Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7):609-615

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of antimicrobial and free radical scavenging potentials of three valuable medicinal plants-*Hyptis suaveolens, Spathodea campanulata* and *Shorea robusta*

Nivetha Thampi[1*] and S. Nandha Kumar[2]

[1]*Department of Biotechnology, Jeppiaar Engineering College, Rajiv Gandhi Salai, Chennai, Tamil Nadu, India*
[2]*Department of Biotechnology, St. Peter's University, Avadi, Chennai, Tamil Nadu, India*

ABSTRACT

The enlarging prevalence of drug resistant bacteria and booming incidences of oxidative stress-related degenerative diseases has paved the way for the identification of novel drugs from natural sources. Even though researches have been conducted in various plant species to exploit their therapeutic applications, there still exist lacunae in the exploration of the medicinal properties of authentic plants that could be a rich source of treasured phytocompounds. In this study, the antibacterial, antifungal and antioxidant activities of Shorea robusta, Hyptis suaveolens, Spathodea campanulata were evaluated. The fresh leaves (H. suaveolens, S. campanulata) and powdered oleoresins (S. robusta) were extracted using methanol, petroleum ether, acetone, chloroform and water to test their efficacies as antimicrobial agents against strains of Micrococcus luteus, Proteus vulgaris, Aspergillus niger and Candida albicans. The aqueous and methanolic extract of all the three plants exhibited good antibacterial activity, while the chloroform extracts proved its potential as a better antifungal agent. The ability of all the extracts from H. suaveolens, S. campanulata and S. robusta to scavenge free radicals was ascertained by hydrogen peroxide method. The methanolic and aqueous extracts of H. suaveolens and S. campanulata displayed promising results where as S. robusta extracts showed moderate activity. The observations made in this study support the use of these plants as a natural remedy and as a low cost intervention in the enhanced therapy for drug resistance and other diseases. It can be concluded that this study would definitely lead to the establishment of new and more potent drugs from cheaper native plants from natural origin.

Key words: Antimicrobial activity, Free radical scavenging activity, *Hyptis suaveolens*, *Spathodea campanulata* and *Shorea robusta.*

INTRODUCTION

Medicinal plants are described as any plant with one or more of its organs containing therapeutic phytoconstituents that can be used as precursors for the synthesis of antimicrobial drugs [1] antioxidant, anti-infectious and anti-tumor activities [2]. Due to the presence of such components with therapeutic values, medicinal plants have been used for centuries as remedies for human diseases [3]. Undoubtedly, medicinal plants are the prime source of drugs in both developing and developed nations and about 2000+ plant species are known to possess medicinal value in the Asian traditional system of medicine [4]. Hence the use of plant derived natural compounds used as alternative sources of medicine continues to play a crucial role in the general wellness of the people all over the world.

Recently, antibiotic resistance has become a global concern [5]. This ever-increasing incidences of antibiotic resistance is largely due to the indiscriminate use of commercial antimicrobial drugs employed for the treatment of infectious diseases [6,7]. The ultimate goal is to offer an appropriate and efficient antimicrobial drug to the patients that ultimately leads to the rise in the universal demand for natural antimicrobial therapeutics [8] and in order to meet such increasing demand, further exploration of plant antimicrobials are essential.

An antioxidant is any compound that slows down or prevents the oxidation of molecules. Antioxidants act by terminating the chain reactions through the removal of free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Hence, antioxidants are often considered as reducing agents such as polyphenols, thiols, ascorbic acid etc.[9]. An increase in free radical generation or impaired endogenous antioxidant mechanism is the important causes of oxidative stress leading to the development of chronic diseases. The reactive oxygen species include such as hydroxyl radicals, superoxide anion radicals and free radicals species such as hydrogen peroxide [10]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) are widely used in the food industry. However, due to the increased toxicity and carcinogenicity of synthetic antioxidants, a restriction has been imposed for their usage [11,12]. Therefore, the development and utilization of more effective antioxidants of natural origins are desired. The secondary metabolites of plants were identified as the source of various phytochemicals that could be directly utilized as intermediates for the production of novel drugs. Natural medicines are believed to be more acceptable and compatible to the human body when compare to synthetic drugs. Thus it is extremely beneficial to derive the maximum sources of drugs from the traditional system of medicine for providing adequate healthcare services to the rural people [13] as well as conserve and limit the over-exploitation of several authentic medicinal plants.

Hyptis suaveolens commonly known as "Wilayati tulsi" belongs to the family Lamiaceae and is an ethnobotanically important medicinal plant. Almost all parts of this plant are being used in traditional medicine to treat various diseases [14]. *Spathodea campanulata Beauv*. commonly known as the Fountain Tree or African Tulip belongs to the family Bignoniaceae which is native to Africa and also in India [15]. The plant drug selected for this study has a broad spectrum of proven biological activity [16] and chemically interesting compounds [17] such as iridoid glucoside and phenolic benzoic acids. *Shorea robusta* Gaertn (Dipterocarpaceae) is widely distributed in India, Nepal and Bhutan. The oleoresin (gum) of the aerial parts has been reported in indigenous system of medicine as it also used as an ingredient of ointments to heal wounds, burns, pains, skin diseases and to control diarrhea and dysentery [18-20].

Considering the vast potentiality of the three medicinal plants, the present study involved a systematic investigation to screen the antimicrobial and free radical scavenging efficacy from the leaves of *Hyptis suaveolens, Spathodea campanulata* and from the oleoresins of *Shorea robusta*.

EXPERIMENTAL SECTION

Chemicals

All the chemicals used in this study were of analytical grade and were purchased from Hi-Media lab. Ltd., Mumbai, India.

Collection of Plants and Test Organisms

Hyptis suaveolens and *Spathodea campanulata* were collected from the local areas in and around Chennai and the oleoresins of *Shorea robusta* were commercially purchased from Central Siddha Research Institute, Chennai (Fig 1). Bacterial cultures of gram positive *Micrococcus luteus* (*M. luteus*) and gram negative *Proteus vulgaris* (*P. vulgaris*) and fungal strains *Aspergillus niger* (*A. niger*) and *Candida albicans* (*C. albicans*) were procured from the Laboratory of Department of Microbiology, Central Leather Research Institute (CLRI) Campus, Adyar, Chennai.

Maintenance of Cultures and Preparation of Inoculums

The Bacterial cultures were maintained at 37ºC in Nutrient Broth (NB) and the Fungal Strains were maintained at 28ºC in Potato Dextrose Agar (PDA).

The gram positive *M. luteus* and *P. vulgaris* was cultured in NB medium by inoculating a loopful of culture from the mother culture and serial diluted to obtain 10^{-3} dilution factor and stored at 37ºC overnight for 24 hours in rotary shaker incubator.

The fungal strains *A. niger* and *C. albicans* were sub cultured in PDA plates by using cork-borer and grown at 27ºC for 5 days in Incubator. Then 8 ml of sterile water was poured over the grown fungus, the spores were removed and inoculated in PDB medium with serial dilution to obtain 10^{-3} dilution factor and incubated at 27ºC for 2 days.

Preparation of Plant Extracts

The collected leaves of *Hyptis suaveolens* and *Spathodea campanulata* were washed thoroughly 2-3 times in running tap water followed by sterile distilled water and were dried in hot air oven at 60 ºC overnight. The dried leaves and the oleoresins (*S. robusta*) were grounded well with the help of mortar and pestle. 5g each from the powdered dried leaves and oleoresin were mixed with 100ml of five solvents such as Water, Methanol, Chloroform,

Acetone and Petroleum Ether (50mg/ml) and was kept in a rotary shaker at 37 ºC for 48 hrs. The solution was filtered through Whatmann filter paper no.1 and was stored in air tight bottle at 4 ºC.

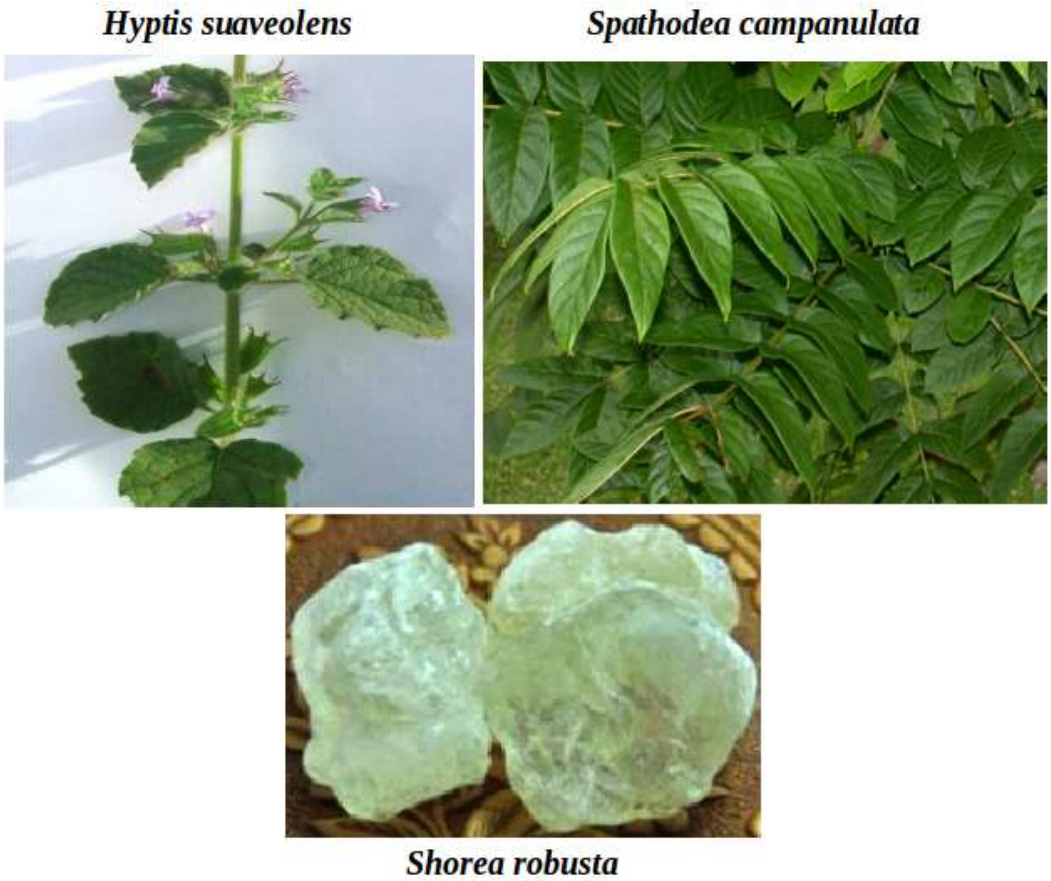

Fig 1: shows the leaves of *Hyptis suaveolens* and *Spathodea campanulata* and the oleoresins of *Shorea robusta*

***In vitro* Antimicrobial Assays**

The crude extracts of five solvents from *H. suaveolens, S. campanulata* and *S. robusta* were tested for antibacterial activity using agar disc diffusion technique following the Kirby-Bauer method [21]. 200 µl of each bacterial subculture was micropipetted onto the solidified agar plates separately and the cultures in each plate were evenly spread over the agar medium. Sterile discs of 6mm diameter were cut out using a Holing Pincer, from a Whatmann filter paper Grade 1. 150 µl of each extracts and solvents were impregnated into the disc respectively. Antibiotic gentamycin was used as positive control and the solvents as negative control. All the discs were placed in the centre of the respective plates using a pair of sterile forceps. The plates were kept at room temperature for 2 hours to allow diffusion and then incubated at 37°C for 24 hours in an incubator. Next day the diameter of the Zone of Inhibition was measured in millimetres (mm).

The crude extracts were also tested for antifungal activity by disc diffusion method [22]. The PDA plates were inoculated with each fungal culture by point inoculation. Sterile discs of 6mm diameter were impregnated with 150 µl of each extracts and solvents and were placed on test organism-seeded plates. The solvents were used as negative control and Nystatin (10 µg disc) were used as positive control. The activity was determined after 72 hours of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

Free Radical Scavenging Activity by H_2O_2 Assay

The ability of the extracts to scavenge hydrogen peroxide was determined by the method of Ruch *et al* [23]. A solution of hydrogen peroxide (H_2O_2) (40 mM) was prepared in phosphate buffer (pH 7.4). 4 ml of each extracts from different solvents (4 mg/ml) were added to 0.6ml of previously prepared H_2O_2 solution. The absorbance of the solution was measured at 230 nm after 10 min against a blank containing phosphate buffer without H_2O_2 using Varian Cary 100 UV-Visible Spectrophotometer. Ascorbic Acid was used as the control. The percentage of H_2O_2 scavenging by the extracts was calculated using the formula

% Scavenging [H_2O_2] = 1- Abs (sample) /Abs (control) x100

Where, Abs (control) was the absorbance of the control at 230nm and Abs (sample) was the absorbance of the extracts at 230nm.

RESULTS AND DISCUSSION

In vitro Antimicrobial Assays

The *in vitro* antibacterial and antifungal activity of the extracts from the test plants *S.robusta*, *H. suaveolens* and *S.campanulata* against bacterial species of *M. luteus* and *P. vulagris* and fungal strain of *A. niger* and *C. albicans* were determined. Table 1 shows the different inhibition levels of *H. suaveolens* extracts against the bacterial and fungal strains. The maximum zone of inhibition was exhibited by the aqueous extract of *H. suaveolens* agianst *M. luteus* and *P. vulagris* with 20mm diameter for the bacterial strains and by the acetone extract against *C. albicans* with 18mm. Table 2 shows the zone of inhibition values produced by *S.campanulata* extracts against the bacterial and fungal strains. The aqueous extract of *S.campanulata* showed maximum inhibition with 17mm against *M. luteus*, while the chloroform extract displayed a zone with 16mm diameter against *C. albicans*. Table 3 shows the different inhibition levels of *S.robusta* against the test bacterial and fungal pathogens. The maximum zone of inhibition was exhibited by the aqueous and methanolic extracts of *S.robusta* against gram negative *P. vulagris* whereas *A. niger* was inhibited with a maximum of 18mm. Fig 2 shows the antibacterial plates that displayed the highest inhibition zones by the test plant extracts. Fig 3 displays the antifungal plates that show the maximum zone of inhibition by the three medicinal plants. The positive control gentamycin inhibited *M. luteus* and *P. vulagris* with 24mm and 22mm respectively and nystatin inhibited *A. niger* and *C. albicans* with 18mm and 24mm respectively.

Table 1: Zone of Inhibition (in mm) exhibited by *H. suaveolens* extracts against various pathogens

Solvents	Zone of Inhibition (mm)			
	M. luteus	***P. vulgaris***	***A. niger***	***C. albicans***
Water	20	20	10	10
Chloroform	10	10	10	16
Acetone	15	13	16	18
Petroleum Ether	0	8	0	10
Methanol	18	16	13	12

Table 2: Zone of Inhibition (in mm) exhibited by *S. campanulata* extracts against various pathogens

Solvents	Zone of Inhibition (mm)			
	M. luteus	***P. vulgaris***	***A. niger***	***C. albicans***
Water	17	16	15	10
Chloroform	8	0	12	16
Acetone	10	14	10	0
Petroleum Ether	0	10	10	8
Methanol	16	14	14	16

Table 3: Zone of Inhibition (in mm) exhibited by *S. robusta* extracts against various pathogens

Solvents	Zone of Inhibition (mm)			
	M. luteus	***P. vulgaris***	***A. niger***	***C. albicans***
Water	15	16	12	10
Chloroform	0	5	18	15
Acetone	12	10	12	10
Petroleum Ether	0	8	10	12
Methanol	14	16	12	14

Free Radical Scavenging Activity by H_2O_2 Assay

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. H_2O_2 probably reacts with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical which may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The present study assessed the five different solvents of *S.robusta*, *H. suaveolens* and *S.campanulata* for its free radical scavenging activity by H_2O_2 method. Table 4 provides the % scavenging data from the different solvents of the three plants. It was shown that the highest % scavenging was produced by the methanolic extract of *H. suaveolens* with 71% followed by the aqueous extract of *S.campanulata* with 70.2% scavenging. The methanolic extract of *S.robusta* showed a moderate level of scavenging of 64.7%. Fig 4 illustrates the various levels of H_2O_2 activity by *S.robusta*, *H. suaveolens* and *S.campanulata*. The % scavenging of ascorbic acid standard was observed to be 70%.

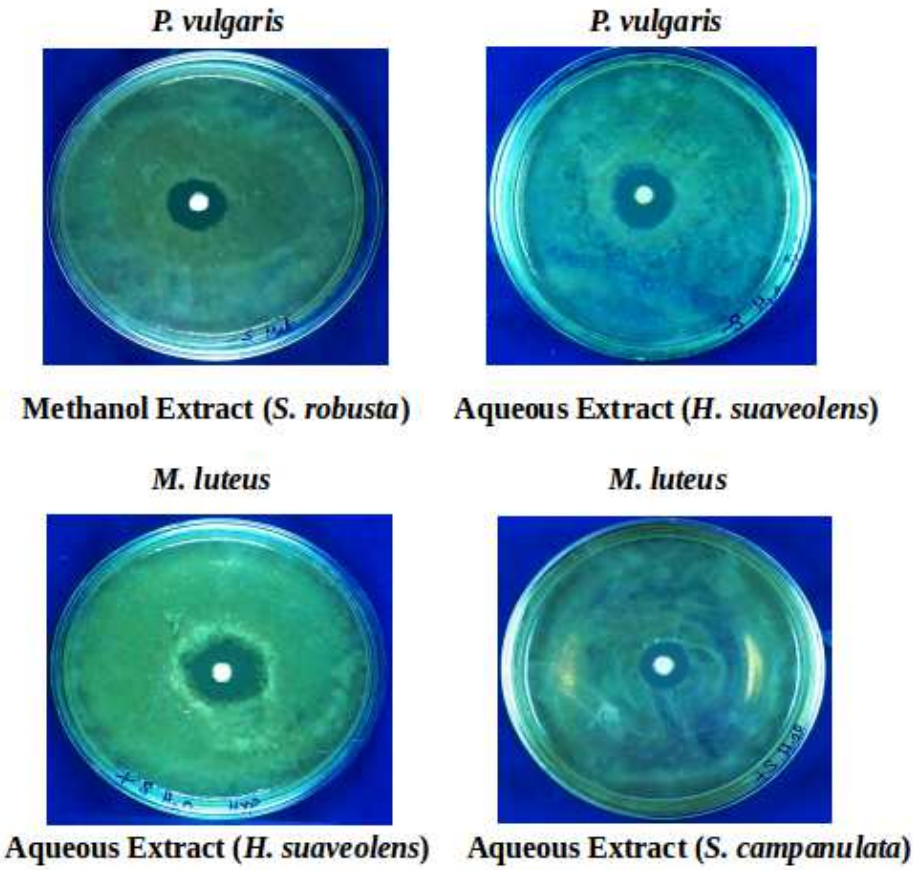

Fig 2: Antibacterial plates showing the maximum inhibition by the three medicinal plants tested by disc diffusion assay

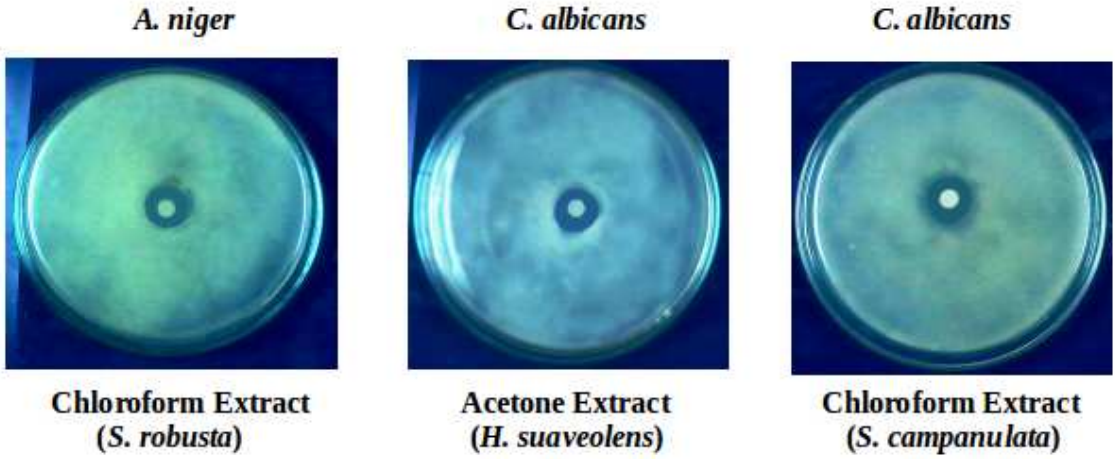

Fig 3: Antifungal plates showing the maximum inhibition by the three medicinal plants tested by disc diffusion assay

Table 4: H_2O_2 radical scavenging activity of the various extracts from *S.robusta*, *H. suaveolens* and *S.campanulata*

Solvents	% Scavenging *H. suaveolens*	% Scavenging *S. campanulata*	% Scavenging *S. robusta*
Water	66.2	70.2	59
Methanol	71	69.7	64.7
Acetone	47	55.6	44.4
Chloroform	30.6	39.7	30.1
Petroleum Ether	31.3	42	36.3

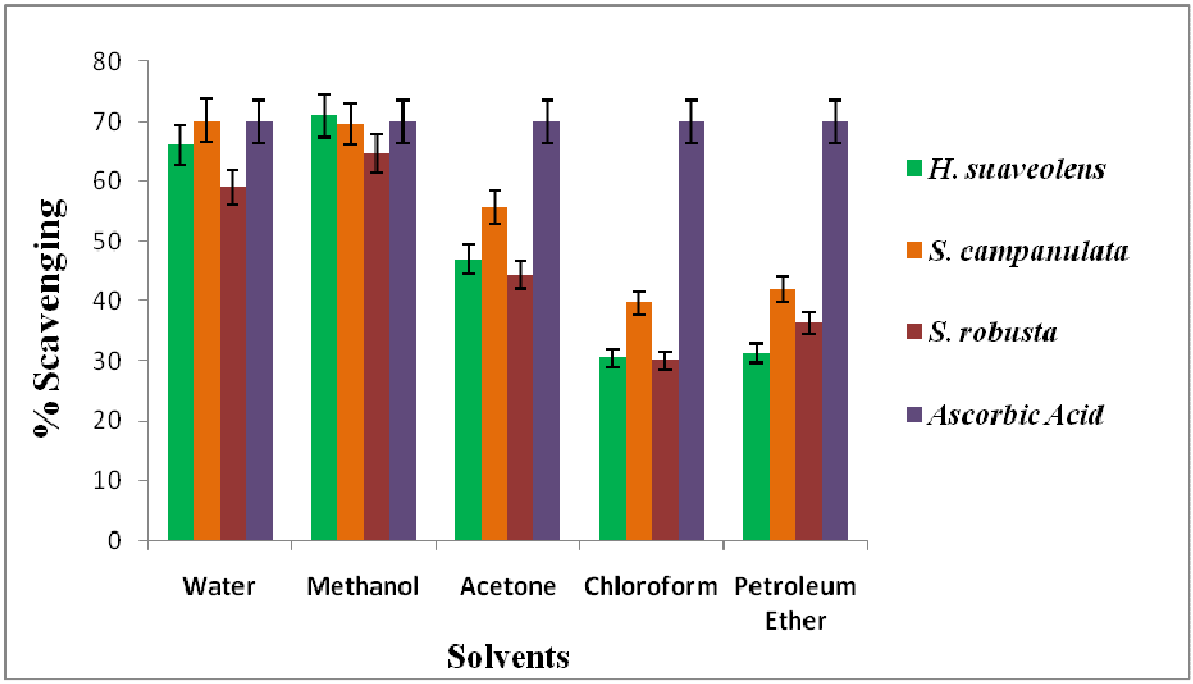

Fig 4: Various levels of percentage scavenging of the plant extracts with reference to Ascorbic acid

Currently, much attention has been projected towards the biologically active compounds isolated from popular plant species. The use of medicinal plants plays an indispensable role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [24,25]. The results from the antimicrobial activities of the crude extracts demonstrated significant inhibition against the tested pathogenic bacterial and fungal isolates. The aqueous and methanolic extracts showed high inhibitory potency against the bacteria while the chloroform extract showed good potency against the fungal species than petroleum ether. This effectiveness may be due to the cumulated action of different compounds present in the plant parts [26]. They include flavonoids, alkaloids, triterpenoids and other compounds of phenolic nature and are classified as active anti-microbial compounds [27]. The inability of petroleum ether to display as an effective inhibition activity might be of its weak extraction nature of bioactive components from these plants majorly for antimicrobial purposes. Despite its weak extraction ability, some of the isolates were susceptible, ascertaining that it is a solvent not to be neglected though may be less considered where more potent solvents are available.

The result obtained from the free radical activity of the plants shows that they have appreciable amount of bioactive components. On the bases of these results, the methanol extract showed good scavenging activity compared to the Ascorbic acid and this may be due to the higher amounts of saponins and flavonoids like quercetin and polyphenols which could be extracted only with non polar solvent like methanol. Many researches showed that phytochemical constituents such as flavonoids and other phenolic compounds which have been reported to have multiple biological effects such as antioxidant activity, anti-inflammatory actions, inhibition of platelets aggregation and antimicrobial activities [28]. Flavonoids and phenolic compounds have good antioxidant potentials and mechanism of action of flavonoids is through scavenging or chelation [29], while phenolic compound are important because of their hydroxyl groups which posses scavenging ability [30]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals. 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 pandemic and resources are limited, such challenge can assume overwhelming proportions, hence the resurgence in the use of herbal preparations to treat diseases should occur. Therefore, plants evaluations in antimicrobial and antiradical properties are important majorly in managing diseases by those that can accept their innumerable values for alternative therapy.

CONCLUSION

The present study justifies the claimed uses of *S.robusta*, *H. suaveolens* and *S.campanulata* in the traditional system of medicine to treat various infectious diseases caused by the microbes. Our study emphasizes that these plants would become good bactericide and fungicide and the utilization of their secondary metabolites for the development

of traditional medicines and further investigations is necessary for the production of novel plant based compounds like antiseptics or disinfectants. From our results, it appeared that the crude extracts of these traditional medicinal plants have good free radical scavenging effect when compared with the standard that can ameolirate the fatal effects of free radical causing various diseases. Further chemical and pharmacological investigations to isolate and identify the phytoconstituents in *S.robusta*, *H. suaveolens* and *S.campanulata* and to screen other potential bioactivities may be recommended. This study also encourages cultivation of these highly valuable plants in large scale to increase the economic status of the cultivars in the country.

Acknowledgements
The authors owe great many thanks to our family members for their never-ending support and our friends for their help, encouragement and suggestions. We thank God Almighty who showered his blessings throughout our project work.

REFERENCES

[1] J Bouayed; A Djilani; H Rammal; A Dicko; C Younos; R Souliman. *J. Life Sci*., **2008**, 2, 7-14.
[2] S Akroum; S Dalila; L Kirrich. *Eur. J. Sci. Res*., **2009**, 31, 289-295.
[3] A Nostro; MP Germano; VA Amgelo; A Marino; MA Cannatelli. *Lett. Appl. Microbiol*., **2000**, 30, 379 -388.
[4] AM Agnese; C Perez; Carbrera. *Phtyomed*., **2001**, 7(5), 389-394.
[5] H Westh; CS Zinn; VT Rosdahl. *Microb. Drug Resist*., **2004**, 10, 169-176.
[6] J Davis. *Sci*., **1994**, 264, 375-382.
[7] RF Service. *Sci*., **1995**, 270, 724-727.
[8] JR Soberon; MA Sgariglia; DA Sampietro; EN Quiroga; MA Vattuone. *J. Appl. Microbiol*., **2007**, 102, 1450-1461.
[9] NS Praveen; N Shweta; S Ranjan; P Das. *Res. Rev. J. Pharmacogn. Phytochem*., **2010**, 2(1),
[10] L Barros; MB Ferreira. *Food Chem*., **2006**, 103, 413–419.
[11] HC Grice. *Food Chem. Toxicol*., **1986**, 24, 1127-1130.
[12] HP Wichi. *Food Chem. Toxicol*., **1988**, 26, 717-723.
[13] A Ghani. In: Traditional Medicine., Dhaka: 1990; 15-40.
[14] GF Asprey; P Thornton. *West Indian Med J*., **1953**, 2(4), 233–52.
[15] Z Zahid; PP Aniruddha; DD Sagar; K Subur. *African J Pharm Pharmacol*., **2011**, 5, 2226-31.
[16] P Adriana; PP Jurandir; TF Dalva; KI Noemia; BF Raimundo. *Ciências Agrárias*, Londrina., **2007**, 28, 251-256.
[17] P Kumaresan; PN Palanisamy; PE Kumar. *J. Nat. Prod. Plant Resour*., **2011**, 1, 14-17.
[18] A Saraswathy; KK Purushothaman; A Patra; AK Dey; AB Kundu. *Phytochem*., **1992**, 31, 2561-2562.
[19] OP Upadhyay; K Kumar; RK Tiwari. *Pharm. Biol*., **1998**, 36, 167-172.
[20] LN Misra; A Ahmad. *Phytochem*., **1997**, 45, 575-578.
[21] WMM Kirby; AW Bauer; JC Sherris; M Turck. *Am. J. Clin. Pathol*., **1966**, 45, 493-496.
[22] RSL Taylor; NP Manandhar; JB Hudson; GHN Towers. *J. Ethnopharmacol*., **1995**, 546, 153-159.
[23] RJ Ruch; SJ Cheng; JE Klaunig. *Carcinogenesis*., **1989**, 10, 1003-1008.
[24] D Mun oz-Mingarro; N Acero; F Llinares; JM Pozuelo; A Gala n de; JA Mera Vicenten. *J. Ethonopharmacol*., **2003**, 87, 163-167.
[25] G Coelho de Souza; APS Haas; GL Von Poser; EES Schapoval; E Elisabetsky. *J. Ethnopharmacol*., **2004**, 90, 135-43.
[26] D Bai. *Plant Medica*., **1990**, 56, 5002.
[27] A Rojas; Hernandez; R Pereda-Miranda; R Mata. *J. Ethonopharmacol*., **1992**, 35, 275-285.
[28] D Venkatanarayana; SS Kumar; M Lakshni. *Int. J. Phytopharmacy Res*., **2010**, 1(1), 1-4.
[29] A Yildirim; A Mavi; M Oktay; AA Kara; OF Algur; V Bilaloglu. *J Agric Food Chem*., **2000**, 48(10), 5030-5034.
[30] NC Cook; S Samman. *J Nutr. Biochem*., **1996**, 7, 66-76.