

Original Research Article

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Comparison of Total Phenolic Content, Total Flavonoid Content, Antioxidant capacity and Free Radical Scavenging Activity of Leaves of *Elaeocarpus sphaericus* and Roots of *Pelargonium zonale*

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A B S T R A C T

Elaeocarpus sphaericus is commonly known as Rudraksha belonging to family Elaeocarpaceae and *Pelargonium zonale* is a perennial small shrub, belonging to family Geraniaceae are one of the oldest and popular medicinal plants, rich in antioxidants and phytonutrients. In this study, Methanolic leaf extract of *E. sphaericus* and root extract *P. zonale* were prepared for the comparative determination of total phenolic content, total flavonoid content, total antioxidant capacity and free radical scavenging activity by using Folin-Ciocalteau method, aluminium chloride colorimetric method, phosphomolybdenum method, DPPH and ABTS assay respectively. According to results, TPC and TFC was higher in leaf extract of *E. sphaericus* with the value of 37.5mg GAE/g of DW(dry weight) and 53.3mg QE/g of DW of extract as compare to *P. zonale* (TPC: 24mg GAE/g of DW and TFC: 21.05 QE/g of DW of extract). TAC of root extract of *P. zonale* (25mg AAE/g of DW) was higher than leaf extract of *E. sphaericus* (14.28mg AAE/g of DW). In DPPH assay, % inhibition was higher in *E. sphaericus* with IC₅₀ value of 477.125µg/ml in comparison to *P. zonale* with IC₅₀ value of 659.89µg/ml. ABTS assay showed that the free radical scavenging activity of both *E. sphaericus* (IC₅₀ value = 2.96µg/ml) and *P. zonale* (IC₅₀ value = 2.96µg/ml) was lower than the synthetic antioxidant BHT (IC₅₀ value = 12.56µg/ml). It was observed that both medicinal plants can be used in pharmaceutical and food industry in order to replace synthetic antioxidants with natural antioxidants.

Keywords

Elaeocarpus sphaericus,
Pelargonium zonale,
Antioxidants, Total phenolic
content (TPC), Total
flavonoid content (TFC),
Total antioxidant capacity
(TAC) and free radicals

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Introduction

It has been established that antioxidants play a vital role in preventing the oxidative stress and also certain degenerative diseases including atherosclerosis, heart diseases, ageing, diabetes mellitus, cancer, immune-suppression, neurodegenerative diseases and others (Bhatt *et al.*, 2013). The relationship between free radicals production and disease occurrence can be best explained by the

oxidative stress (Sies, 1996). Oxidative stress signifies the imbalance between free radical production and antioxidant defense lead to high concentration of free radicals causes oxidation of biological molecules such as DNA, RNA, Proteins, carbohydrates and lipids (Betteridge, 2000). Free radicals are highly reactive chemical species (ROS and RNS) having unpaired electrons in their outer shells. Under physiologic and pathologic state, both ROS and RNS are generated in animals

and human cells (Fang *et al.*, 2002). Oxidation process induced by ROS known to be the main reason behind the tissue damage and death and various cardiovascular and neurodegenerative diseases (Battin *et al.*, 2009). ROS are not only associated with tissue damage and diseases but also involved in lipid peroxidation causes food deterioration. It has been estimated that 70–80 % of the world's population cannot afford modern medicine; use of medicinal plants can be an important source of natural antioxidants. Therefore, medicinal plants being viewed as potent source of antioxidants and used as an alternative source of medicine to combat the diseases associated with oxidative stress. Because these plants contain different chemical compounds such as phytochemicals, phenolic compounds, tannins, flavonoids and have pharmacological, antibacterial and antifungal properties that work as natural antibiotics (Roja and Rao, 2000 and Miguel *et al.*, 2010).

Antioxidant defense mechanism is present in all, the aerobic organism including human beings help to cure diseases, improve health and protect against oxidative stress by eliminating and repairing damaged molecules (Yildirim *et al.*, 2001). Antioxidant is a substance which causes scavenging of free radicals and their actions (Sies, 1996). There are many synthetic antioxidants *i.e.*, butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) is available in food industry used to prevent unwanted deterioration of food products. Moreover, synthetic antioxidants possess moderate antioxidant activity, less solubility, high volatility, and instability at high temperature as compare to natural antioxidants (Barlow, 1990 and Branen, 1975). It has been reported that these synthetic antioxidants has adverse effects on human's health (Siddhuraju and Becker, 2003). So, recent research has been shifted towards, the use of naturally occurring

antioxidants present in medicinal plants as therapeutics antioxidants.

In this study, we quantify antioxidants in medicinal plants selected from north Indian region *i.e.*, methanolic leaf extract of *E. sphaericus* and roots of *P. zoanle* for different properties like total phenolic content, total flavonoid content, antioxidant capacity and free radical scavenging activity. Roots of *Pelargonium* species are used by people of South Africa for treatment of tuberculosis and coughs, as roots known to possess antibacterial, antifungal and anti-tubercular activity (Mativandlela *et al.*, 2006). To the best of our knowledge, this is first study to check antioxidant properties on leaf extracts of *E. sphaericus* and roots of *P. zoanle* from north Indian region.

Materials and Methods

Chemicals

Chemicals used in this study included: - 2, 2-diphenyl-1-picryhydrazyl (DPPH) and TROLOX from Sigma-Aldrich, St. Louis, USA. Butylated Hydroxy Toluene (BHT), Ascorbic Acid, Quercetin, EDTA, Folin Ciocalteau Reagent, sodium carbonate, potassium acetate, potassium persulphate, sulphuric acid, ammonium molybdate, ABTS and Gallic acid from Hi-media, Mumbai, India.

Collection, drying and preparation of samples

E. sphaericus and *P. zoanle* was obtained from Botanical garden, Panjab University, Chandigarh. Leaves from *E. sphaericus* and roots from *P. zoanle* were used for sample preparation. Leaves and roots were washed with water followed by drying at 37°C in incubator for 3-4 days, and then grounded into fine powder in electronic grinder. After this,

powder of leaves and roots immersed in 100% methanol in 1:10 ratio. The mixture was kept in shaker at 37°C at 180 rpm for 24 hours and filtered using whatman no.1 paper. Then, this methanolic mixture was concentrated, dried and stored in methanol at 4°C for further antioxidant analysis.

Chemical analysis

Determination of total phenolic content (TPC)

The total phenolic content was determined by using Folin-Ciocalteu method given by Demiray *et al.*, 2009. Stock solution of 1mg/ml was used to prepare different concentrations (0.2to18 µg/ml) of 100µl. To each concentration, 500µl of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 400µl of sodium carbonate (7.5%w/v) was added to make volume of 1 ml. Thereafter, the mixture was vortexed and incubated in dark for 30 minutes at room temperature. Then absorbance was measured at 765nm in double beam spectrophotometer. The TPCs of the sample extracts were expressed as gallic acid equivalents (GAE) in mg per g of dry weight of the extracts.

Determination of total flavonoid content (TFC)

Aluminium chloride (Chang *et al.*, 2002) is a colorimetric method used to determine the flavonoid content of sample extracts. Different dilutions (100 to 8500µg/ml) of sample extracts were prepared from stock solutions of 10mg/ml. In 100µl of each dilution, 300µl of 100% methanol, 20µl of 10% aluminium chloride, 20µl of 1M Potassium acetate was added and 560µl of distilled water was added for volume make up of 1000µl. Thereafter, the mixture was vortexed and incubated in dark for 30 minutes at room temperature. Then absorbance was measured at 420nm. The

TFCs of the sample extracts were expressed as Quercetin equivalents (QE) in mg per g of dry weight of the extract.

Determination of total antioxidant capacity

Phosphomolybdenum assay (Prieto *et al.*, 1999) was used to measure total antioxidant capacity of sample extracts in which ascorbic acid was used as a control. Stock of each sample (10mg/ml) was used to prepare different concentrations (20-1000µg/ml). To 500µl of each dilution, 1.5ml of TAC reagent (10ml concentrated sulphuric acid + 1.005g sodium dihydrogen monohydrate + 1.47g ammonium molybdate, dissolved in 290ml of distilled water) was added. Thereafter, the mixture was kept in water bath at 95°C for 1 hour; the absorbance was measured at 695 nm. The results were expressed as µg Ascorbic Acid Equivalent (AAE)/10 mg extract.

Determination of free radical scavenging activity by DPPH assay

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Surinrut *et al.*, 2005). 500µl of different concentrations of (40-900µg/ml) each sample extract were added, to 500µl of 0.004% methanolic solution of DPPH and 500µl of 100% methanol. After 15 min at room temperature, the absorbance was recorded at 517 nm. Negative control was prepared using 1000µl of methanol and 500µl of DPPH. The experiment was repeated for three times. BHT and quercetin were used as standard controls. IC₅₀values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. The free radical scavenging activity was calculated using following formula:-

$$\text{Free radical scavenging activity (\%)} = [(A_0 - A_S) / A_0] \times 100$$

A_0 = absorbance reading of negative control
 A_S = absorbance reading of sample extracts

Determination of free radical scavenging activity by ABTS assay

The total antioxidant activity of the samples was measured by [2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)] ABTS•+ radical cation decolorization assay given by Re *et al.*, (1999). Equal quantities of both, 7mM ABTS+ aqueous solution and 2.4mM potassium persulfate react in the dark for 12-16 hours at room temperature to produce ABTS•+ solution. Before performing assay, ABTS•+ solution was diluted with methanol to obtain an absorbance of 0.700±0.01 at 734nm. 500µl of ABTS•+ solution was added to 500µl different dilutions (0.25-5.5µg/ml) of sample extracts. Then absorbance was measured after 7 minutes at 734nm. The free radical scavenging activity was calculated using following formula:-

$$\text{Free radical scavenging activity (\%)} = [(A_0 - A_S) / A_0] \times 100$$

A_0 = absorbance reading of negative control
 A_S = absorbance reading of sample extracts

Results and Discussion

Statistical Analysis of Total Phenolic content, Total Flavonoid content, Total Antioxidant capacity and Free radical scavenging activity of samples were carried out using Microsoft Excel 2007.

The total phenolic content of leaves of *E. sphaericus* and roots of *P. zonale* was calculated using from the Regression equation of calibration curve and expressed as gallic acid equivalents (GAE) per gram of the extract mg/g. The results are represented in fig 1&2. Our study showed that the total phenolic content in methanolic leaf extract of *E.*

sphaericus was 37.5mg GAE/g of dry weight of the extract. Kumara *et al.*, 2008 showed that the ethanolic leaf extract of *E. sphaericus* was found to be 56.79±1.6 mg GAE/g of dry material. Another paper stated that total phenolic content in methanolic leaf extract of *E. sphaericus* showed high phenolic content 247.6 + 3.91 mg GAE/g (Pandey *et al.*, 2014). The considerable difference between results of phenolic content may be due to environmental related factors like temperature, climate, location, soil conditions and rainfall. It has been reported that rainfall has a considerable effect on phenolic content (Manach *et al.*, 2004). The total phenolic content of root extract *P. zonale* (24mg/GAE in per gram of dry weight of extract) has not been analysed before and ours is the first study to do it.

Total flavonoid content of methanolic extracts was calculated from the regression equation of calibration curve and expressed as quercetin equivalents (QE) per gram of the extract mg/g. The results are represented in fig 3&4.

The total flavonoid content in methanolic leaf extract of *E. sphaericus* showed high flavonoid content 61.9 + 2.83 mg QE/g in study done by Pandey *et al.*, 2014 as compare to the present study in which the total flavonoid content of *E. sphaericus* was 53.3mg QE/g dry weight. Total phenolic content in ethanolic leaf extract of *E. sphaericus* was found to be 18.53±0.3 mg Quercetin equivalents/g of dry material (Kumara *et al.*, 2008). The total flavonoid content of root extract of *P. zonale* has not been analysed before. According to our study, the total flavonoid content of root extract of *P. zonale* was 21.05mg/QE dry weight of the extract. It has been reported that flavonoid content is always higher in upper parts of the plants as compare to roots (J.S Kim ; 2016) and here it has been clearly shown that flavonoid content of roots of *Pelargonium zonale* is lower than leaf extract of *E. sphaericus*.

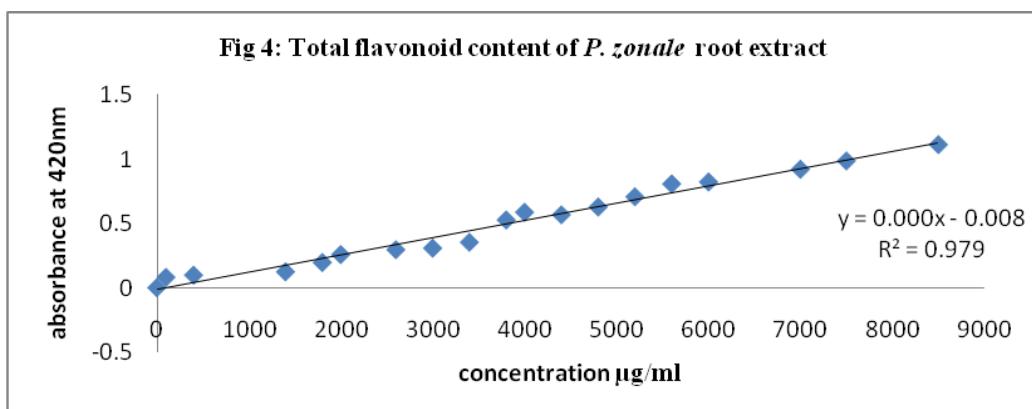
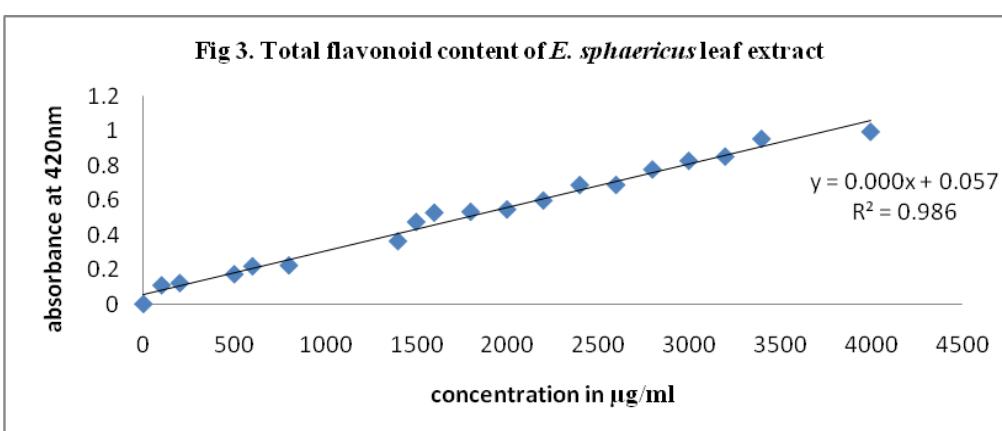
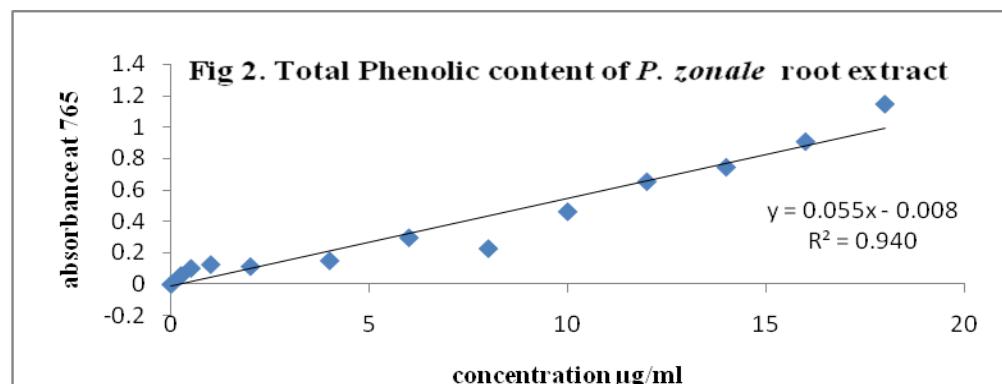
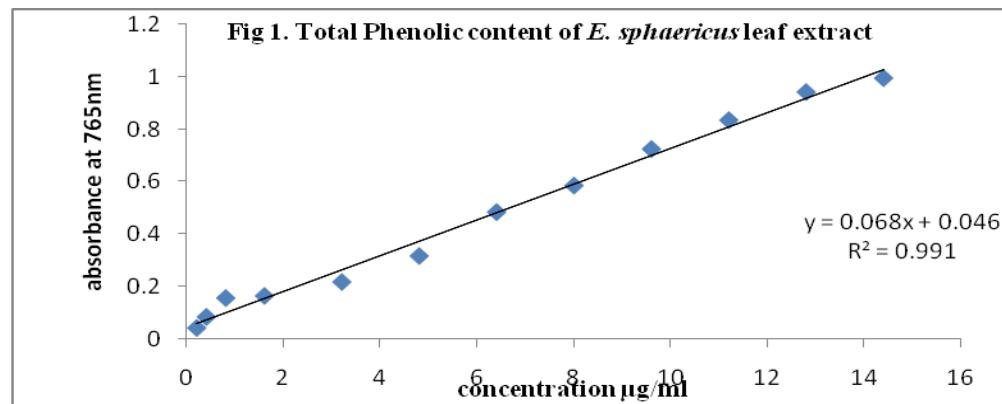


Fig 5. Total Antioxidant activity of leaf extract of *E. sphaericus* & root extract of *P. zonale*

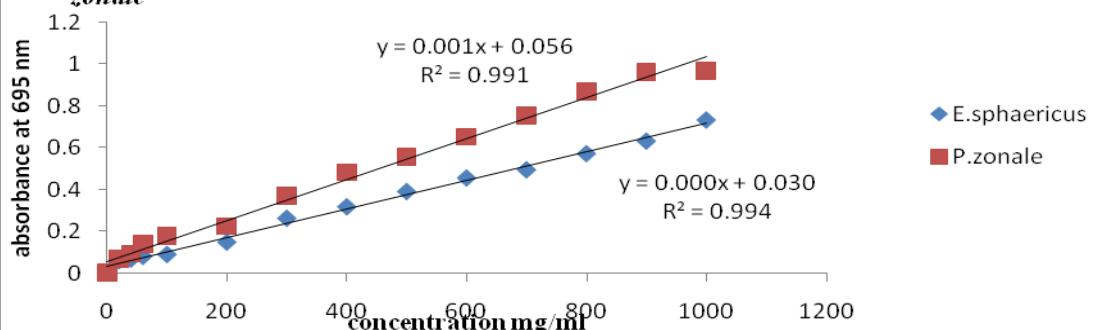


Fig 6. DPPH Free Radical scavenging activity of leaf extract of *E. sphaericus*

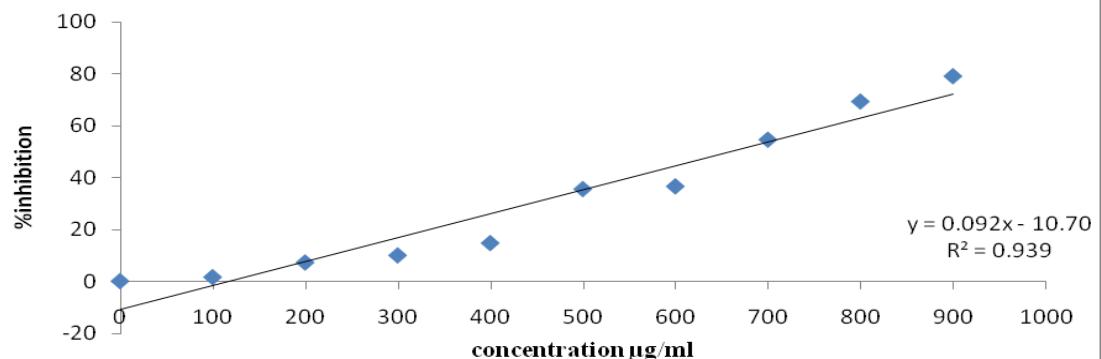
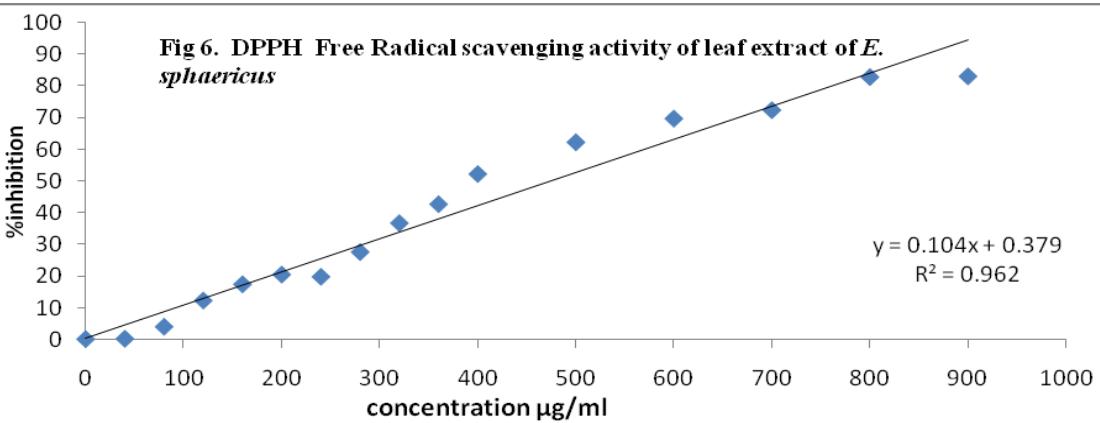
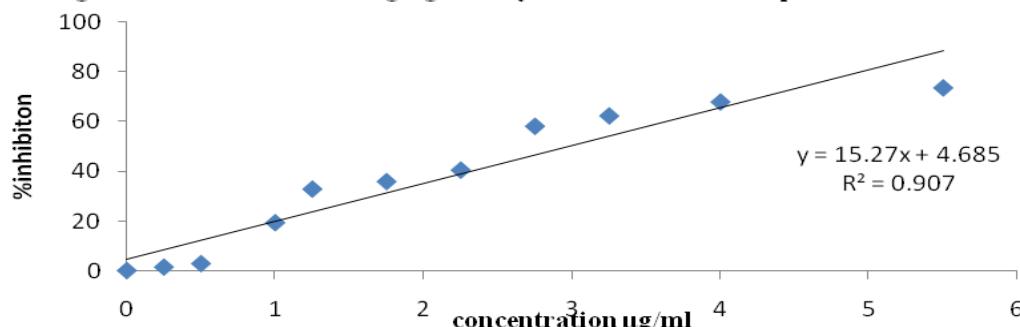
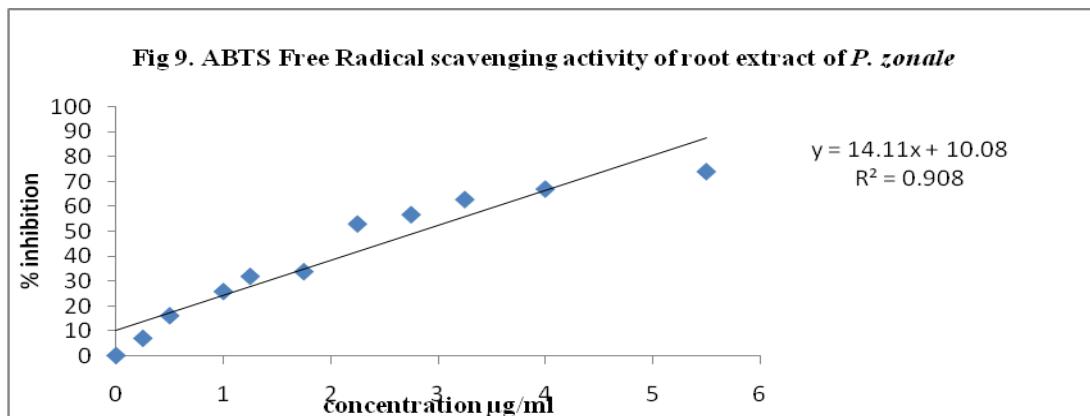


Fig 8. ABTS Radical scavenging activity of leaf extract of *E. sphaericus*





Our study showed the total antioxidant capacity of *P. zonale* was found to be 25.0 mg ascorbic acid equivalents at 500 $\mu\text{g/ml}$ extract concentration which is higher than *E. sphaericus* (14.28 mg ascorbic acid equivalents at 500 $\mu\text{g/ml}$ extract). Total antioxidant capacity of *E. sphaericus* was found to be 24.18 mg ascorbic acid equivalents at 500 $\mu\text{g/ml}$ extract concentration (Kumar *et al.*, 2008). This good antioxidant activity in *P. zonale* might be attributed to the presence of phytochemicals, such as flavonoids and biflavones. The results for total antioxidant activity are shown in fig 5.

DPPH assay is an important assay for the determination of free radical scavenging property of the plant extract and lower IC₅₀ means good radical scavenging activity. In one study, DPPH radical scavenging activity was seen in 70% acetone extract of *E. sphaericus* and IC₅₀ 34.00 $\mu\text{g/ml}$ was observed (Pandey *et al.*, 2014). Free radical scavenging activity of root extract *P. zonale* has not been analyzed before. Present study showed that *E. sphaericus* (IC₅₀ value = 477.125 $\mu\text{g/ml}$) has higher free radical scavenging activity as compare to *P. zonale* (IC₅₀ value = 659.89 $\mu\text{g/ml}$). Thus present investigation shows that leaves of *E. sphaericus* have higher radical scavenging activity as compare to roots of *P. zonale*.

As per our study, shown in fig. 8, 9 both *P. zonale* (IC₅₀ value = 2.82) and *E.*

sphaericus (IC₅₀ value = 2.96) has highest free radical scavenging activity as compare to BHT ((IC₅₀ value = 12.56). This point is very important as BHT is very commonly used as an antioxidant in food preservation and it is known to be harmful to the human health (Sharla *et al.*, 2009).

Therefore, BHT can be replaced with these medicinal plants for their use in food and pharmaceutical industry. So there is great need to focus on natural or plant derived antioxidants. The free radical scavenging activity of roots of *P. zonale* gives a hope for investing our time and resources into further analysis of plant based antioxidants. Our results are novel as ABTS assay has not been performed on *P. zonale* root extract before this study.

The present study demonstrates that both medicinal plants possess good antioxidant capacity and free radical scavenging activity and provide information about antioxidant properties and polyphenolic content of selected Indian medicinal plants which could be used in pharmaceutical and food industry in place of synthetic antioxidants. The finding of this study supports the fact that *P. zonale* root extracts and *E. sphaericus* leaf extract can potentially be used as novel antioxidants in the food, cosmetic and pharmaceutical industries.

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