FORMULATION AND EVALUATION OF OINTMENT CONTAINING CHLOROXYLON SWIETENIA LEAF EXTRACT FOR ANTIMICROBIAL ACTIVITY

Author: Diksha Gautam*, Vijayshree Patle, Dr. T. P. Nimbekar

SHRI LAXMANRAO MANKAR INSTITUTE OF PHARMACY, AMGAON

DIST- GONDIA-441902

Corresponding Author: Diksha Gautam*

ABSTRACT

The present study of a *Chloroxylon swietenia* ointment might summarize its formulation, therapeutic uses, and antibacterial properties. The ointment was formulated from the leaves extract of *Chloroxylon swietenia* plant with various excipients to enhance its topical application. *Chloroxylon swietenia*, commonly known as East Indian Satinwood, has garnered attention for its potential therapeutic properties. Traditionally used in Ayurvedic medicine. *Chloroxylon swietenia* is renowned for its antimicrobial, antibacterial, anti-inflammatory, anti-fertility, analgesic and insecticidal properties. Under this research work the phytochemical screening, antimicrobial activity of hydroalcoholic extract was evaluated and herbal ointment was formulated. The result showed that the phytochemicals present in the extract of *Chloroxylon swietenia* are carbohydrates, proteins, phenols, flavonoids, saponin and tannins. However, glycoside and alkaloids were found absent in leaf extract. In vitro antimicrobial activity of hydroalcoholic extract against *Staphylococcus aureus* and *Escherichia coli* has shown effective results. The antimicrobial activity was maintained when hydroalcoholic extract was incorporated into the ointment base against *S. aureus*, *E. coli*. The herbal ointment was measured the antimicrobial activity by zone of inhibition using methods like agar well diffusion and has shown effective results for antibacterial activity. The current study deals with the determination of formulation and evaluation of herbal ointment using *Chloroxylon swietenia*.

KEYWORDS- *Chloroxylon swietenia*, Phytochemical, Antimicrobial, Herbal.

INTRODUCATION

Ayurveda has long utilized herbal therapies, with plants like *Chloroxylon swietenia* being used to treat various ailments, including treating wounds, cuts, burns, skin diseases, rheumatism, snakebites, and as an astringent, with some extracts showing antimicrobial, anti-inflammatory, and anti-diabetic properties [4]. It is commonly known as East Indian Satinwood come from Rutaceae family native to Asia [1]. The plant ranges in Sri Lanka, India, Madagascar. Leaves, bark, roots, flower of *Chloroxylon swietenia* are full of medicinal property. It is found that extract of various parts of plants is used for their antimicrobial activity [3]. The *Chloroxylon swietenia* leaves extract were screened for their phytochemical content. Quantitative test was used to detect the presence of are carbohydrates, proteins, phenols, tannins, flavonoids, saponins. Presence of these phytochemicals in the medicinal plants indicates the presence of antimicrobial properties against *S. aureus* and *E. coli* [5]. This study aims to establish the pharmacognostic profile and ethnomedicinal values of *Chloroxylon swietenia*, aiding in its identification, standardization, and conservation. The findings suggest that *Chloroxylon swietenia* leaf extracts are a rich source of bioactive compounds. The study also evaluates the phytochemical and antibacterial potential of *Chloroxylon swietenia*, which may yield novel phytoconstituents for preventing infections caused by clinical pathogens [5].

IMPORTANCE

- *Chloroxylon swietenia* leaf extracts exhibit significant antimicrobial activity, traditionally used for treating various ailments like skin infections, rheumatism, and coughs, and have shown potential as a natural source for antimicrobial agents [9].
- Studies have shown that extracts of *Chloroxylon swietenia* possess antimicrobial activity against various pathogens [6].
- The hydroalcoholic extract of *Chloroxylon swietenia* has shown high antibacterial activity against various bacterial species [12].
- The leaf crude extract and leaf essential oil are used to kill mosquito larvae and microbial infections.
- Leaves are used for the treatment of inflammation-associated disorders such as rheumatism [7].
- The plant contains various phytochemicals, including tannins, flavonoids, and alkaloids, which may contribute to its antimicrobial properties.
- The plant extracts can be used in the synthesis of silver nanoparticles (AgNPs) for antimicrobial applications.
- The plant extracts have potential applications in the development of natural antimicrobial agents for treating various infections [8].

CHLOROXYLON SWIETENIA

Chloroxylon swietenia, the Ceylon satinwood or East Indian satinwood [9], is a tropical hardwood, the sole species in the genus *Chloroxylon* (from the Greek "green wood") [11].

Botanical name: *Chloroxylon swietenia.*

Vernacular Names: in different areas different names are used by the people. It is commonly called as bherul in Sanskrit and satinwood in English [4].

Synonyms: Satinwood, East Indian satinwood, Ceylon Satinwood.

Plant profile:-

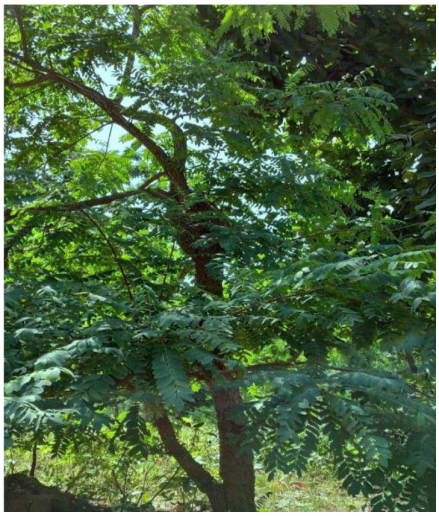

Fig.01: *C. swietenia* A) A complete plant

Fig.02: B) The single compound leaf

Table 1: Vernacular Names [4]

Common name (English)	Ceylon satinwood, East Indian Satinwood, Buruta
Telugu	Billu, Bilydu, Billudu, Bella
Hindi	Bhirra, Bhivia, Dhoura, Girya
Tamil	Vaaimaram, Porasu, Mammarai, Porinja maram
Malayalam	Varimaram
Kannada	Bittula, Huragalu, Hurihuli, Masula
Sanskrit	Bhillotaka, Bimbilota
Oriya	Bheru gatcho

Marathi	Behru, Halda, Bheria, Hulda

Taxonomical classification

Table 2: Taxonomical classification of *Chloroxylon swietenia.*

Kingdom	Plantae, Plant
Sub-kingdom	Tracheophyta
Division	Angiosperma
Class	Eudicots
Sub-class	Rosids
Order	Sapindales
Family	Rutaceae
Genus	*Chloroxylon*
Species	*Chloroxylon swietenia*

Habitat and distribution

Chloroxylon swietenia is a small to medium-sized tree native to India, Sri Lanka, and Malaysia. In India, it is found in various states, including Maharashtra, Orissa, Madhya Pradesh, Andhra Pradesh, Karnataka, Kerala, and Tamil Nadu. The tree thrives in poor, laterite soils and is also found in tropical dry evergreen forests. It is characterized by its moderate size, glaucous pinnate leaves, and straight, cylindrical stem, typically reaching a girth of 3-4 feet [2].

Microscopic characteristics

The plant is extensively used in traditional medicine, with various parts exhibiting medicinal properties. The essential oil extracted from its leaves and stems possesses antibacterial and antifungal properties. The dried leaves are used to alleviate pain, while crushed leaves are applied to treat wounds, snake bites, and rheumatism. A paste made from leaves and roots can be taken internally or applied externally to relieve headaches. The seeds yield oil, and the wood is valued for its durability and hardness, making it suitable for decorative timber, heavy construction, agricultural equipment, boat building, and railway sleepers[3]. Additionally, it is used as fuelwood. The tree's physical characteristics include:

- Height: up to 18-25 meters tall
- Bole: straight, cylindrical, and branchless for up to 4.5 meters
- Diameter: up to 45-90 cm
- Bark surface: yellowish or pale greyish-brown, rough, and corky
- Crown: spreading
- Branchlets: greyish hairy [5]

Phenology

The tree is usually leaf less from February to April or May, flowers appearing during March-April; the fruits generally ripen during May-August [7].

Table 3: Morphology

Leaves	15 - 23cm long and abruptly pinnate. The leaflets (10 - 20 pairs) are sub-opposite or alternate, oblong, obtuse, glabrous and glaucous.
Stem	Straight cylindrical stem generally attaining a girth of 3-4 feet.
Flowers	Small white or cream in colour and present in terminal or axillary panicles 10-20cm long. The tree is usually leafless from February to May, flowers appear during March-April, and fruits generally ripen during May-August and produce seeds profusely almost every year. Buds are round. Inflorescence a terminal or axillary pyramid-shaped panicle up to 15 cm long, short-hairy.
Fruit	Oblong three-segmented capsule 2.5-4.5cm long, containing 1-4 seeds in each segment.
Wood	Produced by the tree is often a golden colour with a reflective sheen. It is used for small luxury items and as a veneer in wooden furniture. It is one of the best-known satinwoods.
Seeds	About 4 in each cell, imbricate, compressed, winged especially above, their margins are angular, attached to the edges of the septa. 1 cm long, with oblong wing on one side, up to 1.5 cm long.

PHARMACOLOGICAL ACTIVITIES

Chloroxylon swietenia has been traditionally used as an antioxidant due to its perceived medicinal properties. This has prompted numerous in vivo and in vitro studies, which have yielded positive results for various activities. Some of the pharmacological properties exhibited by *Chloroxylon swietenia* include:

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of *Chloroxylon swietenia* leaf extract has been studied for its potential to inhibit the growth of various microorganisms. The extract has shown promising results against a range of bacteria, fungi, and other pathogens. The leaf extract has demonstrated antibacterial activity against several Gram-positive and Gram-negative bacteria. Studies have reported significant inhibition of bacterial growth, including species such as Staphylococcus aureus, Escherichia coli.

Mechanism of Action

The antimicrobial activity of *Chloroxylon swietenia* leaf extract is attributed to the presence of bioactive compounds, including flavonoids, phenolic acids, and terpenoids. These compounds may interact with microbial cell membranes, disrupting their structure and function, ultimately leading to the inhibition of microbial growth.

ANTI-INFLAMMANTORY ACTIVITY

A study by Kumar et al. (2006) found that the chloroform extract of *Chloroxylon swietenia* leaves exhibited significant anti-inflammatory activity in rat models. The extract was tested at doses of 50, 100, and 200 mg/kg and showed notable effects in reducing inflammation induced by carrageenan, histamine, serotonin, and cotton pellet-induced granuloma when administered orally [13].

ANALGESIC ACTIVITY

A study by Kumar et al. (2006) found that the chloroform extract of *Chloroxylon swietenia* leaves exhibited significant anti-inflammatory activity in rat models. The extract was tested at doses of 50, 100, and 200 mg/kg and showed notable effects in reducing inflammation induced by carrageenan, histamine, serotonin, and cotton pellet-induced granuloma when administered orally [14].

ANTIOXIDANT ACTIVITY

The ethanolic extract of the whole plant has been shown to exhibit antioxidant activity against acetaminophen-induced hepatic injury in rats. Oxidative stress contributes to hepatotoxicity by causing tissue damage and impairing the body's natural antioxidant defences, leading to an overproduction of free radicals. This is characterized by decreased activity of key enzymes such as superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, and glutathione-S-transferase. However, treatment with the

ethanolic extract at a dose of 500 mg/kg orally significantly reversed these changes, suggesting its potential as an antioxidant agent [15].

HEPATOPROTECTIVE ACTIVITY

The ethanolic extract of the whole plant demonstrated hepatoprotective activity against acetaminophen-induced hepatic injury in rats. The liver damage led to significant increases in serum levels of SGOT, SGPT, and total bilirubin. However, administration of the ethanolic extract at doses starting from 25 mg/kg via oral gavage resulted in a dose-dependent restoration of these enzyme levels, indicating its potential as a hepatoprotective agent [16 &17].

ANTHELMINTIC ACTIVITY

The anthelmintic activity of various extracts from the roots of *Chloroxylon swietenia* was evaluated against Indian earthworms (Pheretima posthuma). The chloroform and methanol extracts showed notable anthelmintic effects, particularly at the highest concentration of 100 mg/mL [18].

MOSQUITOCIDAL ACTIVITY

The essential oil and sesquiterpenes isolated from *C. swietenia* leaves were evaluated for their mosquitocidal activity using a fumigant toxicity model against three mosquito species: Anopheles gambiae, Culex quinquefasciatus, and Aedes aegypti. The essential oil demonstrated mosquitocidal activity against all three species. Among the major sesquiterpenes tested, germacrene D exhibited the most potent mosquitocidal activity, followed by pregeijerene and geijerene. Notably, the oil and isolated compounds showed particularly high efficacy against Anopheles gambiae [19].

OTHER ACTIVITIES

The secondary metabolites of *Chloroxylon swietenia* crude extract and its fractions, such as hexane and n-butanol fractions showed good tyrosinase inhibition activity [20].

MATERIAL AND METHODS

Instruments, Reagents and Chemicals

Soxhlet apparatus, Ethanol, Distilled water, Wool fat, Nutrient agar, Cetostearyl alcohol, Hard paraffin and Yellow soft paraffin.

Collection and Authentication

The leaf of Chloroxylon swietenia was collected from nearly areas of Amgaon, Gondia during the month of February and Authenticated by Dr. M.G. Awale, Head of Department of Botany Bhawbhuti Mahavidyalaya Amgaon. The collected plant material was primarily washed with tap water, followed by a rinse with distilled water. It was then air-dried under shade and powdered into a coarse texture using a mechanical blender. The coarse powdered passed through sieve 100 mesh sizes and stored in air-tight containers. The plant C. swietenia was collected from various tribes living in tribal pockets of zone of Gondia. All other chemicals and reagents used were of analytical grades.

EXTRACTION OF C. SWIETENIA LEAVES

The fresh leaves were collected, washed with water and sun dried.The dried leaves were powdered. Powdered leaves (47.5gm) were extracted with hydroalcoholic extraction using a solvent mixture of ethanol and water (80:20 ratio) in Soxhlet apparatus. The extraction was carried out at 40°C until the solvent becomes colourless in the Soxhlet loop. Extract was concentrated by keeping them at room temperature. Allow the solvent to evaporate and the residue obtained, stored at 4°C in refrigerator which was further used.

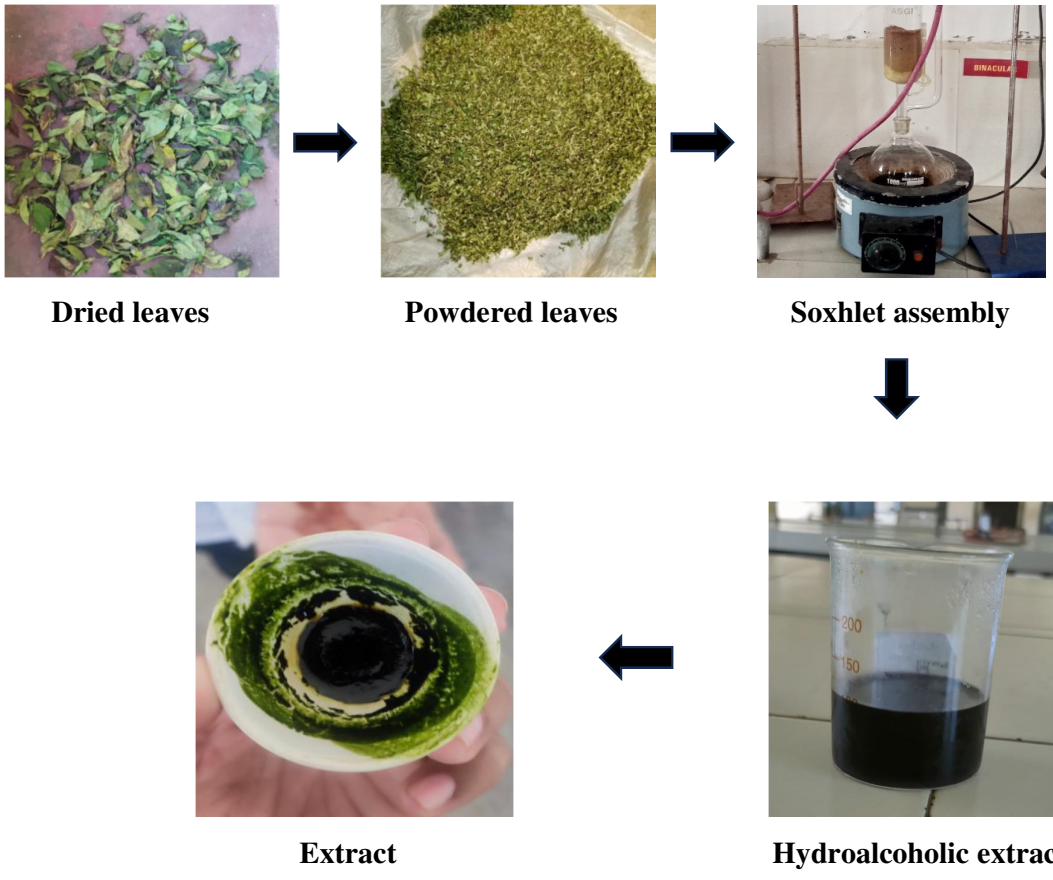

Fig 03. Extraction Process

PHYTOCHEMICAL SCREENING

Qualitative Analysis

The *Chloroxylon swietenia* extracts under the phytochemical screening to detect the presence of various compounds, including alkaloids, flavonoids, terpenoids, saponins, tannins, phenolic compounds, and glycosides, using established standard procedures.

Quantitative Analysis

Quantitative analysis was conducted on all extracts to determine the total content of phenolics, alkaloids, and terpenoids.

Test for alkaloids

A) Wagner's test:

1 ml of extract add 5-6 drops of Wagner's reagent, brown colour precipitate indicates the presence of alkaloids. (Iodine potassium iodide solution).

B) Hager's test:

Few drops of Hager's reagent were added in 1 ml of extract, formation of yellow colour indicates the presence of alkaloids. (saturated solution of picric acid).

Test for Flavonoids

A) Lead Acetate Test: A yellow or green precipitate indicates the presence of flavonoids. (Lead acetate solution).

Test for Phenols

A) Ferric Chloride Test: A green, blue, or purple colour indicates the presence of phenols.

Test for Proteins

A) Ninhydrin Test: A blue or purple colour indicates the presence of proteins or amino acids. (Ninhydrin solution).

B) Xanthoproteic Test: A yellow colour indicates the presence of proteins. (Nitric acid).

Test for Carbohydrates

A) Benedict's Test: Detects reducing sugars, indicated by a colour change (green, yellow, orange, or red). (copper (II) sulfate, sodium carbonate, and sodium citrate)

B) Fehling's Test: Detects reducing sugars, indicated by a brick-red precipitate. (copper(II) sulfate and sodium hydroxide).

Test for Saponins

A) Foam Test:

1ml of extract, 5ml water was added and shake well in the test tube shaker. Formation of foam layer on the top of test tube showed the presence of saponins.

Test for Diterpenes

A) Copper acetate test: The Copper Acetate Test detects diterpenes, indicated by a colour change or precipitate formation (Emarald green colour), using copper (II) acetate as the reagent.

Test for Glycosides

A) Borntrager's test

1gm of drug sample + 5-10 ml of dilute HCl + 10 min. boil on water bath and filter + extract of filtrates with CCl4 or benzene + equal amount of ammonia solution to filtrates + shake → appearance of pink to red colour → indicate presence of anthraquinone moiety.

OINTMENT

An ointment is type of topical medication used to treat and protect the skin. It is typically composed of oil-base substances making it greasy and thick. Topical ointment containing antimicrobial properties can be used in the treatment and prevention of infections caused by different kind of microbes.

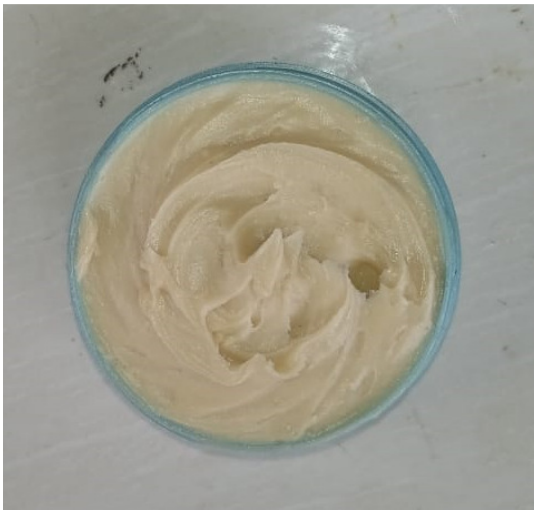

Fig 04: Herbal Ointment

FORMULATION OF HERBAL OINTMENT

The constituents of base were wool fat, yellow soft paraffin, Cetostearyl alcohol, and hard paraffin. All the ingredients were weighed and melted in a beaker at 70°C using heating mantle. The ingredients were stirred gently for 5 min by maintaining the temperature at 70°C.Then the hydroalcoholic extract of *Chloroxylon swietenia* leaves was added and stirred well until homogenous mass is formed and transferred in the bottle.

Procedure for preparation of herbal ointment

To prepare the ointment base, accurately weigh grated hard paraffin and place it in an evaporating dish on a water bath. Once melted, add the remaining ingredients and gently stir to facilitate homogeneous mixing. Finally, allow the ointment base to cool. To prepare the herbal ointment, accurately weigh the *Chloroxylon swietenia* leaves extract and mix it with the ointment base. Start by blending the extract with 2-3 times its weight of base to form a smooth paste, then gradually incorporate more base until a homogeneous ointment is formed. Finally, transfer the ointment to a suitable container.

Table 4: Formation of ointment bases

Sr. No.	Ingredients	Function/ Purpose	Quantity to be taken (gm)
1	Hard paraffin	Enhance texture, stability	1gm
2	Cetostearyl alcohol	Emulsifier, thickening agent	1gm
3	Yellow soft paraffin	Emollient	1gm
4	Wool fat	Moisturizer, emollient	17gm
5	*Chloroxylon swietenia* leaf extract	Antimicrobial agent	-

Table 5: Formulation of Herbal ointment.

Formulation code	**Prepared *C. swietenia* extract (g)**	**Ointment base q.s. (g)**
F1	2	20
F2	2.5	20
F3	3	20

Fig: F1

Fig: F2

Fig: F3

PHYSICOCHEMICAL PARAMETERS OF HERBAL OINTMENT

Colour and Odour

Physical parameters like colour and odour were examined by visual examination.

Consistency

Smooth and no greediness was observed.

pH

The pH of the prepared herbal ointment was measured using a digital pH meter. A solution was prepared by dissolving the ointment in 100ml of distilled water and allowed to stand for 2 hours. The pH was determined in triplicate, and the average value was calculated to be 5.

Spreadability

Spreadability was assessed by compressing a sample between two slides under a set weight for a specified time, creating uniform thickness. The time taken for the slides to separate was measured, with shorter times indicating better spreadability. Spreadability was calculated by following 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

It was found to be 5 seconds.

Loss on Drying

The Loss on Drying (LOD) was determined by placing the formulation in a petri dish on a water bath and drying it at 105°C. The LOD was found to be 20%.

Solubility

Soluble in water, alcohol and chloroform.

Washability

The formulation was applied to the skin, and its ease of removal with water was evaluated by checking how easily it washed off.

Non-irritancy Test

Prepared herbal ointment was applied to the skin of human being and observed for the effect.

Stability study

The physical stability of the herbal ointment was evaluated over a four-week period at various temperatures, including 20°C, 25°C, and 35°C. The results showed that the ointment remained physically stable at all tested temperatures throughout the four-week duration.

ANTIMICROBIAL ASSAY OF OINTMENT

Antimicrobial activity of hydroalcoholic extract was determined by cup plate method. Sterilization of Petri plates and Nutrient agar in autoclave at 121°C for 15 min Allow the sterile molten nutrient agar to cool and then inoculate test microorganism. Pour agar into the plates and allow to solidify. Place a sterile well on agar plate with the help of cork borer. Add dilutions to the wells of test sample. Incubate the plates for 24-48 hours at appropriate temperature. Measure the zone of inhibition. Microbial assay of ointment by using cup plate method in which the nutrient agar was used as a culture media and this assay was evaluated against *E. coli* bacteria to determine the antibacterial activity of ointment. The microorganism was inoculated in the molten nutrient agar, then this agar was poured into the petri plate and allow to solidify. The sterile wells were made with the help of cork borer. The ointment and reference were added into the wells and incubate the plates for 24 – 48 hours. Measure the diameter of zone of inhibition to determine the antibacterial activity.

RESULT

A) PHYTOCHEMICAL SCREENING

Table 6: Phytochemical screening of leaves extract of Chloroxylon swietenia

Sr.no	Chemical constituent	Test performed	Observation	inference
1	Alkaloid	a. Hager's test	No yellow cream ppt observed	Absent
		b. Wagner's test	No reddish brown ppt observed	Absent
2	Flavonoid	Lead acetate test	Brown ppt	Present
3	Saponin	Froth formation test	A small height froth was formed	Present
4	Carbohydrate	a. Fehling's test	Brown-red ppt	Present
		b. Benedict's test	Bluish-green ppt	Present
5	Phenol	Fecl3 test	Bluish-black	Present
6	Protein	a. Xanthoproteic test	Yellow Colour observed	Present
		b. Ninhydrin test	No purple Colour observed	Present
7	Diterpene	Copper acetate test	No emerald green colour	Present
8	Triterpene	Salkowski test	No yellow colour observed	Absent
9	Glycoside	Borntrager's test	No appearance of pink to red Colour	Absent

B) PHYSIOCHEMICAL PARAMETER OF OINTMENT

Table 7: Physicochemical parameters.

Sr. No.	Parameters	F1	F2	F3
1	Colour	Pale yellow	Pale yellow	Pale yellow
2	Odour	Characteristics	Characteristics	Characteristics
3	Texture	Smooth	Smooth	Smooth
4	State	Semi-solid	Semi-solid	Semi-solid
5	pH	5.5	5.5	5.5
6	Irritancy	Irritant	Non-irritant	Non-irritant
7	Consistency	Thick & greasy	Thick & greasy	Thick & greasy
8	Washability	Washable	Washable	washable
9	Solubility	Soluble in water, alcohol & chloroform	Soluble in water, alcohol & chloroform	Soluble in water, alcohol & chloroform
10	Spreadability	7 seconds	6 seconds	5 seconds

C) ANTIMICROBIAL ASSAY OF OINTMENT

After the incubation period, the zone of inhibition was measured to assess the antimicrobial activity of the prepared ointments.

Table 8: Zone of inhibition of standard, F1, F2, F3.

Formulation code	**Zone of inhibition *Escherichia coli***
F1	1cm
F2	1.4cm
F3	2cm

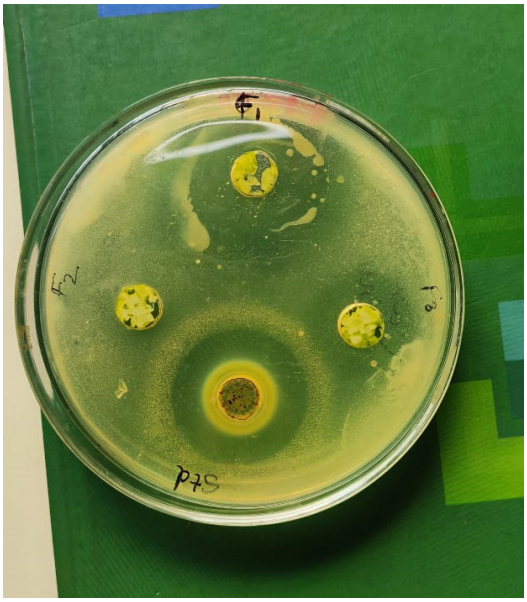

Fig: Zone of inhibition of Standard, F1, F2 and F3.

DISCUSSION

The hydroalcoholic extract of *Chloroxylon swietenia* leaves underwent preliminary phytochemical screening, revealing the presence of carbohydrates, proteins, phenols, tannins, flavonoids, and saponins, while glycosides and alkaloids were absent. The extract's physiochemical parameters, such as pH and solubility, were evaluated, and the formulated ointment showed no colour change. The study demonstrated that the extract exhibited antimicrobial activity against Escherichia coli, which was maintained when incorporated into an ointment base for topical use. The final product spread easily on the skin surface without causing irritation, making it suitable for wound healing, boils, skin rashes, and other skin infections due to its antibacterial properties. The ointment's physicochemical properties, including spreadability, washability, solubility, and loss on drying, were satisfactory. A stability study showed no changes in spreading ability, diffusion, or irritant effects over three weeks at various temperatures. Formulation F3 exhibited the largest zone of inhibition against E. coli, indicating maximum antimicrobial activity compared to F1 and F2. Therefore, the hydroalcoholic extract of *Chloroxylon swietenia* was effectively used as an active component in preparing an antibacterial ointment, with F3 being the most potent formulation.

CONCLUSION AND FUTURE PERSPECTIVE

The present study concluded that *Chloroxylon swietenia* leaf hydroalcoholic extract showed antimicrobial activity *Escherichia coli*. This activity was maintained, when extract was incorporated into the ointment base for topical use. The final product readily spread on skin surface, showed no irritant effect. It can be topically used for wound healing, boils, skin rashes and other skin infection for its antibacterial activity. Research going on it would be easier to develop potent, stable and more effective new formulations.

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