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EVALUATION OF ANTI-ACNE POTENTIAL OF *IMPATIENS WALLERIANA* EXTRACT

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ABSTRACT: Objective: Knowledge about medicinal plants can contribute to modern therapeutics. *Impatiens walleriana* [Family: Balsaminaceae] an ornamental, nutritive herb has relevance in African and Asian traditional medicines. Traditionally *I. walleriana* is used for anti-inflammatory, anti-dermatitis effects and is known to relieve skin irritation. It is applied on skin to treat bacterial and fungal infection. Acne is a common skin disorder mainly seen in adolescents. Treatment for acne revolves around anti-bacterial, anti-inflammatory and antioxidant effects of drugs. Anti-bacterial and antioxidant activities of *I. walleriana* have been reported previously reported. In the present study anti-acne potential of *I. walleriana* extract is evaluated against *Propionibacterium acne* and an attempt has been made to correlate findings with traditional claims. **Methods:** Hydroalcoholic extract of aerial parts of *I. walleriana* is evaluated for effectiveness against *P. acne* by measuring zone of inhibition using well diffusion method and by finding MIC. Tetracycline is used as standard. **Results:** Results of Zone of Inhibition are promising with an average of 11mm, 08mm and 4mm in the duplicate readings obtained. MIC results with 1.6µl/ml concentration reinforces the effectiveness of *I. walleriana* extract against *P. acne* organism. **Conclusion:** Our hypothesis is met with positive results. *I. walleriana* extract can be a good source for supplementing existing anti-acne medicines.

INTRODUCTION: Acne is a common skin affliction, encountered by adolescents and many adults continue to struggle even after their teenage years. Though acne is not a lethal or debilitating disorder, it is painful and disfiguring ¹. It affects confidence in individuals,, reducing self-esteem and causing emotional distress ². Acne is a common skin disorder, almost affects everybody during lifetime ³. Acne is characterized by inflammatory lesions or comedones in the face, back and trunk regions ⁴. *Acne vulgaris* is multifactorial in origin ⁵.

Genetics plays an important role, and acne develops as a result of follicular epithelium hyperkeratinization, increased sebum production, proliferation of *P. acne* like bacteria and inflammation. *P. acne*, *Staphylococcus epidermidis* and *S. aureus* are involved in the bacterial colonization of the follicle. Among the three organisms *P. acne* plays an important role in pathogenesis and progression of acne ⁶.

The other two organisms mainly contribute by forming a biofilm which provides anaerobic conditions for *P. acne* to thrive ⁶. Reactive oxygen species (ROS) are subsequently generated from hypercolonization of *P. ance* and from UV exposure ⁷. Sebum also contains ROS and these free radicals also contribute to irritation of acne ⁸. Current therapies for acne by topical and systemic routes employ use of antibiotics, comedolytics, anti-inflammatory drugs, zinc in addition to laser

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treatment. The currently used drugs and approaches have limitations in the form of toxicity, side effects and high costs⁸. Among the prevailing treatments not a single is full-proof and satisfactory³. *P. acne* is also showing resistance to the currently used antibiotics¹. Owing to these problems novel new therapies are in demand.

Traditionally medicinal plants have been used for managing a number of dermatological conditions¹. Valuable leads can be obtained from herbal resources¹. Folklore use of medicinal plants can serve as a guide to identify bioactive compounds and is useful compared to random search.¹ Traditional herbs are also unexplored sources for developing new drugs².

Herbs can play an important role in finding new lead compounds for acne. Natural remedies are blooming as they target multiple factors related to acne⁹. Recent trends show use of plant-based products as adjunctive and alternatives to current therapies¹⁰. Herbal approaches are also relatively free of side effects and are economical¹¹. It is estimated that nearly 20% of all visits to dermatologists are related to the treatment of acne¹².

Genus *Impatiens* is mainly known for antioxidant and antimicrobial activity. It is also recognized for antiallergic, antipruritic, antidermatitic, antihistaminic and COX-2 inhibitory activities¹³. *I. walleriana* is less researched compared to other *Impatiens* species. *Impatiens* treats bruises. *I. walleriana* leaves are used to treat chicken pox in Indonesia¹³. In Costa Rica flowers are used to heal wounds by rubbing onto the wound¹³.

I. walleriana Hook.f is an ornamental plant belonging to family *Balsaminaceae*. It is both nutritive and medicinal. It finds use in Brazilian folk medicine¹⁴. Antioxidant, antibacterial and antifungal activities of the plant are reported but it has not been screened for effectiveness against *P. acne* hence the present study.

MATERIALS AND METHODS¹⁵:

Collection of Plant and Extraction: The fresh leaves, stem and pink flowers of *I. walleriana* were collected from a local garden in Belagavi during the month of July 2024. Authentication for the plant was carried out at Regional Medical Research

Centre, Belagavi and specimen was deposited in the said research centre herbarium (Accession number RMRC – 1341). The fresh plant parts were first washed using tap water and soiled portions were cleaned to exclude root portion followed by drying in shade at room temperature. The dried plant material was size reduced to coarse powder and stored in an air tight bottle.

Extraction was done using 50:50 Distilled water and 95% alcohol as solvent by maceration for 72 hours. After extraction the solvent was evaporated, and residue was collected then % yield was calculated using the formula:

$$\% \text{ Yield} = W_1 / W_2 \times 100$$

Where, W_1 = weight of the extract after extraction, W_2 = weight of plant powder.

Well Diffusion Assay Procedure: The well diffusion test was performed to evaluate the antimicrobial activity of the compounds. Initially, colonies from the agar plate were transferred to plates using a swab. The inoculum was prepared by adjusting the turbidity to equal that of a 0.5 McFarland turbidity standard visually.

Within 15 minutes, a sterile cotton swab was dipped into the inoculum, excess removed, and the agar plate was swabbed three times, rotating 60° between streaks. The inoculated plate stood for 3-15 minutes before making wells.

Stock solutions were prepared by dissolving 10mg of compound in 1ml of Dimethyl sulfoxide. Four wells (5mm diameter) were created on each plate using a heated hollow tube. Using a micropipette, 200µl, 100µl and 50µl of the stock solution were added to each well and tetracycline was added to the fourth well.

Plates were incubated within 15 minutes, inverted, stacked (no more than five high), and incubated for 18-24 hours at 37°C. After incubation, plates with confluent or nearly confluent lawns were read. The diameter of the inhibition zone was measured to the nearest whole millimeter using a measuring device.

MIC Test Procedure: The Minimum Inhibitory Concentration (MIC) test was performed using the broth dilution method. For *I. walleriana* extract,

nine serial dilutions were prepared in Brain Heart Infusion (BHI) broth.

Initially, 20μL of the drug was added to 380μL of BHI broth in the first tube. Then, 200μL of BHI broth was added to each of the remaining nine tubes. Serial dilutions (10-1 to 10-9) were prepared by transferring 200μL from the previous tube to the next, starting from the initial tube.

5μl of the test organism containing stock culture was inoculated into 2ml of BHI broth and incubated. Subsequently, 200μl of this culture

suspension was added to each serially diluted tube. The tubes were incubated for 24 hours and observed for turbidity to determine the MIC.

RESULTS AND DISCUSSION:

Extract Yield: *I. walleriana* extract exhibited consistency that was resinous and appearance was brown with earthy odour. The yield of the extract was found to be 15.36 g%. Zone of inhibition has been carried out twice and duplicate readings are depicted as IWA I and IWA II:

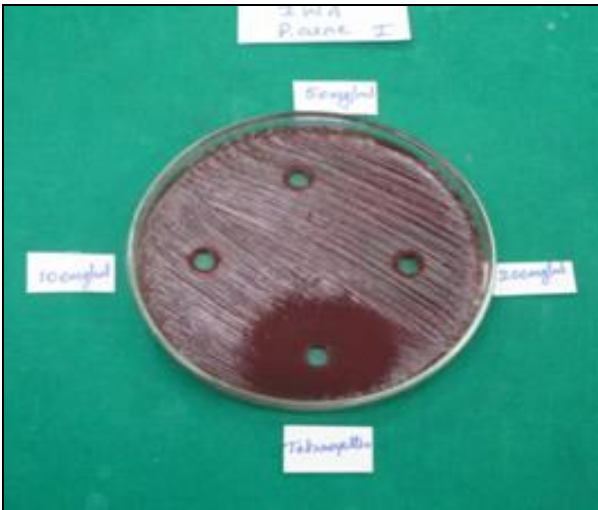


FIG. 1A: IWA I FRONT VIEW

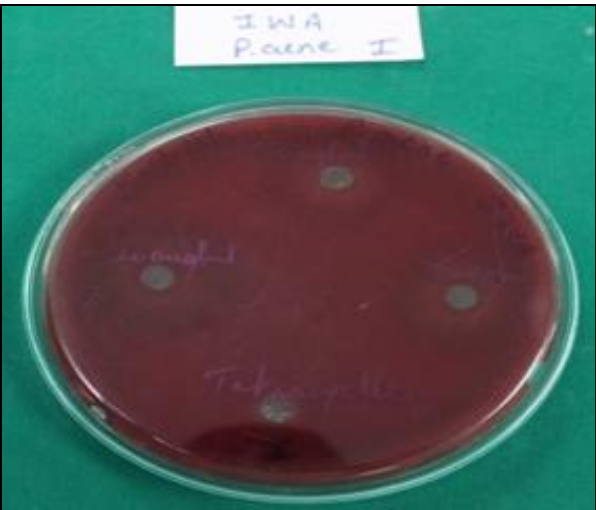


FIG. 1B: IWA I BACK VIEW

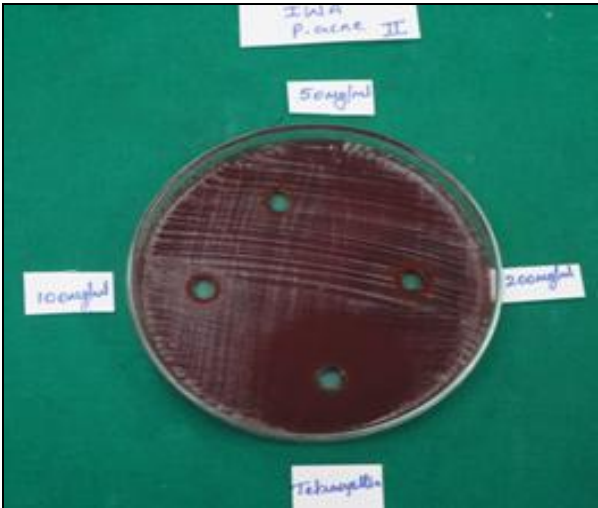


FIG. 2A: IWA II FRONT VIEW

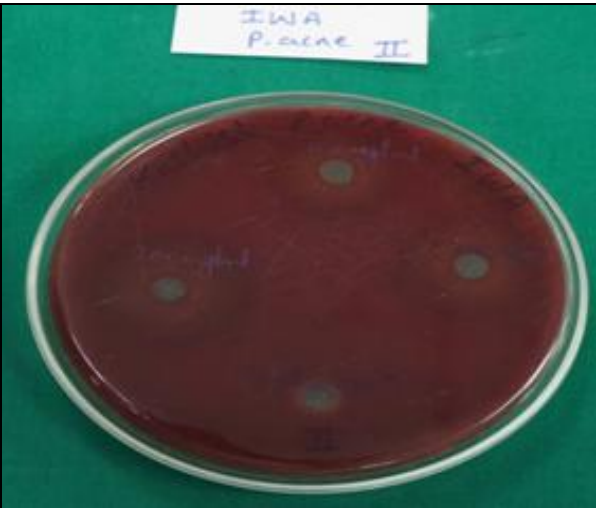


FIG. 2B: IWA II BACK VIEW

TABLE 1: RESULTS OF WELL DIFFUSION ASSAY

Treatment	IWA I	IWA II
200 mg/ml	10mm	11mm
100 mg/ml	8mm	8mm
50 mg/ml	8mm	R
Tetracycline 50μg/ml	38mm	35mm

R= Resistant.

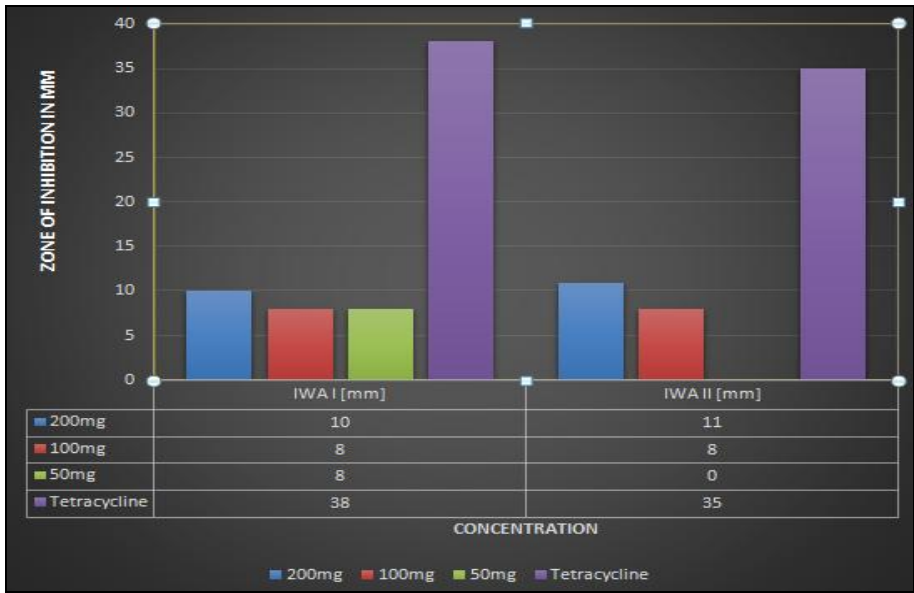


FIG. 3: WELL DIFFUSION METHOD

MIC Results:

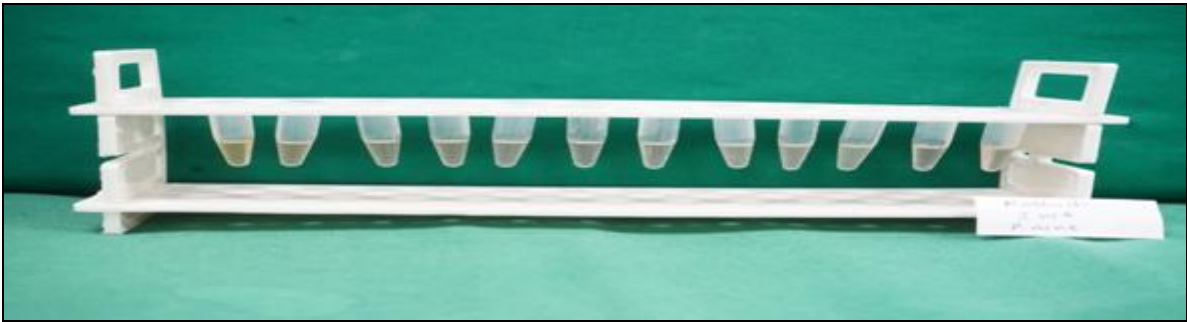


FIG. 4: MIC TEST RESULTS

TABLE 2: RESULTS OF MIC

Samples	<i>P. acne</i>	IWA
100 µl/ml	P	S
50 µl/ml	P	S
25 µl/ml	P	S
12.5 µl/ml	P	S
6.25 µl/ml	P	S
3.12 µl/ml	P	S
1.6 µl/ml	P	S
0.8 µl/ml	P	R
0.4 µl/ml	P	R
0.2 µl/ml	P	R
B+C	P	S
B+O	P	R

S=Sensitive; R=Resistant; B+C = Broth + Control; B+O = Broth + Organism, P= Present.

Medicinal plants with their chemical constituents offer high therapeutic benefits. Several plants have been researched for treatment of diseases. To resolve concerns like antibiotic resistance herbal Plants with improved safety and efficacy are handy. If proven effective the plant can become a supplement to the current antibacterial therapy¹¹.

P. acne is slow growing, aero tolerant–anaerobic rod shaped gram positive bacterium associated with acne. It resides deep inside in follicles and pores where these bacteria use sebum and metabolic by-products for their survival. Hyperactive sebaceous gland, obstruction of the follicle like factors can significantly increase growth of *P. acne*. In addition to *P. acne*, *M. furfur*, an yeast and *S. epidermidis* are also present in acne lesions. *S. epidermidis* is gram positive, aerobic and found in affected acne pores in addition to *P. acne*¹⁰. *S. aureus* is also involved in triggering inflammatory acne flare-ups⁶. Reactive oxygen species are generated from hyper-colonization of *P. acne* and from exposure to UV-rays. Herbals inhibiting these organisms and having antioxidant activity are of great interest in treating acne⁷. Herbals with healing multiple factors related to acne are in demand³. The objective of the present study was to investigate effectiveness of *I. walleriana* extract against acne causing *P. acne* organism and confirm

the folklore use of *I. walleriana* for various skin related afflictions. Such studies backed by folk use and traditional medicinal claims are better than random screening of plant extract to find new medicinal alternatives. Our hypothesis that *I. walleriana* extract being antioxidant, antibacterial, anti-inflammatory might be effective against *P. acne* is well substantiated in our present investigation wherein antibacterial effect is confirmed through MIC as well as Zone of inhibition study. In our previous findings we learnt about the effectiveness of *I. walleriana* extract against *S. epidermidis* and *S. aureus* the other organisms involved in acne development^{11, 16}. *I. walleriana* extract is effective against all the three bacteria causing acne namely *P. acne*, *S. epidermidis* & *S. aureus* making it an interesting contender for developing an alternative for acne. This is also the first study to document *I. walleriana* extract effect against *P. acne*.

Results of Zone of Inhibition are promising with an average of 11mm, 8mm and 4mm in the duplicate readings obtained. Effectiveness of the standard tetracycline is much higher than *I. walleriana* extract nevertheless *I. walleriana* extract in principle can constitute adjunctive to currently used antimicrobials for the treatment of acne. A dose dependent increase in antibacterial effect has been observed in the study. MIC results with 1.6µl/ml concentrations show the effectiveness of *I. walleriana* extract against *P. acne* organism. According to Gibbson and researchers isolated phytochemicals should have MIC < 1mg/ml⁷. In this study the constituents have not been isolated as such but extract has been studied. MIC results of *I. walleriana* reinforce antibacterial effectiveness. The plant is reported to have Flavonoids, Alkaloids, Carbohydrates, Tannins, Anthocyanins, Caffeic acid, minerals such as calcium, phosphorous, potassium, sodium, chloride, magnesium, iron, zinc, iodine, chromium, copper, fluoride, molybdenum, manganese and selenium etc^{11, 13}. In the present study the observed effect may be due to these constituents showing effect including synergistic effect which cannot be ruled out. Presence of zinc like constituents in the extract may be contributing to antiacne effect by anti-inflammatory action. Generally polar organic solvents are used to obtain extract high in Phenolic compounds supporting antioxidant effect, hence in

the present study hydroalcoholic (50:50) solvent has been employed for extraction. Anti-acne drugs have side effects like skin drying and also have other toxicities. Acne infection is also associated with irritation. *I. walleriana* as per traditional claims offers advantage in terms of resolving skin irritation. Further studies can be taken up to validate the claim. Additionally effectiveness of *I. walleriana* extract against the yeast implicated in acne *M. furfur* can be taken up. As *I. walleriana* extract effectiveness against *P. acne* is learnt through this study, clinically resistant strains of *P. acne* can be subjected to testing specifically. *I. walleriana* extract being effective against *S. epidermidis*, *S. aureus* and *P. acne* may benefit inflammatory acne in papules, pustules and reduce bacterial infection which leads to abscess development. *I. walleriana* extract can serve as an alternative to supplement existing antibacterials for acne treatment following evaluation of all parameters of anti-acne efficacy.

CONCLUSION: Our hypothesis is met with positive results for anti-acne activity of hydroalcoholic extract of *I. walleriana*. *I. walleriana* extract can be a good source to supplement existing anti-acne medicines. Further studies are required to evaluate other parameters of anti-acne efficacy and find the exact mechanism of action.

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CONFLICT OF INTEREST: Nil

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