Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(3): 142-145
Received: 28-03-2016
Accepted: 30-04-2016

Khursheed Ahmad Ansari
Assistant Professor, Faculty of Unani Medicine, Jamia Hamdard, New Delhi, India

Correspondence:
Khursheed Ahmad Ansari
Assistant Professor, Faculty of Unani Medicine, Jamia Hamdard, New Delhi, India

To determine the anti-bacterial properties of Reihan (*Ocimum sanctum*) and its efficacy in reduction of cariogenic pathogen

Khursheed Ahmad Ansari

Abstract
Aim: The aim of this study was to determine the anti-bacterial properties of tulsi (*Ocimum sanctum*) and its efficacy in reduction of cariogenic pathogen namely *Streptococcus mutans* in oral cavity under *in vitro* conditions.
Methods: The study was carried out to determine and compare the anti-bacterial properties of three extracts obtained from Tulsi on *Streptococcus mutans*, a cariogenic pathogen. A total of 48 culture plates per plant including 16 plates per extract served as sample size for the study.
Results: From total 30 samples, Tulsi categorized into 10 leaf extract samples, 10 stem extract samples and 10 root extract samples. The result depicted the zones of inhibition (mm) for leaf, stem and root extracts of Tulsi and Positive control against *Streptococcus mutans*, with mean values equivalent to 22.80 mm, 20.40 mm and 21.20 mm (for Tulsi) and 23 mm, respectively for positive control (Cefotaxime).
Conclusion: Tulsi (*Ocimum sanctum*) is most sacred and valuable medicinal plant which is used for treatment of bacterial, viral, fungal and insecticidal diseases from long time in the whole world. *O. sanctum* has many beneficial properties which are useful for human health without showing any side-effect.

Keywords: Phytoextract, *Ocimum sanctum*, antimicrobial

Introduction
Oral diseases are among the major health issues wherein dental caries and periodontal diseases are the most commonly occurring problems. Oral cavity is cohabitated by over 750 bacterial species of which only 50% related to the pathogenesis of oral diseases such as dental caries. There has been a rise in the oral disease incidence since 1998, particularly in the developing nations like India due to dietary habits, low awareness among the population regarding the oral health, increased antibiotic resistance exhibited by the pathogenic bacteria, opportunistic oral infections in subjects with compromised immunity and low per capita income [1].
Normal commensals of the oral cavity such as *Streptococcus mutans* (MS) species actively participate in the initiation and progression of dental caries [2]. The bacterial population in oral cavity undergoes constant changes because of changes in local environment (pH, for example) of the oral cavity, salivary effects and changes in colony population mainly due to weak or suppressed immunity [3].
The Tulsi (*Ocimum sanctum*) is one of the most valued and holistic medicinal plant which is having medicinal importance and is used for the preparation of traditional medicines from many years in India. Tulsi has been described as 'Queen of Herbs' and 'Mother of Medicine of Nature' because of many useful medicinal properties [4]. This plant is widely growing in India and many other countries of South-East Asia. *O. sanctum* is commonly known as Tulsi in India. It is traditionally important medicinal herb containing many useful compounds [5]. About 85% population of the whole world partially or wholly is dependent on herbal medicines for the treatment of primary health related issues. According to traditional medical system, herbal medicines are the major remedies.
Tulsi has been used in medical practices from thousands of years and it has a great contribution to maintaining human health [6]. The whole plant of Tulsi is used in medicines and has been found to possess various therapeutic properties and many useful phytochemicals which act as antimicrobial agents against pathogenic microbes. Tulsi has therapeutic application in cardiovascular disorders according to the ancient system of medicines including Greek, Ayurveda, Siddha, Unani and Roman [7].

Tulsi is used in different forms such as extract and essential oil in medicines from ancient in India. Tulsi extract is commonly used for various medicines for therapeutic uses to treat many diseases such as fever, headache, malaria, heart diseases and inflammation. Tulsi leaves and inflorescence are mostly used in the extraction of oil, this Tulsi oil have numerous properties such as hepatoprotective, immune-modulatory, hypotensive, stress reducer, inflammation reliever and anti-asthmatic [8]. The extract and essential oil of *O. sanctum* is found to possess insecticidal and antibacterial properties [7]. In recent years, Tulsi is used in different forms in Indian medicines such as the aqueous extract from leaves (fresh and dried powder) or oil of extract. Tulsi extract is commonly used for various medicines for therapeutic uses for treatments of many diseases such as fever, headache, malaria, heart diseases and inflammation. Aqueous Tulsi extract and essential oil of Tulsi seed has been used as an antimicrobial ingredient in 'Food Preservation' to inhibit the bacterial and fungal infestation [9, 10].

The aim of this study was to determine and compare the anti-bacterial properties of tulsi (*Ocimum sanctum*) to evaluate the efficacy of these medicinal plant extracts in effective reduction of cariogenic pathogen namely *Streptococcus mutans* in oral cavity under *in vitro* conditions.

Methods

The study was carried out to determine and compare the anti-bacterial properties of three extracts obtained from Tulsi on *Streptococcus mutans*, a cariogenic pathogen. A total of 30 culture plates per plant including 10 plates per extract served as sample size for the study.

For extract preparation

1. Shade dried plant parts (leaves, stems and roots)
2. Mortar and pestle
3. 80% ethanol
4. Conical glass flasks
5. Sonicator bath (ELMASONIC)
6. Whatman's filter paper (A1, 125mm)
7. Spherical evaporating flasks
8. Rotary vacuum Evaporator (BUCHI INDIA PVT. LTD.)
9. Eppendorf tubes

For determination of Zones of inhibition

1. Culture petriplates
2. Inoculating loops, tips
3. Hot air oven
4. Incubator
5. Culture media (Brain Heart Infusion Broth, Blood Agar and Mueller Hilton Blood Agar) (Hi-MEDIA, India)
6. Hydrogen peroxide (H_2O_2).

Methodology

The plant materials belonging to study plants (*Ocimum sanctum*) were collected. The main reason for selection of the above-mentioned plants for the study were:

1. Easy availability of the plants in the Indian subcontinent.
2. Cost effectiveness.

Streptococcus mutans was selected as the test organism because it plays a major role in the progression of the process of formation of dental caries and many other dental ailments.

Preparation of extract

The leaves, stems and roots of both the plants were shade dried and powdered using mortar and pestle and then, extracted by process of successive extraction. Firstly, 60 gms each of the powdered plant materials was put into 50 ml of 80% ethanol in individual conical flasks. The flasks were rested for 30 minutes and then, subjected to sonication for 30 minutes at 45 °C three times to extract the constituents from the plant materials. Prepared extracts were filtered using Whatman's filter paper all three times. The extracts obtained by filtration were transferred into spherical flasks and concentrated to dryness by evaporating the solvent using reduced pressure and heat in a rota-vapour apparatus. Pressure dried extracts were taken out of the flasks by scraping and transferred to Eppendorf tube.

Chemicals and culture media

Bacterial cultivation was done on Brain Heart Infusion Broth (Hi-MEDIA, India) and Blood Agar (Hi-MEDIA, India). Thereafter, culturing on Mueller Hilton Blood Agar (Hi-MEDIA, India) was done for analyzing extract's sensitivity.

Culture preparation methods

For brain heart infusion broth

1. Dissolved 37 g media in 1000 ml of distilled water.
2. Mixed well and distributed into final containers.
3. Sterilized by autoclaving at 121 °C for 15 minutes
4. Introduced test organism into each container with the help of the pure culture sample stick of *Streptococcus mutans* species (ATCC 25175) manufactured by Hi- MEDIA.
5. Kept overnight for growth of the test organism at 37 °C.

For Blood Agar (Test Organism Sub-culturing)

1. Prepared the blood agar base as per manufacturer's guidelines.
2. Autoclaved at 121 °C for 15 minutes at 15 lbs pressure and transferred to 50 °C water bath to cool it down.
3. Added sterile blood and mixed gently to avoid air bubble formation.
4. Dispensed 15 ml of prepared agar into sterile petriplates, aseptically.
5. Labeled the petri-plates and let the agar material to set.
6. The opalescent growth of the test organism obtained after overnight incubation in BHI broth was transferred to Blood agar for sub-culturing for 24 hours at 37 °C.

For Mueller Hilton Blood Agar

1. Suspended 38.0 grams of commercial media in 1000 ml of distilled water.
2. Heated to boil and to dissolve the medium completely.
3. Sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.
4. Cooled to 45-50 °C.
5. Added 50 ml Human blood obtained
6. Mix well and pour into sterile petri-plates.

7. Replace the lid of each petriplate and store the plates in a refrigerator.
8. The colonies obtained by the overnight growth on blood agar were then transferred to MHB agar and incubated for 1 hour.
9. Three wells of 6mm diameter were bored [Picture 3] in the medium with the help of sterile borer having 6mm diameter and were labeled properly and ten microliter of the working suspension i.e., 0.1gmof crude extract/100ml of DMSO (Picture 2) of different crude extracts of both Neem and Tulsi along with 30mcg of Cefotaxime (positive control) and ten micro-litre of DMSO (negative control) in different petri-plates, were filled in the wells with the help of micropipette.
10. Plates were left for some time (5-10 minutes) till the extract diffuse in the medium with the lid closed and incubated at 37 °C for 48 hours and zone of inhibitions were measured using scale and mean were recorded.

Results

Table 1: Distribution of total sample

Total no of sample	Nature of phytoextracts
30	Tulsi (*Ocimum sanctum*) 10 – Leaf sample 10 – Stem sample 10 – Root sample

From total 30 samples, Tulsi categorized into 10 leaf extract samples, 10 stem extract samples and 10 root extract samples.

Table 2: Antibacterial activity of different Tulsi extracts concentration 0.1gm/ml (10ml) on *Streptococcus mutans* in agar well diffusion method

S. No.	Tulsi Leaf (mm)	Tulsi Stem (mm)	Tulsi Root (mm)	Positive Control (Cefotaxime)
1	22	22	20	22
2	18	22	22	22
3	26	20	26	20
4	22	22	24	23
5	32	16	18	27
6	20	20	18	23
7	26	20	28	20
8	16	20	18	26
9	24	22	20	24
10	22	20	18	23
Mean	22.80	20.40	21.20	23

Table 2 depicts the zones of inhibition (mm) for leaf, stem and root extracts of Tulsi and Positive control against *Streptococcus mutans*, with mean values equivalent to 22.80 mm, 20.40 mm and 21.20 mm (for Tulsi) and 23 mm, respectively for positive control (Cefotaxime).

Discussion

Tulsi (*O. sanctum*) plant has the property to inhibit the growth of pathogenic microbes such as bacteria, fungus and viruses. Antimicrobial properties of *O. sanctum* (Shyam Tulsi) have been found to be higher as compared to commonly available other species i.e. *O. gratissimum, O. canum* and *O. basilicum*, etc. in India [12]. The aqueous extract, seed oil and alcoholic extract of *O. sanctum* exhibited antimicrobial properties against enteric pathogens [11, 13]. The Tulsi extract and essential oil is effective against gram-positive and gram-negative bacteria [14]. Tulsi extract has also shown significant antimicrobial properties against some of the multi-drug resistant and clinical isolates of Neisseria gonorrhoeae [15].

Asper estimation (World Health Organization), at least 25% of all modern drugs are synthesised either directly or indirectly from plants and their products playing an important role in treatment of diseases worldwide and affecting millions of people. A wide range of various plant and herb species with great medicinal potential are found in the tropical and subtropical regions of India. Among the vast variety of secondary metabolites found in these plants are compounds such as flavonoids, terpenoids, tannins, glycosides and alkaloids. These metabolites serve as important agents of antimicrobial crude drugs and source of other anti-infection compounds [16]. Development of antibacterial drug resistant microorganisms is leading to the occurrence of many bacterial diseases in south Asia, particularly in India. To handle this problem it has become a global challenge to use newer sources to develop other therapeutic reforms [17].

In an earlier study, Agarwal *et al*. in 2010 [18] demonstrated that among all the extracts of *Ocimum sanctum*, extract at 4% concentration showed highest antibacterial activity against MS with an inhibition zone of 22mm, almost equivalent to the results of our study where ZOI was found to be 22.80mm for Tulsi. In another study by Ali H *et al*. (2012) [19], it was concluded that Tulsi at a concentration of 400mg/ml showed inhibitory zones ranging from 19.55 mm to 20.95 mm against E.coli, Proteus and other strains of Staphylococcus, showing almost similar results when compared to the current study.

Goyal P *et al*, 2011 [20] demonstrated the antibacterial activity of the steam distilled extract of Tulsi and concluded that the extracts showed zones of inhibition ranging from 30mm – 39 mm against different bacteria. However, this study showed significantly greater results when compared to the results of our study (ZOI= 22.80 mm). The reason for greater zones of inhibition can be attributed to the technique of steam distillation and the bacterial species studied. Steam distillation leads to the extraction of only the active volatile aromatic compounds like essential oil and eugenol that are responsible for greater antibacterial potential.

Conclusion

Tulsi (*Ocimum sanctum*) is most sacred and valuable medicinal plant which is used for treatment of bacterial, viral,

fungal and insecticidal diseases from long time in the whole world. *O. sanctum* has many beneficial properties which are useful for human health without showing any side-effect. Thus, these beneficial properties made to this plant unique from others. Tulsi leaves extract contain active components which act as an antimicrobial agent.

References

1. Bhardwaj A, Bhardwaj S. Ethno-dentistry: popular medicinal plants used for dental diseases in India. J Intercult Ethnopharmacol. 2012;1(1):62-65.
2. Loesche WJ. Role of *Streptococcus mutans* in Human Dental Decay. Microbiol Rev; c1986. p. 353-380.
3. Fani M, Kohanteb J. Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria. J Oral Sci. 2012;54(1):15-21.
4. Anbarasu K, Vijayalakshmi G. Improved shelf life of protein-rich tofu using *Ocimum sanctum* (tulsi) extracts to benefit Indian rural population. Journal of food science. 2007 Oct;72(8):M300-5.
5. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacognosy reviews. 2010 Jan;4(7):95.
6. Rahman S, Islam R, Kamruzzaman M, Alam K, Jamal AH. *Ocimum sanctum* L.: A review of phytochemical and pharmacological profile. American journal of drug discovery and Development. 2011;1:15.
7. Sethi J, Sood S, Seth S, Talwar A. Protective effect of Tulsi (*Ocimum sanctum*) on lipid peroxidation in stress induced by anemic hypoxia in rabbits. Indian J Physiol Pharmacol; c2003.
8. Shokeen P, Bala M, Singh M, Tandon V. *In vitro* activity of eugenol, an active component from *Ocimum sanctum*, against multiresistant and susceptible strains of Neisseria gonorrhoeae. International journal of antimicrobial agents. 2008 Aug 1;32(2):174-179.
9. Raghav PK, Agarwal N, Saini M. Herbal edible coatings of fruits & vegetables: A newer concept. International Journal of Advanced Research. 2016 Jun;4(6):1452-1458.
10. Prakash PA, Gupta N. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian journal of physiology and pharmacology. 2005 Apr 1;49(2):125.
11. Williamson EM. Major herbs of Ayurveda, Londen: Churchill Livingstone; c2002.
12. Sharma A. MVSc., Thesis. Department of Veterinary Microbiology and Immunology, DUVASU, Mathura, UP, India; c2010.
13. Sinha GK, Gulati BC. Antibacterial and antifungal study of some essential oils and some of their constituents. Indian perfumer. 1990;34(2):126-129.
14. Uma Devi P, Ganasoundari A, Vrinda B, Srinivasan KK, Unnikrishnan MK. Radiation protection by the ocimum flavonoids orientin and vicenin: mechanisms of action. Radiation research. 2000 Oct;154(4):455-460.
15. Prasad G, Kumar A, Singh AK, Bhattacharya AK, Singh K, Sharma VD. Antimicrobial activity of essential oils of some Ocimum species and clove oil. Fitoterapia. 1986;57(6):429-432.
16. Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK, *et al*. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evidence-Based Complementary and Alternative Medicine, 2013, Oct.
17. Dias DA, Urban S, Roessner U. A Historical Overview of Natural Products in Drug Discovery. Metabolites. 2012;2(2):303-336.
18. Agarwal P, Nagesh L, Murlikrishnan. Evaluation of the antimicrobial activity of various concentrations of Tulsi (*Ocimum sanctum*) extract against *Streptococcus mutans*: an *in vitro* study. Indian J Dent Res. 2010;21(3):357-359.
19. Ali H, Dixit S. *In vitro* antimicrobial activity of flavanoids of *Ocimum sanctum* with synergistic effect of their combined form. Asian Pacific Journal of Tropical Disease. 2012 Jan 1;2:S396-S398.
20. Goyal P, Kaushik P. *In vitro* Evaluation of Antibacterial Activity of Various Crude Leaf Extracts of Indian Sacred Plant, *Ocimum sanctum* L. Br Microbiol Res J. 2011;1(3):70-78.