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Research Article

Antiacne Potential Of Impatiens Walleriana Hook.F. Phytochemical Analysis And Antibacterial Efficacy: Ethnopharmacological Study

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ABSTRACT

Herbal medicines from plants are being increasingly used to treat a number of ailments and number of culinary herbs have been evaluated for their beneficial effects in the management of ailments. Plant based antiacne are in demand for its cosmetic or pharma industry. In pursuit of finding such safe drug presents antiacne activity employing a hydro alcoholic extract of plant namely Impatiens walleriana. Antiacne effect of Impatiens walleriana species is not reported, present study was undertaken to investigate the antiacne effect on gram positive organisms. The effect of 50mg/ml, 100mg/ml and 200mg/ml of the hydroalcoholic extract on gram +ve organisms has been tested using the disc diffusion method. Finding confirmed the antiacne potential as the organism used in the study were hydroalcoholic extract. Further phytochemical studies reveal the presence of proteins, phenolics, tannins, steroids and flavonoids. The observed antiacne activity could be attributed to the presence of any of these synergistic effect due to these constituents. Ethnopharmacological claim for using this plant to treat these severe acne is substantiated and further studies can be taken up using clinical and resistant isolates furthering the investigation.

INTRODUCTION

A member of the "Balsaminaceae" family of ornamental plants, which has more than 1200 species scattered globally, is Impatiens walleriana. The plant Impatiens walleriana, sometimes known as "Busy Lizzy," thrives best in damp, humid environments. It includes phenolic substances, flavonoids, anthocyanins, steroids, and carbs, among other things. It is a garden plant that may

be eaten, and traditional medicine uses it for its abortifacient, antibacterial, anticancer, and antioxidant properties [1] [2]. It is well known fact that several plants possess healing properties. Ethnopharmacological knowledge is useful tool to search and find species having bioactive potential and develop herbal remedies. The world health organization estimates that 65%-80% of world's

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population in developing countries prefer to use plants for their health care. There is an increasing resistance to antibiotics coupled with increased incidences of ADRs to the current drugs used in treatment of Infections. [3] Native to the mountains of East Africa. Named "Sultana" after the Sultan of Zanzibar, it was first introduced in 1896 in the cold marshes of Zanzibar [4] Their apparent impatience to expel the ripe seeds, hence the botanical name 'impatiens', they are continuously blooming and often referred to as the 'busy lizzie'. It is naturalized throughout the world, including North America, Australia and the Pacific Islands, and grows in scrub along waterways. It grows in rich, moist soil and prefers shade. Impatiens grows up to 30-38 cm and has broad leaves, often alternating near the top of the plant. The flowers are abundant, with five petals and spores. Hybrid strains of impatiens plants have white, red, pink, orange, and sometimes bicolor flowers that are known to have one or more petals. The leaves can be oval or heart-shaped green. Sometimes there is a brown shield with red or brown spots underneath, [5] Impatiens have a variety of uses. It has bactericidal activity and reduces the problem of chemical control of fungal diseases in agriculture. [6] Using genetically modified toxic proteins delivered to mosquitoes through nectar, impatiens can be turned into a nectar plant that kills mosquitoes, which are known to live in a plant cage. [7] Toxic metal pollution in the environment has increased. Impatiens tend to concentrate heavy metals, such as lead, from the soil. [8] This aspect should be taken into account before exercising. Impatiens used in phytoremediation experiments have been shown to be effective in removing minerals from contaminated soil. [9] Its flowers are edible and have different flavors and colors depending on the variety. Impatiens (pink, sweet) is not toxic as no daily intake limits have been established. [10] Impatiens walleriana Hook.f. has

both nutritional and therapeutic benefits in Brazilian traditional medicine. [10] Impatiens walleriana Hook.f. is used medicinally by the "Oromo" ethnic group, which is located in southern Ethiopia and the Bale area [11] In particular, the Oromo women utilize the roots to strengthen their hair. [12] In Africa, uterine infections are typically treated with impatiens walleriana Hook.f. [13] Amenorrhea and dysphagia are recognized conditions for therapy in Chinese medicine. [14] The root is dried and ground into "salep," a sweet-tasting, pungent-smelling powder that is used in cooking. It is nourishing and used to make drinks or as a cereal addition while making bread. "Salep" relieves GI irritations since it is demulcent. A jelly made from "Salep" is used as a treatment for tension and stress in the mind. Impatience and irritation are reduced [15] You can add sweet-tasting edible flowers to salads or to upscale cocktails. Flowers that can be eaten contain 14.75 w/w Dry matter content, 4.60g/kg crude protein, fresh mass of phenolics and flavonoids, as well as elements such as phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, copper, zinc, and molybdenum. As an abortifacient, liquid produced by crushing together water and dried roots and plants is consumed Pelargonidin. [16] complex 3:5-dimonoside is a pigment found in this plant's blooms; bluish rose blossoms specifically contain it. [17] Antibiotics that are more modern and secure are required. It is unknown whether Impatiens walleriana Hook.f. species have antibacterial properties. Because ethnopharmacological research reveals that it is effective in treating polymicrobial uterine infections, the current study has been undertaken. Acne, sometimes known as acne vulgaris, [18] is a common skin disorder characterized by oil glands located at the base of hair follicles. Acne is a skin illness. The growth of sebaceous (oil) glands typically occurs throughout puberty. Male hormones secreted by both male and



female adrenal glands activate the aforementioned organs. Acne comes in this way. Acne vulgaris is a phrase for "common acne." It is a skin disease caused by sebaceous gland changes. The color is caused by an underlying ailment, in which the skin gets inflamed and changes color. Pimples are a common skin condition among adolescents. Acne is a common skin disorder that usually affects teenagers due to hormonal fluctuations. The best acne therapy depends on the severity of the disease, which can range from light (a few odd pimples) to moderate (inflammatory papules) to severe (nodules and cysts). Acne is a common condition during puberty. When a person matures from a child to an adult as a result of the release of several hormones. Acne is more common among people who reach maturity. Human skin contains pores (small openings) that link to the sebaceous glands situated beneath the skin. The glands are connected to the pores by follicles, which are tiny channels. These glands create sebum, an oily fluid that carries dead skin cells from the hair follicles to the skin's surface. A tiny hair grows through the hair follicle and out of the skin. Pimples form when these follicles get blocked, allowing oil to accumulate beneath the skin. Skin is made up of microscopic holes known as pores, which can

become blocked with oil, debris, and bacteria, causing acne or "pimples" to appear. Under closed pores, oil accumulates. The bacteria on the skin is repeatedly affected by such a situation; acne may emerge, but it is not a life-threatening condition, however uncomfortable when severe. Acne is most commonly found on the face, neck, chest, back, and biceps. Chronic face acne can leave lifelong scars but is not hazardous. Multiple mechanisms occurring within the pilosebaceous unit lead to bacterial proliferation and inflammation, which is the etiology of acne. When changes in the body's hormonal environment affect the function of the pilosebaceous gland, this ailment often manifests around the time of the pubertal transition. Follicular epithelial cells initially develop improperly, form tighter intracellular adhesions, and are consequently less easily removed. This procedure causes the growth of hyperkeratotic plugs, also known as microcomedones, which gradually enlarge to produce non-inflammatory, closed or open comedones. [19] Circulating and cutaneously generated androgens, which are frequently cited as the main cause of acne, stimulate sebum production, which aids in the growth of comedones. [20]

MATERIALS AND METHODS:

Materials:

Table 1: List of chemicals and reagents used in the study

Sr. no.	Chemicals
1	Ethanol
2	Potassium dichromate
3	Sulfuric acid
4	Sodium hydroxide
5	Iodine
6	α -Naphthol
7	Fehling A&B
8	Benedict's reagent
9	Millen's reagent
10	Lead acetate
11	Chloroform
12	Acetic anhydride
13	Conc. Hydrochloride



14	Magnesium turnings
15	Acetic acid
16	Dragendroff's reagent
17	Mayer's reagent
18	Hager's reagent

Equipment's:

Table 2: List of Equipment used in the study

Sr. no.	Equipment's
1	Magnetic stirrer
2	Water bath Rectangular

Magnetic stirrer:



Figure 1: Magnetic stirrer

Model: 1-MLH REMI ELEKTROTECHNIK LTD.

PRINCIPLE:

An external magnetic field is used by an electromechanical device called a magnetic stirrer to rotate a stir bar in a mixture. Up to one liter of material can be mixed by the stir bar, which spins at different speeds. Glass jars work well with magnetic stir bars. Comedone growth is aided by the production of sebum. [20]

Procedure:

Ensure that the working bench is clean, and stirrer speed control knob is in off position. Keep the beaker containing 500ml solution of hydro-

alcoholic mixture of each plant (I.W. & S.A.) on the centre of the magnetic stirrer. Switch on the power supply. It contains two knobs- one is Temperature controlling knob and another one is Stirring controlling knob. Rotate the stirring knob slowly to adjust the speed of rotation to 500-600rpm, the bar magnet starts to rotate, magnetic bead also starts to rotate with the bar magnet. We have not used the temperature controlling knob because its cold maceration. After stirring is completed, decrease the stirring speed slowly by rotating the speed control knob anticlockwise and keep the knob in off position. Remove the magnetic bead.

Water bath evaporator:



Figure 2: Water Bath Evaporator

Model : WATER BATH RECTANGULAR

PRINCIPLE:

A water bath is an electromechanical device that helps to remove the alcoholic contents from extract, warming reagents, melting of substrates or incubation of cell cultures.

Procedure:

Water bath evaporator is an device that evaporates the alcoholic contents from the plant extract.

Fill the water bath with water, set the temperature at 300 c & turn on the start button and keep the extract on water bath to evaporate alcohol and water.

Collection of plant:

Impatiens walleriana Hook.f. flower-bearing plants were picked at random from a single garden source in Belagavi in the month of January 2016. For the investigation, the entire plant with the exception of the roots—was utilized. The specimen has been deposited in the RMRC herbarium with the accession number RMRC-1341 after being authenticated at the RMRC (Regional Medical Research Centre, Belagavi). Before being ground up with a mechanical grinder, the fresh plant material was sorted, cleaned to remove soil, and shade dried for two weeks.



Collection of plant



Drying

Figure 3: Collection and drying of *Impatiens walleriana*

Extraction of *Impatiens walleriana*

Maceration method

Fresh *impatience walleriana* leaves and stems were collected and cleaned with distilled water. The fresh leaves were dried and pulverized to a coarse powder. The coarse powder was steeped in a 50/50 ethanol/distilled water solution for three days. The

mixture was then filtered via muslin cloth. The solvent in the extract was evaporated by keeping the temperature below 50 °C. The dried powder of *impatience walleriana* was stored in an airtight container. (Plan for standardization, extraction, and pre-phytochemical screening of herbal medications). Use 108g of powder with analytical

grade hydroalcoholic as the solvent. The extraction process was then completed. [21] The dried powder of *Impatiens walleriana* was kept in an airtight container.



Fig 4: Extraction of *Impatiens walleriana* by maceration method

Percentage (%) yield:

The percentage (%) yield of hydroalcoholic extract of *Impatiens walleriana* is given below. [21].

Formula:

$$W2/W1 \times 100$$

where,

W2 is the weight of extract against the container.

W1 is the weight of the container itself and

W0 is the weight of the dry powder used for extraction

Analysis of phytochemicals:

Preliminary tests with extract have been conducted to find the presence of phytoconstituents like carbohydrates, proteins, steroids, flavonoids, alkaloids, tannins, and phenolic compounds. [22]

Qualitative phytochemical analysis:

The extract was tested for the presence of bioactive compounds by using following standard methods.

Table 3: Qualitative phytochemical analysis of *Impatiens walleriana*

Test	Procedure	Observations (Indicating Positive test)	References
Test for Carbohydrate			
Molish's test	2mL filtrate + 2 drops of alcoholic α -naphthol + 1mL conc. H ₂ SO ₄ (along the sides of test tube)	A violet ring	[23, 24]
Test for Reducing sugar			
Fehling's test	1mL each of Fehling's solution A & B + 1mL filtrate + boiled in water bath	A red precipitate	[23, 24]
Benedict's test	0.5mL filtrate + 0.5mL Benedict's reagent + Boiled for 2 min.	Green/yellow/red color	[23, 24]
Test for Protein			
Millon's test	2mL filtrate + few drops of Millon's reagent	A white precipitate	25,23

Xanthoprotein test	Plant extract + Few drops of conc. Nitric acid	A yellow-colored sol.	25,26
Ninhydrin test	2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone)	A purple-colored sol. { Amino acids}	23, 25
Test for Alkaloids			
Dragendroff's/ Kraut's test	Few mL filtrate + 1-2 mL Dragendorff's reagents	A reddish-brown precipitate	24,25
Mayer's/ Bertrand's/ Valser's test	Few mL filtrate + 1-2 drops of Mayer's reagent (Along the sides of test tube)	A creamy white/yellow precipitate	23,26,27
Hager's test	Few mL filtrate+ 1-2 mL Hager's reagents	A creamy white precipitate	25,23
Test for Flavonoids			
Shinoda's test/ Mghydrochloride reduction test	Plant extract is dissolved in 5mL alcohol + Fragments of magnesium ribbon + few drops of conc. HCl	A pink to crimson colored solution {flavonal glycosides}	23, 28
Sulfuric acid test	Plant extract + conc. H ₂ SO ₄	An orange colour	29
Lead acetate test	Plant extract+ few drops of lead acetate (10%) solution	Yellow precipitate	30
Test for tannins			
Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl	A white precipitate	26,31
10% NaOH test	0.4mL plant extract + 4mL 10% NaOH + shaken well	Formation of emulsion {Hydrolysable tannins}	24
Test for steroid			
Liebermann's-Buchard test	Plant extract+ few drops of acetic anhydride, boiled and cooled.+conc. H ₂ SO ₄ added from the sides of the test tube	Formation of a brown ring at the junction of two layer. Green coloration of the upper layer and formation of deep red color in the lower layer	30
Test for steroid	Plant extract + 2 ml of acetic anhydride + 0.5 g of the extract of each with 2 ml of H ₂ SO ₄	color changed from violet to blue or green	32

Antibacterial activity(Bacterial resistance):

By using the standard techniques outlined in the Manual of Clinical Microbiology of the research of 50 mg/ml, 100 mg/ml, and 200 mg/ml, a natural product diffusion method was employed to test the antibacterial activity.

Zone of Inhibition**Disc Diffusion Test Procedure**

- Using a loop or swab, transfer the colonies to the plates.
- Visually adjust turbidity with broth to equal that of a 0.5 McFarland turbidity standard that



has been vortexed. Alternatively, standardize the suspension with a photometric device.

Inoculation of Agar plate:

- Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum.
- Swab entire surface of agar plate three times, rotating plates approximately 60° between streaking to ensure even distribution. Avoid hitting sides of petriplate and creating aerosols.
- Allow inoculated plate to stand for at least 3 minutes but no longer than 15 min before making wells.

Stock solution preparation:

- Prepare the stock solution weighing 10mg of compound and dissolve it in 1ml of DMSO

RESULTS AND DISCUSSION:

Yield of extract:

Table 4: Percentage (%) yield of extract

Sr.no	Plant name	Method of extraction	Color of extract	Yield(%w/w)
1.	Impatiens walleriana	Maceration	Brown	5.29%

Phytochemical studies:

Table 5: Test for presence of Phytochemicals in Impatiens walleriana plant extract

Sr. no.	Phytoconstituents	Present/Absent
1	Carbohydrate	Absent
2	Protein	Present
3	Alkaloid	Absent
4	Flavonoids	Present
5	Steroids	Absent
6	Phenolic and tannins	Present

Addition of compound into plate:

- Take hollow tube of 5mm diameter, heat it. Press it on above inoculated Agar plate and remove it immediately by making a well in the plate. Likewise, make five well on each plate.
- With the help of micropipette add 50µl of each concentration in each well.

Incubation:

- Incubate plates within 15 min of compound application.
- Invert plates, and stack them no more than five high.
- Incubate for 18-24hrs at 37 °C in incubator.

Reading plates:

- Read plates only if the lawn of growth is confluent or nearly confluent.
- Measure diameter of inhibition zone to nearest whole millimeter by holding the measuring device. [33]





Figure 5: Showing presence of reducing sugar

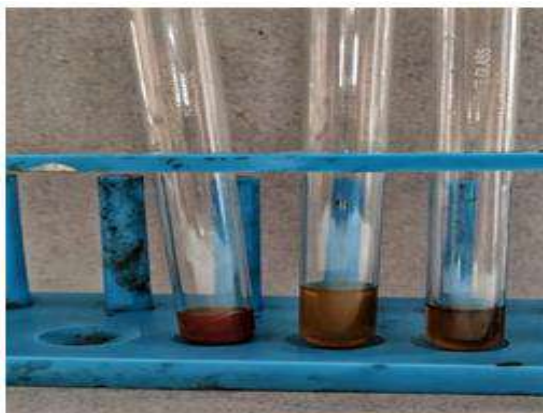


Figure 6: Showing presence of protein



Figure 7: Test for Flavonoids

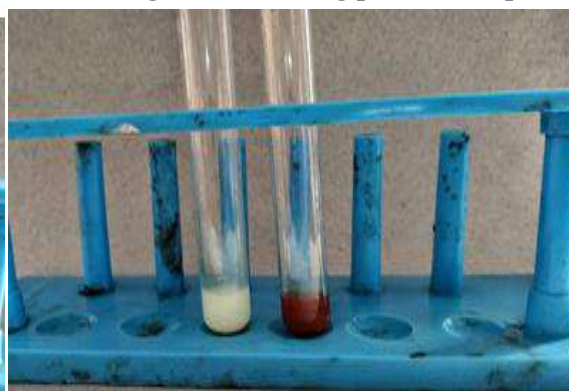


Figure 8: Test for Tannins

Antiacne activity:

Table 6: Zone of inhibition in millimeters obtained for the 3 concentration of impatiens walleriana extract.

S. No	Samples	200	100	50	Erythromycin
	Impatiens walleriana				
1	Staph epidermidis	R	R	R	23mm
2	Propionibacterium Acne	8mm	R	R	40mm

Note:

S-Sensitive

R-Resistant

Standard Values

Erythromycin

Staph epidermidis-26mm

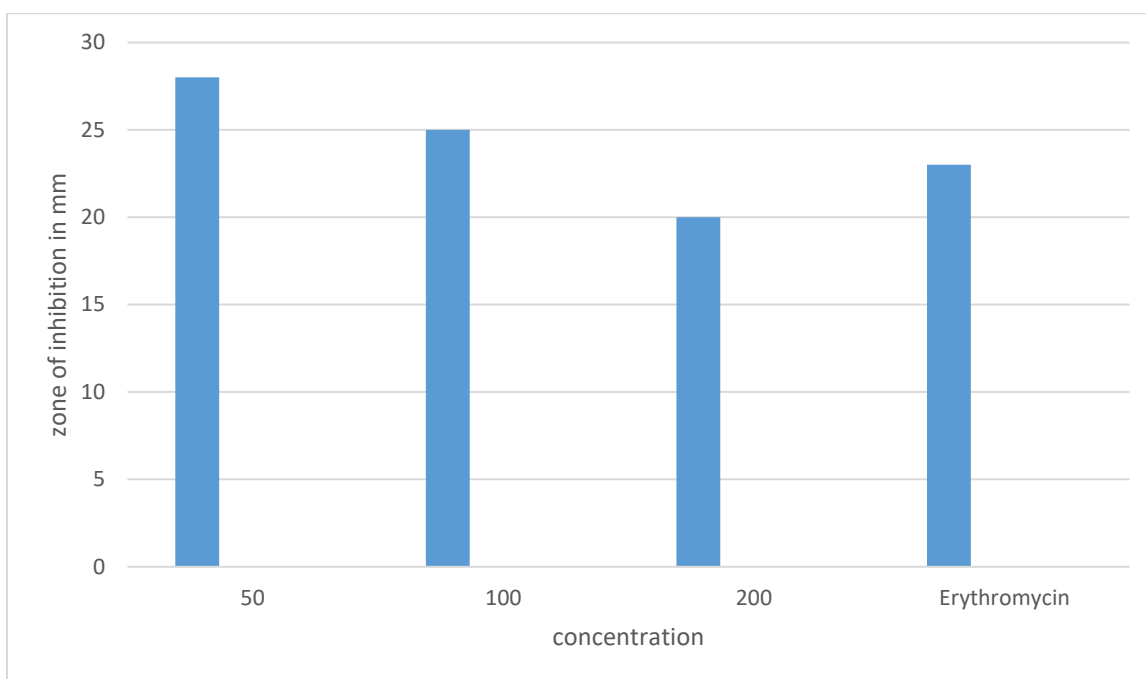


Figure 9: Antiacne Activity of Impatiens Walleriana and Erythromycin Estolate

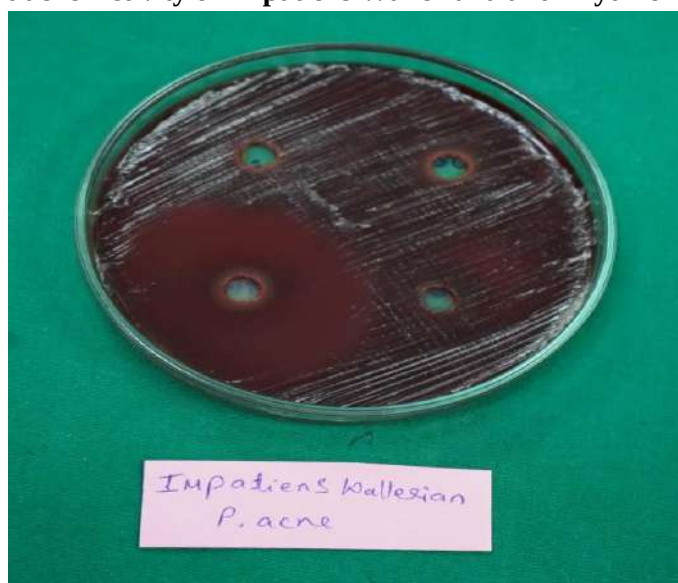


Figure 10: Staphylococcus epidermidis susceptibility to the extract

DISCUSSION:

Results in Table.5 depict presence/absence of phytoconstituents. Phytochemical studies on the whole plant extract got revealed for the very first time using the entire plant extract except root showed the presence of tannins, phenolics and flavonoids. Plants as such may provide alternate sources of antiacne agents with novel mechanisms and new treatments.[34] As active principles are more soluble in alcohol, in the present

study extraction has been carried out using magnetic stirrer and hydroalcoholic employed as solvent. Results are as depicted in Table.6 wherein susceptibility along with the diameter of the zone of inhibition in comparison to the standard are mentioned. Findings confirmed antiacne potential as the organism used in the study were sensitive to hydroalcoholic extract. Screening methods employed for natural products include disc diffusion methods with diffusion method variant

"Disc" more suitable as it is simple and less time consuming. In the "Disc" variant natural product's diffusion by carriers like DMSO.[35] Hence, the study has been carried out using the agar disc diffusion method. Acne is a common skin condition that affects the oil glands at the base of hair follicles. Skin illness known as acne. Acne is produced by a number of factors that occur within the pilosebaceous unit, including bacterial proliferation and inflammation. As a result, the study looked into the effectiveness of the hydroalcoholic extract against gram-positive organisms, which are known to cause acne and have ethnopharmacological properties. *Impatiens walleriana* extract is efficient against the anaerobic bacteria used in the study. The medicinal value of plants is derived from chemical components that have unique physiological actions. Previous research has found flavonoids, phenolics, proteins, and minerals in *Impatiens walleriana* flowers. Pigments are also reported in the flowers. Constituents present in the whole plant of this species are not reported. In the present study extract has shown the presence of proteins, flavonoids, tannins and phenolics. Phytochemicals like tannins exert antimicrobial activity by iron deprivation, hydrogen bonding or by non-specifically interacting with vital proteins/enzymes, [36] Phenolics are known to cause membrane rupture and flavonoids much like tannins bind to enzymes, form complexes with the cell wall or bind to adhesions. [37] The antiacne activity of this extract may be due to the presence of either tannins/ phenolics/ flavonoids or is due to the synergistic effect of these constituents. Since bacterial growth inhibition does not mean bacterial death, the method used does not distinguish bactericidal and bacteriostatic effects.

CONCLUSION

The study investigated the antiacne properties of *Impatiens walleriana* using a hydroalcoholic extract. The results confirmed the plant's antiacne

potential against gram-positive organisms, as demonstrated by significant zones of inhibition in the disc diffusion method. Phytochemical analysis revealed the presence of flavonoids, phenolics, proteins, and tannins, which are known to possess antimicrobial properties. The antiacne activity could be attributed to these constituents, either individually or through synergistic effects. The findings support the ethnopharmacological use of *Impatiens walleriana* in treating acne, suggesting it as a promising alternative source of antiacne agents. Further studies, including clinical trials and research on drug-resistant bacterial strains, are recommended to substantiate these results.

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