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Phytochemical screening, antimicrobial, antioxidant and anticancer activity of O*cimum Sanctum* plant extract

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Abstract: *Ocimum sanctum*(tulsi) leaves were collected and extracted in water and methanol and evaluated for its phytochemical constitutes.Antibacterial activity of *Ocimum sanctum* extract against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae* were determined. Antifungal activity was carried out against *Tricodermaviridae*. Antioxidant method is based on the scavenging of DPPH through the addition of radical species or an antioxidant that decolourizes the DPPH solution. Anticancer activity was studied in HeLa cell line by MTT assay. *Ocimum sanctum* extract were characterized by GCMS(Gas Chromatography with Mass Spectrometer).

Keywords: *Ocimum sanctum,* Phytochemical, Antimicrobial, antioxidant and anticancer activity.

1. INTRODUCTION

*Ocimum sanctum*species often referred the "King of the herb". It is commonly known as 'Tulsi'.Different parts of plant are used in Ayurveda and Siddha systems of Medicine for prevention and cure of many illnesses. The medicinal values of these plants lie in their phytochemical components, which produce definite physiological actions on the human body, and leaves possess antimicrobial activity. In traditional systems of medicine, different parts such as leaves, stem, flower, root, seed and whole plant of *O. sanctum* have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, chronic fever and insect bite.Recently, due to indiscriminate use of commercial antimicrobial drugs, multiple drug resistance human pathogens have developed [Archana Sharma *et al.*,2012].Plants produce compounds which though have no apparent function in the primary metabolism of the plant, had an extensive history of use as therapeutic agents [Chetia*et al.*, 2014]. The herbs are being used by the people as drugs, condiments, dyes, perfumery material and many other purposes from ancient ages[Devesh and Tewari*et al.,*2012]. In addition, many phenolics have been identified, which also exhibit antioxidant and anti-inflammatory activities.[Robin Sharma *etal.,* 2013].DNA damage by Oxygen Derived Free

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Radicals (ODFR) is important contributors to cancer development. Earlier works state that antioxidant defense system function inefficiently in tumor cells leading to accumulation of Reactive Oxygen Metabolites (ROM) which further enhance the hazardous effect. [Reshma *et al.,*2005]**.** The nano-particles have come forward as the capable approach in drug delivery systems for the well-organized delivery of drugs utilized in the treatment of various diseases such as cancer by crossing the reticula endothelial system, enhanced permeability and retention effect, and tumor-specific targeting.[SatyanarayanaRentala*et al.,* 2015].

2. MATERIALS AND METHODS

2.1 Collection of Leaves samples and Test Organism:

Leaves samples;

Fresh leaves of *Ocimum sanctum*were collected from local farm of Coimbatore district, in different area such as Ondipudur (sample1), Machampalayam (sample2), Eachanari (sample3). Fresh plant leaf materials were washed thrice under running tap water.

Test Organism;

The bacterial species *Escherichia coli, Staphylococcus aureus, Klebsiellapneumoniae* and the fungus *Trichodermaviridae*were selected..

2.2 Preparation of Extract

About 1-2gm of leaf samples were washed and smashed at mortar and pestle and separately mixed with water and methanol in a conical flask with tight cover, then the sample were incubated in Orbital shaker at 37^0c for 24 hours, at 60 - 70 rpm. After incubation the sample were filtered through WhatmannNo1 filter paper. The extracts were stored in air-tight bottles.

2.2 Phytochemical screening:

The presence of various phytochemicals in *Ocimum sanctum* leaf was analyzed by standard method. The compounds analyzed were alkaloids, terpenoids, phenol & tannin, sugar, saponins, flavonoids, quinines, proteins and steroids.

2.2.1 Test for alkaloids: Mayer's test: To 1 ml of the leaf extract, add few drops of Mayer's reagent, formation of white or pale yellow precipitate confirmed the presence of alkaloids

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2.2.2 Test of Terpenoids:To 2ml of Leaf extract 2ml of chloroform was added and evaporated to dryness. To add, 2ml of con H_2So_4 was added and heated for about 2minutes. A grayish color indicates the presence of terpenoids.

2.2.3 Test for Phenol and Tannin: To 1ml of leaf extract, 2ml of distilled water followed by few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green color indicated the presence of phenols. To 1-2ml of leaf extract, few drops of 5% aqueous ferric chloride solution were added. A bluish black color, which disappeared on addition of few ml of dilute sulphuric acid, was followed by the formation of a yellowish brown precipitate which indicated the presence of tannins.

2.2.4 Test for Carbohydrates (Fehling's test): To 5 ml of leaf extract add 1 ml of Fehling's solution, the contents were then boiled for few minutes. Formation of red or brick red precipitate confirmed the presence of carbohydrate.

2.2.5 Test for Saponins: Froth test: To 5ml of leaf extract, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like froth was formed and it showed the presence of saponins.

2.2.6 Test for Flavonoids: To 0.5ml leaf extract, 5-10 ml of diluted HCl and a small amount of zinc or magnesium powder was added and the solution was boiled for few minutes. In the presence of flavonoids, reddish pink or dirty brown color was produced.

2.2.7 Test for Quinones: To the 1% leaf extract 2% sodium hydroxide was added. Blue green (or) red color indicates the presence of quinones.

2.2.9 Test for Protein: To 1ml of leaf extract of the leaves, 5-8 drops of 10% sodium hydroxide solution and add 1 or 2 drops of 5% copper sulphate. Formation of red or violet color confirmed the presence of proteins.

2.2.10Test for steroids: To 2ml of chloroform extract, 1 ml of concentrated sulphuric acid was added along the sides of the test tube. The presence of steroids was confirmed by the presence of red color in the chloroform layer.

2.3. Antimicrobial Activity

The leaf extracts were tested for their antibacterial activity against the test organisms in the Muller Hinton Agar by agar well diffusion method. Log phase test specimens of *Escherichia coli,*

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*Staphylococcus aureus*and*Klebsiella pneumonia* were swabbed over the surface using the sterile cotton swab. Wells were made with the gel puncture and 20µl of the herbal extracts were loaded on to the well. All the plates were incubated at 37°C for 24 hours.The size of the clear zone was measured to evaluate the inhibitory action of the herbal.

The leaf extracts were tested for their antifungal activity against the test organisms in the Malt agar by agar well diffusion method. 50µl of test fungi *Tricodermaviridae*was swabbed over the surface using the sterile cotton swab. Wells were made with the gel puncture and different volumes of leaf extracts were loaded and they were incubated in room temperature for 2 to 5 days at 37^0C.

2.4. AntioxidantActivity (DPPH (Di Phenyl PicrylHydrazyl) radical scavenging activity)

Antioxidant activity is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolorizes the DPPH solution. 0.5ml of extract was mixed with 1ml of 0.1mM DPPH and 0.4ml of 50mM Tris-Hcl was added.The reaction mixture was incubated at room temperature for 30minutes. After incubation the reaction mixtures were measured at 517nm in spectrometer.

2.5. Anticancer Activity

To DMEM medium (Dulbecco's modified Eagle medium), 0.195ml of DMEM, 0.045mg of $C_6H_{12}O_6$, 0.037mg of Na_2CO_2 was added and HeLa cell lines were inoculated and incubated at 37^0C for 72hours.After 72hours 500 micro liters of the plant extract and MTT dye was added and kept for 30 minutes at room temperature.Then analyzed in spectrophotometer at 450nm and 540nm.

2.6. GCMS (Gas Chromatography with Mass Spectrometer)

GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with an Elite-1, fused silica capillary column (30 mm/0.25 mm) composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionizing energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 ml was employed (Split ratio of 10:1) injector temperature 250°C; ion-source temperature280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 2000°C, then 5°C/min to 280°C, ending with a 9 min isothermal at280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak

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area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

3. RESULTS AND DISCUSSION

Fresh plant leaves of *Ocimum sanctum*were collected from three places (Ondipudur, Machampalayam, Eachanari). The leaves were washed thoroughly with normal tap water. Then the leaves were smashed at mortar and pestle and it stored air-tight bottles.

3.1Phytochemical screening

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical characteristics of the leaves extract of *Ocimum sanctum* investigated were summarized in table-1. The results revealed the presence of medicinally active constituents like Alkaloids, Terpenoids, saponins, steroids, Quinones, Proteins. While tannins, sugar and Flavonoids were absents in the plants leaves. The results obtained in the study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants were studied and tabulated.

3.2 Antimicrobial Activity

The antibacterial activity of *Ocimum sanctum*leaf extracts against bacterial strains was evaluated by the agar well diffusion method. Antibacterial activity of different *Ocimum sanctum*extracts against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* were studied. However some reports on higher antibacterial activity against gram positive bacteria than gram negative bacteria by *Ocimum* extract also available [Mann *et al.,* 2000]. The antibacterial activity of *Ocimum sanctum* were shown in plate 1 and results were tabulated in table 2.

The antifungal activity of *Ocimum sanctum*leaf extracts against fungi *Tricodermaviridae*was evaluated by the agar well diffusion method.The result of antifungal activity assay showed that essential oils of the *Ocimum*species had inhibitory effect of the growth of *R. solani* in dose dependent manner[Sethi*et al.,* 2013].The antifungal activity of *Ocimum sanctum* were shown in plate No2 and results were observed from table 3.

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3.3. Antioxidant Activity

After incubation the mixture of DPPH solution is red at 517nm in spectrometer.The antioxidant activity is 17.80%.The works revealed that the extract of *Ocimumgrastissimum* leaves possesses good antioxidant potential presumably because of its phytochemical constituents' [Thabrew*et al.,* 1998]. As a reported by [Dhawan*et al.,* 1977] the DPPH scavenging activities of *Ocimumgratissimum* exert good correlation with its reductive potentials thereby establishing its medicinal use for the treatment of various maladies.

3.4. Anticancer Activity

Anticancer activity was carried out by MTT assay. The MTT (tetrazolium) dye was reduced from purple to yellow formazan on the viable cell. The percentages of viable cell were determined by the UV-Vis spectrophotometer. The absorbance was measured at 450nm and 540nm.The anticancer activity of *Ocimum sanctum* was shown in plate 3 andthe results were tabulated in table4. Similar results were reported by Karthikeyan *et al.*, 2008 The *Ocimum sanctum* has been investigated against human fibro sarcoma cells (HFS) line and induced cytotoxicity at 50μg/ml and above.

3.5. GC-MS (Gas Chromatography with Mass Spectrometer)

The GC-MS analysis of *Ocimum sanctum*leaves revealed the presence of 3 compounds. The identified decanoic acid, meristic acid and Bis(2- ethylhexyl) phthalate. The GC-MS analysis of *Ocimum sanctum*was shown in Fig.1 and the results were observed from table 5.

4.CONCLUSION

The present study provides evidence that aqueous extract of *Ocimum sanctum* contains medicinally important bioactive compounds and justifies the use of plant species as traditional medicine for treatment of various diseases. The various compounds present in the leaves were determined by phytochemical screening. The antimicrobial activity of the *Ocimum sanctum* leaves shows higher bactericidal and fungicidal effect. The sample has cytotoxicity activity against HeLa cell line was observed.

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Table1: Phytochemical analysis of *Ocimum sanctum*

Chemical constituent	Plant 1 (Methanol)	Plant 1 (Water)	Plant 2 (Methanol)	Plant 2 (Water)	Plant 3 (Methanol)	Plant 3 (Water)
Alkaloids	+	+	+	+	+	_
Terpenoids	+	+	+	+	+	+
Tannin	_	_	_	_	_	_
Sugar	_	_	_	_	_	_
Saponins	_	+	_	+	_	+
Flavonoids	_	_	_	_	_	_
Quinines	+	_	+	_	+	+
Proteins	+	_	+	_	+	+
Steroids	_	+	_	+	_	+

Table2: Antibacterial activity of *Ocimum sanctum*

Microorganisms	Antibacterial activity of *Ocimum sanctum*		Disk
	10µl	20µl	
Escherichia coli	2mm	3mm	Nil
Staphylococcus aureus	3mm	4mm	Nil
Klebsiella pneumonia	1mm	2mm	4mm

Table3: Antifungal activity activity of *Ocimum sanctum*

Fungi	Antifungal activity of *Ocimum sanctum*		
	10µl	20µl	30µl
Tricoderma viridae	2mm	3mm	4mm

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Table4: Anticancer activity of *Ocimum sanctum*

Wavelength in nanometer	Control	Extracts of *Ocimum santum*	
		100µl	500µl
450nm	0.274	0.239	0.261
540nm	0.326	0.269	0.242

Table5: GC-MS results of *Ocimumsanctum*

No	Retention Time	Molecular Formula	MW	Peak Area %
1.	10.60	$C_{10}H_{20}O_2$	172	145.67
2.	11.58	$C_{14}H_{20}O_2$	228	189.36
3.	12.56	$C_{24}H_{38}O_4$	390	289.65

Figure 1: GC-MS peaks of *Ocimum sanctum*Extract

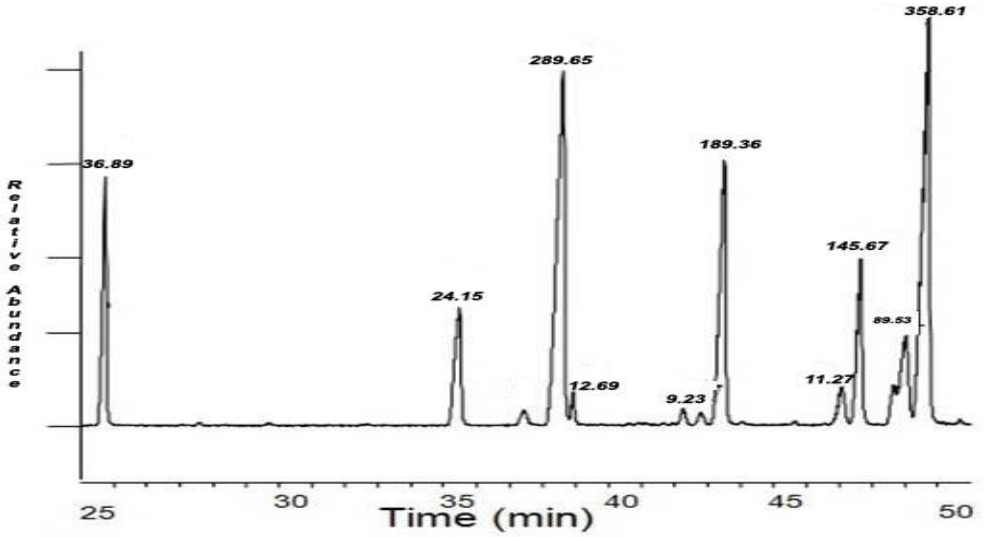

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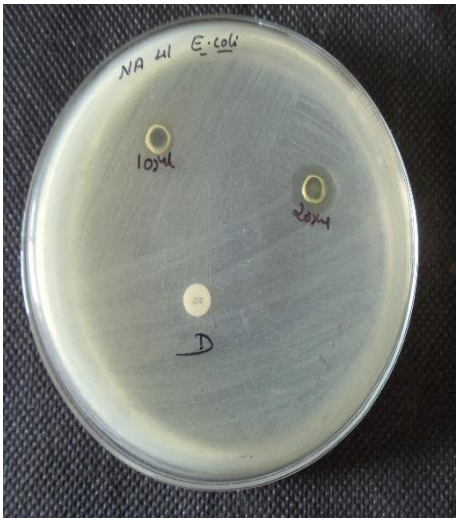
Plate 1: Antibacterial activity of Ocimum *sanctum*

Escherichia coli ***Staphylococcus aureus*** ***Klebsiella pneumonia***



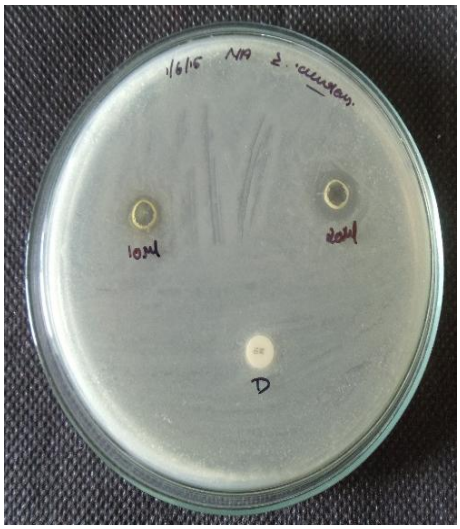
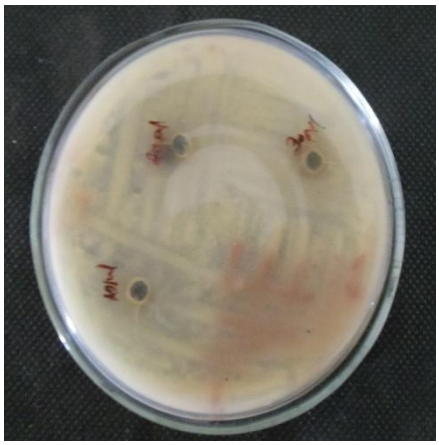


Plate 2: Antifungal activity of *Ocimum sanctum*

Tricodermaviridae



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