Research Article

Evaluation of Antioxidant Activity using by H_2O_2, DPPH, ABTS Methods and Phytochemical Tests, TPC & TFC of Commonly Used Medicinal Plants of West Bengal

[1]Mradu Gupta*, [1]AnumitaDey, [1]Sushmita Majumder, [1]Sukumar Ghosh, [2]Ananya Barui
[1]Department of Dravyaguna, Institute of Post Graduate Ayurvedic Education & Research, kol-700009, India.
[2]Center of Healthcare Science and Technology, IIEST, Shibpur, India.
***Corresponding author's E-mail:** mradu_gupta@hotmail.com

Received: 22-10-2021; **Revised:** 26-12-2021; **Accepted:** 03-01-2022; **Published on:** 15-01-2022.

ABSTRACT

Medicinal plants are very useful in folk medicine due to present different phyto-components whose properties show medicinal importance. Antioxidant is one of them important properties which can protect cells from various types of cell damage by capturing free radicals. Various phyto-compounds are relatable for showing antioxidant activity of plants. The main aim of this study is that to investigate whether the relation between phenolic compound, flavonoid compound and antioxidant property is present or not. So, in this study was performed on twelve medicinal plant. Various phytochemical study is performed such as carbohydrate by Fehling's, Benedict's test, alkaloid test by dragendroff's and Mayer's method, protein by biuret and ninhydrin test, tannin by lead acetate, ferric chloride methods, and glycoside test. Besides phyto-chemicals test, total phenolic content (TPC), total flavonoid content (TFC) is performed by Folin Ciocalteau method, aluminium chloride colorimetric technique respectively. Antioxidant activity is measured by H2O2, DPPH, and ABTS method. Ascorbic acid and trolox are used as a standard of antioxidant test. Mandukparni and Bramhi show strong antioxidant property who's TPC and TFC value falls in vicinity of highest range. According to the H_2O_2 method, IC_{50} value of Mandukparni is 50.06 mg/ml. Mandukparni show high antioxidant activity by using DPPH method, with IC_{50} value 50.22 mg/ml. In ABTS method, Bramhi showed highest antioxidant property with IC50 value 49.98mg/ml. There is a relation between TPC, TFC and antioxidant. But only the TPC, TFC is responsible for antioxidant that is not validate reasons. Various others phyto-compound synergistically or individually can influence the antioxidant properties.

Keywords: Antioxidant, phytochemical, DPPH, ABTS, total phenolic compound, total flavonoid compound.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2022.v72i01.018

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v72i01.018

INTRODUCTION

Since the primitive time, the purpose of use of medicinal plants is in folk medicine[1]. All over world for thousands of years, these medicinal plants are used as a natural medicine[1]. Today, according to the World Health Organization (WHO) primary health-care is associated with traditional medicine which is used by 80% of world's people[1]. The medicinal properties of plants are explored due to presence of their potent pharmacological activities[2]. Various type of free radical scavenging molecules is found in plants which act as a natural product, rich in antioxidant properties[3]. That's why plants extract is displayed various biological activities such as antioxidant properties[4]. Antioxidant molecules can be suppressed the oxidative reaction which is instigated by the free radicals[5]. Polyphenolic compound mainly phenols group are responsible for the antioxidant feature[6]. Innumerable physiological and biochemical reaction is happened in human body and produced free radicals which can damage to biomolecules (lipids, protein, DNA), and eventually these are related to different disease like as atherosclerosis, cancer, diabetes, aging and other degenerative diseases[7]. Natural antioxidant can inhibit lipid peroxidation and to chelate heavy metal ions[8].Besides this phenolic compound, other phyto-compound is also responsible for antioxidant property. Twelve plants are studied under the antioxidant study.

These plants are *Euphorbia thymifolia* Linn. (Dugdhika), *Centella asiatica* (Mandukparni), *Sesbania grandiflora* Pers. (Agastya), *Tylophora indica* Merrill. (Anantamool), *Clitoria ternatea* Linn. (Aparajita), *Cissus quadrangularis Linn* (Asthisrimkhala), *Tinospora cordifolia* Willd (Guduchi), *Piper nigrum* Linn (Marich), *Gymnema sylvestre* R.Br. (Mesasringi), *Vitex negundo* Linn. (Nirgundi), *Eupatorium triplinerve* Vahl (Ayapan), *Bacopa monnieri* Linn (Bramhi). According to the ethnobotanical information, Dugdhika (*Euphorbia thymifolia* Linn.) is a one kind of medicinal plants which is used in purpose of different medical issue[9]. Various phyto-compound like as alkaloid, carbohydrates, glycosides, steroids, tannin, flavonoids, triterpenes are present and may be this compound is played a vital role in medicinal purpose[9]. Above mention plants, all are more or less medically important such as Agastya related to antiuroithiatic, anticonvulsive, anti-arthritic, anti-inflammatory, anti-bacterial etc[10]. Anantamool is also pharmaceutically importance like as anti-allergic activity, anti-asthmatic activity, anti-fungal activity, anti-diabetic activity, anti-bacterial activity, Hepatoprotective activity,

Diuretic activity, anti-Tumour Activity[11]. Prevention of neurodegenerative disease and diabetes mellitus, Aparajita is one of the recommended option among the medicinal plants[12]. Asthisrinkhala is one of the most important medicinal plants which is known as a "Hadjod", basically it is important in promotion of fracture healing and also has analgesic, ant osteoporotic activity, gastro protective activity etc[13]. Guduchi (*Tinospora cordifolia* Willd) is medically important due to have anti-cancer, anti-tumour, anti-toxin, anti-diabetic, anti-oxidant, immunomodulatory activity[14]. Another medically important plant is Marich (*Piper nigrum* Linn) and show the cardio protective effect, antiulcer activity, antidiarrheal activity, analgesic activity, aphrodisiac property, immunomodulatory activity, antidyslipidemic activity, neuroprotective effect, anti-inflammatory activity, gastro protective activity, antioxidant activity, radio protective and cytoprotective activity, ameliorative effect[15].

Sl No	Name	Scientific Name& Family	Part use	Ayurvedic indication	Chemical compound	Pharmacological action
1	Dugdhika	*Euphorbia thymifolia* Linn. &Euphorbiaceae	Leaf, Whole plant	Used in kasa, swas, hridyarogas etc.	Alkaloid, carbohydrate, steroid etc.	Antibacterial, anti-inflammatory, antioxidant.
2	Mandukparni	*Centella asiatica* & Umbelliferae	Leaf& whole plant	Used in unmad, stress Shotha, Pandu, Jvara, and Chardirogas.	Alkaloid, glycosidic, flavonoid etc.	Effect on nerves and brain cells.
3	Agastya	*Sesbania grandiflora* Pers & Leguminosae	Leaf& seeds & Flowers	Kasa, Jvara, respiratory & skin diseases,	Alkaloid, tannin, saponin, flavonoid etc.	Hepatoprotective, antioxidant, anxiolytic.
4	Anantamool	*Tylophora indica* Merrill &Asclepiadaceae	Leaf& Roots	Swas, Kasa, pratishyay&krimirogas.	Alkaloid, Flavonoid, carbohydrate, tannin etc.	Effect on asthma, bronchitis, rheumatism.
5	Aparajita	*Clitoria ternatea* Linn &Leguminosae	Leaf, Seeds & Flowers	Jwar, Kustha, udarsula, Varna, Sotharogas.	Alkaloid, tannin, glycoside etc.	Antioxidant, analgesic, antipyretic.
6	Ashathisrankshala	*Cissus quadrangularis Linn* &Vitaceae	Leaf& Stem	General weakness, diseases of bones, bone fracture &Netraroga.	Potassium, Calcium, Zinc etc.	Repair bone fracture healing, antioxidant, antihemorrhoidal.
7	Guduchi	*Tinospora cordifolia*Willd &Menispermaceae	Leaf, Stem	Used in kasa, swas, skin and liver diseases, Prameha, Joints pain rogas.	Alkaloid, lactone, lignin, aliphatic compound etc.	Jaundice, rheumatism, urinary disorder, skin diseases.
8	Marich	*Piper nigrum*Linn &Piperaceae	Leaf & Fruits, roots	Indigestion, loss of appetite, krimi, skin& respiratory diseases.	Alkaloid, Pentose, hexose sugar etc.	antihypertensive, antiplatelet, antioxidant, anti-asthmatics.
9	Meshashrangi	*Gymnema sylvestre*R.Br &Asclepiadaceae	Leaf	Used in Madhumeha, Abdominal discomfort, constipation & Swasraogas.	Alkaloid, cardiac glycoside, anthraquinone etc.	Anti-obesity, anti-hyperglycemic.
10	Nirgundi	*Vitex negundo* Linn. &Verbenaceae	Leaf, Roots & Seeds	Shotha, Joint pains, Krimi, Kustha, Aruchi, and Netraroga.	Phenolic compound, alkaloid, Saponins etc.	Anti-arthritic, anxiolytic, anti-amnesic.
11	Ayapan	*Eupatorium triplinerve* Vahl & Compositae	Leaf& Whole plant	Arsha, Raktapitta, aruchi, Jwar& bleeding disorders Kaphapittashamaka, arsha, and jwararogas.	Tetradecanoic acid, Bicyclo, Hexadecanoic acid, 1,14-tetradecanediol	Hepatoprotective, anti-bacterial, neuroprotective.
12	Brahmi	*Bacopa monnieri* Linn. & Scrophulariaceae	Leaf & Whole plant	Memory enhancer, Pandu, Apasmara, Sotha, Jvara, kasa, stress Sleeplessness	Alkaloid, flavonoid, phenol, tannins etc.	Protect brain from oxidative damage.

Besides these clinical problems, obesity is also a major problem. Mesashrimgi (*Gymnemasylvestre*R.Br.) can help to maintain weight control which is related to anti-obesity[16]. Besides this clinical role, anti-microbial and anti-hyperglycaemic activity is also present in this plant[16]. Pharmacologically important another plant is Nirgundi (*Vitex negundo* Linn.) which play vital role against pain, headache, inflammation, leukoderma, enlargement of the spleen, rheumatoid arthritis, gonorrhoea etc[17]. Ayapan (*Eupatorium triplinerve* Vahl.) is one type of ornamental perennial her. It's medically important due to have anti-cancer, anti-bacterial, anti-fungal, hepatoprotective and also neuroprotective activity[18]. Bramhi (*Bacopa monnieri* Linn) is an Indian herb, act as a dietary antioxidant with various mode of action by which it can help to protect the brain from oxidative damage and age related cognitive decline[19]. Here we study these plants to check antioxidant property which can modulate various pharmacological activity and that are related to different phyto-compounds.

The aim of the present study to find out the maximum antioxidant activity in comparison of standard chemical compounds and correlation with the Total phenolic and total flavonoid compounds of the specific plant part leaf following the standard methods which can reduce various factors which are responsible for oxidative damage. These antioxidant medicinal plants may be prescribed to reduce the oxidative stress and act as free radical scavenger for Ayurvedic system of medicines. The detailed literature review of the above-mentioned plants is given below including plants local name, scientific name, which parts are generally used for research purpose, and also mentioned their Ayurvedic indication, chemical compound, and pharmacological action.

MATERIALS AND METHODS

The pharmacognostic and antioxidant was performed at department of Dravyaguna, Institute of Post Graduate Ayurvedic Education & Research, kol 09.

Collection of plants: The plants materials (leaf) were collected by following the guideline of good agricultural and collection practices (GACP) for medicinal plants from herbal garden of this institute, Eco-Park, Now town, Kolkata.

Processing of plant materials: Plants materials were collected from the gathering field. Then it had been washed out through the running water to get rid of the soil particles and other foreign particles. Then wet plant leaves are allowed to dry in shade. After drying process, leaves were dig many pieces and grinded for powder making. This powder was preserved in air-tight polythene bag for qualitative analysis further.

Chemicals and reagent: Methanol, Fehlings A and Fehlings B, Benedict's solution, Dragendroff's, Mayer's reagent, HCl, sodium hydroxide, copper sulphate, Ninhydrin, ferric chloride, lead acetate, benzene, ammonia, Folin Ciocalteau, sodium carbonate, gallic acid, aluminium chloride, potassium acetated, quercetin, phosphate buffer, H2O2, ascorbic acid, DPPH, ethanol, ABTS, potassium persulfate, these all are used and purchase from DST-BT granted fund.

Processing of plant extract: hydro alcoholic (water 70: methanol 30) solution was used for extract preparation from the powder of plant leaves and stay at overnight. Then it was often filtered and filtrate was stored in Refrigerator for further studies. This extract was getting to under anti-oxidant test and total phenol and total flavonoid test.

Qualitative phytochemical analysis: The extract was examined to check presence of phyto-compound by using standard methods[20, 21, and 22].

- **Test for Carbohydrates**

Fehling's test: Equivalent measure of Fehlings A and Fehlings B was added to the 2ml of plant unrefined concentrate and delicately shake for great blending, at that point it is permitted to bubble. After couple of mints, a brick red accelerate was showed up at the lower part of the test tube which demonstrates that carbohydrate is available.

Benedict's test: Few drops of Benedict's solution was mixed to the 2 ml of plant extract and permit heating up, a reddish or brown colour precipitation was showed which indicate the presence of carbohydrate.

- **Test for Alkaloid**

Dragendroff's test: 1ml of Dragendroff's reagent and 1ml of dil HCl are blended in with 1ml of plant extract and permit to bubble and appear the orange red precipitation which indicate the presence of alkaloid.

Mayer's test: 3ml of Mayer's reagent and 1ml of dil HCl were blended with 1ml of plant extract, formation of cream colour precipitated. This is recommended the presence of alkaloid.

- **Test for protein**

Biuret test: 3ml of plant extract was mixed with 1ml of 4% sodium hydroxide solution and few drop of 1% copper sulphate solution was added. After reaction violet pink colour is seen that is indicated the presence of protein and amino acids.

Ninhydrin test: Two drops of freshly prepared 0.2% Ninhydrin reagent was added with plant extract and then allow to heat. Development of blue colour revealed the presence of proteins, peptides, or amino acids.

- **Test for tannin**

Ferric chloride test: Few drops of 5% ferric chloride (FeCl3) is permitted to react with 2-3 ml of extract and boiled for few mints. Develop deep blue black colour reveal that tannin is present.

Lead acetate test: Few drops of 10% lead acetate is allowed to react with 2-3 ml of extract and boiled for few mints. Appear white or yellow precipitated indicate that tannin is present.

➢ **Teat for glycoside:** Extract were resolved with dil hydrochloric acid and then it was going to test for glycosides. Extract was treated with ferric chloride solution immersed in boiling water for about 5 mints. The mixture was allowed to cool and benzene was added equal volumes of extract. The benzene layer was distinguished and treated with ammonia solution. Formation of rose pink in the ammonium layer indicates the presence of glycosides.

Estimation of total phenols: Total phenols were determined by Folin Ciocalteau method[23]. 0.5 ml of sample were blended with 2.5 ml 10 fold diluted Folin Ciocalteau then give in 2 ml of 7.5% sodium carbonate (Na_2CO_3). Then this mixture was allowed to dark incubate up to 30 min at room temperature. After incubation, take absorbance at 765 nm against blank. The phenolic content was calculated from standard graph of Gallic acid [Fig1A]. The outcome data were expressed as mg/g of Gallic acid equivalents in milligrams per gram (mg GAE/g) of extract.

Estimation of total flavonoids: Total flavonoids were evaluated by the aluminium chloride colorimetric technique[23]. 0.5 ml extract was amalgamated with 1.5 ml methanol, 0.1 ml 1% aluminium chloride ($AlCl_3$), 0.1 ml of 1M potassium acetated and the finally added 2.8 ml distilled water. This mixture was permitted to incubate for 30 min at room temperature. After incubation, absorbance was noted at 415 nm. The flavonoids content was determined by the standard curve of quercetin [Fig 1B]. The outcome data were communicated as mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of extract.

Antioxidant activity of plant extracts:

Antioxidant activity of hydro alcoholic extract was evaluated by the free radical scavenging methods like as the H2O2 scavenging method, DPPH scavenging method, and ABTS radical cation decolourization assay.

- **H_2O_2 scavenging method**: Antioxidant activity of individual extract was evaluated by using H_2O_2method[23]. 0.1ml of sample added with 3.4 ml of 0.1 M phosphate buffer and 0.6 ml of 40 mM H_2O_2. This mixture was incubated 10 mints at room temperature. After incubation, absorbance was note down at λmax 230 nm against blank solution. Ascorbic acid was used as standard [Fig 1C]. The percentage scavenging of H2O2 was calculated using the equation.

$$\%\ scavengingofH2O2 = \frac{A_0 - A_1}{A0} \times 100$$

- **DPPH scavenging method**: DPPH scavenging activity assay of plant extract was estimated following by the standard protocol[23]. 0.1 mM DPPH solution in ethanol was made ready. 3 ml of DPPH stock solution was added with 1 ml of extract at different concentration and equal volume of ethanol. It was incubated for 30 mints and after incubation absorbance was noted at 517 nm. Antioxidant activity of sample is measured by the standard curve of ascorbic acid [Fig 1D]. The DPPH scavenging capacity was represented by the following equation

$$\%\ inhibition = \frac{blank - sample}{blank} \times 100$$

- **ABTS radical cation decolourization assay**: antioxidant activity was determinate by ABTS protocol[24]. For ABTS assay, working solution was made up by mixing equal amount of 7.4 mM ABTS and 2.45 mM potassium persulfate and this solution was stored in dark 12-16 hrs incubation to react and produce active ABTS radical cation which is react with plant solution to determine antioxidant activity. ABTS solution was diluted by the ethanol. Then, 50µl of sample was blended with 1.9 ml of ABTS solution and allow to dark incubate for 6 mints. After incubation, absorbance was taken at 734 nm. Sample result was compared with trolox standard (Y= 0.0379x-0.0015, R2 = 0.9872,). Result was expressed by mmol Trolox equivalents/g dry extract (mmol TE/g DE).

RESULTS AND DSCUSSION

The collected plants sample were identified and standardized by using the pharmacognostical method and prepared hydro-alcoholic extract of each plant sample for qualitative like as phytochemical and quantitative analysis like as Total phenolic compound (TPC), Total flavonoid compound (TFC) and antioxidant activity (H2O2, DPPH, ABTS method).

Qualitative phytochemical screening: The phytochemical analyses of twelve medicinal plants are given in concise form in the table 1. This result can help to conclude that which active compounds are present in the twelve plants. From the table 1, carbohydrate, alkaloid and tannin are present in all twelve plants. Protein and amino acids are present in five plants among the twelve plants, which are *Sesbania grandiflora* Pers(Agastya), *Clitoria ternatea* Linn (aparajita), *Piper nigrum*Linn (marich), *Gymnema sylvestre* R.Br (meshashringi), *Bacopa monnieri* Linn(bramhi).Glycoside is found in all twelve plants except *Centella asiatica*(mandukparni) and *Vitex negundo* Linn. (Nirgundi).

Determination of TPC: The concentration of TPC is variable among the twelve plants. *Vitex negundo* Linn. (Nirgundi) show the highest TPC value among the twelve plants. Unit of TPC is represented by (µg gallic acid equivalent/mg of extract). 282.67 µggallic acid equivalent/mg of extract are the TPC of *Vitex negundo* Linn (Nirgundi). Besides *Vitex negundo* Linn (nirgundi) plant, *Euphorbia thymifolia* Linn (dugdhika) show 242.222 µg gallic acid equivalent/mg of extract, *Centella asiatica* (mandukparni) show 180.285 µg gallic acid equivalent/mg of extract, *Tylophora indica* Merrill (anantamool) show 149.94 µggallic acid equivalent/mg of extract, *Gymnemasylvestre*R.Br (meshashringi) show 165.284 µg gallic acid equivalent/mg of extract, *Eupatorium triplinerve* Vahl (ayapan) [174.3762 µggallic acid equivalent/mg of extract] are also showed maximum TPC value may influence antioxidant activity and other physiological functions.

Determination of TFC: *Vitex negundo* Linn. (Nirgundi) show the highest TFC value among the twelve plants. Unit of TFC is represented by (µg quercetin equivalent/mg of extract). 205.42µg quercetin equivalent/mg of extract is the TFC of *Vitex negundo* Linn. (Nirgundi). Besides *Vitex negundo* Linn. (Nirgundi), other few plant among the twelve plants are also showed tends to maximum value that can modulate antioxidant properties. TFC value pf Bramhi (76.43 µg quercetin equivalent/mg of extract), Mesasringi (70.78 µg quercetin equivalent/mg of extract), Guduchi (59.20 µg quercetin equivalent/mg of extract), Aparajita (45.61 µg quercetin equivalent/mg of extract), Mandukparni (48.79 µg quercetin equivalent/mg of extract) are towards the high range.

Table 2 represent the summary of TPC and TFC of twelve medicinal plants. **Fig 2** is represent the comparison of TPC and TFC of plants through the graphical diagram.

Table 1: Phytochemicals analysis of twelve mention medicinal plants

Plant Name	Carbohydrate		Alkaloid		Protein and amino acid		Tannin		Glycoside
	Felhings	Benedict	D.D	Mayer's	Biuret	Ninhydrin	FeCl3	Lead acetate	
Dugdhika	+	+	+	+	-	-	+	+	+
Mandukparni	+	+	+	+	-	-	+	+	-
Agastya	+	+	+	+	-	+	-	+	+
Anantamool	+	+	+	+	-	-	+	+	+
Aparajita	+	-	+	+	-	+	+	+	+
Asthisrinkhala	+	+	+	+	-	-	-	+	+
Guduchi	+	+	+	+	-	-	+	+	+
Marich	+	+	+	+	-	+	+	-	+
Mesasringi	+	+	+	+	+	+	+	+	+
Nirgundi	+	+	-	-	-	-	+	+	-
Ayapan	+	+	-	-	-	-	+	+	+
Bramhi	+	-	+	-	-	+	+	+	+

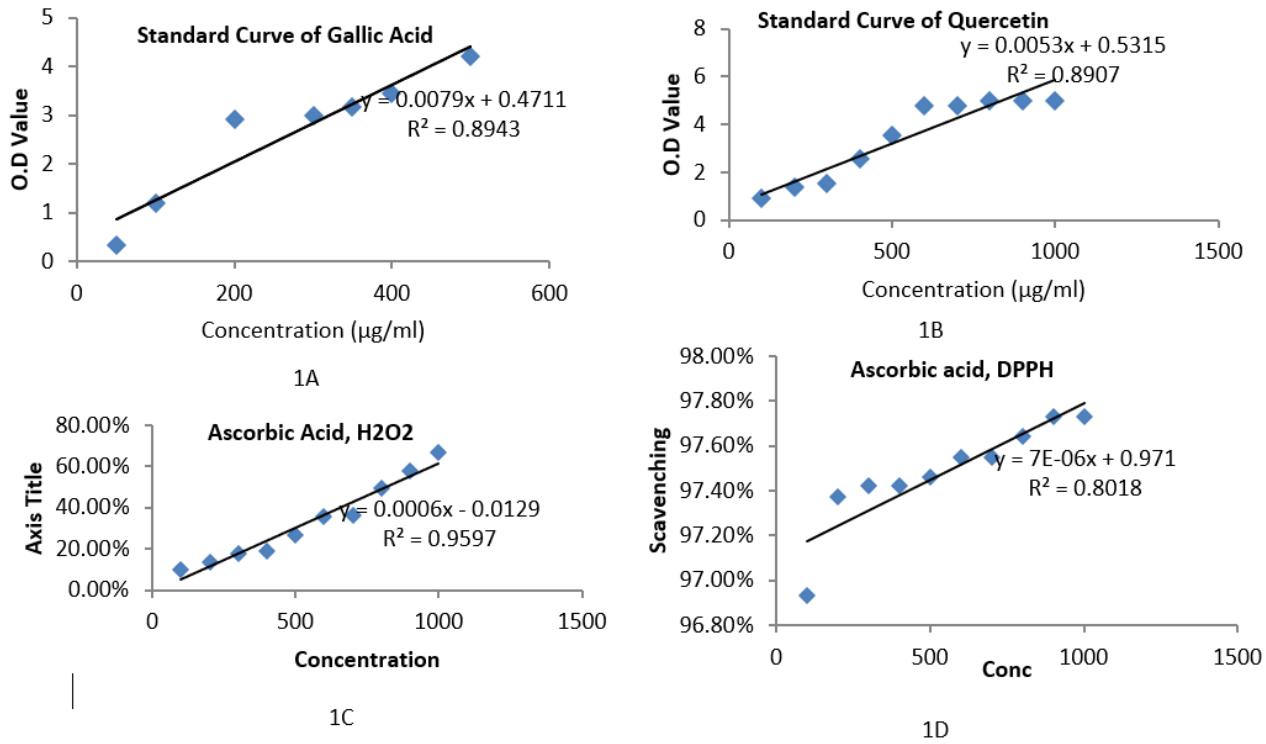

Figure 1: 1A. Standard curve of Gallic acid for TPC, 1B. Standard curve of Quercetin for TFC, 1C. Standard curve of ascorbic acid of H2O2 scavenging method, 1D. Standard curve of ascorbic acid of DPPH scavenging method.

Table 2: Total Phenolic Compound (TPC) and Total flavonoid Compound (TFC) of twelve medicinal plants and Comparative study of anti-oxidant of twelve plants on the basis of IC_{50} value by three scavenging method.

Plants Name	TPC (µg gallic acid equivalent/mg of extract)	TFC (µg quercetin equivalent/mg of extract)	IC50 (mg/ml)		IC50 (mmol TE/g DE)
			H_2O_2 Method	DPPH Method	ABTS Method
Dugdhika	242.222	28.98	50.49	50.93	50.41
Mandukparni	180.2857	48.79	50.06	50.22	53.64
Agastya	32.6616	11.80	51.42	51.31	78.34
Anantamool	149.94	17.91	51.32	60.47	56.59
Aparajita	113.8037	45.61	50.11	78.13	51.15
Asthisrinkhala	0.4089	16.00	52.38	54.27	52.33
Guduchi	92.3249	59.20	54.22	61.89	51.51
Marich	90.05204	16.90	50.90	57.50	55.75
Mesasringi	165.2847	70.78	50.23	62.92	50.91
Nirgundi	282.6794	205.42	52.96	50.55	53.75
Ayapan	174.3762	22.10	52.08	58.64	56.64
Bramhi	240.844	76.43	52.75	56.42	49.98

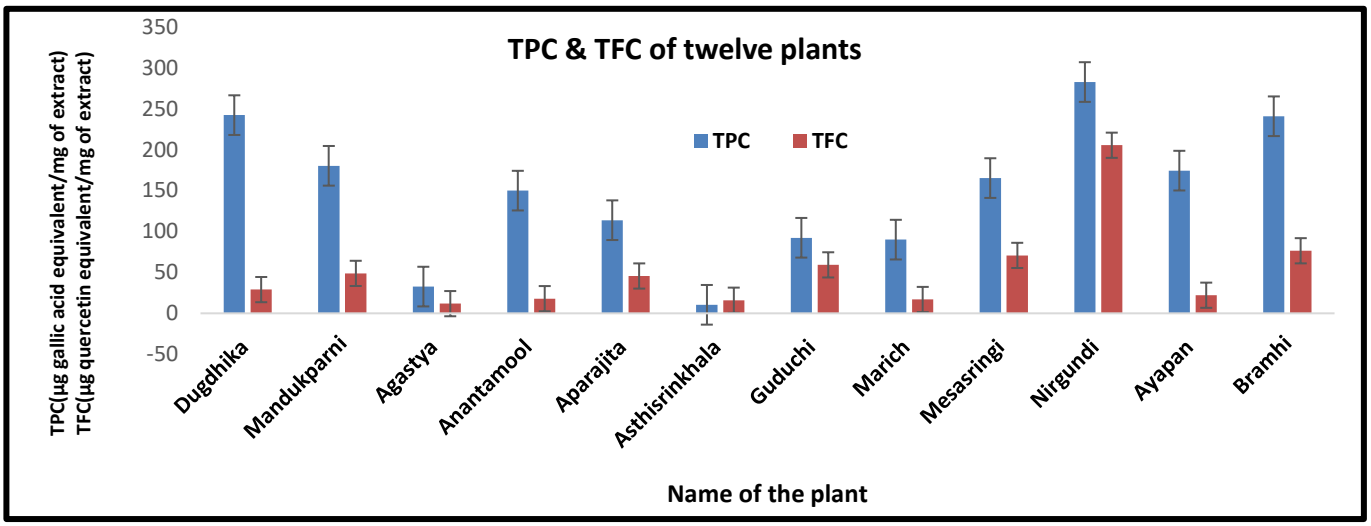

Figure 2: Graphical representation of TPC & TFC of selected twelve medicinal plants.

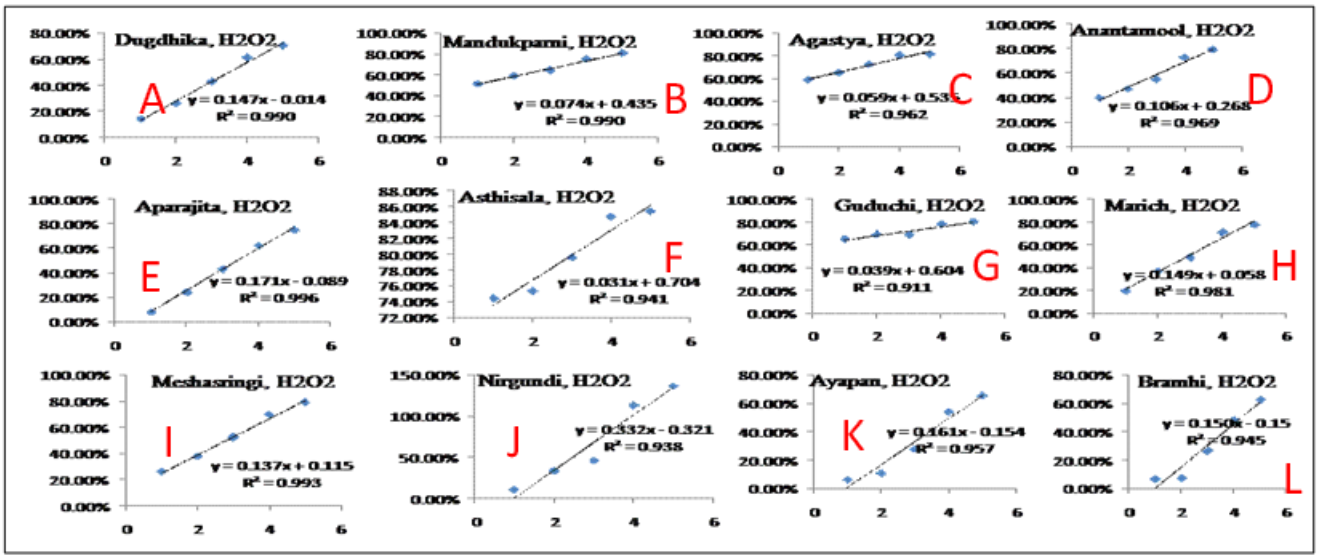

Figure 3: Estimation of antioxidant potentiality of twelve plant by H2O2 method (Y axis = Scavenging inhibition percentage, X axis = Concentration of Plant extract)[A- Dugdhika, B-Mandukparni, C-Agastya, D-Anantamool, E-Aparajita, F-Asthisrinkhala, G-Guduchi, H-Marich, I-Meshasringi, J-Nirgundi, K-Ayapan, L-Bramhi]

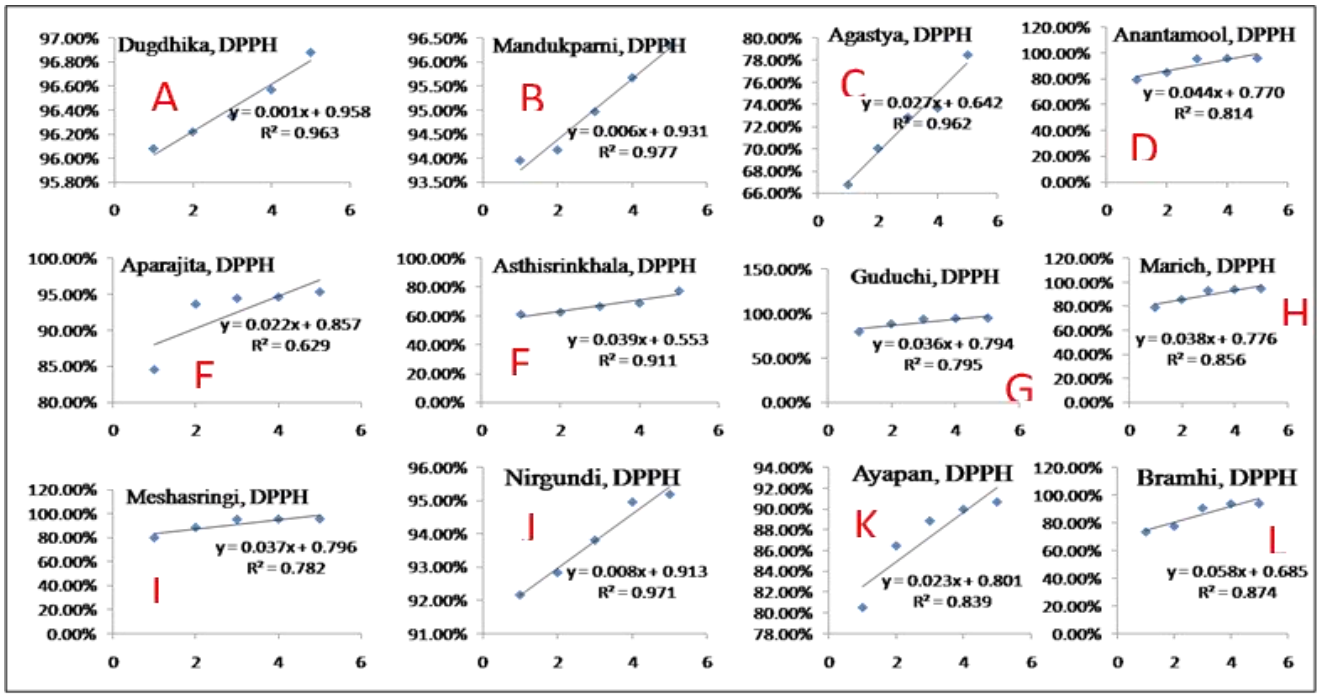

Figure 4: Estimation of antioxidant potentiality of twelve plant by DPPH method (Y axis = Scavenging inhibition percentage, X axis = Concentration of Plant extract)[A- Dugdhika, B-Mandukparni, C-Agastya, D-Anantamool, E-Aparajita, F-Asthisrinkhala, G-Guduchi, H-Marich, I-Meshasringi, J-Nirgundi, K-Ayapan, L-Bramhi]

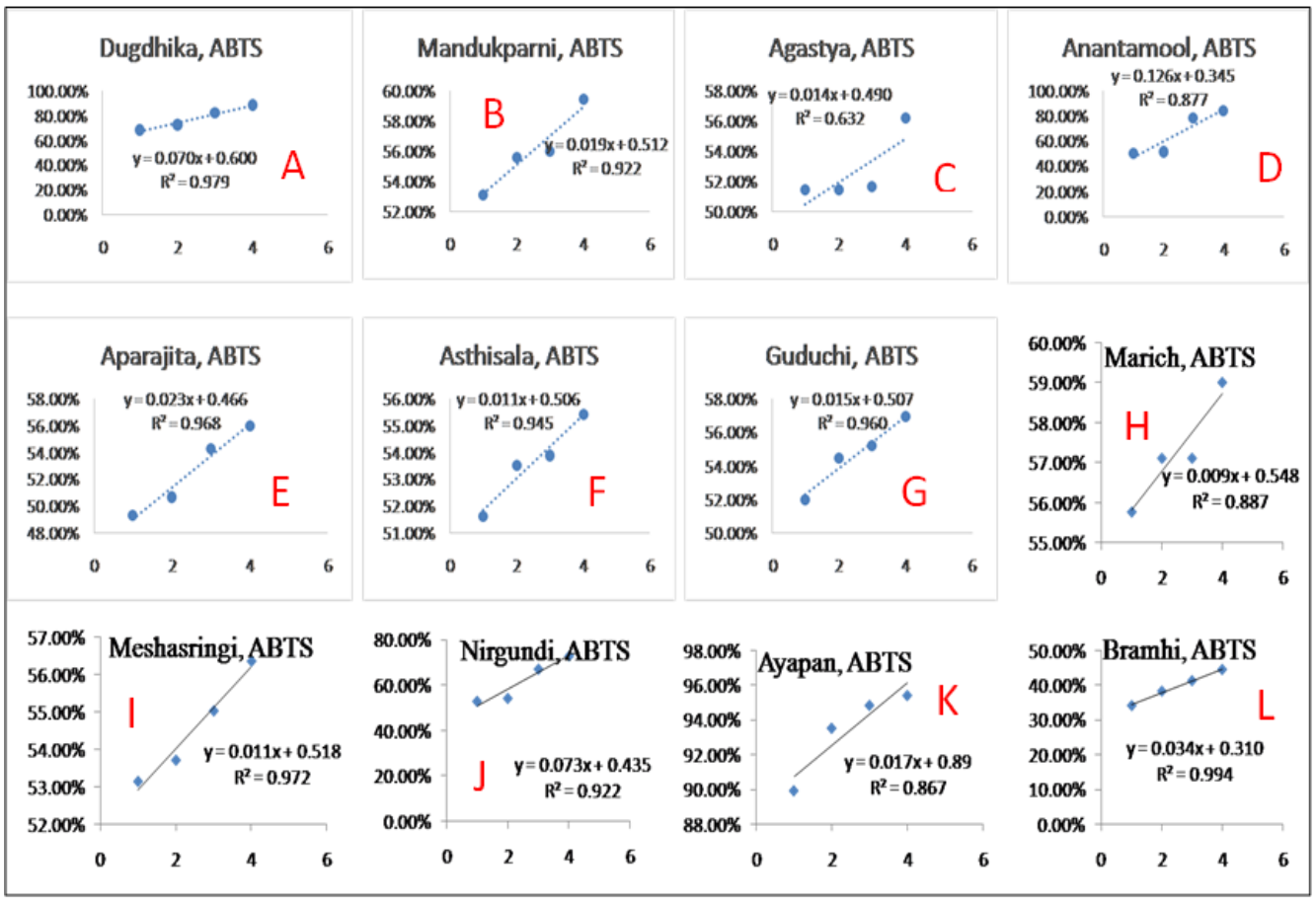

Figure 5: Estimation of antioxidant potentiality of twelve plant by ABTS method (Y axis = Scavenging inhibition percentage, X axis = Concentration of Plant extract) [A- Dugdhika, B-Mandukparni, C-Agastya, D-Anantamool, E-Aparajita, F-Asthisrinkhala, G-Guduchi, H-Marich, I-Meshasringi, J-Nirgundi, K-Ayapan, L-Bramhi].

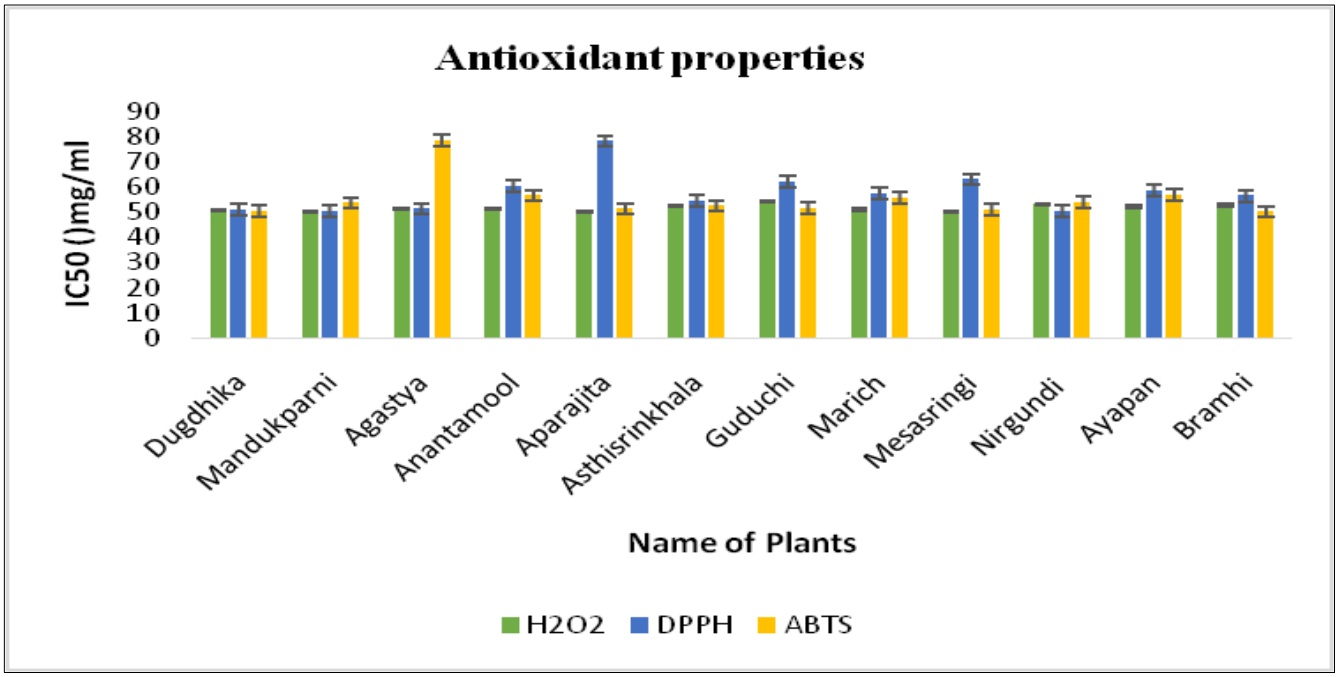

Figure 6: Antioxidant study of twelve plants by three methods (H2O2, DPPH, ABTS)

Antioxidant properties: Antioxidant properties of twelve medicinal plants are evaluated by three scavenging method, H2O2 scavenging method, DPPH method, ABTS methods **(Fig 3, 4, 5)**. Antioxidant activity help to protect cell from various oxidative damage. IC50 value can determined the antioxidant measurement. Low IC50 value can interpret the high antioxidant activity.

Centella asiatica (mandukparni) show highest antioxidant properties with low IC50 value (50.06 mg/ml) through the H2O2 method. Besides *Centella asiatica* (mandukparni)**,** Aparajita (50.11 mg/ml), Dugdhika (50.49 mg/ml), Marich (50.90 mg/ml) show high antioxidant property which IC50 value falls towards the low range. [Fig 6].

Centella asiatica (mandukparni) show highest antioxidant properties with low IC50 value (50.22 mg/ml) in DPPH methods and as well as other plants also show high antioxidant activity with low range IC50 value. These plants are Nirgundi (50.55 mg/ml), Dugdhika (50.93 mg/ml), and Agastya (51.31 mg/ml). Besides these plants, other plants also have antioxidant activity according to their IC50 value [Fig 6].

Bacopa monnieri Linn (Bramhi) show highest antioxidant properties with low IC50 value (49.98 mg/ml) in ABTS method [Fig 6]. IC50 value of Dugdhika (50.41 mg/ml), Mesashringi (50.91 mg/ml), Aparajita (51.15 mg/ml), and Gudhuchi (51.51 mg/ml) falls toward the low range and show high antioxidant activity. Besides these plants, other mentioned plants are also showed antioxidant activity according to the IC50 value.

Table 2 also represent the comparative study of anti-oxidant of twelve plants on the basis of IC50 value by three scavenging method.

CONCLUSION

Antioxidant properties play an important role in plant physiology. In medicinal plant, different type of bioactive compound can influence the anti-oxidant activity[25]. These bioactive compounds play a vital role in blocking of free radical generation[25]. Mainly phenolic compound are involved in antioxidant property. Not only phenolic compound, other phytocompounds are also influence antioxidant activity. Like as glycoside have a therapeutic value in treatment of inherited deficiencies in man[26]. This study reveals that polyphenolic compound can influence antioxidant but other phytocompounds is also important in antioxidant activity. Antioxidant activity is measured by IC50 values. IC50 value is negatively co-related with antioxidant activity. Basically IC50 value defined that how much concentration of antioxidant is needed to decrease the initial concentration of DPPH, and active ABTS+ radicals by 50%[27]. So lower range of IC50 value is indicated high antioxidant properties. *Centella asiatica* (mandukparni) show lowest IC50 value through H2O2 and DPPH method. This plant may be having high antioxidant activity. *Bacopa monnieri* Linn (Bramhi) show lowest IC50 value through ABTS method and may be having more antioxidant property compare to other plants. *Centella asiatica* (mandukparni), *Bacopa monnieri* Linn (Bramhi), is not show highest TPC and TFC value but range of TPC, TFC value of these plant falls in the vicinity of highest value. Only *Vitex negundo* Linn (nirgundi) show highest TPC, TFC value. Only the TPC, TFC are correlated with antioxidant activity that statement is not validated[28]. Solely phenolic and flavonoids are not only responsible compound to modulate the antioxidant activity. Other various phyto-compounds which are present in plants, play a role to show antioxidant activity either synergistically or individually[28]. For this reason Nirgundi show highest TPC, TFC value but not show highest antioxidant property among the selected mentioned twelve

plants but antioxidant activity of Nirgundi is towards the high range. On the other hand, through the H2O2 and DPPH method Mandukparni show the highest antioxidant activity who's TPC, TFC value is not highest but towards the high range. Same as Bramhi whose TFC value is not highest nut tends to high range, show highest antioxidant activity according to the ABTS method. This result may be conclude that TPC, TFC may be modulate the antioxidant activity but these are not only thing to responsible the antioxidant property. Antioxidant play a vital role in human health like obesity, aging. Antioxidant generally two type, enzymatic and other is non-enzymatic. Non-enzymatic antioxidant is also categorized into two group that are hydrophilic and hydrophobic[29]. Hydrophobic can help to protect cell membrane from lipid peroxidation and hydrophilic can dissolve blood and cytoplasm which are capable to react with the free radicals[29]. This study show that these medicinal plants have antioxidant potentiality by which this plant derive component can maintain good health condition. Antioxidant activity may be influenced by poly-phenolic compounds.

ACKNOWLEDGEMENT: The authors are thankful to Department of Dravyaguna, I.P.G.A.E&R.

FUNDING: This work was supported by the Department of Science &Technology and Biotechnology, Memo No: 810(Sanc.)/ST/P/S&T/2G-20/2018, dated 17.06.2019 stating the Commission's approval and sanction of an on account grant for the Major Research Project titled "Sequence based DNA bar-coding of fresh leaves of medicinal plants for Authentication" in favour of this Institute.

REFERENCES

1. Škrovánková S, Mišurcová L, Machů, L, Antioxidant activity and protecting health effects of common medicinal plants, Adv. in food and nutri. Res., 2012; 67:75-139.
2. Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F. et al., Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases, J. of ethnopharm, 2003; 84(2-3):131-138.
3. Choi C W, Kim S C, Hwang S S, Choi B K, Ahn H J, Lee M Y. et al., Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison, Plant sci, 2002; 163(6):1161-1168.
4. Jaberian H, Piri K, Nazari J, Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants, Food chem, 2013; 136(1):237-244.
5. Biapa P C, Agbor G A, Oben J E, Ngogang J Y, Phytochemical studies and antioxidant properties of four medicinal plants used in Cameroon, Afr J Tradit Complement Altern Med, 2007; 4(4):495-500.
6. Agbor G A, Kuate D, Oben J E, Medicinal plants can be good source of antioxidants: case study in Cameroon, Pak. J. Biol. Sci, 2007; 10(4):537-544.
7. Cai Y, Luo Q, Sun M, Corke H, Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, Life Sci, 2004; 74(17):2157-2184.
8. Veeru P, Kishor M P, Meenakshi M, Screening of medicinal plant extracts for antioxidant activity, J. Med. Plant Res, 2009; 3(8):608-612.
9. Shrivastava R, Mishra J, Extraction, phytochemical screening, isolation and identification of bioactive compounds from extract of the plant Euphorbia Thymifolia Linn., J. drug Deliv. Ther, 2019; 9(3):107-113.
10. Mohiuddin A K, Medicinal and Therapeutic values of Sesbania grandiflora. J Pharm Sci Exp Pharmacol. 2019; 81-86.
11. Priya RV, Vijaya Kumar S, Manigandan LS, Tylophora indica–A mini review, Intern. J. Front. Sci. Tech, 2015; 2:58-66.
12. Chakraborthy GS, Kumar V, Gupta S, Kumar A, Gautam N, Kumari L, Phytochemical and pharmacological aspects of ClitoriaTernatea-a review, J. Appl Pharm. Sci. Res, 2018; 3-9.
13. Mishra G, Srivastava S, Nagori BP, Pharmacological and therapeutic activity of Cissus quadrangularis: an overview, Int. J. pharmtech Res, 2010; 2(2):1298-1310.
14. Tiwari P, Nayak P, Prusty SK, Sahu PK, Phytochemistry and pharmacology of Tinospora cordifolia: A review, Syst. Rev. Pharm, 2018; 9(1):70-78.
15. Khushbu C, Roshni S, Anar P, Carol M, Mayuree P, Phytochemical and therapeutic potential of Piper longum Linn a review, Int. J Res Ayurveda pharm, 2011; 2(1):157-61.
16. Gurav S, Gulkari V, Duragkar N, Patil A, Systemic review: Pharmacognosy, phytochemistry, pharmacology and clinical applications of Gymnemasylvestre R Br., Pharmacogn Rev, 2002; 1(2).
17. Tripathi YB, Tiwari OP, Nagwani S, Mishra B. Pharmacokinetic-interaction of Vitex negundo Linn. & paracetamol, Indian J Med Res, 2009; 130(4):479.
18. Krishnan M, Jayaraj RL, Megala J, Elangovan N. Antioxidant mediated antiulcer effect of Eupatorium triplinerve Vahl against acetic acid induced ulcerative colitis in mice, Biom. Aging Pathol, 2014; 4(2):153-160.
19. Simpson T, Pase M, Stough C, Bacopa monnieri as an antioxidant therapy to reduce oxidative stress in the aging brain, Evid Based complementary Altern med, 2015; 2015.
20. Yadav RNS, Agarwala M, Phytochemical analysis of some medicinal plants, J. Phytol, 2011; 3(12).
21. Nandagoapalan V, Doss A, Marimuthu C, Phytochemical analysis of some traditional medicinal plants, Biosci. Discov, 2016; 7(1):17-20.
22. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N, Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal, Plants, 2019; 8(4):96-103.
23. Mohan B, Kakkar A, Evaluation of phenolic, flavonoid contents and antioxidant activity in leaves, stem and roots of Solanum indicum Linn. Environ. Conserv. J, 2020; 21(1&2):167-172.
24. Stanković N, Mihajilov-Krstev T, Zlatković B, Stankov-Jovanović V, Mitić V, Jović J. et al., Antibacterial and

antioxidant activity of traditional medicinal plants from the Balkan Peninsula, NJAS-WAGEN J Life Sc, 2016; 78:21-28.

25. Basma AA, Zakaria Z, Latha LY, Sasidharan S, Antioxidant activity and phytochemical screening of the methanol extracts of Euphorbia hirta L., Asian Pac. J. Trop. Med, 2011; 4(5):386-390.

26. Kytidou K, Artola M, Overkleeft HS, Aerts J M, Plant glycosides and glycosidases: a treasure-trove for therapeutics, Front. Plant Sci, 2020; 11.

27. Rivero Cruz JF, Granados Pineda J, Pedraza Chaverri J, Pérez Rojas JM, Kumar Passari A, Diaz Ruiz G. et al., Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of Mexican brown propolis, Antioxidants, 2020; 9(1):70-76.

28. Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S, Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants, Pak J. Pharm. Sci, 2009; 22(1):55-62.

29. Lakhtakia R, Ramji MT, Lavanya K, Rajesh K, Jayakumar K, Sneha C. et al., The role of antioxidants in human health maintenance: Small molecules with infinite functions, Int. j. Pharm. Sci. res, 2011; 2(6):1395-1402.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.