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Effect of *Pergularia daemia* (Forssk) and *Momordica charantia* L. (bitter gourd) leaf extracts on some enteric bacterial species

Abstract

Medicinal plants are potential sources of new antimicrobial molecules. This study was undertaken to determine the effects of *Pergularia daemia* (Forssk) and *Momordica charantia* (bitter gourd) leaf extracts on some enteric bacterial species. Plant leaves were collected from Kerker village of Jos East Local Government Area of Plateau State. Plants were identified, the leaves were shade-dried, pulverized into fine powder. Proximate analysis was carried out using AOAC methods. Extracts were obtained through cold maceration techniques. The extracts were used for phytochemical screening; antibacterial activity was carried out using well diffusion methods. Minimum inhibitory concentration and maximum bactericidal concentration was determined using broth dilution method. The results of proximate analysis showed that the plants powder were rich in carbohydrates, *M. charantia* 55.59± 0.02 and *P. daemia* 31.38 ± 0.03, followed by crude fiber and crude fat 19. 62± 0.04 and 16.69± 0.22 in *P. daemia* compared to 10.66 ± 0.03 and 10.78± 0.03 in *M. charantia* while ash content and moisture were moderate with 12.86±0.12 in *M. charantia* and 7.53 ± 0.02 in *P. daemia.* The phytochemicals showed the presence of alkaloids, phenols, flavonoids, tannins and terpenoids in all plant extracts. The antibacterial activity showed that the extracts have a broad spectrum of activity on the test organisms as compared to the standard drug gentamicin. There was a significant difference at $p<0.05$. *Shigella flexneri* was the most susceptible to methanolic and aqueous extracts with 22.53± 0.10 at 500 mg/ml as compared to standard drug gentamicin 32.33± 0.18, followed by *E. coli* with 21.13± 0.13 at 500 mg/ml as compared to the control 29.60±0.15 and *Salmonella typhi* with 20.47±0.14 at the concentration of 500 mg/ml as compared to standard drug with 38.50±0.36. The results of minimum inhibitory concentration showed that *Shigella flexneri* was the most susceptible with the MIC of 31.25 mg/ml and 62.5 mg/ml for both methanolic and aqueous extracts of *P. daemia* while *salmonella typhi* was the most susceptible with the MIC of 62.5 mg/ml and 125 mg/ml for *M. charantia* respectively. The presence of alkaloids, saponins, tannins and terpenoids showed that the plants leaves have antibacterial activity. The results of this study support the use of these plants as therapeutic agents for diarrhoea caused by *S. flexneri* and *E. coli*.

Keywords: *pergularia daemia*, *mormordica charantia*, leaf extracts, phytochemicals, antibacterial activity

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Introduction

Traditional medicine has been practiced for many centuries in many parts of the world, including Nigeria, and most especially in rural areas due to its availability and low cost. Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of such based on their use in traditional medicine.[1] Most of the plant materials used in traditional medicines are readily available in rural areas and this has made traditional medicine relatively cheaper than modern medicine. The global market for herbal drugs is lucrative and the world herbal trade is expected to reach USD 7 trillion by 2050.[2]

Pergularia daemia (Forssk.) (Asclepiadaceae) is a well-known herbal drug used in ancient medicines. It is mainly found in tropical and sub-tropical areas, secreting milky latex. Leaves are thin, broadly ovate and heart-shaped, covered with soft hairs. The leaves were used in folk medicine to treat various diseases including liver disorders, diabetes and fungal infection.[3] Bioactive compounds of *P. daemia* such as quercetin, α- sitosterol, βamyrin, betaine, isorhamnetin, chrysoeriol, taxifolin, naringenin. are responsible for its wonderful therapeutic potential and free radical scavenging activity.[4] *Momordica charantia* L. (bitter melon), a member of the Cucurbitaceae family, has long been used as a food and medicine.[5] Bitter melon is known as bitter gourd, balsam pear, karela, and pare since it grows in tropical regions. Antioxidant, anti-diabetes, anti-inflammatory, anti- bacterial and anti-cancer effects *of M. charantia* have been reported.[6] Fruits and seeds of *M. charantia* possess medicinal properties such as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial and antitumor. The plant was generally used to investigate for immunostimulant activity, chemotaxis stimulation, treating ulcers, anti-hyperglycemic and hypoglycemic activity and antioxidant enzyme activities.

During the last few decades, it has been possible to combat the bacterial and fungal infections through major improvement in early detection techniques, and newly developed antibiotics. Nevertheless, many of the currently used antimicrobials often lead to toxicity and undesirable side effects, or drug-drug interactions. The problem of antibiotic resistance has become particularly alarming due to the emergence of multi-drug resistant strains exhibiting simultaneous resistance to two or more classes of antibiotic.[6]

World Health Organization[7] reported that 80% of African populations use traditional medicine to meet their primary healthcare needs, most of which involve the use of plants. There has been an increasing research on medicinal plants to validate their folkloric uses. These plants have been a valuable source of medicinal agents with proven potential of treating infections with minimal side effects when used cautiously. Plants are the treasure homes of potential drugs that could be the source of obtaining variety of future drugs (Figure 1, 2).[8]

Figure 1 Picture showing *Pergluria daemia* plant.

Figure 2 Picture showing *Mormodica charantia* plant.

Materials and methods

Study area

The research was carried out in Jos North Local Government area of Plateau state. The experiment was carried out in the Department of Microbiology University of Jos, Jos, Nigeria.

Collection of plant materials

Fresh and mature leaves of *Pergularia daemia* and *Momordica charantia* L. were collected from full grown plants in Kerker village of Jos East Local Government Area, Plateau state, Nigeria. The plant leaves were taken to the herbarium at University of Jos for proper identification by the curator in charge. The leaves were then cleaned and washed with tap water and rinsed with distilled water, the leaves were shade-dried at room temperature for 12 days and the dried leaves were pulverized to fine power using mortar and pestle.

Preparation of plant materials

The extraction was carried using cold maceration method using the ratio 1:10 grams to volume of 70 % methanol. 50g of the powder was weighed using a top loading balance and it was then transferred into a large extracting flask (bottles), the content was soaked with 500ml of 70 % methanol and allowed to stand for three (3) days at room temperature. The suspension was then filtered with a sterile muslin cloth and then filtered again using sterile Whatman No.1 filter paper inserted in a funnel. The plant residue was subjected to several parts of rinsing and filtration to attain an exhaustive level of extraction.

Phytochemical determination

The plant fractions were screened for their phytochemical constituents to determine the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, anthraquinones, cardiac glycosides and terpernoids using standard phytochemical screening procedures. Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins, saponins, terpenes, steroids, carbohydrates, cardiac glycosides and anthraquinone were carried out on the extracts as described by Sofowora.[9]

Test for alkaloids

About 2ml of each plant extract was stirred with 3ml of 1% aqueous hydrochloric acid on steam bath and filtered. lml of the filtrate was treated with a few drops of Mayer's reagent and a second portion treated similarly with Dragedorffs reagent. Precipitation with either of those reagent was taken as evidence for the presence of alkaloids.[9]

Test for saponins

About 2ml of each plant extract was added to 4.0ml of distilled water in a test tube and the test tube was shaken vigorously for about 30 seconds. The test tube was allowed to stand for half an hour. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.[9]

Test for flavonoids

Lead sub-acetate test 2ml of each plant extract was dissolved in 5ml of distilled water, heated for 5 minutes and filtered. The filtrate was allowed to stand for 5 minutes to cool and about 2-3 drops of lead acetate solution was added to the filtrate. A yellow colored precipitate indicated the presence of flavonoids.[9]

Test for tannins

About 2ml of each plant extract was stirred with 1ml of distilled water, filtered and few drop of ferric chloride were added to the filtrate. A blue-black, green precipitate was taken as evidence for the presence of tannins.[9]

Tests for anthraquinones

About 2ml of each plant extract was taking into a test tube and 5ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate shaken with an equal volume of 100% NH_3 solution. A pink –violet color in the ammonical layer indicate the presence of free anthraquinones.[9]

Test for cardiac glycosides.

About 3ml of each plant extract was dissolved in 1 ml of glacial acetic acid containing one drop of $FeCl_2$(Ferric chloride) solution. It was then under-layered with 1 ml of concentrated H_2SO_4. A brown ring was observed at the interphase, which is a positive reaction for the presence of a deoxy-sugar characteristic of cardiac glycosides.[9]

Test for carbohydrates

About 1ml of each plant extract was dissolved in 3ml of distilled water and mixed with a few drops of Molisch reagent (10% solution

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of α- naphthol in alcohol). Then 1ml of concentrated sulphuric acid was carefully added down the side of the inclined tube so that the acid forms a layer beneath the aqueous solution without mixing it. A reddish or violet ring at the junction of the liquids was observed indicating the presence of carbohydrate.[9]

Proximate analysis

Moisture content

About 2grams of moisture free sample was placed in each dish and dried in an oven at 105ºC for 24hours until constant weight was achieved; the dishes with samples were then cooled in a desiccator and weighed.

Crude protein

Digestion: About 2grams of the sample was weighed into a conical flask, 0.2 grams of catalyst made up of anhydrous sodium sulphate, copper(ii) sulphate and selenium dioxide in the ratio of (98:1:1).

Exactly 10mls of analar sulphuric acid was added with gentle heat until foaming reduce, heat continue strongly until the solution became clear.

Crude fibre

Two (2) g of moisture free sample was weighed and 200ml of 1.25% H2SO4 was added and boiled for 30minutes. It was then washed with distilled water to free sulphate. The residue was transferred into another beaker. 200ml of 1.25% sodium hydroxide was added and boiled for another 30minutes and washed again with distilled water to free the alkaline. Then samples were transferred into petri dishes and dried in oven for 24hrs at 105ºC.After cooling the samples were weighed again on the porcelain crucible and ignited for 6hrs at 600 ºC to obtain the ash content which was cooled and re-weighed. The loss in weight on ignition was expressed as the percentage crude fiber.

%crude fibre= loss of weight after ignition/weight of original sample*100.

a) Digestion= about 2grams of sample was weighed into a conical flask 0.2g catalyst (made up of anhydrous sodium sulphate, copper II sulphate and selenium dioxide with ratio 98:1:1). 10ml of annular sulphuric acid was added with gentle heating until foaming radical, heating continued strongly until the solution became clear. A blank containing the digestion reagent was treated same as sample.

b) Distillation= A 100mL conical flask (receiving flask) containing 20ml of 2% boric acid solution with two drops of screen indicator was placed under condenser exactly 10ml of sample and 20ml of NaoH was pipette into distillation set through the small funnel. Heating of the distillation flask commenced until 80ml of distillation was obtained. The receiving flask followed by titration with 0.05m sulphuric acid, (H_2SO_4) to a Pink colour end point.

(%N) = vs-vb * Nacid * 0.04/W *Vl/V2*100. Where vs= volume of acid required.

Vb= volume of acid required to titrate the blank Nacid= Normality of acid

W= weight of sample in grams Crude protein=%N * 6.25(convertion)

Ash content

About 2grams of moisture free sample was weighed in to pre heated and transferred into a muffle finance at 55ºC for 6hrs until white or light grey color was obtained. It was then cooled in a desiccator and reweighed.

%Ash content= W3-W1/W2-W1*100/1 Where W1= weight of empty crusible

W2= weight of crusible+sample before ashing

W3= weight of crusible+weight of sample after ashing.

Fat content

Fat free thimble was weighed (W1). 2g sample was added into the thimble and weighed again (W2). A 500ml round bottom flask was also weighed fat free (W3). Two third (2/3) of pet ether or n-hexane was transferred into the flask, the extraction set up was expressed to a heating device and allowed to boil for 6hrs after which the flask containing the fat residue was allowed to cool in a desiccator and weighed (W4), The percentage was calculated.

%fat= W4 - W2/W2 -W1*100/1

Where W1= weight of empty thimble W2= weight of thimble+sample W3= weight of empty flask

W4= weight of flask+sample after extraction.

Carbohydrate

Carbohydrate content of the sample was determined by the different percentage of carbohydrate = (100- moisture + fat content + crude protein + ash content + crude fiber).

Preparation and re-constitution of plant extracts

For the preparation of dilutions of crude extracts for antibacterial assay, the extracts were reconstituted by dissolving in 10% DMSO (Dimethyl Sulphur oxide) solvent. 0.8g of the solid plant extract was dissolved in 2ml of 10% DMSO in distilled water to make a stock of 400 mg/ml and further double dilutions were made to obtain 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml respectively. The reconstituted extracts were maintained at a temperature between 2-8°C under refrigerated condition until they were used for the experiment.

Source of microorganisms

Clinical isolates of the bacteria *Escherichia coli*, *Salmonella typhi*, and *Shigella* spp. were obtained from the Veterinary Research Institute Vom. The organisms were collected in suspension of nutrient broth.

Antimicrobial susceptibility testing

Agar well diffusion techniques

The antimicrobial susceptibility test was performed with the clinical isolate using the Agar well diffusion technique. The bacteria inoculum was prepared from subculture as follows: 4-5 colonies of the isolates were emulsified in sterile nutrients both and the turbidity adjusted to 0.5 Mcfarland standard. A sterile cotton swap was dipped into the standardized bacterial suspension and used to evenly inoculate the nutrient agar plates. The plates were allowed for 5 minutes. Wells of about 6mm in diameter were aseptically punched with a sterile cork-borer (5 holes per plates) and the wells were filled with 100 micro liter of the different percentage of the oil. The plates were left for 30 minutes before incubation in order for the extracts to diffuse into the agar. After incubation at 37°C for 24 hours, the average diameter of three readings of the clear zone around the hole were recorded as the measure of inhibitory level of the extract against the test bacteria and reported as mean±SEM. The plates were inoculated with the same standardized inoculum to check for the activities of standard drugs against the tested organisms using standard antibacterial drug gentamycin.

MIC and MBC determination

The minimum inhibitory concentration which is the concentration giving the least inhibitory activity was determined using the broth dilution method. Standardized inoculate of 1 ml of broth containing the organism was introduced into a test tube containing 5ml of sterile broth, 200ul of the re-constituted extract at various concentrations was introduced into the test tubes and it was incubated at 37ºC for 24 hours and observed the growth in the form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered as the MIC's value.

Data analysis

Data obtained was subjected to statistical analyses using Graph Pad Prism version 8.2. The results are expressed as Mean ± SEM, $p<0.05$ was taken as accepted level of significant difference.

Results

The proximate composition of *P. daemia* and *M. charantia*

The results of the proximate composition showed significant difference with *M. charantia* having the highest carbohydrates, ash and moisture content of 55.59±0.02, 12.86±0.12 and 9.90±0.01% respectively, whereas *P. daemia* had the highest crude fiber, crude protein and fats of 19.62±0.04, 17.23±0.03 and 16.69±0.22% respectively. As seen in Table 1.

Table 1 The proximate composition of *P. daemia* and *M. charantia*

Parameters (%)	P. *daemia*	M. *charantia*
Moisture	7.83± 0.03^{b}	9.90± 0.01^{a}
Ash	7.53± 0.02^{b}	12.86± 0.12^{a}
Fats	16.69±0.22^{a}	10.78± 0.03^{b}
Crude protein	17.23±0.03^{a}	12.33± 0.19^{b}
Crude fiber	19.62±0.04^{a}	10.66± 0.03^{b}
Carbohydrates	31.38±0.03^{b}	55.59± 0.02^{a}
L.S.D	0.08	
P-value	<0.0001	

At P≤0.05 there was a significant difference in the proximate analysis of the two plants. Values are presented as mean±standard error of means. Ranking was done across the plants and values with the same super script are not significant.

Phytochemical screening of different plant extracts

The results of the phytochemical screening showed the presence of secondary metabolites such as alkaloids, saponin, tannin, flavanoid, phenols, steroids, anthraquinones, cardiac glycosides, and terpenoids. Alkaloids highly present for *P. daemia* aqueous and methanolic extracts and more present for *M. charantia* aqueous and methanolic extracts, tannins slightly present for both *P. daemia* aqueous and methanolic extracts and more present for *M. charantia* aqueous and methanolic extracts, Flavonoids slightly present for *P. daemia* aqueous while more present for *P. daemia* methanolic extracts and slightly present for *M. charantia* aqueous and more present for *M. charantia* methanolic extracts, phenols more present for *P. daemia* aqueous and methanolic extracts and highly present for *M. charantia* aqueous and more present for *M. charantia* methanolic extracts, Saponins absent for both *P. daemia* aqueous and methanolic extracts and slightly present for both *M. charantia* aqueous and methanolic extracts, Steroids slightly present for *P. daemia* aqueous while absent for *P. daemia* methanolic extracts and slightly present for *M. charantia* aqueous and absent for *M. charantia* methanolic extracts, Anthraquinones slightly present for *P. daemia* aqueous while absent for *P. daemia* methanolic extracts and absent for both *M. charantia* aqueous and methanolic extracts, Cardiac glycosides more present for *P. daemia* aqueous while absent for *P. daemia* methanolic extracts and more present for *M. charantia* aqueous and absent for *M. charantia* methanolic extracts, Terpenoids absent for *P. daemia* aqueous while highly present for methanolic extracts and slightly present for *M. charantia* aqueous while highly present for *M. charantia* methanolic extracts and carbohydrates is absent for both *P. daemia* and *M. charantia* extracts (Table 2).

Table 2 Phytochemical Screening of different plant extracts

Constituents	P. *daemia*	P. *daemia*	M. *charantia*	M. *charantia*
	Aqueous	Methanolic	Aqueous	Methanolic
Alkaloids	+++	+++	++	++
Saponins	-	-	+	+
Tannins	+	+	++	++
Flavonoids	+	++	+	++
Carbohyrdrates	-	-	-	-
Phenols	++	++	+++	++
Steroids	+	-	+	-
Anthraquinones	+	-	-	-
Cardiac glycosides	++	-	++	-
Terpenoids	-	+++	+	+++

- =absent; += slightly present; ++= more present; +++=highly present

Table 3 shows that *Salmonella typhi* was the most susceptible to the methanolic extracts of *P. daemia* with zones of inhibition of between 4.33±0.10-20.47±0.14mm, while *E. coli* with the zones of inhibition of between 5.30± 0.17b-21.13± 0.13b, and *Shigella flexneri* with zones of inhibition of between 8.67± 0.09a-22.53± 0.10a compare to standard drug gentamicin.

The antibacterial activity of *P. daemia* aqueous extract on selected bacteria

Salmonella typhi was the most susceptible to the *P. daemia* aqueous extracts with zones of inhibition of between 1.67± 0.15c-12.26± 0.15c while *E. coli* with the zones of inhibition of between 2.37± 0.10b-14.70± 0.12b and *Shigella flexneri* with the zones of inhibition of between 3.43± 0.14a-16.47± 0.10a compare with the standard drug gentamicin (Table 4).

The antibacterial activity of *M. charantia* methanolic extract on selected bacteria

E. coli was the most susceptible to the *M. charantia* extracts with the zones of inhibition of between 3.44± 0.10c-17.13± 0.09c, followed by *Salmonella typhi* with zones of inhibition of between 9.33± 0.10b -23.46± 0.15b and *Shigella flexneri* with the zones of inhibition of between 10.23± 0.09a-25.30± 0.30a compare to the standard drug gentamicin (Table 5).

The antibacterial activity of *M. charantia* aqueous extract on selected bacteria

E. coli was the most susceptible to the *M. charantia* aqueous extracts with the zones of inhibition of between 3.46± 0.09c-15.37± 0.12c, followed by *Salmonella typhi* with the zones of inhibition of between 4.20± 0.12b-17.43± 0.09b and *Shigella flexneri* with the zones of inhibition of between 5.20± 0.06a-19.20± 0.12a compared to the standard drug gentamicin (Table 6).

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The MIC & MBC for the antibacterial activity of *P. daemia* aqueous extract on some selected bacteria.

The results of minimum inhibitory concentration showed that *Salmonella typhi* was the most susceptible with MIC at concentration of 250 mg/ml, followed by *E. coli* 125 mg/ml and *Shigella flexneri* is 62.5 mg/ml. while their maximum bactericidal concentration *Salmonella typhi* showed significant difference at concentration 500 mg/ml, followed by *E. coli* 250 mg/ml and *Shigella flexneri* showed at 125 mg/ml (Table 7).

The MIC & MBC for the antibacterial activity of *P. daemia* methanolic extract on some selected bacteria.

The results of minimum inhibitory concentration showed that *Salmonella typhi* and *E. coli* was the most susceptible with MIC both at 62.5 mg/ml and *Shigella flexneri* only showed at 31.25 mg/ml. In maximum bactericidal concentration *E. coli* and *Salmonella typhi* showed significant difference at 125 mg/ml, and *Shigella flexneri* at 125 mg/ml respectively (Table 8).

The MIC & MBC for the antibacterial activity of *M. charantia* aqueous extract on some selected bacteria.

The results of minimum inhibitory concentration *E. coli* showed turbidity at 125 mg/ml both *Salmonella typhi* and *Shigella flexneri* showed a turbidity at 62.5 mg/ml. While maximum bactericidal concentration *E. coli* showed no turbidity at 250 mg/ml and *Salmonella typhi* and *Shigella flexneri* showed no turbidity at 125 mg/ml (Table 9).

The MIC & MBC for the antibacterial activity of *M. charantia* methanolic extract on some selected bacteria.

The results of minimum inhibitory concentration of *Salmonella typhi* showed turbidity at 125 mg/ml followed by *E. coli* at 62.5 mg/ml and *Shigella flexneri* show only at 31.25 mg/ml respectively. Maximum bactericidal concentration *Salmonella typhi* showed no turbidity at 250 mg/ml followed by *E. coli* from 125 mg/ml and *Shigella flexneri* at 250 mg/ml respectively (Table 10).

Table 3 The antibacterial activity of *P. daemia* methanolic extract on selected bacteria

Organism	31.25 mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	500 mg/ml	Control
E. coli	5.30± 0.17^{b}	9.50± 0.09^{b}	13.23±0.15^{b}	16.70±0.12^{b}	21.13± 0.13^{b}	29.60± 0.15^{c}
Salmonella Spp	4.33± 0.10^{c}	7.47± 0.18c	10.30±0.10^{c}	14.27±0.15^{c}	20.47± 0.14^{c}	38.50± 0.36^{a}
Shigella spp.	8.67± 0.09^{a}	12.27± 0.12^{a}	14.23±0.09^{a}	18.47±0.09^{a}	22.53± 0.10^{a}	32.33± 0.18^{b}
LSD		0.34				
P-value		<0.0001				

At P≤0.05 there was a significant difference in the antibacterial activity of the methanolic extracts of *P. daemia* on the selected bacteria. Values are presented as mean±standard error of means. Ranking was done across the organisms and values with the same super script are not significant.

Table 4 The antibacterial activity of *P. daemia* aqueous extract on selected bacteria

Organism	31.25mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	500 m/ml	Control
E. coli	2.37± 0.10^{b}	4.37± 0.09^{b}	7.63± 0.12^{b}	10.53±0.15^{b}	14.70± 0.12^{b}	29.60± 0.15^{c}
Salmonella spp	1.67± 0.15^{c}	2.37± 0.20^{c}	5.33± 0.10^{c}	8.57± 0.09^{c}	12.26± 0.15^{c}	38.50± 0.36^{a}
Shigella spp	3.43± 0.14^{a}	5.37± 0.12^{a}	9.70± 0.15^{a}	12.46±0.12^{a}	16.47± 0.10^{a}	32.33± 0.18^{b}
LSD	0.31					
P-value	<0.0001					

At P≤0.05 there was a significant difference in the antibacterial activity of the aqueous extract of *P. daemia* on the selected bacteria. Values are presented as mean±standard error of means. Ranking was done across the organisms and values with the same super script are not significant.

Table 5 The antibacterial activity of *M. charantia* methanolic extract on selected bacteria

Organism	31.25mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	500 m/ml	Control
E. coli	3.44± 0.10^{c}	7.43± 0.10^{c}	10.40±0.12^{c}	14.37±0.12^{c}	17.13± 0.09^{c}	29.60± 0.15^{c}
Salmonella spp	9.33± 0.10^{b}	13.23± 0.12^{b}	18.27±0.15^{b}	20.60±0.11^{b}	23.46± 0.15^{b}	38.50± 0.36^{a}
Shigella spp	10.23±0.09^{a}	14.70± 0.20^{a}	19.30±0.11^{a}	22.50±0.10^{a}	25.30± 0.30^{a}	32.33± 0.18^{b}
LSD	0.36					
P-value	<0.0001					

At P≤0.05 there was a significant difference in the antibacterial activity of the methanolic extract of *M.charatia*on the selected bacteria. Values are presented as mean±standard error of means. Ranking was done across the organisms and values with the same super script are not significant.

Table 6 The antibacterial activity of *M. charantia* aqueous extract on selected bacteria

Organism	31.25mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	500 m/ml	Control
E. coli	3.46± 0.09^{c}	6.20± 0.12^{c}	8.93± 0.10^{c}	12.27±0.15^{c}	15.37± 0.12^{c}	29.60± 0.15^{c}
Salmonella spp.	4.20± 0.12^{b}	8.37± 0.09^{b}	11.27±0.12^{b}	15.36±0.12^{b}	17.43± 0.09^{b}	38.50± 0.36^{a}
Shigella spp.	5.20± 0.06^{a}	9.36± 0.07^{a}	13.23±0.09^{a}	16.77±0.09^{a}	19.20± 0.12^{a}	32.33± 0.18^{b}
LSD		0.4				
P-value		<0.0001				

At P≤0.05 there was a significant difference in the antibacterial activity of the aqueous extract of *M.charatia*on the selected bacteria. Values are presented as mean±standard error of means. Ranking was done across the organisms and values with the same super script are not significant.

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Table 7 MIC & MBC for the antibacterial activity of *P. daemia* aqueous extract on some selected bacteria

Organism	31.25mg/ml	62.5mg/ml	125mg/ml	250mg/ml	500mg/ml	MIC	MBC
E. coli	+	+	+	-	-	125	250
Salmonella Typhi	+	+	+	+	-	250	500
Shigella flexneri	+	+	-	-	-	62.5	125

Table 8 MIC & MBC for the antibacterial activity of *P. daemia* methanolic extract on some selected bacteria

Organism	31.25mg/ml	62.5mg/ml	125mg/ml	250mg/ml	500mg/ml	MIC	MBC
E. coli	+	+	-	-	-	62.5	125
Salmonella Typhi	+	+	-	-	-	250	500
Shigella flexneri	+	-	-	-	-	62.5	125

Table 9 MIC & MBC for the antibacterial activity of *M. charantia* aqueous extract on some selected bacteria

Organism	31.25mg/ml	62.5mg/ml	125mg/ml	250mg/ml	500mg/ml	MIC	MBC
E. coli	+	+	+	-	-	125	250
Salmonella Typhi	+	+	-	-	-	62.5	125
Shigella Flexneri	+	+	-	-	-	62.5	125

Table 10 MIC & MBC for the antibacterial activity of *M. charantia* methanolic extract on some selected bacteria

Organism	31.25mg/ml	62.5mg/ml	125mg/ml	250mg/ml	500mg/ml	MIC	MBC
E. coli	+	+	-	-	-	62.5	125
Salmonella Typhi	+	+	+	-	-	125	250
Shigella flexneri	+	-	-	-	-	31.25	62.5

Discussion

The proximate composition of *P. daemia* and *M. charantia* revealed significant differences in their nutritional content. Moisture content compared between the two plants showed *P. daemia* exhibiting lower moisture content (7.83%) compared to *M. charantia* (9.90%). Ash content was notably higher in *M. charantia* (12.86%) compared to *P. daemia* (7.53%). Ash content represents the mineral content of plant materials and can be an indicator of nutritional value. Higher ash content in *M. charantia* suggests a richer source of minerals. Fats content was higher in *P. daemia* (16.69%) compared to *M. charantia* (10.78%).

Dietary fats are a source of energy and essential fatty acids. The higher fat content in *P. daemia* may make it a more energy-rich plant. Crude protein content was also higher in *P. daemia* (17.23%) compared to *M. charantia* (12.33%). Protein is essential for growth and repair processes in the human body. The higher protein content in *P. daemia* indicates its potential as a protein source. Crude fiber content differed significantly, with *P. daemia* (19.62%) having a higher content than *M. charantia* (10.66%). Crude fiber is important for digestive health and can influence the dietary fiber content of plant-based foods.

Carbohydrate content was substantially higher in *M. charantia* (55.59%) compared to *P. daemia* (31.38%). Carbohydrates are a primary source of energy in the human diet. The higher carbohydrate content in *M. charantia* suggests it may be a valuable energy source. These findings demonstrate the nutritional diversity between *P. daemia* and *M. charantia,* highlighting their potential for various dietary and medicinal applications. These findings agree with the work of Edeoga *et al.,* 2005. The phytochemical screening of the aqueous and methanolic extracts of *P. daemia* and *M. charantia* revealed the presence of several bioactive compounds. Both plant species exhibited the presence of alkaloids, tannins, flavonoids, phenols, and cardiac glycosides in their extracts These compounds are known for their potential medicinal properties, including antioxidant and antibacterial activities reported by.[10]

The presence of these phytochemicals suggests that *P. daemia* and *M. charantia* have therapeutic potential. Saponins were absent in *P. daemia* but present in *M. charantia*, indicating differences in the chemical composition of the two plants. Saponins are known for their surfactant properties and have been associated with various biological activities, including antimicrobial effects. The absence of saponins in *P. daemia* may contribute to variations in its biological properties compared to *M. charantia*. Carbohydrates were found to be absent in both plants, terpenoids were present in *M. charantia* for both aqueous and methanolic extracts, while they were absent in *P. daemia* for aqueous extracts but present in the methanolic extracts. Terpenoids are a diverse group of compounds with various biological activities, including antimicrobial properties.[11] Anthraquinones were present in *P. daemia* for aqueous extracts but absent in methanolic extracts, and also absent in *M. charantia* for both aqueous and methanolic extracts. Anthraquinones are known for their laxative and antibacterial properties.[11] The presence of anthraquinones in *P. daemia* aqueous extracts suggests potential medicinal applications. Steroids were found to be present in *P. daemia* for the aqueous extracts but absent in methanolic extracts. Steroids were also found to be present in *M. charantia* for the aqueous extracts but absent in the methanolic extracts. Steroids are a diverse group of compounds with various biological activities, including anti-inflammatory and antimicrobial effects.[12] The variations in steroid content between extraction methods and plant species may influence their pharmacological properties. The antimicrobial activity in both methanolic and aqueous extracts of *P. daemia* and *M. charantia* against *E. coli*, *Salmonella typhi,* and *Shigella flexneri.* In the case of *P. daemia*, both methanolic and aqueous extracts exhibited differential responses among the bacteria at lower concentrations. However, as the extract concentration increased, the zones of inhibition increased revealing concentration dependence. This suggests a concentration-dependent antimicrobial effect *Shigella flexneri* was more susceptible than *E. coli* and *Salmonella typhi* at 500 mg/ml concentration. The MIC and MBC results showed that *P. daemia* extracts have effective antibacterial agents. Similarly, *M. charantia* methanolic and aqueous extracts

showed significant antibacterial activity against the selected bacterial strains. The MIC and MBC values corroborated the potency of these extracts. These findings indicate that *M. charantia* extracts also have the potential to serve as effective antibacterial agents.The presence of bioactive compounds, such as alkaloids, tannins, flavonoids, and phenols, in the phytochemical screening of both plants contributed to their antibacterial properties. These compounds have been reported to exhibit antimicrobial activity. The differences in antimicrobial activity between the two plants and their extracts may be attributed to variations in phytochemical profiles and concentrations. Moreover, the choice of extraction solvent (methanol or water) can influence the types and quantities of bioactive compounds extracted.[13-15]

Conclusion

In conclusion, this study provides a comprehensive evaluation of *P. daemia* and *M. charantia*, encompassing phytochemical screening, proximate composition, and antimicrobial activity assessment. The findings offer valuable insights into the potential applications of these two plant species in various domains, including traditional medicine, nutrition and ethnobotanical research.

Recommendations

Quantitative analysis of the plant is needed to isolate the phytochemicals contained therein and to test the antimicrobial activity of the respective phytochemical constituents in order to identify the active components. Also, more attention should be given to the plant kingdom because a variety of plants contain promising secondary metabolites that may help curb the issue of antimicrobial drug resistance in the society.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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