Research Article

Phytochemical Analysis, Antioxidant and Antimicrobial Activity and Nutrient Content analysis of *Ocimum gratissimum* linn. From dibrugarh, N.E. India

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Accepted on: 07-01-2014; **Finalized on:** 28-02-2014.

ABSTRACT

Young and mature leaves and inflorescence of *Ocimum gratissimum* was analyzed for determination of phytochemical constituents, antioxidant and antimicrobial activity and nutritive value. The study confirms the presence of tannins, flavonoids, terpenoids, steroids, glycosides, cardiac glycosides, saponins, carotenoids, alkaloids and reducing sugars in the samples. Carotenoids could not be detected in mature inflorescence. Phenol was recorded highest in ethanol extract of young leaves while highest flavonoid was recorded in petroleum ether extract of mature leaves. DPPH and ABTS radical scavenging activity were recorded highest (84.88 % and 86.79 % respectively) in methanol extract of mature leaves. Ethanol extract of young leaf showed the maximum inhibition (14mm) against *Enterococcus faecalis.* The highest nutrient value (370.16 cal/100gm) was recorded in the mature leaves.

Keywords: Antioxidant and Antimicrobial activity, Nutritive value, Phytochemicals.

INTRODUCTION

North eastern region of India is endowed with rich vegetation and medicinal plants. The ethnic groups of the region have long experience in plant based traditional medicinal practices.[1-4] Plants produce compounds which though have no apparent function in the primary metabolism of the plant[5], had an extensive history of use as therapeutic agents.[6] Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant properties and can protect the human body against both cellular oxidation reaction and pathogen. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential.[7-9] The medicinal values of these plants lie in their phytochemical components, which produce definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds.[10]

Oxidative stress is an important contributor to the pathophysiology of a variety of pathological conditions including cardiovascular dysfunction, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury and neurodegenerative diseases.[11] The fruits, vegetables, medicinal herbs contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, that can act as strong antioxidant agent.[12- 15] Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissue.[16] Antioxidants are considered as possible protective agents reducing oxidative damage to the human body[17] and many of them are naturally abundant in plants and are able to neutralize free radicals by donating an electron and converting them to harmless molecules.[18] It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect.[19,20] The antioxidants protect other molecules (*in vivo*) from oxidation when they are exposed to free radicals and reactive oxygen species have been implicated in the aetiology of many diseases, in food deterioration and spoilage.[19,21-23] The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants.[24]

Ocimum gratissimum Linn (Lamiaceae) is grown for the essential oils in its leaves and stems and eugenol, thymol, citral, geraniol and linalol have been extracted from the oil.[25] The plant is found in hot and temperate regions of India. It is a woody herb grows to a height of one to two feet. The leaves taste like cloves, hence they are widely used for flavouring of vegetables.[26-28] Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties.[29] Whole plant and the essential oil are used in traditional medicine especially in Africa and India. The oil is also an important insect repellent and germicidal.[30-32] It is used in toothpastes, mouth washes and some topical ointments, also used as an excellent gargle for sore throats and tonsillitis, expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animals and man[33, 34]; treatment of rheumatism, paralysis, epilepsy, high fever, diarrhoea, sunstroke, influenza, gonorrhoea and mental illness.[35-37,25]

Synthesis of secondary metabolites including phenolic compounds in plants may be stimulated by the action of different parameters like environmental factors, use of precursors of the targeted molecules, use of elicitors and genetic transformation of the plants.[38] There has been

little focus on investigating the effect of climate conditions on production of secondary metabolite in medicinal plants.[39] Various studies on the use of this plant by the ethnic group of North East India was reported [40-42], but a little studies has been carried out on the secondary metabolite content in *O. gratissimum* from Assam.[43] On the above context, a study was carried out on qualitative, phytochemical analysis, antioxidant and antimicrobial activity and nutrient content of the leaves and inflorescence of *O. gratissimum* collected from Dibrugarh, Assam.

MATERIALS AND METHODS

Sample collection

Young and mature leaves and inflorescence were collected from household premises of Dibrugarh. The materials were shade dried and grounded to fine powder using electric grinder.

Sample extraction

Samples were macerated separately with water, methanol, ethanol and petroleum ether for 48 hours and filtered through Whatman No 1 filter paper. The filtrate was then evaporated at a constant temperature (60°C) until a semi dried powder/sticky mass of crude extract was obtained. The crude extract was dissolved in Dimethyl sulphoxide (DMSO) as neutral solvent to make final concentration for biochemical analysis.

Experimental

Following methods were used for the phytochemical analysis, antioxidant & antimicrobial activity and nutrient content of leaves and inflorescence of *O. gratissimum*- Phytochemical analysis, total phenol and flavonoid, antioxidant activity.

The qualitative phytochemical analysis was performed following the standard laboratory methods described by Edeoga et al.,[44]; Aja et al.,[45] and Ajayi et al.,[46]. Quantitative estimation of phenol was done by the method described by Malik and Singh [47] and the flavonoid by the method described by Mervat and Hanan.[48] Antioxidant activity study was performed using DPPH and ABTS radical scavenging method as described by Anti-Stanojevic et al., [49] and Re et al., [50] respectively.

Antimicrobial activity study

The antimicrobial test was carried out by agar well diffusion method described by Nair et al.,[51] using 6mm borer. The activity was determined by measuring the diameter of zone of inhibition (ZOI) exhibited by the extract.

Selected strains for antimicrobial study

Five Gram Positive bacterial strains viz, *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744); two Gram Negative strains viz, *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439) and two fungal strains viz- *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947) were used in the study. Strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference of bacterial strains were maintained on nutrient agar slants and fungal strains on PDA slants and stored in freeze. Strains were regularly subcultured using nutrient broth for bacterial strains and PDB for fungal strains.

Standard antibiotics

Standard antibiotics viz, Chloramphenicol (C) 30mcg, Tobramycin (TOB) 10mcg, Clotrimazole (CC) 10mcg, Ampicillin (AP) 10mcg, Streptomycitin (ST) 10mcg, Imipenem (IPM) 10mcg, Ciprofloxacin (CI) 30mcg, Streptomycin (S) 25mcg, Gentamycin (GEN) 30mcg, Erythromycin (E) 15mcg and Co-trimaxazole (COT) 25mcg, were taken for bacterial strains and for fungi, Nystatin (NS) 50mcg, Clotrimazole (CC) 10mcg, Ampicillin (AP) 10mcg, were employed for comparision of ZOI with sample.

Determination of Nutrient Content

Moisture, ash and fat content were determined by the method described by Indrayan.[52] Protein content was determined following the method described by Lowry[53] using methanol extract. The, nutritive value was determined by the following formula;

Nutritive value = 4 x percentage of protein + 9 x percentage of fat + 4 x percentage of Carbohydrate.[52]

Statistical analysis

All the experiments were performed in triplicate and the results were expressed in mean ± S.D.

RESULTS AND DISCUSSION

The phytochemical quality results analysis are presented in the Table 1. Presence of tannin, flavonoid, terpenoid, steroid, glycoside, cardiac glycoside, alkaloid, saponin, carotenoid and phenol were recorded in the samples. These phytochemicals either singly or in combination of two or more, perhaps give therapeutic effect of the plant against various diseases. The studies of Obho[54] from Nigeria revealed that ethanolic extracts of *Ocimum gratissimum* contains tannin, saponin, anthraquinone, alkaloid and glycoside, but the presence of anthraquinone has not been traced out in the present samples. This may happens because of habitat differences which play significant role in production of secondary metabolites in plants.[55-57]

Table 2 presents the phenol and flavonoid content of various extracts of leaves and inflorescence. Both ethanol and methanol extracts showed a decreasing order of phenol and flavonoid from young leaves to mature inflorescence. In case of phenol, ethanol extract of young leaves showed the highest and water extract of mature inflorescence showed the least amount. In case of

flavonoid, petroleum ether extract of mature leaves shows the highest and water extract of mature inflorescence showed the least amount. The studies of Mahapatra,[58] clearly proposed that methanolic extract of *O. gratissimum* contains high phenolic and flavonoid compound that was measured by spectrophotometric method. Methanolic extract of *O. gratissimum* contains 61.72 mg phenolic compound/g of *O. gratissimum* powder, and 251.83 mg flavonoid/g of *O. gratissimum* powder. Obho.[54] reported that the ethanolic extract of the plant has total phenolic content of 3.6 g/100 g,

The radical scavenging activity of different solvent extracts is presented in Table 3.

In both DPPH and ABTS tests all other extracts showed less amount of antioxidant activity than Ascorbic acid except the methanol extract of mature leaves. The highest ABTS and DPPH radical scavenging activity were recorded in the methanol extract of mature leaves and the lowest in water extract of mature inflorescence. The works revealed that the extract of *O. gratissimum* leaves possesses good antioxidant potential presumably because of its phytochemical constituents.[59,19] As reported by Dhawan[35], the DPPH scavenging activities of *O. gratissimum* exert good correlation with its reductive potentials thereby establishing its medicinal use for the treatment of various maladies. Obho[54] reported that the ethanolic extract of the plant has reducing power and free radical scavenging ability were 2.4 OD_{700} and 51.2%, respectively.

Table 4 presents the zone of inhibition of different solvent extracts of leaves and inflorescence in comparison to certain standard antibiotics. The ethanol extract of young leaf showed the highest inhibition (14mm) against *Enterococcus faecalis*. This is followed by ethanol extract of young inflorescence against *E. faecalis (*12mm). The water extract of mature inflorescence against *B. cereus* (11mm), ethanol extract of mature leaf against *E. faecalis* (10mm), ethanol extract of mature inflorescence against *E. faecalis (*10mm) and methanol extract of mature inflorescence against *B. cereus* (10mm) shows inhibition. Verma[60], observed maximum inhibition against *Staphylococcus aureus* (16mm) followed by *Streptococcus mutans* (14mm), *Enterococcus faecalis* (10mm) and *Staphylococcus epidermis* (8mm). Borkotoky[43] reported that the methanol extract of *O. gratissimum* L. showed significant activity against *B. subtilis* (21mm) and least activity recorded in *Enterococcus cloacae* measured 14mm.

Table 1: Qualitative analysis of phytochemicals in the leaves and inflorescence of O. gratissimum

Phytochemicals/ Samples	Young leaves	Mature leaves	Young inflorescence	Mature inflorescence
Tannin	+	+	+	+
Phlobatannin	-	-	-	-
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Glycosides	+	+	+	+
Cardiac glycosides	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Carotenoids	+	+	+	-
Reducing sugar	+	+	+	+
Phenol	+	+	+	+
Free anthraquinone	-	-	-	-

+ indicates presence of constituents and – indicate absence of constituents

Table 2: Total Phenolic and total flavonoid content of leaves and inflorescence of O. gratissimum

Samples	Phenol (mg catechol equivalent/gm dry material)				Flavonoid (mg quercetin Equivalent/gm dry material)			
	Ethanol extract	Methanol extract	Petroleum ether extract	Water extract	Ethanol extract	Methanol extract	Petroleum ether extract	Water extract
Young leaves	4.39± 0.05	4.27± 0.12	1.64± 0.23	3.31± 0.01	3.45± 0.23	3.04± 0.33	1.48± 0.01	1.51± 0.13
Mature leaves	3.63± 0.23	4.10± 0.34	3.84± 0.63	2.98± 0.23	2.96± 0.11	2.11± 0.11	3.63± 0.01	1.49± 0.32
Young inflorescence	2.29± 0.05	2.53± 0.22	2.50± 0.02	2.76± 0.66	2.14± 0.22	1.39± 0.19	2.11± 0.18	1.23± 0.32
Mature inflorescence	1.61± 0.55	2.49± 0.08	2.61± 0.08	1.09± 0.07	1.39± 0.12	1.38± 0.29	1.08± 0.13	1.00± 0.03

Table 3: Antioxidant activities of leaves and inflorescence of O. gratissimum

Sample	Antioxidant activity (% inhibition in mg/ml)							
	DPPH radical scavenging activity				ABTS radical scavenging activity			
	Ethanol extract	Methanol extract	Petroleum ether extract	water extract	Ethanol extract	Methanol extract	Petroleum ether extract	Water extract
Young leaves	87.24± 0.33	84.21± 0.13	39.36± 0.41	50.74± 0.09	80.58± 0.43	83.10± 0.34	74.17± 0.34	71.45± 0.19
Mature leaves	87.70± 0.34	84.88± 0.33	43.00± 0.14	53.00± 0.41	80.97± 0.16	86.79± 0.23	79.22± 0.11	73.12± 0.45
Young inflorescence	67.20± 0.45	57.97± 0.23	39.89± 0.35	32.98± 0.32	80.00± 0.32	74.27± 0.21	67.18± 0.13	61.20± 0.07
Mature inflorescence	36.87± 0.34	34.55± 0.43	32.20± 0.19	29.01± 0.27	45.04± 0.14	68.05± 0.22	52.03± 0.24	43.18± 0.16
Ascorbic acid	88.20±0.10				83.00±0.00			

Table 4: Antibacterial activity of crude extracts of leaves and inflorescence of O. gratissimum.

Test sample		Diameter of inhibition of zone (mm)						
		B.subtilis	*B.cereus*	*S.aureus*	*S.epidermis*	*P.vulgaris*	*E.faecalis*	*E.coli*
Young leaf	Water	-	-	-	-	-	-	-
	Methanol	8	-	-	8	8	-	-
	Ethanol	-	-	-	-	-	14	-
	Petroleum ether	-	-	-	-	-	-	8
Mature leaf	Water	-	10	-	8	-	-	-
	Methanol	8	8	-	8	8	-	8
	Ethanol	-	-	-	8	8	10	8
	Petroleum ether	-	8	8	-	8	8	8
Young inflorescence	Water	-	-	-	-	-	-	-
	Methanol	8	-	8	8	8	-	8
	Ethanol	-	-	-	8	8	12	8
	Petroleum ether	-	-	-	-	8	-	-
Mature inflorescence	Water	-	11	-	-	-	-	-
	Methanol	8	10	-	8	8	-	-
	Ethanol	-	-	-	8	-	10	8
	Petroleum ether	-	-	-	-	8	-	-
Chloramphenicol (C) 30mcg		15	-	-	30	-	8	-
Tobramycin (TOB) 10mcg		44	24	32	-	40	42	35
Clotrimazole (CC) 10mcg		20	10	14	20	8	-	26
Ampicillin (AP) 10mcg		-	-	-	-	12	10	10
Strptomycitin (ST) 10mcg		18	-	10	-	-	10	12
Imipenem (IPM) 10mcg		66	-	-	-	32	-	30
Ciprofloxacin (CI) 30mcg		44	32	40	-	40	36	22
Streptomycin (S) 25mcg		-	32	28	-	22	60	28
Gentamycin (GEN) 30mcg		40	32	30	-	-	-	24
Erythromycin (E) 15mcg		32	30	28	30	12	48	12
Co-trimaxazole (COT) 25mcg		46	-	-	-	30	-	24

*- No activity; Zone of inhibition includes the diameter of well (6mm).

Table 5: Antifungal activity of crude extracts of leaves and inflorescence *of O. gratissimum.*

Test sample		Diameter of inhibition of zone (mm)	
		C.albicans	*P.crysogenum*
Young leaf	Water	-	-
	Methanol	-	10
	Ethanol	-	14
	Petroleum ether	-	-
Mature leaf	Water	-	-
	Methanol	8	12
	Ethanol	8	-
	Petroleum ether	8	
Young inflorescence	Water	-	-
	Methanol	-	-
	Ethanol	-	-
	Petroleum ether	-	-
Mature inflorescence	Water	-	-
	Methanol	-	-
	Ethanol	-	10
	Petroleum ether	-	10
Nystatin (NS) 50mcg		-	24
Clotrimazole (CC) 10mcg		11	32
Ampicillin (AP) 10mcg		-	46

*- No activity; Zone of inhibition includes the diameter of well (6mm).

Table 6: Nutritive value of leaves and inflorescence of *O. gratissimum*

Sample	Ash (%)	Moisture content (%)	Crude fat (%)	Protein (%)	Carbohydrate (%)	Nutritive value (cal/100g)
Young leaf	5.49	7.55	3.10	16.90	66.96	363.34
Mature leaf	4.25	8.96	4.60	16.80	65.39	370.16
Young inflorescence	4.23	15.06	1.80	13.90	65.01	331.84
Mature inflorescence	4.59	8.16	0.90	12.80	73.55	353.50

Table 5 presents the antifungal activity of the extracts against two fungal strains. The ethanol extract of young leaves showed highest ZOI against *P. crysogenum* measuring 14 mm. Keita[61] reported that essential oil of *O. gratissimum* showed antifungal activity against pathogenic fungi *Botryosphaeria rhodina* and *Alternaria* spp. Faria[62] evaluated antifungal activity of eugenol by agar well diffusion assay against *Alternaria* spp. and *P. crysogenum* and the minimal inhibitory concentrations of eugenol were 0.16 and 0.31 mg for *Alternaria* spp and *P. crysogenum* respectively.

Table 6 presents the nutritive value of leaves and inflorescence of *O. gratissimum*. The order of nutritive value of different parts was- mature leaf (370.16 cal/100gm)> young leaf (363.34 cal/100gm)> mature inflorescence (353.50 cal/100mg)> young inflorescence (331.84 cal/100gm).

From the above results and discussion, we found the scientific basis of use of this plant in traditional health-care system. The results are encouraging, therefore the plant extracts may serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants and antimicrobial agents. The variation in the secondary metabolite content of the plant in the present study is may be due to the different geographical location, climate and habitat condition of the study area.

Acknowledgment: Authors are thankful to the DST for financial support and Dibrugarh University, Assam for providing necessary facilities.

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Source of Support: Nil, **Conflict of Interest:** None.