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Antimicrobial and Antioxidant Studies of the Leaf Extract and Fractions of *Sabicea brevipes* Wernham (Rubiaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors COU and JNI designed the study and wrote the protocol. Authors COU, MOA, NRE and FIN performed the laboratory and statistical analysis. Author COU wrote the first draft of the manuscript. Authors FIN and EIO managed the analyses of the study. Author COU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Phytochemical analysis, antimicrobial and antioxidant properties of the leaf extract and fractions of *Sabicea brevipes* were studied. The leaves were defatted and the marc extracted with methanol. The extract was further purified by solvent-solvent partitioning using *n*-hexane, ethyl acetate and *n*-butanol to obtain the three solvent fractions. They were screened for phenolics, flavonoids, tannins, saponins, terpenoids, glycosides, and steroids. Total phenolics, flavonoids and tannins were determined quantitatively. The antimicrobial test was screened *in vitro* by agar diffusion method. Analysis of variance (ANOVA) was used to test for significant difference at $p \leq 0.05$ in all study groups. The methanol extract exhibited the most significant amount of phenolics (110.78 ± 1.06 mg

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GAE/g) while ethyl acetate fraction had the least total phenolics content (50.55 ± 2.91 mg GAE/g). The same trend was observed for the total flavonoids content whereas the methanol extract measured (418.40 ± 14.03 mg QE/g) while ethyl acetate fraction had 192.40 ± 3.06 mg QE/g. Total tannins contents were: methanol extract (102.22 ± 7.58 mg GAE/g) and ethyl acetate (27.33 ± 0.77 mg GAE/g). The antioxidant results showed that the methanol extract had the highest DPPH free radical scavenging ability (93.69%) with half maximal inhibitory concentration (IC_{50}) of 0.601± 0.02 and also highest ferric ion reducing power (50.381 ± 1.56 µmol Fe^{2+}/g). Also, the methanol extract showed high total antioxidant capacity (96.79 ± 0.31 mg AAE) and IC_{50} of 0.798± 0.01. The antimicrobial results revealed that the methanol extract showed better activity against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Candida albicans* than the solvent fractions at concentrations of 200, 100, 50, 25, 12.5, mg/mL having various inhibition zone diameters (IZDs). The methanol extract and fractions of *S. brevipes* compared favourably in terms of zone of inhibition and minimum inhibition concentration (MIC) with the standard drug disc (Gentamycin and Ketoconazole) against the tested microorganisms. The MIC of the extract and solvent fractions ranged from 6.31 mg/mL to 50.12 mg/mL. The continual use of the extract of *Sabicea brevipes* in preventing oxidative stress and in the treatment of common infection is justified by these results.

Keywords: Sabicea brevipes; phenolic content; flavonoid content; tannin content; antimicrobial and antioxidant activity.

1. INTRODUCTION

Medicinal plants have been used from ancient time for their medicinal values as well as to impart flavor to food. Nowadays, crude extracts and dry powder samples from medicinal and aromatic plants and their species have shown interest for the development and preparation of alternative traditional medicine and food additives [1].Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, some people still prefer using traditional folk medicines because of their less harmful effects [2]. Plants and plant parts are known source of herbal medicine and natural health- enhancing products for many centuries**.** Various plant parts such as leaves, fruits, seeds, bark, flowers, rhizomes and roots have at one time or the other been utilized for medicinal purposes. It is estimated that about 75% of useful bioactive plant-derived pharmaceuticals used globally are discovered by systematic investigation of *leads* from traditional medicines [3]. The search for antimicrobial agents have over the years led researchers to in-depth study and analysis of various plants and their parts [2]. Over the years, infections caused by strains of bacteria that are resistant to orthodox drugs, also called multi-drug resistant (MDR) bacteria, have either found cure or control by the use of bioactive compounds isolated from plants [4]. These phytochemicals can either be used alone as antimicrobial agents or in combination with commercially available antibiotics as studies have shown that a higher activity against microorganisms can be achieved by combining certain phytochemicals with commercially available antibiotics [4]. For example, *Pseudomonas aeruginosa*, a microorganism which has exhibited resistance to 19 different antibiotics was observed for synergistic effects when phytochemical extracts from clove, jambolan, pomegranate and thyme were used together with known antibiotics [5]. Oxidative damage is caused by free radicals and reactive oxygen species, mostly generated endogenously**.** Free radicals are atoms or group of atoms that have at least one unpaired electron, which make them highly unstable and reactive. Living organisms accumulate free radicals through both normal metabolic processes and exogenous sources. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating [6]. *Sabicea brevipes* (Wernham) commonly known as "Susu" plant belongs to the *Rubiaceae* family that has more than 6,500 species out of which 152 are members of the Sabicea genus [7]. "The root has stimulating and tonifying effects on the muscles when consumed and this probably accounts for their use in enhancing male potency and the metabolites found in the root extracts are known to have curative activity against several pathogens and for the treatment of several diseases" [8]. Information from the local people of Nsukka, Enugu State, Nigeria has it that the leaves are used in ethnomedicine to heal

wounds and to treat bacterial and fungal infections (Oral Communication). However, no scientific work has been published to validate these claims. In this study, we report the first biological activities from the leaves of *S. brevipes.*

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The plant *S. brevipes* was collected in June, 2016 from Lejja in Nsukka Local Government Area, Enugu State and was identified and authenticated by Mr. Felix Nwafor a taxonomist in the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka, Enugu State.

2.2 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) bought from Sigma-Aldrich (Germany). Folin–Ciocalteu reagent, Na_2CO_3, gallic acid and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA), Potassium ferric cyanide ($K_3F(CN)_6$), phosphate buffer, ferric chloride ($FeCl_3.6H_2O$), Trichloroacetic acid, weighing balance (Scout pro u401 made in China), Incubators, Dimethylsulfoxide (DMSO) , aluminium chloride ($AlCl_3.6H_2O$), Sodium phosphate (NaH_2PO_4) and ammonium molybdate were bought from JHD (China). All reagents were of analytical grade.

2.3 Extraction of the Phytochemicals

2.3.1 Extraction procedure

One kilogram (1000 g) of the powdered material was defatted with petroleum ether (40 – 60°C, 5.0 L), the marc air dried and extracted with methanol (5.0 L) by cold maceration at room temperature for 48 hours with continuous agitation. The mixture was filtered and the filtrate concentrated *in vacuo* at room temperature to obtain the dry extract. The weight of the dried extract was expressed as percentage yield.

2.3.2 Solvent-solvent partitioning procedure

The purification of the extract was done using solvent-solvent partitioning method as previously reported by Agbo et al. [9]. Briefly, the crude extract (10 g) was dissolved in 200 mL of 10% methanol and the aqueous portion successively partitioned against *n*-hexane (250 mL *x* 10), ethyl acetate (250 mL *x* 10), *n*-butanol (250 mL *x* 4) to obtain the solvent fractions *viz: n*-hexane (SB-HF), ethyl acetate (SB-EF) and *n*-butanol (SB-BF) respectively.

2.4 Acute Toxicity Studies of the Extract

Determination of the acute toxicity and lethality (LD_{50}) of the methanol extract (ME) was performed in mice using the method described by Lorke [10]. Adult mice (18-30g) of either sex were used for the test. The test was divided into two stages.

2.5 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the extract and fractions were done using standard methods [11].

2.6 Quantitative Analysis of Phytochemicals

The quantitative phytochemical analysis of the extract and fractions were performed to determine the quantity of total phenolics content (TPC), total flavonoids content (TFC) and total tannins content (TTC) of the extract and fractions.

2.6.1 Determination of total phenolic contents

Folin-Ciocalteau method was used for the determination of the total phenolics content of the extract using gallic acid as an internal standard with slight modification [12]. Briefly, the extract (1 mg/mL) was mixed with distilled water (9 mL) in a 20 mL volumetric flask. Two and half milliter (2.5 mL) of a 10 fold dilute Folin-Ciocalteau phenol reagent (FCPR, 1:10) was added. After 5 minutes 10 mL of 7.5% of sodium trioxocarbonate (IV) (Na_2CO_3) solution was added to the mixture and made up to the mark with distilled water. The mixture was incubated in the dark for 90 mins at room temperature. A set of standard solutions of gallic acid (20, 40, 60, 80, 100 μg/mL) were prepared in the same manner as described for the extract. The absorbance of the extract and standard solutions were read against the reagent blank at 760 nm with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The determination of the total phenolics in the extract was carried out in triplicate. The Total Phenolics content was determined from the calibration curve and expressed as milligrams of gallic acid equivalent (GAE) per gram of the extract [12].

2.6.2 Determination of total flavonoid contents

Aluminium-Chloride colourimetric assay was used to determine the total flavonoids content in the extract [13]. Briefly, 1 mL of the extract (1 mg/mL) was mixed with 4 mL of distilled water in 20 mL volumetric flask. 0.30 mL of 5% sodium nitrite was added to the flask. After 5 mins, 0.30 mL of 10% $AlCl_3.6H_2O$ solution was added to the mixture, followed by addition of 2 mL of 1.0 M sodium hydroxide (NaOH) after another 5 minutes and diluted to the mark with distilled water. A set of standard solutions of quercetin (20, 40, 60, 80, 100 *µ*g/mL) were prepared in the same manner as described for the extract. The absorbance of the extract and standard solutions were read against the reagent blank at 510 nm with a UV/Visible spectrophotometer. The determination of total flavonoids in the extract and standards were carried out in triplicates. The total flavonoids content was expressed as milligram of quercetin equivalent (QE) per gram of extract.

2.6.3 Determination of total tannin contents

Folin-Ciocalteau method was used in the determination of the total tannins content of the extract using gallic acid as an internal standard [14]. Briefly, a 0.1 mL of the extract (1mg/mL) was mixed with 7.5 mL of distilled water in a 10 mL volumetric flask. 0.5 mL of a 10 fold dilute Folin-Ciocalteau phenol reagent (FCPR, 1:10) was added. 1.0 mL of 35% Na_2CO_3 solution was added to the mixture and made up to the mark with distilled water. The mixture was incubated in the dark room for 30 minutes at room temperature. A set of standard solutions of gallic acid (100, 80, 60, 40, and 20 *µ*g/mL) were prepared in the same manner as described for the extract. The absorbance of the extract and standard solutions were read against the reagent blank at 725 nm with a UV/Visible spectrophotometer. The total tannins content was determined from the calibration curve and milligram of gallic acid equivalent (GAE) per gram of the extract [14]. The determination of the total tannins content in the extract was carried out in triplicate.

2.7 Antimicrobial Assay of the Extract and Fractions

2.7.1 Microorganisms

24-hour Cultures of six human pathogenic bacteria made up of both gram positive (*Staphylococcus aureus,* and *Bacillus subtilis*), gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria and fungi (*Candida albicans* and *Aspergilus niger*) were used for the *in-vitro* antimicrobial assay. All microorganisms were obtained from the clinical stock of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka. Agar well diffusion method was used for the antimicrobial assay.

2.7.2 Positive and negative control

Gentamicin (50 *µ*g/mL) and Ketoconazole (50 *µ*g/mL) were used as positive control for the tested bacteria and fungi strains. Sterilized distilled water and dimethyl sulfoxide were used as negative control.

2.7.3 Evaluation of the minimum inhibitory concentration

The MIC was determined for each organism and extract/ fractions by plotting the graph of Inhibition Zone Diameter (IZD^2) against Log of concentration and the antilog of the point of intersection on the x- axis was read and taken as the MIC [15].

2.8 Antioxidant Activity

The *in- vitro* antioxidant assays of the extract were carried out by dissolving 0.2 g of the extract in 10 mL of distilled water and then 1 in 20 dilutions to form stock solutions of 1 mg/mL (1000 *µ*g/mL). Serial dilutions (15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/mL) of each extract were made from the stock solution. Ascorbic acid and Gallic acid were used as standard for the antioxidant assays.

2.8.1 DPPH free radical scavenging assay

The radical scavenging activity of the extract and fractions was determined using DPPH assay with slight modifications [9]. A fresh stock solution of DPPH was first prepared by dissolving 4.5 mg of DPPH in 100 mL of methanol. A volume, 1 mL of sample solution and 3 mL of DPPH stock solution were mixed, and was incubated at room temperature for 30 minutes in the dark and the absorbance was read at 517 nm. The DPPH radical scavenging activity of ascorbic acid was also determined for comparison and all tests were performed in triplicate. The inhibition percentage (%) of radical scavenging activity was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times \frac{100}{1}$$

2.8.2 Ferric Reducing Antioxidant Power (FRAP) assay

The reducing power of the crude extract and its fractions were determined [16]. A 2.0 mL of sample was mixed with 2.0 mL of 0.2 M of phosphate buffer of 6.6 and 2.0 mL of 10 mg/L potassium ferricyanide in a water bath at 50°C for 20 mins. Following this, 2.0 mL of 100 mg/L trichloroacetic acid solution (10% (w/v)) was added. An aliquot of 2.0 mL of the mixture was combined with 2.0 mL of distilled water and 0.4 mL of 0.1%(w/v) ferric chloride ($FeCl_3.6H_2O$) solution. The absorbance of the reaction mixture was measured at 700nm after 10 minntes of the reaction. The ferric reducing antioxidant power of extract and fraction was expressed as micromole of Fe^{2+} per gram of the extract (μmol/g).

2.8.3 Total Antioxidant capacity (TAC) assay using phosphomolybdate method

The total antioxidant capacity assay of the extract was carried out by the Phosphomolybdate method [17,18]. Briefly, 0.1 mL aliquot of different concentrations (15.63, 31.25, 62.5, 125, 250, 500, 1000 μg/mL) of the extract, fractions and ascorbic acid was mixed with 1 mL of reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate, 1:1:1). The test tubes were covered with aluminium foil and incubated in a water bath at 95°C for 90 minutes. After the extract was cooled to room temperature, the absorbance of the mixture was determined at 765 nm against a blank containing 1 mL of the reagent solution. Ascorbic acid was used as the positive standard. The assay was carried out in triplicate. The Total antioxidant capacity (TAC) was expressed as milligram Ascorbic acid equivalent per gram of the extract (mgAAE/g). The antioxidant capacity was estimated using the following formula:

$$\text{Total antioxidant capacity (\%)} = \frac{\text{Absorbance of control - Absorbance of extract}}{\text{Absorbance of control}} \times \frac{100}{1}$$

2.9 Statistical Analysis

Experimental results were expressed as Mean ± SEM. All measurements were replicated three times. The IC_{50} values were calculated using linear regression analysis from the graph of scavenging percentage against extract Log concentration. Significant difference were established by one-way analysis of variance (ANOVA) using Duncan and LSD multiple comparison statistics. Values with ($p < 0.05$) were regarded as significant, while values with ($p > 0.05$) were regarded as non-significant.

3. RESULTS

3.1 Percentage Yield

The extractive yield of the extract and various fractions are presented in Table 1.

3.2 Result of the Acute Toxicity Test of the Methanol Extract

The acute toxicity test showed that the methanol extract caused no death in the two stages of the test. The oral LD_{50} of the methanol extract in mice is greater than 5000 mg/kg. No sign of acute intoxication was observed.

3.3 Phytochemical Analysis of the Extract and Fractions of *S. brevipes*

The results of the qualitative phytochemical analysis of *S. brevipes* as seen in Table 2 shows the presence or absence of the secondary metabolites. The quantitative determination of total phenolics, total flavonoids and tannins content are also shown in Tables 3, 4 and 5 respectively. The results show that methanol extract had the most significant amount of phenolics (110.78 ± 1.06 mg GAE/g) while ethyl acetate fraction had the least total phenolics content (50.55 ± 2.91 mg GAE/g). The same trend was observed for the total flavonoids content whereas the methanol extract measured (418.40 ± 14.03 mg QE/g) while ethyl acetate fraction had 192.40 ± 3.06 mg QE/g. Total tannins contents were: methanol extract (102.22 ± 7.58 mg GAE/g) and ethyl acetate (27.33 ± 0.77 mg GAE/g).

3.4 Quantitative Analysis of the Crude Extract and Fractions of Sabicea *brevipes*

The quantitative phytochemical study showed that phenolics and tannins were absent in the *n*-hexane fraction while they were most abundant in the methanolic fractions but, total flavonoids were present in all the fractions. It also showed that the phytochemicals were dose-dependent as higher doses had higher concentrations of total phenolics (Tables 3-5).

Table 1. The percentage yield of the crude methanol extract and fractions of *S. brevipes* are shown below

Extraction/Fractions	Yield (g)	Percentage yield (%w/w)
ME	200.85	20.09
EAF (from 20 g extract)	5.80	29.00
HF (from 20 g extract)	5.60	28.00
BF (from 20 g extract)	6.50	32.50

Key: ME -Methanol Extract, EAF -Ethyl acetate Fraction, HF-Hexane Fraction BF –Butanol Fraction

Table 2. Qualitative phytochemical analysis of the extract and fractions of *Sabicea brevipes*

Extract/ fractions screened	Alkaloids	Phenols	Tannins	Glycosides	Saponins	Terpenoids	Steroids	Flavonoids
Methanol extract	Nd	+++	+++	++	++	++	++	+++
n-Butanol	Nd	+++	++	++	++	++	++	+++
n-Hexane	Nd	Nd	Nd	+	+	+	+	+++
Ethyl acetate	Nd	++	+	+	+	++	++	+++

Key: +++ = High in abundance, ++ = Moderate abundance, + = Low abundance, Nd = Not detected

Table 3. Results of total phenolics content (mg GAE/g) of extract and fractions of *Sabicea brevipes*

Conc (*μ*g/mL)	Methanol	*n*-butanol	Ethylacetate
25	0.78 ± 0.22^{e1}	0.89 ± 0.29^{f1}	1.33 ± 0.51^{e2}
50	5.00 ± 1.35^{e2}	7.00 ± 0.84^{e3}	2.45 ± 0.78^{e1}
100	12.11 ± 2.69^{d1}	28.00 ± 2.91^{d2}	11.56 ± 1.09^{d1}
200	47.22 ± 2.80^{c2}	51.67 ± 2.01^{c2}	25.22 ± 0.59^{c1}
250	76.33 ± 1.45^{b2}	76.56 ± 1.49^{b2}	42.22 ± 0.97^{b1}
300	110.78 ± 1.06^{a3}	91.55 ± 0.78^{a2}	50.55 ± 2.91^{a1}

Results expressed in Mean ± SEM (n = 3). Mean values having different letters as superscripts across the rows are significantly different at (p < 0.05) while mean values having different numbers as superscripts along the column are significantly different at (p < 0.05)

Table 4. Results of total flavonoids content (mg QE/g) of extract and fractions of *Sabicea brevipes*

Conc (*μ*g/mL)	Solvent type			
	Methanol	*n*-butanol	Ethyl acetate	*n*-hexane
25	15.07 ± 2.90^{d1}	12.40 ± 4.16^{e1}	4.40 ± 2.31^{f1}	13.06 ± 4.67^{f1}
50	45.73 ± 16.34^{d1}	45.73 ± 1.33^{d1}	58.40 ± 2.00^{e1}	54.40 ± 3.46^{e1}
100	121.73 ± 18.98^{c2}	73.73 ± 4.06^{c1}	93.73 ± 2.40^{d12}	73.73 ± 2.91^{d1}
200	128.40 ± 16.65^{c2}	93.07 ± 4.67^{c1}	117.73 ± 3.53^{c12}	93.73 ± 2.91^{c1}
250	325.70 ± 2.91^{b3}	133.73 ± 6.36^{b1}	153.73 ± 1.76^{b2}	137.07 ± 5.81^{b1}
300	418.40 ± 14.03^{a2}	255.73 ± 4.67^{a1}	192.40 ± 3.06^{a1}	249.73 ± 14.53^{a1}

Results expressed in Mean ± SEM (n = 3). Mean values having different letters as superscripts across the rows are significantly different at (p < 0.05) while mean values having different numbers as superscripts along the column are significantly different at (p < 0.05)

3.5 Results of Antimicrobial Analysis

The *S. brevipes* extract had activity against *Pseudomonas aeruginosa* with MIC values of 6.76 mg/mL, *Escherichia coli* 6.31 mg/mL, *Staphylococcus aureus* 7.76 mg/mL, *Bacillus subtilis* 7.08 mg/mL and the *Candida albicans* 47.86 mg/mL, the *n*-butanol fraction had activity

against *Pseudomonas aeruginosa* with MIC values of 12.59 mg/mL, *Escherichia coli* 15.85 mg/mL, *Staphylococcus aureus* 17.78 mg/mL, *Bacillus subtilis* 15.85 mg/mL and *Candida albicans* 47.86 mg/mL. The ethyl acetate fraction had activity against *Pseudomonas aeruginosa* with MIC values of 50.12 mg/mL, *Escherichia coli* 28.18 mg/mL, *Staphylococcus aureus* 25.12 mg/mL, *Bacillus subtilis* 26.92 mg/mL and *Candida albicans* 50.12 mg/mL., while for *n*-hexane fraction, the *S. brevipes* had activity against *Pseudomonas aeruginosa* with MIC values of 48.98 mg/mL, *Escherichia coli* 47.86 mg/mL, *Staphylococcus aureus* 50.12 mg/mL, *Bacillus subtilis* 50.12 mg/mL and *Candida albicans* 50.12 mg/mL respectively (Table 6).

3.6 Result of Antioxidant Assays

For DPPH assay, the methanol extract and fraction were significantly higher ($p < 0.05$) than the ascorbic acid standard in an increased dose dependent (concentration) manner whereas the gallic acid standard was significantly higher ($p < 0.05$) than the methanol extract and fraction for Ferric reducing antioxidant power (FRAP). The IC_{50} of methanol extract and butanol fraction of *S. brevipes* and that of the standard (ascorbic acid) for DPPH model were 0.867*μ*g/mL,1.417 *μ*g/mL, and 1.539 *μ*g/mL respectively. The IC_{50} of methanol extract and *n*-butanol fraction of *S. brevipes* and that of the standard (ascorbic acid) for TAC model were 0.798 *μ*g/mL, 1.352 *μ*g/mL and 1.563 *μ*g/mL respectively (Fig. 1).

Table 5. Results of total tannin content (mgGAE/g) of extract and fractions of *Sabicea brevipes*

Conc (μg/mL)	Solvent type		
	Methanol	***n*-butanol**	**Ethylacetate**
25	1.56 ± 0.48^{e2}	1.33 ± 0.58^{f1}	0.89 ± 0.22^{c1}
50	4.56 ± 1.16^{e2}	7.89 ± 0.49^{e3}	2.11 ± 0.29^{c1}
100	23.00 ± 1.68^{d3}	13.89 ± 0.78^{d2}	4.11 ± 0.67^{c1}
200	42.22 ± 0.97^{c2}	37.78 ± 1.35^{c2}	11.22 ± 2.58^{b1}
250	63.45 ± 2.48^{b3}	49.89 ± 2.15^{b2}	13.67 ± 1.15^{b1}
300	102.22 ± 7.58^{a3}	60.67 ± 1.34^{a2}	27.33 ± 0.77^{a1}

Results expressed in Mean ± SEM (n = 3). Mean values having different letters as superscripts across the rows are significantly different at (p < 0.05) while mean values having different numbers as superscripts along the column are significantly different at (p < 0.05)

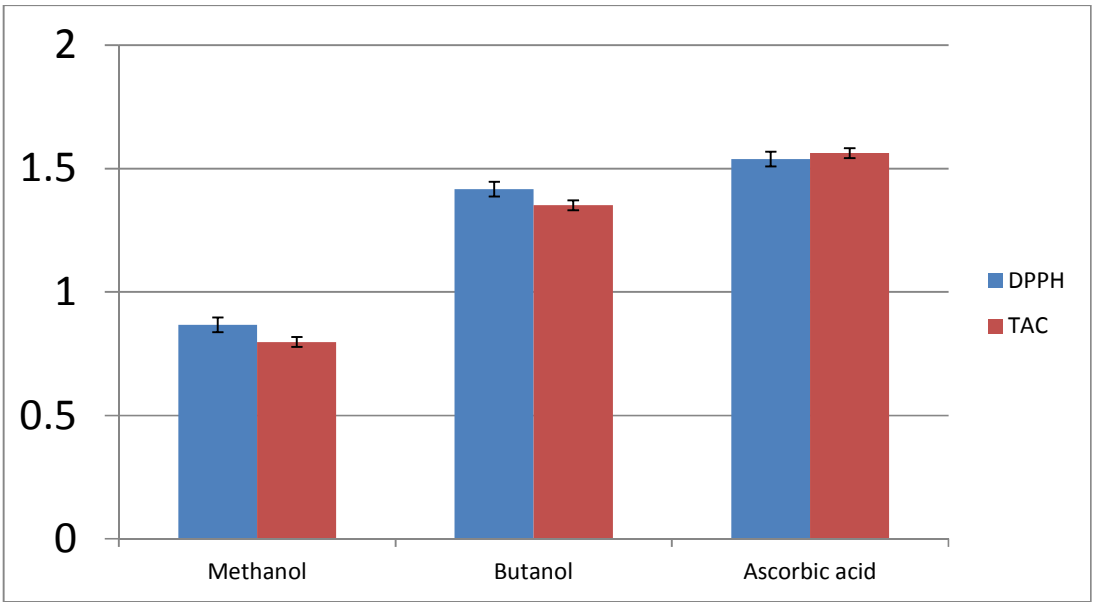

Fig. 1. IC_{50} values for the *in vitro* antioxidant tests of the fractions compared with ascorbic acid

Table 6. Minimum Inhibitory Concentration (MIC) of methanol extract and fractions on test organism

Test organism	ME (mg/mL)	BF (mg/mL)	EAF (mg/mL)	HF (mg/mL)	GENT. (µg/mL)	KETO. (µg/mL)
P. aeruginosa	6.76	12.59	25.12	48.98	1.78	-
E. coli	6.31	15.85	26.92	47.86	1.78	-
S. aureus	7.76	17.78	50.12	50.12	1.25	-
B. subtilis	7.08	15.85	26.92	50.12	1.25	-
C. albicans	47.86	47.86	50.12	50.12	-	1.58

Table 7. Result of the *in vitro* antioxidant tests for the DPPH, FRAP and total antioxidant capacity (TAC)

Model	Extract/Sample	Varying concentrations (µg/mL)						
		15.63	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
DPPH	MeOH	75.76 ± 0.67^{d2}	85.33 ± 0.70^{c2}	91.47 ± 0.27^{b2}	92.21 ± 0.16^{ab2}	92.65 ± 0.68^{ab3}	93.17 ± 0.11^{a3}	93.69 ± 0.34^{a3}
	n-butanol	61.66 ± 1.64^{c1}	62.14 ± 0.33^{c1}	62.27 ± 1.12^{c1}	64.49 ± 2.29^{c1}	72.38 ± 0.85^{b2}	75.72 ± 3.08^{b2}	84.25 ± 1.06^{a2}
	Standard	62.61 ± 1.14^{a1}	62.78 ± 0.56^{a1}	63.02 ± 0.45^{a1}	63.22 ± 0.31^{a1}	64.13 ± 0.41^{a1}	64.19 ± 0.78^{a1}	64.38 ± 0.28^{a1}
FRAP	MeOH	0.219 ± 0.01^{d1}	0.383 ± 0.04^{d1}	1.009 ± 0.05^{d1}	2.431 ± 0.18^{d1}	6.176 ± 0.72^{c1}	16.729 ± 0.68^{b1}	50.381 ± 1.56^{a2}
	n-butanol	0.339 ± 0.02^{f2}	0.650 ± 0.02^{f1}	1.263 ± 0.02^{e1}	2.472 ± 0.11^{d1}	5.398 ± 0.08^{c1}	10.666 ± 0.36^{b1}	20.165 ± 0.17^{a1}
	Standard	0.845 ± 0.04^{d3}	3.015 ± 0.51^{d2}	4.619 ± 0.68^{d2}	10.455 ± 1.29^{d2}	31.318 ± 4.11^{c2}	60.938 ± 4.21^{b1}	187.325 ± 4.90^{a3}
TAC	MeOH	84.18 ± 1.08^{d3}	88.83 ± 0.27^{c3}	89.96 ± 0.27^{c3}	90.42 ± 0.37^{c3}	93.37 ± 0.09^{b2}	95.28 ± 0.67^{a3}	96.79 ± 0.31^{a3}
	n-butanol	58.17 ± 0.31^{f2}	63.49 ± 0.04^{e2}	64.34 ± 0.15^{e2}	68.74 ± 0.31^{d2}	77.69 ± 0.22^{c1}	86.51 ± 0.92^{b2}	90.21 ± 0.15^{a2}
	Standard	48.17 ± 2.19^{e1}	53.63 ± 0.09^{d1}	56.27 ± 0.34^{d1}	64.20 ± 0.21^{c1}	76.04 ± 1.27^{b1}	79.42 ± 0.26^{a1}	80.55 ± 0.22^{a1}

Results expressed in Mean ± SEM (n = 3). Mean values having different letters as superscripts across the rows are significantly different at ($p < 0.05$) while mean values having different numbers as superscripts along the column are significantly different at ($p < 0.05$). FRAP was expressed in micro mole of Fe^{2+} per gram of the extract (µmole Fe^{2+}/g). Total antioxidant capacity was expressed as mg Ascorbic acid equivalent per gram of the extract (mgAAE/g)

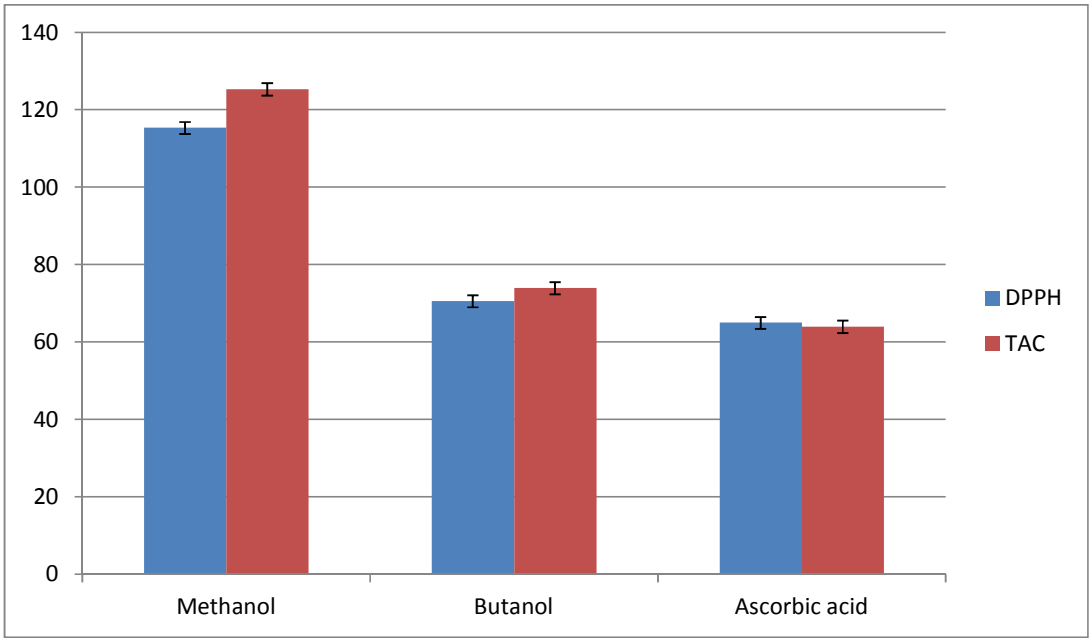

Fig. 2. Anti radical power of the samples compared with ascorbic acid

4. DISCUSSION

The result of the phytochemical analysis carried out on the powdered leaf of *Sabicea brevipes* showed that the plant contained phenolic compounds and this is line with previous report [8]. It was observed that the phytochemical constituents were solvent dependent. Methanol extract contains high amounts of phenols, tannins and flavonoids, the *n*-hexane and ethylacetate fractions had high amount of flavonoids while the *n*-butanol fraction had high amounts of phenols and flavonoids. Quantitative analyses showed the methanol extract had the highest amounts of total phenolics (110.78 ±1.06 mg GAE/g), total flavonoids (418.40 ±14.03 mg QAE/g) and tannins (102.22 ±7.58 mg GAE/g) followed by the *n*-butanol fraction (91.55 ±0.78 mg GAE/g, 255.73 ±4.67 mg QAE/g, 60.67 ±1.34 mg GAE/g respectively).

This result may be ascribed to antioxidant and antimicrobial activities of the plant [1,19,20]. Glycosides, steroids, saponins and terpenoids are also present but in little quantity while alkaloids were not present. A study has implicated saponin component of plants in enhancing aphrodisiac properties due to its androgen increasing property [21]. Saponins present in the methanol extract and fractions of this plant might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the level of Leutinizing Hormones (LH). The LH release normally by the pituitary gland helps to maintain testosterone levels, as LH increases, so does the testesterone [21]. The increase in testosterone seemed to have translated into the male sexual competence.

The antimicrobial activity of the methanol extract and fractions can be related to the presence of tannins, flavonoids and phenols. It is believed that flavonoids are capable of complexing with the bacterial cell wall, causing the death of the microorganism and the tannins are able to inactivate enzymes, transport proteins and microbial adherence [22]. Medicinal plants having tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery which form their therapeutic application [3]. The results obtained showed reasonable inhibitory effect of the extracts on *S. brevipes*. The presence of these phytochemicals may be responsible for the antimicrobial effect. The methanol extract and fractions had varying degree of antimicrobial activity against the test organisms. From the result of the minimum inhibitory concentration (MIC), it shows that *P. aeruginosa*, *S. aureus* and *B. subtilis* had the lowest susceptibility and *Escherichia coli* had the highest susceptibility to the extract. The activities of the extract were compared to those of the standard antimicrobial drugs gentamycin and ketoconazole. The antimicrobial potentiality can

be said to be due to the phytochemicals present such as tannins, flavonoids and phenols. The results of the three antioxidant models were presented and analysed using IC_{50}. It is considered that the lower the IC_{50} value the higher the antioxidant activity and also the more powerful or potent the antioxidant. Thus, the IC_{50} of methanol extract and butanol fraction of *S. brevipes* and that of the standard (ascorbic acid) for DPPH model were 0.867*µ*g/mL,1.417 *µ*g/mL, and 1.539 *µ*g/mL respectively. This shows that the methanol extract and *n*-butanol fraction had better antioxidant property than the standard. Ferric reducing antioxidant power (FRAP) assay depends on the reduction of ferric ion into ferrous ion [23]. Increased absorbance of the reaction indicated a high reducing power. The absorbance increases with increase in concentration. This shows that the methanol extract and *n*-butanol fraction had promising antioxidant property. The IC_{50} of methanol extract and *n*-butanol fraction of *S. brevipes* and that of the standard (ascorbic acid) for TAC model were 0.798 *µ*g/mL,1.352 *µ*g/mL and 1.563 *µ*g/mL respectively. This shows that methanol extract and butanol fraction of *S. brevipes* had better antioxidant property than the standard when analysed using TAC model. The IC_{50} of the methanol extract and *n*-butanol fraction showed a lesser value compared to the standard (ascorbic acid). This indicates that the methanol extract and the *n*-butanol fraction are more potent since IC_{50} and potency are inversely related. The extract worked in synergy that is, it may have ascorbic acid and other natural antioxidant like vitamin E etc. The extract was able to mop up DPPH radical (visible deep purple colour) to the yellow coloured diphenyl picryl hydrazyl. The decrease in absorbance of DPPH caused by antioxidants is due to the reaction between the antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation [24]. Therefore the effect of *Sabicea brevipes* extract on DPPH scavenging was thought to be due to the hydrogen donating ability of the plant extract. Also from the result of the phytochemical analysis, total phenol contents of the extract was high, suggesting that the phenolic compounds present in the extract could be responsible for the observed DPPH radical scavenging activity, since they can readily donate hydrogen atom to the radical. It therefore suggests that the extract could be used as a natural antioxidant source to limit free radical damage occurring in the human body. The methanol extract and fraction were significantly higher ($p < 0.05$) than the ascorbic acid standard in an increased dose dependent (concentration) manner whereas the gallic acid standard was significantly higher ($p < 0.05$) than the methanol extract and fraction for Ferric reducing antioxidant power (FRAP). The antioxidant activity of plant extract of *S. brevipes* is due to the presence of flavonoids, phenolic, and tannin compounds and it shows that the extract from the leaves of *S. brevipes* could be a potential source of natural antioxidant.

5. CONCLUSION

The result of the current study shows that the extract and fractions from the leaves of *S. brevipes* could be a potential source of natural antioxidant and antimicrobial drugs which can be pharmaceutically exploited as an effective antioxidant source, also the plant could be exploited in the development of phytomedicines for drug development. Therefore further studies are required towards purifying and characterizing the biological activities of the compounds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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