EVALUATION OF ANTIOXIDANT ACTIVITY OF TERMINALIA ARJUNA LEAVES EXTRACT

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Summary

Current research on *Terminalia arjuna* is directed towards finding naturally-occurring antioxidants of plant origin that provided efficacy by additive or synergistic activities because antioxidants from plant origin are essential to prevent the progression of free radical mediated disorders. Antioxidant activity of *Terminalia arjuna* methanolic extract (TAME) and *Trminalia arjuna* ethyl acetate extract (TAEAE) find out by using different in-vitro models. It includes Free radical scavenging activity of DPPH, Ferric reducing antioxidant power, Total flavonoid content and Total phenolic content. The plant contain much amount of Phenolic compounds and Flavonoids. Plant shows significant antioxidant activity.

Keywords: *Terminalia arjuna, antioxidant, phenolic content, flavonoid content.*

Introduction

*Terminalia arjuna (*Combretaceae) is about 60-80 feet height. Arjuna is large, evergreen with a spreading crown and dropping branches. In favorable localities and especially along the banks of streams, the tree attains very large sizes. Two trees of 26 feet and 32 feet in girth at 5 feet from the ground have been recorded in the village of Manipur in Jammu and Kashmir. Leaves sub-opposite, oblong or elliptic, coriaceous, cordate, shortly acute and obtuse at the apex. Flowers in panicled spikes. Fruits ovoid or ovoid-oblong, 2.5-5.0 cm long, nearly glabrous, with 5-7 hard, winged angles.[1,2,3]

Stem bark used as Astringent, cooling, aphrodisiac, cardiotonic, expectorant, alexiteric, in fractures, ulcers, diabetes, anemia, cardiac disorders, cough, tumor, excessive perspiration, fatigue, asthma, bronchitis, intrinsic hemorrhage, otalgia, diarrhea associated with blood, cirrhosis of liver, hypertension and skin disorders. Fruits used as Tonic and deobstruent**.** Leaves are used as Juice for earache.[4,5]

Materials and methods

Plant material

The leaves of *Terminalia arjuna* were collected from Jaipur, Rajasthan, India and a voucher specimen (RUBL 20845) for this plant material was preserved in the herbarium of Department of Botany, Rajasthan University, Jaipur. The leaves, dried in shade were powdered and subjected to soxhlet with methanol and ethyl acetate for 72 hr. The extract collected was evaporated (yield 2.30% w/w ethyl acetate and 4.58% w/w methanol), and stored in a vacuum desiccator. The preliminary phytochemical investigations with the TAME revealed the presence of alkaloids, carbohydrate, flavonoids, flavones, phenolic compound, tannins, volatile oil, triterpenoids, proteins, saponins, glycosides and TAEAE revealed the presence flavonoids, flavones, phenolic compound, volatile oil, fixed oil and fats, triterpenoids.[6,7]

Chemical

Chemical required namely methanol, ethanol, tri-chloro acetic acid, ascorbic acid, BHA, rutin, Folin-Ciocalteu's phenol reagent, potassium ferricynide, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), aluminium chloride, sodium nitrite, sodium hydroxide were used during the experimental study.

Method

Free Radical Scavenging Activity Measured by 1,1-Diphenyl-2-picryl-hydrazil

The free radical scavenging activity of TAME and TAEAE was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH·) using the method of Blois[8]. Briefly, a 0.1 mM solution of DPPH· in ethanol was prepared and 1 ml of this solution was added to 3 ml of TAME and TAEAE solution in water at different concentrations (20-60 µg ml-1). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity.

DPPH· scavenging effect (%) = [(Ao − A1/Ao) × 100]
Where,
Ao was the absorbance of the control reaction and
A1 was the absorbance in the presence of the sample of TAME and TAEAE [9].

Ferric reducing antioxidant power (FRAP)

The total reducing power of the TAME and TAEAE was determined according to the method of Oyaizu [10,11]. Briefly, different concentrations of BL extracts (20, 40, and 60 µg ml-1) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50 oC for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm using a UV-Vis spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of total flavonoid content

The total flavonoid content was determined with aluminium chloride (AlCl3) according to the known method of Zhishen et al., (1999)[12] using Rutin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 0.03 ml NaNO2 (5%) and incubated for 5 min at 25°C. Later 0.03 ml AlCl3 (10%) was added and further after 5 min, the reaction mixture was treated with 0.2 ml (1mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. All tests were performed six times. The flavonoid content was calculated from a Rutin standard curve.

Estimation of total phenolic content

The total phenolic content of the extract was estimated according to the method described by Singleton and Rossi [13]. From the stock solution (1 mg/ml) of the TAME and TAEAE, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of Folin Ciocalteu's reagent. After 5 min, 4 ml of 20% (w/v) sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The absorbance was recorded at 765 nm, after 30 min. Percentage of total phenolics was calculated from calibration curve of Gallic acid (50-250 µg) plotted by using same procedure and total phenolics were expressed as % Gallic acid.

Result

Free Radical Scavenging Activity Measured by 1,1-Diphenyl-2-picryl-hydrazil

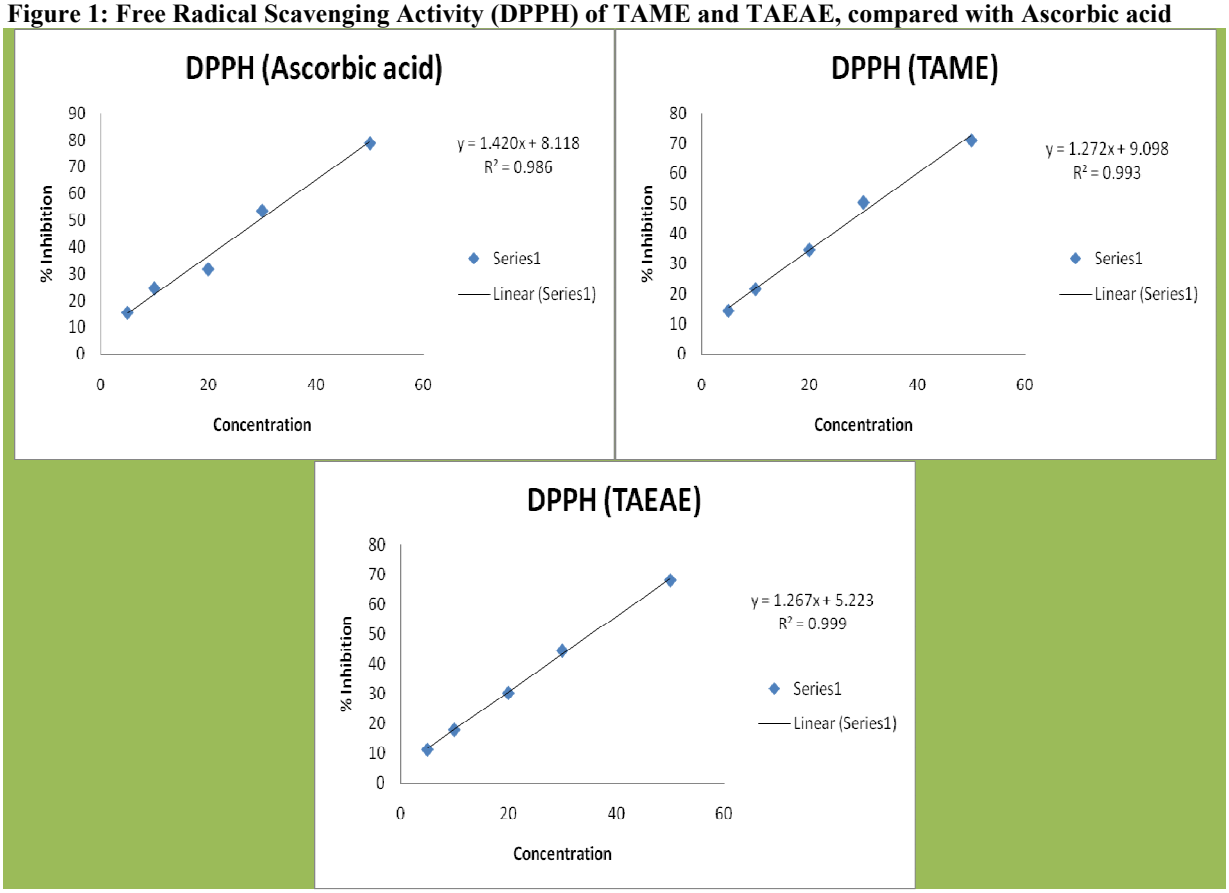
TAME and TAEAE free radical scavenging activity are given in the Table 1 and Figure 1, IC_{50} of Ascorbic acid against DPPH is 29.43 µg/ml, IC_{50} of TAME against DPPH is 32.16 µg/ml, IC_{50} of TAEAE against DPPH is 35.33 µg/ml.

Table 1: Free Radical Scavenging Activity (DPPH) of TAME and TAEAE, compared with Ascorbic acid

s.no.	Group	Concentration (µg/ml)	Absorbance (700 nm)	% Inhibition
	Control		0.9873±0.002	-
	STD (Ascorbic acid)			
1.		5	0.8362±0.001	15.30
2.		10	0.7457±0.001	24.47
3.		20	0.6747±0.001	31.66
4.		30	0.4583±0.001	53.58
5.		50	0.2077±0.003	78.96
	TEST (TAME)			
1.		5	0.8460±0.001	14.31
2.		10	0.7747±0.002	21.53
3.		20	0.6457±0.001	34.60
4.		30	0.4898±0.002	50.39
5.		50	0.2861±0.001	71.02
	TEST (TAEAE)			
1.		5	0.8761±0.006	11.26
2.		10	0.8113±0.004	17.82
3.		20	0.6876±0.001	30.36
4.		30	0.5487±0.001	44.42
5.		50	0.3155±0.001	68.04

Data presented in (MEAN ± SD), n=3

Figure 1: Free Radical Scavenging Activity (DPPH) of TAME and TAEAE, compared with Ascorbic acid



Ferric reducing antioxidant power (FRAP)

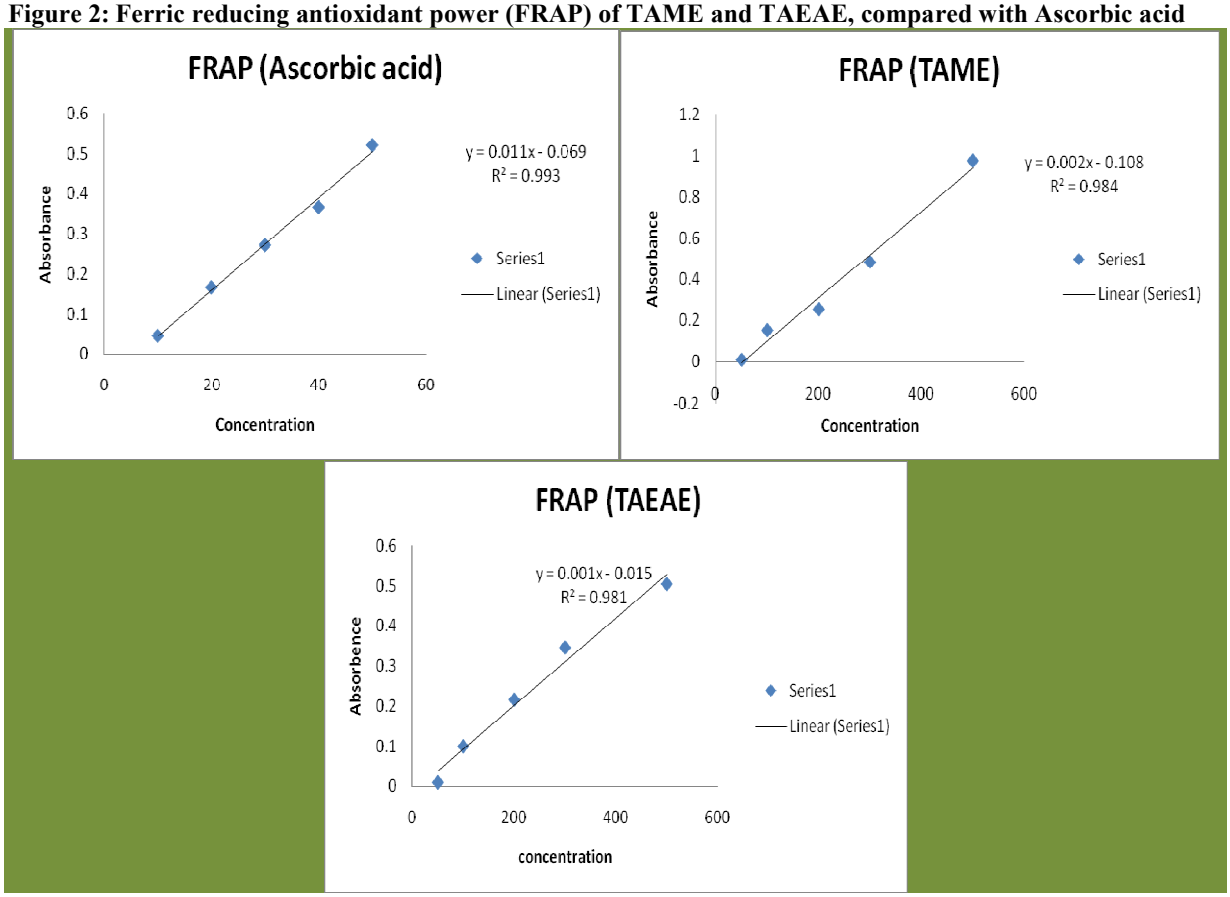
TAME and TAEAE Ferric reducing antioxidant power given in the Table 2 and Figure 2, Ascorbic acid used as a reference drug and TAME and TAEAE given significant activity.

Table 2: Ferric reducing antioxidant power (FRAP) of TAME and TAEAE, compared with Ascorbic acid

s.no.	Group	Concentration (µg/ml)	Absorbance (700 nm)
	STD (Ascorbic acid)		
1.		10	0.0464±0.001
2.		20	0.1670±0.002
3.		30	0.2722±0.008
4.		40	0.3665±0.001
5.		50	0.5205±0.002
	TEST (TAME)		
1.		50	0.0078±0.001
2.		100	0.1514±0.001
3.		200	0.2529±0.003
4.		300	0.4846±0.001
5.		500	0.9764±0.003
	TEST (TAEAE)		
1.		50	0.0088±0.001
2.		100	0.099±0.003
3.		200	0.2156±0.004
4.		300	0.3459±0.002
5.		500	0.5044±0.002

Data presented in (MEAN ± SD), n=3

Figure 2: Ferric reducing antioxidant power (FRAP) of TAME and TAEAE, compared with Ascorbic acid



Determination of total flavonoid content

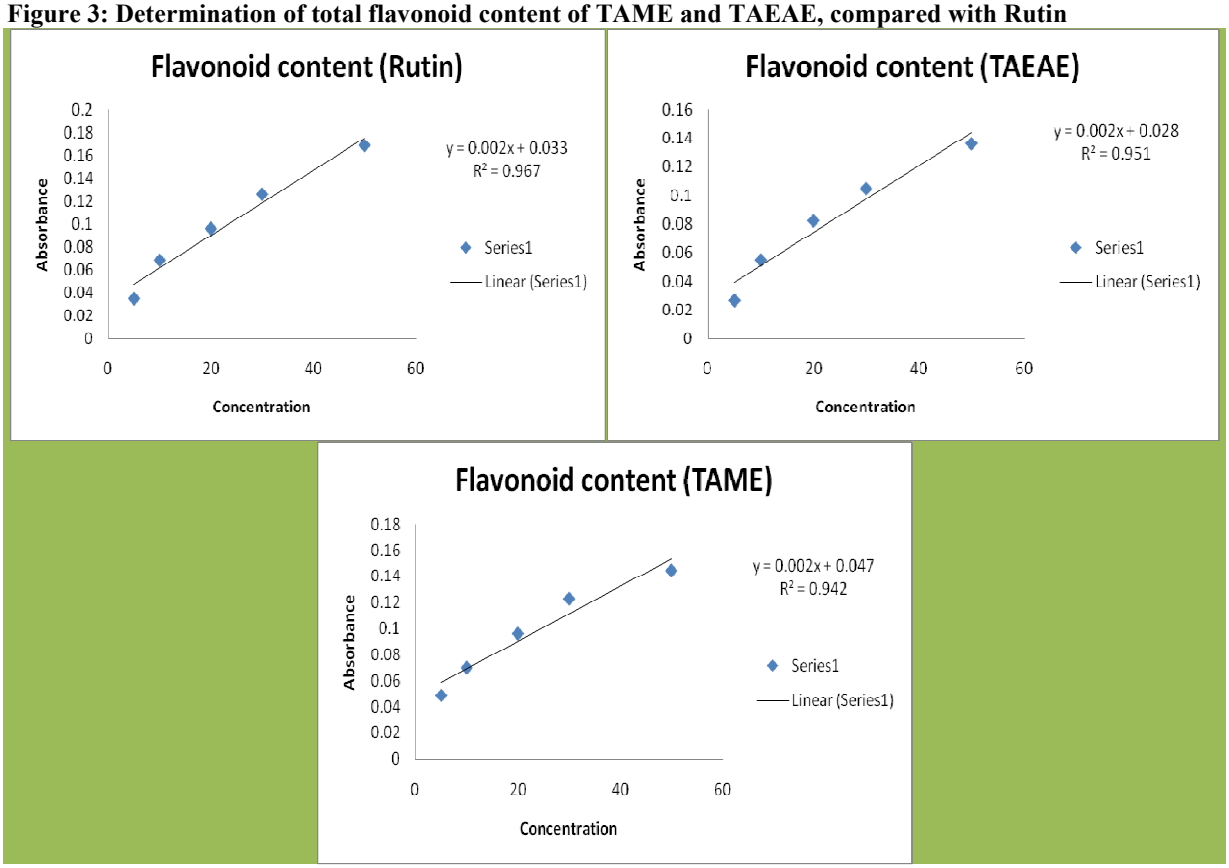
TAME and TAEAE flavonoid content are given in the Table 3 and Figure 3, Rutin used as a reference drug and approximately 20 µg/ml rutin = 20 µg/ml TAME = 25 µg/ml TAEAE.

Table 3: Determination of total flavonoid content of TAME and TAEAE, compared with Rutin

s.no.	Group	Concentration (µg/ml)	Absorbance (700 nm)
	STD (Rutin)		
1.		5	0.03470±0.001
2.		10	0.0683±0.001
3.		20	0.0960±0.001
4.		30	0.126±0.001
5.		50	0.1686±0.002
	TEST (TAME)		
1.		5	0.0485±0.001
2.		10	0.0698±0.001
3.		20	0.0960±0.001
4.		30	0.1230±0.001
5.		50	0.1448±0.001
	TEST (TAEAE)		
1.		5	0.0271±0.001
2.		10	0.0553±0.002
3.		20	0.0827±0.002
4.		30	0.1052±0.001
5.		50	0.1366±0.003

Data presented in (MEAN ± SD), n=3

Figure 3: Determination of total flavonoid content of TAME and TAEAE, compared with Rutin



Estimation of total phenolic content

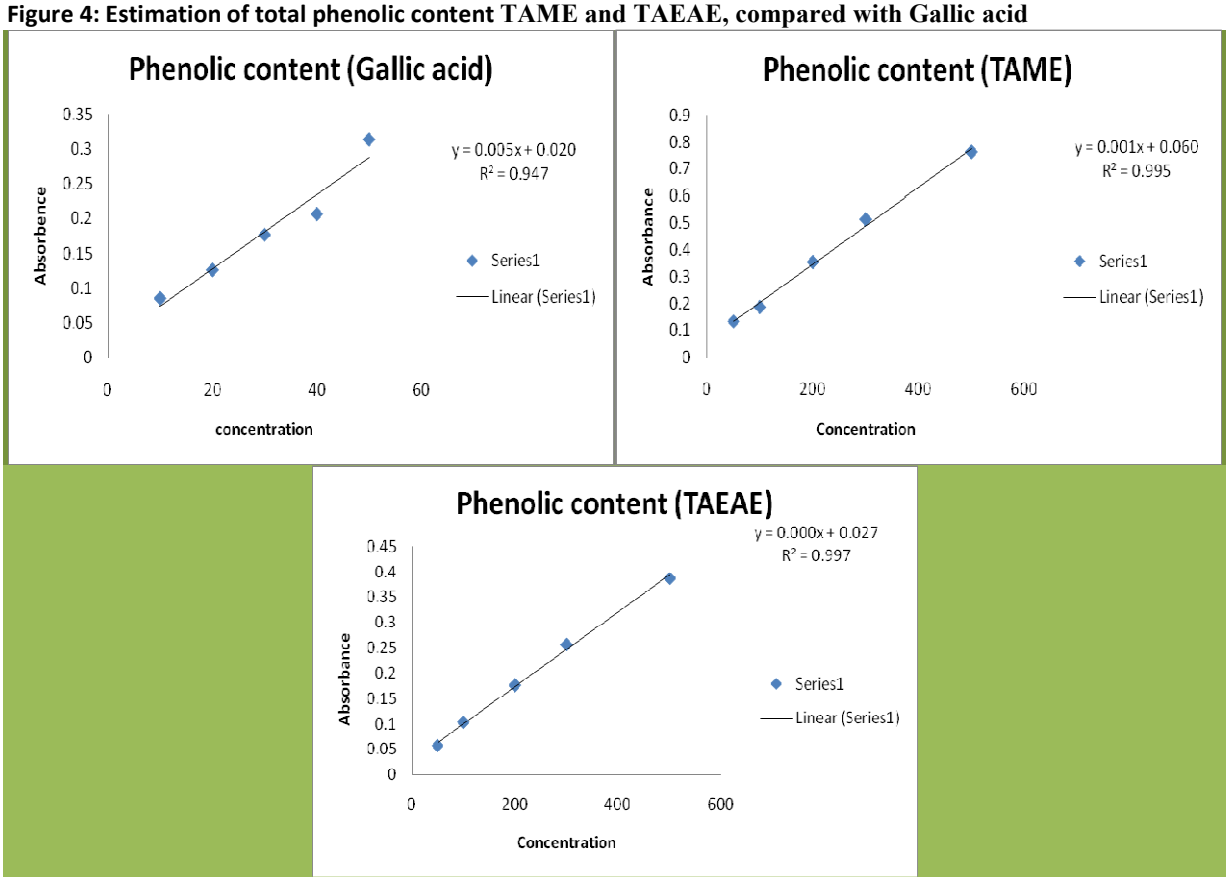
TAME and TAEAE phenolic content are given in the Table 3 and Figure 3, gallic used as a reference drug and approximately 30 µg/ml gallic acid = 100 µg/ml TAME = 200 µg/ml TAEAE.

Table 4: Estimation of total phenolic content TAME and TAEAE, compared with Gallic acid

s.no.	Group	Concentration (µg/ml)	Absorbance (760 nm)
	STD (Gallic acid)		
1.		10	0.0854±0.002
2.		20	0.1261±0.003
3.		30	0.1766±0.002
4.		40	0.2063±0.004
5.		50	0.3134±0.004
	TEST (TAME)		
1.		50	0.1319±0.001
2.		100	0.1855±0.001
3.		200	0.3537±0.001
4.		300	0.5129±0.003
5.		500	0.7613±0.020
	TEST (TAEAE)		
1.		50	0.0569±0.001
2.		100	0.1031±0.001
3.		200	0.1764±0.001
4.		300	0.2570±0.001
5.		500	0.3876±0.002

Data presented in (MEAN ± SD), n=3

Figure 4: Estimation of total phenolic content TAME and TAEAE, compared with Gallic acid



Discussion

2,2-diphenyl-1- picrylhydrazyl (DPPH) is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products[14]. In this study, DPPH scavenging activity has been observed in TAME and TAEAE. DPPH radical scavenging activities of TAME and TAEAE varied from 11.26 to 71.02%. All of the extracts tested possess radical scavenging activity. This activity was increased by increasing the concentration of the sample extract.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [15]. For the measurements of the reductive ability, it has been found that the Fe^{3+}-Fe^{2+} transformation occurred in the presence of extract samples which was postulated previously by Oyaizu [10] Tanaka et al. have observed a direct correlation between antioxidant activities and reducing power of certain plant extracts [16]. The reducing properties are generally associated with the presence of reductones [17], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[18]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In this assay, depending on the reducing power of antioxidant compounds, the yellow color of the test solution changes into various shades of green and blue. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, we can monitor the Fe^{2+}

concentration. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. TAME and TAEAE indicates significant antioxidant activity.
Phenolic compounds are known powerful chain breaking antioxidants[19], important plant constituents because of their scavenging ability due to their hydroxyl groups and contribute directly to antioxidative action[20] Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants[21] It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1g daily with a diet rich in fruits and vegetables[22]. Further research on this plant helpful to find out more potent antioxidant.

Conclusion

The present study provides an evidence that TAME and TAEAE even though having more amount of flavonoid and phenolic content, shows potential antioxidant and free radical scavenging activity. These *in vitro* assays demonstrate that plant extracts are important sources of natural antioxidants, which might be useful as preventive agents against oxidative stress. To elucidate the prime source of antioxidant properties further studies should be carried out with isolate active principles.

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