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Evaluation of some Medicinal Plants for their Antioxidant Properties

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Abstract: The present investigation was undertaken to appraise the antioxidant properties and the total phenolic contents of 13 important medicinal plants. The herbal extracts of the plants were prepared with the solvents namely water and ethanol at 5 % concentration levels. Radical attenuating abilities of 13 plant extracts were ascertained by 2, 2-diphenyl 1-picryl hydrazyl (DPPH) radical scavenging assay. The DPPH scavenging potential of the aqueous extracts of the herbs ranged from 25%-98% whereas the ethanolic extracts of the medicinal plants showed the percentage inhibition in the range of 38 % -95%. The highest inhibition of DPPH radical was observed in the aqueous extract of *Hyptis suaveolens* leaf followed by *Alpina calcarata* leaf. Among the medicinal plants examined in ethanolic solvent system, *Ocimum basillicum* leaf exhibited greater antioxidant potential. *Ocimum basillicum* leaf, *Alpina calcarata* leaf, *Jatropa multifida* flower, *Hyptis suaveolens* leaf, *Solanum indicum* leaf and *Clitorria ternate* leaf & flower possessed higher DPPH radical scavenging activity in both the solvent systems. The total phenolic contents of herbs were also determined by Folin–Ciocalteu method. *Ocimum basillicum* (16.08 mg of catechol/g of plant tissue) and *Clitorria ternate* (10.64 mg of catechol/g of plant tissue) contained the maximum amount of phenols. The results of these findings revealed that the radical scavenging activity of medicinal plants may be due to the hydrogen donating ability of phenolics .
Key words: 2, 2-diphenyl 1-picryl hydrazyl, *Hyptis suaveolens, Alpina calcarata, Ocimum basillicum, Jatropa multifida,* Folin–Ciocalteu method.

1. INTRODUCTION

Medicinal plants play a pivotal role in the health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various human ailments because they contain the components of therapeutic value [1]. In addition, plant based drugs remain an important source of therapeutic agents because of the availability, relatively cheaper cost and non-toxic nature when compared to modern medicine [2]. Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species.

Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, carcinoma and parkinson's disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources [3].

Some of the *in vivo* free radicals play a positive role in phagocytosis, energy production and regulation of cell growth etc. However, free radicals may also be damaging. Free radicals produced in the body react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources [4].

Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals [5]. They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers [6].

Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases [7]. There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants. Hence, the present study was aimed at measuring the relative content of phenolics and antioxidant capacities of 13 important medicinal plants.

2. MATERIALS AND METHODS

2.1. Medicinal plants

The medicinal plants were obtained from the botanical gardens of Mount Carmel College, Bangalore. The plant materials were authenticated by the Botanists, Mount Carmel College, Bangalore. The following list of medicinal plants was used for the study.

2.2. Plant extract preparation

0.5 gram of the dried herbal powder was taken and dissolved in 10ml of water or ethanol. The solution was heated using a water bath maintained at 80 degree Centigrade for 15 minutes. The mixture was cooled at room temperature and centrifuged at 6000 rpm for 10 minutes. The supernatant solution was filtered and the filtrate was collected and used for the analysis.

2.3. Antioxidant assays

The antioxidant activity of plant material was assayed by employing the following methods described in 2.3.1.

2.3.1. DPPH radical scavenging assay [8].

DPPH (2, 2-diphenyl picryl hydrazyl) is a commercially available stable free radical, which is purple in colour. The antioxidant molecules present in the herbal extracts, when incubated, react with DPPH and convert it into di-phenyl hydrazine, which is yellow in colour. The degree of discoloration of purple to yellow was measured at 520 nm, which is a measure of scavenging potential of plant extracts. 10 µl of plant extract was added to 100 µl of DPPH solution (0.2mM DPPH in methanol) in a microtitre plate. The reaction mixture was incubated at 25 ^{0} C for 5 minutes, after that the absorbance was measured at 520 nm. The DPPH with corresponding solvents (without plant material) serves as the control. The methanol with respective plant extracts serves as blank. The DPPH radical scavenging activity of the plant extract was calculated as the percentage inhibition.

$$\% \text{ Inhibition of DPPH radical} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

2.3.2. Determination of total phenolics [9].

Phenols react with phosphomolybdic acid in Folin-ciocalteau reagent in alkaline medium and produce a blue colored complex (molybdenum blue) that can be estimated colorimetrically at 650 nm. Weighed exactly 0.5 to 1.0 g of the plant sample and ground it with a pestle and mortar in 10X volume of 80% ethanol. Centrifuge the homogenate at 10000 rpm for 20 minutes. Save the supernatant. Re-extracted the residue with five times the volume 80% ethanol, centrifuged and pool the supernatants. Evaporated the supernatant to dryness. Dissolve the residue in a known volume of distilled water. Pipetted out different aliquots (0.2 to 2 ml) into test tubes. Made up the volume in each tube to 3.0 ml with water. Added 0.5 ml of Folin-Ciocalteau reagent. After 3 minutes, added 2.0 ml of 20% sodium carbonate solution to each tube. Mixed thoroughly, placed the tubes in a boiling water bath for exactly 1 minute, cooled and measured the absorbance at 650nm against reagent blank.

Sr.No	Botanical name	Family	Part used
1	*Alpina calcarata*	Zingiberaceae	Leaf
2	*Ocimum basillicum*	Lamiaceae	Leaf
3	*Jatropa curcas*	Euphorbiaceae	fruit
4	*Acorus calamus*	Araceae	Leaves
5	*Verbascum thapsus*	Scrophulariaceae	Leaves
6	*Jatropa gossipifolia*	Euphorbiaceae	Leaves
7	*Jatropa multifida*	Euphorbiaceae	Flower
8	*Strebilis aspera*	Moraceae	Leaves
9	*Hyptis suaveolens*	Lamiaceae	Leaves
10	*Solanum indicum*	Solanaceae	Leaves
11	*Clitorria ternate*	Papilionaceae	Leaf and flower
12	*Passiflora edulis*	Passifloraceae	Fruit
13	*Sauropus androgynous*	Euphorbiaceae	Leaves

2.4. Statistical analysis:

Samples were analyzed in triplicate and the results were given as Mean ± S.D.

3. RESULTS AND DISCUSSION

Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So the medicinal plants are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress [10].

3.1. DPPH radical scavenging assay

DPPH is a protonated radical having the characteristic absorption maxima at 517 nm which decreases with the scavenging of the proton radical by natural plant extracts. Hence, DPPH finds applications in the determination of the radical scavenging activity of plant materials [11]. DPPH scavenging ability of thirteen medicinal plants were screened in both aqueous and ethanol solvent system as shown in Figure 1. In Aqueous system, *Hyptis suaveolens* leaf (98.06 %) did show the strong inhibition of DPPH radical followed by the leaf extract of *Alpinia calcarata* with 97.4% inhibition. Aqueous extract of *Ocimum basillicum* leaf (95.08 %) and aqueous extract of *Passiflora edulis* fruit (95.68%) possessed almost the similar percentage inhibition of DPPH radical. *Jatropha curcas* (fruit), *Acorus calamus* (leaves), *Jatropha gossipifolia* (leaf), *Jatropha multifida* (flower), *Solanum indicum* (leaf), *Clitorria ternate* (leaf and flower) and *Sauropus androgynous* (leaf) exhibited the DPPH radical scavenging capacity ranged from 70-90 % in aqueous solvent.

Out of the 13 medicinal plants screened in ethanolic solvent system, *Ocimum basillicum* leaf ranked first with the percentage inhibition of DPPH radical (96.18%). This is followed by *Alpinia calcarata* leaf with the percentage inhibition of 94.63 %. The plant extracts namely *Verbascum thapsus* leaf, *Jatropa gossipifolia* leaf, *Jatropa multifida* flower, *Hyptis suaveolens* leaf, *Solanum indicum* leaf, *Clitorria ternate* leaf & flower exhibited similar antiradical effect. The plant extracts showed weak inhibition of DPPH radical were *Jatropha curcas* fruit, *Acorus calamus* leaf, *Strebilis aspera* leaf, *Passiflora edulis* fruit and *Sauropus androgynous* leaf in ethanol solvent system.

Ocimum basillicum leaf, *Alpina calcarata* leaf, *Jatropa multifida* flower, *Hyptis suaveolens* leaf, *Solanum indicum* leaf and *Clitorria ternate* leaf & flower possessed higher DPPH radical scavenging activity in both the solvent systems. The antioxidant capacity of plant extract may be due to the hydrogen donating ability of phenols and flavonoids present in it.

3.2. Determination of total phenolics

Antioxidant activity of the plant extract is often associated with the phenolic compounds present in them. Plant phenols constitute the major group of compounds that act as primary antioxidant [12]. They can react with active oxygen radicals, such as hydroxyl radicals [13], superoxide anion radicals [14] and lipid peroxyl radicals and inhibit the lipid peroxidation at an early stage. This is because of their scavenging ability due to their hydroxyl groups.

Figure 2 demonstrates the total phenolic content of some herbs. It was found that hydroethanolic extract of *Ocimum basillicum* leaf and *Clitorria ternate* leaf & flower possessed higher phenolic content while the lowest phenolic content was observed in all other plant extracts. This shows that There exists a good correlation between phenolic contents and the antioxidant activities in the plant species namely *Ocimum basillicum* and *Clitorria ternate* .

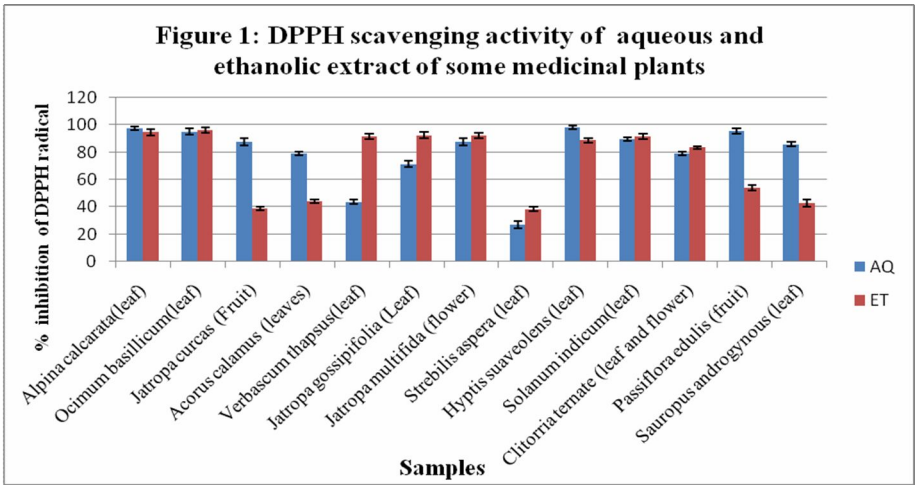
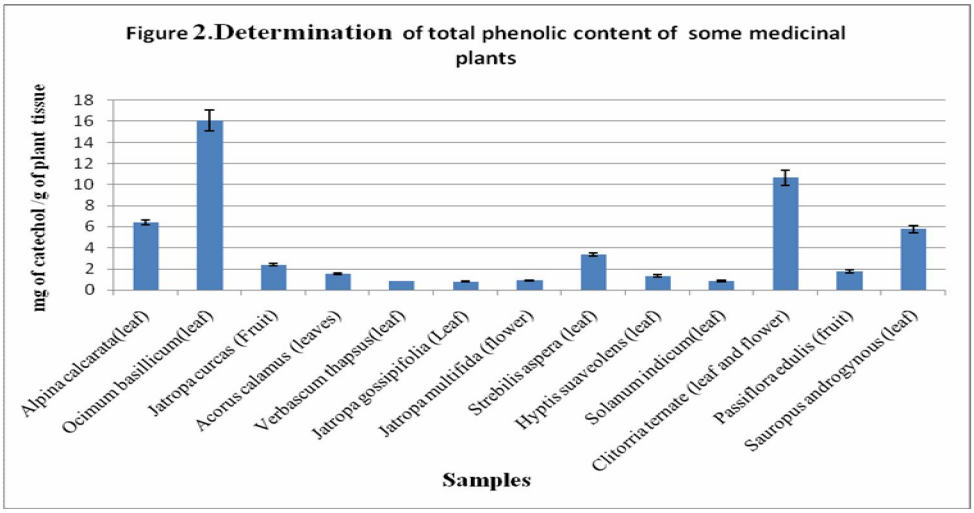


Figure 1: DPPH scavenging activity of aqueous and ethanolic extract of some medicinal plants



CONCLUSIONS

Out of the 13 herbs selected for the evaluation of antioxidant activities, *Ocimum basillicum*, *Alpina calcarata, Jatropa multifida, Hyptis suaveolens*, *Solanum indicum and Clitorria ternate* exhibited the greater antioxidant capacity. The phenolic content was in good correlation with antioxidant abilities of the species namely *Ocimum basillicum* and *Clitorria ternate*. The antioxidant activities of medicinal plants may be due to the presence of phenolic compounds, containing the hydroxyl groups that confers the hydrogen donating ability. The present investigation suggests that medicinal plants which possess good antioxidant potential are the best supplements for the diseases associated with oxidative stress.

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