EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 12
529-534
Year: 2017

COMPARATIVE STUDIES ON PHENOLIC CONTENT, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY IN SELECTED SPECIES OF *OCIMUM* FROM CENTRAL REGION OF GUJARAT

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Article Received on 01/10/2017 **Article Revised on 22/10/2017** **Article Accepted on 12/11/2017**

ABSTRACT
The objective of the present study was comparative studies on phenolic content, flavonids content and antioxidant activity of selected species of *Ocimum*. Crude methanolic extract of dried leaves of selected species viz., *Ocimum basilicum* L., *O. gratissimum* L. and *O. sanctum* L. were used and it's showed variation in terms of phytochemical screening. Qualitative analysis of phytochemical constituent's viz. alkaloids, tannins, flavonoids, phenol steroids and saponins were found to be present in all the tested species. However, cardiac glycosides, triterpens and phlobatannins were absents in all the tested species of *Ocimum*. Quantitative analysis of total phenolics and flavonoids had revealed that *O. gratissimum* possessed maximum phenolic content (4.14 mg/g dry weight basis of leaf sample). However, minimum content was reported in *Ocimum basilicum* (1.92 mg/g dry weight basis of leaf sample). For flavonoids content, maximum result was showed by *O. sanctum* (6.86 mg/g dry weight basis of leaf sample) while minimum content was found in *O. basilicum* (2.96 mg/g dry weight basis of leaf sample). Antioxidant activity was determined by DPPH radical scavenging and reducing power assays. The results suggested that maximum antioxidant activity by DPPH method as well as reducing power assay were showed by *O. gratissimum* (67.42% and 5.65 mg/g dry weight basis of leaf sample). Overall results has indicate that *O. gratissimum* has maximum phytochemical contents in compare to rest of tested species.

KEYWORDS: Antioxidant, DPPH, Phytochemicals and *Ocimum*.

1.0 INTRODUCTION
In Ayurveda, the uses of different species of *Ocimum* (Tulsi) are still alive in Indian system of medicines. The genus of *Ocimum* is belonging to Lamiaceae family which is well known for its aromatic compounds. The genus *Ocimum*, comprising of more than 150 species grows widely and is distributed throughout temperate regions of the world [1-3]. *Ocimum basilicum* L. is commonly known as sweet basil which is important medicinal herb. Simon *et al*., (1999) reported that sweet basil used for treatment of headaches, cough, diarrhoea, constipation, warts, worms and kidney malfunctions [4]. Leaves and flowering parts showed perceived as carminative, galactogogue, stomachic and antispasmodic in folk medicine [5]. *Ocimum gratissimum* L. is herbaceous plant which is indigenous to tropical areas of India. In India, Ram tulis (Hindi) is one of common name used for this species. It has been extensively used in the traditional system of medicine and also be used for the treatment of sunstroke, headache and influenza as a diaphoretic, antipyretic and for its anti-inflammatory activity. The infusion of leaves is used as pulmonary antisepticum, antitussivum and antispasmodicum [6]. *Ocimum sanctum* L. is known by shyam tulsi in India. In traditional system of medicine this herbs also used as expectorant, analgesic, anticancer, antiemetic, diaphoretic, antifertility, hypotensive and antistress agents [7]. There are numerous phytochemicals were reported by Dhar *et al*., (1968) in Holy basil leaves which shows an essential oil, contains eugenol, eugenal, carvacrol, methylchavicol, limatrol and caryophylline. Seeds oil composed of fatty acids and sitosterol where roots contain sitosterol and three triterpenes such as A, B, and C [8]. The leaves also contain ursolic acid and n-triacontanol.

Since form the time of evolution, lots of genetic diversity of medicinal plants helpful in developing the human culture. Now a day's many ethnopharmacologists and researcher seeks to explore the novel compounds derived from plants species grown in different geographical localities which shows quantitative variation in their chemical constituents. Variation in biological compounds leads to explore the potential of genetic

constituents of plants at a particular ecological region. Looking to the demand and authenticity of medicinal plants in current time, it's a time to not only to explore more and more genetic diversity shown by valuable indigenous medicinal species but also its an urgent needs to ethanopharmcological based studied so that selection of superior medicinal plants for sustainable conservation, and development of new pharmaceutical drugs can be workout.

In order to investigate the comparative studied on variability of phytochemical constituents in crude methanolic leaves extracts of selected species of *Ocimum*, viz., *Ocimum basilicum* L., *Ocimum gratissimum* L. and *Ocimum sanctum* L. which were collected from central region of Gujarat State of India. Present investigation not only helps to estimate the phytochemical variability among tested species of *Ocimum* but also helps to identify the superior species having better yield of active phytochemicals for herbal drugs formulation.

2.0 MATERIALS AND METHODS

2.1 Plant Materials

Selected species of *Ocimum* viz. *Ocimum basilicum* L.; *Ocimum gratissimum* L. and *Ocimum sanctum* L. were collected from Anand District of Gujarat, India. Fresh and mature leaves were washed thoroughly and chopped into small pieces shade dried and grinded into powdered for phytochemical test, phenolic content, flavonoid content and antioxidant activity. Figure 1 shows Geographical position of places where the *Ocimum* samples were taken.

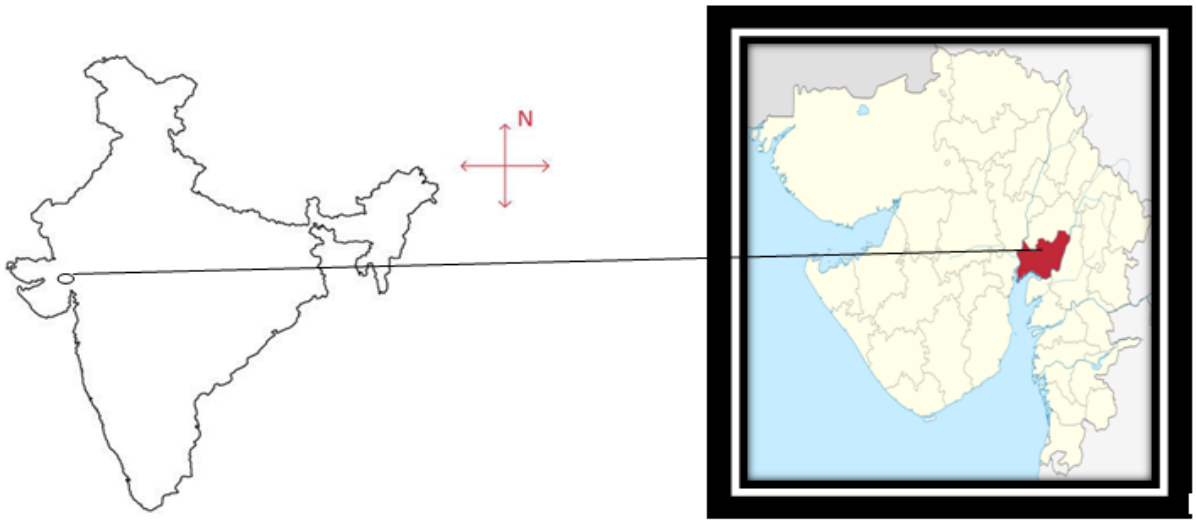

Figure 1. Geographical position of study area and *Ocimum* sampling points.

2.2 Preparation of Plant Extract

For the phytochemical screening and analysis of phenolics, flavonoids and total antioxidant activity the dried leaves samples were extracted by soaking 10 g dry leaves powdered material in 200 ml of methanol solvent at its respective boiling temperature for 24 hrs. in soxhlet apparatus, filtered through Whatman no.1 filter paper. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40^{o}C.The dried filtrates (crude methanolic extracts) were reconstituted in known amount of methanol to obtain methanol extract of known concentration. The stock solution of crude leaves extracts (1mg/ml) was prepared by dissolving a known amount of dry extract in 100 ml of methanol. The different working solutions of tested species extracts were prepared from the stock solution using suitable dilution for further analysis of phytoconstituents.

2.3 Preliminary phytochemical analysis

The crude methanolic leaves extracts were subjected to various phytochemical tests to determine the active constituents present in methanolic solvents of selected species of *Ocimum*. The details of the tests are as follows:

2.3.1 Hager's test for alkaloids

To the leaves extracts, saturated solution of picric acid was added. Yellow colors indicate the presence of alkaloids.

2.3.2 Braemer's test for tannins

Take a 2–3 ml of leaves extracts, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicate the presence of tannins in leaves extracts.

2.3.3 Shinoda test for flavonoids

To 2–3 ml of leaves extracts, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. Pink red or red coloration of the solution indicate the presence of flavonoids in the leaves extract.

2.3.4 Folin test for phenol
To 2–3 ml of leaves extracts, 1 ml of Folin's reagent was added. Violet/brown coloration of the solution indicates the presence of phenol in the leaves extracts.

2.3.5 Sterols (Salkowski test)
Ferric chloride and glacial acetic acid was added to the leaves extracts, after mixing, appearance of brown colors indicates the presence of sterols in it.

2.3.6 Saponins (form test)
Known amount of water was added in the leaves extracts and properly shaken, appearance of froth indicates the presence of the saponins.

2.3.7 Cardiac glycosides (Keller-kiliani test)
Add the glacial acetic acid, $FeCl_3$, and H_2SO_4, in the extracts appearance of the green blue color indicates presence of cardiac glycoside.

2.3.8 Triterpenes (Chloroform test)
Add chloroform in leaves extracts, warm it for 30 minutes and add concentrated sulfuric acid, after mixing it, appearance of red color will indicate the presence of triterpenes.

2.3.9 Phlobatannins test
To leaves extracts add 1% sulfuric acid, properly shake the mixture, appearance of the red precipitates will indicate the presence of phlobatanins

2.4 Determination of total phenolics
The amount of total phenol content in crude methanolic leaves extracts from selected species of *Ocimum* leaves were determined by spectrophotometrically using Folin–Ciocalteu reagent (McDonald *et al*., 2001) with slight modifications[9]. To 0.5 ml of each sample (0.1 mg/ml), 2.5 ml of 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of 20% Na_2CO_3 were added and incubated at 37°C for 15 min. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Optizen 3220, Mecasys, Kore). For standard reading gallic acid (standard phenolic compound 0.1 mg/ml) was used. Standard graph was compared with samples reading. The analysis was performed in triplicate and the results were expressed as the gallic acid equivalent (on dry weight basis of leaves).

2.5 Determination of total flavonoids
The aluminum chloride colorimetric method (Chang *et al*., 2002) was used to determine the flavonoid content in crude methanolic extracts from selected species of *Ocimum* leaves[10]. To 1.0 ml of leaves extracts (1mg/ml) was mixed with 1.5 ml of methanol, 0.1ml of 10% aluminium chloride, 0.1 ml of 1M sodium acetate and 7.3 ml of distilled water was added then mixture was allowed to stand for 30.0 minutes at room temperature. The absorbance of reaction mixture was measured at 415 nm. Quercetin (standard flavonoid compound 0.1 mg/ml) was used as standard for the calibration curve. Standard graph was compared with samples readings. Total flavonoid content of samples was expressed in mg/g (on dry weight basis of leaves). All samples were analyzed in triplicates.

2.6 *In vitro* antioxidant assay
2.6.1 DPPH free radical scavenging assay
DPPH radical is a widely used method to evaluate the free radical scavenging ability of natural compounds. The DPPH scavenging activity was determined using the method described by Shimada *et al*., (1992) with slight modification [11]. Each methanolic extracts of tested samples (1ml at 1mg/ml) was mixed with 1 ml of a 0.1 mM DPPH (Merk). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. L-Ascorbic acid (0.1mg/ml) was used as the standard. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated as follows:

% DPPH radical scavenging activity = A_C-A_S/A_C X 100

Where A_C = absorbance of the control and A_S = absorbance of sample.

All tests were run in triplicates (n = 3), and the average values were calculated.

2.6.2 Reducing power assay ($FeCl_3$ method)
The Fe^{3+} reducing power of the extract was determined by the method of (Dinis *et al*., 1994*)* with slight modifications[12]. In ferric chloride method, 0.5 ml of crude methanolic leaves extracts was mixed with 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.8) and 0.5 ml of 1% ferricyanide. The reaction mixture was incubated in a temperature controlled water bath at 50°C for 20 minutes, followed by addition of 0.5 ml of 10% trichloroacetic acid. The mixtures were centrifuged for 10 min at 3000 rpm. The supernatant obtained (0.5 ml) was added with 100 µl of 0.1% $FeCl_3$.The blank was prepared in the same manner as the samples were prepared. The absorbance of the reaction mixture was measured at 700 nm. Ascorbic acid (0.1mg/ml) was used as standard compound.

3.0 RESULT AND DISCUSSION
3.1 Extraction yield of phytochemicals
Results for percentage of crude methanolic extraction yield of phytochemicals constituents on dry weight basis (w/w) from selected species of *Ocimum* leaves in methanol as an extracting solvent showed that there were differences in yield of crude extracts of tested species Table 1. Maximum yield was exhibited in crude methanolic extract of *Ocimum sanctum* leaf that is 194.2 mg/g of dried leaf powder. However, similar type of result were obtained in *Ocimum basilicum* and *Ocimum gratissimum*, where both of them showed similar amount of yield from crude methanolic extracts of leaves (181.0 mg/g of dried leaf powder). Variability of extracted phytochemical yield in selected species of *Ocimum* might be due to its genetic constituents as well as its

adaptability in give environmental condition. Mallavarapu *et al*., (1995) stated that a plant species grown in different geographical localities also show quantitative variation in their chemical constituents [13].

Table 1: Extraction yield of phytochemical constituents in selected species of *Ocimum*.

Sr. no	Name of tested species	Extraction yield of phytochemicals constituents
01	*Ocimum basilicum* L.	181.0 ± 0.08 mg/g of dried leaf powder
02	*Ocimum gratissimum* L.	181.0 ± 0.03 mg/g of dried leaf powder
03	*Ocimum sanctum* L.	194.2 ± 0.05 mg/g of dried leaf powder

3.2 Preliminary phytochemical screening

The data shown in Table 2 shows screening of crude methanolic extracts of leaves from selected species of *Ocimum* viz., *Ocimum basilicum*, *Ocimum gratissimum* and *Ocimum sanctum* based on phytochemical tests. These tests showed the presence of wide range of phytoconstitutes including alkaloids, tannins, flavonoids, phenols, steroids and saponin, supporting the reasons for its wide range of biological activity. All the tested species of *Ocimum* showed same trend for presence and absence of phytoconstituents in crude methanolic extracts of respective leaves. Alkaloids, tannins, flavonoids, phenols, sterols and saponins were found to be universally present in *Ocimum basilicum* L., *Ocimum gratissimum* L. and *Ocimum sanctum* L., whereas cardiac glycosides, triterpene and phlobatanins were found to be altogether absent in all the tested species of *Ocimum*.

Similar line of result were also reported by Akinmoladum *et al*., (2007) in which extracts from the leaves of *Ocimum gratissimum* showed positive test for phytochemicals constituents such as tannins, terpenoids, flavonoids and cardiac glycosides in both methanolic and aquous extracts[14]. Rana *et al*., (2015) also reported the presence of alkaloids, flavonoids, saponins, and tannins of *O. sanctum* leaf extract [15]. Shukla, *et al*., (2015) carried out similar line of research work where he reported the presence of various phytoconstituents in *Ocimum gratissimum* [16]. Thus, the preliminary screening test may be useful in the detection of the bioactive principle and subsequently may lead to the drug discovery and development.

Table 2: Preliminary phytochemical analysis of methanolic crude leaves extracts from selected species of *Ocimum*.

Sr. no	Components	*O. basilicum* L.	*O. gratissimum* L.	*O. sanctum* L.
01	Alkaloids	+	+	+
02	Tannins	+	+	+
03	Flavonoids	+	+	+
04	Phenols	+	+	+
05	Steroids	+	+	+
06	Saponins	+	+	+
07	Cardiac glycosides	-	-	-
08	Triterpenes	-	-	-
09	Phlobatanins	-	-	-

Key: - = absent and + = present

3.3 Determination of total phenolic and flavonoids content

Total phenolic content of the selected species of *Ocimum* were measured using the Folin-Ciocalteu method, and the results showed significance difference in terms of phenol content among them are shown in Table 3. Calculated from the calibration curve (y=0.478 x+0.011, R^2 = 0.976), in tested samples phenolic content ranged from 1.97 to 3.21 mg/g dry weight basis of leaf sample, expressed as gallic acid equivalents. *Ocimum gratissimum* showed the highest phenolic content (4.14 ± 0.02 mg/g dry weight basis of leaf sample), followed by *Ocimum sanctum* (3.21 ± 0.05 mg/g dry weight basis of leaf sample), whereas *Ocimum basilicum* showed the lowest phenolic content (1.97 ± 0.03 mg/g dry weight basis of leaf sample) of these dried leaves samples. Similarly for quantification of total flavonoids content in crude methanolic extracts of dried leaves samples from selected species of *Ocimum* was calculated from the regression equation of the standard plot (y=0.467 x-0.001, R^2=0.999) and is expressed as quercetin equivalents (QE). Maximum result was found in methanolic crude leaves extract of *Ocimum sanctum* that was 6.86 ± 0.1 mg/g dry weight basis of leaf sample compare to other. This was followed by *Ocimum gratissimum* in which 6.14 ± 0.05 mg/g dry weight basis of leaf sample reported. However, minimum amount of total flavonoid content was found in *Ocimum basilicum* where 2.96 ± 0.02 mg/g dry weight basis of leaf sample was noted (Table 3). These results are also in agreement with Uyoh *et al*., (2013) who reported similar result where total phenol and flavonoid contents of the spice extracts ranged from 9.09-27.41 µg GAE/mg and 5.38 - 22.88 µg RE/mg respectively [17]. The total flavonoids

content of leaf-derived callus cultured showed 2.2 times higher than leaf-derived callus, whereby the former produced 0.733 ± 0.077 mili gram catechin equivalent per gram fresh weight (mg CE/g fresh weight), while the latter only yielded 0.333 ± 0.043 mg CE/g fresh weight[18].

The presence of flavonoids confirms that the plant has high antioxidant activity value, as well as justify its antimicrobial, antimutagenic, antiviral and anti allergic actions. Flavonoids are a group of polyphenolic compounds, it has properties like free radical scavenging, inhibition of hydrolytic and oxidative enzymes; anti-inflammatory action.[19]

Table 3. Total phenol and flavonoids contents in selected species of *Ocimum*.

Extract	Selected species of *Ocimum*	Total phenolic content (mg/g dry weight basis of leaf sample)	Total flavonoid content (mg/g dry weight basis of leaf sample)
Crude methanolic Extract of leaf	*Ocimum basilicum* L.	1.92 ± 0.03	2.96 ± 0.02
	Ocimum gratissimum L.	4.14 ± 0.02	6.14 ± 0.05
	Ocimum sanctum L.	3.21 ± 0.05	6.86 ± 0.1

3.4 *In vitro* antioxidant assay

3.4.1 DPPH free radical scavenging assay

Result for total antioxidant activity of methanolic crude leaves extracts of *Ocimum basilicum* L., *Ocimum gratissimum* L. and *Ocimum sanctum* L. showed there were variation in terms of total antioxidant activity (Table 4). Among all the tested species of *Ocimum*, maximum amount of total antioxidant activity was found in *Ocimum gratissimum* where 67.42% was recorded. This was followed by *Ocimum basilicum* L. in which 66.45 % was noted. However, minimum total antioxidant activity was found in case of *Ocimum sanctum* where 58.04 % was noted. Thus it is clear that polyphenolic antioxidant in leaves of tested species of *Ocimum* play an important role as bioactive principles and the scavenging effect can be attributed to the presence of active phytoconstituents in them.

Similar kind of research work was also done by the total antioxidant capacities and reducing powers of the extracts (measured as absorbance values) ranged from 0.137 - 0.160 and 0.130 - 0.158 respectively. Generally, *Ocimum basilicum* maintained superior antioxidant activities to *O. gratissimum* in all the test assays, and all the extracts showed dose-dependent antioxidant activities[17].

3.4.2 Reducing power assay ($FeCl_3$ method)

In case of ferric reducing antioxidant power, result revealed that maximum reducing power was noted in methanolic crude extract of *O. gratissimum* leaves where 5.65 ± 0.07 mg/g dry weight basis of leaf sample which was followed by *O. sanctum* in which 4.14 ± 0.04 mg/g dry weight basis of leaf sample was recorded. However, minimum result was found in case of *O. basilicum* where 2.54 ± 0.02 mg/g dry weight basis of leaf sample was reported. Similar type of work was also done by Agbor *et al*., (2007) where the leaves of *Alchornea cordifolia* showed the highest antioxidant properties as determined by both Folin and FRAP free antioxidant, followed by *Dacryodes edulis* and *Ocimum basilicum* in FRAP and by *Dacryodes edulis*, *Harungana madagascariensis* for Folin and DPPH method (Table 4)[20].

Table 3. Antioxidant activity in crude methanolic extracts of tested species of *Ocimum*.

Extract	Selected species of *Ocimum*	Total Antioxidant content (%)	Ferric reducing antioxidant power (mg/g dry weight basis of leaf sample)
Crude methanolic extract	*Ocimum basilicum* L.	66.45	2.54 ± 0.02
	Ocimum gratissimum L.	67.42	5.65 ± 0.07
	Ocimum sanctum L.	58.04	4.14 ± 0.04

4.0 CONCLUSION

It could be concluded form present study that in crude methanolic leaves extracts of selected species of *Ocimum* showed phytochemical constituents and antioxidant activity were found to be present in good quantity. Among the tested species of *Ocimum*, maximum potential in terms of phytochemicals constituents was showed by *Ocimum gratissimum* L. Due to presence of its good amount of antioxidant, reducing power, total phenolic and flavonoid content in crude leaves extracts will help in new drug development, and use as a herbal medicine to cure disease instead of antibiotics. This current investigation leads to fresh sources of new antibacterial activity in future. Further phytochemical studies are required to establish the types of compounds responsible for the antibacterial activity.

ACKNOWLEDGEMENT

The authors are thankful to the P.G. Department of Genetics, ARIBAS, New V.V. Nagar, Anand and

C.V.M., V.V. Nagar, Anand for providing necessary facilities to carry out this work.

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