



Analysis of Active Pharmaceutical Ingredients and Antioxidant Potential of Ayurvedic Medicinal Plants

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Abstract: Traditional medicine is very popular medicine system from last decade and in future because of their beneficial effects against various diseases. It is mainly formulated from one or more than one plant parts. Various plant extracts were screened for total Polyphenolics, Flavonoids tartaric ester and antioxidant properties. Maximum content of poly phenolics are found in *Dendrophythoe falcate* and *Adhantoda zeylanica* 22.43 and 19.27 mg of gallic acid/gm of dry weight of medicinal plant materials. Maximum Quercetin content found in *Acorus calamus*, *Fiscus religiosa* 672.5 and 566.98 $\mu\text{g/gm}$ of sample. Higher Naringin contents found in *Acorus calamus*, *Fiscus religiosa* *Tridux procumbens*; 700.5, 590.9 and 408.76 $\mu\text{g/gm}$ of sample. Antioxidant activity by ferric reducing antioxidant potential is higher in *Syzygium cuminii* L, *Wthanic somanifere*, *Acmella oleracea*, *Lagenaria siceraria*, *Acorus calamus* L. 713.331, 119.33, 99.110, 80.661 and 75.20 mM/gm of sample. High radical scavenging and phosphomoydat antioxidant activity found in *Dendrophythoe falcate* and *Acorus calamus* plant extracts.

Keywords: Antioxidant, medicinal plants, polyphenolics, FRAP, Flavonoids

INTRODUCTION

Antioxidant are compound that inhibit or delay the oxidation of other molecules by inhibiting the initiation of propagation of oxidizing chain reaction involved in living as well as nonliving (Brewer, 2011). There are two basic categories of antioxidants namely endogenous and exogenous antioxidants. Exogenous antioxidants are having natural and synthetic occurrence (Agustyniak et al., 2010). In general, synthetic antioxidant are compound with phenolic structure of various degree of alkyl substitution where as natural antioxidants are plant secondary metabolite which can be polyphenolics compound (chalcone, tartaric easter, anthiocynin, tocopherol, Flavonoids and polyphenolic acids) nitrogen compound (alkaloids chlorophyll derivatives, amino acids and amines) as well as carotinoids (Liu et al., 2015; Rodil, Quintana and Cela, 2012). Natural antioxidant had tremendous biological applications like anticancer antidiabetic anti CVD anti HIV anti protozoal antimicrobial etc. as compare to the synthetic antioxidants (Andrade, Fasolo, 2014; Kaliora and George, 2007; Bansal et al., 2012). Previous reports suggest that traditional medicinal plants are the good source of natural antioxidants.

Generally plants contains secondary metabolite get vary from one region to other region observed in some species. Such change is responsible due to variation in of climate type of soil water stress and other environmental factors. These environmental factors are responsible for the change in primary and secondary metabolite biosynthesis and biotransformation (Martinez-Dominguez et al., 2008). Variation in natural product may responsible in change in formulation of ayurvedic medicine. The present study elaborates on the chemical composition of medicinal plant as well as on the total antioxidant activity of methanol extract.

Material and Method

Chemicals

Methanol (MeOH), Himedia, Quercetin, Caffeic acid, Catechin, Gallic acid, Ascorbic acid, Quercetin, Naringenin, Naringin, Rutin, Epigallocatechin, Curcumin, Hydrochloric acid (HCl), Ferrous sulphate(FeSO₄), Sulfuric acid(H₂SO₄), Sodium phosphate, Ammonium molybdate, Ammonium ferrous sulphate (NH₃(FeSO₄)), Trolox, Diphenyl picryl hydrazine (DPPH), Ferric chloride (FeCl₃), Folin ciocalteu reagent (FC reagent), TPTZ, pyrogallol acid (Sigma Germany). Methanol (HPLC gradient grade Merck Germany). Orthophosphoric Acid (OPA), RANKEM, Acetic acid (HPLC grade) was purchased from J. T. Baker (Netherlands). Milli-Q water was used in all experiments. Acetonitril, Sodium Citrate, Sodium Hydroxide, Boric Acid Ethanol, Perchloric Acid, Sodium Carbonate, Potassium Sulphate, Caprylic Acid, Mercapto Ethanol, Brij 35 solution, Sodium Hypochlorate, Tricarboxylic Acid, Aspartic Acid, Threonine, Serine, Glutamic Acid, Proline, Glycine, Alanine, Cysteine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenyl Alanine, Histidine, Lysine, Arginine, Glutamic acid were purchased from himedia.

Plant material

Various plant materials were obtained from western ghat region, India. Dried at 37°C and stored in plastic bag in the dark atmosphere till use. Plant identification was conducted at the herbarium in the department of botany university of Pune and national botanical survey of India, Pune.

Extraction of plant material

The different parts of the various plants taken for the study were: *Tridax procumbens* (Leaves), *Dendrophthoe falcate* (Leaves), *Withania somnifera* (whole plant), *Acacia catechu* (root), *Ricinus communis* (root), *Adhatoda zeylanica* (Leaves), *Acmella oleracea* (root), *Ficus religiosa* (root) and *Acorus calamus* (whole plant) 1 gm powder was mixed with 15 mL of methanol and kept slow stirring for 24 h. at 37 °C. Keeping the final volume were constants 15 mL, The supernatant was collected after filtration.

Phytochemicals Analysis

- **Analysis of Amino Acids**

The details of the methods are mentioned below as per the Shimadzu protocol (Shimadzu protocol for amino acid analysis; Ishida, Fujita and Asai, 1981).

Preparation of Mobile Phase

Chemical Composition of Mobile Phase are mentioned in Table 1.

Table 1 - Chemical Composition of Mobile Phase of Amino acid analysis.

Chemical	Liquid A	Liquid B
Sodium Normality (N)	0.2	0.6+α(about 0.1)
pH	3.2	10.0
Sodium citrate.2H ₂ O (g)	58.8	58.8
Sodium hydroxide (g)	-	-
Boric Acid (g)	-	12.4
Ethanol (99.5%) (ml)	210	-
Perchloric Acid (60%) (ml)	50	-
4N NaOH (ml)	-	30
Final Volume (liter)	3	1

Liquid A and B filtered through 0.45μ membrane filter.

Preparation of fluorescence reaction reagent

Preparation of Boric acid–Carbonic acid Buffer, pH 10 mentioned in table 2.

Table 2 Preparation of Boric acid–Carbonic acid Buffer, pH 10

Chemical	Quantity (g)	Molarity (M)
Sodium Carbonate	122.20	0.384
Boric acid	40.70	0.216
Potassium Sulphate	56.40	0.108

Above reagent were dissolved in water to make 3.0 liter solution. pH adjusted to 10.

Brij-35 (polyoxyethylene lauryl ether) Solution (10%)

Preparation of fluorescence reaction Solution A (Sodium hypochlorite (NaClO) solution)

0.2 ml commercially available sodium hypochlorite aqueous solution was added to 500 ml boric acid–carbonic acid buffer. The solution was mixed thoroughly and filtered through 0.45µ membrane filter.

Table - 3 Preparation of fluorescence reaction Solution B (Ortho phthalaldehyde (OPA) solution 0.08%)

Chemical	Quantity
OPA	400 mg
Ethanol	7.0 ml
2- Mercaptoethanol	1.0 ml
10% Brij – 35 solution	2.0 ml

Buffer additions were carried out to make it 500 ml solution. Solution was stirred till crystals dissolve completely and filtered through 0.45µ membrane filter.

Diluent preparation: 0.2N Na+ (sodium citrate), pH 2.20

9.8g sodium citrate was dissolved in 400 ml distilled water. To this made to addition of 8 ml perchloric acid and 0.05 ml n-caprylic acid; distilled water was used to make 500 ml solution. pH 2.20 of solution adjusted by adding perchloric acid.

Preparation of standarad

Different essential and non essential amino acid (Cysteine, Tyrosine, Glycine, Histidine, Arginine, Serine, Glutamic acid, Aspartic acid, Threonine, Methionine, Glutamic acid, Lysine, Alanine, Isoleucine, Leucine, Valine and Phenyl alanine) standard preparation was carried out in 4 ml ethanol. From that 20 µL of this filtered solution injected into the HPLC system.

Instrumentation

Name of Instrument: Shimadzu 20A instrument

Column: Na+ type strongly acidic cation exchange resin Shim pack sodium column (ISC-07/S1504 Na).

Detector: RF–10A XL SHIMADZU Fluorescence detector. Software: LC solution

Program set up for HPLC

Table 4 Gradient program for the HPLC for amino acid analysis

Time (min)	Conc. of mobile phase A (%)	Conc. of mobile phase B (%)	Flow rate
9.00	100	0	0.6
13.00	93	7	0.6
17.20	92	8	0.6
17.21	89	11	0.6
20.80	89	11	0.6

20.81	50	50	0.6
22.00	42	58	0.6
22.01	000	100	0.6
29.30	000	100	0.6
29.31	100	000	0.0
35.00	100	000	0.6
36.50	100	000	0.7
43.30	100	000	0.7
44.00	100	000	0.6
60.00	Stop	Stop	Stop

The reagents were passed simultaneously for fluorescence reaction through HPLC column at the flow rate of 0.4 ml/min throughout the process, the reaction solution contains 0.2 ml fluorescence reaction Solution A and 0.2ml fluorescence reaction Solution B.

Preparation of sample for amino acids analysis

Different part of plant (1 - *Tridax procumbens* 2- *Dendrophthoe falcate* 3 - *Withania somnifera* 4 - *Acacia catechu* 5- *Ricinus communis* 6 - *Adhatoda zeylanica* 7- *Acmella oleracea* 8 - *Ficus religiosa* 9- *Acorus calamus*) powder (1 gm) was mixed with 15 mL of Ethanol: Water (70:30) solvents reflux over a period of 2 hr. Then mixture was filtered through whatman filter paper Final volume was made to 15 mL in which 2.5 mL of 20 % TCA was added and kept overnight at 4 °C. The extract is centrifuged and filter through whatman paper. 20 µL filtrate was used for the analysis of essential and non essential amino acids.

- **Determination of polyphenolic content**

Total polyphenolic content was analyzed by the Folin–Ciocalteu method (Fukumoto et. al., 2000). The reaction mixture (3.6 mL) contains 20µL of sample, 3.2 ml of distilled water, 100µL of FC reagent; 300 µL of 60µg of saturated sodium carbonate was incubated at 37°C for 30 min in water bath the absorbance was read at 765 nm was measured in triplicate. Gallic acid (100µg/mL) was used for calibration of standard curve. The results were expressed as mg of gallic acid equivalent (mg GAE)/g of dry plant material.

- **Chromatographic analysis of polyphenolic and flavonoids**

A simple and quick reversed phase HPLC method used for determination of phenolic acids (Klejdus et al., 2005; Rostagno, Palma and Barroso, 2007). Chromatographic analysis was with the use of liquid chromatographic system, which consisted of P680 HPLC Pump, ASI-100 manual sample injector, thermostat column compartment C18, UVD170U detector. Chromatographic system was connected through the water universal chromatography interface to the computer. Software used for data acquisition and evaluation was Water. The separation was carried out on inertsil ODS 3V, 250*4.6*5 micron reversed phase column. Column temperature was maintained at 40 °C. Elution was performed by using gradient with the mobile phase consisting of mixture of buffer and acetonitrile (buffer 0.1M KH₂PO₄, PH 3.5 with OPA). Mobile phase A (buffer: ACN = 900:100), Mobile phase B (buffer: ACN = 450:550) and the flow rate was 1 ml/min, with following gradient program (Table 5).

Table 5 – Gradient program of solvent system for HPLC

Time in Min	Mobile Phase A	Mobile Phase B
00	95	05
08	95	05
20	50	50

35	44	56
45	44	56
50	10	90
75	10	90
80	95	05
90	95	05

The injection volume for all samples was 20 μ L. For detection, chromatograms were monitored at 275 nm. Identification of phenolic acids was based on retention times in comparison with standards. The quantification was carried out using the external standard method. Stock solution of standard compounds at concentration 1 mg/ml each was prepared in methanol. The solution of standards was injected into the HPLC system.

Antioxidant Activity

- **Total antioxidant potential**

Total antioxidant potential of crude extract was determined by the Phosphomolybdate method (Germano et al., 2002). Final volume of the reaction 3.010 ml containing 3.0ml of (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) 10 μ L of the respective extract incubated at 95 $^{\circ}$ C for 90 min. After the samples were cooled at room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank which contains 10 μ L of extracting solvent. Antioxidant potential of extracts were expressed as mM of Vit. C /g of dry plant material. The estimation was carried out in triplicate.

$$mg\ of\ Ascorbic\ acid = \frac{(O.D.\ of\ Standard - O.D.\ of\ Sample)}{(O.D.\ of\ Standard)} \times Conc.\ of\ Standard$$

- **Ferric-reducing antioxidant potential assay**

The FRAP assay was carried out according to the procedure of Benzie and Strain with modification (Benzie and Strain, 1996). FRAP reagent was prepared from sodium acetate buffer (300 mMol/L, pH 3.6), 10 mMol/L TPTZ solution in 40 mMol/L HCl and 20 mMol/L FeCl₃ in 40 mMol/L HCl solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was stored in cooled condition at 0-4 $^{\circ}$ C for five days. Before use the reagent was warmed to 37 $^{\circ}$ C in a water bath. 25 μ L of each extracts was added to 1.475 mL of sodium acetate buffer and 1.5 mL of FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 5 min. The standard curve was constructed using FeSO₄ solution (10–100 μ Mol/L). The results were expressed as μ Mol Fe (II)/g dry weight of sample.

$$\mu\ Mol\ of\ Fe(II) = \frac{(O.D.\ of\ Standard - O.D.\ of\ Sample)}{(O.D.\ of\ Standard)} \times Conc.\ of\ Standard$$

- **Free radical scavenging ability by DPPH radical**

The DPPH assay was carried out by 96 well plate method (Bozin, Mimica-Dukic and Samojlik, 2008). DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical. The free radical DPPH, which shows absorption at 517 nm, is reduced to the corresponding hydrazine when it reacts with hydrogen donors. Stock solution of DPPH 1 mM was prepared in methanol and working solution 0.1mM prepared in methanol. 10 μ L of 1:4 diluted plant extract was added in 100 μ L of methanol and 100 μ L of 0.1mMol of DPPH. The mixture was shaken and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 10 min. The results were expressed in % of inhibition by following formula.

$$\% \text{of inhibition} = \frac{(O.D. \text{ of control} - O.D. \text{ of Sample})}{(O.D. \text{ control})} \times 100$$

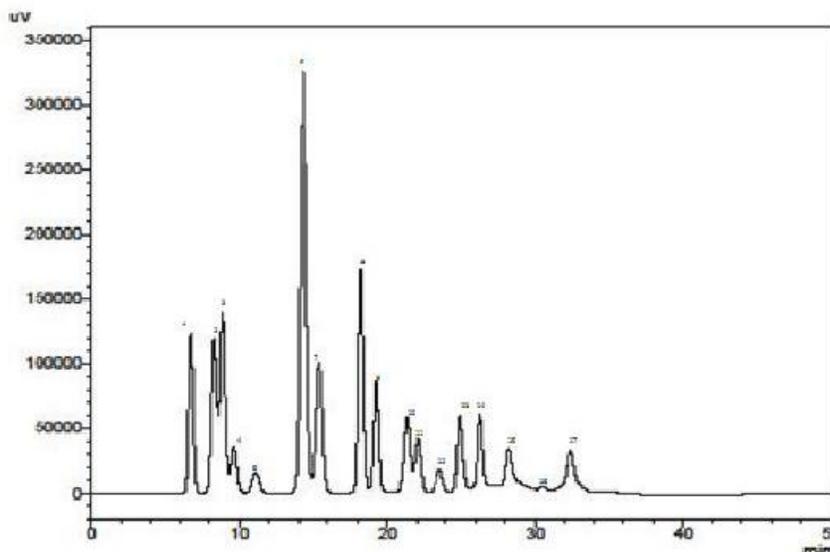
Results

Antioxidants are complex chemicals (polyphenol, flavonols, chalcone minerals and other) found in plants (Fukumoto et. al., 2000, Bozin, Mimica-Dukic and Samojlik, 2008). Around 9 different types of plant sources were quantitatively analysed for the phytochemical (polyphenolics and flavonols) and antioxidant activities (FRAP, phosphomolybdate and DPPH). The result obtained from medicinal and vegetable were obtained are mentioned below.

Phytochemicals analysis

• **Amino acids**

Different amino acids (Cysteine, Tyrosine, Glycine, Histidine, Arginine, Serine, Glutamic acid, Aspartic acid, Threonine, Methionine, Glutamic acid, Lysine, Alanine, Isoleucine, Leucine, Valine, Phenyl alanine) are determined by using amino acid analyzer. The quantities of 17 amino acids estimated are given in table 6. Essential amino acids are necessary for growth and maintains of the body. They cannot be synthesized by the human system. There are eight amino acids generally considered essential for adults while ten for children. Tyrosine and tryptophan are the precursors of neurotransmitters while Phenylalanine acts as analgesic and antidepressant (Beckmann et al., 1979; Meyers, 2000; Beckmann et al., 1979).



Spectra – 1: Standard amino acid spectra of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenyl alanine, histidine, lysine, arginine, glutamic acid.

Table 6 – Conc. of free amino acids of different traditional medicinal plants

	<i>Acacia catechu</i> µg/gm	<i>Dendrophthoe falcate</i> µg/gm	<i>Withania somnifera</i> µg/gm	<i>Ricinus communis</i> µg/gm	<i>Tridax procumbens</i> µg/gm	<i>Acmella oleracea</i> µg/gm	<i>Ficus religiosa</i> µg/gm	<i>Adhatoda zeylanica</i> µg/gm	<i>Acorus Calamus</i> µg/gm
Aspartic acid	0.069	0.000	0.879	0.287	0.381	0.244	0.067	ND	ND
Threonine	0.282	0.488	4.182	0.375	2.744	0.279	12.038	0.065	0.337
Serine	0.176	0.100	1.115	0.455	1.045	ND	ND	ND	ND

Glutamic acid	0.192	1.516	0.908	0.627	2.516	0.147	ND	ND	0.169
Proline	0.485	2.586	92.771	1.786	3.535	0.537	0.279	0.674	0.397
Glycine	0.007	0.071	0.348	0.168	0.090	0.012	0.008	0.007	0.011
Alanine	1.200	0.240	0.862	0.163	1.396	0.018	0.168	0.020	0.476
Cystine	0.621	2.455	5.244	1.895	2.440	0.206	0.141	0.156	0.060
Valine	1.855	0	6.169	3.154	2.557	0.226	0.333	0.271	0.132
Methionine	0.990	1.356	3.289	0.000	1.987	0.155	ND	0.066	ND
Iso- leucine	6.911	3.281	26.062	7.973	10.468	0.175	ND	ND	ND
Leucine	21.666	3.086	ND	ND	ND	1.260	2.773	0.647	16.267
Tyrosine	24.869	14.654	27.258	15.765	17.520	1.006	0.935	0.448	1.568
Phenyl alanine	53.898	102.740	133.171	56.264	121.895	ND	ND	ND	ND
Histidine	0.000	113.299	92.106	100.178	88.295	ND	ND	ND	ND
Lysine	ND	645.056	ND	ND	ND	23.109	28.638	32.250	17.848
Arginine	69.418	61.111	36.908	66.752	31.338	ND	ND	ND	8.109

• **Total Phenolic Compounds**

Results obtained from the analysis of polyphenolics are mentioned in Fig – 1. The amounts of phenolic compounds are varied from plant to plant. Among all of the extracts, the highest amount was found in the *Dendrophthoe falcate* and *Acorus calamus* (22.43 and 19.27 mg gallic acid / g of sample) plant extracts were as the lowest amount was measured in the *Withania somnifera* extract (6.36 mg gallic acid / g of sample).

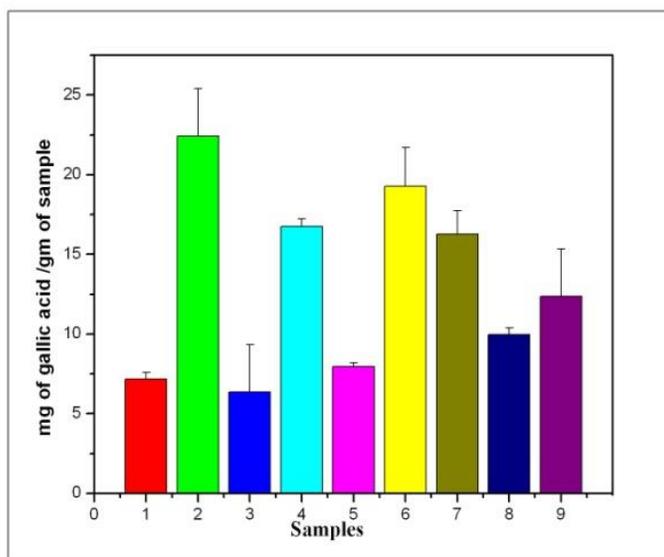
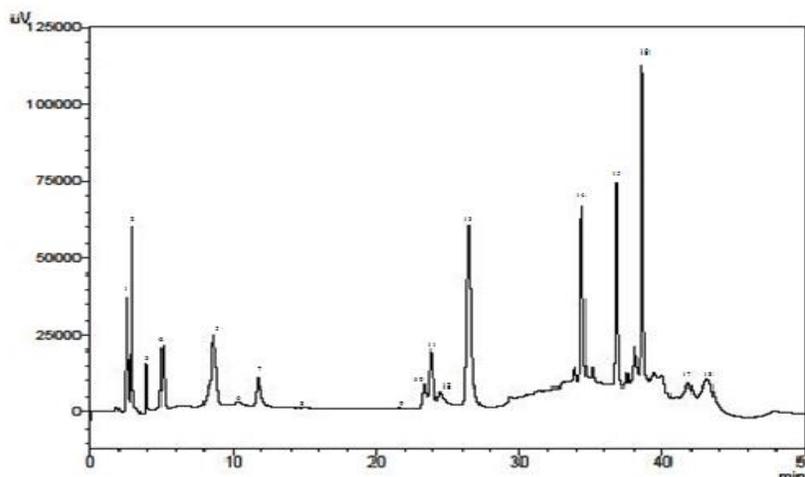


Fig. 1: Polyphenolic contents of medicinal plants extract.

1 - *Tridax procumbens* 2- *Dendrophthoe falcate* 3 -*Withania somnifera* 4 -*Acacia catechu* 5- *Ricinus communis* 6 -*Adhatoda zeylanica* 7 -*Acmella* 8 -*Ficus religiosa* 9- *Acorus calamus*. n (sample size) = 3.

• **Chromatographic analysis of polyphenolic and flavonoids**



Spectra – 2: Chlorogenic acid, imidazol, gallic acid, quinine, hydraquinone, catachin hydrate, vanillin, epigallacto catechin, rutin, naringenin, quercetin, naringin, couymarin, indole, 2-methyl napthaquinone, hydroxy diphenyl phenol, cucumin, quinoline, tannic acid standard hplc spectra.

Phytochemicals such as flavonoids (quercetin, naringenin, naringin and rutin) and polyphenolics (gallic acid, epigallactocatechin and curcumin) were determined by HPLC (Table 7). *Acorus calamus*, *Ficus religiosa* and *Acmella oleracea* contained higher level of flavonoids likes Quercetin, Rutein, Naringenin, and naringin shown in Table-7.

Table 7: analysis of Phytochemicals ($\mu\text{g/ gm dry wt.}$) of different traditional medicinal plants by HPLC.

Plants Compounds	<i>Tridax procumbens</i>	<i>Dendrophthoe falcate</i>	<i>Ficus religiosa</i>	<i>Adhatoda zeylanica</i>	<i>Acacia catechu</i>	<i>Acmella oleracea</i>	<i>Withania somnifera</i>	<i>Ricinus communis</i>	<i>Acorus Calamus</i>
Quinone	417.27	ND	232.94	547.9	ND	261.44	251.8	28.45	1527
Quercetin	392.30	143.04	566.98	280.96	ND	380.8	32.78	ND	672.52
Naringin	408.76	149.08	590.93	292.83	ND	396.88	34.16	ND	700.59
Naringenin	56.24	48.87	92.66	60.57	7.12	112.64	12.42	18.47	4.607
Rutein	198.06	240.12	413.8	573.08	ND	237.17	120.08	11.91	207.34
Chlorogenic acid	28.92	64.5	75.78	48.48	32.6	10.01	89.2	22.18	2278
Gallic acid	50.65	94.98	174.02	392.32	157.62	135.32	107.22	13.17	ND
Epigallacto Catechin	284.08	ND	66.98	233.12	ND	570.78	ND	36.35	36.04
Curcumin	246.14	22.5	545.84	111.78	348.23	197.17	310.8	254.4	50.88

Antioxidant Activity

- **Total antioxidant potential**

Medicinal plant methanol extracts (1–9) were evaluated for their phosphomolybdate antioxidant potential activity. Results are compared with the standard ascorbic acid. All plants showed phosphomolybdate antioxidant potential (Fig. 2).

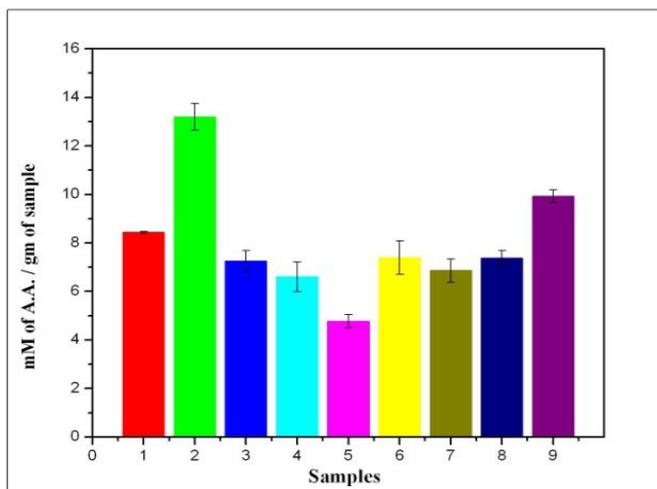


Fig – 2. Total antioxidant activity of medicinal plants extract.

1 - *Tridax procumbens* 2- *Dendrophthoe falcate* 3 -*Withania somnifera* 4 -*Acacia catechu* 5- *Ricinus communis* 6 -*Adhatoda zeylanica* 7 *Acmella oleracea* 8 -*Ficus religiosa* 9- *Acorus calamus*. Data are shown as mean±S.D. one way ANOVA $p < 0.001$ which represents the significance n (sample size) = 3.

The methanol extract of plant *Dendrophthoe falcate* and *Acorus calamus* (13.198 and 9.926 mM of ascorbic acid/ gm of sample) was found to exhibit the highest antioxidant activity, followed by *Tridax procumbens* and *Withania somnifera* activity as compare to *Acacia catechu*, *Ricinus communis*, *Acmella oleracea*, *Adhatoda zeylanica* and *Ficus religiosa*.

- **Ferric-reducing antioxidant potential**

The antioxidant potential of methanol extracts for plants part were estimated from their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II).The reducing ability of the medicinal plant extracts *Acmella oleracea*, *Adhatoda zeylanica*, *Ricinus communis*, *Acorus calamus*, *Ficus religiosa*, *Acorus calamus* and *Dendrophthoe falcate* was higher than that of the other extracts (Fig 3). The FRAP value of the various extract was shown in fig 3.

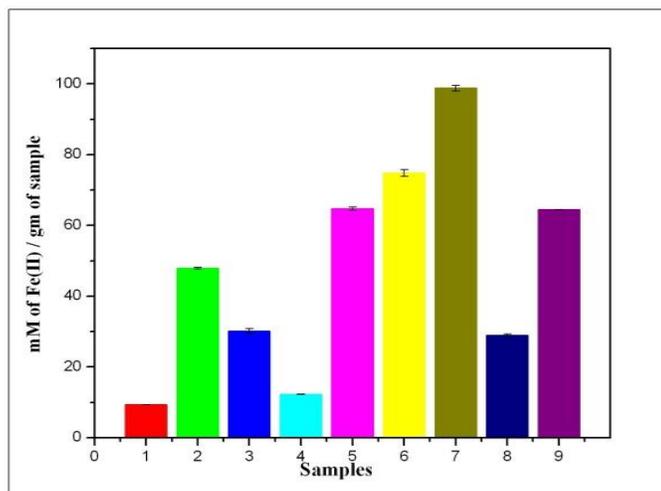


Fig – 3. Ferric reducing antioxidant potential of medicinal plants extract.

1 - *Tridax procumbens* 2- *Dendrophthoe falcate* 3 -*Withania somnifera* 4 -*Acacia catechu* 5- *Ricinus communis* 6 -*Adhatoda zeylanica* 7 -*Acmella oleracea* 8 -*Ficus religiosa* 9- *Acorus calamus*. Data are shown as mean±S.D. one way ANOVA $p < 0.001$ which represents the significance, n (sample size) = 3.

- **DPPH radical**

DPPH assay is considered a valid and easy assay to evaluate the radical scavenging activity of antioxidants. Medicinal plants *Dendrophthoe falcate*, *Ficus religiosa* and *Acorus calamus* showed maximum radical scavenging ability (Fig. 4).

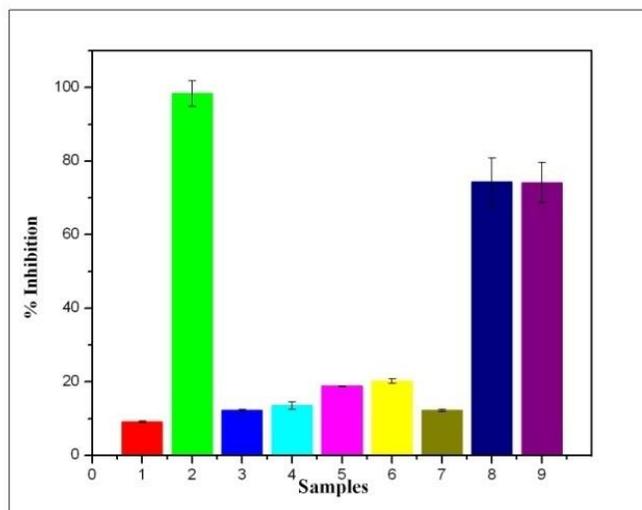


Fig – 4 - Radical scavenging antioxidant activity of medicinal plants extract.

1 - *Tridax procumbens* 2- *Dendrophthoe falcate* 3 -*Withania somnifera* 4 -*Acacia catechu* 5- *Ricinus communis* 6 -*Adhatoda zeylanica* 7- *Acmella oleracea* 8 -*Ficus religiosa* 9- *Acorus calamus*. Data are shown as mean±S.D. one way ANOVA $p < 0.001$ which represents the significance, n (sample size) = 3.

Discussion

The presence of different antioxidant components in the plant tissues makes it relatively hard to quantify each antioxidant component separately. The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metal chelating potential.

The unclear relationship between the antioxidant activity and the total phenolics may be explained in numerous ways, in fact, the total phenolics content incorporate all the antioxidants (Fukumoto et. al., 2000; Bozin, Mimica-Dukic and Samojlik, 2008; Adedapo et al., 2008). In addition, the synergism between the antioxidants in the mixture makes the antioxidant activity not only dependant on the concentration, but also on the structure and the interaction between the antioxidants. This is the reason why samples such as *Tridax procumbens*, *Withania somnifera* and *Ricinus communis* with similar concentrations of total phenolics, may vary in their antioxidant activities. The results suggest that the phenolic compounds contribute significantly to the antioxidant capacity of the medicinal plants.

Antioxidant activity of phenolics mainly depends on the number and the position of hydrogen-donating hydroxyl groups on the aromatic cycles of the phenolic molecules (Fukumoto et. al., 2000). It has been shown that the presence of the CH=CH, COOH, OH and phenyl group in the polyphenolics ensures greater H-

donating ability and subsequent radical stabilization (Rice-Evans, Miller and Paganga, 1996; Heim, Tagliaferro and Bobilya, 2002).

Conclusion

The purpose of this study was the evaluation of the antioxidant capacity and phenolic compounds of medicinal plants. These medicinal plants showed stronger antioxidant activity and phenolics content than the common nutritional plants. It has been also noted in this study that these plants are strong radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial uses. However, due to the diversity and complexity of the natural mixtures of phytochemical compounds in these plant extracts, it is not easy to characterize every compound and assess their antioxidant activities. Each plant contains generally different phytochemical compounds with different amount of antioxidant activity. Upon this study, we can state that more pharmacological studies are needed to further confirm the advantageous quality of these extracts.

References

1. A. A. Adedapo, F. O. Jimoh, S. Koduru, P. J. Masika, A. J. Afolayan, Evaluation of the medicinal potentials of the methanol extracts of the leaves and stems of *Halleria lucida*, *Bioresource Technology*. 99(2008) 4158-4163.
2. A. Agustyniak, G. Bartosz, A. Cipak, G. Duburs, L. Horakova, W. Luczaj, M. Majekova, A.D. Oddesseos, L.Rackova, E. Skrzydlewska, M. Stefek, M. Strosova, G. Tirzities, P. R. Venskutonis, J. Viskupicova, P. S.Vraka, N. Zarkovic, Natural And Synthetic antioxidants: and update overview, *Free Radic Res*. 44 (2010) 1216-1262.
3. A.C. Kaliora, V. Z. George, Dedoussis, Natural Antioxidants Compound in risk factor of CVD, *Pharmacology research*, 56(2007) 99-109.
4. B. Bozin, N. Mimica-Dukic, I. Samojlik, A. Goran, R. Igc, Phenolics as antioxidants in garlic (*Allium sativum* L. ,Alliaceae), *Food Chemistry*. 111 (2008) 925–929.
5. B. Klejdus, R. Mikelova, J. Petrlova, D. Potesil, V. Adam, M. Stiborova, P. Hodek, J.Vacek, R. Kizek, V. Kuban, Determination of isoflavones in soy bits by fast column high-performance liquid chromatography coupled with UV–visible diode-array detection, *Journal of Chromatography A*. 1084 (2005) 71–79.
6. C. A. Rice-Evans, N. J. Miller, G. Paganga, Structure – antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*.20 (1996) 933-956.
7. D. Martinez-Dominguez, M. A. Heras, F. Navarro, R. F.Torrnteras, Efficiency of antioxidant response in *spartina densiflora*: An adaptative success in a polluted environment, *Environmental and Experimental Botony*. 62 (2008) 69- 77.
8. H. Beckmann, D. Athen, M. Olteanu, R. Zimmer R, dl-Phenylalanine versus imipramine: A double-blind controlled study. *European Archives of Psychiatry and Clinical Neuroscience*. 227(1979) 49-58.
9. H. Beckmann, D. Athen, M. Olteanu, R. Zimmer, dl-Phenylalanine versus imipramine: A double-blind controlled study. *European Archives of Psychiatry and Clinical Neuroscience*. 227)1979 (49-58.
10. I. F. F. Benzie, J. J. Strain, The ferric reducing ability of plasma as a measure of antioxidant power the FRAP assay. *Analytical Biochemistry*. 239 (1996), 70-76.
11. J.M. Andrade, D. Fasolo, Chapter 20- Polyphenol Antioxidants from natural sources and contribution to Health promotion polyphenols in Human Health and Disease. 1(2014) 253-265.
12. K. E. Heim, A. R. Tagliaferro, D. J. Bobilya, Flavonoids antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*. 13(2002) 572-584.
13. L. R. Fukumoto, G. Mazza, Assessing antioxidant and prooxidant activities of phenolic compounds, *J. Agric. Food Chem*. 48 (2000) 3597–3604.

14. M. A.Rostagno, M. Palma, C. G. Barroso, Fast analysis of soy isoflavones by high- performance liquid chromatography with monolithic columns, *Analytica Chimica Acta.* 582)2007 (243–249.
15. M. P. Germano, D.E. R.Pasquale , V. Angelo, S. Catania, V. Silvaria, C. Costa, Evaluation of extracts and isolated fraction from *Capparis spinosa* L. Buds as an antioxidant source, *J. Agric. Food Chem.* 50(2002) 1168–1171.
16. M.S. Brewer, Natural Antioxidants: Sources, Compounds, Mechanism of Action and Potential Applications *Comprehensive Reviews in food Science And food Safety.* 10(2011) 221– 247.
17. P. Bansal, J. Mudgal, P. G. Nayak, S. T.Pannakal, K. I. Priyadarsini, M. K. Unnikrishnan, Antidiabetic, antihyperlipidemic and antioxidant effects of the flavonoid rich fraction of *pile microphylla* (L.) in high fat diet/streptozotocin-induced diabetes in mice, *Experimental and Toxicologic pathology,* 64(2012) 651-658
18. R. Liu, T. Ruan, S. Songs, Y. Lin, G. Jiang, Determination of synthetic phenolic Antioxidants and relative metabolites in sewage treatment plant and recipient river by hiperformance liquid chromatography-electrospray tandem mass spectrometry, *J Chromatogr A.* 13(2015) 13-21.
19. R. Rodil, J. B. Quintana, R. Cela, Oxidation of synthetic phenolic antioxidants during water chlorination, *J Hazard Mater.* 15(2012) 73-81.
20. S. Meyers, Use of neurotransmitter precursors for treatment of depression, *Altern.Med. Rev.* 5(2000) 71 – 64.
21. Shimadzu protocol for amino acid analysis by amino acid analyzer . Pages 1 – 60.
22. Y. Ishida, T. Fujita, K. Asai, Report. *J. chromatography.* (1981) 204.