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ANTIOXIDANT POTENTIAL OF ESSENTIAL OILS OF TWO SPECIES OF EUCALYPTUS FROM PAKISTAN

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ABSTRACT

Essential oils of *Eucalyptus Citreodora* and *Eucalyptus Camaldulance* were obtained through hydro-distillation for six hours. After rectification antioxidant activity of these oils was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Spectrophotometerically λ_{max} was determined. BHT was used as reference material. Concentrations 200µl, 400 µl, 600 µl, 800 µl, 1000 µl of both oils were used. It was observed that both oils gave maximum activity at 1000 µl concentrations, which were 62.67% and 69.06% for *E.Ccitreodora* and *E. Camaldulences* respectively. Furthermore it was also observed that BHT showed (90.09%) more activity than the oils.

INTRODUCTION

Eucalyptus belongs to plant family Myrtaceae comprises of 3800 species distributed in 140 genera occurring along tropical and subtropical regions of the world (Mabberly, 1997).[1] Within the family, the Eucalyptus genus has been cultivated and exploited on large scale for many years. (Estanislau et al., 2001).[2] Eucalyptus oils are found in leaves, fruits buds and barks of the tree but most rich oil found in leaves.[3] (Zrira et al., 2004).

Several species of eucalyptus are used in folk medicines as an antiseptic and against infections of the upper respiratory tracts such as cold, influenza and sinus congestion (Harborne and Baxter, 1995).[4] Myrtaceae species possess a strong antimicrobial potential and their volatile oils are used as antimicrobial and antifungal agents in creams, lotions, soaps and toothpastes. (Lis-Balchin et al., 2000).[5] Essential oil is also used in perfumery and

flavour preparations (Zrira et al.,1992).[6] Eucalyptus Camaldulensis is traditionally used for the treatment of wounds, boils and other ailments. (Babayi et al., 2004).[7]

Some studies have demonstrated that the oil and leaf extracts of Eucalyptus spp. have antifungal and repellent activity.[1-2][8,9] Crude methanolic extract of *E. Camaldulensis* has been reported to inhibit the growth of *Candida albicans.*[5][10] Also, it has been shown that ethanolic leaf extract of *Eucalyptus camaldulensis* had marked fungicidal effect against clinical dermatophytic fungal isolates; *Microsporium gypseum* and *Trichophyton mentagrophytes.*[6][11] Citrus and eucalyptus essential oil were compared for antifungal and antibacterial activity. It was found that eucalyptus has better antimicrobial activity as compared to citrus fruit essential oil.[12]

The extraction characterization and utilization of natural antioxidants that may serve as potent candidates in combating carcinogenesis and aging process are in progress. Namolo, (Mathew and Abraham, 2006). [12] The human body is equipped with an inherent defense system which can quench free radical present in almost all cells (Zarkovic, 2003).[13] An imbalance between free radical production and their removal by the body's antioxidant system leads to a phenomena known as oxidative stress (McCord, 2000 and Abdollahi et al., 2004).[14,15] In this situation an external supply of antioxidant is necessary to regain a balance between free radicals and antioxidants. Antioxidant activity of essential oils can not be attributed to the presence of phenolic compounds, monoterpenes, alcohols, aldehydes, ketones hydrocarbons and ethers also contribute to the free radical scavenging activity.(Edris, 2007).[16] Synthetic antioxidant BHA and BHT are being used but now their side effects has been well documented (Sing et al., 2005, 2008)[17,18] So there is a strong need to explore some alternative source of natural antioxidant that can fulfill the requirement of body safely.

Chemical constituents of *E. citriodora and E. Comaldulence* leaf essential oil from Pakistan have been reported by Zafar et al in 2013.[13] Antioxodant activities of Eucalyptus species from various part of the world have been reported, but antioxidant activity of these species from Pakistan area has not been reported. However, it has also been established that the composition pattern of essential oil is affected by factors such as geographical location (20,21), which consequently influence their biological activities.[22] It is on this basis, we investigated the chemical composition, antioxidants effects of essential oil extracted from the leaves of *Eucalyptus citriodora and E. Camaldulences* grown in Punjab,Pakistan.

MATERIAL AND METHOD

Eucalyptus globules and camaldulensis leaves were collected from the area of PCSIR Laboratories complex, Lahore-Pakistan. Leaves of both species were collected in the month of Feb at full maturity and dried under shade for ten days. Five hundred gram of each species were taken and subjected to steam distillation by using dean stark apparatus for eight hours. Essential oils obtained were rectified with petroleum ether (200ml) and then dried over anhydrous sodium sulphate. Yield was calculated on dried basis.

Antioxidant activity

Antioxidant activity was evaluated by measuring the scavenging activity of the examined tangerine peel oil on the 2, 2-diphenyl-1-picrylhydrazil (DPPH) radical. The DPPH assay was performed as described by Epsin *et al.2000*.[19] The samples (100 µl each) of different concentrations of 20%, 40%, 60%, 80% and 100% were mixed with 3 ml of DPPH solution. The absorbance of the resulting solutions and the blank (with only DPPH and no sample) were recorded after an incubation time of 30 minutes at room temperature against ascorbic acid as a reference positive control. For each sample, 3 replicates were recorded. The disappearance of DPPH was measured spectrophotometrically at 517 nm. The percentage of radical scavenging activity was calculated using the following equation;

DPPH scavenging effect (%) = $(A_0-A_1)/A_0 \times 100$

Where A_0 is the absorbance of the control at 30 minutes and A_1 is the absorbance of the sample at 30 minutes.

RESULTS AND DISCUSSION

The free radical scavenging activity of two species of Eucalyptus plant growing in Pakistan were estimated using a stable DPPH free radical. The DPPH scavenger capacity of the essential oils was compared with known antioxidant active substance BHT.

The results indicate that antioxidant activity of essential oil of Eucalyptus Globulus was 32.22%, 38.55%, 46.12%, 53. 40% and 62.67% against the concentrations of 200 µl, 400 µl 600 µl, 800 µl and 1000 µl respectively. While antioxidant activity of Eucalyptus citiodora was 35.44%, 44.70%, 52.70%, 63.73% and 69.06% against the concentrations of 200 µl 400 µl600 µl,800 µl and 1000 µl. The antioxidant activity of standard BHT was 90.05% at 1000 µl. The difference in antioxidant activity of essential oils is because of difference in their chemical constituents. Eucalyptus globules has 1, 8 cineol (26.57%) as major constituent

while Eucalyptus citriodora has citronellal (74.65%) as major constituents (Zafar et al 2003). Antioxidant activity mainly is due to the polyphenols, flavonoids and anthocyanins. Phenolic compound are known as powerful chain-breaking antioxidant (Pamda et al., 2006). [20]

CONCLUSION

Essential oils of Eucalyptus Citreodora and Eucalyptus Camaldulance were obtained through hydro-distillation for six hours. After rectification antioxidant activity of these oils was determined by 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Spectrophotometerically λmax was determined. BHT was used as reference material. Concentrations 200µl, 400 µl, 600 µl, 800 µl, 1000 µl of both oils were used. It was observed that both oils gave maximum activity at 1000 µl concentrations, which were 62.67% and 69.06% for E.Ccitreodora and E. Camaldulences respectively. Furthermore it was also observed that BHT showed (90.09%) more activity than the oils.

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