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Anioxidant activity of palmarosa essential oil (*Cymbopogon martini*) grown in north indian plains

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ABSTRACT

Objective: The present study deals with the *in vitro* study of antioxidant activity of essential oil from Palmarosa (*Cymbopogon martini*) of family gramineae. **Methods:** The *in vitro* study of antioxidant activity of Palmarosa essential oil has been done by using DPPH assay, Nitrogen Oxide assay, reducing power assay, β -carotene bleaching assay and FRAP method. **Results:** IC₅₀ values observed for DPPH and NO assay are 0.125 mg/ml and 12.5 μ g/ml respectively. In beta carotene bleaching method the oil showed 93.15% bleaching for the first hour and it increased to 51.1% in second hour. There was a constant increase in the reducing activities with the increase in concentrations in both reducing activity and FRAP methods. In all the methods BHT and Gallic acid were kept as standards. **Conclusions:** The results clearly indicate that Palmarosa essential oil is effective in scavenging free radical and has the potential to be powerful antioxidant. An essential oil is a liquid that is generally distilled (more frequently by steam or water) from the leaves, stems, flowers, bark or roots or other elements of the plant [1]. These are also known as volatile or ethereal oils. Medicinal applications of essential oils range from skin treatment to remedies for cancer.

1. Introduction

Palmarosa oil is extracted from *Cymbopogon martini* of family gramineae and is also known as Turkish geranium as well as Indian rosha and motia. It is a sweet floral with a hint of rose smell and is pale yellow in color with nearly watery viscosity. The yield of oil is generally 1–1.5%. Essential oil is extracted from inflorescence (2%), leaf lamina (1.4%), whole herb (0.75%) and leaf sheath (0.33%). The major constituents of oil of whole herb are linalool, β -caryophyllene and E,Z-farnesol [2].

Palmarosa oil had a long history of use in India and Turkey. In aromatherapy the oil is reputed to be regenerative and extremely effective against pathogenic intestinal flora to turn them back to normal cell or intestinal mucous membrane [3,4]. Its most important use is in the treatment of skin infections like acne and also to stimulate cell regeneration while regulating the production of sebum.

It may be beneficial for cardiovascular system, circulation, digestion, infection, nervous system and rashes.

Apart from all the above use the essential oil has been shown to be effective insect repellent [5] when applied to stored grains and beans and as antihelminthic against nematodes [6]. There are other activities also reported for oil like antifungal [7] and antimicrobial [8–10].

Antioxidants in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acid, protein lipid or DNA and can initiate degenerative diseases. Antioxidant (compounds) like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxides, hydroperoxides or lipid peroxyl and thus inhibit the oxidative mechanism that lead to degenerative diseases.

Although there are some synthetic antioxidants also like BHT and BHA but all these are associated with some side effects. A majority of plant species has been investigated time to time for their use as antioxidant and antimicrobial agents. Since the biological activities of essential oils usually

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varies depending on the place where they are grown so our study aims at the study of antioxidant activities of essential oil obtained from palmarosa plant grown in North Indian plains [11,12]. For this purpose antioxidant activity has been checked by the following methods: DPPH assay, Reducing activity assay, β -carotene bleaching assay, FRAP method and Nitrogen oxide method.

2. Materials and methods

ESSENTIAL OIL—The essential oil has been obtained from CIMAP (Central Institute of Medicinal and Aromatic Plants (CSIR) in April 2011. The specimen voucher has been deposited at our University herbarium.

THE CHEMICALS: All the chemicals used in the work were purchased from HI-MEDIA Pvt. Ltd, Bombay. The chemicals used were of analytical grade.

2.1. Determination of antioxidant activity

The antioxidant activity was evaluated by four methods which are as follows;

2.2. Free radical scavenging activity (dpph method)

The hydrogen atom or electron donating ability of essential oil and standards – Gallic Acid and BHT was determined from bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent. The diluted working solution of essential oil were prepared in methanol (2.0, 1.0, 0.5, 0.25, 0.125 and 0.0625 mg/ml). DPPH was prepared at a concentration of 0.002%. Different concentrations of essential oils was taken in each test tube and volumes was made upto 2 ml . Then 2ml of DPPH solution was added in each test tube and these solutions were kept in dark for thirty minutes. The same procedure was followed for BHT and Gallic Acid .All the samples were tested in triplicate. Later optical density was recorded at 517nm using UV-Visible spectrophotometer. Methanol with DPPH was used as control. The method was same as used by Kahalaf et al [13] with slight modification . The formula used for calculation is

$$\% \text{ Inhibition of DPPH activity} = (A-B/A) \times 100$$

Where A- Optical Density of control

B-Optical density of sample

2.3. Reducing power

The reducing antioxidant activity of the essential oil has been analyzed by the method given by Huda Fajan et al [14] with slight alterations. In this method different concentrations of essential oil (20, 10, 5, 2.5 and 0.125 μ g/ml) were taken in different tubes and volume of all the working solutions is made upto 1ml by adding distilled water, in these added 2.5 ml Phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated for 20 min at 50 °C. Then 2.5 ml TCA (Trichloroacetic acid, 10%) was added to each mixture and these were centrifuged for 10 min. at 3000 rpm. Then 2.5 ml of the upper layer was mixed with distilled water (2.5 ml) and 0.5 ml FeCl_3 (0.1%) solution.

Then absorbance was measured at 700 nm against a blank using UV-Visible spectrophotometer. The same procedure was repeated with gallic acid and BHT used as standard and sample without the oil was used as control. Increased absorbance of reaction mixture indicated increase in reducing power.

2.4. Nitric oxide scavenging activity method

Nitric oxide was generated from sodium nitroprusside (SNP) and was measured by Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ion that can be estimated by use of Griess Reagent, Sodium nitroprusside (5 mM) in phosphate buffer saline (PBS) was mixed with different concentrations (100, 50, 25, 12.5 and 0.62 μ g/ml) respectively and volume was made upto 3.0 ml. The solution was kept at 25 °C for 180 min. Then the sample from the above were reacted with Griess reagent (a solution of 1% sulphanilic acid in 2% phosphoric acid and 0.1% naphthylamine in distilled water). The absorbance of the chromophore produced by diazotization of nitrite ion with sulphanilic acid and subsequent coupling with naphthylamine was read a 546 nm. BHT and gallic acid were used as standards. The method used has been taken from Ghosh et al [15]

The formula used for calculation is

$$\text{Nitric Oxide scavenged (\%)} = (A-B/A) \times 100$$

Where A- Optical Density of control.

B- Optical density of sample of essential oil.

2.5. β -Carotene bleaching method

The method followed is same as used by Geckil et al [16] with some moderations. 0.02 mg of crystalline β -carotene was dissolved in 10 ml of chloroform and then added in this 20 mg linoleic acid and 200 mg of Tween-80 reagent (Merck). Chloroform was removed in rotary evaporator under vacuum at 40 °C for 5 min and then 50 ml of dist. Water was added with vigorous stirring to form an emulsion. Five ml of this emulsion was taken and added in 0.1ml of essential oil extract (1 μ g/ml). BHT was used as standard. The test tube containing sample and standard were kept in water bath in incubator at 50°C and absorbance was recorded at an interval of 15 min till 2 h at 470nm.

2.6. Ferric reducing antioxidant power (frap) assay

The method for determining the ferric reducing ability of the essential oil has been taken in modified form from the method used by Chaiyasut et al (2010)[17] and Jukic et al (2005)[18]. Briefly FRAP reagent has been prepared by mixing 0.1 M Acetate buffer (pH 3.6) with 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM ferric chloride in a ratio of 10:1:1 (v:v:v), Then 3ml of the Frap reagent has been added to 0.1, 0.2, 0.4, 0.6, 0.8 and 1mM of samples of essential oil (taken in triplicate), and left at room temperature for 5 min. until the reaction was complete. The reacted sample were read at 593 nm using standard ferrous sulphate (with same concentration) as standard. An increase in the absorbance of the reaction mixture indicate an increase in the reducing

power of the sample (as indicated in Fig.5)

3. Results

In order to determine the effect of concentration on radical scavenging power by DPPH method, five different working solutions were used (2.0,1.0,0.5,0.25 and 0.125 mg/ml).Results showed (Fig.1) that percentage inhibition is in increasing order with the increase in concentration, more precisely 49.5,56,61.6,64.8,65.8 and 68.4 respectively. Similar concentration standards (BHT and gallic acid) exhibited percentage inhibition of 50.2,54.1,58,61.2,68.2 and 76.1 and 18, 20.1, 23.2,27.3,31.1,and 36 for Gallic acid. IC50 for Palmarosa oil was found to be 0.125 mg/ml.

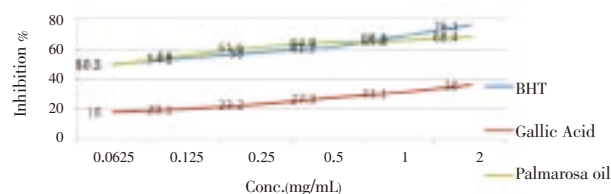


Figure 1. DPPH scavenging activity of BHT, Gallic acid and essential oil of Palmarosa.

Reducing power characteristic of any compound serves as a significant indicator of its potential as antioxidant and is a supporting feature for its antioxidant activity. The concentrations used were 20,10,05,2.5 and 0.125 μg/ml and absorbance was read at 700nm. Reducing power was found to be significant (P<0.01)and the values (Fig. 2) were found to be 0.41,0.52,0.78,0.92 and 1.02 respectively. The results were found to be better as compared to the standards gallic acid (0.12,0.23,0.45,0.637 and 0.712).The result for BHT are 0.415,0.56,0.87,1.227 and 1.625) for the same concentration. The activities were statistically (Fig–2) significant when compared with control.

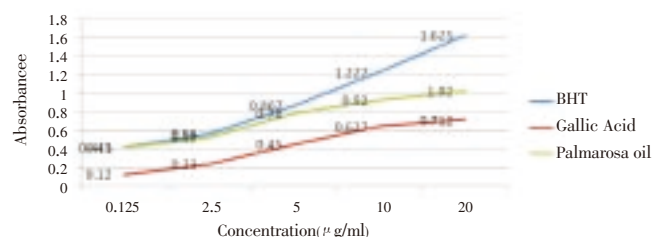


Figure 2. Reducing activity assay with BHT, gallic acid and Palmarosa oil.

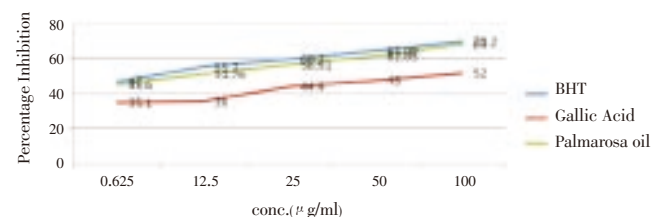


Figure 3. Nitric oxide scavenging assay with BHT, gallic acid and essential oil of Palmarosa.

Nitric oxide radical generated from Nitroprusside at physiological pH was found to be inhibited by the essential

oil as shown in Fig.3.the concentrations were 0.625,12.5,25,50 and 100 μg/ml and inhibition percentage were found to be 45.6,51.76,56.91,62.05 and 69. Gallic acid and BHT were used as reference.IC50 value has been found to be 12.5 μg/ml for the sample.

The anti-bleaching activity of sample of β-carotene was studied by monitoring the color intensity of emulsion at 470nm for every 15 min for 2 h. The concentration taken was 1 μg/ml for the sample as well as standard (BHT). The initial concentration was considered to be 100%. In the first 15 minutes the sample showed 93.1% bleaching as compared to 86% to that of standard. In one hour of incubation, percentage decrease was found to be 71.8% and 55.1 % for oil and standard respectively. During the second hour it came to 51.1% and 25 % for the oil and standard respectively.

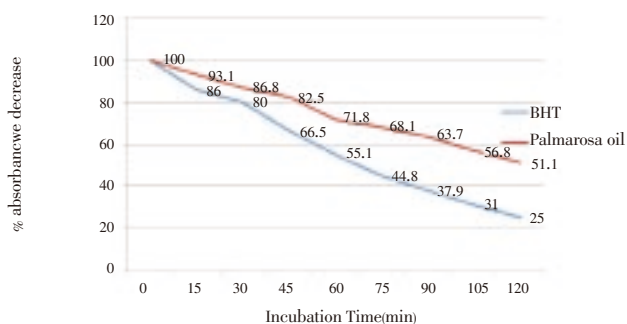


Figure 4. Relative changes in absorbance of beta carotene emulsions containing BHT and Palmarosa essential oil.

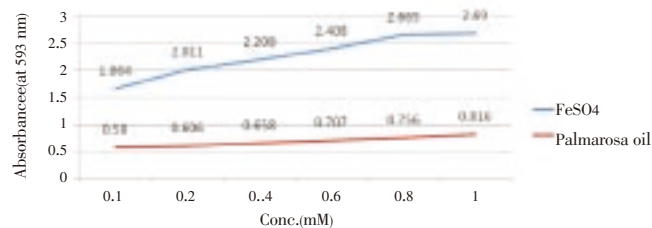


Figure 5. FRAP assay showing Antioxidant activity of Palmarosa oil and FeSO4.

For FRAP assay concentration of the sample and standard FeSO4 were taken to be 0.1,0.2,0.4,0.6,0.8 and 1.0 mM , the increase in absorbance with the concentration indicated increase in antioxidant activity . The FRAP assay measures the change in absorbance at 593nm due to the formation of the blue colored Fe(II)–tripirydyltriazine compound from the colorless oxidized Fe(III) form by the action of electron donating antioxidants.

4. Discussion

Since the study has been done on Palmarosa essential oil and the grass is basically grown in Northern part of India and is indigenous so not much work has been done on the oil. One of the report has been given by Scherer R *et al* (2009) [12] who worked on the chemical composition, antioxidant and antimicrobial activity of the oil of clove (*Caryophyllum aromaticum* L.), Citronella (*Cymbopogon winterianus*) and Palmarosa (*Cymbopogon martini*).Antioxidant activity has been evaluated by DPPH (2,2–diphenyl–1–picryl hydrazyl) method.Clove oil showed the highest antioxidant activity, Citronella and Palmarosa oil presented low antioxidant

activity.

Not much work has been done either on the essential oil or extracts from Palmarosa but antioxidant activity has been reported for different members of family Graminaea for example the antioxidant activity has been reported for Vetiver (*Vetiveria zizanioides*) oil by Kim HJ et al (2005) [19] by two methods namely DPPH free radical scavenging assay and Fe²⁺ metal chelating assay and the results show a strong free radical scavenging activity when compared to standard BHT and α -tocopherol. For example Free radical scavenging activity for α -tocopherol is 97% and 93% by metal chelating capacity and DPPH methods and same is 34% and 89% for Vetiver essential oil respectively.

Another team work on Vetiver root oil has been done by Luqman et al (2009)[20], they have taken essential oil from Vetiver root of two species KSI and Gulabi. The antioxidant activity has been checked by FRAP, DPPH and reducing power assay. Out of the two KSI showed higher FRAP values, DPPH inhibition and reducing power.

Antioxidant activity has also been studied on another member of Graminaea i.e lemon grass (*Cymbopogon citratus*) which is also a native of north India. Study has been done on Citral which is the major constituent of lemongrass by Rabbani SI et al (2006) [21] who have tested antioxidant activity by superoxide scavenging method, the result indicated that citral significantly ($P < 0.01$) inhibited the formation of micronuclei induced by Nickel and superoxide scavenging activity ($EC_{50} = 19$ mcg/ml) was observed in citral treated groups.

Another scientist Salem AM et al [22] compared the antioxidant and antibacterial activity of garlic, thyme and lemongrass oils. Antioxidant activity has been tested by TBA method it was found that best results were obtained at 1.5% and activity was in the order lemongrass oil > thyme > garlic oil.

From the discussion it can be seen that this is the first report on antioxidant activity of Palmarosa oil obtained in north Indian plains. Palmarosa oil showed strong antioxidant activity by inhibiting DPPH and NO radicals. Reducing power assay, FRAP and beta-carotene bleaching assay also showed similar results. The activities are comparable to standard antioxidants. So by such study it can be seen that Palmarosa oil can be taken as easy source of natural antioxidants

Conflict of interest statement

We declare that we have no conflict of interest.

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