Research Article

Comparative Antioxidant Activity of Twenty Traditional Indian Medicinal Plants and its Correlation with Total Flavonoid and Phenolic Content

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Accepted on: 24-10-2014; **Finalized on:** 31-12-2014.

ABSTRACT

In nature, plants have capacity to synthesize various secondary metabolites like alkaloids, steroids, terpenoids, lignin, tannins, phenolic compounds and flavonoids for their defense purpose and benefited to humans. Naturally occurring phenolic compounds and flavonoids have high potential as antioxidant principle to restore, conserve and repair cellular damage. Cellular damage caused by reactive oxygen species (ROS) has been implicated in several diseases, and hence natural antioxidants have significant importance in human health. Investigation has been carried out on different parts of twenty Indian medicinal plants belonging to various families for their possible antioxidation properties. The methanolic extract of various plant parts were analyzed for total phenol, flavonoid content and *in vitro* antioxidant, radical scavenging activity. Total phenols ranged from 12.21 to 355.25 mg gallic acid equivalents per gm and flavonoids from 3.37 to 147.79 mg rutin equivalents per gm of dry plant extract. All the extracts showed different level of antioxidant activities as evaluated by different *in vitro* assays such as total antioxidant activity, ferric ion reducing power, nitric oxide radical scavenging and DPPH radical scavenging activity. Total flavonoid content correlated with antioxidant activity to lower extent as compared to phenolic content. In general, the samples with the highest total phenol values had the highest antioxidant activities. The results reveal that *Terminalia arjuna* Roxb., *Terminalia chebula* Retz., *Terminalia bellirica* Roxb., *Phyllanthus emblica* Linn. and *Curcuma longa* Linn. species showed remarkable antioxidant activities, consequently representing promising plant source of phytomedicine. The members of combretaceae occupy first rank as antioxidant agent among selected twenty Indian medicinal plants. Thus, these plants would be considered as promising sources of antioxidant phytochemicals.

Keywords: Indian medicinal plants; Antioxidant activity; Phenolic; Flavonoids; Correlation

INTRODUCTION

Free radicals and other reactive oxygen species are produced in the human body during various physiological and biochemical processes. Increase production of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as cardiovascular diseases, cancer, atherosclerosis, diabetes, aging, and other degenerative diseases in humans[1].

Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other metabolites, which are rich in antioxidant activity[2,3].

There is increasing interest in naturally occurring anioxidants for use in foods to replace synthetic antioxidants. Many of the therapeutic actions of phytochemicals are ascribed to their biologically active polyphenol components, such as phenolic acids and flavonoids, which have potent antioxidant activities[4]. Earlier literature data have shown that many of these antioxidant compounds possess anti-inflammatory, cardioprotective antitumor, anticarcinogenic, hepatoprotective, and antibacterial activities to a greater or lesser extent[5-8].

Consequently, the search and research for natural antioxidants present in food and other biological materials have received much more attention because of their presumed safety, nutritional and therapeutic value[9,10]. Over the past few years, the search for natural antioxidants for dietary, cosmetic and pharmaceutical purpose has become a major industrial and scientific research challenge.

Therefore, efforts to acquire extensive knowledge regarding the power of antioxidants from plants are increasing to tap their potential. In this regard large number of medicinal plants has been investigated worldwide for their antioxidant activities[11]. Fortunately, India has diverse flora comprising several traditionally used medicinal plants, which are an integral part of Indian Ayurveda[12]. These plants are the potential sources for various naturally occurring non-toxic antioxidants. Several reports are available on the health benefits and antioxidant potential of individual plant species. However, information regarding the comparative study on antioxidant activities of traditional Indian medicinal plant is not available. Additionally studies regarding quantitative correlation of antioxidant activities and total phenolic, flavonoid content of these plants are scanty. Therefore, this study was undertaken to compare the selected twenty traditional Indian medicinal plant species in terms of its potential antioxidant activity. Further quantitative correlations between the antioxidant

properties and the total phenolic/flavonoid content of methanolic extracts of these plants were studied expecting with high biological potential.

MATERIAL AND METHODS

Chemicals and Reagents

All chemicals used were of high grade and purchased from either Sigma chemicals or HiMedia Ltd. (Mumbai, India).

Collection of Plant Materials

Literature survey was carried out in order to select the 20 traditional Indian medicinal plants. Selection of the plants was based on the importance for nutrition and traditional use for the Indian Community. The plant species used were collected in the vicinity of Jalgaon city, Maharashtra, India during December – February 2012. They were identified from the expert taxonomist.

Preparation of Methanolic Extract

Extraction of plant secondary metabolite of selected plant parts was done by Soxhlet extraction method. Thirty gram of finely ground plant part powder was placed in porous bag made of muslin cloth, which was loaded into the main chamber of the Soxhlet extractor. The extraction was carried out with methanol as extraction solvent in 1:10 powder to solvent ratio at temperature 65 °C.

Determination of Yield of Plant Extract

The yield of dried extracts based on dry weight basis was calculated using the following equation:

$$Yield\ (g\ per\ 100\ g\ of\ dry\ plant\ material) = \frac{W1}{W2} \times 100$$

Where, W1 - weight of the extract after the solvent evaporation and W2 - weight of the dry plant material

Determination of Total Phenolic Content (TPC)

TPC in methanolic extract of selected medicinal plants was determined with the Folin-Ciocalteu phenol (FC) reagent based colorimetric assay[13]. The 0.1 mL of the methanolic extracts was mixed with 0.9 mL distilled water to which 0.1 mL of Folin Ciocalteu reagent (1:1 diluted) was added and incubated for 5 minutes in the dark at room temperature. To this mixture 1 mL of sodium carbonate (7% w/v) was added. The tubes were incubated in the dark for 90 min at 23 °C, after which the absorbance was read at 750 nm. Gallic acid (20-100 µg/mL) was used to construct the standard curve. TPC was expressed as mg of gallic acid equivalents (GAE) per g of dry extract.

Determination of Total Flavonoid Content (TFC)

TFC in methanolic extract of selected medicinal plants was estimated by a colorimetric assay[14]. The 1 mL aliquot of appropriately diluted sample or standard solutions of rutin (20, 40, 60, 80 and 100 µg/mL) was added to a 10 mL volumetric flask containing 4 mL distilled water. To this mixture 0.3 mL 5% $NaNO_2$ was added. After 5 minutes of incubation, 0.3 mL 10% $AlCl_3$ and at 6 minutes, 2 mL 1 M NaOH was added. Immediately, the reaction mixture was diluted to volume 10 mL with the addition of 2.4 mL of distilled water and thoroughly mixed. Pink color of the mixture was developed of which absorbance was determined at 510 nm against blank. TFC was expressed as mg of rutin equivalents (RE) per g of dry extract.

Evaluation of Antioxidant Property

Total Antioxidant Capacity (TAC)

The antioxidant activity of methanolic extract of selected medicinal plants was evaluated by phosphor-molybdenum method[15,16]. An aliquot of 0.3 mL of properly diluted sample or standard solutions of ascorbic acid (25, 50, 100, 200 and 400 µg/mL) was added to vial containing 3.0 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vial was capped and incubated in a water bath at 95 °C for 90 minutes. After the incubation, samples were cooled to room temperature, and the absorbance of the mixture was measured at 696 nm against blank. Total antioxidant capacity was expressed as mg of ascorbic acid equivalents per g of dry extract.

Ferric Ion Reducing Power (FIRP)

The reducing ability of methanolic extract of test samples was determined by using ferric ion reducing assay [17,18]. The 1 mL aliquot of properly diluted sample or standard solutions of ascorbic acid (3.125, 6.25, 12.5, 25 and 50 µg/mL) was added to 1 mL of 0.1 M sodium phosphate buffer (pH 6.6) and 1 mL of $K_3Fe(CN)_6$ (1%, w/v). Then reaction mixture was incubated at 50 °C for 20 minutes. After adding 1 mL of trichloroacetic acid (10%, w/v), the upper layer (1 mL) was mixed with 1 mL distilled water and 0.2 mL of fresh $FeCl_3$ (0.1%, w/v), and the absorbance was measured at 700 nm against prepared water blank. Ferric ion reducing power was expressed as mg of ascorbic acid equivalents per g of dry extract.

DPPH Radical Scavenging Activity

The DPPH radical scavenging capacities of methanolic extract of selected medicinal plants were performed according to a previous report[19]. Briefly, 200 µL of serially diluted standard (10-100 µg/ml) crude extracts (10-200 µg/mL) or methanol (control) were added to 2.8 mL of methanolic solution of 70 µM DPPH. The mixtures were shaken vigorously and placed in the dark at room temperature for 30 minutes, and then absorbance (Abs) was read at 517 nm using the Shimadzu UV-1800 spectrophotometer. Antioxidant activity was expressed as inhibition percentage (I%) and calculated using the following equation:

$$I\% = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100$$

Results were represented as IC50 ± standard deviation.

Nitric Oxide Radical Scavenging Activity (NORS)

The activity was measured according to the standard method[20]. To 0.5 mL of the extract having different concentrations (10-200 µg/mL), 1mL of sodium nitroprusside (SNP) solution (5mM) in 0.1 M saline phosphate buffer pH 7.4 was added and incubated for 30 minutes at room temperature. Followed by addition of 1.5 mL of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride) and incubated for 15 minutes at room temperature.

The absorbance of the chromophore was read immediately at 546 nm and compared with standard, Ascorbic acid. NORS activity was expressed as inhibition percentage (I%) and calculated using the following equation:

$$I\% = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100$$

Results were represented as IC50 ± standard deviation.

Data Analysis

Above experiments were performed in replicates of six. Results were expressed as the mean ± standard error of deviation (SD). The antioxidant test results were investigated with multivariate analysis. The correlation matrix was calculated, giving the correlation coefficients between each pair of variables, i.e. the analytical parameters tested. To identify new meaningful underlying variables and to reduce the dimensions of the data set, we performed a principal component analysis.

The results of the analysis are presented in terms of scree, loading and score plots. All calculations and graphic representations were done using the XLSTAT 5.0 (2014) software (Addinsoft, USA).

RESULTS AND DISCUSSION

Complete details of identified plants with botanical name, abbreviation used, family, local name, part used and traditional use mostly related to the major participation of antioxidant principles is summarized in Table 1. The selection of plant part which is mentioned in Table 1 is based on preliminary experimentation. Methanol is selected for the extraction, as flavonoids and phenolic compounds are more soluble in it.

In this study, three plants each of combretaceae and fabaceae, two plants of rutaceae and a single plant of other plant families are included for studying their various phytochemical properties of various dried plant part material. The extract yields vary from 3.30% to 59.31% (w/w) (Table 2). Among the selected medicinal plants, *T. chebula* had highest extract yields while *B. diffusa* had lowest extract yield.

Phenolic compounds are one of the most effective antioxidative constituent that contributes to the antioxidant activity of plant food[21].

Hence, it is important to quantify phenolic content and to assess its contribution to antioxidant activity. The total phenol and flavonoid contents and antioxidant activity of the different parts of the selected Indian medicinal plants studied are shown in Table 2. The mean values of phenols ranged from 12.21 to 355.25 mg of GAE per g and flavonoids from 3.37 to 147.79 mg of RE per g of dry extract. *T. arjuna* stem bark and the fruits from *T. chebula*, *T. bellirica*, *P. embillica*, *C. longa* rhizome and *M. arvensis* leaves showed highest level of total phenols (355.25 ± 7.46, 333.08 ± 8.45, 278.50 ± 19.28, 210.50 ± 14.77, 181.50 ± 4.08 and 127.63 ± 11.14 respectively). The highest flavonoid content was found in *T. arjuna* stem bark followed by *C. longa* rhizome and *M. arvensis* leaves (147.79 ± 11.68, 124.83 ± 3.40 and 98.92 ± 3.89). The rest of the extracts showed phenol and flavonoid values below 100 mg. The lowest phenolic content was observed in seeds of *T. foenum- graecum* (12.21 ± 0.29) and flavonoids with roots of *A. racemosus* (3.37 ± 0.19).

Table 2 also explains the percentage contribution of flavonoids to total phenols. It is evident from the same table that, high phenol content was not always accompanied with high flavonoid concentrations in the given specific plant such as *T. chebula*, *T. bellirica* and *P. embilica* etc. TAC and ferric ion reducing capacity was expressed in mg of AAE per g of dry plant extract. The nitric oxide and DPPH radical scavenging assay was expressed as IC50 value in µg/mL.

It is evident from Table 2, that all the extracts tested had TAC to different extent (from 10.0 to 213.3 mg AAE per g of dry extract). *T. arjuna* stem bark and the fruits from *T. chebula*, *T. bellirica*, *P. embillica*, *C. longa* rhizome and *M. arvensis* leaves possessed the noticeable TAC values. This statement holds true for total phenols.

The reducing capacity of a sample is regarded as a significant indicator of its potential antioxidant activity[21]. In the ferric-reducing power assay, the production of a blue color is directly proportional to the antioxidant activity of the extracts[22]. Ferric ion reducing results highlighted similar trends to TAC data for the different plants studied (Table 2). Ferric ion reducing values ranged from 3.3 to 308.91 mg AAE per g of dry methanolic plant extract. The fruits of *T. chebula*, *T. arjuna* stem bark, fruits from *P. embillica* and *T. bellirica* showing the highest values of ferric ion reducing potential, where as roots of *W. somnifera* shows lowest among selected plants. The ferric reducing power and total antioxidant capacity values increased concomitantly with polyphenol content of the plant extracts analysed. Our results are good in agreement with previous works[23, 24].

In the DPPH assay, the antioxidants are able to reduce the stable DPPH radical (purple) to the non-radical form DPPH-H (yellow).

Table 1: List of selected 20 Indian traditional medicinal plants

Sr. No	Btanical name	Abb	Family	Local name	Part used	Traditional use
1	*Abrus precatorius* Linn	*Ap*	Fabaceae	Gunj	Lf	Hepatic disorder
2	*Aegle marmelos* Linn.	*Am*	Rutaceae	Bael	Fr	Hepatic disorder
3	*Andrographis paniculata* Nees.	*Ap*	Acanthaceae	Kadu kirayat	Lf	Immunomodulation
4	*Asparagus racemosus* Willd.	*Ar*	Asparagaceae	Shatawari	Rt	Hepatic disorder
5	*Azadirachta indica* A. Juss	*Ai*	Meliaceae	Kadunimb	Lf	Diabetes
6	*Berberis aristata* DC	*Ba*	Berberidaceae	Daru halad	Rt	Hepatic disorder
7	*Boerhaavia diffusa* Linn	*Bd*	Nyctaginaceae	Punarnava	Rt	Anti-aging
8	*Curcuma longa* Linn.	*Cl*	Zingiberaceae	Halad	Rt	Diabetes
9	*Glycyrrhiza glabra* Linn.	*Gg*	Fabaceae	Jeshthmadh	Rt	Immunomodulation
10	*Mentha Arvensis* Linn.	*Ma*	Lamiaceae	Pudina	Lf	Hepatic disorder
11	*Murraya koenigii* Linn.	*Mk*	Rutaceae	Kadhi patta	Lf	Diabetes
12	*Ocimum sanctum* Linn.	*Os*	Lamiaceae	Kali tulas	Lf	Hepatic disorder
13	*Phyllanthus emblica* Linn.	*Pe*	Euphorbiaceae	Awala	Fr	Diabetes
14	*Syzygium cumini* Linn.	*Sc*	Myrtaceae	Jambul	Sd	Diabetes
15	*Terminalia arjuna* Roxb.	*Ta*	Combretaceae	Arjun satada	Br	Cardiac disorder
16	*Terminalia bellirica* Roxb.	*Tb*	Combretaceae	Behada	Sd	Diabetes
17	*Terminalia chebula* Retz.	*Tche*	Combretaceae	Hirda	Sd	Hepatic disorder
18	*Tinospora cordifolia* Miers	*Tcord*	Meninspermaceae	Gulvel	Br	Diabetes
19	*Trigonella foenum-graecum*	*Tf*	Fabaceae	Methi	Sd	Diabetes
20	*Withania Somnifera* Linn.	*Ws*	Solanaceae	Ashwagandha	Rt	Stress

Abb - Abbreviation used, Lf - Leaf, Fr - Fruit, Rt - Root, Sd - Seed, Br - Bark

Table 2 Total Phenol and flavonoid content and antioxidant properties of methanolic extract of selected medicinal plants

Sr. No.	Plant	Yield (%)	TPC[a]	TFC[b]	FIRP[c]	TAC[c]	DPPH[d]	NORS[d]
1	*Ap*	24.82	59 ± 5.51	36.0 ± 4.29	9.7 ± 1.71	20.5 ± 2.53	89.56 ± 12.73	111.10 ± 5.67
2	*Am*	47.67	78.29 ± 4.41	26.13 ± 2.10	13.5 ± 1.14	34.1 ± 4.01	73.01 ± 1.81	96.03 ± 3.18
3	*Ap*	24.60	58.71 ± 4.71	38.92 ± 2.94	27.6 ± 9.71	30.4 ± 2.35	95.97 ± 7.83	90.79 ± 7.56
4	*Ar*	34.45	35.67 ± 3.63	3.37 ± 0.19	47.3 ± 6.24	18.3 ± 1.81	98.00 ± 10.04	110.01 ± 5.24
5	*Ai*	28.22	69.21 ± 2.67	23.33 ± 2.59	20.1 ± 2.36	54.4 ± 4.14	86.63 ± 5.04	121.46 ± 15.58
6	*Ba*	4.18	40.0 ± 3.68	17.88 ± 1.33	30.6 ± 1.77	47.6 ± 4.66	67.07 ± 2.94	80.23 ± 6.46
7	*Bd*	3.30	36.63 ± 3.47	27.00 ± 1.23	24.7 ± 1.09	45.2 ± 3.64	92.18 ± 6.10	90.80 ± 4.56
8	*Cl*	17.10	181.50 ± 4.08	124.83 ± 3.40	75.7 ± 2.59	134.4 ± 7.88	59.63 ± 2.55	60.76 ±4.99
9	*Gg*	16.65	93.25 ± 6.72	25.79 ± 2.71	64.5 ± 5.86	64.8 ± 3.95	56.46 ± 1.07	137.86 ± 12.21
10	*Ma*	23.63	127.63 ± 11.14	98.92 ± 3.89	52.6 ± 3.54	60.2 ± 4.55	77.25 ± 2.45	86.73 ± 6.28
11	*Mk*	7.85	77.04 ± 4.60	32.54 ± 4.85	30.2 ± 1.30	56.6 ± 3.32	99.36 ± 3.41	122.69 ± 12.78
12	*Os*	21.67	15.29 ± 1.66	45.75 ± 1.75	82.1 ±19.56	14.1 ± 2.80	70.08 ± 5.88	85.64 ± 5.43
13	*Pe*	43.04	210.50 ± 14.77	32.08 ± 2.51	264.3 ± 21.75	111.0 ± 6.28	45.87 ± 1.73	47.55 ± 0.92
14	*Sc*	45.39	71.29 ± 1.55	12.65 ± 0.41	73.7 ± 3.65	53.0 ± 1.14	86.59 ± 6.06	113.5 ± 13.71
15	*Ta*	33.96	355.25 ± 7.46	147.79 ± 11.68	265.1 ± 21.12	213.3 ± 8.29	27.49 ± 2.09	35.64 ±1.62
16	*Tb*	52.75	278.50 ± 19.28	30.17 ± 1.63	206.0 ± 12.86	133.8 ± 5.59	36.09 ± 0.78	66.70 ± 5.76
17	*Tche*	59.31	333.08 ± 8.45	31.75 ± 2.84	308.9 ± 14.37	153.8 ± 13.86	28.64 ± 0.22	56.66 ± 4.24
18	*Tcord*	8.12	30.83 ± 1.59	37.42 ± 1.71	14.6 ± 2.10	28.9 ± 3.15	90.44 ± 3.48	108.41 ± 4.47
19	*Tf*	20.47	12.21 ± 0.29	7.33 ± 0.27	5.9 ± 1.55	10.0 ± 1.00	92.00 ± 2.22	99.56 ± 4.16
20	*Ws*	11.34	26.29 ± 0.99	7.42 ± 0.64	3.3 ± 0.26	30.9 ± 2.18	85.12 ± 4.61	117.29 ± 7.33

Data are means ± S.D. of six independent determinations. a- mg of gallic acid equivalents (GAE) per g of dry extract; b- mg of rutin equivalents (RE) per g of dry extract; c- mg of ascorbic acid equivalents (AAE) per g of dry extract; d- represented as IC50 value in µg/mL

The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating abilities. The free radical scavenging activities of methanolic extract of selected plants were evaluated through their ability to quench the synthetic DPPH radical and their activities were compared with that of the ascorbic acid, a standard compound. IC50 value varies from 27.49 - 99.36 µg/mL. *T. arjuna* and *T. chebula* found to have maximum and *M. koenigii* shows minimum DPPH radical scavenging activity.

Nitric oxide (NO) has been associated with a variety of physiological processes in the human body since it was identified as a novel signal molecule[25]. Besides its role in physiologic processes, it also participates in pathogenic pathways underlying a large group of disorders including muscle diseases, inflammatory bowel disease, sepsis and septic shock, primary headaches, HIV-associated dementia, multiple sclerosis and stroke. Additionally, increasing evidence demonstrates that NO modulates neurotoxin induced cell damage and is involved in neuronal cell death in Parkinson's disease (PD) and other neurodegenerative disorders such as Alzheimer disease[26-28]. So, it is very important to investigate the NO scavenging potential of the plant material. The stem bark from *T. arjuna* has potent nitric oxide scavenging activity (IC50 value 35.64 ± 1.62 µg/mL) and *G. glabra* stem has shown the least (IC50 value 137.86 ± 12.21 µg/mL) (Table 2). The scavenging of NO by the extracts was increased in dose dependent manner.

The different parameters were analyzed by a multivariate approach. In order to include in the data set, the IC_{50} values were transformed into their reciprocal (IC_{50} = $1/IC_{50}$).

The significant correlation between all the variables was confirmed by correlation matrix (Table 3).

Antioxidant activities are known to increase proportionally to the polyphenol content, mainly due to their redox properties[29]. Their ability to act as antioxidants depends on their chemical structure and ability to donate/accept electrons, thus delocalizing the unpaired electron within the aromatic structure[30]. The highly significant correlation of TPC for TAC (r = 0.970) and for FIRP (r = 0.911) focused that the reducing power of plant extract is due to phenolic/polyphenolic entities which is in accordance with previous work[19,24]. Similarly, the high correlation between phenols and DPPH activity (r = 0.943) exhibits that phenolic chemicals govern the antiradical potency. Total phenolic content also indicates good correlation with nitric oxide scavenging capacity (r = 0.781).

The significant correlation of TFC for TAC (r = 0.711) and for NORS (r = 0.634) was found, whereas there was a less significant correlation for DPPH (r = 0.451) and for FIRP (r = 0.346). This reveals that total flavonoid content (TFC) is less/weakly correlated with the antioxidant activity as compared to total phenolic content.

Table 3: Correlation Matrix (Pearson (n))

Variables	TPC	TFC	FIRP	TAC	NORS	DPPH
TPC	1	0.556	0.911	0.970	0.781	0.943
TFC	0.556	1	0.346*	0.711	0.634	0.451
FIRP	0.911	0.346*	1	0.885	0.780	0.923
TAC	0.970	0.711	0.885	1	0.846	0.911
NORS	0.781	0.634	0.780	0.846	1	0.762
DPPH	0.943	0.451	0.923	0.911	0.762	1

* except these, all values are different from 0 with a significance level α=0.05

The scree plot (Figure 1) indicates that the first two principal components account for 94% of the total variance. The other principal components most likely absorb numerical noise and/or experimental error. The loadings plot (Figure 2) indicates the direction of each original variable, and the scores plot (Figure 3) the position of each plant extracts in the new experimental space of the two independent coordinates.

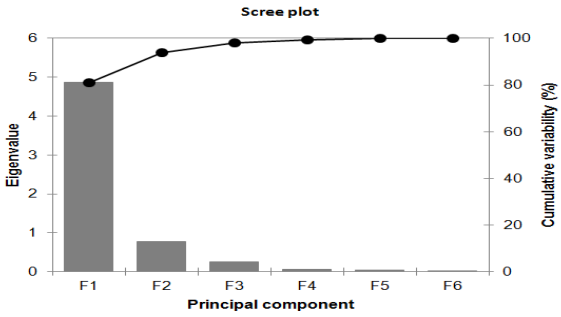

Figure 1: The scree plot of variance explained by each factor of principle component analysis

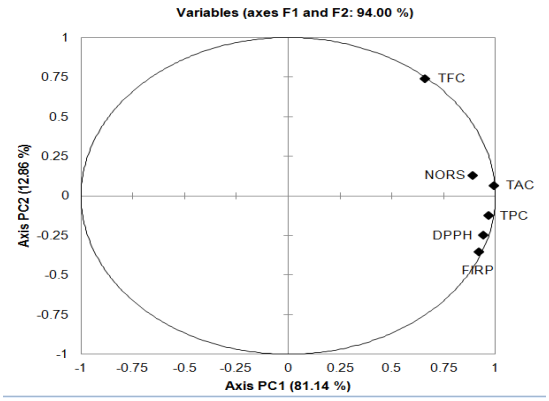

Figure 2: The loadings plot of antioxidant markers

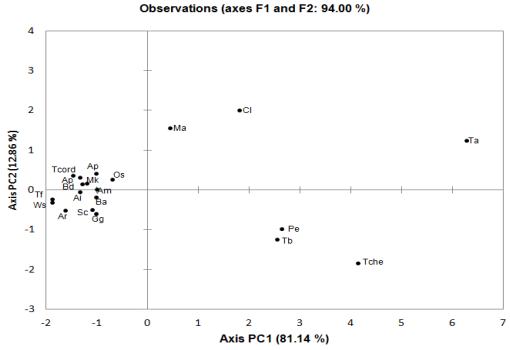

Figure 3: The scores plot of selected Indian medicinal plant extracts

As reported in the loadings plot (Figure 2), the PC1 direction coincides with that of the TPC. Therefore, it is reasonable to assume that PC1 includes most of the information (up to 81.14% of the total variance) due to the phenolic chemicals detected. The high loading values of FIRP, DPPH and TAC confirm, according to multivariate analysis, the major role of phenolic species in the antioxidant capacity of plant extracts. PC2 explains 12.86% of the total variance, with the major contribution of TFC. The score plot reported in Figure 3 shows the antioxidant behavior of plant extract in the space of the two new variables PC1 and PC2. Moving along PC1 from left to right in the graph, we find different patterns of grouping with low-antioxidant extract (Tf, Ws, Ar, Tcord, Ap, Ai, Bd and Mk) while extracts with intermediate antioxidant activity (Sc, Ap, Gg, Ba, Am and Os) separated and grouped in the first quadrant. *T. arjuna*, which had the highest antioxidant activity, is quite far from the others. *T. chebula*, *T. bellirica*, and *P. embillica*, lie in an intermediate position. *C. longa* and *M. arvensis* are sharply separated from the others along the PC2 axis, on account of their unusually high TFC values. Thus, total flavonoid content correlated significantly with antioxidant activity, but to a lower extent compared with total polyphenols as given in Table 3. The most probable reason to this is that phenolic species other than flavonoids, such as coumarins, tannins and/or phenolic acids, could also contribute to the overall antioxidant potential.

CONCLUSION

In this work, the phenol and flavonoid contents of twenty Indian traditional medicinal plants and their related antioxidant activities are evaluated for the first time of this region. *T. arjuna*, *T. chebula*, *T. bellirica*, *P. embilica* and *C. longa* species exhibited noticeable antioxidant activities, thus representing promising sources of plant based medicine. The strong antioxidant properties of these plants highly correlate with presence of phenolic and flavonoid compounds in appreciable amount, supports their uses in traditional medicine particularly stress related disorders. This provides directives for further experimental study in animal model *in vivo*. A detailed analysis of their chemical composition, with regards not only to polyphenols, but also other phytochemicals, as well as their *in vitro* toxicity potential in cell systems merit further investigation at cellular and molecular level.

Acknowledgement: GMC is thankful to UGC, New Delhi, India for providing JRF on major research project. RTM and GMC are thankful to Management and Principal of Moolji Jaitha College, Jalgaon for encouragement and providing necessary facilities for research work. Both authors are grateful to Mr. Sandeep N. Patil and Dr. Bhushan L. Chaudhari, School of life sciences, North Maharashtra University, Jalgaon for giving critical comments and suggestion.

REFERENCES

1. Vaz JA, Barros L, Martins A, Morais JS, Vasconcelos MH, and Ferreira IC, Phenolic profile of seventeen Portuguese wild mushrooms, LWT-Food Science and Technology, 44(1), 2011, 343-346.
2. Masoko P and Elof JN, Screening of Twenty-Four South African Combretum and Six Terminalia Species (Combretaceae) for Antioxidant Activities, African Journal of Traditional, Complementary and Alternative Medicine, 4(2), 2007, 231–239.
3. Bayani U, Singh AV, Zamboni P, and Mahajan RT, Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options, Current neuropharmacology, 7(1), 2009, 65-74.
4. Lizcano LJ, Viloria-Bernal M, Vicente F, Berrueta LA, Gallo B, Martínez-Cañamero M, and Ruiz-Sanz JI, Lipid oxidation inhibitory effects and phenolic composition of aqueous extracts from medicinal plants of colombian amazonia, International journal of molecular sciences, 13(5), 2012, 5454-5467.
5. Muanda FN, Bouayed J, Djilani A, Yao C, Soulimani R, and Dicko A, Chemical composition and, cellular evaluation of the antioxidant activity of *Desmodium adscendens* leaves, Evidence-Based Complementary and Alternative Medicine, Volume 2011, Article ID 620862, 9 pages.
6. Macone A, Fontana M, Barba M, Botta B, Nardini M, Ghirga F, and Matarese RM, Antioxidant properties of aminoethylcysteine ketimine decarboxylated dimer: a review, International journal of molecular sciences, 12(5), 2011, 3072-3084.
7. Halilu ME, Balogun M, Lall N, and Abubakar MS, Studies of In vitro Antioxidant and Cytotoxic Activities of Extracts and Isolated Compounds from *Parinari curatellifolia* (Chrysobalanaceae), Journal of Natural Sciences Research, 3(13), 2013, 149-154.
8. Mahajan RT, and Gajare SM, Manifestation of erectile dysfunction with adaptogenic antioxidant aphrodisiac plants, Int J Pharm Biomed Res, 3(1), 2012, 52-68.
9. Lee OH, and Lee BY, Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract, Bioresource technology, 101(10), 2010, 3751-3754.
10. Jaberian H, Piri K, and Nazari J, Phytochemical composition and *in vitro* antimicrobial and antioxidant activities of some medicinal plants, Food chemistry, 136(1), 2013, 237-244.
11. Subramanian R, Subbramaniyan P, and Raj V, Phytochemical Screening, Total Phenolic Contents and Antioxidant Activity of *Syzygium caryophyllatum* and *Syzygium densiflorum*, Journal of Biologically Active Products from Nature, 4(3), 2014, 224-235.
12. Nadkarni KM, Indian materia medica; Dr. KM Nadkarni's Indian materia medica: with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic and home remedies, appendices and indexes 1 Ed. 1996, Vol. 1, Popular Prakashan, 1996, 487-507.
13. Singleton VL, Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J Enol Vitic. 6, 1965, 144-158.

14. Zhishen J, Mengcheng T, Jianming W, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chemistry, 64, 1999, 555–559.
15. Prieto P, Manuel P, and Miguel A, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Analytical biochemistry 269(2), 1999, 337-341.
16. Albayrak S, Aksoy A, Sagdic O, and Hamzaoglu E, Compositions, antioxidant and antimicrobial activities of *Helichrysum* (Asteraceae) species collected from Turkey, Food Chemistry, 119(1), 2010, 114-122.
17. Oyaizu M, Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine, Jpn J Nutr., 44, 1986, 307–315.
18. Ferreira IC, Baptista P, Vilas-Boas, M, and Barros L, Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity, Food Chemistry, 100(4), 2007, 1511-1516.
19. Zhang R, Zeng Q, Deng Y, Zhang M, Wei Z, Zhang Y, Tang X, Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China, Food Chemistry, 136, 2013, 1169–1176.
20. Mirkov SM, Djordjevic AN, Andric NL, Andric SA, Kostic TS, Bogdanovic GM, and Kovacevic RZ, Nitric oxide-scavenging activity of polyhydroxylated fullerenol, $C_{60}(OH)_{24}$, Nitric Oxide, 11(2), 2004, 201-207.
21. Jungmin O, Heonjoo J, Cho AR, Kim SJ, Han J, Antioxidant and antimicrobial activities of various leafy herbal teas, Food Control, 31(2), 2013, 403-409.
22. Huang D, Ou B, Prior RL, The chemistry behind antioxidant capacity assays, J. Agric. Food Chem., 53, 2005, 1841–1856.
23. Jayasekera S, Molan AL, Garg M, and Moughan PJ, Variation in antioxidant potential and total polyphenol content of fresh and fully-fermented Sri Lankan tea, Food chemistry, 125(2), 2011, 536-541.
24. Wong JY, Matanjun P, Ooi YBH, and Chia KF, Evaluation of Antioxidant Activities in Relation to Total Phenolics and Flavonoids Content of Selected Malaysian Wild Edible Plants by Multivariate Analysis, International Journal of Food Properties, 17(8), 2014, 1763-1778.
25. Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Pourmorad F: Nitric oxide radical scavenging potential of some Elburz medicinal plants, Afr J Biotechnol, 9(32), 2006, 5212-5217.
26. Aliev G, Palacios HH, Lipsitt AE, Fischbach K, Lamb BT, Obrenovich ME, Morales L, Gasimov E, Bragin V, Nitric Oxide as an initiator of brain lesions during the development of Alzheimer disease, Neurotox Res, 16, 2009, 293-305.
27. Zhang L, Dawson VL, Dawson TM, Role of nitric oxide in Parkinson's disease, Pharmacol Ther, 109, 2006, 33-41.
28. Nath AK, Madri JA, The roles of nitric oxide in murine cardiovascular development, Dev Biol, 292, 2006, 25-33.
29. Rasineni GK, and Reddy AR, Free radical quenching activity and polyphenols in three species of Coleus, Journal of Medicinal Plants Research, 2(10), 2008, 285-291.
30. Lin KH, Yang YY, Yang CM, Huang MY, Lo HF, Liu KC, Lin HS, and Chao PY, Antioxidant activity of herbaceous plant extracts protect against hydrogen peroxide-induced DNA damage in human lymphocytes, BMC research Notes, 6(1), 2013, 490.

Source of Support: Nil, **Conflict of Interest:** None.