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Ferric reducing antioxidant power of essential oils extracted from *Eucalyptus* and *Curcuma* species

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ABSTRACT

Objective: *Eucalyptus* and *Curcuma* species are well reputed for their traditional medicinal uses in south east Asia, therefore, the present study was designed to determine reducing potential of their essential oils. **Method:** Essential oils of the selected medicinal species *Eucalyptus sideroxylon*, *E. teriticornis*, *E. citriodora*, *Curcuma longa* and *C. aromatica* were extracted using hydro distillation method, separated with diethyl ether and dried over anhydrous sodium sulphate. Column chromatography of *Curcuma aromatica* was carried out and six fractions were collected using gradient solvent system of n-hexane-ethyl acetate. Ferric reducing antioxidant power (FRAP) of oils were evaluated using standard protocol and results were expressed in μ M equivalent to $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. **Results:** The essential oil of *Eucalyptus sideroxylon* was found to possess highest reducing potential among the *Eucalyptus* species. *Curcuma longa* essential oil showed most significant reducing potential with 138.4 ± 1.1 FRAP equivalents. **Conclusions:** It was concluded that the all essential oil and the column fractions of *C. aromatica* possess significant reducing capacity ranged from 95.8 ± 1.0 to 152.4 ± 1.4 μ M in a dose dependent manner.

1. Introduction

Essential oils are found in various parts of plants, such as leaf, flower, root and are stored in special oil cells and gates. The essential oils extracted from plants are indispensable materials in the pharmaceutical, food, cosmetics sectors because of the increasing concern with harmful synthetic additives [1]. A great majority of the essential oils are used as fragrance in perfumes and aromas in food industry. The essential oils have a number of biological activities, including antibacterial, antifungal and antioxidant properties [2,3]. With the growing interest in the use of essential oils in both food and pharmaceutical industries, a systematic examination of the plant extracts has become increasingly important [4,5].

Eucalyptus is one of the world's most important and most widely planted genera. It includes more than 700 species and belongs to the family of Myrtaceae [6]. Many

species of the genus *Eucalyptus* are used in Chinese folk medicine for a variety of medical conditions. The leaves of *Eucalyptus* species are traditionally used as analgesic, anti-inflammatory and antipyretic remedies for the symptoms of respiratory infections, such as cold and sinus congestion [7]. Its main uses are the production of essential oils, which are used for medicinal and pharmaceutical purposes [8]. In addition, *Eucalyptus* species are also known to contain bioactive products that display antibacterial, antifungal, analgesic antioxidative and anti-inflammatory effects [7, 9, 10].

The genus *Curcuma* (family: Zingiberaceae) consists of about 70 species of rhizomatous herbs most commercially cultivated spice crops of India for the production of turmeric, and are indigenous to Southern Asia. *Curcuma longa* L. var. *Rasmi* and *Curcuma aromatica* Salisb. var. *Bataguda* (Zingiberaceae) are the rhizome, used in the treatment of diabetics, hemorrhoids, anemia, jaundice, cough, asthma, wound healing, colic, gout, renal calculi, poisoning, freckles, skin and neurological disorders [11]. The cured, dried and ground rhizomes provide turmeric powder which is used as a supporting constituent of curry powders

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and as a food colourant. *Curcuma longa* is reputed for several different biological activities including nematocidal, anticancer, topoi–somerase inhibition and protection against alcohol include liver toxicity [12,13]. *Curcuma aromatica* Salisb. (CA), commonly known as ‘Jangli Haldi’ is distributed throughout India and widely used as a flavouring agent, condiment and a source of yellow dye. Medicinally, it possesses strong antimicrobial, anti–inflammatory, anti–tumor and immunological activities [14,15]. It is a well–listed drug in Ayurveda and other indigenous systems of medicine. The aim of the present study was to determine reducing properties of the essential oils of the selected medicinal plants.

2. Materials and Methods

2.1 Plant material

The leaves of *Eucalyptus* species (*Eucalyptus sideroxyylon*, *Eucalyptus citridora* and *Euaclyptus tereticornis*) were collected from Government College University Lahore, while rhizome of *Curcuma longa* and *Curcuma aromatic* were purchased from local market. All plant materials were identified at Sultan Herberium, Department of Botany, GC University, Lahore.

2.2 Extraction of essential oil

The chopped plant material was placed in a round bottom flask and proceeded with steam distillation. The essential oil obtained was separated with diethyl ether through solvent extraction technique, removed under vacuum, dried over anhydrous sodium sulphate and kept at 4°C.

2.3 Column chromatography of *Curcuma aromatica*

Ten grams of the essential oil of *Curcuma aromatica* was loaded on a glass column packed with silica gel. The column was eluted by using gradient mixtures of n–hexane and ethyl acetate. The solvents from elutes were evaporated under vacuum to get six fractions which further subjected to FRAP assay.

2.4 Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power assay of the essential oil was carried out according to the method of Benzi & Stain with some modifications [16]. The TPTZ reagent consist of 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6–tripiryridyl–s–triazine), and 20 mM FeCl₃.6H₂O solution. 150 µl of FRAP reagent was mixed with sample (50 µl) and read absorbance at 595 nm after 15 minutes. The results were expressed in µ M equivalent to FeSO₄.7H₂O by calculating from calibration curve.

3. Results

The essential oil of the selected plants was extracted by steam distillation, % yield was calculated, and the results are shown in Table 1. The highest amount of essential oil was obtained through distillation of the rhizomes of *C. aromatic* (0.91%). The reducing potential of the essential oils was determined by the ferric reducing antioxidant power (FRAP) method and the results are summarized in Table 2. Among the two *Curcuma* species, *C. longa* showed higher results with 138.4±1.1 µ M FRAP equivalents. *E. sideroxyylon* exhibited significant antioxidant potential with 130.5±1.2 FRAP equivalents), followed by *E. tereticornis* and *E. citriodora* (122.1±1.4 and 95.8±1.0 respectively). Silica gel column chromatography of the oil of *C. aromatica* yielded six fractions (GH–01–GH–06), which were subjected to the FRAP assay and the results were found in the range of 95.8 ±1.0 to 152.4±1.4 µ M FRAP equivalents. GH–04, obtained in the system of n–hexane–EtOAc (80:20) showed highest FRAP value (152.4±1.4 µ M FRAP equivalents).

4. Discussion

Plants have been used for the treatment of diseases for a very long time. Recently, interest in drugs of herbal origin has significantly increased due to less harmful side effects of natural products and the fact that plants are easily accessible. Scientific research is being conducted all over the world to determine whether plants that are traditionally used to treat various diseases are actually appropriate for their intended use. The essential oil of the five selected plants was obtained through steam distillation, and % yield was calculated, as shown in Table 1.

Table 1

Yield (%) of the essential oils of the selected plants.

S. No	Plant	Yield (%)
1	<i>Eucalyptus sideroxyylon</i>	0.51
2	<i>Eucalyptus tereticornis</i>	0.28
3	<i>Eucalyptus citridora</i>	0.32
4	<i>Curcuma longa</i>	0.12
5	<i>Curcuma aromatica</i>	0.90

Table 2

The results of FRAP assay of the essential oils of the selected plants.

Genus	Species	FRAP (µ M) equivalent to FeSO ₄ .7H ₂ O
<i>Eucalyptus</i>	<i>Eucalyptus sideroxyylon</i>	130.5±1.2
	<i>Eucalyptus tereticornis</i>	122.1±1.4
	<i>Eucalyptus citridora</i>	95.8±1.0
<i>Curcuma</i>	<i>Curcuma longa</i>	138.4±1.1
	<i>Curcuma aromatica</i>	130.6±1.5

Table 3

The results of FRAP assay of the column fractions of *Curcuma*

aromatica.

Fractions	Solvent systems (n-Hexane-EtOAc)	FRAP (μ M) equivalent to FeSO ₄ .7H ₂ O
GH-01	100:0	138.1 \pm 1.0
GH-02	80:20	130.3 \pm 1.3
GH-03	10:90	137.7 \pm 1.6
GH-04	80:20	152.4 \pm 1.4
GH-05	60:40	122.0 \pm 1.1
GH-06	50:50	127.6 \pm 0.9

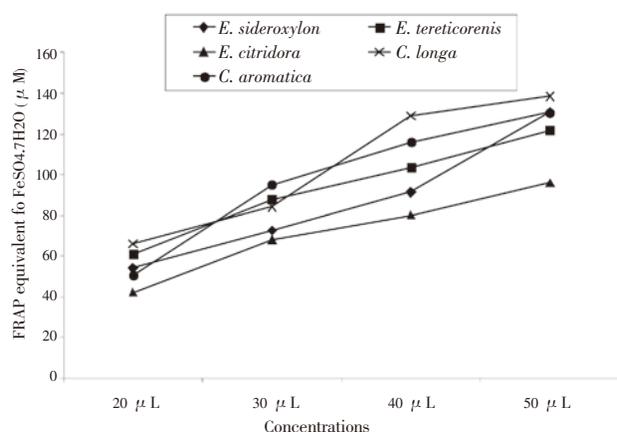


Figure 1. FRAP assay at various concentrations of the essential oil of the selected plants.

Determination of the ferric reducing antioxidant power is a simple direct test of antioxidant capacity. In this study, assay of reducing activity was based on the reduction of ferric to the ferrous form in the presence of reductants (antioxidants) in the tested samples. The Fe²⁺ was then monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. The antioxidant activity of the essential oils and column fractions, determined by the FRAP method are summarized in Table 2 and 3. Significant results were shown by the essential oils of all the selected plants in the decreasing order *C. longa* > *C. aromatica* > *E. sideroxyylon* > *E. tereticornis* > *E. citridora*. The reducing ability of *E. sideroxyylon* was most significant among the three species of *Eucalyptus* (*E. sideroxyylon* = 130.5 \pm 1.2, *E. tereticornis* = 122.1 \pm 1.4 and *E. citridora* = 95.8 \pm 1.0 μ M FRAP equivalents). According to Paula et al., 2006, 1,8 cineol is the major constituent of the essential oil of both *E. tereticornis* and *E. sideroxyylon* [17], while the oil of *E. citridora* contain its characteristic monoterpene citronellal (70–78%) with citronellol (6–7%) and spthulenol (8–9%). It has been reported in the literature that the compounds with highest reducing ability has delocalized chemical bonds [18]. Therefore, significant reducing potential of *E. tereticornis* and *E. sideroxyylon* can be attributed to the synergistic effect of the other components in the oil. The essential oil of *E. citridora* showed moderate results. *Curcuma* species exhibited remarkable results with 138.4 \pm 1.1 and 130.6 \pm 1.5 μ M FRAP equivalents for *C. longa*

and *C. aromatica* respectively. The essential oil of *C. longa* and *C. aromatica* contains 50–80% curcumin and sesquiterpenoids alpha and beta termerone. The phenolic groups in the structure of curcumin describe the ability of this compound to act as a reducing agent. Therefore, the reducing potential can be correlated with the concentration of curcumin in the essential oil of the two *Curcuma* species. Column chromatography of the oil of *C. aromatica* yielded six fractions. The fraction GH-04, obtained in the system of n-hexane-EtOAc (80:20) showed highest FRAP value, which can be attributed to the presence of curcumin as major component in the fraction (Table 3). All fractions exhibited higher reducing potential than the parent oil. It was also noted that FRAP assay depends upon concentration of antioxidant components as shown in Fig. 1.

Conclusions

Our results suggested that the essential oil of the selected plants can be utilized as an effective and safe source of natural antioxidants with consequent health benefits. It is proposed, that the beneficial effects of these essential oils in traditional medicine results from their reducing ability towards reactive oxygen species.

Conflict of interest statement

We declare that we have no conflict of interest.

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