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# EVALUATION OF ANTIOXIDANT PROPERTY OF SELECTED REGIONAL MEDICINAL PLANTS USING DPPH, ABTS AND $H_2O_2$ AND TPC & TFC METHODS

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**Keywords:**

Medicinal plants, Phytochemical, Antioxidant test, TPC, TFC

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**ABSTRACT:** The various medicinal plants have been used in the Ayurvedic medicine system to treat various diseases for a long period. The plant shows first-line defense mechanisms by secondary metabolites against different diseases and foreign particles to build immunity. This metabolite are classified as phyto & polyphenolic compound. Antioxidants play an important role in humans due to the presence of this polyphenolic compound and other phyto-compound. Either body metabolism generates free radical as an endogenous source or different pollution, food intake as an exogenous source. Cellular damage is happened by the excessive production of free radicals. Antioxidants arrest the free radicals by reducing their activity. There are nine medicinal plants such as *Leucas cephalotes* Spreng (Dronapushpi), Andrographis paniculata Nees (Kalmegh), Piper longum (Pippali), *Desmodium gangeticum* DC (Shalmali), *Plumbago indica* Linn (Raktachitrak), *Zingiber officinale* Rosc (Adrak), *Calotropis procera* R.Br. (Arka), *Sesbania sesban* Merrill (Jayanti), *Hemidesmus indicus* R. Br (sariva)] selected on the basis of their Ayurvedic property. Phytochemical analysis, Total phenolic content (TPC), total flavonoid Content (TFC) and antioxidant tests *via* $H_2O_2$, DPPH, and ABTS were performed on these selected medicinal plants. The plant samples Raktachitrak, Kalmegh, Adrak give high antioxidant activity based on low IC50 value. Raktachitrak, Adrak show high concentration of TPC, TFC in comparison to Kalmegh and other plants. Besides TPC, TFC, presence of phyto-compound such as tannin, flavonoids, alkaloid are also found in most plant samples which may be responsible for positive test of antioxidant activity. This antioxidant activity of plant samples may be responsible for increasing strength, immunity and digestive power during treatment of various diseases.

**INTRODUCTION:** The Various physiological and biochemical cycles in the human body may lead to producing oxygen-centered free radicals and other by-products belonging to reactive oxygen species [1]. Excessive production of free radicals leads to oxidative damage of biomolecules such as lipids, protein, DNA *etc.* [1].

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Not only that many chronic diseases are also caused by these free radicals [2]. These free radicals are generated from the crucial element oxygen when the ATP production process goes through the mitochondrial [3]. When the availability of these radicals lies at a low or moderate level that is optimum, free radicals play a positive role in the human body.

When it crosses the optimum level, they act as damaging cellular molecules and generate oxidative stress [3]. Free radicals made up unpaired electron in an atomic orbital [4]. Many radicals belong to the highly reactive and unstable stage. They are cable to donate or accept electron from

the neighbour molecule and act as an oxidant or reactant [4]. Antioxidants work as a scavenger of free radicals by neutralizing process [5]. Source of antioxidant is two types exogenous and endogenous. Endogenous source is body metabolism itself. Besides food, tobacco smoking, fossil fuel burning, different pollutants, ionizing radiation, NO, *etc.*, are endogenous [5]. Naturally these free radicals are controlled by plant products. Many aromatic, medicinal, spice and other types of plants are made up chemical compounds with antioxidant properties [6]. The present study was done to find out the level of grade of antioxidant action of nine selected medicinal plants used for a long time in the ayurvedic system. The plant sample of Dronapushpi (*Leucas cephalotes* Spreng) is used for the treatment of headache, loss of appetite, respiratory diseases, skin disease and worms, and having antipyretic, analgesic, anti-inflammatory, anti-rheumatic activity *etc* [7].

Kalmegh (*Andrographis paniculata* Nees) is a very common medicinal plant used as a medicine in Bangladesh, China, India, Pakistan, Malaysia, Indonesia, Philippines, and Thailand to treat hepatic disorders loss of appetite, digestive and worm's manifestation *etc* [8]. The reported pharmacological action of plant kalmegh is as antidiarrheal, anticancer, anti-hepatitis, antioxidant, hepato-protective, immune stimulatory, sexual dysfunctions [9]. Piper longumplant, known as Pippali is mostly used as a spice and has therapeutic activities against abdominal discomfort, gonorrhea, menstrual pain, sleeping problem, respiratory problems, gut-related problems, *etc* [10]. Shalparni (*Desmodium gangeticum* DC) is an important plant in ayurvedic field for making Dasamula Kwatha, and prescribe for the treatment of arthritis, muscular pains, acne, fever, eye diseases, fever, gout, asthma, bronchitis, infections and liver diseases [11]. Raktachitrak (*Plumbago indica* Linn) is a perennial herb used to treat indigestion, loss of appetite, worm's manifestation and having antibacterial, hepatoprotective, wound healing, anti-fertility and anti-hyperlipidaemia pharmacological reported activities [12]. Adrak (*Zingiber officinale* Rosc) is a well-known plant rich in therapeutic potency for the treatment of cough, cold, rhinitis, bronchial asthma, and loss of appetite and headache and as a food spice, mainly native tropical area [13]. Arka is a very useful medicinal plants from primitive period and used for the treatment of wound healing, cough piles, fistula and anorectal diseases, and abdominal pain [14]. Jayanti (*Sesbania sesban* Merrill) plant is a medium-sized shrub used to treat acne, skin rashes, psoriasis, ulcer, gastritis, and worms due to reported pharmacologic activities like wound healing, hepatoprotective, anti-inflammatory, anti-fertility and antidiabetic *etc*. [15]. Another important plant is Sariva (*Hemidesmus indicus* R. Br) for has various therapeutic values and is prescribed for the treatment of blood disorders, worms, skin diseases, itching, and wounds.

The abovementioned selected plants are very common and prescribed since the Ayurvedic system of medicine as a single form or compound form for the treatment of various types of diseases due to the presence of reported different polyphenolic compounds and other important phyto-compounds [16]. This present study's main aim and objective was to find maximum antioxidant activities based on total phenolic and flavonoid compounds in a hydroalcoholic extract of fresh leaf of nine selected plants. The objective was used to prepare the hydroalcoholic extract after authentication and identification to evaluate the presence of phytochemical compounds, total phenolic content (TPC) and total flavonoid content (TFC) and antioxidant activity using Hydrogen peroxide ($H_2O_2$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. The detailed literature information of selected medicinal plants is given below, including plants' local name, scientific name, which parts are generally used for research purposes, and their Ayurveda indication, chemical compound and pharmacological action.

| S. no. | Name of Medicinal Plant | Scientific Name & Family | Parts used | Ayurvedic indications | Chemical compounds | Pharmacological actions |
|---|---|---|---|---|---|---|
| **1** | **Dronapushpi** | *Leucas cephalotes* Spreng & | Leaf & Whole plant | Vishma Jvara, Kamala, Netraroga, | Carbohydrate, glycoside, tannin steroid etc. | Anti-pyretic, analgesic, anti-rheumatic. |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Labiatae | | Arsha. | | |
| **2** | **Kalamegh** | *Andrographis paniculata* Nees. & Acanthaceae | Leaf | Kamla, Krimi, Pandu, Shotha, Varna, Dipana& hepatic disorders. | Andrograpanin, 7-O-methylwogonin, apigenin, onysilin,Carbohydrate, Glycoside, tannin steroid *etc* | Antidiarrheal, anti-hepatitis, hepato-protective, anti-atherosclerotic |
| **3** | **Pippali** | *Piper longum*Linn. & Piperaceae | Leaf, Fruit7 Rootys | Kasa, Swas, Udar, Kustha, Prameha, Gulma, Aamvata rogas | Piperine, Piplatin, Cepharadione, Cepharanone, Carbohydrate, alkaloid, glycoside, tannins *etc* | Antiasthmatic, hepatoprotective, hypocholesterolemic, anti-inflammatory, antiamoebic activity, antibacterial |
| **4** | **Shalparni** | *Desmodium gangeticum* DC. & Leguminosae | Leaf& Roots | Shotha, Joint pains, Krimi, Jvara, and Chardi rogas | Desmodin, Pterocarpans, Alkaloid, sterol, carbohydrate, tannin, flavonoids etc. | antileishmanial, antiasthmatic, smooth muscle relaxant, anti-inflammatory, anti-ulcer, and cardio-protective activities |
| **5** | **Raktachitrak** | *Plumbago indica* Linn. & Plumbaginaceae | Leaf& roots | Loss of appetite, Arsha, Krimi, Rechana, dipan, pachan | Plumbaginin, plumbagin, chitranone, 3-biplumbagin, Alkaloid, Plumbagic acid, glycoside, reducing sugar, simple phenolics, tannin, lignin, saponins *etc* | antibacterial, antifungal, anti-inflammatory, antidiabetic, anticancer, antioxidant, hepatoprotective, cytotoxic and wound healing |
| **6** | **Adrak** | *Zingiber officinale* Rosc. & Zingeberaceae | Leaf& rhizome | Kasa, Jwar, pratishyaya, Hikka, Agnimonda, Varnarogas | Alkaloid, carbohydrate, tannin, phenol, α-zingiberene, 6- gingerol, β-sesquiphellandrene, 6-shogao, β-bisabolene, *etc* | antibacterial, antidiabetic, antiemetic, hypolipidaemic, hepatoprotective Expectorant anti-inflammatory |
| **7** | **Arka** | *Calotropis procera* R.Br. & Asclepiadaceae | Leaf, seeds, flowers & larex | Arsha, shotha, Krimi, Kandu, Gulma, Varna | Carbohydrate, alkaloids, tannin, phytosterol, flavonoid, polysaccharide containing D-arabinose, D-glucose, D-glucosamine and L-rhamnose, 3-proteinase, and α-calotropeol, 3-epimoretenol, gigantin *etc* | Wound healing, antimicrobial, Hepatoprotective, anti-Alzheimer's disease |
| **8** | **Jayanti** | *Sesbania sesban* Merrill & Leguminosae | Leaf, fruits , flowers | Kasa, Kushtha, krimi, Aruchi & Madhumeha. | Triterpenoids, carbohydrates, vitamins, tannin, steroid, saponinsetc. | Wound healing, Hepatoprotective, Antidiabetic, Anti-inflammatory, fertility. Antipyretic |
| **9** | **Sariva** | *Hemidesmus indicus* R. Br. & Asclepiadaceae | Leaf& roots | Kushtha, Kandu, Prameha, Aruchi, Atisar, Agnimanda. | Hemidesminin Carbohydrate, alkaloids, Tannin, β-sitosterol etc | Antibacterial Wound healing, antipyretic, Anti-inflammatory Anti-cataractous activity, anti-diarrhoeal activity, anti-HIV-1 activity |

**MATERIALS AND METHODS:** The pharmacognostic and antioxidant was performed in the laboratory of the department of Dravyaguna, Institute of Post Graduate Ayurvedic Education & Research, Kolkata.

**Collection of Plants:** The plant materials (leaf) were collected from the herbal garden of this Institute, Eco-Park, New town, Kolkata, following the guideline of good agricultural and collection practices (GACP) for medicinal plants. The collected plant leaf of selected nine medicinal plants are identified and authenticated by the Botanical Survey of India, Howrah, Kolkata. Herbarium sheet were prepared of sample for authentication. Authenticate certificate no is - CNH/Tech.II/2020/1 dated- 25.08.2020.

**Chemicals and Reagent:** The chemicals and standard chemical kits such as Methanol, Fehling's A and Fehling's 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, Hydrogen peroxide ($H_2O_2$), ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate are purchased for the experiments from DST-BT granted fund following the Institutional rules.

**Instrument:** This study has been evaluated by the two types of instruments: a water bath used in phytochemical study and UV-VIS Spectrophotometer: UV-2450 makes SHIMADZU, which is used for the determination of total phenolic content (TPC) and total flavonoid content (TFC) and antioxidant activity.

**Preparation of Plant Samples:** Plants materials were collected in fresh condition from the selected field of the medicinal plants garden. Then it was cleaned out through the running water to remove the dirt particles and other unfamiliar particles. After washing, leaves are cut into many small-sized pieces and allowed to dip 70% hot sterilized water overnight and added 30% methanol to the next day and allowed to stay overnight. Next day, this is filtrated through filter paper. The filtrate was put away in the refrigerator for further examination. The experiments like the antioxidant test, total phenol, and total flavonoid test of each plant sample were performed.

**Qualitative Phytochemical Analysis:** The extract was inspected to check the presence of phytocompound by utilizing standard techniques [17, 18, 19].

**Test for Carbohydrates:**

**Fehling's test:** A comparable proportion of Fehling's A and Fehling's B was added to the 2ml of plant crude extract and carefully shaken for extraordinary mixing.

This mixture was allowed to reach boiling point. After two mines, a red brick appeared at the bottom of the test tube which exhibits that carbohydrate is available.

**Benedict's test:** Few drops of Benedict's solution was added to the 2 ml of plant extract and permitted heating up, a reddish or brown colour precipitation was concluded that presence of carbohydrate.

**Test for Alkaloid:**

**Dragendroff's test:** 1ml of Dragendroff's reagent and 1ml of dilHCl are added on 1ml of plant extract and permit to boil and appear the 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 extricate, development of cream-toned precipitation. This is suggested the presence of alkaloid.

**Test for Protein:**

**Biuret test:** 3ml of plant extract was amalgamated with 1ml of 4% sodium hydroxide solution and few drop of 1% copper sulphate solution was infused. After reaction, appearance of violet-pink colour indicated the presence of protein and amino acids.

**Ninhydrin test:** Two drops of freshly prepared 0.2% Ninhydrin reagent was infused with plant extract and then allow to reach boiling temperature. The formation of blue colour revealed the presence of proteins, peptides, or amino acids.

**Test for Tannin:**

**Ferric Chloride test:** Scarcely any drops of 5% ferric chloride ($FeCl_3$) were permitted to respond with 2-3 ml of concentrate and bubbled for not many mines. The formation of deep blue-black colour reveals that tannin is present.

**Lead Acetate test:** Addition of few drops of 10% lead acetate was performed with 2-3 ml of plant extract and allow to reach at boiling point for few mines. Formation of white or yellow precipitated indicate that tannin is present.

**Teat for Glycoside:** The extract of each plant was digested with dilute hydrochloric acid, and then it was planned to test for glycosides. The extract was treated with ferric chloride solution submerged in boiling water for around 5 mines. After cooling, benzene was added at equal volumes of extract to the mixture. The benzene layer was discriminated and treated with ammonia solution. The development of rose pink in the ammonium layer revealed the presence of glycosides.

**Estimation of Total Phenolic Content (TPC):** Total phenols were estimated by Folin Ciocalteaumethod [20]. 0.5ml of the sample were infused with 2.5 ml 10-fold diluted Folin Ciocalteau then the addition of 2 ml of 7.5% sodium carbonate ($Na_2CO_3$). Then this mixture was incubated up to 30 min at room temperature in a dark place. After incubation, take absorbance at 765 nm against blank. The phenolic content was calculated from the standard graph of Gallic acid **Fig. 1**. The outcome data were communicated as mg/g of Gallic acid equivalents in milligrams per gram (mg GAE/g) of extract.

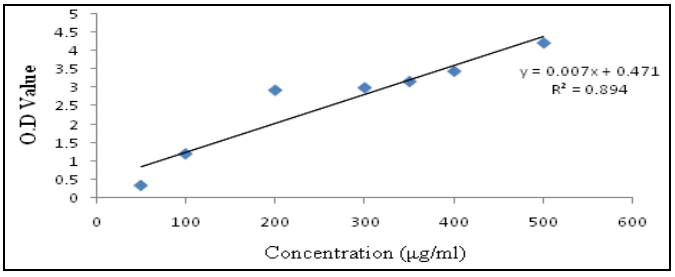



**FIG. 1: STANDARD CURVE OF GALLIC ACID FOR TPC**

**Estimation of Total Flavonoids Content:** Total flavonoids were examined by the aluminum chloride colorimetric technique [20]. 0.5 ml extract was infused with 1.5 ml methanol, 0.1 ml 1% aluminum chloride ($AlCl_3$), 0.1 ml of 1M potassium acetated, and the addition of 2.8 ml distilled water to the mixture. The incubation period of this mixture is around 30 mines at room temperature. After incubation, absorbance was recorded at 415 nm. The calculative data of flavonoid content was purified from the standard curve of quercetin **Fig. 2.** The outcome data were interfaced as mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of extract.

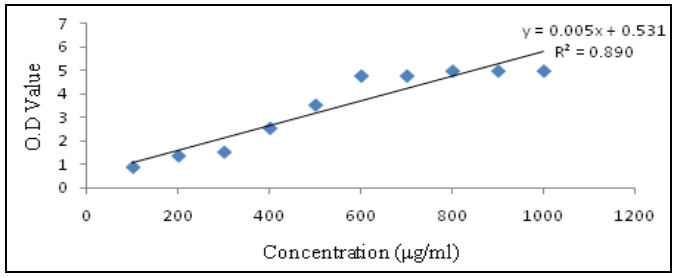



**FIG. 2: STANDARD CURVE OF QUERCETIN FOR TFC**

**Antioxidant Activity of Plant Extracts:** Antioxidant activity of hydroalcoholic extract of each sample was performed by the free radical scavenging methods like the $H_2O_2$ scavenging method, DPPH scavenging method and ABTS radical cation decolorization assay in this present study.

**Hydrogen Peroxide ($H_2O_2$) Scavenging Method:** Antioxidant activity of the individual extract was performed by using $H_2O_2$method [20]. 0.1ml of the sample was amalgamated with 3.4 ml of 0.1 M phosphate buffer and 0.6 ml of 40 mM $H_2O_2$. The incubation period of this mixture is 10 mines at room temperature. After incubation, absorbance was recorded at λmax 230 nm against the blank solution. Ascorbic acid was used as standard **Fig. 3.** The percentage scavenging of $H_2O_2$ was evaluated using the equation.

$$\% \text{ Scavenging of } H_2O_2 = A_O - A_1 / A_O \times 100$$

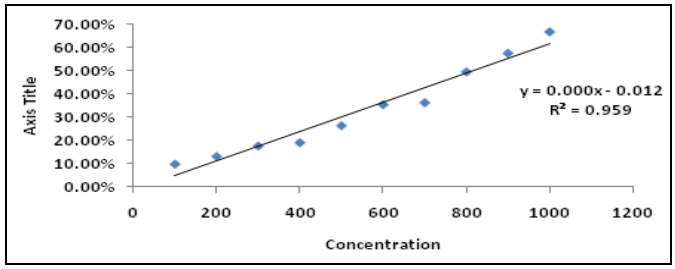



**FIG. 3: STANDARD CURVE OF ASCORBIC ACID OF $H_2O_2$ SCAVENGING METHOD**

**DPPH (2,2-diphenyl-1-picrylhydrazyl) Scavenging Method:** DPPH scavenging activity assay of plant extract was performed following by the standard protocol[20]. Previously 0.1 mM DPPH solution was made in ethanol. 3 ml of DPPH stock solution was infused with 1 ml of extract at different concentrations and added equal ethanol volume. The incubation period is around 30 min, and after incubation, absorbance was recorded at 517 nm. The antioxidant activity of the sample is calculated by the standard curve of ascorbic acid **Fig. 4.** The following equation evaluated the DPPH scavenging capacity.

$$\% \text{ Inhibition} = \text{blank} - \text{sample} / \text{blank} \times 100$$

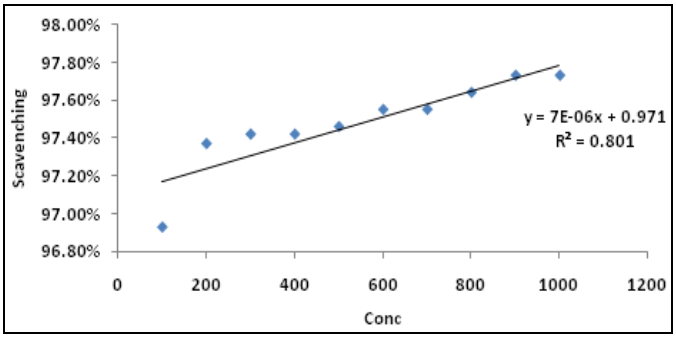



**FIG. 4: STANDARD CURVE OF ASCORBIC ACID OF DPPH SCAVENGING METHOD**

**ABTS (2,2'-azino-bis 3-Ethylbenzothiazoline-6-sulfonic Acid) Radical Cation Decolourization Assay:** Antioxidant activity was performed by ABTS protocol [21]. For ABTS assay, Addition of equal amount of 7.4 mM ABTS and 2.45 mM potassium persulfate were going to prepare working solution that are put away in 12-13 h dark incubation to react and produce active ABTS radical cation which is the reaction solution with plant sample to estimate antioxidant activity. Ethanol was used for making the dilution of ABTS solution. Then, 50µl of samples and 1.9 ml of ABTS solution was poured into a test tube and allowed to dark incubate for 6 mines. After incubation, absorbance was recorded at 734 nm. Sample result was concluded with the help of trolox standard (Y= 0.0379x-0.0015, R2 = 0.9872,). The result was communicated by mmol Trolox equivalents/g dry extract (mmol TE/g DE).

## RESULT:

**Qualitative Phytochemical Screening:** The phytochemical analysis of each plant extract was done to find out the presence of phytocompounds such as alkaloid, tannin, carbohydrate, saponin, and glycosides, *etc*. The result of the phytochemical analysis of selected nine medicinal plant's extract is given in **Table 1**. This outcome can assist with inferring what active compounds are available in the nine plants. This result shows that the plant extract of Dronapushpi, Kalmegh, Pippali, Adrak, and Arka contain carbohydrate, alkaloid, protein & amino acid, tannin, and glycoside *etc*., whereas Shalparni and Raktachitrak contain carbohydrate, alkaloid, tannin and glycoside. It has been observed that the carbohydrate, alkaloid and tannin chemical constituents are present in each of the nine plants, whereas protein and amino acid are absent in only two plant among nine plants, *i.e* Shalparni and Raktachitrak and glycoside is also absent in only one plant i.e Raktachitrak among the nine plants.

**TABLE 1: PHYTOCHEMICALS ANALYSIS OF TWELVE MENTIONED MEDICINAL PLANTS**

| Plant Name | Carbohydrate | | Alkaloid | | Protein and amino acid | | Tannin | | Glycoside |
|---|---|---|---|---|---|---|---|---|---|
| | Fehling's | Benedict | D.D | Mayer's | Biuret | Ninhydrin | $FeCl_3$ | Lead acetate | |
| Dronapushpi | + | + | + | + | - | + | + | + | + |
| Kalmegh | + | + | + | - | - | + | - | + | + |
| Pippali | + | + | + | - | - | + | + | + | + |
| Shalparni | + | + | + | - | - | - | + | + | + |
| Raktachitrak | + | + | + | - | - | - | + | + | - |
| Adrak | + | + | + | + | - | + | + | + | + |
| Arka | + | + | + | + | - | + | + | + | + |
| Jayanti | + | + | + | + | - | + | + | + | + |
| Sariva | + | + | + | + | - | + | + | + | + |

Note: (+) indicates present, (-) indicates absent.

**b. Determination of Total Phenolic Content (TPC):** The TPC was found in highest quantity in plant sample of Sariva among the nine plant samples**.** Unit of TPC is expressed by (µg Gallic

acid equivalent/mg of extract). 452.24 µg Gallic acid equivalent/mg of extract is the TPC of Sariva. Besides sariva plant, Raktachitrak (378.36 µg Gallic acid equivalent/mg of extract), Dronapushpi (194.26 µg Gallic acid equivalent/mg of extract), Shalparni (201.19 µg Gallic acid equivalent/mg of extract), Adrak (105.16 µg Gallic acid equivalent/mg of extract), Arka (107.21 µg Gallic acid equivalent/mg of extract) are also showed high range TPC value which has good impact on antioxidant activity. Not only phenolic compound, other phyto-compounds are also valuable for showing antioxidant properties.

**c. Determination of Total Flavonoid Content (TFC):** The TFC was found in the highest quantity in the plant sample of Raktachitrak among the nine medicinal plants. Unit of TFC is expressed by (µg quercetin equivalent/mg of extract). 152.54µg quercetin equivalent/mg of the extract is the TFC of Raktachitrak. Besides Raktachitrak, TFC value of other some plant among the nine plants are also reached to range of maximum value that can modulate antioxidant properties. TFC value of Shalparni (130.71 µg quercetin equivalent/mg of extract), Dronapushpi (61.89 µg quercetin equivalent/mg of extract), Arka (60.71 µg quercetin equivalent/mg of extract), and Adrak (57.02 µg quercetin equivalent/mg of extract) are towards the high range. **Table 2** is summarized the TPC and TFC value of nine medicinal plants. **Fig. 5** represents the comparable study of TPC and TFC of plants through the graphical diagram.

**TABLE 2: TOTAL PHENOLIC CONTENT (TPC) AND TOTAL FLAVONOID CONTENT (TFC) OF TWELVE MEDICINAL PLANTS**

| Plants | TPC(µgGallic acid equivalent/mg of extract) | TFC(µg quercetin equivalent/mg of extract) |
|---|---|---|
| Dronapushpi | 194.26 | 61.89 |
| Kalmegh | 19.25 | 12.53 |
| Pippali | 136.07 | 19.92 |
| Shalparni | 201.19 | 130.71 |
| Raktachitrak | 378.36 | 152.54 |
| Adrak | 105.16 | 57.02 |
| Arka | 107.21 | 60.71 |
| Jayanti | 80.51 | 37.21 |
| Sariva | 452.24 | 17.06 |

The abovementioned table represents the quantification of the concentration of total phenolic compound (TPC) and total flavonoid compound, which was found present in selected medicinal plants. The hydroalcoholic extract of Raktachitrak, Sariva and Shalparni showed their high level of TPC concentration and simultaneously the hydroalcoholic extract of plant samples Shalparni, Raktachitrak also given high level of TFC concentration among the other medicinal plants which may be directly responsible for their significant antioxidant properties.

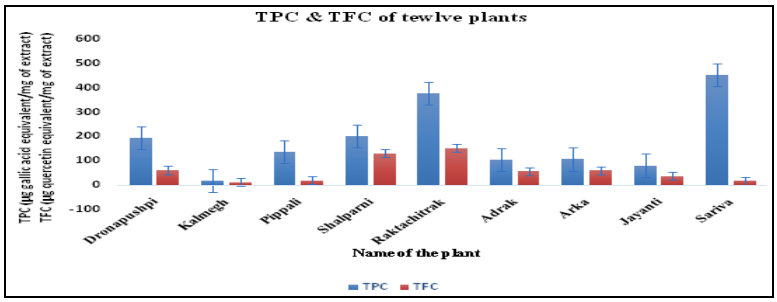



**FIG. 5: GRAPHICAL REPRESENTATION OF TPC & TFC OF SELECTED NINE MEDICINAL PLANTS**

**d. Antioxidant Properties:** Antioxidant properties of nine medicinal plants are tested by three scavenging method, $H_2O_2$ scavenging method, DPPH method, ABTS methods **Fig. 6, 7, 8**). Various types of free radicals are produced by the metabolism process. These free radicals are scavenged by antioxidants and protect the cell from oxidative damage. Antioxidant properties can have evaluated by $IC_{50}$ value. The $IC_{50}$ value is defined that how much concentration is required to decrease the initial concentration of $H_2O_2$, DPPH and ABTS+ active radicals' up to 50%.

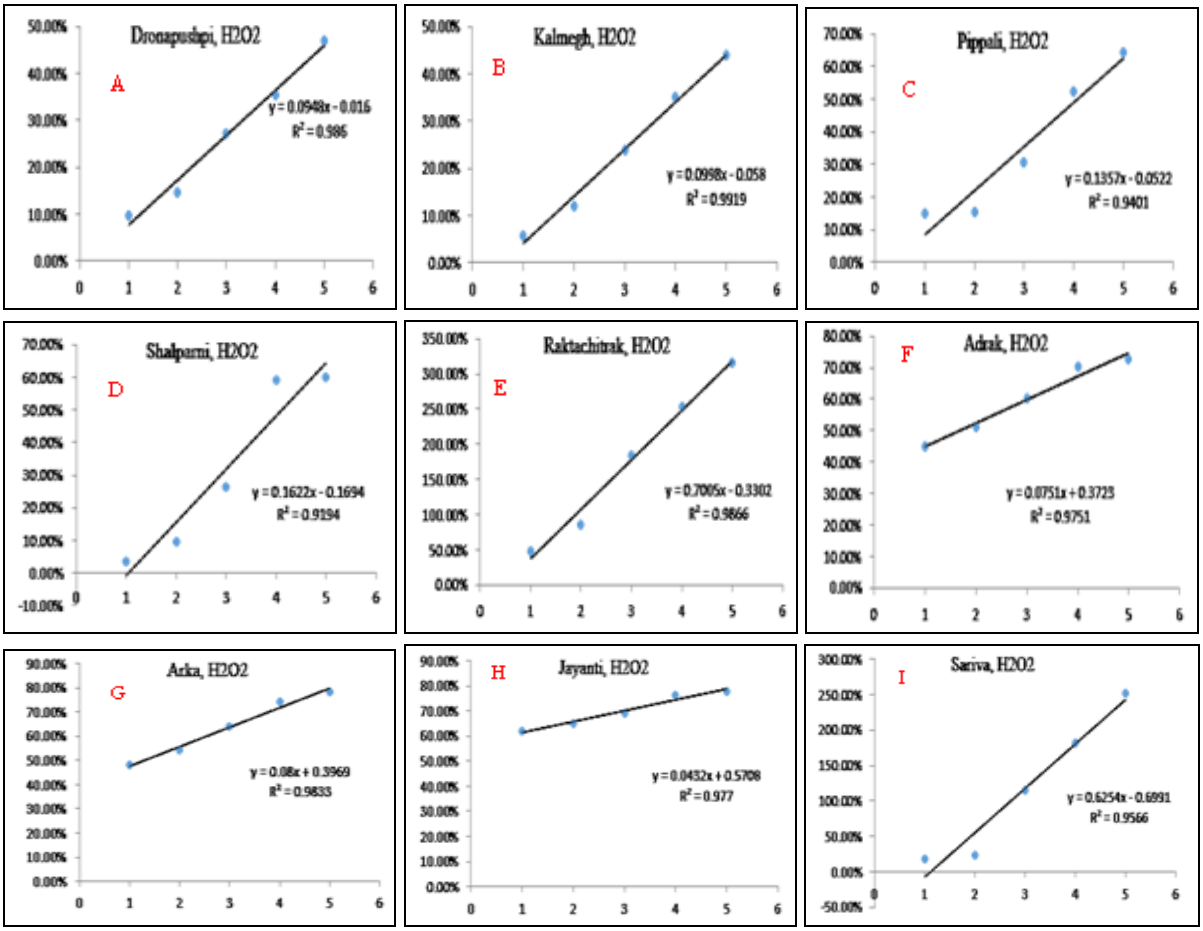
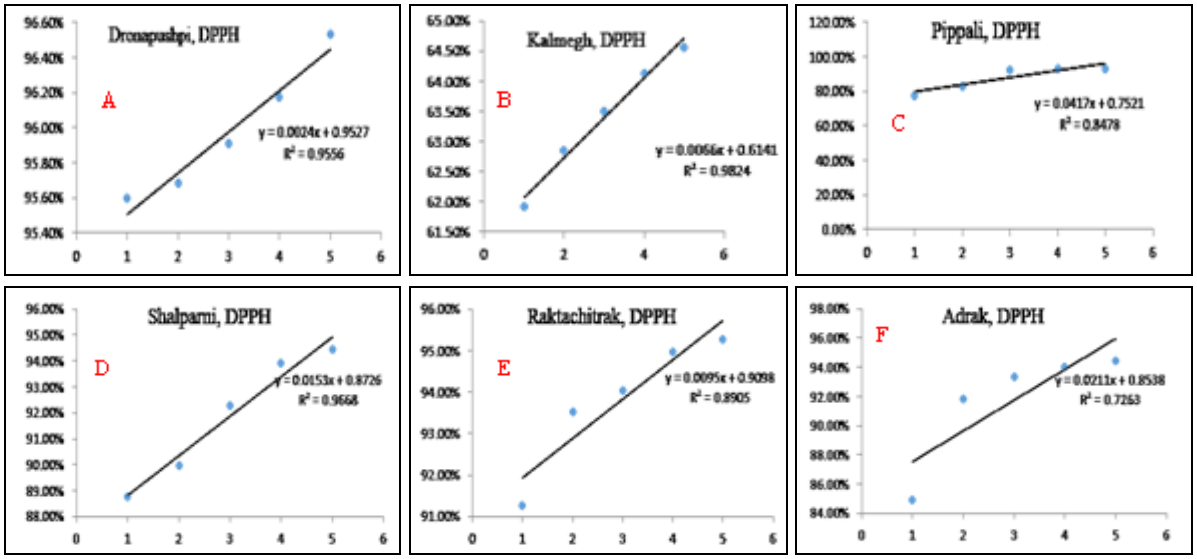



**FIG. 6: ESTIMATION OF ANTIOXIDANT POTENTIALITY OF NINE PLANTS BY H2O2 METHOD (Y AXIS = SCAVENGING INHIBITION PERCENTAGE, X AXIS = CONCENTRATION OF PLANT EXTRACT) [A-DRONAPUSHPI, B-KALMEGH, C- PIPPALI, D- SHALPARNI, E- RAKTACHITRAK, F- ADRAK, G- ARKA, H-JAYANTI, I-SARIVA]**

This **Fig. 6** indicates the graphical representation using the $H_2O_2$ method, which is made up of individual plant scatter plots with a straight-line equation followed by the concentration of plant extract and percentage of scavenging inhibition of free radical. This straight-line equation help to calculate the $IC_{50}$ value of every plant extract to evaluate the antioxidant activity of these selected plant extract.



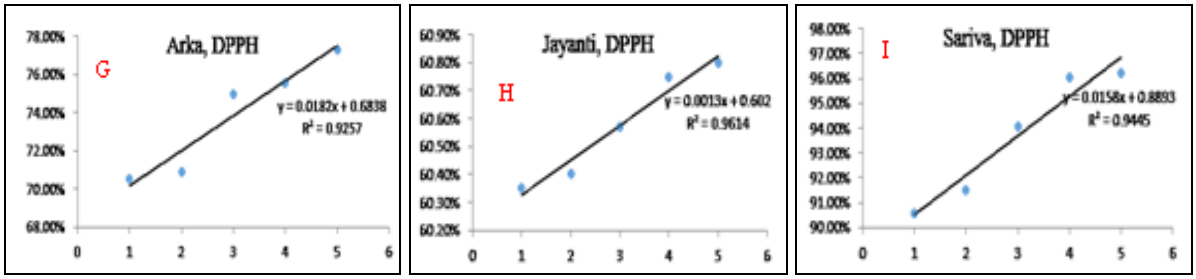



**FIG. 7: ESTIMATION OF ANTIOXIDANT POTENTIALITY OF NINE PLANTS BY DPPH METHOD (Y-AXIS = SCAVENGING INHIBITION PERCENTAGE, X-AXIS = CONCENTRATION OF PLANT EXTRACT) [A-DRONAPUSHPI, B-KALMEGH, C- PIPPALI, D- SHALPARNI, E- RAKTACHITRAK, F- ADRAK, G- ARKA, H-JAYANTI, I-SARIVA]**

This **Fig. 7** indicate the graphical representation using the DPPH method, which is made up of individual plant scatter plot with straight-line equation followed by the concentration of plant extract and percentage of scavenging inhibition of free radical. This straight-line equation help to calculate the $IC_{50}$ value of every plant extract to evaluate the antioxidant activity of these selected plant extract.

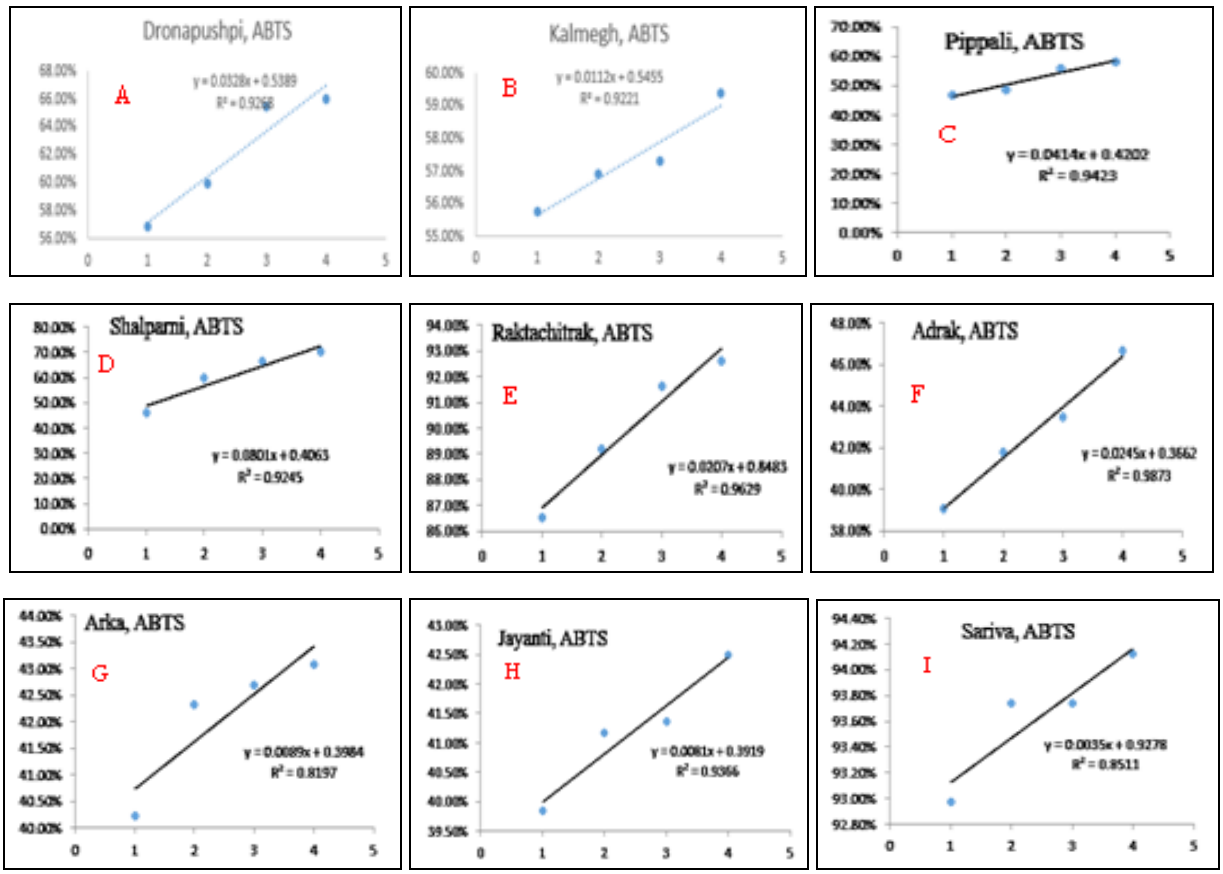



**FIG. 8: ESTIMATION OF ANTIOXIDANT POTENTIALITY OF NINE PLANTS BY ABTS METHOD (Y-AXIS = SCAVENGING INHIBITION PERCENTAGE, X-AXIS = CONCENTRATION OF PLANT EXTRACT) [A-DRONAPUSHPI, B-KALMEGH, C- PIPPALI, D- SHALPARNI, E- RAKTACHITRAK, F- ADRAK, G- ARKA, H-JAYANTI, I-SARIVA]**

This **Fig. 8** indicate the graphical representation using ABTS method, which is made up of individual plant scatter plot with a straight line equation followed by the concentration of plant extract and percentage of scavenging inhibition of free radical. This straight-line equation help to calculate the $IC_{50}$ value of every plant extract to evaluate the antioxidant activity of these selected plant extract.

**TABLE 3: COMPARATIVE STUDY OF ANTIOXIDANTS OF TWELVE PLANTS BASED ON $IC_{50}$ VALUE BY THREE SCAVENGING METHODS**

| Plants Name | IC50 (mg/ml) | | IC50 (mmol TE/g DE) |
|---|---|---|---|
| | $H_2O_2$ Method | DPPH Method | ABTS Method |
| Dronapushpi | 50.69 | 51.36 | 53.36 |
| Kalmegh | 50.39 | 50.29 | 53.63 |
| Pippali | 53.14 | 58.14 | 52.63 |
| Shalparni | 54.22 | 50.85 | 53.67 |
| Raktachitrak | 50.37 | 55.16 | 51.09 |
| Adrak | 50.90 | 67.69 | 50.28 |
| Arka | 50.46 | 53.32 | 60.56 |
| Jayanti | 50.59 | 51.40 | 53.00 |
| Sariva | 51.57 | 52.02 | 57.66 |

**Table 3** represents the comparative study of antioxidant activity of nine plants based on $IC_{50}$ value by three scavenging methods. The antioxidant activity is inversely proportional to the $IC_{50}$ value. The extract of Dronapushpi shows the three $IC_{50}$ values: 50.69 mg/ml, 51.36 mg/ml, and 53.36 mg/ml of $H_2O_2$, DPPH, and ABTS method accordingly. This result shows that Dronapushpi shows better antioxidant activity through the $H_2O_2$ method with low $IC_{50}$ value than the other two methods.

The extract of Kalmegh shows the three $IC_{50}$ values as 50.39 mg/ml, 50.29 mg/ml, and 53.63 mg/ml of $H_2O_2$, DPPH, and ABTS method accordingly. It has been observed that the plant Kalmegh shows better antioxidant activity through the DPPH method with a low $IC_{50}$ value compared to the other two methods. The extract of Pippali shows the three $IC_{50}$ values as 53.14 mg/ml, 58.14 mg/ml, and 52.63 mg/ml of $H_2O_2$, DPPH and ABTS method accordingly. From this result, Pippali shows better antioxidant activity through the ABTS method with low $IC_{50}$ value compared to the other two methods.

The extract of Shalparni shows the three $IC_{50}$ values as 54.22 mg/ml, 50.85 mg/ml, and 53.67 mg/ml of $H_2O_2$, DPPH and ABTS method accordingly. From this result, Shalparni shows better antioxidant activity through the DPPH method with low $IC_{50}$ value compared to the other two methods.

The extract of Raktachitrak has given the three IC50 values as 50.37 mg/ml, 51.16 mg/ml, and 51.09 mg/ml of $H_2O_2$, DPPH and ABTS method accordingly. From this result, Raktachitrak shows better antioxidant activity through the $H_2O_2$ method with low $IC_{50}$ value than the other two methods.

The extract of Adrak shows the three $IC_{50}$ values: 50.90 mg/ml, 67.69 mg/ml and 50.28 mg/ml of $H_2O_2$, DPPH and ABTS method. This result shows that Adrak shows better antioxidant activity through the ABTS method with low $IC_{50}$ value than the other two methods.

The extract of Arka shows the three $IC_{50}$ values: 50.46 mg/ml, 53.32 mg/ml, and 60.56 mg/ml of $H_2O_2$, DPPH and ABTS method. From this result, Arka shows better antioxidant activity through the $H_2O_2$ method with low $IC_{50}$ value compared to the other two methods.

The extract of Jayanti shows the three $IC_{50}$ values, such as 50.59 mg/ml, 51.40 mg/ml, and 53.00 mg/ml of $H_2O_2$, DPPH and ABTS method accordingly. From this result, Jayanti shows better antioxidant activity through the H2O2 method with low $IC_{50}$ value compared to the other two methods. The extract of Sariva show the three $IC_{50}$ values: 51.57 mg/ml, 52.02 mg/ml and 57.66 mg/ml of H2O2, DPPH and ABTS method. From this result, Sariva show their better antioxidant activity through $H_2O_2$ method with low $IC_{50}$ value comparison to other two methods.

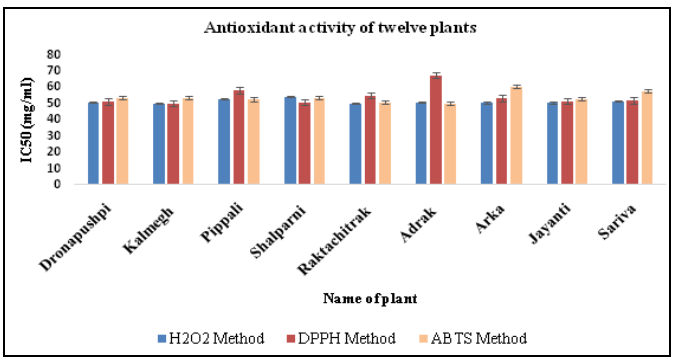



**FIG. 9: ANTIOXIDANT STUDY OF NINE MEDICINAL PLANTS BY USING THREE METHODS ($H_2O_2$, DPPH, ABTS)**

According to the $H_2O_2$ method, the antioxidant activity was observed highest in the sample of hydroalcoholic extract of Raktachitrak among all samples based on obtaining the low $IC_{50}$ value (50.37 mg/ml)[Fig 9]. Besides the hydro-alcoholic extract of Raktachitrak; Kalmegh (50.39 mg/ml), Dronapushpi (50.69 mg/ml), Arka (50.46 mg/ml), Jayanti (50.59 mg/ml), Adrak (50.90 mg/ml) showed high antioxidant property with low $IC_{50}$ value and other plants also have antioxidant property according to the $IC_{50}$ value.

According to the DPPH method, the hydroalcoholic extract of Kalmegh has revealed its highest antioxidant properties with low $IC_{50}$ value (50.29 mg/ml) and as well as the extract of Shalparni (50.85 mg/ml), Dronapushpi (51.36 mg/ml), Jayanti (51.40 mg/ml), Sariva (52.02 mg/ml) have also showed high antioxidant activity with low IC50 value and other plants also have antioxidant property accordingly $IC_{50}$ values **Fig. 9.**

According to the ABTS method, the extract of Adrak showed highest antioxidant properties with low $IC_{50}$ value (50.28 mg/ml) **Fig. 9.** $IC_{50}$ value of Raktachitrak (51.09 mg/ml), Pippali (52.63 mg/ml), Dronapushpi (53.36 mg/ml), Kalmegh (53.63 mg/ml), Shalparni (53.67 mg/ml), Jayanti (53 mg/ml) are falls in vicinity of low range and show high antioxidant activity.

**CONCLUSION:** Presently, medicinal development shows that free radicals are responsible for some diseases. These free radicals help create oxidative stress due to deal an unhealthy lifestyle, chemical exposure, pollution, stress *etc.* These free radicals are unpaired electrons that are highly active and unstable in nature. Therefore, they can accept or donate electrons and act as oxidants or reductants [22]. One kind of substance is responsible for preventing cellular damage; they are called antioxidants. When the level of free radicals is increased, the antioxidant donates an electron and neutralizes the free radicals, and helps prevent any kind of oxidative damage [22]. Antioxidants scavenged the free radicals to prevent any kind of cellular damage. The previous study suggests a relationship between human redox biology and antioxidant metabolism [23]. Plants show non-enzymatic antioxidant activity, which is more efficient than human enzymatic activity [24]. Antioxidant activity is facilitated by reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators and reluctant of ferryl hemoglobin [25]. The property of antioxidants can be controlled by different phytocompound. Phytochemical assay, Total phenolic content (TPC), and Total flavonoid Content (TFC) were performed, and an antioxidant test is also performed. Many types of test for evaluation of antioxidant activity are used, but in this study, three types of antioxidant test are performed such as $H_2O_2$, DPPH and ABTS.

$H_2O_2$ acts as a strong oxidizer molecule and belongs to a group of reactive oxygen species (ROS) called natural chemicals. $H_2O_2$ transmits an oxidizing signal to a specific protein that leads to cell oxidation. Eventually, previous literature suggests that H2O2 molecules also oxidize STAT3 protein.

This $H_2O_2$ molecule are degraded by the enzyme (peroxiredoxins) of antioxidant. Antioxidant activity is measured through the $H_2O_2$ method by the degradative concentration of $H_2O_2$ via UV-Spectroscopy [26]. DPPH is also an important cost-effective assay to measure antioxidant activity. The violated DPPH is reduced by the antioxidant caused by the hydrogen atom transfer mechanism. As a result, color is changed from violate to stable pale yellow color. After the complete reaction time of the DPPH test, the remaining violet part of DPPH is a measure for evaluation of antioxidant activity [27].

ABTS also plays a role the same as DPPH based on electron transfer. ABTS has dark blue color; when antioxidants reduce the ABTS, it turns into a colorless solution[28]. This study reveals that more or fewer plants have good antioxidant properties. Among the nine medicinal plants, Raktachitrak, Kalmegh and Adrak show strong antioxidant activity with the lowest $IC_{50}$ value as per the three methods. Kalmegh is only one plant among the nine medicinal plants who show the highest antioxidant activity with low range $IC_{50}$ value according to both the test H2O2 and DPPH. $IC_{50}$ value is inversely proportional to antioxidant activity. A low-level $IC_{50}$ value indicates high antioxidant activities. $IC_{50}$ value is defined that how much concentration is required to decrease the

initial concentration of DPPH and ABTS+ active radicals 'up to 50%. $H_2O_2$, DPPH, and ABTS methods are used to evaluate the antioxidant properties of selected plants. The TPC and TFC values of Raktachitrak and Adrak showed a high concentration in comparison to the Kalmegh. This study reveals that phenolic and flavonoid compounds and other phyto-compound are responsible for antioxidant properties. Due to that type of phyto-compound, this plant may be used in drug formulation. Phenolic compounds and flavonoids compound cannot modulate solely antioxidant activity. The presence of other phytochemicals may be modulating antioxidant activity either individually or synergistically [24]. For this reason, Despite the TPC and TFC value being low, Kalmegh shows high antioxidant activity through the DPPH method. So many other factors are also responsible for antioxidant activity, not only TPC and TFC.

But the value of TPC and TFC affects antioxidant activity. TPC, TFC value of the extract of Shalparni, Dronapushpi, etc., are towards the high range, and antioxidant activity falls in the high range. Besides phenolic and flavonoids compound, the role of the alkaloid is crucial for enhancing antioxidants [29]. The presence of alkaloids in Kalmegh may be responsible for antioxidant activity and other pharmacological actions. The overall study represent the antioxidant potency of nine plant in an order that is Adrak > Kalmegh > Raktachitrak > Arka > Jayanti > Dronapushpi > Shalparni > Sariva > Pippali in decreasing order on the basis of antioxidant activity. Besides this all compounds, the antioxidant activity of plants may be modulated by many kinds of environmental factors. The antioxidant property of plant help to maintain a good healthy condition and plays a role in anti-inflammatory, antipyretic, and other various kinds of diseases.

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