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APPRAISAL OF ANTIOXIDANT EFFECT OF FRESH AND DRIED LEAVES OF LEMONGRASS (*CYMBOPOGON CITRATUS*)

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Abstract

Lemongrass a commonly used plant in folk medicine for treatment of number of diseases and its leaves are used to make water infusion having a refreshing lemon flavor. The present study was undertaken to evaluate total phenolic content and antioxidant potential of lemongrass tea obtained from fresh and dried leaves. The EC_{50} values of fresh and dried leave extracts were found to be similar in various antioxidant models such as DPPH (10.02 and 9.54mg/ml), ABTS (9.97 and 10.78mg/ml), nitrite radicals (12.38 and 11.30mg/ml) and antilipid peroxidation (1.13 and 1.01mg/ml), respectively. The phytochemical analysis revealed that fresh and dried leaves of lemongrass have TPC (28.1 and 32.1 mg gallic acid equivalent/g) and flavonoid content (16.47 mg QE/g and 14.6 mg QE/g). It was noticed that there is no significant difference between fresh and dried leaves antioxidant capability.

***Keywords*:** Lemongrass, ABTS, DPPH, antilipid peroxidation, total polyphenols, antioxidant.

Introduction

The herbal medicinal plants are dominant on the earth and being used as a source of traditional medicines from ancient times. Extraction of spices, herbs and other related plant material in hot water leads to a preparation known as herbal tea. Herbal teas are consumed all over the world for their aroma and health properties (Naithani *et al.*, 2006; Aoshima *et al.*, 2007; Nankar *et al*., 2017). These medicinal plants may have relieving or preventive effect and can cure diseases or psychological conditions. Plants contain active chemicals in many parts, such as in leaves, stems, roots, fruits etc. Their antioxidant properties and constituents are responsible in disease prevention or control (Ivanova *et al.,* 2005; Kaur *et al*., 2016; Sharma, 2016). Herbs possess large variety of phytoconstituents such as saponins, carotenoids, plant sterol, terpenoids, flavonoids and polyphenolics. Due to the occurence of different phytochemicals having antioxidant properties, these herbs give prominent protection from chronic diseases (Parr and Bolwell, 2000; Malik *et al*., 2013; Malik *et al*., 2016).

Cymbopogon citratus (lemongrass/oil grass) is a perennial aromatic grass with slightly leathery leaves in dense clusters that grow spontaneously around the world, mainly in the tropical regions. Lemongrass is extensively used in herbal teas, baked goods, confectionary items and non- alcoholic drinks. Lemongrass with its aromatic and lemon scented characteristic secure good position in the Asian cooking (Sah *et al*., 2012). Besides cooking, the leaves of lemongrass are also known for traditional remedies and also used in cosmetic industries. Lemongrass tea is known for antidiuretic, mood enhancer, antidepressant and folk remedy for malaria, flu, cough, pneumonia, headache and vascular disorders (Blanco, 2009; Bastos *et al.,* 2010; Prabhakar *et al*., 2011).

The study was conceived to assess and compare the antioxidant potential and phytochemical constitution of lemongrass tea obtained from fresh and dried leaves of lemongrass.

Material & Methods

Reagents and Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), Methanol, Sodium nitroprusside, Sodium nitrite, Sulphanilamide, O-phosphoric acid, Napthylethylene diamine dihydrochloride, 2, 2' azino- bis (3- ethylbenzthiozoline-6- sulfonic acid) ABTS, Potassium persulphate, Ascorbic acid, Ferrous sulphate, Ethylene diamine tetra acetic acid (EDTA), Thiobarbituric acid (TBA), Tri choloro acetic acid (TCA), Hydrochloric acid (HCL), Follin's reagent, Aluminium chloride ($AlCl_3$) and Sodium carbonate (Na_2CO_3) were of analytical grade and were procured from SRL and HiMedia India Ltd.

Sample Preparation

The leaves of the plant *Cymbopogon citratus* were collected from herbal garden, Lovely Professional University, Phagwara, India. Take 5gm of fresh or dried leaves of lemongrass plant put them into the teapot, add 100ml of water, boil it and steep for fifteen minutes. The water infusion is filtered using Whatman's filter paper and stored at 4^{o}C till further use.

Determination of antioxidant activities

The ABTS radical scavenging activity was determined by decolourisation of ABTS solution (Re *et al*., 1999). Radical scavenging activity of fresh and dried lemon grass extracts against stable DPPH was determined spectrophotometrically (Mensor *et al*., 2001; Vyas, 2017). The anti-lipid peroxidation ability of different concentration of lemongrass water extracts employing chick liver homogenate was measured in terms of formation of thiobarbituric acid reacting substances (TBARS) (Ohkawa *et al*., 1979; Sivakumar *et al*., 2011; Saxena, *et al*., 2016). Nitric oxide scavenging potential of fresh and dried leaves of lemongrass tea was determined by the method (Shirwaikar *et al.,* 2006).

Total phenolic and flavonoid assay

Take 1ml of fresh and dried lemon grass extract separately and dissolve in 5 ml of 70% methanol at 70°C for 10 minutes, then cool at room temperature. After cooling the water extracts are centrifuged at 10,000 rpm for 10 min. The supernatant obtained is taken in another falcon tube (Singh *et al*., 2019; Vyas, 2019). The extraction is repeated again with that supernatant. Then the extract is diluted with appropriate amount of water and used for total phenol and flavonoid measurements. Total phenolic content (TPC) estimation was carried out by using gallic acid as standard according to the method (Singleton *et al.,* 1999; Mohan, *et al*., 2011; Chauhan *et al*., 2017).

Statistical analysis

The results were expressed as mean ± S.D. for each sample evaluated in triplicates. The data obtained was analysed employing one way analysis of variance (ANOVA) followed by post hoc test (Tukey's honestly significant difference test) with SPSS software (version 18). If the p-values equal to or less than 0.05 were be considered statistically significant.

Results and Discussion

In vitro antioxidant activity of a *C. citratus* was investigated employing standard battery of antioxidant assays viz. DPPH, ABTS, Nitric oxide and Lipid peroxidation assay. These methods have demonstrated the radical scavenging ability of fresh and dried leave extracts. The infusion obtained from fresh and dry leaves of lemongrass exhibited appreciable antioxidant potential determined experimentally through DPPH, ABTS, nitric oxide radical and lipid peroxidation inhibition.

DPPH is a dark-coloured crystalline powder when we mixed with methanol it gives dark purple color. On contact of antioxidant with DPPH, it transmits electron or hydrogen atom to DPPH. Therefore, it neutralizes DPPH free radical character then transformed it into 1-1 diphenyl-2-picryl hydrazine and amount of decolorization determine scavenging potential of drug (Jayaprakasha and Sakariah, 2004). In another study it was determined that DPPH scavenging activity of ethanolic and aqueous extract of lemongrass at 100µg/ml was 78.21 and 66.90% (Akande *et al*., 2012; Mishra, 2019a, 2019b). The percentage scavenging activity of fresh leaves ranged from 11 to 50% at 2.5-10mg/ml. Whereas, in dried leaves the scavenging activity was observed from 19 to 50% at 2.5-10 mg/ml (Fig. 1a). The EC_{50} value of fresh and dried leave extract of *C. citratus* was calculated to be 10.02 and 9.54 mg/ml ($p \geq .05$).

ABTS assay is used by agricultural researchers and used by food industry to estimate antioxidant capacities of food. ABTS solution reacts with sodium persulfate gives blue color and absorbs light at 734nm (Re *et al.,* 2009; Chakraborty *et al*., 2015). ABTS scavenging activity of hydroalcoholic extract of *C. citratus* was reported (Rao *et al.,* 2010; Nazir *et al*., 2016). In current study the percentage scavenging activity of the fresh leaves ranged from 15 to 50% at 2.5-10 mg/ml. Whereas, in dried leaves the scavenging activity was found to be 18 to 48% at 2.5-10 mg/ml (Fig. 1b). The EC_{50} value of fresh and dried leave extract of *C. citratus* was calculated to be 9.97 and 10.78 mg/ml ($p \geq .05$).

Oxygen reacts with excess nitric oxide (NO) radicals to produce nitrite and peroxy nitrite anions, which act as free radicals (Clancy *et al.,* 1992). NO modulate iron and catalyzed oxidation reactions which produces hydroxyl radical (Dadashpour *et al.,* 2011; Rana and Suttee, 2012). It is reported that methanolic extract of lemon grass exhibited an EC_{50} value 416 mg/g against NO radical (Garg *et al.,* 2012). The percentage scavenging activity of fresh leaves observed from 27 to 52% at 3.75-15 mg/ml. Whereas, in dried leaves the scavenging activity ranged from 19 to 55% at 3.75-15 mg/ml (Fig. 1c). The IC_{50} value of fresh and dried leaves extract of *C. citratus* was calculated to be 12.38 and 11.30mg/ml ($p \geq .05$). Plant extracts demonstrated many biological properties such as antioxidant properties, anticarcinogenic and antidiabetic due to their phytochemical constituents like phenolics and flavonoids. The free radical scavenging ability of phenolics is due to their property to neutralize free radicals (Shah *et al.,* 2012)

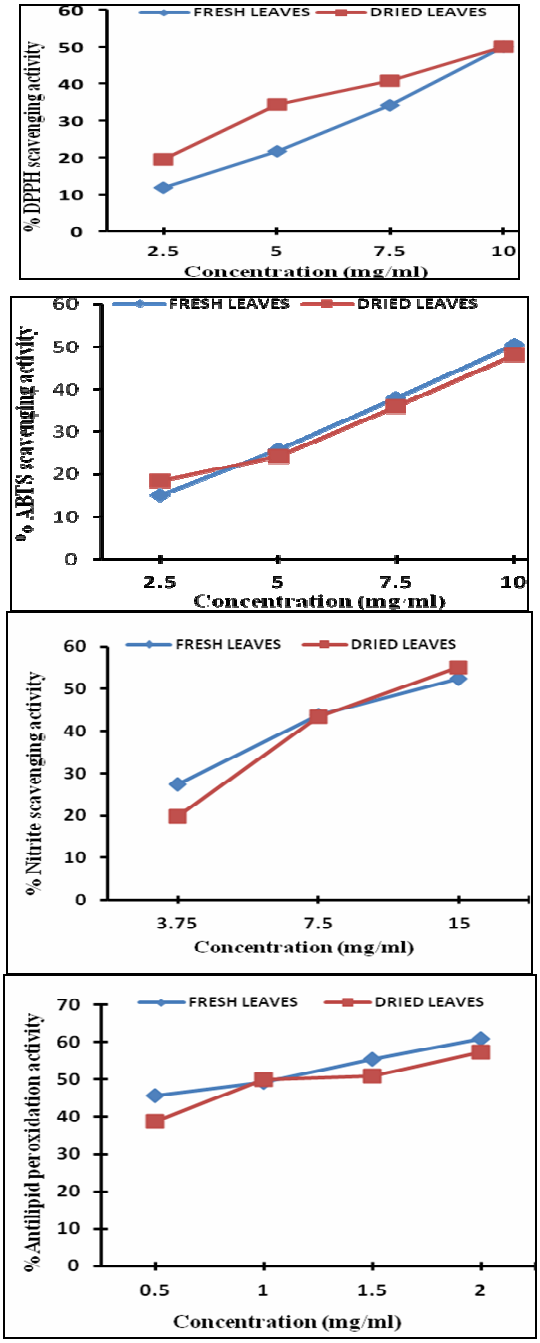

Fig. 1: Antioxidant capacity of fresh and dried leaves of lemon grass (a) DPPH scavenging activity (b) ABTS scavenging activity (c) nitrite radical scavenging activity (d) antilipid peroxidation activity.

The fresh and dried leaves of *C. citratus* showed the total phenolic content (26.1 mg GAE/g and 32.1 mg GAE/g) and total flavonoid content (16.47 mg QE/g and 14.6 mg QE/g) ($p \geq .05$). Earlier reports revealed that phenolic components including flavonoids are potent antioxidants with reported antimutageneic and anticarcinogenic effects (Geetha *et al.,* 2014; Abbasi *et al*., 2018; Parasher *et al*., 2018).

Lipid peroxidation affects colour, flavour, texture and nutritional value of foods (Balu *et al.,* 2005; Sudhakar *et al*., 2015; Prabhakar *et al*., 2020). It is the oxidative degradation of polyunsaturated fatty acid containing any number of C=C bond in which free radical "steal" electrons causing damage to cell membrane. Free radicals react with unsaturated lipid regions in the body and cause lipid peroxidation (Coyle and Puttfarcken, 1993; Kaur *et al*., 2016*)*. A study showed that methanol/water extracts, of *C. citratus* inhibited membrane lipid peroxidation of erythrocytes (Cheel *et al.,* 2005; Mishra *et al*., 2018). The lipid peroxidation inhibition activity of fresh leaves of *C. citratus* ranged from 45 to 60% at 0.5-2 mg/ml. Whereas, in dried leaves inhibition activity was observed from 38 to 57% at 0.5-2 mg/ml increased significantly with increasing concentrations (Fig. 1d). The EC_{50} value of fresh and dried leaves extract of *C. citratus* was calculated to be 0.92 and 1.26 mg/ml ($p \geq .05$). The results indicate that both fresh and dried leaves inhibited lipid peroxidation.

Conclusions

The antioxidant activity of aqueous extract of fresh and dried lemongrass was evaluated using DPPH, ABTS, Nitric oxide and lipid peroxidation methods. The result suggested that both fresh and dried lemongrass extract showed significant antioxidant activity and could serve as a potential source of natural antioxidants. The result indicated that there is no difference in the free radical scavenging potential of fresh and dried lemon grass extracts. Phytochemical analysis also revealed that both the leave extracts of lemongrass tea have similar total phenolic and flavonoid content. Thus, incorporation of these herbal teas which are easily available, cost effective such as lemongrass in our diet may prevent diseases like cardiovascular diseases, cancer, diabetes and neurodegenerative disorders.

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