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COMPARATIVE ANALYSIS AND SIMULTANEOUS QUANTIFICATION OF ANTIOXIDANT CAPACITY OF FOUR *TERMINALIA* SPECIES USING VARIOUS PHOTOMETRIC ASSAYS

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ABSTRACT

Most of the *Terminalia* species are a popular folk medicine and has several proven biological properties. Present study was performed for comparative analysis and simultaneous quantification of antioxidant properties of leaf and stem of four *Terminalia* species belongs to the family Combretaceae. Plant material was extracted by individual cold percolation extraction method using various solvents. The antioxidant activity of all the different solvent extracts of leaves and stem was evaluated using various photometric antioxidant assays like DPPH free radical scavenging assay, superoxide anion radical scavenging assay, ABTS radical cation scavenging assay and Ferric reducing antioxidant power (FRAP). Total phenol and flavonoid content was also measured. The results showed that the extracting solvent significantly altered the antioxidant property estimations of leaf and stem. High correlations between phenol content and antioxidant activities of extracts were observed. High levels of antioxidant activities were detected in *T. chebula* as compared to the other species indicating that *T. chebula* may serve as an excellent source of natural antioxidants.

KEYWORDS: *Terminalia*, leaf, stem, antioxidant, individual extraction, TPC, FC, FRAP, DPPH, ABTS, superoxide

INTRODUCTION

Terminalia arjuna Wight and Arnott, *Terminalia bellerica* (Gaertn.) Roxb., *Terminalia chebula* Retz. and *Terminalia catappa* Linn belong to the family Combretaceae. They are

active ingredients of many traditionally used drugs in alternate system of medicine Ayurveda. Various parts of these plants like bark, leaves and fruits are medicinally important and there are number of reports of their therapeutic importance. Some of the reported activities are antimicrobial (Chanda et al., 2011; Rakholiya and Chanda, 2012a), anticancer (Dinesh et al., 2014), hepatoprotective (Choi et al., 2015), cardiovascular disorders (Dwivedi and Chopra, 2014), antioxidant (Chanda et al., 2013a, b), antitumor (Pettit et al., 1996), etc. Antioxidant substances obtained from natural sources are of great interest. Some species from the Combretaceae family have been found to have antioxidant activities (Masoko et al. 2005; Masoko et al., 2007; Mety and Mathad, 2011).

Major research interests have been towards to oxidative stress in human due to reactive oxygen species (ROS) over the last few decades. Oxidative stress, caused by an unbalanced mechanism between antioxidant protection and the production of excessive free radicals, seems be associated with numerous diseases (Lassoued et al., 2015), especially cancers, cardiovascular diseases and inflammatory disorders (Kaneria and Chanda, 2012; Deng et al., 2013). Accumulative effects tissue destruction caused by ROS lead to pathological conditions (Kaneria et al., 2012a; Xiao et al., 2015).

The natural antioxidants are suggested as a superior alternative for the synthetic ones such as t-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG). Hence, reactive oxygen species induced oxidative cell damage can be prevented by supplementation of naturally occurring medicinal plant, which is one of the most acceptable techniques for modern therapy (Kaneria et al., 2012b; Zhao et al., 2015). The antioxidants play key role to scavenge free radical, hydrogen donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for superoxide and hydroxyl radicals. It has been reported that bioactive plant extracts with high levels of phenolic and flavonoid compounds exhibit strong antioxidant activities and provide a fruitful defense against oxidative stress from free radicals and oxidizing agent (Kaewseejan et al., 2015).

However, no studies have so far been reported comparative analysis and simultaneous quantification on antioxidant properties of different solvent extracts of leaf and stem of these four *Terminalia* species by various photometric assays. Thus, in the present study, we have extensively studied the antioxidant activity of different solvent extracts of four *Terminalia* species using different *in vitro* assays and their correlation with total phenol and flavonoid content.

MATERIALS AND METHODS

Chemicals and reagents: Nitroblue tetrazolium (NBT), Nicotinamide adenine dinucleotide reduced (NADH), Phenazine methosulphate (PMS), 2,4,6-tri-(2-pyridyl)-5-triazine (TPTZ), potassium acetate, potassium persulfate were obtained from Sisco Research Laboratories Pvt. Ltd. (India), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis-(3-ethyl) benzothiazoline)-6-sulfonic acid diammonium salt (ABTS), gallic acid, ascorbic acid, ferrous sulfate ($FeSO_4$), quercetin, sodium carbonate, ferric chloride ($FeCl_3$), Tris-HCl, sodium acetate were obtained from Hi-Media (India), hexane, acetone, ethyl acetate, methanol, hydrochloric acid (HCl), Folin-Ciocalteu's reagent, aluminium chloride ($AlCl_3$) were from MERCK Pvt. Ltd. (India). Water was purified with a Milli-Q system (Millipore, Bedford, 160 USA). All chemicals and reagents used were of analytical grade.

Plant collection: The leaves and stem of four *Terminalia* species (*T. arjuna*, *T. bellerica*, *T. chebula* and *T. catappa*) were collected in July, 2011 from Jamnagar, Gujarat, India. The plant material was washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in air tight bottles.

Extraction: The dried powder was individually extracted by cold percolation method (Parekh and Chanda, 2007, Rakholiya et al., 2014a) using different organic solvents like hexane, ethyl acetate, acetone and water. 10 g of dried powder was taken in 100 ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth, centrifuged at 5000 rpm for (Remi Centrifuge, India) 15 min and the supernatant was collected and the solvent was evaporated using a rotary vacuum evaporator (Equitron, India) to dryness. Hexane was evaporated from the powder. This dry powder was then taken individually in 100 ml of each solvents (ethyl acetate, acetone, water) and was kept on a rotary shaker at 120 rpm for 24 hrs. Then the procedure followed was same as above, and the residues were weighed to obtain the extractive yield of all the extracts and were stored in air tight bottles at 4 °C.

Quantitative phytochemical analysis

Determination of total phenol content: The amount of total phenol content, in different solvent extracts, was determined by Folin-Ciocalteu's reagent method (McDonald et al., 2001). The extract (0.5 ml) and 0.1 ml of Folin-Ciocalteu's reagent (0.5 N) were mixed, and the mixture was incubated at room temperature for 15 min. Then, 2.5 ml of saturated sodium carbonate solution was added and further incubated for 30 min at room temperature, and the

absorbance was measured at 760 nm using a UV–VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. The calibration curve was made by preparing gallic acid (10 to 100 µg ml^{-1}) solution in distilled water. Total phenol content is expressed in terms of gallic acid equivalent (milligrams per gram of extracted compounds) (Kaneria et al. 2012b; Rakholiya et al., 2014b).

Determination of flavonoid content: The amount of flavonoid content, in different solvent extracts, was determination by aluminium chloride colorimetric method (Chang et al., 2002). The reaction mixture (3.0 ml) consisted of 1.0 ml sample (1 mg ml^{-1}), 1.0 ml methanol, 0.5 ml aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM) and was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm using a UV–VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. The calibration curve was made by preparing a quercetin (5 to 60 µg ml^{-1}) solution in methanol. The flavonoid content is expressed in terms of quercetin equivalent (milligrams per gram of extracted compounds) (Kaneria et al., 2012b; Rakholiya et al., 2014b).

Antioxidant assays

Ferric reducing antioxidant power (FRAP): The reducing ability of the different solvent extracts was determined by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996). FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri-(2-pyridyl)-5-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). 0.1 ml of the different solvent extract was added to 3.0 ml FRAP reagent [10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ in 40 mM HCl and 1 part 20 mM $FeCl_3$], and the reaction mixture is incubated at 37 °C for 10 min. And then, the absorbance was measured at 593 nm using a UV–VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. The calibration curve was made by preparing a $FeSO_4$ (100 to 1000 µM ml^{-1}) solution in distilled water (Kaneria et al., 2012b; Rakholiya et al., 2014b). The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as M $FeSO_4$ equivalents per gram of extracted compounds.

DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical scavenging assay: The free radical scavenging activity of different solvent extracts was measured by using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) by the modified method of McCune and Johns (2002). The reaction

mixture (3.0 ml) consisted of 1.0 ml DPPH in methanol (0.3 mM), 1.0 ml methanol, and 1.0 ml of different concentrations (5 to 1000 µg ml^{-1}) of different solvent extracts diluted in methanol, was incubated for 10 min, in dark, after which the absorbance was measured at 517 nm using a UV–VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. Ascorbic acid (2 to 16 µg ml^{-1}) was used as positive control (Kaneria et al. 2012b; Rakholiya et al., 2014b). Percentage of inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = [1 - (A/B)] \times 100$$

Where, B is the absorbance of the blank (DPPH, plus methanol), A is absorbance of the sample (DPPH, methanol, plus sample)

Superoxide anion radical scavenging assay: The superoxide anion radical scavenging activity of different solvent extracts was measured by the method as described by Robak and Gryglewski (1988). Superoxide anion radicals are generated by oxidation of NADH and assayed by the reduction of NBT. The reaction mixture (3.0 ml) consisted of 1.0 ml of different concentrations (5 to 1000 µg ml^{-1}) of different solvent extracts diluted by distilled water, 0.5 ml Tris-HCl buffer (16 mM, pH 8), 0.5 ml NBT (0.3 mM), 0.5 ml NADH (0.936 mM), and 0.5 ml PMS (0.12 mM). The superoxide radical generating reaction was started by the addition of PMS solution to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and then, the absorbance was measured at 560 nm using a UV–VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. Gallic acid (50 to 225 µg ml^{-1}) was used as a positive control (Kaneria et al., 2012b; Rakholiya et al., 2014b). The percentage inhibition was calculated using the formula described as above.

ABTS (2,2'-Azino-bis-(3-ethyl)benzothiazoline)-6-sulfonic acid diammonium salt) radical cation scavenging assay: The ABTS radical cation scavenging activity was measured by the method as described by Re et al. (1999). ABTS radical cations are produced by reacting ABTS (7 mM) and potassium persulfate (2.45 mM) and incubating the mixture at room temperature in the dark for 16 h. The ABTS working solution obtained was further diluted with methanol to give an absorbance of 0.85 ± 0.20 at 734 nm. 1.0 ml of different concentrations (1 to 1000 µg ml^{-1}) of different solvent extracts diluted by methanol was added to 3.0 ml of ABTS working solution. The reaction mixture was incubated at room temperature for 4 min, and then the absorbance was measured at 734 nm using a UV–VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. Ascorbic acid (1 to 10 µg ml^{-}

[1]) was used as a positive control (Zhou *et al*., 2011; Rakholiya et al., 2014b). The percentage of inhibition was calculated using the formula described as above.

Statistical analysis

Each sample was analyzed individually in triplicate and the results are expressed as the mean value (n = 3) ± Standard Error of the Mean (S.E.M.). The correlation coefficients between studies parameters were demonstrated by linear regression analysis.

RESULTS AND DISCUSSION

Extractive yield: The dry powder of four *Terminalia* species leaf and stem was extracted by individual cold percolation method (Chanda et al., 2012; Rakholiya and Chanda, 2012b; Rakholiya et al., 2014c). The results are shown in Table 1. Among four *Terminalia* species, in leaf, maximum extractive yield was in aqueous extract of *T. bellerica* followed by aqueous extracts of *T. chebula* (Table 1) and in stem, maximum extractive yield was in acetone extract of *T. chebula* (Table 1). Among both, leaf and stem of all four *Terminalia* sp., maximum extractive yield was in leaf, except acetone extract of *T. chebula* stem (Table 1). There are many reports in the literature where extractive yield varied with different solvents (Yang et al., 2007; Rakholiya et al., 2011; Chanda et al., 2013c; Rakholiya et al., 2014b).

Total Phenolic and flavonoid content: Phytochemical analysis of plants is necessary as they can be evaluated further from their activities. Phenolic are the most wide spread secondary metabolites in the plant kingdom. Phenolic compounds are a class of antioxidant agents, which act as a free radical scavengers. It is believed that the phenolic and/or polyphenolic compounds biosynthesized in the plant sample might be responsible for antioxidant activity (Di Majo et al., 2008; Cheynier, 2012; Kaneria and Chanda, 2013a; Kaneria et al., 2014). Among all the different solvent extracts of four *Terminalia* species leaf, maximum total phenol content was in acetone extract of *T. arjuna* followed by the acetone extract of *T. chebula*, while in stem, maximum total phenol content was in ethyl acetate extract of *T. chebula* (Table 2). In case of flavonoid content, in leaf, maximum flavonoid content was in ethyl acetate extract of *T. bellirica* followed by acetone extract of *T. chebula*, while in stem, maximum flavonoid content was in acetone and ethyl acetate extracts of *T. chebula* (Table 2). The action of polyphenols is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching single and triplet oxygen, or decomposing peroxide (Itagaki et al., 2009). Many phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower

mortality rates of several human diseases (Rakholiya et al., 2014b; Tee et al., 2015). Polyphenolic compounds like flavonoid have been labeled as "high level" natural antioxidants based on their abilities to scavenge free radicals and active oxygen species (Chanda et al., 2010; Das et al., 2012; Goncalves et al., 2013; Arun et al., 2015).

Antioxidant activities

Antioxidant methods and modifications have been proposed to evaluate antioxidant characteristics and to explain how antioxidant functions. Since different antioxidant assays give widely diverging results, no single method can be used for evaluating antioxidant activity of extracts. Therefore, it is essential to characterize the extract by different antioxidant mechanism (Chanda and Dave, 2009; Chanda et al., 2013a).

Ferric reducing antioxidant power: The antioxidant activity is also determined on the basis of the ability of antioxidant in the plant extracts to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent (Hajimahmoodi et al., 2008; Alothman et al., 2009; Rakholiya et al., 2014b). Generally, FRAP assay is used due to its simplicity and reproducibility. The ferric reducing antioxidant power (FRAP) of leaf and stem of four *Terminalia* species in different solvent extracts, higher FRAP was in *T. chebula* (Table 2). In leaf, maximum FRAP was in acetone extract, while in stem, maximum FRAP was in acetone extract followed by ethyl acetate extract (Table 2). FRAP assay is based on the ability of phenolics to reduce yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue ferrous complex (Fe(II)-TPTZ) by the action of electron-donating antioxidants (Benzie et al., 1996; Kaneria and Chanda, 2013b). The resulting blue color measured spectrophotometrically at 593 nm is taken as linearly related to the total reducing capacity of electron-donating antioxidants. Therefore, reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995; Chanda and Kaneria, 2012).

DPPH free radical scavenging activity: In the present work, three different solvent extracts of four *Terminalia* species were evaluated for their DPPH free radical scavenging activity. Out of 24 extracts investigated, 2 extracts showed IC_{50} value more than 1000 µg ml^{-1} (Table 3), while the remaining 22 extracts showed varied levels of DPPH free radical scavenging activity (Table 3). IC_{50} values ranged from 12 to 480 µg ml^{-1} (Table 3). Ascorbic acid was used as a standard and its IC_{50} value was 11.4 µg ml^{-1} (Table 3). Among all extracts, the lowest IC_{50} value was of acetone extracts of *T. chebula* leaf and stem and highest IC_{50} value

was ethyl acetate extract of *T. catappa* leaf (Table 3). From these results, it can be stated that the extracts were good DPPH free radical scavengers.

Superoxide anion radical scavenging activity: Superoxide anion radical is a weak oxidant but it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Kaneria and Chanda, 2013a). Superoxide anion, which is a reduced form of molecular oxygen, has been implicated in the containing oxidation reactions associated with aging (Alrahmany and Tsopmo, 2012). In the present work, three different solvent extracts of four *Terminalia* species were evaluated for their superoxide anion radical scavenging activity. Out of 24 extracts investigated, 2 extracts showed IC_{50} value more than 1000 µg ml^{-1} (Table 3), while the remaining 22 extracts showed varied levels of superoxide anion radical scavenging activity (Table 3). IC_{50} values ranged from 35 to 800 µg ml^{-1} (Table 3). Gallic acid was used as a standard and its IC_{50} value was 185 µg ml^{-1} (Table 3). Among all extracts, the lowest IC_{50} value was of acetone extract of *T. chebula* stem and highest IC_{50} value was ethyl acetate extract of *T. arjuna* stem (Table 3). Superoxide anion radical is a weak oxidant but it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to the oxidative stress (Rakholiya et al., 2011). This principle is very effective, the present study also indicate that there were out of twenty-four extracts thirteen extracts showed better superoxide anion radical scavenging activity compare to standard gallic acid (Table 3). Antioxidant properties of flavonoid are effective mainly via the scavenging of superoxide anion radical. In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. Antioxidants are able to inhibit the blue NBT formation. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion radical in the reaction mixture (Rakholiya et al., 2011). The results of the present study revealed a direct correlation between phenolic content and antioxidant activity. This is in agreement with many reports in the literature (Babbar et al., 2014; Rakholiya et al., 2014b). The phenolic content of a plant can be a good indicator of its antioxidant capacity.

ABTS radical cation scavenging activity: In the present work, three different solvent extracts of four *Terminalia* species were evaluated for their ABTS radical cation scavenging activity. Out of 24 extracts investigated, 2 extracts showed IC_{50} value more than 1000 µg ml^{-1} (Table 3), while the remaining 22 extracts showed varied levels of ABTS radical cation scavenging

activity (Table 3). IC_{50} values ranged from 4 to 294 µg ml^{-1} (Table 3). Ascorbic acid was used as a standard and its IC_{50} value was 6.5 µg ml^{-1} (Table 3). Among all extracts, the lowest IC_{50} value was of acetone extracts of *T. chebula* leaf, stem and *T. bellirica* stem and highest IC_{50} value was aqueous extract of *T. catappa* leaf (Table 3).

Correlation study between total phenol content and flavonoid content with antioxidant activity: Phenolic compounds are the main class of natural antioxidants and there is a close relationship between the phenolic content and antioxidant activity of plant extracts (Orak et al., 2012). Several studies have shown that higher antioxidant activity associated with medicinal plants is attributed to their total phenolic compounds (Petridis et al., 2012; Rakholiya et al., 2014c). The results of correlation study between total phenol content and flavonoid content with antioxidant activity are presented in Table 4. A significant relationship existed between the antioxidant activity and total phenol content of *Terminalia* extracts, indicating that phenolic compounds in the extracts contributed significantly to their antioxidant capacity. According to the results, it has been stated that individual cold percolation extraction method is an efficient method for the determination of free radical scavenging activity, this method should be considered for extracting higher quality and quantity of antioxidants.

Table 1. Extractive yield (%) of leaf and stem of *Terminalia* species in different solvents with increasing polarity

Plant Name	Parts Used	% Yield (w w^{-1})			
		HE	EA	AC	AQ
Terminalia arjuna	Leaves	0.77	1.03	2.69	6.16
	Stem	0.16	0.34	0.80	1.97
Terminalia bellirica	Leaves	1.89	1.48	5.03	**9.52**
	Stem	0.43	0.52	1.42	3.39
Terminalia chebula	Leaves	0.74	1.88	4.38	**8.45**
	Stem	0.29	0.51	**9.18**	5.65
Terminalia catappa	Leaves	1.58	1.43	1.61	5.03
	Stem	0.36	0.33	0.71	4.71

HE: Hexane; EA: Ethyl acetate; AC: Acetone; AQ: Aqueous

Table 2. Total phenol content, Flavonoid content and Ferric reducing antioxidant power of different solvent extracts of leaf and stem of *Terminalia* species

Plant Name	Parts Used	Extracts	TPC (mg g^{-1})*	FC (mg g^{-1})*	FRAP (M g^{-1})*
Terminalia arjuna	Leaves	EA	127.66 ± 5.33	47.17 ± 0.74	19.95 ± 0.19
		AC	**253.10 ± 9.83**	43.65 ± 0.10	94.19 ± 0.81
		AQ	222.89 ± 10.00	17.07 ± 0.22	72.85 ± 0.89
	Stem	EA	114.96 ± 2.36	16.16 ± 1.82	14.80 ± 0.05
		AC	235.09 ± 3.44	8.60 ± 0.02	82.00 ± 0.73
		AQ	215.52 ± 3.26	1.22 ± 0.06	51.09 ± 1.64
Terminalia bellirica	Leaves	EA	163.93 ± 1.21	**66.55 ± 0.38**	26.56 ± 0.30
		AC	227.64 ± 2.38	28.94 ± 1.21	64.05 ± 0.99
		AQ	220.51 ± 1.80	23.61 ± 0.78	64.08 ± 0.61
	Stem	EA	178.26 ± 1.88	13.59 ± 1.51	30.85 ± 0.03
		AC	241.15 ± 1.42	25.60 ± 0.78	92.83 ± 1.48
		AQ	209.79 ± 0.65	0.46 ± 0.06	53.36 ± 0.44
Terminalia chebula	Leaves	EA	199.30 ± 2.34	44.47 ± 1.21	63.47 ± 2.32
		AC	**252.77 ± 1.13**	**57.94 ± 0.41**	**151.04 ± 19.40**
		AQ	212.32 ± 7.50	39.15 ± 0.39	94.11 ± 1.82
	Stem	EA	**269.97 ± 1.13**	**42.22 ± 0.12**	67.44 ± 2.45
		AC	237.79 ± 1.21	**43.11 ± 0.54**	**299.95 ± 9.30**
		AQ	238.12 ± 1.15	23.64 ± 0.30	**106.93 ± 1.88**
Terminalia catappa	Leaves	EA	26.78 ± 0.71	13.69 ± 0.09	0.24 ± 0.05
		AC	182.03 ± 0.51	42.71 ± 0.69	29.09 ± 1.51
		AQ	184.40 ± 2.42	12.18 ± 0.10	23.39 ± 0.69
	Stem	EA	28.66 ± 0.59	30.01 ± 0.39	1.01 ± 0.12
		AC	222.23 ± 4.12	5.08 ± 0.29	54.67 ± 1.42
		AQ	182.52 ± 1.85	0.00 ± 0.00	35.68 ± 0.49

*Values are expressed in mean ± standard error of the mean (n = 3); EA: Ethyl acetate; AC: Acetone; AQ: Aqueous; TPC: Total Phenol Content; FC: Flavonoid Content; FRAP: Ferric Reducing Antioxidant Power; Bold values indicating potent activity

Table 3. IC_{50} Values DPPH free radical, Superoxide anion radical and ABTS radical cation scavenging activity of different solvent extracts of leaf and stem of *Terminalia* species

Plant Name	Parts Used	IC_{50} Values (µg ml^{-1})								
		DPPH			SO			ABTS		
		EA	AC	AQ	EA	AC	AQ	EA	AC	AQ
Terminalia arjuna	Leaf	85	17	41	500	92	96	25	6	17
	Stem	109	21	290	800	108	110	39	7	109
Terminalia bellirica	Leaf	43	18	30	335	102	110	20	7	11
	Stem	48	16	>1000	320	92	136	17	**5**	>1000
Terminalia chebula	Leaf	29	**15**	27	225	**75**	125	11	**5**	9
	Stem	26	**12**	47	132	**35**	90	9	**4**	20
Terminalia catappa	Leaf	480	38	87	>1000	205	250	>1000	17	38
	Stem	134	20	>1000	>1000	195	230	36	**7**	294
Standard		Ascorbic acid		11.4	Gallic acid		185	Ascorbic acid		6.5

EA: Ethyl acetate; AC: Acetone; AQ: Aqueous; DPPH: DPPH (2,2- diphenyl-1-picryl hydrazyl) free radical scavenging activity; SO: Superoxide anion radical scavenging activity; ABTS: ABTS (2,2'-Azino-bis-(3-ethyl)benzothiazoline)-6-sulfonic acid diammonium salt) radical cation scavenging activity; Bold values indicating potent activity

Table 4. Correlation between total phenol content and flavonoid content with antioxidant activities of different solvent extracts of leaf and stem of *Terminalia* species

Plant Name	Correlation	Fitted equation	r^2	R*
Terminalia arjuna	TPC vs. DPPH	y = -0.3184x + 155.88	0.033	0.18
	TPC vs. SO	y = -4.8398x + 1227.5	0.899	0.95
	TPC vs. ABTS	y = -0.084x + 50.207	0.016	0.13
	TPC vs. FRAP	y = 0.5464x - 50.661	0.940	0.97
	FC vs. DPPH	y = -2.7178x + 154.48	0.248	0.50
	FC vs. SO	y = 2.9166x + 219.25	0.034	0.18
	FC vs. ABTS	y = -1.1147x + 58.706	0.292	0.54
	FC vs. FRAP	y = -0.0954x + 57.941	0.003	0.05
Terminalia bellirica	TPC vs. DPPH	y = -0.4041x + 114.36	0.878	0.94
	TPC vs. SO	y = -3.6702x + 941.79	0.936	0.97
	TPC vs. ABTS	y = -0.1895x + 51.1	0.975	0.99
	TPC vs. FRAP	y = 0.7878x - 107.68	0.926	0.96
	FC vs. DPPH	y = 0.1487x + 26.292	0.044	0.21
	FC vs. SO	y = 2.2886x + 121.95	0.201	0.45
	FC vs. ABTS	y = 0.153x + 7.1567	0.236	0.49
	FC vs. FRAP	y = -0.334x + 64.125	0.092	0.30
Terminalia chebula	TPC vs. DPPH	y = -0.0894x + 47.003	0.035	0.19
	TPC vs. SO	y = -1.3665x + 434.86	0.296	0.54
	TPC vs. ABTS	y = -0.0366x + 18.264	0.028	0.17
	TPC vs. FRAP	y = 0.5313x + 5.6133	0.024	0.15
	FC vs. DPPH	y = -0.9433x + 65.391	0.702	0.84
	FC vs. SO	y = -0.0382x + 115.26	0.0	0.0
	FC vs. ABTS	y = -0.4377x + 27.944	0.711	0.84
	FC vs. FRAP	y = 1.4401x + 70.358	0.032	0.18
Terminalia catappa	TPC vs. DPPH	y = -1.5296x + 348.84	0.576	0.76
	TPC vs. SO	y = -0.8018x + 374.59	0.403	0.63
	TPC vs. ABTS	y = 0.1604x + 52.746	0.010	0.10
	TPC vs. FRAP	y = 0.2255x - 7.056	0.868	0.93
	FC vs. DPPH	y = -2.5213x + 204.07	0.042	0.20
	FC vs. SO	y = -0.3661x + 225.49	0.080	0.28
	FC vs. ABTS	y = -3.7128x + 145.21	0.300	0.55
	FC vs. FRAP	y = -0.5025x + 32.694	0.149	0.39

*n = 6; TPC: total phenol content; FC: flavonoid content; DPPH: DPPH free radical scavenging activity; SO: Superoxide anion radical scavenging activity; ABTS: ABTS radical cation scavenging activity; FRAP: Ferric reducing antioxidant power; R: Correlation coefficient

CONCLUSION

We have studied the comparative analysis and antioxidant capacity using leaf and stem of four *Terminalia* sp. But all of them showed significant antioxidant activity and a positive correlation between total phenol content and antioxidant property. Amongst them of *Terminalia chebula* extracts showed a good antioxidant activity by all antioxidant assay and high inhibitory effect as compared to standard used, probably due to high phenol and flavonoid content. Results from this study also provide a better understanding for the selection of an appropriate solvent and extraction method especially for *Terminalia sp.* so that it's full potential can be utilized. It could also be useful for further investigation and development into valued added "nutraceuticals". Overall, the obtained extracts could be a potential source of natural antioxidants for producing "nutraceuticals" and pharmaceutical applications. Additional studies are currently underway to assess the *in vivo* biological activities and to identify more specific phytochemicals responsible for their antioxidant property.

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Conflict of Interest

We declare that, have no conflict of interest.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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