




Erratum: Phytochemical composition and antioxidant and antimicrobial activities of *Pergularia daemia*

**Authors:**

Omotayo O. Dosumu¹ 
Oluwatoyin O. Ajetunmobi² 
Oluwafemi A. Omole¹
Patricia A. Onocha³ 

Affiliations:

¹Department of Industrial Chemistry, University of Ilorin, Ilorin, Nigeria

²Department of Chemistry, University of Ilorin, Ilorin, Nigeria

³Department of Chemistry, University of Ibadan, Ibadan, Nigeria

Corresponding author:

Omotayo Dosumu,
oltados@unilorin.edu.ng

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In the original article published the second author's last name was spelled incorrectly as Ajetumobi. This has been corrected to Ajetunmobi in the 'Authors' and the 'How to cite this article' sections.

The last author's affiliation was incorrectly indicated as 'University of Ilorin'. This has been corrected in the 'Affiliations' section of the article to the 'University of Ibadan, Ibadan, Nigeria'.

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Phytochemical composition and antioxidant and antimicrobial activities of *Pergularia daemia*

**Authors:**

Omotayo O. Dosumu¹ 
 Oluwatoyin O. Ajetumobi² 
 Oluwafemi A. Omole¹
 Patricia A. Onocha² 

Affiliations:

¹Department of Industrial Chemistry, University of Ilorin, Ilorin, Nigeria

²Department of Chemistry, University of Ilorin, Ilorin, Nigeria

Corresponding author:

Omotayo Dosumu,
 oltados@unilorin.edu.ng

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Background: *Pergularia daemia* of the Asteraceae family has wide use in ethno-medicine. It is established to have pharmacological activity with potential medicinal significance as an antifungal, antioxidant, antimicrobial, anti-inflammatory, analgesic, fertility, hepatoprotective and anticancer agent.

Aim: This study estimated the qualitative and quantitative amount of phytochemicals in *Pergularia daemia* plant and the antioxidant capacity was also determined.

Setting: The fresh plants of *P. Daemia* were collected from Ido-Ekiti, Ekiti State, Nigeria and was identified at Plant Biology Department of the University of Ilorin, Nigeria. The samples were air-dried, pulverized and stored properly for investigation.

Method: Both qualitative and quantitative phytochemical analyses of the leaves and stem were determined through n-hexane, ethyl acetate and ethanol extracts of *P. daemia*. Antioxidant activities of the extracts were evaluated with 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antibacterial and antifungal assay of the extracts were determined by dissolving 1 mg of the extract in 5 mL of the extracting solvent to give a 200 µg/mL solution.

Results: Flavonoids, steroids, terpenoids and glycosides were present in all the stem and leaf extracts of *P. daemia*. The total phenolic content of the extracts ranged from 15.898 ± 0.111 mgGA/g to 54.679 ± 0.675 mgGA/g. The leaf has higher concentration of flavonoids than the stem and the ethanol extracts contain highest content of flavonoids. The concentration of the ethanol extract of the leaf was 400.196 mg QE/g while that of ethyl acetate and n-hexane were 388.627 mg QE/g and 338.725 mg QE/g respectively at 10 mg/mL. Ethanol extracts has the highest concentration of phenolic compounds, flavonoids and antioxidant capacity.

Conclusion: The results derived from this study validate the ethno-medicinal use of the aerial parts of *P. daemia*.

Keywords: *Pergularia daemia*; Phytochemical; Flavonoid concentration; Phenolic content; Antioxidant activity; Antimicrobial.

Introduction

The oldest form of treatment known to humans is the application of herbs to cure ailments and diseases. The prehistoric medical systems such as Ayurveda, Siddha and Homoeopathy use herbal formulations to treat various ailments (Kelly 2009). According to the World Health Organization (WHO), more than 80% of the global population in poor and underdeveloped countries rely primarily on traditional plant-based medicines for their primary health care needs because pharmaceuticals are not available or are relatively expensive (WHO 2002). Even in developed countries where pharmaceuticals are readily accessible, many still prefer herbal drugs over the pharmaceutical drugs (Sridevi, Prema & Sekar 2014).

This development has attracted the interest of pharmaceutical companies and has led to serious research in the field of medicinal plants (Kotnis et al. 2004). In this study, the medicinal plant of interest is *Pergularia daemia*, which belongs to the Asteraceae family. It is a slender, hispid, fetid-smelling perennial climber with small, white flowers and is commonly found in central India and Africa. It is used in ethno-medicine and has been proved to be pharmacologically active with potential medicinal significance as an antifungal, antioxidant, antimicrobial, anti-inflammatory, analgesic, antipyretic, fertility, hepatoprotective and anticancer agent (Karthishwaran & Mirunalini 2010). The plant contains phytochemicals such as alkaloids, glycosides, flavonoids, terpenoids, phenols, steroids and saponins (Sridevi et al. 2014). There is a dearth of scientifically documented literature on the medicinal benefits of this abundant plant; hence, this study was undertaken to estimate the total phenolic and flavonoid concentrations of n-hexane, ethyl acetate and ethanol extracts from the stem and leaves of *P. daemia*. The qualitative phytochemical constituents and antimicrobial activities were also evaluated.

Materials and methods

Plant collection

The fresh plants of *P. daemia* were collected on 02 November 2014 from Ido-Ekiti in Ekiti State, Nigeria. The plant samples were authenticated at the Department of Plant Biology herbarium, University of Ilorin, Nigeria, and a specimen copy with number UIH002/1136 was deposited.

Extract preparation

Stems and leaves of the plant were air-dried separately at room temperature (25 °C) and were pulverised after drying. Of the pulverised sample, 400 g was kept in contact with 1 L n-hexane in a stoppered glass container for 5 days with frequent agitation (Ncube, Afolayan & Okoh 2008). The extract was decanted and filtered through a cotton plug funnel and Whatman no.1 filter paper. The filtrate from the n-hexane extraction was concentrated under vacuum using rotary evaporator. Subsequent extraction using the above procedure was also carried out to obtain crude ethyl acetate and ethanol extracts independently.

Phytochemical screening

Qualitative analysis of phytochemicals

Constituents of the *P. daemia* extracts were screened using standard procedures to identify the metabolites present (Harborne 1973; Okwu 2001; Rahilla et al. 1994; Sofowora 1993). The major metabolites analysed for were alkaloids, tannins, anthraquinones, saponins, flavonoids, terpenoids, glycosides, steroids and phenols.

Quantitative analysis of phytochemicals

Total phenolic content: The total phenolic content in the plant extracts was determined using the spectrophotometric method (Singleton, Orthofer & Lamuela-Raventos 1999). Concentrations of 1 mg/mL, 5 mg/mL and 10 mg/mL of methanolic solution of the extract were prepared and used in the analysis. The reaction mixture was prepared by adding 0.5 mL of methanolic extract to 2.5 mL 10% Folin–Ciocalteu's reagent and 2.5 mL 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 mL methanol, 2.5 mL 10% Folin–Ciocalteu's reagent and 2.5 mL 7.5% of NaHCO₃. The mixture was thereafter incubated in a 45 °C thermo-stated water bath in a dark cupboard for 45 min. The absorbance was then determined at a wavelength of 765 nm using a spectrophotometer (Deckmann GU750). The mixture was prepared in triplicate for each analysis and the mean value of absorbance was obtained. A solution of gallic acid, which serves as a standard, was prepared in methanol to achieve the concentration of 0.5 mg/mL. This solution was then diluted to obtain concentrations of 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.13 µg/mL, 1.56 µg/mL and 0.78 µg/mL, and the calibration line was constructed. Based on the measurement of the absorbance, the concentration of phenolics was determined (µg/mL) from the calibration line and the total phenolic content in the extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Determination of flavonoid content: The total flavonoids in the plant extracts were determined using the spectrophotometric method (Quettier et al. 2000). Methanol was used to prepare the extracts with concentrations of 1 mg/mL, 5 mg/mL and 10 mg/mL, which were used in the analysis. Of each solution of the extracts, 1 mL was added to 1 mL of 2% AlCl₃ solution, which was also prepared in methanol. The samples were incubated for an hour at room temperature and the absorbance was then read using a spectrophotometer at a wavelength of 415 nm (Deckmann GU750). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The quercetin equivalent and calibration curve were evaluated by preparing quercetin solutions at concentrations varying from 10 µg/mL to 100 µg/mL in methanol. Based on the measured absorbance, the concentration of flavonoids was determined (µg/mL) on the calibration line and the flavonoid contents in the extracts were expressed in terms of quercetin equivalent (mg of QE/g of extract).

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl free radical scavenging

The ability of the plant extracts to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was assessed following the method of Chang et al. (2001) and Kumarasamy et al. (2007). The stock solution of the extracts was prepared in methanol to achieve the concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.62 µg/mL, 7.81 µg/mL, 3.90 µg/mL, 1.99 µg/mL and 0.97 µg/mL. The prepared solution (1 mL) was mixed with 1 mL of methanolic solution of DPPH of concentration 1 mg/mL. After 30 min of incubation in the dark at room temperature, the absorbance was recorded at 517 nm with a spectrophotometer. A control sample containing 1 mL of methanol and 1 mL of methanolic DPPH solution was set alongside. Percentage inhibition was calculated using the equation below, while IC₅₀ (inhibitory concentration at 50%) was estimated from the percentage inhibition versus log concentration plot, using a non-linear regression algorithm:

$$\% \text{ inhibition} = \frac{(A \text{ of control} - A \text{ of sample})}{A \text{ of control}} \times 100 \quad [\text{Eqn 1}]$$

Antimicrobial assay: Antibacterial and antifungal activities

Cultures of six human pathogenic bacteria made up of four gram-negative and two gram-positive species were used for the antibacterial assay. *Salmonella typhi* (UCH 4801), *Escherichia coli* (UCH 0026), *Pseudomonas aeruginosa* (UCH 1102) and *Klebsiella pneumoniae* (UCH 2894) were the gram-negative species that were used and *Bacillus subtilis* (UCH 7423) and *Staphylococcus aureus* (UCH 2473) were the gram-positive species. All the microorganisms used were clinical strains from the Medical Microbiology Laboratory of the University College Hospital, University of Ibadan, Ibadan, Nigeria. For the antifungal assay, four fungi, including *Candida albicans*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium notatum*, were used.

The sample extracts that were used for the antimicrobial sensitivity test were prepared by dissolving 1 mg of the extract in 5 mL of the extracting solvents, that is, n-hexane, ethyl acetate and ethanol, to make a 200- $\mu\text{g}/\text{mL}$ solution. By serial dilution, subsequent concentrations of 100 $\mu\text{g}/\text{mL}$, 50 $\mu\text{g}/\text{mL}$, 25 $\mu\text{g}/\text{mL}$, 12.5 $\mu\text{g}/\text{mL}$ and 6.25 $\mu\text{g}/\text{mL}$ were prepared. Gentamycin (5 $\mu\text{g}/\text{mL}$) and tioconazole (0.7 $\mu\text{g}/\text{mL}$) were used as reference antibacterial and antifungal compounds, respectively.

In the antibacterial assay, an overnight culture of the organism was prepared by taking a loop full of the organism from stock and inoculating into a sterile nutrient broth of 5 mL and then incubated for 24 h at 37 °C. From the overnight culture, 0.1 mL of the organism was taken and put into 9.9 mL of sterile distilled water to get 1:100 (10^{-2}) dilution of the organism. From the diluted organism (10^{-2}), 0.2 mL was taken into the prepared sterile nutrient agar, which was maintained at 45 °C, and aseptically poured into sterile petri dishes and allowed to solidify for 60 min. Using a sterile cork borer of 8 mm diameter, wells were made according to the number of graded concentrations. In each well, the different graded extract concentrations were poured, and this was performed in duplicates. The plates were allowed to stay on the bench for 2 h to allow for pre-diffusion. The plates were then incubated upright in the incubator for 24 h at 37 °C (Oloyede et al. 2010; Onocha & Ali 2010).

In the fungal activity inhibition study, surface plate method was used. Sterile sabouraud dextrose agar (62 g/L) was prepared accordingly and aseptically poured into the sterile plates in duplicates and allowed to solidify properly. A volume of 0.2 mL of the organism was spread on the surface of the agar using a petri dish to cover the entire surface of the agar. Then, eight wells were bored using a sterile cork borer of 8 mm diameter. The graded concentrations of the extract were put into the wells accordingly, including the controls. All the plates were left on the bench for 2 h to allow the extract to diffuse properly into the agar, that is, pre-diffusion. The plates were incubated at 25 °C for 72 h. Solvents of extraction were used as control, while gentamycin (5 $\mu\text{g}/\text{mL}$) and tioconazole (0.7 $\mu\text{g}/\text{mL}$) were used as standard reference drugs in the study (Bayer et al. 1986).

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as an average of three analyses \pm standard

error. SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. II, 2004) was used to determine the magnitude of correlation between the variables.

Results and discussion

Phytochemical screening of the crude extract

The phytochemical screening results (Table 1) showed the presence of a high content of glycosides, steroids, terpenoids and flavonoids in all the extracts of both the stem and leaf of *P. daemia*.

Tannins and saponins were observed in the ethanol extracts of the stem and leaf. Anthraquinones were detected in small quantities in n-hexane extracts of both the stem and the leaf. The presence of flavonoids, steroids, terpenoids and glycosides was high in all the extracts. Tannins, alkaloids, saponins and phenols are present in polar solvents extracts, that is, ethyl acetate and ethanol extracts. It is worth mentioning that the high concentration of all the metabolites in the ethanol extract, except for anthraquinone, confirms ethanol as the better extracting solvent of the three solvents. Ethanol, being a polar solvent and close in polarity to water, lends credence to the efficiency of the aqueous extracts of this plant as used in folk medicine. The phytochemicals are responsible for the therapeutic efficiencies and efficacies of medicinal plants (Shoge, Ndukwe & Amupitan 2011) and are present in high concentrations in ethanol, a polar solvent. The presence of this phytochemical in the extracts of the plant may therefore be responsible for the detoxifying action and antimicrobial, antioxidant and anti-inflammatory activities of the plant (Li et al. 2004; Yen, Duh & Chuang 2000). It is therefore not surprising that *P. daemia* has found extensive application as an antioxidant, an analgesic, a hepatoprotective substance and as an anticancer herb in traditional medicine (Karthishwaran & Mirunalini 2010). The presence of some of the above-mentioned metabolites in *P. daemia* had earlier been detected by Sridevi et al. (2014) in a similar study.

Total phenolic content of the plant extracts

The total phenolic content of the *P. daemia* extracts using Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.0052x$, $R^2 = 0.9905$). The total phenolic content in the examined extracts ranged from 15.898 ± 0.111 mg GA/g to 54.679 ± 0.675 mg GA/g. Ethanol extracts from the stem recorded

TABLE 1: Phytochemical composition of *Pergularia daemia* stem and leaf.

Phytochemical constituents	Stem extracts			Leaf extracts		
	n-Hexane	Ethyl acetate	Ethanol	n-Hexane	Ethyl acetate	Ethanol
Alkaloids	-	-	++	-	++	+++
Saponins	-	-	++	-	-	++
Flavonoids	+++	+++	+++	+++	+++	+++
Steroids	+++	+++	+++	+++	+++	+++
Terpenoids	+++	+++	+++	+++	+++	+++
Glycosides	++	+++	+++	++	+++	+++
Tannins	-	-	++	-	-	+++
Phenols	-	++	+++	-	+++	+++
Anthraquinones	+	-	-	+	-	-

+++, high concentration; ++, medium concentration; +, low concentration; -, absent.

the presence of high concentrations of phenolic compounds, while ethyl acetate and n-hexane extracts recorded medium concentration. The polarity of solvents is directly related to extraction efficiency; therefore, polar solvents give better extraction than non-polar solvents (Mohsen & Ammar 2008; Zhou & Yu 2004). Ethanol affords 54.679% and 34.167% GA/g for leaf and stem extracts, respectively, while 18.654% and 15.888% were recorded for n-hexane extracts of leaf and stem, respectively. The total phenolic content of 1 mg/mL, 5 mg/mL and 10 mg/mL of each extract was determined in triplicate.

Phenolic compounds play a key role as antioxidants or free radical scavengers. The antioxidant activity of the phenolic compounds is mainly because of their redox properties, which absorb and neutralise free radicals, triplet oxygen or decomposing peroxides (Osawa 1994). It has also been noted that phenolic compounds provide antimutagenic and anticarcinogenic actions in humans when approximately 1.0 g of mixed antioxidant compounds were consumed daily from vegetable and fruit diet over a short period of time (Tanaka et al. 1998).

The gallic acid equation was used as a standard curve equation for the estimation of the total phenolic compound in the samples. In the standard curve equation $y = 0.0052x$, $R^2 = 0.9905$, y denotes the absorbance and x denotes the concentration of the compound. The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract in Figure 1.

The pairs of n-hexane stem extract and ethyl acetate stem extract were significantly different. Ethyl acetate stem extract was significantly different from that of ethanol stem extract, with $p < 0.05$. All other pairs were not different.

Flavonoid content

The concentration of flavonoids was expressed in terms of quercetin equivalent (mg of QE/g of extract). The concentration of flavonoids in plant extracts from *P. daemia* ranged from 72.549 ± 0.449 mg/mL to 400.196 ± 0.339 mg/mL (Figure 2).

The leaf has a higher concentration than that of the stem. Ethanol extracts of the leaf have the highest concentration of the flavonoids and, generally, ethanol extracts recorded significantly higher concentrations of flavonoids. The concentration of flavonoids in ethanol extracts of the leaf was 400.196 mg QE/g, followed by that of ethyl acetate with 388.627 mg QE/g and the n-hexane extract with 338.725 mg QE/g at 10 mg/mL. The lowest flavonoid concentration was recorded in the n-hexane extract of the stem. The quantities of the flavonoids extracted increases with the polarity of the extraction solvent. This result is in agreement with the observation of Min and Chun-Zhao (2005) when they compared extraction techniques in flavonoid extraction.

Our result revealed that the aerial part of the plant is highly loaded with flavonoids than the stem. Several studies

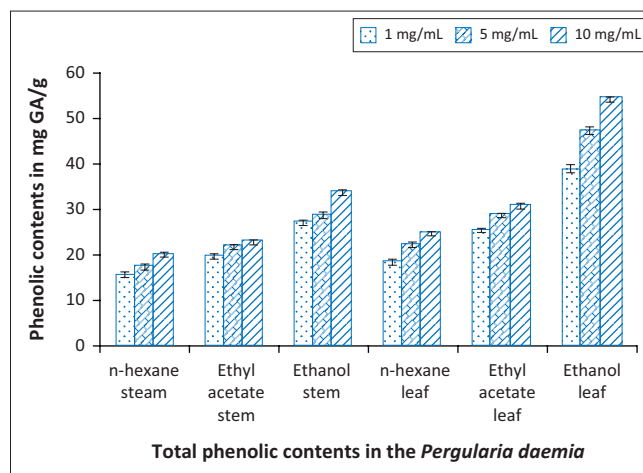


FIGURE 1: Estimated phenolic contents in the stem and leaf extracts of *Pergularia daemia* at 1 mg/mL, 5 mg/mL and 10 mg/mL of each extract.

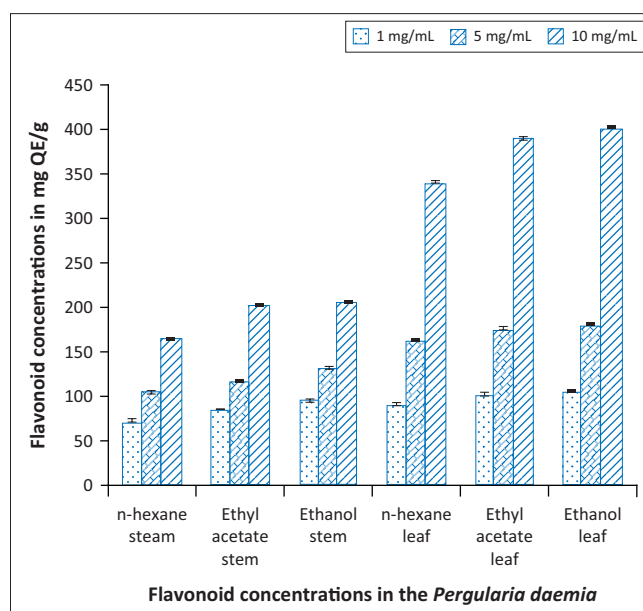


FIGURE 2: Estimated flavonoid concentrations in the stem and leaf of *Pergularia daemia* at 1 mg/mL, 5 mg/mL and 10 mg/mL of each extract.

reported that flavonoids present in herbs significantly contributed to their antioxidant properties and healing ability. Also, flavonoids are found to be effective scavengers of most oxidising molecules, including single oxygen and various free radicals (Manik et al. 2014).

The data obtained were subjected to statistical analysis. It was found that only the n-hexane leaf extract and ethyl acetate leaf extract were different significantly at $p < 0.05$.

Antioxidant activity

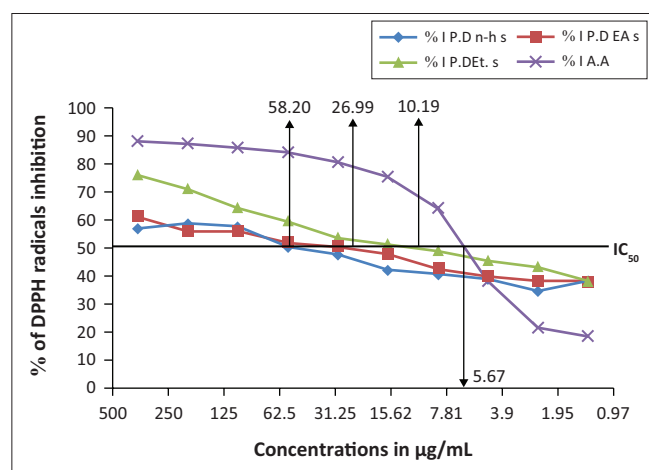
2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The percentage inhibition of *P. daemia* over DPPH free radicals varied from 34.65% to 84.90%. The activity was expressed as IC_{50} (μ g/mL), that is, the minimum concentration of the extract capable of scavenging 50% of the DPPH radicals. The IC_{50} value of ascorbic acid with the minimum concentration

at 5.67 $\mu\text{g}/\text{mL}$ was enough to scavenge the DPPH radical, which is considered to be chemotherapeutically significant ($\text{IC}_{50} < 10 \mu\text{g}/\text{mL}$) with potency that could be used as a reference (Tian et al. 2013). The parallel examination of ascorbic acid as a reference with antioxidant activity of plant extracts is illustrated in Figure 3 and Figure 4.

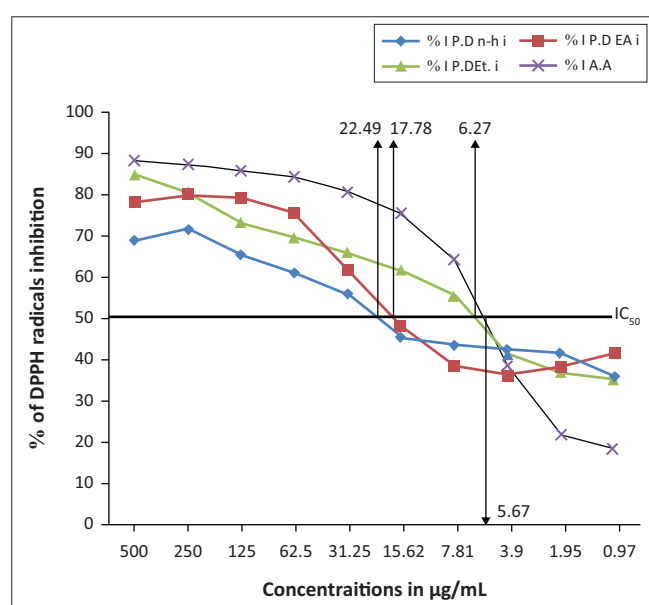
Figure 3 shows the antioxidant activity of an ethanolic stem extract of *P. daemia* at 10.19 $\mu\text{g}/\text{mL}$ in close range with ascorbic acid obtained at 5.67 $\mu\text{g}/\text{mL}$ and 26.99 $\mu\text{g}/\text{mL}$ for ethyl acetate stem extract, followed by 58.20 $\mu\text{g}/\text{mL}$ for n-hexane stem extract, which was observed to be very far from the standard.

Figure 4 shows IC_{50} activity of leaf extracts at 6.27 $\mu\text{g}/\text{mL}$ (ethanol), 17.78 $\mu\text{g}/\text{mL}$ (ethyl acetate) and 22.49 $\mu\text{g}/\text{mL}$ (n-hexane). When compared with the standard at 5.67 $\mu\text{g}/\text{mL}$,



% I, percentage inhibition; P.D, *Pergularia daemia*; n-h, n-hexane; EA, ethyl acetate; Et, Ethanol; A.A, ascorbic acid; l, leaf; s, stem.

FIGURE 3: Percentage inhibition of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical versus concentrations of stem extracts.



% I, percentage inhibition; P.D, *Pergularia daemia*; n-h, n-hexane; EA, ethyl acetate; Et, Ethanol; A.A, ascorbic acid; l, leaf; s, stem.

FIGURE 4: Percentage inhibition of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical versus concentrations of leaf extracts.

the ethanolic extract shows a very close antioxidant activity, followed by ethyl acetate, and the least antioxidant activity was observed in n-hexane. This study reveals that by consuming *P. daemia*, the free radicals that might be present in humans can be eliminated to get relief from oxidative stress and degenerative sicknesses. Ethanolic extract from *P. daemia* manifested the strongest capacity for scavenging DPPH radicals and was even effective at low concentration.

Antimicrobial sensitivity

The antibacterial sensitivity inhibition increases with the polarity of the solvent of extraction and with extract concentration. n-Hexane extract from the stem of *P. daemia* does not show sensitivity on the microorganisms at concentrations lower than 25 $\mu\text{g}/\text{mL}$. Ethyl acetate stem extract shows sensitivity at 12.5 $\mu\text{g}/\text{mL}$ and even at 6.25 $\mu\text{g}/\text{mL}$ on *S. aureus*, *P. aeruginosa* and *K. pneumoniae* (Table 2).

Meanwhile, full sensitivity was observed in ethanolic stem extract at all concentration levels. The ethyl acetate and ethanol extracts showed active inhibition on the growth of all tested microorganisms and the activities were comparable to that of gentamycin, the reference antibacterial drug, particularly at high concentration (Table 2 and Table 3). n-Hexane leaf extract shows sensitivity at 200 $\mu\text{g}/\text{mL}$, 100 $\mu\text{g}/\text{mL}$, 50 $\mu\text{g}/\text{mL}$ and 25 $\mu\text{g}/\text{mL}$ on all organisms but is slightly sensitive at 12.56 $\mu\text{g}/\text{mL}$ on *S. aureus* and *B. subtilis* (Table 3).

Four clinical strains of human pathogenic fungi were used in the study. *Candida albicans*, *A. niger*, *R. stolon* and *P. notatum*. *Candida albicans* and *A. niger* were the only fungi that were sensitive to the n-hexane stem extract inhibitory action at high concentration, although all the four fungi were sensitive to the leaf extract (Table 4 and Table 5). *Rhizopus stolonifera* and *P. notatum* were resistant to n-hexane leaf extract but were inhibited by other extracts, particularly at high concentration. Ethanol extracts for both leaf and stem were active in curbing the activities of the fungi, but they were not comparable to that of tioconazole, even at high concentration of extracts.

All fungi were resistant to the action of extracts at low concentrations, but ethanol extract of the leaf, showed inhibition at all levels. Also, inhibition activity increases with the polarity of extracts as well as concentration.

In the analysis of the zones of inhibition produced by the extracts, it was observed that ethanolic extracts of *P. daemia* showed prominent antimicrobial activity against all microorganisms. Thus, the ethanolic extracts were more potent compared to n-hexane and ethyl acetate extracts. From Table 1, the preliminary phytochemical studies reveal the presence of tannins, terpenoids, alkaloids and flavonoids in high concentration in ethanol extracts, which may contribute to the inhibition of microbial activities as observed. The antimicrobial inhibition results of *P. daemia* extract buttress the literature on the plant usage to combat bacterial

TABLE 2: Antibacterial activities of extracts of *Pergularia daemia* (stem).

Extracts	Extract concentration/ reference/control (mg/mL)	Diameter of zone of inhibition of bacteria (mm)					
		<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>
<i>P. daemia</i> Stem n-Hexane	200	19 ± 1.41	17 ± 1.41	19 ± 1.41	15 ± 1.41	17 ± 1.41	15 ± 1.41
	100	15 ± 1.41	14 ± 0	15 ± 1.41	13 ± 1.41	14 ± 0	12 ± 0
	50	13 ± 1.41	12 ± 0	13 ± 1.41	10 ± 0	12 ± 0	10 ± 0
	25	10 ± 0	10 ± 0	11 ± 1.41	-	10 ± 0	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	n-Hexane	-	-	-	-	-	-
	Gentamycin (mg/mL)	39 ± 1.41	39 ± 1.41	38 ± 0	39 ± 1.41	38 ± 0	40 ± 0
<i>P. daemia</i> Stem Ethyl acetate	200	27 ± 1.41	23 ± 1.41	23 ± 1.41	25 ± 1.41	23 ± 1.41	24 ± 0
	100	23 ± 1.41	18 ± 0	18 ± 0	22 ± 2.83	20 ± 0	20 ± 0
	50	19 ± 1.41	16 ± 0	14 ± 0	18 ± 2.83	18 ± 0	17 ± 1.41
	25	16 ± 2.83	13 ± 1.41	12 ± 0	14 ± 0	14 ± 0	13 ± 1.41
	12.5	13 ± 1.41	10 ± 0	10 ± 0	12 ± 0	12 ± 0	10 ± 0
	6.25	10 ± 0	-	-	10 ± 0	10 ± 0	-
	Ethyl acetate	-	-	-	-	-	-
	Gentamycin (mg/mL)	39 ± 1.41	39 ± 1.41	38 ± 0	39 ± 1.41	38 ± 0	40 ± 0
<i>P. daemia</i> Stem Ethanol	200	31 ± 1.41	30 ± 0	30 ± 0	29 ± 1.41	30 ± 0	28 ± 0
	100	28 ± 0	23 ± 4.24	27 ± 1.41	26 ± 2.83	27 ± 1.41	24 ± 0
	50	24 ± 0	24 ± 0	24 ± 0	22 ± 2.83	24 ± 0	20 ± 0
	25	20 ± 0	19 ± 1.41	20 ± 0	19 ± 1.41	19 ± 1.41	18 ± 0
	12.5	16 ± 2.83	16 ± 2.83	16 ± 2.83	16 ± 2.83	15 ± 1.41	14 ± 0
	6.25	13 ± 1.41	13 ± 1.41	12 ± 2.83	11 ± 1.41	12 ± 0	11 ± 1.41
	Ethanol	-	-	-	-	-	-
	Gentamycin (mg/mL)	40 ± 0	38 ± 0	39 ± 1.41	38 ± 0	39 ± 1.41	39 ± 1.41

P. daemia, *Pergularia daemia*; *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *B. subtilis*, *Bacillus subtilis*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *K. pneumoniae*, *Klebsiella pneumoniae*; *S. typhi*, *Salmonella typhi*.

TABLE 3: Antibacterial activities of extracts of *Pergularia daemia* (leaf).

Extracts	Extract concentration/ reference/control (mg/mL)	Diameter of zone of inhibition of bacterial (mm)					
		<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>
<i>P. daemia</i> Leaf n-Hexane	200	23 ± 1.41	17 ± 1.41	20 ± 0	17 ± 1.41	18 ± 0	17 ± 1.41
	100	19 ± 1.41	14 ± 0	18 ± 0	14 ± 0	16 ± 0	14 ± 0
	50	17 ± 1.41	12 ± 0	15 ± 1.41	12 ± 0	13 ± 1.41	12 ± 0
	25	13 ± 1.41	10 ± 0	12 ± 0	10 ± 0	10 ± 0	10 ± 0
	12.5	11 ± 1.41	-	10 ± 0	-	-	-
	6.25	-	-	-	-	-	-
	n-Hexane	-	-	-	-	-	-
	Gentamycin (mg/mL)	39 ± 1.41	39 ± 1.41	38 ± 0	39 ± 1.41	38 ± 0	40 ± 0
<i>P. daemia</i> Leaf Ethyl acetate	200	29 ± 1.41	25 ± 1.41	26 ± 0	27 ± 1.41	25 ± 1.41	26 ± 0
	100	26 ± 2.83	20 ± 0	21 ± 1.41	24 ± 0	21 ± 1.41	21 ± 1.41
	50	22 ± 2.83	16 ± 0	18 ± 0	20 ± 0	19 ± 1.41	18 ± 0
	25	19 ± 1.41	14 ± 0	14 ± 0	17 ± 1.41	16 ± 2.83	14 ± 0
	12.5	16 ± 2.83	12 ± 0	12 ± 0	13 ± 1.41	12 ± 0	12 ± 0
	6.25	11 ± 1.41	10 ± 0	10 ± 0	10 ± 0	10 ± 0	10 ± 0
	Ethyl acetate	-	-	-	-	-	-
	Gentamycin (mg/mL)	39 ± 1.41	40 ± 0	38 ± 0	39 ± 1.41	38 ± 0	40 ± 0
<i>P. daemia</i> Leaf Ethanol	200	30 ± 0	31 ± 1.41	30 ± 0	32 ± 0	32 ± 0	29 ± 1.41
	100	28 ± 0	28 ± 0	28 ± 0	28 ± 0	28 ± 0	27 ± 1.41
	50	25 ± 1.41	24 ± 0	25 ± 1.41	25 ± 1.41	25 ± 1.41	24 ± 0
	25	22 ± 2.83	20 ± 0	21 ± 1.41	20 ± 2.83	22 ± 2.83	20 ± 0
	12.5	18 ± 0	16 ± 2.83	17 ± 1.41	16 ± 2.83	18 ± 0	18 ± 0
	6.25	14 ± 0	12 ± 2.83	13 ± 1.41	13 ± 1.41	14 ± 0	13 ± 1.41
	Ethanol	-	-	-	-	-	-
	Gentamycin (mg/mL)	40 ± 0	39 ± 1.41	39 ± 1.41	38 ± 0	39 ± 1.41	39 ± 1.41

P. daemia, *Pergularia daemia*; *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *B. subtilis*, *Bacillus subtilis*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *K. pneumoniae*, *Klebsiella pneumoniae*; *S. typhi*, *Salmonella typhi*.

TABLE 4: Antifungal activities of extracts of *Pergularia daemia* (stem).

Extracts	Extract concentration/ reference/control (mg/mL)	Diameter of zone of inhibition of bacteria (mm)			
		<i>C. albicans</i>	<i>A. niger</i>	<i>R. stolon</i>	<i>P. notatum</i>
<i>P. daemia</i> Stem n-Hexane	200	17 ± 1.41	15 ± 1.41	-	-
	100	13 ± 1.41	12 ± 0	-	-
	50	10 ± 0	10 ± 0	-	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
	n-Hexane	-	-	-	-
	Tioconazole (0.7 mg/mL)	38 ± 0	27 ± 1.41	26 ± 0	27 ± 1.41
<i>P. daemia</i> Stem Ethyl acetate	200	17 ± 1.41	20 ± 0	17 ± 1.41	16 ± 0
	100	14 ± 0	18 ± 0	14 ± 0	14 ± 0
	50	12 ± 0	14 ± 0	12 ± 0	11 ± 1.41
	25	10 ± 0	10 ± 0	10 ± 0	-
	12.5	-	-	-	-
	6.25	-	-	-	-
	Ethyl acetate	-	-	-	-
	Tioconazole (0.7 mg/mL)	38 ± 0	27 ± 1.41	26 ± 0	27 ± 1.41
<i>P.D.</i> Stem Ethanol	200	20 ± 0	22 ± 0	20 ± 0	18 ± 0
	100	18 ± 0	19 ± 1.41	18 ± 0	16 ± 0
	50	16 ± 0	17 ± 1.41	16 ± 0	14 ± 0
	25	14 ± 0	14 ± 0	14 ± 0	12 ± 0
	12.5	12 ± 0	12 ± 0	12 ± 0	10 ± 0
	6.25	10 ± 0	10 ± 0	10 ± 0	-
	Ethanol	-	-	-	-
	Tioconazole (0.7 mg/mL)	28 ± 0	28 ± 0	27 ± 1.41	28 ± 0

P. daemia, *Pergularia daemia*; *C. albicans*, *Candida albicans*; *A. niger*, *Aspergillus niger*; *R. stolon*, *Rhizopus stolon*; *P. notatum*, *Penicillium notatum*.

TABLE 5: Antifungal activities of extracts of *Pergularia daemia* (leaf).

Extracts	Extract concentration/ reference/control (mg/mL)	Diameter of zone of inhibition of bacteria (mm)			
		<i>C. albicans</i>	<i>A. niger</i>	<i>R. stolon</i>	<i>P. notatum</i>
<i>P. daemia</i> Leaf n-Hexane	200	14 ± 0	16 ± 0	14 ± 0	13 ± 1.41
	100	12 ± 0	13 ± 1.41	12 ± 0	10 ± 0
	50	10 ± 0	10 ± 0	10 ± 0	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
	n-Hexane	-	-	-	-
	Tioconazole (0.7 mg/mL)	28 ± 0	27 ± 1.41	26 ± 0	27 ± 1.41
<i>P. daemia</i> Leaf Ethyl acetate	200	20 ± 0	20 ± 0	18 ± 0	19 ± 1.41
	100	18 ± 0	18 ± 0	14 ± 0	16 ± 0
	50	14 ± 0	16 ± 0	12 ± 0	14 ± 0
	25	12 ± 0	14 ± 0	10 ± 0	12 ± 0
	12.5	10 ± 0	11 ± 1.41	-	10 ± 0
	6.25	-	-	-	-
	Ethyl acetate	-	-	-	-
	Tioconazole (0.7 mg/mL)	28 ± 0	28 ± 0	26 ± 0	28 ± 0
<i>P. daemia</i> Leaf Ethanol	200	23 ± 1.41	23 ± 1.41	21 ± 1.41	20 ± 0
	100	20 ± 0	21 ± 1.41	18 ± 0	18 ± 0
	50	18 ± 0	19 ± 1.41	16 ± 0	16 ± 0
	25	15 ± 1.41	17 ± 1.41	14 ± 0	14 ± 0
	12.5	13 ± 1.41	13 ± 1.41	12 ± 0	12 ± 0
	6.25	11 ± 1.41	10 ± 0	10 ± 0	10 ± 0
	Ethanol	-	-	-	-
	Tioconazole (0.7 mg/mL)	28 ± 0	28 ± 0	27 ± 1.41	28 ± 0

P. daemia, *Pergularia daemia*; *C. albicans*, *Candida albicans*; *A. niger*, *Aspergillus niger*; *R. stolon*, *Rhizopus stolon*; *P. notatum*, *Penicillium notatum*.

and fungal infections such as sexually transmitted diseases and skin diseases (Kokwaro 1981).

Conclusion

The results show that *P. daemia* has a high content of flavonoids and other phenolic compounds but can only be efficiently extracted with polar solvents. The high content of phenols and flavonoids in the plant extracts contributed to the strong antioxidant activity exhibited by the plant. Consequently, extracts of polar solvent showed more appreciable sensitivity against human pathogens as reported than the less polar extracts. In folk medicine, extraction is carried out with water or liquor (polar solvent), which is highly efficient in the extraction of the active ingredients from the studied plant. The results of this study present *P. daemia* as a potential plant for drug discovery. The plant is therefore recommended for further phytochemical analysis in order to isolate the active compounds responsible for the biological activities exhibited by the *P. daemia*.

Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contribution

O. O. Dosumu designed the research, supervised the research work and did the write up. O. O. Dosumu was the project leader also. O. O. Ajetunmobi carried out the research and reported the results. O. A. Omole did the research experiments with Ajetunmobi and brought out the relevant references. P. A. Onocha did the proof reading and did the final corrections.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Disclaimer

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