Research Article

Formulation and evaluation of a polyherbal ointment for treatment of acne

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Abstract

Objective: Plants represent principle means of therapy for various types of ailments. Investigations on medicinal plants as cosmetics have provided important advances for the therapeutic approach to several skin diseases. Certain herbal drugs are formulated in the form of ointment and are used topically for several purposes, e.g. as protectants, antiseptics, emollients, antipruritic, keratolytics and astringents. **Materials and Methods**: Literature revealed that the crude drugs Liquorice, Turmeric and *Kleinia grandiflora* have protective effect on skin and specifically used to treat acne. In the present work we formulated a polyherbal ointment in different proportions by levigation method using paraffin ointment as the base. The formulations were then evaluated for their physicochemical parameters and their antibacterial activity was compared with a standard skin care ointment Soframycin. **Results:** Physicochemical parameters like colour, odour and consistency were observed and the pH of all the formulations lies in the normal pH range of the human skin (6.5 ± 0.3) indicating better chemical compatibility of ointments with skin. The anti bacterial activity was evaluated using disc diffusion method for the plant extracts and formulated ointment separately and compared with a standard antibiotic, Amikacin and also with Soframycin ointment. **Conclusion:** The results of the physical evaluation of ointment prepared with the extracts of liquorice, turmeric and kleinia indicate the suitability of method for the production of ointments. All three formulations are stable for 60 days even after storing at 20^0C, 25^0C, and 30^0C. Among all the formulations F1, F2 and F3**, F2** showed equivalent activity with that of Soframycin.

Keywords: *Kleinia grandiflora*, turmeric, liquorice, poly herbal ointment, anti-acne

Introduction

Skin is perhaps the most vulnerable part of our body. It is a well known fact that day to day exposure of human skin leads to number of problems such as acne, pimples, pigmentation and sunburn marks (Shweta and Swarnlata, 2009). Acne is one of the most common skin diseases. The effected patients are prone to embarrassment, depression, anger, social withdrawal, anxiety, scorn and stigmatization (Thomas, 2004). The individual lesions of Acne vulgaris are divisible into three types: non-inflamed lesions and scars. Even though Acne vulgaris is the most common type of acne, other forms also exist like Acne conglobata, Acne fulminans, Acne excoriee, Acne mechanica, Acne rosacea (Bettoli et al., 2006). Factors that are responsible to cause acne are hormones, excess sebum, dead cells, bacterial acnes and inflammatory response. Functioning of these five factors is varying from people to people.

A number of people with sensitive skin don't want to use chemical based cosmetics due to concern about skin exposure to unknown chemicals. Now, however, researchers have claimed that cosmetics having herbal components are more suitable for hyper allergic skin because they are less irritant and more easily adjustable to skin (Chermahini et al., 2011). Patients feel more comfortable using topical therapies because they have milder side effects, are easier to use, are generally less expensive and are more readily available. Herbal drugs are formulated in the form of ointment and are used topically for several purposes, e.g. as protectants, antiseptics, emollients, antipruritic, keratolytics and astringents. Herbal cosmetics containing antioxidant properties will also result in good anti-ageing affects (Tengamnuay et al., 2008)

The present study was focused on formulation of a herbal ointment for treatment of acne and its evaluation. Three herbal drugs, *Kleinia grandiflora*, Liquorice and Turmeric

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have been chosen for the present work. These herbs were selected, on the basis of their traditional usage in treatment for different skin ailments.

Kleinia grandiflora (Family: Asteraceae), commonly known as "Common Fleshy Ragweed," has been used for the treatment of ailments, such as aperient and used externally for pimples. *Kleinia grandiflora* is a perennial succulent genus of Asteraceae-Senecioneae (Jeffery, 1992). It is an erect, perennial sub shrub 1–2.5 m high. Stems angled, succulent, 3–6 cm in diam., glabrous, bright deeply green, fleshy with prominent leaf scars. Leaves spiral, fleshy, usually congested at tips of new shoots, pallid milky green, glaucous; blade lanceolate, oblanceolate or obovate, 4–14 × 1.5–4 cm, subsucculent, sparsely pubescent or glabrous, base cuneate, margins entire, apex obtuse; petioles 2–4 cm long, exauriculate, glabrescent. Inflorescence scapose on short lateral or terminal shoots born on mature stems; scape stout, unbranched or branched, up to 15 cm long, 3–6-bracteate beneath capitulum, bracts linear, up to 5 mm long. The plant is grown in Sri Lanka, India, Nepal and Thailand (Pelser, 2007). Traditionally, the plant is named as Kundeluchevviaku (in Telugu). The fresh leaf juice is used to treat earache. However, no scientific data are available to validate the folklore claim. Therefore, this work was aimed at the scientific validation of the ethnopharmacological claim about anti acne activity of the leaf extracts of *Kleinia grandiflora*. In addition, the plant extract was also subjected to preliminary phytochemical analysis.

Glycyrrhiza glabra Linn, (Family: Leguminosae), commonly known as Liquorice, stolon and roots of this herb were used. The plant grows up to 2 m high found cultivated in Europe, Persia, and to little extent in some parts of India. The major constituents are triterpene saponins. Glycyrrhizin (glycyrrhizic acid, glycyrrhizinic acid) is the major component (2–9%); minor components occur in proportions that vary depending on the species and geographical location. Anti ulcer, Anti inflammatory, Hepatoprotective, Expectorant, Antitussive activities were reported with Liquorice (Wang et al., 2015)

Curcuma longa L (Family: Zingiberaceae) commonly known as Turmeric is prevalent in tropical and subtropical regions, mostly in India, South East Asia and China. India is the first producer, consumer and exporter of *Curcuma longa* in the world (Ching et al., 2014). The biological characteristic of turmeric is known to be attributed to curcuminoids which exists in dense structure of turmeric. Curcumin, a yellow crystalline polyphenol with low molecular weight, is extracted from rhizome of turmeric. Curcumin has wide range of applications as a dietary food ingredient, dying agent, therapeutic agent and medicament in different diseases. Curcumin has various pharmacological effects including antioxidant, antibacterial, anti-inflammatory, hepatoprotective, anti-tumor and anti-viral activities (Wanget al., 2013; Tajik et al., 2007).

Materials and methods

Collection of plant materials

The leaves of *Kleinia grandiflora* were collected from the medicinal garden of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences and authenticated by Dr. K. Ammani, Professor, Acharya Nagarjuna University, Guntur, Andhra Pradesh. Turmeric and Liquorice powders were commercially procured from yucca enterprises, Mumbai.

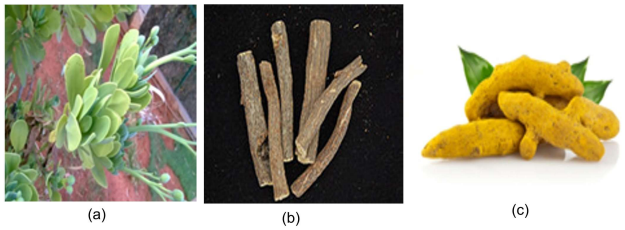

Figure 1. (a) *Kleinia grandiflora* (b) Liquorice roots (c) Turmeric rhizomes

Preparation of extracts of Kleinia, Liquorice and Turmeric

Kleinia grandiflora fresh leaves were collected and extracted with ethanol & water (70:30) as solvent by using maceration for 14 days. Then the extract was further evaporated using simple distillation apparatus to get the concentrate. The residue was stored in refrigerator.

Liquorice and Turmeric powders were dried and extracted with ethanol and water as solvents using soxhelation. The Soxhlet extraction, as the reference method, was performed as follows; 40g of liquorice powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with Ethanol & Water (70:30) as the extraction solvent. The extraction experiment was carried out at 60 °C within 4 h. Upon completion of the extraction, the solvent was evaporated from the extract using a rotary evaporator (Stuart RE300) under vacuum at 35 °C. The concentrated residue was weighed and stored in refrigerator. The same procedure was repeated with turmeric powder to get the extract.

Phytochemical screening of plant extracts

Various qualitative tests were performed for the detection of phytochemical constituents present in the hydroalcoholic extracts. Different tests were performed for the presence of carbohydrates, tannins, flavanoids, steroids, glycosides, alkaloids, saponins etc. (Kokate et al., 2005).

Evaluation of Antimicrobial activity of plant extracts

Anti microbial activity was evaluated using disc diffusion method with a standard antibiotic, 10 units per disc of Amikacin (Rajasree et al., 2012). The microorganisms used are *Staphylococcus epidermidis, Bacillus pumilus (*gram positive) and *Escherchia coli (*gram negative*)*. Nutrient agar medium was used as culture media

Preparation of nutrient agar medium

All the ingredients were weighed and dissolved in water by heating except agar. After all the ingredients were dissolved then finally agar was added and dissolved. Then the pH of the medium was determined by using pH meter. Then the medium was sterilised in autoclave at 15lbs/inch^2 for 20 minutes. The glassware like petriplates, pipettes, empty test tubes were sterilised by dry heat in an oven at a temperature of 160^0 C for 1 hour.

Antimicrobial activity

The hydro-alcoholic extracts of *Kleinia grandiflora*, was dissolved in distilled water in 3 concentrations of 50mg/ml, 100mg/ml, and 200mg/ml to study the antimicrobial activity. The test organisms are seeded into clean test tube containing sterile nutrient agar medium (20ml) for bacterial screening. 1µl of inoculums is mixed uniformly with 20µl of sterile melted nutrient agar and cooled to 48-50^{0}C and then poured into sterile petri dishes. When the agar solidifies, cellulose disc papers prepared by suitable disc cutter were placed on the surface of agar medium which are pre-impregnated in the extract solutions. Reference standard Amikacin was used at a dose of 10mg/ml in sterile water. The bacterial plates were incubated at 37^{0}C for 24hrs and diameters of zones of inhibition were determined as an indication of activity. The antimicrobial activity of Liquorice and Turmeric was evaluated using the same procedure. (Sohel et al., 2014)

Table 1. Composition of Ointment base

S.No.	Name of the Ingredients	Quantity to be taken
1.	Wool fat	0.5g
2.	Cetostearyl alcohol	0.5g
3.	Hard paraffin	0.5g
4.	Yellow soft paraffin	8.5g

Table 2. Formulation of Herbal Ointment

S.No.	Name of the Ingredients	Quantity to be taken		
1.	Kleinia grandiflora extract	0.5%	0.1%	0.2%
2.	Liquorice extract	0.5%	0.1%	0.2%
3.	Turmeric extract	0.5%	0.1%	0.2%
4.	Ointment base	10g	10g	10g

Preparation of herbal ointment

a) Initially ointment base was prepared by weighing hard paraffin and melting it in an evaporating dish on water bath. After melting of hard paraffin remaining ingredients Woolfat, Cetosteryl alcohol and soft paraffin were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.

b) Herbal ointment was prepared by mixing accurately weighed *Kleinia grandiflora*, Liquorice, and Turmeric extracts to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container (Carter et al., 1987).

Evaluation of Ointment

Colour and Odour: Physical parameters like colour and odour were examined by visual examination.

Consistency: Smoothness and consistency of the prepared ointment was observed.

pH: pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. pH was determined in triplicate for the solution and average value was calculated.

Spreadability: The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides indicates better spreadability. Spreadability was calculated by following formula. $S=M\times L/T$

Where,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

Extrudability: The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Diffusion study: The diffusion study was carried out by preparing agar nutrient medium. A hole was bored at the centre

of medium and ointment was placed in it. The time taken by ointment to get diffused through was noted (After 60 minutes).

Solubility: Miscible with alcohol, chloroform, hexane and insoluble in water, slightly soluble in boiling water.

Washability: Formulation was applied on the skin and then ease extent of washing with water was checked.

Stability study: Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2°C, 25°C and 30°C. The herbal ointment was found to be physically stable at different temperature i.e. 2°C, 25°C, 30°C within four weeks.

Antimicrobial activity of ointment

Sample was prepared by taking about 10mg of ointments (0.5%, 1%, 2%w/w) were weighed and dissolved in DMSO (dimethyl sulfoxide) and used for activity studies. All the ingredients of nutrient agar medium were weighed and dissolved in water by heating except agar. After all the ingredients were dissolved then finally add agar and dissolve it. Then the pH of the medium was determined by using pH meter. Then the medium was sterilised in autoclave at 15lbs/inch^2 for 20 minutes. The glassware like petriplates, pipettes, empty test tubes were sterilised by dry heat in an oven at a temperature of 160^0 C for 1 hour.

The bacterial culture was spread on the culture medium and a well was bored in the middle of the agar. Then different samples and standard solutions of 0.05ml was poured inside these wells and plates were incubated at 37°C overnight for observation. The presence of inhibition zone was observed and noted.

Statistical analysis

All values in the results are expressed as an average of three readings. The antimicrobial activity of ointments was evaluated using one-way ANOVA using Graphpad-prism5 software.

Results

Phytochemical analysis

Table 3. Preliminary Phytochemical screening for Hydro-alcoholic extract of *Kleinia grandiflora*

S.No	Plant Constituents	Test Performed	Result
1.	Carbohydrates	Molisch's test	+
2.	Proteins	Ninhydrin test	-
3.	Flavanoids	Lead acetate test	+
4.	Alkaloids	Mayer's test	-
5.	Steroids	Liebermann burchard's test	+
6.	Terpenoids	Salkowski test	+
7.	Saponins	Froth test	+
8.	Tannins	Ferric chloride test	+
9.	Glycosides	Keller- kiliani test	-
10.	Phenolic compounds	Ferric chloride test	-
11.	Fixed oils	Spot test	_

Table 4. Preliminary Phytochemical screening for hydro-alcoholic root extract of liquorice

S.No	Plant Constituents	Test Performed	Results
1.	Carbohydrates	Molisch's test	-
2.	Proteins	Ninhydrin test	-
3.	Flavanoids	Lead acetate test	+
4.	Alkaloids	Mayer's test	+
5.	Steroids	Liebermann burchard's test	+
6.	Terpenoids	Salkowski test	+
7.	Saponins	Froth test	+
8.	Tannins	Ferric chloride test	+
9.	Glycosides	Keller- kiliani test	+
10.	Phenolic compounds	Ferric chloride test	-
11.	Fixed oils	Spot test	-

Table 5. Preliminary Phytochemical screening for hydro-alcoholic extract of turmeric

S.No	Plant Constituents	Test Performed	Results
1.	Carbohydrates	Molisch's test	-
2.	Proteins	Ninhydrin test	-
3.	Flavanoids	Lead acetate test	+
4.	Alkaloids	Mayer's test	+
5.	Steroids	Liebermann burchard's test	+
6.	Terpenoids	Salkowski test	+
7.	Saponins	Froth test	+
8.	Tannins	Ferric chloride test	+
9.	Glycosides	Keller- kiliani test	-
10.	Phenolic compounds	Ferric chloride test	+
11.	Fixed oils	Spot test	+

The results of the preliminary phytochemical screening of extracts of Liquorice, Turmeric and *Kleinia grandiflora* were reported in table 3, 4 and 5 respectively

Evaluation of ointment

The quality control tests were done for the formulated ointment and physical parameters like colour, odour and consistency were observed. Spreadability, pH and extrudabilty were determined and reported in table 9. From

Table 6. Antimicrobial activity of *Kleinia grandiflora* leaf extract

Micro organism	Zone of inhibition (cm)			Standard (10 units/ml)
	50mg/ml	100mg/ml	200mg/ml	
Staphylococcus epidermidis	0.25	0.3	0.34	0.72
Bacillus pumilus	0.17	0.21	0.28	0.53
Escherecia coli	0.21	0.28	0.32	0.5

Table 10. Antimicrobial Activity of Formulated Ointment

Ointments	Zone diameter in cm	
	Staphylococcus epidermidis	Bacillus pumilus
F1 (0.5%)	0.56	0.3
F2 (1%)	0.8	0.5
F3 (2%)	0.83	0.56
Standard	1.2	0.96

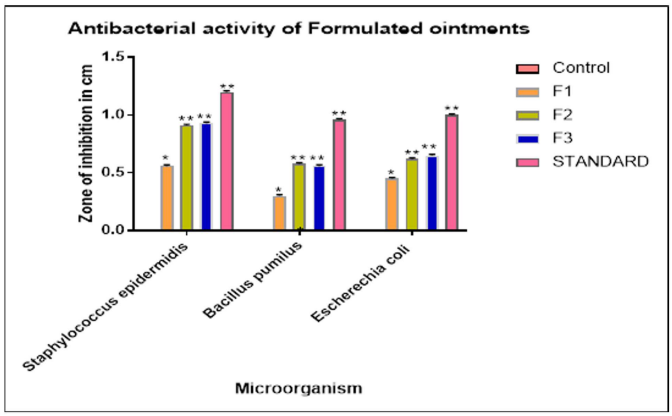

Figure 2. Antimicrobial activity of formulated ointments. All Values in the results are expressed as mean of three readings. *P<0.05, **P<0.01

the stability studies, ointment showed no changes in pH, viscosity, spreadability, consistency and phase separation after keeping at different temperatures for 60 days

Anti-microbial activity of extracts and ointment

The results of antimicrobial activity were evaluated using disc diffusion method for all the extracts of herbs and formulated ointment separately. The results were compared with a standard drug (Amikacin) and also with an antiseptic ointment. The results of extracts were reported in table 6, 7 and 8. The results of antimicrobial activity of formulated ointments were reported in table 10 and figure 2.

Discussion

Literatures revealed that the selected 3 herbs have protective effect on skin and specifically used to treat acne. Hence an attempt was made to formulate a polyherbal ointment as in different proportions were prepared by levigation method using paraffin ointment as the base. The formulations were then evaluated for its physicochemical parameters and its antibacterial activity was compared with a standard skin care ointment soframycin.

The various physicochemical parameters utilized to evaluate the prepared ointment formulations are shown in table 9. The physical parameters like colour, odour and consistency were observed and the pH of all the formulations lies in the normal pH range of the human skin (6.5 ± 0.3).

The physical evaluation parameters like viscosity, spreadability, homogeneity are important tests to evaluate pharmaceutical ointment formulations. The results of viscosity gives an idea about measurement of strength and the result of spreadability denote the extent of area to which the prepared formulations readily spreads on application to skin or affected part and homogeneity confirms no lumps. The results of diffusion study indicate the time taken for the diffusion of all the three formulations is nearly similar. All the three formulations show good diffusion.

The results of stability study indicate that there was no change in results of evaluation parameters of prepared ointments during the treatment period. The results of the physical evaluation of ointment preparation with the extracts of liquorice, turmeric and kleinia indicate the suitability of method for the production of ointments.

Antimicrobial activity was evaluated using disc diffusion method for the extracts of herbs and formulated ointment separately. The results were compared with a standard drug (Amikacin) and also with an antiseptic ointment. The antibacterial activities were found very much promising. Among all formulations F1, F2 and F3**, F2** showed equivalent activity with that of Soframycin.

Therefore the present findings demonstrate that folk medicine can be as effective as modern medicine in treatment of various skin ailments.

Conflict of interest statement

We declare that we have no conflict of interest.

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