indicate consistent activities in the antiparasitic, antibiofilm, anti-inflammatory, and mucilaginous fields, but clinical validation depends on systematic trials that address efficacy, safety, pharmacokinetics, and drug interactions (Mandal et al., 2022; Rizwan et al., 2022). The multifunctional potential of *M. pudica* positions the species as a promising candidate for integrative therapies, especially in gastrointestinal conditions where infection, dysbiosis, and chronic inflammation coexist.

One of the most relevant aspects in translational terms is the definition of standardized extracts. The documented phytochemical variability—determined by the plant part, geographical origin, collection season, and extraction method—implies that two different extracts can have divergent therapeutic and toxicological profiles (Bukhari et al., 2022). Therefore, it is imperative to establish specific chemical markers, such as the quantification of L-mimosine, flavonoids, and mucilage, using techniques like high-performance liquid chromatography (HPLC) and mass spectrometry (LC-MS/MS). This standardization will allow for the correlation of metabolite concentrations with pharmacological effects, ensuring consistency in clinical trials.

Another central point for clinical translation is the understanding of the pharmacokinetic and pharmacodynamic pathways of the active constituents. To date, there are significant gaps regarding the absorption, distribution, metabolism, and excretion of key compounds like L-mimosine and the flavonoids present in *M. pudica*. In vivo assays in animal models suggest adequate intestinal bioavailability, but quantitative data on luminal residence time, interactions with transporters, and impact on hepatic metabolism are still scarce (Jacob et al., 2025). This information is essential to guide the formulation of oral extracts, the determination of safe doses, and the prediction of potential drug interactions, particularly with drugs that depend on cytochrome P450 metabolism.

From an integrative point of view, *M. pudica* offers opportunities for combinatorial applications with probiotics, prebiotics, and other phytotherapeutics. The presence of mucilage rich in glucuronoxylans confers characteristics similar to functional fibers, capable of acting as a substrate for beneficial microbiota and modulating intestinal ecology. Such an effect can be explored in conjunction with

probiotics (e.g., *Lactobacillus* spp. or *Bifidobacterium* spp.), enhancing the restoration of intestinal eubiosis in post-infection dysbiosis contexts (Bukhari et al., 2022; Noor, Muhammad, & Hanif, 2024). Similarly, the association with classic prebiotics, such as inulin and fructooligosaccharides, can create synergies that amplify the effects of restoring the intestinal barrier and immunomodulation.

In the context of parasitic infections, combinations of *M. pudica* and conventional drugs, such as albendazole or ivermectin, represent another promising line of investigation. Recent clinical trials have shown that combinations of antihelminthics increase cure rates and reduce the risk of resistance, especially against *Trichuris trichiura* (Keiser & Utzinger, 2010). The introduction of a phytotherapeutic with multiple mechanisms, like *M. pudica*, can act as an adjuvant, increasing efficacy, reducing the necessary doses of synthetic drugs, and delaying the emergence of resistance. This strategy, however, requires rigorous clinical trials to rule out pharmacodynamic antagonisms and map metabolic interactions.

The antibiofilm activity of *M. pudica* also opens new translational possibilities in scenarios of chronic recurrent infections and in gastrointestinal conditions associated with microbial biofilms, such as irritable bowel syndrome and persistent dysbiosis. Studies have shown that extracts of the plant reduce adhesion, biomass, and morphogenesis of *Candida albicans* and *Streptococcus mutans*, microorganisms that share biofilm principles with gastrointestinal species (Desrini et al., 2023; Hwang et al., 2017). By interfering with quorum-sensing processes and the integrity of the extracellular matrix, *M. pudica* can potentiate the action of conventional antimicrobials, expanding therapeutic efficacy in resistant infections.

In the field of future clinical applications, interesting prospects include the development of green nanoparticles mediated by *M. pudica* extracts. Initial studies demonstrate that the plant can act as a reducing and stabilizing agent in the synthesis of metallic nanoparticles with anti-inflammatory and antimicrobial properties (Abdulmumeen et al., 2024). These nanotechnological platforms can be explored as targeted drug delivery systems or to increase the stability and bioavailability of the plant metabolites themselves.

Another expanding field is the use of omics techniques—metabolomics, proteomics, and metagenomics—to map interactions between compounds of *M. pudica*, the intestinal microbiota, and host cells. These approaches will allow for the elucidation of mechanisms of action at the molecular level, the identification of biomarkers of efficacy and safety, and support the rational design of clinical trials (Tilg et al., 2020). By integrating omics data with computational pharmacological modeling, it will be possible to predict synergies, risks, and priority targets for the development of combinatorial formulations.

In summary, *Mimosa pudica* L. represents a plant with high translational potential, capable of offering integrative solutions for complex gastrointestinal

disorders by acting simultaneously against parasites, biofilms, inflammation, and dysbiosis. The advancement of its clinical application, however, depends on overcoming three key steps: (i) rigorous phytochemical standardization; (ii) randomized clinical trials that validate efficacy and safety in humans; and (iii) innovative integrative strategies, including combinations with drugs, probiotics, and nanotechnology. With adequate investment in translational research, *M. pudica* can evolve from a traditional ethnomedical resource to a reference phytotherapeutic, integrated into modern protocols for intestinal health and integrative medicine (Rizwan et al., 2022; Bukhari et al., 2022; Jacob et al., 2025).

## 3. CONCLUSION

The critical analysis of *Mimosa pudica* L. reveals that this species, beyond its ethnobotanical notoriety and physiological uniqueness, constitutes a medicinal resource with a multifunctional phytochemical framework capable of directly addressing contemporary challenges in intestinal health. The combination of L-mimosine, flavonoids, saponins, tannins, and mucilage represents a natural arrangement of compounds with the potential to modulate distinct pathophysiological axes: intestinal parasitism, microbial biofilm formation, mucosal inflammation, and microbiota disorganization. Such a biochemical configuration lends scientific plausibility to the traditional practices that used the plant as a "cleansing" agent and gastrointestinal regulator, evidencing a continuum of empirical knowledge and mechanistic validation.

Among the axes of greatest translational relevance, the antiparasitic role of *M. pudica* stands out first. L-mimosine, its most characteristic metabolite, acts as an iron chelator and an inhibitor of ribonucleotide reductase, blocking DNA synthesis in protozoa and helminths. In parallel, flavonoids and saponins promote membrane rupture and ionic imbalance, reinforcing the lethal action on parasites. These multifaceted effects give the plant a unique value in facing the growing resistance of intestinal parasites to conventional antihelminthics.

The second axis is the antibiofilm activity, proven in studies demonstrating the plant's interference in critical stages of microbial biofilm formation, including initial adhesion, extracellular matrix biosynthesis, and quorum-sensing signaling. Such properties are of strategic importance in managing recurrent and chronic infections, in which bacterial and fungal biofilms sustain inflammation and relapse. By reducing the biomass and virulence of biofilms, *M. pudica* not only exerts a direct effect but also increases the efficacy of conventional antimicrobials.

The third axis, often overlooked but of great value, is the anti-mucoid action, related to the mucilage rich in glucuronoxylans present in the seeds. This polysaccharide fraction, endowed with high viscosity and bioadhesiveness, favors the aggregation and mechanical removal of luminal residues and muco-biofilm structures, without resorting to the irritative mechanisms typical of aggressive laxatives. In this way, the plant contributes to intestinal cleansing,

modulation of the mucosal barrier, and potential restoration of microbiota homeostasis.

However, the transition of *M. pudica* from the field of ethnopharmacology to clinical practice requires more than scattered evidence of pre-clinical efficacy: it requires methodological rigor and chemical standardization capable of overcoming the intrinsic phytochemical variability of the species. The absence of uniform protocols for extraction, identification of markers, and establishment of safe doses remains an obstacle to the scientific consolidation of its use. In this context, L-mimosine emerges simultaneously as the main active ingredient and a critical risk marker, requiring quantitative monitoring by high-resolution analytical techniques to balance antiparasitic efficacy with long-term safety.

In the translational field, *M. pudica* should be understood not as a panacea or an isolated entity, but as an integrative adjuvant candidate, whose value lies in its ability to potentiate conventional drugs, interact positively with prebiotics and probiotics, and contribute to holistic approaches to restoring intestinal health. The incorporation of omics methodologies, pharmacological modeling, and plant-based nanotechnology represents a promising horizon for transforming the plant into a next-generation therapeutic platform.

Thus, the relevance of *Mimosa pudica* is not limited to the preservation of an ethnomedical tradition, but projects itself as an opportunity for scientific and biomedical innovation. It is up to contemporary research to validate its multifunctionality in robust clinical trials, establish internationally recognized standardization parameters, and delineate its role as a reference phytotherapeutic in the integrated management of parasitic, infectious, and inflammatory diseases of the gastrointestinal tract. The future of *M. pudica* will, therefore, depend on the ability to articulate its ancestral biochemical wealth with a translational agenda that unites science, technology, and integrative medicine for the benefit of human health.

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## CONSENT AND ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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# Fourier Transform Infrared (FTIR) Spectroscopy in Breath Analysis

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## ABSTRACT

There are several potential medicinal and scientific uses for breath analysis, a relatively new field of inquiry. The environment, microorganisms in the gut and airways, and metabolites of ingested precursors all contribute to the body's internal production of volatile organic compounds (VOCs) that are detected in breath. Several recent studies suggest that breath analysis may aid in the diagnosis of illnesses associated with alterations in breath composition. Infrared spectroscopy is a promising analytical method for the metabolic analysis of breath. There are substances in human breath that can be used to assess environmental exposure, diagnose diseases, and monitor physiological conditions. Exhaled breath promotes collaboration and is the ideal biological fluid because it is nearly limitless and causes little to no discomfort for the patient. Breath analysis is a suitable technique for certain applications, as exhaled breath can be captured without the need for medical personnel or privacy, and it typically doesn't produce infectious waste (although airborne infections may be present). Breath analysis is a non-invasive technique that reveals the overall health and condition of the body's metabolism by describing the volatile content of the bloodstream and airways using the volatile composition of exhaled breath (EB). However, because exhaled breath includes relatively little of the metabolites, the absorption strength of the metabolites is still quite moderate. This chapter presents recent applications of the infrared spectroscopic technique published between 2020 and 2025.

*Keywords: FT-IR analysis; breath analysis; infrared spectroscopy; non-volatile compounds; biomarkers; early disease diagnosis applications.*

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## 1. INTRODUCTION

Breath analysis presents a great potential for scientific and clinical research, being one of the oldest medical diagnostic techniques, utilising specific odours to identify diseases (Huh, 1613). Each person has a unique "breath print" that can reveal a lot about their health. Thousands of molecules are released with every breath we exhale (Bunaciu & Aboul-Enein, 2025).

The field of breath analysis is as old as medicine itself. Doctors have known since the time of Hippocrates (460–370 BC) that a person's breath can provide accurate information about health problems and, in some cases, help with evaluation. Hippocrates described fetor oris and fetor hepaticus in his treatise on breath aroma and disease (Sharma et al., 2023, Phillips, 1992). Later, Lavoisier and Laplace studied guinea pigs' $CO_2$ breath for the first time between 1782 and 1783 and showed that the body's combustion produces the exhaled breath, the respiration consumes oxygen, and expels carbon dioxide (Duveen & Klickstein, 1955). Nebelthau demonstrated that diabetics release breath acetone (Hubbard, 1920), while Anstie separated ethanol from breath (the basis for modern breath alcohol testing) (Baldwin, 1977).

In the 1970s, Linus Pauling, using gas-liquid partition chromatography, made a significant contribution to the scientific study of breath by proving that exhaled breath contains more than the traditional gases of carbon dioxide, nitrogen, oxygen, and water vapour (Pauling et al., 1971). At that time, 250 compounds were found, but now it is possible to identify over 1000 distinct chemicals using contemporary technologies (Bunaciu & Aboul-Enein, 2025). A wide range of volatile organic molecules and elemental gases, such as carbon monoxide and nitric oxide, are examples of these substances. Exhaled breath also comprises droplets of aerosols, which have been identified as "exhaled breath condensate" and incorporate proteins that are dissolved in it, as well as other non-volatile compounds. For example, an experienced medical practitioner may easily recognise the musty and fishy smell of advanced liver disease, the urine-like smell of kidney failure, the vile smell of a lung abscess, and the distinctive fruity smell of acetone in diabetes. Breath analysis is therefore a desirable biochemical monitoring method to detect the emergence of other diseases and physical abnormalities.

This technique can also help predict such illnesses because it is non-invasive and applicable to different substances (Bunaciu & Aboul-Enein, 2025). The first review to address VOCs in exhaled breath was published by (Manolis, 1983). The discovery of numerous VOCs in physiological fluids and healthy human breath was demonstrated in a recent review by (Drabińska et al., 2021). By better understanding the metabolic pathways involved in the synthesis of VOCs, this information may aid in the identification of illnesses.

The human body is among the universe's most complex living entities. For a complex metabolism to continue functioning, a wide range of metabolic events must occur. During these metabolic activities, a variety of chemical and biological

components are produced or changed. Monitoring the concentration of the molecules of interest over time allows one to follow the emergence (or recurrence) of a disease and the effectiveness of a treatment (Kim et al., 2009). Thus, a technique to do breath analysis with high-resolution, broad-spectrum coverage, high specificity, and high sensitivity will be more widely available and adopted (Kim et al., 2009).

Due to the numerous metabolic processes involved in the induction, progression, or regression of conditions such as shock, injuries, or diseases, many of these molecular species will enter the blood medium and be carried over to the lungs, where the volatile species among them will be exhaled. Since the processes leading to the production of these molecules will depend on the specific disease and its various stages, they will be "Fingerprints" of such disease conditions. Thus, the study of exhaled air can provide information regarding a person's physiological state and overall health. As a result, it can be used to diagnose many diseases early on, even when they are just starting (Nakhleh et al., 2017). Detection of diseases from exhaled breath has been shown in different fields of medicine, particularly infectiology (Bunaciu & Aboul-Enein, 2025, Shokouhmand et al., 2025) and oncology (Wang & Wu, 2025, Capuano et al., 2025, Ren et al., 2025).

Blood, plasma, faeces, urine, cells, herbal extracts, exhaled breath (EB), and exhaled breath condensate (EBC) represent some of the biological elements used in the most recent health investigation (Khoubnasabjafari et al., 2022). EBC is typically a sample obtained by capturing exhaled aerosols that come from the fluid that lines the lungs (Bunaciu & Aboul-Enein, 2025). It primarily consists of nonvolatile analytes, particularly those that dissolve in aqueous solutions (Hunt, 2007). The term "EB" describes gaseous samples drawn from exhaled breath and primarily comprises volatile analytes with trace amounts of nonvolatile analytes, particularly those with lower boiling temperatures (Dweik & Amann, 2008). Breath is a crucial matrix for VOCs' analysis and non-volatile compounds produced by the body. After passing through the body's bloodstream and arriving at the alveolar interface, these substances are eventually exhaled. Analysing exhaled breath to find VOCs may reveal if a person is healthy or ill. Similar to blood testing in clinical medicine, but quicker and less invasive, identifying the type and concentration of molecules in breath is an effective method of evaluating a person's general health (Bunaciu & Aboul-Enein, 2025). A diagnosis can be made easier if a certain molecule (or combination of molecules) has been detected and is indicative of the existence of an illness or infection (Liang et al., 2021).

With the appearance of new technologies (such as infrared, electrochemical, chemiluminescence, e-nose, and others) and the development of highly sensitive mass spectrometers, breath analysis has advanced significantly in the twenty-first century, and several techniques are currently in clinical use or on the verge of entering that field (Li et al., 2023, Su et al., 2023, Smith et al., 2023, Pu et al., 2023, Stewart et al., 2024, Arachchige & Muller, 2024, Xie et al., 2023, Uthra et al., 2024, Zhang et al., 2025, Dumitras et al., 2020).

Investigating molecular vibrations is a crucial component of vibrational spectroscopy methods, such as Fourier transform infrared (FTIR) spectroscopy. An overview of the vibrational spectroscopic techniques used in breath analysis during the past few years is presented here. There will also be an emphasis on future applications of the advanced spectroscopic techniques. FTIR spectroscopy is a promising alternative to conventional diagnostic methods since it provides label-free, non-invasive bacterial detection, identification, and antibiotic susceptibility testing in a single step, according to the review's conclusions. To minimise the overall burden of outbreaks, prevent resistant germs, decrease the use of needless antimicrobial medications, and enhance patient care and diagnostics, rapid, accurate, and reasonably priced tests are important.

One of the main disadvantages of diagnostic breath analysis is the difficulty of proving the connection between identified marker molecules and pathology, since precise metabolic pathways are frequently unknown. Sampling is a crucial step because the levels of chemicals in exhaled air vary depending on the circumstances and are frequently at a trace level (Di Francesco et al., 2005). Mid-infrared (MIR) spectroscopy is an excellent substitute method for identifying compounds at the trace level with good sensitivity and molecular selectivity. For near real-time analysis evaluating highly discriminative vibrational and rotational chemical fingerprints, MIR spectroscopy appears to be more adapted to integration and downsizing than GC-MS. These are two significant benefits for typical clinical applications (Mansfield et al., 2002, Pebay-Peyroula & Nicaise, 1970).

In human breath, nitrogen, oxygen, water, and carbon dioxide are the most prevalent matrix components (Lide, 2004, Buszewski et al., 2007, Fenske & Paulson, 1999). At this, we can add about 3500 distinct VOCs identified using gas chromatography and mass spectrometry in a study of breath samples from fifty healthy people (Phillips et al., 1999). More than 1000 components are present in exhaled breath at trace levels, at ppm or ppt levels, and several of these substances may be biomarkers for particular illnesses, physiological states, or the effectiveness of treatment (Bunaciu & Aboul-Enein, 2025). Some substances found in exhaled breath (EB) have been thoroughly investigated, and their connections to various disease pathologies have been identified. However, because these biomarkers are often found at low levels below the ppb (v/v) range of EB, molecularly precise identification of these biomarkers in EB at clinically significant levels remains a practical and analytical problem. MIR spectroscopy and sensing techniques need to be significantly improved to be a viable option for breath analysers that may be used in clinical settings. Thus, when a person transitions from a healthy to a diseased condition, a slight but significant change in the VOC spectrum (in concentration and composition) is seen; this phenomenon is called breath metabolomics (breathomics) (Beale et al., 2016). This change can be recognised and used for diagnosis and monitoring.

Infrared spectroscopy's biggest challenge for analysing biological samples is the high concentration of water vapour in gaseous biofluids. For instance, a healthy

person's breath sample normally comprises 5–7% water vapour (Phillips et al., 1999). There are several methods for breath analysis, but this chapter will cover only some of the most recent applications of infrared spectroscopy published between 2020 and 2025.

## 2. BREATH ANALYSIS SELECTED APPLICATIONS

Two significant obstacles must be overcome to create breath diagnostic instruments based on infrared spectroscopy (Bunaciu & Aboul-Enein, 2025). First, a major barrier to using infrared spectroscopy in breath is the high water (Zieliński & Przybylski, 2012) content of an exhaled breath sample. A significant (factor of 2500) decrease in water vapour from the exhaled breath sample at -60$^0$C has been made possible by the recent development of a water suppression approach from gaseous biofluids (Apolonski et al., 2019). A hierarchical correction process was suggested to carry out the baseline corrections (Selvaraj et al., 2020).

There are some other reviews related to breath analysis using infrared spectroscopy, published during this period (Dumitras et al., 2020, Selvaraj et al., 2020, Xia et al., 2024, Khoubnasabjafari et al., 2022, Mortazavi et al., 2023).

Water removal from the sample without altering the constituent molecules is the primary benefit of breath analysis utilising infrared spectroscopy over tissue or liquid-phase biological samples. Recently, a very effective method for removing water was proposed (Maiti et al., 2018).

A typical water-suppressed breath spectrum of a healthy volunteer is presented in Fig. 1a.

The spectra display a wide range of spectral properties. Except for $CO_2$, most of them are obscured by water spectra when water suppression is not present. Although our bodies use it for a variety of biological processes, it is not sufficiently informative because it is difficult to quantify the amount produced by each activity (Bunaciu & Aboul-Enein, 2025). However, many of the tiny VOCs in our breath are produced by a single biological process, particularly when we are ill. Breath analysis's main objective is to identify those particular VOCs and establish their bodily origins.

Generally speaking, C-H stretch vibrational absorptions are present in the spectral range (2800–3200 cm$^{-1}$) (Roy & Maiti, 2018). Since the CH bond is a property of biological molecules, all biological compounds exhibit a high concentration of CH absorption peaks in this spectral region, making them appear as highly crowded spectral features. Consequently, it is almost impossible to use fingerprints to identify individual molecules in the CH stretch vibrational range. But a distinct spectral characteristic at about 3100 cm$^{-1}$ is recognised as the **R** branch of methane. While methane's **P** branch is obscured by other molecules' large C-H absorption spectra, a very strong **Q** branch is visible at 3020 cm$^{-1}$.

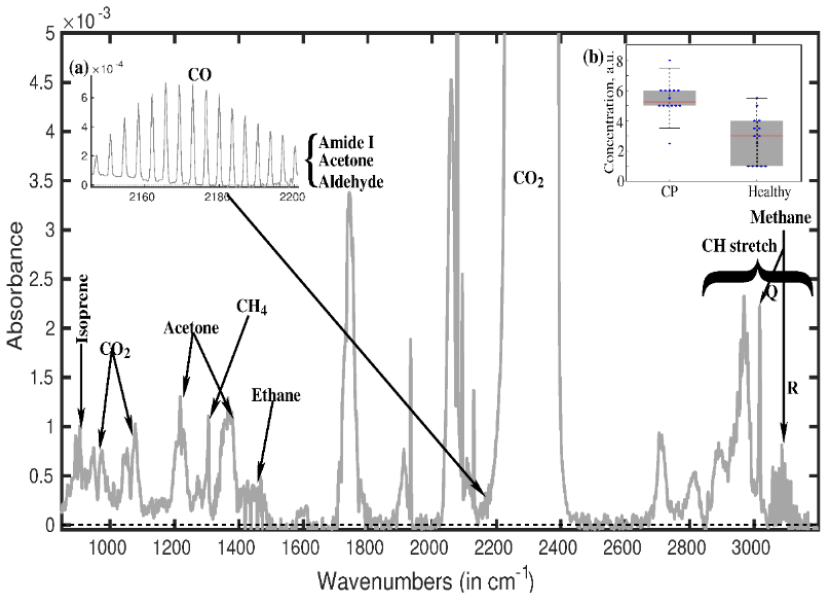


**Fig. 1. A typical water-suppressed breath spectrum (Apolonski et al., 2019)**

Between two groups of volunteers, two distinct spectral characteristics have been identified at 1189 $cm^{-1}$ (**I**) and 1203 $cm^{-1}$ (**II**). The spectral widths of the two features are comparable at 10 $cm^{-1}$. Without any specific exception, the signatures present for 92% (I) and 83% (II) of healthy people and discovered for 100% (I) and 86% (II) of CP patients are, on average, around a factor of two higher in concentration for the former cohort (Bunaciu & Aboul-Enein, 2025). In the inset of Fig. 1(b), the peak's strength at 1189 $cm^{-1}$ is displayed for both volunteer groups. Through statistical analysis using the two spectral features indicated above, we were able to discriminate between the individuals in the groups with an accuracy of over 90%.

Evidence of detectable VOCs in breath linked to lung and breast cancer has been established. Finding every factor affecting the number of VOCs in exhaled air has piqued the curiosity of the scientific community. It has concentrated on standardising the breath sample and analysis methodology in this context. Rapid and non-invasive diagnosis of several illnesses, including diabetes and cancer, may be possible with breath analysis (Pereira et al., 2015).

Normal human subjects' breath has been found to include a variety of chemicals, although it is typically unknown what metabolic pathways these molecules originate from. Certain chemicals have been linked to increased amounts of ovulation, diabetes, cirrhosis, renal disease, and cancer. Yet, many other illnesses have not yet been investigated in this light.

VOC analysis can reveal important details about a person's health, particularly concerning several illnesses. As a result, it can be applied to the early detection of numerous illnesses, even at the initiation (Nakhleh et al., 2017). The primary benefits of breath analysis over other tests currently used to investigate diseases are that it is completely non-invasive, sampling is simple, there are essentially no restrictions on sample size or source, results are available quickly, objective diagnoses can be made using AI/ML (Artificial Intelligence/Machine Learning) methods, requires only reasonably priced equipment, and can be performed by trained technicians without the need for medical professionals (Bunaciu & Aboul-Enein, 2025).

Fig. 2 presents the human body pathways for VOCs (a) and the schematic of the liver's mitochondrial matrix (b), where acetoacetate, beta-hydroxybutyrate, and acetone are formed (Zheng et al., 2024).

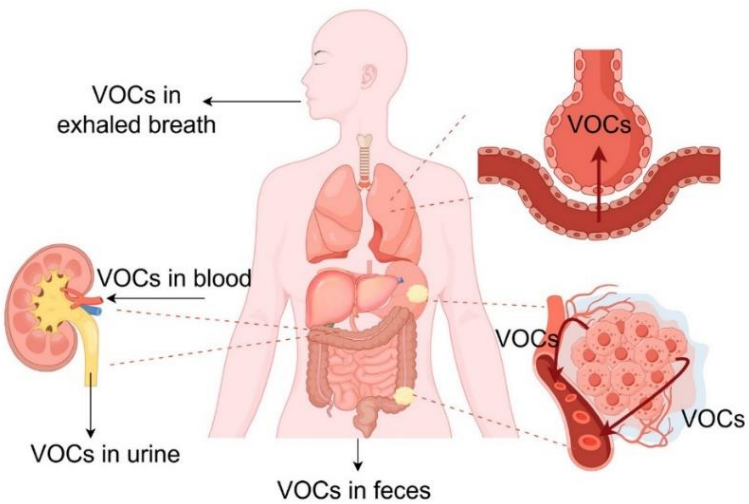


**Fig. 2. Human body pathways for VOCs (Zheng et al., 2024)**

Although there have been some successful attempts to separate cancer samples from healthy controls by analysing blood and tissue VOCs (Mezmale et al., 2023, Astolfi et al., 2023). These techniques serve primarily in research scenarios rather than therapeutic ones. Sample preparation is the first step in VOC analysis. A system's capacity to handle samples easily affects its generalizability, and the quality of the samples determines how accurate the results are. In addition to highlighting their use in medical practice and analysing their advantages and disadvantages, this study focuses on sample preparation methods found in human VOC studies. The cancer-related volatile organic compounds, produced as a result of metabolic alteration in cancer tissues, diffuse into the blood and are carried by the bloodstream to other parts of the body. They cross the alveolocapillary barrier and enter exhaled breath.

A higher degree of physical, emotional, and financial well-being for society is achieved through early diagnosis of diseases like various cancers, resulting in more effective therapy and quicker recovery (Bunaciu & Aboul-Enein, 2025). This allows for significant cost savings in medical care and prevents the loss of human resources. Internal organ cancers (ovarian, liver, pancreatic, etc.), cardiovascular diseases, and communicable diseases of pathogenic origins (like COVID-19) are examples of non-communicable diseases that make up the majority of the health care burden. These diseases require dependable techniques that can detect them early through cost-effective, noninvasive, universally applicable - that is, affordable, acceptable, and accessible - diagnostic methods (V. R et al., 2021).

For neonates' long-term development, it is essential to monitor their health status to provide early therapeutic intervention if physiological circumstances deviate (Batra et al., 2023, Yoo et al., 2024). Preterm newborns require extra caution in physical and neurological diagnostic procedures because of their immaturity. These should ideally be radiation-free, noninvasive, and noncontact. As a noninvasive, noncontact, and radiation-free diagnostic method, exhaled breath from 71 neonates, with a focus on preterm infants, was analysed using infrared spectroscopy (Feddahi et al., 2024). For instance, the risk of cerebral palsy (CP) or other neurological problems is negatively correlated with gestational age (GA); the earlier a child is born, the greater the chance of neurological impairment (Marlow et al., 2005). It was hypothesised that infrared spectroscopy for breath biomarker analysis could contribute to neonatal health monitoring, providing a feasible means of collecting an adequate quantity of exhaled breath.

In Fig. 3, the infrared absorption spectra of ambient air, incubator air (containing a neonate), exhaled air from a neonate with spontaneous (S) respiration, and input and outlet air of a CPAP (Continuous Positive Airway Pressure) system are displayed. Spectra are focused on the carbon dioxide absorption spectra.

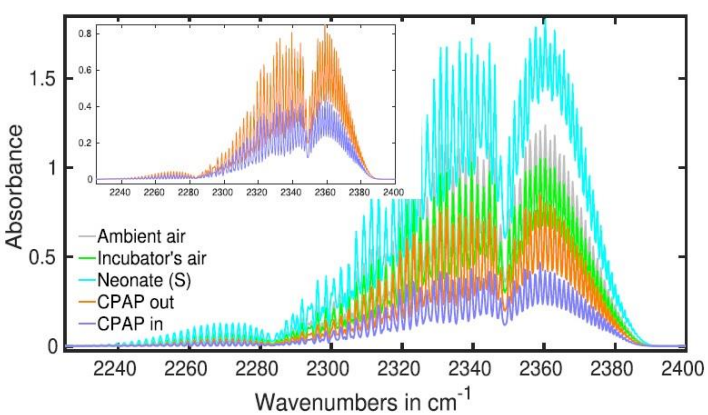


**Fig. 3. FTIR spectra for neonates exhaled breath analysis (Feddahi et al., 2024)**

Studying the obtained spectra, it was concluded that the most prominent $CO_2$ infrared absorption peak is usually seen at about 2350 $cm^{-1}$, whereas the CO peak is found at 2170 $cm^{-1}$. The atmosphere contains several greenhouse gases, including methane. Each person inhales methane from the surrounding air during inhalation, and an equivalent amount is anticipated in exhaled breath (Bunaciu & Aboul-Enein, 2025).

The main cause of periodontitis is the Gram-negative anaerobic bacillus *Porphyromonas gingivalis*. The infrared absorption spectra of the gases released by the cultured bacteria were recorded at a resolution of 0.5 $cm^{-1}$ within the wavenumber range of 500–7500 $cm^{-1}$ using strains of oral bacteria that were cultivated, such as P. gingivalis and the oral commensal bacteria *Actinomyces viscosus* and *Streptococcus mutans (Kaneda et al., 2024)*. A decision tree-based machine learning technique was used to extract the infrared wavenumbers from these spectra, corresponding to distinctive absorptions in the gases that P. gingivalis emitted. Lastly, peaks at comparable locations in the P. gingivalis gases, $NH_3$, and CO spectra were found when the resulting absorbance spectra of ammonia ($NH_3$) and carbon monoxide (CO) were compared using the HITRAN (High-Resolution Transmission Molecular Absorption) database. This technique offers an efficient way to identify P. gingivalis in oral bacteria by differentiating its gases from those of other oral bacteria. The suggested approach may prove useful as a straightforward, noninvasive pathogen diagnosis method in clinical settings.

Alcohol enters the small intestine and stomach when it is consumed. After being absorbed into the blood, it travels throughout the body and into the lungs and brain. Breathing causes it to be exhaled.

For quantitative analysis and ethanol identification are several reviews have been published in this period (Su et al., 2023, Jones, 2022, Gandhi et al., 2024, Mitsubayashi et al., 2022, Paleczek & Rydosz, 2024), and the majority of breath-alcohol instruments currently in use for evidence use infrared (IR) spectrometry as the analytical principle (Harding & Zettl, 2008).

A common quick test for excessive alcohol use in forensic science and legal medicine is the measurement of ethanol in exhaled breath. To maintain sobriety in individuals suspected of driving while intoxicated, police officers employ breath analysers (Bunaciu & Aboul-Enein, 2025). A recent paper discusses the physiological underpinnings, historical evolution, and real-world uses of breathalysers in legal medicine and forensic research (Jones, 2016).

However, several nations chose to employ different BBRs (blood-breath ratio), which varied from 2000:1 to 2400:1, when calculating statutory BrAC (breath-alcohol concentration) limitations (Jones, 2011).

Because of the global pandemic caused by COVID-19, early detection techniques are desperately needed. Breath analysis has demonstrated

significant promise as a quick and non-invasive method of COVID-19 detection (Laird et al., 2023, Sharma et al., 2023, Liang et al., 2023).

Fourier transform infrared spectroscopy (FTIR) (Ruszkiewicz et al., 2020) was one of the techniques utilised recently to analyse exhaled air or exhaled breath condensate to detect COVID-19.

Fig. 4 presents the FTIR spectra for a breath infrared analysis of patients with COVID-19 virus infection.

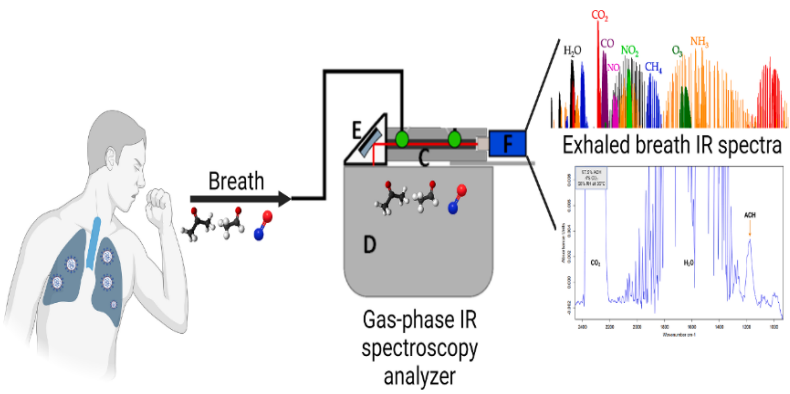


**Fig. 4. The FTIR spectra for a breath infrared analysis of patients with COVID-19 virus infection (Glockler et al., 2023)**

It was demonstrated that a unique approach of using a panel of thirty-four carbonyl compounds detected in all exhaled breath samples for the detection of COVID-19 can be used for the differentiation of the Alpha from the Delta variant and the detection of asymptomatic COVID-19 infection (Xie et al., 2024). Carbonyls are an organic molecule class derived from lipid oxidation (Ratcliffe et al., 2020) and are essential for many biological processes, including oxidative stress and inflammation, which are dramatically triggered by SARS-CoV-2 infection (Fu et al., 2020).

The most often detected VOCs in the exhaled breath of people infected with COVID-19 include nitric oxide, alcohols, aldehydes, and ketones (Glockler et al., 2023). As for acetaldehyde, a recent investigation using GC-MS found that patients with COVID-19 had simultaneous increases in acetaldehyde and acetone in their exhaled breath (Török et al., 2022). Additionally, acetaldehyde, propanal, and n-propyl acetate abundances in the exhaled breath of children infected with COVID-19 were found to rise during acute infection and fall as the illness subsided, according to another study (Berna et al., 2021).

Infection with *Helicobacter pylori (H. pylori)* has been widely linked to gastrointestinal disorders, including gastritis, peptic ulcers, and gastric cancer. Therefore, a precise diagnosis of H. pylori infection is necessary to stop the condition from getting worse and to direct treatment, especially for patients who have had ulcer disease in the past, when immediate eradication therapy (Cho et al., 2021, Magalhães Queiroz & Luzza, 2006) is crucial.

A recent study examines the potential value of MIR exhaled breath sensors using substrate-integrated hollow waveguide (iHWG) technology for the accurate measurement of the isotopic ratio of $^{13}CO_2$ vs. $^{12}CO_2$, simulating conditions pertinent to the exhaled breath analysis method of detecting Helicobacter pylori in the upper gastrointestinal tract. Optimised light-gas interaction and adequate sensitivity are necessary for future integration of such a sensing module, for example, into a cell phone attachment, since the diagnosis relies on detecting the presence of $^{13}CO_2$ 30 minutes after the administration of $^{13}C$-labeled urea via a gel or pill, which is metabolised by H. pyl. (Flores Rangel et al., 2025).

The system's ability to distinguish and measure this isotopologue in simulated breath gas mixtures is confirmed by the linear calibration curve based on the $^{13}CO_2$ peak in the right panel of Fig. 5 (left panel), which shows how the peaks corresponding to both isotopologues change with changing $^{13}CO_2$ concentrations. The spectrum of $^{13}CO_2$ at increasing concentrations of the latter has at least a minor impact on the $^{12}CO_2$ signature, even though a threshold evaluation for an H. pylori infection is undoubtedly achievable.

Identifying chemical components in exhaled human breath offers a chance to evaluate environmental exposure, diagnose illness, or ascertain physiological conditions. Metabolic profiles can be obtained from a range of biological sample types, which can be collected non-invasively (such as faeces, urine, sputum, or breath) or invasively (such as blood, serum, or tissue biopsies) (Pham & Beauchamp, 2021).

Exhaled breath is the perfect biological fluid because it is nearly limitless and causes little discomfort for the patient, promoting collaboration (Bunaciu & Aboul-Enein, 2025). Breath analysis is a desirable method for a variety of applications since exhaled breath can be sampled without requiring privacy or medical professionals, and it usually does not produce infectious waste (despite airborne pathogens) (Pleil et al., 2020).

Early cancer detection is one of the most important factors in saving many lives. In this way, there are several reviews related to cancer screening using breath analysis (Grooms et al., 2024, Le & Priefer, 2023, Chaudhary et al., 2024). Tragically, it might be challenging to identify urogenital malignancies early on. Even at advanced stages, the accuracy of current noninvasive prostate cancer (PCa) diagnoses is limited (less than 70%) (Maiti et al., 2021).

Fig. 6 presents the spectral range, centred at 1005 $cm^{-1}$, that differentiates healthy patients from different cancer groups, including kidney (KC), prostate (PCa), and bladder cancer (BC), due to the presence of acetic anhydride (AA) in exhaled breath.

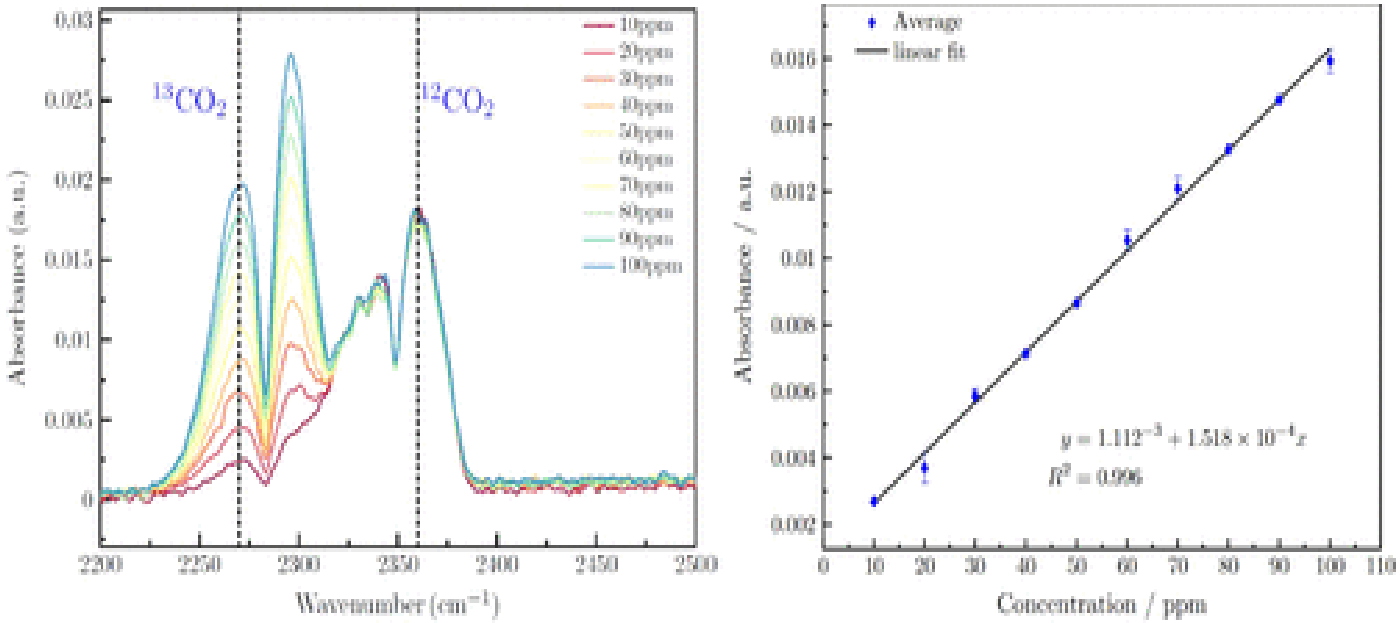


**Fig. 5. Exemplary IR spectra show the $^{13}CO_2/^{12}CO_2$ ratios in 10-cm iHWGs, with $^{12}CO_2$ fixed at 200 ppm and varying $^{13}CO_2$ concentrations from 10 to 100 ppm (Left). The calibration curve was derived from the mixture as a function of $^{13}CO_2$ concentration (Right) (Flores Rangel et al., 2025)**

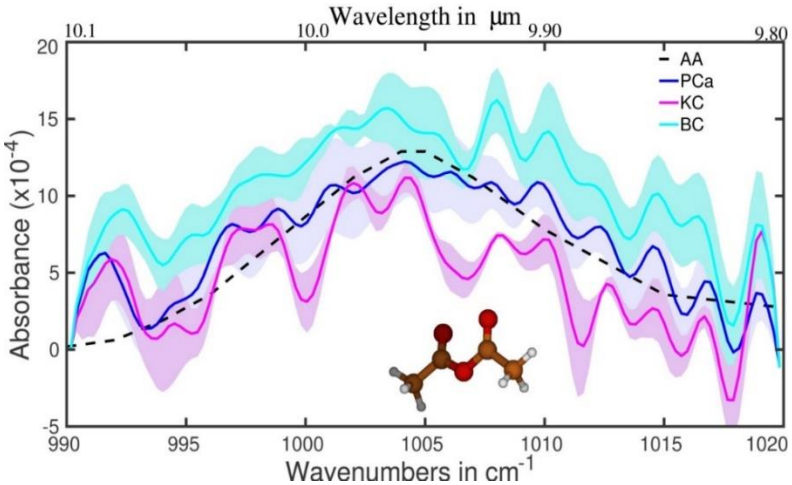


**Fig. 6. The average absorption spectra for healthy and different cancer groups (Maiti, 2023)**

Oscillations visible for the range >1010 $cm^{-1}$ (especially at 1015, 1019 and 992 $cm^{-1}$) are due to the contribution of $CO_2$ (Rothman et al., 2013).

Although AA is not an end product, it can only remain in the body for a few minutes before converting to acetic acid through a reaction with water or other products through acetylation.

Now, let's note that mass spectrometry, the most practical approach for analysing metabolites in gas and liquid phases, was not previously used to detect AA in patient biofluids. First, ten molecules with a molecular mass of 102.09 amu and the formula $C_4H_6O_3$ are identical. These chemicals can be easily distinguished using mid-infrared spectroscopy, which we used in the fingerprint region, but they are difficult to differentiate using GC-MS. The identification process is further complicated by the fact that, in addition to those 10, there are several molecules whose molecular mass differences are less than 0.1%. The FTIR method is better suited for light volatile metabolites in gaseous and liquid phases than the GC method, which is better suited for heavier ones.

The sensitivity and specificity of > 95% offer an excellent scenario for early cancer detection (Bunaciu & Aboul-Enein, 2025). A further reason in favour of this confidence is the patient who was diagnosed with cancer but did not have the original tumour (i.e., T0; following resection during the biopsy, with additional surgery). The methods used here must be changed to show early (pre-symptomatic) cancer detection.

The prognosis is very bad for lung cancer patients who have malignant pleural effusions (MPE). Making the distinction between MPE and benign pleural

effusion (BPE) is essential. Based on FTIR near-infrared spectroscopy (NIRS) in conjunction with a machine learning approach, a recent study attempts to create a quick, practical, and affordable diagnostic tool for classifying clinical pleural effusions (Chen et al., 2021).

Fig. 7 presents the average NIR spectra for MPE (red) and BPE (blue) samples.

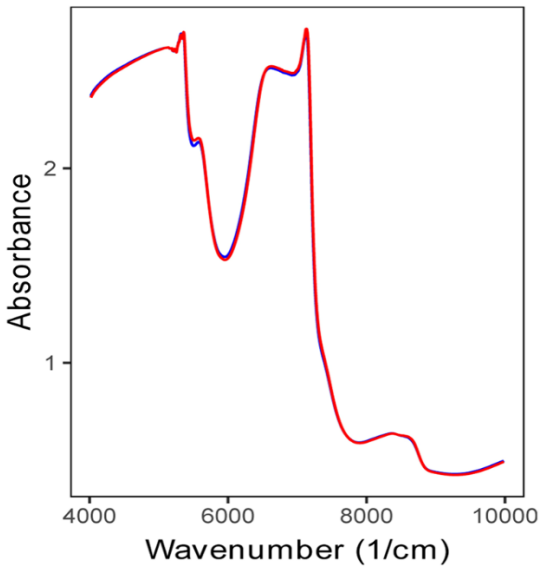


**Fig. 7. Average NIR spectra for MPE (red) and BPE (blue) samples (Chen et al., 2021)**

Even so, the NIR spectra provide a wealth of information about the chemical makeup of the pleural effusion, even in the absence of feature peaks (Bunaciu & Aboul-Enein, 2025). The first overtone of CH is indicated by wavenumbers between 4200 and 5500 $cm^{-1}$, the first overtone of OH, NH, and CH is indicated by wavenumbers between 5400 and 6100 $cm^{-1}$, wavenumbers between 6200 and 7600 $cm^{-1}$ indicate the first overtone of CH, and wavelengths between 7900 and 9000 $cm^{-1}$ indicate the second overtone of CH. Wavelengths of 6200 to 7600 $cm^{-1}$ were used to indicate NH and CH combinations, whereas wavelengths of 7900 to 9000 $cm^{-1}$ were used to indicate the second overtone of CH (Chen et al., 2015, Li et al., 2016).

This is the first study to use NIRS for pleural effusion categorisation. MPE typically denotes advanced cancer growth, which helps create a distinct malignant microenvironment that differs greatly from the surrounding healthy tissues and has different metabolites, such as proteins and lipids (Chen et al., 2015, Li et al., 2016, Zhou et al., 2012).

According to some other results, the IR-CRDS (infrared cavity ring-down spectroscopy) classification of alveolar breath is a potentially effective method for breast cancer screening (Naz et al., 2022).

## 3. CONCLUSIONS

The primary benefits of breath analysis over other tests currently used to investigate diseases are that it is non-invasive, sampling is relatively simple, results are available quickly, objective diagnoses can be made using AI/ML (Artificial Intelligence/Machine Learning) methods that require only relatively inexpensive equipment, and trained technicians can perform the test without the assistance of medical professionals such as radiologists, pathologists, oncologists, etc., particularly for preliminary screening of susceptible subjects (smokers, females over a certain age group, etc.).

Early disease identification, such as that of many types of cancer, results in more effective treatment and quicker recovery, which allows for significant cost savings in healthcare and prevents the loss of human resources. It also raises the standard of physical, emotional, and financial well-being in society (Bunaciu & Aboul-Enein, 2025). Non-communicable diseases, which make up the majority of healthcare costs, such as cancers of the internal organs (ovarian, liver, pancreatic, etc.), cardiovascular conditions, and communicable diseases of pathogenic origin (like COVID-19), require dependable methods that can identify them early using noninvasive, cost-effective, and universally applicable - that is, accessible, affordable, and acceptable - diagnostic techniques.

Breath analysis is not yet a diagnostic tool that clinicians may use, despite several VOC-based detection methods being available and considerable efforts. Given the tens of VOCs found in breath, infrared absorption spectroscopy is a promising method to close this gap.

## AUTHORS' CONTRIBUTIONS

Author AAB contributed to conceptualisation, methodology, sampling and sample analysis, and wrote the first draft of the manuscript. Author HYAE contributed to data curation, supervision and wrote, review and edited the manuscript.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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