



## Phytochemical Characterization and Antioxidative Property of *Ocimum canum* Sims.

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### Abstract

Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. In the present study aimed to find out the phytochemicals present in the various solvent leaf extracts of *Ocimum canum* Sims and all the fractions of *O. canum* demonstrated H-donor activity. The highest DPPH radical scavenging activity was detected in chloroform fraction (IC<sub>50</sub> 0.145 mg/ml), followed by ethyl acetate, pet-ether and residual fractions (IC<sub>50</sub> 0.164, 0.29 and 0.6 mg/ml respectively). The residual fraction of *O. canum* showed the highest reducing ability (absorbance 0.620) than all the other fractions tested. The scavenging activity of the pet-ether fraction (IC<sub>50</sub> 0.141mg/ml) was higher than that of quercetin (0.308 mg/ml). The residual, chloroform and ethyl acetate fractions also showed significant scavenging activities (IC<sub>50</sub> of RF, CF and EAF were 0.125, 0.186 and 0.232 mg/ml respectively) when compared to the standard. The chloroform fraction showed strong nitric oxide scavenging activity (IC<sub>50</sub> 0.183 mg/ml) and that of standard curcumin was 0.078 mg/ml. The total antioxidant activity of the fractions of *O. canum* was determined by the thiocyanate method and compared with the standard, α-tocopherol. The phosphomolybdate method is quantitative, since the total antioxidant capacity is expressed as α-tocopherol equivalents. Among the fractions tested, the chloroform fraction showed the highest ferrous ion chelating ability (IC<sub>50</sub> 0.276 mg/ml). Moreover, total phenolics concentration equivalents to gallic acid was found in the range of 59.50 to 109.0 mg/g of plant extracts, which correlated with antioxidant activity. The findings indicated promising antioxidant activity of crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines.

**Keywords:** medicinal plants, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates

### 1. Introduction

Medicinal plants are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health Dabelstein *et al.*, (2007) [3]. Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter Kokate, (2004) [13]. Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So the medicinal plants with antioxidant property are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress Nithya and Balkrishnan, (2011) [20]. Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease and fibrosis etc. Antioxidants play many vital functions in a cell and have many beneficial effects when present in foods Sharma *et al.* (2013) [27]. The *Ocimum canum* belongs to the Lamiaceae. It is commonly known as Nai tulasi is a rich source of aromatic compounds. These compounds are being used as antimicrobial, antiemetic, antidiabetic, antifertility, antiasthmatic, antistress and anticancer activity (Makker *et al.*, 2007) [15]. The plant

shows a pungent, aromatic flavour and is commonly cultivated for culinary purposes. *O. canum* is used specially for treating various types of diseases and lowering blood glucose and also treats cold, fever, parasitic infestations on the body and inflammation of joints and headaches (Ngassoum *et al.*, 2004) [22]. The findings indicated promising antioxidant activity of crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines.

### 2. Materials and Method

#### Collection and extraction of plant

Fresh *O. canum* plants were collected from the Botanical garden of Ayya Nadar Janaki Ammal College, Sivakasi. Leaves were separated from the plant and dried under shade. Then the leaves were powdered.

#### Preparation of the extract and fractionation

The air-dried powdered leaves of *O. canum* (100 g) was extracted with methanol-water (7:3) mixture using a mechanical shaker for 4 h. The resultant extract was concentrated under reduced pressure to yield a residue. The hydromethanolic extract was then extracted successively with equal volumes of petroleum-ether, chloroform and ethyl acetate. Each fraction was then concentrated under reduced pressure to obtain the pet-ether fraction (PEF1.6% w/w), chloroform fraction (CF-1.8% w/w), ethyl acetate fraction (EAF-0.8% w/w) and residual fraction (RF5.3% w/w).

### Drugs and chemicals

DPPH was obtained from Hi Media Laboratories Pvt. Ltd., Mumbai. Quercetin and pyrocatechol were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai. Folin-Ciocalteu reagent was obtained from SD Fine Chemicals Pvt. Ltd, Mumbai. Calf thymus DNA was purchased from Genei Chemicals, Bangalore. Ferrozine was obtained from Sigma Aldrich, USA. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

### Phytochemical characterization

Leaf samples of *O.canum* extracted with different solvents were subjected to various preliminary phytochemical analysis for the presence or absence of various phytoconstituents by the following tests adapted by Rai *et al* (2016) [26].

### In vitro antioxidant activity

The free radical scavenging activity of the fractions was measured in vitro by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Mensor *et al.*, 2001) [18]. The reducing power was investigated by the Fe<sup>3+</sup>-Fe<sup>2+</sup> transformation in the presence of the fractions as described by Fejes *et al.*, (2000) [5]. The Fe<sup>2+</sup> can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Meir *et al.*, 1995) [17]. Hydroxyl radical scavenging activity was measured by the ability of the different fractions of *O. canum* to scavenge the hydroxyl radicals generated by the Fe<sup>3+</sup>-ascorbate-EDTA-H<sub>2</sub>O<sub>2</sub> system, Fenton reaction (Halliwell *et al.*, 1987; Ilavarasan *et al.*, 2005) [7, 11]. The percentage scavenging activity at different concentrations of the fractions was determined and the IC<sub>50</sub> values were compared with the standard, α-tocopherol (Oktay *et al.*, 2003) [23]. Nitric oxide radical scavenging assay was performed according to the method described by Sreejayan *et al.*, (1997) [28]. The peroxy radical scavenging activity was determined by thiocyanate method using α-tocopherol (50-800 μg/ml) as standard (Yildirim *et al.*, 1999). The total antioxidant capacity of the fractions was determined by phosphomolybdate method using α-tocopherol as the standard (Jayaprakasha *et al.*, 2002) [12]. The percentage chelating effect on ferrozine-Fe<sup>2+</sup> complex was calculated. The IC<sub>50</sub> values were compared with ascorbic acid (Huang and Kuo, 2000) [9]. The pro-oxidant activity of the fractions was determined by bleomycin-dependent DNA damage. All the determinations were carried out in triplicate (Ng *et al.*, 2003). Total soluble phenolics of the fraction were determined with Folin-Ciocalteu reagent using pyrocatechol as the standard (Gulcin *et al.*, 2004). Total soluble flavonoid content of the fractions was determined with aluminium nitrate using quercetin as the standard (Hsu, 2006) [8].

### Statistical analysis

All experiments were performed in triplicate (n=3) and results were expressed as mean ± SE. Statistical analysis was carried out with (SPSS package version 17.10) using ANOVA followed by Turkey's test (P<0.05).

## 3. Results

### Phytochemical screening

Phytochemical screening of the crude extracts of different fractions of the leaves of *Ocimum canum* was represented in Table 1. Carbohydrates were observed in the alcoholic and

aqueous extracts of leaves whereas phyosterols were present in the chloroform and petroleum ether extracts of leaves. Volatile oils showed their presence when the leaves are extracted with chloroform and petroleum ether. Tannins were observed in the alcoholic and chloroform extracts. Flavonoids showed their presence when the leaves were extracted with either alcohol or water. Terpenoids were present in the chloroforms and petroleum ether leaf extracts. Alkaloids, saponins, glycosides, gums and mucilage were not observed in any of the solvent extracts examined.

### DPPH Assay

All the fractions of *O. canum* demonstrated H-donor activity. The highest DPPH radical scavenging activity was detected in chloroform fraction (IC<sub>50</sub> 0.145 mg/ml), followed by ethyl acetate, pet-ether and residual fractions (IC<sub>50</sub> 0.164, 0.29 and 0.6 mg/ml respectively) (Table 2). These activities are less than that of ascorbic acid. The scavenging ability increased towards the ethyl acetate fraction with increasing polarity of the solvent.

### Reducing power ability

Table 3 shows the reductive capabilities of different fractions of *O. canum* when compared to the standard, BHT. Like the antioxidant activity, the reducing power increased with increasing amount of the fractions. The residual fraction of *O. canum* showed the highest reducing ability (0.620nm) than all the other fractions tested. However, the activity was less than the standard, BHT (1.092nm). The pet-ether, chloroform and ethyl acetate fractions also showed significant activity indicating its reductive ability.

### Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of deoxyribose by the free radicals generated by the Fenton reaction. All the fractions of *O. canum* and the standard (quercetin) inhibited the production of hydroxyl radicals. The scavenging activity of the pet-ether fraction (IC<sub>50</sub> 0.141mg/ml) was higher than that of quercetin (0.308 mg/ml). The IC<sub>50</sub>s values of the chloroform, ethyl acetate and residual fractions were 0.348, 0.565 and 0.625 mg/ml respectively (Table 2).

### Hydrogen peroxide scavenging assay

All the fractions of *C. grandis* scavenged hydrogen peroxide in a concentration-dependent manner. The pet-ether fraction (PEF) of *O. canum* showed strong H<sub>2</sub>O<sub>2</sub> scavenging activity (IC<sub>50</sub> 0.092 mg/ml) whereas that of the standard, α-tocopherol was 0.065 mg/ml. The residual, chloroform and ethyl acetate fractions also showed significant scavenging activities (IC<sub>50</sub> of RF, CF and EAF were 0.125, 0.186 and 0.232 mg/ml respectively) when compared to the standard (Table 2).

### Nitric oxide radical scavenging assay

The fractions of *O. canum* effectively reduced the generation of nitric oxide from Sodium nitroprusside. The chloroform fraction showed strong nitric oxide scavenging activity (IC<sub>50</sub> 0.183 mg/ml) and that of standard curcumin was 0.078 mg/ml. The residual fraction (0.37 mg/ml), pet-ether fraction (0.384 mg/ml) and ethyl acetate fraction (0.553 mg/ml) also showed good scavenging activities (Table 2).

### Thiocyanate method

The total antioxidant activity of the fractions of *O. canum* was determined by the thiocyanate method and compared with the standard,  $\alpha$ -tocopherol. The absorbance decreased with the increasing concentrations of the fractions, which indicate that the fractions could effectively decrease the amount of formed peroxides. The total antioxidant activity of the pet-ether and chloroform fractions were almost similar (IC<sub>50</sub> 0.278 and 0.271 mg/ml respectively) and that of the standard,  $\alpha$ -tocopherol was 0.096 mg/ml. The ethyl acetate and residual fractions also showed good antioxidant activity but at higher concentrations (IC<sub>50</sub> 0.405 and 0.46 mg/ml respectively) (Table 2).

### Phosphomolybdate method

The phosphomolybdate method is quantitative, since the total antioxidant capacity is expressed as  $\alpha$ -tocopherol equivalents. Among the fractions tested, the chloroform fraction contains 31.66 $\mu$ g vitamin E equivalent/ 100  $\mu$ g. The antioxidant activity increased in the order of chloroform fraction > residual fraction > ethyl acetate fraction > pet-ether fraction (Table 4).

### Ferrous chelating ability

Addition of the fractions of *O.canum* interferes with the ferrous-ferrozine complex and the red colour of the complex decreased with the increasing concentrations of the fractions. All the fractions captured ferrous ions before ferrozine and thus have ferrous chelating ability. Among the

fractions tested, the chloroform fraction showed the highest ferrous ion chelating ability (IC<sub>50</sub> 0.276 mg/ml). The abilities shown by pet-ether, ethyl acetate and residual fractions were almost similar (IC<sub>50</sub> 0.393, 0.433 and 0.405 mg/ml respectively). Ascorbic acid (IC<sub>50</sub> 0.017 mg/ml) showed the highest ferrous ion chelating ability (Table 2).

### Bleomycin-dependent DNA damage

The pro-oxidant activity of the fractions of *C. grandis* was assessed by their effects on damage to DNA in the presence of a bleomycin-Fe<sup>3+</sup> complex. The absorbance of all the fractions decreased with increasing concentrations, which proves that none of the fractions exhibited pro-oxidant activity (Table 5).

### Total phenolic and flavonoid content

Total phenolic content was estimated by using Folin-Ciocalteu reagent. Total phenolic content of the different fractions of *O. canum* were solvent dependent and expressed as  $\mu$ g pyrocatechol equivalent. The content of the total phenolics in the fractions decreased in the order of pet-ether > chloroform > ethyl acetate > residual fractions. The total flavonoid content in the fractions was expressed as  $\mu$ g quercetin equivalent. The pet-ether fraction of *O.canum* showed highest amount of flavonoids among the fractions tested. The content of total flavonoids in the fractions decreased in the order of pet-ether fraction > residual fraction > ethyl acetate fraction > chloroform fraction (Table 4).

**Table 1:** Preliminary phytochemical analysis of *Ocimum canum* leaves

Test	Alcoholic extract	Aqueous Extract	Chloroform extract	Petroleum Ether Extract
Alkaloid	-	-	-	-
Carbohydrates	+	+	-	-
Glycosides	-	-	-	-
Phytosterols	-	-	+	
Volatile oils	-	-	+	+
Saponins	-	-	-	-
Tannins	+	-	+	-
Proteins and aminoacids	-	-	-	-
Gums and mucilages	-	-	-	-
Flavonoids	+	+	-	-
Terpenoids	-	-	+	+

**Table 2:** Antioxidant Activities of the fractions of *Ocimum canum* leaves

Fraction	IC <sub>50</sub> (mg/ml)					
	DPPH	OH <sup>•</sup>	NO <sup>•</sup>	H <sub>2</sub> O <sub>2</sub>	Fe <sup>2+</sup> chelating	Thiocyanate method
Pet-ether	0.29±0.83	0.141±2.1	0.384±.14	0.092±0.01	0.393±0.12	0.278±0.6
Chloroform	0.145±0.01	0.348±0.6	0.183±0.21	0.186±0.76	0.276±0.2	0.271±0.4
Alcohol	0.164±0.83	0.565±0.36	0.553±0.74	0.232±0.03	0.433±0.37	0.405±0.3
Residual	0.6±0.02	0.625±0.7	0.370±0.37	0.125±0.09	0.405±0.3	0.460±0.12
Ascorbic acid	0.03±0.01				0.017±0.01	
Quercetin		0.308±0.2				
Curcumin			0.078±0.8			
$\alpha$ -tocopherol				0.065±0.21		0.96±0.12

**Table 3:** Reducing power ability of different fractions of *Ocimum canum*

Fractions	Absorbance at 700nm				
	50 $\mu$ g/ml	100 $\mu$ g/ml	150 $\mu$ g/ml	200 $\mu$ g/ml	250 $\mu$ g/ml
Pet-ether	0.067±0.003	0.077±0.004	0.092±0.001	0.112±0.008	0.142±0.05*
Chloroform	0.062±0.002	0.114±0.004	0.173±0.002	0.221±0.023	0.432±0.002*
EthylAcetate	0.242±0.005	0.269±0.007	0.345±0.005	0.426±0.006	0.486±0.006*
Residual	0.432±0.04	0.507±0.004	0.544±0.004	0.586±0.006	0.620±0.020*
BHT	0.092±0.002	0.214±0.004	0.314±0.004	0.640±0.001	1.092±0.002

**Table 4:** Total antioxidant activity (phosphomolybdate method) and total phenolic and flavonoid contents of different fractions of *Ocimum canum* leaves

Fractions	Total antioxidant activity ( $\mu\text{g}$ vitamin E equivalent/100 $\mu\text{g}$ )	Total phenolic content ( $\mu\text{g}$ pyrocatechol equivalent/mg)	Total flavonoid content ( $\mu\text{g}$ quercetin equivalent/mg)
Pet-ether	6.46 $\pm$ 0.03 <sup>a</sup>	63.3 $\pm$ 0.88 <sup>a</sup>	92.02 $\pm$ 0.4 <sup>a</sup>
Chloroform	31.66 $\pm$ 0.33 <sup>b</sup>	47.66 $\pm$ 0.88 <sup>b</sup>	12.01 $\pm$ 0.6 <sup>c</sup>
Ethyl acetate	7.23 $\pm$ 0.36 <sup>a</sup>	32.50 $\pm$ 0.28 <sup>c</sup>	14.33 $\pm$ 0.13 <sup>c</sup>
Residual	8.10 $\pm$ 0.05 <sup>c</sup>	27.01 $\pm$ 0.37 <sup>c</sup>	25.66 $\pm$ 0.32 <sup>b</sup>

Values are expressed as mean  $\pm$  SE of 20 replicates. Values within a column followed by different letters are significantly different ( $P < 0.05$ ).

**Table 5:** Pro-oxidant activity of different fractions of *Ocimum canum* leaves

Fractions	Absorbance at 532nm				
	25 $\mu\text{g}/\text{ml}$	50 $\mu\text{g}/\text{ml}$	100 $\mu\text{g}/\text{ml}$	150 $\mu\text{g}/\text{ml}$	200 $\mu\text{g}/\text{ml}$
Pet-ether	0.153 $\pm$ 0.003 <sup>a</sup>	0.096 $\pm$ 0.001 <sup>a</sup>	0.064 $\pm$ 0.002 <sup>a</sup>	0.044 $\pm$ 0.002 <sup>a</sup>	0.018 $\pm$ 0.1 <sup>c</sup>
Chloroform	0.143 $\pm$ 0.002 <sup>a</sup>	0.083 $\pm$ 0.002 <sup>a</sup>	0.067 $\pm$ 0.002 <sup>a</sup>	0.045 $\pm$ 0.003	0.035 $\pm$ 0.3
Ethyl acetate	0.064 $\pm$ 0.002 <sup>a</sup>	0.045 $\pm$ 0.001 <sup>a</sup>	0.034 $\pm$ 0.002 <sup>a</sup>	0.021 $\pm$ 0.007 <sup>a</sup>	0.017 $\pm$ 0.24 <sup>b</sup>
Residual	0.084 $\pm$ 0.001 <sup>a</sup>	0.064 $\pm$ 0.002 <sup>a</sup>	0.041 $\pm$ 0.001 <sup>a</sup>	0.032 $\pm$ 0.004 <sup>a</sup>	0.027 $\pm$ 0.7 <sup>c</sup>
Ascorbic acid	0.904 $\pm$ 0.003 <sup>a</sup>	0.780 $\pm$ 0.002 <sup>a</sup>	0.511 $\pm$ 0.004 <sup>a</sup>	0.277 $\pm$ 0.002 <sup>a</sup>	0.121 $\pm$ 0.6 <sup>c</sup>

Values are expressed as mean  $\pm$  SE of 20 replicates. Values within a column followed by different letters are significantly different ( $P < 0.05$ ).

#### 4. Discussion

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms. DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts (Nanjo *et al.*, 1996). DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the fractions in a concentration-dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. All the fractions showed significantly higher inhibition percentage and positively correlated with total phenolic content.

The transformation of  $\text{Fe}^{3+}$  into  $\text{Fe}^{2+}$  in the presence of various fractions was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of reductions (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom (Meir *et al.*, 1995) [17]. The antioxidant principles present in the fractions of *O. canum* caused the reduction of  $\text{Fe}^{3+}$ /ferricyanide complex to the ferrous form, and thus proved the reducing power ability.

Hydroxyl radical is the most deleterious and reactive among the ROS and it bears the shortest half-life compared with other free radicals. The oxygen derived hydroxyl radicals along with the added transition metal ion ( $\text{Fe}^{2+}$ ) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid (Halliwell *et al.*, 1987). All the fractions of *O. canum* when added to the reaction mixture, scavenged the hydroxyl radicals and prevented the degradation of deoxyribose.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radicals which is very toxic to the cell (Halliwell, 1991). Thus, scavenging of  $\text{H}_2\text{O}_2$  is a measure of the antioxidant activity of the fractions. All the fractions of *O. canum* scavenged hydrogen peroxide

which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralising it into water.

*In vitro* inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. Scavenging of nitric oxide radical is based on the generation of nitric oxide from Sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent (Marcocci *et al.*, 1994). The absorbance of the chromophore is measured at 546 nm in the presence of the fractions. All the fractions of *O. canum* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles in the fractions which compete with oxygen to react with  $\text{NO}\cdot$  thereby inhibiting the generation of nitrite.

The amount of formed peroxides was measured by the thiocyanate method. The fractions were incubated with linoleic emulsion in dark at 37°C and the amount of peroxides was determined spectrophotometrically by measuring the absorbance at 500 nm (Yen and Chen, 1995). A decrease in absorbance indicated the antioxidant activity of the fractions which might be due to the inactivation of the free radicals and the presence of flavonoid like phytochemicals.

The phosphomolybdate method has been routinely used to evaluate the total antioxidant capacity of the extracts (Prieto *et al.*, 1997). In the presence of the fractions, the Mo(VI) is reduced to Mo(V) and forms a green coloured phosphomolybdenum V complex which shows maximum absorbance at 695 nm. All the fractions possessed antioxidant activity.

The metal chelating ability of the fractions of *O. canum* was measured by the formation of ferrous ion-ferrozine complex. Ferrozine combines with ferrous ions forming a red coloured complex which absorbs at 562 nm (Yamaguchi *et al.*, 2000). It was reported that the chelating agents which form bond with a metal, are effective as secondary antioxidants, because they reduce the redox potential thereby stabilising the oxidised form of the metal ion (Duh

*et al.*, 1999). The results of our study demonstrate that the fractions have an effective capacity for iron binding, suggesting its antioxidant potential. In addition, the metal chelating ability of the fractions demonstrated that they reduce the concentration of the catalysing transition metal involved in the peroxidation of lipids.

Bleomycin-dependent DNA damage has been adopted as a sensitive and specific method to examine the potential pro-oxidant drugs. Degradation of DNA occur if the samples to be tested reduce the bleomycin-Fe<sup>3+</sup> to bleomycin-Fe<sup>2+</sup> resulting in the formation of a product similar to MDA which reacts with TBA to give a pink colour (Liu and Ng, 2000) [5]. All the fractions decreased the absorbance and bleomycin-Fe<sup>3+</sup> is not converted into bleomycin-Fe<sup>2+</sup>, thereby preventing the DNA degradation. These results confirm that the fractions of *O. canum* are devoid of pro-oxidant activity.

Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities and also decrease cardiovascular complications (Yen *et al.*, 1993) [30]. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. Total phenolic assay by using Folin-Ciocalteu reagent is a simple, convenient and reproducible method. It is employed routinely in studying phenolic antioxidants (Huang *et al.*, 2005) [9]. Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, anticancer activities. They also inhibit enzymes such as aldose reductase and xanthine oxidase. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidants (Cao *et al.*, 1997) [2]. In view of their wide pharmacological and biological actions, they have a greater therapeutic potential. The presence of high phenolic and flavonoid content in the fractions has contributed directly to the antioxidant activity by neutralising the free radicals. Based on the results obtained, it may be concluded that all the fractions of the leaves of *O. canum* showed strong antioxidant activity, reducing power ability, free radical scavenging activity, metal chelating ability and inhibition of  $\beta$ -carotene bleaching when compared to standards such as ascorbic acid,  $\alpha$ -tocopherol, curcumin, and butylatedhydroxytoluene. As the various fractions of *O. canum* exhibited different reactive oxygen species scavenging activities, there may be different percentages of phytochemical constituents present in the fractions. Further studies to evaluate the *in vivo* potential of the fractions in various animal models and the isolation and identification of the antioxidant principles in the leaves of *Ocimum canum* are being carried out.

## 5. References

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