



# Antioxidative potential of two chemically characterized *Ocimum* (Tulsi) species extracts

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## Abstract

**Background:** *Ocimum sanctum* and *Ocimum kilimandscharicum* are cultivated in Indian subcontinent both for the religious and medicinal properties. Traditionally, the leaves have been reported for their enormous therapeutic potentials but the roots which are otherwise considered as a waste part have not been explored for their pharmacological activity. **Methods:** Total phenolic content, free radical scavenging and ferric reducing antioxidant potential of various extracts from *Ocimum sanctum* and *Ocimum kilimandscharicum* were assessed and compared. *In vitro* antioxidant activity was estimated spectrophotometrically and the most potent ethyl acetate extract was chemically characterized by developing the chemical fingerprint and quantifying the probable constituents with the help of HPLC/LC-MS techniques. **Results:** The ethyl acetate extract of both the species exhibited significant free radical scavenging potential and also reduced the ferric ions. It was observed that ethyl acetate extract have superior ferric reducing potential than other tested extracts, which were evidenced by high ferrous sulphate equivalent value of  $77.05 \pm 1.54$  and  $80.98 \pm 0.80$  at 100  $\mu\text{g/ml}$  for *O. sanctum* and *O. kilimandscharicum* respectively. The ferric reducing capacity of ethyl acetate extract for both the species was also evidenced by an elevated optical density of  $1.64 \pm 0.12$  and  $2.14 \pm 0.08$ . *Ocimum sanctum* exhibited better antioxidant capacity ( $11.31 \pm 0.20$  AScE) as compared to *Ocimum kilimandscharicum* ( $9.08 \pm 0.27$  AScE). The total phenolic and flavonoid content were estimated by spectrophotometric method and tentatively characterized by HPLC/LC-MS profiling which revealed the presence of rosmarinic acid, caffeic acid along with its derivatives such as caffeoyl-dihydroxyphenyllactoyl-tartaric acid.

**Conclusion:** The ethyl acetate extract of both the species being rich in phenolic and flavonoid contents exhibited potent antioxidant activity. The presence of flavonoid in ethyl acetate extracts further co-relates the antioxidative properties of roots extracts. To the best of our knowledge and understanding this is the first report of comparative chemical profiling by RP-HPLC/LC-MS and antioxidant potential of roots of two *Ocimum* species.

## Keywords

Flavonoids, HPLC profiling, *Ocimum kilimandscharicum*, *Ocimum sanctum*, Phenolics, Rosmarinic acid

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## Introduction

Traditionally, medicinal plants are known to possess enormous therapeutic potentials and have been used for treating various ailments and diseases. In recent years, phytochemicals with antioxidant properties have received great consideration mainly due to their role in preventing diseases which are caused by oxidative stress (Uyoh et al., 2013). Damage to the cells caused by free radicals is believed to play a central role in aging and in progression of many diseases and disorders. Antioxidants are believed to be the first line of defense against free radical damage and are critical for maintaining optimum health and well-being (Bozin et al., 2006). Due to the presence of many active molecules, various parts of plants, such as leaves, stem, bark and roots are now being used in formulations as natural antioxidants for prevention and treatment of various complex conditions in humans (Katalinic et al., 2006). Many phytochemicals have revealed to support in maintaining the balance between oxidants and antioxidants in humans and natural antioxidants are gaining significant attention amongst food dieticians, pharmacists, manufacturers and consumers because of their enormous therapeutic potential and safety (Ito et al., 1986). Several findings have proven that many medicinal plants and food crops are a rich source of many phytochemicals which have excellent antioxidant potentials (Pizzale et al., 2002; Vieira et al., 2014).

*Ocimum*, belonging to the family Lamiaceae, also known as the queen of herbs, has been used for centuries in the different traditional systems of medicines. The genus *Ocimum* consist of about more than 140 different species of aromatic plants and generally found in tropical and sub-tropical regions of the world (Devi, 2001). The *O. sanctum*, *O. basilicum*, *O. gratissimum*, *O. kilimandsachricum* and *O. americanum* are some common examples of the various species being grown in various parts of world for numerous medicinal

values. The essential oils from different *Ocimum* species have been reported to possess antimicrobial, anticonvulsant and antioxidant activities (Akinmoladun et al., 2007; Balaji et al., 2011; Singh et al., 2010; Vieira et al., 2014). A recent study by Ahmad et al., (2012) revealed the anti-diabetic activity of new constituents characterized as urs-12-en-3 $\beta$ ,6 $\beta$ ,20 $\beta$ -triol-28-oic acid, 1''-menthyl-2-glucopyranosyloxybenzoate and n-decanoyl- $\beta$ -D-glucopyranosyl-(2a $\rightarrow$ 1b)- $\beta$ -D-glucopyranosyl-(2b $\rightarrow$ 1c)- $\beta$ -D-glucopyranosyl-(2c $\rightarrow$ 1d)- $\beta$ -D-glucopyranosyl-2d-2-hydroxybenzoate, along with ursolic acid and palmityl glucoside (Ahmad et al., 2012). In another study by Maity et al., (2000) the effect of methanolic root extract of *Ocimum sanctum*, on mouse swimming performance was reported (Maity et al., 2000). The antimicrobial activity and HPLC fingerprinting of crude methanolic and aqueous extracts of *O. sanctum* and *O. kilimandscharicum* have also been revealed (Deo et al., 2011). The antioxidant properties of leaves extracts of *O. onites*, *O. vulgare* and *O. basilicum* along with total phenolic and rosmarinic acid content have been also reported (Lagouri and Nisteropoulou, 2009). There are very few reports related to potential bioactivities and pharmaceutical applications of *Ocimum* root. The roots of *Ocimum* species which are otherwise considered as a waste by product and are generally used as mulching, manure or as a fuel by the farmers after cultivation can thus be a potential source of many active phytochemicals possessing novel biological activities. Hence, in the present study, antioxidant potential and chemical characterization of *Ocimum* (Tulsi) species were carried out by using *in vitro* assay procedure and HPLC-UV/LC-MS analysis respectively. The roots of *Ocimum sanctum* and *Ocimum kilimandscharicum* were subjected to extraction and further evaluated for their antioxidant prospective. The chemical profile of an active fraction by HPLC method was also developed to characterize the extracts.

## Materials-Methods

### Plant materials

The fresh roots of *O. sanctum* and *O. kilimandscharicum* after harvesting were collected from the experimental fields of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India. The voucher specimen samples of both the species were authenticated and deposited in the herbarium unit under reference number 2013-12598 and 2014-12934 respectively in the Botany and Pharmacognosy Department of CSIR-CIMAP, Lucknow, Uttar Pradesh, India.

### Preparation of extracts

The fresh roots of both the species of *Ocimum* were washed in warm water and dried under the controlled conditions in a hot air oven at 40-42°C. The roots were then segregated and grounded in a hammer mill into a coarse powder of 30-40 mesh size. 500 g each of the dried and powdered roots were extracted

exhaustively with methanol in a soxhlet type apparatus. The methanolic extract was concentrated under controlled conditions to get a viscous mass. The extracts were re-dissolved in double distilled water and successively partitioned with hexane, chloroform, ethyl acetate and butanol. All the extracts were concentrated under low temperatures and vacuum to obtain the different fractions. The fractions were air dried at room temperature and stored at 4°C till further use.

### **Solvents and chemicals**

Hexane, methanol, butanol, ethyl acetate and chloroform (all LR grade) were purchased from Molychem (India) Ltd. Gallic acid, ferrous sulphate, tris-[hydroxymethyl] aminomethane, sodium nitropruside dihydrate, N-(1-naphthyl) ethylenediamine dihydrochloride, sulphanilamide, sodium carbonate, potassium ferricyanide, ferric chloride, tripyridyl-s-triazine (TPTZ), hydrogen phosphate, and m-phosphoric acid aluminum chloride, potassium dihydrogen phosphate, potassium phosphate monobasic, phosphate buffer saline (PBS), ammoniummolybdate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), Folin–Ciocalteureagent, sodium acetate quercetin, and ascorbic acid were bought from Sigma Aldrich, Bangalore, India. HPLC grade solvents were procured from Merck Pvt. Ltd, Mumbai, India, Ultrapure water was prepared using a Milli-Q System (Millipore, Milford, MA, USA).

### **Antioxidant Activity Evaluation**

#### **DPPH radical scavenging activity**

DPPH radical scavenging assay is a widely used method for the *in vitro* estimation of antioxidant potential of extracts or phyto-molecules. DPPH radicals produces a dark purple colour in methanol and its characteristic deep purple colour bleaches on accepting hydrogen from a corresponding donor from active extracts (Luciana et al., 2001). The antioxidant potential of the different extracts of *Ocimum* root were assessed according to the method described by Chung et al. (2002) with some variations as reported by Luqman et al. (2009) (Chung et al., 2002; Luqman et al., 2009). According to the described method, different concentrations of the samples (10, 25, 50, and 100 µg/ml) were added to 100µl of methanol followed by 500µl of Tris HCl buffer (pH 7.4) and 500µl of the DPPH reagent. The reaction mixture was then incubated at 37°C for 20 min in the dark and the absorbance was measured at 517nm against a blank buffer and percent DPPH inhibition was calculated with respect to reagent control.

#### **Ferric reducing antioxidant power (FRAP) assay**

FRAP assay is based on the principle of reduction of ferric tripyridyl triazine (Fe<sup>3+</sup> TPTZ) complex to ferrous form (Benzie and Strain, 1996). The change in absorption is directly proportional to the sum total of reducing capacity of the

electron donating antioxidants present in the reaction mixture. A reagent mixture consisting of 20 mM ferric chloride 10 mM TPTZ, and 300 mM acetate buffer (pH 3.6) in ratio of 1:1:10 was prepared. The *Ocimum* extracts (10, 25, 50 and 100 µg/ml) were taken and 50 µl of water and 1.5 ml of FRAP reagent were added to it. The reaction mixture was then incubated at 37°C for 5 min and the absorbance was measured at 593 nm. The results were expressed in terms of ferrous sulphate equivalents (FSE) which were obtained from the standard curve of ferrous sulphate (Luqman et al., 2011).

### Reducing power assay

Phytochemicals have the ability to reduce iron like radicals reacts with potassium ferricyanide ( $\text{Fe}^{3+}$ ) to form potassium ferrocyanide ( $\text{Fe}^{2+}$ ), which further reacts with ferric chloride to form ferric-ferrous complex. The reducing power potential was measured according to the method of Yen and Chen (1995) with some modifications (Negi et al., 2011; Yen and Chen, 1995). Various concentrations *Ocimum* root extracts (10, 25, 50 and 100 µg/ml) were pooled with phosphate buffer (200 mM, pH 6.6) and potassium ferricyanide. The mixture was heated on water bath at 50°C for 20 min. The supernatant was then transferred to micro plate and to it distilled water and freshly prepared 0.10%  $\text{FeCl}_3$  was added and optical density was read at 700 nm against reagent blank.

### Total antioxidant capacity (TAC) estimation

The estimation of the total antioxidant capacity (TAC) is based on method reported by Prieto et al., (1999) where the Mo (VI) is reduced to Mo (V) in suitable acidic medium that lead formation of a greenish complex of phosphate and Mo (V) (Prieto et al., 1999). For the experiment different concentration of the *Ocimum* root extracts (10, 25, 50 and 100 µg/ml) were mixed with 1 ml of the TAC reagent (0.605 M ammonium molybdate, 25 mM phosphate mono-basic and 0.60 M concentrated sulphuric acid). The reaction mixture was heated on boiling water bath for 90 min. The optical density of the formed complex was noted at 695 nm against a reagent blank and results were expressed as ascorbic acid equivalent.

### Total flavonoids (TF) content

The estimation of the TF content in the extract was performed by colorimetric method as described by Meda et al., 2005 (Meda et al., 2005). In this phytochemical estimation, various concentrations of the *Ocimum* roots extract (10, 25, 50 and 100 µg/ml) were dissolved in 50 L of double distilled water with the successive addition of 10 µl of aluminum chloride (10%), 150 µl of methanol, 1 M potassium acetate, and 280 µl of double distilled water. Optical density at 415 nm was measured and compared to blank reagent. The total flavonoid content in the different *Ocimum* root extracts were articulated as quercetin equivalent (QCE) as mentioned recently (Ahmad et al., 2014).

### **Total phenolic (TP) estimation**

Total phenolic compounds present in the plant extracts was estimated by the assay described by Singleton and Rossi, 1965 with some modifications (Luqman et al., 2011; Singleton and Rossi, 1965). Different concentrations of the *Ocimum* root extracts (10, 25, 50, and 100 µg/ml) were taken to which folins reagent was added followed with addition of 7.5 % sodium bicarbonate. The reaction cocktail was then incubated at 37°C for 90 min and the optical density was measured at 765 nm against a reagent blank. The results were expressed as gallic acid equivalent (GAE) which was obtained from a standard curve of gallic acid.

### **HPLC-UV/PDA fingerprint analysis**

As the ethyl acetate extract exhibited the most potent antioxidant activity, so it was taken for further analytical studies. The extraction was carried out in triplicate, filtered, collected and concentrated under vacuum. The uniformity in the three batches of extracts preparation was assured by their reproducible characteristic chemical chromatographic fingerprint. The phytochemical fingerprinting was done on a chromatographic system from Shimadzu (Shimadzu Corporation, Kyoto, Japan) which consist of model LC-20AD pumps, a rheodyne manual injector, a model CTO-20A oven and a model SDP-M20 diode array detector. For controlling the LC system, LC-MS Solution 3.21 software from Shimadzu was used on a computer system (3000H Series, Lenovo). A model of CBM-20 Shimadzu interface was used to send the signals from the detector to the computer. The separation was achieved by a gradient elution using X select C18 (4.6 × 250 mm, 5µm) at 35°C. The mobile phase consisting, acetic acid (0.5%, v/v) in water (solvent A) and in acetonitrile (solvent B) was used (Kumar et al., 2015). Prior to the use, mobile phase was degassed for 15 min using ultrasonication technique (Oscar Ultrasonics, Mumbai, India). The samples and mobile phase was filtered through a 0.45 µm membrane filter in solvent filtration apparatus (Millipore, USA). The gradient elution was started with 5% B with a flow rate of 1.0 ml/min. Detector wavelength was set at 254nm and the injection volume was 20µl. The data acquisition was performed in the range of 200–400 nm to monitor any possible co-elution in plant sample solution. Therefore, considering maximum chromatographic signal response for fingerprint, wavelength 254 nm was selected.

### **Statistical analysis**

All the data are expressed as mean± SD (n=3) which were computed with the help of MS Office 2007. Comparisons were made between test compounds and reference control for antioxidant activity by one-way analysis of variance (ANOVA) followed by post hoc Dunnet test, all vs control, and a *p* value of <0.05 was considered statistically significant.

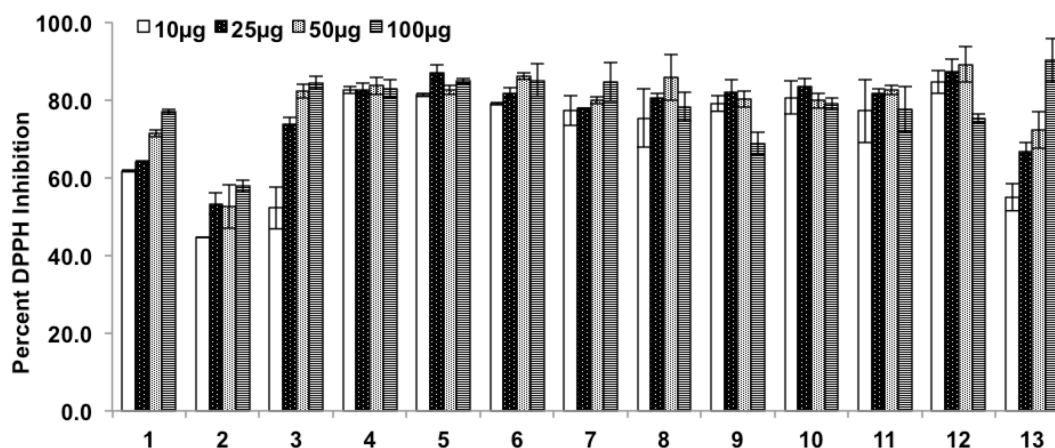
## Results-Discussion

### Extraction and fractionation in different solvents

The crude methanolic extract was prepared and fractionated with the solvents of varying polarity to get different extracts containing mixture of compounds. Better yield was obtained in aqueous fraction for both the species of *Ocimum* which was followed by hexane fraction. The results of extraction procedure are presented in **Table 1**.

**Table 1.** Dried weight of extracts/fractions and percent yield

Extract / Fractions	<i>Ocimum sanctum</i>		<i>Ocimum kilimandscharicum</i>	
	Weight (g)	Yield (%)	Weight (g)	Yield (%)
Crude Methanol	28.57	5.71	32.00	6.40
Hexane	5.71	1.14	8.24	1.64
Chloroform	4.28	0.85	2.44	0.48
Ethyl acetate	2.85	0.57	2.46	0.49
Butanol	4.28	0.85	4.22	0.84
Water	7.14	1.42	10.50	2.1



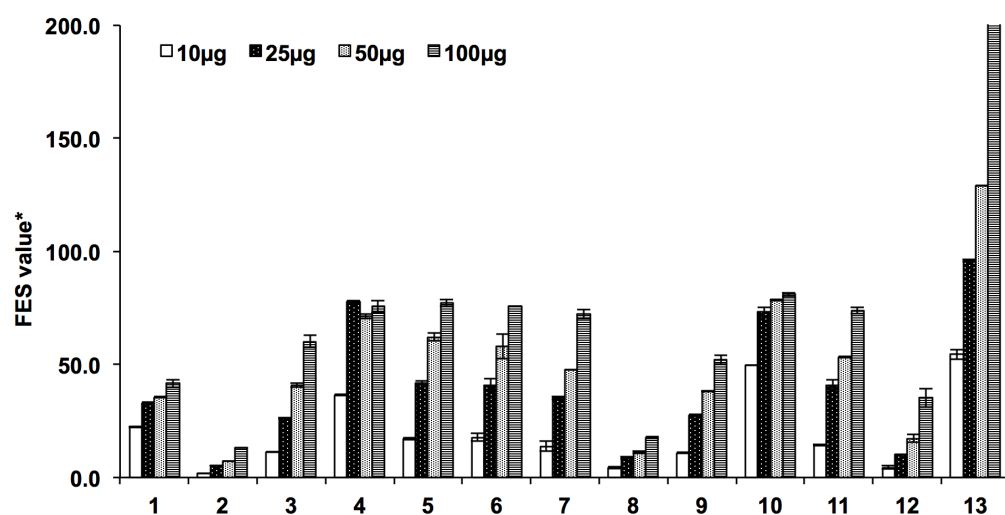
**Figure 1.** DPPH free radical scavenging assay, All values are expressed as mean  $\pm$  SD (n=3). (1) Aqueous extract; (2) Hexane extract; (3) Chloroform extract; (4) Ethyl acetate extract; (5) Butanol extract; (6) Methanolic extract of *O. sanctum*; (7) Aqueous extract; (8) Hexane extract; (9) Chloroform extract; (10) Ethyl acetate extract; (11) Butanol extract; (12) Methanolic extract of *O. kilimandscharicum*; (13) Positive control (Ascorbic acid).

## Antioxidant activity evaluation

A concentration-dependent evaluation of anti-oxidative properties was studied for all the extracts of two *Ocimum* species. The potential of the extracts were tested using several well established *in-vitro* assays, such as reducing power, DPPH, FRAP, and total antioxidant capacity. Total flavonoid and total phenolic contents were also estimated by colorimetric procedure following established protocols.

### DPPH radical scavenging assay

Various root extracts of two *Ocimum* species were evaluated for their free radical scavenging activity by DPPH assay. Our finding showed that methanolic root extract of *O. kilimandscharicum* has the better scavenging potential followed by the methanolic extract of *O. sanctum*, order of their DPPH radical scavenging potential was 6>5>3>4>1>2 (*O. sanctum*) and 12>10>8>7>11>9 (*O. kilimandscharicum*). Overall, it was also observed that methanolic extract of *O. kilimandscharicum* exhibited low IC<sub>50</sub> value (7.02- 24.19 µg/ml) compared to *O. sanctum* (12.93- 35.46 µg/ml) for DPPH radical scavenging (Fig. 1).



**Figure 2.** Ferric reducing antioxidant power assay; All values are expressed as mean  $\pm$  SD (n=3). (1) Aqueous extract; (2) Hexane extract; (3) Chloroform extract; (4) Ethyl acetate extract; (5) Butanol extract; (6) Methanolic extract of *O. sanctum*; (7) Aqueous extract; (8) Hexane extract; (9) Chloroform extract; (10) Ethyl acetate extract; (11) Butanol extract; (12) Methanolic extract of *O. kilimandscharicum*; (13) Positive control (Ascorbic acid).

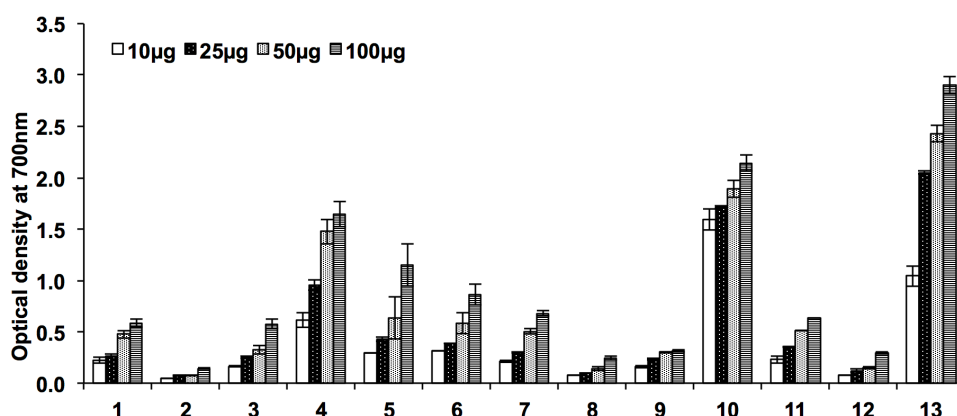


### FRAP assay

The ferric reducing antioxidant power of the *Ocimum* extracts was expressed as equivalence of ferrous sulphate and it was observed that reducing power of the extracts increases as the concentration increases. The ethyl acetate extract of *O. sanctum* and *O. kilimandscharicum* exhibited better ferric reducing activities among all the tested extract ( $77.05 \pm 2.67$  and  $80.98 \pm 3.50$  FSE respectively) which was followed by butanolic extracts ( $75.84 \pm 4.85$  and  $73.91 \pm 4.52$  FSE respectively). The ethyl acetate extracts of both the *Ocimum* species showed the maximum potency to donate electrons to the free radical thus terminating the reactive free radical chain reaction. Interestingly, it was also observed that methanolic and butanolic extracts of *O. sanctum* exhibited similar ferric reducing activity indicated by  $75.84 \pm 4.85$  and  $75.61 \pm 3.25$  FSE respectively. The order of their ferric reducing potential were found as 4>6>5>3>1>2 for *O. sanctum* and 10>11>7>9>12>8 for *O. kilimandscharicum* (Fig. 2).

### Reducing power assay

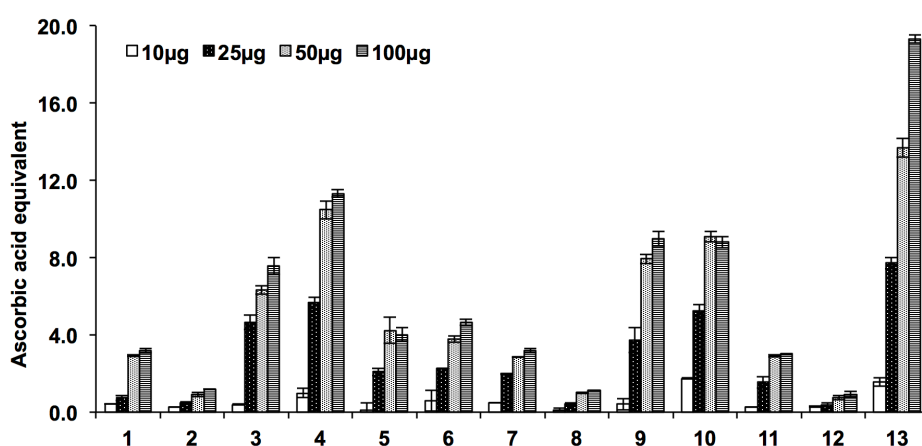
The ethyl acetate root extracts of both the species of *Ocimum* were found most active in iron reducing capacities as compared to other tested extracts, evidenced by a higher optical density recorded at 700 nm ( $1.64 \pm 0.23$  and  $2.14 \pm 0.36$  respectively). The reducing power of these extracts rises as the concentration of the extracts increased and was found to follow the similar pattern as that of ferrous reducing power in FRAP assay. The results of iron reducing assays of various extracts are shown in Fig. 3. The optical density of *Ocimum* extracts ranged from  $0.14 \pm 0.01$  to  $2.14 \pm 0.36$  at  $100 \mu\text{g/ml}$  and the order of their iron reducing property was noted as 4>5>6>1~3>2 for *O. sanctum* and 10>7>11>9>12>8 for *O. kilimandscharicum*.



**Figure 3. Reducing power assay; All values are expressed as mean  $\pm$  SD (n=3). (1) Aqueous extract; (2) Hexane extract; (3) Chloroform extract; (4) Ethyl acetate extract; (5) Butanol extract; (6) Methanolic extract of *O. sanctum*; (7) Aqueous extract; (8) Hexane extract; (9) Chloroform extract; (10) Ethyl acetate extract; (11) Butanol extract; (12) Methanolic extract of *O. kilimandscharicum*. Positive control (Ascorbic acid).**

### Total antioxidant capacity

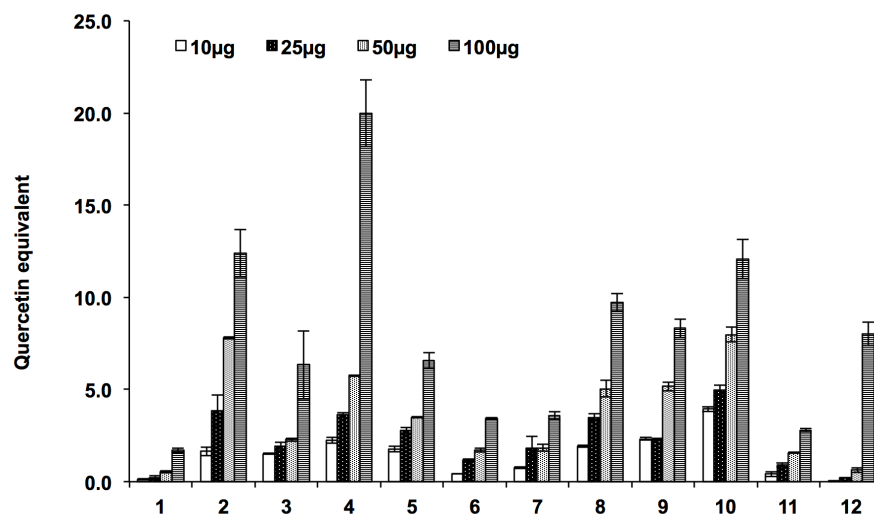
The total antioxidant capacity was determined in terms of ascorbic acid equivalent and amongst the tested species of *Ocimum*, ethyl acetate extract of *O. sanctum* showed maximum antioxidant capacity. The total antioxidant capacity of chloroform and ethyl acetate extracts of both the species exhibited better total antioxidant potential compared to other which ranges from  $7.56 \pm 0.47$  to  $11.31 \pm 1.02$   $\mu\text{g AsE/mg}$  of dried extracts. The order of antioxidant capacities was observed to be  $4 > 3 > 6 > 5 > 1 > 2$  for *O. sanctum*  $10 > 9 > 7 > 11 > 8 > 12$  for *O. kilimandscharicum* (Fig. 4).



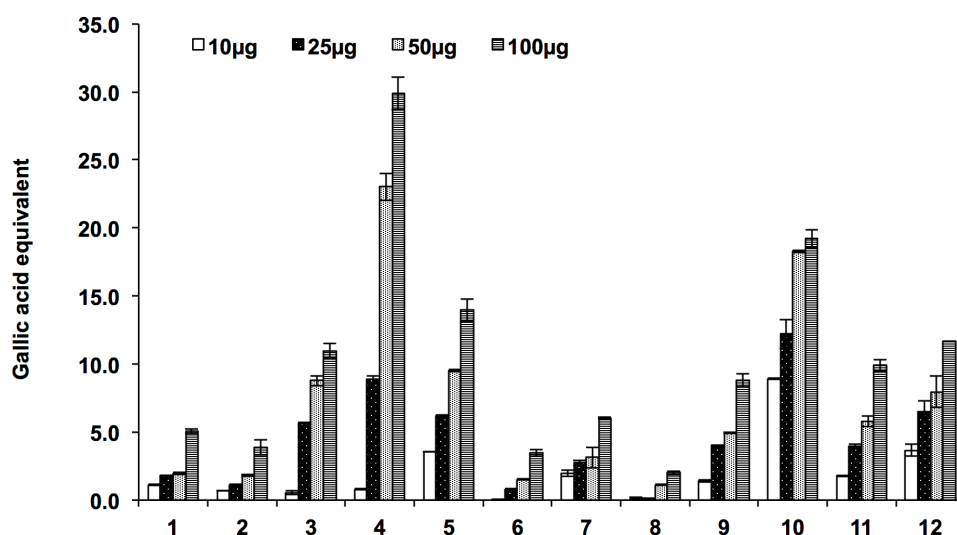
**Figure 4.** Total antioxidant capacity; All values are expressed as mean  $\pm$  SD (n=3). (1) Aqueous extract; (2) Hexane extract; (3) Chloroform extract; (4) Ethyl acetate extract; (5) Butanol extract; (6) Methanolic extract of *O. sanctum*; (7) Aqueous extract; (8) Hexane extract; (9) Chloroform extract; (10) Ethyl acetate extract; (11) Butanol extract; (12) Methanolic extract of *O. kilimandscharicum*. (13) Positive control (Ascorbic acid).

### Total flavonoids estimation

Flavonoids are the important secondary metabolites reported for various biological activities. Most of the plant extract reported elsewhere for their antioxidant activities are found to contain flavonoids, therefore we have also quantified the total flavonoids content in the extracts. High flavonoid content was reported in ethyl acetate extracts which was followed by hexane extracts of both the *Ocimum* species. The aqueous and methanolic extracts of *O. sanctum* and aqueous and butanolic extract of *O. kilimandscharicum* were found to possess poor amount of total flavonoids. The presence of high amount of flavonoids in ethyl acetate extracts was positively co-related with total antioxidant capacity and the order of flavonoid content were found to be as  $4 > 2 > 5 \sim 3 > 6 > 1$  for *O. sanctum* and  $10 > 8 > 9 > 12 > 7 > 11$  *O. kilimandscharicum* (Fig. 5).



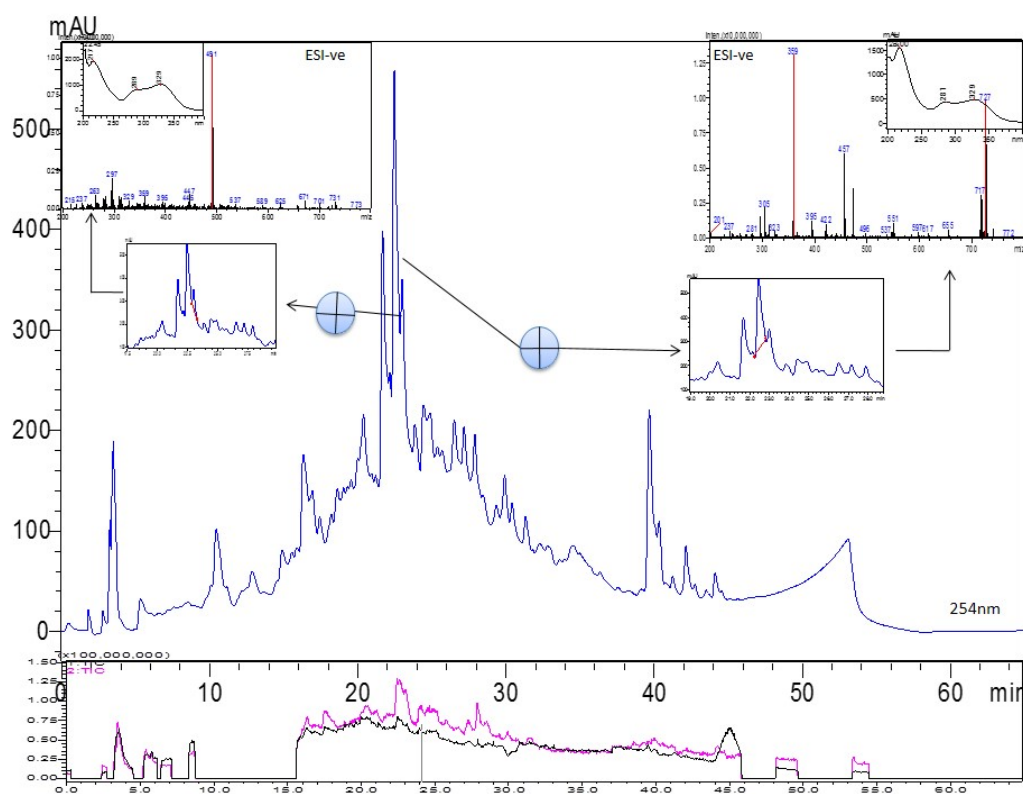
**Figure 5. Total flavonoids estimation; All values are expressed as mean  $\pm$  SD (n=3).** (1) Aqueous extract; (2) Hexane extract; (3) Chloroform extract; (4) Ethyl acetate extract; (5) Butanol extract; (6) Methanolic extract of *O. sanctum*; (7) Aqueous extract; (8) Hexane extract; (9) Chloroform extract; (10) Ethyl acetate extract; (11) Butanol extract; (12) Methanolic extract of *O. kilimandscharium*.



**Figure 6. Total phenolic estimation; All values are expressed as mean  $\pm$  SD (n=3).** (1) Aqueous extract; (2) Hexane extract; (3) Chloroform extract; (4) Ethyl acetate extract; (5) Butanol extract; (6) Methanolic extract of *O. sanctum*; (7) Aqueous extract; (8) Hexane extract; (9) Chloroform extract; (10) Ethyl acetate extract; (11) Butanol extract; (12) Methanolic extract of *O. kilimandscharium*.

## Total phenolic content assessment

The presences of phenolic compound in plant extract also contribute to its antioxidant activity. The total phenolic content was estimated in terms of gallic acid equivalent. The total phenolic content of the two *Ocimum* species ranges from  $3.46 \pm 0.49$  to  $29.88 \pm 2.89$   $\mu\text{g}$  GAE/mg of dried extracts and the ethyl acetate fraction of both the species showed better phenolic content with respect to other extracts which was positively co-related with the total antioxidant capacity. The order of the total phenolic content of different extracts was found to be as 4>5>3>1>2>6 for *O. sanctum* and 10>12>11>9>7>8 for *O. kilimandscharicum* (Fig. 6).



**Figure 7.** Comparative HPLC profile of active fraction of two *Ocimum* species with peak identification using characteristic UV and MS spectra.

## Phytochemical Profiling

Since the ethyl acetate extract of both the species have shown the potent antioxidant activity, the phytochemical profiling of the ethyl acetate extracts with HPLC-PDA and LC-MS was carried out. The presence of various compounds and the hydroxyl cinnamic acid derivatives was observed. It has already been reported that other species of *Ocimum* contains many phenolics like rosmarinic

acid, caffeic acid, etc. The RP-HPLC chromatogram of the ethyl acetate fraction of *O. sanctum* revealed eight major peaks which were characterized as flavonoids on the basis of UV-spectra matching (Fig. 7). The presences of UV active chromophore due to the presence of two aromatic rings provided great support to the flavonoids aglycones which consequently absorb UV absorption peaks. The first maxima found at 240-285 nm range due to the presence of the ring-A benzoyl system and second maximum at 300-400nm range, to the substitution pattern and conjugation of the B-ring cinnamoyl system (Mabry et al., 1970; Markham, 1982). Our previous studies on roots of *O. sanctum* have already revealed the presence of ocimol, galactose, arabinose,  $\beta$ -sitosterol, ocimic acid, ursolic acid, trihydroxy ursolic acid, palmityl glucoside, menthylsalicylic glucoside and capryl tetraglycosidic salicylate (Ahmad et al., 2012). The UV spectra of eight major peaks showed two absorptions at the max range of 238-240 nm of band-II and another at the range of 307-354 nm of band-I indicated that these compounds tentatively belongs to 3-substituted flavonol or flavones. According to available characteristic, UV spectra of each peak can be used as an indicative tool for the characterization of C-ring, whereas the MS spectra could provide additional, significant information in prediction of active molecules (Fig. 7).

## Conclusion

Our investigation on two species of *Ocimum* root extracts revealed that the ethyl acetate extracts of both the species revealed potent antioxidant potential and possess elevated phenolic and flavonoid content. The presence of polyphenolic compounds such as rosmarinic acid, caffeic acid and its derivatives were further tentatively characterized by HPLC/LC-MS profiling of the extract which strengthens the antioxidant potential and usefulness of the waste roots for tapping its prospective commercial utilization.

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## Abbreviations

DPPH: 2, 2-diphenyl-1-picrylhydrazyl.  
FRAP: Ferric reducing antioxidant power  
FSE: Ferrous sulphate equivalent.  
GAE: Gallic acid equivalent.  
LC/MS: Liquid chromatography-mass spectrometry.  
QCE: Quercetin equivalent.  
RP-HPLC: Reverse phase high performance liquid chromatography.  
TAC: Total antioxidant capacity  
UV: Ultraviolet

## Author Contribution

Envisaged and designed the experiments: ST, KS, SL; Root collection and extract preparation: KA, AA; Antioxidant Test: DKS, SL; HPLC/LC-MS Characterization: J, KS; Data Analysis: KA, DKS, AA, KS, ST, SL; Manuscript writing: KA, DKS, J, KS, ST, SL. All authors reviewed and commented on final draft.

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