

Phytochemical Constituents and Antioxidant Activities of Dichloromethane-Methanol Leaf Extract and Fractions of *Sabicea Brasiliensis* (Rubiaceae)

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Abstract

The objectives of this study were to determine the phytochemical components included in the dichloromethane-methanol (DCM-MeOH) extract of *Sabicea brasiliensis* and to assess the extract's impact on various antioxidant models. Fresh leaves of *S. brasiliensis* were processed to a fine powder using a milling machine. A 1000 g of the powdered leaves was extracted with 5 L of a 1:1 dichloromethane-methanol (DCM-MeOH) mixture for 72 h using cold maceration with intermittent vigorous shaking every 6 h. The extract was further fractionated using solvent-solvent partitioning method into *n*-hexane (SB-HF), ethyl acetate (SB-EF) and *n*-butanol (SB-BF) with SB-BF exhibiting the highest percentage yield of 62%. The leaf extract of *S. brasiliensis* was screened for the presence of phytochemicals and the *in-vitro* antioxidant assays were determined using standard methods. Phenols, tannins, glycosides, saponins, terpenoids, steroids and flavonoids were detected except alkaloids. The DCM-MeOH extract exhibited the most significant amount of phenolics (120.78 ± 1.06 mg), total flavonoids content (318.40 ± 4.03 mg), total tannin content (102.22 ± 7.58 mg) while *n*-hexane fraction had the least total phenolics content (38.73 ± 4.53 mg), total flavonoids content (149.73 ± 4.53 mg), and total tannin content (30.73 ± 1.53 mg). The IC₅₀ of the anti-radical powers of the extract ranged from 0.943 ± 0.01 µg/mL, 1.060 ± 0.01 ; to 1.127 ± 0.01 µg/mL, 0.887 ± 0.01 in the methanol and butanol subfractions respectively compared to the ascorbic acid standard (1.164 ± 0.01 µg/mL), the subfractions were more potent. This study provided a scientific validation for the folkloric claims regarding *S. brasiliensis* with novel anti-oxidative potentials.

Keywords: Phytochemical, Antioxidant models, Dichloromethane-Methanol extract, Butanol subfractions, *Sabicea brasiliensis*

Introduction

Traditional medicine is used by more than 80% of the global population, and current research on antioxidants found in plants is the focus of current investigations [1]. Chronic illnesses like atherosclerosis, cancer, aging, and neurological disease are less common when these antioxidants are present [2]. Medicinal plants are a key natural resource used in medicine formulation and human health care. The ability of plants to produce bioactive secondary metabolites is a gift from nature [3]. Numerous chemicals, particularly secondary metabolites, have been identified and extracted from plants. Research has demonstrated that these compounds, in varying degrees, possess antiviral, analgesic, anticancer, antibacterial, analgesic, and anti-inflammatory properties in addition to many other activities [4, 5]. Flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides, stilbenes, tannins, nitrogen compounds (alkaloids, amines, and betalains), terpenoids, and certain other endogenous metabolites are notable examples of these phytochemical substances [4, 6].

Antioxidants play a crucial role in mitigating oxidative stress, which has the potential to impact and harm biological molecules [7]. The imbalance between oxidants and antioxidants that favors oxidants potentially causing harm is known as oxidative stress. One group of substances produced by oxygen metabolism is known as reactive oxygen species (ROS). These extremely reactive chemicals, which include peroxide (ROO⁻), superoxide radicals (O₂⁻), and hydroxyl radicals (OH⁻), can seriously harm cells and tissues during a variety of disorders that are connected to heart disease, cancer, and numerous other health problems.

Phytochemicals, often known as plant chemicals, are a wide range of naturally occurring molecules that are made by plants during their primary and secondary metabolism. Even though their active ingredients were unknown when they were first employed, many have

been utilized as traditional treatments since ancient times. The woody deciduous liana plant *Sabicea brasiliensis* is a member of the Rubiaceae family [8]. Figure 1 depicts the picture of *Sabicea brasiliensis* leaves used in the study. The woody liana plant *Sabicea brasiliensis*, sometimes referred to as Brazilian Sabicea, is indigenous to the coastal rainforests of southeast Brazil [9]. Within the Mata Atlantica biome, it is mainly found growing in the states of Rio de Janeiro, São Paulo, and Paraná [10]. One of the planet's most biodiverse forests was once found in this biologically rich area, but widespread deforestation has put much of its flora and fauna in jeopardy [11]. This study therefore aimed to identify major phytoconstituents in the plant's extract and then evaluating the antioxidant activity through distinct *in vitro* assays. Additionally, the research provided fresh insights into the characteristics of the phytochemicals found in *Sabicea brasiliensis* leaves, which may be crucial in the development of novel medications that could more effectively combat illness and enhance overall health.



Figure 1: Picture of the *Sabicea brasiliensis* plant

Materials and Methods

Collection and identification of plant material

Sabicea brasiliensis leaves were collected in January 2024 from Lejja in Nsukka LGA, of Enugu State and the plant was identified and authenticated by Mr Felix Nwafor, a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The voucher number is UNN/03261.

Chemicals and reagents

The phytochemicals were tested both qualitatively and quantitatively using the following reagents: Lobal Chemie (India) provided the ferric chloride solution (FeCl_3), Wagner's reagent (iodine with potassium iodide in water), and Folin-Ciocalteu reagent. Sodium hydroxide (NaOH), quercetin, sulphuric acid (H_2SO_4), ascorbic acid, dichloromethane, methanol, *n*-butanol, ethyl acetate (EAA), and *n*-hexane were purchased from Sigma-Aldrich (Germany). The source of gallic acid was Qualikems, India. Purchased from Sigma-Aldrich (Germany) are sodium carbonate (Na_2CO_3) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). Each and every reagent was analytical grade.

Equipment used

These consist of the following: rotary evaporator, soxhlet apparatus, steam bath, 55675 Gallenkamp magnetic stirrer, and UV-Visible spectrophotometer (UV-1800, CAT Japan, Shimadzu Corporation, Japan).

Extraction procedure

Fresh leaves of *Sabicea brasiliensis* were cleaned, cut into smaller pieces and dried under the shade to minimize the loss of volatile compounds and reduced to a fine powder. The powdered plant material (1000 g) was extracted with 5 L of a 1:1 DCM-MeOH mixture for 72 h using cold maceration with intermittent vigorous shaking every 6 h. The mixture was filtered and the filtrate concentrated *in vacuo* at room temperature to obtain the dry extract.

Solvent-solvent partitioning procedure

According to [13], the solvent-solvent partitioning approach was used to purify the extract. To obtain the solvent fractions, *n*-hexane (SB-HF), ethyl acetate (SB-EF), and *n*-butanol (SB-BF), respectively, the crude extract (10 g) was dissolved in 200 mL of 10% methanol. The aqueous portion was then partitioned against *n*-hexane (250 mL x 10), ethyl acetate (250 mL x 10), and *n*-butanol (250 mL x 4).

Qualitative phytochemical analysis of the extract and fractions of *S. brasiliensis*

Qualitative phytochemical analysis of DCM-MeOH extract and the fraction was done to determine the presence of phenols, tannins, terpenoids, alkaloids, steroids, saponins, flavonoids and glycosides using standard methods described by [14] with slight modifications.

Quantitative phytochemical analysis of the extract and fractions

Using established procedures, a quantitative phytochemical analysis of the extract and its constituent fractions was conducted to determine the distribution and amount of total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC).

In-vitro antioxidant assays of the extract and the most active fraction of *S. brasiliensis*

From the stock solution, serial dilutions of each extract (15.63, 31.25, 62.5, 125, 250, and 500 µg/mL) were prepared. Gallic acid and ascorbic acid (AsA) were the standard utilized in the antioxidant experiments.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

The extract and fractions' ability to scavenge radicals was assessed using the DPPH test, albeit with some adjustments [16]. First, 4.5 mg of DPPH were dissolved in 100 mL of methanol to create a new stock solution. After mixing 1 mL of the sample solution and 3 mL of the DPPH stock solution, the mixture was let to sit at room temperature for 30 minutes in the dark. The absorbance at 517 nm was then measured. All assays were run in triplicate, and ascorbic acid's DPPH radical scavenging activity was also measured for comparison. Equation 1 was utilized to determine the inhibition percentage (%) of radical scavenging activity.

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

Total antioxidant capacity (TAC) by the phenomolybdate method

As previously described, the phosphomolybdate technique was used to assess the extract's total antioxidant capability [17, 18]. In summary, 0.1 mL aliquots of the extract, fractions, and ascorbic acid at various concentrations (15.63, 31.25, 62.5, 125, 250, and 500 µg/mL) were combined 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 placed in a water bath set at 95°C for ninety minutes after being covered with aluminum foil. The mixture's absorbance at 765 nm was measured after the extract had cooled to room temperature and compared to a blank that contained 1 mL of the reagent solution. The standard was ascorbic acid. The experiment was run in duplicate. The antioxidant capacity was estimated using the following formula (Equation 2):

$$\text{TAC} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}} \dots \dots (\text{Equation 2})$$

Where TAC is the Total Antioxidant Capacity

Statistical analysis

Data obtained were expressed as Mean ± SEM and were analysed for the significance of disparity using one-way analysis of variance (ANOVA) with repeated measures. Statistical Product and Service Solution (formerly known as Statistical Package for Social Sciences - SPSS) version 23 was employed. Values with $p < 0.05$ were regarded as significant, while values with $p > 0.05$ were regarded as non-significant.

Results

Percentage yield

The percentage yield of the plant samples is percentage in Table 1. It ranges from 25% to 62%.

Table 1: The Percentage yield of the crude methanol extract and fractions of *Sabicea brasiliensis*

Extraction/Fractions	Yield (g)	Percentage yield (%w/w)
Crude extract	250.85	25.09
EAF	5.20	52.00
HF	5.40	54.00
BF	6.20	62.00

Keys EAF – Ethyl acetate Fraction, HF-Hexane Fraction, BF – Butanol Fraction

Phytochemical analysis

Table 2 displays the findings of the qualitative phytochemical study of *S. brasiliensis*. The keys used in the (Table 2) also provided an explanation for the presence or absence of the secondary metabolites and their associated relative abundance in the different solvents used. The findings indicated that while alkaloids were absent from the extract and fractions, phenols, tannins, flavonoids, glycosides, saponins, terpenoids, and steroids were present in the DCM-MeOH extract. The amounts of the phytochemical components in the fractions varied. Alkaloids, phenols, tannins, glycosides, and terpenoids were absent from the *n*-hexane fraction, but flavonoids, sterpenoids, saponins, and steroids were present. Alkaloids were not found in the *n*-butanol fraction, but flavonoids, phenols, tannins, glycosides, terpenoids, saponins, and steroids were. Flavonoids, phenols, tannins, terpenoids, saponins, and steroids for the ethyl acetate fraction.

Table 2: Qualitative phytochemical analysis of the extract and fractions of *S. brasiliensis*

Extract/Fractions Screened	Alkaloids	Phenols	Tannins	Glycosides	Saponins	Terpenoids	Steroids	Flavonoids
DCM-MeOH Extract	Nd	+	+	+	+	+	+	+
<i>n</i> -hexane	Nd	Nd	+	Nd	+	+	+	+
<i>n</i> -butanol	Nd	+	+	+	+	+	+	+
Ethyl acetate	Nd	+	+	Nd	+	+	+	+

Keys: + = Present, Nd = Not detected

Tables 3-5 show the result of the quantitative analysis of the crude extract and fractions of *S. brasiliensis*. The absorbance against the reagent blank was determined at 760 nm with a UV-visible spectrophotometer for phenolic. Total phenolics content was expressed as mg gallic acid equivalents (GAE). The Total Phenolics Content shown in (Table 3) was determined using Folin Ciocalteu reagent which was expressed in Gallic Acid Equivalent per gram with a standard curve ($Y = 0.003x + 0.074$; $R^2 = 0.992$). Gallic acid was used as an internal standard for phenolics and tannins and it was measured at wavelength of 760nm. The results showed that DCM-MeOH extract had the highest phenolics (120.78 ± 1.06 mg GAE/g), the *n*-butanol fraction was (95.35 ± 0.72 mg GAE/g) and lastly, ethyl acetate fraction (58.25 ± 0.91 mg GAE/g).

Table 3: Total phenolics content (mgGAE/g) of *S. brasiliensis* extract and fractions

Conc (µg/ml)	Solvent			
	DCM-MeOH	<i>n</i> -butanol	Ethyl acetate	<i>n</i> -hexane
25	0.88 ± 0.22^{e1}	0.99 ± 0.29^{f1}	1.53 ± 0.51^{e2}	0.56 ± 4.67^{f1}
50	7.00 ± 1.35^{e2}	9.00 ± 0.84^{e3}	2.65 ± 0.78^{e1}	1.40 ± 3.46^{e1}
100	15.11 ± 2.69^{d1}	29.00 ± 2.91^{d2}	14.56 ± 1.09^{d1}	9.73 ± 2.91^{d1}
200	52.22 ± 2.80^{c2}	54.67 ± 2.01^{c2}	27.22 ± 0.59^{c1}	20.73 ± 2.91^{c1}
250	78.33 ± 1.45^{b2}	78.56 ± 1.49^{b2}	45.22 ± 0.97^{b1}	30.07 ± 5.81^{b1}
300	120.78 ± 1.06^{a3}	95.35 ± 0.72^{a2}	58.25 ± 0.91^{a1}	32.45 ± 2.53^{a1}

Results expressed in Means $\pm$ 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 ($p < 0.05$).

The Total Flavonoid Content of DCM-MeOH extract, *n*-butanol, ethyl acetate, and hexane fractions are presented in Table 4. The DCM-MeOH extract are in the range of (13.07 ± 2.90) to (318.40 ± 4.03) which had the highest flavonoids while among the fractions, *n*-butanol had the highest flavonoids (272.73 ± 2.34 mg QAE/g) when compared to other fractions which had (186.20 ± 2.03 and 126.33 ± 2.15 mg QAE/g).

Table 4: Total flavonoids content (mgQE/g) of *S. brasiliensis* extract and fractions

Conc (µg/ml)	Solvent			
	DCM-MeOH	<i>n</i> -butanol	Ethyl acetate	<i>n</i> -hexane
25	13.07 ± 2.90^{d1}	10.40 ± 4.16^{e1}	4.40 ± 2.31^{f1}	3.06 ± 4.67^{f1}
50	40.73 ± 1.34^{d1}	25.73 ± 1.33^{d1}	18.40 ± 2.00^{e1}	14.40 ± 3.46^{e1}
100	72.73 ± 1.98^{c2}	43.73 ± 4.06^{c1}	33.73 ± 2.40^{d12}	23.73 ± 2.91^{d1}
200	110.40 ± 1.65^{c2}	73.07 ± 4.67^{c1}	64.73 ± 3.53^{c12}	53.73 ± 2.91^{c1}
250	225.70 ± 2.91^{b3}	133.73 ± 6.36^{b1}	123.73 ± 1.76^{b2}	117.07 ± 5.81^{b1}
300	318.40 ± 4.03^{a2}	272.73 ± 2.34^{a1}	186.20 ± 2.03^{a1}	126.33 ± 2.15^{a1}

The results ($n = 3$) are presented as Means $\pm$ SEM. There is a significant difference ($p < 0.05$) between mean values with different letters as superscripts across the rows and mean values with different numbers as superscripts along the column.

The total tannin content of methanol extract, *n*-butanol and ethyl acetate fractions are presented in Table 5. The results showed that DCM-MeOH extract possessed the highest tannins (102.22 ± 7.58 mg GAE/g) followed by the *n*-butanol fraction that possessed (60.67 ± 1.34 mg GAE/g) and finally, ethyl acetate fraction that possessed 37.33 ± 0.77 mg GAE/g. It was determined to increase from (1.16 ± 0.48 to 102.22 ± 7.58 GAE/g) at different concentrations.

Table 5: Total tannin content (mgGAE/g) of *S. brasiliensis* extract and fractions

Conc (µg/ml)	Solvent			
	DCM-MeOH	<i>n</i> -butanol	Ethylacetate	<i>n</i> -hexane
25	1.16 ± 0.48^{e2}	1.23 ± 0.58^{f1}	1.09 ± 0.22^{c1}	1.06 ± 4.67^{f1}
50	4.56 ± 1.16^{e2}	7.89 ± 0.49^{e3}	3.11 ± 0.29^{c1}	2.40 ± 3.46^{e1}
100	23.00 ± 1.68^{d3}	13.89 ± 0.78^{d2}	4.11 ± 0.67^{c1}	3.73 ± 2.91^{d1}
200	42.22 ± 0.97^{c2}	37.78 ± 1.35^{c2}	18.22 ± 2.58^{b1}	13.73 ± 2.91^{c1}
250	63.45 ± 2.48^{b3}	49.89 ± 2.15^{b2}	23.67 ± 1.15^{b1}	20.07 ± 5.81^{b1}
300	102.22 ± 7.58^{a3}	60.67 ± 1.34^{a2}	37.33 ± 0.77^{a1}	30.73 ± 1.53^{a1}

The results ($n = 3$) are presented as Means $\pm$ SEM. There is a significant difference ($p < 0.05$) between mean values with different letters as superscripts across the rows and mean values with different numbers as superscripts along the column.

Results of antioxidant analysis

The results of *in vitro* antioxidant analysis are presented in Table 6 and Table 7. Inhibition in the DPPH model ranges from 71.66±1.64% to 94.17±0.11% (Table 6) for the extract and *n*-butanol fraction but ranges from 70.24±1.60 (%) to 93.15±0.11(%) in the TAC model (Table 7).

Table 6: Antioxidant activity of extract and fraction by DPPH scavenging free radical capacity

Extract/Sample	Varying Concentrations (µg/mL)					
	15.63	31.25	62.5	125	250	500
DCM-MeOH	72.76±0.67 ^{d2}	82.33±0.70 ^{c2}	90.47±0.27 ^{b2}	92.21±0.16 ^{ab2}	93.65±0.68 ^{ab3}	94.17±0.11 ^{a3}
<i>n</i> -Butanol	71.66±1.64 ^{c1}	72.14±0.33 ^{c1}	75.27±1.12 ^{c1}	78.49±2.29 ^{c1}	79.38±0.85 ^{b2}	81.72±3.08 ^{b2}
Standard	72.61±1.14 ^{a1}	72.78±0.56 ^{a1}	73.02±0.45 ^{a1}	73.22±0.31 ^{a1}	74.13±0.41 ^{a1}	76.19±0.78 ^{a1}

The results (*n* = 3) are presented as Means ± SEM. There is a significant difference (*p* < 0.05) between mean values with different letters as superscripts across the rows and mean values with different numbers as superscripts along the column. DPPH was expressed as percentage (%) inhibition.

Table 7: The extract and fraction of *Sabicea brasiliensis* Total Antioxidant Capacity (%)

Extract/Sample	Varying Concentrations (µg/mL)					
	15.63	31.25	62.5	125	250	500
DCM-MeOH	75.67±0.61 ^{d2}	77.13±0.50 ^{c2}	81.75±0.27 ^{b2}	84.21±0.16 ^{ab2}	90.65±0.68 ^{ab3}	93.15±0.11 ^{a3}
<i>n</i> -Butanol	70.24±1.60 ^{c1}	71.36±0.33 ^{c1}	72.37±1.10 ^{c1}	74.94±2.29 ^{c1}	78.83±0.80 ^{b2}	80.72±3.08 ^{b2}
Standard	68.16±1.24 ^{a1}	70.18±0.55 ^{a1}	71.02±0.45 ^{a1}	73.22±0.31 ^{a1}	74.13±0.21 ^{a1}	76.19±0.72 ^{a1}

Results expressed in Means ± 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.

The IC₅₀ values for the extract and butanol fraction were lower than those for ascorbic acid, as can be shown in (Table 8) above. Given that IC₅₀ and potency have an inverse relationship, this suggests that the extract 0.943 and the butanol fraction 1.127 are more potent. It seems that the extract functioned in concert with other natural antioxidants, such as vitamin E and ascorbic acid.

Table 8: IC₅₀ values for the extract, butanol fraction and the standard (ascorbic acid) of the leaves of *S. brasiliensis* for DPPH model

Extract/Fraction	IC ₅₀ values (µg/mL)	Anti-Radical Power (mL/µg)
DCM-MeOH	0.943 ± 0.01	1.060 ± 0.01
<i>n</i> -Butanol	1.127 ± 0.01	0.887 ± 0.01
Ascorbic acid	1.164 ± 0.01	0.859 ± 0.01

Discussions

Antioxidants have recently become a topic of increasing interest to health, food science researchers and medical experts [27]. The phytochemical screening of crude DCM-MeOH extract and fractions of *Sabicea brasiliensis* leaves revealed the presence of some secondary metabolites as indicated in (Table 2) while alkaloids were not present in the crude extract and fractions of *Sabicea brasiliensis*, the phytochemical compounds found are known to have medicinal importance. For instance, research has shown that tannins have antibacterial [29], antitumor, and antiviral activities [28]. Tannins, on the other hand, are known to have antibacterial [29], antitumor, and antiviral properties; they function by precipitating microbial protein, which prevents them from obtaining nutritional protein. Other phytochemicals like glycosides have been used to treat congestive heart failure and cardiac arrhythmia [32]. Their mode of action starts by inhibiting Na⁺/K⁺ pump which then increase the level of calcium ion, thus more Ca⁺ would be accessible for the contraction of heart muscles which recover cardiac output and lower the distension of heart [30]. Similarly, steroids generated from plants are known to have cardiogenic impact and also feature antibacterial and insecticidal effects [31]. Because of their widely recognized biological functions, they are frequently utilized in medications. It's possible that the phytochemicals found in the crude extract and fractions are what give *S. brasiliensis* its biological activity. These findings are consistent with past research on the chemical composition and medicinal use of *Sabicea brasiliensis* [18], as well as the plant's methanolic crude extract [13].

Like *Sabicea brasiliensis*, several other plants naturally synthesize a diverse array of secondary metabolites like phenolics, flavonoids, terpenoids, tannins and glycosides that influence their interactions with the environment and serve ecological functions [19]. Many of these compounds present in variable quantities across species also impact human nutrition and medicine when consumed. Phenolic acids and flavonoids typically accumulate in high concentration in fruits and vegetables, providing antioxidant effects that help prevent chronic diseases [20]. Moderate phenolic levels afford health protection, while excessive quantities may pose risks including cytotoxic, estrogenic or pro-oxidant activities depending on compound and dose [20]. Terpenoids constitute the largest class of plant secondary metabolites and occur highly in many essential oils [19]. Tannin consumption may decrease iron bioavailability or causes gastrointestinal irritation [21] while glycosides present at moderate levels allow storage and transport of compounds within plants [19]. The principle of antioxidant activity is their interaction to produce oxidative free radicals [13]. IC₅₀ was used to display and analyze the antioxidant models' findings. The anti-radical power is the inverse of the IC₅₀ value, which is a measure of how well a substance inhibits a certain biological or biochemical activity. The IC₅₀ value is also known as the half maximal inhibitory concentration. An antioxidant is considered more powerful or potent if its anti-radical potency is higher and its IC₅₀ value is lower. It is believed that an antioxidant's potency and antioxidant activity increase with a decreasing IC₅₀ value [13]. Therefore, for the DPPH model, the IC₅₀

values for the Dichloromethane-Methanol extract, butanol fraction, and standard (ascorbic acid) were, respectively, 0.943 µg/mL, 1.127 µg/mL, and 1.164 µg/mL.

This demonstrates that compared to the standard, the DCM-MeOH extract and *n*-butanol fraction have superior antioxidant properties. When the DCM-MeOH extract and *n*-butanol fraction were compared to the standard (ascorbic acid), the IC₅₀ values were lower. Given that IC₅₀ and potency have an inverse relationship, this suggests that the extract and the *n*-butanol fraction are more potent. Because the extract contains ascorbic acid together with other natural antioxidants like vitamin E, etc., it may have had a synergistic effect. The extract was able to absorb the diphenyl picryl hydrazyl, which has a yellow color, and the DPPH radical, which is evident as a deep purple color.

Because of the interaction between the antioxidant molecules and the radical, which leads to the radical's scavenging by hydrogen donation, antioxidants reduce the absorption of DPPH [24]. Therefore, it was believed that the plant extract's capacity to donate hydrogen was the reason for *Sabicea brasiliensis* action on DPPH scavenging. The extract's total phenolic content was found to be high based on the phytochemical analysis results. This indicates that the phenolic compounds in the extract may be the cause of the observed DPPH radical scavenging activity because they were able to donate hydrogen atoms to the radical.

Therefore, it was believed that the plant extract's capacity to donate hydrogen was the reason for *Sabicea brasiliensis*'s action on DPPH scavenging. The extract's total phenolic content was found to be high based on the phytochemical analysis results. This indicates that the phenolic compounds in the extract may be the cause of the observed DPPH radical scavenging activity because they are easily able to donate hydrogen atoms to the radical. Thus, it implies that the extract might be utilized as a natural supply of antioxidants to reduce the harm that free radicals do to the human body. A commonly used efficacy metric in pharmacological research is the IC₅₀ value, which is defined as the concentration of a material needed to inhibit a biological process by half [23].

Lower IC₅₀ values indicate higher antioxidant potency and effectiveness for radical scavenging. In phytochemical studies, determining IC₅₀ against reactive oxygen/nitrogen species using assays like DPPH, ABTS and nitric oxide helps quantify antioxidant strength of plant extracts and isolates [24]. Along with IC₅₀, antiradical power expresses the ability of a compound or extract to donate electrons or hydrogen atoms which quenches free radicals and halts oxidative chain reactions linked to diseases [25]. In (Table 8), the IC₅₀ of the extract and butanol fraction showed lower values after calculations in comparison to ascorbic acid, this implies that the extract and butanol fraction possess greater antioxidant potency than the standard. Both metrics were important for objectively assessing active constituents from *Sabicea brasiliensis* and elucidating their potential as nutraceuticals or drug leads against conditions arising from oxidative stress [26].

There is also a confirmation that the plant has anti-inflammatory properties which was reported by [18]. There is a close connection between oxidative stress and inflammatory processes in the body. Reactive oxygen species (ROS) generated during normal aerobic metabolism act as signaling molecules that propagate inflammation if levels exceed antioxidant defenses. At low concentrations, ROS function in immune responses by activating pathways like nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs) which induce expression of pro-inflammatory cytokines and mediators of the acute phase response [25]. However, when oxidative burden overwhelms antioxidants, a state of oxidative stress emerges that sustains excessive production of cytokines, chemokines and lipid inflammatory markers. Oxidation of lipids, proteins and nucleic acids during this stage further amplifies the inflammatory reaction [25]. Compounds exhibiting antioxidant effects by neutralizing free radicals or scavenging peroxides therefore hold potentials for mitigating inflammation. Some antioxidants elicit anti-inflammatory properties through directly targeting redox-sensitive transcription factors pivotal in immune signaling pathways. For example, certain polyphenols suppress activation of NF-κB by preventing degradation of its inhibitor [25]. Down regulation of this pro-inflammatory transcription factor and related MAPKs diminishes synthesis of inflammatory proteins and cytokines [18]. Phytochemicals may also exert indirect anti-inflammatory actions by protecting cellular macromolecules from oxidation and associated tissue damage [25]. Therefore, maintaining redox balance through antioxidant consumption represents an important strategy for modulation of chronic inflammation, as oxidative stress which serves both as a trigger and perpetuator of inflammatory cascades.

Conclusion

From the foregoing, the findings of this investigation provide scientific validation for the medicinal claims regarding *S. brasiliensis* and offer novel anti-oxidative compounds with pharmaceutical promise. The results demonstrated that the Dichloromethane-Methanol extract of *S. brasiliensis* had better antioxidant properties even when compared with the standard. Investigating molecular mechanisms of action against markers of oxidative stress and inflammation in cell-based or animal models could substantiate the phytomedicine's therapeutic potential. As a result, *S. brasiliensis* leaves have the potential to be a useful source of antioxidants in medicine, particularly when it comes to the DCM-MeOH extract and butanol fraction. It can also be used as a source of these pharmacologically important compounds which have promising medicinal properties; this suggests that the plant may be utilized in the production of phytomedicines for the creation of new drugs. Purification and characterization of the compounds' biological activity hence need more research.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Authors' contributions

This work was carried out in collaboration among all authors. Authors COU and DPB designed the study and wrote the protocol. Authors COU, PC, EJM and SIO performed the laboratory and statistical analysis. Author COU wrote the first draft of the manuscript. Authors COU, COO, NRN and GNU managed the analyses of the study. Author COU managed the literature searches. All authors read and approved the final manuscript.

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References

1. Kakouri E, Daferera D, Paramithiotis S, Astraka K, Drosinos EH, Polissiou MG, 2017. *Crocus sativus* L. Tepals: The natural source of antioxidant and antimicrobial factors. Journal of Applied Research on Medicinal Aromatic Plants, 4:66-74.
2. Akoué GN, Obame LC, Ondo JP, Brama I, N'Nang ESO, Tapoyo SY, 2013. Phytochemical composition and antiradical activity of *Sakesia africana* Hook. F. Medicinal Plant from Gabon. International Journal of Biomolecules and Biomedicine, 3(3): 1-8.
3. Rahman MA, Imran TB, Islam S, 2013. Antioxidative, antimicrobial and cytotoxic effects of the phenolics of *Leea indica* leaf extract. Saudi Journal of Biological Sciences, 20(3): 213-25.
4. Ajitha B, Reddy AK, Reddy PS, 2015. Green synthesis and characterization of silver nanoparticles using *Lantana camara* leaf extract. Materials Science and Engineering, 49:373-381.
5. Zulfayy LOMA, Lukitaningsih ER, Rumiya R, 2021. GC-MS analysis of bioactive compounds in ethanol and ethyl acetate fraction of grape fruit (*Citrus maxima* L.) Rind. Borneo Journal of Pharmacy, 4(1): 29-35.
6. Salisbury BH, Tadi P, 2021. 5 alpha reductase inhibitors. In: Stat pearls (internet). Stat Pearls Publishing, Treasure Island (FL), pp. 4.
7. Kim S, Kim DB, Jin W, Park J, Yoon W, Lee Y, 2017. Comparative studies of bioactive organosulphur compounds and antioxidant activities in garlic (*Allium sativum* L.), elephant garlic (*Allium ampeloprasum* L.) and onion (*Allium cepa* L.). Natural Product Research, 32 (10):1193-1197.
8. Heer A, Guleria S, Razdan VK, 2017. Chemical composition, antioxidant and antimicrobial activities and characterization of bioactive compounds from essential oil of *Cinnamomum tamala* grown in north-western Himalaya. Journal of Plant Biochemistry and Biotechnology, 26:191-198.
9. Bashir L, Saidu S, Amos S, Onikanni D, Yunusa OI, Abdulhakeem RA, Halimat YL, Femi O, Ali AJ, Gaber EB, Shukurat BB., Gomaa M, Clara MGL, Alexander TH, Wu HH, Carlos, AC, 2022. Preclinical anti-inflammatory and antioxidant effects of *Azanza garckeana* in STZ-induced glycemic-impaired rats, and Pharmacoinformatics of it major phytoconstituents: Biomedicine & Pharmacotherapy, 152:113-196.
10. Baba H, Ibe AC, Kashimawo AJ, 2021. Phytochemical Evaluation and GC-MS Profiling of the Dichloromethane and Ethanol extracts of *Ocimum gratissimum* L. and *Lasianthera africana*. Vol 20(2): 640- 655.
11. Agbo MO, Uzor PF, Akazie-Nneji UN, Eze-Odurukwe CU, Ogbatue UB, Mbaaji EC, 2015. Total Phenolics and flavonoid contents in selected Nigerian Medicinal Plants. Dhaka Journal of Pharmaceutical Sciences, 14 (1): 35-41
12. Ugorji CO, Ihedioha JN, Agbo MO, Ekere NR, Nwafor, FI, Odoemelam EI, 2019. Antimicrobial and Antioxidant Studies of the Leaf Extract and Fractions of *Sabicea brevipes* Wernham (Rubiaceae), International Journal of Biochemistry Research & Review, 27 (2): 9-10.
13. Harbone JB, 1984. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 2nd edition; London: Chapman and Hull, p. 287.
14. Sahreen S, Muhammad RK, Rahmat AK, 2011. Phenolic compounds and antioxidant activities of *Rumex hastatus* D.Don. Leaves. Journal of Medicinal Plants Research, 5 (13): 2755-2765.
15. Biju J, Sulaiman CT, Sathesh G, Reddy VRK, 2014. Total phenolics and flavonoids in selected medicinal plants from Kerala. International Journal of Pharmaceutical Sciences, 6: 406 408.
16. Abdelwahab SI, Abdul AB, Elhassan MM, Mohan S, Mariod AA, 2010. Phenolic Content and antioxidant activities of *Goniobalanus umbrosus* extracts. International Journal of Natural Product of Pharmaceutical Sciences, 2: 1-6.
17. Farhat MB, Landoulsi A, Chaouch-Hamada R, Sotomayor JA, María JJ, 2013. Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats. Industrial Crops Products, 49: 904-914.
18. Aftab S, Krupadam RJ, Rao CK, 2018. Phytochemistry: A succinct overview. Phytochemistry: Research Progress, 1-11.
19. Alisi CS, Ojiako OA, Osuagwu CG, Onyeze GOC, 2011. Free Radical Scavenging and *In-vitro* Antioxidants Effects of Ethanol Extract of the Medicinal Herb *Chromolaena odorata* Linn, British Journal of Pharmaceutical Research, 1: 141.
20. Mythili K, Reddy CU, Chamundeeswari D, Manna PK, 2014. Determination of Total Phenol, Alkaloid, Flavonoid and Tannin in different Extracts of *Calanthe triPLICATE*. Journal of Pharmacognosy and Phytochemistry, 2 (2): 40-44.
21. Singh R, Verma PK, Singh G, 2012. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. Journal of Intercult Ethnopharmacology, 1 (2): 101-104.
22. Silva KC, Matos MCS, Maciel MAM, Rolim Neto PJ, Rolim LA, Rolim CM, 2010. Medicinal uses and chemical constituents of *Sabicea brasiliensis* (Vell.) B. Gates (Rubiaceae). Brazilian Journal of Pharmaceutical Sciences, 46(2): 263-269.
23. Pérez-Jiménez J, Saura-Calixto F, 2006. Effect of solvent and certain food constituents on different antioxidative activity determinations. Journal of Agricultural and Food Chemistry, 54(13): 4942-4948.
24. Pérez-Jiménez J, Neveu V, Vos F, Scalbert A, 2010. Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: An application of the Phenol-Explorer database. Journal of Agricultural and Food Chemistry, 58(8): 4959-4969.

25. Igbiosa OO, Igbiosa IH, Chigor VN, Uzunugbe OE, Oyedemi SO, Odjajare EE, 2011. Polyphenolic contents and antioxidant potential of stem bark extracts from *Jatropha curcas* (Linn). International Journal of Molecular Sciences, 12(5): 2958-71.
26. Arunkumar E, Muthuselvam M, 2009. Analysis of phytochemical constituents and antioxidant activities of Aloe vera gel extracted from plants in southern Tamil Nadu, India. Plants foods for human nutrition, 64(1): 55-60.
27. Oboh G, Puntel RL, Rocha JB, 2016. Hot pepper (*Capsicum* spp.) in polyphenol-rich diets protects brain and key biomolecules against nitrosodiethylamine (NDEA)-induced oxidative stress in rats. Phytotherapy Research, 30(5): 838-847.
28. Pohanka M, 2021. The Concept of Antioxidant Capacity: From Definition to Implementation. Oxidative Medicine and Cellular Longevity.
29. Ben MA, Porter EA, Kite GC, Simmonds MS, Abdelhedi R, Bouaziz M, 2015. Phenolic profile characterization of Chemlali olive stones by liquid chromatography-ion trap mass spectrometry. Journal of Agriculture and Food Chemistry, 63(7): 1990-1995.
30. Alexei YB, Joseph IS, Olga VF, 2009. Endogenous cardiotonic steroids: Physiology, Pharmacology and novel therapeutic targets. Pharmacological Reviews, 61: 9–38.
31. Vladimir K, Ludmila M, 2001. Glycosides in Medicine: “The role of glycosidic residue in biological activity”. Current Medicinal Chemistry, 8: 1303-1328.

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