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Research Article

BIOACTIVITY SCREENING OF SELECTED TRADITIONAL MEDICINAL PLANTS OF KERALA

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ABSTRACT

Objectives: Traditional medicines all over the world is revaluing nowadays by extensive research programs. To validate the traditional use, the active components in them need to be identified, characterized, and biologically evaluated. *Stereospermum suaveolens*, *Hygrophila spinosa,* and *Naravelia zeylanica* are important medicinal plants used by the ethnic people of Kerala against various ailments. The present study validates the ethnomedical uses of *S. suaveolens*, *H. spinosa*, and *N. zeylanica* by screening their antimicrobial, anthelmintic, and antioxidant properties.

Method: All the analyses were done according to standard protocols.

Results: The ethanolic extracts of their useful parts were investigated for antimicrobial activity against 10 human pathogenic microorganisms. All the three plants had shown prominent antimicrobial activities, and *S. suaveolens* exhibits comparatively more antifungal activity in their higher concentration (500 µg/mL). Anthelmintic efficiency of the plants was screened using Indian adult earthworm *Pheretima posthuma*. All of them had shown significant activity, and the highest was observed in *S. suaveolens* leaves. Antioxidant potential of the plants was screened using 2,2 diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging assay and superoxide anion scavenging assay. In DPPH free radical scavenging assay, maximum radical scavenging was shown by *S. suaveolens* with IC50 value 61.6±2.3 µg/mL, and in superoxide anion scavenging assay, maximum activity was in *N. zeylanica* with IC50 value of 74.66±8.5 µg/mL.

Conclusion: This study provides scientific evidence on the traditional use of *S. suaveolens* (leaves), *H. spinosa* (leaves), and *N. zeylanica* (aerial part) in treating microbial diseases, worm disturbances, and their potential as an antioxidant agent.

Keywords: *Stereospermum suaveolens*, *Hygrophila spinosa*, *Naravelia zeylanica*, Antimicrobial, Anthelmintic, Antioxidant, Traditional medicine.

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INTRODUCTION

Ayurvedic system, tribal medicine, and folk medicines are the three plants based on healing systems in Kerala. Ayurvedic system possesses a very good written documents and history. Tribal medicine is the medicinal knowledge of forest-dwelling tribals, which is not having any documentation and procedure. Folk medicine is the orally transmitting knowledge on medicinal plants for the primary health care [1]. The wide acceptance of traditional medicine as an alternative form of health care and the alarming increase in the incidence of new and reemerging infectious diseases bring about the necessity to investigate these medicinal plants. Medicinal values of various plants are due to the presence of various bioactive compounds such as phenolic compounds, alkaloids, flavonoids, and tannins that produce definite physiological action in the human body [2].

Stereospermum suaveolens DC is a medicinal tree species native to India, Bangladesh, and Myanmar. The Bignoniaceae family having about 100 genera with 800 species is known for their antimicrobial and antiprotozoal properties [3].

Naravalia zeylanica DC belonging to the family Ranunculaceae is a woody climber in habit with tuberous roots, opposite and cordate leaflets, and flowers are small and arranged in panicles. Red-colored achenes with long feathery styles occur in the hot to warm regions in India [4]. The plant is traditionally used by the healers to treat pitta, vitiated vata, inflammations, skin diseases, headache, arthritis, colic, wounds, and ulcers [5]. Leaf paste is consumed to treat chest pain. The vines when crushed give a pungent odor which is inhaled to cure a cold and all types of headaches including migraine [6].

Hygrophila spinosa Anders belongs to the family Acanthaceae. The parts of this plant are widely used in traditional medicine for the treatment of various disorders, which include anasarca, diseases of the urinogenital tract, dropsy from chronic Bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leukorrhea, gonorrhea, asthma, blood diseases, gastric diseases, inflammation, cancer, rheumatism, painful micturition, and menorrhagia [7-10].

Thus, this study aimed to evaluate the potential antimicrobial, anthelmintic, and antioxidant properties of *S. suaveolens* leaves, *H. spinosa* leaves, and *N. zeylanica* aerial part and to provide scientific evidence for its folk claim.

METHODS

Collection of plant material and preparation of the extract

The fresh leaves of *S. suaveolens* and *H. spinosa* and aerial parts of *N. zeylanica were* collected in March 2014 from Mannamangalam village of Thrissur District and shade dried for several days. The coarse powder of the dried plant materials was prepared, and 50 g of the powder was soaked in 95% ethanol (1:5) for 72 h. Using a rotary evaporator, the solvents were removed. For the future studies, the concentrated extracts were refrigerated [11].

Antimicrobial assay

Organisms and culture media

The microorganisms for the antimicrobial screening were collected from the Microbiology Laboratory, St. Mary's College, Thrissur. The pathogenic organisms used were *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Salmonella*

typhi, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *Penicillium notatum.* Nutrient agar (NA) was used to maintain bacterial cultures, and fungal cultures were maintained on Sabouraud Dextrose Agar (SDA).

Antibacterial and antifungal activity of the plant extract

Well diffusion assay [12] on NA and SDA plates was used to determine the antibacterial and antifungal properties, respectively. To prepare the microbial suspension, bacteria inoculated into nutrient broth (NB) and fungus into Sabouraud dextrose broth (SDB) and incubated at 37°C for 6 h. The transmittance of the resulting suspension was diluted using NB and SDB for getting the value 74.3% (absorbance of 0.132) at 600 nm. This percentage is comparable to 0.5 McFarland turbidity standards. This level of turbidity is equivalent to approximately 1.5×10^8 CFU/mL [13]. On the surface of NA plates, bacterial cultures were inoculated and fungal cultures were inoculated on SDA plates. Subsequently, wells with a diameter of 6 mm were prepared on NA and SDA plates using sterile cork borer, and 25 µL of sample in different concentrations (100 µg/mL, 250 µg/mL, and 500 µg/mL) was loaded in each well. Antibiotics were used as positive control (chloramphenicol for bacteria and fluconazole for fungus) [14]. The tests were carried out in triplicates. The plates were incubated at 37°C for 24 h. Zone of clearing was measured at the end of the incubation period using a transparent ruler. Zones of inhibition >6 mm were taken as susceptible to the extracts.

Anthelmintic property

The standard albendazole (25 mg/mL) and the test solutions of *S. suaveolens*, *H. spinosa,* and *N. zeylanica* (25, 50, and 100 mg/mL) were evaluated for anthelmintic activity with Indian adult earthworm *P. posthuma*. Time taken for paralysis and time of death of each worm were observed for 4 h. Time of paralysis was noted when the worms did not show any signs of movement. Death of each worm was confirmed by vigorous shaking and by dipping in warm water of 50°C [15].

Antioxidant property screening

2,2 Diphenyl 1-picrylhydrazyl (DPPH) radical scavenging assay

Free radical scavenging activity of the plant extracts was assessed on the basis of the radical scavenging effect of the stable DPPH, by a modified method [16]. The diluted working solutions of the test extracts (10–1000 µg/mL concentration) and 6.34 µM solution of DPPH were prepared in methanol, and 100 µL of drug to be tested, 100 µL DPPH solution, and 800 µL of methanol were taken in a test tube and mixed well. These solution mixtures were incubated in the dark for 20 min. Optical density was measured after incubation at 517 nm using Cecil-elect spectrophotometer. Methanol (900 µL) with DPPH solution (6.34 µM, 100 µL) was taken as control and methanol as blank. The optical density was recorded, and to calculate the percentage of inhibition, the following formula was used:

Percent (%) inhibition of DPPH activity = A-B/A ×100

Where A = optical density of the control and B = optical density of the sample.

Super oxide radical scavenging assay

In vitro superoxide radical scavenging activity was measured by NBT reduction method [17]. In the presence of light, riboflavin undergoes auto-oxidation and forms superoxide radicals, and it reduces NBT to a blue-colored formazan which can be measured at 590 nm.

200 µL EDTA, 100 µL riboflavin solution, 200 µL ethanol, and 100 µL NBT solution were mixed in a test tube and made up to 3 mL using phosphate buffer. The solution was incubated in light for 15 min, and the absorbance of the resulting solution was measured at 590 nm using phosphate buffer as blank. This was taken as control reading. For screening of test sample, along with the above solutions, added 100 µL sample of varying concentrations (10–1000 µg/mL), and finally, the volume was made up to 3 mL using phosphate buffer and the reading was taken after 15 min of illumination. The formula given below was used to find out the percentage of inhibition:

Percent (%) inhibition = A-B/A ×100

Where A = optical density of the control and B = optical density of the sample.

RESULTS AND DISCUSSION

Antimicrobial screening

The results of the study showed that the ethanolic extracts of *S. suaveolens* leaves, *H. spinosa* leaves, and aerial parts of *N. zeylanica* had prominent antimicrobial activity against the human pathogenic bacteria and fungi studied [Tables 1-6]. All the medicinal plants screened were effective against all the bacterial and fungal species studied, while *S. suaveolens* shows more prominent antifungal activity with maximum zone of growth inhibition against *Aspergillus flavus* and *C. albicans* (23.3±0.57 mm and 23.6±0.57 mm at 500 µg/mL concentration). *C. albicans* was resistant to fluconazole, but it showed promising activity with plant extracts, and the maximum zone of inhibition against *C. albicans* was observed in *N. zeylanica* (24±1 mm at 500 µg/mL).

Table 1: Antibacterial property of *S. suaveolens* leaves

Organism	Zone of inhibition			
	Chloramphenicol (25 µg)	100 µg	250 µg	500 µg
K. pneumoniae	25±1	7.3±0.1.15	9.6±0.57	11.3±1.15
S. typhi	23.6±0.57	9.6±0.57	13±1	16.6±0.57
P. aeruginosa	11.6±0.57	6.6±0.57	8.6±0.57	10.6±1.15
B. cereus	20.6±0.57	7.3±1.15	11±1	12.6±1.15
S. pyogenes	18.6±1.15	6.6±1.15	9.6±0.57	12.3±0.57
S. aureus	23.3±0.57	7.6±0.57	10±1	12.6±1.15

S. suaveolens: Stereospermum suaveolens, K. pneumoniae: Klebsiella pneumoniae, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. cereus: Bacillus cereus, S. pyogenes: Streptococcus pyogenes, S. aureus: Staphylococcus aureus

Table 2: Antifungal property of *S. suaveolens* leaves

Organism	Zone of inhibition (mm)			
	Fluconazole (15 µg)	100 µg	250 µg	500 µg
A. niger	12.7±1.15	15.3±0.57	19.6±0.57	21±1
A. flavus	13.7±1.15	16.7±1.15 1.2	20.7±0.57	23.3±0.57
P. notatum	9.3±0.57	11.6±0.57	13.7±1.15	18.6±0.57
C. albicans	R	17.3±0.57	20.6±0.58	23.6±0.57

R: Resistant. *S. suaveolens: Stereospermum suaveolens, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, P. notatum: Penicillium notatum, C. albicans: Candida albicans*

Table 3: Antibacterial property of *H. spinosa* leaves

Organism	Zone of inhibition			
	Chloramphenicol (25 µg)	100 µg	250 µg	500 µg
K. pneumoniae	24.7±1.15	8.6±1.15	10.3±1.15	13.3±0.57
S. typhi	20.3±0.57	6.3±0.57	9.6±1.15	12.3±0.57
P. aeruginosa	13±0.83	7.6±0.57	9.6±1.15	14±1
B. cereus	29±0.93	8.3±0.57	10.3±1.15	14.3±0.57
S. pyogenes	17.6±2.5	7.7±0.57	11.6±1.2	13.6±0.57
S. aureus	24±1.7	7.6±0.57	9.3±0.57	12.5±1.52

H. spinosa: Hygrophila spinosa, K. pneumoniae: Klebsiella pneumoniae, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. cereus: Bacillus cereus, S. pyogenes: Streptococcus pyogenes, S. aureus: Staphylococcus aureus

Table 4: Antifungal property of *H. spinosa* leaves

Organism	Zone of inhibition (mm)			
	Fluconazole (15 µg)	100 µg	250 µg	500 µg
A. niger	7.6±0.57	8.3±0.57	11.7±1.15	15.3±0.57
A. flavus	7.6±0.57	9.6±0.57	12.6±1.15	14.3±0.57
P. notatum	7.6±0.57	8.3±0.57	9.6±0.57	12.6±1.15
C. albicans	R	9.7±1.15	14.3±0.58	16.6±0.57

H. spinosa: Hygrophila spinosa, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, P. notatum: Penicillium notatum, C. albicans: Candida albicans

Table 5: Antibacterial property of *N. zeylanica* areal part

Organism	Zone of inhibition			
	Chloramphenicol (25 µg)	100 µg	250 µg	500 µg
K. pneumoniae	18.7±1.2	7.6±1.2	9.6±0.57	11.7±1.2
S. typhi	16.6±0.57	7.7±1.15	9.7±1.15	12.6±0.57
P. aeruginosa	9.3±1.2	7.6±0.57	10.6±0.57	13.4±0.92
B. cereus	16.7±1.15	8.7±1.15	11.6±0.57	13.3±0.57
S. pyogenes	17.6±2.5	8.7±0.57	10.6±1.2	12±0.57
S. aureus	14.6±0.72	9.6±0.57	12.6±0.57	14.2±0.57

N. zeylanica: Naravelia zeylanica, K. pneumoniae: Klebsiella pneumoniae, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. cereus: Bacillus cereus, S. pyogenes: Streptococcus pyogenes, S. aureus: Staphylococcus aureus

Table 6: Antifungal property of *N. zeyanica* areal part

Organism	Zone of inhibition (mm)			
	Fluconazole (15 µg)	100 µg	250 µg	500 µg
A. niger	12.5±0.76	16.6±2.5	23.3±1.15	23.4±1.15
A. flavus	14.3±0.64	11±1	12.6±1.15	18.6±1.15
P. notatum	8.6±0.57	14.6±1.15	17.6±1.15	19.3±0.57
C. albicans	R	18.6±0.57	21.6±0.58	24±1

N. zeylanica: Naravelia zeylanica, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, P. notatum: Penicillium notatum, C. albicans: Candida albicans

Due to the reported development of resistance by bacteria and fungi to various commercially available antimicrobial agents, the plant extracts are potential sources of new compounds, which may be developed as effective drugs against the infectious microorganisms. Further, the use of these plants may offer a new source of antifungal agent against the pathogenic fungus such as *C. albicans* which inhibited by the crude drugs in dose-dependent manner.

Anthelmintic property screening

It was seen that the ethanolic extracts of leaves of *S. suaveolens* and *H. spinosa* and aerial parts of *N. zeylanica* possess dose-dependent antihelmintic activity when compared to a standard drug albendazole. The mean paralyzing time of *P. posthuma* with the dose of 25 mg/mL concentration in *S. suaveolens*, *H. hygrophilla,* and *N. zeylanica* was found to be 38±1.53, 119.7±3.5, and 35±2.3 min, respectively, and the mean death time of *P. posthuma* with the dose of 25 mg/mL of plant extracts was 78.6±2.3, 119.7±3.5, and 35±2.3 min. All the three plants possess significant activity, and among them, *N. zeylanica* shows the highest activity by causing paralysis and mortality within minimum time. Albendazole, the commercially used anthelmintic drug at a dose of 25 mg/mL, causes only paralysis, and no death was observed during the experimental period of 4 h [Tables 7-9].

Antioxidant property screening

The action of antioxidant compounds may be an initiator of the complexes of pro-oxidant metals, free radical scavengers, reducing agents, and quenchers of singlet oxygen formation [18]. Therefore, the importance of search for natural antioxidants has increased in the recent years, so many researchers focused on the same [19].

DPPH radical scavenging assay

In many disorders like cancer, neurodegenerative diseases, and AIDS-free radicals are playing important role [20]. For the management of these diseases, antioxidants are playing a vital role due to their radical scavenging activity. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts [20]. By measuring the decrease in the absorbance at 517 nm, the reduction capacity of DPPH radicals by the antioxidants can be measured. The percentage of DPPH radical scavenging activity of the ethanolic extracts of *S. suaveolens*, *H. spinosa,* and *N. zeylanica* is presented in Tables 10-12, and they shows prominent activity with IC50 values 61.6±2.3, 67.5±3.5, and 373±2.81, respectively. *S. suaveolens* is more promising among them with least IC50 value.

Table 7: Anthelmintic property of *S. suaveolens* leaves

Observation	Distilled water	Albendazole (25 mg/mL)	Drug (25 mg/mL)	Drug (50 mgmL)	Drug (100 mgmL)
Time taken for paralysis (min)	-	27±0.72	38±1.53	25.7±2.08	13±1.19
Time taken for death (min)	-	-	78.6±2.3	63.3±2.5	27.3±2.08

S. suaveolens: Stereospermum suaveolens

Table 8: Anthelmintic property of *H. spinosa* leaves

Observation	Distilled water	Albendazole (25 mg/mL)	Drug (25 mg/mL)	Drug (50 mg/mL)	Drug (100 mg/mL)
Time taken for paralysis (min)	-	32.4±2	56.3±0.57	32.7±3.21	24±1.73
Time taken for death (min)	-	-	119.7±3.5	53±2	43.7±1.53

H. spinosa: Hygrophila spinosa

Table 9: Anthelmintic property of *N. zeylanica* aerial part

Observation	Distilled water	Albendazole (25 mg/mL)	Drug (25 mg/mL)	Drug (50 mg/mL)	Drug (100 mg/mL)
Time taken for paralysis (min)	-	29±2	28±1.5	21±2	16±3
Time taken for death (min)	-	-	35±2.3	31±2.6	21±2

N. zeylanica: Naravelia zeylanica

Table 10: Antioxidant property of *S. suaveolens* leaves

Concentration of plant extract (µg/L)	Percentage of inhibition	
	DPPH	NBT
10	14.47±1	15.07±1.51
15	17.48±0.92	31.5±2.12
25	35.05±0.90	34.37±1.27
50	42.02±2.68	38.11±1.1
75	60.65±3.85	42.16±1.18
100	88.16±2.91	46.93±1
250	91.07±0.25	58.16±1.10
500	90.20±0.20	69.53±1.47
750	93.27±0.25	77.9±0.75
1000	94.44±0.15	79.9±0.45
IC 50 value	61.6±2.3	146.6±3.6

DPPH: 2,2 Diphenyl 1-picrylhydrazyl, *S. suaveolens: Stereospermum suaveolens*

Table 11: Antioxidant property of *H. spinosa* leaves

Concentration of plant extract (µg/L)	Percentage of inhibition	
	DPPH	NBT
10	11.5±2.1	17±0.7
15	15.5±0.7	21.6±3
25	18±1.4	25.6±1.15
50	38	40.3±0.57
75	56±1.8	44.6±2
100	78±1.4	50.3±2.5
250	93.3±1.15	56.6±1.5
500	96.6±0.57	59.6±2.08
750	98.6±0.57	64.6±1.15
1000	99	69.6±0.57
IC 50 value	67.5±3.5	95.5±6.3

DPPH: 2,2 Diphenyl 1- picrylhydrazyl, *H. spinosa: Hygrophila spinosa*

Table 12: Antioxidant property of *N. zeylanica* aerial part

Concentration of plant extract (µg/L)	Percentage of inhibition	
	DPPH	NBT
10	3.6±0.51	15.3±0.34
15	8.86±2.57	24.58±0.29
25	12.1±1.9	35.68±0.30
50	16.03±4.2	44.12±0.45
75	21.63±2.6	49.84±2.09
100	23.9±2.12	52.84±1.13
250	38.5±1.49	68.34±0.68
500	60.9±1.27	77.41±0.29
750	79.77±2.18	81.46±1.81
1000	93.71±1.39	84.52±1.23
IC 50 value	373±2.81	74.66±8.5

DPPH: 2,2 diphenyl 1- picrylhydrazyl, *N. zeylanica: Naravelia zeylanica*

Superoxide radical scavenging assay

The superoxide radical scavenging assay also shows significant radical scavenging property with IC50 values 146.6±3.6, 95.5±6.3, and 74.66±8.5, respectively, in *S. suaveolens*, *H. spinosa,* and *N. zeylanica*. The activity was increasing with the increasing concentrations of test solution and shows least IC50 value in *N. zeylanica.*

The present study indicates that the studied medicinal plant extracts could inhibit the oxygen radicals as seen from scavenging superoxide and DPPH radicals, and it could reduce the oxygen radicals and subsequently reduce the harmful effects. The literature supports that phytoconstituents such as polyphenolic compounds in drugs are responsible for the antioxidant potential [22,23]. Further, phenolic compounds are effective hydrogen donors, which make them antioxidant [24]. Leaves of *S. suaveolens* and *H. spinosa* and aerial parts of *N. zeylanica* possess valuable secondary metabolites such as phenols, flavonoids, alkaloids, and tannins [3,25,26], the observed activity may be due to the presence of these phytoconstituents.

CONCLUSION

All the extracts showed varying degrees of antimicrobial, anthelmintic, and antioxidant properties. All these plants were more effective than the commercial antibiotics in combating the helminth studied. To combat pathogenic microorganisms, the folk medicines screened in this *in vitro* study can be used as effective agent due to the efficiency and less side effects. The luxuriant use of these medicinal plants in folk medicine proves that they represent a safe and economic alternative to treat various infectious diseases and oxidative stress damages.

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AUTHORS' CONTRIBUTION

Alby Alphons Baby has performed all the experiments in the laboratory. Regi Rahael K has provided the design, intellectual content to choose the plant, and act as a mentor for the works.

CONFLICTS OF INTEREST

The authors declare that they do not have any conflicts of interest.

REFERENCES

1. Beegam AR, Nayar TS. Plants used for natal health care in folk medicines of Kerala, India. Indian J Tradit Knowledge 2011;10:523-27.
2. Nastro A, Germano M, Marino DV, Cannatelli M. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 2000;30:379-84.
3. Meena K, Yadav AK, Panda P, Preet K, Rao MM. Review on *Stereospermum suaveolens* (Roxb.) DC; a potential herb. Drug Invent Today 2010;2:238-39.
4. Gamble JS. Flora of the Presidency of Madras. Dehradun: Bishen Singh and Mahendra Pal Singh; 1915.
5. Udayan PS, George S, Tushar KV, Balachandran I. Medicinal plants used by malayali tribes of Sevarayan hills Yercad, Salem district, Tamilnadu, India. Zoo Print J 2006;21:2223-4.
6. Anonymous. The Useful Plants of India. New Delhi: NISCAIR; 1992. p. 392.
7. Asolkar LV, Kakkar KK, Chakre OJ. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles. New Delhi: NISCAIR, CSIR; 2005.
8. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. India: Springer Publications; 2007.
9. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants used in Ayurveda. Vol. 4. New Delhi: Central Council for Research in Ayurveda and Siddha; 2002.
10. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. Vol. 3. New Delhi: Publication and Information Directorate, CSIR; 1993.
11. Baby AA, Raphael KR. First step towards unravelling the medicinal properties of an endemic traditional medicine *Bauhinia phoenicea* Wight and Arn bark. Int J Pharm Pharm Sci 2015;7:403-5.
12. Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of nosocomial infections. BMC Complement Altern Med 2006;6:2.
13. du Toit EA, Rautenbach M. A sensitive standardized micro-gel well diffusion assay for the determination of antimicrobial activity. J Microbiol Methods 2000;42:159-65.
14. Vital PG, Velasco RN, Demigillo JM, Rivera WL. Antimicrobial activity, cytotoxicity and phytochemical screening of *Ficus septica* Burm and *Sterculia foetida* L leaf extracts. J Med Plant Res 2010;4:58-63.
15. Baby AA, Raphael KR. Potential antimicrobial, anthelmintic and antioxidant properties of *Areca catechu* L root. Int J Pharm Pharm Sci 2014;6:486-89.
16. Braca A, Sortino C, Politi M. Anti-oxidant activity of flavonoids from

Licania licaniaeflora. J Ethnopharmacol 2002;79:379-81.
17. Deb L, Dubey SK, Jain A, Jain AK, Pandian GS. Preventive effect of *Thuja occidentalis* (Linn) on gastric ulcer-a novel role of free radical scavenger. J Nat Remedies 2009;9:152-8.
18. Andlauer W, Furst P. Antioxidative power of phytochemicals with special reference to cereals. Cereal Foods World 1998;43:356-9.
19. Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape (*Vitis vinifera)* seed extract. Food Res Int 2003;36:117-22.
20. Uttara B, Ajay VS, Zambi P, Mahajan RT. Oxidative stress and neurodegenerative disease; A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 2009;7:65-74.
21. Koleva II, Van Beek TA, Linseen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal 2002;13:8-17.
22. Khushad MM, Masiunas MA, Smith W, Eastmank K. Health promoting phytochemicals invegetable. Hortic Rev 2003;28:125-85.
23. Gooijer CR, Evade H, Zappey H. Liquid chromatography with atmospheric pressure chemical ionization and electroscopy ionization mass spectroscopy of flavonoids with triplequadrupole and ion trap instruments. J Chromatogr 1997;984:45-8.
24. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activity of plant derived polyphenolic flavonoids. Free Radic Res 1995;22:375-83.
25. Sutharsingh R, Kavimani S, Jayakar B, Uvarani M, Thangathirupathi A. Quantitative phytochemical estimation and Antioxidant studies on aerial parts of *Naravelia zeylanica* DC. Int J Pharm Stud Res 2011;2:52-6.
26. Kshirsagar AD, Ingale KG, Vyawahare NS, Thorve VS. *Hygrophila spinosa*: A comprehensive review. Pharmacogn Rev 2010;4:167-71.