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# A comparative evaluation of antioxidant activity in some Indian medicinal plants and their phytochemical analysis

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## Abstract

*Methanolic crude extracts of five Indian medicinal plants were screened for their antioxidant activity by free radical scavenging method using ascorbic acid as positive control. The activity was measured with DPPH (2,2-diphenyl-1-picryal-hydrazyl) and nitric oxide methods. In DPPH assay, the overall maximum activity was shown by Mentha piperita, followed by Punica granatum, Syzygium cumini, Ocimum sanctum and Azadirachta indica in decreasing order of activity. The $IC_{50}$ values of ascorbic acid and plant extracts ranged from 18 to72 ìg/ml. The extract concentrations showing 50% inhibition of nitric oxide radical ($IC_{50}$) were found to be as 123.23, 269.40, 284.64, 302.19, 771.90 and 63.94 ìg/ml for Syzygium cumini, Punica granatum, Mentha piperita, Ocimum sanctum, Azadirachta indica and for ascorbic acid respectively. The present study indicates that these plants are of therapeutic potential due to their antioxidant activity.*

**Keywords:** *Antioxidant activity, medicinal plants, DPPH radical scavenging assay, Nitric oxide assay*

## Introduction

Antioxidants help organism deal with oxidative stress, caused by free radical damage. Free radicals are potentially important in a number of ailment states that can have severe effects on the cardiovascular system, either through lipid peroxidation or vasoconstriction (Lachance et. al 2001). Reactive oxygen species (ROS) are entire classes of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radicals and hydrogen peroxide are often being generated as byproducts of biological reactions or from exogenous factors Ames (1998) and Finkel and Holbrook (2000). Due to biochemical processes occurring in the body, it is normal for free radicals to be present in the body at all times, however when the free radicals increase to a level, the danger begins. Antioxidant-based drug formulations with free radical chain reaction breaking properties are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer Devasagayam et. al (2004) and Padma et al (2006). Some of the compounds having antioxidant properties are obtained from food, such as tocopherol, b-carotene, and ascorbic acid, and micronutrient elements as zinc and selenium. A large number of medicinal plants like *Ocimum sanctum, Piper cubeba* Linn., *Allium sativum* Linn., *Terminalia bellerrica, Camellia sinensis* Linn., *Zingiber officinale* and their purified constituents have been reported to possess therapeutic potentials. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Aqil et. al (2006). Therefore, the development and utilization of better effective antioxidants of natural origin is required. The aim of the present study was to investigate and to evaluate the antioxidant activity of five medicinal plants by using two different methods *viz.* the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging method and the Nitric oxide scavenging method.

## Materials and Methods

The reagents and chemicals DPPH (2,2-Diphenyl-1- picryl-hydrazyl) and Sulfanilamide (Sigma-Aldrich, Germany), Naphthyl ethylenediamine dihydrochloriode(Central Drug House (P) Ltd, New Delhi) and Sodium nitroprusside (Merck) were

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obtained. TLC Aluminium silica gels 60F254, 20x20 cm plates (Merck) were used.

*Preparation of crude plant extracts*

The medicinal plants used in this study, were collected from local region of Agra and Mathura district of country. Plant material consisting of mature leaves of (*Punica granatum*) Anar, (*Ocimum sanctum*) Tulsi, (*Mentha piperita*) Peppermint, (*Azadirachta indica)* Neem and (*Syzygium cumini*) Jamun were collected and shade dried for 3-4 days. The dried plant materials were powdered using grinder. The extraction was done at room temperature with methanol in soxhlet apparatus for up to 24 cycles. The methanolic extracts were evaporated to dryness in a vacuum rotary evaporator (Heidolph, Germany) at set bath and cooling temperature of 35°C and 4°C respectively along with 147 bar vacuum pressure.

*DPPH radical scavenging activity*

Antioxidant activity of plant extracts was evaluated on the basis of the radical scavenging effect of the stable 2, 2-diphenyl-1-picryal-hydrazyl (DPPH) radical using modified method described by *Braca et al* (2002). The dilutions of the test extracts ranging 7.8-1000 µg/ml concentrations were prepared in methanol. Ascorbic acid was used as standard in 7.8-1000 µg/ml concentrations. A 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 1.0 ml of sample solution at different concentrations (7.8-1000 µg/ml) and standard separately. These solution mixtures were kept in dark for 30 min. and optical density was measured at 517 nm. Methanol (1ml) with DPPH solution (0.1mM, 1ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below Bors et. al (1992). $IC_{50}$ values were calculated at different intervals for test samples and standard using Finney, 1962.

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

Where A= optical density of the blank and B= optical density of the test sample.

*Nitric oxide scavenging activity*

Nitric oxide scavenging activity was measured by the spectrophotometry method Madan et. al (2005). Sodium nitroprusside (5mmol) in phosphate buffered saline was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentrations (7.8-1000 µg/ml) were dissolved in methanol and incubated at 25º C for 30 min. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% Phosphoric acid, and 0.1% Naphthyl ethylenediamine dihydrochloriode). The absorbance of the chromophores formed during the subsequent coupling with Naphthyl ethylenediamine dihydrochloriode was measured at 546 nm.

*Phytochemical screening of extract*

Phytochemical analysis of the major phytoconstituents of the plant extracts were undertaken using standard qualitative methods (color tests and /or TLC) as described earlier (Ahmad and Beg, 2001).

*TLC-DPPH separation and determination of radical scavenging activity*

Qualitative assay was performed using some modifications according to *Sadhu et al* (2003). Crude extracts of plants were subjected to thin layer chromatography. The solvent system of toluene and ethyl acetate was used in 7:2 ratios. The plates were developed in an unsaturated chamber. After 15 min air- drying, the plates were sprayed with 0.004% DPPH solution for 5 seconds and spots were observed under visible light at exactly 2 min after spraying. The area of bright yellow bands against the purple background determined the radical scavenging activity.

## Results and Discussion

Methanolic crude extracts of five plants and standard tested for them *in vitro* antioxidant activity using DPPH and Nitric oxide method. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl stable free radical, to decolorize in the presence of antioxidants Hagerman et. al (2007). The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also visible as dark purple colour. When DPPH accepts an electron donated by compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The TLC based qualitative DPPH spray revealed the presence of significant antioxidant activity in the methanolic extracts of all plant samples indicated by the presence of a yellowish spot on the reddish purple background of the TLC plate.

In the quantitative assay among the five extracts and standard tested for the in vitro antioxidant activity using the DPPH method, the crude methanolic extracts of *Punica granatum*, O*cimum sanctum*, *Mentha piperit*a, *Azadirachta indica* and *Syzygium cumini* showed antioxidant activity, with $IC_{50}$ values of 24, 55, 18, 72 and 39 ìg/ml (Table 1). The results indicate that the antioxidant actiivity of crude

Table 1: DPPH Scavenging activity

| Concentration (ug/ml) | Ascorbic Acid % inhibition | *Mentha piperita* % inhibition | *Syzygium cumini*% inhibition | *Punica granatum*% inhibition | *Ocimum sanctum*% inhibition | *Azadirhacta indica*% inhibition |
|---|---|---|---|---|---|---|
| 7.8 | 21.7±.036 | 28.2±.273 | 20.1±.231 | 22.6±.505 | 21.0±.727 | 10.6±.328 |
| 15.6 | 38.4±.036 | 48.7±1.17 | 26.2±.305 | 41.0±.694 | 22.2±.1.16 | 15.6±.934 |
| 31.2 | 45.4±.073 | 57.4±.520 | 48.1±.273 | 59.3±.669 | 31.1±.379 | 33.7±.521 |
| 62.5 | 54.3±.036 | 80.2±.208 | 61.3±.524 | 73.0±.694 | 55.6±.769 | 58.8±.410 |
| 125 | 64.0±.055 | 83.7±.437 | 74.6±.289 | 82.5±.404 | 78.7±.537 | 70.7±.674 |
| 250 | 75.4±.055 | 87.6±.549 | 88.1±.202 | 86.1±.473 | 80.4±.379 | 77.1±.491 |
| 500 | 86.2±.092 | 92.1±549 | 88.5±.115 | 87.9±.586 | 81.1±.493 | 79.1±.208 |
| 1000 | 93.4±.055 | 95.1±.208 | 88.7±.240 | 92.8±.433 | 83.3±1.04 | 80.7±.185 |
| $IC_{50}$(ug/ml) | 42 | 18 | 39 | 24 | 55 | 72 |

Data are mean±SEM, *n*=3

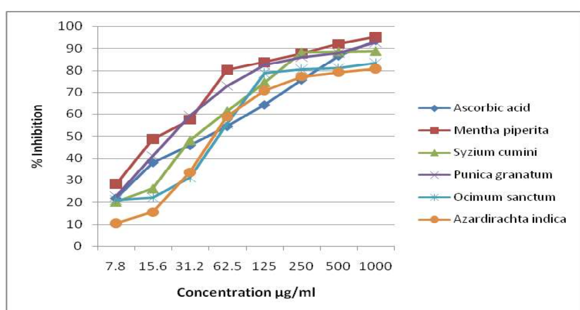


Fig. 1: DPPH free radical scavenging actiivity of Standard Ascorbic acid Methanolic plant extracts

methanolic extracts of *Mentha piperit*a, *Punica granatum* and *Syzygium cumini* were higher than that of ascorbic acid. The $IC_{50}$ value for ascorbic acid was 42 ìg/ml. O*cimum sanctum* and *Azadirachta indica* both found to be less active than ascorbic acid. The antioxidant activity is presented in the figure 1 which showed that the percentage inhibition of 1000 ìg/ml of Peppermint (*Mentha piperit*a) extract was 95.1%, which is comparable with standard antioxidant activity of ascorbic acid (93.4%).

Nitric Oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling and inhibition of platelet aggregation and of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities Hagerman et. al (2007).

The scavenging of nitric oxide by the extracts was showed and scavenging was increased in dose dependent manner. Figure 2 illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The methanolic extracts of *Syzygium cumini, Punica granatum*, *Mentha piperita, Ocimum sanctum* and *Azadirachta indica* showed inhibition 72.9%, 64.4%, 62.7%, 60.5% and 52.8% respectively at 1000 ìg/ml, where as ascorbic acid at the same concentration exhibited 75.2 % inhibition. The $IC_{50}$ values were found to be 123.23, 269.40, 284.64, 302.19, 771.44 and 63.94 ìg/ml for *Syzygium cumini, Punica granatu*, *Mentha piperita,* O*cimum sanctum, Azadirachta indica* and ascorbic acid respectively (Table 2). The free radical scavenging activity of medicinal plant extracts were confirmed in the present investigation.

The phytochemical analysis of these plants extracts showed the presence of alkaloids and flavanoids were present in *Mentha piperita* and *Ocimum sanctum*. Glycosides were present in *Azadirachta indica*, *Mentha piperita*, *Ocimum sanctum*, *Punica granatum* except *Syzygium cumini*. Tannins, phenols and carbohydrates were present in all plant extracts except *Ocimum sanctum.* Hence, the observed antioxidant activity may be due to the presence of any of these constituents.

## Conclusion

In conclusion, the results of the present study showed that the extracts of *Mentha piperita, Punica granatum* and *Syzygium cumini* have high 2, 2-diphenyl-1-picryal-hydrazyl (DPPH) free radical scavenging activity than standard ascorbic acid. The methanolic extracts of *Syzygium cumini* show better results for Nitric oxide analysis in comparison to ascorbic acid.

Table 2: Nitric Oxide assay

| Concentration (ug/ml) | Ascorbic Acid % inhibition | *Mentha piperita* % inhibition | *Syzygium cumini*% inhibition | *Punica granatum*% inhibition | *Ocimum sanctum*% inhibition | *Azadirhacta indica*% inhibition |
|---|---|---|---|---|---|---|
| 7.8 | 21.6±.055 | 20.3±.073 | 22.2±.036 | 21.6±.042 | 18.7±.073 | 15.3±.055 |
| 15.6 | 34.4±.055 | 25.0±.111 | 27.2±.036 | 30.1±.021 | 22.4±.021 | 19.1±.055 |
| 31.2 | 41.5±.063 | 26.6±.111 | 36.8±.055 | 34.6±.063 | 28.2±.042 | 22.3±.036 |
| 62.5 | 56.0±.092 | 35.4±.180 | 44.9±.096 | 37.7±.042 | 32.5±.036 | 29.6±.036 |
| 125 | 62.5±.128 | 39.9±.073 | 46.0±.055 | 38.9±.021 | 41.1±.036 | 36.3±.055 |
| 250 | 66.4±.055 | 47.5±.096 | 58.3±.055 | 42.1±.021 | 50.0±.021 | 40.4±.036 |
| 500 | 70.1±.055 | 58.0±.111 | 64.8±.073 | 59.1±.055 | 56.1±.055 | 44.0±.055 |
| 1000 | 75.2±.073 | 62.7±.146 | 72.9±.036 | 64.4±.042 | 60.5±.073 | 52.8±.036 |
| $IC_{50}$ (ug/ml) | 63 | 284 | 123 | 269 | 302 | 771 |

Data are mean±SEM, *n*=3

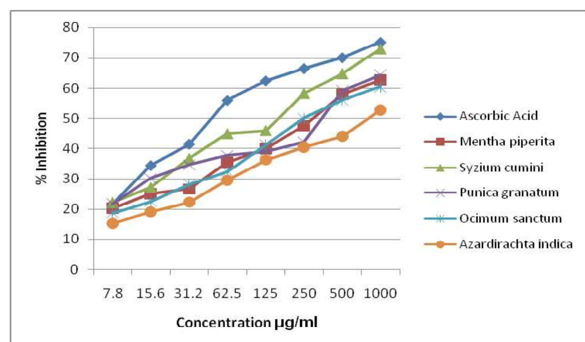


Fig. 2: Nitric Oxide assay of Standard Ascorbic acid Methanolic plant extracts.

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