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Antioxidant activity of Geraniol, Geranial acetate, Gingerol and Eugenol

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Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular and anti-inflammatory diseases. Natural antioxidants have been used instead of synthetic antioxidants to retard lipid oxidation in foods to improve their quality and nutritional value. Apart from their use as aroma additives in food, essential oils from aromatic plants have antioxidant properties..In present study the Antioxidant activity of Geraniol, Geranial acetate, Gingerol and Eugenol was employed by two complementary test systems, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging and Super Oxide Dimutase (SOD) activity. Antioxidant activity of Gingerol was found to be higher than those of the others in DPPH assay. While Eugenol has more antioxidant activity in Super Oxide Dimutase activity. These essential oils showed a promising sources of natural antioxidant activity and these findings give scientific bases to their ethno pharmacological uses.

Key words: Antioxidant activity, DPPH, SOD, essential oils, Geraniol, Geranial acetate, Gingerol and Eugenol.

Reactive oxygen species (ROS) including singlet oxygen ($^1O^2$), superoxide ion ($O_2^{-\cdot}$), hydroxyl ion ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) are highly reactive and toxic molecules generated in cells under normal metabolic activities. However, in response to a variety of factors including tobacco smoke, pollutants, ionising radiations, alcohol, synthetic pesticides, and solvent, their production increases (Halliwell et al., 1989). ROS can cause oxidative damage to proteins, lipids, enzymes, and DNA, and they have also been linked to pathogenesis of oxidative diseases (Halliwell, 1997).Living cells possess an excellent scavenging mechanism to avoid excess ROS-induced cellular injury; however, with ageing and under influence of external stresses, these mechanisms become inefficient, and dietary supplementation of synthetic antioxidants is required. In recent years, due to toxicological concerns associated with the use of synthetic substances in food and increasing awareness about natural foods, there has been an increased interest in the use of natural substances as food preservatives and antioxidants (Peschel et al., 2006). In this context, aromatic plants, particularly their essential oils, are being evaluated for antioxidant activity. It is, thus, pertinent to evaluate the natural antioxidant activity of essential oils, since they find extensive use in the food and beverage industry (Bozin et al., 2006, Vagionas et al., 2007, Wei et al., 2007). The main constituents of essential oils, for example, monoterpenes and sesquiterpenes and phenylpropanoids including carbohydrates, alcohols, ethers, aldehydes

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and ketones, are responsible for the fragrant and biological properties of aromatic and medicinal plants (Reichling, 1999) Various essential oils and their components possess pharmacological effects, demonstrating anti-inflammatory, antioxidant and anti-cancerogenic properties (Golab et al., 2005; Naser et al., 2005; Ito et al., 2008).

In this study, antioxidant tests carried out by two complementary test systems, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging and Super Oxide Dismutase activity. DPPH is the best, easiest and widely used method for testing preliminary free-radical scavenging activity of a compound or a plant extract. The effects of antioxidants in the DPPH-radical-scavenging test reflect the hydrogen-donating capacity of a compound. When the radical form of DPPH is scavenged by an antioxidant through the donation of a hydrogen to form a stable DPPH molecule, this leads to a colour change from purple to yellow, and a decrease in absorbance (Choi et al., 2002). In the Superoxide dismutases are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen.

The main advantage of essential oils is that they can be used in any foods and are considered generally recognized as safe (GRAS) as long as their maximum effects is attained with the minimum change in the organoleptic properties of the food. The current study aimed at exploring the antioxidant activity of the essential oils, geranial, geranial acetate, gingerol and eugenol.

The aim of this study is to evaluate the *in vitro* antioxidant activity of the essential oils with methnol extracts at various concentrations.

Materials and Methods

Essential oil compounds

Four essential oil compounds such as Geraniol, Geranial acetate, Gingerol and Eugenol were obtained from commercial producers of plant essential oils and aromatic substances were used in this study. Quality of the oils was ascertained by GC to be more than 98% pure. The oil was stored in the dark at 4°C until used within a maximum period of one week. (Adams et al., 2001)

DPPH (2,2-Diphenyl-0 picrylhydrazyl) scavenging activity (Qualitative)

Two dimensional thin layer chromatography (TLC) was carried out, on silica gel plate with the following solvents:

1. 2% acetyl alcohol and
2. n-butanol: acetyl alcohol: H2O(4:1:5)

The radical scavenging compounds on the chromatogram were detected by spraying with 0.2%DPPH (2, 2-Diphenyl-0 picrylhydrazyl) solution in methyl alcohol. The paper was examined after 30 minutes.

DPPH (2,2-Diphenyl-0 picrylhydrazyl) scavenging activity (Quantitative)

In order to measure quantitative antioxidant activity, DPPH free radical scavenging assay was carried out as described by (Ref). Test extract (Methanol extract) at various concentrations (0.8, 4, 20, 100 µL/mL) were added to a solution of 1.5×10m DPPH in methanol and the reaction mixture was then shaked vigorously. The amount of DPPH remaining was determined at 520 nm, and radical scavenging activity was obtained from the equation:

(%) = OD Radical scavenging activity control – OD test/ OD control × 100

The antioxidant activity is expressed as IC50. It is the concentration (µL/mL) of the extract inhibiting the formation of DPPH by 50% (Sarker et al., 2006).

Lipid peroxidation inhibition assay

The reaction mixture contained 0.2 mL of liver homogenate in a glass tube incubated with a 0.1 mL KCL and 0.4 mL Tris-buffer (pH 7.5). To this solution, 20 µM of adenine diphosphate was added followed by ascorbic acid (100 miM). Extract (50 µL) was added in various concentrations followed by ferrous sulphate (10µM) and the reaction mixture was incubated at 37oC for 1h. To this reaction mixture 75 µL of 1.3% thio barbituric acid (TBA) was added and reaction mixture was boiled for 15 min. The tubes were then centrifuged at 4000 rpm for 10 min and cooled. Absorbance of the resulting chromogen was recorded at 532 nm.

Determination of antioxidant enzymes

Animals

Male rats (10 weeks old and between 220 and 260 g in body weight) were randomly assigned to two groups. One group (Test group) of rats received oral dose of essential oil compounds in various concentration in ethanol. Another group (control group) received an equivalent volume of ethanol alone. Control and test group rats were caged separately but housed under similar conditions. Both groups of animals were fed with the same diet and water ad libitum. All experimental manipulations were carried out with the animals under diethyl ether anesthesia. On the day of the experiments, blood samples were collected and catalase activities were determined.

Preparation of blood samples and lysates

Blood was collected into heparinized syringes through puncture of the left heart ventricle or tail. Erythrocytes were obtained after centrifugation at 600-1400 x g for 10 min. Erythrocytes were washed twice with 0.9% sodium chloride (Physiological saline) and were centrifuged under the same conditions. A 5% erythrocyte suspension in 0.15 M NaCl – 10 mM sodium phosphate buffer, pH 7.4 was lysed by freezing (- 20°C) for 24 h and was used for catalase measurement.

Super Oxide Dismutase (SOD) activity

Reactive oxygen species (ROS), such as superoxide (O2-) and hydrogen peroxide (H2O2), are constantly produced during metabolic processes in all living species. Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of antioxidant enzymes and other redox molecules. However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, protein, and lipidmembrane. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by a variety of antioxidant defense mechanisms. Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. SOD enzymes are classified into three groups: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular Ec-SOD. In Superoxide Dismutase Activity xanthine/xanthine oxidase (XOD) system was used to generate superoxide anions.

Liver tissue was lysed and processed in isotonic buffer (10 mM Tric-HCl (pH 7.4), 200 mM mannitol, 50 mM sucrose, 1 mM EDTA). Briefly the organ was perfused with PBS containing 0.16 mg/mL of heparin. The tissue was weighed, minced, and homogenized in ice-cold isotonic buffer (10 mL/g of tissue) by means of a Teflon pestle in a Potter-Elvejhomomogenizer. The crude nuclear fraction was isolated by centrifugation at 1000 x g at 2 - 8° C for 10 min, heavy mitochondria at 3000 x g at 2 - 8° C for 10 minutes, light

mitochondria at 20,000 x g at 2 - 8°C for 20 min, and microsomes at 144,000 x g at 2 - 8° C for 90 min. The final supernatant is the cytosolic fraction. Processed samples were stored on ice and assay for SOD immediately or aliquot and stored at -80°C.

Results and Discussion

Antioxidant Activity

Antioxidant tests are designed to mimic the oxidation-reduction reactions popularly occurring in the live biological systems and are used to estimate the antioxidant potentials of various chemical and biological samples. In this research, two most widely used such assays, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Super Oxide Dimutase activity, were applied to evaluate the antioxidant capacities of essential oils.

DPPH Assay

The DPPH-radical-scavenging activity of the essential oils is shown in table 1 and table 2. The Antioxidant activity in qualitative analysis shows a positive result with appearance of yellow spots against purple background and the results are tabulated in table 1.

Table 1- Qualitative identification of DPPH scavenging activity of selected Essential oil compounds

Plant Source	Qualitative DPPH Scavenging activity
Geraniol	Positive
Geranial acetate	Positive
Gingerol	Positive
Eugenol	Positive

The DPPH-radical-scavenging activity in qualitative method of the essential oils and the methanolic extracts are shown in table 2. Lower IC_{50} value indicates higher antioxidant activity. Among the total essential oils, Geraniol shows 24.6μg/mg, Geranial acetate shows 4.2μg/mg, Gingerol shows 68.4μg/mg and Eugenol shows 0.9μg/mg. The results are tabulated in table 2.

Table 2- Quantitative DPPH scavenging activity of selected essential oil Compounds

Plant Source	DPPH Scavenging activity Inhibition %				IC50 μg/ml
	0.8 μg/ml	4 μg/ml	20 μg/ml	100 μg/ml	
Geraniol	0.0	0.0	40.6	NN	24.6
Geranial acetate	0.0	46.8	NN	NN	4.2
Gingerol	0.0	0.0	0.0	73.0	68.4
Eugenol	44.2	ND	NN	NN	0.9

Super Oxide Dismutase (SOD) activity

The results of essential oils on SOD are presented in table 3. The antioxidant activity of the essential oils increases gradually with the concentration. Geranial acetate and Eugenol has got a good oxidation activity compared to others with 454.6 and 454.0 respectively.

Table-3 Effect of selected essential oil compounds on SOD (in units/mg of protein) production

Concentration of essential oil compounds	Essential oil compounds/SOD (in units/μg of protein				Standard Control
	Geraniol	Geranial acetate	Gingerol	Eugenol	
1.84	330.0 ±0.3	313.8±1.5	359.8±3.03	358.6±4.4	302.1±0.6
3.75	357.2±0.2	324.0±0.9	357.0±0.2	355.8±3.4	
5.62	365.4±0.3	336.2±2.4	351.1±3.2	356.1±2.5	
7.5	379.6±0.5	343.4±0.3	353.2±4.4	356.6±0.8	
9.4	392.8±0.6	454.6±2.4	352.4±3.3	454.0±2.2	

Conclusion

In the present study, essential oils and methanol extracts showed similar or near to similar antioxidant activities compared to the standard BHT. Nowadays, there is great worldwide interest in finding new and safe antioxidants from natural sources to prevent oxidative deterioration of foods and to minimise oxidative damage of living cells. The essential oils geranial, geranial acetate, gingerol and Eugenol has antioxidant properties.

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