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Research Article

Free Radical Scavenging Activity, TPC, TFC and Phytochemical Analysis of selected Medicinal Plant Leaf

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ABSTRACT
The present study was taken on to find out the antioxidant value of certain medicinal plant in west Bengal region. In this study, twelve medicinal plants are used to find out antioxidant activity which is correlate with the presence of phenolic and flavonoid quantity in individual plant. Selected twelve plants are *Solanum indicum Linn.* (Brihati), *Asparagus racemosusWilld* (Shatavari), *Rauwolfia sepentinaBenth* (Sarpagandha), *Boerhavia diffusaLinn* (Punarnava), Acorus calamusLinn. (Vach), *Aloe vera Linn* (Aleovera), Trigonella foenumgraecumLinn (Methi), Solanum nigrumLinn. (Kakmachi),1q2we43 Sida cordifolia Linn (Bala), *Datura metel Linn*. (Dhatura), *Cyperus rotundusLinn.* (Mustak), *Phyllanthus urinaria Linn*. (Bhuiamlaki). Selected plants are chosen due to have maximum therapeutic dose and usually used in making formulation in a way of single or combination pattern in Ayurveda science. Total phenolic content (TPC), Total flavonoid content (TFC) and different scavenging methods are studied for checking anti-oxidant activity. Anti-oxidant activity prevents the oxidative cellular damage which is control by free radicals. Besides oxidative damage, cardiovascular disorder, inflammation, neuro-disorder etc are cured by these medicinal plants. Besides phenolic and flavonoid component, other phyto-compounds are also present in these plant extract which are also responsible different pharmacological activity. TPC is expressed by mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of extract and TFC is expressed by mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of extract. Antioxidant activity is measured by H_2O_2 scavenging method, DPPH and ABTS method. Ascorbic acid is used as a standard of H_2O_2 and DPPH method. Result of plant sample using ABTS method is expressed as mmolTrolox equivalents/g extract. There is a correlation between phenolic, flavonoid compounds and antioxidant properties. Among the mentioned twelve medicinal plants, *Cyperus rotundus Linn.* (Mustak), *Boerhavia diffusa Linn* (Punarnava) show highest antioxidant activity. Other plants are also showed antioxidant activity. These plants have other clinical importance also.
Keywords: Antioxidant, Phytochemical analysis, Medicinal plants, Total phenolic content (TPC), Total flavonoid content (TFC).

INTRODUCTION

Many years ago, medicinal plants are very valuable due to present different important properties[1]. Among these properties, anti-oxidant is one of the most important properties in medicinal field. Free radical can cause to create several disorders in human body[2]. Free radicals are responsible for damage of cellular bio-molecules like nucleic acids, proteins, lipids, and carbohydrates[3]. Basically free radicals cause the oxidative damage that is prevented by anti-oxidant property of plant [1]. Free radicals help to formation of reactive oxygen species (ROS). The generated ROS is detoxified by the antioxidants which is present in body[1]. Free radicals release due to the environmental pollutants, various chemicals, toxins, deep fried and spicy food, physical stress etc[2]. Free radicals are produced by the most common popular oxidation process. Catalase and hydroperoxidase help to conversion of peroxide and hydroperoxide to nonradicals forms that play a natural antioxidant role in human body[2].

Large amount of antioxidants is present in many medicinal plants such as polyphenol which play a crucial role in chemisorptions and abrogating free radicals, quenching singlet and triplet oxygen, breaking down of peroxides[4]. Therefore, there is an important issue that which antioxidant compounds are responsible. To identify the antioxidants is still remaining unclear[4]. Some review study support that polyphenol show the antioxidant activity via free radical scavenging and inhibition of lipid peroxidation. This phenolic compound show the antioxidant activity just because due to present their redox properties which interact and play as a reducing agent, hydrogen donors, metal chelators[5]. Now a day's antioxidants are synthesized such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which is exclusively added into foodstuff but there is a doubt about this product toxicity[5]. That's why medicinal plants are used for curing human disease. Few studies are communicated with plant based pharmacological activity. Flavonoid and phenolic compounds are related to plant antioxidant activity[6]. The objective of this study was to determine the total phenolic and flavonoid content and the antioxidant activity of hydroalcoholic extract of selected twelve medicinal plants such as *Solanum indicum Linn.* (Brihati), *Asparagus racemosus Willd* (Shatavari), *Rauwolfia sepentina Benth* (Sarpagandha), *Boerhavia diffusa Linn* (Punarnava),

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Acorus calamus Linn. (Vach), *Aloe vera Linn* (Aleovera), Trigonella foenumgraecum Linn (Methi), Solanum nigrum Linn. (Kakmachi), Sida cordifolia Linn (Bala), *Datura metel Linn*. (Dhatura), *Cyperus rotundus Linn.* (Mustak), *Phyllanthus urinaria Linn*. (Bhuiamlaki).

Solanum indicum Linn. is known as 'Brihati' local name. It has potential therapeutic efficiency due to presence of different active compound. Acetic acid, tartaric acid, malic acid and citric acid are present in this plants as a major organic acid[7]. This plant can survive in stress condition due to presence of Tartaric acid and citric acid [7]. Not only this component, glycol-alkaloid is also found in high concentration. The maturity of the tree also increases its level in solanine[7]. it is prescribed for inflammatory disease, liver disease, pain relief, urine infection, ulcerogenic etc diseases.

Asparagus racemosus Willd (Shatavari) contain sapogenins and saponins in rich quantity. Mainly steroidal saponins are present[8]. it is prescribe for the treatment of diabetic disorder, inflammatory disease, rheumatoid arthritis, neurogenic disorder etc.

Rauwolfia sepentina Benth (Sarpagandha) is a plant source of Vit mainly ascorbic acid and others are riboflavin, thiamine, and niacin. This plant contains phenols, flavonoids, alkaloids tannin[9]. Besides this component, some macroelements such as calcium, phosphorus, magnesium, potassium, sodium are available and few microelements like as zinc, iron are found in this plants [9]. it is good for the treatment of high blood pressure, stomach pain, liver pain, diarrhea etc.

Flavonoids, alkaloids, steroids, triterpenoids, lipid, lignins, carbohydrates, proteins, and glycoproteins are found in *BoerhaviadiffusaLinn* (Punarnava)[10]. Arachidic acid, β-Sitosterol, α-2-sitosterol, palmitic acid, ester of β-sitosterol, tetracosanoic, hexacosonoic, stearic, urosilic acid, Hentriacontane, β-Ecdysone, triacontanoletc are available in the plant[10]. It used for the treatment of reproductive problems, respiratory problem, gastrointestinal problems, live disease and cardiac disorder.

Phytochemically importance of Acorus calamusLinn. (Vach) is due to present of glycosides, flavonoids, saponins, tannin, polyphenolic compounds, mucilage, and volatile oil. Glucosides, alkaloid, and essential oil containing calamen, clamenol, calameon, asaroneandsesquiterpenes[11]. It also contains one type of bitter glycoside such as acorine along with eugenol, pinene and camphene[11]. It is prescribe neurogenic disorder, cardiac disorder, diarrhea, bacterial disorder, urinal disorder etc.

Aloe vera Linn (Aleovera) is medicinally important due to presence of various phytocompound and show different medicinal activity[12]. Vit A, C, E and also contain B12, folic acid, choline. Calcium, Chromium, copper, selenium, magnesium, manganese, potassium, sodium, and zinc are present in this plant. This plants provide steroids such as cholesterol, campesterol, βsisosterol and lupeol[12]. It is prescribed for the treatment of tumour, inflammatory diseases, gastro-intestinal disorder etc.

Phytochemically important one of the plant is Trigonella foenum-graecum Linn (Methi) for having secondary metabolites include saponins, flavonoids, alkaloids and also present VitB1, iron, silicon, sodium, protein, amino acids, fatty acids[13]. It is good for the treatment of inflammatory diseases, fungal disease, live disease, carcinogenic pathogenesis etc.

Main phytocomponents of Solanum nigrum Linn. (Kakmachi) are alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins & phytosterols[14]. It is prescribed for the treatment of inflammation, liver disease, microbial disease, ulcer etc.

Various literatures are reported that above mention phytocompound are also present in Sida cordifolia Linn (Bala); it is good for the treatment of neurogenic disease, inflammatory disease, wound healing etc, *Datura metel Linn*. (Dhatura); it is good for the treatment microbial disease, pain relief, wound healing etc, *Cyperus rotundus Linn.* (Mustak); it is good for GI tract anf prevent the gastrointestinal disorder, prescribe for any kind of pain etc other diseases also, *Phyllanthus urinaria Linn*. (Bhuiamlaki); it is prescribed for the treatment of diabetics, liver disease, pain relief etc. Phytocompounds are responsible for showing different properties[15-17].

The aim of the present study to find out the more medically potential plant among the selected plants on the basis of antioxidant activities and what kind of relationship is conducted between TPC, TFC and antioxidant. Antioxidant potential can prevent oxidative damage of cell and help to reduce oxidative stress. From the review and literature study, little information of the above mentioned plants is given below including plants local name, scientific name, which parts are generally used for research purpose and clinical purpose, and their Ayurvedic indication, chemical compound, and pharmacological action.

Table 1: Information of plants along with pharmacological action.

Sl No	Name	Scientific Name & Family	Parts used	Ayurvedic indication	Chemically Compound	Pharmacological action
1	**Brihati**	*Solanum indicum* Linn. & Solanaceae	Leaf, root, whole plant	Kasa, Swas, Shoth Jvara, Chhardi, Kandu, , Krimi rogas,.	gentisic acid, luteolin, apigenin, kaempferol, and m-coumaric acid glycoalkaloids, steroidal oligoglycosides,	Hepato-protective, anti-tumor, cytostatic, anti-ulcerogenic and anti-inflammatory, antioxidant, anti-cardiac disorder etc.

					pregnane saponins, non-saponins.	
2	**Shatavari**	*Asparagus racemosus*Willd & Liliaceae	Leaf, roots	Amlapitta, general weakness, Raktapitta, Arsha, Atisar, Gulma,Stanyakasay & Shukrakshaya.	sapogenins and saponins, kaempferol, rutin.	Anti-diabetic retinopathy, anti-inflammatory, anti-rheumatoid, neuropathy etc.
3	**Sarpagandha**	*Rauwolfia sepentina Benth* & Apocynaceae	Leaf & roots	Raktachap, Unmad, Anindra, Bhrama, Rakatasamdrak & Krimi roga.	Vit, Phenols, Flavonoids, alkaloids.	Treatment in high blood pressure, Relief stomach pain, liver pain, dysentery.
4	**Punarnava**	*Boerhavia diffusa*Linn & Nyctaginaceae	Leaf	Shotha, Pandu, Hridroga, Mutra roga,Krimi,Raktapitta.	rotenoids, flavonoids, xanthones, purine nucleoside, lignans, and steroids, alkaloids.	Anti-reproductive problem, anti-respiratory problems, anti-gastrointestinal disorder, anti-respiratory disorder, hepatoprotective, anti-cancer, anti-cardiac disorder.
5	**Vach**	*Acorus calamus* Linn. & Araceae	Leaf	Unmad, Jvara, Apasmara, hiccough, & speech disorders, Hoarsness of voice, Anindra	*β*-asarone, glycosides, flavonoids, saponins, tannin.	sedative, CNS depressant, behavior modifying, anticonvulsant, acetylcholinesterase inhibitory, memory enhancing, anti-inflammatory, antioxidant, antispasmodic, cardiovascular, hypolipidemic, immunosuppressive, cytoprotective, antidiarrheal, antimicrobial, anthelmintic, insecticidal, adulticidal, diuretic, antioxidant, genotoxic, and mutagenic activities.
6	**Aleo vera**	*Aloe vera*Linn & Liliaceae	Leaf	Twak roga, Gulma, Varna, Grandhi, vivantha,krimi, udar & skin rogas,	Vit-A, Vit-C, Vit-F, Vit-B_{12}, Vit-B_2, anthraquinones, sterols, salicylic acid, folic acid, cholesterol, campesterol.	Antioxidant, antitumor, anti-inflammatory, hypoglycemic, hypolipidaemic, anti-mutagenic, gastroprotective activity.
7	**Methi**	*Trigonella foenumgraecum* Linn & Leguminosae	Leaf & seeds	Prameha, Jvara, Krimi, Sula & medoroga.	N,N'-dicarbazyl, glycerol monopalmitate, stearic acid, beta-sitosteryl glucopyranoside, ethyl-alpha-D-glucopyranoside, D-3-O-methyl-chiroinsitol and sucrose, saponins, flavonoids, alkaloids.	hypocholesterolemic, antilipidemia, antioxidant, hepatoprotective, anti-inflammatory, antibacterial, antifungal, antiulcer, antilithigenic, anticarcinogenic.

8	**Kakamachi**	*Solanum nigrum* Linn & Solanaceae	Leaf, seed & whole plant	Agnimanda, Krimi, Hridroga, Prameha, Arsha, Hikka, Chardi,Netraroga.	gentisic acid, luteolin, apigenin, kaempferol, and *m*-coumaric acid, Glycosides, proteins, carbohydrate.	Anti-oxidant, hepatoprotective, diuretic, anti-inflammatory, anti-ulcerogenic, anti-consulsant.
9	**Bala**	*Sida cordifolia* Linn & Malvaceae	Leaf, Roots & whole plant	Prameha, Raktapitta, Kasa, Vatavayadhi& general weakness, Relief on Head ache, nasal congestion, joint bones pain.	Phytosterol, Lignin, Alkaloid, flavonoid.	CNS depressant, Analgesic and anti-inflammatory, Hypotensive, Hepatoprotective, Anti microbial activity, Wound healing activity etc
10	**Dhatura**	*Datura metel* Linn & Solanaceae	Leaf & seeds	Kustha, Jvara, Vishroga, Krimi, Varna & udarshula.	Alkaloid, Carbohydrate, Protein.	Antioxidant, analgesic, wound healing, analgesic, antipyretic etc.
11	**Mustak**	*Cyperus rotundus* Linn & Cyperaceae	Leaf & Rhizome	Jvara, Krimi, Atisar, Kundu, Kustha.	Furochromnes, monoterpenes, sesquiterpenes, sitosterol, alkaloid, flavonoid, starch.	Analgesic, sedative, gastro-protective etc.
12	**Bhuiamalaki**	*Phyllanthus urinaria* Linn & Euphorbiaceae	Leaf & Whole plant	Agmimandha, Kamala, Pandu, Jwar, prameha, Kasa & Swasa rogas.	lignans, tannins, flavonoids, phenolics, terpenoids, alkaloid.	Anti-diabetic, hepatoprotective, analgesic, anti-pyretic etc.

MATERIALS AND METHODS

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

a. 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, New town, Kolkata.

b. 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, FolinCiocalteau, sodium carbonate, gallic acid, aluminium chloride, potassium acetated, quercetin, phosphate buffer, H_2O_2, ascorbic acid, DPPH, ethanol, ABTS, potassium persulfate, these all are used and purchase from DST-BT granted fund.

c. Instrument: Water bath and UV-VIS Spectrophotometer (UV-2450 makes SHIMADZU) were used for evaluation of this study. Water bath is used for phytochemical evaluation and UV-VIS Spectrophotometer is used for antioxidant, TPC, TFC study.

d. Preparation of hydro-alcoholic extract of plant leaf's sample: The plant's leaf samples were collected in fresh condition and then it was washed by sterilized water to remove foreign particle and the collected plant's leaf was surface sterilized by 70% alcohol then samples are allow to dry in air condition at room environment. After that these plant's leaf samples were cut in to small pieces and these are allow to submerge into 70% hot water to stay for an overnight and next day put in 30% of methanol of total solvent volume was added to the previous water-plant's leaf mixture solution and allow to stay for an overnight. After that this was allow to filter through filter paper and take filtrate which is hydro-alcoholic, made up 70:30 ratio of water: methanol and it is stored in refrigerator for doing experiment. The some experiments was performed on this extract such as phytochemicals, total phenolic content (TPC), total flavonoids content (TFC), antioxidant tests via H2O2 scavenging, DPPH scavenging, and ABTS scavenging method.

e. Qualitative phytochemical analysis: The extract was evaluated for the presence of bioactive compounds by using standard methods [18-20].

- **Test for Carbohydrates**

Fehling's test: equal amount of Fehlings A and Fehlings B wad added to the 2ml of plant crude extract and gently shake for good mixing, then it is allowed to boil. After few mins, a brick red precipitate was appeared at the bottom of the test tube which indicate that carbohydrate is present.

Benedict's test: Few drops of Benedict's solution was added to the 2 ml of plant extract and allow boiling, a reddish or brown colour precipitation was appeared which indicate the presence of carbohydrate.

- **Test for Alkaloid**

Dragendroff's test: 1ml of Dragendroff's reagent and 1ml of dilHCl are mixed with 1ml of plant extract and allow to boil and formation of orange red precipitation which indicate the presence of alkaloid.

Mayer's test: 3ml of mayer's reagent and 1ml of dilHCl were mixed with 1ml of plant extract, formation of cream colour precipitated. This indicate the presence of alkaloid.

- **Test for protein**

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

Ninhydrin test: Two drops of freshly prepared 0.2% Ninhydrin reagent was mixed with plant extract and then heated. Development of blue color revealed the presence of proteins, peptides, or amino acids.

- **Test for tannin**

Ferric chloride test: Few drops of 5% ferric chloride (FeCl3) is allowed to react with 2-3 ml of extract and boiled for few mins. Appear deep blue black color indicate 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 mins. Appear white or yellow precipitated indicate that tannin is present.

- **Teat for glycoside:** Extract were hydrolysed with dil hydrochloric acid and then subjected to test for glycosides.

Extract was treated with ferric chloride solution immersed in boiling water for about 5 mins. The mixture was allowed to cool and benzene was added equal volumes of extract. The benzene layer was separated and treated with ammonia layer indicates the presence of glycosides.

f. Estimation of total phenolic content (TPC): Total phenols were estimated by Folin Ciocalteau method [21]. 0.5 ml of sample were mixed with 2.5 ml 10 fold diluted Folin Ciocalteau then added 2 ml of 7.5% sodium carbonate (Na_2CO_3). Then this mixture was allowed up to 30 min dark incubation at room temperature. After incubation, take absorbance at 765 nm against blank. The phenolic content was calculated from standard graph of Gallic acid [Fig1]. The outcome data were expressed as mg/g of Gallic acid equivalents in milligrams per gram (mg GAE/g) of extract.

g. Estimation of total flavonoids content (TFC): Total flavonoids were estimated by the aluminium chloride colorimetric technique[22]. 0.5 ml extract was mixed 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 allowed for 30 min incubation at room temperature. After incubation, absorbance was taken at 415 nm. The flavonoids content was measured by the standard curve of quercetin [Fig 2]. The outcome data were expressed as mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of extract.

h. Antioxidant activity of plant extracts: Antioxidant activity of hydro alcoholic extract was determined by the free radical scavenging methods like as the H2O2 scavenging method, DPPH scavenging method, and ABTS radical cation decolourization assay in this present study.

- **Hydrogen peroxide (H_2O_2) scavenging method**: Antioxidant activity of individual extract was examined by using H_2O_2 method[22]. 0.1ml of sample mixed with 3.4 ml of 0.1 M phosphate buffer and 0.6 ml of 40 mM H_2O_2. This mixture was allowed to incubate 10 mins at room temperature. After incubation, absorbance was taken at λmax 230 nm against blank solution. Ascorbic acid was used as standard [Fig 3]. The percentage scavenging of H_2O_2 was calculated using the equation.

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

- **DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method**: DPPH scavenging activity assay of plant extract was done following by the standard protocol[22]. 0.1 mM DPPH solution in ethanol was prepared. 3 ml of DPPH stock solution was mixed with 1 ml of extract at different concentration and equal volume of ethanol. It was allowed to incubate 30 mins and after incubation absorbance was taken at 517 nm. Antioxidant activity of sample is compared by the standard curve of ascorbic acid [Fig 4]. The DPPH scavenging capacity was represented by the following equation

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

ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolourization assay: antioxidant activity was measured by ABTS protocol[23]. For ABTS assay, working solution was prepared by mixing equal amount of 7.4 mM ABTS and 2.45 mM potassium persulfate and this solution kept 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 mixed with 1.9 ml of ABTS solution and allow to dark incubate 6 mins. 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 mmolTrolox equivalents/g dry extract (mmol TE/g DE).

RESULT AND DISCUSSION

Pharmacognostical method were used for the identification and standardization of collected plants samples. Qualitative like as phytochemicals and quantitative like as Total phenolic compound (TPC), Total flavonoid compound (TFC) and antioxidant activity (H2O2, DPPH, ABTS method) were performed by the prepared hydro-alcoholic extract.

Qualitative phytochemical screening: The phytochemical analysis of twelve medicinal plants are summarized and represented in the table 1. This result helps to reveal that some medically active important compounds 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 *Solanum indicum* Linn (Brihati), *Boerhavia diffusa* Linn (Punarnava), *Trigonella foenumgraecum* Linn (Methi),

Solanum nigrum Linn (Kakmachi), *Sida cordifolia* Linn (Bala), *Datura metel* Linn (Dhatura), *Cyperus rotundus* Linn (Mustak) except *Asparagus racemosus* Willd (Shatavari), *Rauwolfia sepentina Benth* (Sarpagandha), *Acorus calamus* Linn (Vach), *Aloe vera*Linn (Aleovera), *Phyllanthus urinaria* Linn (Bhuiamlaki). These twelve medicinal plants contain glycoside except *Rauwolfia sepentina Benth* (Sarpagandha), *Sida cordifolia* Linn (Bala).

Determination of Total Phenolic Content (TPC): *Phyllanthus urinaria* Linn (Bhuiamlaki), *Solanum indicum* Linn. (Bhrihati) show the highest TPC value among the twelve plants. Data of TPC is represented by (µggallic acid equivalent/mg of extract). 219.15 & 207.45 µggallic acid equivalent/mg of extract are the TPC of *Phyllanthus urinaria* Linn (Bhuiamlaki), *Solanum indicum* Linn. (Brihati) respectively. TPC value of these plants like Bala (115.51 µg gallic acid equivalent/mg of extract), Punarnava (113.69 µg gallic acid equivalent/mg of extract), Sarpagandha (128.13 µg gallic acid equivalent/mg of extract), Mustak (89.71 µg gallic acid equivalent/mg of extract), Satavari (66.30 µg gallic acid equivalent/mg of extract) Dhatura (61.98 µg gallic acid equivalent/mg of extract) is toward the high range. TPC of all plants are represented through the graphical diagram [Fig 5].

Determination of Tota Flavonoid Content (TFC): *Solanum indicum* Linn. (Bhrihati), *Solanum nigrum* Linn (Kakmachi) show the highest TFC value among the twelve plants. Data of TFC is represented by (µg quercetin equivalent/mg of extract). 289.56 & 215.83 µg quercetin equivalent/mg of extract are the TFC of *Solanum indicum* Linn. (Brihati), *Solanum nigrum* Linn. (Kakmachi) respectively. Besides these plants, Punarnava (69.86 µg quercetin equivalent/mg of extract), Dhatura (32.57 µg quercetin equivalent/mg of extract), Vach (22.29 µg quercetin equivalent/mg of extract), Sarpagandha (16.82 µg quercetin equivalent/mg of extract) show high range TFC value comparatively other plants. TFC of all plants are represented through the graphical diagram [Fig 6].

Table 2 represent the summary of TPC and TFC of twelve medicinal plants.

Antioxidant properties: Antioxidant properties of twelve medicinal plants are tested by three scavenging method, H_2O_2 scavenging method, DPPH method, ABTS methods (Fig 7, 8, 9). Free radical molecules are generated during various metabolism process. The antioxidant scavenged the free radicals and protect from the cell damage. IC50 value of extract on the basis of antioxidant test help to evaluated antioxidant property which is defines as how much concentration is needed to decrease of initial concentration of H_2O_2, DPPH, ABTS active radicals up to 50%.

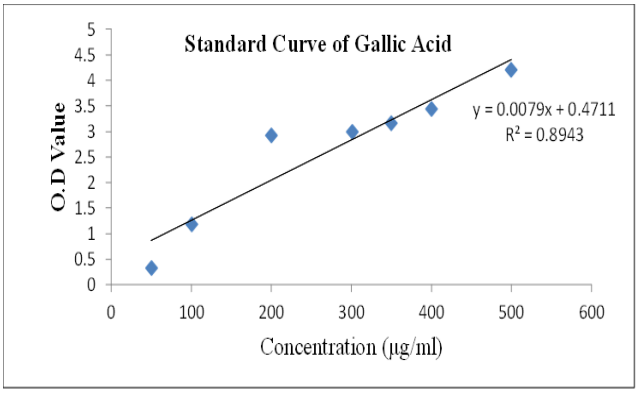


Fig 1: Standard curve of Gallic acid for TPC

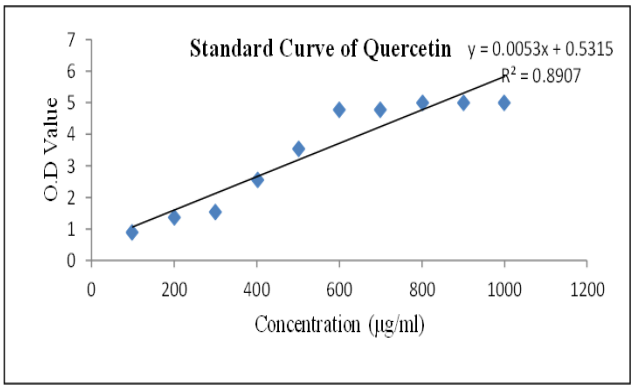


Fig 2: Standard curve of Quercetin for TFC

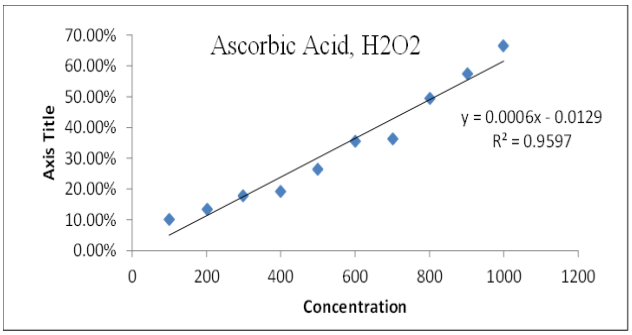


Fig 3: Standard curve of ascorbic acid of H_2O_2 scavenging method

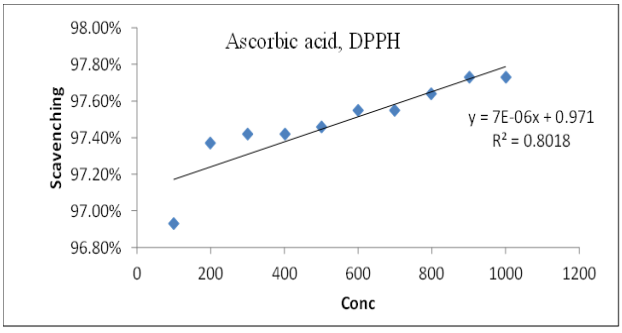


Fig 4: Standard curve of ascorbic acid of DPPH scavenging method.

Fig 5: Estimation of TPC of twelve medicinal plants.

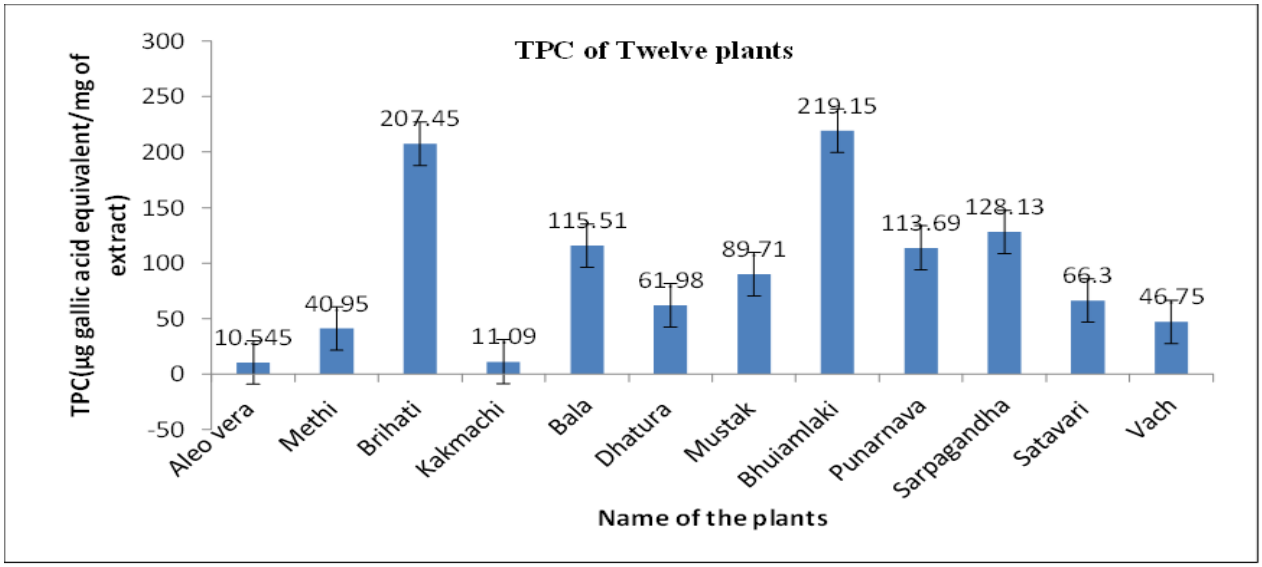
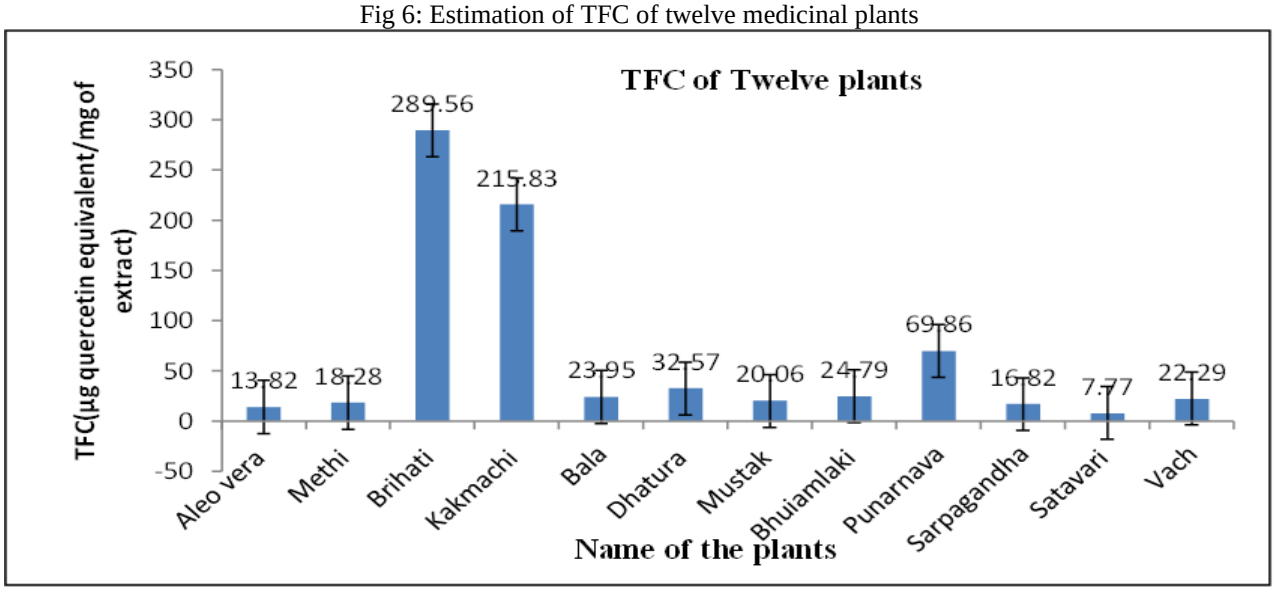


Fig 6: Estimation of TFC of twelve medicinal plants



The three IC50 value of the extract of **Brihati** are 52.91 mg/ml, 53.46 mg/ml, and 51.38 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Brihati show their better antioxidant activity through ABTS method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Satavari** are 50.96 mg/ml, 53.64 mg/ml, and 50.47 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Satavari show their better antioxidant activity through ABTS method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Sarpagandha** are 50.64 mg/ml, 68.81 mg/ml, and 53.52 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Sarpagandha show their better antioxidant activity through H2O2 method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Punarnava** are 53.11 mg/ml, 50.11 mg/ml, and 49.86 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Punarnava show their better antioxidant activity through ABTS method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Vach** are 50.38 mg/ml, 59.35 mg/ml, and 50.26 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Vach show their better antioxidant activity through ABTS method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Aleo Vera** are 53.04 mg/ml, 53.48 mg/ml, and 53.63 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of **Aleo** Vera show their better antioxidant activity through H2O2 method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Methi** are 52.55 mg/ml, 52.32 mg/ml, and 55.49 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Methi show their better antioxidant activity through DPPH method with low IC50 value which is comparable to other two methods.

Fig 7: Estimation of antioxidant potentiality of twelve plant by H2O2 method (Y axis = Scavenging inhibition percentage, X axis = Concentration of Plant extract) [A- Brihati, B- Satavari, C-Sarpagandha, D-Punarnava, E-Vach, F-Aleo vera, G-Methi, H-Kakmachi, I-Bala, J-Dhatura, K-Mustak, L- Bhuiamlaki]

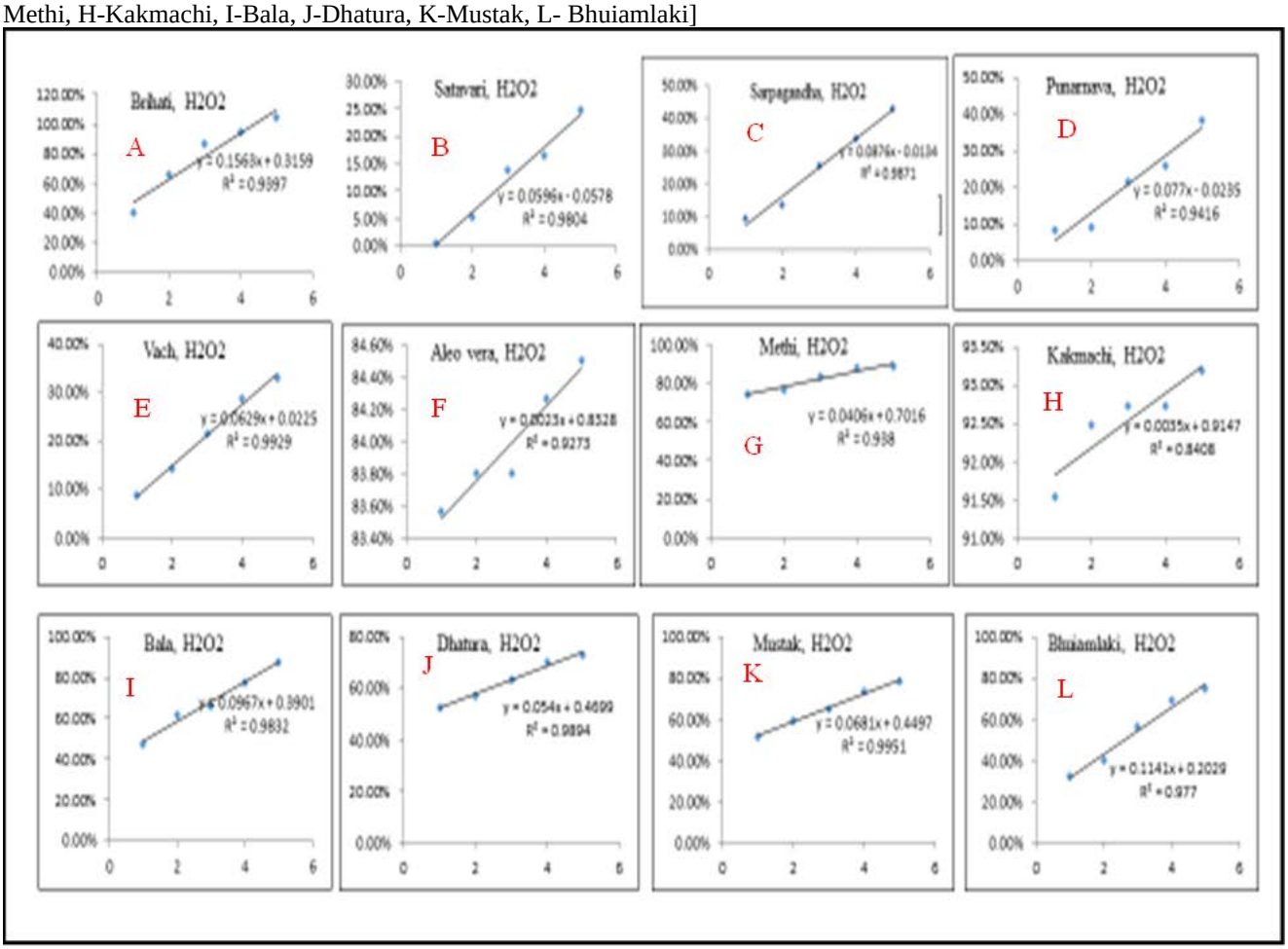


Table 1: Phytochemicals analysis of twelve mention medicinal plants

Plant Name	Carbohydrate		Alkaloid		Protein and amino acid		Tannin		Glycoside
	Felhings	Benedict	D.D	Mayer's	Biuret	Ninhydrin	$FeCl_3$	Lead acetate	
Brihati	+	+	+	-	-	+	+	+	+
Satavari	+	+	-	+	-	-	-	+	+
Sarpagandha	+	+	+	+	-	-	+	+	-
Punarnava	+	+	+	+	-	+	+	+	+
Vach	+	+	+	+	-	-	-	+	+
Aleovera	+	+	+	+	-	-	-	+	+
Methi	+	-	-	+	-	+	-	+	+
Kakmachi	-	+	+	+	-	+	-	+	+
Bala	+	+	+	+	-	+	+	+	-
Dhatura	+	+	+	+	-	+	-	+	+
Mustak	+	+	-	+	-	+	+	+	+
Bhuiamlaki	+	+	+	+	-	-	+	+	+

Table 2: TPC and TFC of twelve medicinal plants

Plants Name	TPC (µg gallic acid equivalent/mg of extract)	TFC(µg quercetin equivalent/mg of extract)
Aleovera	10.545	13.82

Methi	40.95	18.28
Brihati	207.45	289.56
Kakmachi	11.09	215.83
Bala	115.51	23.95
Dhatura	61.98	32.57
Mustak	89.71	20.06
Bhuiamlaki	219.15	24.79
Punarnava	113.69	69.86
Sarpagandha	128.13	16.82
Satavari	66.30	7.77
Vach	46.75	22.29

Table 3: Comparative study of anti-oxidant of twelve plants on the basis of IC50 value by three scavenging method.

Plants Name	**IC50 (mg/ml)**		**IC50 (mmol TE/g DE)**
	H2O2 Method	DPPH Method	ABTS Method
Brihati	52.91	53.46	51.38
Satavari	50.96	53.64	50.47
Sarpagandha	50.64	68.81	53.52
Punarnava	53.11	50.11	49.86
Vach	50.38	59.35	50.26
Aleo Vera	53.04	53.48	53.63
Methi	52.55	52.32	55.49
Kakmachi	58.43	50.93	51.05
Bala	50.46	79.09	56.14
Dhatura	50.08	50.13	55.31
Mustak	49.8	66.08	51.77
Bhuiamlaki	50.97	55.35	51.61

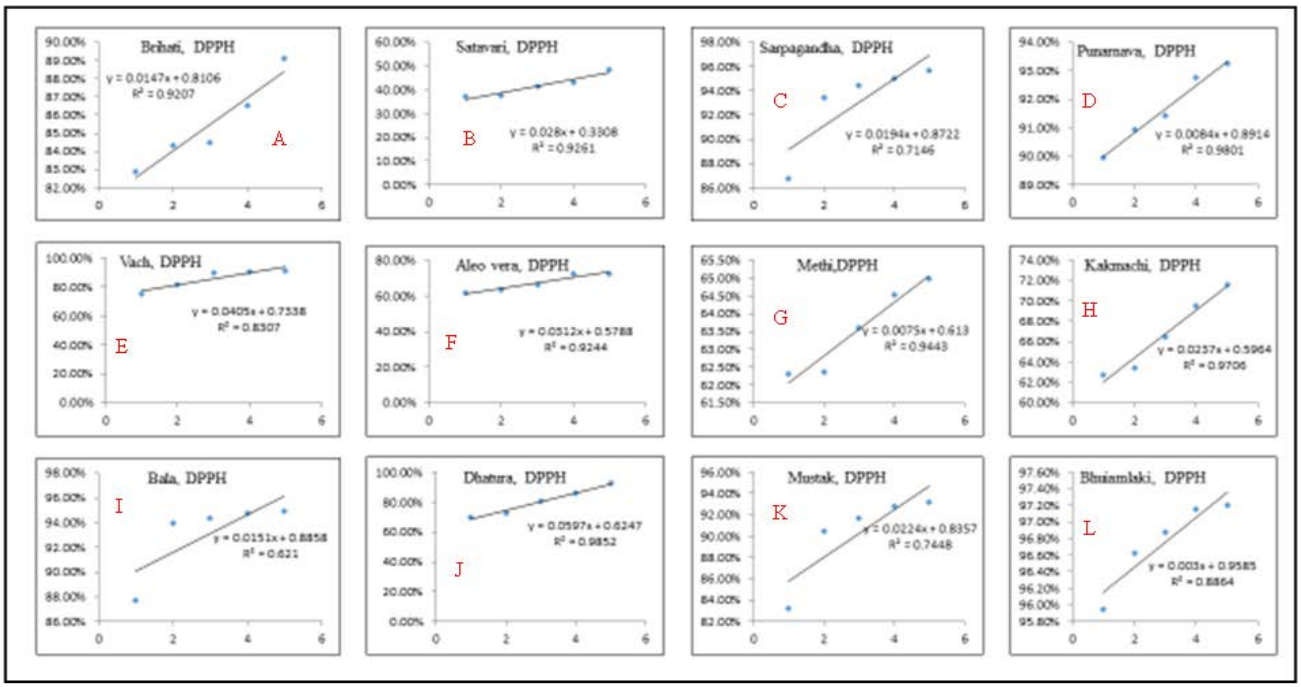


Fig 8: Estimation of antioxidant potentiality of twelve plant by DPPH method (Y axis = Scavenging inhibition percentage, X axis = Concentration of Plant extract) [A- Brihati, B- Satavari, C-Sarpagandha, D-Punarnava, E-Vach, F-Aleo vera, G-Methi, H-Kakmachi, I-Bala, J-Dhatura, K-Mustak, L- Bhuiamlaki]

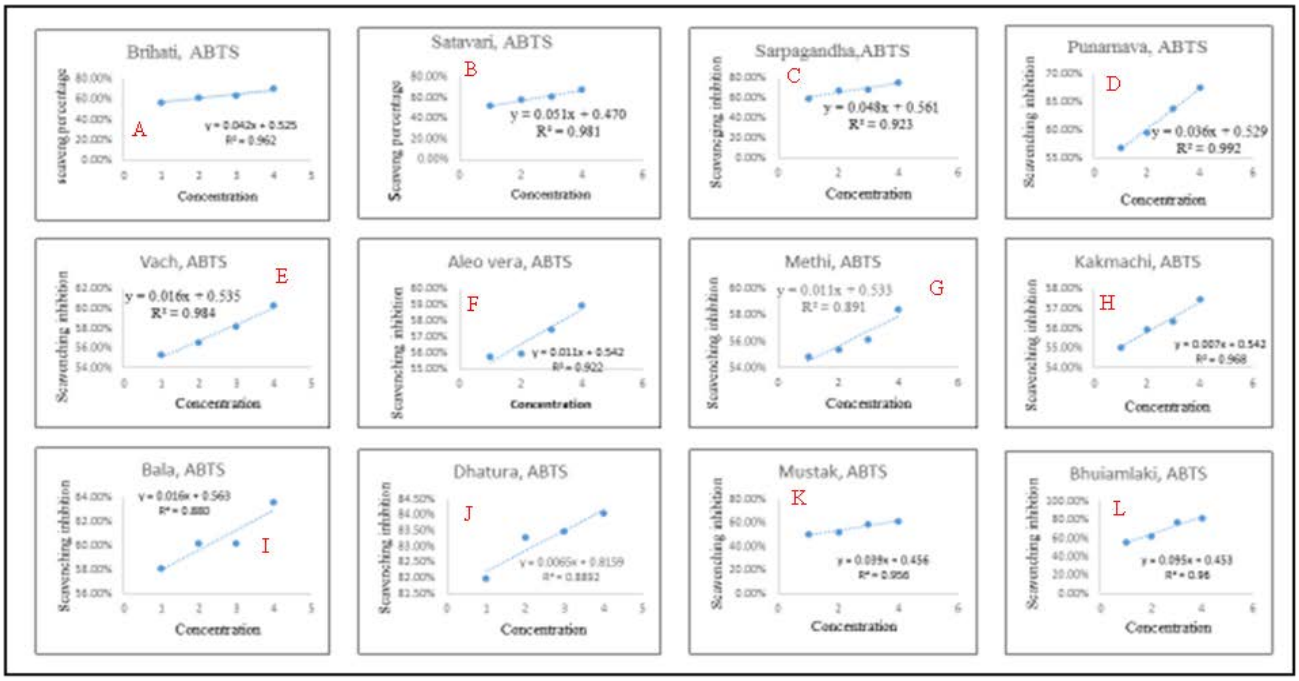


Fig 9: Estimation of antioxidant potentiality of twelve plant by ABTS method (Y axis = Scavenging inhibition percentage, X axis = Concentration of Plant extract) [A- Brihati, B- Satavari, C-Sarpagandha, D-Punarnava, E-Vach, F-Aleo vera, G-Methi, H-Kakmachi, I-Bala, J-Dhatura, K-Mustak, L- Bhuiamlaki]

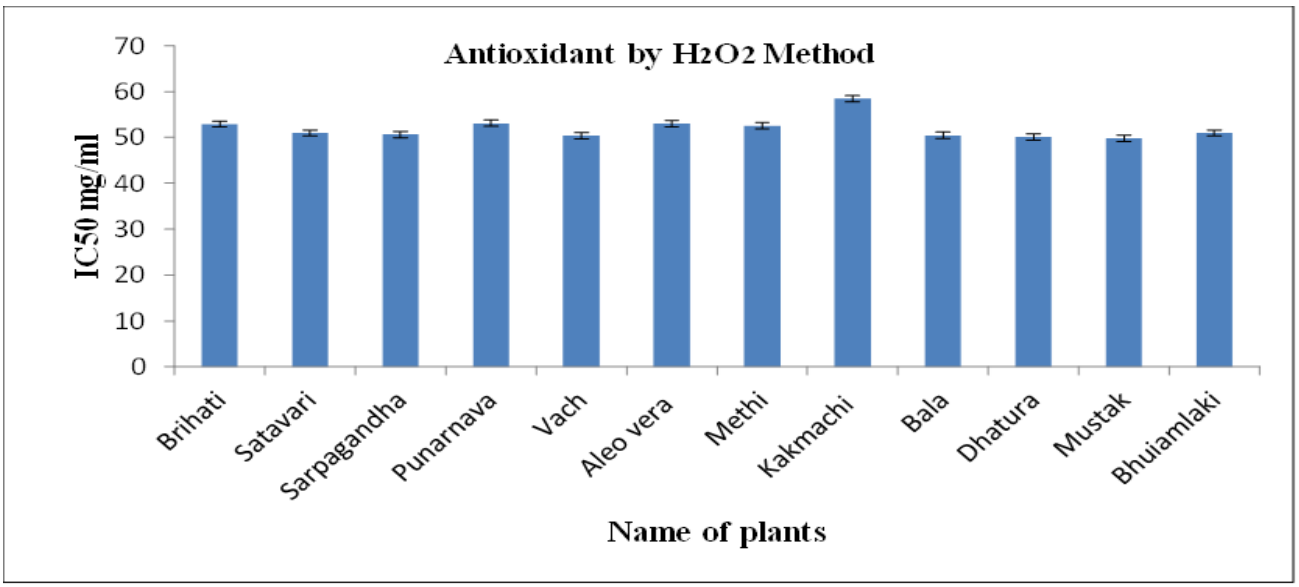


Fig 10: Comparative study of anti-oxidant of twelve medicinal plants by H_2O_2 method on the basis of IC50 values.

The three IC50 value of the extract of **Kakmachi** are 58.43 mg/ml, 50.93 mg/ml, and 51.05 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Kakmachi show their better antioxidant activity through DPPH method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Bala** are 50.46 mg/ml, 79.09 mg/ml, and 56.14 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Bala show their better antioxidant activity through H2O2 method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Dhatura** are 50.08 mg/ml, 50.13 mg/ml, and 55.31 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Dhatura show their better antioxidant activity through H_2O_2 method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Mustak** are 49.8 mg/ml, 66.08 mg/ml, and 51.77 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Mustak show their better antioxidant activity through H2O2 method with low IC50 value which is comparable to other two methods.

The three IC50 value of the extract of **Bhuiamlaki** are 50.97 mg/ml, 55.35 mg/ml, and 51.61 mg/ml of H_2O_2, DPPH, and ABTS method accordingly. This result convey that the plant extract of Bhuiamlaki show their better antioxidant activity through H2O2 method with low IC50 value which is comparable to other two methods.

Cyperus rotundus Linn. (Mustak) show highest antioxidant properties with lowest IC50 value (49.8 mg/ml) through the H_2O_2 method [Fig 10]. *Boerhavia diffusa Linn* (Punarnava) show highest antioxidant properties with lowest IC50 value in two methods such as ABTS (49.86 mg/ml) and DPPH (50.11 mg/ml) method [Fig 11, 12]. Table 3 represent the comparative study of anti-oxidant of twelve plants on the basis of IC50 value by three scavenging method.

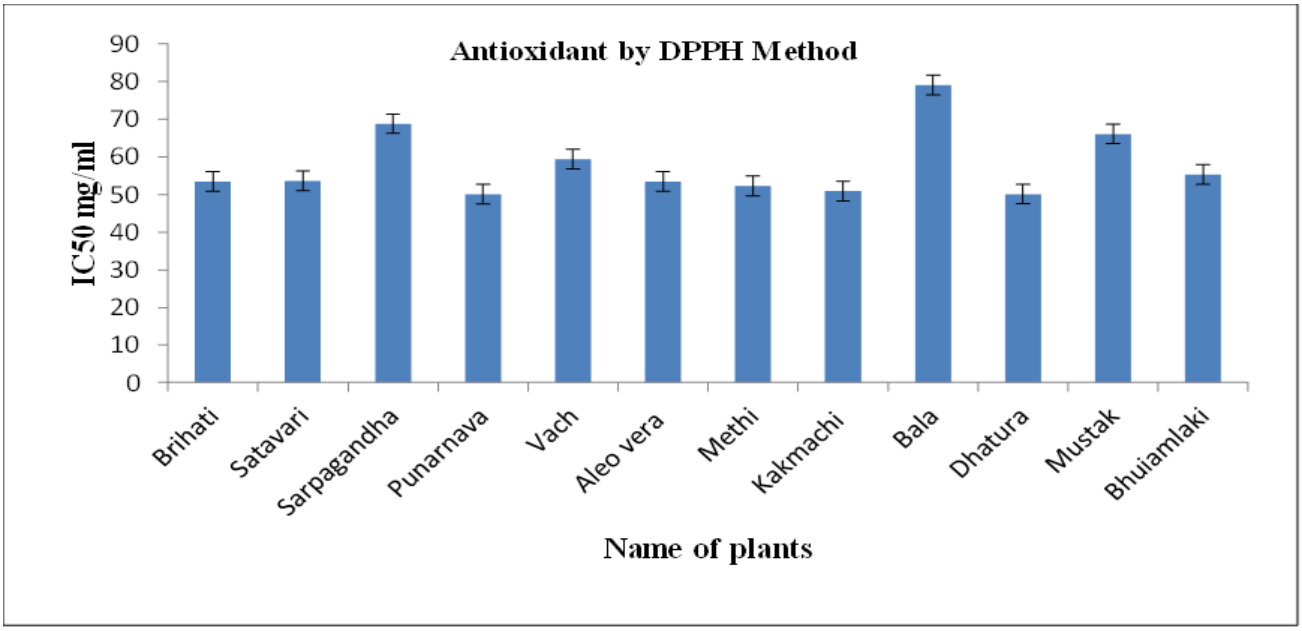


Fig 11: Comparative study of anti-oxidant of twelve medicinal plants by DPPH method on the basis of IC50 values.

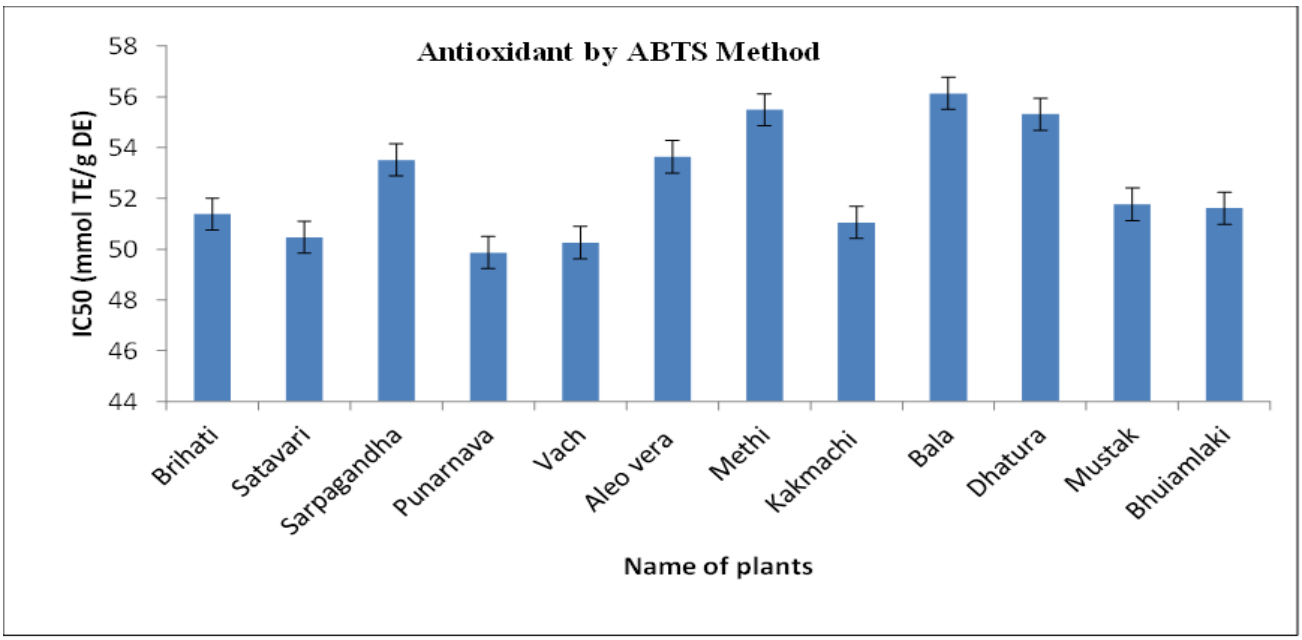


Fig 12: Comparative study of anti-oxidant of twelve medicinal plants by ABTS method on the basis of IC50 values.

According to the H_2O_2 method, a sequence is made on the basis of IC50 value of antioxidant properties, i.e Mustak > Dhatura > Vach > Bala > Sarpagandha > Satavari > Bhuiamlaki > Methi > Brihati > Aleovera > Punarnava > Kakmachi.

According to the DPPH method, a sequence is made on the basis of IC50 value of antioxidant properties, i.e Punarnava > Dhatura > Kakmachi > Methi > Aleovera > Brihati > Satavari > Bhuiamlaki > Vach > Mustak > Sarpagandha > Bala.

According to the ABTS method, a sequence is made on the basis of IC50 value of antioxidant properties, i.e Punarnava > Vach > Satavari > Kakmachi > Brihati > Bhuiamlaki > Mustak > Sarpagandha > Aleovera > Dhatura > Methi > Bala.

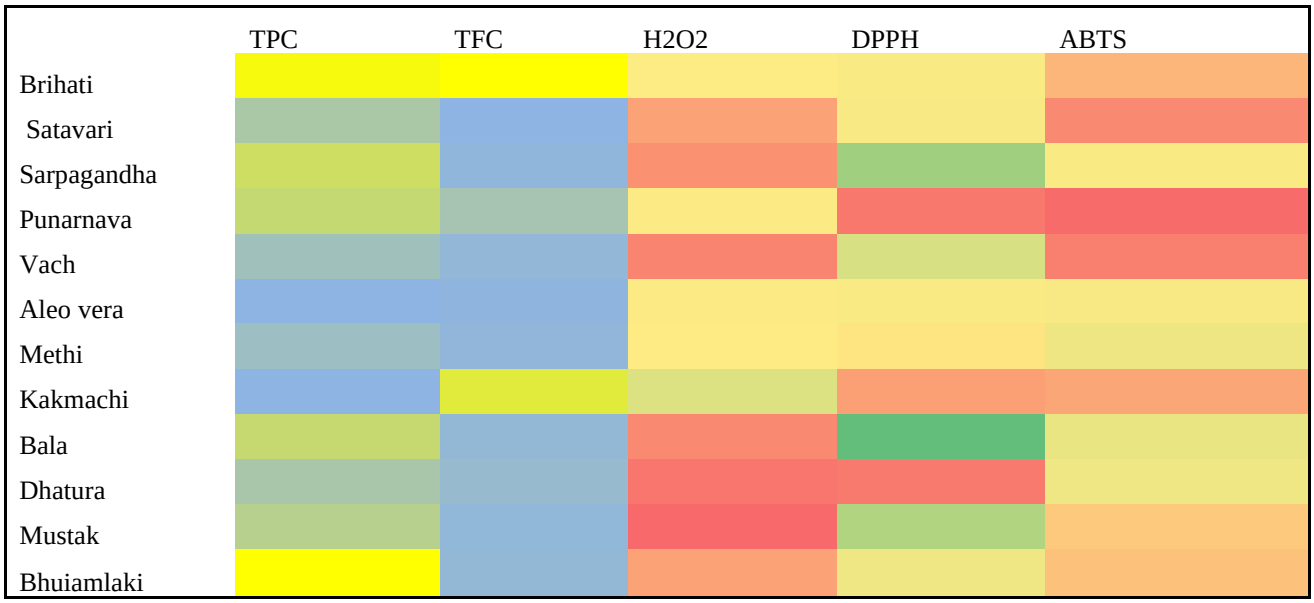


Fig 13: Heatmap of the polyphenolic profiles and antioxidants activities of twelve medicinal plants. In respect of TPC, TFC, yellow represent high value, blue represent low value. In respect of antioxidant activity by H2O2, DPPH, ABTS method, orange represent high activity, yellow represent medium activity and green represent low activity.

Heatmap clustering: Heatmap is a correlative study of twelve medicinal plants between TPC, TFC and antioxidant activities (Fig 13). Many plant natural components regulate the antioxidant activity. Mainly phenolic compounds are more responsible for antioxidant properties. Besides phenolic compounds, flavonooid may be responsible for anti-oxidant but comparatively less than phenolic.

DISCUSSION

Phytochemical analysis can have revealed that which components are responsible for their medicinal as well as physiological activities[24]. Phytochemical analysis of plant extract indicates that carbohydrate, alkaloid, protein & amino acid, tannin, glycoside is present.

Polyphenolic compound is plant component which is responsible for showing various properties. Polyphenolic group is categorized into different compound such as phenols and flavonoid group. Among the polyphenolic group, phenolic and flavonoid group are responsible for showing antioxidant properties[24]. Besides these two groups, other group of polyphenolic compounds are also provide different type of properties. Above mentioned twelve plants are equally importance in medicinal background due to presence of various active natural component like as tannin, flavonoids, alkaloid, different types of protein, glycosides etc. These natural active compounds are involved in different plant metabolism. During protein synthesis, tannin binds with proline rich region and interfere the reaction of protein synthesis[24]. Sometimes flavonoids act as an antimicrobial agent indirectly by hydrolyzed the phenolic compounds which interact with microbial agent that is cause of infection [24]. Alkaloid is also play an important role in human disease[25]. Mainly alkaloid can control neurodegerative disease like as Alzheimer, Parkinson's, Huntington etc disease. Glycosides like as other component play important medicinal role in antioxidant, anti-inflammatory, apoptosis, neuro-disease, diabetes, and cardiac vascular system[26]. These twelve plants play role in various field like as digestion, reproductive system, cardiac system, nervous system, anti-inflammatory, wound healing, anti-cancer, anti-tumour, hepato-protective etc. Presence of various phyto-compounds may be responsible for showing this kind of medicinal properties. Anti-oxidant is also related to the phtocompounds which are presence in plants and anti-oxidant help in prevention of aging, obesity and sometimes in cancer. It is also maintain proper metabolism and health is maintained in good condition.

Antioxidant activity can evaluated by the IC50 value. IC50 value is negatively correlated with antioxidant property. Plant have low IC50 value that is indicate that high antioxidant activity. IC50 value is explained by a certain concentration of plant extract. Requisite of plant extract is such needed that aids to enable at least 50% amount reduction of initial concentration of DPPH and ABTS+ active radicals. From the above result mustak and punarnava show highest antioxidant property who's TPC and TFC value falls in the vicinity of high range not exact highest value. So, from this result, it is concluded that phenolic and flavonoids compound may be influenced the antioxidant activity. But not only phenolic and flavonoids compound are responsible, other phytochemicals are also responsible either individually or synergistically. For this reason, antioxidant activity of Kakmachi is low but its TFC is high and TPC is low through the H2O2 method. On the

other hand, in DPPH method, antioxidant activity of Sarpagandha is low but it's TFC value is low and TPC value is high. In ABTS method, Bala show low antioxidant activity with high range of TPC value and low range of TFC value. So, this result can help to conclude that not only TPC and TFC is responsible for antioxidant activity. Other various phyto-compounds which are present in plants, may be influenced the antioxidant activity. Besides these two plants like as Punarnava and mustak, other plants also have good potentiality of antioxidant property. These plants are recommended as a good natural resources for different kind of disease treatment and maintain health in good condition, can relief from the oxidative stress. These plants are good resources for the new herbal drug development against various health issues due to having various medicinal property like as antioxidant activity, anti-inflammatory activity and other different kind of clinical properties.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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