

Gas Chromatography-Mass Spectrometry Analysis, Phytochemical Screening, Antioxidant and Antibacterial Activity of Methanol Root Extract of *Rhaphiostylis beninensis* (Hook.f.) Planch. [family ICACINACEAE] against Uropathogens

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In Nigeria, urinary tract infections (UTIs) are amid the most prevalent bacterial contagions, with a higher incidence rate in women. Depending on the severity of the infection, different antibiotics are used to treat UTIs. Herbal remedies have, on the other hand, been seen as the paranormal means of treating UTIs while antibiotic resistance has emerged as a problem for medics. This study examined the methanol extract of *Rhaphiostylis beninensis* (MRB) for its phytochemical and bioactive components, antioxidant, and antibacterial activities. From the study, the methanol extract's antioxidant activities grew stronger as concentration increased. Alkaloids, saponin, tannins, cardiac glycosides, flavonoids, and reducing sugar—giving the extract its pharmacological potential—were among the phytochemicals identified from the phytochemical screening. The GC-MS Chromatogram showed that there are 42 bioactive compounds in the MRB with the most prominent compounds being (Z,Z)-9,12-Octadecadienoic acid 19.96%, Lupeol 18.96%, cis-13-Octadecenoic acid 9.96%, n-Hexadecanoic acid 9.87%, 3',5'-Dimethoxyacetophenone 6.67%, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol 3.21%, Octadecanoic acid 2.98%, Capsaicin 2.58%, α -Amyrin 2.53%, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester 2.49%, and Vitamin E 2.45%. The MRB showed significant minimum inhibitory concentration values against *Pseudomonas putida* PA25T, *Escherichia coli* EC3AT, *Pseudomonas aeruginosa* PA129AT and *Serratia marcescens* and a significant MBC value against *Escherichia coli* EC3AT. This study emphasizes the medicinal efficacy of *R. beninensis* root and indicated that the plant could make a promising candidate for the isolation of bioactive compounds that could be synthesized as medications for the treatment of bacteria in uropathogenic disorders.

Keywords: Urinary tract infections, Antibiotics, phytochemicals,
GC-MS analysis, bioactive compounds, antioxidant.

One of the most ubiquitous bacterial illnesses worldwide is urinary tract infections

(UTIs). About 50–60% of women may experience UTIs at some point in their lives, making it a

predominant bacterial infection responsible for close to 25% of all infections in women. About 150 million cases per year, as an estimate, was indicated in 2016. Although both men and women can become seriously ill from these diseases, women are more likely to contract them¹. Women who are sexually active, especially those who use spermicidal products and diaphragms for contraception, are more likely to develop UTIs due to immune suppression in such females, even though the spread of these infections to the kidney is more proliferating in pregnant women owing to their enfeebled immune systems in gestation. While UTIs in pregnant women may result in early labor and high blood pressure², post-menopausal women experience higher rates of UTIs because of the pelvic prolapse, deficiency of estrogen, amplified peri-urethral colonization by *Escherichia coli*, diabetes mellitus, and reduction of *Lactobacilli* spp. amongst the vaginal flora³.

Although *Staphylococcus* spp., *Proteus vulgaris* and *Pseudomonas* spp. are infrequent bacteria responsible for UTIs⁴, *Proteus mirabilis*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* have been identified as the causative agents of UTIs^{5,6}. While *E. coli* and *K. pneumoniae* are the most common uropathogens and UTI indicator organisms, respectively, according to the World Health Organization's Global Antimicrobial Surveillance System (GLASS)^{7,8}, over and above 80% of all unsophisticated community acquired urinary tract infections (CA-UTIs) are triggered by *E. coli* alone⁹. In general, *E. coli* in the intestine are usually normal flora and not detrimental, but if it gets to the vaginal hole during washing or after sexual activity, it could cause infections on entering and colonizing the bladder. For instance, some *E. coli* pathotypes such as uropathogenic *E. coli* (UPEC) in immunocompromised individuals have developed enabling capabilities to breach the characteristically sterile urinary system and cause both symptomatic and asymptomatic infections⁹. Numerous virulence factors, including polysaccharide capsule, lipopolysaccharide (LPS), flagella, pili, non-pilus adhesins, outer-membrane proteins, outer-membrane vesicles, secreted toxins and TonB-dependent iron-uptake receptors such

as siderophore receptors are used to colonize the bladder by this organism. All of these elements offer great potential for the establishment of treatments and vaccines^{10,11}. Amphipathic molecules called LPS are composed of lipid A or endotoxin, core phosphorylated oligosaccharide and a lengthy side chain known as O antigen¹². LPS structural components, rapid bladder colonization, reservoir formation, and induction of innate and adaptive immune responses have roles to play in facilitating various phases of the UPEC life cycle¹³. When there is a decrease in the quantity of LPS at the surface of the cell, LPS confers resistance to hydrophobic antibiotics and hypersensitivity to hydrophobic harmful molecules such as bile salts and certain antibiotics¹⁴.

Since many of the agents causing UTIs have developed resistance to majority of the antibiotics used to treat them^{15,16}, diverse guidelines for treating UTIs have been adopted in innumerable regions of the world¹⁰ based on noteworthy scientific evidence. According to Magyar *et al.*¹⁵, resistance of *E. coli* to ciprofloxacin increased remarkably from 19% to 25%, resistance of *K. pneumoniae* to cephalosporins was 60%, and the percentage of carbapenem-resistant *P. aeruginosa* also increased noticeably. While multidrug-resistant pathogens are increasingly responsible for UTIs worldwide¹⁷, antibacterial resistance has led to higher rates of morbidity and mortality, higher resource use, increased costs, decreased hospital activity, and antibiotic treatment guidelines that favor broad-spectrum empiric therapy^{18,19}. A great impediment to using these empirical therapy options is provided by the rise in antibiotic resistance²⁰. Due to the fact that herbal medications are secure, affordable, and simple to use, concentrated attention has been directed to the therapeutic potential of medicinal plants². Consequently, medicinal plants have been widely investigated²¹, and various essential chemical constituents with tremendous therapeutic potential have been identified to indicate their pharmacological properties²²⁻²⁴. While antimicrobial, anti-inflammatory, and antioxidant activities of plant extracts have been documented^{25,26}, a wide variety of their chemical components have therapeutic potentials *in vitro*^{27,28}. Since there are no credible scientific data indicating that bacteria have evolved a resistance to therapeutic plants, these phytochemicals

could serve as the basis for the manufacture of secure ground-breaking medications for treating infections and diseases. Thus, by using these natural medications, the issue of resistance resulting from the usage of conventional treatment might be resolved.²⁹

Rhaphiostylis beninensis Planch ex Benth (Icacinaeae) is a woody climber with a wide variety of curative and culinary uses. It grows in the West African subcontinent and South-Western Nigeria. It goes by many different names in Nigeria depending on one's location and its usage. Atapata (Yoruba), osumadin (Benin), kpolokoto (Ibos), umeni (Urhobos), and kumeni (Itsekiris) are all names for the same plant. *R. beninensis* belongs to the Icacinaceae family, which contains a variety of bioactive phytochemicals³⁰. Innumerable pharmacological and biological properties of the plant's root extract have also been reported. Antibacterial, analgesic, and anti-inflammatory properties have been reported for the plant³¹. The leaf and root are used in folkloric treatment of arthritis, rheumatism, skin illnesses, mental disorders, convulsions, and eye problems, while the leaf decoction is used as a mouthwash and a sore wash^{32,33}. Adjanooun *et al.*³⁴ reported mosquito repellent activities of its aqueous leaf extract. Gram-positive and Gram-negative bacteria as well as fungus were found to be resistant to the antibacterial activities of the oil extracted from the root, stem bark extracts, and fruit³⁵. While anthraquinones, flavonoids, and triterpenes were discovered in the plant³⁶ and a thiourea derivative, N, N-di (4-methoxybenzyl) thiourea, with anti-inflammatory activity has been isolated from the root of the plant³⁷, information on the chemistry, pharmacology, and antibacterial potential of oils produced from the methanol extract of *R. beninensis* roots is minimal. So, in response to its folkloric use, this study investigated the antioxidant, and antibacterial activities of the methanol extract of *R. beninensis* against uropathogenic bacteria as well as identify bioactive phytochemicals crucial in developing novel drugs from natural products.

MATERIALS AND METHODS

Collection of Plant materials

The roots of *Rhaphiostylis beninensis* were harvested from its natural habitat in Ijebu-

Ode, Ogun State, Nigeria, rinsed with sterile distilled water and allowed to air-dry. The roots were identified and deposited at the Nigerian Forestry Herbarium in Ibadan (FHI) under voucher number FHI 113354.

Plant Extraction

The extraction was done using the method of Adeshina *et al.*³⁸. The roots were pulverized before being sealed up in an airtight container free of grease. Four hundred grams of the sample was soaked in 1000 mL of methanol, left to stand for 72 h while being stirred frequently, sieved with three folds sterile muslin cloth and then filtered with Whatman No. 1 filter paper²⁹. The extracting solvent was effectively evaporated from samples with a rotary evaporator at 68°C. The extract was properly stored pending additional analyses³⁹.

Qualitative Phytochemical analysis

Using standard methods outlined by Sofowora⁴⁰ and Trease & Evans⁴¹, the methanol extract was examined for the occurrence of several phytochemicals.

Test for Saponins

The extract was mixed briskly with 20 mL of water in a graduated measuring cylinder to dilute it, and the concoction was allowed to stand for 15 min to test for the presence of saponin. It was clear that saponin was present because foam was formed. The foam height (H_2-H_1) was then determined.

Test for Alkaloids

A percentage of the extract was dispensed in a test tube, along with ethanol and diluted hydrochloric (HCl) acid (50:50). The concoction was heated in boiling water for 10 min before filtering to check for alkaloids. Mayer's Test: When Mayer's reagent (1.36 g of mercuric chloride and 5.00 g of potassium iodide were mixed in 100 mL of water) was applied to the filtrate, a buff-white yellow precipitate formed, indicating the occurrence of alkaloids.

Test for Tannins

A beaker containing 0.30 g of the methanol extract was boiled in a water bath for 10 min to test for tannins. Boiling was followed by filtering with Whatman number 42 (125 mm) filter paper. Then, 3 drops of 1% ferric chloride were added to 5 mL of the filtrate. The presence of tannins was suggested by the development of brownish-green or blue-black coloring.

Test for Steroids

To test for steroids, chloroform (3 mL) was added to methanol extract (5 mg) before filtering into test-tube. The test-tube was tilted and concentrated Sulphuric acid (H_2SO_4) (2 mL) was carefully added through the side of the test-tube. The presence of brown and reddish color at chloroform phase showed existence of steroid.

Test for Terpenoids

For the terpenoids test, 0.30 g of the methanol extract was extracted for 2 h in a beaker with sterile distilled water (30 mL). To partition the aliquot, the extract (5 mL) was added to chloroform (2 mL) combined with concentrated H_2SO_4 (3 mL). Reddish brown coloring at the border line confirmed the existence of terpenoids.

Test for Phenols

For phenols test, 2 mL of 1% $FeCl_3$ was mixed with the extract. The presence of blue-black (violet) or blue green coloration confirmed the availability of phenol.

Test for Phlobatannins

To test for phlobatannins, sterile distilled water (30 mL) was added to 0.30 g of powdered sample in a beaker. After extracting for 24 h, the aqueous extract (10 mL) was boiled with 1% aqueous hydrochloric acid (5 mL). Red precipitate indicated the presence of phlobatannins.

Test for Flavonoids

To test for flavonoids, 0.30 g of powdered plant material was extracted for 2 h with 30 mL of distilled water before filtering with Whatman filter paper number 42 (125 mm). Five milliliters of 1.0 M diluted ammonia solution were added to 10 mL of the aqueous filtrate of extract. This was followed by adding 5 mL of H_2SO_4 . The presence of yellow colour that vanished upon standing indicated the occurrence of flavonoids.

Test for Reducing Sugars

A test tube containing 2 mL of aqueous extract and 1 mL of each of Fehling solutions A and Solution B was boiled for 10 min to check for reducing sugars. The yellow or brick-red precipitate produced indicated occurrence of reducing sugar.

Test for Glycosides

In this test, 20 mL of water and 2 g of the extract were combined, heated for 5 min in a water bath and then filtered through Whatman number 42 filter paper (125 mm). To detect glycosides, two tests were run with the filtrate. First, each of

Fehling's solution A and solution B (0.2 mL) were mixed with 5 mL of the filtrate until an alkaline mixture was obtained. Positive results were seen when brick-red coloring was present on heating. Second, the aforesaid test was repeated using 15 mL of 1.0 M H_2SO_4 instead of water, and the quantity of precipitate obtained was compared to those obtained from the initial test. The presence of glycosides was confirmed by high precipitate content and low precipitate content confirmed the nonexistence of glycosides.

Phytochemical analysis**Quantitative determination of flavonoid concentration**

The total flavonoid content of the methanol extract of *R. beninensis* was determined as described by Ejikeme *et al.*⁴². Fifty milliliters of 80% aliquot were added to 2.50 g of the plant material in a 250 mL beaker. The mixture was covered and allowed to stand for 24 h at room temperature. The extraction was repeated three consecutive times by adding the same volume of methanol after removing the supernatant. The mixture was filtered before transferring to a crucible and dried in a water bath. The crucible and its content were weighed after being cooled down in a desiccator.

$$\% \text{ Flavonoid} = \left[\frac{\text{Weight of flavonoid}}{\text{Weight of sample}} \right] \times 100$$

Quantitative determination of alkaloid concentrations

The alkaloid was determined quantitatively as described by Ezeonu & Ejikeme⁴³. The extract (2.50 g) was liquefied in 200 mL of 10% acetic acid in methanol, and permitted to stand for 4 h. The filtrate was concentrated in a water bath to a quarter of its initial volume before 15 drops of concentrated ammonium hydroxide (NH_4OH) was added until a precipitate was formed. After 3 h of sedimentation, the supernatant was removed by filtration, and the precipitate was washed with 20 mL of 0.1 M ammonium hydroxide before filtering. After drying the residue in the oven, the percentage of alkaloid was estimated.

$$\% \text{ Alkaloid} = \left[\frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \right] \times 100$$

Quantitative determination of total saponin

The saponin was determined quantitatively as described by Ejikeme *et al.*⁴² and Obadoni & Ochuko⁴⁴. Here, 5 g of powdered sample was added to 100 mL of aqueous ethanol (20%) in a conical flask (250 mL) before being heated with constant stirring at a temperature of 55°C in a water bath for 4 h. After filtration, the procedure was repeated two more times. The extract was vaporized to 40 mL at 90°C in a water bath. In a separating funnel, diethyl ether (20 mL) was added to the concentrate before shaken strongly. The aqueous layer was recovered and the ether layer was discarded. This purification process was repeated twice. *n*-butanol (60 mL) was added to the aqueous layer recovered before extracting twice with 10 mL of 5% sodium chloride. The residual solution was heated in a water bath for 30 min after discarding the sodium chloride layer, transferred to a crucible and dried in an oven to a constant weight.

$$\% \text{ Saponin} = \left[\frac{\text{Weight of saponin}}{\text{Weight of sample}} \right] \times 100$$

Quantitative determination of total tannins

The tannin content of the methanol extract of *R. beninensis* was determined as described by Ejikeme *et al.*⁴² and Amadi *et al.*⁴⁵. Folin-Denis reagent was prepared by liquefying sodium tungstate (Na_2WO_4) (50 g) in distilled water (37 mL) while phosphomolybdic acid ($\text{H}_3\text{PMo}_{12}\text{O}_{40}$) (10 g) and orthophosphoric acid (H_3PO_4) (25 mL) were added to this reagent. This mixture was refluxed for 2 h, cooled, and made up to 500 mL with distilled water. The extract (1 g) was added to distilled water (100 mL) in a conical flask, boiled for 1 h on an electric hot plate and filtered using Whatman number 42 (125 mm) filter paper in a volumetric flask (100 mL). Folin-Denis reagent (5 mL), saturated Na_2CO_3 (10 mL) solution, diluted extract (10 mL) and distilled water (50 mL) were added together in a conical flask (100 mL) for a change in colour. After constant stirring, the aliquot stood for 30 min in a water bath at 25°C before determining the optical density at 700 nm using a UV/VIS spectrophotometer, and its results were compared to a typical tannic standard curve obtained by dissolving 0.20 g of tannic acid in distilled water before being diluted up to 200 mL

mark (1 mg/mL). Five different test tubes were filled with varied amounts of the standard tannic acid solution (0.2-1.0 mg/mL), followed by adding Folin-Denis reagent (5 mL), saturated Na_2CO_3 (10 mL), and distilled water to bring the total volume to 100 mL. The solution was allowed to stand for 30 min in a water bath at 25°C. Optical density (absorbance) versus tannic acid concentration was plotted on a graph.

$$\text{Tannic acid (mg /100 g)} = C \times \text{extract volume} \times \frac{100}{\text{Aliquot volume} \times \text{weight of sample}}$$

where C is concentration of tannic acid read off the graph.

Quantitative determination of total phenolic concentration

Quantitative determination of phenol was carried out as described by Ezeonu & Ejikeme⁴³. Two grams of powdered sample were defatted for 2 h in ether (100 mL) using a soxhlet apparatus. The defatted sample (0.50 g) was boiled in ether (50 mL) for 15 min to extract the phenolic compounds. For colour development, distilled water (10 mL), 0.1 N ammonium hydroxide (2 mL) solution and concentrated amyl alcohol (5 mL) were added to the extract (5 mL) and left to react for 30 min. The optical density was measured at 505 nm while tannic acid (0.20 g) was dissolved in distilled water (200 mL) mark (1 mg/mL) to prepare the phenol standard curve. Varied concentrations (0.2–1.0 mg/mL) of the standard tannic acid solution were pipetted into five different test tubes to which NH_4OH (2 mL), amyl alcohol (5 mL), and water (10 mL) were added for colour development after reacting for 30 min. The optical density was measured with a UV/VIS TG 50 spectrophotometer at 505 nm.

GC-MS Quantification

GC-MS is one of the best, fast and most accurate approach to detect various compounds including alcohols, nitro compounds, alkaloids, organic acids, steroids, long chain hydrocarbons, esters and amino acids, and requires little amount of plant extracts. In this study, the GC-MS analysis detected and identified the chemical compounds present in *R. beninensis* as described by Fagbemi *et al.*²⁴.

Determination of antioxidant Activity by DPPH Radical Scavenging Activity Assay

The DPPH radical scavenging assay, as described by Chen *et al.*⁴⁶, was used to examine the extract's capacity to scavenge free radicals. Briefly, 0.1 mM DPPH (1 mL) in methanol was thoroughly mixed with extract (1 mL) in methanol at different concentrations (25–100 µg/mL), allowed to stand for 30 min at room temperature before measuring the absorbance with a UV-Vis Spectrophotometer at 517 nm. The decreased absorbance in the reaction mixture indicated a higher free radical scavenging capacity. DPPH scavenging effect's percentage inhibition was calculated using the equation:

$$\text{DPPH Scavenging effect (\%)} = \frac{[(A_0 - A_1)/A_0]}{x} \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the standard sample or extract. The IC_{50} value represented the concentration of the extract that inhibited production of DPPH activities by 50%. The experiment was repeated three times at different concentrations.

Determination of Antioxidant Activity by Ferric Reducing Antioxidant Power Assay (FRAP)

Using Otang *et al.*⁴⁷ approach, the ferrous reducing antioxidant capacity of the extract was assessed. One milliliter of the extract (25-100 µg/mL) was mixed with 0.1 M sodium phosphate buffer (2.5 mL) (pH 6.6) and 1% w/v potassium ferrocyanate (2.5 mL) [$K_3Fe(CN)_6$] in a 250 mL conical flask before incubating at 50°C for 20 min. Following this, trichloroacetic acid (2.5 mL) (10% w/v) was added to the mixture before centrifuging at 5000 rpm for 10 min. The upper layer (5 mL) was mixed with of fresh $FeCl_3$ (0.1%, w; 0.5 mL), and the absorbance was measured at 700 nm in comparison to a blank. Gallic acid was used as the control. The percentage inhibition in FRAP was calculated using the following equation:

$$\text{FRAP percentage inhibition (\%)} = \frac{[(A_0 - A_1)/A_0]}{x} \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the standard sample or extract. All tests were performed in triplicate. Higher absorbance of the reaction mixture indicated great reducing power.

Determination of Antioxidant Activity by Nitric Oxide Radical Scavenging Assay

The nitric oxide radical scavenging activity was evaluated as previously described by Silva and Soysa⁴⁸. Different concentrations (25–100 µg/mL) of the extract and the standard ascorbic acid were prepared by serially diluting them with distilled water. These were kept for subsequent use and stored at 4°C. Griess reagent was prepared by mixing equal volumes of sulphanilamide (1%) in phosphoric acid (2.5%) and naphthylethylene diamine dihydrochloride (0.1%) in phosphoric acid (2.5%) proximately before use. Following this, sodium nitroprusside (10 mM) in phosphate buffered saline (0.5 mL) was mixed with 1 mL of the different concentrations of the methanol extract (25–100 µg/mL) and incubated at 25°C for 180 min. The extract and the ascorbic acid used as control were mixed with an equivalent volume of recently prepared Griess reagent. The tubes used as control contained the same concentrations of methanol extract without sodium nitroprusside while 150 µL of the reaction mixture was transferred to a 96-well plate. Using a UV/VIS TG 50 Plus UV-VIS microplate reader, the absorbance at 546 nm was determined (Molecular Devices, GA, USA). The percentage inhibition of the extract and the standard was calculated and recorded. The inhibition percentage of the nitrite radical scavenging activity of both methanol extract and ascorbic acid were calculated using the following formula:

$$\text{Nitric oxide scavenged (\%)} = \frac{[(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}]}{x} \times 100$$

Where A_{control} = absorbance of the control sample and A_{test} = absorbance in the presence of the extract or standard. The experiment was conducted three times at different concentrations.

Determination of Antioxidant Activity by Lipid Peroxidation Assay

The lipid peroxidation assay was valued as described by Murugan & Parimelazhagan⁴⁹. Ten microliters of extract and a standard solution containing 1,1,3,3-tetramethoxypropane (TEP) at varying concentrations of 25, 50, 75 and 100 µg/mL and phosphate buffer (20 mM; 40 µL) (pH 7.0) were added to test tubes in an ice bath. In a tightly sealed test tube, sodium dodecyl sulfate (SDS) (3%; 50 µL), HCl (0.1 N; 200 µL), phosphotungstic

acid (10%; 30 μ L) and 2-thiobarbituric acid (TBA) (0.7%; 100 μ L) were combined and boiled at 100°C for 30 min in a water bath. The reaction mixture was mixed with n-butanol (400 μ L) and then centrifuged at 3000 rpm for 10 min. Supernatants were collected and pass through a UV/VIS spectrophotometer at a wavelengths of 515/555 nm.

$$\text{Lipid Peroxidation (\%)} = \frac{(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}}{\text{] x 100}}$$

Where A_{control} = absorbance of the control sample and A_{test} = absorbance in the presence of the extract or standard. The experiment was conducted three times at each of the concentrations.

Determination of Total Antioxidant Capacity

Total antioxidant capacity (TAC) of the sample was evaluated as indicated by Kattamis *et al.*⁵⁰. Aliquots (100 μ L) of KMnO_4 solution (5 mmol/L) made by dissolving 79 mg of KMnO_4 in distilled water (100 mL) were added to the extract and evenly mixed by shaking. After warming for 30 min in a water bath at 37°C, the mixture's absorbance was measured using a UV/VIS spectrophotometer (optical density, OD, of 570 nm).

$$\text{Total Antioxidant Capacity (\%)} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100$$

Where A_{control} = absorbance of the control sample and A_{test} = absorbance in the presence of the samples of extracts or standards. The experiment was conducted three times at each of the concentrations.

Antimicrobial activity

Bacterial isolates used in this study included *Pseudomonas putida* PA25T, *Pseudomonas aeruginosa* PA129T, *Pseudomonas aeruginosa* PA29T, *Morganella morganii* PA17T, *Serratia marcescens* PA18T, *Klebsiella pneumoniae* KB24AT, *Proteus mirabilis* EC28T, *Escherichia coli* EC3AT, *Acinetobacter indicus* KB45AT and *Acinetobacter indicus* EC41TT isolated from the urinary tract infection of patients at Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria, were used for sample collections between June and October, 2017. The ethical approval to carry out the study was granted with a certificate indicating National code

for Health Research Ethics NHREC/24/01/2020 and Babcock University Health Research Ethics Committee BUHREC 651/21 number from Babcock University, Ilisan Remo, Ogun State, Nigeria. Susceptibility of the uropathogens to the methanol extract of *R. beninensis* was evaluated by determining the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the extract with macrobroth dilution methods²⁶.

Determination of minimum inhibitory concentration (MIC) of the extracts

Minimum inhibitory concentrations (MICs) of the extract, defined as the lowest concentrations maintaining or reducing of inoculums viability⁵¹, was determined by serial tube dilution technique²⁶ against the bacterial isolates. For antibacterial assay, concentrations of extract that ranged between 20 μ g/mL and 10,000 μ g/mL and those of erythromycin used as positive control ranged between 0.0122 μ g/mL and 50 μ g/mL were prepared by serial dilutions in tubes containing double strength Mueller Hinton broth and inoculated with 100 μ L of each of the bacterial isolates. Blank broth of Mueller Hinton was used as negative control.

Determination of minimum bactericidal concentration (MBC) of the extracts

For the MBC assay, one loopful of culture from each of the first five broth cultures without bacterial growth in the MIC tubes was inoculated into fresh nutrient agar plates²⁶ and incubated at 37°C for 24 h. After the incubation periods, the lowest concentration of the extract that showed no sign of bacterial growth on the solid medium was regarded as the MBC value for this extract. This finding was supported by the MIC test tube, which showed no growth after 48 h of the incubation period.

RESULTS

The qualitative phytochemical analysis of the methanol extract of *R. beninensis* indicated alkaloids, saponin, tannins, cardiac glycosides, flavonoids and reducing sugar which are of pharmacological importance (Table 1).

Quantitative phytochemical analysis

The quantitative phytochemical analysis of the methanol extract of *R. beninensis* in

Table 2 showed that the total reducing sugar content was 31.27 ± 0.08 mg/g, alkaloid content was 28.99 ± 0.03 mg/g, terpenoid content was 25.96 ± 0.06 mg/g and saponin content was 22.73 ± 0.19 mg/g while those of tannins and phlobatannins in the extract were 12.39 ± 0.04 and 6.73 ± 0.07 mg/g, respectively. However, the cardiac glycoside, and steroid content were absent.

Table 1. Qualitatively determined phytochemicals in the methanol extract of *R. beninensis*

Phytochemical compounds	Methanolic extract
Saponins	+
Tannins	+
Phlobatannins	-
Flavonoids	+
Cardiac Glycosides	+
Alkaloid	+
Reducing Sugar	+
Terpenoid	+
Phenol	+
Steroids	-

Gas Chromatography Mass Spectrometry (GCMS) Analysis

Figure 1 shows the GCMS chromatogram of the chemical compounds in the extract of *R. beninensis*. The GCMS analysis showed 42 chemical compounds in the methanol (MRB) extract of *R. beninensis*. The name, chemical structure and pharmacological activities of each of the chemical compounds in the methanol extract are presented in Table 3. The most notable compounds with chemical composition's percentages $\geq 1.0\%$ are (Z,Z)-9,12-Octadecadienoic acid

Table 2. Quantitatively determined phytochemicals in the methanol extract of *R. beninensis*

Phytochemical compounds	MRB (mg/100g)
Saponins	22.73 ± 0.19
Tannins	12.39 ± 0.04
Phlobatannin	6.73 ± 0.07
Cardiac Glycosides	0
Alkaloid	28.99 ± 0.03
Reducing Sugar	31.27 ± 0.08
Terpenoid	25.96 ± 0.06
Steroids	0

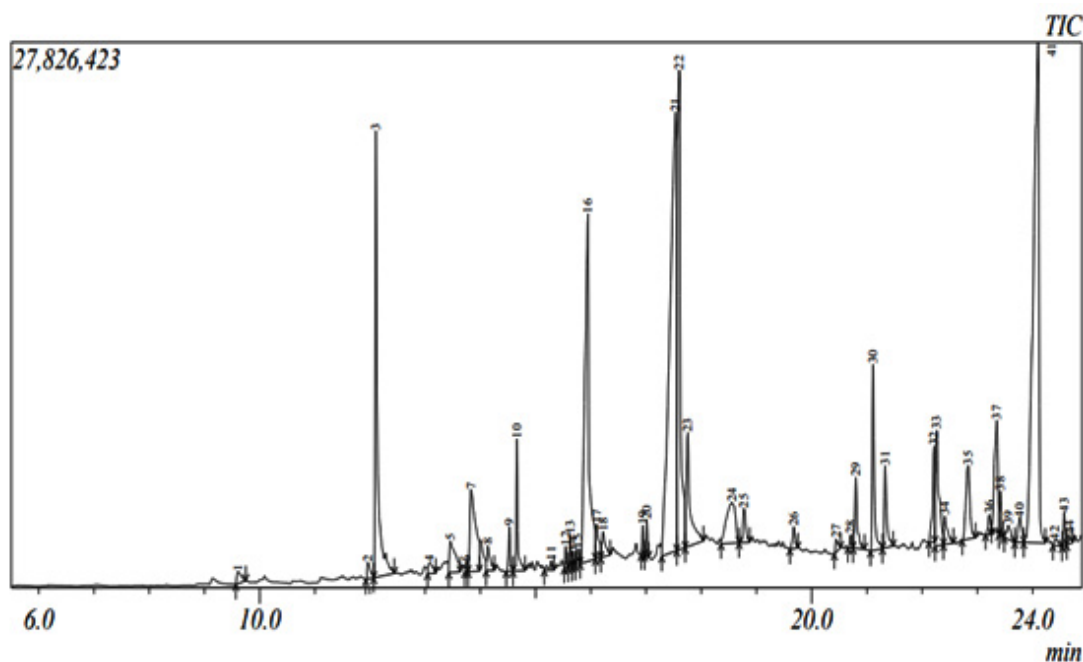

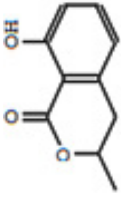
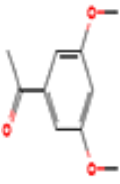
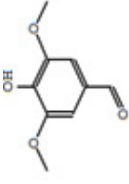
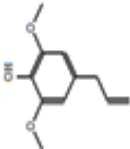
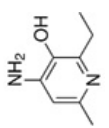
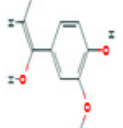

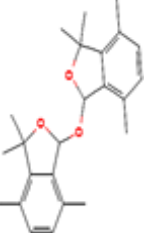



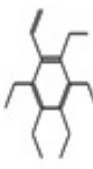
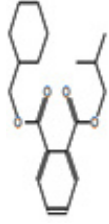
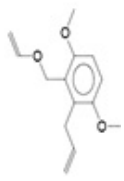


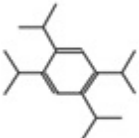








Fig. 1. Chromatogram of the GC-MS analysis of methanol extract of *Rhapsiostylis beninensis*

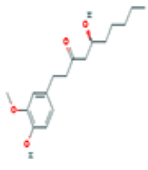
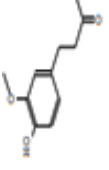
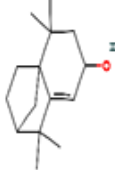


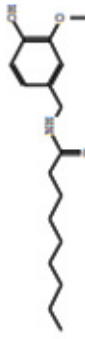
Table 3. GC-MS Analysis of the bioactive compounds of methanol extract of *R. beninensis* (MRB)

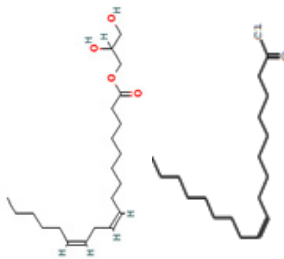

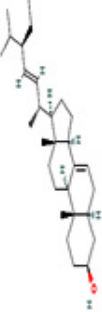
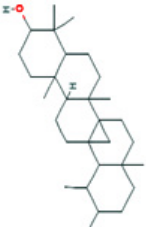
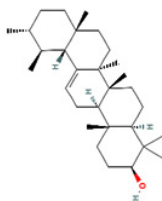

P/N	Rt	Name of the Compound	Chemical Structure	Molecular Formula	Molecular Weight	Area (%)	Pharmacological Properties
1.	9.613	2-Methoxy-4-vinylphenol		C ₉ H ₁₀ O ₂	150	0.35	Antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination ⁵²
2.	11.962	3,4-dihydro-8-hydroxy-3-methyl-1H-2-Benzopyran-1-one		C ₁₀ H ₁₀ O ₃	178	0.35	Cytotoxic activity ⁵³
3.	12.108	3',5'-Dimethoxyacetophenone		C ₁₀ H ₁₂ O ₃	180	6.67	No activity reported ⁵⁴
4.	13.076	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-		C ₉ H ₁₀ O ₄	182	0.35	Anti-edemic, anti-inflammatory, antioxidant, cancer preventive ⁵⁵
5.	13.455	2,6-dimethoxy-4-(2-propenyl)-phenol		C ₁₁ H ₁₄ O ₃	194	1.23	Anti-fungal and Anti-helminthic ⁵⁶

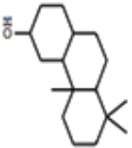
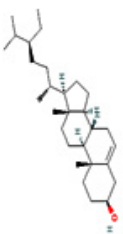
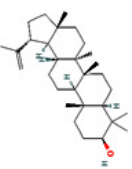
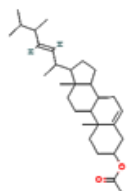
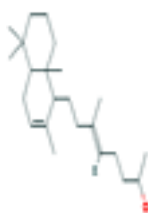
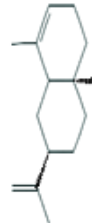
6.	13.743	3-Pyridinol, 4-amino-2-ethyl-6-methyl-		$C_8H_{12}N_2O$	152	0.13	No activity reported
7.	13.829	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol		$C_{10}H_{12}O_3$	180	3.21	Antimicrobial, antioxidant, anti-inflammatory, analgesic ⁵⁷
8.	14.136	Tetradecanoic acid		$C_{14}H_{28}O_2$	228	0.56	Antimicrobial, antispasmodic and anti-inflammatory effects ⁵⁸
9.	14.525	Bis[3,3,4,7-tetramethyl-1,3-2H-benzofuran-1-yl] ether		$C_{24}H_{30}O_3$	366	0.50	Antifungal activity ⁵⁹
10.	14.659	4,7-Dimethoxy-2-methylindan-1-one		$C_{12}H_{14}O_3$	206	1.38	Antibacterial activity ⁶⁰
11.	15.286	5-Nonadecen-1-ol		$C_{19}H_{38}O$	282	0.19	Antimicrobial and antioxidant activity ⁶¹
12.	15.544	Hexadecanoic acid, methyl ester,		$C_{17}H_{34}O_2$	270	0.18	Antioxidant, Insecticide, hemolytic, Hypocholesterolemic ⁶²

13.	15.627	Benzene, ethenylpentaethyl-		$C_{18}H_{28}$	244	0.30	No activity reported
14.	15.675	1,2-Benzenedicarboxylic acid		$C_{20}H_{16}O_4$	334	0.16	Antioxidant and antibacterial activity ⁶³
15.	15.757	2-Allyl-1,4-dimethoxy-3-vinyloxymethylbenzene		$C_{14}H_{18}O_3$	234	0.22	No activity reported
16.	15.946	n-Hexadecanoic acid		$C_{16}H_{32}O_2$	256	9.87	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha-reductase inhibitor ⁶⁴
17.	16.106	Hexadecanoic acid, ethyl ester		$C_{18}H_{36}O_2$	284	0.73	Lubricant, antiandrogenic, antioxidant, 5- alpha-reductase inhibitor ⁶⁵
18.	16.222	Benzene, hexaethyl-		$C_{18}H_{30}$	246	0.60	No activity reported

19.	16.939	9,12-Octadecadienoic acid, methyl ester, (E,E)-		$C_{19}H_{34}O_2$	294	0.25	Hepatoprotective, Anti-histaminic, Antieczemic, Hypocholesterolemic ⁶²
20.	17.016	11-Octadecenoic acid, methyl ester, (Z)-		$C_{19}H_{36}O_2$	296	0.31	Antioxidant and antimicrobial activities ⁶⁵
21.	17.532	9,12-Octadecadienoic acid (Z,Z)-		$C_{18}H_{32}O_2$	280	19.96	Anti-inflammatory, Antibacterial, Antiarthritic, Hepatoprotective, Anti-histaminic, Anticoronary ⁶²
22.	17.602	cis-13-Octadecenoic acid		$C_{18}H_{34}O_2$	282	9.96	Therapeutic uses in medicine, surgery ⁶⁶
23.	17.755	Octadecanoic acid		$C_{18}H_{36}O_2$	284	2.98	Antifungal, Antitumor, Antibacterial ⁶²
24.	18.556	Vitamin E		$C_{29}H_{50}O_2$	430	2.45	Anti-ageing, anti-inflammatory, analgesic, hypoglycaemic, antioxidative, antitumor, anticancer, antispasmodic, antileukemic, vasodilator, anti-bronchitic and anti-coronary ⁶⁷⁻⁶⁹

25.	18.775 19.676 22.408	Gingerol		$C_{17}H_{26}O_4$	294	0.68, 0.38, 0.64 (1.70)	Biological activities including anti- inflammatory and anti-oxidative ⁷⁰
26.	20.444	Nonivamide		$C_{17}H_{27}NO_3$	293	0.22	Flavouring agent, Anti-inflammatory activity ⁷¹
27.	20.706	Isolongifolene, 9-hydroxy-		$C_{15}H_{24}O$	220	0.18	Antioxidant and scavenging activities ⁷²
28.	20.799	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester		$C_{19}H_{38}O_4$	330	1.15	Antioxidant, anti-inflammatory and anthelmintic activities ⁷³
29.	21.111	Capsaicin		$C_{18}H_{27}NO_3$	305	2.58	Anticancer, anti-obesity and anti-inflammatory ⁷⁴
30.	21.331	Dihydrocapsaicin		$C_{18}H_{29}NO_3$	307	1.53	Anticancer, anti-obesity and anti-inflammatory ⁷⁴

31.	22.215	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester		$C_{21}H_{38}O_4$	354	1.45	Anti-inflammatory, hypocholesterolemic, cancer preventive, insectifuge, antiarthritic, anticarcinogenic hepatoprotective, antiandrogenic, nematocidal, antihistaminic ⁷⁵ Antibacterial and anticancer agent ⁷⁶
32.	22.263	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester		$C_{21}H_{40}O_4$	356	2.49	
33.	22.833	Chondrillasterol		$C_{29}H_{48}O$	412	1.98	Cytotoxicity ⁷⁷
34.	23.218	6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-		$C_{30}H_{50}O$	426	0.37	Cytotoxic and anti-arthritis ⁷⁸
35.	23.351	.alpha.-Amyrin		$C_{30}H_{50}O$	426	2.53	anti-obesity ⁷⁹ , anxiolytic ⁸⁰ , antidepressant and antiplatelet ⁸¹ , anti-inflammatory ⁸² , antiulcer, antihyperlipidemic, and antitumor ⁸³ Antibacterial, antitumor, cancer preventive, chemo
36.	23.417	Squalene		$C_{30}H_{50}$	410	0.43	

37.	23.561	3-Phenanthrenol, tetradecahydro-4b,8,8-trimethyl-, [3S-(3.alpha.,		$C_{17}H_{30}O$	250	0.37	preventive, lipoxygenase-inhibitor, pesticide ⁶⁴ Cytotoxic activity ⁸⁴
38.	23.768	gamma.-Sitosterol 4a.beta.,4b.alpha., 8a.beta.,10a.alpha.)],		$C_{29}H_{50}O$	414	0.61	Antimicrobial, antioxidant, anti-inflammatory, anti-arthritis, anti-asthma, diuretic ⁸⁵
39.	24.098	Lupeol		$C_{30}H_{50}O$	426	18.96	Anti-tumor, Cancer preventive, inhibit intestinal cholesterol absorption.
40.	24.400	Ergosta-5,22-dien-3-ol		$C_{30}H_{48}O_2$	440	0.16	Antiinflammatory ⁸⁶ Antituberculosis ⁸⁷
41.	24.574	8-(1,4,4a,5,6,7,8, 8a-octahydro-2,5,5, 8a-tetramethyl naphth-1-yl)-6-methyl-Oct-5-en-2-ol		$C_{23}H_{40}O$	332	0.42	Anticancer ⁸⁸
42.	24.667	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-		$C_{15}H_{24}$	204	0.20	Antibacterial ⁸⁹

(19.96%), Lupeol (18.96%), *cis*-13-Octadecenoic acid (9.96%), *n*-Hexadecanoic acid (9.87%), 3',5'-Dimethoxyacetophenone (6.67%), 4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol (3.21%), Octadecanoic acid (2.98%), Capsaicin (2.58%), α -Amyrin (2.53%), 9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester (2.49%), Vitamin E (2.45%), Chondrillasterol (1.98%), Gingerol (1.70%), Dihydrocapsaicin (1.53%), (*Z,Z*)-9,12-Octadecadienoic acid, 2,3-dihydroxypropyl ester (1.45%), 2,6-dimethoxy-4-(2-propenyl)-phenol (1.23%), 4,7-Dimethoxy-2-methylindan-1-one (1.38%), Hexadecanoic acid, and 2-hydroxy-1-(hydroxymethyl)ethyl ester 1.15%. From

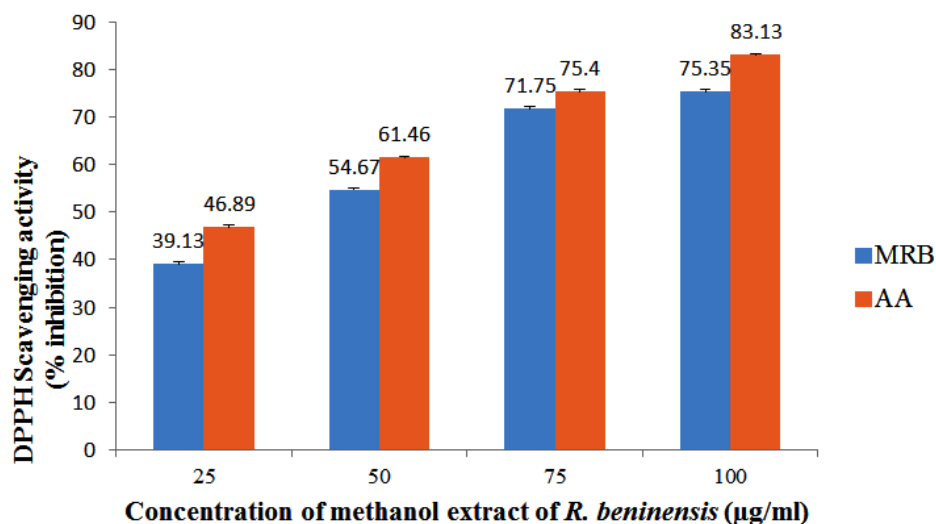


Fig. 2. DPPH radical scavenging activity (%) (Mean \pm Standard deviation) of methanol extract of *R. beninensis*. Key: MRB = Extract with Methanol, AA= Ascorbic acid used as control.

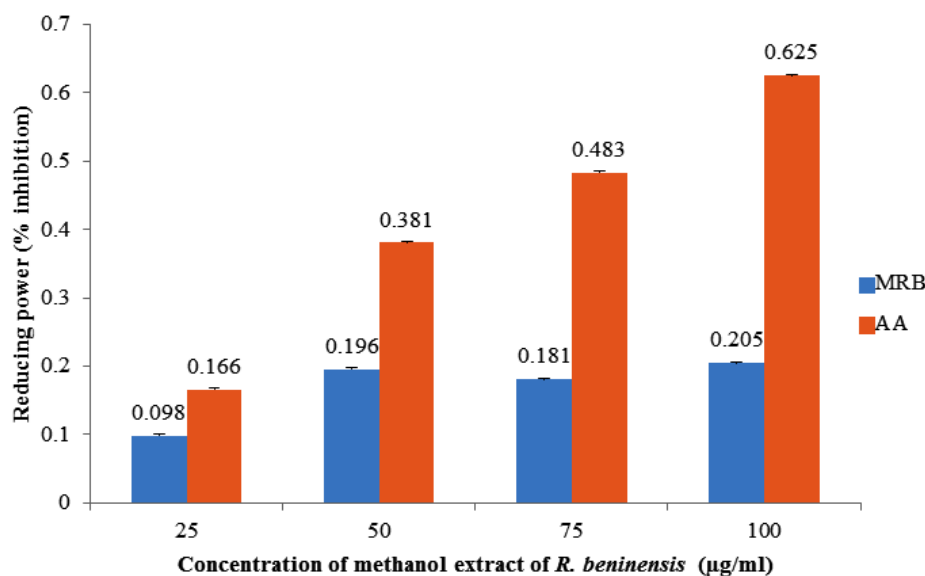


Fig. 3. Ferric reducing antioxidant power (FRAP) activity of methanol extract of *R. beninensis*. Key: MRB = Extract with Methanol, AA= Ascorbic acid.

the literature search, most of these bioactive compounds exhibited varied pharmacological activities and other therapeutic potentials except 3',5'-Dimethoxyacetophenone, 4-amino-2-ethyl-6-methyl-3-Pyridinol, Benzene, ethenylpentaethyl-, 2-Allyl-1,4-dimethoxy-3-vinyloxymethylbenzene Benzene, hexaethyl- and Benzene, hexaethyl-, having no biological activities reported in literatures.

DPPH radical scavenging activity

In this study, the radical scavenging activity of the methanol extract of *R. beninensis* was concentration-dependent. The capacity of the

extract to mop up DPPH radicals increases as its concentration increases as shown in Figure 2. At all the studied concentrations, the extract produced a noticeably high DPPH radical scavenging action. Ascorbic acid, however, used as control showed higher radical scavenging action and an IC_{50} value of 28.42 $\mu\text{g/mL}$ compared with that of the extract having a higher IC_{50} value of 42.19 $\mu\text{g/mL}$.

Ferric reducing power antioxidant assay (FRAP)

Figure 3 illustrates the dependence of the reducing power of the methanol extract of *R. beninensis* (25–100 $\mu\text{g/mL}$) on concentration.

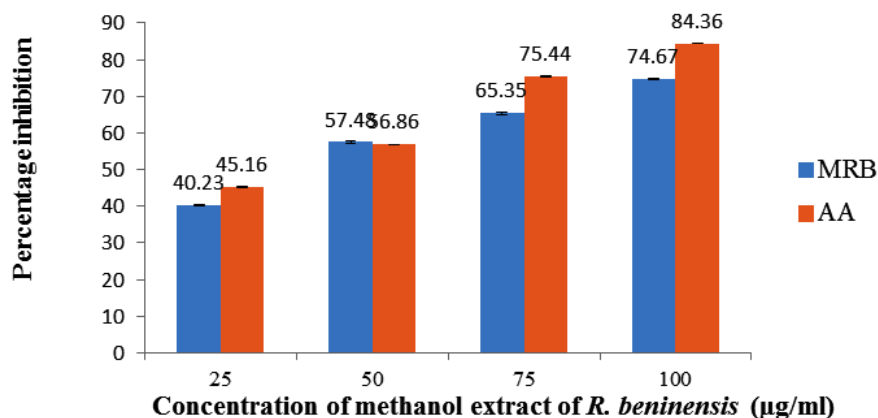


Fig. 4. Nitric oxide radical scavenging activity of methanol extract of *R. beninensis*. Key: MRB = Extract with Methanol, AA= Ascorbic acid used as control.

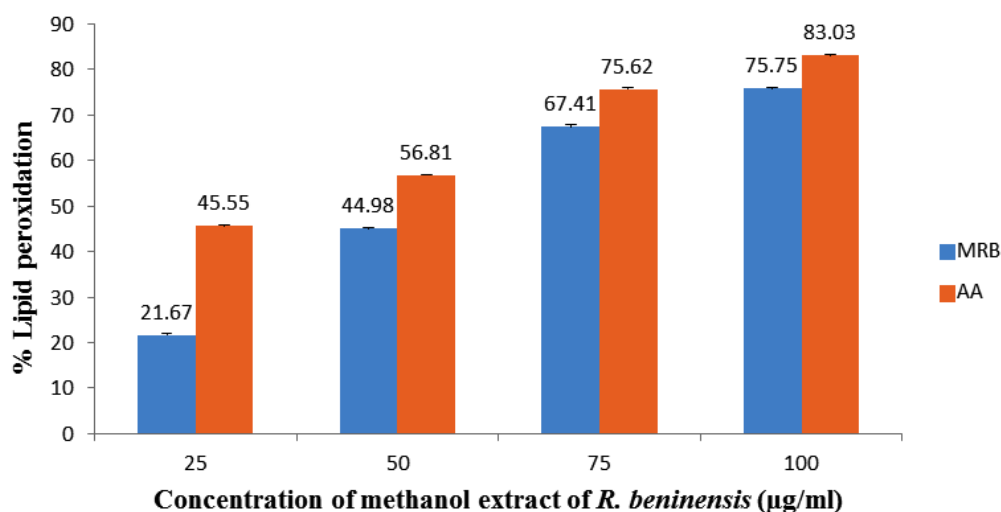


Fig. 5. Lipid peroxidation activity of methanol extract of *R. beninensis*. Key: MRB = Extract with Methanol, AA= Ascorbic acid used as control.

Table 4. Total antioxidant capacity of methanol extract of *R. beninensis*

	Total Antioxidant Capacity (mg/100g)	Total Flavonoid (mg/100g)	Total Phenol (mg/100g)
MRB	42.11 ± 1.13	28.42 ± 0.44	40.38 ± 0.19

Table 5. Antimicrobial activity of methanol extract of *R. beninensis*

Organism code	Antibacterial activity of <i>R. beninensis</i>	
	MIC (mg/ml)	MBC (mg/ml)
<i>E. coli</i> EC3AT	12.5	25
<i>P. mirabilis</i> EC28T	6.25	12.5
<i>A. indicus</i> KB45AT	6.25	12.5
<i>K. pneumoniae</i> KB24AT	6.25	12.5
<i>A. indicus</i> EC41TT	6.25	12.5
<i>P. aeruginosa</i> PA29T	3.125	6.25
<i>M. morgani</i> PA17T	6.25	12.5
<i>P. aeruginosa</i> PA129AT	3.125	6.25
<i>S. marcescens</i> PA18T	12.5	12.5
<i>P. putida</i> PA25T	>50	ND

KEYS: MICs = Minimum Inhibitory Concentrations, MBCs = Minimum Bactericidal Concentrations, ND = Not determined

As concentrations increased, so did the extract's FRAP activity. At all the studied concentrations, the methanol extract of *R. beninensis* produced a considerably high FRAP activity. The ascorbic acid was used as control and it showed FRAP activity and an IC₅₀ value of 77.29 µg/mL in comparison with the methanol extract of *R. beninensis* having an IC₅₀ value of 332 µg/mL.

Nitric oxide radical scavenging assay

The nitric oxide scavenging effect of the methanol extract of *R. beninensis* (25–100 µg/mL) was concentration-dependent. The effects of the methanol extract's nitric oxide scavenging activity at various doses were depicted in Figure 4. Nitric oxide scavenging effects for *R. beninensis* had an IC₅₀ value of 41.29 µg/mL, whereas ascorbic acid demonstrated potent nitric oxide inhibition with an IC₅₀ value of 34.13 µg/mL.

Lipid peroxidation assay

As demonstrated in Figure 5, concentration has an effect on the lipid peroxidation assay of the methanol extract of *R. beninensis* (25–100 µg/mL). In this investigation, the concentration of the plant extract that can scavenge 50% of the lipid peroxidation radicals (IC₅₀) was established.

The results indicated that the IC₅₀ value for the methanol extract of *R. beninensis* was 59.18 µg/mL as opposed to 33.44 µg/mL for the ascorbic acid used as standard.

Total antioxidant capacity

Table 4 showed methanol extract of *R. beninensis* has a higher total antioxidant capacity (TAC) content (42.11 ± 1.13 mg/100 g) when compared with the total flavonoid content of 28.42 ± 0.44 mg/100 g and total phenolic content of 40.38 ± 0.19 mg/100 g.

Antimicrobial activity

The antibacterial activity of the extract was presented in Table 5. The result indicated that the extract inhibited the isolates at minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) ranging from 3.125 - 50 mg/mL and 6.25 - 25 mg/mL, respectively.

DISCUSSION

Using medicinal plants to treat a variety of infections, including UTIs, has received increasing attention in recent years. Consequently, seventy

percent of the population of the developing world use traditional systems of healthcare^{39,90}. Although various antibiotics have been administered to patients, it is necessary to seek for and use “non-antibiotic” approaches for therapeutic purposes due to development of antibiotic resistance, which is a somber concern in the worldwide healthcare arena⁹¹. In order to efficiently treat bacterial infections, particularly urinary tract infections, it is essential to use bioactive compounds possessing therapeutic efficacy. Numerous studies have demonstrated that plant bioactive compounds may meaningfully add to the development of new and potent medications that can modify bacterial resistance as an alternate and complementary method of addressing microbial resistance^{23,24,92}.

In this study, the phytochemical analysis of the root extract of *R. beninensis* revealed that reducing sugar, alkaloids, and terpenoids were found in higher concentrations than phlobatannins, tannins, and saponins. These phytochemicals ensure diverse biological effects including antioxidant activity⁹³. The relative conformation of phytochemicals obtained diverges from phytochemicals in *R. beninensis* stem extract as reported by Lasisi *et al.*³¹. The differences could result from dissimilarities in the phytochemical concentrations in different parts of the plant^{94,95}. However, the existence of these chemical constituents in methanol root extract of *R. beninensis* suggests that, if judiciously screened, the studied plant could provide pharmacologically significant plant-derived pharmaceuticals. This could further bolster the fact that different parts of this plant have been employed in ethnomedicine in the treatment of a number of diseases. Alkaloids are nitrogen-based compounds and are known to have antimalarial, antihypertensive, antiarrhythmic, and anticancer effects⁹⁶ and work as potent analgesics and stimulants for the central nervous system⁹⁷. While saponins have antimalarial properties⁹⁸, antioxidant, anti-inflammatory, anticancer, anti-allergic, and antiplatelet activities have been reported for flavonoids⁹⁹. The availability of these biologically important chemical compounds in *R. beninensis*, therefore, underscores its medicinal values. Thus, the therapeutic potential of various extracts of *R. beninensis* may be due to these phytochemical compounds.

The therapeutic properties of this plant

species are influenced by the different bioactive compounds identified in the methanol extracts of *R. beninensis*. While several prominent bioactive compounds from this extract included (*Z,Z*)-9,12-Octadecadienoic acid (19.96%), Lupeol (18.96%), *cis*-13-Octadecenoic acid (9.96%), *n*-Hexadecanoic acid (9.87%), 3',5'-Dimethoxyacetophenone (6.67%), and 4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol (3.21%) have been known to have various inhibitory effects and therapeutic uses, the pharmacological activities of some chemical compounds identified in this study have been corroborated by Fagbemi *et al.*²⁴ who identified 36 bioactive compounds and many of them possessed antioxidant, antibacterial, antiasthma, antifungal, antidiabetic, anti-cancer and anti-inflammatory activities. Kuete¹⁰⁰ and Dzotam & Kuete¹⁰¹ indicated that antimicrobial activities of studied Cameroonian plants depended on the occurrence of alkaloids, flavonoids, phenolics, steroids and triterpenes. While the antimicrobial activity of methanol extracts of *Artemisia vulgaris*, *Cinnamomum tamala*, *Oxalis corniculata*, and *Ageratina adenophora* was against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter koseri* and *Salmonella typhi*¹⁰², hydromethanol extracts of *Cistus monspeliensis*, *Punica granatum* and *Berberis vulgaris* indicated tremendous antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Enterobacter cloacae*¹⁰³. These identified phytochemicals in the extract are believed to be a constituent of defense mechanisms in plant, and they can be categorized as protective substances present in this plant as “phytoanticipins” and “phytoprotectants”¹⁰⁴. Despite the low percentages of some of the detected chemical compounds in this study, scientific reports indicated that each of the compounds had considerable medicinal importance, and the presence of the identified chemical compounds may have an impact on the antibacterial and antioxidant activities of *R. beninensis*. The chemical compounds identified underscore the veracity of this plant’s usefulness in traditional medicine while identified compounds without biological activities found in literature could also contribute individually or synergistically to the pharmacological activities of the extract.

Disproportionate free radical production damages biomolecules during cellular metabolism

causing few disorders including diabetes, cancer, chronic inflammation, and neurodegenerative illnesses¹⁰⁵. The most practical method in managing oxidative stress-related diseases is antioxidant therapy¹⁰⁶. Despite the availability of various synthetic medications for managing oxidative stress, their high costs and unfavorable side effects have reduced their effectiveness¹⁰⁷. As a result, alternative, low-cost, non-toxic antioxidants are required to fight oxidative stress and the diseases associated with it¹⁰⁶. Consequently, the high potency and effectiveness at low concentrations exhibited by the methanol extract of *R. beninensis* implied that its redox properties are dependent on its polyphenolic contents satiating singlet and triplet oxygen and decomposing peroxides, adsorbing and neutralizing free radicals¹⁰⁸ and directly scavenging free radicals¹⁰⁹.

The methanol extract of *R. beninensis* roots was more effective against *Pseudomonas* spp. PA29T. At low concentrations, strong antibacterial activity was recorded against all the Gram-negative bacteria. The MBC values revealed that the extract could be more bactericidal against the Gram-negative bacteria. This was in consonance with the findings of Semwal *et al.*¹¹⁰, Manivannan *et al.*¹¹¹, and Kouadri¹¹². While antibacterial agents with lower MICs and MBCs are thought to be more effective, Tripathi¹¹³ indicated that antibacterial agents are more bactericidal when the MIC is closer to the MBC. However, the variations in bacterial susceptibility could be attributed to the differences in microorganisms' intrinsic tolerance or the physico-chemical properties of phytochemicals contained in plant extracts¹¹² and phospholipid membranes that contain structural lipopolysaccharide components making their cell walls impermeable to antimicrobials¹¹⁴. While the antibacterial activity of the methanol extract could depend on the most prominent chemical compounds, the therapeutic potential of the chemical compounds with lesser percentages and those without pharmacological activities identified in literature could not be undermined.

CONCLUSION

In conclusion, this study confirmed the presence of different phytochemical compounds and chemical compounds with

diverse pharmacologically important principles or activities in the aqueous methanol extract of *R. beninensis* roots. The extract's antioxidant and antibacterial effects against the test bacterial isolates might be accredited to the occurrence of the aforementioned chemicals found in the extract. This study demonstrates the medicinal value of *R. beninensis*' root, identifies the plant as a significant source of innovative drug compounds, and suggests that this plant may be a viable alternative for treating urinary tract infections, supporting its use in the folkloric treatment of these diseases. Further studies on isolation of bioactive compounds of therapeutic values to determine their biological activities are ongoing in our research laboratory.

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