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Phytochemical and Antioxidant Activities of Leaf extracts of *Ocimum americanum*

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Abstract
Presence of antioxidant activity, polyphenolic compounds and reducing potential activities were assessed in the extracts of Ocimum americanum. Extracts of dry and green leaves in different solvents (ethanolic, methanolic and aqueous) were used for phytochemical assays through standard methods. Maximum antioxidant activity was observed in methanolic extract of green leaf i.e. 335.461µg mL^{-1}. Total phenolic content was maximum in ethanolic extract of green leaf (282.67±2.17mgg−1 of gallic acid equivalent) and total flavonoid content was maximum in methanolic extract of dry leaf (946.67 ± 0.88 mgg−1 of rutin equivalent). Aqueous extract of green leaf showed highest reducing potential. Positive correlations were observed between antioxidant activity and polyphenolic compounds (total phenolic and flavonoid content). Similarly, significant correlation was found between antioxidant activities and reducing potential.

Keywords
Antioxidant activity; Polyphenolic compounds; Ethanolic compounds, Methanolic compounds, Aqueous compounds.

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INTRODUCTION
Plants are valuable sources of several chemicals and therefore, plants are being used as medicines since ancient times[9,21]. Presence of various bioactive compounds in plants such as alkaloids, terpenoids, flavonoids, saponins, etc. have drawn attention of scientists in formulating safe, less toxic and cost-effective medicines. A free radical is defined as any atom or molecule possessing unpaired electrons. Generally, free radicals are formed due to environmental stress, unhealthy food uptake or illness[20].Free radicals are very unstable, and they try to gain an electron to attain stability. Thus, in this process a chain reaction gets started, which may damage biomolecules, DNA and cells. Antioxidants are the substances, which inhibits or delays the process of oxidizing chain reactions even at low concentrations[10]. Both natural and synthetic antioxidants are being used in the treatment of various human diseases[22]. Synthetic antioxidants are costly and sometimes release toxic substances during the production[15]. Whereas, natural antioxidants are more efficient, cost- effective and have negligible side effects. So, scientists are exploring different plant sources to get better natural antioxidants[17]. Antioxidant property of a plant depends upon the presence and concentration of different types of phenolic compounds[26]. Major types of phenolic compounds are flavonoids, anthocyanins, tannins and phenolic acids[16]. Flavonoids are powerful antioxidants against free radicals, because they show various pharmaceutical

activities such as free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action[28].

Ocimum americanum belongs to family Lamiaceae and it is known as limehairy, rosary basil or "hoary basil". It is an annual herb with white or lavender flowers. It is native to Africa, the Indian Subcontinent, China, and Southeast Asia. The plant was identified by BSI (Botanical Survey of India, Allahabad) and it was given accession no.97235.*Ocimum americanum* is valuable for its culinary and pharmaceutical properties. The juice of the leaves is very useful in the treatment of catarrh, cold, bronchitis, dysentery, migraine and skin infections. The plant also contains a volatile oil which is useful in soap and cosmetics industries for fragrance. The oil shows antibacterial, antifungal and anaesthetic properties[24]. The seeds of the plants are diuretic[19].The main chemical constituents of volatiles oils of *O. americanum* are methyl cinnamate, methylheptenone, methylnanylketone, d-camphor, citral, ocimin, methylchavicol, linalool, nevadensin, salvigenin, beta-sitosterol, betulinic, ursolic, oleanolic acids, flavanoids and pectolinarigenin-7-methylether[23]. Nineteen different flavonens have been identified in 111 leaf specimens of *Ocimum americanum* by HPLC[30]. The present work was undertaken to study phytochemical and antioxidant properties of *Ocimum americanum.*

MATERIAL AND METHODS

Chemicals:

1,1-Diphenyl,2-picryl hydrazyl (DPPH), phosphate buffer, potassium hexaferricyanide ($K_3[Fe(CN)_6]$), trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), rutintrihydrate, Folin-Ciocalteu's phenol, sodium carbonate (Na_2CO_3) and gallic acid.

Material collection and preparation of extracts:

From green leaves:

Green young leaves of *Ocimum americanum* were collected from the campus of Banaras Hindu University (BHU), Varanasi, India. Leaves were washed under tap water and dried by blotting papers. 1g of leaves was crushed in pestle and mortar in 5 ml of different solvents like ethanol, methanol and double distilled water separately. Solutions were centrifuged at 3000 rpm for 10 minutes to remove all cell debris. Supernatant was taken and final volume was maintained at 10 ml. The extracts were stored at 4^0C for further use.

For dry leaves:

Leaves were shade dried for 6-7 days, oven dried at 45-50 ^{0}C for 2-3 hrs and then grinded in mechanical grinder to make coarse powder. 20 g of leaf powder was added in 200 ml of solvent and extracts were prepared by using soxhlet apparatus. Ethanol, methanol and DDW (Double distilled water) were used as solvents for the extraction. Extracts were then filtered and dried at 45 ˚C on rotary evaporator. Extracts were stored at -20 ˚C for further use.

Evaluation of antioxidant activity by using DPPH:

This experiment was based on the procedure of [34] with slight modifications. DPPH was prepared by dissolving 25µg/ml in methanol. 3 ml of DPPH was added to 1 ml of sample and was incubated for 30 min at room temperature. OD (optical density) was measured at 517 nm by spectrophotometer (UV1, Thermo Scientific, US). Methanol was used as blank. Solution of DPPH without sample was used as control. Antioxidant activity (%) was calculated by using following equations.

$$\text{Antioxidant activity } (\%) = \frac{Ac-As}{Ac} \times 100$$

Where: Ac = OD of control

As = OD of sample

Measurement of Total Phenolic Content:

TP concentration was measured by **Folin-Ciocalteu** assay[25]. The reaction mixture was prepared by mixing 0.5 ml of plant extract, 2.5 ml of Folin reagent (1:10 DDW), 2.5 ml of sodium carbonate (10.6 g in 100 ml Water). It was incubated for 30 minutes at room temperature and its absorbance was measured at 765 nm on a Spectrophotometer. Aqueous solution (DDW) without any reagent was used as control. Gallic acid was used as standard.

Measurement of Total Flavonoid content:

Total flavonoids content was based on the method given by[14].The reaction mixture was prepared by mixing 2 ml of plant extracts,0.6 ml of sodium nitrite (5%, w/v), 0.5 ml of aluminum chloride (10%,w/v), 3 ml of sodium hydroxide (4.3%, w/v) and final volume was made up to 10 ml with water (DDW). Time period of approximate 6 min was required for shaking after every step to complete the reaction at room temperature. The solution was incubated for 15 min and absorbance was measured at 500 nm on a Spectrophotometer. DDW was used as control. Rutin was used as a standard.

Measurement of Reducing Potential:

Reducing potential of the extract was measured by the procedure given by[16] with slight modification. The reaction mixture containing 1 ml of plant extract with different concentrations was mixed with 2.5 ml of phosphate buffer solution (0.2 M, pH =6.6) and 2.5 ml potassium hexaferricyanide (1%). The reaction

mixture was incubated at 50 °C in water bath for20 minutes. After 20 minutes, 2.5 ml trichloro acetic acid (TCA, 10%) was added to terminate the reaction and centrifuged for 10 minutes at 3000 rpm. The supernatant (2.5 ml) was mixed with 2.5 ml of double distilled water and 0.5 ml ferric chloride (0.1%) solution. The absorbance was measured at 700 nm. Ascorbic acid was used as standard.

RESULTS:

Antioxidant Activity:

All the extracts of green and dry leaf of *Ocimum americanum* have significant free radical scavenging activity. The methanolic extract of green leaf showed maximum scavenging activity (IC_{50}= 335.461 µg mL^{-1}) whereas other extracts of green leaf ethanolic (IC_{50} = 425.588 µg mL^{-1}) and aqueous (IC_{50}= 563.802 µg mL^{-1}) showed comparatively lower activity. The ethanolic extract of dry leaves shows higher scavenging activity i.e. (IC_{50}= 381.695µg mL^{-1}) than methanolic (IC_{50}= 437.438 µg mL^{-1}) and aqueous (IC_{50}= 604.352 µg mL^{-1}) extracts of dry leaves **(Table: 1)**.

Table 1. Antioxidant activity of *Ocimum americanum* green and dry leaf extract by DPPH method.

Conc.	Green leaf extract			Dry leaf extract		
	Percentage inhibition (Mean ± SE)					
	Aq	Mth	Eth	Aq	Mth	Eth
100	33.41 ± 0.18	42.49 ± 0.15	33.45 ± 0.22	20.32 ± 0.35	30.81 ± 0.20	35.14 ± 0.34
200	38.92 ± 0.19	45.05 ± 0.25	36.18 ± 0.21	23.78 ± 0.23	37.08 ± 0.20	39.35 ± 0.19
300	43.53 ± 0.25	48.43 ± 0.26	44.54 ± 0.21	30.16 ± .026	43.03 ± 0.27	46.05 ± 0.24
400	45.22 ± 0.26	52.15 ± 0.22	48.12 ± 0.23	39.02 ± 0.22	48.32 ± 0.15	50.59 ± 0.16
500	49.83 ± 0.19	54.48 ± 0.12	56.31 ± 0.25	45.41 ± 0.14	52.76 ± 0.18	58.05 ± 0.27
600	52.64 ± 0.19	58.91 ± 0.25	60.07 ± 0.24	47.35 ± 0.18	59.14 ± 0.22	63.24 ± 0.24
700	53.09 ± 0.17	64.96 ± 0.24	64.33 ± 0.19	55.89 ± 0.20	66.16 ± 0.21	66.59 ± 0.23
800	56.81 ± 0.22	68.57 ± 0.22	68.94 ± 0.25	59.78 ± 0.14	68.86 ± 0.21	70.59 ± 0.22
900	60.52 ± 0.23	69.85 ± 0.22	74.23 ± 0.19	68.11 ± 0.21	75.57 ± 0.24	77.84 ± 0.20
1000	61.75 ± 0.19	71.13 ± 0.22	77.47 ± 0.24	76.43 ± 0.16	79.78 ± 0.09	80.11 ± 0.24
IC_{50}	563.802	335.461	425.588	604.352	437.438	381.695

IC_{50} of Ascorbic Acid is 54.424. All data is highly significant at $p \leq 0.05$

Total Phenolic Content:

TPC was determined by Folin-Ciocalteu assay and reported as gallic acid equivalents (GE) in reference to standard curve (y = 0.003x+0.013, R^2 = 0.994). Phenolic content was maximum in methanolic extract of dry leaf (248.33 ± 2.17 mg/g) but lower in ethanolic extract (195.00 ± 1.79 mg/g of GE) followed by aqueous (163.00 ± 2.50 mg/g of GE) extracts. Similarly, phenolic content of ethanolic extract of green leaf was higher (282.67 ± 2.15mg/g) than other two extracts i.e. methanolic (237.33 ± 1.94 mg/g of GE) and aqueous (148.33 ± 1.8 mg/g of GE) Fig.1 (a).

Total Flavonoid Content:

Total flavonoid content was assessed by Rutin Equivalent (RE) by reference to standard curve (y = 0.0005x-0.021, R^2 = 0.994). In green leaf, TFC was higher in ethanolic extract (858.67 ± 2.91 mg/g) than methanolic (640.00 ± 3.48 mg/g) and aqueous (568.33 ± 2.73 mg/g) extracts. However, flavonoid content was observed maximum in methanolic extract of dry leaves (946.67 ± 0.88 mg/g). Other two extracts of dry leaves aqueous extracts: (719.00 ± 1.73 mg/g) and ethanolic (558.00 ± 1.15 mg/g) of RE also showed high presence of flavonoids compared to various extracts of green leaves Fig.1 (b).

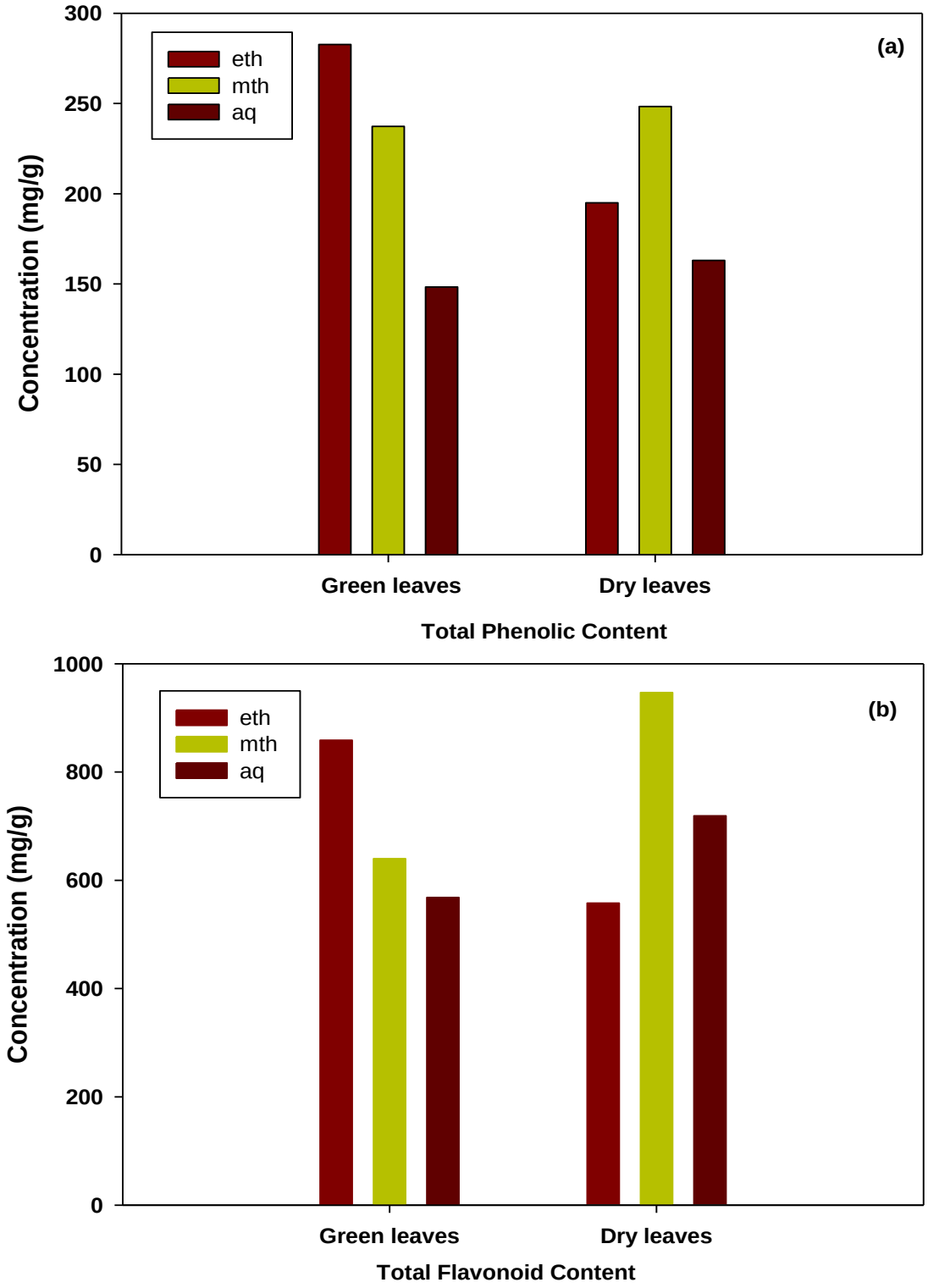

Fig. 1. Comparative (a) Total Phenolic Content (TPC) (b) Total Flavonoid Content (TFC)

Reducing Potential:

The reducing power capacity of the extracts (ethanolic, methanolic and aqueous) of both green and dry leaf increased in a concentration dependent manner from lower to higher concentrations. Maximum reducing power was observed in aqueous extract of green leaf. Ethanolic and methanolic extracts showed comparatively less reducing power. But in contrast, better reducing power was seen in ethanolic extract of dry leaf than its other extracts (aqueous and methanolic) Fig.2 (a) and (b).

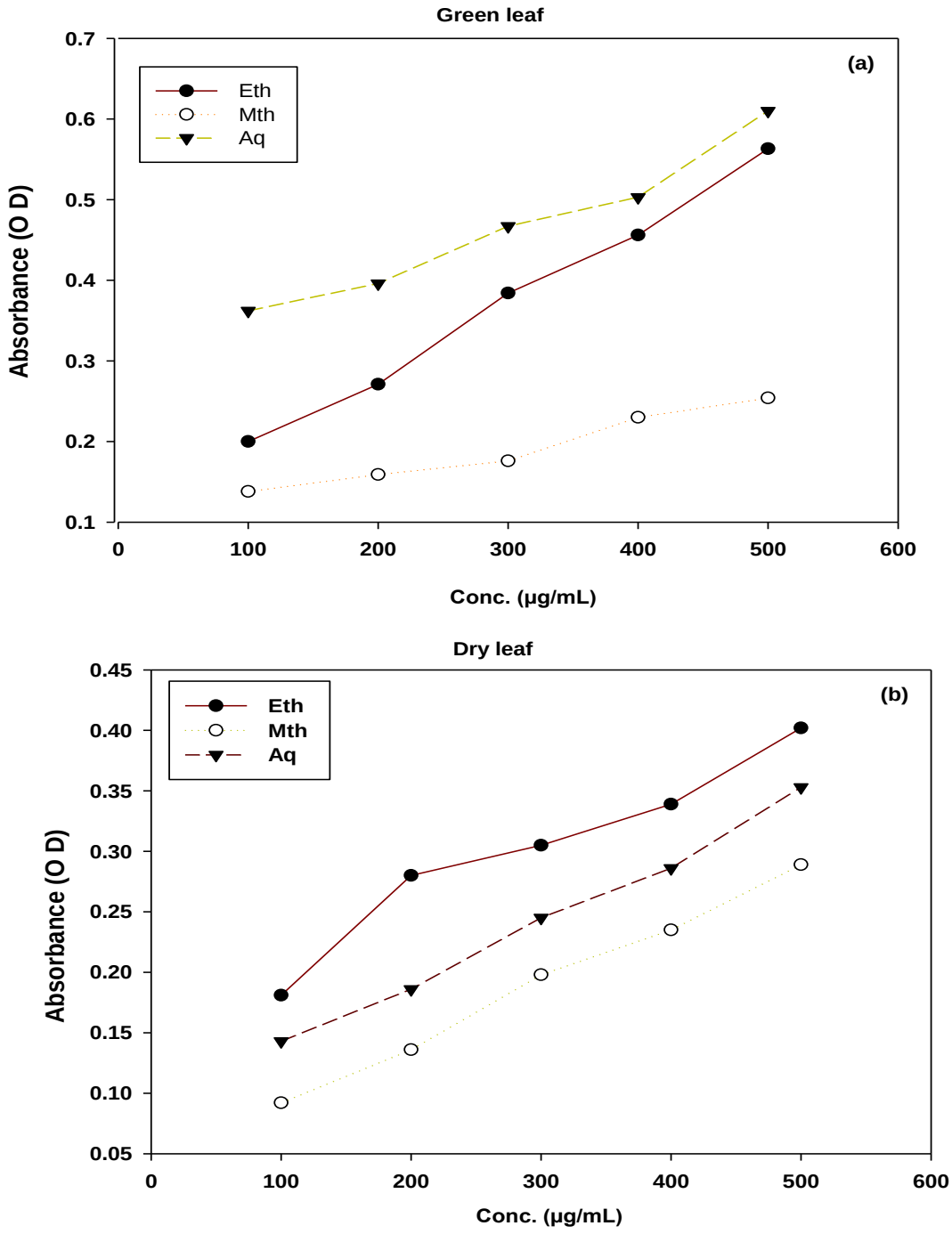

Fig. 2. Reducing power capacity of extract of (a) green leaf and (b) dry leaf

Correlation between antioxidant activity and polyphenolic compounds:

Total antioxidant activity and polyphenolic contents (TPC & TFC) of various extracts showed significant and linear correlation. Correlation coefficient (R^2) of various extracts also showed close relationship between antioxidant activity and polyphenolic contents (Fig.3 a-f).

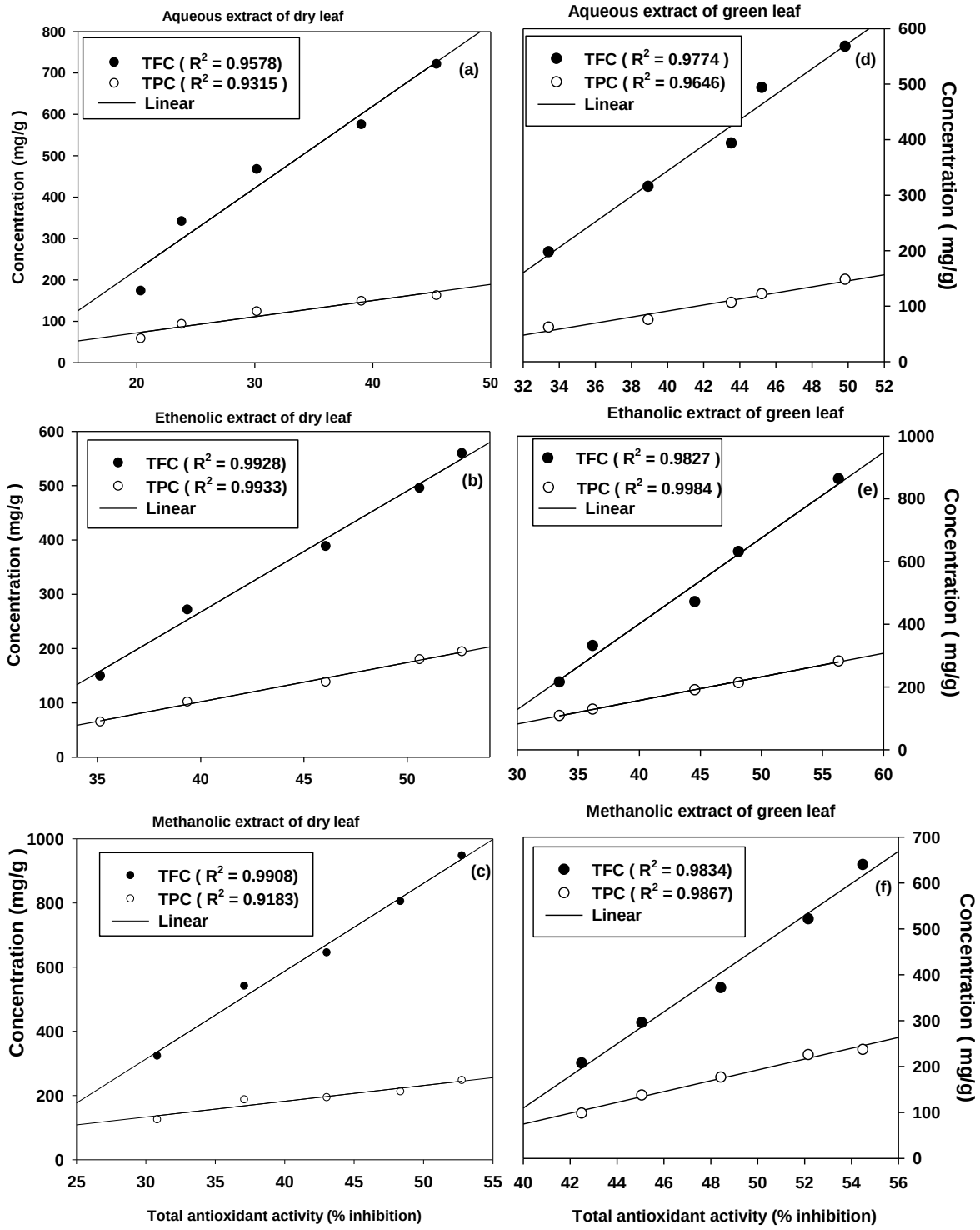

Fig. 3. Total antioxidant activity of dry leaf (a) Aqueous extract (b) Ethanolic extract (c) Methanolic extract and green leaf (d) Aqueous extract (e) Ethanolic extract (f) Methanolic extract

DISCUSSION:

For the assessment of phytochemical activities, different solvents were tried here. Effect of different solvents on extract preparation was also studied by other researchers and it was found that during extraction process solvents solubilise chemicals of similar polarity present in the extract[27]. Therefore, plant extracts in different solvents show different medical efficacies similar to our observation. The presence of significant secondary metabolites like alkaloids, flavonoids, glycosides, tannins, steroids have been observed in many plants which make them medicinally important. Such plant extracts are being used as natural antioxidants which have been helpful in reducing the risk of many disease such as cancer, stroke and heart diseases[1].

DPPH method is an easy and rapid technique to assess antioxidant activity of plant extracts or a specific compound[17]. Antioxidant activity of DPPH is due to presence of their hydrogen donating ability or radical scavenging activity, this method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant[31].The result of DPPH radical scavenging activities depend on concentrations of plant extracts. Its scavenging activity increase with the increase in the concentration of extracts[18]. Methanolic extract of the green leaf has stronger antioxidant activity in comparison to other extracts. Free radicals are known to play a very important role in a wide variety of pathological manifestations. Antioxidant have potential to fight against free radicals and protect us from various diseases. They perform their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanism[29].

The reducing potential of any compound play important role in its antioxidant activity. Many authors have reported positive correlation between antioxidant activity and reducing potential of plant extracts[7, 8, 32]. Similarly, present study shows significant correlation between total antioxidant activity and reducing potential of extracts.

Phenylalanine or its precursor shikmic acid produces plant polyphenols. Plant polyphenols are important dietary antioxidant because of their ideal structural chemistry for free radical scavenging activity[1]. It has been reported that flavonoids have significant antioxidant activity which effect human health and fitness. Flavonoids can perform by scavenging or chelating process[3,12]. Phenolics have high potential to scavenge free radicals due to presence of many phenolic hydroxyl groups[17].The compounds like flavonoids and phenolics which contains hydrolics groups are responsible for radical scavenging activity in the plants[4,33].

In plants it is found that phenolic acids are conventionally coupled with the cell wall complexes or construct ester and glycosidic linkages with organic compounds, like glucose, quinic, maleic and tartaric acid and terpenes. Flavonoids are present in the plants in form of aglycones and glycosides. Degradation of the ester and glycosidic bonds of polyphenolic compounds takes place by acid hydrolysis, which provide a rapid estimation of the amounts of free and bound polyphenols in plant samples[13].

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well-being. Various diseases which are mainly associated with free radicals they prevent and cure by polyphenolic and flavonoids compounds[5, 11]. In most of the plants, it has been reported that compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects[4]. In general, it is well known that plant phenolics, are highly effective in free radical scavenging and they have antioxidants properties[2].

CONCLUSION:

Presence of polyphenolic compounds and reducers in the extract shows its high potential as natural antioxidant. In conclusion, it was found that ethanol, methanol, and aqueous extracts of dry leaves and green leaves of *O. americanum* showed good antioxidant activity and reducing potential. The extracts were also rich source of polyphenolic compounds like phenolics and flavonoids. Methanolic extract had the highest antioxidant activity in case of green leaves while in case of dry leaves ethanolic extract showed the highest antioxidant activity, it showed that extracts were more soluble in organic solvent than aqueous one. So, we can say that plant is a good source of natural antioxidants and has good medicinal properties.

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