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Research Article

ANTI-ACNE ACTIVITY OF ACETONE EXTRACT OF *PLUMBAGO INDICA* ROOT

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ABSTRACT

Objective: In the present study, an attempt has been taken to investigate the *in vitro* anti-acne activity of acetone extract of root of *Plumbago indica*.

Methods: The anti-acne activity was investigated against two acne causing bacteria, i.e., *Propionibacterium acnes* and *Staphylococcus epidermidis* and yeast *Malassezia furfur* by the well diffusion method.

Results: The result showed that drugs were active against all microorganisms. The minimum inhibitory concentration of the *P. indica* root extract against test *P. acnes*, *S. epidermidis*, and *M. furfur* was found to be 600, 200, and 300 µg/ml, respectively. However, the extract acts in a dose-dependent manner against a causative microorganism.

Conclusion: P. indica has potential activity against acne-causing microorganism, and hence this can be used in topical anti-acne preparations.

Keywords: *Plumbago indica*, Acne, *Propionibacterium acnes, Staphylococcus epidermidis, Malassezia furfur,* Skin disorder.

INTRODUCTION

The search for pharmaceuticals and cosmeceuticals to combat skin disorder continues to be a major research and development initiative in the pharmaceutical and personal care industries. Herbal approach with a history of use in traditional cultures has entered the growing "cosmeceuticals" market [1]. The world is now moving toward the herbal medicine or phytomedicine: An ancient approach turning into a future potential source of therapeutics. The new found popularity is due to their proven efficacy for primary health care because of safety and lesser side effects. Thus, natural medicine is attracting renewed attention from both practical and scientific view even though the mode of action of folk herbal medicines and related products from nature is more complex than a mechanistic clarification of a single bioactive factor [2]. 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 [1]. Acne usually affects almost everybody during the life [3]. It 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* [4]. Natural treatments for acne vulgaris have much to offer besides many mainstream dermatology treatment options available. All these prevailing treatments carry risks, and none is completely satisfactory. Therefore, natural alternatives are gaining greater research support. Much disparate and introductory research exists on the effects of herbs on multiple aspects of acne. There is a need for a comprehensive approach combining multiple herbs that could help people with acne in preliminary stages. Moreover, the possibility of this topical approach may serve patients significantly by natural therapies despite continued resistance from mainstream dermatology [5]. Topical treatment is widely useful in topical skin infections confined to the stratum corneum, squamous mucosa, etc. Such diseases include acne, dermatophytosis, candidiasis, tinea nigra, and fungal keratitis [6]. Ancient records show the use of herbals in various indigenous systems of medicine including Indian subcontinent which is a vast repository of medicinal plants being used in traditional medical treatments and providing an outstanding contribution to modern therapeutics. The urge is advancement and harmonization of rich sources of knowledge leading to the integration of phytomedicine into the health system that should be developed in such a way to bring harmony between the traditional and modern system of health care with a minimum threat to each other [7]. The proposed research work is designed to study the impact of topical herbal approach to combat acne, also termed acne vulgaris, an extremely common cutaneous inflammatory disorder of multifactorial origin with prevalence in adolescents. 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. 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 [8]. However, these agents are often accompanied by severe side effects and drug resistance. Therefore, phytotherapeutic approaches with high antibacterial activity and without side effects have been extensively studied as an alternative. In this context, acetone extract of root of *Plumbago indica* has been screened for the aforesaid anti-acne activity [9].

MATERIALS AND METHODS

Plant materials and extract preparation

Dried roots of *P. indica* were procured from the local commercial suppliers of Jalandhar, Punjab. Authentication of *P. indica* was done by Thukral, Professor, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, 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 [10].

Determination of *in-vitro* anti-acne activity

Collection of bacterial strains

Aerobic bacteria: *Staphylococcus epidermidis* (MTCC 3382) and anaerobic bacteria: *P. acnes* (MTCC 1951) were obtained from the

Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh.

Growth conditions and culture medium

Fresh cultures of the isolates of aerobic and anaerobic bacteria were suspended in nutrient broth and incubated. *S. epidermidis* was cultured in Mueller-Hinton (MH) agar medium and incubated for 24 hrs at 37°C in aerobic conditions, and *P. acnes* was cultured in brain heart infusion (BHI) agar medium and incubated anaerobically with 1% glucose at 37°C for 48 hrs [11,12].

Collection of fungal strain

Malassezia furfur (MTCC 1765) was obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh.

Growth conditions and inoculum preparation

The strain was grown on potato dextrose broth (PDB) or potato dextrose agar (PDA) following incubation at 30°C in aerobic conditions during 2-7 days [13].

Anti-microbial activity of plant extracts

Anti-microbial activity of the extract was tested using agar disc diffusion method. To evaluate the anti-microbial activity of plant extracts, *P. acnes*, *S. epidermidis,* and *M. furfur* were incubated in BHI agar, MH agar, and PDA media, respectively. Uniform sized wells were made with sterile borer on agar plates and were impregnated with plant extracts of various concentrations. Anti-microbial activity was defined by measuring the diameter of the growth inhibition zone (mm) [14]. For each isolated bacteria, seven plates were prepared from respective plant extracts and control. Incubation was done for 24 hrs. Similarly, the antifungal activity of plant extracts against *M. furfur* formerly called *Pityrosporum ovale* [15], which is a lipid-dependent yeast commonly found on the skin of adolescents responsible for superficial skin infection, was tested using agar disc diffusion method. The agar plates were impregnated with plant extracts of various concentrations and incubation was done for 2-7 days [16].

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 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 [14]. Similarly, *S. epidermidis* was incubated in MH agar for 24 hrs under aerobic conditions. Controls were also run simultaneously. The anti-microbial agent erythromycin (15 µg/disc) was included in the assays as a positive control and respective solvents (acetone and ethanol) which were 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 anti-microbial activity of plant extracts [17].

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 [16]. Control was also run simultaneously. The antifungal agent fluconazole (1 mg/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.

Concentration of *P. indica* extract (mg/ml)	Zone of inhibition against *S. epidermidis*	Zone of inhibition against *P. acnes*	Zone of inhibition against *M. furfur*
1	9.86±0.06	6.10±0.05	10.00±0.00
2	13.00±0.05	7.93±0.03	10.00±0.05
4	15.06±0.06	8.90±0.03	10.00±0.00
6	16.96±0.08	10.13±0.06	10.06±0.03
8	18.06±0.03	12.06±0.0	11.93±0.03
10	18.13±0.06	12.06±0.03	14.03±0.03

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. SEM: Standard error of mean, *P. indica: Plumbago indica, S. epidermidis: Staphylococcus epidermidis, P. acnes: Propionibacterium acnes, M. furfur: Malassezia furfur*

Determination of minimum inhibitory concentration (MIC) of plant extracts

- Collection and preservation of culture
- Determination of MIC of plant extracts.

The MIC is defined as the lowest concentration of the extract at which the bacterium does not demonstrate visible growth [17].

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 [18]. 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 the microbes does not demonstrate visible growth [4,19].

RESULTS AND DISCUSSION

The extract of *P. indica* was of brownish black with a characteristic odor. The extractive value was found to be 4.46%. The phytochemical analysis of acetone extract of *P. indica* showed the presence of flavonoids, tannins, saponins, steroids, naphthoquinones, resins, and alkaloids, etc. These secondary plant metabolites specially polyphenolics are known to possess various pharmacological effects and may be responsible for actions of *P. indica*.

The MIC value of the *P. indica* root extract against test *P. acne*, *S. epidermidis,* and *M. furfur* was found to be 600 µg/ml, 200 µg/ml, and 300 µg/ml, respectively.

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 byproducts 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, S. epidermidis* are present in acne lesions [3]. *M. furfur* is lipid-dependent yeast commonly found in the skin disorders such as pityriasis versicolor, pityriasis capitis, seborrheic dermatitis, and folliculitis [20,21]. 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 *P. indica* extract show prominent result against *P. acnes, M. furfur* (yeast), and *S. epidermidis,* so *P. indica* extract could be a good source for the anti-acne medicine. 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 anti-microbial activity but on their antioxidant and anti-inflammatory properties by which they inhibit neutrophil migration and generation of reactive oxygen species. *P. indica* is used in acne due to their skin detoxification property.

CONCLUSION

In this study, we evaluated the anti-acne activity acetone extract of root of *P. indica* commonly used traditional medicinal plants from India. Acetone extract displayed a potent antibacterial activity in the dose-dependent manner. MIC of acetone extract of *P. indica* 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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