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FORMULATION AND EVALUATION OF EUPATORIUM ODORATUM HERBAL OINTMENT

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ABSTRACT
The enormous surface area and permeability of the skin play a significant influence in the distribution of drugs through it. The medications like ointments, creams are used for the treatment and caring of the skin. The aim present research study is to prepare the wound healing ointment and to evaluate the medicinal property of the Eupatorium odoratum (EO) Ointment. Eupatorium odoratum is rapidly growing natural herb which have the wound healing property is used in this study for the preparation of the herbal ointment. The extract collected form Soxhlet extractor was used for the analysis of phytochemicals. EO ointment is prepared by adding coconut oil and honey wax. The prepared ointment is evaluated by pH, separability and antimicrobial activity. The positive result of Tannins and Saponins supports the wound healing property of the sample and the presence of Diterpenes supports the antimicrobial property of the plant leaves. The ointments had a good appealing appearance with green color and smooth texture, and they were all homogenous with no signs of phase separation. The pH and spreadability were found to be 6.90±0.172 and 109.20±3.17 respectively. Antimicrobial activity of the EO ointment in different assay organisms and the inhibition of EO ointments is compared with the standard positive control. The result showed that EO ointments has the good antimicrobial properties. This study shows that EO has high potential as antimicrobial agent when formulated as wound healing ointment.

KEYWORDS: Ointment, Eupatorium odoratum, Phytochemical analysis, antimicrobial activity, wound healing.

INTRODUCTION
The delivery of drug through the skin plays major role because of the large surface area and permeability conditions.[1] Ointments are viscous, unctuous, semisolid preparations containing either dissolved or suspended functional ingredients.[2] Majority of ointments consist of a base, which mainly acts as a carrier or vehicle for the medicaments. Waxes such as white wax, carnauba wax, beeswax, and Candelilla wax are the natural waxes commonly used in cosmetic and pharmaceutical products.[3] Herbal ointments are natural without any adverse effect to the skin.[4] Eupatorium odoratum (EO) is a fast-growing herb. It is a multi-stemmed shrub about 2.5 m tall.[5] In Sanskrit it is Ropani, Seekhrasarpi, in English called as Eupatorium, Eupatorium odoratum, natural floss herb and Bug, Dhoka, Tivra Gandha in Hindi.[6] Stems reach 2 cm in diameter. The plant is maintained by a system of numerous thin yellowish lateral roots. Several shoots develop from the leading of the root and the lower stem. Individual branches are long, with relatively few branches. When the leaves are crushed, they give off a sweet scent.[7] It belongs to the following families: Asterale, genus: Cro morena, Kingdom: Plantae, scientific name: Eupatorium odoratum/Chromolaena odorata.[8] The aim present research study is to prepare the wound healing Ointment and evaluate the medicinal property of the Eupatorium odoratum Ointments.

MATERIALS AND METHODS
Materials
Eupatorium odoratum leaves, Honey wax and coconut oil obtained from local area.

Methods
Soxhlet Extraction
The thimble is filled with the sample. Typically, a thick filter paper thimble produced from a solid material containing some of the target component is fed into the Soxhlet extractor's main chamber. The extraction solvent methyl alcohol is taken into a distillation flask and the Soxhlet extractor is now placed onto this flask. The Soxhlet extractor is then equipped with a condenser. The solvent is reflux-heated. The solvent vapour travels up through a distillation arm, and floods into the chamber

housing the thimble of solid. Any solvent vapour is made to cool and drip back down into the chamber containing the solid substance by the condenser.

Warm solvent is gradually poured into the chamber containing the solid substance. The chosen component will then partially dissolve in the heated solvent at that point. A side arm siphon automatically empties the Soxhlet chamber when it is almost full, and the solvent flows back down to the distillation flask. The thimble makes sure that no solids are transported to the still pot by the solvent's rapid velocity. Over the course of a day, this cycle may be allowed to repeat numerous times. A fraction of the non-volatile component dissolves in the solvent throughout each cycle. The target component is concentrated in the distillation flask after numerous cycles. The benefit of this method is that only one batch of warm solvent is recycled, as opposed to numerous portions being passed through the sample.

The extracted component is obtained after the solvent has been evaporated, often using a rotary evaporator. The non-soluble portion of the extracted solid remains in the thimble, and is discarded.[9]

Phytochemical Analysis

Phytochemicals, or naturally occurring chemical substances in plants. Some are in charge of color and other organoleptic qualities, like the smell of garlic and the rich purple of blueberries. The term is generally used to refer to those chemicals that may have biological significance, for example antioxidants, but are not established as essential nutrients. The extract collected form Soxhlet extractor was used for the analysis of phytochemicals.[10]

Preparation of EO Ointment

Accurately weighed 100mg of Eupatorium odoratum extract collected. This extract is boiled by adding coconut oil till the water content evaporates. Filter the mixture and directly pour it to a melt of honey wax and allow it to cool. Perfume (Rose oil) is added for good odour.[11]

Table I: Formulation of EO ointment.

Ingredients	Formula
Eupatorium odoratum extract	100
coconut oil (ml)	50
honey wax (mg)	10
Water	QS

Evaluation of EO Ointment

Organoleptic characteristics

Physical appearance, color, texture, phase separation, and uniformity of the formulation were all examined. Visual observation was used to assess these qualities. Homogeneity and texture were tested by pressing a small quantity of the formulated ointment between the thumb and index finger. The consistency of the formulations was used to evaluate the texture and homogeneity of the formulations. Immediate skin feel was also evaluated.[12]

pH

About 2.0 g of formulation was taken in dry beaker and 50 ml of water was added. The pH of ointments determined using a pH meter EQ-610 by Equip-Tronics. The determinations were carried out in three trials and the averages of three readings were noted.[13]

Spreadability

Spreadability of the formulation was determined by an apparatus suggested by Multimer which is modified according to usage. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. Weighed Ointment was added and the time required to separate the two slides was noted.

$S = M.L/T$ was used to calculate spreadability.

Where M is the weight fastened to the higher slide, L is the length of the glass slides, and T is the time it takes to separate the slides.[14]

Antibacterial activities

The antibacterial activities of the Ointments were determined using agar Well diffusion techniques. Preparation of the test organisms: Standard bacterial organisms from the ATCC were obtained from the Department of microbiology, Srinivas college of pharmacy, Mangalore. The strains employed were P. aeruginosa (ATCC 27853), E. coli (ATCC 25922), and Staphylococcus aureus (ATCC 25923). The organisms were first isolated on nutrient broth for 24 hrs. and then diluted to 1:1000 with the sterile nutrient dextrose broth. The dilutions formed were used as bacterial stock solutions for the agar-well diffusion assays.

Preparation of media (Direct sensitivity testing): Mueller Hinton agar was used. The media was prepared by adding 3.80 g of agar powder to 100 ml of distilled water and the mixture was boiled. The solution was autoclaved at 121°C for 15 min and cooled to 50°C in a water bath. It was then transferred into sterile plates. It was allowed to cool and solidify under sterile conditions, and then incubated for 24 hrs. at 37°C to ensure that there was no bacterial contamination. A sterile borer was used to create wells in the solidified agar that were 10 mm in diameter and 5 mm deep.

Agar-well diffusion assay: Cultures of E. coli, S. aureus and P. aeruginosa were inoculated. About 10, 20, 30 mg/ml of the test extracts and 100 µg/ml of Neomycin sulphate USP (positive control) were dispensed into the wells. For 24 hours, the plates were incubated at 37°C. The sensitivity of the test organisms to the extracts were determined by measuring the diameters of the zone of inhibition surrounding the wells.[15]

Stability testing

The developed ointment formulation was subjected to stability study as per the International Conference on Harmonization (ICH) guidelines. The formulated ointment was filled in the collapsible tubes and stored at different temperatures and humidity conditions for a period of 3 months and studied for appearance, pH and spreadability.[16]

RESULTS AND DISCUSSION

Phytochemical analysis

The positive result of Tannins and Saponins supports the wound healing property of the sample. The presence of Diterpenes supports the antimicrobial property of the plant leaves. This shows the medicinal property of the EO leaves.

Table II: Phyto-constituents of Eupatorium odoratum.

Chemical constituents	Tests	Alcoholic
Carbohydrates	1. Molisch's test	+
	2. Benedict's test	+
	3. Fehling's test	+
	4.Barfoed's test	+
Glycosides	1. Brontrager's test	+
	2. Legal test	+
Saponins	1. Foam test	+
	2.Froth test	+
Resins	1.Acetone water test	+
Phenols	1.Ferric chloride test	+
Tannins	1.Alkaline reagent test	+
	2. Gelatin test	+
Diterpenes	1.Copper acetate Test	+

Organoleptic characteristics

The organoleptic properties, including physical appearance, color, texture, phase separation, homogeneity, and immediate skin feel of the ointment formulation was analyzed. Results showed that the ointments had a good appealing appearance with green color and smooth texture, and they were all homogenous with no signs of phase separation.

pH

pH of all formulations was found to be between 6.90±0.172 that is within the range. The pH of formulation lies in the normal pH range of the skin.

Spreadability

The spreadability of formulation was determined and it was observed that formulation has better spreadability (109.20±3.17) as compared prototype formulations IP. By increasing the quantity of wax the density of the Ointments will be increased.

Antimicrobial activity

Antimicrobial activity of the EO ointment in different assay organisms are shown in table III. The inhibition zone diameter was produced by the EO ointment in bacteria like Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The inhibition of EO ointments is compared with the standard positive control. The result showed that EO ointments has the good antimicrobial properties.

Table III: Diameters of inhibition zone.

Assay organisms	Mean inhibition zone diameter (mm) produced by Ointments			Neomycin sulphate (positive control)
	10 mg/ml	20 mg/ml	30 mg/ml	100 (µg/ml)
Staphylococcus aureus	14	16	17	18
Escherichia coli	-	11	12	14
Pseudomonas aeruginosa	14	16	18	20

Stability testing

All the ointment formulations were subjected to stability study as per ICH guidelines and results are shown in Table IV. During the stability studies, the appearance of formulations was unchanged and no significant variation in pH and spreadability of formulation for the period of 3 months.

Table IV: Stability study.

Sl. No.	Observation	Before stability testing (mean ± SD)	After stability testing (mean ± SD)	
			1 month	3 months
1	pH	6.90±0.172	6.90±0.198	7.01±0.136
2	Spreadability	109.20±3.17	109.10±3.06	108.78±3.53

CONCLUSION

For the production of herbal extracts with a high yield, the Soxhlet extraction method was used. Phytochemical analysis was carried out and the positive result of Tannins and Saponins supports the wound healing property of the sample. The formulated ointment showed acceptable physical properties, and hence, were compatible with the skin. EO ointment shows satisfactory results for spreadability and pH. It shows more zone of inhibition against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa compared to standard. This study shows that EO has high potential as antimicrobial agent and good wound healing property when formulated as ointment.

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