

## *In Vitro* Anti Acne Activity of Ethanolic Extract of Stem of *Berberis aristata*

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### ABSTRACT

Current research work is intended to study the impact of herbal approach to treat acne, an extremely common cutaneous inflammatory disorder of multifactorial origin with prevalence in adolescents. Acne is common disease of skin and is usually treatable. An attempt had been taken to investigate the in vitro antiacne activity of ethanolic extract of stem of *Berberis aristata*. The MIC value of the *B. aristata* extract against *S. epidermidis*, *P. acnes* and *M. furfur* were found to be 600 µg/ml, 200 µg/ml and 100 µg/ml respectively. In vitro antimicrobial screening using erythromycin as a positive control clearly indicated that ethanolic extract of *B. aristata* is promising antimicrobial against the test microorganisms.

### Keywords:

### INTRODUCTION

The skin is perhaps the most vulnerable part of our body. It is a well-known fact that day to day exposure of human skin to the external environment leads to a number of problems such as acne, pigmentation and sunburn marks<sup>1</sup>. Acne is a common skin disorder encountered in the age group of 15-25 years owing to increased production of sebum followed by the attack of *Propionibacterium acnes*<sup>2-4</sup>. The proposed research work is designed to study the impact of topical herbal approach to combat acne. The work emphasizes on the topical treatment of acne, based on reported scientific data of various herbs and herbal extracts, for future development of dermato-cosmetic herbal formulation which could provide complementary and alternative therapy for acne<sup>5-7</sup>.

The treatment modalities for acne are usually aimed at decreasing the *P. acnes* population, producing an anti-inflammatory effect, and decreasing the sebaceous gland activity. Since inflammatory conditions are associated with free radical production, plant materials possessing antioxidant activity are beneficial. For many years, antibiotics and hormones were usually applied to treat acne<sup>8</sup>. However, these agents are often accompanied by severe side effects and drug resistance. Therefore, phyto-therapeutic approaches with high antibacterial activity and without side effects have been extensively studied as an alternative<sup>9</sup>. In this context, alcoholic extract of root of *B. aristata* has been screened for the aforesaid anti-acne activity.

### MATERIALS AND METHODS

#### *Plant materials and extract preparation*

Dried roots of *Berberis aristata* were procured from the local commercial suppliers of Jalandhar, Punjab.

Authentication of *Berberis aristata* was done by Thukral, Professor, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar and the voucher specimens have been deposited at the school of pharmaceutical sciences, Lovely professional University. The crude plant material was pulverized in coarse powder form for the purpose of extraction. Coarsely powdered dried plant drug material was extracted by Soxhlet's apparatus using acetone as solvent.

#### *Phytochemical screening*

The prepared extract was subjected to phytochemical screening to detect the presence/absence of phytoconstituent<sup>10-12</sup>.

#### *Collection of microbial strains*

Aerobic bacteria: *Staphylococcus epidermidis* (MTCC 3382), Anaerobic bacteria: *P. acnes* (MTCC 1951), Fungal strain: *Malassezia furfur* (MTCC 1765) was obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh.

#### *Growth conditions and culture medium*

*S. epidermidis* was cultured in Mueller-Hinton (MH) agar medium and incubated for 24 hrs at 37°C in aerobic conditions.

*P. acnes* was cultured in brain heart infusion (BHI) agar medium and incubated anaerobically with 1% glucose at 37°C for 48 hrs.

*M. furfur* was grown on potato dextrose broth (PDB) or potato dextrose agar (PDA) following incubation at 30°C in aerobic conditions during 2-7 days.

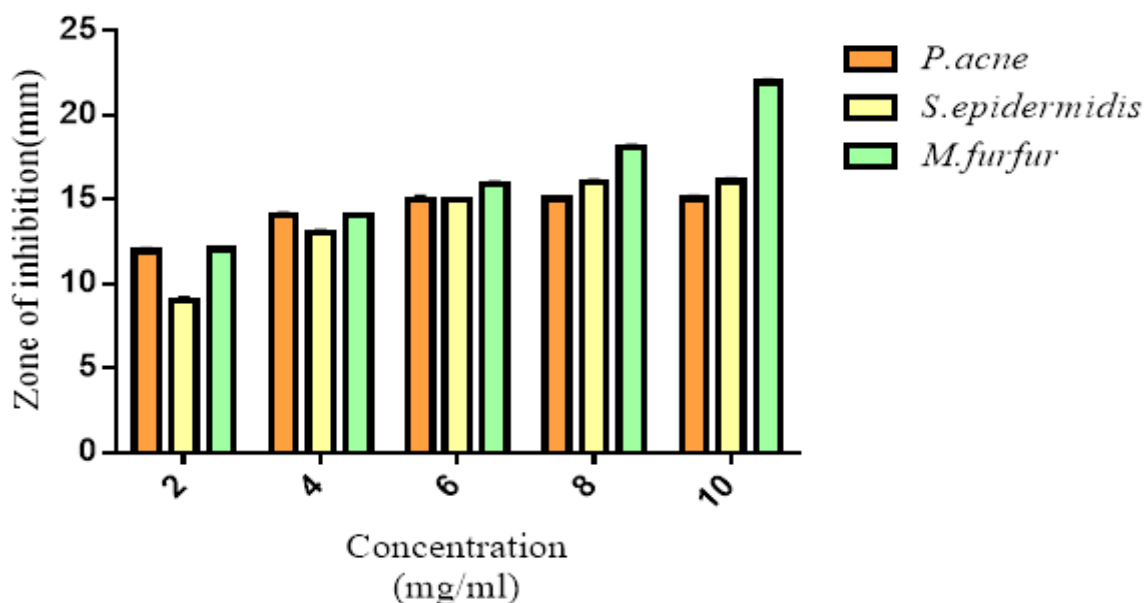
#### *Antibacterial screening by disc diffusion method*

Bacterial suspensions were uniformly spread on each agar plates. *P. acnes* was incubated in BHI medium with 1% glucose for 24 hrs under anaerobic conditions and was spread on BHI agar plate. Two uniform sized wells were

Table 1: Antiacne activity of Ethanolic extract of *B.aristata*.

Conc. of extract (mg/ml)	Zone of inhibition against		
	<i>S. epidermidis</i>	<i>P.acne</i>	<i>M.furfur</i>
1	7.03 ± 0.08	7.96 ± 0.65	10.00 ± 0.03
2	9.00 ± 0.11	11.93 ± 0.06	12.03 ± 0.03
4	13.03 ± 0.03	14.03 ± 0.08	14.00 ± 0.00
6	15.00 ± 0.00	15.00 ± 0.11	15.90 ± 0.05
8	16.03 ± 0.03	15.03 ± 0.03	18.06 ± 0.06
10	16.06 ± 0.06	15.03 ± 0.08	21.93 ± 0.06

### Antimicrobial screening of *Berberis aristata*



The MIC value of the *B.aristata* root extract against test *P. acne*, *S. epidermidis*, and *M. furfur* was found to be 200 µg/ml, 600 µg/ml, and 100 µg/ml, respectively.

made with sterile borer on agar plates that had been seeded with the organism to be tested and in each well 50 µl of plant extracts of various concentrations (1 mg/ml, 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml) were added. Plates were then incubated at 37°C for 48 hrs under anaerobic conditions. Similarly, *S. epidermidis* was incubated in MH agar for 24 hrs under aerobic conditions. Controls were also run simultaneously. The antimicrobial agent erythromycin (15 µg/disc) was included in the assays as a positive control and ethanol which was used for extraction were served as negative control. The plates were sealed and kept in an incubator for 24 hrs. Zone of inhibition in mm was measured to determine the antimicrobial activity of plant extracts<sup>13</sup>.

#### Antifungal activity by disc diffusion method

Fungal suspensions were uniformly spread on PDA plates. Three uniform sized wells were made with sterile borer on agar plates that had been seeded with the organism to be tested and in each well 100 µl of plant extracts of various concentrations (2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml) were added. Plates were then incubated at 30°C for 2-7 days under aerobic conditions. Control was also run simultaneously. The antifungal agent fluconazole (1mg/ml) was included in the assay as a positive control. The plates were sealed and kept in an incubator for 2-7

days. Zone of inhibition in mm was measured to determine the antifungal activity of plant extracts<sup>14</sup>.

#### Determination of minimum inhibitory concentration (MIC) of plant extracts

The MIC is defined as the lowest concentration of the extract at which the bacterium does not demonstrate visible growth. Protocol for evaluation of MIC by broth dilution method Cultures of each aforesaid bacterium and fungal strain were prepared separately in an aseptic area. The medium, i.e. nutrient broth and PDB was poured into the test tubes and sterilized by autoclave using 15 lb pressure at 121°C for 30 minutes. The tubes were then inoculated with 200 µl of each standardised culture of aforesaid microbes. Followed by addition of different concentrations of plant extracts using auto micropipettes and further incubated at 37°C and 30°C for specified period of time and observed for any microbial growth in the form of turbidity. The test procedure was carried out by preparing test samples containing different concentrations, i.e., (10, 100, 1000 µg/ml), (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg/ml), and (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml) of plant extract and observed for the lowest concentration of the extract amongst them at which microbes does not demonstrate visible growth<sup>13-14</sup>.

## RESULTS AND DISCUSSION

The phytochemical analysis of the ethanolic extract of *Berberis aristata* showed the presence of alkaloids, flavonoids, phenol, sterols, terpenes, saponins, and tannins.

The antimicrobial activity of extract was evaluated using zone of inhibition against microorganism. The result obtained is reported in table 1. Zone of inhibition of plant extracts (mm) against microorganism in triplicate (mean±SEM), zone of inhibition of erythromycin is 20 mm, Zone of inhibition of fluconazole is 10 mm.

*P. acnes* is the comparatively slow-growing, characteristically aerotolerant anaerobic, Gram-positive bacterium (rod shape) associated with a skin condition of acne. *P. acnes* bacteria reside deep inside follicles and pores, away from the surface of the skin. In these follicles, *P. acnes* bacteria use sebum, cellular debris, and metabolic by-products from the surrounding skin tissue as their primary sources of energy and nutrients. Elevated production of sebum by hyperactive sebaceous glands (sebaceous hyperplasia) or obstruction of the follicle can lead *P. acnes* bacteria to grow and multiply. In addition to *P. acnes*, as the foremost causative microorganism, *M. furfur* (yeast), *S. epidermidis*, are present in acne lesions. *M. furfur* is lipid-dependent yeast commonly found in the skin disorders such as pityriasis versicolor, pityriasis capitis, seborrheic dermatitis, and folliculitis<sup>1,3,5</sup>. Preliminary research also indicates *S. epidermidis* is universally found inside affected acne vulgaris pores, where *P. acnes* is normally the sole resident. Preliminary research also indicates *S. epidermidis* is Gram-positive, aerobic and universally found inside affected acne vulgaris pores, where *P. acnes* is normally the sole resident. As *B. aristata* extract show prominent result against *P. acnes*, *M. furfur* (yeast), and *S. epidermidis*, so *B. aristata* extract could be a good source for the anti-acne medicine<sup>6</sup>. Herbal extracts and have negligible adverse effects compared with modern medicine are commonly indicated for moderated and several forms of acne. The efficacy of these herbal agents in acne treatment is not only based on antimicrobial activity but on their antioxidant and anti-inflammatory properties by which they inhibit neutrophil migration and generation of reactive oxygen species. *B. aristata* is used in acne due to its skin detoxification property<sup>2</sup>.

## CONCLUSION

In this study, we evaluated the anti-acne activity alcoholic extract of root of *B. aristata* commonly used traditional medicinal plants from India. Extract displayed a potent antibacterial activity in the dose-dependent manner. MIC of extract indicating that these plants could be a good source for the anti-acne medicine. Further studies are necessary for these potent plant extracts to evaluate the other parameters of anti-acne efficacy (e.g. in vivo efficacy and toxicity).

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