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Research Article

FORMULATION AND EVALUATION OF ANTIMICROBIAL OINTMENTS FROM *EUPATORIUM GLANDULOSUM* HORT. EX KUNTH

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ABSTRACT

Objective: The present study was undertaken to evaluate the antimicrobial activity of an ointment prepared from alcohol, ethyl acetate, and chloroform extracts of *Eupatorium glandulosum* Hort. ex Kunth.

Methods: The dried plant materials of *E. glandulosum* were individually extracted using alcohol, ethyl acetate, and chloroform by Soxhlet method. These three different types of extracts were used for the preparation of ointment and evaluated for the phytochemicals, pH, homogeneity, spreadability, tube extrudability, stability, and antimicrobial activity. The activity of prepared ointments was evaluated against *Escherichia coli* and *Bacillus subtilis* and the *in vitro* antimicrobial activity of ointments of plant extracts was performed by cup plate methods. The most effective antimicrobial ointment was determined by comparing the results of the zone of inhibition of all the ointments of each solvent extract.

Results: The antimicrobial activity of optimized formulation was showed significant activity against the tested bacterial pathogens.

Conclusion: The present study confirmed the antimicrobial activity of the formulations. Furthermore, it can use as a potential dosage form for clinical utility. The results are the justification for the use of the plant in folk medicine.

Keywords: *Eupatorium glandulosum*, Herbal ointment, Cup plate method, Stability.

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INTRODUCTION

The bacterial infections mainly occur with the injuries or ulcers or after the surgical treatments. Antibacterial substances help to inhibit or kill bacterial cells [1]. Infectious diseases are an increasingly greater extent in recent years. Furthermore, antibiotic-resistant becomes a major therapeutic problem [2]. The infectious diseases killing 50,000 people every day, and now, this is a world-leading cause for premature death [3]. Natural products of higher plants may possess a new source of an antimicrobial agent with a possible novel mechanism of action [4,5]. The researchers reported the antimicrobial activity of many plants and secondary metabolites of plants such as alkaloids, tannins, terpenoids, and flavonoids mainly responsible for these activities [6].

Medicinal plants used for the antimicrobial treatment started in ancient times. Several medicinal plant extracts and its phytochemicals have shown activity against all types of microorganisms including both Gram-negative and Gram-positive bacteria [7].

Ointments are semisolid preparation. They may or may not be containing medication used for the external application. Medicated ointments are intended to be applied externally in the body or to the mucous membrane. Non-medicated ointments commonly used as a base for the preparation of medicated ointments or used as such for lubricating or emollient effects. Plant drugs can also be formulated in the form of ointment [8,9]. The effective ratio of active ingredients incorporating with ointment base by trituration and after completion of the formulation, the ointment quality is assessing in terms of diffusion, irritancy, stability, and spreadability [10].

Eupatorium glandulosum popularly known as cat weed, Nilgiri weed, goat weed, or Mexican devil belongs to the family Asteraceae. *E. glandulosum* grows up to 1–3 m in height and it as an erect herb or occasionally it may be a shrub [11]. A simple, opposite, glabrous, decussate, and deltoid-ovate-shaped leaves with purple underneath and it grows 10 cm in length. Flowers are white [12]. Tribal people of Nilgiris used the leaves paste to treat cuts and wounds [13].

E. glandulosum is rarely used for the studies and till now the plant not yet explored, consider the medicinal properties, it can be well used for commercial product.

METHODS

The aerial parts of *E. glandulosum* Hort. ex Kunth were freshly collected from Ooty (Nilgiri hills), Tamil Nadu, India, in August 2017. The plant was identified and authenticated by Dr. M N Naganandini, Department of Pharmacognosy, JSS College of Pharmacy, Mysuru, India. Further, the plant material was washed under tap water and dried under shade, coarsely powdered using a mechanical grinder, then passed through 40 meshes and stored in a well airtight container till further use.

Method of extraction

The coarsely powdered *E. glandulosum* was extracted separately with alcohol, ethyl acetate, and chloroform using Soxhlet apparatus. The extract was collected, concentrated under reduced pressure, stored in an airtight container, and kept in desiccator for further use.

Preparation of herbal ointment

Specific quantities of all the ingredients such as plant extracts, stearic acid, white wax, yellow paraffin, triethanolamine, methylparaben, propylparaben, and water were taken for the preparation. The bases were melted together at 70°C in a basal. After that, all the ingredients were stirred gently and maintained the same temperature for a certain period, then cooled with continuous stirring. Different proportions of extracts were incorporated into the base by trituration using mortar

and pestle. The prepared ointments filled into the tube and stored at room temperature [14-16]. The formulation from different solvent extracts of *E. glandulosum* is tabulated in Tables 1-3.

Evaluation of ointments

Organoleptic parameters such as color, odor, and texture were inspected through visual inspection.

Determination of pH

Pre-calibrated digital pH used for the determination of pH of ointments. One gram of ointment dissolved in distilled water (100 ml) and keeps it aside for 2 h. Standardization of the pH meter done with possible buffer solutions. Measurement of each sample solution done triplicate. The average values calculated and noted [17-19].

Spreadability

The spreadability of formulations determined using apparatus consists of a wooden block having a pulley at one end with a fixed glass slide on the block. Around 3 g of ointment placed on the ground plate and in between these plates and another glass plate having the same dimension of the fixed ground plate, the ointment sandwiched. On the top of these two plates, 1 kg weight placed for 5 min to expel air and to provide uniform of the ointment between the plates. The excess of ointments scrapped off from the edges. Two hundred and forty grams pull off with top plate and time required by the top plate to cover a distance of 10 cm noted. The spread capacity expressed in terms of times in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of a certain load [17,20-22]. The spreadability determined using the equation,

$$S=m\times l\div t$$

Where,

S – Spreadability

m – Weight tied to the upper slide

l – Length of glass slides

t – Time is taken to separate the slide.

Separately measured the spreadability of each ointment and repeat 3 times for getting exact values and the average was taken as a consideration.

Viscosity

The viscosity of the formulation was done using Brookfield viscometer. The test sample is taken into a dry and clean 250 ml of the beaker. The viscosity of the sample determined at 25°C using viscometer. The viscosity measured in cps [18,23].

Homogeneity

Based on their appearance, all the developed ointments were tested for homogeneity by visual inspection [18].

Tube extrudability

It is a common test to obtain the force required to extrude the substance from the tube. The method applied for finding the applied shear in the region of rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow. In the present study, the method used for evaluating ointment formulation for extrudability based on the quantity in percentage ointment and extruded the ointment from the tube on a single application of pressure. More quantity of ointment extruded considered a better extrudability. The clean lacquered aluminum collapsible tube used to fill the ointment and the tube has a nozzle tip of 5 mm opening and applied the pressure on the tube with the help of a finger. It depends on the amount of ointment extruded from the tip when an external pressure applied on the tube is considered for the determination of the tube extrudability [17,18].

Antimicrobial studies

Bacillus subtilis and *Escherichia coli* used for the determination of the antimicrobial activity of various ointment formulations and standard cup plate methods used for the study. The nutrient agar solution poured into the previously sterilized Petri dishes up to 5 mm thickness. Each microorganism added to Petri dishes with a sterilized loop and plates were allowed to solidify for 5 min. On each Petri dish, five perforations made with a metal tube with a 4 mm diameter to receive test materials. The standard (neomycin sulfate ointment) and test materials (prepared formulations) added immediately into the wells and kept for incubation at 37°C for 24 h to allow the microorganism to grow and reagents to diffuse through the culture medium. At the end of the incubation, the zone diameter measured with the help of a zone reader [17,24,25]. All the experiments repeated 3 times to get accurate results.

Stability studies

In different temperature conditions, the stability studies carried out for all formulations (4°C, 25°C, and 37°C) for 3 months. All the evaluation parameters viscosity, pH, consistency, spreadability, and phase

Table 1: Composition of ethyl acetate extracts ointment

Ingredients	FEA1	FEA2	FEA3	FEA4	FEA5
Drugs (g)	1.5	2.0	2.5	4.0	5.0
Stearic acid (g)	8	8	8	8	8
White wax (g)	4	4	4	4	4
Yellow paraffin (g)	16	16	16	16	16
Triethanolamine (g)	1	1	1	1	1
Methyl paraben (g)	0.2	0.2	0.2	0.2	0.2
Propyl paraben (g)	0.1	0.1	0.1	0.1	0.1
Water (ml)	qs	qs	qs	qs	qs

FEA: Formulation of ethyl acetate

Table 2: Composition of alcoholic extracts ointment

Ingredients	FA1	FA2	FA3	FA4	FA5
Drugs (g)	1.5	2.0	2.5	4.0	5.0
Stearic acid (g)	8	8	8	8	8
White wax (g)	4	4	4	4	4
Yellow paraffin (g)	16	16	16	16	16
Triethanolamine (g)	1	1	1	1	1
Methyl paraben (g)	0.2	0.2	0.2	0.2	0.2
Propyl paraben (g)	0.1	0.1	0.1	0.1	0.1
Water (ml)	qs	qs	qs	qs	qs

FA: Formulation of alcohol

Table 3: Composition of chloroform extracts ointment

Ingredients	FC1	FC2	FC3	FC4	FC5
Drugs (g)	1.5	2.0	2.5	4.0	5.0
Stearic acid (g)	8	8	8	8	8
White wax (g)	4	4	4	4	4
Yellow paraffin (g)	16	16	16	16	16
Triethanolamine (g)	1	1	1	1	1
Methyl paraben (g)	0.2	0.2	0.2	0.2	0.2
Propyl paraben (g)	0.1	0.1	0.1	0.1	0.1
Water (ml)	qs	qs	qs	qs	qs

FC: Formulation of chloroform

Table 4: Percentage yield of extracts

Type of extract	Alcoholic (%) w/w	Ethyl acetate (%) w/w	Chloroform (%) w/w
Yield of extract	19.32	10.85	7.24

Table 5: Evaluation of ointments

Formulations	Appearance	Homogeneity	Spreadability in 1 min (g)+SD*	Viscosity (cps)+SD*	pH+SD*	Tube extrudability (g)=SD*
FEA1	Greenish-brown	Good	4.4±0.28	5898	6.10±0.55	0.69±0.35
FEA2	Greenish-brown	Good	4.7±0.31	6200	6.65±0.45	1.4±0.28
FEA3	Brown	Good	5.0±0.19	5725	6.83±0.56	1.30±0.32
FEA4	Dark brown	Good	5.1±0.58	6133	7.10±0.49	0.85±0.19
FEA5	Dark brown	Good	4.8±0.26	5914	6.96±0.42	0.91±0.61
FA1	Greenish-brown	Good	4.2±0.41	6157	6.26±0.41	1.70±0.51
FA2	Greenish-brown	Good	4.9±0.61	5725	6.44±0.21	0.74±0.67
FA3	Greenish-brown	Good	4.5±0.28	5689	6.67±0.86	0.82±0.42
FA4	Brown	Good	4.8±0.54	6183	7.00±0.62	0.64±0.12
FA5	Dark brown	Good	5.0±0.18	6458	6.92±0.35	0.78±0.57
FC1	Greenish-brown	Good	4.2±0.62	5421	6.59±0.33	1.20±0.35
FC2	Greenish-brown	Good	4.9±0.34	5800	6.62±0.54	0.69±0.24
FC3	Brown	Good	4.4±0.32	6400	6.70±0.23	0.79±0.19
FC4	Brown	Good	5.1±0.33	5715	6.79±0.29	0.98±0.35
FC5	Dark brown	Good	5.0±0.21	6521	6.96±0.48	1.10±0.54

SD*: Standard deviation*, n=3

Table 6: Antimicrobial study

S. no.	Formulations	Zone of inhibition for *Escherichia coli* (mm) SD*	Zone of inhibition for *Bacillus subtilis* (mm) SD*
1.	FEA1	12.60±0.18	10.20±0.41
2.	FEA2	14.70±0.15	18.50±0.52
3.	FEA3	12.00±0.17	11.50±0.24
4.	FEA4	13.80±0.12	19.80±0.17
5.	FEA5	18.50±0.17	16.50±0.15
6.	FA1	19.31±0.12	19.25±0.21
7.	FA2	20.54±0.23	17.19±0.16
8.	FA3	18.15±0.15	20.40±0.17
9.	FA4	21.20±0.17	23.20±0.11
10.	FA5	17.80±0.14	24.90±0.23
11.	FC1	09.80±0.16	07.20±0.22
12.	FC2	11.06±0.17	08.00±0.15
13.	FC3	10.24±0.22	08.21±0.13
14.	FC4	12.60±0.25	11.10±0.20
15.	FC5	14.70±0.55	11.50±0.14

SD*: Standard deviation*, n=3

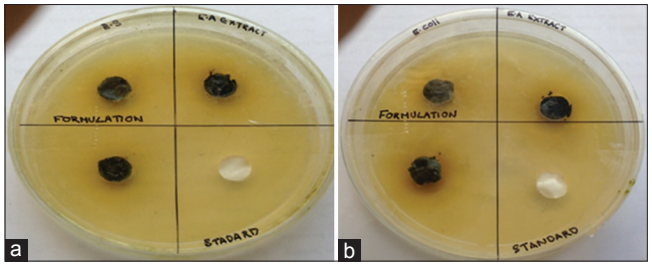

Fig. 1: Zone of inhibition shown by the ethyl acetate extract formulations for *Escherichia coli* (a) and *Bacillus subtilis* (b)

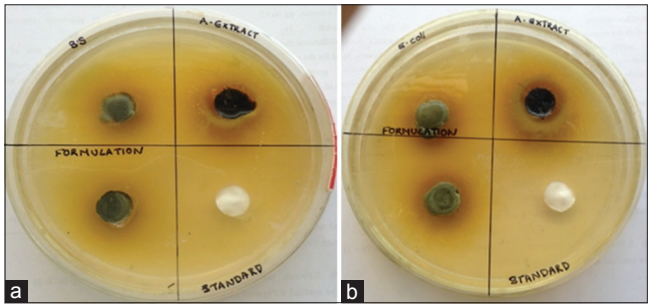

Fig. 2: Zone of inhibition shown by the alcohol extract formulations for *Escherichia coli* (a) and *Bacillus subtilis* (b)

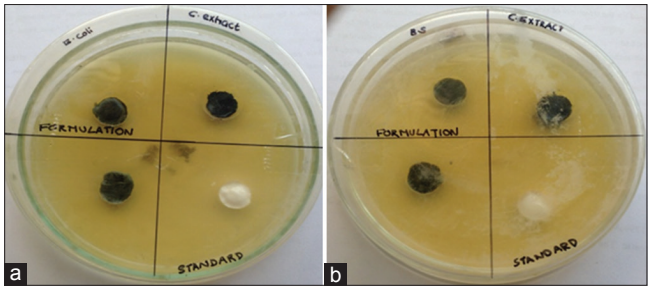

Fig. 3: Zone of inhibition shown by the chloroform extract formulations for *Escherichia coli* (a) and *Bacillus subtilis* (b)

separation studied at different time intervals such as 15, 30, 60, and 90th days [22-27].

RESULTS AND DISCUSSION

Plant material extraction

The yield of alcohol, ethyl acetate, and chloroform extract of aerial parts of *E. glandulosum* was found to be 19.32% w/w, 10.85% w/w, and 7.24% w/w, respectively, as shown in Table 4.

Formulation of *E. glandulosum* ointments

The ointment from *E. glandulosum* prepared using the fusion method. The prepared ointment filled into the tube and stored at room temperature.

Table 7: Stability studies of ointments (FEA4, FA4, and FC5)

Physicochemical parameters	Formulations		
	FEA4	FA4	FC5
Color	Dark brown	Brown	Dark brown
Odor	Characteristic	Characteristic	Characteristic
Spreadability + SD*	5.1±0.51	4.8±0.55	5.0±0.23
pH + SD*	7.10±0.24	7.00±0.62	6.96±0.46
Storage (4°C, 24°C,37ºC)	Stable	Stable	Stable

SD*: Standard deviation*

Evaluation of *E. glandulosum* ointments

The evaluation of ointments such as organoleptic parameters, homogeneity, spreadability, viscosity, pH, and tube extrudability results is shown in Table 5.

Antimicrobial study

Prepared formulations have shown good activity against both *B. subtilis* and *E. coli* and it was found that the formulation of ethyl acetate-4, formulation of alcohol-4, and formulation of chloroform-5 (FEA4, FA4, and FC5) were showing more zone of inhibition comparing to all other formulations. The result of antimicrobial studies shown in Table 6 and the antimicrobial activity of all the extracts reported in Figs. 1-3.

Stability studies

The optimized formulations stability studies carried out at a different temperature and there is no marked change in the appearance of prepared ointments. The pH remains the same as the original pH of the ointments. The product is stable in the base of the ointment. The results are shown in Table 7.

Evaluations of the prepared ointments showed good results in spreadability, homogeneity, viscosity, tube extrudability, and antimicrobial activity and stable at different temperatures (4°C, 25°C, and 37°C). Based on these evaluation parameters and antimicrobial activity, it was found that the optimized formulations of FEA4, FA4, and FC5 were showing good results. When compared to these three formulations, FA4 showing more antimicrobial activity than the other two.

CONCLUSION

The study is concluded as the optimized formulations of different solvent extracts of *E. glandulosum* are efficient antimicrobial formulations and might be useful for related disorders. Further, isolation is required for the researchers to establish potential activities.

AUTHORS' CONTRIBUTIONS

We declare that this work was done by the authors named in this article Miss Silpa performed the experiment and collected the data; Hamsalakshmi helps in writing and designing the manuscript. Dr. J Suresh proofread the whole manuscript and suggested the necessary changes.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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