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Phytochemical Evaluation, Antioxidant and Antimicrobial Activities of Various Extracts from Leaves and Stems of *Bryophyllum pinnatum*

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Abstract

Antioxidant and antimicrobial activities of different extracts (methanol and ethyl acetate) of leaf and stem of *Bryophyllum pinnatum* were studied. The screening for the secondary metabolites was carried out using the standard methods. The antioxidant capacities of the different extracts were assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals and Ferric reducing antioxidant power (FRAP) while the antimicrobial activity of the extracts obtained were screened against Gram-positive, Gram-negative bacteria and fungi (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella* spp., *Vibrio cholerae*, *Candida albicans* and *Aspergillus niger*) using Agar well diffusion method. Both extracts obtained from leaf and stem of *B. pinnatum* contained most of the phytochemical compounds tested for. However, anthocyanins and anthraquinone were not detected in leaf extracts while coumarin was absent in stem extracts. Quantification of bioactive compounds showed that both extracts contained the highest concentration of polyphenols (34.49 ±0.47 mg GAE/g and 32.32 ±1.2 mg GAE/g for methanol leaf and stem extracts respectively) while the least concentration was recorded for alkaloids (0.03±0.02 mg/g for methanol stem extract). Results revealed that the extracts showed dose-dependent scavenging of DPPH as well as the ability of the extracts to reduce $FeCl_3$ solution, with methanol extracts exhibiting the highest scavenging and reducing capacity. However the leaves of *B. pinnatum* had greater antioxidant activity than the stem by DPPH and ferric reducing assays, with IC_{50} values ranging from 3.147µg/ml to 3.80µg/ml for DPPH and 331.9 - 451 µg/ml for FRAP assays. The antimicrobial activity of various solvent extracts of leaf and stem reveal that microorganisms exhibited different sensitivities towards these extracts in a dose-dependent manner. Methanol leaf extract showed no activity against *E. coli* while *P. aeruginosa* was insensitive to ethyl acetate leaf extract. For stem extracts, *A. niger, V. cholerae* and *P. aeruginosa* were resistant to methanol extract while *A. niger*, *Salmonella* spp. and *P. aeruginosa* was resistant towards ethyl acetate stem extract. The results obtained in this study showed that *B. pinnatum* is a reservoir of bioactive compounds and both extracts exhibited significant antimicrobial and antioxidant activity.

Keywords: *Bryophyllum pinnatum*, Antioxidant activity, Agar well diffusion, *Aspergillus niger,* Polyphenols, Ferric reducing power assay.

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Introduction

A medicinal plant is any plant in which one or more of its organs contain substances that can be used for the synthesis of useful drugs, and also serve as a major lead for modern drug design [1, 2]. Numerous researchers on medicinal plants and herbal drug production reported that bioactive components of medicinal plant occur in the leaves, flowers, roots, stem, bark or wood. These bioactive compounds commonly known as phytochemicals include terpenes, alkaloids, flavonoid, bioflavonoid, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthraquinones [3, 4, 5, 6]. Several plants containing secondary metabolites possess antioxidant, antimicrobial and other biological potentials [7]. Natural antioxidants, such as phenolic compounds are gaining importance due to their benefits for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation [8, 9]. Several studies indicate that medicinal plants which are rich reservoir of bioactive compounds contain compounds that are significant in therapeutic application against human and animal pathogen, including bacteria, fungi and viruses [10, 11].

Studies show that nearly 80% of the world's population still relies on traditional medicines for primary health care, most of which is the use of plant extracts [12]. In the past few years, uncertainties concerning the safety of synthetic antimicrobial drugs have led to an increase in the demand for natural compounds such as plants rich in antimicrobials [13]. This is because herbal medicines have been reported to be safe, affordable, acceptable, available and without any adverse side effects especially when compared with synthetic drugs [14, 15]. In developing countries, there is a gradual revival of interest in the use of medicinal plants especially herbal preparations in the local healthcare systems because of the increasing problems of Multi-Drug Resistance (MDR) to human pathogenic bacteria [16, 17]. One of such plants used in the treatment of a wide range of ailments is *Bryophyllum pinnatum* (*B. pinnatum*).

B. pinnatum belongs to the family *Crassulaceae* and the common names include life plant, love plant, miracle leaf, and Canterbury bells. It is widely distributed in tropical Africa, America, Hawaii, India, China, Australia, and Madagascar [18]. *B. pinnatum* is a succulent plant, 50 – 200 cm tall and about 3.2 cm wide, and reproduces via seeds and also vegetatively from leaf bulbils [19, 20]. They are medium green above blotched with purple underneath. It has flashy, dark green leaves. Its flower is in paniculate cymes 20 – 80 cm long. It has fruit whose follicles are 10 -14 mm long enclosed in the persistent papery calyx. The seeds are numerous in each fruit. The leaves and leaf juice have been used traditionally as anti-inflammatory, antipyretic, antimicrobial, antioxidant, antitumor, antidiabetic, anti-ulcer, antiseptic, hypocholesterolemic, and cough suppressant [21]. In Nigeria, the plant is particularly known for its effective wound healing properties and detachment of the umbilicus of infants, for the treatment of earache, burns, abscesses, ulcer, insect's bites, whitlow, diarrhea and lithiasis [19, 20]. The lightly roasted leaves are used externally for skin fungus and inflammations and the leaf infusion is an internal remedy for fevers [22]. *B. pinnatum* leaves are used to expel worms, cure acute and chronic bronchitis, pneumonia and other forms of respiratory tract infections [23]. It is used for all sorts of respiratory conditions such as asthma, cough and bronchitis. It is employed for the treatment of kidney stones, gastric ulcers and oedema of the leg [24].

Materials and Methods

Collection of Plant Materials

Fresh leaves and stem of *B. pinnatum* were obtained from Etinan and Ikot Ekpene local government areas in Akwa Ibom State, Nigeria, and authenticated by the Department of Botany, University of Uyo, Nigeria, and a voucher specimen [Voucher No: UUPH27(a)] was kept in the herbarium for future reference.

Extraction Procedure

The leaves and stem were thoroughly washed with distilled water and air-dried for 2 weeks. The dried parts were pulverized and the powdered plant parts were separately divided into portions. 750g of the different plant parts were macerated with 1.7 L each of methanol and ethyl acetate at room temperature for 72 hrs. After 72 hrs, the different extracts were filtered separately off through a cotton plug and finally with a Whatman No. 1 filter paper. The liquid filtrates were concentrated and evaporated to dryness using a rotary evaporator (WG- tv311-V, Wilmad-LabGlass, USA) at 40 °C, and each extract was transferred into well-labeled sterile glass vials and stored at 4 °C before use [25].

Phytochemical Screening of Plant Extracts

The leaf and stem extracts (methanol and ethyl acetate) were screened for the presence of various bioactive components (phytochemicals) using standard procedures [6, 26, 27].

Test for Anthraquinones

To 6g of the different plant parts in this study, 10 ml of benzene was added. After 10 minutes, the solution was filtered and 10 ml of 10% ammonia was added to the filtrates and shaken. The presence of pink, violet, or red color signified the presence of anthraquinones in the ammonia phase [26].

Determination of Tannins

10 ml each of bromine water was added to the 0.5 g of leaf and stem extracts of *B. pinnatum*. The discoloration of bromine water indicated the presence of tannins.

Test for Saponins

5.0 ml of distilled water was added to the different plant extracts in a test tube. The froth formed was

mixed with few drops of olive oil. The formation of foams showed the presence of saponins.

Tests for Flavonoids

ShinodaTest. Magnesium strip and HCl were mixed with plant extracts. The development of pink colour confirmed the presence of flavonoid.

Tests for Glycosides

Liebermann's Test. 2.0 ml of acetic acid and 2 ml of chloroform were added to the different extracts. The mixtures were allowed to cool after which concentrated H_2SO_4 was added. The appearance of green color signified the presence of aglycone, steroidal part of glycosides.

Keller-Kiliani Test. A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture as well as 1 ml H_2SO_4 was added to 10 ml of the different plant extracts. A brown ring formed between the layers indicated the presence of cardiac steroidal glycosides.

Salkowski's Test. 2 ml of Conc. H_2SO_4 was added to the plant crude extract. A reddish-brown color formed indicated the presence of the steroidal aglycone part of the glycoside.

Test for Terpenoid

2.0 ml of chloroform was added to 5 ml plant extracts and evaporated on the water path. The mixture was boiled with 3 ml of H_2SO_4. A grey color formed confirmed the presence of terpenoids.

Test for Steroids

2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml plant crude extract. In the lower chloroform layer, red color appeared that indicated the presence of steroids.

Test for Coumarins

1ml of 10% sodium hydroxide solution was added to 1ml of the different plant extracts. Formation of yellow colour indicated the presence of coumarins

Test for Reducing Sugar (De- Oxy sugars)

0.5g of each extract was macerated with 20 ml of distilled water and filtered. To 1 ml of the filtrates, 1 ml of alkaline copper reagent was added. The mixture was boiled for 5 min and allowed to cool. Then 1 ml of phosphomolybdic acid reagent and 2 ml of distilled water was added and the absorbance read at 420 nm

Test for phenols

The extract (500 mg) was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

Test for Quinone

1ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinones give coloration ranging from red to blue.

Test for amino acids and proteins

Biuret test: To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicated the presence of protein.

Ninhydrin test: About 0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicated the presence of proteins, peptides or amino acids.

Anthocyanins

2 ml of 2 N HCl and ammonia was added to 2 ml of the different extracts. The appearance of pink-red which later turned blue-violet indicated the presence of anthocyanins.

Test for Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Dragendorff's Test: 1ml of the filtrate was treated with few drops Dragendorff's reagent. Formation of orange-brown precipitate indicated the presence of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent. The formation of a yellow cream precipitate indicated the presence of alkaloids.

Quantitative Estimation of Phytoconstituents

The phytochemicals which are present in the methanol and ethyl acetate stem and leaf extracts of *B. pinnatum* was quantitatively determined using standard Protocols.

Determination of Polyphenols

The total phenolic content in the extracts were determined by the modified Folin-Ciocalteu method as described by Singleton and Rossi [28] and modified by Ayoola et al. [29]. Sample extract was dissolved in methanol (1 mg/ml). An aliquot of 0.5

ml of each plant extract (1 mg/ml) was mixed with 5 ml of Folin- Ciocalteu reagent which was previously diluted with distilled water (1:10 v/v). The mixture was shaken slightly and allowed to stand at 22 °C for 5mins. After, 4 ml (75 g/l) of sodium carbonate (Na_2CO_3) was added, and the tubes containing the mixtures were allowed to stand for 30 min at 40 °C to develop colour. Absorbance was then read at 765 nm using the UV spectrophotometer (Shimadzu, Japan). Results were expressed as Gallic acid equivalent in (mg/g) of extracts (10–100 mg/ml). All samples were analyzed in triplicate.

Determination of total flavonoids

Total flavonoid contents were determined using Aluminium chloride colorimetric method [30]. A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. Using UV spectrophotometer (Shimadzu, Japan). Yellow color indicated the presence of flavonoids. Total flavonoid content was calculated as quercetin equivalent (mg/g). The calibration curve ranged from 10 – 100 mg/ml.

Determination of total alkaloids

Total alkaloids were determined according to the standard method as described by Harbone [27]. 200 ml of 10% acetic acid in ethanol was added to 5 g of different plant extracts. The mixtures were covered and allowed to stand for 4 h. The solutions were decanted and filtered and the extracts were further concentrated in a water bath until one-quarter of the original volume was obtained. Concentrated ammonium hydroxide was added dropwise to the concentrated extract until the precipitation was complete. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Determination of total tannins

50 ml of distilled water was added to 500 mg of the plant extracts and was shaken for 1 h in a mechanical shaker. The solution obtained was filtered into a 50 ml volumetric flask and made up to the mark. 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [31].

Determination of total saponins

To 20 g of each plant extracts, 100 cm^3 of 20% aqueous ethanol were added. The samples were heated at 55°C for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. 20 ml of diethyl ether was added to the concentrates and shaken vigorously. The aqueous layer recovered was further purified by adding 60 ml of n-butanol. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride and heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and the saponin content was calculated [32].

In vitro Antioxidant Assay Antioxidant activity by DPPH Assay

The free radical scavenging activity of the different extracts was measured *in vitro* by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) method as described by Brand-Williams et al. [33]. 0.5 Mm solution of DPPH was added to sample solutions at different concentrations (20 – 100 µg/ml). A control (Abs Control) containing methanol and DPPH solution was also prepared. All solutions obtained were then incubated for 1 hour at room temperature. Absorbance was measured at 517 nm. Vitamin C was used as standard and the same concentrations of it were prepared as the test solutions. The percentage of inhibition of samples was calculated from obtained absorbance by the equation:

$$Percentage\ of\ Inhibition = \frac{Abs\ control - Abs\ test/standard}{Abs\ control} \times 100$$

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC_{50} value for each of the test solutions.

Ferric reducing/antioxidant power (FRAP) Assay

The reducing property of the extract was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution [34]. Briefly appropriate concentrations of the extracts were mixed with 2.5 ml of 200 mM of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was incubated at 50°C for 20 min after which 2.5 ml of 10% trichloroacetic acid was added. The mixture was then centrifuged at 650 rpm for 10 min. Supernatant

(The upper layer) (5 ml) was mixed with equal volume of deionized water and 1 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm. The ferric-reducing-power capacities of the plant extracts and standard antioxidants were expressed graphically by plotting absorbance against concentration [34]. Ascorbic acid was used as a positive reference. The experiment was done in triplicate.

Antimicrobial Activity

Collection of Test Organisms

Microorganisms used were obtained from the microbial stock collection unit of the Department of Microbiology, University of Uyo, Akwa Ibom State. The test organisms used were 1 Gram-positive bacterium (*Staphylococcus aureus*), 4 Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa, Salmonella* spp., *Vibrio cholerae*) and two fungi (*Candida albicans, Aspergillus niger*). These organisms were sub cultured to obtain pure and fresh isolates. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Isolates were identified using standard microbiological procedures by carrying out Gram's reaction and biochemical tests to confirm the species.

Preparation of test organisms before inoculation

McFarland standard was used as a reference to adjust the turbidity of bacterial suspensions. The bacterial suspensions were standardized following the CLSI guidelines for aerobic bacteria. All of the test microorganisms were grown in Mueller Hinton broth for 18–24 h, followed by the matching of bacterial suspension to the turbidity equivalent to 0.5 McFarland solutions (1-2×10^8 cfu/ml). Different concentrations (10, 20, 40, 60 and 80 mg/ml) of the extracts were prepared and kept in corked test tubes.

Seeding of Muller – Hinton Agar plates

0.1 ml of each diluted isolates was aseptically transferred into Mueller–Hinton agar (Oxoid, UK) plates and aseptically spread evenly using sterile Hockey stick. The seeded plates were left for 30 mins for the isolates to diffuse into the medium. Sterile cork borer of 5 mm was used to bore holes on the agar plates. 0.1 ml of each of the extracts was then dropped in the holes and labeled accordingly. The diameters of inhibition zones were measured [35].

Results

Phytochemical screening

The results of the qualitative phytochemical screening of methanol and ethyl acetate of leaf and stem extracts of *B. pinnatum* as presented in **Table 1** revealed the presence of amino acids, quinone, phenol, reducing sugar, coumarin, steroids, terpenoids, glycosides, flavonoids, saponins, tannins and alkaloids in both plant parts. However, anthocyanins and anthraquinones were not detected in the methanol and ethyl acetate leaf extracts.

Table 1: Qualitative Analysis of Different Parts of *Bryophyllum pinnatum*

Plant Parts	Leaf Extract		Stem Extract	
Solvents	Methanol	Ethyl Acetate	Methanol	Ethyl Acetate
Anthocyanins	Absent	Absent	Present	Present
Amino acid	Present	Present	Present	Present
Quinone	Present	Present	Present	Present
Phenol	Present	Present	Present	Present
Reducing sugar	Present	Present	Present	Present
Coumarin	Present	present	Present	Present
Steroids	Present	Present	Present	Present
Terpenoids	Present	Present	Present	Present
Glycosides	Present	Present	Present	Present
Flavonoids	Present	Present	Present	Present
Saponins	Present	Present	Present	Present
Tannins	Present	Present	Present	Present
Anthraquinone	Absent	Absent	Present	Present
Alkaloids	Present	Present	Present	Absent

The results of the quantitative phytochemical screening of methanol and ethyl acetate of leaf and stem extracts of *B. pinnatum* in **Table 2** shows that the concentration of alkaloids ranged from 0.03±0.02 to 0.90±0.13 mg/g. alkaloids were not detected in the ethyl acetate stem extract. The concentration of saponins varied from 0.45±0.43 to 1.12±0.21 mg/g, with methanol leaf extracts having the highest concentration (1.12±0.21 mg/g).

Table 2: Quantification of the Phytochemicals in the extracts of *Bryophyllum pinnatum*

Phytochemicals (mg/g)	Leaf extracts		Stem extracts	
	Methanol	Ethyl acetate	Methanol	Ethyl acetate
Alkaloids	0.16±0.01	0.90±0.13	0.03±0.02	ND
Saponins	1.12±0.21	1.02±0.22	1.05±0.11	0.45±0.43
Flavonoids	0.21±0.14	0.11±0.14	0.53±0.13	0.31±0.01
Tannins	4.98±1.31	2.8±±0.21	0.64±0.24	0.25±0.12
Poly phenols	34.49±0.47	21.2±2.2	32.32±1.2	17.9±0.62

ND = not detected

The results of flavonoids (y=0.142x-0.0.177: R^2=0.983) in both plant part extracts showed that the stem extracts contained the highest concentration of flavonoids (0.53±0.13 and 0.31±0.01 mg for methanol and ethyl acetate extracts respectively) while the

Table 3: Antimicrobial Activity of methanol and ethyl acetate leaf extracts of *Bryophyllum pinnatum* against the human pathogenic bacteria by disc diffusion method.

Crude extracts	Isolates	Zone of inhibition (mm)				
		10 mg/ml	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml
Methanol	*S. aureus*	8.00±0.01	10.00±0.3	12.0±1.2	14±0.2	20±0.5
	A. niger	NA	NA	NA	10±0.05	14±0.3
	Candida albican	16±0.34	20±0.21	23±1.2	30±1.2	34±1.41
	Vibrio cholera	8±0.03	10±0.25	11±0.23	15±0.11	22±1.21
	Escherichia coli	NA	NA	NA	NA	NA
	Salmonella spp.	10±0.12	13±0.22	16±1.3	20±1.31	23±1.10
	P. aeruginosa	14±0.01	20±1.22	23±1.2	27±1.22	30±0.9
Ethylacetate	*S. aureus*	8±0.01	9±0.01	10±0.13	13±0.03	14±0.12
	A. niger	8±0.1	9±0.11	10±0.6	12±0.11	13±0.21
	Candida albican	13±0.3	17±0.33	21±0.02	26±0.22	30±0.12
	Vibrio cholera	NA	NA	NA	NA	NA
	Escherichia coli	13±0.01	15±0.01	17±0.03	20±0.41	25±0.61
	Salmonella spp.	NA	9±0.01	10±0.05	12±0.04	14±0.12
	P. aeruginosa	NA	NA	NA	NA	NA

Key: NA= No activity, *S. aureus*= *Staphylococcus aureus, A. niger=Aspergillus niger, P. aeruginosa*= *Pseudomonas aeruginosa*

least concentration was present in the leaf extracts with (0.21±0.14 and 0.11±0.14 mg/g for methanol and ethyl acetate extracts respectively). Tannin content (y=0.168x -0.094: R^2=0.975) was higher in methanol leaf extract (4.98±1.31 mg/g) while the least concentration was recorded for ethyl acetate stem extract (0.25±0.12 mg/g). Methanol leaf extract recorded the highest concentration of polyphenols (34.49±0.47 mg/g) while ethyl acetate stem extract contained the least concentration (17.9±0.62 mg/g). (y=0.149–0.121 x: R^2 = 0.967).

Antimicrobial activity

Tables 3 and **Table 4** show the result of the antibacterial activity of leaf and stem extracts of *B. pinnatum* respectively tested against five bacterial and two fungi strains at different concentrations (10, 20, 40, 60 and 80 mg/ml). All the extracts showed strong antimicrobial activity against test organisms in a dose-dependent manner. Results from **Table 3** showed that *S. aureus, C. albican, V. cholerae, Salmonella* spp. and *P. aeruginosa* were susceptible to the methanol leaf extract of *B. pinnatum* at all concentrations. However, antibacterial activity was not observed against *E. coli.* For ethyl acetate leaf extracts, the tested microorganisms showed varying degree of susceptibility at various concentrations except *V. cholerae* and *P. aeruginosa*, which were resistant to ethyl acetate extract at all concentrations. **Table 4** showed that *A. niger, V. cholera and P. aeruginosa* were resistant to methanol stem extract at all concentrations while *Salmonella* spp. was resistant at 10 mg/ml. the zone of inhibition of *S. aureus* ranged from 7±0.11 to 15±0.01 mg/ml while that of

Table 4*:* Antimicrobial activity of methanol and ethyl acetate stem extracts of *Bryophyllum pinnatum* against the human pathogenic bacteria by disc diffusion method.

Crude extracts	Isolates	Zone of inhibition (mm)				
		10mg/ml	20mg/ml	40mg/ml	60mg/ml	80mg/ml
Methanol	*S. aureus*	7±0.11	10±0.02	11±0.12	13±0.11	15±0.01
	A. niger	NA	NA	NA	NA	NA
	Candida albican	9±0.02	10±0.01	13±0.2	15±0.13	17±0.11
	Vibrio cholera	NA	NA	NA	NA	NA
	Escherichia coli	8±0.22	11±0.03	14±0.15	17±0.21	19±1.2
	Salmonella spp.	NA	6±0.01	9±0.01	11±0.12	13±0.11
	P. aeruginosa	NA	NA	NA	NA	NA
Ethyl acetate	*S. aureus*	NA	9±0.01	10±0.13	11±0.01	12±0.12
	A. niger	NA	NA	NA	NA	NA
	Candida albican	10±0.3	11±0.22	13±0.14	15±0.23	22±1.02
	Vibrio cholera	8±0.01	11±0.5	14±0.22	16±0.13	18±0.54
	Escherichia coli	13±0.11	16±0.13	20±0.01	24±0.62	28±0.43
	Salmonella spp.	NA	NA	NA	NA	NA
	P. aeruginosa	NA	NA	NA	NA	NA

Key: NA = No activity, *S. aureus* = *Staphylococcus aureus, A. niger* =*Aspergillus niger, P. aeruginosa*= *Pseudomonas aeruginosa*

C. albican varied from 9±0.02 to 17±0.11 mg/ml. At 10 mg/ml, the zone of inhibition of *E. coli* was 8±0.22 mg/ml while at 80mg/ml, the zone of inhibition was 19±1.2 mg/ml. Ethyl acetate stem extract did not show any antimicrobial activity against *A. niger, Salmonella* spp. and *P. aeruginosa.* Higher antimicrobial activity was observed against *C. albican* (10±0.3 -22±1.02 mg/ml) and *E. coli* (13±0.11 - 28±0.43 mg/ml).

Antioxidant activity

DPPH free radical scavenging activity

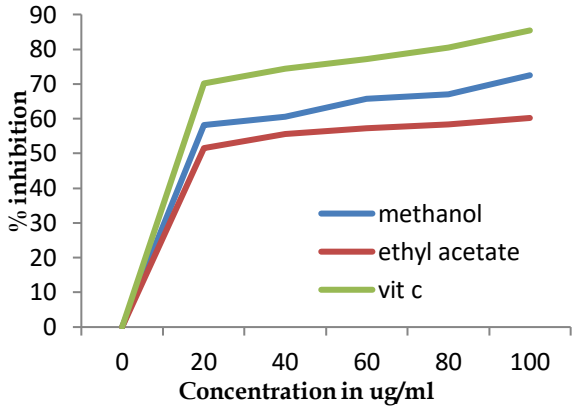

Figure 1: DPPH scavenging activity of leaf extract of *Bryophyllum pinnatum*
Methanol : Y = 11.26x + 14.56: R^2 = 0.612
VIT C: Y =13.16x +18.48: R^2 = 0.590
Ethyl acetate: Y = 9.231x+ 14.84: R^2 = 0.550

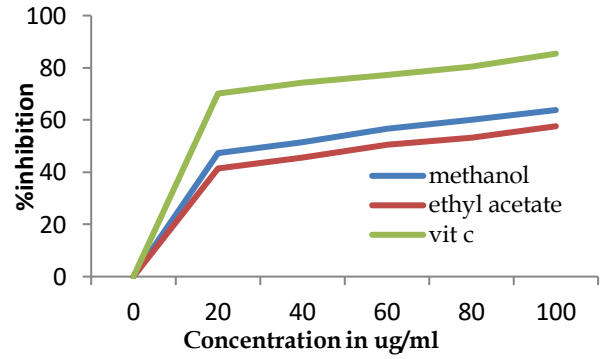

Figure 2: DPPH scavenging activity of stem extract of *Bryophyllum pinnatum*
Methanol: Y = 10.36x + 10.28; R^2 =0.677
Ethyl acetate Y = 9.365X +8.62; R^2 = 0.693

Figures 1 and **Figure 2** show the results of DPPH assay of methanol and ethyl acetate of leaf and stem extracts of *B. pinnatum* respectively. Vitamin C was used as the standard. The extracts have shown dose-dependent scavenging of DPPH radicals. The result also showed that at 100 µg/ml dose, the leaf extracts (methanol and ethyl acetate) inhibited DPPH radical by 72.5% and 60.2% respectively while at the same concentration, methanol and ethyl acetate stem extracts inhibited DPPH radical at 63. 8% and 57.6% respectively compared to the standard drug, vitamin C (85.4%). A graph of % inhibition against various concentrations was plotted and the IC_{50} was calculated from the different regression graphs and the results are presented in **Table 5**. The IC_{50} in DPPH assay ranged from 3.147 to 4.41 µg/ml.

Table 5: IC_{50} in µg/ml for antioxidant activity of *Bryophyllum pinnatum* extracts. IC_{50} for vitamin C in DPPH is 2.39, while that for FRAP is 220.24

Antioxidant activity	leaf extracts		stem extracts	
	Methanol	Ethyl acetate	Methanol	Ethyl acetate
DPPH	3.147	3.80	3.83	4.41
FRAP	331.9	451	428.30	618.38

FRAP Assay

The ferric-reducing-power capacities of the plant extracts and standard antioxidants were expressed graphically by plotting the absorbance against concentration and results presented in **Figures 3** and. All the plant extracts showed concentration-dependent reducing power. The IC_{50} of the ranged from 331.9 µg/ml. to 618.38 µg/ml.

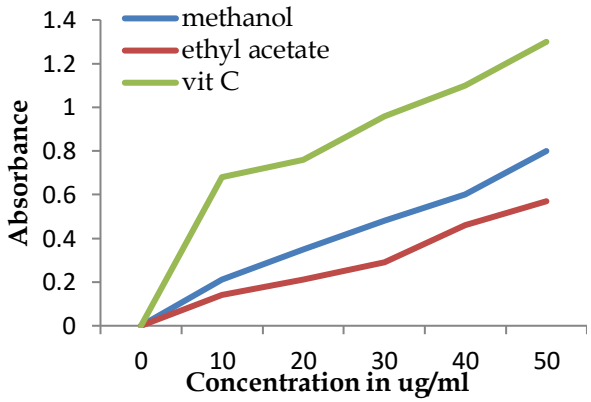

Figure 3: Ferric reducing power activity of leaf extract of *Bryophyllum pinnatum*
VIT C: Y = 0.227X + 0.004; R^2 = 0.886
Methanol: Y= 0.151X – 0.123; R^2 = 0.991
Ethyl acetate Y = 0.- 0.110; R^2 = 0.984

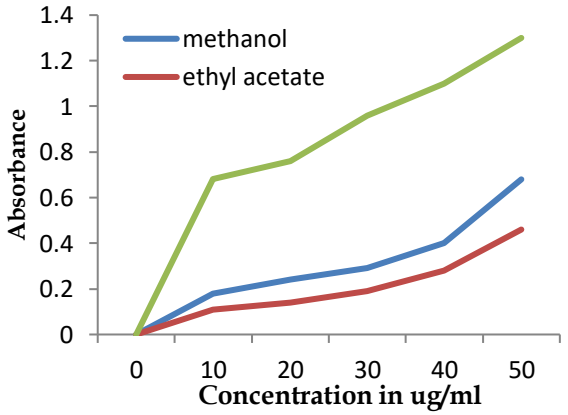

Figure 4: Ferric reducing power activity of stem extract of *Bryophyllum pinnatum*

Methanol Y = 0.117x – 0.112, R^2 = 0.919
Ethyl acetate Y = 0.081X – 0.089; R^2 = 0.929

Discussion

The preliminary phytochemical screening of the different extracts of *B. pinnatum* obtained from leaves and stem was done to assess the presence of bioactive compounds and the results were presented in **Table 1**. The results showed that the leaf and stem extracts contained moderate to high concentrations of most of the phytochemical compounds screened for. However, anthocyanins and anthraquinones were not detected in the leaf extracts while coumarins were not found in the stem extracts. The various phytochemical compounds detected in the different plant extracts (**Table 1**) are known to have health benefits, physiological activities and medicinal importance [36].

Quantitative analysis was done to check the concentration of major bioactive compounds such as polyphenols, flavonoids, alkaloids, saponins, and tannins in the different extracts of *B. pinnatum* and the results is presented in **Table 2**. From the results obtained, the leaf extracts contained the highest concentration of phytochemicals quantified. However, the stem extract contained the highest concentration of flavonoids. This may be attributed to the fact that the stem may be a reservoir of flavonoids. Both extracts followed a similar trend as follows; polyphenols> tannins> saponins> flavonoids > alkaloids.

The polyphenolic content of the different extracts ranged from 17.9±0.62 to 34.49±0.47 mg GAE/g. Many studies have corroborated the anti-carcinogenic, anti-bacterial, anti-viral or anti-inflammatory activities of phenolic compounds [37, 38, 39]. They are also known as a powerful chain-breaking antioxidants. These groups of compounds have received much attention as potential natural antioxidants in terms of their ability to act as efficient radical scavengers and metal chelators. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [40]. The presence of phenolic compounds in *B. pinnatum* indicates that these plants may be used as an antioxidant and anti-microbial agent.

The result of the quantification of tannins indicated that the different extracts of *B. pinnatum* contained varying concentrations of tannin, with methanol leaf extract having the highest concentration (4.98±1.31mg/g). Plant tannins extracted from various sources have been also shown to possess antitumor-promoting effects, analgesic, anti-oxidant, anti-inflammatory and antimicrobial activities [19, 41-44]. Tannin exerts its antioxidant activity by scavenging free radicals, inhibiting lipid peroxidation and chelating of metal [45].

The concentration of flavonoids in this study ranged from 0.53±0.13 mg/g to 0.11±0.14 mg/g. interestingly, the methanol stem extract had the highest concentration of flavonoids while the least was recorded for ethyl acetate leaf extract. Studies have shown that the intake of flavonoid-rich diets lowers heart diseases [46]. This is because Flavonoids lower high blood pressures, as well as cholesterol in animal studies and, have strong anti-inflammatory properties [47]. They also inhibit low density lipoprotein (LDL) oxidation by free radicals Flavonoids exert their antioxidant activity by scavenging free radicals, chelating metals and inhibiting lipid peroxidation. The –OH at C3 of the flavonoid structure plays a role in chelating and scavenging activity [48].

Their antimicrobial properties of flavonoids are probably because they form complexes with both extracellular and soluble proteins, as well as the bacterial cell wall. They could also disrupt cell membranes if lipophilic enough [49]. The presence of flavonoids in extracts of *B. pinnatum* studied enhances its antimicrobial and antioxidant capacity.

Saponins are widely distributed in plants and research has shown that they have many pharmacological actions and biological activities like anti-inflammatory, molluscicidal, antimicrobial, antispasmodic, anti-diabetic and anticancer, hypocholesterolemic, antioxidant, anticonvulsant and analgesic, anthelmintic and cytotoxic activities [21, 50, 51]. The result obtained in this study showed that *B. pinnatum* contained moderate levels of saponins.

The result obtained for the quantification of alkaloids indicated that the extracts contained moderate levels of alkaloids. Ethyl acetate leaf extract contained the highest concentration of alkaloids (0.90±0.13 mg/g) while the lowest concentration was recorded for methanol stem extract (0.03±0.02 mg/g). This may be as a result of the nature of the extractable alkaloids present in the

extract. Naturally occurring alkaloids and their synthetic derivatives have analgesic, antispasmodic and bactericidal activities [44]. The phytochemical compounds present in the different extracts of *B. pinnatum* may be responsible for its antimicrobial and antioxidant properties.

Evaluation of antimicrobial activity of the different extracts of the studied plants parts was determined against different microorganisms. Extent of sensitivity of the test organisms to the plant extracts was assessed by measuring the zone of inhibition after 24 hrs incubation. **Table 3** and 4 showed the antimicrobial activity of *B. pinnatum* leaf and stem extracts respectively using different extracting solvents. All the tested extracts revealed antimicrobial activity showing different selectivity. The result revealed varying degree of inhibition on the different test isolates, with more significant inhibition seen with a higher extract concentration. The results revealed that the methanol leaf extract of *B. pinnatum* was most effective against the test organisms. *P. aeruginosa* showed the highest susceptibility (30±0.9 mm at 80 mg/ml) to *B. pinnatum m*ethanol leaf extract, while *S. aureus* showed the least susceptibility (20±0.5 mm). However, for ethyl acetate leaf extract, the most susceptible bacteria was *E. coli* (25±0.61mm at 80 mg/ml) while the least was *Salmonella* spp. (14 ±0.12 mm). The results also revealed that all the test fungi were susceptible to the leaf extracts. Methanol and ethyl acetate leaf extracts showed maximum inhibition at 34±1.41 mm and 30±0.12 mm against *C. albican* respectively. The result of the antimicrobial activity of the different stem extracts revealed that *E. coli* showed the highest sensitivity to methanol and ethyl acetate extracts at 19±1.2 mm and 28± 0.43 mm, respectively. The least susceptibility was recorded for *Salmonella* spp. and *S. aureus* at 13±0.11 mm and 12± 0.12 mm for methanol and ethyl acetate stem extracts respectively. The results also revealed that all the test fungi were resistant to the different stem extracts except *C. albicans*. It should be noted that the effects of the stem and leaves extract against the bacteria also differed; the variation observed in the phytochemical compounds detected in the extracts may possibly account for their varied bioactivity [52, 53]. The antimicrobial activity could be attributed to some of the detected compounds in these plant extracts such as tannins, saponins, alkaloids, flavonoids and terpenoids [54, 49, 55].

The antioxidant capacity of the different extracts of *B. pinnatum* was evaluated by two different assays: free radical scavenging action on DPPH radicals and Ferric reducing power. This is because no single assay can represent the total antioxidant capacity, and for this reason different and complementary assays were used to evaluate the extract antioxidant activities:

The scavenging ability of *B. pinnatum* extracts (leaves and stem) on DPPH free radical is shown in **Figures 1** and **Figure 2**. The result indicated that all of the assessed extracts of *B. pinnatum* were able to reduce the stable, purple-coloured radical DPPH to the yellow coloured DPPH-H form. The results showed a dose-dependent scavenging power, where activity increased as the concentration increased for both extracts. The result also showed that at 100 µg/ml dose, the leaf extracts (methanol and ethyl acetate) inhibited DPPH radical by 72.5% and 60.2% respectively while at the same concentration, methanol and ethyl acetate stem extracts inhibited DPPH radical at 63.8% and 57.6% respectively compared to the standard drug, vitamin C (85.4%). The IC_{50} for DPPH and FRAP Assays were calculated from a linear regression analysis of the observed inhibition percentage against concentration. The results showed that the IC_{50} value for DPPH assay ranged from 3.142 – 4.441 µg/ml the DPPH radical scavenging ability of the extracts showed the following trend vitamin C<Methanol leaf extract<ethyl acetate leaf extract<methanol stem extract<ethyl acetate stem extract (**Table 5**). It is interesting to note that the lower the IC_{50} value, the higher the scavenging activity of the plant extract. The leaf extracts recorded the lowest IC_{50} values hence its higher antioxidant properties. It was evident that the extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants [56].

Ferric Reducing Antioxidant Power (FRAP) assay is used to evaluate the capacity of natural antioxidant to donate an electron or hydrogen [57]. In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron [58]. The ferric reducing activity of the leaf and stem extracts of *B.*

pinnatum are presented in **Figures 3** and **Figure 4**. All extracts exhibited a similar concentration-dependent activity pattern as given in the DPPH analysis.

The reducing power of the extracts followed the order of methanol leaf extract>methanol stem extract>ethyl acetate leaf extract>ethyl acetate stem extract. The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain and donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation [59-61]. From the results obtained, it can be inferred that the extracts studied possess reducing power and therefore, could serve as electron donors, terminating the radical chain reactions. The IC_{50} value was used as a significant indicator of antioxidant ability. The IC_{50} (**Table 5**) for ferric reducing activity ranged from 331.9 to 618.38 µg/ml. The antioxidant activity of these plants could be due to the ability to scavenge specific free radicals and/or due to the interaction with redox chemistry of iron ions.

Conclusion

Methanol and ethyl acetate extracts of leaf and stem of *B. pinnatum were* obtained in this study. Phytochemical screening revealed the presence of important bioactive compounds such as polyphenols, tannins, saponins, flavonoids and alkaloids. It is worthy of note that all the extracts in this study exhibited broad-spectrum antimicrobial activity on the test microorganisms with highest activity recorded for methanol extracts. The extracts exhibit significant antioxidant activity-DPPH radical scavenging and ferric reducing when compared with standard compounds. Methanol extracts of different plant parts demonstrated the most significant antioxidant activity and also highest polyphenolic content which could be responsible for the activity.

The results obtained in this study showed broad-spectrum antibacterial activity as well as antioxidant potential of *B. pinnatum*; hence further Isolation, purification, identification and structure elucidation of the phenolic phytochemical constituents should be carried out.

Author's contributions

This work was carried out in collaboration with all authors. Author IED designed the study, wrote the procedures and the first draft of the manuscript. Author ECU and ECU managed the literature searches and the experimental process. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Ethical approval and consent

Not applicable.

References

1. World Health Organization (WHO). Resolution-promotion and development of training and research in traditional medicine. WHO document. 1977;30:49-50.
2. Vijayalakshmi R, Ravindhran R. Preliminary comparative phytochemical screening of root extracts of Diospyrus ferrea (Wild.) Bakh and Aerva lanata (L.) Juss. Ex Schultes. Asian J Plant Sci Res. 2012;2(5):581-7. https://doi.org/10.1016/S2221-1691(12)60418-3
3. Iwu MM. Handbook of African Medicinal plants. CRS Press. 1993.
4. Asaolu MF. Chemical compositions and phytochemical screening of the seeds of garcinia kola (bitter kola). Pakistan Journal of Scientific and Industrial Research (Pakistan). 2003.
5. Anon E. Check List of Medicinal Plants of Nigeria and Their Uses. Jamoe Publication, Abakpa, Enugu. 2005.
6. Sofowora EA. Medicinal Plants and Traditional Medicine in Africa. John Willy and Sons Publishers. 1993.
7. Mehrorosh H, Gavanji S, Larki B, Mohammadi MD, Karbasiun A, Bakhtari A. Essential oil composition and antimicrobial screening of some Iranian herbal plants on Pectobacterium carotovorum. Global Nest J. 2014 May 1;16:240-50. https://doi.org/10.30955/gnj.001205
8. Silva BM, Andrade PB, Valentão P, Ferreres F, Seabra RM, Ferreira MA. Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and jam: antioxidant activity. Journal of Agricultural and Food Chemistry. 2004 Jul 28;52(15):4705-12. https://doi.org/10.1021/jf040057v
9. Pulido R, Bravo L. Saura-Calixto (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural Food Chemistry.;48(8):3396-402. https://doi.org/10.1021/jf9913458
10. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of the alkaloidal constituents of the root bark of Eupomatia laurina. Pharmaceutical Biology. 2003 Jan 1;41(4):277-80. https://doi.org/10.1076/phbi.41.4.277.15671

11. Parvez MA, Khan MM, Islam MZ, Hasan SM. Antimicrobial activities of the petroleum ether, methanol and acetone extracts of Kaempferia galanga L. rhizome. J. Life Earth Sci. 2005;1(1):25-9.
12. Sandhya B, Thomas S, Isabel W, Shenbagarathai R. Ethnomedicinal plants used by the Valaiyan community of Piranmalai hills (reserved forest), Tamilnadu, India.-a pilot study. African Journal of Traditional, Complementary and Alternative Medicines. 2006 Jan 12;3(1):101-14. https://doi.org/10.4314/ajtcam.v3i1.31145
13. Hao YY, Brackett RE, Doyle MP. Inhibition of Listeria monocytogenes and Aeromonas hydrophila by plant extracts in refrigerated cooked beef. Journal of Food Protection. 1998 Mar;61(3):307-12. https://doi.org/10.4315/0362-028X-61.3.307
14. Britton RS, Leicester KL, Bacon BR. Iron toxicity and chelation therapy. International journal of hematology. 2002 Oct 1;76(3):219-28. https://doi.org/10.1007/BF02982791
15. Hussain J, Ullah R, ur Rehman N, Khan AL, Muhammad Z, Hussain FU, Anwar S. Endogenous transitional metal and proximate analysis of selected medicinal plants from Pakistan. Journal of Medicinal Plants Research. 2010 Feb 4;4(3):267-70.
16. Njoroge GN, Bussmann RW. Ethnotherapeautic management of skin diseases among the Kikuyus of Central Kenya. Journal of ethnopharmacology. 2007 May 4;111(2):303-7. https://doi.org/10.1016/j.jep.2006.11.025
17. Akinjogunla OJ, Yah CS, Eghafona NO, Ogbemudia FO. Antibacterial activity of leave extracts of Nymphaea lotus (Nymphaeaceae) on methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant Staphylococcus aureus (VRSA) isolated from clinical samples. Annals of biological research. 2010;1(2):174-84.
18. Afzal M, Kazmi I, Anwar F. Antineoplastic potential of Bryophyllum pinnatum lam. on chemically induced hepatocarcinogenesis in rats. Pharmacognosy research. 2013 Oct;5(4):247. https://doi.org/10.4103/0974-8490.118811
19. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology. 2006;5(4):357-61.
20. Okwu DE. Nigerian medicinal plants II. Medicinal and Aromatic Plant Science and Biotechnology. 2007;1(1):97-102.
21. Ali N, Shah SW, Shah I, Ahmed G, Ghias M, Khan I. Cytotoxic and anthelmintic potential of crude saponins isolated from Achillea Wilhelmsii C. Koch and Teucrium Stocksianum boiss. BMC complementary and alternative medicine. 2011 Dec 1;11(1):106. https://doi.org/10.1186/1472-6882-11-106
22. Egereonu UU, Mokwe NR. Characterization of the extract from Bryophyllum pinnatum leaves. J. Chem. Soc. Nig. 2005;30(2):192-6.
23. Mudi SY, Ibrahim H. Brine Shrimp Lethality and antimicrobial susceptibility tests from the leaf extracts of Bryophyllum pinnatum and Cassia occidentalis on respiratory tract infections causing bacteeria. Proc. Chem. Soc. Nig. 2008:592-8.
24. Nassis CZ, Haebisch EM, Giesbrecht AM. Antihistamine activity of Bryophyllum calycinum. Brazilian journal of medical and biological research= Revista brasileira de pesquisas medicas e biologicas. 1992;25(9):929.
25. Okokon JE, Udoh AE, Frank SG, Amazu LU. Anti–inflammatory and analgesic activities of Melanthera scandens. Asian Pacific Journal of Tropical Biomedicine. 2012 Feb 1;2(2):144-8. https://doi.org/10.1016/S2221-1691(11)60209-8
26. Evans WC. Trease and Evans Pharmacognosy. 15th edtion. Edinburgh, Saunders. 2002:249.
27. Harbone JB. Method of plant analysis. Phytochemical methods. 1973. https://doi.org/10.1007/978-94-009-5921-7_1
28. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965 Jan 1;16(3):144-58.
29. Ayoola GA, Folawewo AD, Adesegun SA, Abioro OO, Adepoju-Bello AA, Coker HA. Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. African Journal of Plant Science. 2008 Oct 31;2(10):124-8.
30. Ordonez AA, Gomez JD, Vattuone MA. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food chemistry. 2006 Aug 1;97(3):452-8. https://doi.org/10.1016/j.foodchem.2005.05.024
31. Van Buren JP, Robinson WB. Formation of complexes between protein and tannic acid. Journal of Agricultural and Food Chemistry. 1969 Jul;17(4):772-7. https://doi.org/10.1021/jf60164a003
32. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of pure and applied sciences. 2002;8(2):203-8. https://doi.org/10.4314/gjpas.v8i2.16033
33. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995 Jan 1;28(1):25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
34. Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro. Food chemistry. 2001 May 1;73(3):285-90. https://doi.org/10.1016/S0308-8146(00)00298-3
35. Rahmoun NM, Ziane H, Boucherit-Otmani Z. Antibacterial and antifungal screening of four medicinal plants. Journal of coastal life medicine. 2014;2(12):975-9.
36. Jaberian H, Piri K, Nazari J. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. Food chemistry. 2013 Jan 1;136(1):237-44. https://doi.org/10.1016/j.foodchem.2012.07.084
37. Cassidy A, Hanley B, Lamuela-Raventos RM. Isoflavones, lignans and stilbenes–origins, metabolism and potential importance to human health. Journal of the Science of Food and Agriculture. 2000 May 15;80(7):1044-62. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1044::AID-JSFA586>3.0.CO;2-N
38. Gao D, Kakuma M, Oka S, Sugino K, Sakurai H. Reaction of β-alkannin (shikonin) with reactive oxygen species: detection of β-alkannin free radicals. Bioorganic & medicinal chemistry. 2000 Nov 1;8(11):2561-9. https://doi.org/10.1016/S0968-0896(00)00191-7
39. Tapiero H, Tew KD, Ba GN, Mathe G. Polyphenols: do they play a role in the prevention of human pathologies?. Biomedicine & pharmacotherapy. 2002 Jun 1;56(4):200-7. https://doi.org/10.1016/S0753-3322(02)00178-6
40. Rice-evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free radical research. 1995 Jan 1;22(4):375-83. https://doi.org/10.3109/10715769509145649
41. Mazid M, Khan TA, Mohammad F. Role of secondary metabolites in defense mechanisms of plants. Biology and medicine. 2011;3(2):232-49.
42. Gali-Muhtasib HU, Younes IH, Karchesy JJ, El-Sabban ME. Plant tannins inhibit the induction of aberrant crypt foci and colonic tumors by 1, 2-dimethylhydrazine in mice. Nutrition and cancer. 2001 Jan 1;39(1):108-16. https://doi.org/10.1207/S15327914nc391_15
43. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Critical reviews in food science and nutrition. 1998 Aug 1;38(6):421-64. https://doi.org/10.1080/10408699891274273
44. Okwu DE, Okwu ME. Chemical composition of Spondias mombin Linn plant parts. J Sustain Agric Environ. 2004;6(2):140-7.
45. Perron NR, Brumaghim JL. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell biochemistry and biophysics. 2009 Mar 1;53(2):75-100. https://doi.org/10.1007/s12013-009-9043-x

46. Le Marchand L. Cancer preventive effects of flavonoids—a review. Biomedicine & pharmacotherapy. 2002 Aug 1;56(6):296-301. https://doi.org/10.1016/S0753-3322(02)00186-5
47. Nnam NM, Onyechi JC, Madukwe EA. Nutrient and phytochemical composition of some leafy vegetables with medicinal significance. Nigerian Journal of Nutritional Sciences. 2012;33(2):15-9.
48. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon?. Free Radical Biology and Medicine. 2006 Dec 15;41(12):1727-46. https://doi.org/10.1016/j.freeradbiomed.2006.04.033
49. Cowan MM. Plant products as antimicrobial agents. Clinical microbiology reviews. 1999 Oct 1;12(4):564-82. https://doi.org/10.1128/CMR.12.4.564
50. Huang W, Zou K. Cytotoxicity of a plant steroidal saponin on human lung cancer cells. Asian Pac J Cancer Prev. 2011 Jan 1;12(2):513-7.
51. Alam MS, Quader MA, Rashid MA. HIV-inhibitory diterpenoid from Anisomeles indica. Fitoterapia. 2000 Sep 1;71(5):574-6. https://doi.org/10.1016/S0367-326X(00)00197-0
52. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food chemistry. 2005 Aug 1;91(4):621-32. https://doi.org/10.1016/j.foodchem.2004.06.031
53. Garedew A, Schmolz E, Lamprecht I. Microbiological and calorimetric investigations on the antimicrobial actions of different propolis extracts: an in vitro approach. Thermochimica Acta. 2004 Nov 15;422(1-2):115-24. https://doi.org/10.1016/j.tca.2004.05.037
54. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale pharmaceutica sciencia. 2011 Mar;1(1):98-106.
55. Giteru SG, Coorey R, Bertolatti D, Watkin E, Johnson S, Fang Z. Physicochemical and antimicrobial properties of citral and quercetin incorporated kafirin-based bioactive films. Food chemistry. 2015 Feb 1;168:341-7. https://doi.org/10.1016/j.foodchem.2014.07.077
56. Chung YC, Chien CT, Teng KY, Chou ST. Antioxidative and mutagenic properties of Zanthoxylum ailanthoides Sieb & zucc. Food Chemistry. 2006 Aug 1;97(3):418-25. https://doi.org/10.1016/j.foodchem.2005.05.019
57. Boulekbache-Makhlouf L, Madani K. Antioxidant capacity of crude extracts and their solvent fractions of selected Algerian Lamiaceae. Industrial Crops and Products. 2014 Jan 1;52:177-82. https://doi.org/10.1016/j.indcrop.2013.10.004
58. Antia BS, Essien EE, Udoh BI. Antioxidant capacity of phenolic from seed extracts of Lagenaria siceraria (short-hybrid bottle gourd). European Journal of Medicinal Plants. 2015 May 25:1-9. https://doi.org/10.9734/EJMP/2015/18242
59. Li XM, Li XL, Zhou AG. Evaluation of antioxidant activity of the polysaccharides extracted from Lycium barbarum fruits in vitro. European Polymer Journal. 2007 Feb 1;43(2):488-97. https://doi.org/10.1016/j.eurpolymj.2006.10.025
60. Duh PD. Antioxidant activity of burdock (Arctium lappa Linne): its scavenging effect on free-radical and active oxygen. Journal of the American Oil Chemists' Society. 1998 Apr;75(4):455-61. https://doi.org/10.1007/s11746-998-0248-8
61. Gordon MH. The mechanism of antioxidant action in vitro. InFood antioxidants 1990 (pp. 1-18). Springer, Dordrecht. https://doi.org/10.1007/978-94-009-0753-9_1