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Original Research Paper

ANTIOXIDANT POTENTIAL OF SOME MEDICINAL PLANTS

(Ocimum sanctum, Azadirachta indica and Nigella sativa)

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ABSTRACT

The extracts of leaf, stem and root of *Ocimum sanctum, Azadirachta indica* and *Nigella sativa,* were used in the comparative study of total carotene, flavonoids, phenolics and total antioxidant potential. Leaves of *Ocimum sanctum,* exhibited maximum total carotene content (2.17±0.272 mg /gram fresh weight ±S.D), while root of *Nigella sativa* exhibited the lowest (0.29±0.088 mg /gram fresh weight ±S.D). Flavonoids were found to be maximum in the leaf of *Nigella sativa* (4.93±0.702 mg equivalent QE /gram of tissue ±S.D), while minimum in the root of *Azadirachta indica* (0.16±0.033 mg equivalent QE /gram of tissue ±S.D). A trend similar to total carotene content was exhibited by phenolics with (34.9±2.427 mg equivalent GA /gram of tissue ±S.D) in the leaf of *Ocimum sanctum* as maximum and (6.85±2.005 mg equivalent GA /gram of tissue ±S.D) in the root of *Nigella sativa* as minimum. Total antioxidant potential was observed to be maximum in the root of *Ocimum sanctum* (0.69±0.013 mM equivalent ascorbic acid/g tissue ±S.D), while minimum was recorded in the leaf of *Nigella sativa* (0.22±0.028 mM equivalent ascorbic acid/g tissue ±S.D). The variability when analyzed statistically by two way ANOVA it was found significant with (P-value < 0.05). The present study showed *Ocimum sanctum* to be an excellent source of antioxidants which can be utilized for therapeutic purposes. However, the specific plant part need to be optimally utilized for specific pharmaceutical/neutraceutical formulation.

Keywords: Antioxidant, *Azadirachta indica*, Carotene, Flavonoid, *Nigella sativa*, *Ocimum sanctum*, phenolics.

INTRODUCTION

Plants have been exploited as a substitute remedy for the treatment of variety of diseases ever since beginning of human civilization. In recent years focus has shifted from chemical drugs to exploitation of remedial plants as curative agent to treat numerous stress linked disorders due to production of free radicals. Free radicals are groups or atoms having one unpaired electron, which makes them extremely reactive. The potentially reactive derivatives of oxygen are acknowledged as reactive oxygen species (ROS; e.g., hydrogen peroxide, superoxide anions, hydroxyl, and nitric oxide radicals), which bring on oxidative damage to a variety of biomolecules including lipids, proteins and DNA etc. Free radical mediated damage may cause various diseases such as cancer, diabetes mellitus, arthritis, atherosclerosis, and neurodegenerative diseases, inflammatory diseases and also leads to ageing process (Halliwell and Gutteridge, 1985). In order to guard against free radicals, organisms are gifted with endogenous (superoxide dismutase, catalase, glutathione reductase / peroxidase) and exogenous (vitamins E and C, β-

carotene, flavonoids, uric acid). Although these resistance systems are not adequate in serious condition (oxidative stress and UV exposure, etc). In recent years there has been immense growth of interest in alternative therapies and the remedial use of natural products, particularly those which are derived from plants (Mentz and Schenkel, 1989; Vulto and Smet, 1988; Goldfrank *et al.*, 1982). Therapeutic prospective of plants is primarily due to the existence of bioactive compounds. Antioxidants are crucial biomolecule as they can defend the body from the harm caused by free radicals. Antioxidants aids our body by scavenging the free radicals (i.e. reactive oxygen species (ROS) or reactive nitrogen species) universally occurs in biological systems (Wilson, 1988). Among various bioactive compounds polyphenols are antioxidants accountable for protection from chronic diseases and health care (Sati et al., 2010). In the present study three potential medicinal plants were examined for their antioxidant properties. Firstly, *Ocimum sanctum*, was explored which belongs to the family Lamiaceae which is an inhabitant of Indian Subcontinent (Staples *et. al*., 1999). Some of the important chemical constituents of *Ocimum sanctum* are rosmarinic acid, eugenol, carvacrol, ursolic acid, linalool, oleanolic acid, β-caryophyllene, (Kuhn *et. al*., 2007) β-elemene, and germacrene D (Padalia *et. al*., 2007). Secondly, *Azadirachta indica* of family Meliaceae was taken which is an inhabitant of India and tropical and subtropical countries and is distributed widespread around the world (Ross, 2001). Its chemical constituents contain several biologically active compounds together with triterpenoids, alkaloids, carotenoids, flavonoids, phenolic compounds, and azadirachtin (which is in fact a blend of seven isomeric compounds taged as azadirachtin A-G (Verkerk *et. al*., 1993)). Other compounds that have an organic activity are salannin, volatile oils, meliantriol and nimbin (Ramesh and Balasubramanian, 1998). Lastly the genus *Nigella* (family Ranunculaceae) comprises of 14 species of annual herbaceous plants of Mediterranean and West Asian origin including species of commercial importance for example spices, aromatic, medicinal and ornamental plants (Zohary, 1983). The compounds which have been related to medicinal importance includes alkaloids, terpenes, glycosides, phenolics, flavonoids etc. The compounds isolated from *N. sativa* are mainly, nigellicine, nigellimine, thymols, thymoquinones, sterols, β-amyrine, nigellidine, carvone, tannin, resin and saponines which are of great medicinal value (Paarakh, 2010). The purpose of the present study is to elucidate the antioxidant properties of aforesaid plants and to measure antioxidant capacity of biomass which may facilitate to formulate the composition of pharmaceutical/ neutraceutical and herbal products aiming at healthy individuals.

MATERIAL AND METHODS

Plant Material

Samples were collected from plants growing in the departmental garden of Amity University Uttar Pradesh, Lucknow campus. For extraction and analysis only fresh plant parts viz. leaf, stem and root were taken and washed with detergent followed by sterile water. The plant parts were blot dried and then used in the study. The plant parts were homogenized and extracted differently for different purpose as mentioned below.

Flavonoids

Total flavonoid was extracted by the method of Kevin *et. al.*, 2002; with some modifications. 25-g of tissue was submerged in 100 ml of 80% ethanol for overnight. The extract was filtered twice using Whatman no. 1 filter paper. Filtrate was collected and used for estimation of flavonoids. Ethanolic extract was diluted to 10:1 with 80% ethanol to a total of 3 ml. Absorbance was taken at 362 nm using double beam UV-VIS spectrophotometer (Model: UV-1800, Schimadzu, Japan) in triplicate and was calculated using standard curve of Quercetin (QE) as standard (Figure 1).

Total Phenolics

Total phenolic content was determined by the protocol of Fatma, *et. al*. 2013); with some modifications. 25 gram of tissue was homogenized with 100 ml of 70% ethanol. The

extract was filtered through Whatman no. 1 filter paper. The total phenolic content was determined by using Folin-Ciocalteu reagent. A volume of 0.5 ml of the plant extract was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and then neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min. The absorbance of the resulting color was measured at 765 nm. All the sets were prepared in triplicate. The total phenolic content was determined using standard curve of Gallic acid (G.A) as standard (Figure 2).

Total Carotene Content

Total carotene content was determined spectrophotometrically by the protocol of Sükran *et al.* (1998). The weighed samples were put separately in 100% acetone (100 ml for 2 gram), and homogenized. The homogenate was centrifuged using the REMI cooling centrifuge C-24BL model at 2500 rpm for 10 minutes. The supernatant was separated and the absorbance was taken at 470 nm. The amount of these pigments was calculated according to the formulae of Lichtentaler *et al.* ,(1985).

Total carotene = 1000 A_{470} - 2.270 Ca - 81.4 Cb/227

Where Ca = chlorophyll A $_{662nm}$ and Cb = chlorophyll B $_{646nm}$

Total Antioxidant Activity

Total antioxidant activity was estimated by the protocol of Cacig *et al.*, (2005) with some modifications. 1 gram of tissue was homogenized in 4 ml of double distilled water and incubated for 24 hour at 4°C. It was then filtered twice with Whatman no. 1 filter paper and the collected filtrate was stored at 4°C. 100µl of sample was taken in a 3 ml glass cuvette containing the oxidative mixture of 0.18 ml potassium permanganate 0.01 M; 0.42 ml sulfuric acid 2 M and 2.3 ml distilled water. The decrease in absorbance was measured at 535nm, with standard using ascorbic acid (Figure 3).

In order to quantitatively compare the antioxidant activities, the following formula was used:

$$A50 = \frac{t\ standard}{t\ plant\ sample} \times \frac{C\ standard}{m\ plant} \times \frac{V\ standard}{V\ plant\ sample} \times V\ extract$$

where:

*A*50 — antioxidant activity articulated, reflected in the time until the sample induces a decrease of the oxidizing agent [KMnO4] concentration up to one half, compared against a standard [ascorbic acid] (mmol equivalent / g plant tissue)

t plant sample — the time until the sample induces a decrease of the permanganate concentration up to one half (min)

t standard — the time until the standard (ascorbic acid) persuade a decline of the permanganate concentration up to one half (min) [0.66 minutes as seen in standard curve]

Cstandard — standard (ascorbic acid) concentration [0.01 mmol/ml]

m plant — weight (g) of the plant sample subjected for extraction [1g]

Vplant sample — volume of the plant extract subjected for the analysis [0.1 ml]

Vstandard — volume of the standard subjected for the analysis [1 ml]

Vextract — volume (ml) of the obtained extract [4 ml]

Statistical Analysis

Two way ANOVA (without replication) was done for analysis of variance to establish variation in the antioxidants among respective plants and their different parts.

RESULT AND DISSCUSSION

For many years, the study on plants to expose the mechanism of therapeutic action and to give reason for their claims by conventional healers has been raised. The purpose of this research has been to explore the bioactive components and antioxidant properties of some medicinal plants viz. *Ocimum sanctum*, *Azadirachta indica*, and *Nigella sativa*. The present study has verified that

these remedial plants could be good source of antioxidants. It has been investigated in the present study that antioxidant varied in different parts of same plants viz. leaf, stem and roots. Our results demonstrate that leaves of *Ocimum sanctum*, *Azadirachta indica* and *Nigella sativa* are excellent source of antioxidants and moreover *Ocimum sanctum* plant is a superior source of antioxidant. Many parameters were taken to explore the antioxidant potential namely, total carotene content, flavonoids, phenolics and total antioxidant in these plants and their different parts. The results are given in Table 1. It is evident from the results mentioned in (Table 1) that, leaf of *Ocimum sanctum* shows maximum total carotene content (2.17±0.272 mg /gram fresh weight ±S.D) while minimum was found in the root of *Nigella sativa* (0.29±0.088 mg /gram fresh weight) ±S.D.) The function of carotenoids for plant growth is essential, as two chief phytohormones, strigolactones and abscisic acid, are consequential of carotenoid precursors (Cazzonelli *et al.*,2010). A wide range of carotenoids estimation in different plants have been reported (Prakash, 2009) which are of immense significance as source of vitamin A (Kandlakunta *et al*., 2007) which may help combating against vitamin A deficiency in world population. When flavonoids were examined it was observed that leaves of *Nigella sativa* possess maximum flavonoid content (4.93±0.702 mg equivalent QE /gram of tissue ±S.D)**,** while roots of *Ocimum sanctum* revealed minimum flavonoid content (0.24±0.053 mg equivalent QE /gram of tissue ±S.D). However, (Hertog *et al*., 1992) examined the flavonoid content in 9 fruits and 28 vegetables and recommended that quercetin levels in the edible parts of the majority of vegetables were normally low (4.6-5.6%), although similar results were studied by Pongtip and Wandee (2005) in leaves of *Azadirachta indica* found flavonoid content to range between (110.0 to 511.4 mg Quercetin equivalent per 100 g) of extract. Therefore, leaves of *Ocimum sanctum, Azadirachta indica* and *Nigella sativa* must be considered to hold moderate to high total flavonoid content. As far as total phenolics are concerned leaves of *Ocimum sanctum* contains highest phenolic content (34.9±2.427 mg equivalent G.A /gram of tissue±S.D), while lowest was recorded in the root of *Nigella sativa* (6.85±2.005 mg equivalent G.A /gram of tissue±S.D). In a similar studies *Ocimum sanctum* leaves were used which confirm high (48.93 ± 0.24 mg/g) phenolic content (Prakash, 2009). *Citrus aurantifolia* accounts to possess moderately higher phenolic content as per analyzed by Ahmed et al. (2009). When delimiting to total antioxidant it was observed that roots of *Ocimum sanctum* show maximum antioxidant (0.69±0.013 mM equivalent ascorbic acid/g tissue ±S.D), while minimum was observed in the leaves of *Nigella sativa* (0.22±0.028 mM equivalent ascorbic acid/g tissue ±S.D). In the similar studies (Kaur and Mondal, 2014) accounts the very high antioxidant activity (87.05%) in leaves of *Citrus aurantifolia* and recommends main involvement of non-vitamin aspect to total in vitro antioxidant activity of *Citrus aurantifolia*. Chu *et al.* (2000) also reported to have analogous results concerning antioxidant activity in leaves of *Asparagus racemosus* with more than 85% of activity. It was reported that rosmarinic acid present in the chemical constituent of *Ocimum sanctum* operates as the influential antioxidant (Pattanayak, 2010). However in the present study it was observed that root holds the good source of total antioxidant capacity by reducing potassium permanganate and leading it to decolorization. Furthermore, it was noted that *Ocimum sanctum* roots holds excellent antioxidant activity and total phenolic content in all three genera was at higher side as compared to total carotene content, flavonoids and total antioxidants. However, when all antioxidants statistically compared by two way ANOVA it was observed for total carotene content to have significant variability with (P-value 0.014) i.e. < 0.05, between leaf, stem and root while no significant variability was observed between *Ocimum sanctum, Azadirachta indica* and *Nigella sativa* with (p-value 0.096) i.e. > 0.05. For flavonoids it was calculated to be (P-value 0.04) between leaf, stem and root shows to be significant variable, although no significant

variability was observed between *Ocimum sanctum, Azadirachta indica* and *Nigella sativa* (P-value 0.106). When it comes to total phenolics and total antioxidant potential, significant variability was obtained with (P- values 0.001 and 0.043 respectively) between leaf, stem and root. There was also significant variability between *Ocimum sanctum, Azadirachta indica* and *Nigella sativa* with (P-value 0.005 and 0.002, respectively).

CONCLUSION

Results showed *Ocimum sanctum, Azadirachta indica* and *Nigella sativa* leaves are strong source of carotene, flavonoids and phenolics content and show potential utilization in pharmacological products intended for improving standards of individuals. However roots showed significantly excellent antioxidant activity which can be a potent source to produce therapeutic biomolecules, which can heal ample of diseases. Furthermore, it was observe that *Ocimum sanctum* plant is a superior source of antioxidant in comparison to *Azadirachta indica* and *Nigella sativa* plant. But when it comes to specific group of antioxidants the plant parts play a more decisive role.

Table 1: Antioxidant activity of *Ocimum sanctum*, *Azadirachta indica* and *Nigella sativa*

Types of Antioxidants	*Ocimum sanctum*			*Azadirachta indica*			*Nigella sativa*		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Carotenoid (mg /gram fresh weight) ±S.D	2.17±0.272	1.43±0.230	0.57±0.108	1.89±0.291	1.60±0.469	0.61±0.241	1.30±0.261	0.76±0.120	0.29±0.088
Flavonoids (mg equivalent QE /gram of tissue) ±S.D	1.28±0.300	0.53±0.106	0.24±0.053	2.17±0.418	0.33±0.069	0.16±0.033	4.93±0.702	1.56±0.208	0.90±0.106
Phenolics (mg equivalent GA /gram of tissue) ±S.D.	34.9±2.427	26.8±1.733	14.1±1.079	28.3±3.020	23.4±2.302	13.4±1.160	20.6±0.934	15.6±1.976	6.85±2.005
Total antioxidant (mM equivalent ascorbic acid/g tissue) ±S.D.	0.55±0.029	0.58±0.029	0.69±0.013	0.53±0.031	0.56±0.044	0.59±0.009	0.22±0.028	0.25±0.023	0.44±0.027

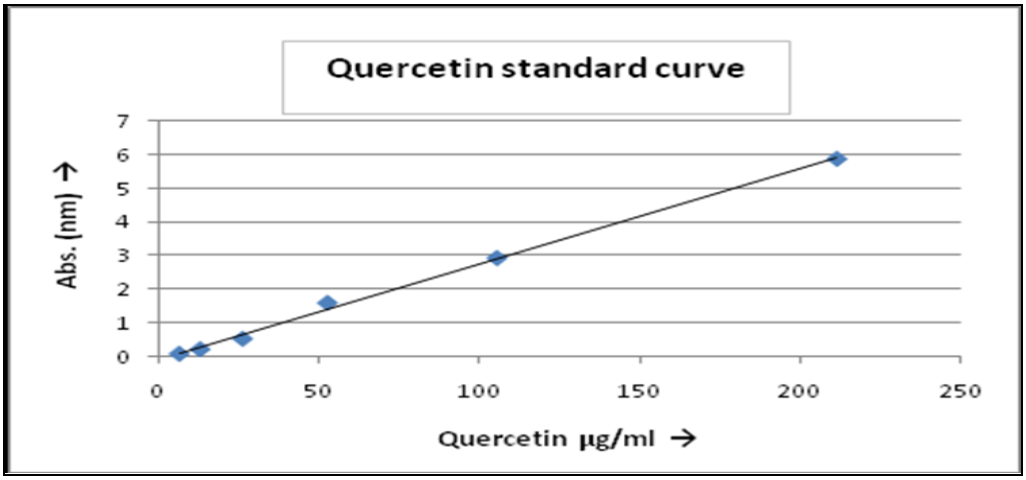

Figure 1

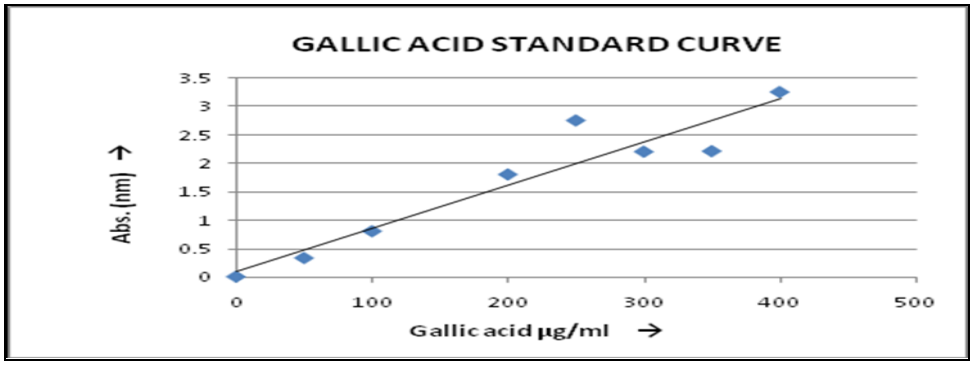

Figure 2

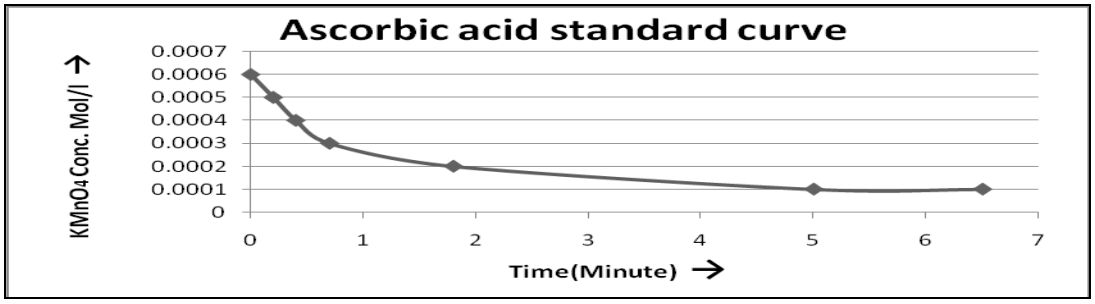

Figure 3: Variation of the potassium permanganate concentration after adding 1 ml of ascorbic acid 0.01 mol/l (mmol/ml)

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