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FORMULATION, PHYTOCHEMICAL SCREENING AND PHYSICO-CHEMICAL EVALUATION OF AN ANTI SEPTIC OINTMENT CONTAINING *AZADIRACTA INDICA* AND *CHROMOLENA ODORATA*

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ABSTRACT

The present study was designed to formulate and evaluate polyherbal ointment with antiseptic activity. Ointments were formulated using methanolic extracts of Azadirachta indica and Chromolaena odorata and were evaluated for its physico-chemical property and antibacterial activity. In the present study, extraction and the phytochemical screening was done using methanol as the solvent. Phytochemical screening confirmed the presence of various phytoconstituent like carbohydrate, glycosides, flavanoids and tannins. The formulation was evaluated for various physico-chemical studies such as colour and odour, pH, spreadability, extrudability, diffusion studies, microbiological study and stability analysis. The physico-chemical parameters are within the acceptable range. The anti-bacterial activity of the prepared ointment was compared with betadine (marketed formulation) using E. coli. Compared to betadine, formulations F2 and F3 showed greater activity on E. coli spp. The release studies confirmed that at 140 minutes, released 90 % of the drug. The stability study confirmed that the formulation was stable. The results concluded that the prepared formulation containing Azadiracta indica and Chromoleana odorata is an effective antiseptic ointment with acceptable characteristics.

KEY WORDS

Azadirachta indica, Chromoleana odorata, spreadability, extrudability, Ointments, antibacterial activity.

1. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years [1]. Various medicinal plants have been used for years in daily life to treat disease all the world [2]. Herbal medicine is making dramatic comeback and increasing number of patients are visiting alternative medicine clinics [3]. Side effects of synthetic medicine are alarming and recent time has seen risk of herbal and herbal-synthetic drug interactions [4].Traditional medicines if used judiciously can save a lot of time spent in the treatment and thus reducing global burden [5].

Uses of plants and traditional practices will continue to play a significant role in the socio-cultural life of village communities [6]. The herbal drugs have more precise action and have no side effects and are economic [5]. Herbal medicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes [7]. Herbal medicine, also called botanical medicine or phyto-medicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes [8]. Long practiced outside of conventional medicine, herbalism is becoming more main stream as up-to-date analysis and research show their value in the treatment and prevention of disease [9]. Plants had been used for medicinal purposes long before recorded history [10].

2. MATERIALS AND METHODS

2.1 Collection of Plants and Extraction

Plants of *Azadirachta indica* and *Chromolaena odorata* were collected from Wayanad district Kerala, India, in the month of May 2014. The plant parts were washed under running tap water, and the leaves were cut into small pieces of 2-3 cm and shade dried (300^{o}C, 50 ± 5% relative humidity) for 15days. The shade dried leaves were powdered using a dry grinder to get the coarse powder (sieve no. 22/8). The powder was stored in air tight container for further use.

The powder of *Azadirachta indica* and *Chromolaena odorata* were subjected to solvent extraction. 480 g of dried powder was taken in the ratio of 1:2 water bath temperature was maintained about 40-45°C. The extraction was carried out by successive method, using methanol. The drug powder and methanol was heated on a water bath for 8 hours and the filtrate obtained was filtered and it was subjected for rotavapour apparatus. The rotations of rotary flash evaporator were 50-60 rpm [11]. The same procedure was repeated for the other two solvents.The extracts obtained were heated to obtain the concentrated extract.

2.2 Phytochemical analysis

The methanolic extract obtained after extraction procedure was subjected to various phytochemical screening as per the standard procedure to reveals the presence of various active phyto-constituents [12].

2.3 Formulation of emulsifying ointment base

Required quantities of emulsifying wax, liquid paraffin and white soft paraffin were weighed and melted. To this, adequate quantities of methanolic extract of the plants were added and using fusion method, formulated herbal ointment obtained. The compositions of different polyherbal ointment are listed in **Table 1**.

Table. 1: The compositions of different polyherbal ointment

Sl. No	INGREDIENTS	QUANTITY
1.	Emulsifying wax	300 g
2.	White soft paraffin	500 g
3.	Liquid paraffin	200 g

2.4 Preliminary and physico-chemical evaluations

The following preliminary evaluations and physico-chemical parameters were carried out for the formulation.

Colour and odour: Visual examination was used to study the colour and odour of the prepared ointment.

Determination of pH: The pH value of a solution was determined by digital pH meter (Mettler Toledo).

Spreadability: Spreadability of the formulation was determined by an apparatus has been designed by Multimer. It consists of a wooden block, which was provided by a pulley at one end. A rectangular ground glass plate was fixed on this block. An excess of ointment under study was placed on this ground plate. The ointment was then sandwiched between this plate and another glass plate having the dimension of fixed ground plate and provided with the hook. Excess of the ointment was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. With the help of string attached to the hook and the time (in seconds) required by the top plate to cover a distance of 10 cm be noted. A shorter interval indicates better Spreadability.

Spreadability is measured as,

$$S = M \times L / T$$

M= weight tide to upper slide

L= length of glass slides

T= Time

Extrudability: The prepared formulations were filled in the collapsible tubes. The extrudability of the different ointment formulations was determined in terms of weight in grams required to extrude a 0.5 cm of ribbon of ointment in 10 second.

2.5 Microbiological studies

The antibacterial activity of various gels formulations evaluated against the strain of *Escherichia coli* microorganism by standard cup plate method and the inhibition zone diameters were measured with the help of zone reader. *Escherichia coli* were used for testing of antibacterial activity. Nutrient agar media was used for bacterial culture and incubated at temperature 37°C±2°C for 48 hrs. The minimum inhibitory concentration (MIC) of ointment was examined using *Escherichia coli spp* at low cell density and high cell density. The experiments were performed in 12 well microtiter plates (in duplicate with three technical replicates). Varying concentrations of prepared formulations were used. After 24 hours growth was measured. Determined MIC was at the lowest test concentration needed to ensure that the culture did not grow over 10% of the relative cell density.

2.6 Diffusion studies

The phosphate buffer with a pH of 6.8 was used for the ex-vivo drug release studies as a receptor medium. The formulated ointment was accurately weighed and placed in the donor part of the Franz diffusion cell and a semi permeable cellulose membrane with 1000 MW cut off. The cell body of diffusion apparatus was filled with degassed phosphate buffer, pH 6.8. The receptor phase was stirred thoroughly. Intervals of 20, 40, 60, 80, 100 and 150 min were chosen for sampling time, and

samples were analyzed for drug content spectrophotometrically at 258 nm. In spectroscopic assay, the amount of the absorbed drug is determined [13].

2.7 Stability studies

The stability study was carried out for the most satisfactory formulation. The most satisfactory formulation was sealed in a glass vial and kept at 4 ± 2°C and 25 ± 2°C at RH 65 ± 5 and 37 ± 5 RH for 2 months. At the end of 1 and 2 months, the samples were analyzed for the drug content and in vitro diffusion study.

3. RESULTS

Many literatures revealed that the selected two herbs *Azadirachta indica* and *Chromolaena odorata* have antioxidant and antibacterial activity. In the present study, polyherbal ointments were prepared by fusion method using emulsifying ointment as the base. Extraction and the phytochemical screening was done using methanol as the solvent. Phytochemical screening confirmed the presence of various phytoconstituent like carbohydrate, glycosides, flavanoids and tannins. The formulation was evaluated for various physico-chemical studies such as colour and odour, pH, spreadability, extrudability, diffusion studies, microbiological study and stability analysis. The results of the formulations are mentioned in **Table 2**, **3**, **4** and **5**.

Table 2: Composition of the formulated ointment

Ingredients	F1 (2%)	F2 (4%)	F6 (6%)
Azadiracta indica Methanolic extract	2 gm	4 gm	6 gm
Chromolena odorata methanolic extract	2 gm	4 gm	6 gm
Emulsifying ointment	q.s to 100 gm	q.s to 100 gm	q.s to 100 gm

Table 3: Phytochemical analysis of extracts

Constituents	Name of test	Methanolic extract of *Azadiracta indica*	Methanolic extract of *Chromoleana odorata*
Carbohydrates and reducing sugars	Molisch's test	-	+
	Fehling's test	-	+
	Benedict's test	-	+
	Barfoed's test	-	-
Proteins	Million's test	-	-
	Biuret test	-	-
	Xanthoprotein test	-	-
	Legal's test	+	-
	Keller killiani test	+	-
Glycosides	Borntrager's test	+	-
	Modified Borntrager's test	+	+
Flavanoids	Shinoda test	+	+
	Lead acetate	+	+
	Sodium hydroxide	-	+
Tannins	5 % $Fecl_3$	+	-
	Lead acetate solution	-	-
	Bromine water	-	-
	Dilute Iodine solution	-	-

Table 4: Physico-chemical evaluations of the formulations

Physicochemical parameters	F1 (2%)	F2 (4%)	F3 (6%)
Colour	Dark green	Dark green	Dark green
Odour	Characteristic	Characteristic	Characteristic
PH	7.00	6.00	6.48
Spreadability (sec)	10	11	13
Extrudability (g)	170	185	187
Diffusion study (cm)	0.4	0.6	0.7
Stability (4^{o}C, 25^{o} C and 37^{o} C)	Stable	Stable	Stable

Table 5: Microbiological studies

Ointments	Zone diameter in cm (*E. coli spp*)
F1 (2%)	0.9
F2 (4%)	2.4
F3 (6%)	2.5
Standard	1.5

4. DISCUSSION

In the present study, *Azadiracta indica* and *Chromolena odorata* were selected for formulating antiseptic ointment. The ointment was prepared by fusion method using emulsifying ointment as base. The physico-chemical parameters are within the acceptable range. The anti-bacterial activity of the prepared ointment was compared with betadine (marketed formulation) using *E. coli*. Compared to betadine, formulations F2 and F3 showed greater activity on *E. coli*. So, the prepared ointment has potent anti-microbial activity against *E. coli spp*. The release studies confirmed that at 140 minutes, released 90 % of the drug. The stability study confirmed that the formulation was stable.

CONCLUSION

The results concluded that the prepared formulation containing *Azadiracta indica* and *Chromoleana odorata* is an effective antiseptic ointment with acceptable characteristics. Hence the study concludes that an efficient antiseptic ointment with anti-microbial activities can be formulated from the methanolic plant extracts of *Azadirachta indica* and *Chromolaena odorata*, which can also be used for wound healing and various skin infections. Further research may be possible in future in the areas of in-vivo studies and wound healing.

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