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Research Article

TOTAL PHENOLIC, FLAVONOID AND MINERAL CONTENTS OF THE METHANOLIC LEAF EXTRACT OF *PARINARI CURATELLIFOLIA* PLANCH. EX BENTH

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ABSTRACT

Objective: *Parinari curatellifolia* is a medicinal plant that possesses multifunctional properties. The aim of this research was to evaluate the mineral, total phenolic and flavonoids contents and some biological parameters of the methanolic leaf extract.

Methods: The leaves were extracted with methanol to obtain the crude extract. Phytochemical screening was carried out using the method described by Harborne, 1999. The extract was subjected to GC-MS analysis, Aluminum colorimetric assay was used to determine the total flavonoid content while Folin-Ciocalteau reagent was used to evaluate the total phenolic content. The antioxidant activity was determined using the DPPH radical scavenging assay. The mineral content was determined via atomic absorption spectrophotometer. Disc diffusion method was used for the antibacterial assay.

Results: The phytochemical screening revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids and cardiac glycosides, while steroids, anthraquinones and cyano glycosides were absent. The GC-MS profile showed catechol as the most abundant phytochemical and the identified phytocomponents were mainly polyphenolics, carbonyls, fatty acids and fatty acid esters. The extract possesses total flavonoid and phenolic contents of 13.46±0.02 mg/g quercetin equivalent and 58.43±0.15 mg/g Gallic acid equivalent, respectively. The extract exhibited a significant antioxidant of 84%. Concentration of the macro and micro elements were quite high in some cases. Na had the highest concentration of 42522.70±2146.50 µg/g, while Cu has the lowest value of 4.18±0.03µg/g. The antibacterial activity of the extract was comparable to that of the standard antibiotic. *Bacillus subtilis* exhibited the highest zone of inhibition of 29±0.10 mm while *Salmonella typii* and *Kliebsiella pneumoniae* gave the lowest value of 14±0.12 mm.

Conclusion: *Parinari curatellifolia* leaves possess tannins, saponins, flavonoids, terpenoids, alkaloids and cardiac glycosides. It has high antioxidant and antibacterial capabilities.

Keywords: *Parinari curatellifolia*, Phytochemicals, Phenolic, Flavonoid, Antioxidant, Antibacterial, Elements, GC-MS

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INTRODUCTION

A large number of synthetic antimicrobial agents are available for the treatment of diseases caused by microorganisms. But researchers have shown that most of these microorganisms are showing resistance to the available synthetic antimicrobial agents. These synthetic antimicrobials also have terrible side effects on the recipients. So there is the need to develop save antimicrobial agents that can effectively combat infective microorganisms [1, 2]. Moreover, endogenous metabolic processes in the human body and exposure to chemicals in our environment lead to the generation of reactive oxygen species such as superoxide radical, hydrogen peroxide, hydroxide radicals and peroxy radicals. These reactive species are involved in the pathogenesis of diseases such as cancer, cardiovascular disorder, atherosclerosis, neurodegenerative disorder and inflammation [3]. Studies on plants that possess medicinal properties show that these plants contain phytoconstituents such as flavonoids, alkaloids, tannins, etc which are responsible for the biological and pharmacological activities of these plants. They have also been shown to be valuable in the prevention of disorders, reduction of diseases and maintenance of health. Some of these compounds have the ability to scavenge free radicals or inhibit electron transfer. They donate hydrogen atom to free radicals and chelate metal ions such as $Fe^{3+}$and Cu^{2+}; which is an important pharmacological activity. They can also up-regulate antioxidant enzymes. The use of medicinal plants in the treatment of ailments is more predominant in underdeveloped countries than in developed countries. This is due to the fact that synthetic drugs are easier and cheaper to obtain in developed countries and more traditional health practitioners' are found in under-developed countries where the synthetic drugs are out of reach of the common man.

Parinari curatellifolia is a medicinal plant that grows well in Africa (west, east, central and south). It is an evergreen tree that stands up to 25 m. The leaves are simple, alternate, elliptical to oblong and leathery. The top of the leaves are darker in green color than the underneath. The apex tapers broadly, base is square, margin entire and the petiole short. The leaves have been reported to possess sedative, antidiabetic properties. Decoction of the leaves is drunk for the treatment of fever, while the crushed leaves are used for dressing of fractures, dislocations, wounds, cuts and sores [4]. The leaf extract could also be used as an expectorant, antibacterial and anti-inflammatory [5]. Other properties include its use for dental hygiene [6], constipation [7] and hypertension [8]. The objective of this research is to carry out both chemical and biological analysis of the leaves of *Parinari curatellifolia* in order to determine the phytochemicals, minerals and some biological parameters of the methanolic leaf extract.

MATERIALS AND METHODS

General

The following chemicals methanol, sodium hydroxide, sodium carbonate, aluminum oxide, sodium nitrite, Folin-ciocalteau reagent, potassium acetate, were obtained from E. Merck (Darmstadt, Germany) while quercetin, Gallic acid, ascorbic acid and 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Absorbance measurements were recorded on a Genseys 10S vI. 200 217H311008 spectrophotometer.

Sample collection and preparation

The plant leaves were collected from an herbalist in Omu aran, Kwara State, Nigeria. Omu aran is located on longitude 5.0963 °E and latitude 8.1402 °N. It was identified by matching the local name of the plant with that found in the book "Vernacular names of Nigerian Plants Yoruba" written by Gbile and Soladoye, 2012 [9]. It was later taken to the department of Pure and applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria for further identification by a plant taxonomist, Prof. A. T. J. Ogunkunle. A voucher specimen was deposited in their herbarium and specimen number of LHO 605 attached to it. The leaves were stripped off the branches, taken to the laboratory and air-dried for two weeks. The dried leaves were pulverized into a fine powder using a Mouliner food blender and stored in an airtight container until further use.

Determination of elements present in the leaves

A mixture of HNO_3 and H_2O_2 in the ratio of 1:1 was introduced into beakers containing 5g of the pulverized leaves. The beakers were placed on hot plates in a fume cupboard and heated until the content turned colorless. The colorless liquid was transferred into a 50 ml volumetric flask; de-ionized water added and made up to the mark. Aliquots of this solution were analyzed for the presence of metals using atomic absorption spectrophotometer Parkin Elmer model 403, USA.

Extraction

500 g of the powdered leaves of *Parinari curatellifolia* was extracted with methanol using a Soxhlet extractor. The extract was concentrated by using a rotary evaporator. The extract obtained was weighed, stored in a sample bottle and placed in a cool dry place.

Phytochemical screening

The methanol extract was screened for the presence of steroids, flavonoids, tannins, saponins, glycosides, terpenoids and alkaloids according to the method described by Harborne, 1999 [10].

Determination of total flavonoid content

10 ml of methanol was added to 10 mg of the extract and mixed thoroughly using a vortex mixer. 0.5 ml of this solution was pipetted into a test tube and 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml potassium acetate and 2.8 ml of distilled water were added to it. The mixture was stirred properly and incubated for 30 min at room temperature. The absorbance was measured at 415 nm against a reagent blank. The total flavonoid content was calculated from the calibration curve of quercetin and expressed as mg of quercetin per gram weight of the dry extract.

Determination of total phenolic content

5 mg of the sample was dissolved in 5 ml of methanol using a vortex mixer. 0.5 ml of this solution was pipetted into a test tube and 3.5 ml of distilled water, 0.25 ml Folin-Ciocalteau reagent added to it. The mixture was incubated for 10 min at room temperature. Later, 0.75 ml of 20% Na_2CO_3 was added to it and left to incubate for 2h. The absorbance was measured at 765 nm against a reagent blank. The total phenolic content was calculated from the calibration curve of Gallic acid and expressed as mg of Gallic acid per gram weight of dry extract.

GC-MS analysis

A gas chromatography (7890A) from Agilent USA hyphenated to a mass spectrophotometer (5975C) with a triple-axis detector equipped with an auto injector (10 µl syringe) was used. Helium gas was used as the carrier gas. The chromatographic separation was performed on a capillary column having the following specifications: length: 30 m; internal diameter: 0.2 µm; thickness: 250 µm, treated with phenyl methyl siloxane. Other GC-MS conditions are ion source temperature (EI): 250 °C; interface temperature: 300 °C; pressure: 16.2 psia; out time: 1.8 min. 1 µl injector in split mode with split ratio of 1:5 and injection temperature of 300 °C were employed. The column temperature was started at 35 °C for 5 min and changed to 150 °C at the rate of 4 °C. The temperature was later raised to 250 °C at the rate of 20 °C/min and held for 5 min. The total elution time was 47.5 min. MS solution software provided by the supplier was used to control the system and acquire data. The identification of the separated compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from NIST library.

Antibacterial analysis

Microorganisms used

The microorganisms used were collected from Bowen teaching hospital, Ogbomoso, Nigeria. These include *Escherichia coli, Salmonella typhi, pseudomonas aeruginosa, Bacillus subtilis, Kliebsella pneumoniae*, *Bacillus aureus* and *Staphylococcus aureus*.

Test samples

1 mg of the methanol extract and the standard antibiotic were separately dissolved in I ml of distilled dimethylsulphoxide.

The antibacterial medium

100 ml of distilled water was added to 2.8 g of nutrient agar, stirred for 5 min and placed in an autoclave at a temperature of 121 °C for 15 min to sterilize. It was later cooled to 45 °C and introduced into sterile petri dishes to solidify.

Disc diffusion test

The nutrient agar plates were inoculated with the test organism and sterile Whatman No. 1 filter paper which has been impregnated with the methanol extract, was placed on the surface of the plates. The plates were incubated at 37 °C and zones of inhibition of the test organisms were determined after 24 h. The same test was carried out for the standard antibiotic, streptomycin and all results obtained as triplicates.

Antioxidant activity by DPPH radical scavenging assay

10 mg of the crude extract was dissolved in 20 ml of methanol using a vortex mixer to give a sample concentration of 0.5 mg/ml. From here different concentrations of the sample were prepared (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, 300 µg/ml, 350 µg/ml).

DPPH was prepared by dissolving 4 mg of DPPH in 100 ml methanol. The control contains no extract, only methanol and 2 ml of DPPH. To each of the different concentrations of the extract was added 2 ml of the prepared DPPH solution in methanol. These were left in the dark for 20 min for color development. Then the absorbance was measured at 517 nm against a reagent blank. Ascorbic acid was used as the standard.

RESULTS AND DISCUSSION

Introduction of very sensitive analytical tools has aided the increased interest in plant's secondary metabolites. Many of these secondary metabolites have been found to possess both biological and pharmacological activities. In this present investigation of *Parinari curatellifolia leaves*, the phytochemical components present in the methanolic leaf extract were determined. The leaves were shown to possess tannins, saponins, terpenoids, cardiac glycosides, alkaloids and flavonoids while steroids, anthraquinones and cyanogenic glycosides were absent, as presented in table 1.

Table 1: Phytochemical screening of methanolic leaf extract of *Parinari curatellifolia*

Extract	Tan	Ste	Sap	Ter	Alk	Anthr	Cy gly	Ca gly	Fla
met	+	-	+	+	+	-	-	+	+

NOTE: met–methanol; tan–tannins; ste–steroids; sap–saponins; ter–terpenoids; alk–alkaloids; Anthr–anthraquinones; Cy gly–cyano glycosides, Ca gly–cardiac glycosides; fla–flavonoids.

This result is in agreement with those of Halilu *et al.*, 2010 [11] but with the exception of terpenoids which was not identified in that of Peni *et al.*, 2010 [12]. Phytochemicals such as alkaloids have been shown to possess physiological activities [13] while flavonoids and tannins possess antioxidant activity [14-16]. Saponins exhibit permeabilizing of cell membrane, lowering of serum cholesterol levels, and stimulation of luteinizing hormone release leading to abortifacient properties and immunomodulatory potential via cytokine interplay [17, 18]. In the determination of the mineral content of the methanolic leaf extract, the concentrations of the macro and trace elements were quite high as shown in table 2. Ca is important in the development of teeth and healthy bones while Na and K are necessary for the repair of worn-out tissues and the building of red blood cells. Zn and Mn are important in enzyme metabolism. Pd and Cd, which are heavy metals, were also detected but in low concentrations as compared to the daily intake of 10 mg/Kg [19]. Although these concentrations are low, one need to be careful in the administration of the plant leaf extract since concentrations of these heavy metals in lower concentrations have been found to be toxic [10].

Table 2: Mineral content of the leaves of *Parinari curatellifolia*

Minerals	Content (µg/g)
Na	42522.70±2146.50
K	3284.36±434.36
Ca	7058.37±615.55
Al	62.51±0.49
Mn	21.74±0.14
Cr	35.14±0.43
Pb	10.78±0.21
Cu	4.18±0.03
Co	26.70±0.42
Cd	6.15±0.40
Ni	26.98±0.21
Zn	100.37±0.69

Note: Results are mean of triplicate and SD

In the determination of the total flavonoid content, an aluminum colorimetric assay was used with quercetin as the standard flavonoid compound. A standard calibration curve was obtained for quercetin ($y = 0.012x–0.011$) with the coefficient of determination $R^2 = 0.992$ (fig. 1). The total flavonoid content of the leaf extract obtained is presented in table 3 and expressed in mg quercetin equivalent/g extract. The total phenolic content of the leaf extract was determined from a linear Gallic acid standard curve ($y = 0.023x+0.292$, $R^2 = 0.946$) (fig. 2) and expressed in mg Gallic acid equivalent/g extract (table 3). In the *in vitro* determination of the antioxidant potential of the methanolic leaf extract, DPPH assay was used. The extract gave a value of 111.17µg/g of ascorbic acid equivalent, while the percentage inhibition of the free radical was 84%. This activity is a result of the presence of polyphenolics in the extract. The free radical scavenging activity was compared to that of ascorbic acid, which is a standard antioxidant. Oxygen plays a very important role in human metabolism and, once inhaled, it undergoes a gradual reduction process and ultimately gets metabolized into water. In this process of absorption, transport, and metabolism, some reactive oxygen species are formed, which can promote lipid peroxidation of unsaturated fatty acids and damage membrane proteins, leading to decreased membrane permeability, decreased activities of enzymes and receptors, and activation of cells. Furthermore, when free radicals react with DNA, cancer-causing mutations may occur. Therefore, antioxidant defense systems in foods and drugs are important in the prevention of many diseases, such as cancer, atherosclerosis, diabetes, and liver cirrhosis etc.

Table 3: Total flavonoid, phenolic and antioxidant activity of the methanol extract of *Parinari curatellifolia*

Total flavonoid content (mg/g quercetin equivalent)	Total phenolic content (mg/g Gallic acid equivalent)	Antioxidant activity of extract (µg/mg Ascorbic acid equivalent)
13.46±0.02	58.43±0.15	111.17±0.03

Note: Results are mean of triplicate and SD

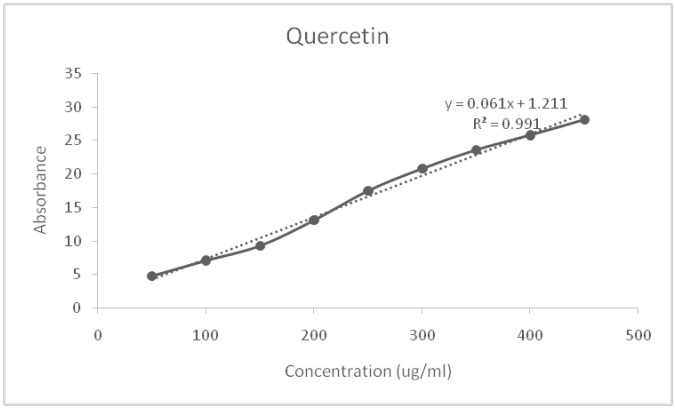

Fig. 1: Standard curve for quercetin

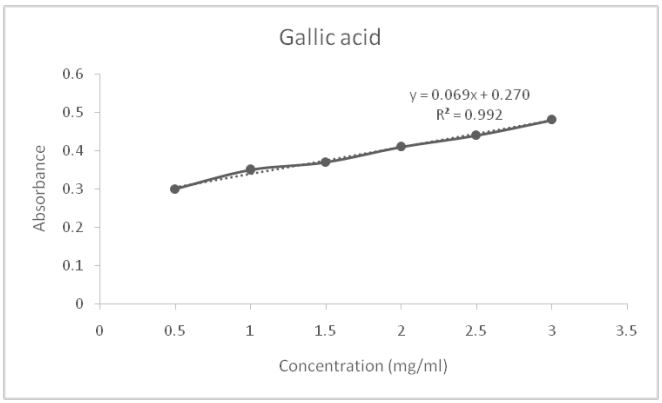

Fig. 2: Standard curve for gallic acid

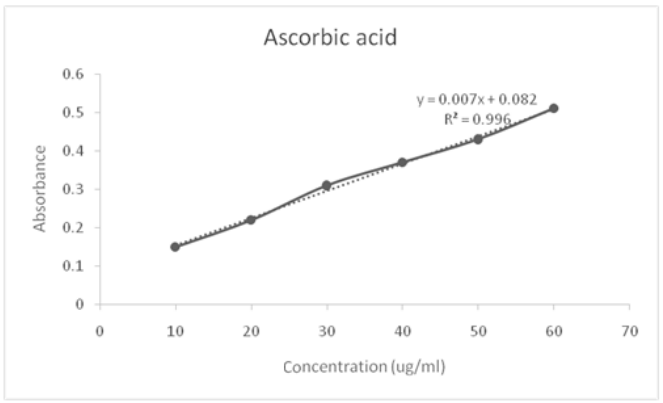

Fig. 3: Standard curve for ascorbic acid

In the last decades GC-MS has been established as a key technological tool for profiling of secondary metabolites in both plants and non-plant species [20-22]. The present study was able to predict, and identify molecular formula and structure of some of the chemicals present in the methanolic leaf extract as presented in table 4. A total of thirty-seven compounds were separated by the GC as shown in the chromatogram in fig. 4. Twenty-five compounds were identified while twelve were unidentified. The MS of the unknown compounds were compared with those of NIST-2011 (National Institute of Standards and Technology) library stored in the computer database of the GC-MS equipment. These compounds were identified based on their molecular weight, peak area in percentage, retention time and molecular formula. The first compound to emerge was 5-methyl-2-furancarboxaldehyde with a retention time 9.53 min while the last to emerge was 2, 2, 4-trimethyl-3-(3, 8, 12, 16-tetramethylheptadeca-3, 7, 11, 15-tetraenyl) cyclohexanol with retention time of 44.75 min. The most abundant compound was catechol (14%) with the retention time of 20.263 min while the least was 2-hydroxy-3-methyl-2-cyclopenten-1-one (0.44%). Other compounds with an appreciable percentage in the methanolic extract are hexadecanoic acid (10.9%), 13-octadecenoic acid (7.9%) and 2-vinylfuran (5.7%). The GC-MS report revealed that the identified compounds are carbonyls, phenolics, fatty acids and fatty acid esters. The presence of these compounds in the plant leaves may be responsible for the plant medicinal properties.

Table 4: GC-MS report of the methanolic leaf extract of *Parinari curatellifolia*

S. No.	Name of compounds	Retention time(min)	Peak height	Molar weight	Molecular formula
1	5-methyl-2-furancarboxaldehyde	9.525	3714336	110	$C_6H_6O_2$
2	2-vinylfuran	11.575	7899062	94	C_6H_6O
3	Acetic acid, bromophenyl ester	11.905	2029060	216	$C_8H_8O_2Br$
4	2-hydroxy-3-methyl-2-cyclopenten-1-one	12.824	7505568	111	$C_6H_7O_2$
5	5, 5-dimethyl-2,4-imidazolidione	13.076	7644985	128	$C_5H_8N_2O_2$
6	Phenol, 2-methoxy	14.592	10189994	124	$C_1H_8O_2$
7	Maltol	15.739	1731317	126	$C_6H_6O_3$
8	Catechol	20.263	11577713	110	$C_6H_6O_2$
9	3,4-dihydroxyacetophenone	21.347	2029316	152	$C_8H_8O_3$
10	2-methoxy-4-vinylphenol	22.581	8704896	150	$C_9H_{10}O_2$
11	Formic acid, 2, 6-dimethoxyphenyl ester	23.924	7020726	182	$C_9H_{10}O_4$
12	2-methoxy-4-(propenyl) phenol	26.862	3058810	164	$C_{10}H_{12}O_2$
13	2, 6-bis(1, 1-dimethylethyl) phenol	29.014	1315212	206	$C_{14}H_{22}O$
14	6-butyl-1,2,3,4-tetrahydronaphthalene	29.462	1573974	188	$C_{14}H_{20}$
15	(z, z)-9,12-octadecadienoic acid	37.482	3672277	280	$C_{18}H_{32}O_2$
16	3,7,11,15-tetramethyl-2-hexadecen-1-ol	37.710	9075171	294	$C_{20}H_{38}O$
17	3,7,11,15-tetramethyl-2-hexadecen-1-ol	37.906	21675194	294	$C_{20}H_{38}O$
18	Hexadecanoic acid, methyl ester	38.370	11895225	270	$C_{17}H_{34}O_2$
19	n-hexadecanoic acid	38.904	36297509	256	$C_{16}H_{32}O_2$
20	Phytol	39.729	8848327	296	$C_{20}H_{40}O$
21	Trans-13-octadecenoic acid	39.980	29164227	282	$C_{18}H_{34}O_2$
22	Octadecanoic acid	40.106	23769888	284	$C_{18}H_{36}O_2$
23	Diisooctylphthalate	42.132	23515947	374	$C_{24}H_{38}O_3$
24	2,2,4-trimethyl-3-(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)cyclohexanol	44.748	7974996	427	$C_{30}H_{51}O$

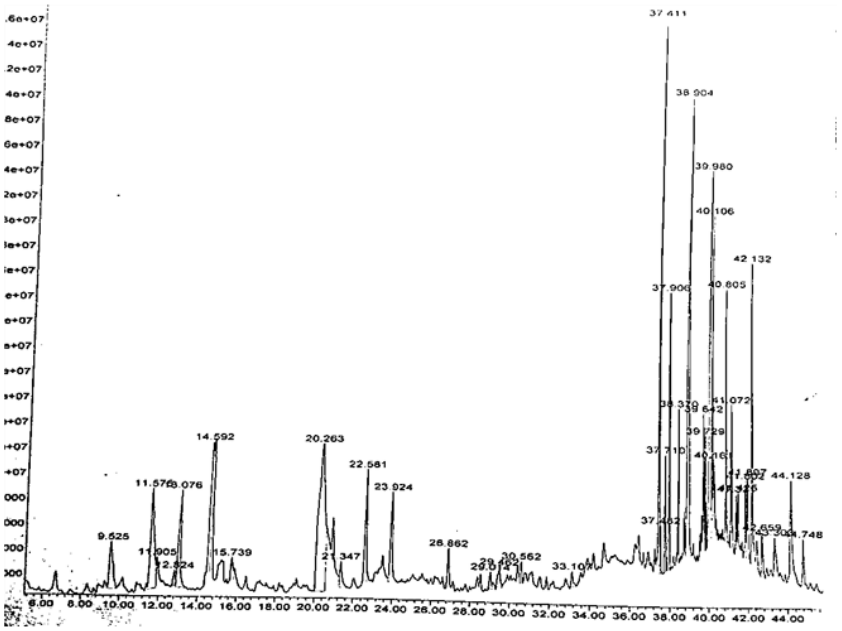

Fig. 4: Chromatogram of methanolic leaf extract of *Parinari curatellifolia*

In the study of the antibacterial potential of the methanolic leaf extract, some selected pathogens were screened as shown in table 5. The activity of the methanolic extract was compared with that of a standard antibiotic, streptomycin. The extract exhibited a significant inhibitory effect on the screened bacteria. It gave the highest activity with *B. subtilis* and the lowest with *S. typii* and *K. pneumoniae*. Comparing its activity with that of streptomycin, it exhibited a higher activity with *E. coli*, *K. pneumoniae* and *B. subtilis* but lower activity with *S. typii*. The activity of *S. aureus* and *B. aureus* were comparable to that of streptomycin.

Table 5: Antibacterial activity of the methanolic leaf extract of *Parinari curatellifolia*

Bacteria	Zones of inhibition (mm)	
	Methanol extract	Streptomycin
Escherichia coli	16±0.10	0.00
Staphylococcus aureus	22±0.14	21±0.30

Pseudomonas aeruginosa	15±0.10	19±0.40
Kliebsiella pneumoniae	14±0.12	10±0.20
Bacillus subtilis	29±0.10	25±0.15
Bacillus aureus	20±0.12	22±0.10
Salmonella typii	14±0.12	26±0.20

NOTE: Results were mean of triplicate analysis and SD.

CONCLUSION

Our study has shown that the plant leaves of Parinari *curatellifolia* have a high level of macro and microelements. They are rich in phytochemicals that possess both pharmacological and biological activities. The presence of these phytochemicals is responsible for its high level of antioxidant activity. The GC-MS profile showed catechol as the most abundant phytochemical and the identified phytocomponents were mainly polyphenolics, carbonyls, fatty acids and fatty acid esters. The leaf extract exhibited significant inhibitory activity against the screened microorganisms. *Parinari curatellifolia* may be considered as a potential source of compounds that may serve as antioxidant, antibacterial and anti-inflammatory agents.

DATA AVAILABILITY

Not applicable

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Edewor T. I designed the research and edited the write-up, Agboola P. O, Amuda M. O and Mmuo A. I carried out the analysis while Owa S. O obtained the plant sample and was responsible for its authentication.

CONFLICT OF INTERESTS

The authors declare that there is none.

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