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Research Article

Phytochemical Profiling and Antibacterial Evaluation of *Polianthes tuberosa* L. (Flowers, Stems, and Tubers) Against Acne-Associated Bacteria

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ABSTRACT

Germes such as Staphylococcus aureus and Cutibacterium acnes are often the causes of the skin disease called acnes vulgaris. The need to find alternative plant-based compounds that could hopefully replace standard antibiotics has increased the pace of search given the increased resistance to these compounds. In the present study, polianthes tuberosa L. extracts of flower, stems, and tubers were examined based on the phytochemical composition and anti-bacterial properties. Solvents extracts were prepared using the traditional methods of extracting solvents, and were initially subjected to phytochemical analysis. Agar well diffusion method was used to assess the antibacterial activity against acnes causing microorganisms and minimum effective concentration of the most active extract was calculated. The results showed that all of the plant components that were examined had antibacterial activity. The flower extracts had the largest zone of inhibition which extended to the tuber extracts and then stem extracts. Phytochemical analysis revealed the presence of bioactive constituents (glycosides, tannins, alkaloids, saponins, and flavonoid, etc.). The antibacterial effect identified could be due to the interaction of these phytoconstituents. These findings support the hypothesis that Polianthes tuberosa L. could be utilized in the development of herbal remedies to fight acnes due to its antibacterial effects and that it can be used to attack the microorganisms that cause the condition. Further studies ought to concentrate on isolating bioactive compounds and authorising their effects to living organisms.

INTRODUCTION

The typical symptoms of acnes vulgaris are comedones, papules, pustules and in the more

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extreme cases nodules and cysts; these symptoms are effects of a chronic inflammation of the pilosebaceous unit. Hundreds of millions of people, mostly young adults and teenagers, suffer from this ailment, making it one of the most frequent dermatological issues in the world.^[1] Inflammation, microbial colonization, follicular hyperkeratinization, and excessive sebum production are the multifactorial pathophysiology of acne. The gram-positive anaerobic bacteria known as *Cutibacterium acnes* is crucial to the development of acne because it induces inflammation, forms biofilms, and releases virulence factors.^[2] Acne lesions are a common site for the isolation of *Staphylococcus aureus* and *Staphylococcus epidermidis*, two bacteria that add to the inflammatory environment and infection persistence.^[3] Topical and systemic antibiotics including clindamycin, doxycycline, and macrolides are part of the conventional approach to acne treatment. Nevertheless, the increasing number of bacteria that cause acne and are resistant to antibiotics has greatly reduced the effectiveness of current treatments, making the search for alternative medicines all the more urgent. Since then, there has been an surge of interest in medicinal plants and the phytochemicals found within them as possible safer and more long-term alternatives to conventional acne treatments due to their antibacterial, anti-inflammatory, and antioxidant capabilities.^[3] The therapeutic qualities of the fragrant herb *Polianthes tuberosa* L., more often known as tuberose, have long been recognized and appreciated. Flavonoids, alkaloids, phenolics, saponins, and glycosides are antimicrobial substances that have been discovered in different plant sections through phytochemical studies.^[4] The antibacterial activity of *P. tuberosa* stem, tuber, and flower extracts against acne-inducing bacteria has not been well studied, though. In order to find natural alternatives to conventional acne

treatments, this study will evaluate the phytochemical profiles and in vitro antibacterial activity of these extracts against *Staphylococcus aureus* and *Cutibacterium acnes*.



Figure 1. An image of a *P. tuberosa* flower. Source: Created by the author.

Role of Acne-Inducing Bacteria

An important step in the development and maintenance of acne vulgaris is the colonization of the pilosebaceous unit by microbes. The main microbe implicated in the etiology of acne is *Cutibacterium acnes*, a gram-positive, anaerobic bacterium. It triggers inflammatory processes and destroys follicular epithelium by disintegrating sebum triglycerides with the aid of lipase and breaking free of free fatty acids. *C. acnes* also enter the activation of the toll-like receptors (TLR-2 and TLR-4) on immune cells and keratinocytes leading to the production of pro-inflammatory cytokines including interleukin-1 β , interleukin-8, and tumor necrosis factor- α . The development of lesions and inflammation inside follicles are facilitated by these mediators. The presence of aerobic bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* along with *C. acnes* is the most common bacterial presence in acne lesion, particularly in the inflammatory and pustular types of lesions. These bacteria can aggravate skin irritation, besides resulting in

secondary infection. Biofilms that are formed by acnes causing bacteria make it more possible that the bacteria survives and becomes harder to kill as a result of the resistance to antibiotics, and subsequently the acnes becomes tougher to treat, and it is more likely to recur or remain.^[5]

Limitations of Conventional Acne Therapies

The primary conventional techniques of acnes care include medications like retinoids, anti-inflammatory agents, topical and systemic antibiotics. The common approach includes the use of antibiotics that include clindamycin, erythromycin, doxycycline and minocycline to reduce the population of the germs and inflammation. An surge in antibiotic-resistant strains of *Staphylococcus species* and *Candida albicans* has been seen due to the extensive and careless use of these medicines, notwithstanding their initial effectiveness.^[6,7] Antimicrobial resistance is just one of several side effects linked to prolonged antibiotic treatment. Other side effects include dry skin, redness, irritation, photosensitivity, and gastrointestinal problems. Patients are less likely to comply with treatment plans and may even stop taking it altogether due to these negative effects. Also, the usual skin flora may get disturbed by traditional treatments, which might make inflammation much worse. Given these restrictions, it is clear that new, better ways to treat *acne vulgaris* are required, methods that are both safe and effective over the long term.

Medicinal Plants as Alternative Anti-Acne Agents

Traditional medical systems have relied on medicinal plants to address skin conditions for ages. Because of their anti-inflammatory benefits, wide-spectrum antibacterial action, and low side effect profiles, medicines derived from plants have recently attracted a lot of attention.^[8] Many of the

secondary metabolites found in plants, including glycosides, tannins, phenolic compounds, alkaloids, saponins, and flavonoids, have strong antibacterial properties and can kill the bacteria that cause acne. The antibacterial actions of these phytochemicals are exerted by many channels, such as bacterial cell membrane breakdown, enzyme activity inhibition, protein synthesis interference, and inflammatory pathway suppression. Another possible mechanism by which plant extracts lessen the probability of resistance development is the synergistic interaction of many bioactive chemicals. Therefore, medicinal plants are great options for creating all-natural, environmentally safe, and economically viable acne treatment.^[9,10]

Botanical Profile of *Polianthes tuberosa* L.

One member of the *Asparagaceae* family is the perennial bulbous plant *Polianthes tuberosa* L., more often known as tuberose.^[11] Perfumery and ornamental horticulture make heavy use of its aromatic blossoms, which are grown abundantly in tropical and subtropical countries. Traditional folk medicine has made use of *P. tuberosa* for more than only its fragrant and aesthetically pleasing qualities; the plant also contains antibacterial, anti-inflammatory, and wound-healing capabilities. The amounts of bioactive chemicals can vary throughout different portions of the plant, including flowers, stems, and tubers. This diversity in phytochemical composition provides more evidence that various plant components may carry out unique biological functions.^[12] Nevertheless, there is still a lack of scientific evidence to support these long-held beliefs, especially when it comes to the bacteria linked to acne.

Phytochemical Constituents of *Polianthes tuberosa* L.



Several physiologically active compounds in *Polianthes tuberosa* L. such as glycosides, tannins, alkaloids, phenolics, and saponins are known to be anti-inflammatory, antioxidant and antibacterial^[13]. Alkaloids and saponins might hamper the growth of microorganisms by destabilising microbial enzyme systems and membrane integrity, and phenolic compounds and flavonoids do so by neutralising reactive oxygen species and damaging bacterial cell membranes respectively. Since these phytochemicals are synergistic and complement each other, *P. tuberosa* extracts have potential to be better against bacteria. It is required to understand the phytochemical composition of various parts of the plants to be used in determining the most effective antibacterial fractions.^[14]

MATERIAL AND METHOD

Plant material

Polianthes tuberosa L. the flowers, stems, and tubers. They (Asparagaceae) were found in the Kolhapur area of Maharashtra, India. The plant materials that were collected were first washed with tap water then rinsed with double distilled water to get rid of dust and foreign contaminants. Shade-drying of the plant parts was done separately at room temperature and over a specified period of time until a constant weight had been attained. The dried flowers, stems and tubers were dried in a dry, clean, airtight glass container and then with the help of a blender or mechanical grinder they were crushed into fine powders which were then stored separately in clean and dry airtight glass containers until further use.

Extraction

The population was dried in an oven and then powdered to 50 g before 1000 mL amounts of the following solvents (n-hexane, ethyl acetate, and

methanol) listed in Table 1 were added to prepare the plant extracts. The extraction was performed by subjecting the mixtures to the cold maceration method where mixtures were allowed to stand at room temperature (72 hours, 3 days) so that phytoconstituents would continuously diffusion in the solvent.

The mixtures were filtered using the muslin cloth and then Whatman No. 1 filter paper after the maceration period in order to eliminate insoluble plant residues. The filtrates were further concentrated by evaporation on a steam bath at 50 °C to eliminate the solvents to get crude extracts. This reduced temperature evaporation process assisted in maintaining thermolabile bioactive constituents. The concentrated extracts were left to cool after which the extracts were transferred into airtight containers and stored at 4 °C till further examination.

Phytochemical screening was then done on the extracts obtained to determine the presence of different secondary metabolites.

Chemicals

In the current study clindamycin hydrochloride was utilized in the role of a standard antibacterial drug. All chemicals, solvents, and reagents utilized were of analytical reagent (AR) grade and were used as received, without further purification. Methanol, n-hexane, and ethyl acetate were employed as extraction solvents, while Tween-80 and various reagents required for phytochemical screening—including Dragendorff's, Wagner's, Mayer's, and Hager's reagents, ferric chloride, lead acetate, Benedict's reagent, Fehling's solution, sodium hydroxide, sulfuric acid, Liebermann–Burchard reagent, potassium permanganate, and iodine solution—were obtained from Merck Life Science Pvt. Ltd. and HiMedia Laboratories Pvt. Ltd., India. Distilled



and triple-distilled water used throughout the experimental procedures was prepared in-house. All chemicals were of verified purity to ensure the reliability and reproducibility of the results.

Microorganisms

Cutibacterium acnes and *Staphylococcus epidermidis* were used the bacterial cultures were maintained under standard laboratory conditions and subcultured prior to use to ensure purity and viability. These microorganisms were selected to evaluate the antibacterial potential of the plant extracts against acne-associated bacteria.

Phytochemical analysis

Phytochemical screening was carried out to qualitatively identify major secondary metabolites, including alkaloids, flavonoids, polyphenols, tannins, monoterpenes and sesquiterpenes, steroids, quinones, and saponins, in the tuber, stem, and flower of *Polianthes tuberosa* L. [16]. This analysis was undertaken to examine differences in the phytochemical composition across the various plant parts..

Determination of Antibacterial activity

Culture media Bacterial inoculum

The bacteria were cultured for twenty-four hours at 37 °C (±1 °C) in sterile Petri plates using nutrient agar (NA). The nutrient agar was purchased from Difco®. A turbidity of 25.0% at 580 nm was achieved by diluting the prepared suspensions with 0.9% (w/v) sodium chloride solution. The turbidity was assessed in comparison with the McFarland turbidity standard using ultraviolet spectrophotometry (Shimadzu® UV-180). The final turbidity corresponded to 3.0×10^8 CFU/mL.

Paper disc diffusion method

A reference drug, clindamycin hydrochloride (P003-WS15021001), was used in the experiments to evaluate the antibacterial activity of *n*-hexane, ethyl acetate, and methanol extracts obtained from the tuberos flower, stem, and tuber. *Staphylococcus epidermidis* and *Cutibacterium acnes* were tested for antibacterial activity using the paper disc diffusion technique. After 15 mL of nutrient agar (NA) medium had solidified in a Petri dish, 0.1 mL of bacterial suspension was evenly spread over the agar surface using a Drygalski apparatus. The plates were then allowed to stand for fifteen minutes to allow absorption. Subsequently, 6 mm diameter paper discs impregnated with the extracts at concentrations of 1000, 500, 250, and 125 mg/mL were placed on the agar surface. A 5% (w/v) Tween 80 solution was used as the negative control, while clindamycin hydrochloride (50 mg/mL) served as the positive control. The plates were incubated at 37 °C for 24 hours under controlled conditions. The experiment was performed in triplicate, and the diameter of the inhibition zones was measured using calipers.

Minimum effective concentration (MEC) evaluation

The plant extract showing the highest antibacterial activity was selected for the determination of the minimum effective concentration (MEC). The assay was carried out using the paper disc diffusion method as described earlier. A series of extract concentrations ranging from 150 to 10 mg/mL was tested against *Staphylococcus epidermidis* and *Cutibacterium acnes*, while Tween-80 (5% v/v) served as the negative control. The MEC was considered to be the lowest concentration of the extract that produced a clearly visible zone of inhibition against the tested bacterial strains.

Cell Morphological Observation



The antibacterial test's treatment, which included soaking the region in a solution of 2% glutaraldehyde overnight, created this clear zone. The test solution was separated using a centrifuge, and the supernatant was removed and discarded. The residue was soaked for a specified period after being added to a solution of 2% tannic acid. Cacodylate buffer was added after the test solution had been centrifuged and the fixative solution had been removed; the mixture was then allowed to soak for twenty minutes. One hour after centrifuging the test solution and collecting the supernatant, 1% osmium tetroxide was added, and the mixture was allowed to soak. After centrifugation, the supernatant was removed and the sample was immersed in 50% ethanol for 20 minutes. The residue was dehydrated over a twenty-minute period using increasing concentrations of ethanol: 70%, 80%, 95%, and 100%. Following a twenty-minute soaking in a mixture of butanol and sample suspension, the mixture was transferred to a coverslip, freeze-dried, and finally coated with gold. The final step was to examine the samples using a scanning

electron microscope (SEM) (JEOL JSM5310LV®).

Bioautography assay

The antibacterial compounds in the flower's methanol extract were identified using the contact bioautography approach, with a few modifications applied. To conduct TLC plating, Silica Gel GF254 (Merck®) was used. An ethyl acetate and ethanol solvent system was employed at a proportion of 7:3. As the solvent evaporated, the chromatogram remained intact. The chromatogram was placed with its bottom facing a 15 mL layer of inoculated nutrient agar and allowed to remain for 30 minutes to permit diffusion to occur. Afterwards, the chromatogram was carefully removed, and the agar layer was incubated for twenty-four hours at 37 °C (± 1 °C). The absence of samples on a TLC plate served as a negative control. [17] The R_f values corresponding to the observed inhibitory zones were recorded.

RESULTS

Table 1. Screening for Phytochemical Varieties in Flowers, Stems, and Tuber using Various Solvents

Plant Part	Extract Type	Alkaloids	Phenolics	Quinones	Saponins	Tannins	Flavonoids
Flower	Crude powder (Simplisia)	+	+	-	-	-	+
Flower	n-Hexane extract	+	-	+	-	-	-
Flower	Ethyl acetate extract	+	+	+	-	-	+
Flower	Methanolic extract	+	+	-	-	-	+
Stem	Crude powder (Simplisia)	+	+	+	+	+	+
Stem	n-Hexane extract	-	-	+	-	-	-
Stem	Ethyl acetate extract	+	+	-	+	-	+
Stem	Methanolic extract	+	+	+	+	+	+
Tuber	Crude powder (Simplisia)	+	-	+	+	+	+
Tuber	n-Hexane extract	+	+	-	-	-	-
Tuber	Ethyl acetate extract	+	+	-	-	-	+
Tuber	Methanolic extract	+	+	+	+	+	+

Note: + = detected, - = not detected



Table 2. The antimicrobial effects of extracts from flowers, stems, and tubers against *Cutibacterium acnes* and *Staphylococcus epidermidis*

Plant Part	Extract	S. epidermidis				C. acnes			
		1000 mg/mL	500 mg/mL	250 mg/mL	125 mg/mL	1000 mg/mL	500 mg/mL	250 mg/mL	125 mg/mL
Flower	Control	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Flower	n-Hexane	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Flower	Ethyl acetate	9.30	8.02	7.42	6.10	7.90	7.10	6.75	6.05
Flower	Methanol	13.60	10.40	9.95	9.70	12.10	10.20	9.95	9.10
Stem	Control	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Stem	n-Hexane	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Stem	Ethyl acetate	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Stem	Methanol	10.40	7.20	6.10	6.10	8.30	7.20	6.05	5.50
Tuber	Control	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Tuber	n-Hexane	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Tuber	Ethyl acetate	10.80	9.70	7.50	6.10	9.10	8.00	6.10	5.50
Tuber	Methanol	11.30	10.40	8.60	6.05	9.60	8.00	6.05	5.50

Note: A zone diameter of 5.50 mm corresponds to the diameter of the paper disc and indicates no antibacterial activity.

Table: 3. The Effect of Various Concentrations of the Extract on Acne-Inducing Bacteria as an Antibacterial Agent

Concentration (mg/mL)	S. epidermidis (mm)	C. acnes (mm)
150	11.75 ± 0.09	10.88 ± 0.30
140	11.02 ± 0.03	9.85 ± 0.20
130	10.12 ± 0.05	9.55 ± 0.02
120	9.20 ± 0.18	9.05 ± 0.05
110	8.90 ± 0.06	8.95 ± 0.11
100	8.68 ± 0.02	8.86 ± 0.14
90	8.55 ± 0.07	8.65 ± 0.12
80	8.44 ± 0.04	8.30 ± 0.15
70	8.30 ± 0.05	8.12 ± 0.04
60	8.18 ± 0.08	8.00 ± 0.06
50	7.85 ± 0.02	7.88 ± 0.04
40	7.70 ± 0.03	7.30 ± 0.18
30	7.10 ± 0.30	6.90 ± 0.20
20	5.50	5.50
10	5.50	5.50

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with statistical significance considered at $p < 0.05$.

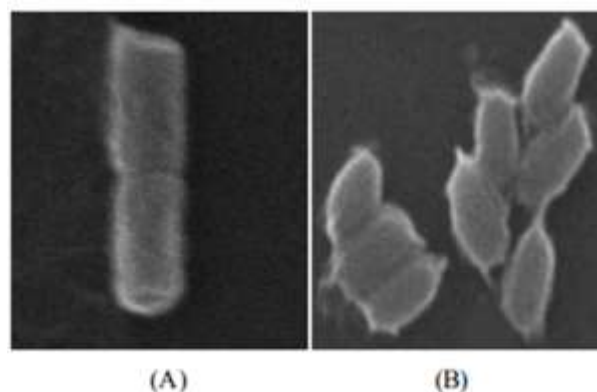


Figure 2. Scanning electron micrographs of untreated and methanolic flower extract–treated *Cutibacterium acnes* showing morphological alterations at 10,000× magnification.
Scale bar: 1 μ m. Source: Created by the author.

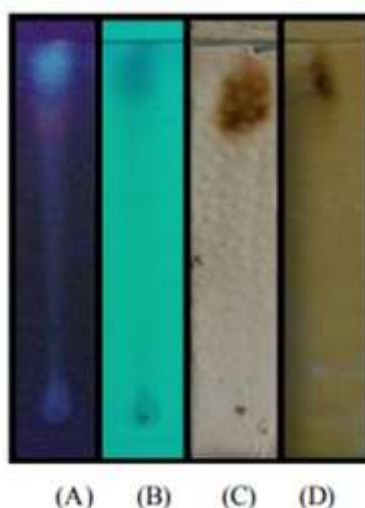


Figure 3. Thin layer chromatography (TLC) profile of methanolic flower extract of *Polianthes tuberosa* L. visualized under UV light at 366 nm (A), 254 nm (B), after spraying with 10% H₂SO₄ (C), and FeCl₃ reagent (D) using silica gel GF254 and ethanol:ethyl acetate (3:7) as mobile phase.
Source: Created by the author.

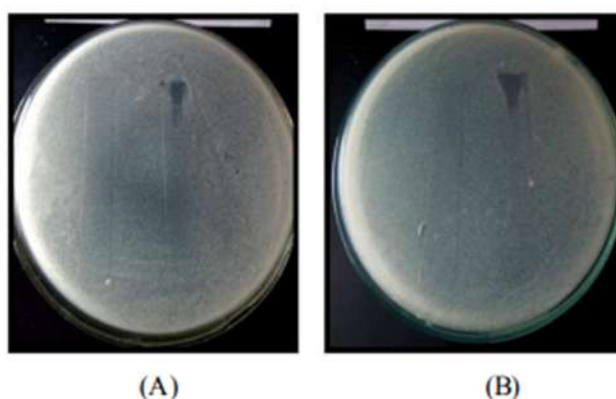


Figure 4 Bioautography of methanolic flower extract of *Polianthes tuberosa* L. against *Staphylococcus epidermidis* (A) and *Cutibacterium acnes* (B) using TLC plates developed with ethanol:ethyl acetate (3:7).
Source: Created by the author.

DISCUSSION

To better understand the presence of bioactive secondary metabolites in plant materials, phytochemical screening is commonly employed. The results indicated that the tuber, stem, and flower of *Polianthes tuberosa* L. contained varying profiles of secondary metabolites. Differences in solvent polarity influenced the types of compounds extracted, as certain metabolites exhibit differential solubility in different solvents. The evaluation of the plant extracts made utilizing the ethyl acetate and methanol revealed the antibacterial activity of the extracts made by the two methods against *Cutibacterium acnes* and *Staphylococcus epidermidis*. Conversely, there was no antimicrobial effect as n-hexane extracts had similar inhibition zones with the control. The methanolic flower extract had the best antibacterial activity of all treatments.

The results of the one-way ANOVA revealed that the inhibitory effect of the floral extracts was significantly different from that of the control group ($\alpha < 0.05$). Moreover, post hoc test with the Tukey test proved that the inhibitory effect of only the methanolic flower extract was statistically significantly significant than that of the control ($\alpha < 0.05$). Due to its high antienzymatic properties, the methanolic compound flower extract was chosen to determine the minimum effective concentration (MEC). MEC against *S. epidermidis* and *C. acnes* was found to be 30mg/mL [18]. In the scanning electron microscopy (SEM) study of *C. acnes* in the presence of the methanolic flower extract, the analysis showed that a considerable morphological change of the bacterial cells was observed in comparison to untreated control, implying the bacterial cell wall was damaged, probably owing to the presence of antibacterial compounds in the study extract. The analysis of

the floral extract was performed on a thin-layer chromatography (TLC), with ethyl acetate ethanol (3:7) as the mobile phase showed two spots with the ultraviolet light at 366 nm with Rf value of 0.79 and 0.87. [19] The spot at Rf 0.87 showed positive reactions with 10% H₂SO₄ and FeCl₃ reagents, indicating the possible presence of phenolic compounds. The antibacterial effect of the compound at Rf 0.87 was further verified by a bioautographic analysis. Phenolic compounds are well known to have antibacterial effects by picking up the hydroxyl group, resulting in suppressed membrane permeability and breaking up the integrity of bacterial cell membranes. [20]

CONCLUSION

This investigation demonstrates that *Polianthes tuberosa* L. as an antibacterial agent has the potential of being used to treat acne-causing bacteria, i.e. *Cutibacterium acnes* and *Staphylococcus epidermidis*. According to the phytochemical screening, it was established that various parts of the plant such as stem, flower and tuber also contained different secondary metabolites. Moreover, the solvent used in the extraction had a significant effect on the antibacterial performance. The antibacterial activity of the methanol flower extract was the best among extracts we studied and the n-hexane extracts were not active. The findings indicated that the methanolic extract of flowers had a greater inhibitory activity as opposed to the control group. The minimum effective concentration (MEC) of the methanolic flower extract was found to be 30mg/mL with a resistance to the two bacterial strains used. The electron microscopes also revealed significant morphological cell damage in *C. acnes* cells after treatment indicating that there were changes in the bacterial cell wall. Thin-layer chromatographic and bioautographic methods indicated that the phenolic constituents of the



floral extract could be linked to the antibacterial effect of the extract. The combination of these results confirms the possibility of the methanolic flower extract of *Polianthes tuberosa* L. to be a natural antibacterial agent in acne. Isolation of bioactive compounds, determination of their toxicity, and in vivo testing are areas that require further research on to promote the designing effective plant-based anti-acne preparations.

CONFLICT OF INTEREST

The author states that there is no conflict of interest about publication of this manuscript.

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ETHICAL APPROVAL:

Not necessary (in-vitro study).

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