Research Article

Antibacterial and Antifungal Activity of Herbal gel from the Ethanolic extract of the Stem bark of *Bauhinia variegata Linn.*

Malarkodi Velraj*, Dhulipalla Sowmya[1], Sindhukavi. D[1]
*Associate Professor, Department of Pharmacognosy, School of Pharmaceutical Sciences (VISTAS), Vels University, Chennai, India.
[1]Student, School of Pharmaceutical Sciences (VISTAS), Vels University, Chennai, India.
***Corresponding author'sE-mail:**malarkodisanna@gmail.com

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ABSTRACT

Bauhinia variegate is an herbaceous medicinal plant which is commonly known as Buddhist Bauhinia, Mountain Ebony and Orchid tree that belongs to the family on Caesalpinaceae (Leguminosae). It has been used as astringent, acrid, cooling, constipating, depurative, antihelminthic, vulnerary, anti-inflammatory and styptic. The major constituents present in *Bauhinia variegata* are flavonoids, tannins, phenolic compounds, alkaloids, carbohydrates, proteins containing sulphur and cardiac glycosides. These phytochemical constituents are the secondary metabolites of plants that serve a defense mechanism against invasion by many microorganisms. The study was conceived to formulate an herbal gel from the Ethanolic extract of stem bark of *Bauhinia variegate linn* and to evaluate its physicochemical parameters and microbiological assay.

Keywords:*Bauhinia variegata,* Herbal gel*,* Antimicrobial activity, Antifungal activity.

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 - *Bauhinia variegata*is an herbaceous medicinal plant that is found throughout India, in the Himalayas region at an altitude of 1300m. The plant is commonly called Sigappumandarai in Tamil and Devakanchanamu in Telugu belongs to the family caesalpiniaceae. The useful parts of the plant are bark, flowers and root.

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

The various topical formulations includes the hydrocarbon based formulations, polar gel formulations, creams, ointments, liposomes etc. 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.

The epidermis of skin provides protection from environmental pathogens and serves as a barrier to infections[5]. There are various skin diseases reported because of various bacteria, fungi as well as virus. Semisolid dosage forms which 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 *"Bauhinia variegatalinn."* 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 *Bauhinia variegate 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 3/4th of the vessel. Then allowed to stand for 72 hours. 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 for the ethanopharmacological screening of the drugs. Hence

chemical tests were carried on the ethanolic extract using standard procedure in order to identify the phytoconstituents.

Formulation of the Gel

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 minutes with stirring. The mixture is stirred for 10 - 15 minutes to avoid air bubbles with glass rod and kept aside for 30 minutes. The mixture was homogenized for 10 minutes and in warm condition methyl paraben 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 glass container and stored at a cool and dry place.

Table 1: Ingredients for Formulation of Gels

S.No.	Ingredients	Quantity for control	Quantity for 1%	Quantity for 2%
1	Carbopol	4 g	4 g	4 g
2	Glycerine	10 ml	10 ml	10 ml
3	Methyl Paraben	50 mg	50 mg	50 mg
4	Propylene glycol	10 ml	10 ml	10 ml
5	Ethanolic extract Stem bark of *Bauhinia variegata*	---	1% (1g)	2% (2g)
6	Tween 80	2 ml	2 ml	2 ml
7	Triethanolamine	2 ml	2 ml	2 ml

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 -ve)

B. *Pseudomonas aeruginosa* (Gram -ve)

C. *Vibrio parahaemolyticus* (Gram -ve)

D. *Klebsiellapneumoniae* (Gram -ve)

E. *Staphylococcus aureus* (Gram +ve)

F. *Bacillus subtilis* (Gram +ve)

The cup-plate method was used for determining the selective effectiveness of the anti-bacterial activity and ciprofloxacin was used as standard.

Preparation of sub-culture[7]

One day prior to this testing, inoculation of the above bacterial cultures were made in the nutrient agar and incubated at 37°C for 18-24 hrs.

Preparation of test solutions

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

Procedure

Weigh nutrient agar and mix in water. Autoclave the mixture to 121°C for 30 minutes 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 borar. Place the test solution in the well. Keep for 4-5 hrs to diffuse. Place in incubator at inverted position for 24 hrs. The zone of inhibition was measured.

Antifungal assay

Preparation of test solutions

Each test compound (250mg/ml) was dissolved in dimethyl sulfoxide (5ml) 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, 15ml of the sterile media was added. Allow it to solidify then. 0.1ml 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 24hrs. Clotrimaxazole was used as standard[8].

RESULTS

Table 2: Percentage Yield of Ethanolic Extract of the Bark of *Bauhinia Variegata*

S.No.	Solvent	Extraction process	% Yield
1	Ethanol	Cold Maceration	13.2%

Table 3: Preliminary Phytochemical Screening of Ethanolic Extract of Bark of *Bauhinia Variegata*

Phytochemical Screening	
Chemical Tests	**Results**
Carbohydrates	+
Proteins containing sulphur	+
Amino acids (cysteine)	+
Steroids	+
Cardiac Glycosides	+
Anthraquinone Glycosides	-
Saponin Glycosides	-
Coumarin Glycosides	-
Flavonoids	+
Alkaloids	+
Tannins & phenolic compounds	+

Formulation of the gel

Formulated gels

Control - Blank gel without any drug.

1 % - 1 g of *Bauhinia variegate* ethanolic extract to make 100 g of gel.

2 % - 2 g of *Bauhinia variegate* ethanolic extract to make 100 g of gel.

A – Control

B - 1%

C - 2%

Figure 1: Formulated Gels A, B and C

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

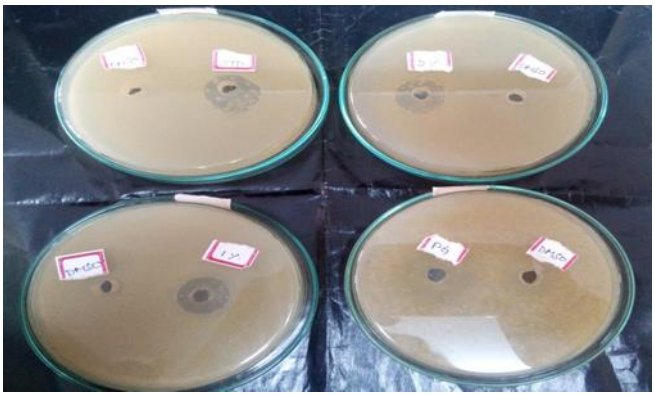

Figure 2: Antibacterial Assay of *Bacillus subtilis*

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

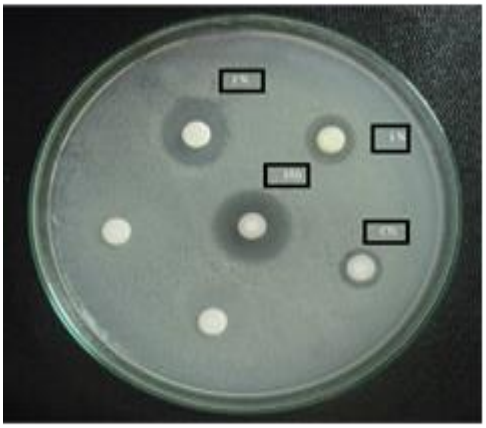

Figure 3: Antifungal Activity of *Candida albicans*

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 *Bacillus subtilis* bacteria and 23.1 mm for *Candida albicans* fungus. The standard used for antibacterial activity was Clindamycin and Clotrimaxazole for antifungal activity. The zone of inhibition for the standard clindamycin was found to be maximum in *Streptococcus aureus* (26.2 mm) bacteria while for fungi, it was maximum for *Candida albicans* (28.9 mm). Significant zone of inhibition was observed in 2% of gel when compared with standard.

CONCLUSION

Bauhinia 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 *Bauhinia variegate* 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 (24.5mm), the 2% ethanolic extract of the herbal gel formulation had shown the maximum effect for *Bacillus subtilis* (22.7mm). In the antifungal studies, a comparison made with the standard clotrimaxazole (28.9 mm), the 2% ethanolic extract of the herbal gel formulation had shown the maximum effect for *Candida albicans* (23.1mm). The phytoconstituents present in the bark might be responsible for the antimicrobial activity.

REFERENCES

1. Pulok K. Mukherjee, Quality control of Herbal Drugs: Natural Products for Modern Medicine, First Edition, Business Horizons Ltd., New Delhi 2002, 13.
2. Rajeev Kumar. Influence of Stem bark Aqueous Extract of *Bauhinia variegate linn*. On Wound Healing In Albino Rats, World Journal Of Pharmacy And Pharmaceutical Sciences, 3(8), 2014, 1128-1140.
3. Indian Medicinal Plants A compendium of 500 species: Vaidyaratnam PS varier Aryavaidyasalai, 3 Volume, Kottakkal Orient long man private Limited, New Delhi, 1994, 256.
4. Amit K Goyal, Gautham Rath, RK Narang, Textbook of Pharmaceutical Dispensing: Semisolid dosage forms, First Edition, New Delhi, 2012, 243.
5. Kuntal Das; Herbal plants and their applications in cosmeceuticals First Edition Paperback press, India, 2014, 6.
6. Gaurav Agarwal, Atul Kaushik; Pharmaceutical Technology First Edition, CBS, Publishers, New Delhi, 2014, 137.
7. Vibhor K Jain. Synthesis, Characterization and Evaluation of Antibacterial and Antifungal activity of Triazole Derivatives of Gallic acid, International Journal of Applied Biology and Pharmaceutical Technology, I(3), 2010, 801,806.
8. Ajinkya M. Bankar, Formulation and Evaluation of herbal antimicrobial gel containing musaacuminata leaves extract, Journal of Pharmacognosy and Phytochemistry, 5 (1), 2016, 01-03.

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