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RESEARCH ARTICLE

Evaluating the total antioxidant capacity of *Ocimum sanctum* Linn by estimating the reduction of molybdate ion (VI to V)

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Abstract

Humans are under constant exposure to free radicals, produced either internally or from the environment. Oxidative stress occurs due to overproduction of these radicals that leads to chronic illness such as cancer, diabetics, atherosclerosis, myocardial infarction, aging, cardiovascular diseases, and other degenerative diseases in humans. Antioxidants are now being looked upon as persuasive therapeutic agents to combat and neutralize free radicals. Medicinal herbs, in particular, are catching special attention as commercial source of antioxidants. Different extracts of *Ocimum sanctum* were evaluated for their molybdate ion reducing capabilities, to evaluate its antioxidant potential. It was found that the ethyl acetate extract of the plant leaves exhibited a strong antioxidant capacity of 83.47 mg AAE/100 mg dry weight of extract, followed by the chloroform extract. The water extract had the minimum capacity to reduce the Mo (VI) to Mo (V). Molybdate ion reduction assay is a quick and convenient method to estimate the total antioxidant capacity of different plants thus depicting their capacity to reduce oxidative stress.

Introduction

Millions of chemical reactions occur in human body. These biochemical reactions require oxygen and produce reactive oxygen species (ROS) such as hydroxyl ions, hydrogen peroxide and superoxide anion as byproducts. Under a state of equilibrium, the antioxidative defense system of humans that comprises of various enzymes and antioxidants scavenge these ROS and prevent the damage or oxidative stress caused by these species. When this equilibrium is disturbed and excessive ROS are generated, oxidative stress occurs and deleterious effects are produced (Wondrak, 2009). In the past few decades, there has been an increase in the knowledge of plants that exhibit considerable antioxidant activity (Khalaf et al., 2008; Saeed et al., 2012; Medini et al., 2014). Increasing doubts towards the synthetic antioxidants for their unstable and carcinogenic nature (Chandra et al., 2014) further reinforce the interest in plant derived antioxidants. Raw extracts or chemical constituents from medicinal plants effectively prevent the damage caused by oxidative stress (Zengin et al., 2011). Although very little knowledge is available about the toxicity profile of most medicinal plants, it is generally accepted that plant based drugs are safer compared to their synthetic counterparts (Vongtau et al., 2005; Oluyemi, 2007).

Ocimum sanctum Linn. commonly called Holy basil in English and Tusli in Hindi, is known for its diverse healing properties. Tulsi is an erect, branched sub-shrub with simple opposite strongly scented green leaves and hairy stems. Leaves are ovate, slightly toothed and have a petiole. Flowers are purple in color and present in elongate racemes in close whorls. The plant grows well in tropical areas worldwide and is cultivated for its religious and medicinal values as well as its essential oil (Pattanayak et al., 2010). Traditionally, the plant is consumed in many forms such

as herbal tea, fresh leaf or dried powder as a remedy for headaches, cold, inflammation, malaria, insect bites, heart diseases and stomach disorders. The plant has been extensively studied for various pharmacological activities such as anti-genotoxic (Siddique et al., 2007), radioprotective (Devi and Ganasoundari, 1995), antimicrobial activities (Amber et al., 2010), neuroprotective (Kaur et al., 2010), antiulcerogenic (Dharmani et al., 2004) and antistress (Tabassum et al., 2010).

The present study aims at investigating the total antioxidant capacity of extracts of the green leaved *O. sanctum* by measuring its ability to reduce the molybdate (Mo) ion from oxidation state VI to V and subsequent formation of a green colored phosphate/Mo (V) complex at an acidic pH, using the molybdate ion reduction assay. The experiment was conducted to find the efficacy of the plant to overcome oxidative stress.

Materials and Method

Fresh and healthy green leaves of *O. sanctum* Linn were collected from the plants growing in the Botanical Gardens of Guru Nanak Dev University, Amritsar. The leaves were shade dried and grinded to fine powder. The powdered leaves were extracted sequentially with hexane, chloroform, ethyl acetate, methanol and water, in order of increasing polarity, filtered through Whatman no.1 sheet and evaporated through Vacuum Rotary Evaporator (Strike 202, Stereo Glass, Italy) to obtain crude extracts. The crude extracts were evaluated for their total antioxidant capacity following the spectrophotometric molybdate ion reduction assay of Prieto et al. (1999) with slight modifications. Briefly, 300 µl of extract solution (100 µg/ml) was added to 3 ml of reagent solution that comprised of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The mixture was incubated at 95 °C for 90 minutes. After incubation, the mixture was cooled at room temperature and the absorbance was measured at 695 nm against blank. Ascorbic acid was used as a standard and a standard curve was obtained using 20-250 µg/ml concentrations. Using the regression equation obtained from the standard ascorbic acid curve, the antioxidant ability of different extracts at 100 µg/ml concentration was calculated and expressed as mg ascorbic acid equivalent (AAE)/100 mg dry weight of extract.

Statistical Analysis

The experiment was conducted in triplicates and presented as mean ± SE. Regression analysis was performed along with one-way analysis of variance (ANOVA) to check statistical significant difference in the total antioxidant capacity between different solvent extracts at $p \leq 0.05$ for their antioxidant potential.

Results

From the standard regression curve (figure 1) of ascorbic acid as constructed using concentrations 20-200 µg/ml, the linear regression equation obtained was $y = 0.004x - 0.059$ ($R^2 = 0.995$). Using this equation the reduction of Mo (VI) to Mo (V) was calculated to estimate the total antioxidant activity of different extracts. The data presented in figure 2 clearly shows that from amongst all the extracts ethyl acetate extract exhibited maximum reduction of Mo (VI) to Mo (V) capability. Therefore the total antioxidant capacity of various extracts was recorded in the order: ethyl acetate > chloroform > hexane > methanol > water extract. Also from the ANOVA summary table 2, it is clear that there is statistical significant difference amongst various solvent extracts in terms of their ability to reduce Mo (VI) to Mo (V) at $p \leq 0.005$.

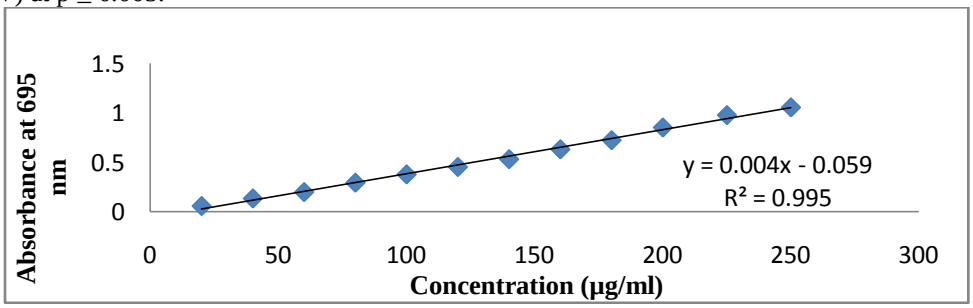

Figure 1. Standard Curve for Molybdate ion reduction assay using Ascorbic acid (20-250 µg/ml)

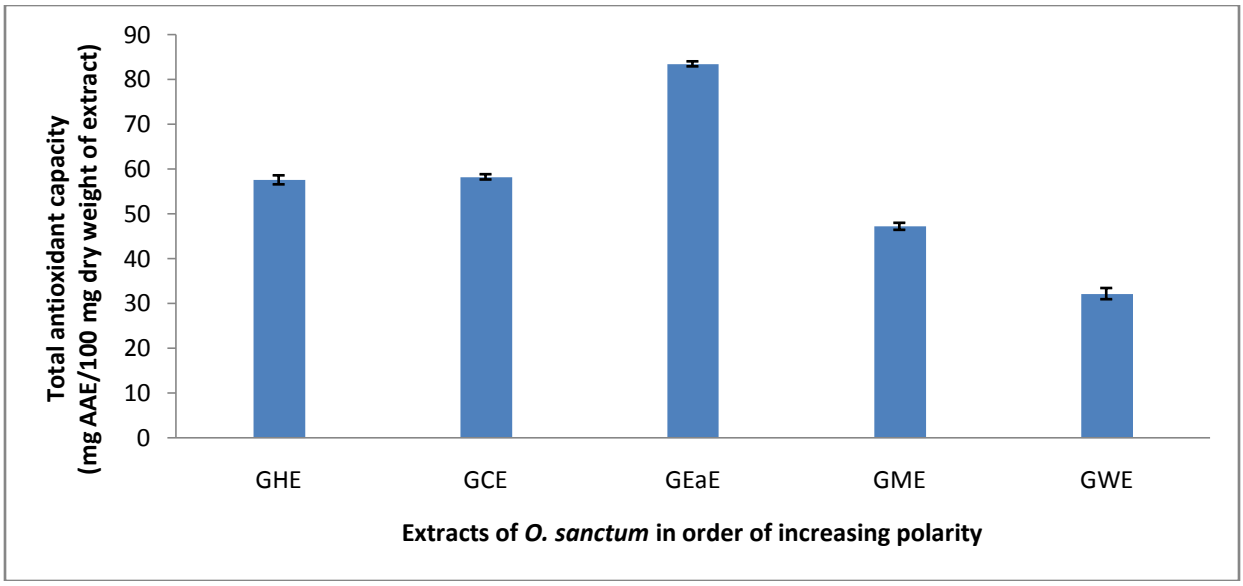

Figure 2. Total antioxidant capacity of different extracts of *O. sanctum* leaves expressed as mean±SE (mg AAE/100mg dry weight of extract). GHE (hexane), GCE (chloroform), GEaE (ethyl acetate), GME (methanol) and GWE (water).

Table 1. ANOVA summary table

Source of Variation	df	MS	F-ratio	HSD
Between groups	4	1055.614	458.0576*	6.1352
Within groups	10	2.304544		
Total	14			

* represents the significance at $p \leq 0.05$
df= degrees of freedom, HSD= honest significant difference

Discussion

Oxidative stress is an implication of excessive reactive oxygen species production in humans. These species react with cellular components such as lipids, proteins and nucleic acids and cause alterations in antioxidant system, lipid peroxidation, liver damage, altered gene expression, DNA damage and even apoptosis (Bersenyi et al., 2008, Pandey et al., 2012). To overcome oxidative damage it becomes necessary to search for antioxidants. Antioxidants are compounds, synthetic or natural, required in low concentrations that inhibit free radical mediated oxidation and either avoid the formation of free radicals or scavenge these radicals. Plants are natural source of antioxidants. Phenols, flavonoids, tannins, terpenoids and curcumin are various types of antioxidants present in plants (Moskovitz et al., 2002). The radical scavenging ability of plants is dependent on both the reactivity and the concentration of the antioxidant (Selvakumar et al., 2011). Medicinal plants are extensively studied for their antioxidative potential (Edziri et al., 2011; Medina et al., 2014) as well as their phytochemical constituents (Rice et al., 1995; Omale and Okafor 2008) that act as antioxidants.

Antioxidants are basically reducing agents. Molybdate ion reduction assay is a quick, reliable, reproducible and cost friendly method to determine the total antioxidant capacity of plants. Total antioxidant capacity can be calculated by this method based on the reduction of Mo (VI) to Mo (V) by the extract/sample and subsequent formation of green phosphate-Mo (V) complex at an acidic pH. This method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid (Preito et al., 1999). In the present study it is clearly seen that all the extracts exhibited reducing ability to various extent. The ethyl acetate extract was the most potent of all the tested extracts. The antioxidant potential may be attributed to various compounds present in *O. sanctum*. Nor and Wagner (1992) earlier reported the presence of rosmarinic acid, cirsilineol, galuteolin, gallic acid and its esters, protocatechic acid, vanillic acid, vanillin, caffeic acid in the ethanolic extract of *O. sanctum* leaves. Also the

essential oil of the plant is a rich source of eugenol, methyleugenol, methylchavicol, β-caryophyllene, β-elemene, β-ocimine, α-humulene, germacrene-D, α-farnesene, farnesol and various other constituents (Zheljazkov *et al*., 2008).

Conclusion

The data obtained in the present study clearly indicated the significant antioxidative potential of *O. sanctum* leaves. The plant can be used in formulations of herbal drugs to reduce the frequency of oxidative stress related disorders as well as reduce the toxicity of Mo (VI) ions in humans.

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References

Amber, K., Aijaz, A., Immaculata, X., Luqman, K.A. and Nikhat, M. (2010). Anticandidal effect of *Ocimum sanctum* essential oil and its synergy with fluconazole and ketoconazole. Phytomedicine. 17: 921–925.

Bersenyi, A., Berta, E., Kadar, I., Glavits, R., Szilagyi, M. and Fekete, S.G. (2008). Effects of high dietary molybdenum in rabbits. Acta Vet. Hung. 56: 41–55.

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., El-Sohly, M. A., Khan I.A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evid. Based Complement. Alternat. Med. 1–9.

Devi, P.U. and Ganasoundari A. (1995). Radioprotective effect of leaf extract of Indian medicinal plant *Ocimum sanctum.* Ind. J. Exp. Bio. 33: 205-208.

Dharmani, P., Kuchibhotla, V.K., Maurya, R., Srivastava, S., Sharma, S. and Patil, G. (2004). Evaluation of anti-ulcerogenic and ulcer-healing properties of *Ocimum sanctum* Linn. J. Ethnopharmacol. 93: 197-206.

Edziri, H.L., Smachd, M.A., Ammarc, S., Mahjoubc, M.A., Mighric, Z., Aounia, M. and Mastourib, M. (2011). Antioxidant, antibacterial, and antiviral effects of *Lactuca sativa* extracts. Ind. Crop. Prod. 34: 1182-1185.

Kaur, G., Jaggi, A.S. and Singh, N. (2010). Exploring the potential effect of *Ocimum sanctum* in vincristine-induced neuropathic pain in rats. J. Brachial Plex. Peripher. Ner. Inj. 5**:** 1-9.

Khalaf, N.A., Shakya, A.K., Al-Othman, A., El-Agbar, Z. and Farah, H. (2008). Antioxidant Activity of Some Common Plants. Turk. J. Biol. 32: 51-55.

Medini, F., Fellah, H., Ksouri, R., Abdelly, C. (2014). Total phenolic, flavonoid and tannin contents and antioxidant andantimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum.* J. Taibah Univ. Sci. 8: 216–224.

Moskovitz, J., Yim, M.B. and Chock, P.B. (2002). Free radicals and disease. J. Biochem. 397: 354-359

Norr, H. and Wagner, H. (1992). New constituents from *Ocimum sanctum*. Planta Med. 58: 574

Oluyemi, K.A., Okwuonu, U.C., Baxter, D.G. and Oyesola, T.O. (2007). Toxic effects of methanolic exract of *Aspilia africana* leaf on the estrous cycle and uterine tissues of Wistar rats. Int. J. Morphol. 25: 609–614.

Omale, J. and Okafor, P. N. (2008). Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata.* J. Afr. Biotech. 7: 3129- 3133.

Pandey, G., Jain, G.C. and Mathur, N. (2012). The effect of sub chronic exposure to ammonium molybdate on hematological and hepatic parameters in albino rats. Asian J. Phar. Biol. Res. 2: 136-142.

Pattanayak, P., Behera, P., Das, D. and Panda, S.K. (2010). *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn. Rev. 4: 95-105.

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantization of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. J. Anal. Biochem. 269: 337-341.

Rice, E. C., Miller, N. J., Bolwell, G. P., Bramley, P. M. and Pridham, J. B. (1995). The relative antioxidant activities of plant derived polyphenolic flavonoids. J. Free Radicals Res. 22: 375-383.

Saeed, N., Khan, M. R. and Maria Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement. Alt. Med. 12: 221-226.

Selvakumar, K., Madhan, R., Srinivasan, G. and Baskar V. (2011). Antioxidant Assays in Pharmacological Research Asian J. Pharm. Tech. 1: 99-103.

Siddique, Y.H., Ara, G., Beg, T. and Afzal, M. (2007). Anti-genotoxic effect of *Ocimum sanctum* L. extract against cyproterone acetate induced genotoxic damage in cultured mammalian cells. Acta Biol. Hung. 58: 397-409.

Tabassum, I., Siddiqui, Z.N. and Rizvi, S.J. (2010). Effect of *Ocimum sanctum* and *Camellia sinensis* on stress-induced anxiety and depression in male albino *Rattus norvegicus*. Ind. J. Pharmacol. 42: 283-288.

Vongtau, H.O., Abbah, J., Chindo, B.A., Mosugu, O., Salawu, A.O., Kwanashie, H.O. and Gamaniel, K.S. (2005). Central inhibitory effects of the methanol extract of *Neorautanenia mitis* root in rats and mice. J. Pharm. Biol. 43:113–120.

Wondrak, G.T. (2009). Redox-directed cancer therapeutics: molecular mechanisms and opportunities. Antioxid. Redox Signal. 11: 3013–3069.

Zengin, G., Cakmak, Y.S., Guler, G.O. and Aktumsek, A. (2011). Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz. Rec. Nat. Prod. 5: 123–132.

Zheljazkov, V.D., Cantrell, C.L., Tekwani, B. and Khan, S.I. (2008). Content, composition, and bioactivity of the essential oils of three basil genotype as a function of harvesting. J. Agric. Food Chem. 56: 380-385.