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Research Article

EVALUATION OF ANTI-MICROBIAL ACTIVITY OF *OCIMUM SANCTUM* METHANOLIC EXTRACT

Bhatt Mehul K[1]*, M. B. Shankar[1], Saluja Ajay K[2], Dholwani Kishor K[2], Captain A. D. [2]
[1]Shivam Pharmaceutical studies & Research center, Valasan, India
[2]A. R. College of Pharmacy, Vallabh Vidyanagar, India
Email: mkb_0999@yahoo.co.in

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ABSTRACT
The present study was conducted to investigate anti-microbial activity of *Ocimum sanctum* methanolic extract against strains of gram positive and gram negative bacteria. Tulsi plant is known to possess therapeutic potentials and have been used, by traditional medicinal practitioners, as an expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic, anti-microbial, antifungal activity against *Asperigillus niger*. The extract was tested for its antimicrobial activity against Gram-positive bacteria like *Bacillus subtilis* and Gram-negative bacteria like *Escherichia coli.* Inhibition of microbial growth was investigated using agar well diffusion method. UV-Visible and HPLC analysis of the extract was carried out for the presence of eugenol.
Keywords: Antimicrobial, Eugenol, *Ocimum sanctum*, Well diffusion method, HPLC.

INTRODUCTION

The anti-biotic era started in the 1950s, and from then onwards the use of plant anti-microbial declined[1]. Although, it was not the case so far as the traditional healing systems that heavily rely on the medicines from the natural sources, especially plants, are concerned. The emergence and spread of microbial resistance is growing each day, thereby necessitating the development of new anti-microbial of natural or synthetic origin[2]. As far as the sources are concerned, apart from the microbial sources, plants appear to be valuable anti-microbial resources. Plants can produce a large number of secondary metabolites that may exceed a hundred thousand molecules[3], all of these don't have anti-microbial potential, but some of them can produce significant activity against the human pathogens. One of the species that emerged from such an inventory is *Ocimum sanctum*(Family: Labiateae)[4].*Ocimum sanctum L.,* known as 'Tulsi' in Hindi and 'Holy Basil' in English, is an erect softy hairy aromatic herb or under shrub found throughout India. Tulsi is commonly cultivated in gardens. Two types of *Ocimum sanctum L.* are met within cultivation: (1) Tulsi plants with green leaves known as Sri Tulsi & (2) Tulsi plants with purple leaves known as Krishna Tulsi. *Ocimum sanctum L.* is held sacred by Hindus and is used as medicinal plants in day to day practice in Indian homes for various ailments. In ayurveda tulsi has been well documented for its therapeutic potentials and described as Dashemani, Shwasaharni (anti-asthmatic) and anti-kaphic drugs (Kaphaghna). Several medicinal properties have been attributed to *Ocimum sanctum* L. Different parts of Tulsi plant e.g. leaves, flowers, stem, root, seeds etc. are known to possess therapeutic potentials and have been used, by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic, anti-microbial, antifungal activity against *Asperigillus niger*. Aqueous extract of Tulsi is found effective in patients suffering from viral encephalitis. Although the traditional medical practitioners in India have been widely using this medicinal plant for management of various disease conditions from ancient time, not much is known about the mode of action of tulsi and a rational approach to this traditional medical practice with modern system of medicine is also not available. In last few decades several studies have been carried out any Indian scientists and researchers to suggest the role of essential oils & eugenol in therapeutic potentials of *Ocimum sanctum* [5-14]. Eugenol is a phenolic compound and major constituent of essential oil extracted from different parts of tulsi plant. The therapeutic potential of tulsi has been established on the basis of several pharmacological studies carried out with eugenol and steam distilled, petroleum ether and benzene extracts of different parts of tulsi plant. The aim of this study was the antimicrobial activities of methanolic extract of *Ocimum sanctum* against range spoilage bacteria, evaluating zone of inhibition.

MATERIAL AND METHODS:

Plant Material:

The whole herb of *Ocimum sanctum* was collected from the botanical garden of the Shivam Pharmaceutical Studies & Research Centre, Valasan. The drug was authenticated at the Pharmacognosy department of the shivam pharmacy college, Valasan. A Voucher specimen (MKB/Os-55/SPSRC-2012) has been deposited to Shivam Pharmacy College, valsan.

Preparation of the Extracts:

The shade dried, powdered whole herb (250gm) of *Ocimum sanctum* were defatted by extracting with petroleum ether (60-80°C), followed by extraction with methanol using Soxhlet's extractor. The methanolic extract was then concentrated using rotary flash evaporator to a syrupy consistency. The residual solvent was removed by drying the extract in vacuum oven (yield - 25.5gm).

Microorganisms:

The following bacterial strains were used in the antimicrobial tests. Gram positive bacteria *Bacillus subtilis* (ATCC 6633). Gram negative bacteria were *Escherichia coli*. All microbial strains were obtained from the Bio-science department Vallabh Vidyanagar. In vitro antimicrobial activity was determined by using nutrient agar (Himedia Laboratories Pvt. Ltd., Mumbai). Each medium was autoclaved at 121° C, 15 psi for 15 min before inoculation. The bacteria used in the tests were obtained from 24 h cultures.

Antimicrobial Activity:

Antimicrobial activity of methanolic extract was determined using agar well diffusion method. About 15ml of sterilized

selective agar based mediums were added aseptically to sterile plates to prepare a basal layer. The plates were incubated at 37° C ±0.5° C for 24 hrs. The basal layer was seeded the next day with 7ml of sterilized selective agar based medium containing 1ml of suspension of standard inoculums. The plates were allowed to set. Each Petri dish was divided into four sectors, and in each sector a bore of 6mm diameter was made using sterilized borer in the solidified medium. Using sterilized dropping pipettes, each bore in different sector was carefully loaded with 75µl of Methanolic extract of *Ocimum sanctum* and allowed to diffuse at room temperature for 2 hrs. The plates were then incubated at 37°C for 24 hrs for bacteria. The zone of inhibition of growth of microorganisms around the well was measured in cm, with the help of a scale.

UV-Visible analysis of the Methanolic extract:

Methanolic extract of *Ocimum sanctum* was subjected to UV-Visible analysis to detect the presence of eugenol (200-400nm).

HPLC analysis of the Methanolic extract:

Apparatus and reagents

The chromatographic system includes an ELICO HL-459 MODEL gradient pump, a stainless steel injector (5 _L loop), and a UVVIS detector (operated at 282 nm for *Ocimum sanctum* extract. A Chromolith RP-18 column (Inertsil 7 ODS-3 4.6 mm i.d., 250 mm, Merck) was used as analytical column. The optimal compositions of the mobile

phase is 80% solution A (Diluted water with 1% acetic acid) and 20% Acetonitrile. The flow rate of the mobile phase was 1 ml/min and the column temperature was kept at 25°C. The sample solution and reagent solution were degassed before each run. The Soxhlet extracting method was used for extracting the eugenol from the desire samples.

UV-Visible Spectra was taken. All reagents, such as methanol, ethanol, acetonitrile, acetic acid, etc, are HPLC grade from Merck (Darmstadt, Germany). Reagents were degassed in an ultrasonic bath as required before injecting into HPLC.

RESULT & DISCUSSION:

Antimicrobial Activity:

The minimum inhibitory concentration (MIC) of the methanolic extract against different microorganisms is tabulated in Table 1. The studied concentration of the methanolic extract was 5 mg exhibited antimicrobial activity against the test microorganisms with zone sizes 2.5cm & 2.3cm respectively. The minimum inhibitory concentration of the methanolic extract was found 65 & 64 mg respectively against the different test organisms. The photographs of the plates exhibiting antimicrobial activity against the different test microorganisms are shown in Figure1 & 2 respectively.

UV-Visible analysis:

UV- Visible analysis of the Methanolic extract of *Ocimum sanctum* revealed presence of eugenol when compared with the standard UV data of the eugenol. (Figure 3 & 4)

HPLC analysis:

HPLC Chromatogram of standard eugenol and *Ocimum sanctum* extract shows the Peak of same retension time. (Figure 5)

CONCLUSION:

Eugenol is the most prominent phytoconstituent present in the Tulsi plant which may be responsible for anti-microbial activity.

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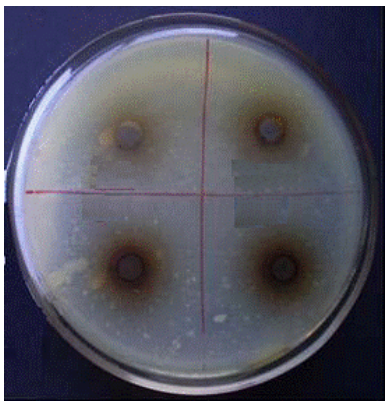

Figure 1: Effect methanolic Extract of *Ocimum sanctum* on *E. coli*

Figure 2: Effect methanolic Extract of *Ocimum sanctum* on *B. subtilis*

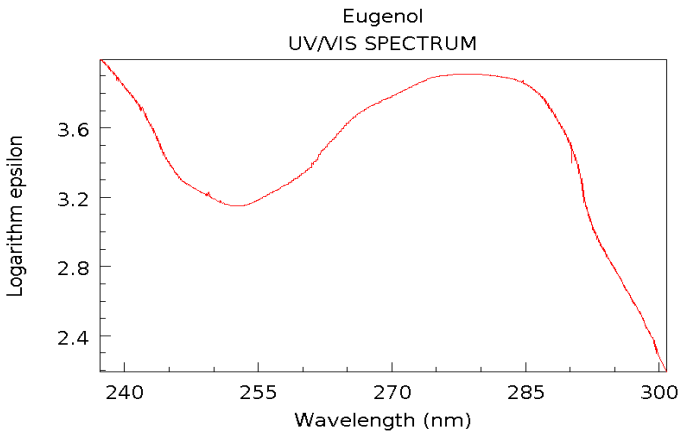

Figure 3: UV-Visible analysis of Standard eugenol, λ_{max} 282nm

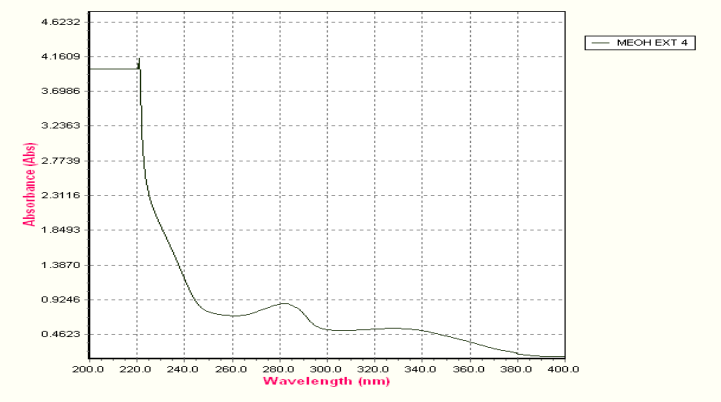

Figure 4: UV-Visible analysis of Methanolic extract of Ocimum sanctum, λ_{max} 282nm

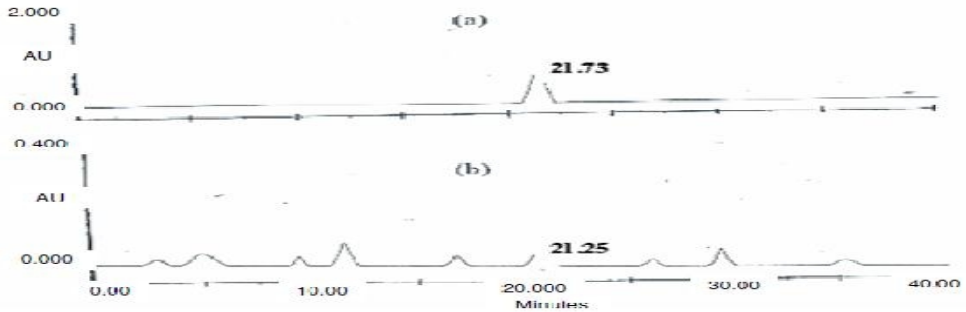

Figure 5. HPLC Chromatogram of (a) eugenol and (b) *Ocimum sanctum*. Mobile phase was 80% Diluted water (with 1% acetic acid) and 20%Acetonitrile at pH 6.5; analytical column: Inertsil 7 ODS-3 4.6 mm i.d. x 250 mm; 1 ml/min; 25°C; injection volume: 5µL; detection at 280 nm.

Table 1: Antimicrobial Activity of Methanolic extract of *Ocimum snactum* against tests organisms

Organism	Zone of Inhibition (cm) at MIC	Minimum inhibitory Concenteration (MIC) mg/ml.
Bacteria(Gram Positive)		
Bacillus subtilis	2.5 cm	65
Bacteria (Gram negative)		
Escherichia coli	2.3 cm	64

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