

Research Article

OVERVIEW ON ANTI-ACNE ACTIVITY OF METHANOL EXTRACT OF DRIED FRUITS OF EMBELIA RIBES

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ABSTRACT

Objective: The present work was undertaken to validate the folk use of traditional Indian medicinal plant in the treatment of skin disorder by using scientific methods. **Method:** The anti-acne potential of methanol extract of the fruits of *Embelia ribes* were evaluated against *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Malassezia furfur* using well diffusion method. **Result:** The result showed that drugs were active against all microorganisms. The minimum inhibitory concentration (MIC) value of the *E.ribes* fruits extract against test *S.epidermidis*, *P. acne*, and *M. furfur* were found to be 500 µg/ml, 600 µg/ml, and 400 µg/ml, respectively. **Conclusion:** It was concluded from the study that the plant materials *Embelia ribes* are effective in acne vulgaris, and hence this can be used in topical anti-acne preparations.

Key words: Acne vulgaris, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Malassezia furfur*.

INTRODUCTION

Medication and cosmetic measures to overcome skin problems continue to be a foremost research and development initiatives by pharmaceutical and personal care industries. Herbal medicines with the history of use from ancient time have entered the growing 'cosmeceuticals' market for combating various skin problems [1]. It is attracting renewed attention from both practical and scientific view even though the mode of action of phytoconstituents from herbal origin is more complex than mechanisms of one bioactive factor. Ancient records show that the varieties of herbal approaches are proven to be effective for primary health care and treatment of various diseases [2].

Skin is most important and sensitive part of the human body. The external environmental exposure leads to many kinds of skin problems and disorders like acne, sunburn and pigmentation [1]. Acne is superficial skin disorder encountered in the age group from 15 to 25 years owing to increased production of sebum followed by the attack of *Propionibacterium acnes* (*P.acne*) [3]. It usually begins at puberty and worsens during adolescent age, around 12 to 13 years in females and 14 to 16 years in males. Statistic study shows that globally around 85% of young adults aged from 12 to 25, 8% of adults aged from 25 to 34, and 3% of adults aged 35 to 44 years experienced certain degree of acne during their lifetime. At age of 20 years both men and women suffers from acne. Recent research shows that, around 30% of women within their fertile period faced persistent acne [4]. One population study in Germany shows that 64% of population aged from 20 to 29 and 43% of aged from 30 to 39 had visible acne. Another study of more than 2000 adults found that 3% of men and 5% of women had mild acne at the age of 40 to 49 years. In USA, 61.9% of population aged 18 years and older were seen in clinics for acne vulgaris [5].

Natural alternatives are blooming as they are being explored for healing multiple factors related with acne [6]. Topical approach is useful in treatment of acne whereas it can also be effectively used for dermatophytosis, candidiasis, tinea nigra and fungal keratitis [7]. Natural products research plays an important role in the identification of bioactive lead molecule for the management of acne [4]. The plants producing antioxidant, antimicrobial,

anticomedogenic activity and, in certain cases hormone balancing properties can be beneficial as acne involve production of free radicals in inflammatory conditions, microorganism invasion and hormone imbalance. But still there is need for comprehensive studies of combining various herbs which can help people at preliminary stages of acne and other skin diseases [8]. The proposed research work is designed to study the impact of topical herbal approach for 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. There are several medications used for its treatment from which topical preparations like creams, ointments, and gels are common. Other oral hormonal, oral antibiotics and antibacterial medications may be prescribed for severity cases. History and cause of acne is important factor of identification before its treatment. These agents have impact on the pathogenetic factors and chosen according to type of acne lesions [9]. For many years, antibiotic and retinoid have been used to treat acne but these drugs produce a number of side effects and develop resistance due to use of antibiotics [8]. Therefore, herbal approaches with high antibacterial activity and without any side effects have been extensively studied as an alternative. In this context, methanol extract of fruits of *Embelia ribes* has been screened for the aforesaid anti-acne activity.

MATERIALS AND METHODS

Plant materials and extract preparation

Dried fruits of *E.ribes* were procured from local commercial suppliers of Jalandhar, Punjab. Authentication of *E.ribes* was done by Dr Javed Ahmad from Department of Botany of Jamia Hamdard in New Delhi. The crude plant material (dried fruits) was pulverized in coarse powder form for the purpose of extraction. Coarsely powdered dried plant material was extracted using Soxhlet apparatus. The Solvent used for extraction was methanol.

Phytochemical screening of methanol extract

The prepared extract was subjected to various chemical tests to detect the presence/absence of chemical constituents [10].

Determination of in-vitro anti-acne activity

Collection of Bacterial strains

Aerobic bacteria: *S. epidermidis* (MTCC 3382) and Anaerobic bacteria: *P.acnes* (MTCC 1951) were obtained from the Microbial Type Culture Collection Centre from Institute of Microbial Technology in Chandigarh.

Growth conditions and culture medium

The Freeze and dried microorganism was activated by suspending bacteria in 0.9% sodium chloride which was kept at 37±1°C for half an hour. The suspension of *S. epidermidis* was cultured in sterile Mueller Hinton (MH) agar medium and incubated for 24 hours at 37°C in aerobic conditions. The suspension of *P. acnes* was cultured in Nutrient agar and incubated anaerobically at 37°C for 48 hours [11].

Collection of Fungal strains

Malassezia furfur (MTCC 1765) was obtained from the Microbial Type Culture Collection

Centre from Institute of Microbial Technology in Chandigarh.

Growth conditions and inoculum preparation

Freeze dried fungal strain was activated by suspending in sterilized double distilled water. The strain was grown on potato dextrose agar (PDA) following incubation at 30°C in aerobic conditions during 2-7 days [12].

Antimicrobial activity of plant extract

Anti-microbial activity of plant extract was tested using agar disc diffusion method. In order to evaluate anti-microbial activity of plant extract *P. acnes*, *S. epidermidis* and *M. furfur* were incubated in Nutrient agar, MH agar and PDA media respectively. Uniform sized wells were made with sterile borer on agar plates and were impregnated with plant extract of various concentrations. Antimicrobial activity was calculated by measuring the diameter of the growth inhibition zone (mm). For each isolated bacteria, three

plates were prepared of given plant extract and control. Incubation was done for 24 hours. Three wells were made in each plate for comparative study [13].

Similarly anti-fungal activity of plant extract against *M. furfur* formerly called *Pityrosporum ovale* was tested using agar disk diffusion method. The agar plates were impregnated with plant extract of various concentrations and incubation was done for 2-7 days [14].

Antibacterial screening by disc diffusion method

Bacterial suspensions were uniformly spread on each agar 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 50µl of plant extract of various concentrations (100mg/ml, 200mg/ml and 300mg/ml) were added. Plates were then incubated at 37°C for 48 hours under anaerobic conditions. *S. epidermidis* was also incubated in MH agar for 24 hours under aerobic conditions. Controls were also prepared and incubated under same condition. The anti-microbial agent clindamycin with concentration of 15µg per disc, was used as a positive control and methanol which was used as solvent for dilutions served as negative control. Zone of inhibition in mm was measured to determine anti-microbial activity of plant extracts [15].

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 50µl of plant extracts of various concentrations (100mg/ml, 200mg/ml and 300mg/ml) were added. Plates were then incubated at 30°C for 2-7 days under aerobic conditions. Control was prepared and incubated at same condition. The anti-fungal agent fluconazole (1mg/ml) served as a positive control in the assay. The plates were sealed and kept in incubator for 2-7 days. Zone of inhibition in mm was measured to determine anti-fungal activity of plant extract [14]

S.no.	Concentration of extract(mg/ml)	<i>E.ribes</i> Zone of inhibition against <i>S. epidermidis</i>	Zone of inhibition against <i>P. acnes</i>	Zone of inhibition against <i>M. furfur</i>
1.	100	7.88 ±0.02	6.08±0.05	10.40±0.03
2.	200	10.92±0.02	10.21±0.02	12.33±0.03
3.	300	10.93±0.05	10.40±0.03	14.67±0.01

Determination of minimum inhibitory concentration (MIC) of plant extract

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

“The MIC is defined as the lowest concentration of the extract at which the bacterium does not demonstrate visible growth” [16].

Protocol for evaluation of MIC by broth dilution method

Evaluation of MIC was done by addition of different concentrations of plant extract in previous cultured bacterium and fungal strain test tubes and incubated at 37°C and 30°C for specified period of time and observed for any microbial growth in form of turbidity. The test procedure was carried out by preparing test samples containing different concentrations of 50,100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg/ml among which the lowest concentration of extract was determined at which bacteria showed no visible growth [3].

RESULT AND DISCUSSION

The extract of *E.ribes* was of Reddish brown with a characteristic odour. The extractive value was found to be 9.2%. The phytochemical screening of methanol extract of *E.ribes* showed presence of alkaloids, flavonoids, tannin, carbohydrates, resin and steroids. These secondary plant metabolites which are known to be possess various pharmacological effects and may be responsible for

actions of *E.ribes*. The MIC value of the *E.ribes* fruits extract against test *S.epidermidis*, *P. acne*, and *M. furfur* were found to be 500 µg/ml, 600 µg/ml, and 400 µg/ml, respectively.

CONCLUSION

From this review, we evaluated the anti-acne activity of Methanol extract of dried fruits of *Embelia ribes*, commonly used traditional medicinal plants from India. Methanol extract displayed a potent antibacterial activity in the dose-dependent manner. MIC of Methanol extract of *Embelia ribes* 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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