

Evaluation of Anti-acne Activity of Hydroalcoholic Extract of *Embelia ribes* Burm.

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ABSTRACT

Medicinal plants play an important role in the development of potent therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics as a source of many valuable secondary metabolites which serves as plant defence mechanisms against predator such as microorganism, insects and herbivores which have been proved to be potentially active compounds. There is a tremendous increase in search of antimicrobial plant extracts due to the fact that the resistance offered against antibiotic by the microorganism, in short the effective life span of any antibiotic is limited. One such folklore plant which has number of traditional uses is *Embelia ribes* Burm of myrsinaceae family. The synergistic action of embelia constituents appears to be superior to that of single constituents. The current research was focused on evaluation of antiacne activity of *Embelia ribes* Burm. extract followed by its biological screening. The fingerprinting and spectroscopic analysis of the extract was determined. The attempt was made to investigate the extract for the said activity with the goal of elucidating the active potential compounds.

Keywords: *Embelia ribes*, Broth dilution, cup plate method, High performance thin layer chromatography

INTRODUCTION

Acne is a chronic inflammatory follicular disorder of the skin, occurring in specialized pilosebaceous units on the face. Comedonal bacteria, "*Propionibacterium acnes*", play an important role in the pathogenesis of acne inflammation by inducing polymorphonuclear leukocytes (PMNL) and monocyte and/or macrophages to produce pro-inflammatory mediators. These organisms produce neutrophil chemotactic factors, which attract neutrophils to release inflammatory mediators such as reactive oxygen species (ROS) and lysosomal enzymes, resulting in disruption of the integrity of the follicular epithelium¹. Acne is a skin disorder characterized by plugged pores or pilosebaceous unit. Pilosebaceous units are made up of sebaceous glands (oil glands) and hair follicles in the dermis or middle layer of the skin. There are lots of oil glands in the body but they are concentrated in the nose and cheek areas. The function of the oil gland is to lubricate and make the skin moist by generating oil or sebum. The sebum then flows through the follicles or tiny tubes that starts beneath the skin and leads to the surface of the skin². The sebum acts as a nutrient for a resident skin bacterium called *Propionibacterium acnes* (or more familiarly the acne bacillus), which grows abnormally in follicles whose pores are blocked³. Research had shown that multi-therapy acne treatment is the best possible option. Multi-therapy involves the use of several anti-acne medicines in such a way that they would

complement each other. The synergistic action of these acne medications result in reduced side effects, faster healing times of acne lesion and lower *P. acnes* bacterial population compared to monotherapy benzoyl peroxide, azelaic acid is a potent oxidizing agent with adverse effects such as bleaching of clothes, transient skin irritation, occasional allergic contact dermatitis, local irritation and photosensitization and presently treatment is limited to a maximum of six months. Topical retinoid can be applied once or twice daily with effects such as erythema, desquamation, occasional hyperpigmentation or hypopigmentation, and sensitization of the skin to sunlight. These products are derivatives of vitamin A, and there have been reports of malformed infants born to women who have used topical retinoids during early pregnancy⁴. Topical antibiotics are particularly useful in mild to moderate acne and in acne which is resistant to benzoyl peroxide. The development of antibiotic resistance in *P. acne* may limit the prescription of topical antibiotics⁵. Systemic antibiotics remain the main line of treatment for acne and tetracycline remains the treatment of first choice. The adverse effects of oral antibiotics including that of tetracycline include gastrointestinal upset, vaginal candidiasis, and hyperpigmentation with high dose of minocycline. The treatment of acne is directed towards correcting the altered pattern of follicular keratinization, decreased sebaceous gland activity, decreasing the formation of causative bacteria *P.*

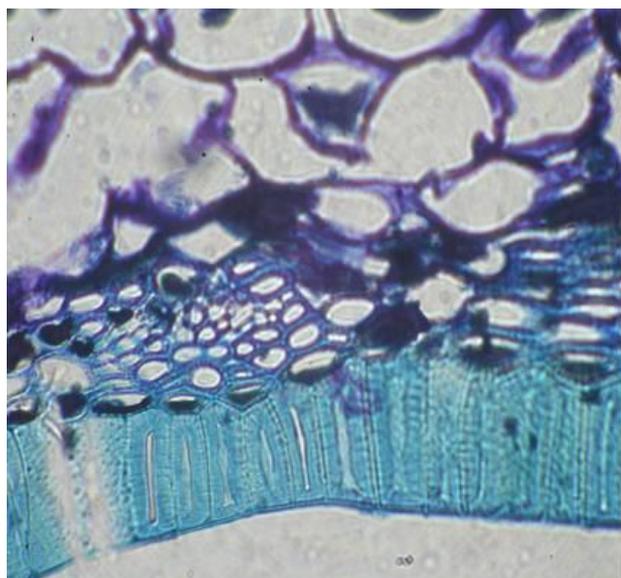


Figure 1 and 2: Histology showing epicarp and mesocarp region

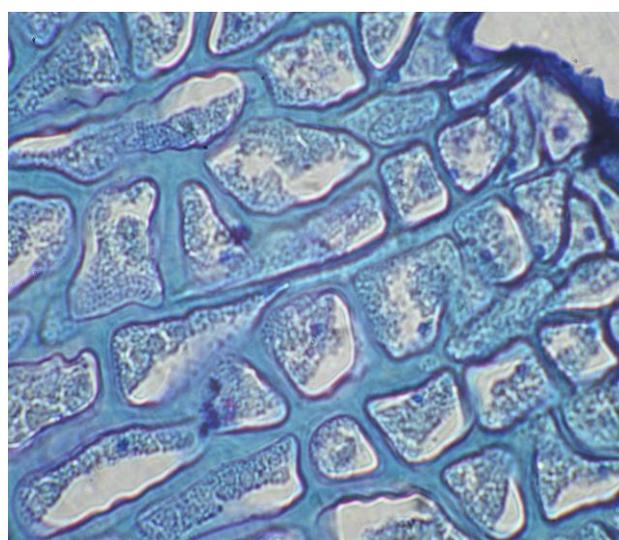
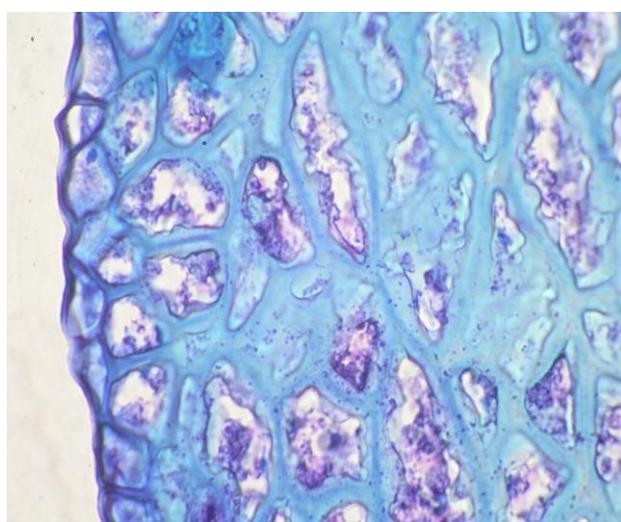


Figure 3 and 4: Histology showing Vascular strands with xylem and Phloem masses and starch grains

acne and finally leads to an anti-inflammatory effect³. Herbal plants used in folk medicine have been accepted as one of the main sources of drug discovery and their development. India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications⁶. *Embelia ribes* Burm, a medicinal woody climber, belongs to the Myrsinaceae family is an ancient, mystical, unique fruit used in several systems of medicine for a variety of ailments. It is also commonly known as false black pepper or vidanga. *Embelia* species identified by *Susruta* as anthelmintic, alternative & tonic. It acts as ascaricidal, anthelmintic, carminative, diuretic, astringent, anti-inflammatory, antibacterial and febrifuge. Active principles are found to be estrogenic and weakly progestogenic. Pulp is purgative. Fresh juice is cooling, diuretic and laxative. The root acts as be antimicrobial and anti-diarrhoeal. The

seeds are spermicidal, oxytocic and diuretic. The plant is also useful and known for its blood purifying properties. The effect of di-isobutyl amino derivatives shows anti-inflammatory, hypotensive and anti-pyretic effects. Aqueous extract of the fruit shows anthelmintic against tapeworms⁷. The traditional claim on this plant for the fruit reveals the anti-acne effect which can reduce the sebum production. That is why the current research work is focused to carry out antiacne activity. The plant contains tannins, alkaloids, triterpenes and saponins and anti-microbial activities of tannins are well documented like *Embelia officinalis* as it contains gallic acid. The growth of many fungi, yeasts, bacteria and viruses were inhibited by tannins. Tannins may serve as a natural defense mechanism against various microbial infections. For example tannin components of epicatechin and catechin (*Vaccinium vitis-idaea* L.) showed strong anti-microbial activity against bacteria and fungi. Methanol extracts of *T. citrina* fruit yielded known tannins such as corilagin, punicalagin and chebulagic acid that were tested for anti-microbial action⁸.

MATERIALS AND METHODS

Plant Collection and Extraction

The fresh fruits of the plant *Embelia ribes* Burm were collected from the local region of Pune. It was authenticated by Department of Botany, University of Pune (Voucher No. Bot /35/11). The powder of fruit prepared was extracted by maceration with hydroalcoholic mixture (60:40) for 72 hours.

Histology

Histology was performed as to know the various structural bodies which are present in the plant such as crystals, grains and also which is a part of the authentication. The plant specimen of fruit were cut and fixed in FAA and then infiltration of specimen is carried out by paraffin wax. The specimens were sectioned with the help of microtome with thickness of 10-12 µm. The sections were stained with toluidine blue⁹. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units¹⁰.

Preliminary Phytochemical Screening

The extract was then subjected to preliminary phytochemical screening to detect the presence of various phytoconstituents by various chemical tests¹¹.

Antiacne activity of the Extract

The lyophilized cultures of bacteria *Propionibacterium acne* (MTCC No. 1951) were procured from Indian Institute of Microbial Technology (IMTECH), Chandigarh. The dilutions of extract were prepared and brain heart infusion broth was prepared. Tween 80 and 0.03ml thioglycolic acid per 100 ml was added in the prepared broth as a reducing agent¹². The 25 ml of the medium was poured in the ten test tubes followed by sterilization with autoclave at 15 lb pressure and 121°C for 30 minutes. Using sterile pipette exact amount of extract was added as indicated in the Table 1 and the final volumes were adjusted to 10ml with medium followed by inoculation of cultures and incubation at 37 °C for 48 hrs. The growth in the tubes was monitored by turbidity method and MIC of the extract was determined^{13, 14}. The extract was also subjected to antiacne activity by Cup plate diffusion method using Clindamycin as internal standard (positive control) and zone of inhibition with MIC was determined¹⁵. Both this analysis was performed thrice to confirm the efficacy of the result.

Fingerprinting and Spectroscopic analysis

The extract and standard (gallic acid) then subjected to fingerprinting analysis. The calibration curves were plotted (for the purpose to know λ max) at λmax 264 nm using UV spectrometer.

RESULTS

Pharmacognostic Study

The quality control parameters were established and proximate analysis found to be significant. Preliminary phytochemical screening revealed the presence of tannins and alkaloids.

Histology

The required samples of different organs were cut and removed from the plant and fixed in FAA. After 24 hrs of fixing, the specimens were dehydrated with graded series

Table 1: Protocol for evaluation of MIC by broth dilution method

Sr. No.	Amount of Extract/ml	Amount of medium	Total Vol of Solution (ml)	Conc. of Extract in final sol (ml)
1	0.1	9.9	10	0.1
2	0.2	9.8	10	0.2
3	0.3	9.7	10	0.3
4	0.4	9.6	10	0.4
5	0.5	9.5	10	0.5
6	0.6	9.4	10	0.6
7	0.7	9.3	10	0.7
8	0.8	9.2	10	0.8
9	0.9	9.1	10	0.9
10	1.0	9.0	10	1.0

Table 2. Zone of inhibition by cup plate method

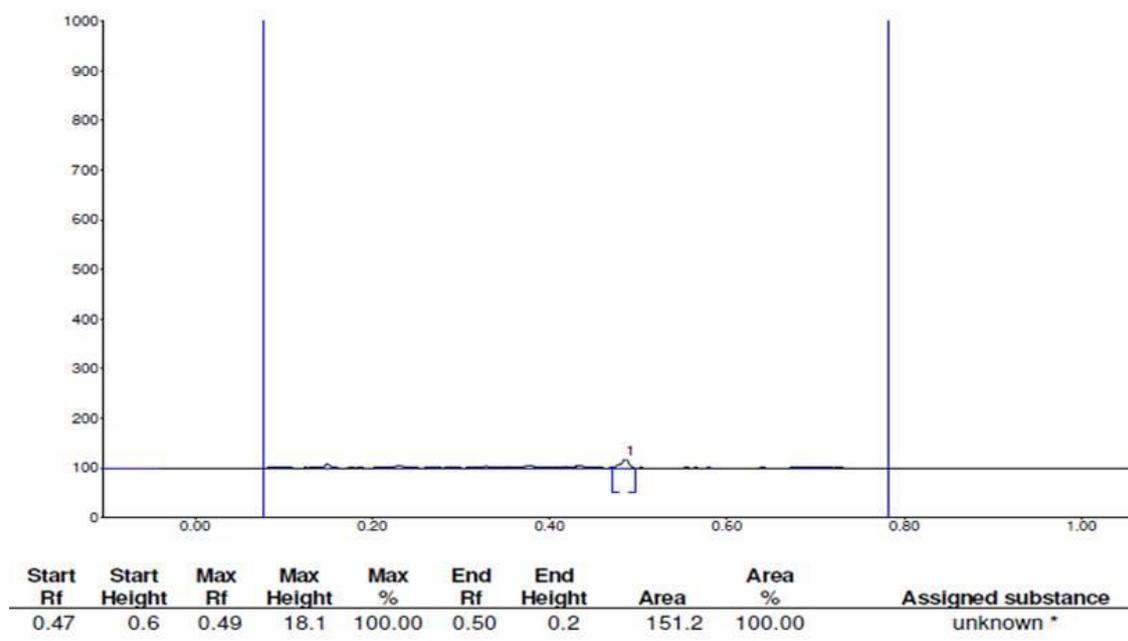
Sr. No.	Amount of Extract/ml	Zone of inhibition in mm for <i>Embelia</i> extract (including borer size)	Amount of Standard drug	Zone of inhibition For Standard drug
1	0.5	14	0.2	11
2	0.6	15.5	0.3	12
3	0.7	18.7	0.4	14.5



Figure 5: Fingerprinting analysis for presence of Tannins (Rf 0.49)

of tertiary -Butyl alcohol. The fruit or the pericarp is thick and fleshy and consist of less prominent epidermis or epicarp. Sclerides are distributed throughout in Mesocarp (Figure 1 and 2). Vascular strands have radial files of small xylem elements with phloem masses (Figure 3, 4)¹⁰

:k 12, ID: E. ribes



Graph 1 and 2: Planar chromatogram for presence of Tannins

Screening of Extract for Antiacne activity

To screen the plant material for their antiacne activity *in vitro* experiments were carried out by using the organism *P. acnes*¹⁶. The culture media was standardized using McFarland turbidity standard. The broth dilution method was used to detect the MIC of the extract. (Table 1).

Diameter of standard borer 6 mm, n= 3

The results as shown in Table 2 depict that the MIC values of hydroalcoholic extract of *E. ribes* was found to be 500 mg/ml¹⁷. The zone of inhibition was determined by cup plate diffusion method where an increase in antiacne activity was observed from zone of lysis emphasizes that the lysis may be due to the active components present in the hydroalcoholic extract of the plant⁵. All this analysis has been carried out thrice to confirm the efficacy of the extract.

Fingerprinting and Spectroscopic analysis. The extract shows RF value 0.19 and 0.49 (Graph 1) after fingerprinting analysis which indicates presence of tannins (Figure 7).

DISCUSSION

The results of the zone of inhibition for *E. ribes* Burm are shown in Tables. The hydroalcoholic extract shows good anti-acne activity when compared to standard drug clindamycin. Thus the targets in the microbial cell could be surface exposed adhesion, cell wall peptides and membrane bound enzymes. Here tannins are the major phytoconstituents present in this plant which is responsible for anti-acne action due to cell lysis with leakage of cytoplasmic constituents as in plant *Portulaca oleracea*, also ethanolic extract of *Vernonia scorpioides* possess anti-acne action by improving regeneration due to the presence of tannins. The probable mode of action may be the formation of irreversible complexes with

nucleophilic amino acids, often leading to a loss of function of vital proteins in the microbial organism⁸.

As infections being a major cause of morbidity and mortality in burn patients, the herbal extract may prevent infection that leads to high risk of sepsis. Thus the experimental findings may suggest the plant was found to be effective as to inhibit the effect caused by the *P. acnes*.

CONCLUSION

This study thus demonstrates the antiacne activity of hydroalcoholic extract which is effective in the treatment acne vulgaris.

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