

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*) of the plant leaf extracts to ascertain its importance in folklore medicine. The leaf extract showed qualitatively the presence of saponin, steroid, flavonoids, glycoside, alkaloids, phenol, tannin, terpenoids, phlobatanin and antraquinone. Valuable 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 while there was increase in lipid peroxidation on compare with control values. However improvement in enzymatic antioxidant levels of mice was observed when treatment with the plant extracts. 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, neurodegeneration, 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].

2. MATERIAL AND METHODS

2.1 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.

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 Dragendoff'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 H_2SO_4 . 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

Phenol was determined by the method of [15], Flavonoids by the methods of [16, 17], Saponin was determined based on the criteria of [18], Tannins by the method of [19], Alkaloids by the method of [20]

2.4. In vitro antioxidant screening

2.4.1. Ferric Reducing Antioxidant Property

The method of [21] 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 picrylhrazyl (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). This 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.

2.7. In vivo antioxidant screening

The antioxidant assay was performed with liver tissues of experimental animals and evaluation of the antioxidant status was carried out by measuring the level of its lipid peroxidation [25] and the amount of enzymatic (Catalase: CAT) and nonenzymatic antioxidant system (reduced glutathione: GSH) by [26,27] methods respectively.

2.8. 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.

2.9. 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 CfU/ml of *Salmonella typhi*. To groups 3, 4, 5, 6 and 7, previously administered with 10^7 CfU/ml of *S. typhi* for 3 days were co administered with 0.5 ml each of varying doses of the 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.

2.10. 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

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).

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

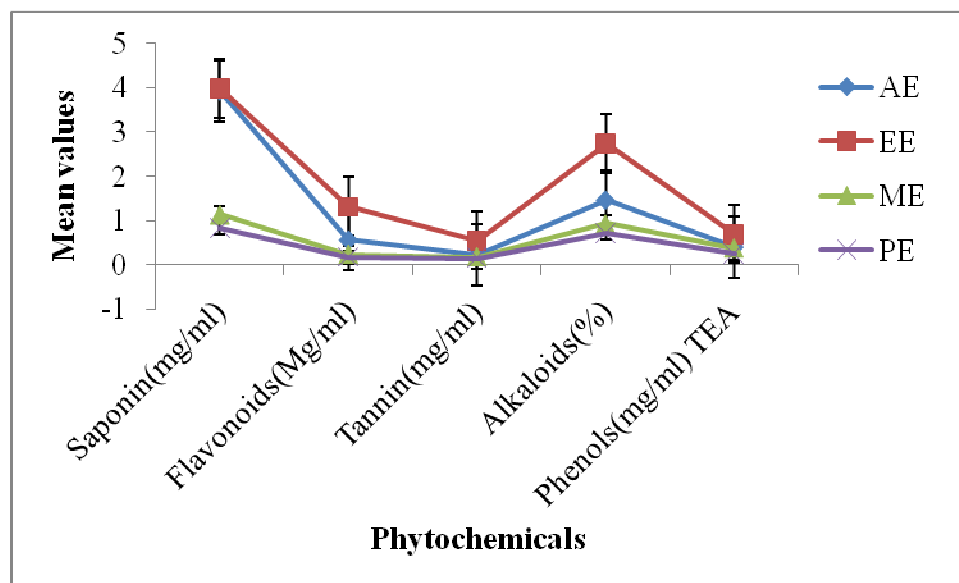
Legend: AE = Aqueous extract, EE = Ethanol extract, ME = Methanol extract, PE = Petroleum ether extract, + = positive, - = negative

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,

197 flavonoids with a value of 0.56 ± 0.2 mg/ml, 0.22 ± 0.4 mg/ml of tannins, 1.46 ± 0.01 % of
 198 alkaloids and 0.40 ± 0.1 mg/ml TEA of phenols. Related phytochemical values were observed
 199 between methanol and petroleum ether extracts where saponin was 1.14 ± 0.3 mg/ml and $0.83 \pm$
 200 2.0 mg/ml in methanol and petroleum ether extracts respectively. Flavonoid values in methanol
 201 extract was 0.24 ± 0.1 and 0.17 ± 0.1 mg/ml in petroleum ether extract, tannin value in methanol
 202 extract was 0.18 ± 1.2 and 0.15 ± 2.4 mg/ml in petroleum ether extract; alkaloids was $0.93 \pm$
 203 1.0% in methanol extract and 0.71 ± 2.1 % in petroleum ether extract, phenols values in
 204 methanol extract was 0.38 ± 0.1 and 0.25 ± 0.1 mg/ml TEA in petroleum ether extract (Figure 1).

205



206

207

208 **Figure 1.** Quantitative estimation of secondary metabolites from the leaf extracts

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

211

212 3.3. In vitro antioxidant activity

213 The in vitro antioxidant assay of the aqueous, ethanol, methanol and petroleum ether extracts in
 214 free radical (DPPH) scavenging, ferric reducing antioxidant property and hydroxyl scavenging
 215 activities is presented in Table 2. Free radical scavenging (DPPH) activities in aqueous, ethanol,
 216 methanol and petroleum ether leaf extracts was 1.44 ± 0.5 , 1.57 ± 1.4 , 0.40 ± 1.4 and 0.30 ± 0.1
 217 (mg of ascorbic acid/1g dry plant material) respectively. Ferric reducing antioxidant property in
 218 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
 219 plant material) respectively. The hydroxyl scavenging activity of the aqueous, ethanol, methanol
 220 and petroleum ether extracts was 0.65 ± 0.90 , 0.43 ± 0.50 , 0.89 ± 0.54 and 0.87 ± 0.42 (mg of
 221 ascorbic acid/1g dry plant material) respectively.

222

223

224 **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

225

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

227

228

229

230

231

232

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 $\pm$ 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

248 traditional medicines. Practices in using one or more of the mentioned methods have helped in
 249 providing lasting solutions for prevention and cure of deleterious diseases in the traditional way.
 250 From the phytochemical results, it is evident that extraction of bioactive compounds from leaves
 251 of *S. campanulata* have notable potentials to strengthen the available methods to address health
 252 problems in urban and rural areas of developing countries where orthodox or modern medicine
 253 are not afforded by many individuals. The chemical methods employed for the qualitative
 254 secondary metabolites identified saponin, alkaloids, phenols and flavonoids among others in the
 255 plant's leaf. However, these phytochemical have been reported to be highly of therapeutic
 256 importance [9; 28]. Medicinal plants management in quality and quantity of administration
 257 could provide effective health care as a challenge under the best economic circumstance. In the
 258 world's poorest countries, where infectious diseases are rife and resources limited, such
 259 challenges can assume over whelming proportions, hence the resurgence in the use of herbal
 260 preparations to treat diseases [29]. Therefore, plants evolutions in phytochemical and antioxidant
 261 properties are important values in prediction of potential drugs or herbal preparation able to be
 262 effective in management of diseases most especially by those that can accept their innumerable
 263 values for alternative therapy. The antioxidant screening result of the extracts showed that it has
 264 appreciable amount of bioactive compounds. This is in correlation with some studies elsewhere
 265 that medicinal plants used in traditional medicine and healing are one of the sources of
 266 antioxidants. The antioxidant activities of the test plant aqueous, ethanol, methanol and
 267 petroleum ether leaf extracts investigated with DPPH free radical scavenging assay showed
 268 valuable results which contributed to the interest of screening further the plants extract for more
 269 medical related indices to evaluate its therapeutic value. Evidence has it that DPPH free radical
 270 scavenging by antioxidants is due to their hydrogen denoting ability [30]. The antioxidant

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

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

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 is oxidized without the release of energy, unsaturated lipid go rancid as a result of deterioration when directly reacted with molecular oxygen on catalyses 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

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

321 Glutathione (GSH) is the major compound in the intracellular redox status regulation and it is
322 considered as an important cofactor in many metabolic reactions [36]. The observed elevation of
323 GSH in liver of mice after extract pre-treatment for seven days may be due to a compensatory
324 response induced by imbalance in the redox status of the cell as a result of excessive hydrogen
325 peroxide (H_2O_2) production and progressive decrease of glutathione peroxide. This result is in
326 agreement with [37, 38, 31, 32] who observed that enzyme activity of glutathione peroxide
327 (GSH-Px) in liver was significantly increased in extract treated mice after injury with toxic
328 substances. Such inhibitory effect could underlay the efficacy of the leaf extracts of *S.*
329 *campanulata* in this study. Increase in GSH value in the bacteria/extract treated mice was also on
330 dose dependant. This is in agreement with the results obtained by [39-41] who assumed that
331 GSH correlates in a time and dose dependant fashion with an increase in levels of extracts.

332 Catalase (CAT) is metallo proteins and accomplished their antioxidant functions by
333 enzymetically detoxifying the peroxide (OH , H_2O_2) and superoxide anion. The CAT increase and
334 LPO decrease in the bacteria/extract treated group, is an indication of effectiveness of the plant
335 antioxidant. This manifestation is evident because catalase converts harmful hydrogen peroxide
336 into water and oxygen which in that order protects the liver tissue from highly reactive hydroxyl
337 radicals able to be generated by *S. typhi* infection at a concentration of 10^7 CfU/ml for the three
338 days induction. The reduction in the activities of this enzyme as observed in the positive control
339 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 (42). 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.

REFERENCES

- [1]. Akroum S. Dalila S, Kirrich L. Antimicrobial, Antioxidant, Cytotoxic activities and Phytochemical screening of some Algerian plants. Eur J Sci Res 2009; 31: 289-295.
- [2]. Amjad K, Dhia SK, Abeer K. Genetic relationship among salvia species and antimicrobial activity of their crude extracts against pathogenic bacteria Asian Jour Plant Sci 2005; 4(5): 544-49.
- [3]. Akharaiyi FC, Boboye B. Antibacterial and phytochemical evaluation of three medicinal plants. J Nat Prod 2010; 3: 27-34.
- [4] Manna A, Abalaka ME. Preliminary screening of the various extracts of *Physalis angulata* for antimicrobial activities. Spectra Journal 2000; 7: 119-125.
- [5]. Farnsworth F, Akerels NR, Bingel O, Soejarto AS, Guo DD, Bull Z. W.H.O.1985; 63:965.

- 364 [6]. Katalynic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for
365 antioxidant capacity and total phenols. Food Chemistry 2006; 94:550.
- 366 [7]. O. Polterait, "Antioxidants and free-radical scavengers of Natural origin," *Curr Org Chem*,
367 vol. 1, pp. 415-440, 1997.
- 368 [8]. Nakayoma J, Yamada M Suppression of active oxygen-induced cytotoxicity by flavonoid.
369 Biochem. Pharmacolog 1995; 45:265-267.
- 370 [9] Auudy BF, Ferreira F, lasina L, Lafon F, Arredondo F, Dajas R, Tripathi PC. Screening of
371 antioxidant activity of three Indian medicinal plants, traditionally used for the management
372 of neurodegenerative diseases. J Ethnopharmacol 2003; 84: 131-138.
- 373 [10]. Miller AL. Antioxidant flavonoids: structure, function and clinical usage. Alt Med Rev
374 1996; 1: 103-111.
- 375 [11]. Meena MK, Kalpesh G, Kori C M., Sharma IS, Nema RA. Jain RK, Jain CP In-vitro
376 antioxidant properties of leaves of Cassia Grandis Linn. Asain J Pharm Clin Res 2009; 2(1): 46-
377 49.
- 378 [12]. Yoganarasmiha SN. Medcinal plants of India, *Karnataka* 2000; 442.
- 379 [13].Trease, GE, Evans WC. Pharmacognsy 11th edn. Brailliar Tiridel Canada" Macmillian
380 Publishers 1989.
- 381 [14].Muchuweti M, Ndhlala A, Kasiamhuru A. Analysis of phenolic compounds including
382 tannins, gallotannins and flavonols of *Uapaca kirkiana* fruit. Food Chem 2006; 94: 415-419.
383
- 384 [15]. Zhishen J, Menghheng T, Jianming W. The determenation of flavonoid contents in
385 mulberry and their scavenging effects on superoxide radicals. Food Chem 1999; 64:. 555-559.
- 386 [16]. Ohkawa H, Oishi N, Yagi K. Assay for lipid peroxides in animal tissue by

- thiobarbituric acid reaction. Anal Biochem 1979; 5: 351-358.
- [17]. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude
extracts of some homeostatic plants in Edo and Delta States of Nig. Glob J Pure Appld Sci 2000;
86: 2003-2008.
- [18]. Van-Burden TP, Robinson WC. Formation of Complexes between protein and tannin acid.
J Agric Food Chem 1981; 1:77.
- [19]. Harbone JB, Williams CA. Advances in flavonoid research since 1992.” *Phytochem*,
Oxford 200; 55: 481-504.
- [20]. Buricova L. Reblova Z. Czech medicinal plants and possible sources of antioxidants. Czek
Jour Food Sci 2008: 26: 132-38.
- [21]. Dornam HJ, Bachmayer O, Kosar M, HilYunen R. Antioxidant properties of aqueous
extracts from selected Laniaceae species grown in Turkey. J Agric Food Chem 2004; 52:. 762-
770.
- [22]. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple “test-tube”
assay for determination of rate constants for reactions of hydroxyl radicals. Analytical
Biochemistry 1987; 165: 215–219.
- [23] Kim DK. Ock M, Young Hae-Yeon, Chang YL. Quantification of polyphenolics and their
antioxidant capacity in fresh plums. J Agric Food Chem 2003; 51: 6509-6515.
- [24]. Ibanez E, Kubatova A, Senorans FJ, Cavero S, Regilero G, Hawthorne SB. Subcritical
water extraction of antioxidant compounds from rosemary plants. J. Agric Food Chem 2003; 51:
375-382.
- [25]. Luck H. Methods of Enzymatic Analysis. Bergmeyer HV, vol. III, Academic press,
New York 1963; 886-888.

- 410 [26]. Ellman GL, Tissue sulphhydryl groups. Arch Biochem Biophys 1959; 82: 70-72.
- 411 [27]. De N, James NE, Antimicrobial spectrum of extracts of *Ocimum gratissimum* L (Basil) and
- 412 *Xylopia aetiopica* A. Rich (Dunal) Nig J Appl Sci 2007; 11: 65-75.
- 413 [28]. El-Mahmood MA, Daughari J, Chanji FJ. *In vitro* antimicrobial activities of *Nauclea*
- 414 *latifolia* and *Daniella oliveri*. Sci Res Ess. 2008; 3: 102-105.
- 415 [29]. Hareesh AR, Rajesh MG, Harsha MG, Ahmed R, Satish kumar BP, Dinesha R, Irfan AM,
- 416 Thammanna Gowda SS. In vitro Antioxidant and Free Radicals Scavenging Activity of Flower
- 417 of *Spathodea campanulata* P. Beauv. Int J Pharm Sci 2010; 2(2): 508-512.
- 418 [30]. Chanda, S, Dave R. In Vitro models for antioxidant activity evaluation and some medicinal
- 419 plants possessing antioxidant properties: an overview. African Journal of Microbiology Research
- 420 2009; 3(13): 981–996.
- 421 [31] Panchal CV, Jyotiram A, Sawale B, Poul N, Khandelwal KR. Hepatoprotective activity of
- 422 *Lageneria Sicerarla* (MOLINA) Standley fruits againstparacetamol induced hapatotoxicity in
- 423 mice. Int J Pharm Sci Res 2013; 4(1): 371-377.
- 424 [32]. Subramanion LJ, Azlan A, Yeng C, Sreenivasan Sasidharan. Antioxidant Activity and
- 425 Hepatoprotective Potential of *Polyalthia longifolia* and *Cassia spectabilis* Leaves against
- 426 Paracetamol-Induced Liver Injury. Evidence-Based. Compl Alt Med 2012; Article ID 561284,
- 427 10 pages doi:10.1155/2012/561284
- 428 [33]. Palanisamy P, Yagneswara RY, Jawahar F, Ganesan S. Oxidative Stress and Cardiac
- 429 Biomarkers in Patients with Acute Myocardial Infarction. Eur J Sci Res, 2009; **27(2)**: 275-285.
- 430 [34]. Gupta VK, Mallika V, Yashik G, Shrivastav DK. Oxygen derived free radicals in clinical
- 431 context. Indian J Clin Biochem 1992; 7(3): 3-10.

- 432 [35]. Van Bladeren PJ. Glutathione conjugation as a bioactivation reaction. Chem-Biol Interact
433 2000; 129: 61-76.
- 434 [36]. Campolo J., De Maria R, Caruso R, Accinni R, Turazza F, Parolini M, Roubina E, De
435 Chiara B, Cighetti G, Frigerio M, Vitali E, Parodi O, Blood glutathione as independent marker of
436 lipid peroxidation in heart failure. Inter J Cardiol 2006; 117(1): 45-50.
- 437 [37]. Girish C, Koner CBC, Jayanthi S,, Rao KR, Rajesh KB,. Pradhan SC. Hepatoprotective
438 activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. Indian J
439 Med Res 2009; 129: 569-578.
- 440 [38]. Pamela M. The effects of stress and aging on glutathione metabolism. Ageing Res. Rev
441 2005; 4: 288-314.
- 442 [39]. Sanapala AK, Kumar KE. Hepatoprotective Activity of Aqueous Fruit Extract of *Coccinia*
443 *indica* against Paracetamol Induced Hepatotoxicity in rats, International Journal of
444 Research in Pharmaceutical and Biomedical Sciences 2013; 1(1): 179-182
- 445 [40]. Maheswari C, Maryammal R, Venkatanarayanan R. Hepatoprotective activity of
446 “*Orthosiphon stami-neus*” on liver damage caused by paracetamol in rats. Jordan J
447 Biological Sciences 2008; 1(3): 105-108.
- 448 [41]. Parmar SR, Vashrambhai PH, Kalia K. Hepatoprotective activity of some plants extract
449 against paracetamol induced hepatotoxicity in rats. Journal of Herbal Medicine and
450 Toxicology 2010; 4(2): 101-106.
- 451 [42]. Pauli-Magnus C, Stieger B, Meier Y, Kullak-Ublick GA, Meier PJ. J. Hepatol 2005;
452 43(2): 342-357.
- 453