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Antibacterial, Phytochemical and Antioxidant Activities of the Leaf Extracts of *Gliricidia sepium* and *Spathodea campanulata*

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Abstract: The methanol, ethanol and petroleum ether soluble crude and fractions extract of *Gliricidia sepium* and *Spathodea campanulata* leaf were examined for antibacterial activities, phytochemicals and possible sources of antioxidant. The antibacterial activity of the crude and fractions were carried out against nine clinical bacteria isolates using the agar well and disc diffusion methods respectively. *S. campanulata* crude and fractions extract possessed higher inhibitory potencies than *G. sepium* extracts. The antioxidant DPPH test was performed where appreciable level of both ferric reducing antioxidant properties and free radical scavenging activities were of better expression in *S. campanulata* than *G. sepium*. However, *G. sepium* extract has the highest concentration of phenol with a value of 1.7mg/ml and flavonoid content with a value of 0.46mg/ml. The highest phenol and flavonoids contents in *S. campanulata* were 1.2mg/ml and 0.56mg/ml respectively. The phytochemical compounds of the extracts such as phenols, alkaloids and saponin could have shown the high value in antibacterial assay. The crude ethanol extracts of the two plants was more potent in inhibiting the organisms, followed by the methanol extract and was least with the petroleum ether extract. *G. sepium* fractions extract inhibited the organisms with halos between 0-48.7mm while *S. campanulata* inhibited the organisms with halos between 0-49.3mm.

Key words: Antibacterial · Clinical · Antioxidant · Plant extracts · Phytochemical · Nigeria

INTRODUCTION

Medicinal plant is defined as any plant with one or more of its organs containing substance that can be used for therapeutic purpose or which can be used as precursors for the synthesis of antimicrobial drugs [1] antioxidant, anti-infectious and anti-tumor activities [2]. Plants are presently the sources of medicines for many people of different age in many countries of the world, where diseases are treated primarily with traditional medicines obtained from plants. The modern pharmaceutical industry itself still relies largely on the diversity of secondary metabolites in plants and secondary metabolites of which at least 12,000 have been isolated; a number estimated to be less than 10% of the total [3].

The synthesized aromatic substances (metabolites) are used by plants as defensive weapon against predation by microorganisms, insects and herbivores. The search for plants with antimicrobial has gained increasing important in recent years due to the development of antimicrobial drug resistance and often the occurrence of undesirable side effect of some antibiotics [4, 5]. With the recent advent of ever-increasing resistant bacteria, there has been a corresponding rise in the universal demand for natural antibacterial therapeutics [6]. Although pharmacological industries and researchers have produced a good number of antibiotics in the last three decade, resistance to these drugs by microorganisms is increasingly high.

Herbal medicines have been widely used and form an integral part of primary health care of many countries [7-11] and may constitute a reservoir of new antimicrobial substances to be discovered. However, the last few years have seen a major increase in their use in the developed world [12, 13]. Nearly all culture and civilizations from ancient times to the present day have depended fully or partially on herbal medicines because of their effectiveness, affordability, low toxicity and acceptability [14]. Antioxidant is a molecule capable of inhibiting the

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oxidation of other molecules. Diets of fruits and vegetables which are high in antioxidants, promote health and reduce the effects of aging, however antioxidant vitamin supplementation has no detectable effect on the aging process, so the effects of fruit and vegetables may be unrelated to their antioxidant contents [15, 16].

Spathodea campanulata P. Beav species popularly known as African tulip tree belong to the Bignoniaceae family. This plant has many uses in folk medicine such as the leaves being employed against kidney diseases, urethra inflammations and as antidote against animal poisons. The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomach aches and diarrhoea [17].

Gliricidia sepium is a plant valued for its ornamental, medicinal and insect repellent purposes in Nigeria. Farmers also use this plant to improve soil fertility and stakes for yam.

MATERIALS AND METHODS

Collection of Plant Samples: *G. sepium* and *S. campanulata* plants were collected from forest in Akure metropolis of Ondo State, Nigeria. The plants were identified by Dr Oyun, M.B. in Department of Forestry and Wood Technology, Federal University of Technology, Akure, Ondo State. The voucher number of the plants are (AF 1503) *G. sepium* and (AF 1504) for *S. campanulata* were deposited in the herbarium of the Department of Forestry and Wood Technology, Federal University of Technology, Akure. The leaves were sorted, air-dried for two weeks at room temperature (25±2°C) and pulverized with a grinder into smooth powder for solvent extractions.

Bacterial Cultures: The bacteria isolates used in this study are clinical isolates from human septic wound, feaces and urine. The clinical isolates include: *Pseudomonas aeruginosa* (wound), *Serretia marcescens, Escherichia coli* (urine), *Staphylococcus aureus* (wound), *Klebsiella pneumoniae* (urine), *Bacillus cereus* (wound), *Proteus mirabilis* (feaces), *Salmonella typhi* (feaces) and *Enterococcus feacium* (feaces). The organisms were maintained on nutrient agar slants at 4°C and purity of the organisms was checked at regular intervals by plating.

Extraction and Preparation of Test Solutions: 1.5kg powder of each plant part (leaf) was extracted at room temperature (25±2°C) with ethanol, methanol and petroleum ether for 24 hours. The extracts were filtered into a clean Pyrex beaker (250ml capacity), labelled and allowed to evaporate by exposure at room temperature. The dried crude extracts labelled appropriately as ethanol, methanol and petroleum ether extracts of *G. sepium* and *S. campanulata* were used for the assay.

Column Chromatography and Fraction Extracts: One gram each of the crude extracts was subjected to column chromatography (30x8 cm column) using 60g of silica gel 60 F_{254} (Merck, 0.020 mm thickness). The column was successfully eluted first with petroleum ether and then with chloroform: methanol (40:1) and finally with 100% methanol. Each 100ml eluent was collected into a round bottom flask (250ml) capacity and distilled to obtain fractions. The fractions collected were numbered. Thin Layer Chromatography (TLC) analyses were carried out on pre-coated plates (20x20 cm) with Merck silica gel 60 F_{254}, 0.2 mm thickness using ethyl acetate: methanol (40:1), butanol: water (1:1), chloroform: pyridine (1:1), methanol: ammonium hydroxide (200:3), acetate: pyridine: water (5:1:4), chloroform: hexane: ethanol: acetic acid (5:4:1:1) as the mobile phases. The spots and developed fractions on TLC plates were visualized under a UV light after spraying with methanolic solution of ferric chloride (50g/100ml). All solvents chemicals used were product of BDH chemicals (Analar) Ltd, Poole England. The fractions showing the same TLC characteristics were pulled together. This was also confirmed by measuring their absorbance with spectrophotometer (Pharmacia, England). All the solvent chemicals were Analar grade. Altogether, fourteen fractions were obtained from *G. sepium* and were numbered from 1-14. *S. campanulata* extract gave twelve fractions and were labelled 15-26. From *G. sepium* fractions 1-4 were same and was pulled together and labelled as GF1; 5-7 were same and was pulled together and labelled as GF2; 8-13 were same and was labelled as GF3 while fraction 14 was labelled GF4. In *S. campanulata*, 15-17 were same and was labelled SF5; 18-20 were same and was labelled as SF6; 21-23 were same and was labelled as SF7 while 24-26 were same and was labelled as SF8.

Antibacterial Assay: The antibacterial activities of the crude plant extracts were evaluated by agar well diffusion method. One ml each of the test bacterial isolates standardized at 10^7cell/ml from a 24 hour broth culture were aseptically pour plated with molten agar cooled to about 45°C, swirled and allowed to stand for 1.5 hours for the test bacterial isolates to be fully embedded. Wells of equal distance were dug on the seeded plates with

previously sterilized cork borer (4mm). Each well was filled up with the crude extracts. The fraction extracts were impregnated to a 5mm size sterile paper discs for one hour and picked to air dry before use. With the help of a sterile forceps the discs were placed on the bacteria seeded plates. The plates were incubated at 37°C for 24 to 48 hours. The sensitivities of the test organisms to the crude and fractions extract were indicated by clear zone around wells and discs. Where applicable, the halos were measured with a transparent ruler and expressed as the degree of sensitivity.

Determination of Total Phenol Content: The criterion of [Muchuweti *et al*., 2006] was adopted and was slightly modified. The extracts (0.1g) of ethanol, methanol and petroleum ether from each plant sample was dissolved in 20ml of distilled water for 10 minutes and filtered. From the solution 0.5ml was obtained and diluted with 0.5ml of distilled water. Folin-Ciocalteu reagent (1:1) (0.5ml) and 2.5mls of 20% sodium carbonate were added. The reaction was kept in the dark for 40 minutes, after which the absorbance was read at 750nm on spectrophotometer against a blank that contained methanol instead of sample. Total phenol compounds were expressed in term of equivalent amount of gallic acid (GAE). All determinations were carried out in duplicates.

Determination of Total Flavonoids Content: The content of flavonoids was determined using quercetin as a reference compound. The stock solution (0.50 µl) of each extract was mixed with 50 µl of aluminium trichloride and potassium acetate. The absorption at 415nm was read in spectrophotometer after 30minutes at room temperature. Standard quercetin solution was prepared by weighing 0.01g quercetin and 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 [19, 20].

Ferric Reducing Antioxidant Property: The method of [Buricova and Reblova, 2008] was adopted but with little modifications. 0.1g each of methanol, ethanol and petroleum ether extract were dissolved separately in 20ml of water and filtered. From the filtrate, 2.5ml was measured, 2.5ml of phosphate buffer (pH 6.6) and 2.5ml of potassium ferrocyanide were added. The mixture was incubated at a temperature of 50°C. Trichloroacetic acid (10%) was added, followed by the addition of 5mls of distilled water and 1ml of 0.1% ferric chloride. The absorbance of the standard and the samples were read at 700nm against reagent blank. The contents of the oxidizable substances were expressed as mg of ascorbic acid /g of dry plant weight material. Calibration of freshly prepared solutions of ascorbic acid in deionised water was used for this purpose (concentration from 0 to 4mg / 100ml). All determinations were carried out in duplicates.

Free Radical Scavenging Activity: The method used was almost the same as used by [22-24] but was modified in details. An aliquot of 0.5ml of 1, 1-diphenyl 1-2 picrylhdrazyl (DPPH) radical (Sigma Aldrich, St Louis, USA) in the concentration of 0.05mg/ml in methanol was added to a test tube with 1ml of the aqueous methanol, ethanol and petroleum ether extract each at a concentration of 20mg/ml in separate vials. The reaction mixture was mixed at room temperature and kept for 20 minutes. The absorbance was read at 520nm with a spectrophotometer. The absorbance of the DPPH radical solution contained in the plants extracts were expresses as mg of L-ascorbic (Sigma Chemical Co, St Louis, USA) per 1g of plant material. Calibration was used in such a case, where the plants extracts were replaced with a freshly prepared solution of ascorbic in deionised water (concentration from 0 to 1.6mg/100ml). All determinations were duplicated.

RESULTS AND DISCUSSION

Differences were noticed in the antibacterial activities of the crude and fractions of the considered plants extracts. These attributes are linked to the differences in the chemical components of the plants extract such as tannins, alkaloids, phenols, flavonoids and saponins. The crude methanol extract of *G. sepium* exhibited antibacterial potency on all the organisms with halos of between 8 to 17.8 mm in diameter, followed by ethanol extracts with inhibitory halo of between 6 to 17 mm in diameter and was least with petroleum ether which inhibited only three out of the nine test bacteria organisms in this study (Table 1). *S. campanulata* methanol crude extract also inhibited all the organisms with inhibitory halos from 10 to 15.5 mm followed by ethanol extract with 9.8 to 23 mm halo of inhibition while petroleum ether inhibited only two of the bacteria species (*E. coli*) with 14.9 mm and (*S. aureus*) with 15.9 mm halo of inhibition (Table 2). Tables 3 and 4 shows the eight fractions obtained from both plants leave extracts. After 24 h of incubation, the fractions extract of *G. sepium* showed inhibitory halos of between 12 to 48.7 mm against the clinical bacteria organisms except GF1 and GF2 fractions extract where *P. aeruginosa* and

Table 1: Zones of inhibition (mm) of crude extracts of Methanol, Ethanol and Petroleum ether of *G. Sepium* leaf

TEST ORGANISMS	MET	ETH	PET
Pseudomonas aeruginosa	16	17	-
Serretia marcescens	20	14.6	14.6
Escherichia coli	16	16	-
Staphylococcus aureus	4.8	14.5	12
Klebsiella pneumoniae	8	6.8	6.8
Bacillus cereus	10	8.5	-
Proteus mirabilis	7.9	6	-
Enterococcus feacium	17.8	10	-
Salmonella typhi	10.3	11	-

Key: MET: Methanol, ETH: Ethanol, PET: Petroleum ether

Table 2: Zones of inhibition (mm) of crude extracts of Methanol, Ethanol and Petroleum ether of *S. campanulata* leaf

TEST ORGANISMS	MET	ETH	PET
Pseudomonas aeruginosa	14.2	18	-
Serretia marcescens	10	16.5	-
Escherichia coli	13	9.8	14.9
Staphylococcus aureus	15.2	12	15.8
Klebsiella pneumoniae	12.7	19	-
Bacillus cereus	11	16	-
Proteus mirabilis	15.5	9.8	-
Enterococcus feacium	12	23	-
Salmonella typhi	11	12	-

KEY: MET: Methanol; ETH: Ethanol; PET: Petroleum ether

Table 3: *G. sepium* fractions extract inhibitory assay (mm)

TEST ORGANISMS	GF1	GF2	GF3	GF4
Pseudomonas aeruginosa	-	-	20	30
Serratia marcescens	13	14.7	22	34
Escherichia coli	12	17	20	21
Staphylococcus aureus	32	43	41	31
Klebsiella pneumoniae	-	-	38	33
Bacillus cereus	33	32	38	35
Proteus mirabilis	32	48.7	45	41
Enterococcus feacium	35	37.3	32	35
Salmonella typhi	29	32	41	30

GF: *Gliricidia sepium* fractions extract.

Table 4: *S. campanulata* fractions extract sensitivity assay (mm)

TEST ORGANISMS	SF1	SF2	SF3	SF4
Pseudomonas aeruginosa	24.8	32.3	31.3	31
Serratia marcescens	18	40	42	39
Escherichia coli	-	30	30.4	32.2
Staphylococcus aureus	23.9	48	40	45.3
Klebsiella pneumoniae	44	49	35	45
Bacillus cereus	35	32.4	40	44.5
Proteus mirabilis	42	43	35	49.3
Enterococcus feacium	43	34	35	36
Salmonella typhi	12	40	42	40

SF: *S. campanulata* fraction extracts

Plate 1: GF2 fraction extract on *P. mirabilis*

Plate 2: SF4 fraction on *P. mirabilis*

K. pneumoniae, showed resistance. Meanwhile, *Proteus mirabilis* was the most inhibited with inhibitory halo of 48.7mm (Plate 1) by GF 2 fraction extract and *S. marcescens* was the least inhibited with inhibitory halo of 14.7mm. GF3 fraction of *G. sepium* displayed inhibitory halo of between 20 to 45 mm where *Proteus mirabilis* was the most inhibited and *Escherichia coli* least inhibited with 20.0 mm as halo of inhibition (Table 3). However, *S. campanulata* fraction (SF4) possessed the highest inhibitory halo of 49.3 mm on *Proteus mirabilis* (Plate 2) and was least on *S. typhi* with inhibitory halo of 12.0 mm (Table 4).

In the concentration of total phenol contents (TPC), *G. sepium* extract has the highest phenol contents with a value of 1.7mg/ml and 0.04mg/ml in *S. campanulata*. Total flavonoids content in *G. sepium* was 0.47 mg/ml and was 0.56mg/ml in *S. campanulata* extracts. Alkaloids yielded as much as 7.5% in *G. sepium* and

Table 5: Qualitative phytochemical screening of *G. sepium* and *S. campanulata* leaf

	G. sepium	*S. campanulata*
Alkaloids	+	+
Flavonoids	+	+
Saponin	+	+
Tannins	+	-
Steroids	+	+
Glycosides	-	-
Phenols	+	+
Terpenoids	+	+

Key: + = Positive,-= Negative

Table 6: Quantative phytochemical screening of *G. sepium* and *S. campanulata* leaf

Phytochemical	GS	SC
Alkaloids (%)	7.5	1.5
Saponin (mg/ml)	5.81	3.92
Tannin (mg/ml)	0.1	0.0
Total phenol (mg/ml)TAE	1.7	0.04
Flavonoids (mg/ml) QE	0.47	0.56

Key: GS = *Gliricidia sepium*, SC = *Spathodea campanulata*

1.46% in *S. campanulata*. Meanwhile, saponin content was the highest yield among the quantitatively assayed phytochemical contents where *G. sepium* valued at 5.81mg/ml and 3.92mg/ml in *S. campanulata* (Table 6).

From the two plants screened, the extracts revealed appreciable ferric reducing antioxidant activity as well as effective free radical scavenging in the DPPH assay. Figure 1 showed the value of ferric reducing antioxidant ability with highest value of 30% in *S. campanulata* petroleum ether extracts, followed by methanol extract and the least value was recorded in *G. sepium* ethanol extract (1.5%.).

Figure 2 expressed the values obtained for the free radical scavenging activities of *G. Sepium* and *S. campanulata*. The ethanol extracts of *G. sepium* had the highest value with 88.2%, followed by methanol extracts of *S. campanulata* (58 %) and *G. sepium* petroleum ether had the least value of 1%.

The results of antibacterial activities of both the crude and fraction extracts demonstrated high inhibitory potency against the tested pathogenic bacteria isolates. The methanol extracts showed higher inhibitory activity than ethanol and petroleum ether. The petroleum ether inability to display effective inhibition activity based it a weak solvent for the extraction of bio-active components

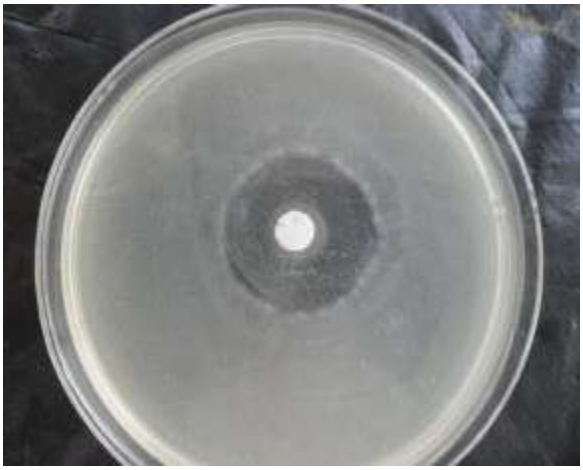

Plate 3: GF 3 fraction on *E. coli*

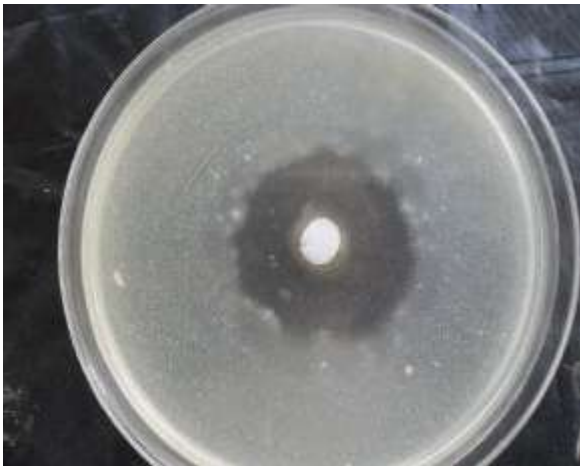

Plate 4: SF2 Fraction on *E. coli*

from these plants majorly for antimicrobial purposes. Despite its weak extraction ability, some of the isolates were susceptible, determining that it is a solvent not to be neglected though may be less considered as effective solvent where more potent solvents are available. The used crude and fraction extracts of the plants were visibly active on the tested bacteria isolates due to the combinative therapeutic action of the various bioactive compounds contained in the plants. This was expected because the various inhibitory components present were not separated into single entities for directional effect on certain bacteria as does by synthesized antibiotics. Some of the test organisms such as *P. aeruginosa, E. coli, K. pneumoniae and S. aureus* have caused a lot of threat in health care system involving individuals and communities. Most appreciably, with the primary screening of these plants, susceptibility of the organisms resulted in both the plants crude and fractions extracts.

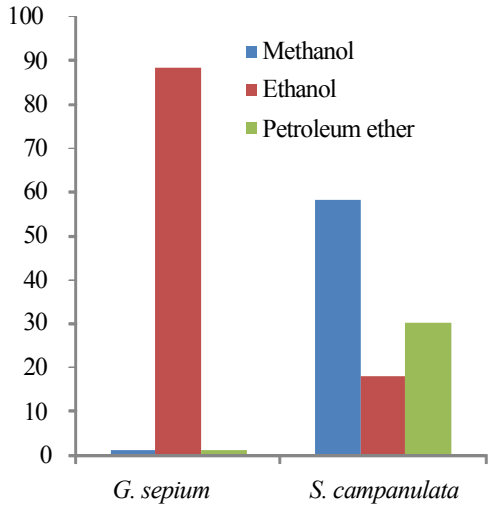

Fig. 1: Ferric reducing antioxidant property (%) of *G. sepium* and *S. campanulata*

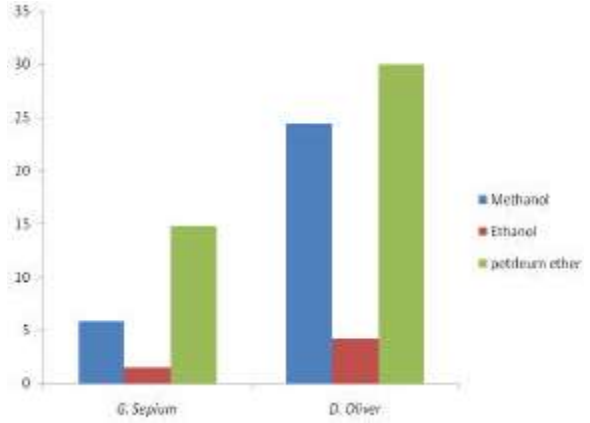

Fig. 2: Free reducing antioxidant property (%) of *G. sepium* and *S. campanulata*

In the earlier study by [14], *S. campanulata* crude extract was effective against *E. coli, S. aureus, B. cereus* and *P. aeruginosa*. 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 challenge can assume overwhelming proportions, hence the resurgence in the use of herbal preparations to treat diseases [25]. Therefore, plants evaluations in antimicrobial and antioxidant properties are important majorly in managing diseases by those that can accept their innumerable values for alternative therapy. The result obtained for the antioxidant screening of the plants shows that they have appreciable amount of bioactive components. This is in correlation with some studies elsewhere that medicinal plants used in traditional medicine and healing are one of the sources of antioxidants. In many countries, screening studies were carried out for the comparison of antioxidant activities of medicinal plants typical for the respective country [26-28]. On the bases of the result obtained, we can conclude that the antioxidant properties were more in the ethanol extract than other solvents employed. The plants contained appreciable amount of saponin, phenol, flavonoids, free radical scavenging and ferric reducing antioxidant properties which could have resulted to the inhibitory activities exhibited by the plants extracts. Antioxidants are secondary metabolites and their contents in plants vary based on the stress conditions of vegetation [29, 30]. Therefore, apparently healthy plants are necessary for valuable results. Although all oxidizable substances do not have antioxidant activity, some compounds without reduction abilities can act as antioxidants, most especially some substances that are able to bind metal ions, a close relation between the antioxidant activity and the content of compounds with reducing properties was described by other authors [31, 32]. In this study, considerable differences were found in the activities of the plants samples in antibacterial, phytochemical and antioxidant. Despite the differences, both plants were found valuable as medicinal plants to be comprehended for utilization in health remedy. It is evident that the flavonoid, phenols, alkaloids and saponin contents in the plants evaluates them strongly for the antibacterial activities observed. Many studies have suggested that flavonoids exhibit antioxidant, anti-inflammatory, antimicrobial, vascular activities and other medicinal properties [33]. Related studies of antimicrobial activity indicates that crude extracts containing flavonoids, triterpenes and steroids have showed significantive activity against various strains of *Staphylococcus aureus* and *Escherichia coli* [34]. The presence of some compounds such as phenol, flavonoids and free radical scavenging properties have been linked to their activities against disease causing bacteria [2, 35, 36]. The effectiveness of the plants crude and fractions extract on the test organisms could be as a result of the crude form in which they were used leading to diverse activities in inhibiting the organisms unlike the purified antibiotics that are directional to the cure and prevention of certain disease(s). The inhibitory properties in crude extracts remain same and work in combined form when used. This however could make them acceptable in some cases than the refined antibiotics because they will address both the problem in question and the minors which might contribute some factors to illnesses.

CONCLUSION

On the basis of the results obtained *S. campanulata* and *Gliricidia sepium* leaf extracts therefore seemed reliable and could be used as prevention, alleviation and curing of diseases of microbial and non microbial origins hence appreciable antioxidants were also evaluated.

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REFERENCES

1. Bouayed, J., A. Djilani, H. Rammal, A. Dicko, C. Younos and R. Souliman, 2008. Qualitative evaluation of the antioxidant properties of *Catha edulis*. J. Life Sci., 2: 7-14.
2. Akroum, S., S. Dalila and L. Kirrich, 2009. Antimicrobial, Antioxidant, Cytotoxic activities and Phytochemical screening of some Algerian plants, Eur. J. Sci. Res., 31: 289-295.
3. Mallikharjuna, P.B., L.N. Rajanna, Y.N. Seetharam and G.K. Sharanabasappa, 2007. Phytochemical studies of *Strychnos potatorum* L.F. A medicinal plant. E. J. Chem., 4: 510-518.
4. Davis, J., 1994. Inactivation of the antibiotics and the dissemination of resistance genes. Sci., 264: 375-382.
5. Service, R.F., 1995. Antibiotics that resist resistance. Sci., 270: 724-727.
6. Soberon, J.R., M.A. Sgariglia, D.A. Sampietro, E.N. Quiroga and M.A. Vattuone, 2007. Antibacterial activity of plant extracts from Northwestern Argentina. J. Appl. Microbiol., 102: 1450-1461.
7. Akinyemi, K.D., O. Oladapo, C.E. Okwara, C. Ibe and K.A. Fasure, 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicilin resistant-Staphylococcus aureus activity. Compl. Alt. Med., 5: 5-8.
8. Chung, P.Y., L.Y. Chung and Y.F. Ngeow, 2004. Antimicrobial activities of Malaysian plant species. Pharm. Biol., 42: 292-300.
9. Nair, R. and S.V. Chanda, 2005. Antibacterial activity of some medicinal plants of Suarashtra region. J. Tissue Res., 4: 117-120.
10. De Boer, H.J., A. Kool and A. Broberg, 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol., 96: 461-469.
11. Nair, R., T. Kalaliya and S. Chanda, 2005. Antibacterial activity of some selected Indian medicinal flora. Turk J. Biol., 29: 41-47.
12. Parekh, J., D. Jadeja and S. Chanda, 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk J. Biol., 23: 203-210.
13. Ellis, J.M. and P. Reddy, 2002. Effects of *Panax ginseng* on quality of life. Ann. Pharmacother, 36: 375-379.
14. Akharaiyi, F.C. and B. Boboye, 2010. Antimicrobial and phytochemical evaluation of three medicinal plants. J. Nat. Prod., 3: 27-34.
15. Thomas, D., 2004. Vitamins in health and aging. Clin. Geriatr. Med., 20: 259-74.
16. Polterait, O., 1997. Antioxidants and free-radical scavengers of natural origin. Curr. Org. Chem., 1: 415-440.
17. Adriana, P., P.P. Jurandir, T.F. Dalva, K.I. Noemia and B.F. Raimundo, 2007. Iridoid glucose and antifungal phenolic compounds from *Spathodea campanulata* roots, Cien. Agrar, 28: 251-256.
18. Muchuweti, M., A. Ndhlala and A. Kasiamhuru, 2006. Analysis of phenolic compounds including tannins, gallotannins and flavonols of *Uapaca kirkiana* fruit. Food Chem., 94: 415-419.
19. Zhishen, J., T. Menghheng and W. Jianming, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.
20. Kim, D., K. Ock, M. Young Hae-Yeon and Y.L. Chang, 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem., 51: 6509-6515.
21. Buricova, L. and Z. Reblova, 2008. Czech medicinal plants as possible sources of antioxidants. Czech J. Food Sci., 26: 132-138.
22. Gadow, A., E. Joubert and C.H.F. Hansmann, 1999. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT and BHA, J. Agric. Food Chem., 45: 632-638.

23. Ibanez, E., A. Kubatova, F.J. Senorans, S. Cavero, G. Reglero and S.B. Hawthorne, 2003. Subcritical water extraction of antioxidant compounds from rosemary plants. J. Agric. Food Chem., 51: 375-382.
24. Dorman, H.J., O. Bachmayer, M. Kosar and R. Hilyunen, 2004. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. J. Agric. Food Chem., 52: 762-770.
25. El-Mahmood, M.A., J. Daughari and F.J. Chanji, 2008. *In vitro* antimicrobial activities of Nauclea latifolia and Daniella oliveri. Sci. Res. Ess., 3: 102-105.
26. Ivanova, D., D. Gerova, T. Chervenkov and T. Yankova, 2005. Poluphenols andantioxidant capacity of Bulgarian medicinal plants. J. Ethnopharmacol., 96: 145-150.
27. Katalinic, V., M. Milos, T. Kulisic and K. Jukic, 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenol. Food Chem., 94: 550-557.
28. Wong, C.H., H.B. LI, K.W. Cheng and F. Chen, 2006. A systemic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem., 97: 705-711.
29. Verpoorte, R., R. Van Der Heijden, H.I.J. Ten Hoopen and J. Memelink, 1999. Metabolic engineering of plant secondary metabolite pathways for the production of fine chemicals. Biotechnol. Lett., 21: 467-479.
30. Bhattacharya, S.K. and S. Ghosal, 2000. Experimental evaluation of the anti-stress activity of herbal formulation. Zeestress J. Nat. Remedies, 1: 1-17.
31. Buratti, S., N. Pellegrini, O.V. Brenna and S. Mannino, 2001. Rapid electrochemical method for the evaluation of the antioxidant power of some lipophilic food extracts. J. Agri. and Food Chem., 69: 5136-5141.
32. Cosio, M.S., S. Buratti, S. Mannino and S. Benedeti, 2006. Use of an electrochemical method to evaluate the antioxidant activity of herbs extracts from the Labia tea family. Food Chem., 97: 725-731.
33. Harbone, J.B. and C.A. Williams, 2000. Advances in flavonoid research since 1992. Phytochem, Oxford, 55: 481-504.
34. Chattopadhyay, D., K. Maiti, A.P. Kundu, M.S. Chakraborty, R. Bhadra, S.C. Maudal and A.B. Maudal, 2001. Antibacterial activity of *Alstonia macrophylla*: A folklore of bay islands. J. Ethnophamacol., 77: 49-55.
35. De, N. and E. Ifeoma, 2000. Antibacterial effects of the components of the bark extract of neem (*Azadirachia indica*). Juss. Tech. Dev., 8: 23-28.
36. Ruiz-Teran, F., A. Medrano-Martinez and A. Navarro-Ocana, 2008. Antioxidant and free radical scavenging activities of plant extracts used in traditional medicine in Mexico. Afri. J. Biotechnol., 7: 1886-1893.