# Antioxidant Studies on Ethanol Extracts from Two Selected Genera of Indian *Lamiaceae*

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**The present work is targeted to evaluate antioxidant activity of ethanol extracts from the leaves of *Plectranthus mollis* and *Salvia officinalis* belonging to family Lamiaceae using nitric oxide scavenging, hydrogen peroxide scavenging, ferric reducing antioxidant power assay and lipid peroxidation methods. The results of the study indicate that the leaf extracts of both the plants possess *in vitro* antioxidant activity. The higher amount of flavanoids and phenolic compounds may correspond to their greater antioxidant activity.**

**Key words: *Plectranthus mollis*, *Salvia officinalis*, ethanol extract, antioxidant activity**

Antioxidants slow down the process of excess oxidation and protect cells from the damage caused by free radicals. When the cells are attacked by free radicals, excess oxidation occurs, which damage and destroy cells. Antioxidants stop this process. The cellular damage caused by free radicals can be responsible for causing or accelerating many diseases[1,2], *Plectranthus mollis* (*P. mollis*) and *Salvia officinalis* (*S. officinalis*) belonging to family Lamiaceae are rich in antioxidants like phenols and flavonoids, which were reported in our earlier studies[3], and so they can be recommended to guard against free radicals and protect from damaging excess oxidation.

In our earlier studies, we have reported flavonoids like quercetin, caffeic acid and luteolin, cinnamic derivatives like chlorogenic acid, triterpenoids and steroids like β-sitosterol and β-amirin by phytochemical screening and thin layer chromatography in both the plants. The total phenolics and flavonoid content showed a positive correlation with total antioxidant activity. Several ethnomedicinal studies have shown that *Plectranthus mollis* is used as a febrifuge[4], as a cure for haemorrahage, as a cardiac depressant, as a smooth muscle and skeletal muscle relaxant and in the treatment of mental retardation[5]. *Plectranthus mollis* have cytotoxic and antitumour promoting activity and can be used in the treatment of cancer[6]. *Salvia officinalis* L is native to Mediterranean region and is commonly known as sage. The infusion and decoction of the leaves have been used as nerve tonic, digestive, antispasmodic and antiinflammatory in Indian traditional medicine[7]. *Salvia officinalis* contains tannic acid, rosmarinic acid, chlorogenic acid, caffeic acid, steroids, flavones and flavonoid glycosides[8]. Hence the present research has focussed to evaluate their *in vitro* antioxidant potential of ethanol extracts of leaves.

The collection and authentication of plant materials, methods of extraction and phytochemical screening were already described in our earlier studies[3]. Scavenging activity of nitric oxide[9] by the extracts and standard were determined by the method of Jaishree *et al*. A solution of sodium nitroprusside (10 mM) prepared in phosphate buffered saline (PBS, pH 7.4) and the test samples at various concentrations (25, 50, 75 and 100 µg/ml) was incubated at 25° for 150 min. After incubation, 0.5 ml of Griess reagent (1% w/v),

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sulfonilamide (2% v/v), orthophosphoric acid (2% v/v) and 1 ml naphthylethylene diamine dihydrochloride (0.1% w/v) was added. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric acid, which reacts with oxygen to produce nitrite ions, which can be estimated at 540 nm. Quercetin was used as reference standard in this study. Scavenging activity of hydrogen peroxide[10] by the extracts and standard were determined by the method of Ruch *et al.* The plant extracts (1 ml) prepared in methanol at different concentrations (25, 50, 75 and 100 µg/ml) was mixed with 2 ml of hydrogen peroxide solution prepared in phosphate buffered saline (0.1 M, pH 7.4) and incubated for 10 min. The absorbance was measured at 230 nm. Rutin was used as reference standard in this study. Lipid peroxidation assay[11] of extracts and standard were determined by the method of Dhu *et al*. The plant extracts (0.1 ml) prepared in DMSO at various concentrations (25, 50, 75 and 100 µg/ml) were added to 1 ml of egg lectin. Lipid peroxidation was induced by adding 0.02 ml of ferric chloride and 0.02 ml of ascorbic acid. After incubation for 1 h at 37°, 2 ml of 15% TCA and 2 ml of 75% TBA were added and the reaction mixture was boiled for 15 min. Then cooled, centrifuged and absorbance of supernatant was measured at 532 nm. Gallic acid was used as reference standard in this study. Ferric reducing antioxidant power assay[12] was carried out by the method of Kuda *et al.* The extracts (1 ml) prepared in dimethyl formamide (DMF) at different concentrations (25, 50, 75 and 100 µg/ml) were mixed with 2.5 ml of phosphate buffer (pH 7.4) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50° for 20 min. After that, 2.5 ml of trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1% w/v). The absorbance was measured at 700 nm. Ascorbic acid was used as reference standard in this study. Increased absorbance of the reaction mixture indicates stronger reducing power.

The nitric oxide radical scavenging activity of extracts and standard were presented in Table 1. Nitric oxide is a potent molecule required for several physiological processes such as smooth muscle relaxation, inhibition of platelet aggregation, neuronal signaling, vasodilation immune response and blood pressure[13]. However, higher concentration of nitric oxide may result in several physiological conditions including cancer and inflammation[14]. Both the extracts showed significant inhibition of NO with $IC_{50}$ values of 37.435±0.246 µg/ml for *P. mollis* and 42.886±0.307 µg/ml for *S. officinalis* whereas the $IC_{50}$ value of quercetin was observed 22.704±0.635. The observed activity may be due to the chemical constituents present in the plant. Hydrogen peroxide is an reactive oxygen species generated *in vivo* by oxidase enzyme like superoxide dismutase. It is a strong oxidizing agent but either directly or indirectly via its reduction product hydroxyl radical causes severe damage to biological systems. In the present study, it was found that the ethanol extracts of *P. mollis* and *S. officinalis* were capable of scavenging hydrogen peroxide in a concentration-dependent manner, which can be attributed to their phenolic content that donated electrons to hydrogen peroxide thus reducing it to water. The results are shown in Table 2. The $IC_{50}$ value of *P. mollis* 23.736±0.327 µg/ml was better than that of *S. offinalis* 29.874±0.391 µg/ml but

**TABLE 1: NITRIC OXIDE SCAVENGING ACTIVITY**

| Samples | Percentage inhibition (µg) | | | | $IC_{50}$ (µg) |
|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | |
| Control | 0.9395 (absorbance) | | | | |
| Quercetin | 95.900±0.131 | 66.303±1.200 | 51.557±0.405 | 43.727±0.265 | 22.704±0.635 |
| Ethanol extract of *Plectranthus mollis* | 89.897±0.137 | 57.973±0.070 | 42.093±0.250 | 34.103±0.263 | 37.435±0.246 |
| Ethanol extract of *Salvia officinalis* | 85.783±0.570 | 54.627±0.110 | 38.413±0.455 | 31.180±0.508 | 42.886±0.307 |

Each value represents mean value±SD of triplicate samples analysis, SD: standard deviation

**TABLE 2: HYDROGEN PEROXIDE SCAVENGING ACTIVITY**

| Samples | Percentage inhibition (µg) | | | | $IC_{50}$ (µg) |
|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | |
| Control | 0.9395 (absorbance) | | | | |
| Rutin | 91.907±0.111 | 67.033±0.436 | 54.597±0.674 | 48.373±0.793 | 15.746±1.524 |
| Ethanol extract of *Salvia officinalis* | 86.827±0.095 | 60.500±0.108 | 47.440±0.225 | 40.920±0.279 | 29.874±0.391 |
| Ethanol extract of *Plectranthus mollis* | 88.890±0.141 | 63.560±0.046 | 50.590±0.221 | 44.223±0.205 | 23.736±0.327 |

Each value represents mean value±SD of triplicate samples analysis, SD: standard deviation

**TABLE 3: LIPID PEROXIDATION ACTIVITY**

| Samples | Percentage inhibition (µg) (*n*=3, mean±SD) | | | | $IC_{50}$ (µg) |
|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | |
| Control | 0.8703 (absorbance) | | | | |
| Gallic acid | 89.603±0.311 | 64.753±0.221 | 52.567±0.649 | 46.043±1.048 | 20.177±1.529 |
| Ethanol extract of *Plectranthus mollis* | 84.127±0.526 | 57.953±0.210 | 45.670±0.190 | 38.183±0.170 | 34.422±0.027 |
| Ethanol extract of *Salvia officinalis* | 81.203±0.735 | 55.207±0.351 | 42.380±0.390 | 35.970±0.542 | 39.740±0.794 |

Each value represents mean value±SD of triplicate samples analysis, SD: standard deviation

**TABLE 4: FERRIC REDUCING POWER**

| Samples | Percentage inhibition (µg) (*n*=3, mean±SD) | | | | $IC_{50}$ (µg) |
|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | |
| Control | 0.1982 (absorbance) | | | | |
| Ascorbic acid | 86.630±0.481 | 64.396±0.379 | 53.313±0.354 | 47.746±0.480 | 17.569±0.917 |
| Ethanol extract of *Plectranthus mollis* | 81.601±0.325 | 61.167±0.811 | 50.303±0.573 | 43.743±0.614 | 25.342±1.503 |
| Ethanol extract of *Salvia officinalis* | 78.120±0.608 | 57.349±0.354 | 45.879±0.304 | 39.674±0.657 | 34.841±1.070 |

Each value represents mean value±SD of triplicate samples analysis, SD: standard deviation

significantly lower than the value obtained for rutin, 15.746±1.524 µg/ml. Both the extracts showed anti-lipid peroxidation activities, which are less than gallic acid. The % antioxidant activity increased in concentration dependant manner. The results are shown in Table 3. The ferric reducing power of the extracts is a measure of the reductive ability of its antioxidants and it is evaluated by the transformation of $Fe^{3+}$ to $Fe^{2+}$ in the presence of sample extracts. The ferric reducing ability of both the ethanol extracts increased with increase in their concentrations, which were observed in a similar report[15] found in the case of methanol extract of *Cosmos caudatus*. The results are shown in Table 4. This activity may be due to the phenolic compounds in the plants, which would have acted as electron donors thereby reducing free radical generation. The results of the present study indicate that the leaf extracts of both the plants possess *in vitro* antioxidant activity. The higher amount of flavanoids and phenolic compounds may correspond to their greater antioxidant activity.

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There are no conflicts of interest.

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