Int J Pharm Bio Sci 2017 Apr; 8(2): (P) 283-291

Original Research Article Pharmacognosy

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

RELATIVE TOTAL PHENOLICS CONTENT, 1,1-DIPHENYL PICRYLHYDRAZYL FREE RADICAL SCAVENGING AND TOTAL ANTIOXIDANT POTENTIALS OF SEVEN INDIAN MEDICINAL PLANT PARTS' AQUEOUS EXTRACTS

SOUREN GOSWAMI AND SANJIB RAY*

Molecular Biology and Genetics Unit, Department of Zoology, The University of Burdwan, Golapbag, Burdwan-713104, West Bengal, India.

ABSTRACT

Here, relative total phenolics, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, total antioxidant potentials and phytochemical profiles of seven ethnomedicinally important, mostly antitumor, plant parts' aqueous extracts (leaves of: *Cordia dichotoma*, *Holoptelea integrifolia, Crinum asiaticum*; aerial parts of: *Croton bonplandianum*, *Cayratia carnosa*, *Scadoxus multiflorus* and seeds of: *Trigonella foenum-graecum*) were analyzed. Data indicate the different extracts with varied amounts of phenolics and their differential capacity to neutralize the DPPH free radicals and total antioxidant activity. Out of the seven extracts, the maximum free radical scavenging, total antioxidant activity and the highest phenolics content was found in *C. asiaticum.* The preliminary phytochemical analysis indicates the prevalent secondary metabolites are alkaloids, terpenoids, tannins and saponins. In summary, considering the relative phenolics abundance and antioxidant activities, it can be concluded that out of seven anticancer plant products tested here *C. asiaticum* leaf aqueous extract possesses the highest efficient free radical scavengers and antioxidant potentials. Therefore, further study demands for *in vivo* antioxidant and cyto-genotoxic potentials of *C. asiaticum.*

KEYWORDS: *Antioxidant, Phenolics, DPPH, Crinum asiaticum, Trigonella foenum-graecum, Scadoxus multiflorus*

SANJIB RAY*

Molecular Biology and Genetics Unit, Department of Zoology, The University of Burdwan, Golapbag, Burdwan-713104, West Bengal, India.

Received on : 31-01-2017
Revised and Accepted on 15-04-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.p283-291

INTRODUCTION

Cancer, rheumatism, AIDS, atherosclerosis, cataracts, hyperglycemia, other auto-immune and old age diseases are associated with oxidative stress.[1] There are diverse groups of antioxidants who scavenge the free radicals by reduction, like tocopherol (vitamin E) and ascorbic acid (vitamin C), polyphenols, thiols, glutathione (a tri-peptide), enzymes (catalase, peroxidase and superoxide dismutase) who prevent proteins, lipids and deoxyribonucleic acids from oxidative damages.[2,3,4] Antioxidants by scavenging reactive oxygen species (ROS) and inhibiting free radicals protect the cellular oxidisable components.[5] Antioxidants are extensively used in foodstuff industries due to their numerous health benefits and are considered as nutraceutical components. Recently the demand of natural antioxidants has increased as they cause lesser liver damage, can prevent carcinogenesis and other pathological conditions due to its protective biochemical functions.[6] The potential sources of natural antioxidants are fruits, seeds, vegetables, leaves, roots, barks, spices and herbs.[7] Plant's major aromatic secondary metabolites, such as phenolics, are accountable for the nutritional, colour and sensory qualities of foods.[8,9] Herbal foods are rich in antioxidant due to their richness in phenolic content and are responsible for their varied medicinal activities like antiviral, antiallergic, antiinflammatory, antimicrobial, anticarcinogenic. Moreover, by their free radical scavenging activities, they can reduce oxidative alteration of LDLs (low density lipoproteins) and lipid peroxidation[10] and thus perform crucial role in our health care.[11,12,13,14,15,16] Positive correlation exists between phenolic content and antioxidant activity like as found in clove, oregano, garden thyme, peppermint, sage, cinnamon and all spices.[17] Flavonoids, the most abundant phenolics are secondary metabolites, derived from malonate, phenylalanine and tyrosine and the well established bioactive flavonoids are flavones, flavanones, isoflavones, flavanonols, flavans, flavanols, flavonols, anthocyanidins and catechins.[18] These are abundant in fruits, vegetables, roots, stems, barks, flowers, tea, grains and wine.[19] The Primary antioxidant function of flavonoids due to having their hydroxyl groups that leads to antiallergic, antiatherosclerotic, antiinflammatory, anticancer, cardio protective[20] and detoxification activities.[21] The search for secondary metabolites having antioxidant potentials continues to be of a great significance in the search for remedies against free radical-mediated diseases.[22,23] Therefore, the keen interest on the antioxidant research is principally due to the fact that the majority of pathological conditions are coupled with the ROS and oxidative stress. In this context, here, we aimed to explore relative free radical (DPPH) scavenging and antioxidant potentials of seven ethnomedicinally important (mostly antitumor) plant products' (leaves of: *Cordia dichotoma*, *Holoptelea integrifolia, Crinum asiaticum*; aerial parts of: *Croton bonplandianum*, *Cayratia crcarnosa*, *Scadoxus multiflorus* and seeds of: *Trigonella foenum-graecum*) aqueous extracts and to correlate with the total phenol and flavonoid contents. *Cordia dichotoma* (family: Boraginaceae) leaves are traditionally used as antidiabetic, antihelmintic, astringent, diuretic, demulcent, expectorant, purgative, tonic, antiulcer and to treat cough.[24,25,26,27,28] In vitro growth inhibition in human cervical cancer cell line (HeLa) and apoptosis induction indicate its anticancer activity.[29] Ethnomedicinally, the leaves of *Holoptelea integrifolia* (family: Ulmaceae) are used for cancer treatment[30] and also used for treating odema, inflammation, jaundice, herpes infection, leprosy and other skin diseases, hair loss, intestinal disorders and piles.[31,32,33,34,35] *Crinum asiaticum* Linn. (family: Amaryllidaceae) is a tuberous herb used to treat fevers, lumbago, headaches, earaches, swellings, aches, sores, piles, haemorrhoids, chaps as a rheumatic remedy to relieve local pain,[36] inflamed joints, injury and fractures,[37] common cold and cough, vomiting, worm infestations, disuria, polyuria, bowel complains, throat disorder, colic, flatulence and even leprosy.[38,39,40] Moreover, cytotoxicity against human tumor cell lines and *in vivo* antitumor activity of it has been reported.[41,42] *Croton bonplandianum* (family: Euphorbiaceae) is one of the most common exotic weeds in wastelands with high medicinal value and is commonly used to control high blood pressure and for the treatment of cholera, liver and skin diseases including cut and wounds. It is also used as antiseptic and antidote.[43,44,45] It has been experimentally proved to be useful in cancer therapy.[46] The aerial parts of *Cayratia carnosa* (family: Vitaceae) are used as diuretic, anticancer, antiviral, antibacterial, antiprotozoal, hypoglycemic, astringent, splenopathy and leucorrhea.[47,48,49] Leaves, roots and seeds are used as blood purifiers and poultice to ulcers, wounds and boils.[48,50,51] *Scadoxus multiflorus* (family: Amaryllidaceae), the poisonous fireball lily, is used to treat any respiratory problems, such as asthma, bronchitis, pneumonia, sinusitis or tuberculosis and also to treat dropsy, scabies and poorly healing wounds. Antimicrobial properties are the reasons to be beneficial in the treatment of several pathogenic afflictions.[52,53] *Trigonella foenum-graecum* (family: Fabaceae) is found to be beneficial against metabolic diseases (diabetes, obesity, hypercholesterolemia, dyslipidemia), inflammation and cancer. It also has antiulcerogenic, antipyretic and immunomodulatory effects.[54] Researchers reported that it has potential to prevent colon cancer[55] and inhibit growth of breast, pancreatic and prostate cancer cell lines.[56] Though several aspects of medicinal properties of the selected seven antitumor plant products are explored but information is limited regarding their relative abundance of phenolics, DPPH free radical scavenging and antioxidant potentials. Therefore, in the present study relative abundance of phenolics and their *in vitro* antioxidant potentials are analysed.

MATERIALS AND METHODS

Chemicals

Tannic acid powder was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Quercetin was purchased from Sigma-Aldrich, St Louis, MO, USA. Ammonium molybdate and sulphuric acid were obtained from Qualigens. Aluminium chloride and sodium phosphate were obtained from Merck Specialities Pvt. Ltd., Mumbai, India. Folin-Ciocalteu and sodium citrate were obtained from BDH Chemicals Ltd., Poole Dorset,

UK. Benzene and ethyl acetate were obtained from SRL, Pvt. Ltd., Mumbai, India. All chemicals used in this study were of analytical grade.

Collection of plants and storage

Fresh leaves of *C. dichotoma*, *H. integrifolia, C. asiaticum*; aerial parts of *C. bonplandianum*, *C. carnosa* and *S. multiflorus* were collected from The University of Burdwan campus in September-October of 2013, washed in tap water, shade dried, grinded into small pieces and pulverized using an electric grinder (Philips Mixer Grinder HL1605, Kolkata, West Bengal, India). Only the seeds of *T. foenum-graecum* were collected from local grocery shop and used intact. The plant species were taxonomically identified by Dr. Ambarish Mukherjee (Taxonomist), Professor, Department of Botany, University of Burdwan. Grinded leaf powder was then stocked in an air tight glass container for future use.

Extract preparation

50 g plant material (dried powder or seeds) was extracted twice in 500 X 2 ml distilled water for 12 X 2 h at 60°C, in a water bath; extracts were filtered through Whatman filter paper #1 (GE Healthcare UK Limited, Buckinghamshire, UK). The filtrate was concentrated in a vacuum hot air oven at 50°C for around 6 h, the obtained final volume was recorded,[57] and the extracts were stored in -20°C for future use. To determine the extract value and the initial extract concentration, 25ml (5 ml X 5) of extract was evaporated to complete dryness in a hot (60°C) air oven. Leaves extracts of *C. dichotoma*, *H. integrifolia, C. Asiaticum;* aerial part's extracts of *C. bonplandianum*, *C. carnosa*, *S. multiflorus* and seeds extract of *T. foenum-graecum* were abbreviated respectively as *Cd*LAE, *Hi*LAE, *Ca*LAE, *Cb*AAE, *Cc*AAE, *Sm*AAE and *Tf*SAE.

DPPH free radical scavenging assay

The relative free radical scavenging capacities of the aqueous extracts of seven different plant products were determined by 1,1-diphenyl-2-picrylhydrazyl, the DPPH, free radical scavenging assay.[58,59,60] DPPH becomes colourless when it accepts hydrogen radical or an electron. Methanolic stock solution of DPPH (0.002%) was freshly prepared. Ascorbic acid was used as standard antioxidant. 1 ml ascorbic acid (5-50 µg/ml) and all the aqueous extracts (50-500 µg/ml) of different concentrations were taken in the respective test tubes. Then in each test tube 3 ml methanol followed by 0.5 ml 1 mM DPPH solution was added. The test tubes were then incubated in darkness for 35 min at room temperature (25 ± 2°C) and the optical density was measured at 517 nm using spectrophotometer (UV-1800 Series, Shimadzu, Japan). Percentage of free radical scavenging activity was calculated as described below-

Scavenging activity (%) = [($Absorbance_{Control}$ - $Absorbance_{Sample}$) / $Absorbance_{Control}$] X 100

Total antioxidant assay

Total antioxidant potentials of the different extract fractions were measured by their ability to reduce Mo (VI) to Mo (V) and the ultimate formation of green Mo complex at acidic pH, which gives the highest absorbance at 695 nm. 0.3 ml of sample (100 µg/ml) was taken in each test tube and then 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphates and 4 mM ammonium molybdate) was added. For 90 minutes the reaction mixtures were incubated at 95°C. The absorbance of the solutions was recorded at 695 nm at room temperature using spectrophotometer. Total antioxidant potentials of the extracts were presented in terms of ascorbic acid (standard reducing agent) equivalent in mg/g dry aquous extract.[61]

Phyto chemical detection

All the seven extracts were qualitatively tested to detect tannins, triterpenoids, flavonoids, phlobatannins, anthraquinones, alkaloids, saponins, steroids, glycosides and carbohydrates following the standard procedures[62,63,64,65] as described earlier in details.[66]

Estimation of total phenol and flavonoid contents

In all the extracts the total phenolic content, as tannic acid equivalent, was estimated following the procedure of Makkar et al[67], with slight modification. Firstly, stock extract solutions were prepared as 1 mg/ml. From this stock 10 µl (=10 µg) was taken in each test tubes and volume was adjusted to 1ml with distilled water. Then, 0.5 ml 1 N Folin-Ciocalteu reagent was added to each tube and immediately mixed thoroughly after adding 2.5 ml 20 % sodium carbonate solution. Next to it, they were kept in dark at room temperature (25 ± 2 ºC) for 40 minutes. At the end OD was recorded at 725 nm using UV-Vis spectrophotometer and phenolic contents were determined comparing with the standard curve. The standard curve of OD was drawn by freshly prepared tannic acid solutions of different concentrations, ranging from 5-50 µg/ml. The total flavonoids content of all the seven extracts were estimated following the aluminium chloride colorimetric method described by Chang et al[68] with slight modification.[69,70] In a test tube 1 ml extract (1 mg/ml) was added to 2 ml of distilled water. 3 ml of 5 % (w/v) sodium nitrite and 0.3 ml of 10 % (w/v) aluminium chloride were mixed to the diluted sample. Just after 6 min 2 ml 1 M sodium hydroxide was added and the final volume was adjusted to 10 ml by adding required amount of distilled water. The OD of the reaction mixtures were measured at 510 nm using the spectrophotometer. The total flavonoid content of the extracts were calculated from the standard OD curve, which was drawn by freshly prepared standard quercetin solutions of different concentrations, ranging from 5-150 µg/ml.

The correlation analysis

The correlation of coefficient(r) and coefficient of determination (r^2) were determined for the different extracts' extract values i.e. extract yield percentages versus their total phenolics and total flavonoids contents; total phenolics contents versus DPPH free radical scavenging EC_{50} values, total antioxidants and total flavonoids; and total flavonoids content versus DPPH scavenging EC_{50} values and total antioxidants. Correlation between total antioxidants and DPPH free radical scavenging EC_{50} values were also analysed using Microsoft Office Excel (2007) software.

RESULTS

DPPH free radical scavenging assay

Data indicate extracts of the different medicinal plant parts shows the differential capacity to neutralize the DPPH free radicals and the variation in the EC_{50} values (Table 1). Here, the maximum free radical scavenging activity was shown by *Ca*LAE. It neutralizes half of the free radicals at a concentration of 196.92±11.97 µg/ml which is approximately 8 times more than ascorbic acid (fig. 1). DPPH free radical scavenging EC_{50} values for the other six extracts were determined respectively as 354.52, 355.19, 408.87, 499.85, 533.54 and 579.08 µg/ml for *Sm*AAE, *Tf*SAE, *Hi*LAE, *Cd*LAE, *Cc*AAE, *Cb*AAE (table 1).

Total antioxidant capacity

Alike DPPH scavenging action the used extracts have shown the differential ability to reduce Mo (VI) to Mo (V) and the ultimate formation of green Mo complex at acidic pH. The maximum total antioxidant activity, 213.78±4.96 mg ascorbic acid equivalent per gram dry leaf aqueous extract, was shown by *Ca*LAE and in the other extracts, *Sm*AAE, *Tf*SAE, *Hi*LAE, *Cd*LAE, *Cc*AAE, *Cb*AAE, the total antioxidant activities were recorded respectively in the decreasing order as 171.11±4.92, 159.89±5.63, 155.78±4.14, 121.11±7.2, 74.56±3.49 and 65.67±1.67 mg ascorbic acid equivalent in each gram of dried aqueous extract (table 1).

Table 1

DPPH free radical scavenging EC_{50} and total antioxidant activities of the seven different plant extracts

Extracts	DPPH scavenging EC_{50} (µg/ml) values	Total antioxidant activity (Ascorbic Acid equivalent mg/g dried aqueous extract)	
Ascorbic acid	25.04±1.4	Range	Average ± SEM
*Cb*AAE	579.1±12.8	64.00-69.00	65.67±1.67
*Cc*AAE	533.5±12.1	69.00-81.00	74.56±3.49
*Cd*LAE	499.9±24.1	107.33-131.67	121.11±7.2
*Hi*LAE	408.9±20.9	148.67-163.00	155.78±4.14
*Tf*SAE	355.2±30.7	151.00-170.33	159.89±5.63
*Sm*AAE	354.5±30.2	163.00-180.00	171.11±4.92
*Ca*LAE	196.9±11.9	206.67-223.33	213.78±4.96

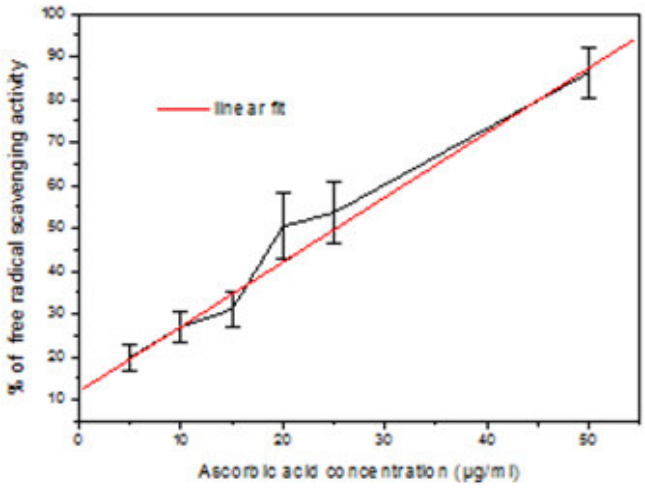

Figure 1

Standard graph, showing percentage of DPPH free radical scavenging activity of ascorbic acid

Phytochemical detection

The preliminary phytochemical analyses indicate the presence of relatively higher quantities of alkaloids, flavonoids, terpenoids, tannins, saponins, glycosides and carbohydrates in *Ca*LAE, the most effective extract and alkaloids, steroids, tannins, saponins were absent in *Sm*AAE, the second highest effective extract. Terpenoids were present in all the test extracts, where as anthraquinones present only in *Hi*LAE, the fourth effective extract. Test for phlobatannin in all the samples results negative. Glycosides were absent only in *Cb*AAE and carbohydrates were not detected in *Hi*LAE and *Tf*SAE (Table- 2).

Table 2
Phytochemical profiling of the seven plant parts' aqueous extracts

PHYTOCHEMICALS (Tests performed)		*Cb*AAE	*Cc*AAE	*Cd*LAE	*Hi*LAE	*Tf*SAE	*Sm*AAE	*Ca*LAE
Alkaloids	Mayer's test	+	+	+	+	+	–	+
	Wagner's test	–	–	–	–	+	–	+
Flavonoids (Alkaline reagent test)		–	+	+	+	+	+	+
Anthraquinones (Borntrager's test)		–	–	–	+	–	–	–
Terpenoids[74]		+	+	+	+	+	+	+
Steroids[74]		+	+	+	+	–	–	–
Tannins	$FeCl_3$ test	+	+	+	+	+	–	+
	Alkaline reagent test	+	+	+	–	+	–	–
Phlobatannins (HCl test)		–	–	–	–	–	–	–
Saponins (Froth test)		+	–	+	+	+	–	+
Glycosides (Alkaline reagent test)		–	+	+	+	+	+	+
Carbohydrates	Benedict's test	+	+	+	–	–	+	+
	Fehling's test	+	–	+	–	–	+	+

'+' indicate presence and '-' indicate absence of the respective phytochemicals

Phytochemical estimation

Total phenol and flavonoid contents

*Ca*LAE contains 294±6.9 mg tannic acid equivalent phenolics per gram of dried aqueous extract which is significantly more than that of the *Sm*AAE (235±6.9 mg). *Cb*AAE and *Cc*AAE contained least phenolics as 85.33±17.2 mg and 101±8 mg, while *Hi*LAE (176±13.9 mg), *Cd*LAE (192±8 mg) and *Tf*SAE (207.67±10.3 mg) contain moderate amounts of tannic acid equivalent per gram of dried aqueous extract (Table 3). Flavonoid content (Quercetin equivalence) was 53.54 % of total phenolics in *Tf*SAE, 50.68 % in *Ca*LAE and 46.34 % in *Cd*LAE. In *Sm*AAE and *Hi*LAE it was respectively 28.77 and 22.99 %, but in *Cb*AAE and *Cc*AAE it was below 15 % (Table 3).

Correlation analysis

Data indicate a linear negative correlation between DPPH free radical scavenging EC_{50} values and the total phenolic contents (fig. 2), a linear positive correlation exists between total antioxidant activities and total phenolic contents (fig. 3), a positive correlation between phenolics and flavonoids, and negative correlation between total antioxidant and DPPH free radical scavenging EC_{50} values (Table 4

Table 3
Showing pooled data of extract value, total phenolic and flavonoid contents in the extracts

Extracts	Extract Yield (%)	Total phenolic content* (mg/g of dae)	Total flavonoid content** (mg/g of dae)
*Cb*AAE	21.9	085.3±17.2	10.1±03.6
*Cc*AAE	17.8	101.0±08.0	14.2±02.9
*Cd*LAE	09.6	192.0±08.0	88.9±12.1
*Hi*LAE	22.7	176.0±13.9	40.5±04.6
*Tf*SAE	29.4	207.7±10.3	111.2±09.3
*Sm*AAE	43.6	235.0±06.9	067.6±02.9
*Ca*LAE	35.5	294.0±06.9	148.9±12.4

Tannic Acid equivalent, ** Quercetin equivalent, dae = dried aqueous extract.

Table 4
Showing results of correlation (r) and coefficient of correlation (r^2) analysis amongst different variable factors

	Correlation Between	The correlation of coefficient (r)	Coefficient of determination (r^2)	Regression equation
1	DPPH scavenging EC_{50} value (µg/ml) Vs Total phenolics (mg/g of dae) in different extracts	-0.937	0.878	y = -1.680x + 728.2
2	DPPH scavenging EC_{50} value (µg/ml) Vs Total flavonoids (mg/g of dae) in different extracts	-0.846	0.715	y = -2.155x + 566.5
3	Total antioxidant in mg/g of dae Vs Total phenolics (mg/g of dae) in different extracts	0.967	0.934	y = 0.708x + 6.828
4	Total antioxidant in mg/g of dae Vs Total flavonoids (mg/g of dae) in different extracts	0.838	0.702	y = 0.872x + 77.41
5	Extract Yield (%) Vs Total phenolics (mg/g of dae) in different extracts	0.603	0.363	y = 0.093x + 8.481
6	Extract Yield (%) Vs Total flavonoids (mg/g of dae) in different extracts	0.381	0.145	y = 0.084x + 19.98
7	Total flavonoids (mg/g of dae) in different extracts Vs Total phenolics (mg/g of dae) in different extracts	0.909	0.825	y = 0.639x - 49.11
8	Total antioxidant in mg/g of dae Vs DPPH scavenging EC_{50} value (µg/ml)	-0.969	0.939	y = -0.395x + 303.0

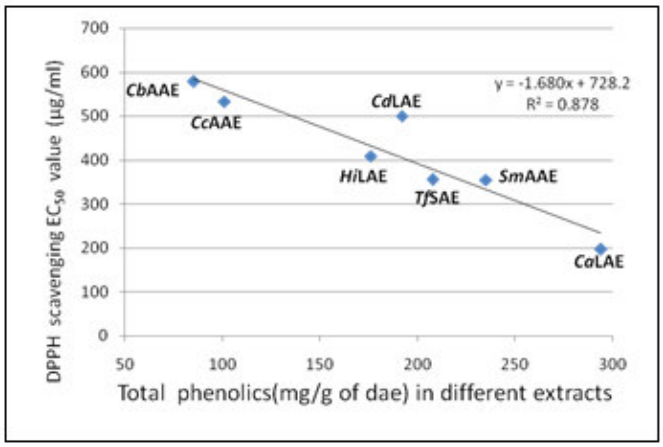

Figure 2
Showing a linear negative correlation between DPPH scavenging EC_{50} value (Y axis) and total phenolics contents (X axis) of the seven test extracts.

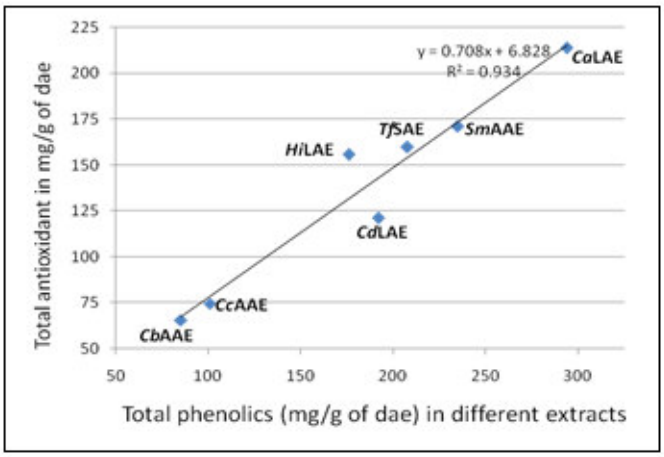

Figure 3
Showing a positive linear correlation between total antioxidant capacity (Y axis) and total phenolics contents (X axis) of the seven test extracts.

DISCUSSION

Screening of medicinal plants, isolation and characterization of antioxidants of natural sources has gained renewed interest to identify suitable antioxidant compounds to replace the synthetic one.[58] Ethnomedicinally important antitumor plant products (leaves of: *C. dichotoma*, *H. integrifolia, C. asiaticum*; aerial parts of: *C. bonplandianum*, *C. carnosa* and seeds of: *T. foenum-graecum*) are frequently used in herbal medicine.[29,30,41,42,46,48,55,56] Several acute diseases are associated to free radical induced cellular damage and oxidative stress. Here, antioxidant potentials in terms of total antioxidant and free radical (DPPH) scavenging activities of seven antitumor plant products' aqueous extracts were evaluated *in vitro* with relative total phenols and flavonoids content. The DPPH free radical scavenging activities of the test extracts were compared to standard curve, prepared from ascorbic acid solutions. Data indicate that different extracts were with differential capacity to scavenge the DPPH free radicals and thus varies in EC_{50} values (table. 1 and fig. 2). The efficiency in free radical scavenging activity, perhaps, related with the total phenolics and flavonoid contents, as they have high redox potentials and is considered as efficient reducing agents, singlet oxygen quenchers and hydrogen donors.[71] Free radical scavenging by reducing potentials and thus stabilizing cellular molecules by blocking the oxidation of organic molecules are an important attribute of phenolics. Due to significant antioxidant activity phenolics have effective role on disease prevention and body maintenance. Positive correlation found between the reduced occurrence of degenerative diseases like arthritis, cancer, heart disease, brain dysfunction, inflammation, cataracts etc., and the habit of polyphenolic compounds containing food consumption.[72,73] Here, out of the seven aqueous extracts tested, *Ca*LAE has shown the most potency in free radicals (DPPH) scavenging and also in total antioxidant activity (ascorbic acid equivalent) in comparison to the other extracts, who were found in decreasing order of potentials, as follows *Sm*AAE > *Tf*SAE > *Hi*LAE > *Cd*LAE > *Cc*AAE > *Cb*AAE. The most effectiveness of *Ca*LAE may be as a result of the presence of relatively higher amount of phenolics, which are the major secondary metabolites of plant, and are the primary antioxidants of their extracts.[74] Several elaborate studies have recognized previously that phytochemicals' antioxidant activities are mainly attributable to its phenolic constituents.[13,14,15] The correlation of coefficient (r) and coefficient of

determination (r^2) of all the extracts for their phenolic or flavonoid contents and antioxidant activities was determined (table 4). Our results indicate (a) linear negative correlation among total phenolic content and DPPH scavenging EC_{50} values (fig. 2), (b) total phenolic contents are correlated to total antioxidant activities in linear positive manner (fig. 3), (c) negative linear correlation among total antioxidant and DPPH scavenging EC_{50} value, (d) positive linear correlation between flavonoids and phenolics quantity, (e) poor correlation between total flavonoids and extract value, (f) poor correlation between extract value and total phenolics of the tested seven different extracts (Table 4). Here, the highest phenolics content of *Ca*LAE correlated to the maximum total antioxidant and DPPH scavenging activity. Moreover, *Ca*LAE shows positive correlation between total phenolics and total flavonoids. There are previous reports of positive correlation in between total phenolics and antioxidant activities of phytochemicals.[75] The differential free radical scavenging and antioxidant property of the extracts tested here and also both maximised in *Ca*LAE, perhaps, due to the presence of corresponding varied degree of flavonoids and/or phenolics. Correlation analysis further indicates polar solvent, water, could extract effective amounts of phytochemicals and the extract values of the different extracts are poorly correlated with their phenols and flavonoids contents. In summary, aqueous extracts of seven Indian antitumor plants were studied for antioxidant potentials and the *Ca*LAE was found to be the most efficient free radical scavenger and it may be due to the highest phenolics content. Now, further work is needed to isolate and characterise the free radical scavenger compounds and *in vivo* assessment for antioxidant and cyto-genotoxic potentials of *Ca*LAE.

FUNDING ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support of the UGC {F.No.42-563/2013 (SR) dt. 22.3.13}, UGC-DRS and infrastructural supports of the Department of Zoology (DST-FIST and UGC-DRS Sponsored Department), The University of Burdwan, West Bengal, India.

ABBREVIATIONS

***Ca*LAE** Leaf aqueous extract of *Crinum asiaticum*
***Cb*AAE** Aerial parts aqueous extract of *Croton bonplandianum*
***Cc*AAE** Aerial parts aqueous extract of *Cayratia carnosa*
***Cd*LAE** Leaf aqueous extract of *Cordia dichotoma*
DPPH 1,1-diphenyl-2-picrylhydrazyl
***Hi*LAE** Leaf aqueous extract of *Holoptelea integrifolia*
ROS Reactive Oxygen Species
***Sm*AAE** Aerial parts aqueous extract of *Scadoxus multiflorus*
***Tf*SAE** Seeds Aqueous Extract of *Trigonella foenum-graecum*

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Majewska M, Skrzycki M, Podsiad M, Czeczot H. Evaluation of antioxidant potential of flavonoids: an *in vitro* study. Acta Pol Pharm. 2011;68:611-5.
2. Sies H. Oxidative stress, oxidants and antioxidants. Exp Physiol. 1997;82:291-5.
3. Devasagayam TPA, Boloor KK, Ramsarma T. Methods for estimating lipid peroxidation: analysis of merits and demerits (minireview). Indian J Biochem Biophys. 2003;40:300-8.
4. Vertuani S, Angusti A, Manfredini S. The antioxidants and pro-antioxidants network, an overview. Curr Pharm Des. 2004;10:1677-94.
5. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer, how are they linked? Free Radic Biol. 2010;49:1603-16.
6. Noda Y, Anzai-Kmori A, Kohono M, Shimnei M, Packer L. Hydroxyl a superoxide anion radicalscavenging activities of natural source antioxidants using the computerized JES-FR30 ESR Spectrometer system. Int J Biochem Mol. 1997;42:35-44.
7. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem. 1998;46:4113-7.
8. Tomas-Barberan FA, Espın JC. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J Sci Food Agric. 2001;81:853-76.
9. Martinez-Tome M, Jimenez A, Ruggieri S, Frega N, Strabbioli R, Murcia M. Antioxidant properties of mediterranean spices compared with common food additives. J Food Prot 2001;64:1412–9.
10. Martin KR, Appel CL. Polyphenols as dietary supplements; a double edged sword. Nutr Dietary Suppl. 2010;2:1-12.
11. Mohammedi Z, Atik F. Impact of solvent extraction type on total polyphenols Content and biological activity from *Tamarix aphylla* L. Karst. Int J Pharmacogn Biol Sci. 2011;2:609-15.
12. Bravo L. Polyphenols, Chemistry, dietary sources, metabolism and nutritional significance. Nutr Rev. 1988;56:317-33.
13. Pietta PG. Flavonoids as antioxidants journals of natural products Review. J Nat Prod. 2000;63:1035-42.
14. Mandal SM, Chakraborty D, Dey S. Phenolic acids act as signaling molecules in plant–microbe symbioses. Plant Signaling Behav. 2010;5:359-68.
15. Medina E, Brenes M, Romero C, Garcia A, De Castro A. A Main antimicrobial compounds in table olives. J Agric Food Chem. 2007;55:9817-23.
16. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. BMC Complementary Altern Med. 2009;30:1-10.

17. Dimitrios B. Sources of natural phenolic antioxidants. Trends Food Sci Tech. 1996;7:505–12.
18. Servili M, Selvaggini R, Esposto S, Taticchi A, Montedoro G, Morozzi G. Health and sensory properties of virgin olive oil hydrophilic phenols, Agronomic and technological aspects of production that affect their occurrence in the oil. J Chromatogr A. 2004;1054:113-27.
19. Brahmachari G. Bio-flavonoids with promising antidiabetic potentials. A critical survey. In: Tiwari VK, Mishra BB, editors. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry. Res Signpost; 2011. p. 187-212.
20. Nijveldt RJ, Nood E, Hoorn DEC, Boelens PG, Norren KV, Paul AM. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr. 2001;74:418–25.
21. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease, the Zutphen Elderly Study. Lancet. 1993;342:1007-11.
22. Lin KH, Yang YY, Yang CM, Huang MY, Lo HF, Liu KC, et al. Antioxidant activity of herbaceous plant extracts protect against hydrogen peroxide-induced DNA damage in human lymphocytes. BMC Res Notes. 2013;6:490.
23. Farombi EO, Hansen M, Ravn-Haren G,Moller P, Dragsted LO. Commonly consumed and naturally occurring dietary substances affect biomarkers of oxidative stress and DNA damage in healthy rats. Food Chem Toxicol. 2004;42:1315–22.
24. Khond M, Bhosale JD, Arif T, Mandal TK, Padhi MM, Dabur R. Screening of some selected medicinal plants extracts for in-vitro antimicrobial activity. Middle-East J Sci Res. 2009;4(4):271-78.
25. Parekh J, Chanda S. *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. Afr J Microbiol Res. 2007;1(6):92-9.
26. Sebastian MK, Bhandari MM. Medico-ethno botany of Mount Abu, Rajasthan, India. J Ethnopharmacol. 1984;12:223-30.
27. Rapisarda A, Iauk L, Ragusa S. Micromorphological study on leaves of some Cordia (Boraginaceae) species used in traditional medicine. Econ Bot. 1997;51(4):385-91.
28. Anjaria J, Parabia M, Bhatt G, Khamar R. Nature heals a glossary of selected indigenous medicinal plants of India. SRITI Innovations. Ahmedabad, India; 1997. p. 23.
29. Rahman MA, Hussain A. Anticancer activity and apoptosis inducing effect of methanolic extract of *Cordia dichotoma* against human cancer cell line. Bangladesh J Pharmacol. 2015;10:27-34.
30. Graham JG, Quinn ML, Fabricant DS, Farnsworth NR. Plants used against cancer - an extension of the work of Jonathan Hartwell. J of Ethnopharmacol. 2000;73(3):347-77.
31. Yoganarasimhan SN. Medicinal plants of India, Vol. II. Cyber Media. Bangalore, India; 2000. p. 273.
32. Nadkarni KM. Indian Materia Medica. Popular Prakashan Pvt Ltd, Mumbai, India; 1976. p. 651-2.
33. Sivarajan VV. Ayurvedic Drug and their plant sources. IBH Publishing, New Delhi, India; 1996. p. 117.
34. Parinitham M, GU Harish, NC Vivek, T Mahesh, MB Shivanna. Ethnobotanical wealth of Bhadra wild life santuary in Karnataka. Indian J of Traditional Knowledge. 2004;3:37-504.
35. Reddy MB, Reddy K R, Reddy MN. A survey of plant crude drugs of Anantapur District, Andhra Pradesh, India. Int J Crude Drug Res. 1989;27(3):145-55.
36. Hutchings A, Scott AH, Lewis G, Cunningham AB. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg; 1996.
37. Wee YC. A Guide to Medicinal Plants. The Singapore Science Centre; 1992. p. 160.
38. Ghani A. Medicinal Plants of Bangladesh: Chemical Constituents and Uses. Asiatic Society of Bangladesh, Dhaka; 1998. p. 142-143; 334-7.
39. Zhanhe Ji, Alan WM. Amaryllidaceae. In: Flora of China. Science Press, Beijing and Missouri Botanical Garden Press; 2000. p. 264.
40. Walter CH. *Crinum asiaticum*. In: Flora of North America. Oxford University Press, Oxford; 1998. p. 278-9.
41. Sun Q, Shen YH, Tian JM, Tang J, Su J, Liu RH, et al. Chemical constituents of *Crinum asiaticum* L. var. sinicum baker and their cytotoxic activities. Chem and Biodiversity. 2009;6:1751-7.
42. Min BS, Gao JJ, Nakamura N, Kim YH, Hattori M. Cytotoxic Alkaloids and a Flavan from the Bulbs of *Crinum asiaticum* var. *japonicum*. Chem Pharm Bull. 2001;49(9):1217-9.
43. Nishanta R, Harris CS, Towers GHN. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharma Bio. 2002;40(3):235–44.
44. Chaudhuri AB. Endangered Medicinal Plants. Daya Publishing House. Delhi, India; 2007. p. 226.
45. Bhakat RK, Sen UK. Ethnomedicinal Plant Conservation through Sacred Groves. Tribes and Tribals. 2008;2:55-8.
46. Islam MS, Rahman MM, Rahman MA, Qayum MA, Alam MF. *In vitro* evaluation of *Croton bonplandianum* Baill. as potential antitumor properties using *Agrobacterium tumefaciens.* J of Agri Tech. 2010;6(1):79-86.
47. Gupta AK, Sharma M. Review on Indian Medical Plants, Vol. 5. ICMR. New Delhi, India, 2007. p. 879–82.
48. Gaur RD, Sharma J. Plants Used in Traditional Healthcare of live stock by Gujjar community of Sub Himalayan tracts, Uttrakhand, India. IJNPR. 2010;2:243–8.
49. Patil DA, Pawar S. Ethnobotany of Jalgaon District, Maharashtra. Daya Publishing House. New Delhi, India; 2006. p. 100,486,513,516,549.
50. Vardana R. Direct use of medical plant and their identification. Vol. 1. SARUP and Sons. New Delhi, India; 2008. p. 177.
51. Kiritikar KR, Basu BO. Indian Medicinal Plants. International Book Distributors, Dehradun, 1975. p. 611-2.
52. Daniel MM, Shem MM, Abdrizak AN. Indigenous knowledge of Taita community in the use and

conservation of medicinal plants: the case of Taita hills, Kenya. J Bio Innov. 2012;1(4):77-86.
53. Saad S, Taher M, Susanti D, Qaralleh H, Fadhlina A, Awang I. *In vitro* antimicrobial activity of mangrove plant *Sonneratia alba.* Asian Pacific J of Tropical Biomedicine. 2012;427-9.
54. Satheeshkumar N, Mukherjee PK, Bhadra S, Saha BP. Acetylcholinesterase enzyme inhibitory potential of standardized extract of Trigonella foenum graecum L and its constituents. Phytomedicine: Int J of phytotherapy and phytopharmacology. 2010;17:292-5.
55. Raju J, Patlolla JMR, Swamy MV, Rao CV. Diosgenin, a steroid saponin of *Trigonella foenum graceum* (Fenugreek), inhibits azoxymethane-induced aberrant crypt foci formation in f344 rats and induces apoptosis in HT-29 human colon cancer cells. Cancer Epidemiol Biomark Prev. 2004;13:1392-8.
56. Shabbeer S, Sobolewski M, Anchoori RK, Kachhap S, Hidalgo M. Fenugreek: a naturally occurring edible spices as an anticancer agent. Cancer Biol Ther. 2009;8:272-8.
57. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr J of Biomedical Res. 2007;10:175–81.
58. Brand-Willims W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft and Technologie. 1995;28:25-30.
59. Ayoola GA, Sofidiya T, Odukoya O, Coker HAB. Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. J Pharm Sci & Pharm Pract. 2008;8:133-6.
60. Dapkevicius A, Venskutonis R, Van Beek TA, Linssen JPH. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J Sci Food Agric. 1998;77:140-6.
61. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;44:307-15.
62. Garrat DC. The quantitative analysis of drugs. Chapman Hall, Japan, 1964. p. 456–8.
63. Harborne JB. Phytochemical methods. Chapman and Hall Ltd., London, 1973. p. 49-188.
64. Trease GE, Evans WC. Pharmacognosy Brailliar Tiridel can, 13th ed. Macmillian Publishers; 1989.
65. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd. Ibadan, Nigeria; 1993. p. 289.
66. Ray S, Chatterjee S, Chakrabarti CS. Antiproliferative activity of allelochemicals present in aqueous extract of *Synedrella nodiflora* (L.) Gaertn. in apical meristems and wistar rat bone marrow cells. Iosr J of Pharmacy. 2013;3(2):1-10.
67. Makkar HPS, Blummel M, Borowy NK, Becker K. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J Sci Food Agric. 1993;61:161-5.
68. Chang C, Ming-Hua Y, Chuan Chen WJ. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Journal of food and drug analysis. 2002;10:178-82.
69. Dutta S, Ray S. Evaluation of antioxidant potentials of leaf aqueous and methanolic extracts of *Calophyllum inophyllum* in relation to total phenol and flavonoid contents. Int J Pharm Bio Sci. 2014;5(3):441–50.
70. Dutta S, Ray S. Evaluation of *in vitro* free radical scavenging activity of leaf extract fractions of *Manilkara hexandra* (roxb) dubard in relation to total phenolic contents. Int J Pharm Pharm. Sci 2015;7(10):296-301.
71. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y. Effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2-picrylhydrazyl radical. Chem Pharm Bull. 1989;37:2016-21.
72. Beevi SS, Narasu M, Gowda BB. Polyphenolics profile, Antioxidant and radical scavenging activity of leaves and stem of *Raphanus sativus* L. Plant Foods Hum Nutr. 2010;65:8–17.
73. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al. Bioactive compounds in foods: their role in the prevention of cardioascular disease and cancer. Am J Med. 2002;113:71–88.
74. Kantamreddi VSSN, Lakshmi YN, Kasapu VVVS. Preliminary phytochemical analysis of some important indian plant species. Int J Pharma Bio Sci. 2010;1:351-7.
75. Khan RA. Evaluation of flavonoids and diverse antioxidant activities of *Sonchus arvensis*. Chem Cent J. 2012;6:126.

Reviewers of this article

Dr Ansuman Chattopadhyay M.Sc., Ph.D

Prof ,Dept of Zoology,Siksha Bhavana visva-Bharati,santiniketan,west bengal,India

G. Bakhya Shree M.S. (Research)

Coordinator and Trainer, Department of Biotechnology and Life Sciences, Dexter Academy, Madurai, Tamilnadu

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript