

## Original article

## Phytochemical paradigm, antioxidant status and their correlation in *Rotheca serrata* (L.) Steane and Mabb.

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### Abstract

Lamiaceae member *Rotheca serrata* (L.) Steane and Mabb. (*Clerodendrum serratum*) is reported as regionally “vulnerable” in northern India and “endangered” in Chhattisgarh and Madhya Pradesh regions. Being highly medicinal, it is used widely in treatment of different diseases. Locally called Bharangi, has antitussive, antioxidant, anticancerous and vasorelaxant properties. Present investigation deals with quantitative study of bioactive compounds like total phenolics and total flavonoids. Four different solvents systems (aqueous, methanol, chloroform, isoamyl alcohol) and fresh leaf and root plant parts were used for extraction. In the various solvent systems used, methanolic extract of root reported highest phenolic content ( $34.3 \pm 0.05$  mg GAE/g FW). The highest flavonoid content also has been found in methanolic extract of root ( $13.8 \pm 0.01$  mg RE/g FW). The antioxidant activities of fresh leaf and root parts of *R. serrata* were determined using 2,2 diphenyl-1-picrylhydrazyl (DPPH), Ferrous Ion Chelating activity (FICA), Superoxide Anion Scavenging (SOAS), Phosphomolybdenum reducing power (PMo) and Ferric Reducing antioxidant power (FRAP) assays of *R. serrata*. The antioxidant activities in leaf were strongly correlated with total phenolics (PMo  $R^2 = 0.433$  and FICA,  $R^2 = 0.326$  both significant), those in root were also correlated with total phenolics (FICA,  $R^2 = 0.798$ , DPPH,  $R^2 = 0.717$ , FRAP,  $R^2 = 0.551$ , PMo,  $R^2 = 0.500$  all values significant). In leaf, the antioxidant activities were correlated with total flavonoid content (PMo,  $R^2 = 0.445$  significant), in roots were strongly correlated with total flavonoid content, with all values significant (DPPH,  $R^2 = 0.532$ , FICA,  $R^2 = 0.840$ , FRAP,  $R^2 = 0.571$ ).

**Key words:** *Rotheca serrata* (L.) Steane and Mabb., phenolics, flavonoids, antioxidant, DPPH, FICA, SOAS, PMo, FRAP

### 1. Introduction

The secondary metabolites present in plants, such as phenolics, flavonoids, tannins and sterols play important role in plant protection and resistance mechanism. Lamiaceae is rich in phenolics and flavonoids. Secondary metabolites protect plant from free radicals like ROS, hydroxyl ions, superoxides, singlet oxygen and UV-B radiations. Antioxidants neutralizes the adverse effect of free radicals by inhibiting the formation of reactive oxygen species (ROS) and scavenging the free radicals. Free radicals tend to be reactive and participate in chain reaction in which single free radical initiation event can be propagated to damage multiple molecules. Radicals such as reactive oxygen species (ROS), superoxide anion ( $O^-$ ), hydroxyl radical ( $OH^\cdot$ ), hydrogen peroxide ( $H_2O_2$ ) and reactive nitrogen species (RNS); nitric oxide (NO), peroxynitrite (ONOO $^\cdot$ ) act as free radicals in cells. In human body under stressful condition, large number of ROS are produced. The oxidative stress causes cellular stress, oxidative damage to DNA, proteins and lipids in humans. Oxygen is an essential element of aerobic life forms. But sometimes, it causes severe damages to DNA and protein due to the formation of reactive oxygen species. ROS are unstable, contains unpaired electron to donate other

molecules or snatch electron from other cellular molecule to attain the stability. ROS causes the activation of oncogenes and enhances aging processes. Antioxidants are helpful to protect plant as well as human body from free radicals. To prevent diseases, now a days plants tend to be the best remedies on oxidative damages of tissues. They are vastly used to scavenge the free radicals.

In absence of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases and Alzheimer’s disease (Di Matteo and Esposito, 2003), neurodegenerative diseases (Shahidi *et al.*, 1992, Liu *et al.*, 2017), cardiovascular diseases, inflammatory diseases (Sreejayan and Rao 1996) and cancers (Gerber *et al.*, 2002). Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been extensively used as antioxidants in the food manufacturing and may be responsible for liver damage and carcinogenesis (Grice, 1986; Wichi, 1988). To overcome side effects of synthetic antioxidants, many medicinal plants are investigated for their antioxidant properties.

Medicinal plants are rich in naturally occurring antioxidants like ascorbic acid, tocopherols, carotenoids and several phenolic compounds. Like, flavonoids, phenolic acids and tannins (King and Young 1999) are shown to scavenge reactive species and convert them into less reactive molecule. Dietary antioxidants can stimulate cellular defences and help to prevent cellular components against

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oxidative damage (Halliwell, 1989, Evans and Halliwell, 2001). *R. serrata* (*C. serratum*) is found more or less right the way through India in forests up to 1500 m elevation. It is a perennial shrub belonging to family Lamiaceae. The plant has an vital role in folkloric system of medicine because of presence of vital phytochemicals. Present study is an endeavour to reveal the antioxidant potential of many compounds of botanical origin and its correlative relevance in *R. serrata*.

## 2. Materials and Methods

### 2.1 Collection of plant material and preparation of plant extracts

The fresh leaves and roots of medicinal plant, *Rothea serrata* (L.) Steane and Mabb. was used for the analysis of antioxidant activity. The plant was collected from different localities like Kolhapur (16° 47'57.4" N 74°08'.16" E), Sangli (16°57' 55.0" N 74° 04' 33.2" E) and Satara (17° 55'35.7" N 73°48'25.9" E). The plant extract (1%) were prepared on fresh weight bases by using four different solvent systems, methanol, chloroform, isoamyl alcohol and distilled water.

### 2.2 Biochemical analysis

#### 2.2.1 Quantification of total flavonoid content (TFC)

The total flavonoids were estimated by using modified colorimetric method (Luximon-Ramma *et al.*, 2002). The reaction mixture had 1.5 ml of extract to 1.5 ml of 2% methanolic AlCl<sub>3</sub>. The mixture was incubated for 10 min at room temperature and absorbance was measured at 368 nm against 2% AlCl<sub>3</sub>, which served as blank. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. A standard calibration curve for rutin (10 µg/ml-100 µg/ml r<sup>2</sup> = 0.964) was obtained by following same procedure. The optical density (OD) measurements of samples were compared to standard curve of rutin and expressed as mg of rutin equivalent (RE)/g fresh weight of plant parts like leaves and roots of *R. serrata*. All the experiments were performed in triplicates and expressed as mean ± Standard Error (SE). The statistical analysis was done using the Graphpad Instat software and MS Excel.

#### 2.2.2 Quantification of total phenolic content (TPC)

The total phenolic contents of *R. serrata* extracts were determined by using modified spectrophotometric Folin-Ciocalteu method (Wolfe *et al.*, 2003). The reaction mixture was prepared by mixing an aliquot of extracts (0.125 ml) with Folin-Ciocalteu reagent (0.125 ml) and 1.25 ml of saturated Na<sub>2</sub>CO<sub>3</sub> solution. Reaction mixture was further incubated for 90 min at room temperature and absorbance was measured at 760 nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was recorded. Calibration curve for standard phenolic compound gallic acid was obtained by using concentration of 10 µg/ml -100 µg/ml (r<sup>2</sup> = 0.993). Results were expressed as of mg gallic acid equivalents (GAE)/g fresh weight of samples of different plant parts of *R. serrata*. All the experiments were expressed as mean ± SE of triplicate measurements. The data were subjected to statistical analysis using the Graphpad Instat software and MS Excel.

#### 2.2.3 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of plant extract was measured (Aquino *et al.*, 2001). Plant extract (25 l) was mixed with 3 ml of

DPPH methanolic solution 25 mM. The reaction mixture was incubated in dark at room temperature for 30 min. The absorbance was measured at 517 nm against blank. Results were expressed as percentage of inhibition of the DPPH radical and percent antioxidant activity of plant extract was calculated using the following formula:

$$\% \text{ DPPH Inhibition} = \frac{\text{Control (abs)} - \text{Sample (abs)} \times 100}{\text{Control (abs)}}$$

#### 2.2.4 Ferrous ion chelating activity (FICA)

Ferrous ion chelating activity was measured by following method described by (Dinis *et al.*, 1994). Assay mixture contained 0.1 ml of 2 mM FeCl<sub>2</sub> and 0.3 ml of 5 mM ferrozine and mixed with 1 ml of plant extract. The mixture was incubated for 10 min at room temperature and absorbance was measured at 562 nm spectrophotometrically. The ability of sample to chelate ferrous ion was calculated as the percent inhibition of Fe<sup>+2</sup> to ferrozine complex. Percentage antioxidant activity of plant extract was calculated using the following formula:

$$\% \text{ Ferrous ion inhibition} = \frac{\text{Control (abs)} - \text{Sample (abs)} \times 100}{\text{Control (abs)}}$$

#### 2.2.5 Superoxide anion scavenging assay (SOAS)

Nitroblue tetrazolium (NBT) prepared in dimethyl sulfoxide (DMSO), was used for assaying SOAS. The reaction mixture was prepared by adding 0.1 ml of NBT (10 mg of NBT in 10 ml DMSO), 0.3 ml of plant extract and 1 ml of alkaline DMSO (1 ml of alkaline DMSO containing 0.1 ml of 5 mM NaOH and 0.9 ml of DMSO) was added to make final volume 1.4 ml and the absorbance was recorded at 560 nm. DMSO solution was used as blank. Decrease in value of absorbance of the reaction mixture designate the increase in superoxide anion scavenging activity (Tiwari *et al.*, 2017). The SOAS activity was calculated by using formula:

$$\% \text{ SOAS inhibition} = \frac{\text{Control (abs)} - \text{Sample (abs)} \times 100}{\text{Control (abs)}}$$

#### 2.2.6 Phosphomolybdenum reducing power assay (PMo)

Antioxidant capacity of the extracts was evaluated by phosphomolybdenum method according to the procedure described by Prieto *et al.* (1999). Plant extract (0.3 ml) was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. The absorbance of the solution was measured at 695 nm using a UV-visible spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank.

$$\% \text{ Phosphomolybdenum inhibition} = \frac{\text{Control (abs)} - \text{Sample (abs)} \times 100}{\text{Control (abs)}}$$

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

The ferric ion reducing capacity was calculated by using assay described by Pulido *et al.* (2000). To 100 l plant extract, 3 ml of FRAP reagent [300 mM sodium acetate buffer at pH 3.6, 10 mM of 2,4,6-Tripyridyl-S-triazine (TPTZ) solution and 20 mM FeCl<sub>3</sub>·6 H<sub>2</sub>O solution (10:1:1)] was added. The reaction mixture was incubated at 37°C for 15 min. The absorbance was measured at 595 nm. A calibration curve was prepared, using an aqueous solution of ascorbic acid. The value of FRAP was expressed as milligrams of ascorbic acid equivalents per gram of plant sample.

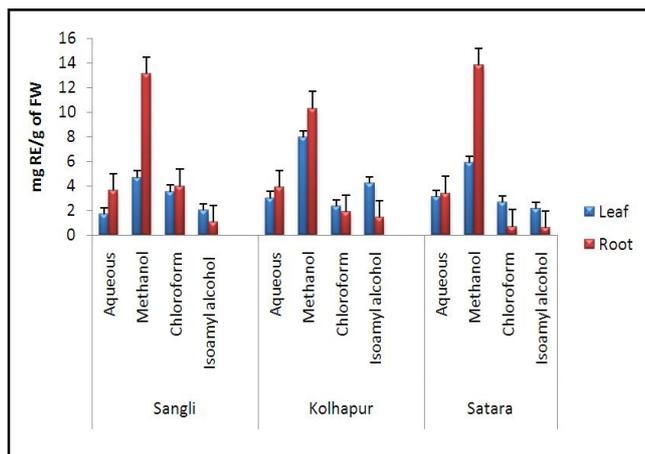
### Statistical Analysis

Statistical data was calculated using Graphpad Instat software and MS Excel. Pearson's correlation analysis was used for the correlation study.

## 3. Results

### 3.1 Total flavonoid content (TFC)

The total flavonoid content of *R. serrata* was evaluated and expressed as mg rutin equivalents/g fresh weight (mg RE/g FW). The total flavonoid content for all solvent systems measured, was varying between  $0.60 \pm 0.01$  to  $13.8 \pm 0.01$  mg of RE/g of FW (Figure 1). The highest flavonoid content was present in methanolic root extract ( $13.8 \pm 0.01$  mg RE/g of FW) (Locality-Satara), while the lowest content was recorded in isoamyl alcohol root extract ( $0.60 \pm 0.01$  mg RE/g of FW) (Locality-Satara). From the results, it was observed that root part of the plant contains higher amount of flavonoids as compared to leaf. A particular trend was observed in the flavonoid content in all the solvent systems used. Flavonoid content was more in polar solvents as compared to non polar solvents (methanol > aqueous > chloroform > isoamyl alcohol).

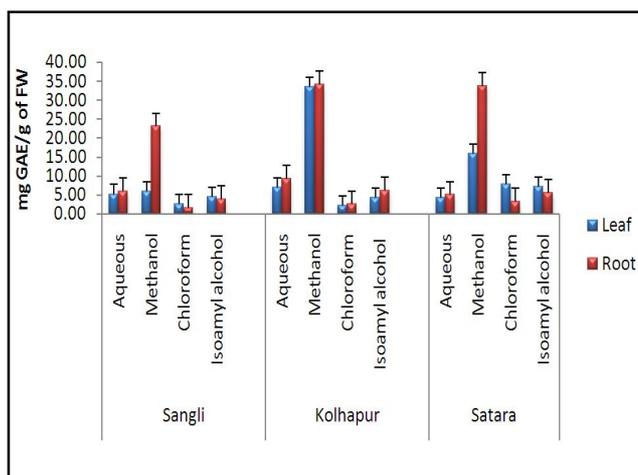


Bar indicates Standard Error (SE)

Figure 1: Total flavonoid content in *R. serrata*.

### 3.2 Total phenolic content (TPC)

The total phenolic content of *R. serrata* was evaluated by using extracts of fresh leaf and root parts in different solvent systems. Total phenolic content was varying between ( $1.57 \pm 0.01$  to  $34.3 \pm 0.05$  mg of GAE/g of FW). From the results, it was observed that root part of the plant contains higher amount of phenolics (Figure 2) as compared to leaf. The highest phenolic content was found in methanolic extract of root ( $34.3 \pm 0.05$  mg GAE/g of FW) (locality-Kolhapur) of plants while the lowest phenolic content was found in chloroform extract of root ( $1.57 \pm 0.01$  mg GAE/g of FW) (locality-Sangli). Polar solvents have a much stronger ability to dissolve secondary metabolites and hence extract in polar solvent showed more content of phytochemicals (methanol > aqueous > chloroform > isoamyl alcohol).



Bar indicates Standard Error (SE)

Figure 2: Total phenolic content in *R. serrata*.

### 3.3 Antioxidant ability

The antioxidant potential of the plant extracts of leaf and root parts of *R. serrata* were measured by using different antioxidant assays like, DPPH free radical scavenging assay, ferrous ion chelating assay, superoxide anion scavenging assay, phosphomolybdenum reducing power assay and FRAP. The results of antioxidant potential of the fresh leaf and root sample varied according to the nature of the solvent used. The antioxidant activities of extracts were measured in terms of inhibition %.

In DPPH radical scavenging activity (Table 1), it was observed that methanolic root extract (Locality-Satara) exhibited maximum radical scavenging activity (96.40%) while the minimum activity (63.01%) was recorded in chloroform leaf extract (locality-Kolhapur). The maximum DPPH radical scavenging potential of root methanolic extract may be due to presence of highest flavonoid and phenolic content. The results for ferrous ion chelating activity are presented in Table 1. The methanolic root extract shows highest (77.37%) FICA activity (Locality-Satara), while in isoamyl alcohol extract of leaf (Locality-Kolhapur), it was lowest (10.17%). The superoxide radical scavenging activity shown in Table 1 revealed that the methanolic root extract (Locality-Satara) showed highest (93.98%) inhibition percentage of superoxide anion scavenging activity, while the lowest (17.97%) superoxide anion scavenging activity was seen in chloroform extract of leaf (Locality-Kolhapur). Phosphomolybdenum reducing power activity results presented (Table 1) was seen highest (63.75%) in methanolic root extract (Locality-Satara). The isoamyl alcohol extract of leaf (Locality-Sangli) showed lowest (23.33%) phosphomolybdenum reducing activity. The ferric reducing antioxidant power assay results (Table 1) showed that the highest (3.48583 mg AAE/g FW) ferric ion reducing activity was seen in root methanolic extract (Locality-Satara), while the lowest (0.217274 mg AAE/g FW) activity was observed in root chloroform extract (Locality-Sangli).

**Table 1:** Antioxidant potential of *Rothea serrata*

Locality	Solvent	DPPH inhibition %*		FICA inhibition %*		SOAS inhibition %*		PMo inhibition %*		FRAP mg AAE/g FW*	
		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Sangli	Aqueous	69.56 ± 1.94	76.43 ± 0.03	31.67 ± 0.01	46.68 ± 0.03	83.85 ± 0.62	85.27 ± 0.54	34.86 ± 0.09	40.86 ± 0.03	0.31 ± 0.00	0.44 ± 0.13
	Methanol	88.41 ± 0.44	95.60 ± 0.05	67.44 ± 0.01	73.83 ± 0.01	91.21 ± 0.23	93.51 ± 0.20	42.03 ± 0.10	43.36 ± 0.07	0.70 ± 0.01	1.07 ± 0.15
	Chloroform	77.55 ± 0.62	64.61 ± 0.07	10.70 ± 0.01	14.79 ± 0.04	50.83 ± 0.54	54.16 ± 0.59	27.20 ± 0.17	35.027 ± 0.03	0.32 ± 0.00	0.22 ± 0.27
	Isoamyl alcohol	79.55 ± 0.68	86.34 ± 0.03	17.53 ± 0.00	19.84 ± 0.00	73.32 ± 0.55	82.50 ± 0.34	23.33 ± 0.01	24.72 ± 0.14	0.39 ± 0.00	0.28 ± 0.23
Kolhapur	Aqueous	68.37 ± 0.49	77.55 ± 0.16	36.08 ± 0.00	41.44 ± 0.00	71.10 ± 0.15	85.51 ± 0.20	46.96 ± 0.01	48.19 ± 0.09	0.34 ± 0.01	0.70 ± 0.09
	Methanol	87.15 ± 0.09	95.05 ± 0.12	58.71 ± 0.00	74.19 ± 0.01	82.90 ± 0.49	92.16 ± 0.23	57.65 ± 0.02	62.36 ± 0.00	0.68 ± 0.00	0.82 ± 0.07
	Chloroform	63.01 ± 0.17	72.36 ± 0.13	25.49 ± 0.02	22.92 ± 0.00	17.97 ± 1.58	52.41 ± 0.07	37.15 ± 0.00	42.37 ± 0.09	0.56 ± 0.00	0.45 ± 0.36
	Isoamyl alcohol	66.37 ± 0.74	77.15 ± 0.09	10.17 ± 0.02	14.81 ± 0.03	66.90 ± 0.55	82.03 ± 0.28	25.48 ± 0.04	27.04 ± 0.10	0.31 ± 0.00	0.39 ± 0.15
Satara	Aqueous	72.76 ± 0.47	78.99 ± 0.14	30.04 ± 0.00	46.13 ± 0.01	91.37 ± 0.07	88.36 ± 0.59	49.20 ± 0.09	53.50 ± 0.18	0.48 ± 0.00	0.62 ± 0.21
	Methanol	88.57 ± 0.25	96.40 ± 0.05	61.21 ± 0.01	77.37 ± 0.00	92.48 ± 0.28	93.98 ± 0.39	57.26 ± 0.25	63.75 ± 0.04	2.01 ± 0.01	3.49 ± 0.06
	Chloroform	72.92 ± 0.37	75.87 ± 0.11	12.51 ± 0.00	13.28 ± 0.00	26.43 ± 0.09	51.07 ± 0.41	39.25 ± 0.10	46.80 ± 0.01	0.30 ± 0.01	0.43 ± 0.14
	Isoamyl alcohol	80.75 ± 0.33	84.58 ± 0.05	21.12 ± 0.00	23.35 ± 0.01	36.98 ± 0.63	82.19 ± 0.20	24.33 ± 0.02	41.24 ± 0.06	0.31 ± 0.00	0.37 ± 0.12

\*Mean of triplicate readings are taken

\*Values are expressed as mean ± SE of triplicate measurements.

#### 4. Discussion

Antioxidant compounds, natural or synthetic have a variety of *in vivo* effects. They may be responsible for *de novo* synthesis of antioxidants or may also generate new mechanism of antioxidation *via* biochemicals present in the plant. These chemicals are in the form of phenolics, flavonoids which have specific roles in phytochemical responses towards stress. Evaluation of antioxidant capacities merely on one common background assay is not sufficient. In fact, the capacities should be explored comprehensively to simultaneously assess the antioxidants. Use of more than a single method is therefore suggested to understand the exhaustive and complete prediction of antioxidant potential from the actual collected data (Luximon-Ramma *et al.*, 2002). The present investigation was aimed to achieve the antioxidant capacities related to DPPH radical scavenging activity, Ferrous ion chelating activity, superoxide anion scavenging activity, phosphomolybdenum reducing power assay and FRAP assay.

Mechanisms of antioxidant action can include suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation. The up regulation or protection of antioxidant defences is done by flavonoid (Mishra *et al.*, 2013). Due to their lower redox potentials, flavonoids (Fl-OH) are thermodynamically able to reduce highly oxidizing free radicals (redox potentials in the range 2.13-1.0V) such as superoxide, peroxy, alkoxy and hydroxyl radicals by hydrogen atom donation (Kumar and Pandey, 2013).

Phenolic compounds are classified into: (i) phenolic acids, (ii) flavonoid polyphenolics (flavonones, flavones, xanthones and catechins) and (iii) non-flavonoid polyphenolics. The role of phenolic compounds as scavengers of free radicals has been emphasized in *Origanum dictamnus* (Moller *et al.*, 1999). Phenolics, saponins and flavonoids have been shown to possess antioxidant properties (Scalbert *et al.*, 2005; Francis *et al.*, 2002; Pietta, 2002). Phenolics, flavonoids, tannins and saponins have the ability to attach to cations and other biomolecules and are able to shield the protein membranes from denaturation (Oyedapo, 2001). Phenolic compounds are a group of antioxidant compounds which act as free radical terminators (Shahidi *et al.*, 1992). Due to the existence of hydroxyl group, phenolics and antioxidant activity are positively correlated. This is due to the efficient scavenging ability of hydroxyl group. (Vinson *et al.*, 1998). By donating an electron a phenolic compound can also scavenge the

hydrogen peroxide and convert it into water (Nabavi *et al.*, 2009). The ethanolic extract and hydroethanolic extract of *Clerodendrum serratum* has effective superoxide scavenging activity (Barua *et al.*, 2014). Phenolics play major significant role in plant defence against pathogens, herbivore predators that is why they are applied in the control of human pathogenic infections (Puupponen-Pimiä *et al.*, 2008). Caffeic acid (Nair *et al.*, 1976), serratagenic acid (Singh *et al.*, 2012), ferrulic acid (Praveen Kumar *et al.*, 2013) and catechin (Murade *et al.*, 2015) are phenolic compounds as well as hispidulin (Agrawal *et al.*, 2013) flavonoids which have been previously reported from *Clerodendrum serratum*.

The majority of flavonoids exist naturally as glycosides. The presence of sugars and hydroxyl groups make them water soluble whereas methyl groups and isopentyl units make flavonoids lipophilic (Crozier *et al.*, 2006). The compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants (Das and Pereira, 1990; Younes, 1981). Flavonoids immediately donate hydrogen atom to free radicals, due to which they interfere in further oxidation of lipids and other molecules (Schroeter *et al.*, 2002). Diverse studies on flavonoids have shown that flavonoids like quercetin with all the glycosides, rutin, luteolin including all its derivatives, have powerful inhibitory activity against lipid peroxidation (Panovska *et al.*, 2005; Cook and Samman 1996). Flavonoids possess capacity to absorb the most energetic solar wavelengths (*i.e.*, UV-B and UV-A), obstruct the production of ROS and quench ROS as soon as they are formed in any process (Agati *et al.*, 2012). The studies have proposed the role of flavonoids in secondary antioxidant defence mechanism in stress exposed plant (Agati *et al.*, 2012). Lipid peroxidation is the common consequence of oxidative stress which disrupts the cell membrane integrity. Quercetin 3-*O*-rutinoside (rutin) interact with the polar head of phospholipids at water lipid interface, enhancing membrane rigidity and consequently protecting membranes from oxidative damage (Erlejman *et al.*, 2004). Chelation of metal ions has an antioxidant effect because the conversion metal ion generates the reactive oxygen species, leading to oxidation of unsaturated lipids and promoting oxidative damage at different levels (Meyer and Frankel, 2001). Presence of flavanoids in *C. serratum* and their role as antioxidant and anti-inflammatory has been successfully studied (Ismail *et al.*, 2011). *C. serratum* is rich source of flavonoids which are responsible for its significant

antioxidant potential and may be helpful in eliciting neuroprotective effects thereby preventing or slowing the progression of various oxidative stress induced diseases (Vazhayil *et al.*, 2017).

The most commonly used method for extrapolating the antioxidant activity of plants. 2,2-Diphenyl-1-picryl-hydrazyl radical is a commercial, stable, common and organic free radical which can be read spectrophotometrically at 517 nm. The antioxidants present in the plant extract arrests these DPPH free radicals, leaving the solution with a colour change from purple to yellow. This change occurs only when the solution loses absorption when the electron from free radical is accepted by plant extract, rendering the colour of solution yellow. Ferrous ion catalysing oxidation exerts tremendous stress on plant cells. To mitigate this stress, an effective and common food pro-oxidant; ferrous ion was used for the assay. The transition metal ferrous  $Fe^{2+}$  is, however trapped by the antioxidants from the plant extracts. The remarkable brick red colour of the ferrozine  $Fe^{2+}$  complexes is decreased which is measured spectrophotometrically. Superoxide radicals are generated in phenazine methosulfate-nicotine amide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium which is converted in to purple formazone where decrease in absorbance of reaction mixture indicates increased superoxide anion scavenging activity. The total antioxidant assay is based on reduction reaction. The antioxidant compounds present in plant extract are autooxidised due to exposure of reaction mixture to high temperature for prolonged time due to which reduction of phosphate molybdenum (VI) to phosphate-molybdenum (V) is observed. The reaction results into bluish green complex of molybdenum (V) which is spectrophotometrically measured. This assay measures the reducing potential of an antioxidant present in plant extract reacting with a ferric tripyridyltriazine ( $Fe^{3+}$ -TPTZ) complex and producing blue coloured ferrous ( $Fe^{2+}$ -TPTZ) through the reduction of ferric iron ( $Fe^{3+}$ ) to ferrous iron ( $Fe^{2+}$ ) by the reductant compounds present in plant extract, indicating antioxidant potential. DPPH and ABTS scavenging assays detect antioxidant such as flavonoids, polyphenols where as the phosphomolybdenum assay usually detects antioxidants like ascorbic acid, alpha tocopherol, some phenolics and carotenoids (Diwan *et al.*, 2012).

### Correlation of antioxidant activity with total flavonoids and total phenolics

Reactive oxygen or nitrogen species under certain conditions can cause an imbalance and lead to oxidative damage to large biomolecules such as lipids, DNA and proteins. Overproduction of oxidants and chronic inflammation are responsible for the pathogenesis of many chronic diseases. Thus, antioxidant phytochemicals are among the most potential agents to treat chronic diseases.

The highly positive relationship between TFC/TPC and antioxidant activity was observed dominantly in roots than in leaves of *R. serrata*. The highest antioxidant activity may probably be due to high flavonoid/phenolic contents present in the plant.

The TFC in leaf were found to be significant with DPPH (Figures 3a-e) (linear regression coefficient 0.381). This showed a weaker correlation between the TFC and DPPH, though significant at  $p < 0.05$ . The linear regression coefficient for TFC with FICA, in leaf was  $R^2=0.44$  which showed good correlation which was significant with  $p < 0.05$ . The TFC in leaf was not significant with SOAS activity ( $R^2 = 0.207$  at  $p > 0.05$ ), thus were not found to be correlated strongly. The correlation between TFC with PMo were found to be significant at  $p < 0.05$  level

( $R^2=0.445$ ). The correlation between TFC and FRAP shows linear regression  $R^2=0.306$  at  $p > 0.05$  which indicates the correlation is not significant.

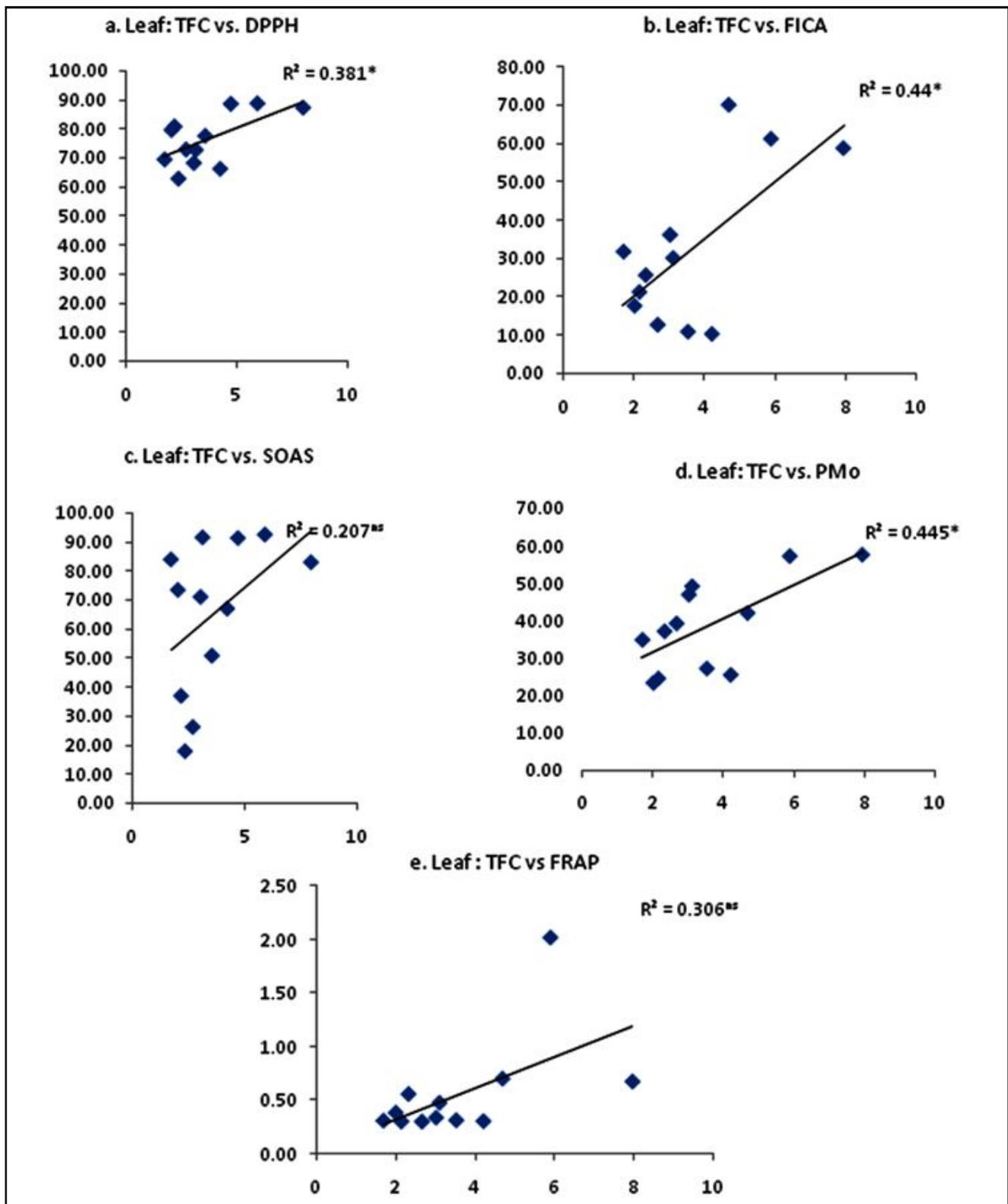
The correlation TPC present in leaf with DPPH shows linear regression coefficient  $R^2=0.327$  at  $p > 0.05$ , indicated the correlation was not significant (Figures 4a-e). The linear regression coefficient for TPC with FICA in leaf was recorded  $R^2=0.326$  (significant at  $p < 0.05$ ) which shows good correlation, while that between TPC and SOAS in leaf was not found significant ( $R^2=0.099$  at  $p > 0.05$ ). The TPC in leaf showed favourable correlation with PMo, which had  $R^2=0.433$  measuring at ( $p < 0.05$ ). The linear regression for TPC and FRAP is  $R^2 = 0.163$  at  $p > 0.05$  which shows that the correlation is not significant.

The TFC in root were found to be significant with DPPH and linear regression coefficient was  $R^2=0.532$  at  $p < 0.01$  which indicated good correlation between the two (Figures 5a-e). TFC and FICA for root was strongly correlated linear regression coefficient  $R^2=0.840$  which was highly significant at  $p < 0.0001$ . Linear regression coefficient  $R^2 = 0.302$  at  $p > 0.05$  indicated non significant fair correlation between the TFC and SOAS activity in roots of *R. serrata*. In root, the TFC were found to be less significant with PMo, linear regression coefficient  $R^2 = 0.397$  at  $p < 0.05$ . The correlation between TFC and FRAP shows linear regression  $R^2 = 0.571$  at  $p < 0.05$  which indicates the significant correlation.

TPC and DPPH in root showed a good correlation and the linear regression coefficient was  $R^2 = 0.717$  at  $p < 0.01$  which was significant (Figures 6a-e). The TPC in root was found to be highly significant with FICA with linear regression coefficient  $R^2 = 0.798$  at  $p < 0.0001$  which indicated a stronger correlation between TPC and FICA. The linear regression coefficient for root,  $R^2 = 0.384$ , indicated TPC and SOAS correlation is significant at  $p < 0.05$ . For root, the TPC was found to be significant with PMo with  $R^2 = 0.500$  linear regression coefficient at  $p < 0.01$ . The correlation between TPC and FRAP showed linear regression  $R^2 = 0.551$  at  $p < 0.05$  which indicates the correlation is significant. For leaf, the correlation between TFC and antioxidant assays DPPH, FICA, PMo is more significant as compared to SOAS and FRAP. While the correlation between TPC and FICA, PMo, FRAP show significance in comparison with DPPH, SOAS.

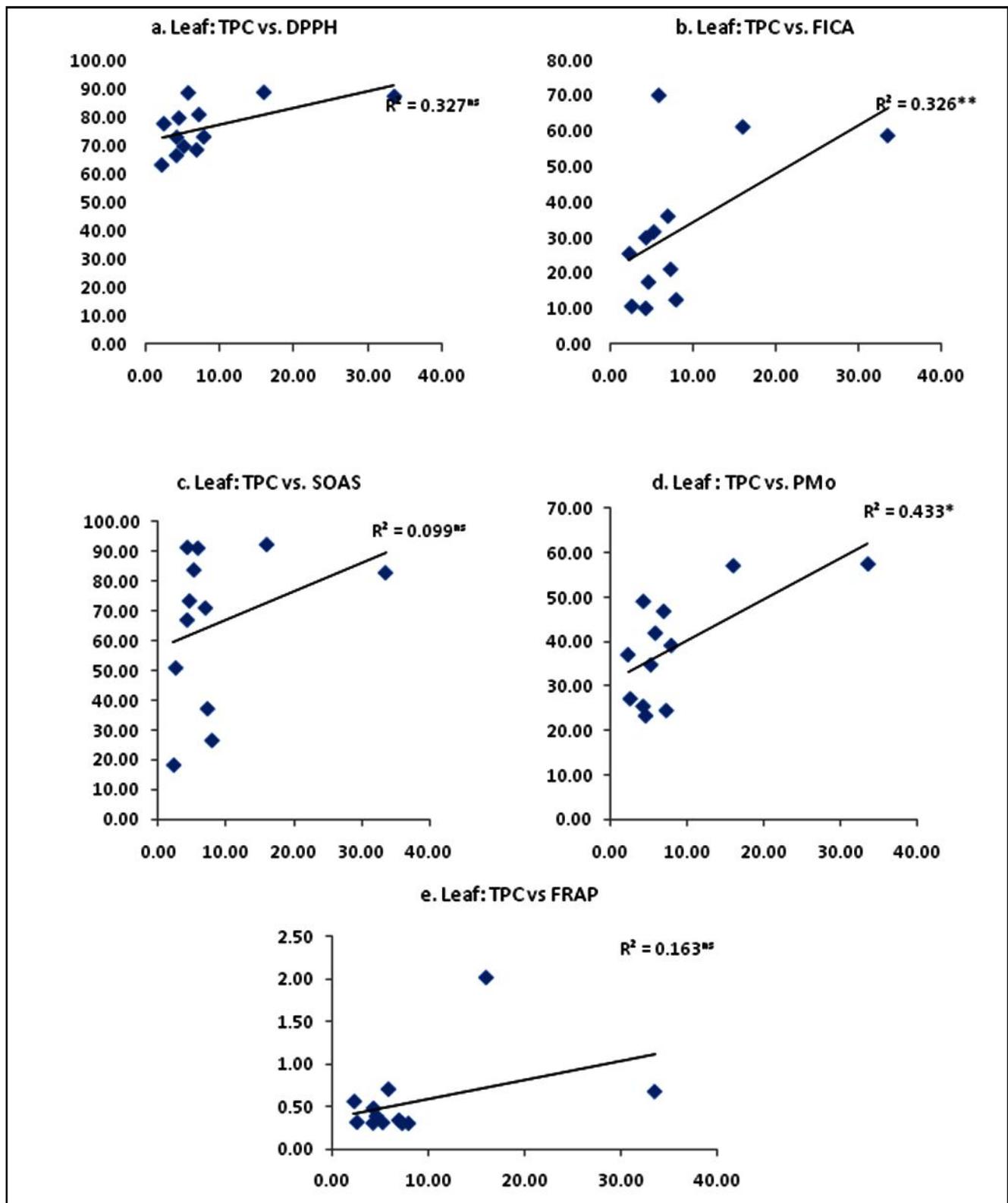
Root shows significant correlation between TFC and DPPH, FICA, FRAP while is correlated less significant with SOAS and PMo. The correlation between TPC and antioxidant assays DPPH, FICA, PMo, SOAS and FRAP is more significant.

The secondary metabolites have important role in antioxidant capacity of any plant (Kudale *et al.*, 2016). TFC and antioxidant activity was previously observed by (Karadeniz *et al.*, 2005). Investigations on the antiradical and antioxidant activities of phenolics including flavonoids have been reported (Heim *et al.*, 2002). There is a positive correlation between TPC and free radical scavenging activity (Oki *et al.*, 2002; Kolar *et al.*, 2011). Presence of large amount of flavonoids in species *L. sidoides* which was concurrent to its high antioxidant activity, was determined by DPPH free radical scavenging assay (Almeida *et al.*, 2010). The antioxidant activity and phenolic compounds shows direct linear correlation with high phenolic content, showing high antioxidant capacity. This was observed in several plants. (Cai *et al.*, 2004; Djerdane *et al.*, 2006, Kolar *et al.*, 2014). As the total phenolic content is higher the plant shows elevated antioxidant capacity which was observed in *Rosmarinus officinalis* (Erken *et al.*, 2008).



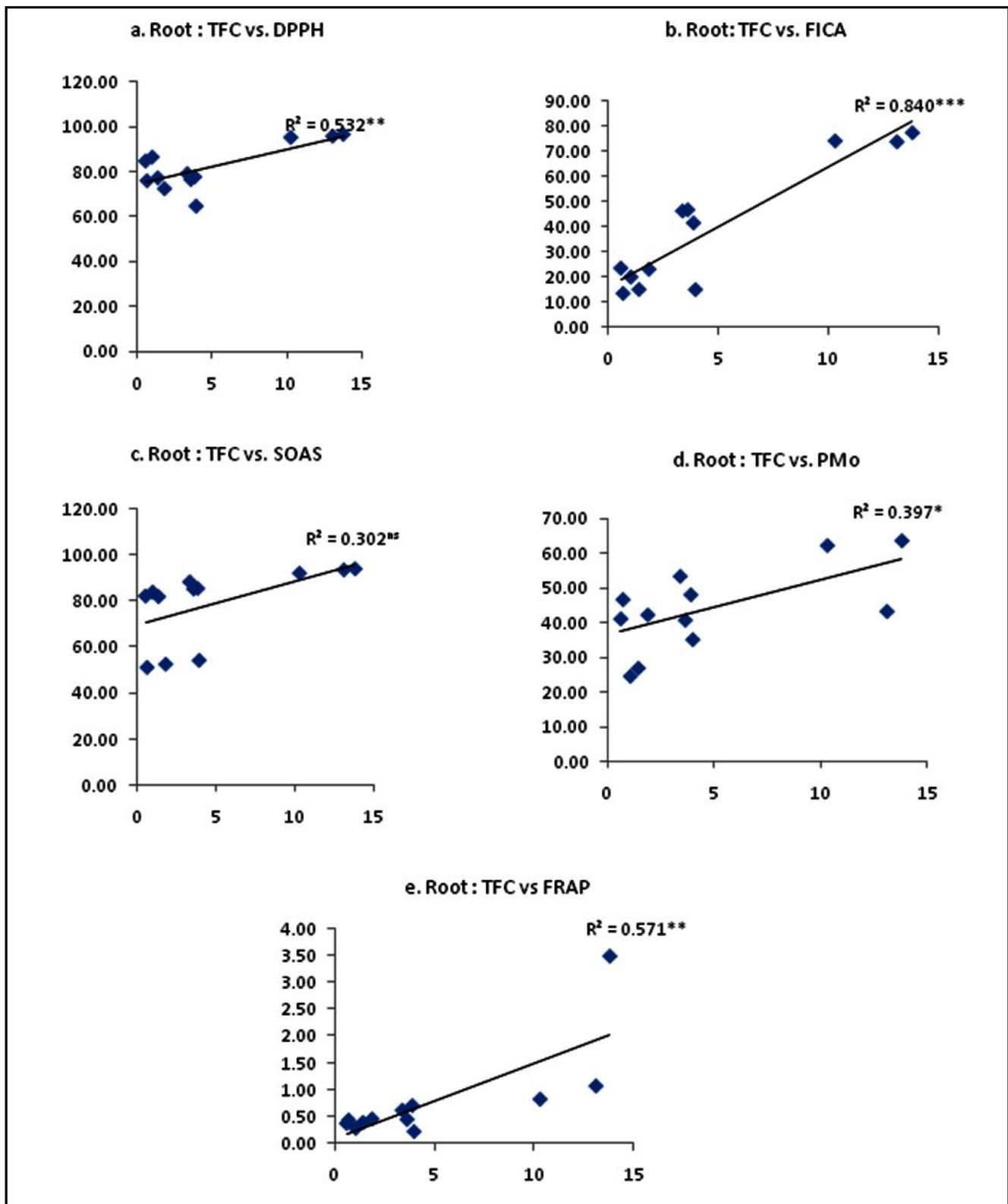
**Figures 3 (a-e):** Correlation between total flavonoid contents (TFC) and radical scavenging activity in leaves of *Rotheca serrata*.

(ns indicates- not significant at  $p > 0.05$ , \*indicates a significant difference at  $p < 0.05$ , \*\*indicates a significant difference at  $p < 0.01$  and \*\*\*indicates a significant difference at  $p < 0.0001$ ).



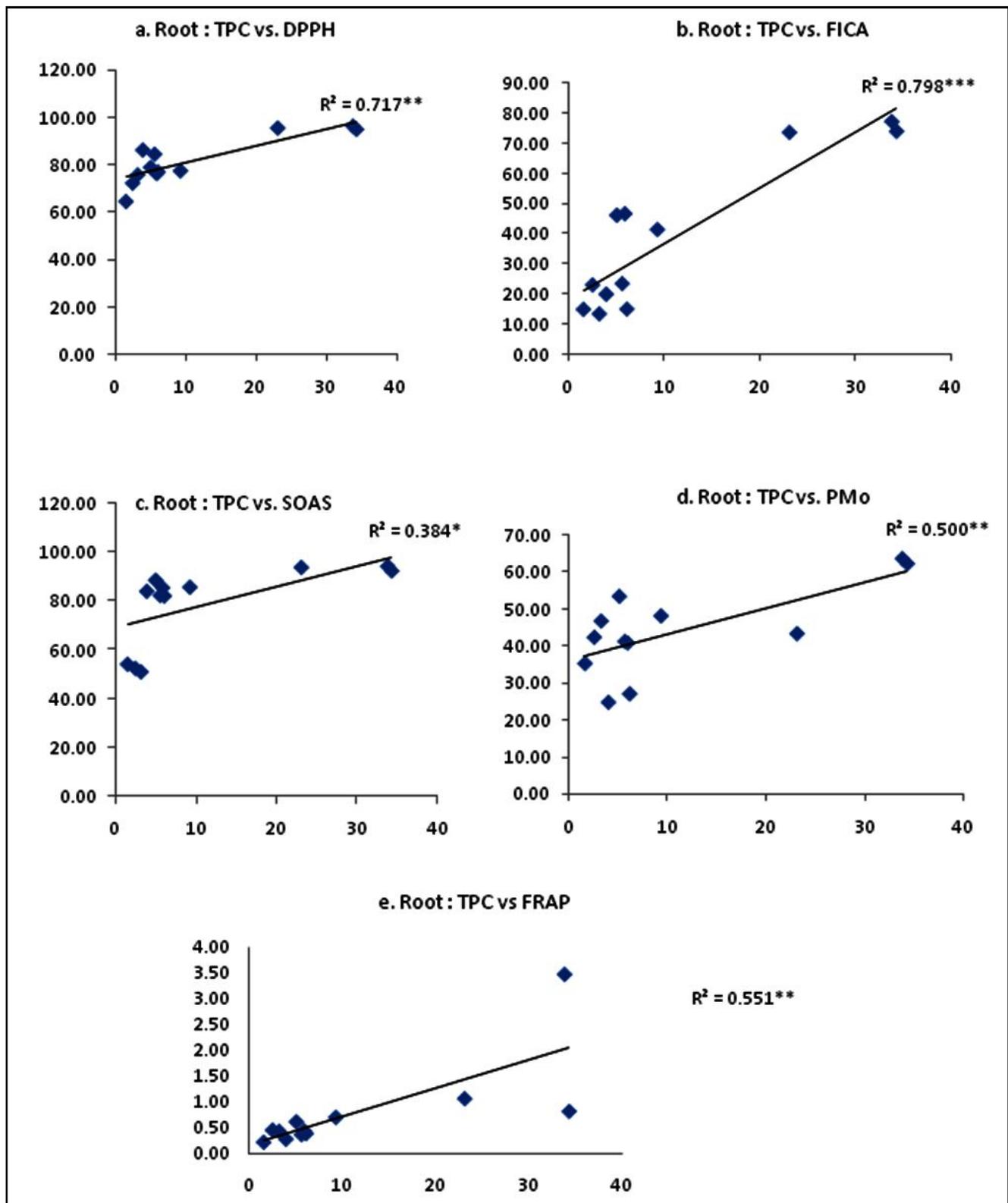
**Figures 4 (a-e):** Correlation between total phenolic contents (TPC) and radical scavenging activity in leaves of *Rothea serrata*.

(ns indicates- not significant at  $p > 0.05$ , \*indicates a significant difference at  $p < 0.05$ , \*\*indicates a significant difference at  $p < 0.01$  and \*\*\*indicates a significant difference at  $p < 0.0001$ ).



**Figures 5 (a-e):** Correlation between total flavonoid contents (TFC) and radical scavenging activity in roots of *Rothea serrata*.

(ns indicates- not significant at  $p > 0.05$ , \*indicates a significant difference at  $p < 0.05$ , \*\*indicates a significant difference at  $p < 0.01$  and \*\*\*indicates a significant difference at  $p < 0.0001$ ).



**Figures 6 (a-e):** Correlation between total phenolic contents (TPC) and radical scavenging activity in roots of *Rothecha serrata*.

(ns indicates- not significant at  $p > 0.05$ , \*indicates a significant difference at  $p < 0.05$ , \*\*indicates a significant difference at  $p < 0.01$  and \*\*\*indicates a significant difference at  $p < 0.0001$ ).

## 5. Conclusion

Based on the foregoing results, presence of phenolics and flavonoids was confirmed in *R. serrata*. In addition to this, the antioxidant potential of the plant was found to be remarkable. The phytoconstituents of the plant may be the reason for high radical scavenging power. The plant may be further explored for quantification of phytochemicals which are economically importance and the present research has opened several facets for same in the light of value added medicinal plants and pharmaceuticals.

## Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. Both the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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