

Phytochemical and antioxidant effect of *Spathodea campanulata* leaf extracts.

ABSTRACT

Spathodea campanulata is a medicinal plant useful in traditional medicine for the treatment and prevention of some diseases of bacterial and non microbial origins. As a result of this, it becomes very important to investigate the phytochemical and antioxidant (in vitro and in vivo) activities of the plant leaf extracts by chemical methods to ascertain its potential role in folklore medicine. 1.5kg each of *S. campanulata* leaves ground to powder was extracted separately with ethanol, methanol and petroleum ether at room temperature ($25 \pm 2^\circ \text{C}$). The leaf extracts showed qualitatively the presence of saponin, steroid, flavonoids, glycoside, alkaloids, phenol, tannin, terpenoids, phlobatanin and antraquinone. Amount of quantitative phytochemicals screened from the extracts was more in ethanol followed by aqueous, methanol and was least in petroleum ether. Valuable in vitro antioxidant activities were exhibited by the aqueous, ethanol, methanol and petroleum ether extracts in free radical (DPPH), hydroxyl scavenging activities and ferric reducing antioxidant properties. Decrease in values was observed in the in vivo antioxidant assay of glutathione and catalase levels in group of mice infected with *Salmonella typhi* for three days while there was increase in lipid peroxidation on comparison with negative control value. However improvement in enzymatic antioxidant levels of mice was observed when treated with the plant ethanol leaf extract. The recorded data in the study proposed the use of leaf extract of *S. campanulata* in traditional medicine hence its inhibition potentials and barrier to generation of free radicals.

Keywords: Medicinal plant, phytochemical, antioxidant, inhibition

1. INTRODUCTION

Medicinal plants are used for the ailment of several microbial and non-microbial originated diseases due to their valuable effects in health care. Several plants have therapeutic and pharmaceutical effects for antimicrobial, antioxidant, anti-infectious and anti-tumour activities [1, 2]. In plants, the synthesized aromatic substances (metabolites) are used as defensive weapons against predation by microorganisms, insects and herbivores. These defensive molecules give plants their medicinal values which are appreciated by human beings because of their importance in health care of individuals and communities [3]. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activities and also facilitates pharmacological studies [4]. A great number of plants worldwide showed a strong antioxidant activity and a powerful scavenger activity against free radicals [5, 6].

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, ageing process and perhaps dementias [7]. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties [8]. Many human diseases are caused or negatively affected by free radicals. The natural defence of human organs against free radicals is not always sufficient mainly due to the significant exposition to free radicals from external sources in the modern world [9], therefore, supplements from natural sources as endowed in medicinal plants and fruits are of necessity.

Plant and plant products are being used as a source of medicine due to their potent antioxidant activities, no side effects and of economic viability [10]. Flavonoids and phenolic compounds widely distributed in plants have been reported to exert

multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc [11]. Phytoantioxidants are readily available, less toxic, serving as food and the medicinal components have been suggested to reduce threat of wide range of ROS [12].

Spathodea campanulata P. Beauv species is belonging to the Bignoniaceae family. The flowers are employed as diuretic and anti-inflammatory, while the leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons. The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomach aches and diarrhoea [13].

In this study, we will investigate the phytochemicals in quality and quantity using chemical methods, in vitro and in vivo antioxidants of the plant extracts by chemical methods and animal model for its validity in folklore medicine.

2. MATERIAL AND METHODS

2.1 Collection of plant samples and extracts preparations

Healthy looking leaves of *S. campanulata* was collected from forest in Akure, Ondo State, Nigeria and identified in Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria. The voucher number of the plant AF 1504 was deposited in the herbarium. The leaves were air dried for 3 weeks at room temperature of $25 \pm 2^\circ \text{C}$ in laboratory and ground to powder with a mechanical grinder. 1.5kg each of the powders obtained was extracted separately with ethanol, methanol and petroleum ether at room temperature ($25 \pm 2^\circ \text{C}$). The resulting crude extracts were filtered with triple layered sterile muslin cloth and concentrated using a rotary evaporator (RE -52 A Union Laboratories, England) at $40\text{-}45^\circ \text{C}$. The water extract was evaporated in a

shaker water bath regulated at 50° C. The concentrated extracts were contained in plastic containers, labeled accordingly as aqueous extract (AE), ethanol extract (EE), methanol extract (ME) and petroleum ether extract (PE) and were used for the phytochemical and antioxidant activities.

2.2. Qualitative phytochemical determination

2.2.1. Alkaloids test

Five grams each of plant extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. One millilitre of the filtrate was treated with few drops of Draggendoff's reagent. Blue-black turbidity serves as preliminary evidence of alkaloids.

2.1.2. Saponin test

Five grams of each extract was shaken with distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence of the presence of saponins.

2.2.3. Tannins test

Five grams of each extract was stirred with 100 ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determines the presence of Tannins [14].

2.2.4. Phlobatannins test

Disposition of red precipitate when an aqueous extract of the test samples was boiled with 1% hydrochloric acid determines the presence of phlobatannins [14].

2.2.5. Flavonoids test

Five millilitres of diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of concentrated H₂SO₄. A yellow coloration observation determines the presence of flavonoids.

2.2.6. Cardiac glycosides (keller-killiani test).

Five grams of each extract was dissolved in 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was under laid with 1 ml concentrated H_2SO_4 . A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form just gradually spread throughout this layer [14].

2.2.7. Anthraquinones test

A total of 0.5 g of the extract was shaken with 100 ml of benzene and filtered. Five millilitres of 10% ammonia solution was added to the filtrate. The mixtures were shaken and the presence of pink, red or violet colour in the lower phase of the ammonia indicates the presence of free anthraquinones.

2.2.8. Terpenoids (Salkowski test)

Five millilitres of each extract was mixed in 2 ml of chloroform, and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.3. Quantitative Phytochemical Determination

Determination of total phenol contents

The total phenolic content of the extracts was determined using a modified Folin–Ciocalteu method [15; 16]. 200 μ l of sample was mixed with 2.6 ml of distilled water, 200 μ l of Folin–Ciocalteu’s phenol reagent was added to each tube. The content was vortexed and incubated for 5 min. Then 2 ml of 7% Na_2CO_3 was added to each tube. The content in the tube was vortexed and incubated for 2 h with intermediate shaker. The absorbance of samples was in spectrophotometer at 752nm. Total phenol contents were expressed as milligrams of Gallic acid per gram of dry extract.

Determination of total flavonoids content

The content of flavonoids was determined using quercetin as a reference compound. Stock solution (0.50 μ l) of each extract was mixed with 50 μ l of aluminium trichloride and potassium acetate. The absorption at 415nm was read after 30minutes at room temperature. Standard quercetin solution was prepared from 0.01g quercetin dissolved in 20ml of ethanol. All determinations were carried out in duplicate. The amount of flavonoids in extracts was expressed as quercetin equivalent (QE) /gram dry weight [17; 18]

Saponin determination

Spectrophotometric method of [19] was used. 2g of finely ground sample was weight into a 250ml beaker and 100ml of Isobutyl alcohol was added. The mixture was shaken in a shaker water bath for 5h to ensure uniformity in the mixture. The mixture was filtered with No 1 Whatman filter paper into 100ml beaker containing 20ml of 40% saturated solution of magnesium carbonate (MgCO_3). The mixture obtained was again filtered with filter paper to obtain a clean colourless solution. 1ml of the colourless solution was pipetted into 50ml volumetric flask, 2ml of 5% ferric chloride (FeCl_3) solution was added and made up to the mark with distilled water. It was allowed to stand for 30minutes for colour development. The absorbance was read against blank at 380nm.

Tannin determination

0.2g of finely ground sample was weighed into a 500ml sample bottle. 100ml of 70% aqueous acetone was added and properly covered. The bottles were kept in shaker water bath for 2h at 30° C. Each solution was then centrifuged and the sediment was stored in ice. 0.2ml of each solution was pipitted into test tubes and 0.8ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5mg/ml of the stock and the solution made up to 1ml with distilled water. 0.5ml of Folin-ciocateau reagent was added to both sample and standard followed

by 2.5ml of 20% Na₂CO₃ the solutions were then shaken vigorously and allowed to incubate for 40 minutes at room temperature, its absorbance was then read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared [20].

Alkaloid determination

5g sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4minutes. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is then considered as alkaloid which was dried and weighed. [21]

$$\text{Alkaloids (\%)} = \frac{W_3 - W_2}{W_1} \times 100$$

2.4. In vitro antioxidant screening

2.4.1. Ferric Reducing Antioxidant Property

The method of [9] was adopted but with little modifications. 0.1 g each aqueous, methanol, ethanol and petroleum ether extract of each (0.1 g) were dissolved in 20 ml of water and filtered. The filtrate (2.5 ml) was taken and 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of potassium ferrocyanide were added. The mixtures was incubated at a temperature of 50° C. 10% Trichloroacetic acid was added, followed by the addition of 5 ml of distilled water and 1 ml of 0.1% ferric chloride. All determinations were carried out in duplicate. The absorbance of the standard and the samples were read in spectrophotometer at 700nm wavelength against reagent blank.

2.4.2. Free Radical Scavenging

The method used was almost the same as used by [22, 23] but was modified in details. An aliquot of 0.5 ml of 0.1 mM 1, 1-diphenyl 1-2 picrylhydrazyl (DPPH) radical (Sigma Aldrich, St. Louis, USA) in the concentration of 0.05 mg/ml. Aqueous, methanol, ethanol and petroleum ether extracts each at a concentration of 20mg/ml were placed in cuvettes. The reaction was mixed at room temperature and kept for 20 minutes. Absorbance was read with a spectrophotometer at a wavelength of 520nm. The absorbance of the DPPH radical solution containing the plant extract was expressed as mg of L-ascorbic (sigma Chemical Co, St. Louis, USA) per 1 g of dry plant material. Calibration was used in such cases, where the plant extracts were replaced with a freshly prepared solution of ascorbic acid in deionised water (concentration from 0 to 1.6 mg/ml – 100 mg/ml). All determinations were replicated. The experiment was performed in triplicate. The percentage of the DPPH free radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the extract or positive control.

2.4.3. Hydroxyl radical scavenging assay

The capacity to scavenge hydroxyl radicals was measured according to the method proposed by [24] with modification. The hydroxyl radicals are generated by iron-ascorbate-EDTA- H_2O_2 , which then react with deoxyribose to form thiobarbituric acid reactive substances (TBARS). These substances yield pink chromogen at low pH while heating with trichloroacetic acid (TBA). The reaction mixture contained 4mM deoxyribose, 0.3 mM ferric chloride, 0.2 mM EDTA, 0.2 mM ascorbic acid, 2 mM H_2O_2 and various concentrations of extracts. The tubes were capped tightly and incubated for 30min at 37° C. Then 0.4 ml of 5% TBA and 0.4ml of 1% TBA

were added to the reaction mixture which was kept in a boiling water bath for 20min. The intensity of pink chromogen was measured spectrophotometrically at 532 nm against the blank sample. Ascorbic acid was used as a positive control. All tests were performed in triplicate. The hydroxyl radical scavenging activity of leaves extract was reported as % inhibition of deoxyribose degradation and calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the extract or positive control.

2.4.4. In vivo antioxidant screening

The antioxidant assay was performed with liver tissues of experimental Swiss albino mice which were anesthetized with chloroform soaked in cotton wool. The mice were dissected openly and the liver was repeatedly washed with ice-cold saline until uniformly pale and was immediately removed. The liver were homogenized with 4 volume of ice-cold 0.1 M potassium phosphate buffer (pH = 7.4) containing 1.15% (w/v) KCl. The homogenate was centrifuged at 10000 g for 60 min. The supernatant was used for the study.

2.4.4.1. Determination of Lipid peroxidation (LPO).

Determination of the antioxidant status was carried out by measuring the level of its lipid peroxidation with the method of [25]. Mice liver microsome (2 mg/ ml) was mixed with 0.1 ml of FeSO₄ (26% mM), 0.1 ml of ascorbate (0.13 mM), 0.1 ml of the sample in 150 mM KCl/Tris-HCl buffer solution (pH = 7.4). The mixture was incubated at 37°C for 60 min in a water bath; 0.75 ml of 2 M trichloroacetic acid/1.7 M HCl was added to stop the reaction, then tubes were centrifuged (4000 rpm, 10 min) and 0.5 ml of the supernatant was mixed with 0.15 ml TBA and was heated at 95°C for 10 min. The level of malondialdehyde was determined by

measuring the absorbance at 532 nm. The percent of lipid peroxidation inhibition was calculated using the Equation:

$$\%I = (A_0 - A_1 / A_0) \times 100$$

Here, A_0 is the absorbance of the control reaction; A_1 is the absorbance in the presence of the agents.

2.4.4.2. Determination of glutathione (GSH)

The amount of non enzymatic antioxidant system (reduced glutathione: GSH) was by the methods of [26]. The principle was based on the determination of reduced glutathione in each dilution by the measurement of the absorbance of colored solution developed within 5 min of the generation of Elman's reagent at 430 nm wavelength.

2.4.4.3. Determination of catalase (CAT)

The enzymatic lipid peroxidation (Catalase: CAT) was determined by the method of [27]. The principle was made use of here to monitoring the rate of enzyme catalyzed decomposition of hydrogen peroxide (H_2O_2) using Potassium tetraoxomanganateVII ($KMnO_4$). 50 microlitre of liver homogenate was added to a test tube. H_2O_2 was then added to the tube and incubated on ice for 3 min. H_2SO_4 was used to stop the reaction. Finally, $KMnO_4$ was added and the absorbance recorded at 480 nm. In this assay,

$$1 \text{ unit of enzyme activity} = \frac{K}{0.00693}$$

Where

$$K = \frac{S_0}{S_2} \times \frac{2.3}{t}$$

Where S_0 = Absorbance of standard-absorbance, S_2 = Absorbance of standard-absorbance of sample. T = Time interval. The measured activities were normalized with the protein content of each sample.

2.5. Experimental animals

Apparently healthy Swiss albino mice of between 23-35 g were used. The animals were contained in a cage and maintained under standard laboratory conditions. They were giving rodent pellets (Vital feeds) and water *ad libitum*. They were acclimatized for 2 weeks and were fasted over night with free access to water prior the experiments. The animals were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals. Before the experiment, the mice were divided into seven groups of five mice per group.

2.5.1. Administration of crude extracts of *S. campanulata*

A total of 35 mice were used. They were divided into seven groups of five mice each. While group 1 served as negative control which was only allowed to normal rat feed and water, group two served as positive control and was administered with 10^7 Cf/ml of *Salmonella typhi*. To groups 3, 4, 5, 6 and 7, previously administered with 10^7 Cf/ml of *S. typhi* for 3 days were co administered with 0.5 ml each of varying doses of **ethanol** leaf extracts at concentrations of 200, 400, 800, 1000 and 2000 mg/kg^{bw} orally as a single daily dose using wash bottle whose dispenser was directly laid on the mice throat. After 7 days of treatment each rat was sacrificed and livers were obtained for in vivo antioxidant activity.

2.6. Statistical Analysis

The results were expressed as mean $\pm$ standard deviation (SD) and were subjected to one way analysis of variance (ANOVA). The least significant difference (LSD) was performed for the pair wise mean comparisons, to determine the significant treatment dose at 95% level of confidence. Values were considered statistically significant at ($P < 0.05$).

3. RESULTS

3.1. Qualitative phytochemical screening

Qualitatively identified phytochemical in leaf extract of *S. campanulata* are saponin, steroids, glycosides, alkaloids, phenols, terpenoids, tannins, anthraquinones, phlobatannin and flavonoids. However, saponin, glycosides, phenols and flavonoids were found to be more in aqueous and ethanol extracts than methanol and petroleum ether extracts. (Table 1).

Qualitative phytochemical screening of plant extracts

Secondary Metabolites	AE	EE	ME	PE
Saponin	++	+++	++	+
Steroids	+	+	+	+
Flavonoids	++	++	++	++
Tannins	+	++	+	+
Glycosides	++	++	++	++
Alkaloids	+	++	+	+
Phenols	++	++	+	+
Terpenoids	++	++	+	+
Phlobatannin	+	+	-	-
Anthraquinones	+	+	-	-

Legend: AE = Aqueous extract, EE = Ethanol extract, ME = Methanol extract, PE = Petroleum ether extract, + = positive, - = negative

3.2. Quantitative phytochemical

Ethanol extract quantified highest amounts of phytochemical where saponin was 3.98 ± 0.3 mg/ml, flavonoid (1.32 ± 0.2 mg/ml), Tannin (0.55 ± 0.6 mg/ml), alkaloids (2.74 ± 0.4 %) and phenols (0.70 ± 0.6 mg/ml TEA). In aqueous extract

saponin was 3.92 ± 0.1 mg/ml, flavonoids with a value of 0.56 ± 0.2 mg/ml, 0.22 ± 0.4 mg/ml of tannins, 1.46 ± 0.01 % of alkaloids and 0.40 ± 0.1 mg/ml TEA of phenols. Related phytochemical values were observed between methanol and petroleum ether extracts where saponin was 1.14 ± 0.3 mg/ml and 0.83 ± 2.0 mg/ml in methanol and petroleum ether extracts respectively. Flavonoid values in methanol extract was 0.24 ± 0.1 and 0.17 ± 0.1 mg/ml in petroleum ether extract, tannin value in methanol extract was 0.18 ± 1.2 and 0.15 ± 2.4 mg/ml in petroleum ether extract; alkaloids was $0.93 \pm 1.0\%$ in methanol extract and 0.71 ± 2.1 % in petroleum ether extract, phenols values in methanol extract was 0.38 ± 0.1 and 0.25 ± 0.1 mg/ml TEA in petroleum ether extract (Figure 1).

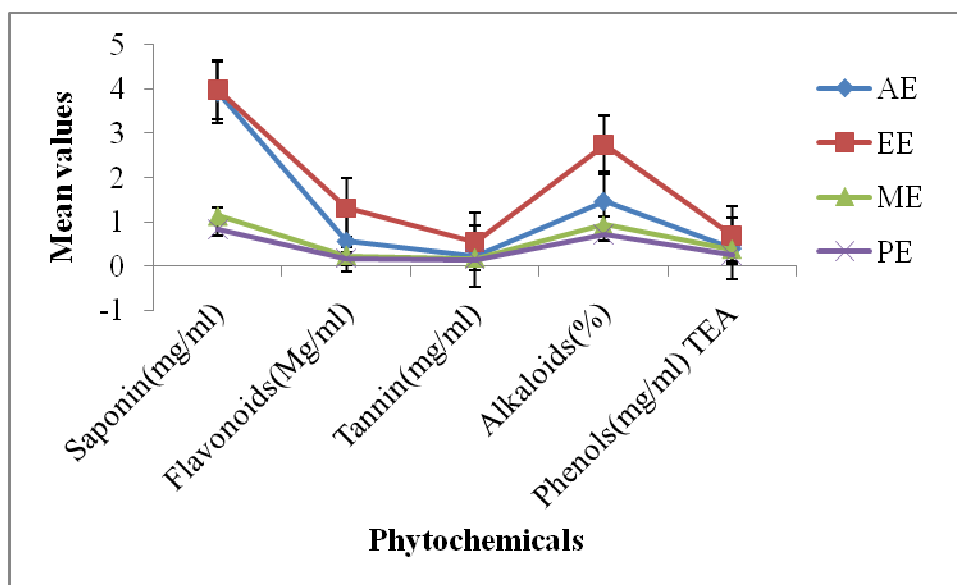


Figure 1. Quantitative estimation of secondary metabolites from the leaf extracts
 Legend: AE = Aqueous extract, EE = Ethanol extract, Me = Methanol extract, PE = Petroleum ether extract

3.3. In vitro antioxidant activity

The in vitro antioxidant assay of the aqueous, ethanol, methanol and petroleum ether extracts in free radical (DPPH) scavenging, ferric reducing antioxidant property and hydroxyl scavenging activities is presented in Table 2. Free radical scavenging (DPPH) activities in aqueous, ethanol, methanol and petroleum ether leaf extracts was 1.44 ± 0.5 , 1.57 ± 1.4 , 0.40 ± 1.4 and 0.30 ± 0.1 (mg of ascorbic acid/1g dry plant material) respectively. Ferric reducing antioxidant property in that order was 1.19 ± 0.4 , 1.69 ± 0.18 , 1.74 ± 0.5 and 2.84 ± 1.8 (mg of ascorbic acid/1g dry plant material) respectively. The hydroxyl scavenging activity of the aqueous, ethanol, methanol and petroleum ether extracts was 0.65 ± 0.90 , 0.43 ± 0.50 , 0.89 ± 0.54 and 0.87 ± 0.42 (mg of ascorbic acid/1g dry plant material) respectively.

346 **Table 2.** In vitro antioxidant

Plant Name	FRAS DPPH (mg of ascorbic acid/1g dry plant material)				FRAP (mg of ascorbic acid/1g dry plant material)				Hydroxyl radical scavenging assay			
	AE	EE	ME	PE	AE	EE	ME	PE	AE	EE	ME	PE
<i>S. campnulata</i>	1.44 ± 0.5	1.57± 1.4	0.40 ± 1.4	0.30 ± 0.1	1.19 ± 0.4	1.69 ± 0.18	1.74 ± 0.5	2.48 ± 1.8	0.65± 0.9	0.43 ± 0.50	0.86 ±0.54	0.87±0.42

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348 Legend: AE = Aqueous extract, EE = Ethanol extract, Me = Methanol extract, PE = Petroleum ether extract

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3.4. In vivo antioxidant activity

Table 3 illustrates the *in vivo* efficacy of *S. campanulata* ethanol leaf extracts on the enzymatic antioxidant in mice. A significant LPO level increase ($P<0.05$) in the positive control was observed when compared with the negative control values. However, decrease in LPO level was observed in bacterial/extract treated mice alongside extract concentrations. While this decrease in LPO values, increased values were observed in GSH and CAT levels in the extract treated groups of mice alongside extract concentrations.

Table 3. *In vivo* antioxidant assay

Group	LPO($\mu\text{M/g}$)	GSH($\mu\text{M/g}$)	CAT($\mu\text{M/g}$)
Control (-)	98.94 $\pm$ 6.66	34.76 $\pm$ 1.40	70.58 $\pm$ 6.66
Control(+)	138.40 $\pm$ 2.10	22.34 $\pm$ 2.00	47.36 $\pm$ 3.76
200mg/kg ^{bw}	128.76 $\pm$ 1.16	25.19 $\pm$ 1.76	48.10 $\pm$ 1.01
400mg/kg ^{bw}	127.63 $\pm$ 1.14	26.30 $\pm$ 2.14	48.56 $\pm$ 2.44
800mg/kg ^{bw}	124.71 $\pm$ 2.65	26.46 $\pm$ 2.11	53.38 $\pm$ 1.13
1000mg/kg ^{bw}	124.03 $\pm$ 3.14	27.19 $\pm$ 1.36	55.24 $\pm$ 1.41
2000mg/kg ^{bw}	122.01 $\pm$ 1.92	28.63 $\pm$ 1.31	58.27 $\pm$ 1.34

4. Discussion

Novel traditional technologies such as infusion, decoction and concoction with water solutions to high polar solvents such as ethanol and methanol have been used to improve herbal therapy in traditional medicines. Practices in using one or more of the mentioned methods have helped in providing lasting solutions for prevention and cure of deleterious diseases in the traditional way.

4.1 Qualitative phytochemical screening

From the phytochemical results, it is evident that extraction of bioactive compounds from leaves of *S. campanulata* have notable potentials to strengthen the available methods to address health problems in urban and rural areas of developing countries where orthodox or modern medicine are not afforded by many individuals. The chemical methods employed for the qualitative secondary metabolites identified saponin, alkaloids, phenols and flavonoids among others in the plant's leaf. However, these phytochemical have been reported to be highly of therapeutic importance [9; 28]. Medicinal plants management in quality and quantity of administration could provide effective health care as a challenge under the best economic circumstance. In the world's poorest countries, where infectious diseases are rife and resources limited, such challenges can assume over whelming proportions, hence the resurgence in the use of herbal preparations to treat diseases [29]. Therefore, plants evolutions in phytochemical and antioxidant properties are important values in prediction of potential drugs or herbal preparation able to be effective in management of diseases most especially by those that can accept their innumerable values for alternative therapy.

4.2. In vitro antioxidant activity

The antioxidant screening result of the extracts showed that it has appreciable amount of bioactive compounds. This is in correlation with some studies elsewhere that medicinal plants used in traditional medicine and healing are one of the sources of antioxidants. The antioxidant activities of the test plant aqueous, ethanol, methanol and petroleum ether leaf extracts investigated with DPPH free radical scavenging assay showed valuable results which contributed to the interest of screening further the plants extract for more medical related indices to evaluate its therapeutic value. Evidence has it that DPPH free radical scavenging by antioxidants is due to their hydrogen denoting ability [30]. The antioxidant

400 properties determined showed that the solvents were able to extract substances
401 with antioxidants potency. However, the petroleum extract was less efficient in the
402 extraction of the substances with antioxidants to other employed solvents. The
403 reducing power of the extracts increased with ethanol extract than other solvents
404 and variations in reducing power ability was noticed among the extracts. Hence the
405 leaf extracts of *S. campanulata* exhibited exciting free radical scavenging activity;
406 the plant extracts may be useful in reducing harmful effects of free radicals in the
407 maintenance of health and management of cancer, aging process, Parkinson's
408 diseases and perhaps dementias.

409 Ferric reducing antioxidant property (FRAP) is used for the determination of
410 reducing power of various samples which is shown by colour change of test
411 samples solutions from yellow to blue and green in proportionate to the reducing
412 power of various samples. The presence of antioxidants in the test sample solutions
413 would result in reducing Fe^{3+} to Fe^{2+} by denoting an electron by the extracts. The
414 extracts with reducing power revealed that they are electron donors, reduced the
415 oxidized intermediates and act as primary antioxidant substrates [31]. Colour
416 change from yellow to blue and green was dependant on the reducing power of
417 each compound present and the chemical solvents used. This reaction of ferric
418 form in ferric tripyridyl triazine complex changes to ferrous form showing the
419 various shades of green and blue which were of different reaction time and colour.
420 The changes in colour were however observed by measuring at wavelength of
421 500nm. Hence the compounds present in the extracts differ in types and quantity;
422 different results were of course envisaged. The compounds with reducing potential
423 will react with potassium ferrocyanide Fe^{3+} to form potassium ferrocyanide Fe^{2+} ,
424 which then reacted with ferric chloride to form ferric ferrous complex that is green
425 in colour. The ferric reducing potential of the plant extracts could be due to their
426 reducing power ability by the bioactive compounds in the extracts. Similar result of

ferric reducing potentials of plant extracts was recorded by [32, 33]. The FRAP results further confirmed the antioxidant properties of the plants extracts observed in the DPPH assay. The correlation between reducing power and the DPPH values of the plant extracts in this study is assumed to be due to the same mechanism on which the employed methods of antioxidant assay rely. From the correlation in results, it could be justified that the plant extracts tested can act as electron donors and react with free radicals conversion to more stable products, thus breaking the radical chain reaction and preventing cardiovascular or carcinogenic related diseases.

4.3 In vivo antioxidant activity

Administration of *S. typhi* at dose concentration of 10^7 CfU/ml daily for three days showed negative reactions in the *in vivo* antioxidant assay on lipid peroxidation (LPO), Glutathione (GSH) and Catalase (CAT). Effects proving these results are the higher value observed in the positive control than the negative control in LPO and the lesser values in GSH and CAT in the negative control than the positive control. In such process where higher value of LPO in positive control than negative control, non-enzymatic lipid peroxidation or enzymatic peroxidation would have occurred because when lipids are oxidized without the release of energy, unsaturated lipids go rancid as a result of deterioration when directly reacted with molecular oxygen catalysed by free radicals which must have been generated in the mice. The increase in lipid peroxidation in the positive control and bacteria/extract treated mice than the negative control group could likely be the increase in free radicals developed during abnormal metabolic activities and the imbalances in antioxidant defense causing an oxidative stress which could have decreased glutathione level leading to oxidative damage and resulting to an increase in lipid peroxidation level. This is in agreement with [34]. Increased lipid peroxidation is a highly destructive process that induces a plethora of structural

and functional alterations of cellular membranes and involves oxidation of fatty acids [35]. Though the reduction in values for the period of pre-treatment with extracts were not same with the negative control, values were comparable, signifying that if pre-treatment is extended the tendency of values becoming equal with the negative control is predictable..

Glutathione (GSH) is the major compound in the intracellular redox status regulation and it is considered as an important cofactor in many metabolic reactions [36]. The observed elevation of GSH in liver of mice after extract pre-treatment for seven days may be due to a compensatory response induced by imbalance in the redox status of the cell as a result of excessive hydrogen peroxide (H_2O_2) production and progressive decrease of glutathione peroxide. This result is in agreement with [37, 32, 33] who observed that enzyme activity of glutathione peroxide (GSH-Px) in liver was significantly increased in extract treated mice after injury with toxic substances. Such inhibitory effect could underlay the efficacy of the leaf extracts of *S. campanulata* in this study. Increase in GSH value in the bacteria/extract treated mice was also on dose dependant. This is in agreement with the results obtained by [38-40] who assumed that GSH correlates in a time and dose dependant fashion with an increase in levels of extracts.

Catalase (CAT) is metallo proteins and accomplished their antioxidant functions by enzymetically detoxifying the peroxide (OH , H_2O_2) and superoxide anion. The CAT increase and LPO decrease in the bacteria/extract treated group, is an indication of effectiveness of the plant antioxidant. This manifestation is evident because catalase converts harmful hydrogen peroxide into water and oxygen which in that order protects the liver tissue from highly reactive hydroxyl radicals able to be generated by *S. typhi* infection at a concentration of 10^7 CfU/ml for the three days induction. The reduction in the activities of this enzyme as observed in the positive control mice may results in number of deleterious effects due to

accumulation of highly toxic metabolites and hydrogen peroxide on *S. typhi* infection. This is liable to induce oxidative stress in the cells (41). The co-administration of the extracts in various concentrations increased the activities of catalase in mice preventing the accumulated excess free radicals thereby protecting the liver from intoxication induced by *S. typhi* infection.

The in vivo antioxidant assay showed that *S. typhi* at concentration of 10^7 Cfu/ml induced oxidative stress in mice by decreasing endogenous antioxidant defence system of ferric reducing with an increase in lipid peroxidation in liver which is likely liable to cell death in the mice liver. Conclusively, it is evidenced that the leaf extracts of *S. campanulata* could be of barrier to generation of free radicals. The appreciable phytochemicals; in vitro and in vivo antioxidants status has established the potential value this plant could play in medicine. It is therefore suggested that its employment in traditional medicine be encouraged.

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