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# INVESTIGATION OF ANTIOXIDANT POTENTIAL OF AN ETHANOLIC EXTRACT OF WHOLE PLANT OF *LEONOTIS NEPETIFOLIA* (L). R.BR.

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## ABSTRACT

In this study antioxidant activity was performed by hydroxyl radicals, DPPH and nitric oxide radical scavenging method for an ethanolic extract of whole plant of *Leonotis nepetifolia* (L). R.Br. An extract was used at different concentrations like 1.25, 2.5, 5.0, 10.0, 20.0 µg/ml and radical scavenging activity was determined in terms of percentage inhibition and $IC_{50}$ values were calculated. The study proved that an ethanolic extract of *Leonotis nepetifolia* found to have highest antioxidant activity against nitric oxide radical (% inhibition = 77.07 ± 1.77; $IC_{50}$ = 2.03 µg/ml) and moderate activity against DPPH radicals (% inhibition = 64.47 ± 1.42; $IC_{50}$ = 3.07 µg/ml) and minimum activity against hydroxyl radicals (% inhibition = 62.96 ± 1.78; $IC_{50}$ = 7.55 µg/ml) at concentration of 20 µg/ml. The strongest antioxidant activity of an ethanolic extract may be due to the presence of flavonoids. The further study has to be focused on isolation and characterization of functional molecules of the extract and their individual activity.

**KEYWORDS:** *Leonotis nepetifolia* (L). R.Br., *in vitro* antioxidant activity, free radicals.

## INTRODUCTION

Free radical-induced oxidative damage is involved in the pathogenesis of many chronic and degenerative diseases, such as cardiovascular disease, cancer, diabetes, neurodegenerative disease and ageing.[1-3] In recent years, much attention has been devoted to natural antioxidant and their association with health benefits.[4]

Consumption of antioxidant constituents reported to have protection against oxidative damage induced degenerative and pathological processes including ageing and cancer.[5]

Harmful effects resulted from the disequilibrium in the antioxidant-prooxidant balance that can be largely prevented by the intake of antioxidant substances.[6] Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease.[7]

Oxidation processes are intrinsic in the energy management of all living organisms and are therefore kept under strict control by several cellular mechanisms.[8]

Antioxidant properties have been studied in several plant species for the development of natural antioxidant formulations in the areas of food, medicine and cosmetics.[9] Health promoting effects of antioxidants in plants have been well documented. Antioxidants work by donating an electron to a molecule that has been compromised by oxidation, bringing it back into a state of proper function.[10]

The present study has therefore been designed to determine the antioxidant activity of *Leonotis nepetifolia* ( Fig.1) also known as Klip Dagga or Lion's Ear, is a species of plant in the *Leonotis* genus and the Lamiaceae family.

Phytochemical examination of this plant indicated the presence of alkaloids (leonurine and stachydrene), iridoid glycoside (leonuride), iridoid glycosides (leonurin and leonuridine), diterpenoids (leocardin), flavonoids (rutin, quercetin, hyperoside, apigenin), volatile oil, tannins and vitamin A. Medicinal uses of the plant are used in Madagasgar, Brazil, Canada, Kenya and many African countries to treat kidney disease, rheumatism, dysmenorrhoea, bronchial asthma, fever and diarrhoea.[11]

The drug has been reported to possess wound healing,[12] antibacterial,[13] antirheumatic,[14] anti-inflammatory,[15] analgesic and anticancer activities.[16]

**Fig.1. *Leonotis nepetifolia* (L). R.Br**

## MATERIALS AND METHODS

### Plant material

The whole plant of *Leonotis nepetifolia* (L). R.Br was collected from the Tirunelveli, Tamilnadu in the month of December 2014. The plant was authenticated by the Dr. V. Chelladurai, M.Sc., Ph.D., Research officer botanist (Scientist - C), Central council for Research in Ayurveda and Siddha, Govt. of India (Retired), Tirunelveli, Tamilnadu, India. The plant material was deposited in our department (Specimen No. SVCP/2014/07). The whole plant material were dried in shade and pulverized. The powder were passed through sieve no. 40 and used for the extraction.

### Preparation of extract

The shade dried whole plant materials were powdered separately by using grinder except leaflets which were manually grinded and defatted with petroleum ether separately. Defatted 500gm of powder was extracted by 95% ethanol in a Soxhlet apparatus for 72h followed by concentrated in rotary evaporator under reduced pressure at temperature 40-50$^0$C and then lyophilized to get a dry residue. The extract was stored in desiccators till further use. The percentage yields of extracts were calculated with reference to air dried powder.[17]

### *In vitro* antioxidant activity

### Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of an ethanolic extract of whole plant of *Leonotis nepetifolia* (L). R.Br was measured according to the method of Kunchandy E et al.,[18] The reaction mixture contains (1.0 ml) 100 µl 2-deoxy-2-ribose (28mM in 20 mM phosphate buffer, pH7.4) different concentrations of extracts (1.25-20µg/ml) in phosphate buffer (20mM, pH 7.4), 200µl 1.04mM EDTA and 200µM $FeCl_3$ (1:1 v/v), 100µl 1.0 mM $H_2O_2$ was incubated at 37$^0$C for an hour. 1ml of 1% TBA and 1.0ml of 2.8% TCA were added and incubated at 100$^0$C for 20 min. After cooling absorbance was measured at 532nm against the control preparation containing deoxyribose and buffer. Reactions were carried out in triplicate. Percentage inhibition was determined by comparing the results of the test and control samples.

### DPPH radical scavenging activity

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of an ethanolic extract of *Leonotis nepetifolia* (L). R.Br.[19] 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol. Different volumes (1.25-20µg/ml) of plant extract were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control. The experiment was repeated in triplicate. $IC_{50}$ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

### Nitric oxide scavenging activity

Nitric oxide scavenging activity of an ethanolic extract of whole plant of *Leonotis nepetifolia* was measured spectrophotometrically.[20] Sodium nitroprusside (10mM), in phosphate-buffered saline, was mixed with different concentrations of extracts (1.25-20µg/ml) dissolved in water and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The experiment was repeated in triplicate.

## RESULTS

The results showed an ethanolic extract of whole plant of *Leonotis nepetifolia* (L). R.Br., is found to have free radical scavenging potential against various radical systems like hydroxyl radical, DPPH and nitric oxide radical. An ethanolic extract at 1.25, 2.5, 5.0, 10.0, 20.0 µg/ml concentrations were used against all the radicals. The radical scavenging activity was expressed in terms of percentage inhibition in the methodological as mentioned part. The increasing concentration of test extract increases the percentage inhibition. The percentage inhibition and $IC_{50}$ values were calculated and presented in the Table. 1 and 2 respectively. The

results showed that an ethanolic extract of *Leonotis nepetifolia* is having highest activity against nitric oxide radical (2.03 µg/ml) and followed by DPPH radicals (3.07 µg/ml) and hydroxyl radicals (7.55 µg/ml).

**Table 1. Percentage inhibition of an ethanolic extract of whole plant of *Leonotis nepetifolia* (L). R.Br. on different radical systems**.

| Ethanolic extract Concentration(µg/ml) | Free radical scavenging activity (% inhibition) | | |
|---|---|---|---|
| | Hydroxyl radical | DPPH radical | Nitric oxide radical |
| 1.25 | 20.48 ± 0.66 | 41.37 ± 1.17 | 42.88 ± 1.06 |
| 2.5 | 36.31 ± 1.26 | 48.03 ± 1.03 | 54.24 ± 1.35 |
| 5.0 | 46.62 ± 1.34 | 52.77 ± 1.31 | 62.95 ± 1.20 |
| 10 | 55.89 ± 1.68 | 58.70 ± 1.53 | 72.11 ± 1.50 |
| 20 | 62.96 ± 1.78 | 64.47 ± 1.42 | 77.07 ± 1.77 |

(Values are expressed as Mean ± SEM, n=3)

**Table. 2. $IC_{50}$ values of an ethanolic extract of whole plant of *Leonotis nepetifolia* (L). R.Br. on different radical systems.**

| S.No. | Assay methods | $IC_{50}$ values [Extract Con. (µg/ml)] |
|---|---|---|
| 1. | Hydroxyl radical scavenging activity | 7.55 |
| 2. | DPPH radical scavenging activity | 3.07 |
| 3. | Nitric oxide scavenging activity | 2.03 |

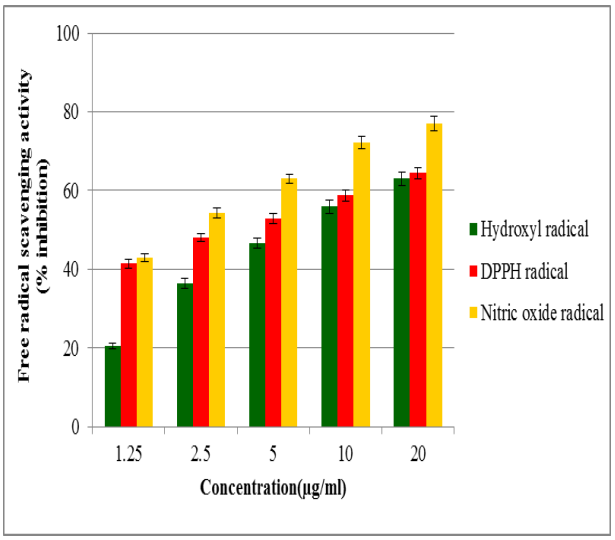


**Fig. 1. Comparative analysis of free radical scavenging activity of an ethanolic extract of whole plant of *Leonotis nepetifolia* (L). R.Br. at different concentration.**

## DISCUSSION

Free radicals are chemical entities that can subsist separately with one or more unpaired electrons. The generation of free radical can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals.

The free radicals are involved in various acute and chronic diseases including cancer, atherosclerosis, ageing etc.,[21] In the present study, ethanolic extract of *Leonotis nepetifolia* (L). R.Br was studied for *in vitro* antioxidant activity by hydroxyl radical, DPPH and nitric oxide radical scavenging methods which are summarized in Table 1.

Hydroxyl radical is one of the most detrimental free radicals formed in biological systems and causes enormous damage on bio molecules of the living cells.[22] In course of the Fenton reaction, hydroxyl radicals are formed that cause 2-deoxy-2-ribose damage and generate malondialdehyde (MDA) like product. As an ethanolic extract of *Leonotis nepetifolia* is added to the reaction mixture the hydroxyl radicals are scavenged and thereby sugar damage can be blocked. The results indicate that the plant extract having hydroxyl radical scavenging activity.

DPPH has been used to evaluate the free radical scavenging activity of natural antioxidants. DPPH which is a radical itself with a purple colour, changes into a stable compound with a yellow colour by reacting with an antioxidant and the extent of the reaction depends on the hydrogen donating ability of the antioxidant.[23] The ability of an ethanolic extract of *Leonotis nepetifolia* scavenge DPPH radicals suggests that it is an electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reactions.

Nitric oxide is produced by several different types of cells, including endothelial cells and macrophages. The chronic emergence of nitric oxide radical is linked with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis.[24] Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radicals. In the present study, an ethanolic extract of *Leonotis nepetifolia* showed better activity in competing with oxygen to react with nitric oxide and thus the inhibition of generation of anions. The

activity of the extract is found to be varying according to the nature of radicals. The reason may be due to different phytomolecules that are present in the crude extract acts on different way against the radicals.

## CONCLUSION

Based on the results of the present study, we conclude that an ethanolic extract of *Leonotis nepetifolia* (L). R.Br exhibits the better *in vitro* antioxidant effect. However, further investigation of individual compounds, there *in vivo* antioxidant activities and in different antioxidant mechanisms is needed.

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