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Determination antioxidant activity of Green tea (*Camellia sinensis*), Thyme (*Thymus vulgaris L*) and *Salvia Officinalis* (Sage)

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Abstract: *The methanolic crude extracts of some commonly used medicinal plants were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The overall antioxidant activity of green tea Camellia sinensis, was the strongest, followed in descending order by Thyme (Thymus vulgaris L.) (Salvia officinalis L.) Showed less free radical scavenging activity with the DPPH method. All the methanolic extracts exhibited antioxidant activity significantly. The IC_{50} of the methanolic extracts ranged between 8.7 ± 0.1 and 783.4 ± 6.5 µg/ml and that of ascorbic acid was 9.1 ± 0.2 µg/ml. The study reveals that the consumption of these spices would exert several beneficial effects by virtue of their antioxidant activity.*

Keywords: DPPH, green tea *Camellia sinensis*, Thyme (*Thymus vulgaris)* (*Salvia officinalis L)*

Introduction

Nowadays, there are increasing consumer demands for foods, which contain ingredients that may impart health benefits beyond basic nutrition, including herbal. They represent not only a suitable medium for the dissolution of functional components, but also a convenient method of consumption **[1]** The potential source of natural antioxidants is plants, fruits and vegetables [**2**]. Alarge number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including bellerica, *Camellia sinensis L*., and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins [**3**].

Green tea and black tea leaves are obtained from dried leaves of *Camellia sinensis L*. belonging to the family Theaceae. Steaming or drying fresh tea leaves at elevated temperature makes the commercial green tea. Its chemical composition is similar to that of fresh tea leaves. Green tea contains polyphenols, which include flavonols, flavandiols, flavonoid and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the green tea catechins are (-) epicatechin-3-gallate, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate, (-)- epicatechin, and (+) catechin. Caffeine, theobromine and theophylline, the principle alkaloids, account for about 4% of the dry weight. In addition, there are phenolic acids such as gallic acids and characteristic amino acids such as theanine (**4**). Thyme contains many flavonoids, phenolic antioxidants like zeaxanthin, lutein, pigenin, naringenin, luteolin and thymonin. Fresh Thyme herb has one of the highest antioxidant levels among herbs. It is packed with minerals and vitamins that are essential for optimum health. Its leaves are one of the richest sources of potassium, iron, calcium, manganese, magnesium and selenium [**5**]. Thymol are the main phenolic components that are primarily responsible for its antioxidative activity [**6**]. Thyme is herbaceous plant of the platoon species, grows in mountainous areas, used as a beverage instead of or with tea, added to some food to give it an acceptable flavour, the plant is used in folk medicine frequently where it is prescribed to treat mouth infections, stomach, intestine and airways, coughing and gastroenteritis and expel intestinal worms, as well as to strengthen the heart [**2**]. Extracts from Thyme have been used in traditional medicine for the treatment of several respiratory diseases like asthma and bronchitis and for the treatment of other pathologies thanks to several properties such as antiseptic, antispasmodic, antitussive antimicrobial, antifungal, antioxidative, and antiviral [**7].** *Salvia* (sage)**,** one of the largest and the most important aromatic and medicinal genera of the Lamiaceae family, comprises about 1000 worldwide-distributed species. Salvia species are reported to have antioxidant, antibacterial, antifungal, antiviral, cytotoxic, neuroprotective, anti-inflammatory and other biological activities [**8**]. *Salvia officinalis*, known as Dalmatian sage, common sage or garden sage, is a perennial subshrub native to the northern coastal region of the Mediterranean, but widely cultivated in many countries [**9**] due to its culinary and medicinal significance. It is used for food preservation, as a spice for flavoring, and for treatment of many diseases **[2]**. Free radicals and reactive oxygen species (ROS) are well-known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, atherosclerosis and cardiovascular diseases [**10**]. S. officinalis is proven to be biologically active, and promising as antioxidant agent of natural origin [**5.11**]. The aim of this study was to determine the antioxidant potential, of extracts obtained from material of green tea Camellia sinensis. and plants Salvia officinalis L. Thyme (Thymus vulgaris L.) originated from continental part of west Libya Aljabal al Alakhdar.

Materials and Method

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. All other chemicals used were of analytical grade

Preparation extract- green tea from local market and Plants samples were collected from Aljabal Alakhdar area. The plant dried and powdered using a grinder. The extraction was done at room temperature. about 100 g of dried, ground plant materials were soaked in methanol (1 L of 98%) for 5-7 days separately. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40 o C and stored at 4 o C for further use. The stock solution of crude extracts (5 mg/ml) was prepared by dissolving a known amount of dry extract in 98% methanol. The working solutions (1, 2, 4, 6, 8, 10, 15, 25, 50, 75, 100, 250, 500 and 750 µg/ml) of the extracts were prepared from the stock solution using suitable dilution.

Antioxidant activity (DPPH free radical scavenging activity) of methanolic extract The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method [**12**]. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below [**13**]:

The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100. Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample.

Results and Discussion

Natural antioxidants that are present in samples are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present paper, we have evaluated

The free radical scavenger activity of methanolic extract of green tea leaves (*Camellia sinensis L.*), and (*Thymus vulgaris and Salvia officinalis L.*)

Among the three extracts and standard tested for the in vitro antioxidant activity using the DPPH method, the crude methanolic extracts of green tea, (*Camellia sinensis L.*) Thyme (*Thymus vulgaris and Salvia officinalis L.*), and ascorbic acid. Showed antioxidant activity, with IC_{50} values of 7.4 ± 0.1, 14.9 ± 0.2, 45.9 ± 0.2, 9.8 ± 0.1, µg/ml, respectively (Table). *Salvia officinalis* showed weak antioxidant activity, with IC_{50} values of 45.7 ±0.1

The IC_{50} value for ascorbic acid was 9.8 ± 0.1 µg/ml. The results indicate that the antioxidant activity of the crude extract of green tea is higher than that of ascorbic acid. The antioxidant of (*Thymus vulgaris and Salvia officinalis L.*) Was less active than ascorbic acid since their IC50 values were found to be higher when compared to ascorbic acid..

Table antioxidant activity of methanolic extract of samples

Test compound (methanolic extract)	IC_{50} (µg/ml) (Mean ± SD)	Weight of extract (g %, w/w)
green tea Camellia sinensis,	7.4±0.1	14.6
Thyme (Thymus vulgaris L.)	14.9±0.2	8.8
Salvia officinalis	45.7±0.1	11.5
Ascorbic acid	9.8±1	-

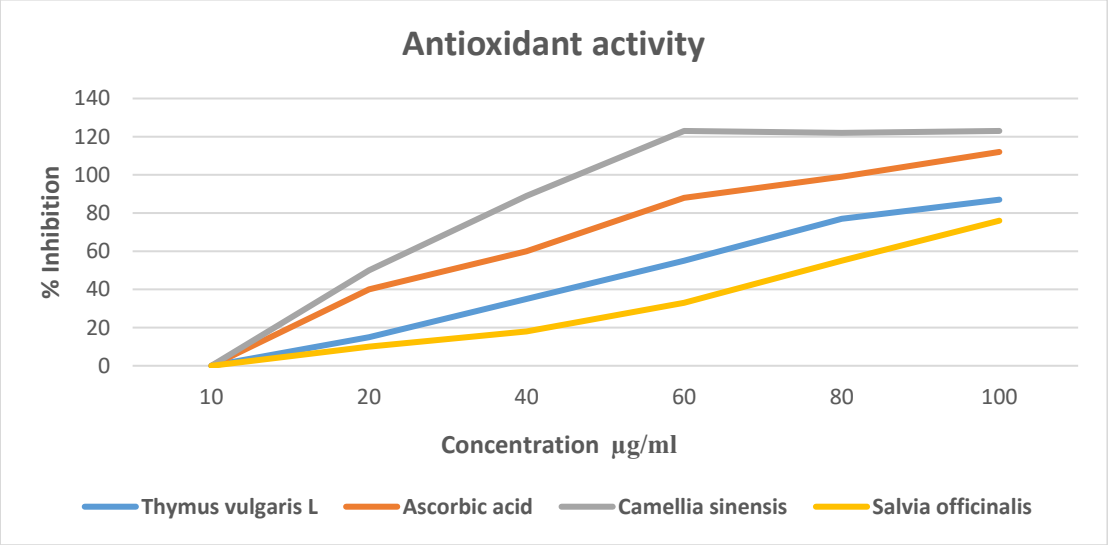

Figure: DPPH free radical scavenging activity of standard ascorbic acid and methanolic extract

However, the chemical constituents present in the extract, which are responsible for this activity, need to be investigated, and it is obvious that the constituents like tannins, reducing sugars and proteins present in the extract may be responsible for such activity. The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the crude methanolic extract. Several of such compounds are known to possess potent antioxidant activity [**14**]. We can conclude oral consumption of these compounds using natural antioxidant products have a positive impact on human health; therefore, we recommend their regular usage as part of the daily diet [**15**]. Some of these constituents have already been isolated from this plant. Hence, the observed antioxidant activity may be due to the presence of any of these constituents. The plant exhibited strong anticancer, hepato-protective, antiviral and several other activities. These properties may be due to its antioxidant activity. The crude methanolic extract merits further experiments in vivo.

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