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DETERMINATION OF ANTIOXIDANT ACTIVITY IN INDIGENOUSLY USED FOLK MEDICINAL PLANTS

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Abstract

Pavetta zeylanica (Hook.) and *Tarenna asiatica* (L.) are used by the indigenous Paliyar tribal for various disorders such as stomach pain, oral ulcer and skin problems. This study was conducted to evaluate the antioxidant potential of the above mentioned two plants with DPPH and FRAP assay. Extracts were prepared from the leaves of plants using three different solvents such as methanol, chloroform and petroleum ether. These extracts were subjected to assess their antioxidant potential in different concentration ranging from 25 to 200 µg/ml. Both plants exhibited antioxidant activities in all concentrations in the range of 6.10% to 48.12%. However the activity was slightly higher in *T. asiatica* leaf samples. The activities were found to be increased in dose dependent manner. Extracts obtained with methanol showed higher activity than the other extracts. Oxidative stress is said to be the reason for many disorders including stomach disorders. Since both plants exhibited noticeable antioxidant activities, their usage as medicine for stomach disorder could be justified.

Key words *: Pavetta zeylanica, Tarenna asiatica*, antioxidant, DPPH, FRAP.

Introduction

Oxidation is a necessary process to all living organisms, through which the energy production takes place for all biological activities. Free radicals or reactive oxygen species (ROS) are also produced during the process of energy production. The production of free radicals during metabolic activity is a normal and essential process. They play many important physiological functions in the organisms. However, when the production of free radicals exceeds the requirement level, oxidative stress is caused. Oxidative stress leads to onset of many disorders like rheumatoid arthritis, atherosclerosis, abdomen disorder, cancer and aging (Turkoglu *et al.,* 2007).

This phenomenon, the oxidative stress, may be due to the highly reactive nature of the free radicals. The free radicals possess an unpaired electron in the outer orbit. They possess the tendency to absorb electron from the nearby molecule for their stability, which makes the nearby molecule to become a free radical and thus start a chain reaction. Because of this tendency, the free radicals are considered as highly reactive atoms or molecules and react with biological molecules like lipids, proteins and DNA, resulting in oxidative stress (Ames *et al.,* 1993; Hanninen *et al.,* 2000; Shenoy and Shirwaikar, 2002; Patil *et al.,* 2003).

All the organisms possess well regulated self defense mechanism to manage the excess production of free radicals. Many enzymes such as superoxide dismutase and catalase or compounds such as glutathiones, ascorbic acids and tocopherols, present in the living organisms play a vital role in controlling the free radical activities (Elmastas *et al.,* 2005). When these self regulating mechanisms are compromised, antioxidant supplements and antioxidant based foods can be used to minimize the oxidative damage in human body (Niki *et al.,* 1994; Cazzi *et al.,* 1997; Roesler *et al.,* 2007).

Studies with synthetic antioxidants to combat the effect of free radicals proved that the synthetics are costly and toxic. Replacement of synthetic with natural antioxidants may be noteworthy in health aspects and functionality (Sherwin, 1990; Wanasundara and Shahidi, 1998; Da silva and Paiva, 2012). Natural antioxidants obtained from medicinal plants not only provide drugs with less side effects, but they are also source of many

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valuable substances such as phytochemicals (Iriti and Faora, 2009). Phytochemicals are considered as a gift to humans from plants as they have many health benefits and may be a new source of natural antioxidants (Liu *et al.,* 2017; Wang *et al.,* 2018). Phytochemicals from plants, such as phenolics, flavonoids and anthocyanins act on free radicals and prevent damage to DNA, proteins and membrane lipids (Elmastas *et al.,* 2006; Li *et al.,* 2009; Gulcin *et al.,* 2011, Rekha *et al.,* 2012; Junaid *et al.,* 2013).

Discovery of new and effective antioxidants from natural source becomes an important research topic at present. Knowledge of indigenous people, who still use medicinal plants to treat diseases, may be used to identify the specific natural source. Indigenous or tribal communities are experts in the usage of numerous medicinal plants to treat wide spectrum of diseases in India (Samvatsar and Diwanji, 2000; Harsha *et al.,* 2002; Hebbar *et al.,* 2004; Chhetri *et al.,* 2005; Saikia *et al.,* 2006). One such indigenous group in Tamil Nadu State in India is Paliyar Tribal. They were leading a nomadic life till the beginning of the last century in Western Ghats of India. Now, they are settled in the vicinity of Western Ghats of Virudhunagar, Theni, Tirunelveli and, Dindigul district in Tamil Nadu State.

Western Ghats still provides them non wood forest products for their monitory needs and medicinal plants for their health needs. Paliyar Tribal are using around 87 medicinal plant species for treating many disorders ranging from leprosy to common cold. Among the 87 plant species, *Tarenna asiatica* and *Pavetta zeylanica* from family Rubiaceae, are selected for the present study. Plants from family Rubiaceae are rich source of substances of phytochemicals. Several plant species from this family are used in traditional system of medicine (Sankhadip *et al.,* 2011). The leaves of *Tarenna asiatica* and *Pavetta zeylanica* are used for the treatment of abdomen pain, skin disorder and oral ulcer by Paliyar tribal (Kalusalingam and Balakrishnan, 2018).

Phytochemicals like phenols, flavonoids and steroids are reported in the leaf extracts of *Tarenna asiatica* and *Pavetta zeylanica.* The leaf extract obtained with methanol from both selected plants is reported to be effective against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* (Kalusalingam and Balakrishnan 2019).

To the best of our knowledge and thorough complete literature survey, we found that no information is available about the antioxidant activities of selected plants. Hence, the present study is conducted to evaluate the antioxidant potential of *Tarenna asiatica* and *Pavetta zeylanica* leaf extracts, prepared with different solvents such as methanol, chloroform and petroleum ether. DPPH assay and Iron chelation assay are used to evaluate the antioxidant activities.

Materials and Methods

Collection of samples

The leaves of *Tarenna asiatica* and *Pavetta zeylanica* were collected from the Sathuragiri Hills, Virudhunagar District, Tamil Nadu State, India during November 2017. The plant was properly identified by Botanical Survey of India (Southern Circle), Regional Office, Coimbatore, Tamil Nadu, India. The specimen also identified with plant Taxonomist. The voucher specimens (BOT-AAGAC – 04/2018 - *Pavetta zeylanica;* BOT-AAGAC – 05/2018- *Tarenna asiatica*) were deposited in the Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India. Freshly collected samples were packed in polythene bags and transferred to laboratory on the same day. They were properly sorted, rinsed with clean water, shade dried till they get a constant weight. Dried samples were ground using mixer grinder, and stored until analysis in brown air tight bottles.

The plant was properly identified by Botanical Survey of India (Southern Circle), Regional Office, Coimbatore, Tamil Nadu, India. The specimen also identified with plant Taxonomist. The voucher specimen (BOT-AAGAC – 04/2018 - *Pavetta zeylanica* (Hook) *&* BOT-AAGAC – 05/2018- *Tarenna asiatica* (L.)) are deposited in the Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India.

Extraction process

Extraction of powdered material was done with Soxhlet apparatus. About 50 g of each leaf sample was extracted using methanol, chloroform and petroleum ether. The extracts were evaporated to dryness at 45°C in hot water bath and re dissolved in concerned solvents and stored at 4°C prior to further use (Barros *et al.,* 2007).

Antioxidant

DPPH Assay

DPPH (1-1 diphenyl-2-picryl hydrazine) assay was used to screen the leaf extracts, obtained with different solvents, to measure the antioxidant activities. The main advantage of using DPPH to evaluate antioxidant activities is that it needs relatively short time compared to other methods. DPPH is a stable free radical and having maximum absorption at 517nm (Apak *et al.,* 2007; Shah *et al.,* 2010). This assay is widely used for

determining the radical scavenging effect of plants extracts. The natural colour of DPPH is purple. It turns yellow, when the free radical nature of DPPH is lost in the presence of an extract capable of donating an hydrogen atom (Coruh *et al.,* 2007; Kaviarasan *et al.,* 2007; Inglet and Chen, 2011; Kekuda *et al.,* 2011).

DPPH radical scavenging activity of extract was determined according to the method reported by Blois (1958) in the present study. An aliquot of 0.5 ml of different concentration of sample solution was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. The experiment was performed in triplicates.

Iron chelating activity (FRAP)

FRAP assay (Ferric reducing antioxidant power) is widely used method to determine antioxidant activity. It has been used in many studies to evaluate antioxidant activity of plant based samples (Yuan *et al.,* 2005; Hinneburg *et al.,* 2006; Poornima *et al.,* 2012). Besides that, the method is simple, quick and reproducible. Results generated from this method are linearly related to molar concentration of antioxidants. FRAP value is based on the reducing power of antioxidants. Antioxidants donate a single electron or hydrogen atom in the reaction. Substance with higher antioxidant capacity gives higher FRAP values (Hodzic *et al.,* 2009).

The method of Benzie and Strain (1996) was adopted for the assay in this study. The principle is based on the formation of *O*-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200ìM) and 2 ml of various concentrations ranging from 25 to 500ìg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Results

Pavetta zeylanica

DPPH assay

The colour bleaching of DPPH radicals in the presence of varying concentration of leaf extracts was monitored at 517 nm and the scavenging activity of extracts was compared with standard ascorbic acid. Free radical scavenging values of *Pavetta zeylanica* extracts as percentage were presented in Table 1. Methanol extract of *P. zeylanica* showed the strongest radical scavenging effect (41. 32%) at 200 ìg/ml concentration. This activity was followed by chloroform extract (31.63%) and petroleum ether extract (20.31%) at same concentration (Figure 1). Reduction of DPPH was found to be increased with increasing sample concentration. The lowest activity was exhibited by chloroform extract (5.07%) at 25 ìg/ml concentration. However the scavenging effect of control ascorbic acid was remarkably high with 79.67% at 200 ìg/ml concentration.

Iron chelating activity (FRAP)

All the three extracts exhibited iron chelation activities in dose dependent manner. The chelating activities were 36.24%, 20.82% and 17.77% in methanol, chloroform and petroleum ether extracts respectively at 200 ìg/ml concentration (Table 2). The lowest chelating activity was recorded in petroleum ether extract (6.53%) at 50 µg/ml concentration. The standard EDTA exhibited highest chelation activity, 77.25 % at 200 µg/ml (Fig. 2).

Tarenna asiatica

DPPH assay

The potential of leaf extracts to scavenge free radicals was determined by DPPH radical assay model and the results are furnished in (Table 3). The scavenging effect was high in the case of methanol extract (42.18%) followed by chloroform extract (39.09%) and petroleum ether extract (30.37%) at 200 ìg/ml concentration. The same pattern of results was observed in other concentration also. However, the scavenging effect of standard ascorbic acid was higher than that of all extracts (Fig. 3), it was 79.67% at 200 ìg/ml. The radical scavenging activity found to be increased with increasing sample concentration in all cases.

Iron chelating activity

Leaf extracts obtained with solvent methanol exhibited significantly higher chelating activity than the two other extracts *i.e.* chloroform and petroleum ether. The highest chelating value, 43.11%, was recorded in methanol extract at 200 µg/ml concentration (Table 4). The chelating values were 28.97% and 21.06% in chloroform and petroleum ether extract respectively at the same sample concentration. Furthermore, petroleum extract possessed lowest antioxidant activity (7.09%) at 25µg/ml concentration. All the tested samples showed lower chelating values than the positive control EDTA (Fig. 4). The highest chelating value of standard EDTA (77.25%) was observed at 200 µg/ml concentrations.

Statistical analysis

Each value is a mean of three replications. Values of different parameters were expressed as the man of $\pm$

Table 1: DPPH values in *Pavetta zeylanica.*

S.No.	Concentration of Sample (µg/ml)	Free radical scavenging activity (%)			
		Methanolic Extract	Chloroform Extract	Petroleum Ether Extract	Ascorbic Acid
1.	25	12.51±1.14	05.07±0.70	06.12±0.16	46.33±0.60
2.	50	20.59±1.56	13.07±0.27	09.42±0.28	56.98±1.54
3.	100	32.01±2.49	19.73±0.35	13.14±0.43	68.46±0.48
4.	200	41.32±2.45	31.63±2.05	20.31±1.16	79.67±0.96

± - Standard Error of triplicates. Difference between values are significant at $p<0.05$ with two way ANOVA test.

Table 2: Iron chelating values of *Pavetta zeylanica.*

S.No.	Concentration of Sample (µg/ml)	Iron chelating activity (%)			
		Methanolic Extract	Chloroform Extract	Petroleum Ether Extract	Ascorbic Acid
1.	25	11.40±0.64	08.93±0.54	06.53±0.89	45.24±1.89
2.	50	16.27±0.01	12.16±1.20	09.60±0.60	60.74±1.36
3.	100	21.62±2.01	16.83±0.59	12.47±1.17	73.33±0.92
4.	200	36.24±1.09	20.82±1.21	17.77±1.19	77.25±1.10

± - Standard Error of triplicates. Difference between values are significant at $p<0.05$ with two way ANOVA test.

Table 3: DPPH values in *Tarenna asiatica.*

S.No.	Concentration of Sample (µg/ml)	Free radical scavenging activity (%)			
		Methanolic Extract	Chloroform Extract	Petroleum Ether Extract	Ascorbic Acid
1.	25	24.26±1.12	18.57±0.49	06.10±0.35	43.33±0.60
2.	50	31.38±1.76	26.18±1.36	14.98±0.76	56.98±1.54
3.	100	37.95±0.38	30.94±1.18	22.49±2.45	68.46±0.48
4.	200	48.12±0.90	39.09±1.30	30.37±1.71	79.67±0.96

± - Standard Error of triplicates. Difference between values are significant at $p<0.05$ with two way ANOVA test.

Table 4: FRAP values in *Tarenna asiatica.*

S.No.	Concentration of Sample (µg/ml)	Iron chelating activity (%)			
		Methanolic Extract	Chloroform Extract	Petroleum Ether Extract	Ascorbic Acid
1.	25	14.59+0.93	09.49+0.89	07.09+0.68	45.24±1.89
2.	50	22.08+0.80	17.36+0.43	10.90+1.32	60.74±1.36
3.	100	31.20+1.04	23.49+1.91	14.97+2.11	73.33±0.92
4.	200	43.11+0.71	28.97+2.24	21.06+1.70	77.25±1.10

± - Standard Error of triplicates. Difference between values are significant at $p<0.05$ with two way ANOVA test.

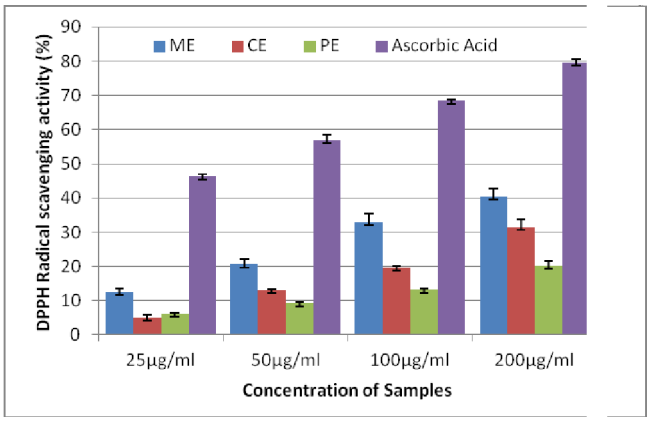
standard error (mean + SE). Two way ANOVA (analysis of variance) test was conducted for all the experiments with P value < 0.05. The difference between the observed values found to be significant. All the statistical analyses were performed using MS Excel software.

Discussion

The phytochemical studies with *P. zeylanica* and *T. asiatica* showed the presence of phenol and flavonoid. The studied plants reported to be effective in the prevention of growth of certain pathogenic bacteria also in culture plates (Kalusalingam and Balakrishnan, 2019). Majority of the plant secondary metabolites belong to the group of flavonoids and polyphenolics. They possess significant health promoting effects including antioxidant activities (Velioglu *et al.,* 1998; Dorman *et al.,* 2004; Choi *et al.,* 2007; Chua *et al.,* 2011; Al-Laith *et al.,* 2019). Based on these reports, the antioxidant activities were studied for the leaf extracts of *P. zeylanica* and *T. asiatica*. The results confirmed that *T. asiatica* extracts exhibited slightly higher antioxidant activities in both DPPH and FRAP test. The quantity of reported phytochemicals might be higher in *T. asiatica*. That may be the reason for the better performance of *T. asiatica*. Further investigation on quantification of phytochemicals is required for arrival of any such conclusion.

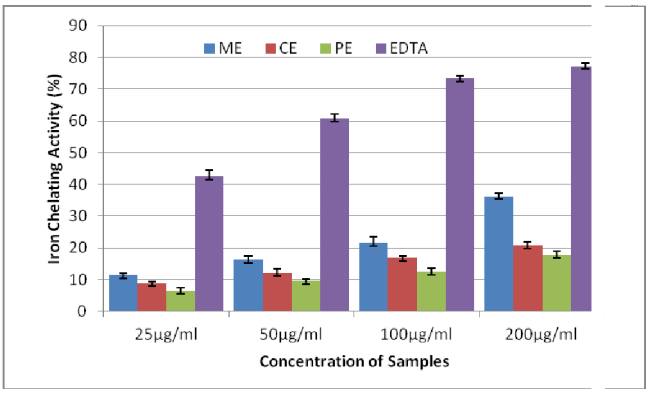
The antioxidant activities of extracts increase in ascending order according to the polarity of the solvents. The results with methanol extracts exhibited the highest antioxidant activities than thee chloroform and petroleum ether extracts in both tested plants. Extracts obtained with high polarity methanol reported to be better in many pharmacological studies. The antioxidant activities found to be increasing with the increase of sample concentrations in both DPPH and FRAP studies. It is in consistent with various previous reports (Sankhadip *et al.,* 2011; Thambiraj *et al.,* 2012; Muhammad *et al.,* 2019).

The antioxidant activity observed with FRAP assay was lesser than that DPPH assay in both plants samples. Though both methods determine the antioxidant activities, they have different mechanism. FRAP assay is based on



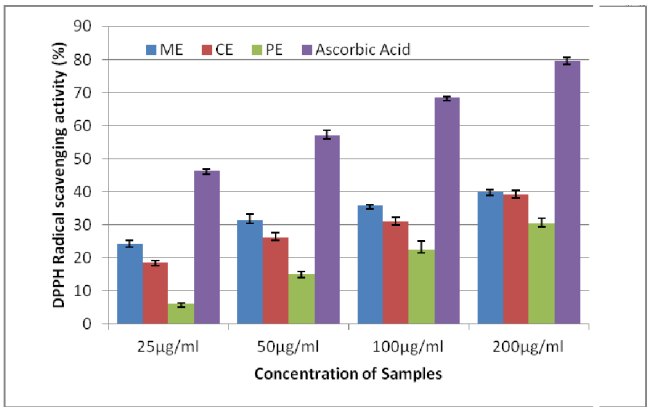
ME-Methanol Extract ; CE-Chloroform Extract ; PE-Petroleum Extract

Fig. 1: DPPH values in *Pavetta zeylanica.*



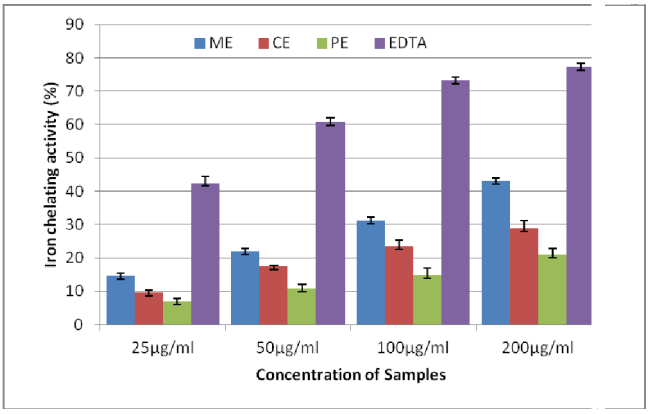
ME-Methanol Extract ; CE-Chloroform Extract ; PE-Petroleum Extract

Fig. 2: Iron chelating values of *Pavetta zeylanica.*



ME-Methanol Extract ; CE-Chloroform Extract ; PE-Petroleum Extract

Fig. 3: DPPH values in *Tarenna asiatica.*



ME-Methanol Extract ; CE-Chloroform Extract ; PE-Petroleum Extract

Fig. 4: FRAP values in *Tarenna asiatica.*

electron transfer reaction, whereas DPPH assay are based on electron and hydrogen atom transfer (Prior *et al.,* 2005). Despite the fact that FRAP and DPPH have different mechanism, the two methods clearly indicated that the studied plants possess uneven but significant antioxidant and chelating activities.

The leaves of both plants are reported to be used by the Paliyar Tribal for stomach disorders (Kalusalingam and Balakrishnan, 2018). Chi *et al.* (2002) reported the association between abdomen disorder and oxidative stress. The results of the present investigation shows the considerable antioxidant capacities of both tested plants, which may indicate the relevance of this capacity to its folk usage.

Conclusion

Pavetta zeylanica and *Tarenna asiatica* are two folk medicinal plants, used by indigenous Paliyar tribal. Leaf samples of both plants exhibited variable but considerable antiradical activities. Among the two studied plants, *T. asiatica* possessed the slightly higher anti radical activities in the DPPH and FRAP methods. Antiradical activities found to be increased with dose dependent manner in all the samples in both DPPH and FRAP studies. Leaf extracts obtained with methanol exhibited higher activities than chloroform and petroleum ether extracts. However, the active component in the extract which is responsible for the observed antioxidant activity is unknown at present. Therefore, further work, involving the isolation and purification of the active compounds from the crude extracts of *Pavetta zeylanica* and *Tarennna asiatica* is required in order to examine the mode of action.

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