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Antibacterial Poly-Herbal Semisolid Formulations Containing Leaves Extracts of *Tectona grandis, Mangifera indica* and *Anacardium occidentale*

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ABSTRACT

Herbal medicine have become an item of global importance both has medicinal and economical value. Plants used for medicinal purposes long before recorded history. Many plant species have been proved to have antibacterial activity. Thus, the main objective of the study was to formulate and evaluate a poly-herbal semisolid dosage forms using ethanolic extracts of frontal leaves of *T. grandis, M. indica* and *A. occidentale.* Formulations were evaluated for its physicochemical properties like color, consistency, pH, spreadability, extrudability and the results were found satisfactory. Antibacterial activity of formulations was studied against *S. aureus*, *E. coli* and *P. aeruginosa* by agar well diffusion method. (*E. coli, P. aeruginosa)* bacteria. The order of activity was as follows: Water Soluble bases > Gel Bases > Hydrocarbon Bases. The zones of inhibition of poly herbal formulations were in between 23-28 mm which can be comparable with standard formulation 24-29 mm. Activity of the formulations may be due to the presence of alkaloids, flavanoids, phenols and tannins in the extracts. The formulations were found to be very efficacious in all the parameters.

Keywords: Extraction from leaves, Poly-herbal Ointment, Evaluation, Antibacterial activity.

INTRODUCTION

Medicinal plants have been known for their disease-curing qualities for centuries. Nature has provided us with a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in the traditional medicine. Natural phytochemicals derived from fruits, vegetables and herbs have been reported to possess a wide range of biological effects, including antioxidant, antimicrobial and anti-inflammatory actions. [1-3] Phytoconstituents have found applications as naturally occurring antimicrobial agents in the field of preservation, pharmaceutics, phytopathology, etc. The most essential phytoconstituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. [4]

Increasing failure of chemotherapeutics and the resistance exhibited by pathogenic microbial infectious agents against antibiotics have led to the screening of medicinal plants for their potential antimicrobial activities. 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. Polyherbal formulations are used in Ayurvedic products and they are found to be pharmacologically active. Some drugs of plant origin used in conventional medical practice are direct plant extracts or plant materials that have been suitably prepared and standardized. Some of the medicinal plants have been employed in folk medicine

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for wound care. Some of these plants either possess prowound healing activities or other related properties which are beneficial in overall wound care. [5-8]

Wound defined simply as the disruption of the cellular and anatomic continuity of a tissue may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. This insult to the tissue is the portal of entry for many bacterial or microbial organisms and there is therefore need to administer substance that would heal rapidly to prevent the ingress of bacterial in tissues through wound openings. Some of the wound healing substances are either inaccessible or expensive. [9-12] There have been concerns related to the conventional topical dosage forms such as lotions, creams, ointments and powder in terms of drug diffusion or release from the vehicle and delivery through the skin. [13-14] Present research work was aimed at formulation and evaluation of Poly-herbal semisolid dosage forms containing frontal leaves extracts of *Tectona grandis,* (Verbenaceae), *Mangifera indica,* (Anacardiaceae), and *Anacardium occidentale* (Anacardiaceae).

MATERIALS AND METHODS

Materials: Gentamycin Sulphate USP gift sample procured from Ranbaxy Laboratories Ltd., Madkaim, Ponda, Goa. All the other chemicals used in the formulations were of analytical grade. Microbiological media, Mueller Hinton Agar (MHA) and Nutrient broth were obtained from the Department of microbiology, Srinivas College of Pharmacy, Mangalore.

Collection, identification and extraction Preliminary Phytochemical Screening

Frontal leaves of Leaves *T. Grandis, M. indica,* and *A. occidentale* were collected from coastal region of Udupi district, Karnataka, India. The plant leaves were authenticated. Leaves were washed with running water, air dried and made in to coarse powder by using grinder, stored in air tight containers in cool place. Dried powders were subjected to soxhlet extraction. Approximately 50 g of the powder was 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 and extracts were labelled and stored at 2-8°C. Extracts from leaves were subjected to qualitative analysis to check for the presence alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils and fats, resins, phenols, tannins, flavonoids, proteins and amino acids, triterpenoids. [15]

Preparation of Poly-herbal semisolid formulations by incorporating all the three extracts

Different semisolid bases were selected based on optimisation studies having desirable characteristics. The following bases [Formulation I, II and III] were selected for the formulation of semisolid dosage forms (Table 1, 2 and 3). Extracts of 10% w/w concentration were incorporated in various bases. [16-19]

Table 1: Formulation I – Hydrocarbon Base

S. No.	Ingredients	Formula
1.	Extract of frontal leaves of *T. grandis*	5.00 g
2.	Extract of *M. indica* leaves	5.00 g
3.	Extract of *A. occidentale* leaves	5.00 g
4.	Stearic acid	7.50 g
5.	White wax	1.00 g
6.	White soft Paraffin	4.00 g
7.	Triethanolamine	0.40 g
8.	Methyl paraben	0.10 g
9.	Propyl paraben	0.05 g
10.	Propylene glycol	4.00 g
11.	Purified Water to	50.00 g

Table 2: Formulation II -Water Soluble Base

S. No.	Ingredients	Formula
1.	Extract of frontal leaves of *T. grandis*	5.00 g
2.	Extract of *M. indica* leaves	5.00 g
3.	Extract of *A. occidentale* leaves	5.00 g
4.	Polyethylene glycol 400	16.00 g
5.	Polyethylene glycol 4000	16.00 g
6.	Propylene glycol	7.25 g
7.	Purified water to	50.00 g

Table 3: Formulation III- Gel Base

S. No.	Ingredients	Formula
1.	Extract of frontal leaves of *T. grandis*	5.00 g
2.	Extract of *M. indica* leaves	5.00 g
3.	Extract of *A. occidentale* leaves	5.00 g
4.	Carbopol 934	2.00 g
5.	Propylene glycol	2.00 ml
6.	Ethanol	5.00 ml
7.	Triethanolamine q. s.	To neutralize the gel base
8.	Water to	50.00 g

Preparation of Formulations I and II: The oily and water soluble ointment bases were prepared by fusion method. In this method constituents of the base 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.

Preparation of Formulation III:

Carbopol 934, 2 g was soaked in water for a period of 2 hours and was then neutralized with triethanolamine (TEA) with stirring. 5 g of extracts were dissolved in 2 g of propylene glycol and 5 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 (6.8-7.0). During P^H adjustment, the mixture was stirred gently with a spatula until homogeneous gel was formed. [20-21]

Evaluation of prepared semisolid formulations [19-21]

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: The antibacterial activity of semisolid dosage forms were evaluated by Agar well diffusion method. 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. The ointment was dissolved in DMSO (200 mg/ml) and added into the agar wells and Gentamycin cream (Tinovate G) used as standard was also added into the wells and kept in the incubator at 37°C for overnight. The plates were incubated at 37°C for 24 hours and the antibacterial activity was checked. The experiments were run in duplicate and the zones of inhibition were determined and recorded.

RESULTS AND DISCUSSION

All the leaves were authenticated and extractions from leaves were carried out. Preliminary phytochemical screening of extracts were done and revealed the presence of alkaloids, glycosides, saponins, resins, tannins, phenols and flavonoids. Three different poly-herbal extracts were incorporated in three different types of semisolid bases and were evaluated for Color, Consistency, pH, Spreadability, and viscosity studies. The results of all formulated preparations were found acceptable (Table 4). The ointments prepared using hydrocarbon bases were found quite greasy and sticky in nature. The ointments prepared using water soluble bases were found to show more time of spread because of their stiffer consistency. The developed herbal gel was translucent in appearance and showed good homogeneity with absence of any lumps. The values of spread ability indicate that the gel is easily spreadable with a small amount of shear. Spread ability of formulated gel formulation was good as compared other formulations. Colour of gel was dark brown compare to ointments which are light brown in colour. All formulations pH lies between 6.30 to 6.90 and viscosity of formulations range from 12600 cps to 13400 cps at 50 rpm. Raw materials of formulation-I are hydrocarbons, this type of bases are being occlusive; increases skin hydration by reducing the rate of loss of surface water, bases of this kind used for skin moisturizing and may increases the drug activity and stability of dosage forms. Formulation II is made up of water soluble bases. This type of base has been selected to maximize drug availability. Major components are Polyethylene Glycols (PEGs), these compounds are innocuous and long term use confirmed their lack of irritation. They are relatively inert, non-volatile and water miscible. To get good consistency blend of high (PEG 4000) and low molecular weight (PEG 400) glycols used. Formulation III is aqueous gel vehicles containing water poly propylene glycol, and gelled with carbomer. Propylene glycol is used for its solvent properties as well as its preservative effect. [22]

Table 4: Evaluation Results of Formulations

Evaluation Parameters	I	II	III
Color	Light Brown	Light Brown	Dark Brown
Consistency	Homogenous, free from lumps	Homogenous, free from lumps	Homogenous, free from lumps
pH (1% w/v solution)	6.90	6.30	6.80
Viscosity (centipoises)	13400	13100	12600
Spread ability (gm.cm/ sec)	12	12	20
Extrudability study (g)	180	200	130

Table 5: Antimicrobial activities of Extracts in Different Bases comparison with Standard

S. No.	Formulation and its code	Minimum inhibitory Zone (mm)		
		S. aureus (G+)	*E. coli (G-)*	*P. aeruginosa (G-)*
1.	Gentamicin ointment (1% w/w)	26-29	24-27	25-29
2.	Formulation I	24-26	21-24	22-25
3.	Formulation II	24-27	24-28	23-27
4.	Formulation III	25-26	24-26	23-26

Preparations showed antimicrobial activity against both Gram positive (*S. aureus)* and Gram-negative (*E. coli*, *P. aeruginosa)* bacteria. The order of antibacterial activity was as follows: Water Soluble base> Gel Base > Hydrocarbon Base. The drug diffusion in water soluble base was better compared to other bases. The zones of inhibition of poly herbal formulations were in between 23-28 mm which can be comparable with standard formulation 24-29 mm (Table 5). The antibacterial activity could be due to different classes of compounds present in leaves extracts, such as alkaloids, flavonoids, phenols and tannins. [23-26] The antimicrobial activity of individual ethanol extracts of frontal leaves of *T.*

grandis, M. indica, and *A. Occidentale* were already investigated. In this study different plant extracts had different active constituents. Hydrophobic ointments are oleaginous, greasy and are inconvenient to patients and also medicated powders for topical application which have short residence time on the skin. The use of semisolid formulations can increase the residence time of drugs on the skin and consequently enhance bioavailability. Gel delivery systems have several advantages such as the ease of administration, non greasy, patient compliance, high residence time on the skin and better drug release.

Poly-herbal ointments prepared by incorporating ethanolic extracts of leaves were effective can be used as antibacterial agent for the treatment of wounds and burns. Water soluble bases and gel bases are better vehicle for the release of extracts. Remaining parameters like drug diffusion, drug content, skin irritation test, and wound healing activity shall be carried out to know the effectiveness of the preparations.

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