Communication in Physical Sciences, 2024, *11(2): 316-331*

Phytochemical, Anti-inflammatory, Antioxidant, Toxicity and Antimicrobial Activities of *Sarcophrynium brachystachys (Benth) K.* Shum Leaves

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Received: 06 March 2024/Accepted: 03 May 2024 /Published: 06 May 2024

Abstract: This study investigates the phytochemical composition and pharmacological properties of Sarcophrynium brachystachys leaf extract. The extract was obtained through ethanol percolation and subjected to qualitative and quantitative phytochemical analyses, revealing significant amounts of flavonoids (5.51 mg/100 g), alkaloids (6.68 mg/100 g), and saponins (4.28 mg/100 g). Antibacterial screening against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa demonstrated notable inhibitory effects, particularly with the methanol fraction showing zones of inhibition ranging from 12±1.03 mm to 15±1.23 mm. Acute toxicity evaluation revealed no mortalities across all dose groups, indicating a favorable safety profile. Anti-inflammatory assessment exhibited dose-dependent reductions in paw circumference post-induction, with percentage inhibition ranging from 34.07% to 40.98% compared to aspirin (56.92%). Furthermore, in vitro antioxidant assays demonstrated dose-dependent scavenging activity against DPPH radicals (18.90% to 73.74%), nitric oxide radicals (13.10% to 73.16%), and ferric ions (3.91% to 53.92%). These findings underscore the therapeutic potential of S. brachystachys leaf extract as a source of natural bioactive compounds with antibacterial, anti-inflammatory, and antioxidant properties, warranting further investigation for pharmaceutical applications.

Keywords: S. *brachystachys, anti-inflammatory, antioxidant, toxicity, antimicrobial, extract*

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1.0 Introduction

The extensive array of naturally occurring chemicals, referred to as secondary metabolites, which are not essential for plant growth, demonstrates the remarkable chemical variety within the plant kingdom (Otuokere *et al*., 2022; Nwankwo *et al*., 2022; Anyanwu *et al*., 2021; Ikpeazu *et al*., 2020; Kwekowe *et al*., 2021). They are built from different kinds of simple chemical scaffolds. Natural products have been highly relevant for infectious diseases and cancer because they have been structurally tuned by evolution to perform certain biological tasks (Atanasov *et al*., 2015), such as the regulation of endogenous defence mechanisms and the interaction (often competition) with other species. It can be difficult to pinpoint the relevant bioactive chemicals. It can also be difficult to get enough biological material for isolation and characterization. Plant-based natural products are widely employed as medications, nutraceuticals, cosmetics, and fine chemicals. They are often categorised into three classes: phenolics, terpenoids, and alkaloids (Wang *et al*., 2019).

Natural products have been used as a key source of both traditional and modern medicine. They have been used as a herbal remedy for thousands of years, changing the path of human civilisation and history and having a broad impact on human health (Satic *et al*., 2017). Our human predecessors and forefathers have long understood and utilised the therapeutic properties of medicinal herbs. They learned to use plant-based folk medicine to treat conditions including headaches, fevers, and pains (Lietara *et al*., 1992). Hundreds of bioactive chemicals derived from plants have been found to date, and many of them—like the anti-tumour ginsenoside, anti-malarial artemisinin, and anti-tumour paclitaxel taxol—are used to treat diseases in humans. The WHO recommends the sesquiterpene endoperoxide artemisinin from Artemisia annua as the most effective medication against malaria (WHO, 2021). Treatments for ovarian and lung cancer, as well as Kaposi's sarcoma, have been licensed for the drug paclitaxel (taxol), which was extracted from the bark of Taxus trees (Weaver, 2014). Plants create a variety of chemicals that perform biological activity as an adaptation against pathogens and environmental stress. These tiny organic compounds serve a variety of biological purposes and are produced by secondary metabolism. Among the acts mentioned are anti-oxidative and anti-inflammatory ones (Locatelli *et al*., 2016; Virshitte *et al*., 2019).

Anti-inflammation is recognized as a vital survival strategy and an evolutionary conserved protective function (Liu *et al*., 2017). It is made up of intricately coordinated tissue alterations aimed at eradicating the source of the cell damage, which could have been physical (radiation, burns, trauma), chemical (caustic substances), or infectious agents or substances from their metabolism (microorganisms and toxins) (Fialho *et al*., 2018; Jang *et al*., 2016). Localized redness, swelling, discomfort, heat, and loss of function are indicators of inflammation (Locatelli *et al*., 2016). It is thus desirable to find a new generation of therapeutic medicines to employ in the resolution of inflammation. Some of the mechanisms involved in the treatment can serve as therapeutic targets (Liu *et al*., 2017). Owing to the generation of secondary metabolites that have therapeutic potential, novel and promising medications have been produced (Li, 2020; Taynab *et al*., 2018). Numerous illnesses and long-term medical issues, including obesity, diabetes, cancer, and cardiovascular diseases, are associated with a

low-grade inflammatory state (Kimy *et al*., 2018; Purohit *et al*., 2018; Clark *et al*., 2017; Espigol *et al*., 2018; Donninelli *et al*., 2017; Kafere *et al*., 2017; Moses *et al*., 2017; Qi *et al*., 2017). However, antioxidants can neutralize reactive species and free radicals, counteracting oxidative damage and, as a result, lowering the frequency of diseases linked to oxidative stress and breaking free radical-mediated chain reactions. The identification of fresh materials and substances for more durable, focused extraction and separation techniques and more reliable and accurate analytical methods is still ongoing, as is the assessment of the role of antioxidants in human health and well-being.

Phenolics make up the majority of natural antioxidants, which may be found in all sections of plants, including fruits, vegetables, nuts, seeds, bark, roots, and leaves (Asif, 2015). These studies have encouraged researchers to concentrate their efforts on investigating natural sources that have a fair potential for antioxidants (Ramalashini *et al*., 2008), which is one of the primary goals of studying S. brachystachys leaves. These sources, whose antioxidant potential can be ranked from highest to lowest, must be identified and publicized. Individuals who have made it a practice to include foods like fruits, vegetables, nuts, and seeds in all of their regular meals are said to be less likely to develop several chronic illnesses (Dembinaica-Iidec *et al*., 2008), and studies have also confirmed the long-term positive effects of the excessive generation of free oxygen and nitrogen species, or their presence in the cell at a very high concentration, causes oxidative stress, which is the primary cause of the development and progression of many human diseases, including cancer, inflammatory diseases, and cardiovascular diseases. As free radical scavengers, endogenous (naturally produced in the body) and exogenous (externally supplied through food antioxidants work to prevent and repair damage caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). As a result, they strengthen the immune system and reduce the risk of degenerative diseases and cancer (Bghonu *et al*., 2006; Halliwell, 2007).

In herbal therapy, the plant S. brachystachys is used to cure bronchitis and cough. Studies have shown that it contains active biochemical constituents from the isolated structural elucidation (Egbucha *et al*., 2023), which are responsible for the antimicrobial properties of the leaves. To the best of our knowledge, there are no reports on the phytochemical and bioactivity of S. brachystachys. Therefore, novel presentation is provided in this report the phytochemical, anti-inflammatory, antioxidant, toxicity, and antimicrobial activities of S. brachystachys.

2.0 Materials and Methods

2.1 Reagents/Solvents

The reagents and solvents used in the research work were analytical-grade reagents from BDH Chemicals London. They were purchased from commercially available stores and made available for use. These commercially purchased chemicals and reagents include analytical-grade chloroform (CHCl3, purity 99.5 %), ethyl acetate (EtOAc; 99.5%), ethanol (EtOH; 96%), n-hexane (purity 99.5%), methanol (MeOH; 99.5%), and distilled water. The silica gel (100–120 mesh) was a product of Merck.

2.2 Collection of plant material

The leaves of *S.brachystachys* were collected in bulk from a forest area in Umuokpo, Emeabiam, Owerri West Local Government Area of Imo State. The fresh leaves of the plant were collected in the early hours of the day between 7 a.m. and 8 a.m. on June 23, 2022, as the temperature ranged from 23 oC to 25 oC with an average humidity of 73%. The sampling of the plant part was done following the traditional uses of the species. The plant was identified by a taxonomist of the Forestry Department, Michael Okpara University of

Agriculture, Umudike, Abia State, and later on, the leaves were air dried at room temperature for one month to ensure proper drying, after which they were ground and sieved into fine particles using a 2 mm mesh size sieve.

2.3 Extraction of plant material

The extraction was performed at room temperature, where 1000 g of the plant materials were percolated with 3.5 L of 96% (v/v) ethanol for two weeks at room temperature with frequent agitation using the modified method (Johnbull *et al*., 2001). The powdered sample was weighed into an amber bottle using a glass funnel. A measuring cylinder was used to introduce the solvent into the bottle gradually with agitation to ensure proper percolation between the sample material and solvent. This was allowed for two weeks for maximum extraction. The percolate was decanted and filtered using Whatman's filter paper, a glass funnel, and a 1000-ml beaker. A dark greenish filtrate was collected. The filtrate was concentrated at reduced pressure using a rotatory evaporator (Buchi, R200) at 40 °C. Before the rotatory evaporator was set to work, it was quarterly filled with the filtrate, and the flask was carefully inserted to fit in properly to avoid spillage during rotation. This was set to work until the ethanol solvent had evaporated into the second flask, leaving the filtrate sample behind. The dark greenish-concentrated sample was then collected and poured into an already-weighed beaker, covered with perforated aluminium foil, and then allowed to further air-dry. The dried crude extract was weighed and labelled as JS01a. The weight of the crude sample was found to be 163.32 g.

2.4 Qualitative and quantitative phytochemical analysis

The ethanol extracts underwent analysis for the presence of alkaloids, saponins, tannins, flavonoids, steroids, cardiac glycosides, phenolic compounds, and terpenoids. Standard methods for preliminary phytochemical analysis were used with some minor modifications (Anyanwu *et al*., 2022; Sofowora, 1994; Trease and Evans, 2002).

2.5 Clinical isolates

S. aureus, E. coli, and P. aeruginosa were obtained from the microbiology laboratory, at Federal Medical Centre, Umuahia, Abia State.

2.6 Antibacterial screening

The antibacterial screening was carried out using the disc diffusion method according to standard procedure (Chessborough, 2000).

2.7 Evaluation of the anti-inflammatory effect of the extract

Twenty-five (25) rats will be divided into 5 groups, each containing 5 rats, and starved for 24 hours before the experiment. Group 1 was treated with only 0.2 ml of normal saline and served as a control, while group 2 was given aspirin (100 mg/kg body weight) and was the standard drug group. Groups 3, 4, and 5 will receive low, moderate, and high dose levels of the extract, respectively. All treatment was done through the oral route. The paw circumferences (PC) of the animals were measured and recorded before these treatments. Thirty minutes after treatment, acute inflammation (paw oedema) was induced by sub-plantal injection of 0.1 ml of λ-carrageenan (in 0.9% saline solution) into the right hind paw of each rat. Thereafter, the PC of the treated rats and control rats were measured and recorded at different time intervals (30 min, 1 hour, and 2 hours) following induction. The degree of oedema was assessed as the difference between the initial and final PC values, while the percentage inhibition of oedema was calculated using equation 1

$$\%\ \boldsymbol{Inhibition\ of\ oedema} = \frac{\boldsymbol{Pc\ in\ control - Pc\ test}}{\boldsymbol{Pc\ in\ control}}\ \boldsymbol{x\ 100} \qquad (1)\backslash$$

2.8 Toxicity Evaluation of the Extract

2.8.1 Animals

A total of 41 adult Wister rats were used for the studies. Twenty-one of the rats were used for

the acute toxicity or lethal dose (LD50) evaluation of the extract, while 20 were used for the subacute toxicity evaluation. The animals were obtained from the Animal House of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, and transported to FUTO, where research was carried out. At the site of the study, the rats were housed in aluminium cages and allowed to acclimatise for two weeks to allow for proper adaptation to their new environment and living conditions before the commencement of the study. Feeding was done libitum with Chikkun finisher's mash (Chukkun Feeds, Nigeria) and clean water, but animals were starved for 12 hours before the commencement of each experiment. All animal experiments were conducted in compliance with international guidelines for the care and use of laboratory animals as stipulated by international regulations and approved by the FUTO animal ethics committee.

2.9 Acute toxicity (LD50) evaluation of plant extract

The toxicity evaluation using Lorke's method (Orieke *et al*., 2019). was adopted with little modification. For each extract, two stages were involved in the experiment. In the first stage, nine Wister rats were assigned to 3 groups (A, B, and C) of 3 rats each and treated with 10, 100, and 1000 mg/kg of the extract, respectively. The animals were thereafter monitored for the manifestation of toxicity signs and deaths within 24 hours. After zero mortality was recorded, the study proceeded to the second phase, which also involved the use of nine rats assigned to groups (A–C). The single treatment doses that were assigned to the group were 1600, 2900, and 5000 mg/kg, respectively. The animals were again monitored for toxicity signs and deaths within 24 hours. At the end of the period, we observed no mortality; we repeated the highest dose (5000 mg/kg) on another set of 3 rats as a confirmatory test, observing them within 24 hours and a further week. Acute toxicity values were calculated using Lorke's formula shown in equation 2 below

$$LD_{50} = (A \; x \; B)^{½} \qquad (2)$$

A is the maximum dose that produced no mortality, B is the minimum dose that kills all animals in a group.

2.10 Design for sub-acute toxicity evaluation

Twenty adult albino rats would be divided into four groups of 10 rats each. Each group would be housed in an aluminium cage measuring 100 x 80 cm and assigned treatment according to the order below; Group 1: Normal control, Group 2: Low-dose extract, Group 3: Moderate dose of extract, Group 4: High dose of extract. Treatment was oral and lasted for twenty-eight (28) days, during which body weights would be taken at the beginning and end of treatment. At the end of treatment, the animals were sacrificed for blood collection into K3EDTA and plane bottles for haematology and biochemical analysis, respectively. Liver and kidney weights were taken and relative organ weights (ROW) were evaluated before being preserved in 10% formalin for histological evaluation.

2.11 In vitro antioxidant assays

2.11.1 Nitric oxide inhibition activity

Nitric oxide, generated from sodium nitroprusside in an aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction (Marcocci *et al*., 1994). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate-buffered saline (PBS) and the extract from 25–400 µg/ml were incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture was removed, and 0.5 ml of Griess reagent (1% (w/v) sulfanilamide, 2% (v/v) H_3PO_4 and 0.1% (w/v) naphthylethylenediamine hydrochloride) was added. The absorbance of

the chromophore formed was measured at 546 nm.

2.12 Photometric assay of 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The free radical scavenging activity of APE was analysed by the DPPH assay (Menser *et al*., 2001), using a spectrophotometer. The crude extract at concentrations (25, 50, 100, 200, and 400 µg/ml was mixed with 1 ml of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage of antioxidant activities was calculated as follows:

$$\% \, \boldsymbol{Antioxidant\ activity}\ (\boldsymbol{AA}) = \mathbf{100} - [(\boldsymbol{ABS\ sample} - \boldsymbol{ABS\ blank})\boldsymbol{x}\ \mathbf{100})/\boldsymbol{ABS\ control}]$$

1 ml of methanol plus 2.0 ml of the test extract was used as the blank, while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as a reference standard (Iwalewa *et al*., 2008; Igwe *et al*., 2024).

2.13 Ferric-reducing antioxidant power

The ferric-reducing antioxidant power was carried out as used (Benzie and Strain, 1999). The protocol involved is as follows:

Reagents:

Acetate buffer (300 mM), pH 3.6 (3.1 g sodium acetate). 3H2O and 16 ml glacial acetic acid in a 1000 ml buffer solution).

2, 4, 6-triphridyl-s-triazine (TPTZ) (10 mM) in 40 mM HCL.

FeCl3 6H2O (20 mM) in distilled water.

The FRAP working solution was prepared by mixing solutions 1, 2, and 3 in a ratio of 10:1:1, respectively. The working solution was freshly prepared for each test. The aqueous solution of a known amount of ascorbic acid was used for calibration.

Assay: Blank FRAP reagent.

Sample: FRAP reagent (3 ml) and 100 µl sample solution at concentrations of 25, 50, 100, 200, and 400 µg/ml were mixed and allowed to stand for 4 minutes. Colometric readings were recorded at 593 nm at 37 °C. The ascorbic acid standard solution was tested in a parallel process. Calculations were made using a calibration curve.

3.0 Results and Discussion

The results of the qualitative and quantitative phytochemical analysis of crude extract are presented in Table 1.

Table 1: Qualitative and quantitative phytochemical results

Phytochemicals	Inference	Quantity (mg/ 100 g)
Saponins	+ +	4.28±0.16
Flavonoids	+ + +	5.51±0.33
Tannins	+	1.04±0.02
Steroids	+	0.89±0.01
Terpenes	+	0.91±0.02
Alkaloids	+++	6.68±0.56
Glycoside	+	0.39±0.01
Phenolic compounds	+	0.79±0.13

****: +=Small amount, ++= Moderate, +++ =Large amount. Data are mean of means three replicate (n=3) ± standard error of the mean.**

The qualitative phytochemical determination (Table 1) showed that flavonoids and alkaloids were present in high quantities. Saponin was found in moderate amounts, while tannins, steroids, terpenes, cardiac glycosides, and phenolic compounds were present in low amounts. In decreasing order, the result follows thus: alkaloid (6.68 mg/100 g) >flavonoids (5.51 mg/100 g)>saponin (4.28 mg/100 g) > tannins (1.04 mg/100 g) terpenes (0.97 mg/100 g) > steroids (0.87 mg/100 g) > phenolic compounds (0.79 mg/100 g) > cardiac glycoside (0.39 mg/100 g). The qualitative phytochemical result, as presented in Table 1 above, captures the high presence of flavonoids, signifying that the plant is a rich source of antimicrobial agents because

flavonoids are synthesised by plants in response to microbial attack (Dixon *et al*., 1983). Hence, it is not surprising that the leaves are effective against microorganisms when tested. The activity is probably due to their ability to react with extracellular and soluble proteins to complex the bacterial cell wall, leading to the death of the bacteria (Cowan and Stell, 1990). The presence of tannins suggests the ability of this plant to play a major role as an antidiarrhoea and antihaemorrhagic agent (Asquith and Butler, 1986), while the high content of alkaloids suggests that the plant is a detoxifier and has high antihypertensive properties (Zee-Cheng, 1997).

The result of the antibacterial activity with zones of inhibition is presented in Table 2.

Table 2: Antibacterial activity with zones of inhibition for the extract

Solvents	Conc. of extracts (µg/disc)	Test	Organisms	
		S. aureus	E.coli	P. aeruginosa
Methanol	1000	15 ±1.23	12±1.03	14±1.04
	100	7±0.82	6±0.12	7±0.22
	10	-	-	-
Chloroform	1000	-	-	8±0.58
	100	-	-	-
	10	-	-	-
n-Hexane	1000	7±0.88	9±1.26	10±1.00
	100	-	6±0.11	-
	10	-	-	-
Streptomycin (control)	10	22±0.00	16±0.00	15±0.00

Diameter zone of inhibition of less than 6mm is represented as Results represents mean triplicate (n=3) ±standard deviation (SD)

The antibacterial test was used to determine the efficiency of plant extracts against some bacteria. It was expressed in terms of zones of inhibition, which showed a significant reduction in bacterial growth, as seen in Table 2. The methanol extract showed a wider zone of inhibition in all the bacteria isolates, indicating that the extract is active against S. aureus, *E. coli,* P. aeruginosa with results ranging from 15±1.23 in *S. aureus* at 1000 µg/disc to 12±1.03 in *E.coli* at 1000 µg/cm3 and 14±1.04 mm in *P. aeruginosa* at 1000 µg/disc. It explains that the bacteria isolates, S. aureus and E.coli were not inhibited by the chloroform extract except for *P. aeruginosa,* where there was inhibition. The n-hexane fraction showed inhibition of the isolates, the zone of inhibition ranged from 7±0.88 for S. aureus at 1000 µg/disc to 9±1.26 for E.coli at 1000 µg/disc and 10±1.00 for *P.aeruginosa* at 1000 µg/disc. The overall results explain that the leaf extract of *S.brachystachys* can be used as an antibacterial agent, considering that it inhibited the growth of these bacteria effectively. The results also showed that the zone of inhibition increased with an increase in the concentration of the extracts. This also explains that inhibition is a concentration-dependent activity consistent with the polarity of the tested fractions, as the methanol extract has the widest zone of inhibition.

Based on the results provided in Table 3, it seems that there were no mortalities observed

in any of the tested dose groups of *Sarcophrynium brachystachys* leaf extract during the acute toxicity evaluation. The acute toxicity of a substance is typically evaluated by administering escalating doses to test subjects and observing their responses, particularly mortality, within a short period, usually 24 to 48 hours. In this case, the doses ranged from 10 mg/kg body weight to 5000 mg/kg body weight. The absence of mortality across all dose groups suggests that the Sarcophrynium *brachystachys* leaf extract, at the doses tested, does not exhibit acute toxicity in the tested animal model, at least within the observed time frame. This is indicative of a favourable safety profile regarding acute toxicity.

Table 3: Results of acute toxicity evaluation of the extract

Group	Dose (mg/kg body weight)	Mortality	Percentage mortality
1	10	0/3	0.00
2	100	0/3	0.00
3	1000	0/3	0.00
4	2900	0/3	0.00
5	5000	0/3	0.00

The results presented in Table 4 demonstrate the anti-inflammatory potential of *Sarcophrynium brachystachys* leaf extract compared to both a control group and a positive control group treated with aspirin. In the control group, there was a significant increase in paw circumference (P.C) post-induction, indicating inflammation, whereas aspirin treatment resulted in a significant reduction in P.C, indicating potent anti-inflammatory effects. Similarly, treatment with the leaf extract at varying doses also led to a dose-dependent reduction in P.C increase post-induction, suggesting anti-inflammatory activity. The percentage inhibition of inflammation ranged from moderate to substantial across the extract-treated groups, indicating its potential therapeutic use in inflammatory conditions. These findings suggest that *Sarcophrynium brachystachys* leaf extract possesses promising anti-inflammatory properties, warranting further investigation into its underlying mechanisms and clinical applications. Results of the anti-inflammatory evaluation of the extract are presented in Table 4, Figures 1a, 1b and 1c.

In Table 5, *Sarcophrynium brachystachys* leaf extract demonstrated varying levels of DPPH radical scavenging activity at different doses. At a dose of 25µg/ml, the extract exhibited a scavenging activity of 18.90%, which was significantly lower than that of vitamin C (26.16%). Similarly, at 50µg/ml, the extract's scavenging activity increased to 23.99%, still lower than vitamin C (38.82%). However, at a dose of 100µg/ml, the extract's scavenging activity significantly increased to 56.53%, approaching that of vitamin C (60.68%). The highest dose tested, 200µg/ml, demonstrated the most significant scavenging activity at 73.74%, comparable to that of vitamin C (78.05%). These results suggest a dose-dependent increase in the antioxidant potential of the extract, with higher doses exhibiting greater scavenging activity. While the extract's activity is generally lower than that of vitamin C, it still shows promising antioxidant effects, particularly at higher concentrations.

Table 4: Anti-inflammatory activity of the extract

Group	1	2	3	4	5
Treatment	Control	Aspirin (100 mg/kg bw)	Extract(250 mg/kg bw	Extract (500 mg/kg bw)	Extract (1000 mg/kg bw)
Initial P.C (mm)	22.67±0.67a	22.00±0.58a	23.33±0.33a	22.67±0.33a	22.67±0.67a
P.C 1H post-induction (mm)	37.00±0.58d	28.33±0.88a	33.67±0.33bc	31.00±0.58b	33.00±1.15bc
P.C 2H post-induction (mm)	37.33±0.33d	28.33±0.33a	33.00±0.58c	31.33±0.33b	32.00±0.58bc
P.C after 2H (mm)	14.67±0.88c	6.33±0.88a	9.67±0.33b	8.67±0.67ab	9.33±1.20b
% inhibition of inflammation	0.00±0.00a	56.92±6.00c	34.07±2.36b	40.98±4.48bc	36.51±8.17b

**** Mean±SEM. Means with same alphabets (such as a,a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05.**

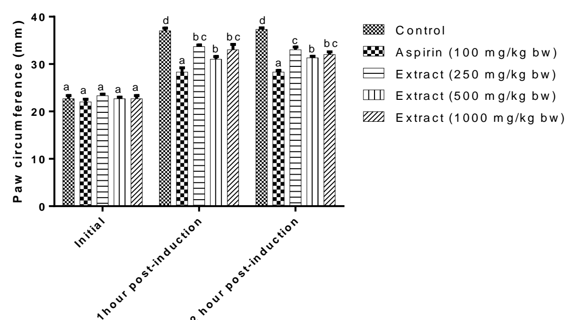

Fig 1a: Anti-inflammatory activity of the extract (initial to 2 hours post induction)

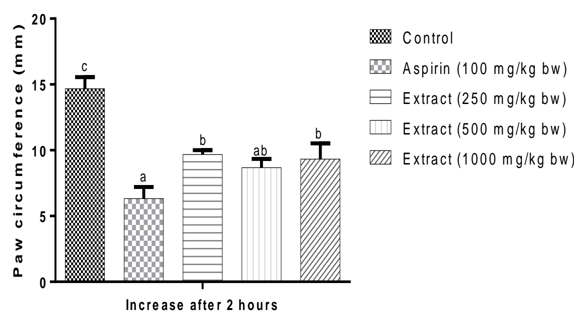

Fig. 1b: Anti-inflammatory activity of the extract (after 2 hours post induction)

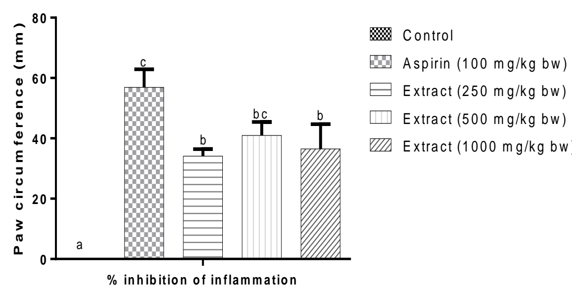

Fig. 1c: Anti-inflammatory activity of the extract (% inhibition of inflammation)

At all doses administered, the extract showed significant anti-inflammatory activity in egg albumin-induced paw inflammation in rats when compared with the control (p<0.05), as 250, 500, and 1000 mg/kg body weight of the extract inhibited inflammation in rats by 34.07±2.36%, 40.98±4.48%, and 36.51±8.17%, respectively, comparing favourably with the 56.92±6.00% inhibitory activity produced by 100 mg/kg body weight of aspirin (Table 4; Figures 1a, 1b, and 1c).

Results of in vitro antioxidant activity of the extract are presented in Tables 5, 6 ,7 and Figures 2a, 2b and 2c.

Table 5: DPPH radical scavenging activity of the extract

Groups	Doses	DPPH radical scavenging activity of the extract (%)	DPPH radical scavenging activity of vitamin C (%)
1	25µg/ml	18.90±0.52a	26.16±0.15a
2	50µg/ml	23.99±0.59b	38.82±0.55b
3	100µg/ml	56.53±0.64c	60.68±0.75c
4	200µg/ml	73.74±0.68d	78.05±0.66d

**** Mean±SEM. Means with same alphabets are not significantly different, however, means with different alphabets are significantly different P<0.05.**

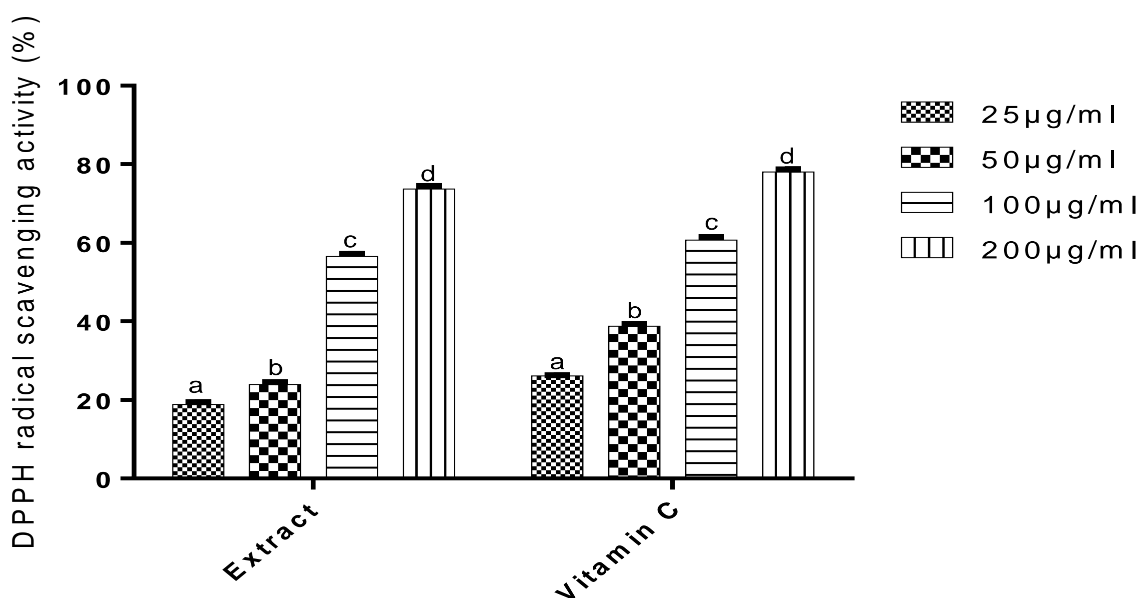

Fig 2a: DPPH radical scavenging activity (%)

Table 6: Nitric oxide radical scavenging activity of the extract

Groups	Concentration (µg/ml)	Nitric oxide radical scavenging activity of the extract (%)	Nitric oxide radical scavenging activity of vitamin C (%)
1	25	13.10±0.41a	14.60±0.36a
2	50	32.00±0.23b	29.68±0.50b
3	100	59.31±0.33c	52.62±0.29c
4	200	73.16±0.46d	67.28±0.46d

****Mean±SEM. Means with same alphabets are not significantly different, however, means with different alphabets are significantly different P<0.05.**

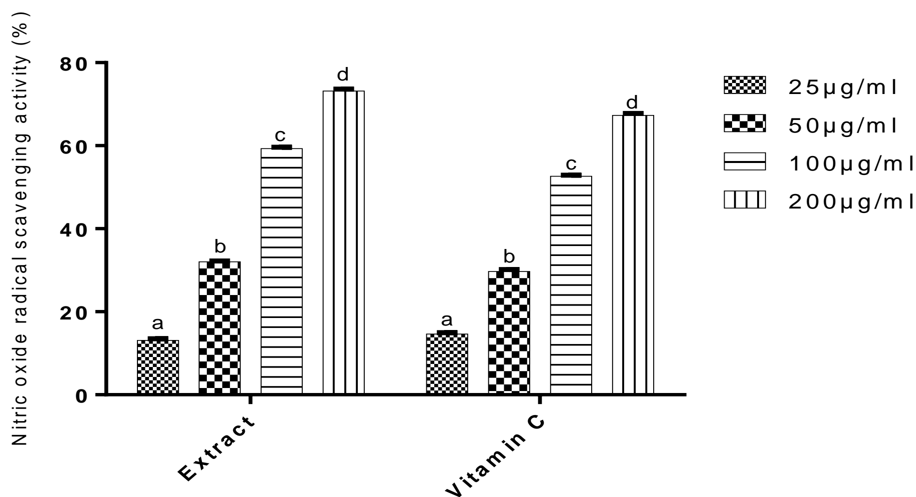

Fig.5: Nitric oxide radical scavenging activity (%)

Table 7: Ferric reducing antioxidant power (FRAP) activity of the extract

Groups	Concentration (µg/ml)	Percentage inhibition (%) for extract	Percentage inhibition (%) for vitamin C
1	25	3.91±0.27a	8.67±0.17a
2	50	11.24±0.16b	23.29±0.26b
3	100	31.14±0.44c	49.93±0.27c
4	200	53.92±0.38d	72.63±0.77d

Results are presented as Mean±SEM. Means with same alphabets are not significantly different, however, means with different alphabets are significantly different P<0.05.

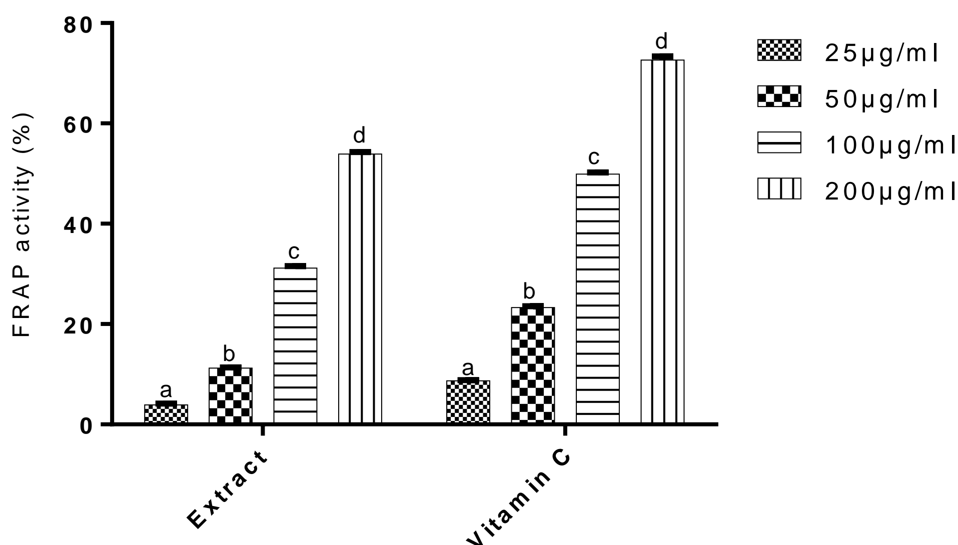

Fig. 2c: FRAP activity (%)

The extract showed significant scavenging effects on DPPH radical in an in vitro medium, producing 56.53±0.64% and 73.74±0.68% inhibitory effects at 100 and 200µg/ml, respectively, and was compared favourably with the activity of vitamin C at the same concentrations (Table 5; Fig. 2a). The same pattern of results were obtained for the nitric oxide scavenging effect of the extract (Table 6; Fig. 2b). The extract also significantly reduced the ferric ion (Fe3+) to the ferrous ion (Fe2+) following in vitro evaluation of its ferric-reducing antioxidant power potential, such that at 200 µg/ml, an activity of 53.92±0.38% was produced (Table 7; Fig. 2c).

4.0 Conclusion

The study explores the phytochemical composition, anti-inflammatory, antioxidant, toxicity, and antimicrobial activities of Sarcophrynium brachystachys, a plant traditionally used for treating bronchitis and cough. The plant contains various bioactive compounds, including flavonoids and alkaloids, indicating its potential therapeutic value. Antibacterial tests reveal the effectiveness of methanol extracts against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Toxicity assessments indicate no acute toxicity in tested doses, suggesting a favourable safety profile. Anti-inflammatory evaluations demonstrate a dose-dependent reduction in inflammation, comparable to aspirin's effects. Furthermore, the plant extract exhibits significant antioxidant properties, scavenging free radicals and potentially reducing oxidative stress-related diseases.

It is evidence from the results and findings of the study that *Sarcophrynium brachystachys* possesses a rich phytochemical profile with notable anti-inflammatory, antimicrobial, and antioxidant activities. The absence of acute toxicity and dose-dependent reduction in inflammation suggest its safety and therapeutic potential. These findings support its traditional use in herbal medicine and warrant further investigation into its clinical applications.

It is hereby recommended that future research should focus on elucidating the underlying mechanisms of the plant's therapeutic effects and conducting clinical trials to validate its efficacy and safety in humans. Additionally, studies exploring potential synergistic effects with conventional drugs and formulation development for optimal delivery are warranted.

5.0 References

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Compliance with Ethical Standards Declarations:

The authors declare that they have no conflict of interest.

Data availability

All data used in this study will be readily available to the public.

Consent for publication

Not Applicable.

Availability of data and materials

The publisher has the right to make the data public.

Competing interests

The authors declared no conflict of interest\

Funding

The author declared no source of funding

Authors' Contributions