Evaluation of Antioxidant activity of Ocimum sanctum extract in comparison with Vitamin C - An In Vitro Study

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Abstract
Introduction: Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called "free-radical scavengers." The sources of antioxidants can be natural or artificial.Periodontitis results from the loss of balance between microbial virulence factors and a proportionate host response.Antioxidant is a substance that is present at low concentrations which significantly delays or prevents oxidation of that substrate.Ocimum sanctum which is a plant extract had a medicinal value and it had been used in asian countries to treat various disease.Ocimum sanctum has an antioxidant agent. Materials and methods: Ocimum Sanctum commercially available powders are used to analyze antioxidant potential. In which three radical scavenging activity analyzed DPPH radical scavenging ,Superoxide anion radical scavenging,Nitric oxide radical scavenging Results: The results from obtained from the reagents shows DPPH radical scavenging activity shows tulsi shows higher activity compared with vitamin c,Superoxide anion radical scavenging,Nitric oxide radical scavenging activity shows vitamin c has higher activity compared with tulsi Conclusion: The above results of the antioxidant activity shows that vitamin c activity is higher in Superoxide anion radical scavenging,Nitric oxide radical scavenging activity of each concentration of tulsi 100$\mu g/ml$,200μg/ml,300μg/ml,400μg/ml,500μg/ml.

1. Introduction

Tulsi is a basil family Lamiaceae (tribe ocimeae) is native to the Indian subcontinent, China, and Southeast Asia and widespread as a cultivated plant throughout the Southeast Asian tropics. [1] Tulsi is known as "Mother Medicine of Nature" with its medicinal properties. [2] Within India, tulsi has been adopted into medicinal value and lifestyle practices that provide the health benefits that are just beginning with modern science. The science on tulsi, in ancient Ayurvedic suggests that tulsi is a tonic for the body, mind and spirit that offers solutions to many modern health problems.Tulsi provides a better lifestyle approach to health. Tulsi which penetrates the deep tissues, dry tissue secretions. Consumption of tulsi gives sweetness to the voice, intelligence, stamina and a calm emotional disposition [3,4,5,6] Tulsi has the properties, which including anxiety, cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, otalgia, indigestion, hiccups, vomiting, gastric, cardiac and genitourinary disorders, back pain, skin diseases, ringworm, insect, snake and scorpion bites and malaria. [7] The seeds, leaves and roots of holy basil traditionally have been ascribed a powerful medicinal value. It is used both internally and externally. Tulsi has the effect of antiseptic and analgesic properties and relieves swelling. The leaves when chewed mitigate gum infections. Fresh juice of the tulsi leaves is an effective domestic remedy for earaches. A tea made with leaves of holy basil is common for cold, cough and mild indigestion. [8]

Periodontitis is a prevalent inflammatory disease, affecting 10% of people worldwide.[9] It can result in the destruction of teeth,pdl and alveolar bone loss that ends up with a loss of teeth. In addition,

doi.org/10.31838/hiv22.02.732 Received: 27.07.21, Revised: 23.08.21, Accepted: 25.09.21

periodontitis also has associations with several systemic diseases, e.g, cardiovascular disease, diabetes, and adverse pregnancy outcomes. [10] Current concept suggests that this inflammatory disease is initiated by bacterial infection and subsequently progressed by aberrant host response, which mainly contributes to periodontal tissue destruction. [11] In recent years, reactive oxygen species (ROS) have gained more and more attention, because of their central role to the progression of many inflammatory diseases. [12] ROS are described as oxygen free radicals and other non-radical oxygen derivatives involved in oxygen radical production. [13]. In which they are involved in normal cellular metabolism. Another category of substances called antioxidants exist in the cells and can effectively delay or inhibit ROS-induced oxidation. ROS are effectively neutralized by antioxidants.When inflammation occurs in the tissues ROS production is drastically increased mainly due to cells of the innate immune system, e.g., neutrophils and macrophages during the process of phagocytosis and respiratory burst. [14] Subsequently, high levels or activities of ROS cannot be balanced by the antioxidant defense system, which leads to oxidative stress and tissue damage. [15] ROS causes tissue damage, involving lipid peroxidation, DNA damage, protein damage, and oxidation of important enzymes; meanwhile, they can function as signaling molecules or mediators of inflammation. [16] Neutrophils have several selective mechanisms for controlling bacterial invasion, including both intracellular oxidative and non-oxidative killing mechanisms. The oxidative killing mechanism of neutrophils and phagocytes involves the formation of reactive oxygen species. ROS generates the neutrophils and requires a minimum oxygen tension of about 1% and a pH of 7.0–7.5. Cells require adequate levels of Antioxidants in order to prevent tissue damage caused by excessive production of reactive oxygen species. [17] Our team has extensive knowledge and research experience that has translated into high quality publications (Choudhari and Thenmozhi, 2016; Govindaraju, Jeevanandan and Subramanian, 2017; Ravi et al., 2017; Vikram et al., 2017; Gupta, Ariga and Deogade, 2018; Hannah et al., 2018; Kavarthapu and Thamaraiselvan, 2018; Pandian, Krishnan and Kumar, 2018; Ramamurthy and Mg, 2018; Ashok and Ganapathy, 2019; Ramesh et al., 2019; Sharma et al., 2019; Venu, Raju and Subramani, 2019; Wu et al., 2019; Samuel, Acharya and Rao, 2020)The aim of this study is to evaluate antioxidant activity of tulsi.

2. Materials and Methods

The commercially available Ocimum sanctum powder was used to identify the antioxidant activity In which three radical scavenging activities were analyzed. DPPH radical scavenging,Superoxide anion radical scavenging,Nitric oxide radical scavenging.

DPPH free radical scavenging activity of plant extract

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al. (1989).

Principle:

The scavenging reaction between DPPH and an antioxidant of the sample (H-A) can be written as:

DPPH + (H-A) DPPH-H + (A)

Figure 1-shows the commercially available Tusli

(Purple) (Antioxidant) (Yellow)

Antioxidants of the sample react with DPPH which is a stable free radical and gets reduced to the DPPH-H and as a consequence the absorbance decreases from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds of the extracts in terms of hydrogen donating ability.

Reagents

1. Methanolic solution of DPPH (0.1mM): DPPH (19.7mg) was dissolved in 500ml of analytical grade methanol.
2. Ascorbic acid (1%): Ascorbic acid (1g) was dissolved in 100 ml of methanol.
3. Extract preparation (Stock): Each extract (50mg) were dissolved in 50 ml of analytical grade methanol. The required concentrations of the extracts were diluted accordingly from the stock.
4. Extract preparation (working) [Eg. 5µl/ml]:

The extract of 0.005ml (5µl) was made up to 1ml (1000µl) by the addition of 995 µl of water.

Procedure

DPPH solution (1.0 ml) was added to 1.0 ml of plant extract different concentrations (100-500µg/ml). The

mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at various concentrations thulsi chooranam (100-500µg/ml) was used as standard. The percentage of free radical inhibition was calculated as IC50. It denotes the concentration of the sample required to scavenge 50% of DPPH free radical. The capability to scavenge the DPPH radical was calculated using the following formula,

DPPH radical scavenging (%) =Control OD-Sample OD/Control ODX100

Figure 2-shows the DPPH solutions

Nitric oxide radical scavenging activity

Scavenging of nitric oxide radical was assayed by the method of Garrat, (1964).

Principle

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 which compete with oxygen, leading to reduced production of nitrite ions.

Reagents

1. Sodium nitroprusside (10 mM):
Sodium nitroprusside (29.79mg) was dissolved in 100 ml of double distilled water.
2. Phosphate buffer saline (0.1M, pH 7.4):
Sodium chloride (0.8g), 0.2g potassium chloride (KCl), 1.44g sodium orthophosphate (NaHPO4) and 0.024 g of potassium dihydrogen phosphate (KH2PO4) were dissolved in 80ml of double distilled water and the pH was adjusted to 7.4 and was made up to 100ml with double distilled water.
3. Sulfanilic acid (0.33% w/v):
Sulfanilic acid (330mg) was dissolved in 100 ml of 20% acetic acid.
4. Naphthyl ethylenediamine dihydrochloride (0.1%, w/v):
Naphthyl ethylenediamine dihydrochloride (100mg) was dissolved in 100ml of double distilled water.
5. Extract preparation (Stock):
Each extract (100mg) were dissolved in 100ml of analytical grade methanol. The required concentrations of the extracts were diluted accordingly from the stock.
6. Extract preparation (working) [Eg. 100µl/ml]:
The extract of 0.1ml was made up to 1ml by the addition of 900 µl of water.

Procedure

The reaction mixture (3ml) containing sodium nitroprusside (10mM, 2 ml), phosphate buffer saline (0.5 ml) and different concentrations of extracts of tulsi Chooranam (100-500µg/ml) were incubated at 25C for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted out and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% acetic acid) and allowed to stand for 5 minutes for completing diazotization. Then, 1 ml of naphthyl ethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 minutes at 25°C. A pink colored chromophore is formed in diffused light. Ascorbic acid at various concentrations (100-500µg) were used as standard. The activity was measured at 550 nm and the results were expressed as % of scavenging using the following formula,

Nitric oxide radical scavenging (%) = Control OD-Sample OD/Control ODX100

Superoxide anion scavenging activity

Scavenging of superoxide anion activity was assessed by the method of Liu et al. (1997).

Principle

Superoxide anion is generated by the Phenazine methosulphate-NADH (PMS-NADH) system by oxidation of NADH and is assessed by the reduction of nitroblue tetrazolium (NBT).

Reagents

1. Tris-Hcl buffer (16µM, pH 8.0):
Tris-HCl (126.08) was dissolved in 40 ml of double distilled water. pH was adjusted to 8.0 and then made up to 50 ml with double distilled water.
2. Nitroblue tetrazolium (NBT) (50µM):
Nitroblue tetrazolium (408.82 mg) was dissolved in 10 ml of double distilled water.
3. Phenazine methosulphate (PMS) (10µM):
Phenazine methosulphate (30.63 mg) was dissolved in 10ml of double distilled water.
4. NADH (78µM) for 10 ml:
NADH (517.48 mg) was dissolved in 10ml of double distilled water.
5. Extract preparation (Stock):
Each extracts (100mg) were dissolved in 100ml of analytical grade methanol. The required concentrations of the extracts were diluted accordingly from the stock.
6. Extract preparation (working) Eg. [100µl/ml]:
The extract of 0.1ml was made up to 1ml by the addition of 900 µl of water.

Procedure

Superoxide anions were chemically generated in a mixture of phenazine methosulphate (PMS) and

NADH. The reaction was quantified by coupling superoxide generation to the reduction of nitroblue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3ml of Tris-Hcl buffer (16mM, pH 8.0) containing 1ml of NBT (50 µM), 1ml of NADH (78 mM) and 1ml of various concentrations (100- 500 µg/ml) of A.cepa varieties extracts. Ascorbic acid at various concentrations (100,200,300,400 and 500µg) were used as standard. The reaction mixture was incubated at 25°C for 5 minutes and the activity was measured at 560 nm. Results were expressed as % of scavenging using the following formula,

Superoxide anion scavenged (%) = Control OD-Sample OD/Control ODX100

3. Statistical Analysis

Antioxidant activity of tulsi was calculated in SPSS 2.0 version in which ANOVA analysis of variances has been detected in DPPH radical scavenging,Nitric oxide radical scavenging activity and Superoxide anion scavenging activity.

4. Results

The results from the antioxidant activity of tulsi of DPPH radical scavenging(Figure 3)shows that in 100μg/ml shows vitamin c has higher concentration compared with tulsi,200μg/ml,300μg/ml,400μg/ml,500μg/ml shows tulsi has higher concentration compared with vitamin C.In superoxide anion radical scavenging (Figure 4) 100μg/ml,200μg/ml,300μg/ml,400μg/ml,100μg/ml show that Vitamin C has higher concentration compared with tulsi.In Nitric oxide radical scavenging(Figure 5) shows that 100μg/ml,200μg/ml,300μg/ml,400μg/ml,100μg/ml show that Vitamin C has higher concentration compared with tulsi.The results of the study shows that DPPH radical scavenging show the tulsi has highest antioxidant activity compared with Superoxide anion scavenging and Nitric oxide radical scavenging.

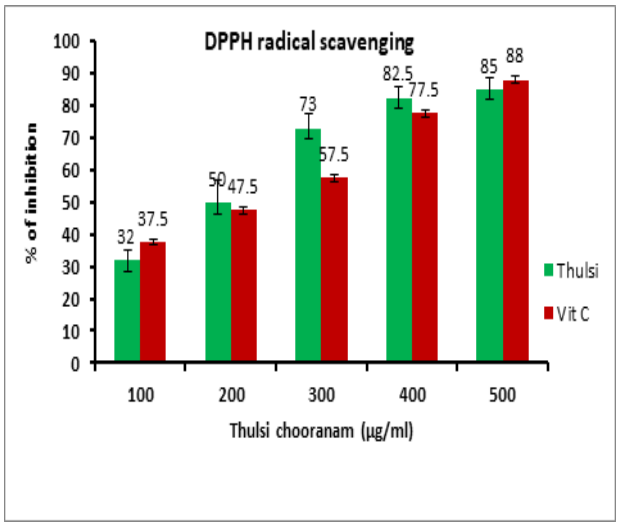

Figure 3-shows in DPPH radical scavenging between Tulsi and VitaminC

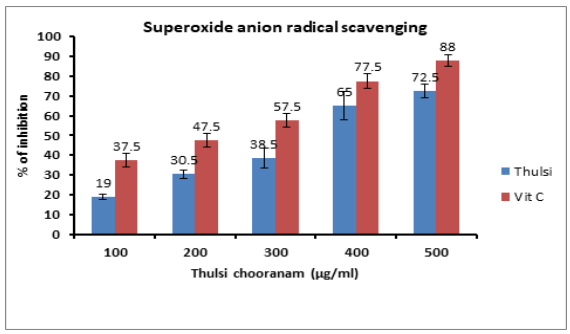

Figure 4-shows the results of Superoxide anion radical scavenging between Tulsi and VitaminC

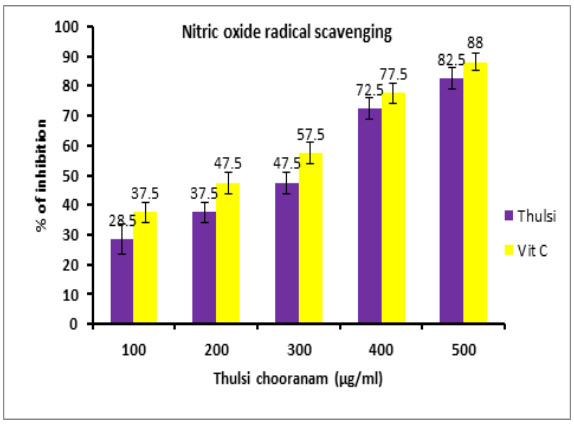

Figure 5-shows Nitric oxide radical scavenging between Tulsi and VitaminC

5. Discussion

Medicinal plants are the sources of natural antioxidants and represent the discovery of new drugs in the therapeutic disease. Most members of the Lamiaceae family have exhibited interesting biological effects due to their antioxidant compounds. [18]Ocimum sanctum has various properties such as antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective and antioxidant. [19] Leaves of Ocimum sanctum contain water-soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophyllene that may act as an immunostimulant. Saponins act as antihyperlipidemic, hypotensive and cardio depressive properties. [20] The accumulations of free radicals in organs or tissues are strongly associated with oxidative damages in biomolecules and cell membranes. This can lead to many chronic diseases, such as inflammatory, cancer, diabetes, aging, cardiac dysfunction, and other degenerative diseases. [21] The relationship between oxidative stress and periodontal disease is quite strong and can be a two-way path. Periodontal inflammation increases the number of oxidative stress markers, and it tends to potentiate aspects of periodontal destruction. [22] Ocimum sanctum has antioxidant activity through the analysis and DPPH radical scavenging analysis. It is concluded that there is a good antioxidant potential of Ocimum sanctum with ethanolic Soxhlet extraction. [23,24] Superoxide is a

reactive oxygen species that can damage cells and DNA, leading to various diseases. This assay was determined by NBT assay and the value ranges from 12.04% to 60.16% methanol leaves extracted respectively at a concentration 10-500 µg/mL. While that of the control, ascorbic acid the inhibition percentage ranges from 10µg/mL to 500µg/mL.The methanolic leaves extracts of O. sanctum had strong antioxidant activity against all the free radicals.The DPPH radical is widely used in assessing free radical scavenging activity was 65.75% in methanol respectively at a concentration of 500µg/mL leaves extracts.

6. Conclusion

Within the limitation of the study we are able to identify the antioxidant activity of ocimum sanctum.From the aqueous extract of ocimum sanctum antioxidant activity were analyzed by DPPH free radical scavenging ,Nitric oxide radical scavenging and Superoxide anion radical scavenging.We have found that Vitamin C has higher activity compared with tulsi.

Further studies has to be done before using this novel product as mouthwash in patients with periodontal disease

7. Acknowledgement

The authors are thankful to the Director of academics,Chancellor and Dean of Saveetha Dental College and Hospitals for providing a platform to do research activities

8. Conflict of Interest

The authors declare no potential conflict of interest

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