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Phytochemical Screening and Antibacterial Activity of Leaf Extracts from *Psiadia Punctulata*

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Abstract

Background: *Psiadia punctulata* (*P. punctulata*) is a delicate medicinal herb commonly used in traditional herbal therapy. **Objective:** To identify the phytochemical constituents and assess the antibacterial efficacy of methanolic extracts of *Psiadia punctulata*. **Materials and Methods:** The plant material was collected, shade-dried, and then powdered. The powder was soaked and macerated in methanol, followed by extraction using the Soxhlet method. Phytochemical tests were conducted qualitatively. Antibacterial activity was evaluated using different types of bacteria and compared with various antibiotics. **Results:** The *P. punctulata* plant showed the presence of alkaloids, tannins, phenols, flavonoids, cardiac glycosides, steroids, and terpenoids. The extract exhibited significant inhibition zones against *Staphylococcus epidermidis* (28 mm) and *Escherichia coli* (30 mm). **Conclusion:** This study revealed that *Psiadia punctulata* contains a wide range of secondary metabolites with antibacterial properties.

Keywords: Antibacterial**;** *Psiadia punctulate***;** Phytocemical; Yemen

1. Introduction

For many decades, humans have relied on medicinal plants to treat a variety of diseases, including infections. Medicinal plants are considered an essential source for treating or preventing many diseases because they contain important vital components used in the medical field [1]. These components can be used in developing different types of medicines [2].

Medicinal plants are widespread across large parts of the world, with Yemen being one of the countries rich in medicinal plants utilized by Yemenis for treating and preventing numerous diseases. *Psiadia punctulata* is one of these medicinal plants found in Yemen, as well as in other African and Asian nations. Belonging to the family Asteraceae, three species—*Psiadia incanao*, *Psiadia punctulata*, and *Psiadia schweinfurthii*—are among the most common in Yemen [3-5]. *Psiadia punctulata* has sticky-textured leaves and shiny foliage, with many secretions on the leaf surface that serve a protective role for the plant [6]. Traditionally, *Psiadia punctulata* is used to treat a wide range of illnesses, including fever, malaria, skin infections, pain, and bone injuries [7]. Phytochemical screening studies have shown *that Psiadia punctulata* contains chemical components such as flavones, diterpenes, phenylpropenoids, and other bioactive compounds [6,8-14]. Research has also been conducted on its cytotoxic, antibacterial, antitrypanosomal, antiplasmodial, and antileishmanial properties [15].

To our knowledge, this is the first time *Psiadia punctulate* has been collected from Bani Omer in the Dhamar government and extracted using methanol, and its phytochemical constituents have been screened. Our work also aims to evaluate *Psiadia punctulate*'s antibacterial properties and compare it with antibiotics.

2. Materials and Methods

2.1 Plant Materials

The resinous leafy branches of *P. punctulata* were collected from Bani Omer village near Dhamar City on January 6, 2024. The plant was identified by Dr. Khalid Imran, an assistant professor in the Faculty of Agriculture at Thamar University.

2.2 Preparation of the Alcoholic Extracts

Up to 1 kg of leaves were separated from the branches of the *P. punctulata* plant and macerated in 2 liters of methanol for 3 days at room temperature. The extracted leaves were then allowed to air-dry to a constant weight of 500 g. The methanol extract was decanted and filtered through glass wool into a 2.5L Winchester bottle. The methanol was evaporated using a rotary evaporator, and the resulting concentrate was preserved in an open glass bottle and stored in a vacuum desiccator for qualitative phytochemical tests and antibacterial activity studies.

2.3 Qualitative Phytochemical Analysis

2.3.1 Alkaloids Test

To 2 mL of the extract, a few drops of Mayer's reagent (prepared by dissolving 5 grams of iodide in 100 ml of mercury iodide solution in 100 ml of distilled water) were added along the side of the test tube. The presence of alkaloids is confirmed by the formation of a white precipitate [16].

2.3.2 Flavonoid Test

To 2 mL of the methanolic extract, add a piece of magnesium ribbon and 1 mL of concentrated hydrochloric acid. The presence of flavonoids is indicated by the appearance of a pink-red or red coloration in the solution [17].

2.3.3 Phenol Test

Add 2 mL of the methanolic extract to 5 mL of distilled water. Then, add a few drops of neutral 5% ferric chloride solution. A dark green color indicates the presence of phenolic compounds [18].

2.3.4 Steroid Test

Dissolve 2 mL of the extract in chloroform and add a few drops of acetic anhydride and concentrated sulfuric acid. The formation of a violet-blue color, which eventually turns green, indicates the presence of steroids [19].

2.3.5 Tannins Test

Add 30 mL of distilled water to 2 mL of the methanolic extract. Heat the mixture, then cool it, and add ferric chloride. The presence of tannins is indicated by the appearance of a brownish-red or dark blue color [20].

2.3.6 Cardiac Glycosides Test

Combine 2 mL of methanolic extract with 1 mL of glacial acetic acid, 1 mL of ferric chloride, and 1 mL of concentrated sulfuric acid. The presence of glycosides is confirmed by the green-blue coloration of the solution [21].

2.3.7 Terpenoids Test

Treat 2 mL of the extract with 2 mL of acetic anhydride. Then add a few drops of concentrated sulfuric acid to this solution. The formation of blue and green rings indicates the presence of terpenoids [22].

2.3.8 Saponins Test

Take 2 mL of methanolic extract and add 20 mL of distilled water. Heat the mixture, then cool and filter it. Transfer the filtrate to a test tube, shake vigorously, and let it sit for 3 minutes. The absence of foam indicates that there are no saponins in the extract [23].

2.4 Antimicrobial Screening

The antimicrobial activity of *P. punctulata* extracts was studied against two bacteria: *Staphylococcus epidermidis* (a Gram-positive bacterium) and *Escherichia coli* (a Gram-negative bacterium). These bacteria were obtained from Medlab Laboratory in Dhamar city, Yemen. The selected strains were tested for their antibacterial activities using the agar well diffusion method [24].

Twenty milliliters of sterilized nutrient agar medium were added to each sterile Petri plate, which were then allowed to solidify. The test bacterial cultures were standardized to the 0.5% McFarland standard and evenly spread on the appropriate media using a swab stick [25]. Six-millimeter sterile cork borer holes were made in the media [26]. Sample solution concentrations were prepared and diluted to the required amount (10 mg/mL). These concentrations (0.1 mL) were added to various wells, and the plates were incubated at 35°C for 24 hours. Zones of growth inhibition (ZI) were measured using a clear ruler.

Standard Ciprofloxacin (5 mcg/disc), Gentamycin (10 mcg/disc), Levofloxacin (5 mcg/disc), and Tetracycline (5 mcg/disc) discs were used as positive controls to verify the activity of the standard antibiotic against the test organisms and to compare the response generated by *P. punctulata* extracts with that of known antimicrobial agents.

3. Result and Discussion

3.1 Qualitative Phytochemical Screening

Phytochemical analysis is crucial for identifying beneficial chemicals with medical and industrial relevance. The significance of medicinal plants lies primarily in their phytochemicals or active molecules, which have strong physiological effects. These potent compounds can help prevent a variety of chronic ailments due to their diverse range of actions.

The phytochemical constituents in *P. punctulata* are summarized in Table 1. Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by various microorganisms, insects, and other herbivores. This study revealed the presence of flavonoids, glycosides, phenols, terpenoids, steroids, and tannins in *P. punctulata*, which could be responsible for its observed antimicrobial properties. Our findings align with similar studies conducted by Dal Piaz *et al.* (2018) [27] and Al-Mahbashi *et al.* (2020) [28].

Table 1: Phytochemical constituents of *Psiadia punctulate* extract.

No	Phytochemical analyzed	*P. punctulata*
1	Alkaloids	++
2	Flavonoids	++
3	Steroids	++
4	Phenols	+++
5	Saponins	---
6	Terpenoids	+++
7	Cardiac Glycosides	+++
8	Tannins	+++

Absent (---), Present (++), Abundant (+++).

A major challenge to public health is the global burden of infectious diseases caused by bacterial organisms. Antibiotic therapy is the recommended course of action for treating bacterial infections. However, the development of antibiotic resistance and concerns about the toxicity of antibacterial agents pose significant challenges. These restraints on antibiotic efficacy and safety have led to increased interest in studying the antibacterial functions of plants, due to their comparable effectiveness and lower toxicity.

A wide range of therapeutic plants are grown organically in Yemen. In this work, we investigated the antibacterial properties of the naturally growing *P. punctulata* plant. The methanol extracts of *P. punctulata* were tested against two pathogenic microbes: *E. coli*, whose virulent strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis, and *Staphylococcus epidermidis*, which causes septicemia and endocarditis in immunocompromised patients.

Findings from the current study revealed that *P. punctulata* extract has potential inhibitory effects on all tested bacteria but was more effective against *E. coli* at a concentration of 1000 mg/mL, with a zone of inhibition of 30 mm, compared to 28 mm for *Staphylococcus epidermidis*. At lower concentrations (500, 250, and 125 mg/mL), *Staphylococcus epidermidis* showed higher susceptibility than E. coli. Among the four antibiotics used in this study, gentamycin exhibited the widest range of inhibition on the two species of human pathogenic bacteria. The maximum zone of inhibition was observed against *Staphylococcus epidermidis* (22 mm) and *Escherichia coli* (28 mm), as shown in Table 2.

The antimicrobial activity reported for *P. punctulata* methanol extract is likely due to the presence of trachylobane diterpenoid compounds. This finding aligns with the study conducted by Mothana *et al.* (2011) [29].

Table 2: Antibacterial activities of *P. punctulata* against *Staphylococcus Epidermides* and *Escherichia coli.*

Plant extract	*Staphylococcus Epidermides*	*Escherichia coli*
1000 mg/ml	28 mm	30 mm
500 mg/ml	26 mm	25 mm
250 mg/ml	23 mm	20 mm
125 mg/ml	20 mm	18 mm
Antibiotic		
Ciprofloxacin	15 mm	23 mm
Gentamycin	22 mm	28 mm
Levofloxacin	20 mm	25 mm
Tetracycline	20 mm	20 mm
Amoxicillin	R	R
Ampicillin	R	R

R = Resistance

4. Conclusions

The current investigation demonstrated the presence of medicinally significant bioactive compounds in the methanol extract of *P. punctulata*, supporting its use in traditional medicine for a range of ailments. This study also assessed the chemical composition of *P. punctulata*. Further research is necessary to identify the active components in these plant extracts, as well as to isolate and characterize each unique bioactive compound.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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References

[1] Rakotoarivelo, N.H., Rakotoarivony, F., Ramarosandratana, A.V., Jeannoda, V.H., Kuhlman, A.R., Randrianasolo, A., Bussmann, R.W. (2015) Medicinal plants used to treat the most frequent diseases encountered in Ambalabe rural community, Eastern Madagascar, *Journal of Ethnobiology and Ethnomedicine* **11**: 1-16.

[2] Yuan, H., Ma, Q., Ye, L., Piao, G. (2016) The traditional medicine and modern medicine from natural products, *Molecules* **21**: 559.

[3] Midiwo, J.O., Owuor, F., Juma, B., Waterman, P.G. (1997) Diterpenes from the leaf exudate of *Psiadia punctulata*, *Phytochemistry* **45**: 117-120.

[4] Mabberly, D. (1987) The Plant-Book: A Portable Dictionary of the Higher Plants, ed., Cambridge University Press, Cambridge, UK, pp. 1021.

[5] Al-Khulaidi, A. (2013) Flora of Yemen. Sustainable Natural Resource Management Project (SNRMP) II, Sana'a, Yemen.

[6] Juma, B.F., Yenesew, A., Midiwo, J.O., Waterman, P.G. (2001) Flavones and phenylpropenoids in the surface exudate of *Psiadia punctulata*, *Phytochemistry* **57**: 571-574.

[7] Mahadeo, K., Grondin, I., Kodja, H., Soulange Govinden, J., Jhaumeer Laulloo, S., Frederich, M., Gauvin-Bialecki, A. (2018) The genus *Psiadia*: Review of traditional uses, phytochemistry and pharmacology, *Journal of Ethnopharmacology* **210**: 48-68.

[8] Juma, B.F., Midiwo, J.O., Yenesew, A., Waterman, P.G., Heydenreich, M., Peter, M.G. (2006) Three ent-trachylobane diterpenes from the leaf exudates of *Psiadia punctulata*, *Phytochemistry* **67**: 1322-1325.

[9] Ogweno Midiwo, J., Yenesew, A., Juma, B.F., Derese, S., Ayoo, J.A., Aluoch, A.O., Guchu, S. (2002) Bioactive compounds from some Kenyan ethnomedicinal plants: Myrsinaceae, Polygonaceae and *Psiadia punctulata*, *Phytochemistry Reviews* **1**: 311-323.

[10] Abou-Zaid, M.M., El-Karemy, Z., El-Negoumy, S.I., Altosaar, I., Saleh, N.A. (1991) The flavonoids of *Psiadia punctulata*, *Bulletin of the Chemical Society of Ethiopia* **5**: 37–40.

[11] Flann, C.(2015) GCC: global compositae checklist (version 5 (Beta). Species 2000 & ITIS Catalogue of Life, 26th August 2015.

[12] El-Domiaty, M.M., El-Feraly, F.S., Mossa, J.S., McPhail, A.T. (1993) Diterpenes from *Psiadia arabica*, *Phytochemistry* **34**: 467-471.

[13] Mossa, J.S., El-Domiaty, M.M., Al-Meshal, I.A., El-Feraly, F.S., Hufford, C.D., McPhail, D.R., McPhail, A.T. (1992) A flavone and diterpene from *Psiadia arabica*, *Phytochemistry* **31**: 2863-2868.

[14] Mothana, R.A., Kriegisch, S., Harms, M., Wende, K., Lindequist, U. (2011) Assessment of selected Yemeni medicinal plants for their in vitro antimicrobial, anticancer, and antioxidant activities, *Pharmaceutical Biology* **49**: 200-210.

[15] Gouda, Y.G., Abdallah, Q.M., Elbadawy, M.F., Basha, A.A., Alorabi, A.K., Altowerqe, A.S., Mohamed, K.M. (2014) Cytotoxic and antimicrobial activities of some Compositae plants growing in Taif area, Saudi Arabia, *International Journal of Pharmaceutical Science Invention* **3**: 43-48.

[16] Egwaikhide, P., Gimba, C. (2007) Analysis of the phytochemical content and anti-microbial activity of *Plectranthus glandulosis* whole plant, *Middle-East Journal of Scientific Research* **2**: 135-138.

[17] Sawant, R.S., Godghate, A.G. (2013) Preliminary phytochemical analysis of leaves of *Tridax procumbens* Linn, *International Journal of Science, Environment and Technology* **2**: 388-394.

[18] Mir, M.A., Sawhney, S., Jassal, M. (2013) Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*, *Wudpecker Journal of Pharmacy and Pharmocology* **2**: 1-5.

[19] Harborne, A.J. (1998) Phytochemical Methods A Guide to Modern Techniques of Plant Analysis, ed., Springer Science & Business Media, London, UK, pp. 302.

[20] Talukdar, A.D., Choudhury, M.D., Chakraborty, M., Dutta, B. (2010) Phytochemical screening and TLC profiling of plant extracts of *Cyathea gigantea* (Wall. Ex. Hook.) Haltt. and *Cyathea brunoniana*. Wall. ex. Hook (Cl. & Bak.), *Assam University Journal of Science and Technology* **5**: 70-74.

[21] Chhetri, H.P., Yogol, N.S., Sherchan, J., Anupa, K., Mansoor, S., Thapa, P. (2008) Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal, *Kathmandu University Journal of Science, Engineering and Technology* **4**: 49-54.

[22] Savithramma, N., Rao, M.L., Suhrulatha, D. (2011) Screening of medicinal plants for secondary metabolites, *Middle-East Journal of Scientific Research* **8**: 579-584.

[23] Njoku, O.V., Obi, C. (2009) Phytochemical constituents of some selected medicinal plants, *African Journal of Pure and Applied Chemistry* **3**: 228-233.

[24] Mbata, T., Debiao, L., Saikia, A. (2008) Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes*, *African Journal of Biotechnology* **7**: 1571-1573.

[25] National Committee for Clinical Laboratory Standards. (1993) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Third Edition: Approved Standard M7-A3.*; NCCLS, Villanova, PA, USA.

[26] Bhargav, H., Shastri, S.D., Poornav, S., Darshan, K., Nayak, M.M. (2016) Measurement of the Zone of Inhibition of an Antibiotic, IEEE 6th International Conference on Advanced Computing (IACC), IEEE, Bhimavaram, India, pp. 409-414.

[27] Dal Piaz, F., Bader, A., Malafronte, N., D'Ambola, M., Petrone, A.M., Porta, A., Ben Hadda, T., De Tommasi, N., Bisio, A., Severino, L. (2018) Phytochemistry of compounds isolated from the leaf-surface extract of *Psiadia punctulata* (DC.) Vatke growing in Saudi Arabia, *Phytochemistry* **155**: 191-202.

[28] Al-Mahbashi, H., Moharram, B.A., Al-Maqtari, T. (2020) Phytochemical, anti-inflammatory, analgesic, antipyretic and acute toxicity of *Psiadia punctulata* growing in Yemen, *Universal Journal of Pharmaceutical Research* **5**: 61-66.

[29] Mothana, R.A.A., Kriegisch, S., Harms, M., Wende, K., Lindequist, U. (2011) Assessment of selected Yemeni medicinal plants for their *in vitro* antimicrobial, anticancer, and antioxidant activities, *Pharmaceutical Biology* **49**: 200-210