Research Article

Formulation and evaluation of herbal gel from the ethanolic extract of the stem bark of *Bauhinia variegata* Linn. for antimicrobial activity

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ABSTRACT

Aim: The study was conceived to formulate an herbal gel from the ethanolic extract of the stem bark of B. variegata Linn. and to evaluate its physicochemical parameters and microbiological assay. **Materials and Methods:** Formulation of 1% and 2% Gel for Antimicrobial Assay. The microbial evaluation was carried out using cup and plate method for all the formulations of gels. The Antibacterial Assay was carried with the Gram negative bacteria *Escherichia coli, Vibrio parahaemolyticus , Klebsiella pneumonia, Pseudomonas aeruginosa* and Gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* compared with the standard ciprofloxacin. Antifungal Assay was performed against *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus,* and *Candida albicans* compared with the standard Clotrimazole. **Results:** The studies had revealed that the developed herbal formulations of 1% and 2% exhibited good minimum inhibitory concentration. In the antibacterial studies, a comparison made with the standard ciprofloxacin (24.5 mm), 2% ethanolic extract of the herbal gel formulation had shown the maximum effect for *Bacillus subtilis* (22.7 mm). In the antifungal studies, a comparison made with the standard clotrimazole (28.9 mm), 2% ethanolic extract of the herbal gel formulation had shown the maximum effect for *Candida albicans* (23.1 mm). **Conclusion:** Herbal formulations have growing demand in the world market. A very good attempt was made to establish the herbal gel containing B. variegata ethanolic extract. The studies had revealed that the developed herbal formulations of 1% and 2% exhibited good minimum inhibitory concentration. The phytoconstituents present in the bark might be responsible for the antimicrobial activity.

KEY WORDS: Antifungal activity, Antimicrobial activity, *Bauhinia variegata*, Herbal gel

INTRODUCTION

Herbal medicine is a triumph of popular therapeutic diversity.[1] *Bauhinia variegata* is a medium-sized deciduous tree with many medicinal properties. The plant – *B. variegata* is an herbaceous medicinal plant that is found throughout India, in the Himalayas region at an altitude of 1300 m. The plant is commonly called Sigappu mandarai in Tamil and Devakanchanamu in Telugu and belongs to the family Caesalpiniaceae. The useful parts of the plant are bark, flowers, and root.

The bark of *B. variegata* is useful in the treatment of skin diseases, leprosy, intestinal worms, tumors, wounds,[2] ulcers, scrofula, proctoptosis, hemorrhoids, hemoptysis, cough, menorrhagia, and diabetes.[3] *B. variegata* bark has an excellent antimicrobial property. The present study was designed to formulate and evaluate a topical gel with ethanolic extract of the bark of *B. variegata* of various concentrations. The gel was evaluated for its basic principle parameters such as pH, viscosity, spreadability, extrudability, and skin irritation studies, stability studies along with antibacterial and antifungal activities.

The various topical formulations include hydrocarbon-based formulations, polar gel formulations, creams, ointments, and liposomes. These topical formulations can be used to manipulate the barrier function of the skin. Gels are the semisolid systems containing either a suspension of small inorganic particles or large organic molecules.[4] Semisolid dosage forms for dermatological drug therapy are intended to produce desired therapeutic action at specific sites in the epidermal tissue.

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The epidermis of skin provides protection from environmental pathogens and serves as a barrier to infections.[5] There are various skin diseases reported due to various bacteria, fungi, as well as virus. Semisolid dosage forms when applied to the skin or accessible mucous membranes tend to alleviate or treat a pathological condition or offer protection against a harmful environment.[6]

MATERIALS AND METHODS

Collection of Plant

The stem bark of the plant "*B. variegata* Linn." was collected in Chennai and authenticated by Dr. Jayaraman (PARC) and the voucher specimen (PARC/2016/3289) was deposited in the Pharmacognosy Laboratory in Vels University for future reference.

Preparation of Plant Extract

The fresh stem bark of *B. variegata* Linn. was collected and dried in shade. Then, the dried bark was powdered to get a coarse powder. About 200 g of the drug was mixed with 99% ethanol to about third-fourth of the vessel and then allowed to stand for 72 h. The ethanolic extract was filtered and concentrated to a dry mass. A dark red color residue was obtained. The marc left after ethanolic extract was taken out and dried under the shade to get a dry mass.

Preliminary Phytochemical Screening of Ethanolic Extract

The present preliminary phytochemical analysis gives the information about phytoconstituents in the crude drug. This information is important in the ethanopharmacological screening of the drugs. Hence, chemical tests were carried on the ethanolic extract using standard procedures to identify the phytoconstituents.

Formulation of the Gel

About 4 g of Carbopol was taken in a beaker and to this 50–60 ml of water was added. Then, the mixture was kept in a hot air oven at 100°C for 30 min with stirring. The mixture is stirred for 10–15 min to avoid air bubbles with glass rods and kept aside for 30 min. The mixture was homogenized for 10 min and in warm condition methylparaben was added.

Weighed quantity of drug was dissolved in small amounts of water and the remaining ingredients are added to the drug solution. Finally, remaining quantity of water was added with triethanolamine to neutralize the pH. Prepared gel was filled in a glass container and stored in a cool and dry place. Ingredients for the formulation of gels are shown in Table 1.

Antimicrobial Assay

The microbial evaluation was carried out using in cup and plate method for all the formulations of gels.

Media used – nutrient agar was used as the media for the study.

Antibacterial Assay

The following bacteria were used:

a. *Escherichia coli* (Gram negative)
b. *Pseudomonas aeruginosa* (Gram negative)
c. *Vibrio parahaemolyticus* (Gram negative)
d. *Klebsiella pneumoniae* (Gram negative)
e. *Staphylococcus aureus* (Gram positive)
f. *Bacillus subtilis* (Gram positive).

The cup-plate method was used for determining the selective effectiveness of the antibacterial activity and clindamycin was used as standard.

Preparation of subculture[7]

One day before this testing, inoculation of the above bacterial cultures was made in the nutrient agar and incubated at 37°C for 18–24 h.

Preparation of test solutions

Each test compound (250 mg/ml) was dissolved in dimethyl sulfoxide (5 ml) to give 1000 µg/ml.

Procedure

Weigh nutrient agar and mix in water. Autoclave the mixture to 121°C for 30 min at 15 lp pressure. Molten agar is poured in Petri dish. After solidification, spread the inoculum on the surface using spreader or loop. Dig well of 6 mm using sterile borer. Place the test solution in the well. Keep for 4–5 h to diffuse. Place in an incubator at inverted position for 24 h. The zone of inhibition was measured.

Table 1: Ingredients for the formulation of gels

Ingredients	Quantity for control	Quantity for 1%	Quantity for 2%
Carbopol	4 g	4 g	4 g
Glycerine	10 ml	10 ml	10 ml
Methyl paraben	50 mg	50 mg	50 mg
Propylene glycol	10 ml	10 ml	10 ml
Ethanolic extract stem bark of *Bauhinia variegata*	-	1% (1 g)	2% (2 g)
Tween 80	2 ml	2 ml	2 ml
Triethanolamine	2 ml	2 ml	2 ml
Distilled water	q.s	q.s	q.s

Antifungal Assay

Preparation of test solutions

Each test compound (250 mg/ml) was dissolved in dimethyl sulfoxide (5 ml) to give a 1000 µg/ml.

Procedure

The assay was performed against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus,* and *Candida albicans*. Nutrient agar was used as the growth media. In each plate, 15 ml of the sterile media was added. Allow it to solidify, then 0.1 ml of the inoculum was spread over media; cavity was made at different positions. Test solution was added and the plate was kept in incubator for 24 h. Clotrimazole was used as standard.[8]

Table 2: Percentage yield of ethanolic extract of the bark of *Bauhinia variegata*

Solvent	Extraction process	% yield
Ethanol	Cold maceration	13.2

Table 3: Preliminary phytochemical screening of ethanolic extract of the bark of *Bauhinia variegata*

Phytochemical screening	
Chemical tests	**Results**
Carbohydrates	+
Proteins containing sulfur	+
Amino acids (cysteine)	+
Steroids	+
Cardiac glycosides	+
Anthraquinone glycosides	-
Saponin glycosides	-
Coumarin glycosides	-
Flavonoids	+
Alkaloids	+
Tannins and phenolic compounds	+

Table 4: Antibacterial assay

Bacteria	Control	1% gel	2% gel	STD
	Diameter of zone of inhibition in mm			
Escherichia coli	6.3	10.5	16.8	20.6
Pseudomonas aeruginosa	6.4	12.1	20.3	25.2
Vibrio parahaemolyticus	6.1	9.1	14.7	18.3
Klebsiella pneumoniae	6.7	10.3	15.2	19.1
Staphylococcus aureus	6.4	13.4	21.6	26.2
Bacillus subtilis	7.1	16.2	22.7	24.5

Table 5: Antifungal assay

Fungi	Control	1% gel	2% gel	STD
	Diameter of zone of inhibition in mm			
Candida albicans	9.3	15.1	23.1	28.9
Aspergillus niger	8.9	12.8	16.7	21.4
Aspergillus flavus	7.6	11.7	15.4	20.8
Aspergillus fumigatus	8.1	12.4	15.9	21.2

RESULTS

The percentage yield of ethanolic extract of the bark of *B. variegata* is shown in Table 2.

Preliminary phytochemical screening of ethanolic extract of the bark of *B. variegata* is shown in Table 3.

Formulation of the Gel

Formulated gels

Control – blank gel without any drug.

1% – 1 g of *B. variegata* ethanolic extract to make 100 g of gel.

2% – 2 g of *B. variegata* ethanolic extract to make 100 g of gel.

- Formulated gels A, B, and C are shown in Figure 1
- Antibacterial assay is shown in Table 4

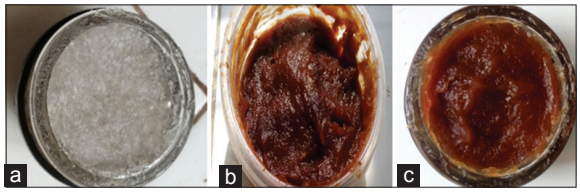

Figure 1: Formulated gels. (a) Control, (b) 1%, (c) 2%

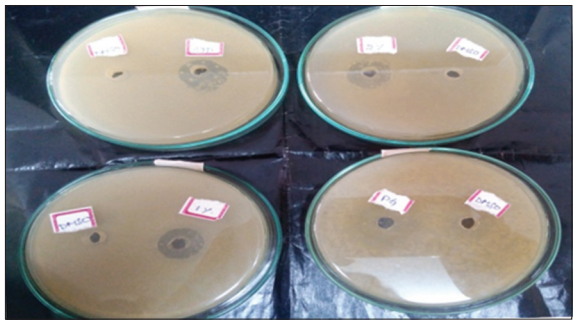

Figure 2: Antibacterial assay of *Bacillus subtilis*

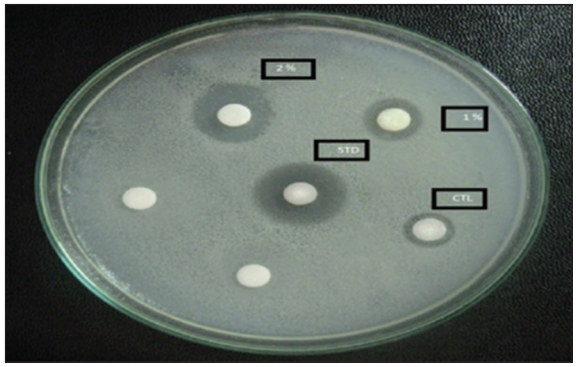

Figure 3: Antifungal activity of *Candida albicans*

- Antibacterial assay of *B. subtilis* is shown in Figure 2
- Antifungal assay is shown in Table 5
- Antifungal activity of *C. albicans* is shown in Figure 3.

DISCUSSION

Both 1% and 2% gel formulations showed significant zone of inhibition for various bacteria and fungi, of which 2% gel formulation showed maximum inhibition of 22.7 mm for *B. subtilis* bacteria and 23.1 mm for *C. albicans* fungus. The standard used for antibacterial activity was clindamycin and clotrimazole for antifungal activity. The zone of inhibition for the standard clindamycin was found to be maximum in *S. aureus* (26.2 mm) bacteria while for fungi, it was maximum for *C. albicans* (28.9 mm). A significant zone of inhibition was observed in 2% of the gel when compared with the standard.

CONCLUSION

B. variegata is an herbaceous medicinal plant having many folklore properties. The phytoconstituents of the plant had major pharmacological importance. Herbal formulations have growing demand in the world market. A very good attempt was made to establish the herbal gel containing *B. variegata* ethanolic extract. The studies had revealed that the developed herbal formulations of 1% and 2% exhibited good minimum inhibitory concentration. In the antibacterial studies, a comparison made with the standard clindamycin (24.5 mm), 2% ethanolic extract of the herbal gel formulation had shown the maximum effect for *B. subtilis* (22.7 mm). In the antifungal studies, a comparison made with the standard clotrimazole (28.9 mm), 2% ethanolic extract of the herbal gel formulation had shown the maximum effect for *C. albicans* (23.1 mm). The phytoconstituents present in the bark might be responsible for the antimicrobial activity.

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