Research Article

Antibacterial Activity of Semisolid Dosage Forms Containing Extracts of Frontal Leaves of *Tectona grandis* (Family: Verbenaceae)

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ABSTRACT

Teak (*Tectona grandis*) is considered as a major constituent in many folklore medicines. Even though some works on pharmacological potential of teak has carried out earlier, a systematic approach is lacking which can bring out a clear picture on medicinal value of the plant. For most of the herbs, the specific ingredient that causes therapeutic effect is not known. Bacterial infections are one of the prominent causes of health problems, Plants have been used in medicine as antimicrobial agents since ancient times could provide a promising solution for drug resistant species. The present study involves formulation and evaluation of antibacterial activity of semisolid dosage forms containing ethanol extract of frontal leaves of *Tectona grandis,* Morphological studies, phytochemical screening and antibacterial activity of extracts were carried out. Extract was incorporated in different semisolid bases and evaluated for various parameters. The qualitative analysis of extracts indicated presence of alkaloids, Tannins and flavonoids. The antibacterial activity showed that ethanol extracts have potential antibacterial activity compared to standard drug. The physicochemical parameters of the formulated ointments were identified and antibacterial activity of semisolid dosage forms found satisfactory. Extracts were incorporated in three different types of (oily/aqueous/ gel) semisolid bases. These were evaluated for preliminary tests like Colour, consistency, pH, Viscosity, Spreadability, Extrudability tests and results were found satisfactory.

Keywords: Teak Leaves Extracts, Phytochemical Screening, semisolid dosage from.

INTRODUCTION

Use of medicinal plants as traditional medicine is one of the common practices in India due to their wide pharmacological activities. Traditional medicines are being used as the primary health care level by many developed and developing countries. Many of currently used drugs are expensive and their continued usage may lead to development of resistance. Some drugs may also have side effects. This situation urgently forced scientists for searching drugs which are inexpensive, safe, have fewer side effects and which will be able to act for longer periods before resistance sets in. Since ancient time in India, herbal medicines have been the basis of treatment and cure for various diseases physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha.[1-2] The WHO estimates that more than 80% of the world's population rely either solely or largely on traditional remedies for health care. Medicinal plants are one of the most sensitive commodity areas of research in the world today. Herbal medicines have various therapeutic uses such as healing wounds, treating inflammations due to infection, skin lesions, leprosy, diarrhoea, scabies, venereal diseases, snake bite and ulcers etc. Many infectious agents such as virus, fungi, and parasites may harm the plants. All plants synthesize a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones which are capable of providing protection against infectious diseases. There are several reports regarding the antimicrobial activity of crude extracts prepared form plants. Some of the active principles of the bioactive compounds are preferred for their therapeutic purposes either as a single entity or in combination, so as to inhibit the life processes of microbes[3-4].

Tectona grandis Linn. (Common name – Teak; Family - *Verbenaceae*) is one of the most famous timbers in the world and is renowned for its dimensional stability, extreme durability

and hard which also resists decay even when unprotected by paints and preservatives. Timber value of teak has been well known from decades. Teak is a major exotic species found in tropical regions. It is commonly found in India and other South-East Asian countries. Teak is also considered as a major constituent in many folklore medicines. Extracts from various parts of teak shows expectorant, anti-inflammatory, anthelmintic properties and dysentery. Even though some works on pharmacological potential of teak has carried out earlier, a systematic approach is lacking which can bring out a clear picture on medicinal value of the plant.[5-7] Considering above facts present research work was aimed at formulation of antibacterial semisolid dosage forms containing frontal leaves extract *Tectona grandis Linn.* Family: *Verbenaceae.*

MATERIALS AND METHODS

MATERIALS

Gentamycin Sulphate USP gift sample procured from Ranbaxy Laboratories Ltd., Madkaim, Ponda, Goa. Microbiological media, Mueller Hinton Agar (MHA) (Himedia) was procured from the department of microbiology, Srinivas College of pharmacy, Mangalore. All the other chemicals used in the formulations were of analytical grade.

Collection of plant material and preparation of plant extracts

The frontal leaves of *Tectona grandis*, were collected from local area of Udupi, Karnataka. The leaves were washed with water to clean the adhering dust particles. Then the leaves were dried in shade for one week and made in to coarse powder by using grinder, stored in air tight container. Approximately 50 g of the powdered crude drug was soxhlet extracted with ethyl alcohol. Evaporation of the solvents from the extracts was done by using rotary vacuum evaporator at 40°C. A sticky mass was obtained after evaporation of extracts, labelled and stored at 2-8°C. The percentage yields were calculated.[8]

Preliminary Phyto-chemical Screening

Extracts from the leaves were subjected to qualitative analysis (Table 1) to check the presence alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils and fats, resins, phenols, tannins, flavonoids, proteins, amino acids, and triterpenoids.[8-10]

Antibacterial activity of plant extract

Ethanol extracts of *Tectona grandis,* were dissolved in a few drops of Di-methylsulphoxide (DMSO) and stock solution of 100 mg/ ml was prepared by using distilled water. The stock solution was kept at 4-8°C. Standard bacterial organisms from the ATCC were obtained from the department of microbiology, Srinivas college of pharmacy, Mangalore. *S. aureus* (ATCC25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used. Cultures of *E. coli*, *S. aureus* and *P. aeruginosa* were inoculated separately in sterile Mueller Hinton agar media. After solidification of the medium wells were bored with the help of sterile borer. 1 ml of extract (100mg/ml) and standard (Gentamycin Sulphate, 10 µg/ml) was loaded in the wells and kept in the incubator at 37˚C for overnight[11-13.]

Formulation of Ointments

Different semisolid bases were selected based on optimisation studies having desirable characteristics. The following different bases [Formulation I, II and III] were selected for the formulation of semisolid dosage forms. Extract containing 10% w/w concentration was incorporated in various bases. The compositions of the herbal semisolid preparations were as follows:

Preparation of Formulations I and II

The oily and water soluble ointment bases were prepared by fusion method. In this method the constituents of the base (Table 1 and 2) were placed together in chaina dish and allowed to melt together at 70° C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for certain period of time and then add the extract, cooled with continuous stirring.[14,22]

Preparation of Formulation III

Carbopol 934, 2.0 g was soaked in water for a period of 2 hours. Carbopol was then neutralized with triethanolamine (TEA) with stirring. Then 10 g of extract was dissolved in 2.0 g of propylene glycol and 5.0 ml of ethanol. Solvent blend was transferred to carbopol container and agitated for additional 20 min. The dispersion was then allowed to hydrate and swell for 60 min, finally adjusted the pH with 98% TEA until the desired pH value was approximately reached (6.8-7). During P^H adjustment, the mixture was stirred gently with a spatula until homogeneous gel was formed.[15]

Evaluation of prepared semisolid formulations[15-17,22]

Determination of clarity and colour

Colour and odour of the prepared ointments were visually examined.

pH determination

pH of the formulation was determined using digital pH meter. One gram of ointment was dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were taken.

Viscosity

Viscosity was measured by Brookfield viscometer which measures the shearing stress on a spindle No. 7 rotating at 50 rpm, constant speed while immersed in the sample.

Spreadability

An excess of sample was placed between the two glass slides and a 1000 g weight was placed in slides for 5 minutes to compress a sample to uniform thickness. Weight (80 g) was added to the pan. The time required to separate the two slides was taken as a measure of spreadability. It was calculated using the formula

$$S = m. l / t.$$

Where S is spreadability, m is weight tied to upper slide, l is the length of glass slide and t is time taken.

Extrudability study

The formulations were filled in collapsible tubes and kept in the container. The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 second.

Antibacterial activity of ointment

The antibacterial activity of semisolid dosage forms were evaluated by Agar well diffusion method. The ointment was dissolved in DMSO (200 mg/ml) and added into the agar wells and Gentamycin cream used as standard was also added into the well. The plates were incubated at 37˚C for 24 hours and the antibacterial activity was checked[16-17].

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening of extracts was done and revealed the presence of alkaloids, glycosides, saponins, resins, tannins and flavonoids as represented in table 4.

The extracts were studied for antibacterial activities of using agar well diffusion method. Gentamycin sulphate USP, used as the positive control, showed sensitivity to test organisms with 22-24 mm of zone diameter showed a maximum inhibitory effect compared to leaves extract 16-19 mm. So the ethanol extract of *T. Grandis* containing 100 mg/ml concentrations were effective. Antibacterial activity could be due to different classes of compounds present in extract. Some of the classes of compounds identified in the crude extract were mainly alkaloids, phenols, flavonoids, and tannins have been reported to possess antibacterial activity.[18-22]

Three different semisolid formulations were formulated and the various physicochemical parameters such as colour, odour and pH, viscosity, Spreadability, Extrudability were evaluated table 5. Also the antibacterial potential of the formulations were determined as shown in table 6. All formulations had different colours and characteristic odour. The pH was in the range of 6.60 – 6.92. The gel base without herbal extract was transparent and had good viscosity. The colour was changed to brown after adding the extract and viscosity also slightly decreased due to addition of extract.

From the data it is clearly evident that physicochemical characteristics of formulations were found satisfactory. The viscosities of the preparations were measured using Brookfield viscometer. The herbal gel was greenish in colour and translucent in appearance and gave smooth feel on application. Spreadability results the formulations were found satisfactory.

Preparations showed antimicrobial activity against both Gram positive (*S. aureus)* and Gram-negative (*E. coli*, *P. aeruginosa)* bacteria. Ethanol extracts of leaves with different bases showed a less antibacterial activity compared with the gentamycin ointment positive control against the study organisms (Table 6). The order of antimicrobial activity is follows: Water Soluble base > Gel Base > Hydrocarbon Base. From this it can be concluded that rate of diffusion of drug from hydrocarbon is less compared to gel and water soluble base. So water soluble base can be selected for the formulation of ointment.

CONCLUSIONS

Semisolid preparations containing ethanolic extract of *Tectona Grandis* were prepared. Extracts were incorporated in three different types of (oily/aqueous/ gel) semisolid bases. These were evaluated for preliminary tests like Colour, consistency, pH, Viscosity, Spreadability and Extrudability tests and results were found satisfactory. Antibacterial activity could be due to different classes of compounds present in extracts. Some of the classes of compounds identified in the crude extract were mainly alkaloids, flavonoids, and tannins have been reported to possess antibacterial activity. Further fractionation of the extracts could yield fractions or

compounds with higher antibacterial activity. This study reports that the frontal leaves of *T. grandis* contain alkaloids, flavonoids and tannins and possess antimicrobial activity.

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Fig. 1: Frontal leaves of *Tectona grandis*[23]

Table 1: Formulation I (Oily Base)

S.No	Ingredients	Formula
1.	Ethanolic extracts of frontal leaves of *Tectona grandis*	10.0 g
2.	Stearic acid	15.0 g
3.	White wax	2.0 g
4.	White soft Paraffin	8.0 g
5.	Triethanolamine	1.0 g
6.	Methyl paraben	0.2 g
7.	Propyl paraben	0.1 g
8.	Propylene glycol	8.0 g
9.	Purified Water to	100.0 g

Table 2: Formulation II (Water soluble base)

S.No	Ingredients	Formula
1.	Ethanolic extracts of frontal leaves of *Tectona grandis*	10.0 g
2.	Polyethylene glycol 400	32.0g
3.	Polyethylene glycol 4000	32.0g
4.	Propylene glycol	7.5 g
5.	Purified water to	100.0 g

Table 3: Formulation III (Gel base)

S.No	Ingredients	Formula
1.	Ethanolic extracts of frontal leaves of *Tectona grandis*	10.0 g
2.	Carbopol 934 (%w/w)	2.0
3.	Propylene glycol	2.0 ml
4.	Ethanol	5.0 ml
5.	Triethanolamine	q. s. to neutralize the gel base
6..	Purified water to	100.0 g

Table 4: Phytochemical Screening of extracts of *T. Grandis*

S.No	Chemical Constituents	Contents
1	Alkaloids	+
2	Glycosides	+
3	Saponionis	+
4	Phytosterols	-
5	Fat and Oils	-
6	Resins	+
7	Phenols	-
8	Tannins	+
9	Flavonoids test	+

Table 5: Physicochemical evaluation of semisolid formulations: I, II, and III

S.No	Evaluation parameters	I (Oily Base)	II (Water soluble Base)	III (Gel Base)
1	Colour	Light brown	Light brown	Brownish
2	Consistency	Homogenous, free from lumps	Homogenous, free from lumps	Clear, Free from lumps
3	pH (1% w/v solution)	6.80	6.60	6.92
4	Viscosity (centipoises)	13400	11200	7400
5	Spreadability (gm.cm/ sec)	12	12	67
6	Extrudability study (g)	250	180	150

Table 6: Antibacterial activity of semisolid formulations: I, II, and III

S.No	Formulation and its code	Inhibitory Zone (mm)		
		S. aureus (G+)	*E. coli (G-)*	*P. aeruginosa (G-)*
1	Gentamicin ointment (1%w/w)	28-30	29-33	30-32
2	I - (Hydrocarbon base)	16-17	15-18	19-20
3	II (Water soluble base)	21-23	20-23	21-23
4	III (Gel Base)	17-19	18-22	19-21

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