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Formulation, Evaluation and Antimicrobial activity of *Evolvulus alsinoides* Cream

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Research Article

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ABSTRACT

The *Evolvulus alsinoides*, commonly known as dwarf morning-glory and slender dwarf morning-glory, is flowering plant from the family *Convolvulaceae*. Traditionally this plant is used as blood purifier, brain tonic, an antimicrobial agent and for memory loss. This work is done for determining whether the cream prepared by the aqueous extract of *Evolvulus alsinoides* has antimicrobial activity. The dried plant leaves are extracted using water as the solvent. The extractive value is calculated and was found to be 12.5% w/w. The phytochemical screening of the extract was performed and it contains slight amount of glycosides, carbohydrates, proteins, alkaloids and tannins. Then using that aqueous extract, a cream was formulated and its evaluation was carried out. From the evaluation the cream was found to be light brown in color with a characteristic odour and have smooth texture. pH and viscosity of the cream are 5.58 and 13254 mpas respectively. Cream was homogeneous by its visual appearance with a smooth and good consistency.

On application, the cream was non-slippery, smooth and easily removable also does not show any irritation on skin. After that the antimicrobial activity of the cream was tested using agar well diffusion method. Gentamicin was used as the standard reference drug. The antimicrobial sensitivity was determined using zone of inhibition. In our study, the cream containing aqueous extract displayed antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The cream was found to be more active against gram-negative (*Escherichia coli*) bacteria. The identified phytochemical compounds *viz.,* glycosides, tannins and alkaloids may be responsible for the antimicrobial activity of *Evolvulus alsinoides.*

INTRODUCTION

Herbalism is the study of pharmacognosy and the use of medicinal plants, which are basis of traditional medicine. Herbal drugs are types of medicine that uses roots, stems, leaves, flowers, seeds, or other parts of plants to improve health, prevent diseases and treat illness. Herbal preparations are formulations made from one or more herbs or processed herbs with the help of different processes like infusion, decoction, maceration, concentration, distillation, purification etc., which provides specific nutritional, cosmetic or other benefits meant for diagnose, treat, mitigate diseases of human beings or animals or to alter the structure or physiology of human beings or animals. Herbal medicines have its roots in every culture around the world. There are many different systems of traditional medicine and practices of each are influenced by social conditions, environmental and geographic location, but these systems all agree on a holistic approach to life. One of the greatest benefits associated with herbal medicine is the non-existence of side effects and also, they tend to offer long lasting benefits in terms of overall wellness, but reports on serious reactions are indicating in some cases which leads to the need for development of effective marker system for isolations and identification of the individual components. Standards for herbal drugs are being developed worldwide but as yet there is no common consensus as to how these should be adopted. Standardization, stability and quality control for herbal drugs are feasible, but difficult to accomplish. Further, the regulation of these drugs is not uniform across countries. There are variations in the methods across medicine systems and countries in achieving stability and quality control the present study attempts to identify the evolution of technical standards in manufacturing and the regulatory guideline development for commercialization of herbal drugs. Also cost effectiveness is another benefit of herbal preparations [1].

In India more than 70% of population use herbal drugs nowadays for their health. There is vast experience-based evidence for many of these drugs. There are also a number of institutes or universities in India carrying research on herbal drugs, medicinal plants and their benefits. These herbal drugs are also rich sources of beneficial compound including antioxidants and components used in functional foods. Newer approaches utilizing collaborative research and modern technology in combination with established traditional health principle will yield rich dividends in the near future in improving health, especially among people who do not have access to the use of costlier western system of medicine [1].

Standardization of herbal drugs is also very important. Standardisation is the first step for the establishment of a consistent biological activity, chemical profile, or simply a quality assurance program for production and manufacturing. Mainly the following parameters like authentication, physical parameters, qualitative and quantitative analysis, microbiological contamination, pesticide residue, heavy metal analysis, etc are considered for standardisation of herbal drugs. Nowadays newer and advanced methods are also available like fluorescence quenching, the combination of chromatographic and spectrophotometric methods, biological assays, use of biomarkers in fingerprinting etc [1].

Cream is defined as semisolid emulsions which are oil in water or water in oil type which are intended for external application on skin or accessible mucous membrane which provide localized or systemic effects at site of applications. Its main ability is to remain for a longer period of time at the site of application. Cream protects the skin from various environmental conditions and also provides soothing effect to the skin. Different varieties of creams like cleansing cream, cold creams, vanishing creams, foundation creams, night creams, body creams etc are available in the markets [2]. Herbal creams are creams which contains one or more herbal constituents in it.

Nowadays herbal creams have considerably wide attention because of their good activity and comparatively lesser or nil side effects and skin irritations. General ingredients used in the preparation of skin creams include water, oil, fats or waxes, mineral oil like liquid paraffin or liquid petroleum, glyceride oil, vegetable oil, colourants, emollients, humectants, perfumes, preservatives, etc. In addition to these ingredients active pharmaceutical ingredients should also be included if the cream is intended for any disease condition. The general processes of preparation of a cream includes four individual operations like preparation of oil phase, hydration of aqueous phase ingredients, forming the emulsion and finally dispersion of the active ingredients [3].

Evolvulus alsinoides, commonly known as dwarf morning-glory and slender dwarf morning-glory, is flowering plant from the family *Convolvulaceae*. It has a natural pantropical distribution encompassing tropical and warm-temperate regions of Australasia, Indomalaya, Polynesia, Sub-Saharan africa and the Americas. It is one of the plants included in Dasapushpam, the ten sacred flowers of Kerala. Chemical compounds isolated from *E. alsinoides* include scopoletin, umbelliferone, scopolin and 2-methyl-1,2,3,4-butanetetrol (Figure 1) [4].

The scientific classification of *Evolvulus alsinoides* is as follows:

- Kingdom: Plantae
- Class: Tracheophytes, Angiosperms, Eudicots, Asterids
- Order: Solanales
- Family: *Convolvulaceae*
- Genus: Evolvulus
- Species: *Alsinoides*
- Varieties: *E. alsinoides, E. decumbens, E. rotundifolia*

Figure 1. The *Evolvulus Alsinoides.*

Therapeutic uses of this plant include:

- Whole plant is used in form of decoction for nervous disabilities and memory loss.
- Used as blood purifier and in bleeding piles.

- The fresh flowers with sugar are eaten as a brain tonic.
- The leaves are made into cigarettes and smoked in chronic bronchitis and asthma.
- It also improves complexion, voice and cures from intestinal worms.
- It promotes 'medha', the power of memory (Tables 1 and 2) [5].

METHODS AND METHODOLOGY

Table 1. List of instruments.

Sl. No	Instruments	Purpose
1	Electronic weighing balance	Weighing ingredients
2	Heating mantle	Evaporating filtrate to obtain crude extract
3	Brookfield viscometer	Determining viscosity of cream
4	pH meter	Determining pH of cream
5	Laminar air flow	Creating aseptic environment
6	Hot air oven	Sterilizing glassware
7	Incubator	Growing cell cultures
8	Autoclave	Sterilizing media

Table 2: List of chemicals.

Sl.No	Chemicals	Use
1	Beeswax liquid paraffin	For preparing oil phase of cream
2	Water Borax	For preparing aqueous phase
3	Peptone water	Preparing bacterial culture
4	Muller-Hinton agar	Preparing solid culture media

Other requirements

Mortar and pestle, China dish, beaker, funnel, filter paper, iodine flask, desiccator, water bath, thermometer, glass slide, petri plates, test tube, micropipette, antibiotic disk.

Extraction

Extraction is the method of separation of medicinally active constituents of a plant or animal tissue from an inert or inactive components by using a selective liquid solvents. Extracts are preparations of crude drugs which contains all constituents which are soluble in solvents. Solid residue obtained after extraction is called marc and the solvent used for extraction is called menstrum. The main steps involved in the extraction are size reduction, extraction,

filtration, concentration and drying. The commonly used extraction methods are infusion, decoction, percolation, maceration etc [6-8].

Preparation of aqueous extract

12.5 g of powdered leaves and twigs of *Evolvulus alsinoides* was dispensed in 250 ml of distilled water in a 250 ml conical flask and the same procedure is followed for the rest 87.5 g of powder. The mixture was shaken vigorously and then allowed to stand for 24 hours. It was shaken again and filtered through What'smann filter paper No.1 into a beaker. Filtrate was evaporated at 40 °C on a water bath to obtain the solid crude extract (Figure 2).

Figure 2. Aqueous Extract of *E. Alsinoides.*

Extractive value

Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method. Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs. There are 3 types of extractive values and they are water soluble, alcohol soluble and ether soluble extractive values [9].

Tests for alkaloids

Dragendroff's test: To 2 to 3 ml extract, add few drops of Dragendroff's reagent. Orange-brown precipitate is formed.

Mayer's test: To 2 to 3 ml extract with few drops Mayer's reagent gives precipitate.

Hager's test: To 2 to 3 ml extract with Hager's reagent gives yellow precipitate.

Wagner's test: To 2 to 3 ml extract with few drops Wagner's reagent gives reddish brown precipitate.

Tests for flavonoids

Shinoda test: To extract, add 5 ml 95% ethanol, few drops conc. HCl and 0.5g magnesium turnings.

Orange, pink, red to purple colour appears.

Sulphuric acid test: To extract, add 66% sulphuric acid, a deep yellow solution.

Lead acetate test: To small quantity of the extract, add lead acetate solution. A yellow colored precipitate is formed.

Test for tannins

Ferric chloride test: To 2 to 3 ml of aqueous extract, add 5% ferric chloride solution. A deep blue black colour.

Lead acetate test: To 2 to 3 ml of aqueous extract, add lead acetate solution. A white precipitate.

Bromine test: To 2 to 3 ml of aqueous extract, add bromine water. Decolouration of bromine water.

Nitric acid test: To 2 to 3 ml of aqueous extract, add dilute HNO_3. A reddish to yellow colour.

Test for steroids

Salkowski test: To 2 ml of extract, add 2 ml chloroform and 2 ml conc H_2SO_4. Shake well. Chloroform layer appear red and acid layer shows greenish yellow fluorescence (Table 3) [10,11].

Table 3. Formulation of cream.

Sl. No	Ingredients	Official formula (100 g)	Working formula (400 g)
1	Bees wax	16 g	64 g
2	Liquid Paraffin	50 g	200 g
3	Borax	0.8 g	3.2 g
4	Water	33.2 g	132.8 g
5	Extract	0.5 g	2.0 g
6	Perfume	Quantity sufficient	Quantity sufficient

Procedure

Cream was prepared by using fusion method. Whenever a cream consists of solid ingredient as component of the base, it is necessary to melt them. If the ingredients have different melting points, melting should be done in decreasing order of the melting point. This method is quicker and required less heat and avoids overheating of medicaments with low melting point. Medicaments are added slowly and stirred until the mass cools down completely and a homogenous product is formed. Bees wax and liquid paraffin are melted to 75°C in a beaker by stirring. This is the oil phase. In another beaker borax and water are taken and heated to same temperature as the oil phase. Plant extract is added to the aqueous phase and mixed well. Aqueous phase is added to the oil phase and stir continuously until a homogenous product is formed. Finally, perfume is added and packed in a suitable container (Figure 3).

Figure 3. *E. Alsinoides* Cream.

Evaluation of cream

Physical appearance: The physical appearance of the cream can be observed by its colour, odour and roughness.

Determination of pH: The pH of the cream can be measured on a standard digital pH meter at room temperature by taking adequate amount of the formulation diluted with a suitable solvent in a suitable beaker.

Spreadability: Adequate amount of sample is taken between two glass slides and a weight of 100 gm is applied on the slides for 5 minutes. Spreadability can be expressed as,

$S = m \times l/t$

Where,

m = weight applied to upper slide. l = length moved on the glass slide. t = time taken.

Viscosity: Viscosity of formulated creams can be determined by using Brookfield Viscometer.

Principle

Brookfield viscometers are often used within the sectors where accurate bench-top analysis of product viscosity is needed. These viscometers use the principle of 'rotational viscometry', i.e., their measurement of product viscosity is based upon immersing a specifically selected spindle within a sample of the product followed by measurement of the torque required to rotate the spindle at a set speed whilst immersed within the product sample. As the torque required will be proportional to the quantity of viscous drag upon the spindle, this therefore provides an assessment of the product viscosity, reported in Centipoise Units (CP) [12].

Procedure

Sample was filled in a beaker without any air bubble entrapment. If any air bubbles get entrapped the readings may become very high than the original value. With the motor off attach the spindle by gently lifting up the coupling nut on the viscometer with one hand and screwing the spindle on with the other hand. Turn the gear knob to move the instrument up and down until the sample is level with the spindles immersion groove [13]. After attaching spindle a spindle entry code must be entered. To enter the spindle entry code push the 'select spindle' button scroll using the up or down arrows to the correct spindle code and push the 'select spindle' button again to lock in the code. To set

the speed scroll using the up/down arrows to the speed of the choice and press the 'set speed' button. Press the motor on button to rotate the spindle. Here we used spindle 4 and is rotated at 50 rpm for 15 min.After 15 min the reading is taken and noted (Figure 4) [14].

Figure 4. Brookfield Viscometer.

Homogeneity: The formulation was tested for the homogeneity by visual appearance and by touch.

Removal: The ease of removal of the creams applied was examined by washing the applied part with tap water.

After feel: Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

Irritancy study: Mark an area of 1 sq cm on the left hand dorsal surface and then the cream was applied to the specified area and time was noted. Irritancy, erythema, edema was checked, for regular intervals upto 24 hrs and reported [15].

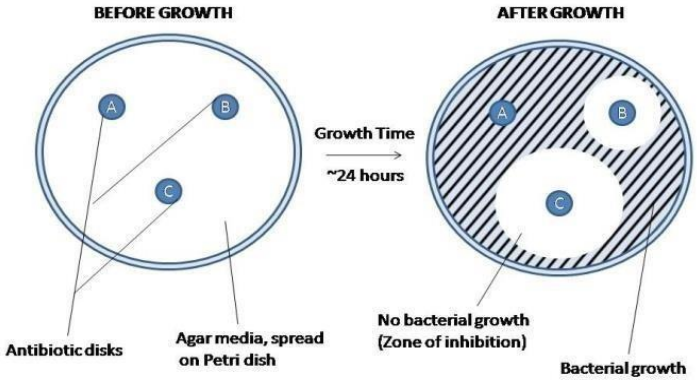
Antimicrobial activity

Agar well diffusion method: The disk diffusion method is used to determine the susceptibility of clinical isolates of bacteria to different antibiotics. An effective antibiotic will produce a large zone of inhibition (disk C), while an ineffective antibiotic may not affect bacterial growth at all (disk A). Antibiotics to which a bacterial isolate is partially susceptible will produce an intermediate size zone of inhibition (disk B) [16].

Principle

The bacterial culture is suspended in a saline solution and its turbidity is standardized. Then the bacterial inoculum is uniformly spread on agar plate and then the antibiotic disk containing a defined concentration of antibiotic is applied. The antibiotic in the disk diffuses through the solid medium, so that the concentration is highest near the site of application and decreases gradually away from it. A well is made on the solid medium using a micropipette and the sample is introduced into it. The sample also diffuses through the medium similar to the antibiotic. Sensitivity is determined by the zone of inhibition of bacterial growth (Figure 5) [17].

Figure 5. Agar well diffusion method.



Procedure

Medium preparation: Muller-Hinton Agar (MHA) is considered as the best medium to use for routine susceptibility testing of bacteria because it shows acceptable batch-to-batch reproducibility for susceptibility testing.

Composition of Muller-Hinton agar includes:

- HM infusion B from*- 300.00 gm/litre
- Acicase- 17.50 gm/litre
- Starch- 1.50 gm/litre
- Agar- 17.00 gm/litre Final pH (at 25°C) –7.3 ± 0.1

Note: *Equivalent to beef infusion

Equivalent to beef infusion

Directions: Suspend 38 g in 1000 ml of purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C-50°C. Mix well and pour into sterile petri plates.

Culture preparation

The organisms are isolated in pure culture on a solid medium. Isolated colonies are inoculated in a suitable broth medium (peptone water) and incubated at 35°C-37°C for 1-2 hours or continue to the process until the medium is turbid. Composition of peptone water includes:

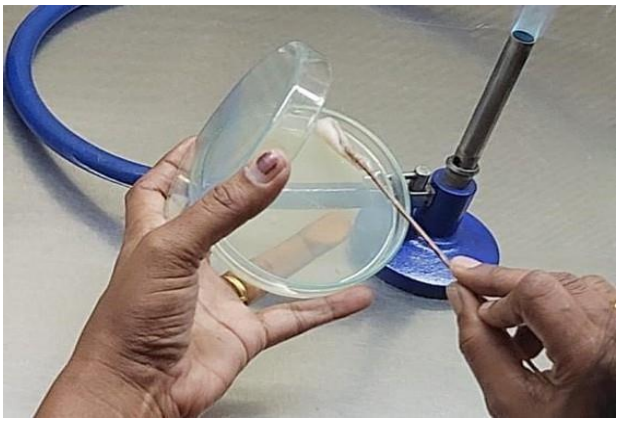
- Peptone - 10g
- Sodium chloride - 5g
- Distilled water -1000 ml

Culture of both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) was prepared for this study [18].

Inoculation

Inoculation is the process of introducing an organism or a disease-causing pathogen into a culture medium. The inoculation method mostly used for sensitivity testing is lawn culture method. Lawn culture medium is prepared by uniform spreading of bacterial culture on the solid medium using a cotton swab. Lawn culture provides a uniform surface growth of the bacterium in Figure 6.

Figure 6. Lawn culture.



- A well is then made on the culture medium using micropipette and sample is introduced into it.
- An antibiotic disc (Gentamicin) is placed parallel to the well.
- The medium is incubated for 24 hours and antimicrobial sensitivity is determined using zone of inhibition.

RESULTS AND DISCUSSION

The leaves of *Evolvulus alsinoides* were collected and authenticated. Then the leaves were dried and the dried powdered crude drug was extracted using water as the solvent. The extractive value was calculated and found to be 12.5% w/w. The phytochemical screening of the extract was carried out and it was found to contain carbohydrates, proteins, glycoside, alkaloids and tannins as shown in Tables 4 and 5.

Table 4. Preliminary phytochemical screening.

Sl.No	Test	Inference
1	Carbohydrates	+
2	Proteins	++
3	Glycosides	++
4	Alkaloids	+++
5	Flavonoids	-
6	Tannins	+++
7	Steroids	-

Note : Strongly present +++, Present ++, Weekly present +, Absent -

Cream was prepared by the fusion method. Then it was evaluated for its physical features (colour, odour, texture), pH, viscosity, homogenecity, consistency, after feel, irritancy and spreadibility (Figure 6) [19,20] .

Table 5. Evaluation of cream.

Sl.No	Parameter	Observation
1	Physical appearance	
	a) Colour	Light brown or cream
	b) Odour	Characteristic odour
	c) Texture	Smooth
2	pH	5.58
3	Viscosity	13254 mpas (15 min at 50 rpm using spindle 4)
4	Homogeneity	
	a) By visual	Homogenous
	b) By touch	Smooth and consistent
5	Removal	Washable
6	Consistency	Good
7	After feel	Smooth, non- slippery and no residue left after application
8	Irritancy	No irritations
9	Spreadibility	17

Antimicrobial activity of the cream against gram-negative and gram-positive organism was evaluated by agar well diffusion method, using Gentamicin as the standard reference drug [21]. The antibacterial activity was measured individually and compared the zone of inhibition of sample (*E. alsinoides* cream) against standard (Gentamicin antibiotic). The results are shown in Table 6.

Table 6. Observation of Anti-microbial activity.

Organism	Zone of inhibition	
	Sample	Standard
Staphylococcu saureus (Gram positive)	28mm	37mm
Escherichia coli(Gram negative)	34mm	21mm

Figure 7. Zone of inhibition against *S. aureus*.

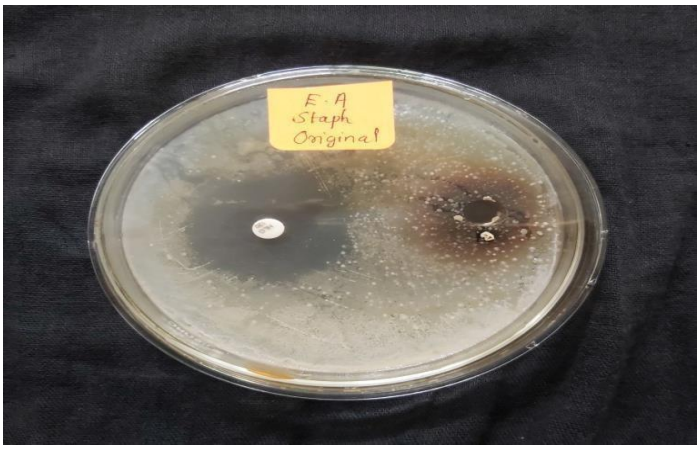
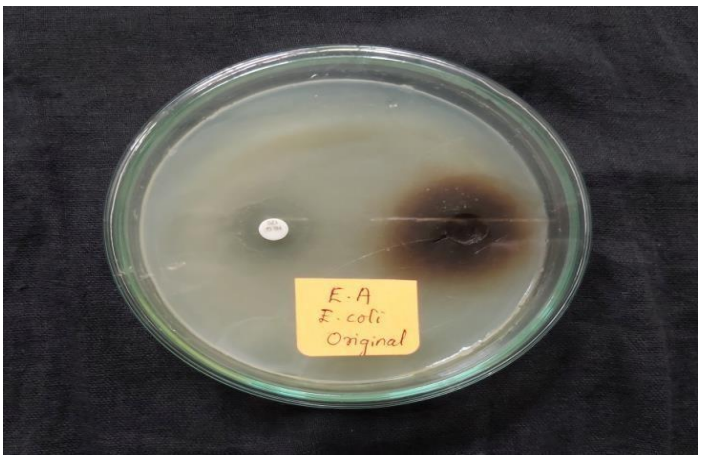


Figure 8. Zone of inhibition against *E. coli*.



The result shows that cream has antibacterial activity against both gram positive and gram-negative organisms. In case of *E. coli*, the zone of inhibition produced by sample was more than that of the standard. Thus, gram-negative bacteria (*E. coli*) show more sensitivity to the cream compared to gram-positive bacteria (*S. aureus*) (Figures 7 and 8).

CONCLUSION

The traditional methodologies utilized a wide variety of medicinal herbs for various treatments and *Evolvulus Alsinoides* is one of the notable herbs among them. As it is widely available it has been employed for clinical use from centuries. The present study concludes that the cream prepared from the aqueous extract of *Evolvulus Alsinoides* have the anti-microbial activity against both gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* and among them more activity is shown against the gram-negative organism. Thus, the anti-microbial activity of *Evolvulus Alsinoides* indicates the therapeutic potential for the treatment of various infectious diseases and supports the traditional use of medicinal herb. Since the plant extracts present various components

like carbohydrates, proteins, glycoside, tannins, alkaloids etc. therefore, at this stage, it is difficult to say which components are responsible for antimicrobial activity and it requires further phytochemical studies to isolate the active components responsible for the pharmacological activities. Hence, medicinal plant extract and formulations made from it could be an answer to those people who needs better therapeutic agents from natural sources which are more efficient with no or little side effects when compared to other synthetic agents. Further clinical studies are required to validate the efficacy of this medicinal plant against various strains of bacteria.

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