Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 1995-1999
Received: 22-09-2018
Accepted: 24-10-2018

Loganathan V
Department of Chemistry, Periyar University, Salem, Tamil Nadu, India

Devi Kaniakumari M
Department of Chemistry, Quaid-E-Millath Govt. College for Woman, Chennai, Tamil Nadu, India

Selvakumar P
Department of Chemistry, Periyar University, Salem, Tamil Nadu, India

Correspondence
Devi Kaniakumari M
Department of Chemistry, Quaid-E-Millath Govt. College for Woman, Chennai, Tamil Nadu, India

In-Vitro assessment of antimicrobial and antioxidant activities of different extracts of *Mallotus rhamnifolius*

Loganathan V, Devi Kaniakumari M and Selvakumar P

Abstract
Antimicrobial activity of different solvent leaf extracts from *Mallotus rhamnifolius* was evaluated against certain pathogenic gram positive and gram negative bacteria as *Bacillus cereus*, *Staphylocous aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and fungal pathogens such as *Aspergillus niger* and *Fusarium oxysporum* by Agar well diffusion method. It was showed that the different extracts had potent antimicrobial activity against both Gram positive and Gram negative bacterial pathogens and fungal pathogens. Gentamicin was used as positive control for bacteria and Ketoconazole was used for fungi. In this study, quantitative values of antioxidant activity such as ABTS, FRAP and nitric acid radical scavenging activity were analyzed to determine both scavenging ability and the reducing properties of different solvent leaf extracts of *Mallotus rhamnifolius*. The free radical scavenging activities were compared with standard antioxidants like Rutin.

Keywords: *Mallotus rhamnifolius*, antimicrobial activity, antioxidant activity

Introduction
In recent years, multiple drug resistances in human pathogenic microorganisms have developed due to the indiscriminate use of antimicrobial drugs generally employed in the treatment of infectious diseases. The undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are the disadvantages of commercial antimicrobial drugs [1, 2]. The screening of plant extracts and products of plant for antimicrobial activity has shown that higher plants represent a potential source of new anti–infective agents [3].
Many medicinal plant contain a wide variety of phenolic compounds (flavonoids, tannins etc.,) act potentially as antioxidants to scavenge free radicals and inhibit lipid peroxidation [4].The enhanced wound healing potency of numerous herbal extracts may be attributed to antioxidants and the antimicrobial property of the phytoconstituents present. Hence the therapeutic benefit of medicinal plants is often characteristic to their antioxidant property [5]. Antioxidants are important to prevent tissue damage that stimulates wound healing process. Botanicals with free radical-scavenging activity or antioxidant thus can play a significant role in healing of wound [6]. There has been an increasing importance in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Many vegetables, fruits and other plant species are already exploited commercially either as antioxidant additives or a nutritional supplements [7] or have been investigated in the search for new antioxidants [8, 9].
Radicals are chemical species having one or more unpaired electrons, and free radicals are radicals that have moved out of the immediate molecular environment of their generation. There are several sources of oxidants in the body reduction of molecular oxygen in mitochondria during cellular respiration takes place in several sequential steps, giving the radical by-products superoxide O2•, hydrogen peroxide H_2O_2, and hydroxyl HO• degradation of fatty acids and other molecules in peroxisomes produce H_2O_2 phagocytosis results in an oxidative burst of nitric oxide (NO•), which also reacts with superoxide to produce the oxidizing and nitrating species peroxynitrite ($ONOO^-$) [10]. The genus *Mallotus rhamnifolius* belongs to the family Euphorbiaceae. Plants of this genus has been used in folk medicine such as topical antiseptic and using in treatment of bronchitis, abdominal diseases, anthelminthic, spleen enlargement as well as to treat chronic hepatitis in traditional Vietnamese folk medicine [11, 12]. The genus as a whole is significantly efficacious against helminthes parasites infections [13, 14, 15], especially its fruit used to cure worm constipation, Infestation as well as abdominal diseases [16].

Materials and Methods

Culture and Maintenance of microorganisms

Pure cultures of all experimental bacteria and fungi were obtained from the Department of Microbiology, Hindustan College of Arts and Science, Coimbatore. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by sub-culturing regularly on the same medium and stored at 4°C before use in experiments.

Table 1: For the present study following pure Bacterial pathogens were taken

S. No.	Name	Type	MTCC No.
1	*Bacillus cereus*	Gram positive	MTCC4317
2.	*Staphylocous aureus*	Gram positive	MTCC3160
3.	*Escherichia coli*	Gram negative	MTCC1652
4.	*Pseudomonas aeruginosa*	Gram negative	MTCC4676

Table 2: For the present study following pure fungal pathogens were taken

S. No.	Name	MTCC No.
1	*Fusarium oxysporum*	MTCC6659
2.	*Aspergillus niger*	MTCC282

Preparation of plant extract

About 80 gm of the dried leaves pulverization was extracted with hot solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetate and ethanol: water (95:5) for 24 hours with each solvent, using by hot continuous extraction method [17] in Soxhlet apparatus at a temperature of 30˚C to 85˚C. Each time before extracting with next solvent, the powdered material was air dried and then subjected to further extraction. After the effective extraction, they were filtered using Whatman filter paper No.1. Then the extracts was evaporated to solventlessness by using vacuum rotary evaporator to yields a soxhlet crude extracts to produce a sticky material, and further transferred into sterile bottles and refrigerated until use screened [18, 19].

Microbiological screening

Antimicrobial activities of different extracts were evaluated by the agar well diffusion method (Murray *et al*.) [20] Modified by (Olurinola) [21]

Media Preparation and its Sterilization

In agar well diffusion method antimicrobial susceptibility was tested on solid (Agar-agar) media in petri plates. For bacterial assay nutrient agar (NA) (40 gm/l) and for fungus PDA (39 gm/l) was used for producing surface colony growth.

Agar well diffusion method

Agar well-diffusion method was employed to measure the antimicrobial activity. Potato Dextrose Agar (PDA) and Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria and fungi. Wells (10 mm diameter and about 2 cm a part) were formed in each of these plates using sterile cork borer. Stock solution was prepared at a concentration of 10 mg/10ml by ethanol in different plant extacts viz. Petroleum ether, Chloroform, Ethyl acetate and Ethanol: Water. About 500 µl of different concentrations of plant solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without extract of plant were set up. The plates were incubated for 18-24 hours for bacterial pathogens and 28°C for 48 hours fungal pathogens at 37°C. Measured the diameter of the inhibition zone in mile meter and the activity index was also calculated. For maintained the triplicates, the experiment was repeated thrice. For each replicates the readings were taken in three different fixed directions and the average values were recorded.

Measurement of antimicrobial activity using Agar well diffusion Method

The antimicrobial potential of the experimental plants was calculated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards. Gentamicin and Ketoconazole. (100µg /disc) were used as positive control sample. The results showed that all the extracts are potent antimicrobials against all the microorganisms studied. Among the different solvents extracts studied method ethanol: water showed high degree of inhibition followed by ethyl acetate, chloroform and petroleum ether extract for all the tested microorganisms.

Antioxidant activity

Stock solution was prepared at a concentration of 10 mg/10ml by ethanol in different plant extact viz. Petroleum ether, Chloroform, Ethyl acetate and Ethanol: Water. Rutin used as positive control in 100µg/500µl concentration with ethanol solvent.

Nitric oxide radical scavenging activity

The nitric oxide scavenging activity of different solvent extracts of leaves of *Mallotus rhamnifolius* on nitric oxide radical was measured according to the method [22] of Sodium nitroprusside (10 mM) in phosphate buffered saline, was mixed with 500µl different extractions of *Mallotus rhamnifolius* extracts and incubated at room temperature for 150 min. Griess reagent (0.5mL), containing 1% sulphanilamide, 2% H_3PO_4 and 0.1% N- (1 – naphthyl) ethylene diaminedihydrochloride was added to the mixture after incubation time. The absorbance of the chromophore was read at 546 nm. Gallic acid and quercetin and the same mixture of the reaction without *Mallotus rhamnifolius* extracts were employed as positive and negative control. Radical scavenging activity was calculated as the inhibition percentage of free radical by the sample.

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) assay

For ABTS assay [23, 24] the procedure followed. The 500µl stock solutions included 7.4 mM $ABTS^{\cdot+}$(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate))solution and 2.6 mM Potassium persulfate solution. Then the working solution was prepared by mixing equal quantities of the two stock solutions and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^{\cdot+}$ solution with 60 mL methanol to obtain an absorbance of 1.1 units at 734 nm using the spectrophotometer. Fresh $ABTS^{+}$ solution were prepared for each assay. 500µl different extractions of *Mallotus rhamnifolius* extracts were allowed to react with 2850 µl of the $ABTS^{\cdot+}$ solution for 2 h in a dark condition. Then the absorbance was taken using the spectrophotometer at 734 nm. The standard curve was linear between 25 and 600 mM Trolox. Results are expressed in µM Trolox equivalents (TE)/g fresh mass.

FRAP (Ferric reducing ability of plasma) assay

Total antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) assay [25, 26, 27]. The principle of this method is based on the reduction of a ferric tripyridyltriazine complex to its ferrous, colored from in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 ml of a 10 mmol/L, TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mmol/L HCl plus 2.5 ml of 20 mmol/L $FeCl_3.6H_2O$ and 25 ml of 0.3 mol/L acetate buffer, pH 3.6 and was prepared freshly and warmed at 37 °C. Working FRAP reagent 2850 µl is mixed with 500µl different extractions of *Mallotus rhamnifolius* extracts were allowed to react for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm using the spectrophotometer. The final result was calculated as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$. Results are expressed in µM Trolox equivalents (TE)/g fresh mass.

Results and Discussion

In the present investigation, different solvent extracts of *Mallotus rhamnifolius* was evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria, fungus which was regarded as human pathogenic microorganism.

Our preliminary investigation showed that all Petroleum ether, Chloroform, Ethylacetate and Ethanol: Water extracts of *Mallotus rhamnifolius* were active against the locally isolated human pathogens. During the incubation, the maximum zone of inhibition was formed around the disc and transparent millimeter ruler used to measure inhibition zone that clearly shown in Table -3 and Figure-1.

Table 3: Antimicrobial activity of different solvent extracts of leaf of the *Mallotus rhamnifolius*

Microorganisms	Inhibition zone in diameter (mm)				
	Control	Pet. ether	Chloro form	Ethyl acetate	Ethanol: water
Bacillus cereus	22	10	14	14	16
Staphylocous aureus	26	11	16	17	17
Escherichia coli	29	14	18	18	19
Pseudomonas aeruginosa	28	10	14	16	21
Fusarium oxysporum	32	12	15	16	18
Aspergillus niger	32	14	16	17	20

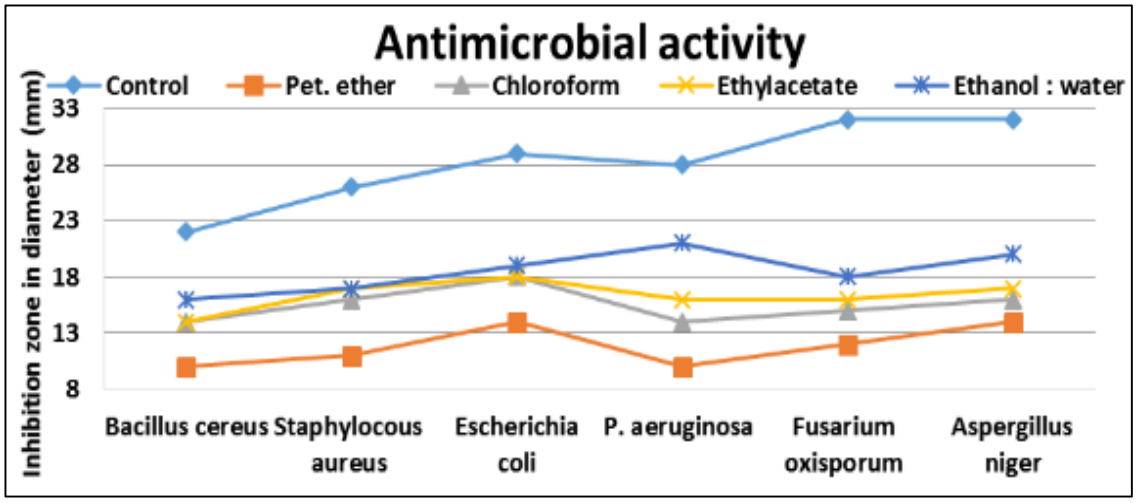

Fig 1: Antimicrobial activity of different solvent extracts of leaf of the *Mallotus rhamnifolius*

Here bacteria and fungus are tested *Staphylocous aureus*, Bacillus cereus are gram positive *Escherichia coli* and *Pseudomonas aeruginosa* gram negative bacteria, *Fusarium oxysporum*, *Aspergillus niger* as fungus culture. In the Pet. ether extracts of *Mallotus rhamnifolius* the high antimicrobial zone was formed around (14 mm) in *Escherichia coli* and *Aspergillus niger* followed by *Fusarium oxysporum* (12 mm), in *Staphylocous aureus* (11 mm), *Bacillus cereus* and *Pseudomonas aeruginosa* affected only (10 mm) its low zone compare other organisms. In the extracts of Chloroform and Ethyl acetate *Fusarium oxysporum* followed by *Escherichia coli* has high zone of inhibition around 16 mm and 18 mm. Finally in the extracts of Ethanol: water good
inhibition zone was formed in all tested microorganisms, maximum antimicrobial zone were formed in *Fusarium oxysporum* (18 mm) of fungus culture followed by gram negative and positive bacteria *Escherichia coli* (19 mm), *Pseudomonas aeruginosa* (21mm), *Staphylocous aureus* (17mm) and small inhibition zone found in *Bacillus cereus* (16mm) and *Aspergillus niger* (20mm).

So the Ethanol: water extracts of *Mallotus rhamnifolius* plant leaves have excellent antimicrobial activity against all tested microorganisms like gram positive, gram negative and fungus cultures.

Nitric acid radical scavenging activity assay

The results of the free radical scavenging potential of the different extracts tested by Nitric acid radical scavenging activity assay is given in the Table 4 and Figure-2. Ethanol: water extract of the leaf was found to have the most potent antioxidant property among all the other extracts. The high activity of water: Ethanol extracts are generally attributed to the presence of alkaloids and phenols, as the majority of active antioxidant compounds are observed in these classes of phytochemical compounds.

ABTS Assay

The Ethanol: water extracts of the leaf of *Mallotus rhamnifolius* were found to be more effective scavengers of the ABTS radical compared to the other extract (Table-4 and Figure-2). The petroleum ether extracts of the leaf showed lower ability in scavenging the ABTS radical. Proton radical scavenging is an important characteristic of antioxidants. ABTS, a protonated radical, has absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals [28].

Table 4: Percentage of scavenging activity of different solvent extracts of leaf of the *Mallotus rhamnifolius*

Extraction on Medium	% of Scavenging activity		
	Nitric acid radical scavenging activity	ABTS	FRAP
Pet. Ether	19.44±0.93	33.73±.062	49.9±0.4
Chloroform	27.78±1.85	46.23±1.04	55.8±0.5
Ethyl acetate	33.04±1.07	58.16±1.16	62.3±2.1
Ethanol : water	41.98±1.41	64.88±0.44	64.2±0.7
Rutin	81.25±1.56	98.88±2.53	84.23±1.86

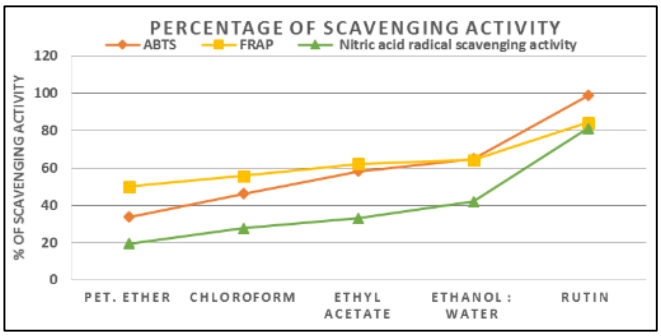

Fig 2: The percentage of antioxidant scavenging activity different solvents extracts of *Mallotus rhamnifolius*

The percentage of antioxidant scavenging activity different solvents extracts of *Mallotus rhamnifolius* are presented in the figure: 2. The ethanol: water extracts shows the maximum activity in all the three experiments. Comparative the nitric acid radical scavenging activity, ABTS and FRAP methods shows significantly good values of 64.88±0.44 and 64.2±0.7 respectively.

The scavenging of the FRAP radical by the solvent extracts of *Mallotus rhamnifolius* was found to be much higher than that of ABTS radical. Although many methods are available to determine antioxidant activity, it is important method a consistent and rapid method. While each method has its own merits and drawbacks, it has been found that the most common and reliable methods are the ABTS and FRAP methods; these have been modified and improved in recent years [29].

Conclusion

The crude extracts were used for testing their antimicrobial activity and antioxidant activity using ABTS, FRAP and Nitric acid radical scavenging activity assays. Ethanol: water extract of the leaves was found to exhibit maximum antimicrobial activity and antioxidant activity among all the extracts showed the possibility for isolation and characterization of specific molecules from the crude extract, which could be purified and used for further studies. The ability of the extracts to scavenge different free radicals in different systems indicated that they may be useful therapeutic agents for treating radical related pathological damage.

References

1. Dubey R, Dubey K, Sridhar C, Jayaveera KN. Human Vaginal Pathogen Inhibition Studies on Aqueous, Methanolic And Saponins Extracts of Stem Barks of *Ziziphus Mauritiana*. Int. J Pharm. Sci. Res. 2011; 2(3):659-663.
2. Kamali HH EL, Amir MYEL. Antibacterial Activity and Phytochemical Screening of Ethanolic Extracts Obtained from Selected Sudanese Medicinal Plants. Curr. Res. J of Bio. Sci. 2010; 2(2):143-146.
3. Lalitha P, Arathi KA, Shubashini K, Sripathi, Hemalatha, S, Jayanthi P. Antimicrobial Activity and Phytochemical Screening of an Ornamental Foliage Plant, *Pothos aureus* (Linden ex Andre). An Int. J of Chem. 2010; 1(2):63-71.
4. Hussain H, Badawy A, Elshazly A, Elsayed A, Krohn K, Riaz M, *et al*. Chemical Constituents and Antimicrobial Activity of *Salix subserrata*. Rec. Nat. Prod. 2011; 5(2):133-137.
5. Preethi R, Devanathan VV, Loganathan M. Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants against Food Borne Pathogens. Adv. in Bio. Res. 2010; 4(2):122-125.
6. Sharma A. Antibacterial activity of ethanolic extracts of some arid zone plants. Int. J of Pharm. Tech. Res. 2011; 3(1):283-286.
7. Enne VI, Livermore DM, Stephens P, Hal LMC Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. The Lancet. 2001; 28:1325-1328.
8. Westh H, Zinn CS, Rosdahl VT. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microb. Drug Resist. 2004; 10:169-176.
9. Kim H, Park SW, Park JM, Moon KH, Lee CK. Screening and isolation of antibiotic resistance inhibitors from herb material Resistant Inhibition of 21 Korean plants. Nat. Prod. Sci. 1995; 1:50-54.
10. Alagesaboopathi C. Antimicrobial Potential and Phytochemical Screening of *Andrographis Affinis* Nees an Endemic Medicinal Plant from India. Int. J of Pharma. and Pharmaceutical Sci. 2011; 3 (2):157-159.
11. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multiple drug resistant human pathogens. J Ethanopharma. 2001; 74:113-123.
12. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S, Verma RS. Antibacterial activity of the plant used in Indian herbal medicine. Int. J of green pharma. 2010; 10:22-28.
13. Zgoda JR, Porter JR A convenient micro dilution method screening natural products against bacteria and fungi. Pharm. Biol. 2001; 39:221–225.
14. Warrier PK, Nambiar VPK, Ramankutty C. Indian medicinal plants, a compendium of 500 species. Hyderabad, India: Orient Longman Ltd, 1995, 10-12.
15. Ntalli NG, Cottiglia F, Bueno CA, Alché LE, Leonti M, Vargiu S, Bifulco SUM, *et al*. Cytotoxic Tirucallane Triterpenoids from *Melia azedarach* Fruits. Molecules. 2010; 15:5866-5877.
16. Vishnukanta, RAC Evaluation of Hydroalcoholic Extract of *Melia Azedarach* Linn Roots for Analgesic and Anti Inflammatory Activity. Int. J of Phytomed. 2010; 2:341-344.
17. Tasneef Ahmad, Swatantra Bahadur Singh, Shivshankar Pandey, Phytochemical Screening and Physicochemical Parameters of Crude Drugs: A Brief Review International Journal of Pharma Research & Review. 2013; 2(12):53-60.
18. Subhashini R, Mahadeva-Rao US, Sumathi P, Gunalan G. A comparative phytochemical analysis of cocoa and green tea, Indian J Sci., Technol. 2010; 3 (2):188192.
19. Sofowora A, Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, 1993, 289-300.
20. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken

HR. Manual of Clinical Microbiology, 6th Ed. ASM Press, Washington DC, 1995, 15-18.
21. Olurinola PF. A laboratory manual of pharmaceutical microbiology, Idu, Abuja, Nigeria, 1996, 69-105.
22. Sreejayan and Rao. Sreejayan N, Rao MNA. Nitric oxide scavenging by cucuminoids. J Pharma Pharmacol. 1997; 49:105-107.
23. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chemistry. 2002; 76:69-75.
24. Miller, Rice-Evans. Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays, Freem Radic. Res 1997; 26(3):195-9.
25. Benzie FF, Strain JJ. Ferric reducing/ antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology. 1999, 299:15-23.
26. Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research. 2003; 23:1719-1726.
27. Jimenez-Escrig A, Rincon M, Pulido R, Saura-Calixto F. Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. Journal of Agricultural and Food Chemistry. 2001; 49:5489-5493.
28. Mathew S, Abraham ET. *In vitro* antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food Chem. Toxicol. 2006; 44:198-206.
29. Ravi Shankar K, Ramesh KV, Naveena P. Free radical scavenging activity of the flower and fruit extracts of Alstonia scholaris. Biosci. Biotechnol. Res. Asia. 2008; 5:493-494.