

FORMULATION AND EVALUATION OF ANTIBACTERIAL HERBAL OINTMENT CONTAINING *THESPESIA POPULNEA* BARK EXTRACT.

Submitted in partial fulfillment of the requirement for the award of

BACHELOR OF PHARMACY

BY

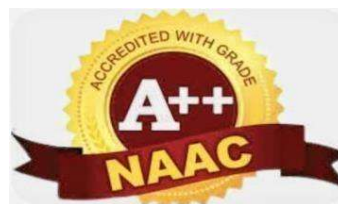
ALLAN BRITTOBHEN .P (40050004),

KARTHIKEYAN .M (40050023),

MOHANA SUNDARAM .S (40050030),

PRETHEES .M (40050037),

RAJAPANDIAN .M (40050039).



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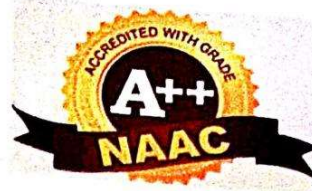
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SCHOOL OF PHARMACY

Bonafide Certificate

Project Guide: Mrs Kokila .S, M. Pharm,

Assistant Professor.

This is to certify that the thesis entitled "**Formulation and Evaluation of Anti-bacterial herbal ointment containing *Thespesia populnea* bark extract**", submitted by the following candidates "**Allan Brittabhen P (40050004), Karthikeyan M (40050023), Mohana Sundaram S (40050030), Prethees M (40050037), Rajapandian M (40050039)**" to the **SATHYABAMA Institute of Science & Technology, Chennai – 600119** for the award of the degree of **Bachelor of Pharmacy by Research** is a Bonafide record of research work carried out by him/her under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree, diploma, associate ship, fellowship or any similar title and it represents entirely an independent work on the part of the candidate.

Place: Chennai

Date: 02/04/2024

Project Guide: Mrs. Kokila .S

Designation: Assistant Professor

Approved by Dean

Authenticated by Examiners

1. 17/04/2024
- 2.



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Declaration

We hereby declare that the work presented in the thesis entitled “**Formulation and Evaluation of Anti-bacterial herbal ointment containing *Thespesia populnea* bark extract**”, submitted by us to the **SATHYABAMA Institute of Science and Technology** Chennai – 600119, for the award of **Bachelor of Pharmacy (2020-2024)** by research is an original record of research work carried out by us under the supervision of “**Mrs.Kokila.S, Associate Professor, School of Pharmacy**”. The contents of this dissertation, in full or in parts, have not been submitted to any other institute or university for the award of any degree, diploma, membership, fellowship, associate-ship etc.. In keeping with the general practicing reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

Place: Chennai

Date: 02/04/2024

Brittobhen .

ALLAN BRITTOBHEN .P (40050004),

Karthikeyan

KARTHIKEYAN .M (40050023),

Mohana Sundaram .

MOHANA SUNDARAM .S (40050030),

Prethees .

PRETHEES. M (40050037),

M. Rajapandian .

RAJAPANDIAN .M (40050039).

Acknowledgement

First and foremost, praises and thanks to the God, the Almighty, for his showers of blessings throughout our research work to complete the research successfully.

We are grateful to, **Dr. Mariazeena Johnson**, Chancellor; **Dr. Marie Johnson**, President; **Mr. J. Arul Selvan**, Vice President; **Mrs. Maria Bernadette Tamilarasi**, Vice President; **Ms. Maria Catherine Jayapriya**, Vice President; **Dr. T. Sasipraba**, Vice Chancellor; **Dr. E. Logashanmugam**, Pro Vice Chancellor; **Dr. S.S. Rao**, Registrar; **Dr. G. Sundari**, Director Administration and to all our Faculty members of Sathyabama Institute of Science and Technology, for their cooperation extended by them in all possible ways during the Programme.

We would like to express our deep and sincere gratitude to our research supervisor, **Mrs. Kokila S**, Assistant Professor, School of Pharmacy, Sathyabama Institute of Science and Technology, for giving us the opportunity to do research and providing invaluable guidance throughout this research. Her dynamism, vision, sincerity and motivation have deeply inspired us. She has taught us the methodology to carry out the research and to present the research works as clearly as possible. It was a great privilege and honor to work and study under her guidance.

We are extremely grateful to **Dr. P. Shanmugapandiyan** Professor and Dean, School of Pharmacy, for providing all the necessary facilities that helped us to balance the competing demands of our research work in a successful manner. We take this opportunity to thank all our staffs **Dr. M. Arun Sundar** (Associate Professor), **Mrs. G. Shanthini Nachiar** (Assistant Professor), **Dr. R. Joan Vijetha** (Associate Professor), **Mrs. S. Monisha** (Assistant Professor), **Dr L. Kaviarasan** (Assistant Professor), School of Pharmacy Sathyabama institute of Science and Technology for all their guidance and our lab technicians **Mrs. S. Subashini**, **Ms. R. Meena**, **Ms. B. Indumathi** for their help during our research work.

We would also like to thank, Dr. **M. Radhakrishnan**, Centre in-charge of CDDD and **Mr. A. Kishore Kumar**, technical assistant, Centre for Drug Discovery and Development, Dr. **L. Stanley Abraham** and **Mrs. Steffy Joseph**, Centre for Ocean Research of Sathyabama Institute of Science and Technology for permitting us to do our research work and providing all the necessary facilities that helped us to complete my research.

We shall be failing in our duty if we don't express heartfelt gratitude to our parents. We cannot imagine a life without their love, blessings and valuable prayers.

Finally, our thanks go to all the people who have supported us to complete the research work directly or indirectly.

ABSTRACT

BACKGROUND: Bacterial skin infection is a contagious skin disease which affects million of people all over the world. Excessive usage of topical antibiotics is known to be key driver of Anti-microbial resistance (AMR) and is directly responsible for increasing anti-bacterial resistance. The use of herbal medicines and the interest in them has grown significantly in recent years, even in places where access to modern medicine is available. Herbal formulation are considered to be more safe with no or less side effect compared to the marketed product.

OBJECTIVE: To formulate and evaluate Anti-bacterial herbal ointment containing *Thespesia populnea* bark extract.

METHODS: *Thespesia populnea* bark was collected, dried coarsely powered and macerated by petroleum ether solvent for 3 days. The extract obtained was filtered and used for phytochemical screening. The result showed the presence of phytosterols and further the plant extract were evaporated by using rotary evaporator. The evaporated plant extract were freeze dried (lyophilization) in order to obtain dry powder of the plant. Four different formulations of ointment was prepared i.e., F1, F2, F3, F4 by varying the drug concentration. All the prepared formulations were evaluated for different parameters such as pH, colour, odour, consistency, solubility, washability, spreadability and extrudability study. Further all the prepared formulation were tested for anti-bacterial activity against *Staphylococcus aureus* (gram +ve) and *Pseudomonas aeruginosa* (gram -ve) by well diffusion assay.

RESULT: Formulation F4 was found to have optimum physicochemical characteristics and also showed the anti-bacterial activity against the *Staphylococcus aureus* bacteria. Further formulation F4 was evaluated for its stability at different temperature i.e, 2°C, 25°C, and 37°C for four weeks and was found to be stable.

CONCLUSION: Overall it can be concluded that the ointment prepared using *Thespesia populnea* bark extract was stable, showed satisfactory physicochemical properties and possessed anti-bacterial activity against *Staphylococcus aureus*. Hence the study showed that it is possible to develop *Thespesia populnea* ointment for the treatment of bacterial skin infections caused by *Staphylococcus aureus* which provides safety and less side effects compared to allopathic drugs. However, further investigations are required to determine the therapeutic efficiency of the prepared *Thespesia populnea* ointment formulation.

KEYWORDS: Bacterial skin infection, *Thespesia populnea*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Well Diffusion Assay.

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LIST OF ABBREVIATION

Q FEVER	Query Fever
pH	Potential Of Hydrogen
MRSA	Methicillin-resistant Staphylococcus aureus
ALTP	Alcoholic <i>Thespesia populnea</i>
AQTP	Aqueous <i>Thespesia populnea</i>
DNSA	3,5- Dinitro Salicylic Acid
IC50	Half- Maximal Inhibitory Concentration
HRBC	Human Red Blood Cell
MTCC	Microbial Type Culture Collection
T.populnea	<i>Thespesia populnea</i>
S.aureus	<i>Staphylococcus aureus</i>
P.aeruginosa	<i>Pseudomonas aeruginosa</i>
AMR	Anti-Microbial Resistance

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1. INTRODUCTION

BACTERIA INFECTIONS

Bacteria are ubiquitous. They play an important role in maintaining the environment in which we live. Only a small percentage of the world's bacteria cause infection and disease. These bacterial infections have a large impact on public health. As a general rule, bacterial infections are easier to treat than viral infections, since the armamentarium of antimicrobial agents with activity against bacteria is more extensive. More so than with infectious diseases caused by viruses and parasites.

Bacteria are unique among the prokaryotes in that so many of them are normal flora that colonize the host without causing infection. Once a person is infected, clinically apparent disease may or may not be seen, and only in a small subset of infections we see clinically significant disease. Bacterial infections can be transmitted by a variety of mechanisms.

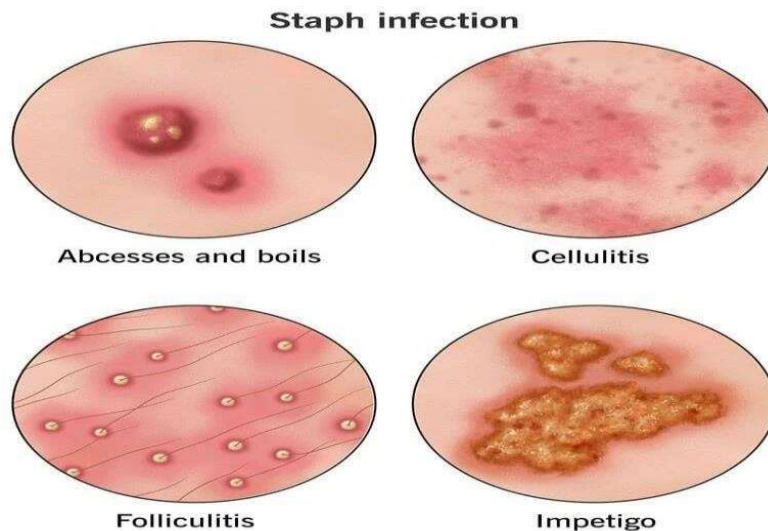


Fig 1.1 Staph infection

MODES OF TRANSMISSION

There are five principal modes by which bacterial infections may be transmitted:

- i. Contact,
- ii. Airborne,
- iii. Droplet,
- iv. Vectors, and
- v. Vehicular (including food, water, and fomites)

- i. Contact: Transmission via contact includes direct skin-to-skin or mucous membrane-to-mucous membrane contact or fecal-oral transmission of intestinal bacteria. Transfusion of contaminated blood products also transmits several bacterial infections, such as syphilis.

Example: Streptococcal impetigo, gonorrhea

- ii. Airborne: Some bacteria are carried on air currents in droplet nuclei. Q fever, tuberculosis, and Legionella travel great distances from their origin. Animals with Q fever have been known to transmit infection to other animals as far as 10 miles away.

Example: Tuberculosis, Q fever

- iii. Droplet: When an infection is spread via droplets greater than 5 μm in diameter, this type of spread is not considered airborne given that the droplet is unlikely to travel through the air for more than 1 m. They are generally more susceptible than airborne droplet nuclei to filtering in the nose via nasal hairs or to removal by nasal or facial masks.

Example: Pertussis, meningococcus.

- iv. Vectors: Typically, the arthropod (mosquito, tick, louse) takes a blood meal from an infected host (which can be human or animal) and transfers pathogens to an uninfected individual. Bacteria such as Shigella can

adhere to the foot pad of house flies and be transmitted in this manner.

Example: Lyme disease, bubonic plague

- v. Vehicular (including food, water, and fomite transmission): Bacterial infection due to food and water generally develops when bacteria enter the intestine via the mouth. Those organisms that survive the low pH of the stomach and are not swept away by the mucus of the small intestine adhere to the cell surfaces.

There they may invade the host cells or release toxins, causing diarrhea.

Example: Campylobacter, trachoma.^[1]

BACTERIAL SKIN INFECTIONS AND THEIR AGENT ^[2]

Bacteria	Mucocutaneous infections(or infections with skin signs)
<i>Staphylococcus aureus</i> (common)	Cellulitis, Folliculitis, Impetigo (school sores) and ecthyma, Methicillin-resistant Staphylococcus aureus (MRSA), Toxic shock syndrome, Tropical pyomyositis,
<i>Streptococcus pyogenes</i> (common)	Cellulitis, Erysipelas, Impetigo.
<i>Corynebacterium</i> species	Erythrasma, Pitted keratolysis, Trichomycosis axillaris
<i>Neisseria</i> species	Gonorrhea, Meningococcal disease
<i>Erysipelothrix insidiosa</i>	Erysipeloid
<i>Pseudomonas aeruginosa</i>	Wound infections Athlete's foot.

PREVENTION OF BACTERIAL INFECTION

Prevention of infection, e.g., through a vaccine, is generally called primary prevention. Clearly, measures to prevent infection have a dramatic impact on morbidity and mortality. Prevention is especially important in this age of increasing antibiotic resistance, because treatment can be so difficult to achieve. There are three major principals of control of bacterial infection:

- Eliminate or contain the source of infection,
- Interrupt the chain of transmission, and
- Protect the host against infection or disease.

Treatment of infected people to prevent symptomatic infection is called secondary prevention, and treatment of infected people to prevent transmission to other humans is called tertiary prevention. ^[3]

TREATMENT OF BACTERIAL INFECTIONS:

Recently, several new drugs for the treatment of bacterial infections have been developed. Quinupristin/dalfopristin, moxifloxacin and gatifloxacin have been approved throughout the world for clinical use. Levofloxacin has been approved for the treatment of community-acquired pneumonia caused by penicillin-resistant *Streptococcus pneumoniae*. The Food and Drug Administration has approved linezolid for clinical use, and new drug applications for gemifloxacin and telithromycin were filed. Other new targets have surfaced in the quest for novel antibacterial agents.

Although a plethora of antibacterial agents seems to exist in the pharmaceutical repertoire, some bacterial infections have become virtually impossible to treat with currently approved agents. Pathogens resistant to more than one class of agent are becoming increasingly common. ^[4]

SOME COMMON DRUGS USED IN BACTERIAL INFECTIONS

- Staphylococcal folliculitis -Cloxacillin, dicloxacillin, erythromycin.
- Furuncles - Cloxacillin, dicloxacillin, erythromycin, nafcillin, methicillin
- Staphylococcal scalded skin syndrome - Oxacillin, methicillin, nafcillin.
- Actinomycosis - Penicillin, tetracycline, erythromycin
- Pitted keratolysis - Topical erythromycin solution or Whitfield ointment
- Skin tuberculosis, localized form -Isoniazid. ^[5]

ANTIBACTERIAL OR ANTIMICROBIAL AGENTS

An ideal antimicrobial agent acts at a target site that is present in the infecting organism but not the host cells. Four major sites in the bacterial cell can be targeted by antibiotics because they are sufficiently different from human cells. These are the cell wall, the cell membrane, the nucleic acid synthetic pathway, and the ribosome. Antibacterial agents, or antibiotics, are typically products of other microorganisms, elaborated by them to compete for space and resources. There are three ways to classify an antibacterial agent:

- Based on whether it is bactericidal (kills bacteria) or bacteriostatic (inhibits growth of bacteria); By its chemical structure;
- By its target site.

TOPICAL ANTIBACTERIALS

Topical antibacterials are commonly used for the prevention and treatment of skin infections. Additionally, acne vulgaris, whose pathogenesis involves bacterial infection, is also treated with topical antibacterials.

Staphylococcus aureus and group A Streptococci are the two most frequently encountered pathogens causing primary and secondary infections of the skin and minor skin wounds.

Topical antibacterials are eminently suitable for targeted drug delivery by taking the drug directly to the site of action, thus ensuring excellent bioavailability of antimicrobials at the infected tissue.

ADVANTAGES OF TOPICAL ANTIBACTERIAL THERAPY

- Targeted delivery of a high concentration of drug to the site of infection.
- Small amounts of drugs used.
- Less potential for systemic side effects and toxicity.
- Need for systemic antimicrobials may be avoided.
- Ease of administration particularly for children.

DISADVANTAGES OF TOPICAL ANTIBACTERIAL THERAPY

- Many agents associated with contact dermatitis.
- Minimal depth of penetration.
- Used for superficial infection only.
- Concerns about impairment of wound healing.
- Possibility of recurrent use and contamination after storage in patients' home

BACTERIAL RESISTANCE TO TOPICAL ANTIBACTERIALS

Development of bacterial resistance to topically applied antibiotics is a growing concern. In a study of susceptibility of 218 isolates of *S. aureus*, resistance was found to macrolide (48.6%), clindamycin (6%), and mupirocin (10.6%)

It is commonly assumed that bacteria will have difficulty developing resistance to botanical extracts which potentially contain thousands of active ingredients.^[6]

HERBAL DRUG PREPARATION

They are processed forms of herbs. They are derived from herbal drugs by various techniques like extraction, fractionalization, purification, concentration, fermentation and may be in the form of powders, extracts, tinctures, fixed oil, volatile oil, resins, gums, etc. They contain a mixture of various constituents.

HERBAL MEDICINAL PRODUCTS

These are the medicinal products which contain exclusive herbal drugs or herbal drug preparations which are made from one or more herbs. They may contain excipients in addition to active ingredients. ^[7]

MEDICINAL PLANTS FOR BACTERIAL SKIN INFECTION

Chemical compounds derived from medicinal plants have been used as models for many clinically effective medications for over a century; these compounds are currently being reevaluated as antibacterial agents. Antimicrobial resistance is on the rise, there are fewer new antibacterial medications in the pipeline from pharmaceutical companies, and there is an increased need for therapies for newly developing diseases. Numerous therapeutic plants are effective against a variety of gram positive and gram negative bacteria, according to testing done in vitro on literally thousands of plant species. ^[8]

Because of the development of antibiotic resistance in virtually all clinically important pathogens, alternatives to conventional antibiotics are urgently needed. Natural products have proven to be highly efficient for the treatment of bacterial infections and not surprisingly, the variety of drugs based on natural products is enormous. ^[9]

OINTMENT

Ointment are a class of semi-solid dosage forms meant for external application to the skin or mucous membrane. Ointments contain medicament or medicaments, suspended, emulsified or dissolved in base.

The ointment should be stable, smooth, free from grittiness, melt or soften at body temperature and be easily applied. The medicament used must be finely divided and uniformly distributed throughout the bases. The ointment base should be non-irritating and should have no therapeutic action. ^[10]

HERBAL OINTMENT

Along with other dosage forms, herbal drugs are also formulated in the form of Ointment.

An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. These include the skin and the mucus membranes of the eye, vagina, Anus, and nose. An ointment may or may not be medicated. Medicated ointments Contain a medicament dissolved, suspended or emulsified in the base.

Ointment are Used topically for several purposes, e.g. as protectants, antiseptics, emollients, Antipruritic, keratolytics and astringents.^[11]

ADVANTAGES OF OINTMENT

- Increases hydration and temperature of the skin.
- Ointments are used most effectively in glabrous areas namely, the palms and soles, or on skin with short or sparse hair.
- Ointments are also very well tolerated in drier areas of the body.

DISADVANTAGES OF OINTMENT

- Greasy texture, so less cosmetically appealing
- Preparation is difficult to wash off
- Not easily spreadable.^[12]

REASONS FOR TOPICAL DELIVERY SYSTEM

Dosage forms are dermatological use for intended to produce the desired therapeutic effect at specific sites in the epidermal tissues

The main advantages of topical drug delivery system are the extended duration of action and consequently, the reduction of dosing frequency. Furthermore, topical delivery is an useful protection tool for potent drug as it provides a reduction of systemic adverse effects.^[13]

2. LITERATURE SURVEY

1. **G.L.Viswanatha et al.,(2008)** worked to investigate the phytochemical constituents and antimicrobial activity of alcoholic *Thespesia Populnea* (ALTP) and aqueous extracts of stem barks of *Thespesia populnea* (AQTP) by Cup plate technique. The extraction was done with petroleum ether, alcohol (70%) in soxhlet apparatus. The ALTP contained alkaloids, Carbohydrates, glycosides, saponins, proteins, steroids, flavonoids, tannins and phenolic compounds. AQTP was formed to contain alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins and phenolic compounds. The aqueous extract showed the significant Anti-microbial activity against the tested bacterial organisms compared to ethanolic extract. The percentage yield of petroleum ether, alcoholic and aqueous extracts was found to be 2.3%, 5.33% and 2.5% respectively. It was revealed that zone of inhibition exhibited by the test extracts was found to be less than that of the reference standard drug Ciprofloxacin.^[14]
2. **Saxena Prasoon Kumar et al.,(2010)** worked on phytochemical investigation and antimicrobial activity of stem bark extracts of *Thespesia populnea*. They are commonly used for herbal preparations in the treatment of toothache, gastrointestinal disorders, dysentery, diarrhoea, sore gums and sore throats. Different bacterial isolates comprising of both gram negative and gram positive organisms were used. The results showed that the extracts exhibited antimicrobial activities at a concentration of 20 mg/ml. The zones of inhibition exhibited by *Thespesia Populnea* extract ranged between 5 mm and 10 mm. However the zone of inhibition exhibited by the test extracts was found to be less than that of the reference standard drug (Gentamycin).^[15]
3. **R.Parthasarathy et al.,(2010)** researched on preliminary phytochemical screening of *Thespesia Populnea* and determination of its diuretic activity. The shade dried bark and Leaves were cut it into small pieces and made

into coarsely powdered using mechanical grinder and preserved in air tight container. Then it was subjected to successive extraction using different solvents (Petroleum ether, chloroform, alcohol and water).The volume of urine, urinary concentration of Na⁺, K⁺ and Cl⁻ ions were the parameters of the study Furosemide (100 mg/kg) was used as standard. The extract (400 mg/kg) show significant increase in volume of urine of Na⁺, K⁺, Cl⁻. This study revealed that the extract of *Thespesia populnea* acted as an diuretic in rats almost to an extent similar to that produced by furosemide.^[16]

4. **Aravinda Nalla et al.,(2017)** worked on formulation and evaluation containing the herbal ointment of garlic bulb extract for anti-microbial activity. The Soxhalation process was used to create the benzene extract. Four different formulations of ointments were made by integrating the extract in the base using the levigation process after the ointment base had been prepared the physical-chemical characteristics of each formulation, including colour, flavour, pH, spreadability, extrudability, consistency, diffusion studies, solubility, and washability were evaluated, where the irritancy, spreadability, and diffusion testing reveals no change. This study concluded that it is possible to develop and evaluate garlic ointment with benzene extract of garlic bulbs for anti-microbial activity.^[17]
5. **R.Kavitha et al.,(2021)** worked to investigate the inhibitory effect of alpha-amylase of aqueous bark and leaf extract of three medicinal plants *Polyalthia longifolia* Benth. And Hook., *Solanum Torvum* Swartz. And *Thespesia populnea* (L.) Soland. Ex Correa. For assessing anti diabetic properties, inhibitory effect on alpha-amylase were carried out using DNSA(3,5 – dinitro salicylic acid) method and anti inflammatory studies (Human red blood cell) membrane stabilization activity was done. These extracts caused a significant decrease in releasing maltose during the experiment. Among these *Thespesia Populnea* showed the highest activity. The Ic50 value suggests that extract of *Thespesia Populnea* was more effective in inhibitory HRBC hemolysis than *solanum Torvum* and *polyalthia*

longifolia. This study revealed that leaf and bark extract of those three plants showed anti diabetic and anti inflammatory activity.^[18]

6. **Muhammad Asif et al.,(2014)** worked on investigation of the invivo analgesic effect of *Thespesia Populnea* extract. The drug was screened to identify the antinociceptive property by reducing the writhing acid time difference in tail flick latency of mice. For chemical method acetic acid writhing test and tail flick test were performed. The extract at all doses 100,150,200mg/kg inhibited the abdominal construction induced by acetic acid. Aspirin was used as standard. The result of acetic acid writhing showed reduced in acetic acid induced number of writhes in mice and tail flick model produced marginal increase in tail flick latency. This study revealed that *Thespesia Populnea* bark extract exhibited profound antinociceptive activity by central and Peripheral mechanism.^[19]
7. **Himal Paudel Chhetri et al.,(2009)** worked on formulation and evaluation of antimicrobial herbal Ointment from the local medicinal plant. The antimicrobial test of the mixture was conducted after the ethanolic extract of the chosen plants were ingested in various ratios. The findings of the zone of inhibition provided by the different extract ratios on Staphylococcus Aureus, Escherichia coli and Klebsiella species were then compared to determining the most efficient combination. The formulation of the ointment was completed by triturating the prepared ointment base and adding the active component in the most efficient combination ratios. After the formulation, the ointment quality was evaluated in terms of irritancy, spreadability diffusion and stability.^[20]
8. **Sharma Praveen et al.,(2013)** worked on pharmacological evaluation of bark extract of *Thespesia Populnea* for antiulcer activity in rats. *Thespesia populnea* bark extract's antiulcer properties were tested using rat models of acute gastric lesions brought on by pylorus ligation and ethanol ingestion.

Using ethanol induced and pylorus ligation methods, animals were pretreated with dosages of 100 mg/kg 200 mg/kg and 400 mg/kg of ethanolic bark extract of *Thespesia populnea*. It showed substantial decrease in lesion index of total affected area and percentage of lesion was compared to the control group in the ethanol induced pylorus ligation. These results support the traditional usage of *Thespesia populnea* preparation and add to its pharmacological value by showing that an aqueous extract of the plants bark had good antiulcer efficacy.^[21]

9. **Mr. Mayur Mohan Jagtap et al.,(2023)** had researched on the development and assessment of antimicrobial herbal ointments. The majority of chemotherapeutic medications are derived from plants, and antibiotics are made from bacteria. A plant's flowers, roots, berries, bark, leaves, or seeds can be used to make herbal medicine. In addition to other dosage forms, ointment can also be prepared from plants. A cream glutinous semisolid mixture was applied topically. This study concluded that herbal antibacterial ointment can be prepared by using a locally assessed plant and after evaluation the least inhibitory concentration of the plant were identified.^[22]

10. **T Shekshavali et al.,(2012)** studied Antimicrobial activity of *Thespesia populnea* Soland. Ex Correa bark extracts. The antimicrobial activity of this plant's bark was examined in the current study using the well diffusion method against two fungus and four bacteria [*Staphylococcus Aureus* MTCC87, *Streptococcus pyogenes* (Gram+ve), *Escherichia coli* MTCC46, *Pseudomonas aeruginosa* MTCC442(Gram-ve), and fungi *Candida albicans* MTCC 227 and *Aspergillus flavus* MTCC 277]. Compared to ethanolic and aqueous extracts, petroleum ether extract had significant efficacy against all organisms. Extracts exhibited mild to moderate activity. This study was possible to draw the conclusion that *T. populnea*'s petroleum ether extract have significant anti-microbial action against the tested bacterial and fungal species compared to ethanolic and waterbased extracts.^[23]

11. **Shubhangi E. Sawant *et al.*, (2015)** worked on formulation and evaluation of herbal ointment containing neem and tumeric extract. Using the maceration process, the ethanolic extracts were prepared. The formulation of the ointment was completed by integrating the extract into the base using the levigation process. Following formulation, the product was evaluated for its physicochemical properties, including color, flavor, pH, spreadability, extrudability, consistency, diffusion studies, solubility, and washability. This study showed that ointment could become a media to use these medicinal properties effectively and easily as a simple dosage form.^[24]

3. AIM AND OBJECTIVE OF WORK

AIM

To formulate and evaluate herbal ointment containing *Thespesia Populnea* stem bark extract.

OBJECTIVE

- ☐ To prepare the extract of *Thespesia Populnea* using petroleum ether by maceration process.
- ☐ To perform lyophilization of plant extract.
- ☐ To prepare herbal ointment of *Thespesia Populnea* by varying the concentration of extract.
- ☐ To evaluate the herbal ointment for different parameters.
- ☐ To perform *In vitro* antibacterial activity screening of ointment by Disc diffusion method.

4. EXPERIMENTAL WORK

MATERIALS

Selection of plant materials:

The selection of *Thespesia populnea* was based on literature review and research-based articles. The plant materials has shown the antibacterial - activity against various microorganisms.

Thespesia populnea:

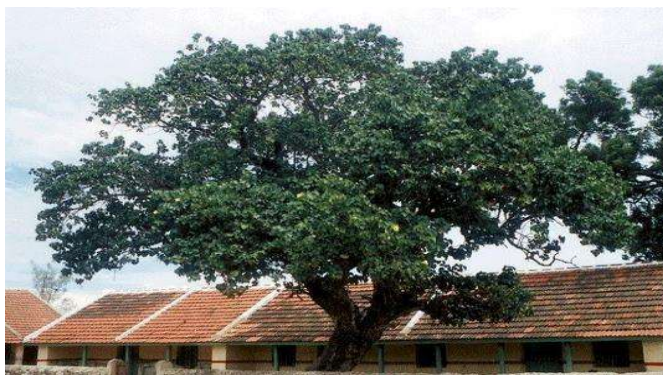


Fig 4.1 *Thespesia populnea*

Plant profile:

Botanical name – *Thespesia populnea* (L.) Soland ex Correa

Common name – Portia tree, Pacific rosewood

Indian names – Puvarasu (Tamil)

Indian tulip tree (English)

Parshwapippal (Sanskrit)

Taxonomical Classification:

Kingdom – Plantae

Division – Tracheophytes

Order – Malvaceae

Family – Malvaceae

Genus – Thespesia

Species – *Thespesia populnea*^[25]

BOTANICAL DESCRIPTION:

- It is an evergreen tree (6-12 m in average height) with a broad dense crown with often crooked stem.
- The tree grows in short twists and turns with numerous limbs, therefore lumber is only found in short lengths.
- Clear bole around 2-2.5 m, Girth (at breast height around 0.61.2m.
- It has fissured grey / dark brown bark, and a dense crown of green glossy heart shaped leaves.
- It is easily recognized by its large yellow flowers with purple Centre, its heart shaped leaves and the turban shaped capsules.
- Leaves are glossy, green heart shaped resembling those of poplar 8-15 cm x 6-10 cm.
- Broadly ovate with the lower corners rounded off, cordate at the base and acuminate.
- Flowers are bisexual, axillary, solitary, yellow, bell shaped, 5 to 7.5 cm in length with five overlapping broad and rounded petals.
- Maroon / purple spot can be observed at the base of each petal with star shaped hairs on outer surface.
- The yellow flowers turn dark red, purple or pink as the day progresses.

- Fruits, five celled globose capsules 2.5 to 5 cm in diameter, flattened covered with minute peltate scales with disc like persistent calyx at the base, black when ripe. Seeds about 1 cm long, ovoid (Troup, 1921).



Fig 4.2 *Thespesia populnea* - Flower



Fig 4.3 *Thespesia populnea* - Fruit



Fig 4.4 *Thespesia populnea* - Bark



Fig 4.5 *Thespesia populnea* – Leaf

Geographical Distribution:

Coastal areas of Indian and Pacific oceans from East Africa and India to mainland South- east Asia, Indonesia and the Philippines. In the Pacific it grows from Papua New Guinea and the northern coast of Australia through the Solomons, Vanuatu and Fiji. In India it occurs in the littoral forests of Andaman's and Sunderbans.

Ethnomedical uses:

- Extracts from roots are applied externally for scabies, Psoriasis and related skin diseases.
- Heart wood is used in Indigenous medicine for skin diseases (Girach et al., 1994).
- In Mauritius, the bark is described as depurative and as a cure for Dysentery and Hemorrhoids.
- Decoction of the bark is used for washing skin diseases.
- Ground bark mixed with coconut oil is also applied for skin diseases.
- Bark extracts have been reported to possess antioxidant and hepatoprotective activity (Ilavarasan et al., 2003).
- Leaf extracts are applied on inflamed and swollen joints.
- Cytotoxic activity of methanol extracts of leaves has been reported on human leukaemia cells (masuda et al., 2002) .
- Bioassay results have showed that the extracts of flowers have anti-bacterial activity.
- Flavonoids have been isolated from the ether and ethyl acetate fractions of the ethanol extract of the flowers (Shirwaikar and Srinivasan, 1996).
- Antihepatotoxic (Shirwaiker et al., 1995) and antisteroidogenic activities (Kavimani et al., 1999) of flower extract have been reported.
- The fruit abounds in a viscid, yellow juice which the natives of south India use as an excellent application in psoriasis.
- It is also used to treat insect bites, gonorrhoea, ringworm, migraine, fistula,

Sprains and wart removal.

- Fruit extracts has wound healing properties also (Nagappa and Cheriyan, 2001).
- Irular tribal community of Anaikkatty Hills, Kerala use the seed extracts for Prevention of pregnancy (Nadanakunjidam, 2003).
- Plant extracts have been reported to have significant anti-malarial activity (vasanth et al., 1990).
- Vasudevan et al. (2007) has proved the antinociceptive and anti-inflammatory effects of its bark extract^[26]

METHODOLOGY:

Collection of Plant materials and Authentication:

Thespesia populnea bark was collected from college campus and authenticated in Siddha Central Research Institute, Arumbakkam, Chennai-600106. Authentication Form Number - PCOG002-ACF

Selection of solvents:

Petroleum ether was selected based on literature and can be easily used for phytochemicals screening of plant materials.

Drying and powdering of Plant materials:

Thespesia populnea bark were washed thoroughly with water for few times and shade dried under good ventilation of air for 1 week and pulverized into coarse powder.



Fig 4.6 Dried Bark



Fig 4.7 Bark Powder

Preparation of plant extracts:

150 grams of *Thespesia populnea* bark were powdered and sieved using sieve number: 10. Extracts were prepared by using petroleum ether solvent.



Fig 4.8 Preparation of plant extract



Fig 4.9 Extraction of plant

Extract:

100g of coarsely powdered *Thespesia Populnea* bark were transferred into the beaker containing 500ml of petroleum ether with frequent agitation, then beaker was closed with aluminum foil and macerated for 3days.

After 3 days of maceration, the content of extracts was filtered by using filter paper. The obtained petroleum ether extracts of *Thespesia Populnea* were used for phytochemicals screening and the remaining extracts were evaporated by using rotary evaporator at 75°C. Evaporated extracts obtained from the rotary evaporator was then lyophilized at 18°C by using Freeze dryer.

PHYTOCHEMICAL SCREENING OF PLANT EXTRACT:

Phytochemicals are regarded as secondary metabolites. The plant extract were screened for the presence of biologically active components. The medicinal value of plants depends on numerous active compounds which are helpful in improving the life and treatment of diseases. The sample was extracted using petroleum ether and that extract were analyzed for Carbohydrate, Alkaloids, Phenols, Phytosterols, Saponins, and Flavonoids. Presence of phytochemical was determined on the basis of standard qualitative test procedures.

CARBOHYDRATES**a) Fehling's test:**

The plant extract was boiled with Fehling's solution I and II. The appearance of red coloration indicates the presence of carbohydrates. ^[27]

ALKALOIDS**a) Dragendroff's Test:**

To the plant extract a few drops of Dragendroff's reagent was added and shaken well. The formation of orange red precipitate indicates the presence of alkaloids.

b) Wagner Test:

To the plant extract few drops of Wagner's reagent was added and shaken well. The formation of reddish - brown precipitate indicates the presence of alkaloids.

c) Mayer Test:

To the plant extract few drops of Mayer's reagent was added and shaken well. The formation of white precipitate indicates the presence of alkaloids.

PHENOLS:**a) Ferric chloride test:**

To the plant extract a few drops of 5 % Ferric chloride solution was added. The appearance of deep blue or black colour indicates the presence of phenol. ^[28]

FLAVONOIDS:**a) Shinado's Test:**

To the plant extract 1 ml of lead acetate solution was added. The formation of yellow precipitate indicates the presence of flavonoids. ^[29]

SAPONINS:**a) Foam test:**

The plant extract was shaken vigorously with 5ml of water. Persistent foam formation indicates the presence of saponins. ^[30]

PHYTOSTEROLS:**a) Libermann – Burchard test:**

The plant extract was mixed with 5 ml of chloroform, 1-2 ml of acetic anhydride then 2 drops of concentrated sulphuric acid were added from the sides of test tube. The appearance of bluish green colour indicates the presence of steroids.

b) Salkowski reaction:

The plant extract was mixed with 5 ml of chloroform and 2 ml of concentrated sulphuric acid was added along the sides of test tube. The appearance reddish brown coloration of the interface indicates the presence of steroids. ^[31]

FORMULATION OF OINTMENT:

Preparation of ointment base:

The ointment base was prepared by precisely weighing hard paraffin that had been grated and was transferred into a china dish. The china dish was placed over a water bath. The remaining ingredients were added and gently swirled and continuously mixed until a homogeneous mixture was obtained. The ointment base was then allowed to cool.

Table 4.1: Formulation of ointment base

S.NO	Name of Ingredient	Quantity to be taken
1	Wool fat	0.5gm
2.	Cetostearyl alcohol	0.5gm
3.	Hard paraffin	0.5gm
4.	Yellow soft paraffin	8.5gm

Procedure for preparation of herbal ointment:

Herbal ointment was prepared by mixing accurately weighed Thespesia populnea extract to the ointment base by levitation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container. Four different formulation were prepared i.e., F1, F2, F3, F4 by varying the drug concentration.

Table 4.2: Formulation of herbal ointment ^[32]

Formulation code	Prepared plant extract (g)	Ointment base q.s (g)
F1	0.5g	10
F2	0.75g	10
F3	1g	10
F4	1.5g	10 ^l

EVALUATION PARAMETER:

Colour and Odour:

Visual assessment was conducted to evaluate parameters such as colour and odour.

Consistency:

Consistency was assessed, ensuring a smooth texture without any greasiness.

Solubility:

Solubility was tested, checking for its ability to dissolve in boiling water and its miscibility with alcohol, ether, and chloroform.

Washability:

Washability was determined by applying the formulation to the skin and assessing the ease of washing with water. ^[33]

pH:

The pH of the prepared herbal ointment was measured using digital pH meters. One hundred milliliters of distilled water was used to create the ointment solution, left for two hours, and the pH will be measured three times, with the average value computed. ^[34]

Spreadability:

Spreadability was evaluated by applying a specific weight for a predetermined duration to compress the extra sample between two slides. The spreadability between the two slides will be assessed, and improvements will be sought by reducing the time needed to separate the slides. Spreadability will be determine using this formula.

$$S=M \times L/T$$

Where,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

Extrudability:

The formulation was filled into a collapsible tube container, and extrudability was determined by assessing the weight of ointment needed to extrude a 0.5cm ribbon within 10 seconds. ^[35]

Stability study:

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2°C, 25°C and 35°C.

Non irritancy test:

Herbal ointment prepared was applied on the skin of human beings, and its effects was observed. ^[36]

ANTIBACTERIAL ACTIVITY:

All the prepared formulations with different concentration of plant extract were checked for its anti-bacterial activity.

Materials required:

Sterile micropipette, sterile filtered micropipette tips, sterile cork borer/ well puncher, sterile cotton swab, Disposable Petri plates, Sterile media, Sterile distilled water, Standard drug – drug control.

Indicator organisms:

Pseudomonas aeruginosa (ATCC), *Staphylococcus aureus* (ATCC)

Sample preparation:

The ointment was dissolved in DMSO and mixed properly to get a uniform suspension.

Preparation of pathogens suspension:

- In a sterile 2 ml centrifuge tube, 1ml of Nutrient broth was added and inoculate a loopful of colonies into them.
- Vortex thoroughly and keep it undisturbed for 10 minutes to allow the clumps to settle down.
- Nutrient broth was added to make up the desired volume.

Assay procedure:

- Mueller Hinton agar plates were prepared (pH 7.2- & 4-mm depth).
- The test organisms *Pseudomonas aeruginosa* (ATCC), *Staphylococcus aureus* (ATCC) were inoculated by streaking the loop in a back-and-forth motion over the entire agar surface.
- The hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer or a well puncher.
- After that the prepared ointment of different concentration were filled in it.
- Then the petri plates were placed into incubator and allow it for growth of microorganisms for 24hrs.
- After the incubation period, the zone of inhibition was measured which includes the wells' diameter, to identify antibacterial activity of the prepared ointment.

5. RESULT AND DISCUSSION

RESULT:

A. Phytochemical Screening:

The result obtained after the qualitative screening of phytochemical components from the petroleum ether extract of the plant are presented in table 5.1.

Table 5.1 Phytochemical analysis

Test Name	Phytochemical	Petroleum ether extract
Fehling's test	Carbohydrates	-
Dragendroff's test	Alkaloids	-
Wagner test	Alkaloids	-
Mayer test	Alkaloids	-
Libermann– Burchard Test	Phytosterols	+
Salkowski reaction	Phytosterols	+
Shinado's test	Flavonoids	-
Lead acetate test	Flavonoids	-
Ferric chloride test	Phenols	-
Foam test	Saponins	-

The petroleum ether extract of the plant showed the presence of phytosterols as shown in the Figures 5.1 and 5.2.



Fig 5.1 Salkowski reaction

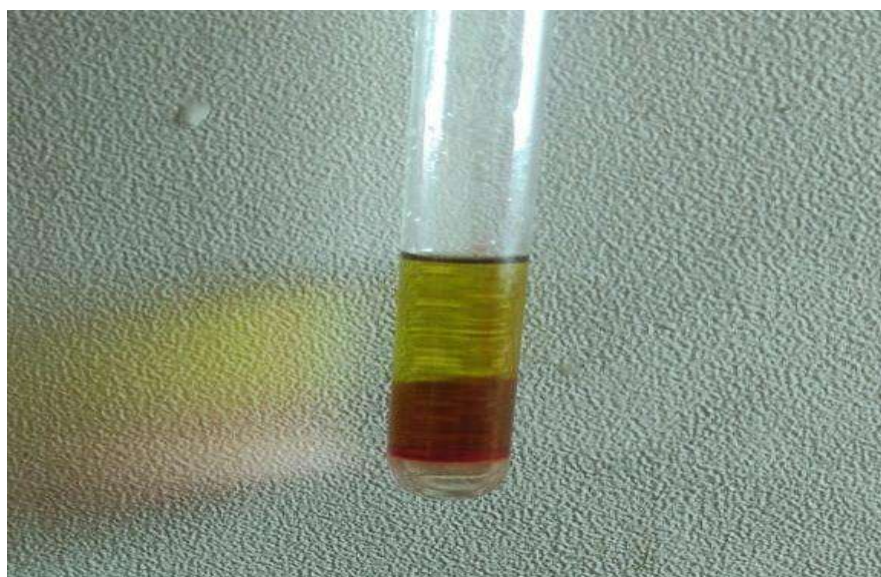


Fig 5.2 Libermann butchard

B. Evaluation Parameter:

Colour and Odour:

After visual examination, formulation F1 and F2 was observed to be yellow in Colour whereas F3 and F4 was yellowish brown Colour.

Consistency:

All the formulation were found to be smooth in consistency and no greediness was observed.

Solubility:

the prepared formulation were soluble in boiling water, miscible with alcohol, ether, chloroform.

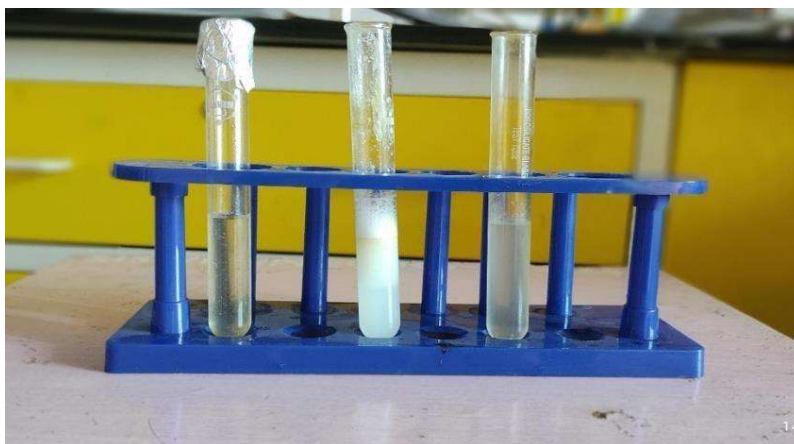


Fig 5.3 Solubility

Washability:

The prepared formulations were found to be easily washable with water and did not leave any residue on the skin.

pH:

The pH of prepared formulations F1, F2, F3, F4 were measured and was found to be within the range as shown in Table 5.2.

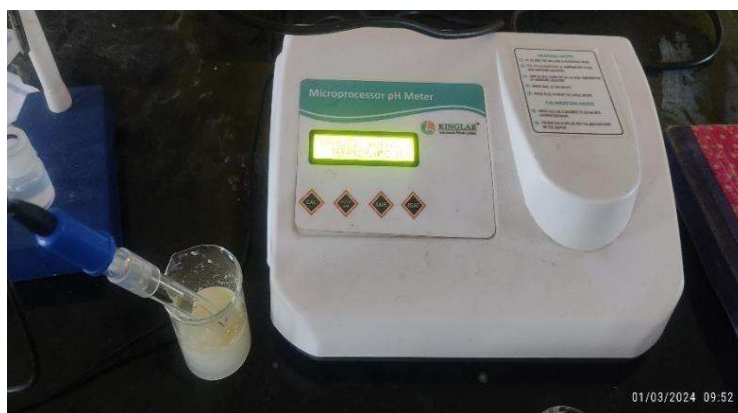


Fig 5.4 pH

Spreadability:

After evaluation, the formulation F4 was found to be easily spreadable compared to other formulation and the result are present in Table 5.2.



Fig 5.5 Spreadability

Extrudability:

All the prepared formulations were filled in collapsible tube container and extrudability of ointment was measured and result are present in Table 5.2.

Non irritancy:

All the prepared formulations showed no irritation when applied on the skin.

Table 5.2 Physicochemical evaluation of formulated ointment

Evaluation parameter	observation	observation	observation	observation
Formulation	1	2	3	4
colour	yellow	yellow	Yellowish brown	Yellowish brown
odour	characteristics	characteristics	characteristics	characteristics
consistency	smooth	smooth	smooth	smooth
washability	good	good	good	good
Non irritancy	Non irritant	Non irritant	Non irritant	Non irritant
PH	6.20	6.32	6.48	5.91
Spreadability seonds	6.2	6.5	5.8	5.1
Extrudability	0.65g	0.6g	0.4g	0.5g

C. Anti-bacterial activity:

All the prepared formulation with different concentration of plant extract were checked for its anti – bacterial activity. And the result revealed that, formulation F4 showed the anti-bacterial activity against the bacteria *S.auresus* with the zone of inhibition of 12mm.

Table 5.3 Anti – bacterial activity:

organism	Formulation code	Formulation code	Formulation code	Formulation code
	F1	F2	F3	F4
Pseudomonas aeruginosa	-	-	-	-
Staphylococcus aureus	-	-	-	+

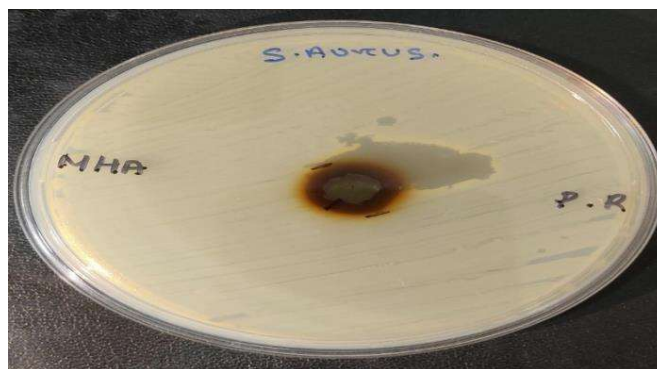


Fig 5.6 Anti-bacterial activity of formulation F4

Stability study:

The formulation F4 was found to be physically stable at different temperature i.e. 2°C, 25°C and 35°C after four weeks.

5.2 Discussion:

Bacterial skin infection is a contagious skin disease which affects million of people all over the world. Excessive usage of topical antibiotics is known to be key driver of Anti-microbial resistance (AMR) and is directly responsible for increasing Anti-bacterial resistance in *S.aureus*. Herbal formulation are considered to be more safe with no or less side effect compared to the marketed product. **Saxena prasoon Kumar et. al., (2010)** have worked on phytochemical investigation and Anti-microbial activity of skin bark extract of *Thespesia populnea*. Based on their studies we have performed the phytochemical investigation. The selected plant material were macerated by using petroleum ether. Evaluation of phytochemical analysis of petroleum ether extract of *T.populnea* showed the presence of phytosterols. **Shubhangi E. Sawant et. al., (2016)** have worked on formulation and evaluation of herbal ointment containing neem and turmeric extract. Based on their study we have formulated the ointment using *T.populnea* bark extract and evaluated for different parameters. Four different formulation of ointment F1, F2, F3, F4 was prepared by varying drug concentration. All the prepared formulation were evaluated for physicochemical parameters and Anti-bacterial activity against *S.aureus* and *P.aeruginosa* by well diffusion assay. The formulation F4 showed zone of inhibition against the *S.aureus*.

6. SUMMARY AND CONCLUSION

SUMMARY:

Bacterial skin infection affects about 155 million people all over the world. Only a small percentage of the world's bacteria cause infection and disease. These bacteria infection have a large impact on public health. Currently available allopathic ointment used for treatment of bacterial skin infection cause adverse effect and with continuous use can lead to Anti-microbial resistance (AMR). Because of this issue many people prefer medicinal plant for treatment of bacterial infection. *Thespesia populnea* was selected for the present study.

The literature Survey showed the information regarding the Anti-bacterial activity of *Thespesia populnea*. Based on literature survey *Thespesia populnea* were selected for further studies and aimed to formulate into a topical dosage form to treat bacterial skin infections.

In our present study, *Thespesia populnea* bark was collected, dried coarsely powered and macerated by petroleum ether solvent for 3 days. The extract obtained was filtered and used for phytochemical screening. The result showed the presence of phytosterols and further the plant extract were evaporated by using rotary evaporator. The evaporated plant extract were freeze dried (lyophilization) in order to obtain dry powder of the plant. Four different formulations of ointment was prepared i.e, F1, F2, F3, F4 by varying the drug concentration. All the prepared formulations were evaluated for different parameters such as pH, colour, odour, consistency, solubility, washability, spreadability and extrudability study. Further all the prepared formulation were tested for anti-bacterial activity against *S.aureus* (gram +ve) and *P.aeruginosa* (gram -ve) by well diffusion assay. Formulation F4 showed the zone of inhibition of 12mm against *S. aureus* bacteria. After all the evaluation test performed formulation F4 was found to have optimum physicochemical characteristics and also showed the anti-bacterial activity against the *S.aureus* bacteria. Further formulation F4 was evaluated for its stability at different temperature i.e, 2°C, 25°C, 37°C for four weeks and was found to be stable.

Overall it can be concluded that the ointment prepared using *Thespesia populnea* bark extract was stable, showed satisfactory physicochemical properties and possessed anti-bacterial activity against *S.aureus*. Hence the study showed that it is possible to develop *Thespesia populnea* ointment for the treatment of bacterial skin infections caused by *S.aureus* which provides safety and less side effects compared to allopathic drugs. However, further investigations are required to determine the therapeutic efficiency of the prepared *T.populnea* ointment formulation.

CONCLUSION:

Herbs have been used in medicine for centuries due to their inexpensive cost, no or less side effects, and ease of availability. In this study *T.populnea* bark was selected for its anti-bacterial activity. Phytochemical screening was done for the *T. Populnea* bark extract obtained using petroleum ether as a solvent and the results revealed the presence of phytosterols. Further an attempt was made to formulate four different ointment formulations with different concentrations of *T.populnea* bark extract. All the formulations were evaluated for physicochemical parameters and Anti- bacterial activity was tested using well diffusion assay. The results indicated that formulation F4 exhibited anti-bacterial activity against *S.aureus* bacteria and was found to be physically stable of different temperature condition after the stability study.

The present experimental work showed that it is possible to formulate ointment from *T.populnea* bark extract and as it showed anti-bacterial activity against the bacteria *S.aureus*, it could be used as a simple topical dosage form in order to treat the bacterial skin infections.

Anyway, further investigation and comparative studies with other allopathic formulations are required in order to determine the therapeutic efficiency, safety of the herbal ointment

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ANNEXURE

AUTHENTICATION CERTIFICATE



சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம்
சித்த மருத்துவ ஆராய்ச்சி துறாம். ஆயுஷ் அமைச்சகம், இந்தியா

सिद्ध केंद्रीय अनुसन्धान संस्थान

(सी.सी.आर.एम., चेन्नई, आयुष मंत्रालय, भारत सरकार).

அணா சர்க்காரி அஸ்தானல பரிஸர், அம்மாக்கம், சென்னை - 600106

SIDDHA CENTRAL RESEARCH INSTITUTE

(Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India)

Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106.

E-mail: crisiddha@gmail.com/stores.cris@gmail.com Phone: 044-26214925, 26214809, Web: http://crisiddha.tn.nic.in

Form No. PCOG002-ACF

06.07.2023

AUTHENTICATION CERTIFICATE FOR 562.06072301

Certified that the sample submitted by M. Prethees, M. RajaPandian, M. Karthikeyan and S. Mohana Sundaram and P. Allan Britto bhen Sathyabama Institute of Science and Technology, Semmencheri, Chennai – 119 was identified as:

Code
T06072304P

Botanical Name
Thespesia populnea Sol. ex Corrêa. *

Part
Stem bark



* WFO (2023): *Thespesia populnea* Sol. ex Corrêa. Published on the Internet:
<http://www.worldfloraonline.org/taxon/wfo-0000455933>; Accessed on 06 Jul 2023

Dr KN Sunil Kumar
Research Officer/Sci-II and HOD
Department of Pharmacognosy

डॉ. के.एन. सुनील कुमार/Dr. K.N. Sunil Kumar
अनुसंधान अधिकारी (कार्मकोशाधि)/RO(Pharmacognosy)
सिद्ध केंद्रीय अनुसंधान संस्थान,
(सी.सी.आर.एम., चेन्नई, आयुष मंत्रालय, भारत सरकार)
अण्णा सरकारि अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
SIDDHA CENTRAL RESEARCH INSTITUTE
(Central Council for Research in Siddha, Chennai, Ministry of Ayush, Govt. of India)
Anna Govt. Hospital Campus, Arumbakkam, Chennai-600106

Dr P Elankani
Research officer (Siddha)
Sci-IV/In charge

Dr. P. Elankani / डॉ. पी. इलंकानी
अनुसंधान अधिकारी (सिद्धा) (सैनिक-IV)
Research officer (Siddha) (Sci-IV) I/C
सिद्ध केंद्रीय अनुसंधान संस्थान,
(सी.सी.आर.एम., चेन्नई, आयुष मंत्रालय, भारत सरकार)
अण्णा सरकारि अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
SIDDHA CENTRAL RESEARCH INSTITUTE,
(Central Council for Research in Siddha,
Ministry of Ayush, Govt. of India),
Arumbakkam, Chennai-600 106.