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Research Article

ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF SELECTED MEDICINAL PLANTS OF *LAMIACEAE* FAMILY

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ABSTRACT

This research evaluated antioxidant and anti-inflammatory activity in a methanol extract of four medicinal plants of *Lamiaceae* family. Folin–Ciocalteu reagent assay was used to estimate the phenolic contents of extracts. The antioxidant activities of the samples were measured by two different methods (DPPH and ABTS) while the anti-inflammatory activity was studied by LOX assay. *Thymus vulgaris* extract showed the highest total phenolic content followed by *Ocimum canum, Ocimum adscendens* and *Leucas linifolia* respectively. The strongest antioxidant scavenging activity was shown in *Ocimum canum* extract by DPPH assay while the highest antioxidant capacity was found in *Thymus vulgaris* extract by ABTS assay. *Thymus vulgaris* extract showed the highest anti-inflammatory activity followed by *Ocimum canum, Leucas linifolia* and *Ocimum adscendens* respectively.

Keyword: Antioxidant, Anti-inflammatory, Phenolic compound, Radical scavenging

INTRODUCTION

The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000–5000 B.C. and Chinese used first the natural herbal preparations as medicines. In India, however, earliest references of use of plants as medicine appear in Rigveda, which is said to be written between 1600-3500 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians in Ayurveda (an indigenous system of medicine) which is a basic foundation of ancient medical science in India[1]. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%[2]. It is estimated that there are over 7800 medicinal drug-manufacturing units in India, which consume about 2000 tonnes of herbs annually. Several investigations have shown that many of these plants have antioxidant activities that could be therapeutically beneficial and it has been mentioned that the antioxidant potential of plants might be due to their phenolic components[3,4].

Different forms of free radicals such as nitric oxide and the alkoxy radicals were produced through aerobic respirations and increase the risk of chronic diseases. Application of a healthy diet including edible antioxidants can help human body to neutralize these free radicals and reduce the oxidative stress damages. Plants are good source of edible antioxidants such as ascorbic acid, tocopherols, carotenoids and several phenolic compounds[5]. Flavonoids, a group of polyphenolic compounds with known properties, such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and anti-inflammatory action have been isolated from plants[4,6]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant[7].

In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids which are capable of producing definite physiological action on body[8].The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in different ailments are their safety besides being economical, effective and their easy availability Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day-to-day practice[1]. Among all plant secondary metabolites which act as antioxidants phenolic compounds form a large and varied group. Phenolic compounds contribute significantly to the antioxidant potential of several plant species. A positive linear correlation between total phenolic contents and antioxidant activities for aqueous and methanol extracts of different Chinese medicinal plants and different Jordanian plant species was shown[5].

Most people especially in rural areas depend on herbal medicines to treat many diseases including inflammation-related ailments such as rheumatism, muscle swelling, cut wound, accidental bone fracture, insect bites, pains and burn by fire and hot water[9]. Plants containing polysaccharides are the most potent in curing inflammatory diseases[10].

In the present study, we selected 4 different medicinal plants of *Lamiaceae* family. The *Lamiaceae* family (*Labiatae*) or the mint family is one of the largest and most distinctive families of flowering plants, with about 236 genera and almost 7200 species worldwide[11]. We focused on preliminary screening of some wild medicinal plants species of *Lamiaceae* such as *Ocimum canum, Ocimum adscendens, Thymus vulgaris and Leucas linifolia* for their bioactive potential specially antioxidant and anti-inflammatory potential. Assays that can be used for antioxidant capacity are: DPPH, ABTS, FRAP (Ferric reducing antioxidant power), Lipid peroxidation, SOD (superoxide dismutase) and ROS (Reactive oxygen species). In this research we used DPPH, ABTS and LOX assay for measuring antioxidant and anti-inflammatory activity.

MATERIALS AND METHODS

Chemicals

1, 1-diphenyl, 2-picrylhydrazyl radical (DPPH), 2, 2-Azinobis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma, USA. Lipoxygenases enzyme and linoleic acid were purchased from Himedia, Mumbai, India. Ascorbic acid, methanol, ethyl acetate, DMSO and hexane were purchased from Fisher Scientific Bangalore India.

Collection of plant samples

Plants belonging to *Lamiaceae* family were identified based on taxonomical parameters and collected. Whole plant was washed in tap water and dried under shade.

Extraction of compounds from plant material

The dried plants were ground to fine powder and extracted in sonication apparatus with methanol. Methanol extract was dried under fume hood and used for further studies.

In vitro bioassays on crude extracts

Total Phenolic Content

Estimation of the total phenolic contents of the different **e**xtracts was determined using the Folin-Ciocalteu reagent method[12].To 50 µl **o**f each extract, 2.5 ml of Folin-Ciocalteu reagent(1/10 dilution) and 2 ml of 7.5% Na_2CO_3 (w/v) was added and mixed well. The blend was incubated at 45°C for 15 min. The absorbance of all

samples was measured at 765 nm withNa_2CO_3 solution (2 ml of 7.5% Na_2CO_3 in 2.55 ml of distilled water) as blank. The results were expressed as gallic acid equivalence (GAE) in micrograms.

Antioxidant assay

The antioxidant potential of *Lamiaceae* plants was tested by DPPH and ABTS free radical scavenging assay

DPPH assay

Antioxidant scavenging activity was studied by using 1,1-diphenyl, 2- picrylhydrazyl free radical (DPPH)[13]. Various concentrations of test solutions in 5µl are added to 95 µl of 0.3 mM solution of DPPH in methanol. Methanol was used as experimental control. After 30 minutes of incubation at room temperature, the reduction in the number of free radical was measured reading the absorbance at 517nm. Due to the presence of an odd electron it gives a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured.

Ascorbic acid was used as reference standard. The percent inhibition is calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample) / Absorbance control] x100

ABTS assay

ABTS radical cations are produced by reacting ABTS (7mM) and potassium persulfate (2.45mM) on incubating the mixture at room temperature in dark for 16 hours. The solution thus obtained was further diluted with methanol to give an absorbance of 0.700. Different concentrations of the test sample in 10µl are added to 990 µl of ABTS working solution. The absorbance was recorded immediately at 734 nm. Trolox is used as reference standard[14].

The $ABTS^{\bullet+}$ scavenging capacity of extract compared with Trolox and percentage inhibition was calculated as ABTS radical scavenging activity.

Radical scavenging (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$

Where $Abs_{control}$ is the absorbance of ABTS free radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract/standard.

Anti- inflammatory assay

Inhibition of lipoxygenases (LOX) enzyme, lipoxygenase from soybeans was used for peroxidation of linoleic acid, and inhibition was carried out as described by Lyckander and Malterud[15]. To a solution of linoleic acid (final concentration 134 µM) in borate buffer (0.2 M, pH 9.00), 5 µl of test substance dissolved in DMSO or DMSO alone (for blanks) was added and mixed with lipoxygenases solution in borate buffer (200 U/ml) and the increase in absorbance at 234 nm for 30–90 seconds was measured. Quercetin is used as positive control. The percentage inhibition of enzyme activity was calculated as;

$$(\%) = [(A_1 - A_2)/ A_1] \times 100$$

Where A_1 and A_2 are values for increase in absorbance at 234 for sample without test substance and with test substance, respectively.

RESULTS

Total Phenolic Content

Total phenolic content of the four selected plants was measured using the Folin-Ciocalteu method, and the results are shown [Figure 1]. As seen, the total phenolic content of these plants ranged from 62 to 350 µg GAE/ml. *Thymus vulgaris* showed the highest phenolic content, followed by *Ocimum canum, Ocimum adscendes* and *Leucas linifolia* showed the lowest phenolic content of these plants.

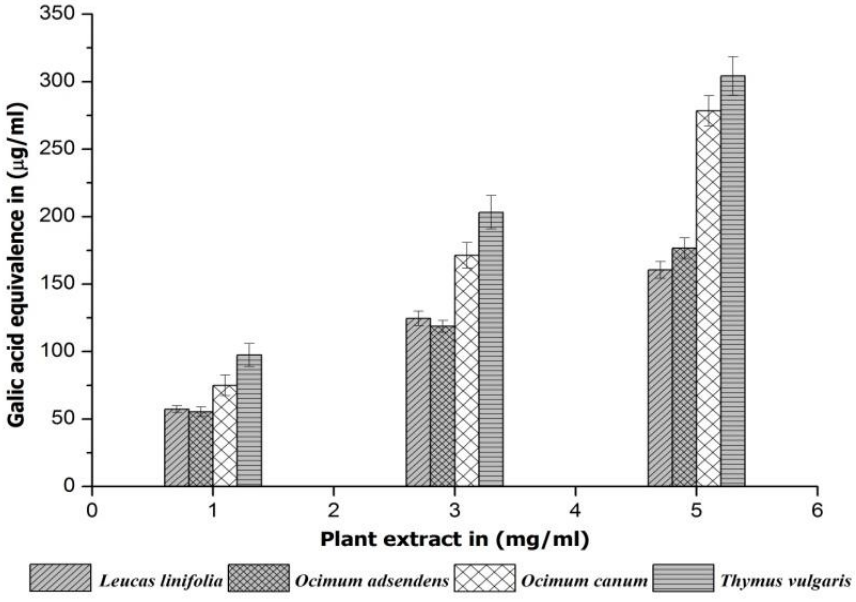

Fig. 1: Total phenolic content of methanol crude extracts of four different plants from *labiatae* family

DPPH assay

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to discolor in the presence of antioxidants.The antioxidant activity of the plant extracts and the standard (Ascorbic acid) was assessed based on the radical scavenging of effect of the stable DPPH free radical. DPPH is a stable free radical that shows a maximum absorption at 517 nm. When DPPH encounters proton donating substances such as an antioxidant and a radical species, the absorbance at 517 nm disappears because the DPPH radical is scavenged. Based on this principle, the radical scavenging effect of each extraction was measured and the results are presented in [Figure 2]. The strongest activity was shown in *Ocimum canum* in comparison to *Thymus vulgaris, O. adscendes* and *L. Linifolia*.Total antioxidant capacity of the extracts is expressed as the number of equivalents of ascorbic acid. The antioxidant activity of *O. Canum* was approximately 65%, *Thymus vulgaris* 62%, *L. Linifolia* 58% and *O. adscendes* 35%.

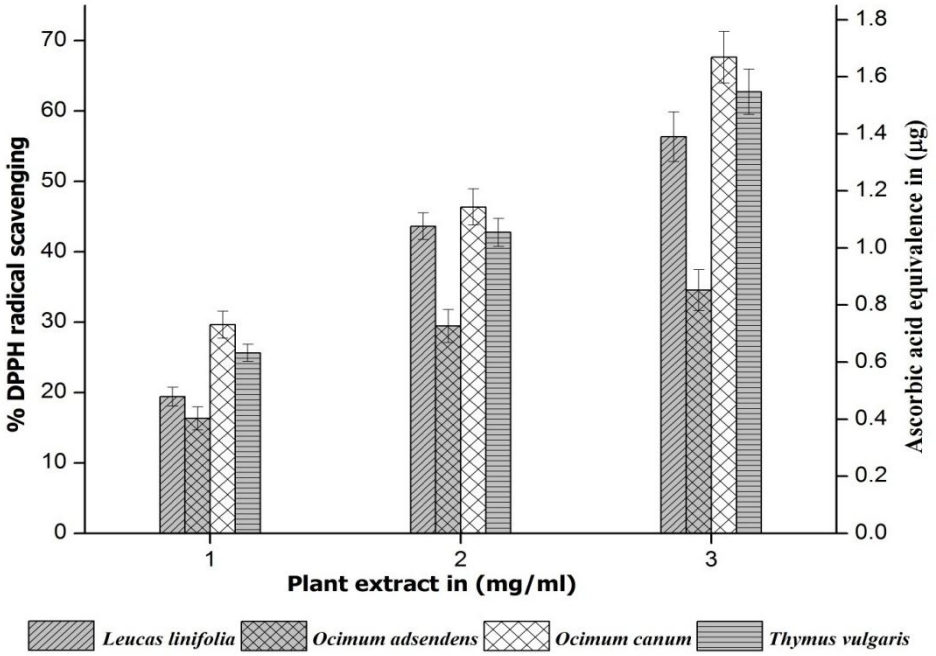

Fig. 2: Free radical scavenging activity by DPPH for methanol crude extracts

ABTS assay

ABTS$^{\bullet+}$ radicals are widely used to screen antioxidant activity of fruits, vegetables, foods and plants and is applicable to both lipophilic and hydrophilic antioxidants. In particular, it is recommended to be used for plant extracts because the long wavelength absorption maximum at 734 nm eliminates color interference in plant extracts. In this study, antioxidant capacities of the methanol extracts from four selected medicinal plants were measured by using Trolox equivalence as shown in [Figure 3].

Thymus vulgaris showed the highest antioxidant capacity, followed by, *Ocimum canum and Ocimum adscendens. Leucas linifolia* exhibited the lowest antioxidant capacity.

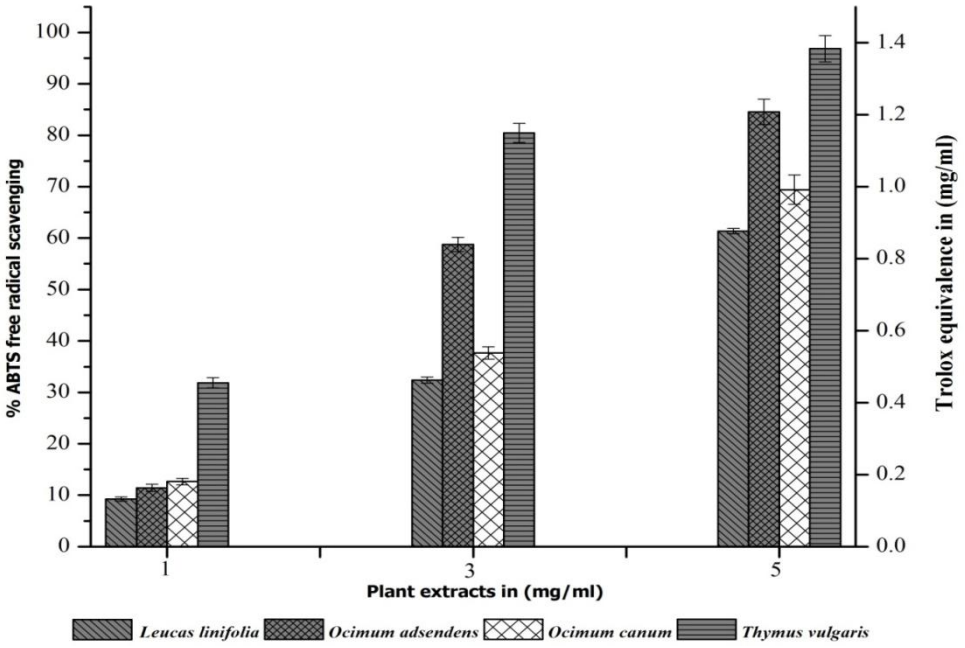

Fig. 3: ABTS free radical scavenging activity for methanol crude extracts

LOX assay

Three different levels of activity have been determined. An inhibition below 40% is considered to be low or insignificant at the dose tested. Samples which showed inhibition between 40% and 70% is regarded to be moderate while inhibition above 70% is expressed as high. The results obtained in this study [Figure 4] indicate a difference in anti-inflammatory activity between extracts. The methanol crude extracts of different plants from *Labiatae* family displayed low and moderate activity. *Thymus vulgaris* showed the highest anti-inflammatory activity with 62%inhibition, followed by, *Ocimum canum* and *Leucas linifolia* with 32 and 34% inhibition, respectively. *Ocimum adscendens* exhibited the lowest anti-inflammatory activity with 30% inhibition.

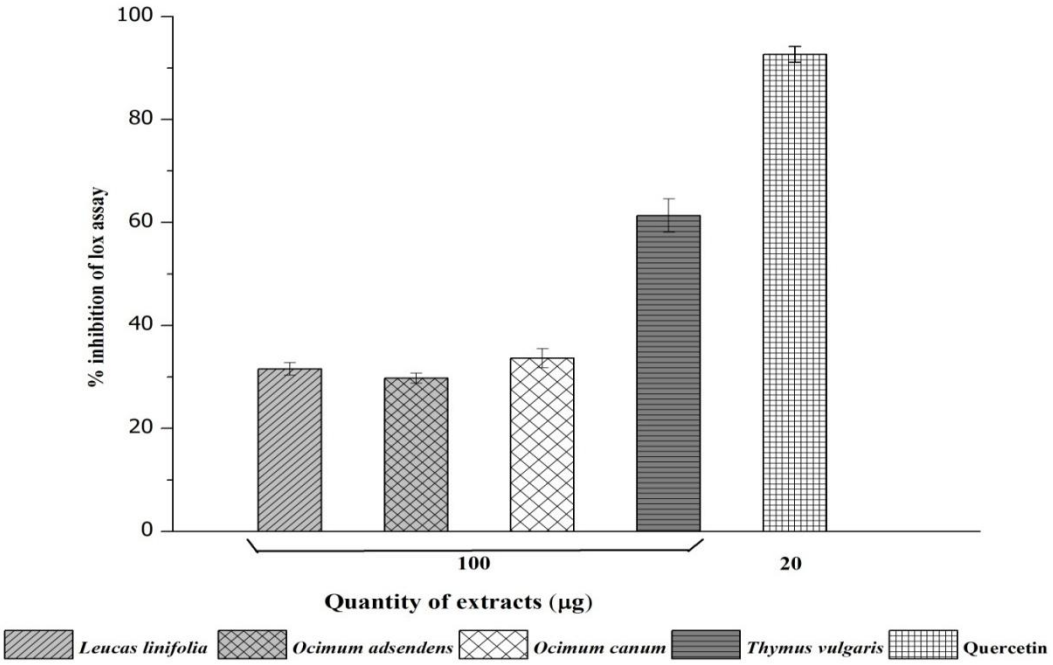

Fig. 4: Inhibition of soybean 15-lipoxygenase by methanol crude extract

DISCUSSION

Ocimum canum (Hoary basil) is an indigenous plant, widely available in India and other tropical and sub-tropical areas of the world. Different parts of the plant e.g. leaves, flower, seed, oil and etc are reported to have significant pharmacological properties. Though various preparations of other species of the same genus have use in traditional medicine and for treatment of variety of ailments, very few reports are available regarding the anti-inflammatory activity of *Ocimum canum*[16].

It is worth noting that the total phenol content of *O. canum* was higher than that of the total phenol content of broccoli, spinach, onion, carrot, cabbage, potato, lettuce, celery and cucumber[17]and as well some commonly consumed green leafy vegetables in Nigeria[18]and green and hot red pepper[19]. In addition, the total phenol content of *O. canum* was higher than that of some commonly consumed fruit (apple, red grape, strawberry, peach, lemon, pear, banana, orange, grapefruit and pineapple)[20]. The present study shows that the total phenol content of *Thymus vulgaris* is higher than *Ocimum canum.*

Thymus vulgaris is an aromatic plant belonging to the *Lamiaceae* family, used for medicinal and spice purposes almost everywhere in the world. In Romania, *Thymus* genus contains one cultivated species as aromatic plant (*Thymus vulgaris*) and other 18 wild species. *Thymus vulgaris* shows a polymorphic variation in monoterpene production, the presence of intraspecific chemotype variation being common in the genus *Thymus*. Each of the six chemotypes, geraniol (G), α-terpineol (A), thuyanol-4 (U), linalool (L), carvacrol (C), and thymol (T), is named after its dominant monoterpene. Many pharmacological *in vitro* experiments carried out during the last decade revealed well defined pharmacological activities of both, the thyme essential oil and the plant extracts. The non-medicinal use of thyme is worthy of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect. Thyme essential oil constitutes raw material in perfumery and cosmetics due to a special and characteristic aroma[21].

In the present study on comparing the total phenol content, ABTS assay and Lox assay on all four chosen species for study, *T. vulgaris* reveals the highest antioxidant and anti-inflammatory activity.

The *Lamiaceae* family demonstrates antioxidant and anti-inflammatory activity. It shows they have pharmacological properties.

In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation. The present study supports the studied plant and suggests that the plant extract possesses certain constituents with antioxidant and anti-inflammatory properties can be used for treatment of diseases such as aging, cancer and atherosclerosis and Cardiovascular disease.

Ocimum adscendens and *Leucas linifolia,* both are belonging to *labiatae* family. There is no information available about medicinal properties of these plants. In this project work, I have done some assays for checking medicinal property. These plants showed moderate antioxidant and anti-inflammatory activity compared to other plants.

The methanol extract of *O. canum* was used for TLC purification and after checking different mobile phase the solvent system hexane and ethyl acetate (7:3) was selected for extraction. TLC results shows that there are more than eight bands more of which are polar that are synergistically giving the antioxidant property.

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