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# EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME MEDICINAL PLANTS

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## ABSTRACT

The present study was carried out to determine the antioxidant activity in the *Acacia nilotica, Solidago virgaurea, Coriandrum sativum, Murrya koenigii, Azadirachta indica, Spinacia oleracea, Ocimum tenuiflorum, Trigonella foenum, Saraca asoca and Aloe barbadensis* medicinal plants. Out of ten plant samples tested, leaf extract of *Solidago virugaurca* was found to have maximum percent inhibition value of 130% for free radical scavenging capacity and *Aloe barbadensis* was found to have maximum superoxide anion radical scavenging activity (78.5%). Hydroxyl radical activity was found to be maximum for *Corriandrum sativum* (117%). Also, Hydrogen peroxide activity for *Murraya konengii* was found to be maximum at 900%. Reducing power capacity of *Azadirachta indica* was found to be 11.1 mg/ml and Nitric Oxide activity for *Solidago virgaurea* and *Spinacia oleracea* possessing highest activity (12.2 mg/ml). It can be inferred that the parts of plant having high content of phytochemicals may serve as a good source of nutraceuticals which have potential for use in health care formulations.

**Keywords:** Phytochemicals, Nutraceuticals, Antioxidant, Phenolics, Healthcare.

## INTRODUCTION

Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities. As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, and

catalytic metals and also by acting as oxygen scavengers [1, 2]. The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defence can easily affect and persuade oxidative damage to various bio molecules including proteins, lipids, lipoproteins and DNA [3]. This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process.

Antioxidants (endogenous as well as exogenous) may, for example, donate one of its electrons to the free radicals to make them stable molecules. If no antioxidants are present, the free radicals take an electron from vital cell structures, damaging the cells leading to several chronic diseases. This requirement is fulfilled by the supplementation of phytochemical rich diets. Medicinal plants containing phytochemicals with antioxidant potential have strong protective effect against major diseases[4]. It has been reported that the diets rich in vegetables, fruits and medicinal plants provide a wide range of antioxidant phytochemicals such as polyphenolics, carotenoids, terpenoids, flavonoids, vitamins like E and C, glutathione and vegetable pigments. These phytochemicals offer protection against cellular damage due to their ability to quench oxygen-derived free radicals by donating electrons, chelating redox active metals and by inhibiting lipooxygenases [5]. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of diseases. Phenolic compounds have the potential to function as antioxidants by scavenging the superoxide anion, hydroxyl radical, peroxy radical or quenching singlet oxygen and by inhibiting lipid peroxidation in biological systems [6].

According to a WHO survey, 80% of the population living in the developing countries are now showing faith in traditional medicine for their primary health care needs. It is well known that the available synthetic drugs may not be able to completely cure the fatal diseases. It is already known that some treatments for these diseases produce side effects. So there is a need to find out other safer alternatives for the treatment of certain diseases. In view of increasing awareness towards health and increased use of phytochemicals in the prevention and treatment of common as well as serious diseases, there is a need to explore the possibilities of including phytochemicals as well as health care products in our daily diet.

In the present study, medicinal plants have been screened and evaluated for their Free radical scavenging activity, Hydrogen peroxide scavenging activity, Hydroxyl radical scavenging activity, Nitric oxide-scavenging activity, Superoxide anion scavenging activity and Reducing power.

## MATERIALS AND METHODS

### Plant material collection and preparation of the extracts

The leaf parts of plants *Acacia nilotica, Solidago virgaurea, Coriandrum sativum, Murrya koenigii, Azadirachta indica, Spinacia oleracea, Ocimum tenuiflorum, Trigonella foenum, Saraca asoca and Aloe barbadensis* were collected from the city of Jaipur, Rajasthan. The samples were dried at room temperature and further ground in a mortar. About 10 grams of each plant powder was extracted in 100 ml of methanol by maceration (48h). The solvent was concentrated at temperature below 40°C and the resulting extracts were used for determination of antioxidant activity.

### Antioxidant activity

**Free radical scavenging activity**: The free radical scavenging activity of methanol extract was evaluated using 1,1-diphenyl-2-picryl-hydrazil (DPPH) [7], where the stock solution of methanolic extract (1 mg/mL) was prepared. Aliquot of 400µl of 0.1µM of DPPH solution was added to 1 mL cuvette. Extract solutions at different doses (1 to 50 µg) were added. A volume of 600 µl of ethanol was added and the mixture was shaken vigorously and allowed to stand in dark place at room temperature for 5 min. Then the absorbance was measured at 517 nm in UV-Visible-NIR spectrophotometer. The radical scavenging activity of the tested samples were calculated according to the equation one and expressed as percentage of inhibition [8, 9]:

$$Percent\ of\ DPPH\ inhibition = [(AA - AB)/AB] \times 100$$

AA and AB are the absorbance values of the test and the blank samples, respectively.

### Hydrogen peroxide scavenging activity

Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of different fractions was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4) After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and ab-sorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank [10]. The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

$$Hydrogen\ peroxide\ scavenging\ assay = \frac{1 - absorbance\ of\ sample}{absorbance\ of\ sample}\ X\ 100$$

**Hydroxyl radical scavenging activity**

Hydroxyl radical scavenging activity was measured by the ability of the different fractions of extract to scavenge the hydroxyl radicals generated by the $Fe^{3+}$ -ascorbate-EDTA- $H_2O_2$ system (Fenton reaction) [11]. The reaction mixture contained; 500 µl of 2-deoxyribose (2.8 mM) in phosphate buffer (50 mM, pH 7.4), 200 µl of premixed ferric chloride (100 mM) and EDTA (100 mM) solution (1:1; v/v), 100 µl of $H_2O_2$ (200 mM) with or without the extract solution (100 µl). The reaction was triggered by adding 100 µl of 300 mM ascorbate and incubated for 1 h at 37°C. 0.5 ml of the reaction mixture was added to 1 ml of TCA (2.8%; w/v; aqueous solution), then 1 ml of 1% aqueous TBA were added to the reaction mixture. The mixture was heated for 15 min on a boiling water bath. After the mixture being cooled the absorbance at 532 nm was noted against a blank (the same solution but without reagent). The scavenging activity on hydroxyl radical was calculated as follows:

$$Hydroxyl\ radical\ scavenging\ assay = \frac{1 - absorbance\ of\ sample}{absorbance\ of\ sample}\ X\ 100$$

**Nitric oxide scavenging activity**

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of each extracts 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 546 nm. Quercetin was used as positive control [12].

**Superoxide anion scavenging assay**

The assay for superoxide anion radical scavenging activity was supported by riboflavin-light-NBT system [13]. Briefly, 1 ml of sample was taken at different concentrations (25 to 500 µg/ml) and mixed with 0.5 ml of phosphate buffer (50 mM, pH 7.6), 0.3 ml riboflavin (50 mM), 0.25 ml PMS (20 mM), and 0.1 ml NBT (0.5 mM). Reaction was started by illuminating the reaction mixture using a fluorescent lamp. After 20 min of incubation, the

absorbance was measured at 560 nm. Ascorbic acid was used as standard. The scavenging ability of the plant extract was determined by the following equation:

$$Superoxide\ anion\ scavenging\ activity = \frac{1 - absorbance\ of\ sample}{absorbance\ of\ sample}\ X\ 100$$

**Reducing power**

The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the solvent fractions [14]. The Fe (II) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Various concentrations of the sample (2 ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/l). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml from each of the mixture earlier mentioned was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power.

**RESULTS AND DISCUSSION**

DPPH scavenging ability of ten medicinal plants were screened in methanol solvent system as shown in Figure 1. Among ten crude extracts, the methanol extract of *Murraya konengii* was the most active with a percent inhibition value of 142% followed by the methanol extracts of *Solidago virugaurca* and *Oscimum tenuiflorum* with percent inhibition value of 130% and 120% respectively. Methanol extracts of *Acacia nilotica* and *Spinacia oleracea* exhibited moderate DPPH activity with percent inhibition value of 106% respectively (Table 1). 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 [15].

The antioxidant capacity of plant extract is due to the hydrogen donating ability of phenols and flavonoids present in it. 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 [16]. This could be due to the high flavonoid contents of leaf extract. It has been recognized that flavonoids, which contain hydroxyls, are responsible for the radical

scavenging effects of most plants. They show antioxidant activity and their effects on human nutrition and health is considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [17, 18].

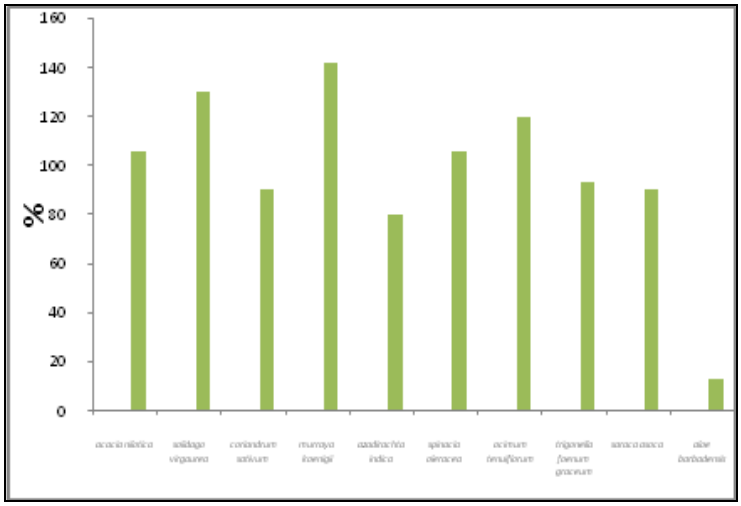


**Fig 1: Free radical Scavenging Activity of Medicinal Plants**

**Table 1: Antioxidant activity of medicinal plants calculated in percent inhibition value**

| PLANTS | $H_2O_2$ TEST % | FREE RADICAL TEST % | SUPEROXIDE TEST % | HYDROXYL TEST % |
|---|---|---|---|---|
| *Acacia nilotica* | 455 | 106 | 66.6 | 29.9 |
| *Solidago virgaurea* | 334 | 130 | 9.8 | 36.9 |
| *Coriandum sativum* | 163 | 90 | 35.1 | 117 |
| *Murraya koenigii* | 900 | 142 | 8.6 | 75.4 |
| *Azadirachta Indica* | 669 | 80 | 12.3 | 81.2 |
| *Spinacia oleracea* | 334 | 106 | 66.6 | 25 |
| *Ocimum tenuiflorum* | 0 | 120 | 17.6 | 81.2 |
| *Trigonella foenum graceum* | 132 | 93 | 25 | 44.2 |
| *Saraca asoca* | 733 | 90 | 3 | 61.1 |
| *Aloe barbadensis* | 455 | 13 | 78.5 | 82 |

Super oxide anions damage biomolecules directly or indirectly by forming $H_2O_2$, OH, peroxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation [19].The result of superoxide anion radical scavenging activity of the leaf extract of ten medicinal plants is

shown in the Table 1 and Fig 2. Thus, higher inhibitory effects of the leaf extracts on superoxide anion formation shown herein possibly renders them as a promising antioxidants. The result suggests that the percent inhibition value of plant extract of *Aloe barbadensis* has a potent super oxide radical scavenging effect followed by *Spinacia oleracea and Corriandum sativum* (Fig.2).

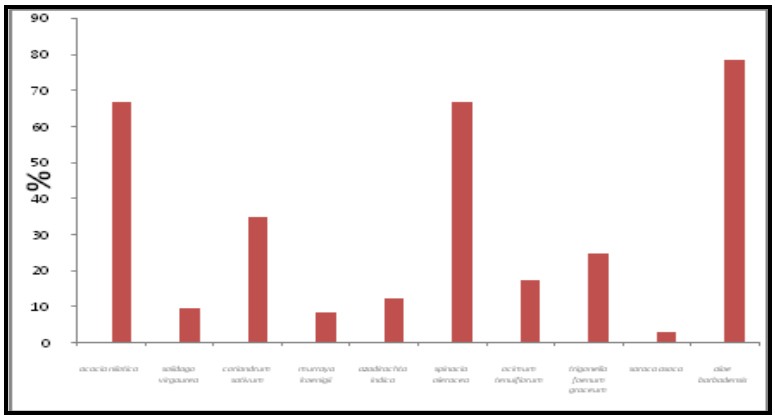


**Figure 2: Superoxide Anion Radical Scavenging activity of Medicinal Plants**

Hydroxyl radical is highly reactive oxygen centred radical formed from the reaction of various hydro peroxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acids in membranes and most biological molecule it contacts (Aruoma, 1999) and is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxidic reaction of lipids (Yen and Duh, 1994). Activity of the leaf extract on hydroxyl radical has been shown in Table 1 and Figure 3. The plant extract exhibited scavenging activity against hydroxyl radial generated in a Fenton reaction system (Figure 3). The highest activity was found in *Corriandrum sativum*(117%) *f*ollowed by *Azadirachta indica* (81.2%) and *Ocimum tenuiflorum*(81.2%).

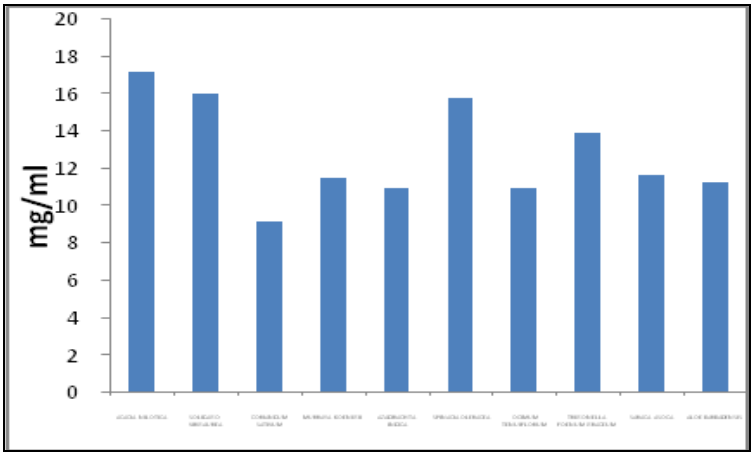


**Fig 3: Hydroxyl Radical activity of medicinal plants**

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, $H_2O_2$ can probably react with $Fe^{2+}$ and possibly $Cu^{2+}$ ions to form hydroxyl radical and this may be the origin of many of its toxic effects [20]. It is therefore biologically advantageous for cells to control the amount of $H_2O_2$ that is allowed to accumulate. As shown in the Figure -4, the leaf extract of *Murraya konengii* was found to be most active with a percent inhibition value of 900% which has demonstrated hydrogen peroxide decomposition activity among all plants (Table 1 and Fig 4). The decomposition of $H_2O_2$ by the leaf extract may at least partly result from its antioxidant and free radical scavenging activity. The findings of Ramalingam *et al*., (2011) [21] agree with these results on hydrogen peroxide scavenging activity.

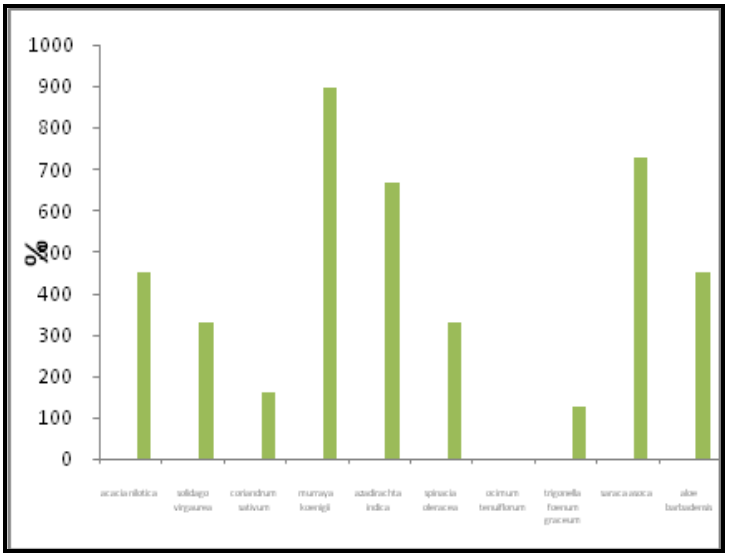


**Fig 4: $H_2O_2$ Scavenging Activity of Medicinal Plants**

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [22]. The reducing power activity in *Azadirachta indica* was found to be 11.1 mg/ml and *Solidago virgaurea* with 9.2 mg/ml respectively (Fig 5).

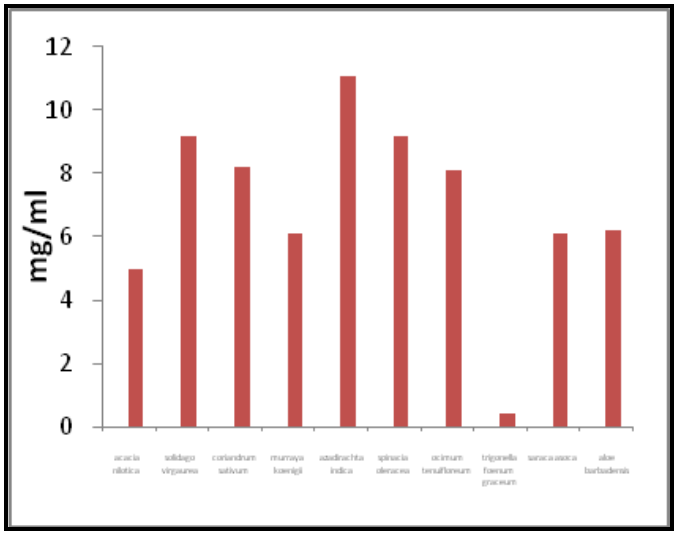


**Fig 5: Reducing Power activity of Medicinal plants**

The Nitric Oxide activity generated from sodium nitroprusside in aqueous solution at pH interacts with oxygen to produce nitrite ions were measured using Griess reaction reagent [12]. From the reaction it was observed that *Solidago virgaurea* and *Spinacia oleracea* were possessing highest activity (12.2 mg/ml) followed by *Trigonella foenum* (8.2 mg/ml). (Fig 6).

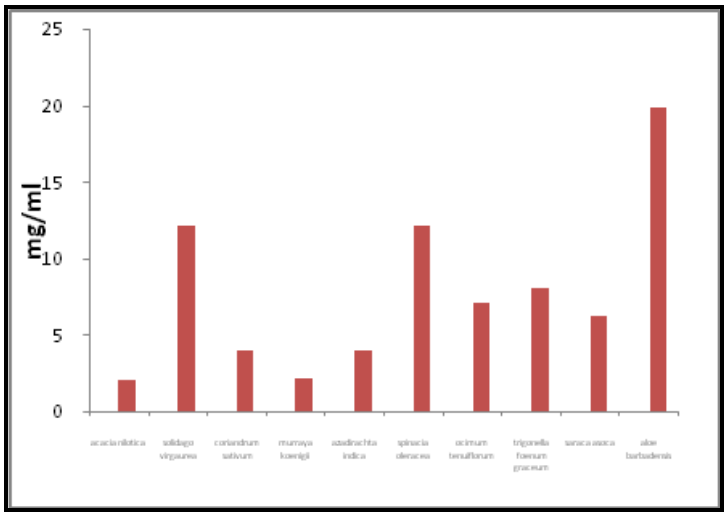


**Fig 6: Nitric Oxide radical scavenging activity of Medicinal plants**

## CONCLUSION

From the above study it may be concluded that the *Spinacia oleracea, Solidago virgaurea, Azadirachta indica and Murraya konengii* are the good sources of natural antioxidants and might be useful in treating the diseases associated with oxidative stress. These plants, rich in flavonoids and phenolic acids could be a good source of natural antioxidants. Therefore, the qualitative and quantitative analysis of major individual phenolics in the species could be

helpful for explaining the relationships between total antioxidant activity and total phenolic content of the extracts. Obviously, to confirm the beneficial effects of these extracts, it is necessary to carry out further studies about their in vivo activity and bioavailability.

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