World Journal of Pharmaceutical research
Volume 1, Issue 3, 705-716. Research Article ISSN 2277 – 7105

EVALUATION OF ANTIBACTERIAL AND DPPH RADICAL SCAVENGING ACTIVITIES OF THE LEAF EXTRACTS AND LEAF ESSENTIAL OIL OF *CORIANDRUM SATIVUM* LINN.

[*]L. Joji Reddy[1], Reshma Devi Jalli[1], Beena Jose[2], Spandana Gopu[1]

[1] Department of Biotechnology, Loyola Academy Degree & P.G. College, Alwal, Secunderabad, Andhra Pradesh, 500010, India

[2]Department of Chemistry, Vimala College, Thrissur, Kerala, 680009, India

Article Received on
24 May 2012,

Revised on 05 June 2012,

Accepted on 12 June 2012

***Correspondence for Author:**

*** L. Joji Reddy**

Department of Biotechnology, Loyola Academy Degree & P.G. College, Alwal, Secunderabad, Andhra Pradesh, 500010, India

jojilrsj@gmail.com

ABSTRACT

The antibacterial and DPPH radical scavenging activities of the leaf extracts and leaf essential oil of *Coriandrum sativum* (Coriander) were investigated. The antibacterial potential of the leaf essential oil, petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Coriandrum sativum* were studied against human pathogenic bacteria viz. *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* by 'agar well diffusion' method. Leaf essential oil as well as leaf ethyl acetate, chloroform and methanol extracts of *Coriandrum sativum* exhibited pronounced activity against Gram-positive and Gram-negative bacteria and their activity is quite comparable with the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin screened under similar conditions.

Among the leaf essential oil and leaf extracts of *C. sativum* studied, methanol extract and leaf essential oil showed potent scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The remarkable antibacterial and antioxidant activity exhibited by the plant extracts and essential oil can be attributed to the synergic effect of the active compounds present in it. The results obtained showed that the leaf methanol extract and leaf essential oil of *C. sativum*

can be considered as good sources of natural antioxidants and antimicrobial compounds and can be incorporated into the drug formulations.

Key words: *Coriandrum sativum* , antibacterial activity, agar well diffusion method, DPPH radical scavenging activity, drug formulations

INTRODUCTION

Coriandrum sativum (family Umbelliferae) is highly reputed ayurvedic medicinal tree commonly known as the Dhanya. It is a glabrous, aromatic, herbaceous annual plant, small sized tree growing throughout India, Italy, Netherlands, Central and Eastern Europe, China and Bangladesh. Essential oil, flavonoids, fatty acids, and sterols have been isolated from different parts of *C. sativum*. The different parts of this plant contain monoterpenes, α-pinene, limonene, γ-terpinene, p-cymene, borneol, citronellol, camphor, geraniol, coriandrin, dihydrocoriandrin, coriandrons A-E, flavonoids and essential oils. Various parts of this plant such as seed, leaves, flower and fruit, possess antioxidant activity, anti-diabetic activity, anti-mutagenic activity, anti-helmintic activity, sedative-hypnotic activity, anticonvulsant activity, diuretic activity, cholesterol lowering activity, protective role against lead toxicity, antifungal activity, anti-feeding activity, anticancer activity, anxiolytic activity, hepatoprotective activity, anti-protozoal activity, anti-ulcer activity, post-coital anti-fertility activity, heavy metal detoxification[1]. In the Indian traditional medicine, a coriander is used in disorders of digestive, respiratory and urinary system, as it has diaphoretic, diuretic, carminative and stimulant. In Iranian traditional medicine, coriander has been indicated for a number of medical problems such as dyspeptic complaints, loss of appetite, convulsion and insomnia [2,3]. Coriander has been reported has been reported to exhibit several pharmacological effects such as antioxidant activity[4,5], anti-diabetic activity[6], anti-mutagenic activity[7], anthelmentic activity[8], sedative-hypnotic activity[9], anticonvulsant activity[10], diuretic activity[11], cholesterol lowering activity[12], protective role against lead toxicity[13], anti-feeding activity[14], anticancer activity[15], post-coital anti-fertility activity[16], heavy metal detoxification[17].

Based on review of literature no reports are available regarding antibacterial and DPPH radical scavenging activities of *Coriandrum sativum* leaf essential oil and leaf extracts. In this work, the antibacterial property of the *Coriandrum sativum* leaf oil and leaf extracts were checked against various multi-drug resistant Gram positive and Gram negative bacterial strains by ‘agar well diffusion method’. The antioxidant activity of the leaf essential oil and

extracts were studied by DPPH radical scavenging assay. The results showed that the leaf essential oil and methanolic extracts of the leaves of *Coriandrum sativum* is a good source of active compounds and antioxidants.

MATERIALS AND METHODS

Plant Material

The leaves of *Coriandrum sativum* were collected from Thrissur district of Kerala, South India and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur.

Essential oil extraction

Fresh leaves of *Coriandrum sativum* (250g) were ground to a paste using an electric mixer grinder and subjected to steam distillation for three hours. About 2 liters of the distillate were collected and extracted with diethyl ether (3X100 mL) and dried using anhydrous sodium sulphate. The dry ether extract on evaporation yielded 0.75g (0.30% of fresh weight of the sample) of pale yellow leaf oil.

Preparation of Plant Extracts

Fifty grams of the powered plant material were extracted successively with 150mL of petroleum ether, chloroform, ethyl acetate and methanol as solvents for 24hours by Soxhlet equipment.

Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India), which were maintained on Nutrient broth media. They were Gram-positive bacteria such as *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-96) and *Enterobacter faecalis* (MTCC-5112) and Gram-negative bacteria such as *Salmonella paratyphi* (MTCC-735), *Escherichia coli* (MTCC-729), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647), *Proteus vulgaris* (MTCC-426) and *Serratia marcescens* (MTCC-86).

Culture medium and inoculums

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^0C. Inoculum was prepared by suspending a loop full of bacterial cultures into

10mL of nutrient broth and was incubated at 37^0C for 24hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50^0C was distributed by pipette (20mL) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1mL of bacterial suspension was taken and poured into Petri plates containing 20mL nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

Antibacterial activity assay

The agar well diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50µL of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Coriandrum sativum* were added in each well. Wells introduced with 50µL of pure petroleum ether, chloroform, ethyl acetate and methanol served as negative controls. The plates were incubated at 37^0C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm[18]. All the assays were performed in triplicate and expressed as average values.

Preliminary Phytochemical analysis

The sample extracts were analysed for the presence of various phytoconstituents like flavonoids, alkaloids, glycosides, steroids, phenols, saponins and tannins according to standard methods[19].

DPPH free radical scavenging assay

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants[20]. Hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometer assay uses the stable radical DPPH as a reagent. The sample solution of material (50 µL) at four concentrations (1.0, 0.5, 0.25 and 0.125 mg/mL) was mixed with freshly prepared methanolic solution of DPPH (634 μM) and allowed to stand for 30 min at room temperature. The

absorbance was then measured at 515nm using a spectrophotometer and the inhibition of free radical DPPH in percent (%) was calculated using the formula below:

The percent of inhibition of DPPH reduction (decolourization)

$$\% \text{ of inhibition} = \frac{A_0 - A_{sample}}{A_0} \times 100$$

where (A_0) is the absorbance of the control (blank) and (A_{sample}) is the absorbance of the test compound. The compound concentration demonstrating 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against sample concentration. Tests were carried out in triplicate. Samples and DPPH were dissolved in methanol. L-ascorbic acid was used as positive control.

RESULTS

Antibacterial screening

The leaf extracts and leaf essential oil of *Coriandrum sativum* showing the zone of inhibition in millimeters, for Gram positive and Gram negative bacteria are summarized in Table1. In addition, the inhibition zones formed by standard antibiotics and those of negative controls are listed in Table 2.

Phytochemical screening

Phytochemical evaluation was performed with methanol, ethyl acetate, chloroform and petroleum ether extracts of the leaves of *Coriandrum sativum* (Table 3).

Antioxidant activity

The antioxidant activity of *Coriandrum sativum* leaf essential oil and leaf extracts in solvents of varying polarity were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. The method is based on the reduction of alcoholic DPPH· solutions in the presence of a hydrogen donating antioxidant. DPPH· solutions show a strong absorption band at 515 nm appearing as a deep violet color. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining DPPH·, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant. The results of the free radical scavenging activity of the leaf extracts and leaf essential oil of *C. sativum* assessed by DPPH assay and amount of the sample needed for 50% inhibition of free radical activity, IC_{50} values were summarized in table 4.

Table 1: Inhibition zones formed by *Coriandrum sativum* leaf essential oil and leaf extracts

	Diameter of inhibition zones(mm/50µL)							
	C.sativum leaf oil				*C.sativum* leaf extracts			
Microorganisms	**20%**	**10%**	**5%**	**1%**	**A**	**B**	**C**	**D**
1. *Bacillus cereus*	20	17	15	13	29	20	17	15
2. *Enterobacter faecalis*	20	19	16	14	29	21	17	14
3. *Salmonella paratyphi*	29	22	19	16	20	19	16	15
4. *Staphylococcus aureus*	20	18	15	12	29	20	19	15
5. *Escherichia coli*	32	30	18	15	19	16	15	13
6. *Proteus vulgaris*	26	20	19	13	26	20	17	12
7. *Klebsiella pneumoniae*	20	17	15	13	23	21	19	16
8. *Pseudomonas aeruginosa*	24	21	19	16	20	15	13	11
9. *Serratia marcescens*	28	22	19	17	23	20	18	14

A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether

Used concentrations: 50µL of 20%, 10%, 5% and 1% essential oil samples in DMSO & 50µL of 10mg/mL of plant extracts

Table 2: Inhibition zones formed by the standard antibiotics and negative controls

	Diameter of inhibition zones (mm/50µL)				
Microorganisms	**Tob 10µg**	**Gen 10µg**	**Oflo 10µg**	**Cip 10µg**	**Control A, B, C, D**
1. *Bacillus cereus*	28	32	34	30	--
2. *Enterobacter faecalis*	26	32	32	26	--
3. *Salmonella paratyphi*	25	30	28	30	--
4. *Staphylococcus aureus*	26	28	24	24	--
5. *Escherichia coli*	30	36	32	34	--
6. *Proteus vulgaris*	26	30	24	32	--
7. *Klebsiella pneumoniae*	26	32	32	36	--
8. *Pseudomonas aeruginosa*	26	24	32	28	--
9.*Serratia marcescens*	24	32	30	30	--

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether

Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip:ciprofloxacin

Table 3: Phytochemical screening of *Coriandrum sativum* leaf extracts

Phytoconstituents	Methanol extract	Ethyl acetate extract	Chloroform extract	Petroleum ether extract
Alkaloids	-	-	-	-
Phenolics	+++	++	_	_
Saponins	-	-	_	_
Tannins	-	-	_	_
Glycosides	-	_	_	_
Steroids	+++	++	+	+
Flavonoids	+++	++	+	+

+ Present *++Moderately present* *+++Appreciable amount*

Table 4: DPPH free radical scavenging activity of the leaf essential oil and leaf extracts of *Coriandrum sativum*

	Concentration(mg/ml)				
Samples	**1.0**	**0.5**	**0.25**	**0.125**	
	Radical scavenging effect (%)				**IC_{50}/DPPH (µg/mL)**
C. sativum leaf oil	89.55	87.63	85.93	81.02	71.4
C. sativum leaf methanol extract	95.95	92.32	89.77	87.63	67.3
C. sativum leaf ethyl acetate extract	84.86	81.02	80.39	75.91	83.3
C. sativum leaf chloroform extract	82.52	79.32	78.89	74.84	89.2
C. sativum leaf pet. ether extract	76.75	73.13	53.30	49.25	125
L-ascorbic acid	98.93	94.66	93.18	90.83	58.3

DISCUSSION

Antibacterial screening of leaf essential oil

Coriandrum sativum leaf oil at various concentrations was evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria strains and the oils exhibited marked activity against all tested microorganisms. The leaf oil (20%) showed pronounced activity against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *Klebsiella pneumonia* (20-32mm/50µL inhibition zone).

The inhibitory effect of 20% leaf oil of *C. sativum* on *Bacillus cereus, Enterobacter faecalis, Staphylococcus aureus* and *Klebsiella pneumonia* was comparatively less than that of standard antibiotics tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin whereas the activity of the leaf oil (20%) against other tested bacteria like *Escherichia coli* (32mm/50µL inhibition zone), *Proteus vulgaris* (26mm/50µL inhibition zone), *Pseudomonas aeruginosa* (24mm/50µL inhibition zone), *Salmonella paratyphi* (29mm/50µL inhibition zone), *Serratia marcescens* (28mm/50µL inhibition zone) was found to be equal to the standard antibiotics such as tobramycin, ofloxacin (10µg each) screened under similar conditions.

The activities of 10% (17-30mm/50µl inhibition zone), 5% (15-19mm/50µL inhibition zone) and 1% (12-17mm /50µL inhibition zone) of the leaf oil samples were also studied against various pathogenic bacteria and were found to be active on all microorganisms tested.

The remarkable antibacterial activity exhibited by the *C. sativum* leaf oil can be attributed to the synergic effect of the antimicrobial agents present in the oil. The leaf oil contains 44 compounds mostly of aromatic acids of which the major are 2-decenoic acid, E-11-tetradecenoic acid, capric acid, undecyl alcohol and tridecanoic acid. The high concentration of 2-decenoic acid in leaf oil makes it potentially useful in the medicines and perfumery purposes[6].

Antibacterial screening of leaf extracts

Among the leaf extracts, methanol extract exhibited higher activity than the other extracts and petroleum ether extract showed least activity. Methanol (19-29mm/50µL inhibition zone), ethyl acetate (15-21mm/50µL inhibition zone), chloroform (13-19mm/50µL inhibition zone) and petroleum ether (11-16mm/50µL inhibition zone) extracts of the leaf exhibited marked activity against all the tested organisms.

The leaf methanol extract exhibited significant activity against *Proteus vulgaris* (26mm/50µL inhibition zone). The results obtained were compared with the standard antibiotics and it was observed that the *C. sativum* leaf methanol extract at a concentration of 1mg/mL showed marked activity against *Proteus vulgaris* its activity is similar to that of the standard antibiotics such as tobramycin and ofloxacin. Methanolic leaf extract of *C. sativum* was also found to be effective on *Bacillus cereus* (29mm/50µL inhibition zone), *Enterobacter faecalis* (29mm/50µL inhibition zone) and *Serratia marcescens* (23mm/50µL inhibition zone) and its

activity is quite comparable with the standard antibiotic tobramycin screened under the similar conditions. The inhibitory effect of leaf methanol extract on *Staphylococcus aureus* was comparable with all the antibiotics tested (10µg each).

The antimicrobial potency of *C. sativum* leaf extracts is due to the presence of flavonoids, terpenoids in it [21]. It is interesting to note that even crude extract of this plant showed prominent activity against various pathogenic bacteria where modern therapy has failed.

Phytochemical analysis

Phytochemical studies revealed the presence of various secondary metabolites in the leaf extracts of *C. sativum*. Various phytochemical compounds detected are known to have beneficial importance in medicinal sciences[22]. Leaf methanol extract showed appreciable amount of phenolics, steroids and flavonoids. Steroids, phenolics and flavonoids are present in moderate amount in ethyl acetate extract. Chloroform and petroleum ether extracts gave positive test for steroids and flavonoids. Antibacterial and antioxidant potential of leaf extracts can be attributed to the presence of these phytochemicals.

DPPH free radical scavenging activity assay

The DPPH free radical scavenging activity of the leaf essential oil and leaf extracts of *C. sativum* are sorted in descending order: *C. sativum* leaf methanol extract> leaf oil > leaf ethyl acetate extract > leaf chloroform extract > leaf petroleum ether extract.

Out of the five samples tested, *C. sativum* leaf methanol extract showed the highest scavenging activity (% inhibition 95.95, 92.32, 89.77 and 87.63 at 1.0, 0.5, 0.25 and 0.125mg/mL respectively), followed by *C. sativum* leaf essential oil. Leaf petroleum ether extract exhibited least DPPH radical scavenging ability with % inhibition 76.75, 73.13, 53.30 and 49.25 at 1.0, 0.5, 0.25 and 0.125mg/mL respectively.

By comparing the IC_{50} value of the leaf extracts and leaf essential oil of *C. sativum* with that of the authentic antioxidant L-ascorbic acid, it was found that the antioxidant activity of *C. sativum* leaf methanol extract (IC_{50}: 67.3 µg/mL) was quite comparable with that of L-ascorbic acid (IC_{50}: 58.3µg/ml). IC_{50} value of *C. sativum* leaf essential oil (71.4µg/mL) is not significantly different from that of L-ascorbic acid (IC_{50}: 58.3µg/mL).

CONCLUSIONS

The essential oil and extracts from the leaves of *C. sativum* showed varying degrees of antibacterial activity on the microorganisms tested. From the above experiment it can be inferred that *Coriandrum sativum* leaf essential oil as well as leaf methanol extract showed significant activity against Gram-positive and Gram-negative bacteria. The activity of leaf oil and leaf methanol extract was found to be quite comparable with the standard antibiotics screened under similar conditions. So they can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by various pathogenic bacteria. This study demonstrated that the essential oil and methanolic leaf extracts of *Coriandrum sativum* is as effective as modern medicine to combat pathogenic microorganisms.

Among the leaf essential oil and leaf extracts of *Coriandrum sativum* studied, the leaf methanol extract and leaf essential oil showed potent scavenging activity on DPPH free radical. Antioxidant activity of the leaf methanol extract can be attributed to the presence of phytochemicals such as flavonoids, phenols and sterols. Phenolic compounds are reported to have antioxidant activities [23,24]. Antioxidant activities of essential oils from medicinal plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them. The results obtained showed that the leaf methanol extract and leaf essential oil of *Coriandrum sativum* can be considered as good sources of natural antioxidants.

REFERENCES

1. Momin AH, Sawapnil SA, Amit VG. Coriandrum sativum- Revew of advances in phyto pharmacology. Int J Pharm Sci. Res, 2012; 3(5): 1233-1239.
2. Benjumea D, Abdala S, Hernandez LF, P´erez-Paz P, Martin-Herrera D. Diuretic activity of Artemisia thuscula, an endemic canary species. J Ethnopharmacol, 2005;100:205–209.
3. Maghrani M, Zeggwagh N, Haloui M, Eddouks M. Acute diuretic effect of aqueous extract of Retamaraetam in normal rats. J Ethnopharmacol, 2005; 99:31-35.
4. Wangensteen H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from coriander. Food Chem, 2004; 88:293–297.

5. Meloa EA, Filhob JM, Guerrac NB. Characterizations of antioxidant compounds in aqueous coriander extract (*Coriandrum sativum* L.). Lebensm.-Wiss u.-Technol, 2005; 38:15-19.
6. Matasyoh JC, Maiyo ZC, Ngure RM, Chepkorir R. Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. Food Chem, 2009; 113:526–529.
7. Cortes EJ, Gómez AS, Villalobos PR. Antimutagenicity of coriander (*Coriandrum sativum*) juice on the mutagenesis produced by plant metabolites of aromatic amines. Toxicol Lett, 2004; 153:283– 292.
8. Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E. In vitro and in vivo anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus*. J Ethnopharmacol, 2007; 110:428–433.
9. Emamghoreishi M, Heidari HG. Sedative-Hypnotic Activity of extracts and essential oil of coriander seeds. Iran J Med Sci, 2006; 31(1):22-27.
10. Hosseinzadeh H, Madanifard M. Anticonvulsant effect of *Coriander sativum* L. seed extracts in mice. Iran J Pharm, 2005; 3:1-4.
11. Abderahim A, Hilaly EJ, Israili ZH, Lyoussi B. Acute diuretic effect of continuous intravenous infusion of an aqueous extract of *Coriandrum sativum* L. in anesthetized rats. J Ethnopharmacol, 2008; 115:89–95.
12. Dhanapakiam P, Joseph JM, Ramaswamy VK. The cholesterol lowering property of coriander seeds (*Coriandrum sativum*): Mechanism of action. J Environ Biol, 2008; 29(1):53-56.
13. Leena K, Veena S, Arti S. Protective role of *Coriandrum sativum* (coriander) extracts against lead nitrate induced oxidative stress and tissue damage in the liver and kidney in male mice. Int J Appl Pharm Technol, 2011; 2(3):65-83.
14. Catherine JD, Ian FH, Peter W, Lucy DL. Action of extracts of apiaceae on feeding behavior and neurophysiology of the field slug *Deroceras reticulatum*. J Chem Ecol, 1999; 25(9):2127-2147.
15. Chithra V, Leelamma S. *Coriandrum sativum* -Effect on lipid metabolism in 1, 2-dimethyl hydrazine induced colon cancer. J Ethnopharmacol, 2000; 71:457–463.
16. Mansoor SA, Al-khamis KI, Mohammad WI. Post-coital antifertility activity of the seeds of *Coriadrum sativum* in rats. J Ethnopharmacol, 1987; 21:165-173.
17. Karunasagar D, Balarama Krishna MV, Rao SV, Arunachalam J. Removal and preconcentration of inorganic and methyl mercury from aqueous media using a sorbent

prepared from the plant *Coriandrum sativum*. J Hazardous Materials, 2005; 118:133–139.

18. Omar S, Lemonnier B, Jones N, Ficker C, Smith ML, Neema C. Antimicrobial activity of extracts of eastern North American hardwood trees and relation to traditional Medicines. J Ethnopharmacol, 2000; 73: 161-170.
19. Harborne JB. Pytochemical Methods: A guide to Modern techniques of plant analysis. London; Chapman and Hall: 1973.
20. Fenglin H, Ruili L, Bao H, Liang M. Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. Fitoterapia, 2004; 75:14-23.
21. Ali SS, Kasoju N, Luthra A. Indian medicinal herbs as sources of antioxidants. Food Res Int, 2008; 41:1–15.
22. Cowan MM. Plant products as antimicrobial agents .Clin Microbiol Rev,1999; 12: 564-582.
23. Raquibul SM, Mokarram H, Akter R, Jamila M, Mazumder EH, Rahman S. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. J Med Plants Res, 2009, 3(11): 875-879.
24. Shyamala SG, Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. Int J Pharm Tech Res, 2010, 2(2): 1569-1573.