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Non-enzymatic Antioxidant Potentialities of *Ocimum Sanctum* and *Ocimum Gratissimum*- a Comparative Study

KEYWORDS: *O.sanctum, O.gratissimum,* Total phenolic content , Flavanoid content, Ferric reducing Antioxidant potential (FRAP)

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ABSTRACT *Medicinal plants are potential sources of new drugs to improve the treatment of diseases whose treatment is associated with anti-oxidative agents. The genus Ocimum is a member of the lamiaceae family. There are two main species grown in India, green-leaved holy basil (Sri Tulsi) and purple-leaved holy basil (Krishna Tulsi). These are the sources of aroma compounds and essential oils associated with diverse biological activities. The antioxidant potential of these herbs is well correlated with the presence of phenolic compounds. In the present study, O.sanctum (OS) and O.gratissimum (OG) have been compared for their antioxidant activity by Ferric Reducing Antioxidant Power (FRAP) and total phenols. Flavonoid contents were measured to assess the medicinal prospective. Both the species were found to possess the antioxidant activity. However, O.gratissimum showed significantly highest ferric reducing power (>30%) than O.sanctum. This shows a good correlation with the presence of high phenolic content than flavonoids in O.gratissimum than O.sanctum.*

Introduction

Atharvaveda (an Indian religious book), Ayurveda (Indian traditional system of medicine) etc, are the important sources for precautionary and remedial medicines. Worldwide, people are still depending on plant based medicines from times immemorial, as plants are the primary source of secondary metabolites and oils that possess therapeutic potentialities. The most important advantage of phytomedicines are their availability, less cytotoxicity, and less cost expensive (Warrier PK,1995). Plants and plant products are considered as safer components for self-medicating group (Biswas NP&Biswas AK,2005;Sarahroodi S,2012). The beneficial effects of plant based medicines in therapy are mainly due to the action of a combination of different secondary metabolites present in different parts of the plant.(Gordon MC& David JN,2001). An epidemiological research demonstrates that elderly people who consumed vegetarian diet showed an improvement in cognitive function. For example, ageing women who consume cruciferous vegetables, which are rich in antioxidant potentials showed less cognitive decline than the others who didn't add it in their diet (Rubio J et al.2011)So far many plants have been recognized as medicinal plants in folk medicine from ancient time [Prakash P&Neelu G,2005)].

In traditional system of medicine leaves, stem, flower, root, seeds or whole plant of *Ocimum sanctum* L (commonly known as Tulsi) have been used for the treatment of common colds, bronchitis, arthritis, headaches, stomach disorders, diarrhea, dysentery, inflammation, heart disease, various forms of poisoning, malaria, skin disease, eye diseases, insect bites etc .,for thousands of years. It is mentioned by Charaka in the Charaka Samhita- an Ayurvedic text, that tulsi is considered to be an adaptogen, balancing different processes in the body and helpful for adapting to stress(Prakash P&Neelu G,2005) .

Tulsi is native to the tropics of Asia and Africa, and is widespread as a cultivated plant and weed. The two main varieties grown in India are green-leaved holy basil (Sri Tulsi) and purple-leaved holy basil (Krishna Tulsi). The chemical composition of Tulsi is highly complex, containing many nutrients and bioactive compounds (Lalit Mohan,2011). Many studies indicate that O.*sanctum* and O.*gratissimum* possess antistress, antioxidant, hepatoprotective, immunomodulatory, anti-inflammatory, antibacterial, antifungal, antiviral, antipyretic, antidiuretic, antidiabetic, antimalarial and antilipedem ic,(Hallivel&Gutteridge,1992; Kasaikina,1997; Farombi,2000; Koleve et al. 2000) antinociceptive property (Rabelo et al. 2000), germicidal properties (Nakamura et al.1999;Pessoa et al.2003;Holets et al.2003) and is also used for the treatment of rheumatism, paralysis, epilepsy, high fever. (Dhawan et al.1977; Abdul rahman,1992; Sofowora,1993; Sulistiarini,1999) . Flavonoids and phenolic compounds of the plants share most of the pharmacological properties. Flavonoids and phenolic compounds are known to associate with strong antioxidant properties (Rice-Evans CA,1996).In this paper, we aim to compare the total antioxidant activity, using FRAP assay with the total flavonoid and phenolic content of *O. sanctum* and O.*gratissimum* to assess their medicinal prospective.

MATERIALS AND METHODS

Sample extraction:

Fresh leaves of O.*sanctum* and O.*gratissimum* were obtained from our institute's garden. Leaves were washed in running water and dried to desiccate under shade. Dried leaves were ground to fine powder. This powder was soaked in 80% aqueous methanol and kept in a shaking incubator for 24 h at room temperature. Later, the methanolic extract of both species was concentrated under reduced pressure in a rotary evaporator. The percent yields from O.*sanctum* and O.*gratissimum* were 6.0 and 10.22gm respectively. These extracts were stored in glass containers at 4°C for further studies.

Determination of total antioxidant power:

The total antioxidant power of the two extracts were measured by using standard FRAP method -Benzie and Strain. (Benzie IFF.&Strain JJ,1999) Fresh FRAP reagent was prepared by mixing 10mM 2, 4, 6-tripyridyl triazine (TPTZ) and 20mM ferric chloride in 0.25M acetate buffer, pH 3.6. The temperature of the solution was maintained to 37°C before its use. 200µL of test samples (1mg/ml) were added to 3mL of freshly prepared FRAP reagent in separate test tubes and incubated at 37°C for 30min. The absorbance was measured at 593nm against a blank, as FRAP reagent is responsible for the formation of a blue colored FeII-tripyridyltriazine compound from colorless oxidized FeIII form by the action of electron donating antioxidants. The standard curve was prepared by using 1000 µM $FeSO_4$ solution. Average of the

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triplicates was expressed as mmol of Fe^{2+} equivalents per 100gm (dw) of sample.

Estimation of total phenols:
Total phenolic contents of the extracts were determined by Folin-Ciocalteu method (Singleton VL, 1999)1ml of crude extract(1mg/ml) was mixed with 5 ml Folin-Ciocalteu reagent and 4ml of 7.5%sodium carbonate. Tubes were thoroughly vortexed and incubated for 30min at room temperature for colour development. Absorbance of the tubes were measured at 765 nm using Systronics UV-VIS spectrophotometer. The standard curve was prepared by using 250µg/ml of gallic acid in ethanol as standard.. The total Phenolic content values were expressed in terms of gallic acid equivalent (mg/g of dry mass).

Estimation of total flavonoids:
Total flavonoid content in the plant extracts were measured following the aluminium chloride method . (Marinova D et al.2005).1ml of plant extract (1mg/ml) was diluted with 4ml of distilled water in a flask. To this, 0.3ml of 5% $NaNO_2$ was added and at the end of 5min incubation 0.3ml of 10%$AlCl_3$ was added and re-incubated for another one minute. Finally, 2ml of 1M NaOH was added and then the contents were made up to 10ml with distilled water. All the contents were mixed and the absorbance was measured at 510nm. The standard curve for total flavonoids was made using Quercetin (0 to 100 µg/ml) in methanol. The total flavonoids were expressed as milligrams of quercetin equivalents per g of dried fraction.

Statistical analysis:
The experimental data was expressed as mean ± standard deviation (SD) of triplicates.

RESULTS AND DISCUSSION
Total Phenolic and Flavonoid Contents
Non-enzymatic antioxidant activities of natural sources are endorsed to their total phenolic and flavonoid content. Total phenolic and flavonoid contents in OS and OG were estimated by FC and $AlCl_3$ methods. The total phenolic content in OS and OG leaves were 58.06 ± 0.245 mg and 91.68 ± 0.825mg gallic acid equivalent ,GAE/g of dried leaf extract as shown in Table-1.(table 1 about here). These results also indicate OG to possess significantly high amount of TPC. Total Flavonoid content of OS and OG were expressed as Quercetin equivalents, also shown in Table-1. These results demonstrate OS to contain significantly high amount of flavonoids than OG.

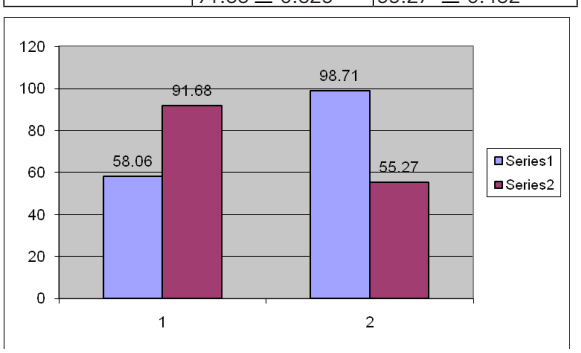
Total antioxidant activity:
Total antioxidant potentials of OC and OG extracts were estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The results of the FRAP assay of OC and OG were shown in Table 2. (table 2 about here). The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to that of the standard FeSO4 (1000 µM). This data demonstrated OG to possess considerably high amount of antioxidant activity (0.105 ± 0.005 µM) than OC (0.009 ± 0.001 µM).

Discussion:
Removal or preventing the development of reactive free radicals is a positive signal in the treatment of many disorders associated with tissue degeneration. In other words, antioxidant agents are closely associated with the prevention of degenerative diseases (Krishnaiah D et al .2009). Most of the pharmacological properties of the medicinal plants were anticipated to be due to their antioxidant activities (Halliwell B.GutteridgeJMC,1992.). This activity is due to the presence of poly phenols and flavonoids associated with radical scavenging activity(OsawaT,1994). Phenols and flavonoids are important components of the human diet. Ocimum species are consumed either fresh or dried (Lako B,EJ&Glennie VL,2001). Surveys on literature showed Ocimum species to contain high level of secondary metabolites such as terpenoids, polyphenols and flavonoids like quercetin, kaempferol and myricetin; tannins like catechins(Shahidi F&Wanasundara PKJPD,1992; Kandasamy Selvam et al.2013). These are strong antioxidants and might prevent oxidative damage to bio-molecules such as DNA, lipids and proteins. Plant phenols may interfere with all stages of the cancer process (Dillard CJ & German JB,2000;CaragayAB,1992; Percival M,1997). OC and OG are found to associate with high levels of phenols and flavonoids as shown in tables 1 and 2. Antioxidant activities are directly proportional to the phenolic content (Sarahroodi S,2012). Our data in table 2 demonstrates OG to exhibit significantly high antioxidant activity than OC .This can be related to the fact that OG showed high levels of phenols. Our results support the use of OG for human and animal disease therapy over OC.

Conclusion:
Our study concludes O.*gratissimum* to be associated with high levels of ferric reducing antioxidant potential than O.*sanctum* and this might be due to the presence of high phenolic content. Hence, it is supported for its medicinal activities.

Table - I: Total phenolic content and Flavonoid content of O.sanctum & O.gratissimum

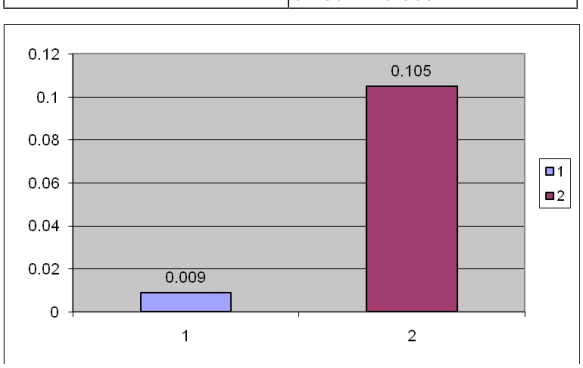
Plant Species	Total Phenolic Content (mg GAE/g)	Flavonoid Content (mg GAE/g)
O.*sanctum*	58.06 ± 0.245	98.71 ± 1.144
O.*gratissiumum*	91.68 ± 0.825	55.27 ± 0.462



TPC & FLAVANOID CONTENTS OF O.*sanctum* (Blue) & O.g*ratissimum* (Red)

Table - II: Ferric Reducing Anti Oxidant Potential (FRAP assay) of O.*sanctum* **& O**.*gratissiumum*

Plant Species	**FRAP values** (µM)
O.sanctum	0.009 ± 0.001
O.gratissimum	0.105 ± 0.005



FRAP CONTENT OF O.*sanctum* **(Blue) & O.**g*ratissimum* **(Red)**

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REFERENCE

1. Abdulrahman ,F .(1992). Studies in natural products: *The Moraceae in* | African traditional medicine and management of psychiatry in Bornu | State. M. Sc thesis, Department of Chemistry, University of Maiduguri | 2. Biswas ,NP.,& Biswas, AK. (2005)Evaluation of some leaf dusts as grain protectant against rice weevil Sitophilus oryzae (Linn.) Environ Ecol. ;23:485–8. | 3. Benzie,I.F.F.,.&Strain J.J.(1999).Ferric reducing/antioxidant power assay:direct measure of total antioxidant activity of biological fluids and modified version of simultaneous measurement of antioxidant power and ascorbic acid concentration.*Methods Enzymol.,299:15-27.* | 4. Chang,SS.,Ostric-Matijasevic,B.,Heisho,AL.,&Haung,CL.(1977).Natural antioxidant from rosemary and Sage.*J.Food sci.42:1102-1076.* | 5. Caragay,AB.(1992).Cancer preventive foods and ingredients.*J.Food Technol.56:65-68.* | 6. Dillard CJ & German JB.*Phytochemicals:nutrace uticals and human health;Journal of the science of Food Agriculture:(2000),80,1744-1756.* | 7. Dhawan, BN., Patnik, G.R., Rastogy, RAT., Singh, K.K.,&Tandol ,TS. (1977). | Screening of Indian plants for biological activity. *YL India Exp. B. 15:108.,* | 8. Farombi ,E.O. (2000). Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. *Pharmacol. Res 42:75-80.* | 9. Gordon, M.C., David, J.N.(2001) Naturan product drug discovery in the next millennium. *Pharm Boil. 2001;39:8–17.* | 10. Halliwell B .Gutteridge .,JMC.(1992).Free radicals,antioxidants and human diseases:*where are we now?J.Lab.Clin.Med.119:598-620.* | 11. Holets FB, Ueda-Nakamura T, Filho BPD, Cortez DAG, Morgado-Diaz JA&Nakamura CV (2003). Effect of essential oil of Ocimum gratissimum on the trypanosomatid *Herpetomonas samuelpessoai.* Act. Protonzool 42: 269-276. | 12. Krishnaiah ,D.,Devi ,T.,Bono, A.,&Sarbatly, R.(2009).Antioxidant properties and stability of ethanolic extracts of holy basil.*J.Med.Plants Res.3(2):67-72* | 13. Kasaikina ,O.T., Kortenska, V.D., Marinova ,EM.,&Rusina IF Yarisbheva NV (1997). *Russ. Chem. Bull. 46: 1070-1073.* | 14. Koleva II, Niederlander HAG & Van Beek TA (2000). An online HPLC method for detection of radical scavenging compounds in complex mixtures. *Anal. Chem. 72: 2323-2328. olic extracts of Holy basil.J.Med.Plants Res.3(2):67- 72.* | 15. Kandasamy Selvam, Rathika Rajinikanth, Muthusamy Govarthanan, AgastianPaul, Thangasamy Selvan Kumar & Arumugam sengottaiyan.(2013)Antioxidant potential and secondary metabolites in Ocimum sanctum at various habitats..*J.Med.Plants Res.Vol.7(12),pp.706-712,2013* | 16. Lalit Mohan,Amberkar,MV.,&Meenakumari. (2011)-Ocimum sanctum Linn.(Tulsi)-an Overview.*Review article Volume 7, Issue 1, March – April 2011; Article-009* | 17. Lako B,EJ.,&Glennie ,VL.(2001).Antioxidant properties of phenolic compounds. *J.Agric.FoodChem.49:1702-1706* | 18. Marinova ,D.,Ribarova, F.,&Atanassova ,M.(2005).Total phenolics and total flavonoids in Bulgarian fruits and vegetables.*J.Univ.Chem.Technol.Mettaurgy 40:255-260.* | 19. Nakamura ,CV., Nakamura ,TU., Bando, E., Melo ,AJN, Cortez DAG & Dias Filho BP (1999). Antibacterial activity of Ocimum gratissimum essential oil. *Mem. Inst. Oswaldo Cruz 94: 675-678.* | 20. Osawa,T.(1994).Novel natural antioxidants for utilization in food and biological systems.In I.Uritiani,V.V.Garcia &E.M.Mendoza(Eds.),Post harvest biochemistry of plant food materials in the tropics(pp.241-251).Tokyo,Japan:-Japan scientific societies press. | 21. Prakash, P.,&Neelu G.(2005). Therapeutic uses of Ocimum sanctum Linn(Tulsi) with a note on eugenol and its pharmacological actions:a short review. *Indian J.Physiol. Pharmacol.49(2):125-131.* | 22. Pessoa, L.M., Morais ,SM., Bevilaqua ,CML.&Luciano ,JHS. (2002). Antihelmintic activity of essential oil of Ocimum gratissimum Linn and eugenol against Haemoachus contortus. *Vet. Parasitol. 109: 59-63.* | 23. Percival ,M.(1997) Nutritional support for connective tissue repair and wound healing:*Clinical Nutrition Insights(1997),5,1-5.* | 24. Rubio J, Qiong W, Liu X, Jiang Z, Dang H, Chen SL et al.(2011) Aqueous extract of black maca (Lepidium meyenii) on memory impairment induced by ovariectomy in mice. *Evid Based Complement Alternat Med. 2011;2011:253958. [PMC free article] [PubMed]* | 25. Rabelo M, Souza EP, Soares PMG, Miranda AV& Matos FJA Criddle DN (2003). Antinociceptive properties of the essential oil of Ocimum gratissimumI L. (Labiatae) in *ice. Bra. J. Med. Biol. Res. 36: 521-52* | 26. Rice-Evans Catherine A.Miller NJ & Pagang G(1996):Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Rad.Biol.Med.20:933-956:* | | 27. Sofowora LA (1993). Medicinal plants and traditional medicine in Africa. | Spectrum Books Ltd, Ibaban. pp. 55-71. | 28. Sulistiarini D, Oyen LPA& Nguyen Xuan Dung (1999). Ocimum gratissimum | L. In*: Plant Resources of South-East Asia. No. 19: Essentialoils | Plants. Prosea Foundation, Bogor, Indonesia. pp. 140-142.* | 29. Shahidi F,Wanasundara PKJPD(1992)Phenolic antioxidants . | Crit.Rev.Food Sci.Nutri 32:67-103. | 30. Sarahroodi S. Self-medication(2012) Risks and benefits. *Int J Pharmacol. 2012;8:58–9.* | 31. Sarahroodi S, Arzi A.(2009) Self-medication with antibiotics, *is it a problem among Iranian College students in Tehran. J Biol Sci. 2009;9:829–32.* | 32. Sarahroodi S, Arzi A, Sawalha A, Ashtarinezhad A. Antibiotics self-medication among Southern Iranian University students. Int J Pharmacol. 2010;6:48–52. | 33. Sarahroodi ,S., Maleki-Jamshid, A., Sawalha, A., Mikaili ,P.,& Safaeian ,L.,(2012) Pattern of Self-Medication with analgesics among Iranian University Students in central Iran. *J Family Community Med. 2012;20:59–63. [PMC free article] [PubMed]* | 34. Sarahroodi, S.,(2006) *Traditional medicine. 2nd ed. Tehran: Hayyan; 2006* | 35. Singleton, V.L., Orthofer, R., and Lamuela-Ravento's, R.M.,(1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Coicalteau reagent. In*: Methods in enzymology (Packer L. ed), San DiegoCA. Academic Press, 299: pp 152-177 (1999).*. | 36. Warrier, PK., (1995)In: *Indian Medicinal Plants. Longman O, editor. New Delhi: CBS publication; 1995. p. 168.* |