Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8):1052-1056

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytochemical screening, total phenolic content determination and antimicrobial activity of *Ocimum gratissimum* root

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ABSTRACT

In present study, phytochemical screening, total phenolic content, and antimicrobial activity were carried out. Extraction was performed successively with petroleum ether, diethyl ether, acetone and distilled water. Phytochemical screening indicates the presence of various phytoconstituents. Petroleum ether and diethyl ether extracts are rich in triterpenoids while acetone extract has carbohydrate, inulin, flavonoids and phenolic compounds. Diethyl ether extract has highest concentration of total phenol (48.823%) which is responsible for its excellent antioxidant activity. Antimicrobial activity was performed against four bacterial and two fungal strains by agar well diffusion method which showed that diethyl ether extract is more prominent against all strain. These results revealed that diethyl ether extract could be used as an antimicrobial agent of natural origin in pharmaceutical industry.

Keywords: *Ocimum gratissimum*, Phytochemical screening, Total phenolic content, Antimicrobial activity

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INTRODUCTION

Ocimum gratissimum is herbaceous plant belonging to *Lamiaceae* family. The plant is indigenous to tropical areas especially India and West Africa [1]. It is known by different names in various parts of the world. In India, the most commonly used ones being Vriddhu tulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada) [2]. *Ocimum gratissimum* has been used extensively in the traditional system of medicine in several countries. It is used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of tea and infusion [3]. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, eye and skin diseases, pneumonia, cough, fever and conjunctivitis. In India, the whole plant has been used for the treatment of sunstroke, headache and influenza as a diaphoretic, antipyretic and for its anti-inflammatory activity. The infusion of *Ocimum gratissimum* leaves is used as pulmonary antisepticum, antitussivum and antispasmodicum [4]. Since no work has so far been cited on *Ocimum gratissimum* root, the present study deals with the investigation of medicinally useful bioactive phytoconstituent in root extracts and their antimicrobial activity along with total phenolic content.

EXPERIMENTAL SECTION

Collection of Plant material

The *Ocimum gratissimum* root was collected from Haridwar region in month of June 2012 which was authenticated by Botanical Survey of India (BSI), Dehradun under accession no. 114456. Two voucher species of herbarium were prepared in which one was submitted to botanical survey of India and other has been submitted to Department of

chemistry, Gurukula Kangri Vishwavidyalaya, Haridwar under registry no. 25/15. Collected material was washed and shade dried and crushed in to fine powder and stored in poly bags for further use.

Extraction of Plant material

200 g of crushed root was successively extracted by petroleum ether, diethyl ether, acetone and distilled water in a soxhlet extractor in increasing order of polarity. At least 60 cycle of siphoning was completed with each solvent and extraction was continuing until siphon tube becomes colorless. Extracts were concentrated under reduced pressure with vacuum rotary evaporator and placed in refrigerator for further analysis.

Phytochemical screening

All plant extracts were screened for the presence of various secondary metabolites as alkaloid, glycoside, steroid, terpenoid and total phenolic compound. The screening of these parameters performed by using standard qualitative method [5, 6].

Total phenolic content

The total phenolic content present in various extract of *Ocimum gratissimum* root was measured spectrophometrically with small modification of Folin–Ciocalteu method [7]. Gallic acid is used as a standard whose calibration curve is plotted in between 100µg/ml to 800µg/ml concentration which follow **y = 0.0014x + 0.0278** linear equation. Briefly, in this method 1 ml of sample or standard is taken and added 38.5 ml of distilled water with 2.5 ml of Folin–Ciocalteu reagent. The reaction mixture was shaken properly and incubated for 8 min at room temperature. After incubation 8 ml of 20% of Na_2CO_3 solution was added and the mixture was allowed to stand for 2 hours. After the completion of reaction the absorbance of this mixture is taken at 765 nm by UV spectrophotometer. Distilled water was used as a blank determination and concentration of total phenol in various samples were calculated by calibration curve and expressed in terms of mg GAE/100g dw.

Antimicrobial activity

The root extracts were evaluated for antibacterial and antifungal activity which was performed by agar well diffusion method [8]. Two gram positive *S. aureus* (MTCC-737), *B. cereus* (MTCC-430) and two gram negative *P. aeruginosa* (MTCC-1688), *S. entric* (MTCC-98) while *A. flavus* (MTCC-277) and *C. albicans* (MTCC-227) were used as fungal strains. Muller Hinton agar and Sabourd dextrose agar were used as culture media for bacterial and fungal strain respectively. Appropriate amount of culture media was dissolve in distilled water and autoclaved at 121°C for 15 minute. Culture suspension equivalent to 0.5 McFarland standard was prepared in saline in case of bacterial strain and in distilled water in case of fungal strain. A 1% of culture suspension was mixed in sterile culture media and shaken properly. Now poured 25 ml of this suspension media in sterile petriplate and stand for solidification. After solidification 5 wells cut in plate by sterile cork borer. 40µl of extract and standard antibiotic solution in DMSO was poured in this well and sealed with parafilm and these plates were kept undisturbed for 1 hour for proper diffusion of samples. After diffusion bacterial plates were kept in BOD incubator for 18-24 hours at 35-37°C while fungal plates for 3-4 days at 20-25°C. Ofloxacin and Fluconazole were used as standard antibiotic drug for bacterial and fungal strain respectively.

Statistical analysis

All analysis results are processed as mean ± standard deviation of triplicate measurements. The data were statistically analyzed using the statistical program (sigmastat ver. 2.0.).

RESULTS AND DISCUSSION

Extractive Yield

The percentage extractive yield of *O. gratissimum* root extracts was illustrated in Table No. 1 which showed that aqueous extract has maximum yield while petroleum ether and diethyl ether extract have low extractive yield. Although most of the compound presents in plant were isolated with water in salt form yet it has weekly effective phytoconstituents. Most of the mid polar phytoconstituent were isolated in diethyl extract. The concentration of higher polar constituent is maximum in acetone extract. The pie chart representation of extractive yield was shown in Figure No. 1

Table No. 1:- The percentage yield, colour and physical state of concentrated different

Extract	Weight of sample (g)	Weight of extract (g)	% Yield (w/w)	Colour	Consistency
PE	200	1.0	0.51	Brownish	Waxy
DE	200	0.9	0.45	Brownish	Waxy
AT	200	4.4	2.24	Dark Brown	Fludy sticky
AQ	200	18.2	9.10	Black	Solid

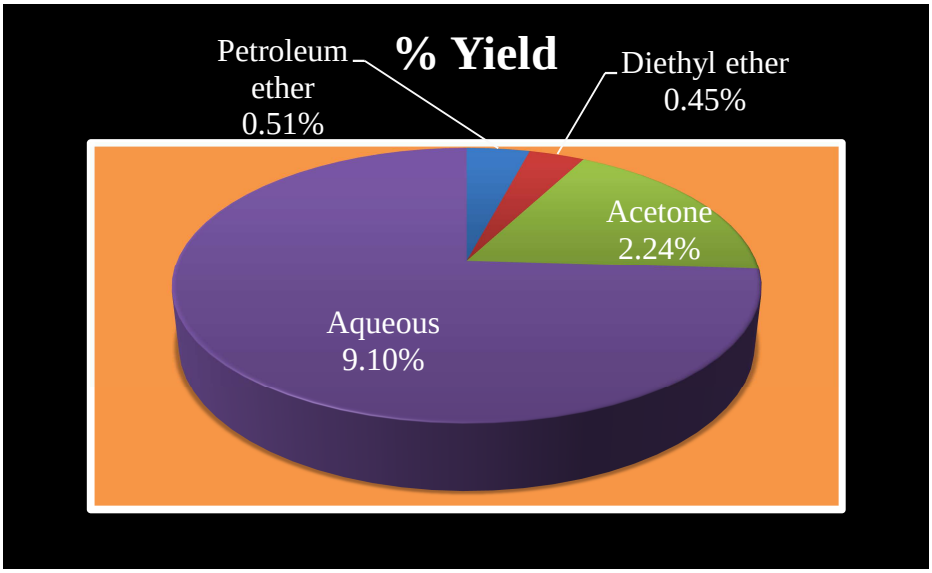

Figure no: - 1 Percentage yield of different extract of *Ocimum gratissimum* root (w/w)

Table No. : - 2 The phytochemical screening of the various extract of *Ocimum gratissimum* root

Phytoconstituents and Test performed			Extracts			
			PE	DE	AT	AQ
1. Alkaloids	Mayer's Test		-	-	-	-
	Wagner's Test		-	-	-	-
	Hager's Test		-	-	-	-
	Tannic acid Test		-	-	-	-
2. Carbohydrate	Molisch's Test		-	-	++	-
	Fehling's Test		-	-	++	-
	Benedict's Test		-	-	++	-
	Selivanoff's Test		-	-	++	-
3. Glycosides	***Anthraquinone glycosides***	Borntrager's Test	-	-	-	-
		Test for Hydroxy-anthraquinones	-	-	-	-
		Keller-Killiani Test	-	-	-	-
	Cardiac glycosides	Legal's Test	-	-	-	-
		Baljet's Test	-	-	-	-
	Saponin glycosides	Froth formation Test	-	-	-	++
	Flavanol glycosides	Mg and HCl reduction	-	-	-	-
4. Inulin			-	-	++	-
5. Protein	Heat Test		-	-	-	-
	Biuret Test		-	-	-	-
	Xanthoproteic Test		-	-	-	-
6. Amino Acid	Ninhydrin Test		-	-	-	-
7. Steroids and Triterpenoids	Salkowski Test		+++(T)	++(T)	-	-
8. Fixed oils and Fats	Spot Test		++	++	+	-
	Saponification Test		++	++	+	-
9. Flavonoids	Shinoda Test		-	-	+	-
	Alkaline reagent Test		-	-	++	-
	Zinc hydrochloride Test		-	-	-	-
10. Phenolic compounds and Tannins	Lead Acetate Test		-	-	+	+
	Ferric chloride Test		-	-	+	+
	Test for Catechin		-	-	-	-
	Test for Chlorogenic acid		-	-	+	-
11. Gums and Mucilage			-	-	-	-
12. Naphthoquinone	Juglone Test		-	-	-	-
	Dam-Karrer Test		-	-	-	-

Phytochemical screening

The presence of various secondary metabolites screened under phytochemical screening was shown in Table no. 2. It is clear from the screening that petroleum ether and diethyl ether extract are rich for triterpinoids and fixed oil. Carbohydrates, Inulins, flavonoids, tannins and phenolic compounds are present in acetone extract. The presence of flavonoid and tannins in acetone extract is responsible for free radical scavenging activity because flavonoids, phenolic compounds and tannins are major group of compounds that acts as primary antioxidants or free radical scavengers [9]. Result shows that aqueous extract has saponin glycosides. Medicinal value of any plant material is due to presence of secondary metabolite and other constituent [10]. Since root of *Ocimum gratissimum* has secondary metabolite in appreciable concentration so it demonstrated its medicinal value for pharmaceutical

industries and also in prevention of colorectal carcinoma, hyperchloestrolcamia and renal calculi [11]. Woody plants are versatile plant material show a wide range of their therapeutic application as root *Ocimum gratissimum* may be antipyretic, laxative, analgesic, antifungal, antibacterial and non inflammatory [12, 13].

Total phenolic content

The concentration of total phenols in various extracts determined by folin Ciocalteau method was shown in Table No. 3. It is clear that diethyl ether extract has excellent amount of total phenol while acetone has fair concentration. Moderate concentration of total phenols was found in petroleum ether and aqueous extract. It has been reported that phenolic compounds have been recognized as antioxidant agent who act as free radical oxidation terminator and also show their medicinal activity as well as exhibiting physiological functions and contribute to human health [14]. Phenolic compounds also act as antimutagens and anticariogens [15] metal chelators, antimicrobial agents and clarifying agents [16].

Table No. 3:- Concentration of total phenolic content in various extract of *Ocimum gratissimum* root

ABSORBANCE	CONCENTRATION (mg GAE/100g dw)
PE	3.418±0.17
DE	48.823±0.10
AT	15.442±0.14
AQ	8.418±0.10

Antimicrobial activity

The extracts are screened for their antibacterial and antifungal activity by agar well diffusion method. The results were recorded in terms of zone of inhibition and demonstrated in table no. 4. The bar graph presentation of extract with standard is shown in Figure no. 2. The result showed that diethyl ether extract is more prominent against all microorganisms. Petroleum ether extract also active against all microorganisms. Phytochemical screening showed that triterpenoids present in petroleum ether and diethyl ether extract are responsible for their antimicrobial activity. Acetone and aqueous extract are inactive against gram negative bacteria *S. entric* while other extracts are weekly active. All extracts also show weak zone of inhibition against *P. aeruginosa.* Extracts showed their good antifungal activity against filamentous fungi *A. flavus* and non filamntous fungi *C. albicans.* The antimicrobial activities of plant extracts are due to the presences of secondary metabolites such as saponins, flavonoids, tannins, carbohydrate etc. [17, 18]. Generally, the synthetic drugs are more potent than a complex mixture of components such as plant extracts but it should remembered that synthetic drugs have undesirable side effects than natural drugs of plant origin [19].

Figure no: - 2 Graphical representation of extracts against selected bacterial strains

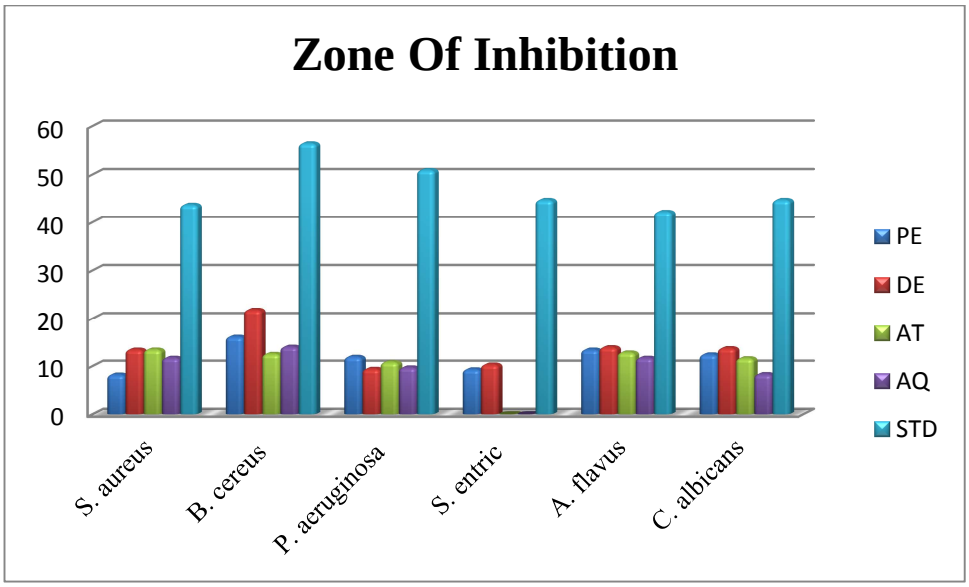

Table No. 4:- Antimicrobial investigation of different extract of *Ocimum gratissimum* root against selected microorganism

Bacterial strain	Zone of inhibition in mm. (Mean ± SD)				
	Std. drug (Of)	PE	DE	AT	AQ
S. aureus	43.8±0.76	8.3±0.57	13.5±0.50	13.5±0.50	11.8±0.76
B. cereus	56.5±0.86	16.2±0.72	21.9±0.90	12.6±0.52	14.1±0.41
P. aeruginosa	51.0±1.00	12.0±0.50	9.5±0.50	10.9±0.65	9.8±0.52
S. entric	44.8±0.80	9.4±0.40	10.4±0.52	-	-
A. flavus	42.3±0.57	13.5±0.50	14.0±0.50	12.9±0.36	11.8±0.28
C. albicans	40.1±0.76	12.5±0.50	13.8±0.76	11.7±0.64	8.4±0.40

CONCLUSION

Ocimum gratissimum is well known plant which is widely used due to their medicinal value. So, the present work is focused to study the medicinal value of root of this plant. *Ocimum gratissimum* root have excellent amount of total phenols which conclude that the total phenols act as antimicrobial agent. Phytochemical screening indicates root has phytoconstituent such as terpenoids, flavonoids and glycosides etc. due to which root may used as natural antimicrobial, antidiabetic drug and responsible for their antioxidant activity. However, the active phytoconstituent in extract can be isolated and characterized by various spectral techniques for further research.

Acknowledgement
The author's thankful to Department of Chemistry, Gurukul Kangri vishwavidyalaya, haridwar for providing all facilities for research work. The authors are great thankful to Head, Department of Zoology and Environment, Gurukul Kangri vishwavidyalaya, haridwar to laboratory facility for microbial activity.

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