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In vitro Antioxidant Activities and Antimicrobial Efficacy of Asian Snakewood; *Colubrina asiatica* (L.) Brong.

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ABSTRACT

The present study evaluated antimicrobial efficacy of essential oil and *in vitro* antioxidant activities of the aqueous extract from *Colubrina asiatica* (L.) Brong. as well as the chemical composition of essential oil. In the present investigation, *Colubrina* Water Extract (CWE) was studied for its antioxidant activity and *Colubrina* Essential Oil (CEO) for anti microbial properties. The antioxidant properties of CWE were evaluated using different free radical scavenging assays, such as reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities. We found that CWE had powerful antioxidant activity. The different concentrations (50, 100 and 250 g) of CWE showed 39, 66 and 98% inhibition on peroxidation of linoleic acid emulsion, respectively, while 60 g mL^{-1} of ascorbic acid, exhibited only 30% inhibition. Moreover, CWE had effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities at the same concentrations. Those various antioxidant activities were compared to standard antioxidants such as ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), gallic acid and quercetin. In addition, total phenolic compounds in the CWE were determined as Gallic acid equivalent. The *Colubrina* Essential Oil (CEO) content quantified showed presence of 10 compounds in which, dodecamethylcyclohexasiloxane has showed the highest (17%) and dehydro-N-[4,5-methylenedioxy-2-nitrobenzylidene]-tyramine showed the lowest percentage (1.9%). Cubebene, comprised of 14%. The antimicrobial activity of oil was studied on gram negative and gram positive bacterial.

Key words: Essential oil composition, phytochemical screening, *Colubrina asiatica* Brong

INTRODUCTION

Medicinal plants play an important role in human life to combat diseases since time immemorial. The villagers and the tribals in India even today largely depend on the surrounding plants/forests for their day-today needs. Medicinal plants are being focused upon not only as a source of health care but also as a source of income. The Ministry of Environment and Forests, Government of India, reveals that there are over 8000 species of medicinal plants grown in the country. About 70% of these plants are found in the tropical forest; spread across the Western and Eastern Ghats. The Export-Import Bank of India, in its report for the year 1997, puts medicinal plants related trade in India at $.5.5 billion and the same is growing rapidly (Kumar and Janagam, 2011). Free radicals are highly reactive species produced in the body during normal metabolic

functions. These are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reactive. Though Oxygen, is essential to life, but it is the source of the potentially damaging free radicals. Antioxidants counteract these cellular by-products, called free radicals and bind them before they can cause damage (Pandey *et al*., 2005). Fruits and vegetables are major source of dietary antioxidants and their precursors (Block *et al*., 1992). Recently, various phytochemicals and their effects on health, especially the suppression of active oxygen species by natural antioxidants from teas, spices and herbs, have been intensively studied (Ho *et al*., 1994). The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) (Sherwin, 1990).

Among the plants with promising biological activities employed by the traditional people, *Colubrina asiatica* (Brong.), is a Rhamnaceae species popularly known as "Asian snake wood" with a glabrous, scandent or sprawling shrubby nature. In India the species is wide spread in littoral scrub forests, tidal forests of Orissa and Ghats of Konkan (Thaman, 1992). In some regions of Northeast and Southeast it is popularly employed in medicinal preparations for the treatment of digestive aid, antiscorbutic (counteracts scurvy), tonic, laxative, a febrifuge, medicinal bath and a vermifuge and skin diseases (Burkill, 1966; Morton, 1981; Austin, 1999). The plant is economically valued for its leaf saponins used in soap substitute, used to wash and whiten textile kilts and garments (Richardson *et al*., 2000).

In this sense, this study aimed the phytochemical investigation and *in vitro* evaluation of antioxidant activities of the aqueous extract and antimicrobial efficacy of essential oil of *Colubrina asiatica.*

MATERIALS AND METHODS

Antioxidant activities

Chemicals: Ammonium thiocyanate and gallic acid were purchased from E. Merck. Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), ascorbic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2, 4-triazine (ferrozine), nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trichloroacetic acid (TCA) were purchased from Sigma (Germany).

Plant material and extraction: *Colubrina asiatica* Brong. (whole plant) was collected from coastal area of Goa (coordinates 15°27'7" N 73°50'6" E), in December 2011 and identified by Dr. MayurNandikar, a plant taxonomist of Department of Botany from Shivaji University of Kolhapur. A specimen herbarium deposited in Shivaji University, Kolhapur (NMDESAI 001 SUK). The plant material was initially dried in sunlight and then in oven. The dried sample was chopped into small parts with a blender. For water extraction, 20 g dried leaves of *Colubrina* ground into a fine powder in a mill and was mixed with 400 mL boiling water by magnetic stirrer during 15 min. Then the extract was filtered over Whatman No.1 paper. The filtrate was frozen and lyophilized in a lyophilizator at 5 μm Hg pressure at -50°C (Labconco, Freezone 1L). The extract was placed in a plastic bottle and then stored at -20°C until further use.

Total antioxidant activity determination: The antioxidant activity of CWE was determined according to the thiocyanate method (Mitsuda *et al*., 1996). For stock solution; 20 mg lyophilized *Colubrina* Water Extract (CWE) was dissolved in 20 mL water. Then the solution, which contains

different amount of stock CWE solution or standards samples (50, 100 and 250 μg) in 2.5 mL of 0.04 M potassium phosphate buffer (pH 7.0) was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). Each solution was then incubated at 37°C in a glass flask in the dark. At intervals during incubation, each solution was stirred for 3 min, 0.1 μL this incubation solution, 0.1 mL $FeCl_2$ and 0.1 mL thiocyanate were transferred to the test tube, which containing 4.7 mL ethanol solution incubated for 5 min. Finally, the peroxide value was determined by reading the absorbance at 500 nm in a UV-1800 UV-Vis spectrophotometer (Shimadzu). During the linoleic acid oxidation, peroxides formed and these compounds oxidize Fe^{2+}-Fe^{3+}. The latter Fe^{3+} ions form complex with SCN-, which has a maximum absorbance at 500 nm. Therefore higher absorbance values indicate higher linoleic acid oxidation. The solutions without added CWE or standards were used as blank samples. Five millilitres linoleic acid emulsion is consisting of 17.5 μg Tween-20, 15.5 μL linoleic acid and 0.04 M potassium phosphate buffer (pH 7.0). On the other hand, 5 mL control composed of 2.5 mL linoleic acid emulsion and 2.5 mL potassium phosphate buffer (0.04 M, pH 7.0). All data about total antioxidant activity is the average of duplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of CWE (Duh *et al*., 1999).

Reducing power: The reducing power of CWE was determined according to the method of Oyaizu (1986). The different doses of CWE (50, 100 and 250 μg) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at 1000×g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Superoxide anion scavenging activity: Measurement of superoxide anion scavenging activity of CWE was based on the method described by Liu *et al*. (1997) with slight modifications (Gulcin *et al*., 2003). Superoxide radicals are generated in phenazine methosulphate (PMS)-nicotinamide adenine dinucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 mL of Tris-HCl buffer(16 mM, pH 8.0) containing 1 mL of NBT (50 μM) solution, 1 mL NADH (78 μM) solution and 1 mL sample solution of CWE (100 μg mL^{-1}) were mixed. The reaction was started by adding 1 mL of PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured against blank samples. l-ascorbic acid was used as a control. Decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control (l-ascorbic acid) and A_1 was the absorbance of CWE or standards (Ye *et al*., 2000).

Free radical scavenging activity: The free radical scavenging activity of CWE was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) using the method of Shimada *et al*. (1992). Briefly, 0.1 mM solution of DPPH• in ethanol was prepared. Then, 1 mL of this solution was added to 3 mL of CWE solution at different doses (50-250 μg). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH• concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (R2: 0.9678).

Absorbance = 104.09×[DPPH•]. The DPPH radical concentration was calculated using the following equation:

$$\text{DPPH} \bullet \text{ scavenging effect } (\%) = 100 - \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of CWE (Oktay *et al*., 2003).

Metal chelating activity: The chelating of ferrous ions by the CWE and standards was estimated by the method of Dinis *et al*. (1994). Briefly, extracts (50-250 μg) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm in a spectrophotometer. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula:

$$\text{Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of CWE and standards. The control contains FeCl2 and ferrozine (Ilhami *et al*., 2003).

Scavenging of hydrogen peroxide: The ability of the CWE to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer. Extracts (50-250 μg) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of CWE and standard compounds was calculated using the following equation:

$$\text{Percent scavenged } (H_2O_2) = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of CWE and standards (Ilhami *et al*., 2003).

Determination of total phenolic compounds: Total soluble phenolic compounds in the CWE were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using pyrocatechol as a standard phenolic compound. Briefly, 1 mL of the CWE solution (contains 1000 µg extract) in a volumetric flask diluted with distilled water (46 mL). One milliliter of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, 3 mL of Na2CO3 (2%) was added and then was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The total concentration of phenolic compounds in the CWE determined as microgram of gallic acid equivalent by using an equation that was obtained from standard pyrocatechol graph (Gulcin *et al*., 2002):

Absorbance = 0.0053×total phenols [gallic acid equivalent (µg)]-0.0059

Extraction of oil and GC MS analysis: Hydrodistillation of the plant material was performed in a clevenger-type apparatus for 210 min. The oil obtained was light yellow, liquid at room temperature with an agreeable odor. After isolation, the Essential Oil (EO) was collected and stored in steeled glass vials in refrigerator at 4-5°C. The samples were analysed by GC-MS (Schimadzu) using capillary column. The GC-MS conditions were as follows; injection volume (1 mL), temperature programme 80-160°C for 5 min at 10°C min^{-1}; 160-235°C for 5 min at 5°C min^{-1} and 235-290°C for 5 min at 50°C min^{-1}; injector temperature (280°C), MS transfer line (290°C), ion source (200°C) spit ratio (1: 10) and mass range at 50-450. Data was analysed by comparing it with SI (standard index) from the NIST library available.

Antimicrobial activities

Preparation of test microorganisms: For the purpose of antimicrobial evaluation ten microorganisms were used. *Pseudomonas aeruginosa* (ATCC 9027, gram-negative), *Escherichia coli* (ATCC 9837, gram-negative), *Staphylococcus aureus* (ATCC 6538, gram-positive) and *Streptococcus pneumoniae* (ATCC 49619, gram-positive) microorganism strains were employed for determination of antimicrobial activity. Microorganism strains were obtained from the stock cultures of Microbiology Laboratory, Department of Microbiology, Shivaji University, Kolhapur.

Antimicrobial activity determination: Agar cultures of the test microorganisms were prepared as described by Mackeen *et al*. (1997). Three to five similar colonies were selected and transferred with loop into 5 mL of tryptone soya broth. The broth cultures were incubated for 24 h at 37°C. For screening, sterile, 6 mm diameter lter paper disc were impregnated with 250 µg of the CEO. Then the paper discs were placed onto Mueller Hinton agar. The inoculum for each organism was prepared from broth cultures. The concentration of cultures was adjusted to 108 colony forming units (1×108 CFU mL^{-1}). The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity the average of triplicate analyses. Netilmicin (30 µg per disc), amoxicillin-clavulanic acid (20-10 µg per disc) were used as reference standards, which as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

Statistical analysis: Experimental results concerning this study were Mean±SD of three parallel measurements. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests. p values<0.05 were regarded as significant and p values<0.01 very significant.

RESULTS AND DISCUSSION

Antioxidant capacity: Antioxidants are the compounds which helps to delay or inhibit the oxidation of lipids and other molecules through the inhibition of either initiation or propagation of oxidative chain reactions (Jaleel *et al*., 2007). Antioxidants can act as either reducing agents, or by free radical scavengers or singlet oxygen quenchers (Chanwitheesuk *et al*., 2005). Recent studies focused on several antioxidant methods and its modifications to evaluate antioxidant activity and to explain how antioxidants function. Among these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, $O_{\bullet}^{2-}$ and $OH^{\bullet}$ quenching assays are most commonly used for the evaluation of antioxidant activities of extracts (Duh *et al*., 1999; Amarowicz *et al*., 2000; Chang *et al*., 2002). Total antioxidant activity of CWE was determined by the thiocyanate method. The CWE exhibited effective antioxidant activity at all the studied doses. The effects of different amounts of CWE (from 50-250 µg) on peroxidation of linoleic acid emulsion are shown in Fig. 1. The antioxidant activity of CWE was found concentration dependently. The CWE (50, 100 and 250 µg) showed higher antioxidant activities than that of 100 µg concentration of standard antioxidant ascorbic acid. After incubation times the percentage inhibition of peroxidation in linoleic acid emulsion was 34, 62 and 91%, respectively and greater than that of ascorbic acid (30%).

The reductive capabilities of CWE compared to ascorbic acid was shown in Fig. 2. For the measurements of the reducing power ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of CWE samples using the method of Oyaizu (1986). Like the antioxidant activity, the reducing power of CWE increased concentration dependently. All of the concentrations of CWE showed higher activities than the control in a statistically significant ($p<0.05$) manner. In the PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. Superoxide anion is an initial free radical and plays an important role in the

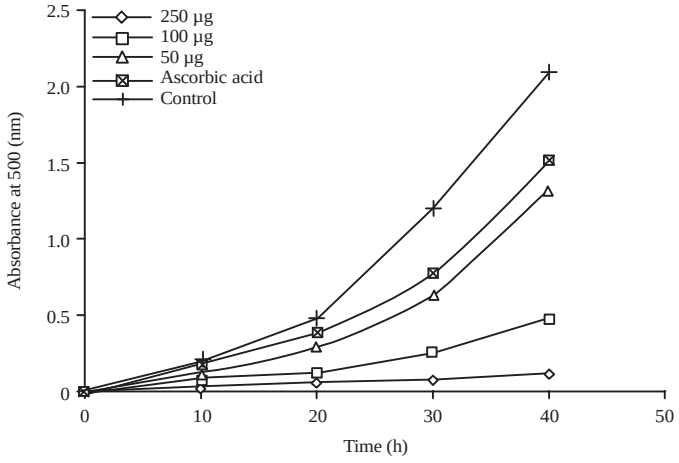

Fig. 1: Antioxidant activity of different doses of CWE and ascorbic acid in the linoleic acid emulsion

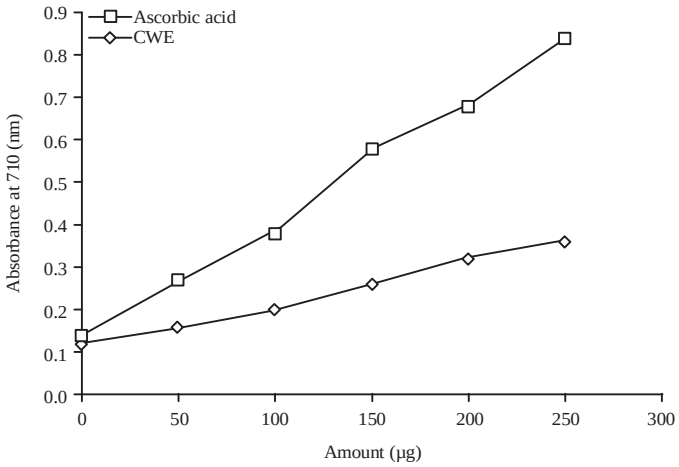

Fig. 2: Reducing power ability of CWE compared with ascorbic acid using spectrophotometric detection of the Fe^{3+}-Fe^{2+} transformation

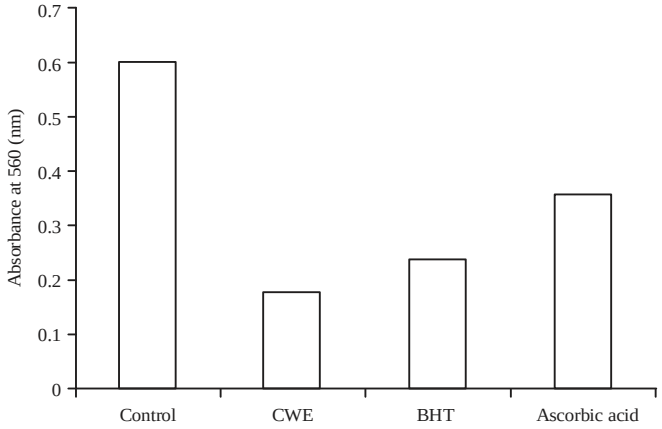

Fig. 3: Superoxide anion radical scavenging activity of 100 g of WEN, BHA, BHT and ascorbic acid by the PMS-NADH-NBT method

formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, or singlet oxygen in living systems (Stief, 2003). It can also react with nitric oxide and from peroxynitrite, which can generate toxic compounds such as hydroxyl radicals and nitric dioxide (Halliwell, 1997). Figure 3 shows the percentage inhibition of superoxide radical generation by 100 µg of CWE and comparison with same doses of BHT and ascorbic acid. The CWE exhibited higher superoxide radical scavenging activity than BHT and ascorbic acid ($p<0.01$). The percentage inhibition of superoxide generation by 100 µg amount of CWE was found as 90% and greater than that of some doses of BHT and ascorbic acid (89, 80 and 61%), respectively. Superoxide radical scavenging activity of those samples followed the order: CWE>BHT >ascorbic acid. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Themodel of scavenging the stable DPPH radical is a widely used method to evaluate

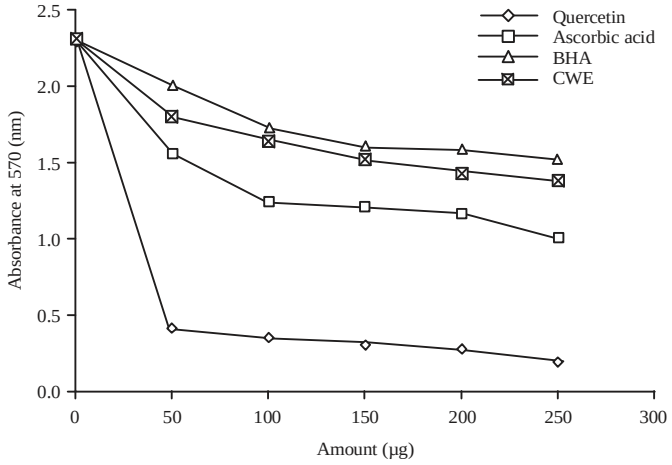

Fig. 4: Free radical scavenging activity of quercetin, ascorbic acid, BHA and CWE on DPPH

antioxidant activities in a relativelyshort time compare to other methods (Soare *et al*., 1997). The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The maximum absorption of a stable DPPH radical in ethanol is at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results inthe scavenging of the radical by hydrogen donation.The DPPH radical scavenging assay depends on the decoloration of the purple coloured methanolic DPPH solution to yellow by the radical scavengers present in the sample extracts (Blois, 1958). The result of DPPH scavenging activity implies that the plant extract may be useful for treating radical related pathological damages (Wang *et al*., 1998). A significant ($p<0.01$) decrease in the concentration of DPPH radical due to the scavenging ability of the CWE and standards was observed (Fig. 4). The CWE and BHA showed almost equal DPPH scavenging activity, however, significantly are lower than that of quercetin. The scavengingeffect of CWE and standards on the DPPH radical decreasedin the order of quercetin>ascorbic acid >CWE>BHA and were 89, 45, 36 and 31% at the concentration of 60 µg mL^{-1},respectively. Uncontrolled generation of ROS can lead to their accumulation causing oxidative stress in the cells (Kunwar and Priyadarsini, 2011). Severe oxidative stress causes cell damage and death (Aruoma, 1998). Superoxide anion radical scavenging activity of 100 µg of CWE, ascorbic acid, BHT and ascorbic acid by the PMS-NADH-NBT method obtained from this study, CWE exhibits free radical scavenging activity as well as a primary antioxidant thatreacts with free radicals, which may hampers the damages caused due to free radical in the human body (Fig. 3). The chelating of ferrous ions by CWE was estimatedwith the method of Dinis *et al*. (1994). In the presence ofchelating agents, Ferrozine can quantitatively form complexes with Fe^{2+} is interrupted and ultimately diminishes the red colour of the complex. The actual mechanism of antioxidant action is chelation of transition metals thus preventing catalysis of hydroperoxidedecoposition and fenton type reactions (Gordon, 1990). Iron can stimulate lipid peroxidation by the Fenton reaction and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell, 1991). In this assay CWE and standard antioxidant compound interfered with the formation of ferrous and ferrozine complex, suggesting its potent chelating

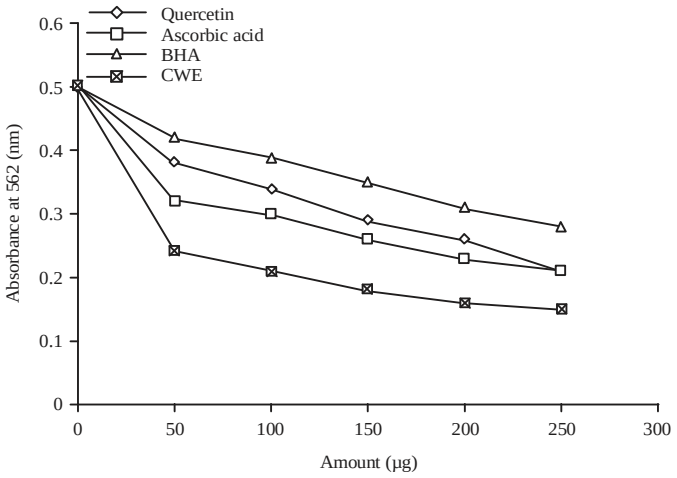

Fig. 5: Metal chelating effect of different amount of *Colubrina* water extract and standards on ferrous ions

activity which capture ferrous ion before ferrozine. The absorbance of Fe^{2+}-ferrozine complex was linearly decreased dose-dependently (from 50-250 µg) (Fig. 5). The difference between CWE and the control was statistically significant ($p<0.01$). The percentages of metal chelating capacity of 250 µg concentration of CWE, ascorbic acid, BHA and quercetin were found as 84,40, 58 and 34%, respectively. The metal scavenging effect of CWE and standards decreased in the order of CWE>ascorbic acid>quercetine>BHA metal chelating capacity is important since it reduced theconcentration of the catalysing transition metal in lipid peroxidation (Duh *et al*., 1999). The data obtained from Fig. 5 revealed that CWE demonstrate a marked capacity for iron binding, revealing that their action as peroxidation protector may berelated to its iron binding capacity. Hydrogen peroxide scavenging activity of CWE may be endorsed to their phenolic content, which could bestow electrons to H_2O_2, thus counter acting it to water.

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (Arulmozhi *et al*., 2008). Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water (Nabavi *et al*., 2008; Ebrahimzadeh *et al*., 2009). The ability of CWE to scavenge H_2O_2 was determined according to the method of Ruch *et al*. (1989). The scavenging ability of CWE on H_2O_2 is shown in Fig. 6 and compared with BHA, BHT and ascorbic acid as standards. The CWE was capable of scavenging H_2O_2 in a dose-dependent. Two hundred and fifty micrograms of CWE exhibited 20% scavenging activity on H_2O_2. On the other hand, at the same concentration; BHA, BHT and ascorbic acid showed 32, 80 and 51% activity, respectively. These results indicated thatCWE posses potent H_2O_2 scavenging activity but had lower than the BHA, BHT and ascorbic acid. However, statistically significant correlation between those valuesand control ($p<0.01$) was observed. The H_2O_2 scavenging effect ofsame dose (250 µg) of CWE and standards decreased in the order of BHT>ascorbic acid>BHA>CWE.

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources and they have been shown to possess significant antioxidant activities (Nabavi *et al*., 2009). The 25.3 µg gallic acid equivalent of phenols was detected in 1 mg of CWE.

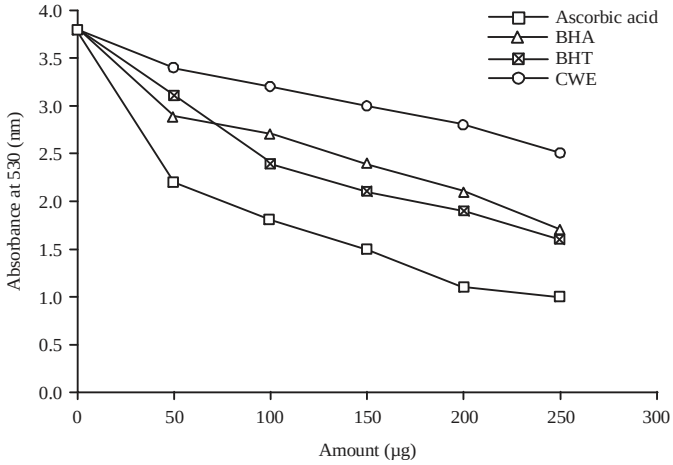

Fig. 6: Hydrogen peroxide scavenging activity of different amount of CWE, BHA, BHT, quercetin and ascorbic acid

Table 1: Composition of essential oil in *C. asiatica* leaves

Peak	Rt	Compound name	Percentage
1	13.4	Dodecamethylcyclohexasiloxane	19.0
2	20.1	Tetradecamethyl-cycloheptasiloxane	14.3
3	16.2	α-cubebene	14.0
4	2.5	2,4-dimethylhexane	13.0
5	5.3	6,6-methylenebicyclo[3.1.1]heptane	12.0
6	21.9	Cadina-1(10),4-diene	6.2
7	27.1	Hexadecamethyl-cyclooctasiloxane	4.9
8	6.6	Octamethylcyclo-tetrasiloxane	4.1
9	18.1	Isocaryophillene	3.2
10	6.9	Dehydro-N-[4,5-methylenedioxy-2- nitrobenzylidene]-tyramine	2.3

The phenolic compounds may contribute directly to theantioxidative action (Duh *et al*., 1999). The interest on these compounds is related with their antioxidant activity and promotion of health benefits (Ryan *et al*., 2002).

Essential oil composition and antibacterial efficacy: In the present study, the components present in the essential oil are identified using NIST library. The composition of essential oils showed dodecamethylcyclohexasiloxane the highest (18%) and decamethylcyclopentasiloxane showed the lowest percentage (1.6%) (Table 1). These compounds were reported to be in many personal care products such as toiletries. Volatile compounds is essential to determine the predominant components and their composition in order to investigate their bioactivity including antioxidant and antibacterial activities. A number of reports have shown that plant volatile compounds exhibited potent antioxidant and antibacterial activities (Choi and Hwang, 2005). Cubebene was reported to show potent antibacterial properties (Prabuseenivasan *et al*., 2006) and antioxidant properties (Ruberto and Baratta, 2000). Essential oils produced by plants have been traditionally used for respiratory tract infections and are used nowadays as ethical medicines for colds (Federspil *et al*., 1997). In this study, three different microbial and one yeast species were used to screen the possible antimicrobial activity of *Colubrina* Essential Oil (CEO). In the present study, 4 strains of bacterial strains representing gram negative and gram positive were used to screen the possible antimicrobial activity of CEO. Water extract of *Colubrina* exhibited antimicrobial activity against all tested microorganisms. Amongst these *Staphylococcus aureus*

Table 2: Antimicrobial activities of CEO (250 µg per disc) and standard antimicrobial agents

Tested microorganisms	Diameter of zone of CWE (mm)	Antimicrobial agents (mm)	
		Netilmicin (25 µg/disc)	Amoxicillin-clavulanic acid (15 µg/disc)
Escherichia coli	8	18	21
Pseudomonas aeruginosa	-	-	7
Staphylococcus aureus	9	17	13
Streptococcus pneumoniae	10	14	21

CEO: *Colubrina* essential oil, ND: Not detected activity at this amount of CWE or standards

gram positive bacteria responsible for food poisoning. Interestingly CEO showed antibacterial activity against this bacterium. Table 2 showed appreciable inhibitory activity of CEO. Escherichia coli, belonging to the normal ora of humans, is a gram negative bacterium. Amoxicillin-clavulanic acid (15 µg per disc) and netilmicin (25 µg per disc) were used as positive controls. Gram positive bacteria are known to be more susceptible to essential oils than gram negative bacteria (Smith-Palmer *et al*., 1998). Gram positive bacteria are more sensitive to plant oils and extracts than gram negative bacteria (Karaman *et al*., 2003).

CONCLUSION

Present findings clearly indicate that CWE has a powerful antioxidant activity against various oxidative systems *in vitro*; moreover, CWE can be further used as accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. The various antioxidant mechanisms of CWE may be accredited to strong hydrogen donating ability, a metal chelating ability and their effectiveness as scavengers of hydrogen peroxide, superoxide and free radicals. The essential oil of *Colubrina* possessed noticeable antimicrobial activity against gram positive and negative bacteria when compared with standard and strong antimicrobial compounds such as amoxicillin clavulanic acid and netilmicin. We believe that the present investigation together with previous studies provide support to the antibacterial properties of *Colubrina* essential oil as well as potent natural antioxidant source. It can be used as antioxidant and antibacterial supplement in the developing countries towards the development of new therapeutic agents. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent and natural antioxidant compounds with health benefits in topical or oral applications.

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