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COMPARATIVE EVALUATION OF FOUR MEDICINAL PLANTS FOR TOTAL PHENOLS, FLAVONOIDS, *IN-VITRO* ANTIOXIDANT AND ANTI-HEMOLYTIC ACTIVITIES FOR THERAPY

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ABSTRACT: Antioxidants from plant sources have gained acclaim as an excellent source for living a healthy life in recent times. The present study investigates comparative quantitative characterization of phytochemicals, in-*vitro* antioxidant and anti-hemolytic activities among aqueous and ethanolic extracts of four Indian medicinal plants *viz. Berberis aristata, Cedrus deodara, Vitex negundo* and *Tinospora cordifolia*. Quantitative estimation of phenolics, flavonoids, and alkaloids was done by colorimetric estimation. *In-vitro* antioxidant capacities were evaluated by hydrogen peroxide radical scavenging assay and ferric reducing antioxidant potential assay, while total antioxidant capacity was evaluated through phosphomolybdate assay. Anti-haemolytic activity of plant extracts was assessed using H_2O_2 induced hemolysis of human RBCs. The aqueous extract of *V. negundo* and ethanolic extract of *C. deodar* resulted in significantly higher phenolic content amongst all plant extracts. The greatest flavonoid content was observed with an aqueous extract of *V. negundo*, and ethanolic extract of *C. deodara* demonstrated the highest alkaloid content. High antioxidant capacities were reported for the ethanolic extract of *V. negundo* and *C. deodara*. The ethanolic extract of *C. deodara* afforded significant protection to human RBC against the free radical damaging effect of H_2O_2 with 82.8 ± 0.231% hemolytic inhibition as compared to other plant extracts. The aqueous and ethanolic extracts of *Tinospora cordifolia*, *Cedrusdeodara*, and *Vitex negundo* are effective in scavenging free radicals and could be a potential source of natural antioxidants. Our future work will focus on the identification and validation of bioactive antioxidants for their use in modern medicine or food products.

INTRODUCTION: Medicinal plants offer tremendous opportunities for therapeutics due to low cost, effectiveness, and no or low side effects. 80% of the world's population, particularly in the third world, are fully Dependent on medicinal plants for meeting their health care needs. According to one study, about 80% of present-day drugs are either directly or indirectly obtained from medicinal plants [2].

India is a country rich in indigenous herbal resources, which grow on their varied topography and under changing agro-climatic conditions permitting the growth of almost 20,000 plant species, of which about 2,500 are of medicinal value. Most of the observed therapeutic effects of plants have been linked to their potent antioxidant activity.

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Research on antioxidant properties of plants has gained much attention in recent years in the field of medicine as well as in the food industry. Antioxidants prevent the slow damage to cells caused by the release of free radicals or reactive oxygen species (ROS), which are unstable molecules produced in the body in reaction to defective metabolism, environmental, and other related stress on the body [3]. Such oxidative species initiate peroxidation of membrane lipids, aging, cell membrane disintegration, membrane protein damage, DNA mutations, and various other diseases. Both enzymatic and non - enzymatic antioxidants defend to minimize the cellular damage [4].

Non-enzymatic antioxidants are obtained from natural sources and include vitamin – C, E, flavonoids, tannins, and carotenoids. Antioxidants like flavonoids, phenols, alkaloids, polyphenols, *etc*., naturally distributed in plants, are very effective against oxidative damage. Polyphenols and flavonoids have potent anti-inflammatory activity, anti-carcinogenic, and immune-modulatory properties [5]. Saponins are popular for their hemolytic and foaming properties but also have significant anti-cancer and anti-tumorigenic activity [6]. Alkaloids have a wide range of physiological and pharmacological effects, mainly antibiotic and anticancer activity [6]. *Tinospora cordifolia*, *Vitex negundo*, *Cedrusdeodara,* and *Berberisaristata* are the Indian medicinal plants used in Ayurveda since ages and are rich in such antioxidants. *Tinospora cordifolia,* also known as guduchi (Sanskrit) or giloy (Hindi), is a bulky, smooth, climbing deciduous shrub, lacking bristles. It is also referred to as "amrita" due to its ability to impart youthfulness, vitality, and longevity.

It is well known in modern medicine for its adapt-ogenic, immune-modulatory, anti-oxidant, anti-inflammatory, anti-arthritic, and anti-allergic properties [7]. *Cedrus deodarais* a large evergreen, dioecious tree also known as deodar, cedar, devdar *etc*. It is useful in inflammations, dyspepsia, insomnia, cough, fever, urinary discharges, ozoena bronchitis, itching, leucoderma, plies, disorders of the mind, skin, and blood [8]. *Berberisaristata*, commonly known as "Daruhaldi and chitra", is spinous shrub native to the northern Himalaya region. The plant is used traditionally in inflammation, wound healing, and infection of eyes [9]. *Vitex negundo,* commonly known as Nirgundi, belongs to family Verbenaceae. Its leaves have maximal medicinal use.

It is used for treatment of inflammation, eye-disease, toothache, leucoderma, ulcers, cancers, rheumatoid arthritis, gonorrhea, sinuses, bronchitis and as tonics, antibacterial, antipyretic, anti-histaminic, analgesic, insecticidal, and ovicidal agents [10].

Preliminary investigations about the phyto chemicals (qualitative) found in these plants have been reported earlier [11]. In our previous study, different extracts (aqueous, ethanolic, methanolic, chloroform, hexane) of these four medicinal plants viz, *Tinospora cordifolia*, *Berberis aristata*, *Vitexnegundo*, *Cedrusdeodara* were screened for their phytochemical identification and anti-microbial property along with the thin layer chromatographic profiling in different solvent systems [11].

Many studies have been conducted on these four medicinal plant extracts individually, however, there is no documented report that evaluates and provides information on their comparative quantitative phytochemical analyses and anti-oxidant activities [12, 13]. The comparative analysis is required in order to investigate the major bioactive phytochemical which acts in combinations or in solitary mode against various diseases.

The identified phytochemical then can be used in the development of new drugs. The present study deals with the estimation of total phenolic, flavonoid, alkaloid contents, as well as antioxidant and anti-hemolytic activities associated with the aqueous and ethanolic crude extracts of four medicinal plants *viz, Tinospora cordifolia, Berberis aristata, Vitexne gundo, Cedrusdeo dara*.

MATERIALS AND METHODS:

Collection of Plant Materials: The dried stem of *Tinospora cordifolia* and *Berberis aristata*, the stem woods of *Cedrus deodara*, leaves of *Vitex negundo*, were collected from different Ayurveda shops (SEVA Aushadhi and Gangaram Mohanlal) from RAJWADA (local market of Indore) where the head Vaidyarajji identified and validated them on 11th June 2019.

Plant Extract Preparation: The extract of the plant samples was prepared by soaking 20 gms of finely ground dried powder in 80-100 ml of aqueous and ethanolic solvent for 24 h. The extracts were then filtered using Whatman filter paper No. 42 and used for carrying out different assays.

Total Phenolic Content: Total phenolic content was determined using the Folin- Ciocalteu reagent [14]. To 20 µl of aqueous and ethanolic extract, 1 ml distilled water, 1 ml of Folin- Ciocalteu reagent (10%), and 0.8 ml of 7.5% sodium carbonate were added and mixed properly. The absorbance was read after 30 min incubation at room temperature at 750 nm spectrophotometrically against a blank. A calibration curve of gallic acid was generated at 750 nm for concentrations ranging from 6 µg/ml to 48 µg/ml. The concentration of phenolics in the test samples was calculated from the calibration plot. Total phenolic content was expressed as mg of gallic acid equivalents (GAE)/mg of extract. All measurements were done in triplicates.

Total Flavonoid Content: The standard Aluminium Chloride method [15] was used to determine the total flavonoid content (TFC) in a crude extract of four medicinal plants. An aliquot of 0.05 ml of the test sample (1 mg/ml) was mixed with 0.5 ml of distilled water, 0.1 ml of 1% aluminium chloride, and 0.1 ml of potassium acetate solution (1M). In the mixture, 4.25 ml of distilled water was added to bring up the total volume to 5 ml. The test solution was shaken vigorously, and absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin ranging from 6 µg/ml to 36 µg/ml against blank. The concentration of flavonoids in the test samples was calculated from the calibration plot.

Total Alkaloid Content: The analysis is based on the reaction between alkaloids and bromocresol green (BCG) to form a yellow color complex, which can be extracted with chloroform at pH 4.7 and measured at λ_{max} of 415 nm spectro-photometrically [16]. 20 µl of plant extract was diluted with distilled water to bring up the volume upto 2 ml. 50 µl of phosphate buffer (pH 4.7) and 50 µl of bromocresol green (BCG) solution were added to diluted plant extract. The mixture was shaken, and the complex formed was extracted with 5 ml of chloroform. The chloroform layer was collected in 10 ml of the volumetric flask, and the volume was made up to the mark with chloroform. Absorbance was taken at 415 nm against a blank. A set of reference standard solutions of atropine was prepared to range from 50 µg /ml to 800 µg /ml. The rest of the procedure was followed in the same manner as described above. All experiments were performed in triplicates.

Hydrogen Peroxide Scavenging Assay: Hydrogen peroxide is found in biological systems, including the human body. It enters the human body through inhalation of vapour or mist or through skin or eye contact. When H_2O_2 enters the body, it decomposes into oxygen and water, producing hydroxyl radicals (OH-) and initiates lipid peroxidation, which causes damage to the DNA [17]. The ability of plant extracts to scavenge hydrogen peroxide can be determined through UV spectrophotometry [18]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). Hydrogen peroxide's concentration was determined by absorption at 230 nm using a UV spectrophotometer. 1 ml of 6% plant extracts (aqueous & ethanolic) in distilled water was added to 0.6 ml of hydrogen peroxide solution. The absorbance of the reaction mixture was recorded at 230 nm against phosphate buffer without H_2O_2 as blank after 10 min incubation in the dark. Ascorbic acid was taken as a control for this assay. From a stock of 1mg/ml ascorbic acid, different concentrations ranging from 12 µg/ml -48 µg/ml were added to H_2O_2 solution and placed in the dark for 10 min incubation, and the absorbance was noted for preparation of calibration graph. The percentage of hydrogen peroxide scavenging of extracts and standard compounds were calculated as follows:

% scavenged (H_2O_2) = [(Absorbance of control - Absorbance of test) / Absorbance of control] * 100

Ferric Reducing Power Assay (FRPA): Ferric ion reducing power was measured by colorimetric method [19]. Reducing power is a reflection of antioxidant activity of any compound. 0.1ml of aqueous and ethanolic extracts were mixed with 1 ml sodium phoshphate buffer (0.2 M; pH = 6.6)

and 1 ml potassium ferricyanide and incubated at 50 °C for 20 min. After that, 1ml of 10% TCA was added to the mixture and centrifuged at 3000 r.p.m. for 10 minutes. 1.5 ml supernatant was then added to 1.5 ml of distilled water and 0.1 ml of 0.1% ferric chloride ($FeCl_3$). The mixture was left aside at room temperature for 10 min, and then O.D. was recorded at 700 nm using a spectrophotometer. Ascorbic acid (1 mg/ml) was used as positive control and analyzed in a similar manner as discussed above. Higher reducing power is indicated by high absorbance. All assays were run in triplicates.

Total Antioxidant Capacity (Phosphomolybdate Assay): This assay is based on the reduction of phosphate-Mo (VI) to phosphate-Mo (V) by the sample and subsequent formation of a bluish-green colored phosphate-Mo (V) complex at acidic pH. The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard. An aliquot of 0.1 ml of sample solution was mixed with 1 ml distilled water and 2 ml phoshphomolybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 °C for 90 min. After cooling, the absorbance of the mixture was measured at 765 nm against a blank. A typical blank contained 1 ml of distilled water in place of the plant sample and incubated under the same conditions. Ascorbic acid was used as standard in concentrations of 10-100 ug /ml. The antioxidant capacity was reported as mg of ascorbic acid equivalents (AAE) per gram of extract.

***In-vitro* Anti-hemolytic Assay of Extracts:** Erythrocytes were obtained from healthy donors and processed for the study of anti-hemolytic activity [21]. Whole blood was centrifuged at 1500 rpm for 10 min and washed three times with phosphate buffer saline (0.2M, pH 7.4) and re-suspended in the same buffer to make 2% RBC suspension. For haemolysis the protocol was modified [22] and performed as follows. The reaction mixture contained 1.5 ml of RBC suspension and 20 µl of aqueous and 5 µl of ethanolic plant extracts. The mixture was incubated for 30 min at room temperature. Haemolysis was induced by the addition of 100 ul of H_2O_2 (100 uM) followed by incubation at 37 °C for 5 min. The samples were centrifuged at 1000 rpm for 10 min, and the absorbance of the supernatant was measured at 540 nm. An increase in absorbance indicates greater haemolysis. For this experiment, the absorbance obtained from H_2O_2 alone without the plant extract was taken as 100% haemolysis. Hence, the absorbance values obtained at 540 nm were expressed as % haemolysis inhibition. Positive and negative controls were prepared by adding 1.5 ml distilled water and 1.5 ml PBS respectively in 1.5 ml RBC suspension. The percent haemolysis was calculated using the following formula:

Percent haemolysis = Absorbance of test / Absorbance with 100% haemolysis × 100

Statistical Analysis: All the experiments were carried out in triplicate, and the results were given as the mean ± standard deviation (SD). The data were analyzed for statistical significance using Student's t-test, and differences were considered significant at $p < 0.05$. The significant difference between multiple groups was validated using one-way ANOVA.

RESULTS:

Plant Extracts: The collected plant parts were processed and extracted with water and ethanol. The yield of various extracts from the desired parts of medicinal plants is presented in **Table 1**.

TABLE 1: YIELD OF PLANT EXTRACTS

S. no	Plants→	*Tinosporacordiofolia*	*Cedrusdeodara*	*Vitexnegundo*	*Berberisaristata*
	Extracts↓	**Yield→**			
1	Ethanol	41 ml	28 ml	38 ml	42 ml
2	Aqueous	34 ml	33 ml	60 ml	39 ml

Total Phenolic Content (TPC): The total phenol content of aqueous and ethanolic extracts of medicinal plants was estimated using gallic acid (GA) as standard from the calibration curve as shown in **Fig. 1**. They were 0.8 ± 0.002, 1.6 ± 0.002, 1.2 ± 0.003, 0.2 ± 0.003 mg gallic acid equivalents/gm of sample in aqueous extract of *Tinospora cordifolia, Vitex negundo, Berberies aristata and Cedrusdeodara* respectively. The content of phenolic compounds in ethanol extracts

ranged from 0.5 to 1.3 mg GAE/gm sample. Among the four plants, the greatest phenolic content in aqueous extracts was found in *Vitex negundo* (1.6 ± 0.002 mg gallic acid equivalents/gm), while in ethanolic extracts, it was found in *Cedrus deodara* (1.3 ± 0.002 mg gallic acid equivalents/gm). The minimum phenolic content (0.2±0.003 mg GAE/gm) was observed in an aqueous extract of *Cedrusdeodar*. The experimental results obtained from the various extracts are presented in **Table 2**.

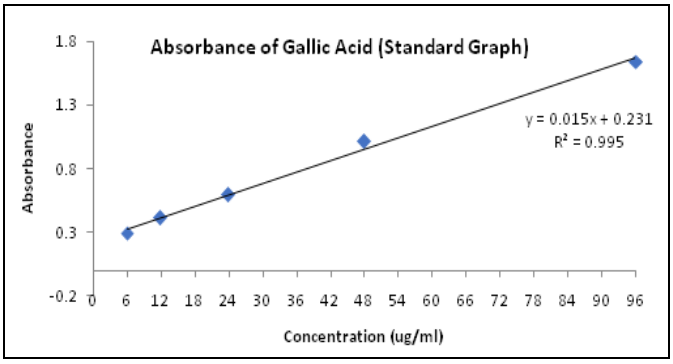

FIG. 1: STANDARD CURVE OF GALLIC ACID

TABLE 2: TOTAL PHENOL CONTENT OF EXTRACTS OF MEDICINAL PLANTS

Plant Extracts	**Aqueous** (mg GAE/gm extract)	**Ethanolic (**mg GAE/gm extract)
Tinospora cordifolia	0.8±0.002	0.9±0.002
Vitex negundo	1.6±0.002	1.0±0.003
Berberis aristata	1.2±0.003	0.5±0.002
Cedrus deodara	0.2±0.003	1.3±0.002

Values are means ± SD (n=3). Within rows, mean values are significantly different (P<0.01; Student's t-test)

Total Flavonoid Content: Total flavonoid contents were extrapolated from the straight line equation of quercetin standard curve **Fig. 2**. TFC obtained for aqueous extracts of *Tinospora cordifolia*, *Vitex negundo*, *Berberies aristata* and *Cedrus deodara* are 0.5 ± 0.017, 0.7 ± 0.015, 0.1 ± 0.003 and 0.3 ± 0.038 mg quercetin equivalent (QE)/g extract respectively.

The flavonoid content of ethanolic plant extracts of four plants ranged from 0.2-0.5 mg quercetin equivalent/g extract. The significant amount of total flavonoids was found in aqueous extract of *Vitex negundo* and the minimum amount was in aqueous extract of *Cedrus deodar*a. The experimental results obtained from the various extracts are presented in **Table 3**.

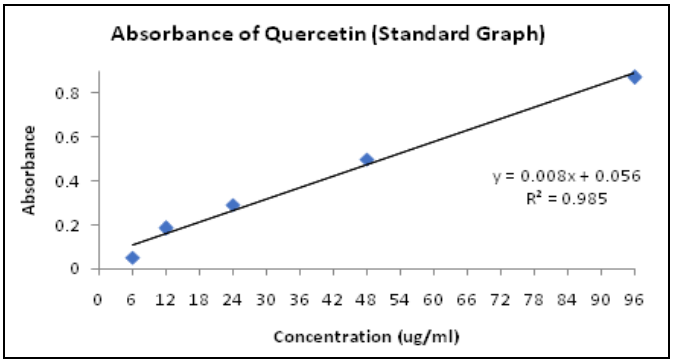

FIG. 2: STANDARD CURVE OF QUERCETIN

TABLE 3: TOTAL FLAVONOID CONTENT OF EXTRACTS OF MEDICINAL PLANTS

Plant Extracts	Aqueous (mg QE/gm extract)	Ethanolic(mg QE/gm extract)
Tinospora cordifolia	0.5 ± 0.017	0.5 ± 0.003
Vitex negundo	0.7 ± 0.015	0.3 ± 0.027
Berberis aristata	0.3 ± 0.038	0.4 ± 0.025
Cedrus deodara	0.1 ± 0.003	0.2 ± 0.011

Values are means ± SD (n=3). Within rows, mean values are significantly different (P<0.01;Student's t-test)

Total Alkaloid Content: The alkaloid content was examined in plant extracts and expressed in terms of atropine equivalent as mg of AE/gm of extract (the standard curve equation from **Fig. 3**, y= 0.008 × + 0.107, R^2 = 0.993).

The highest concentration of alkaloid was measured as 7.2 ± 0.125 mg of AE/g of ethanolic extract of *Cedrus deodara* as demonstrated in **Table 4**.

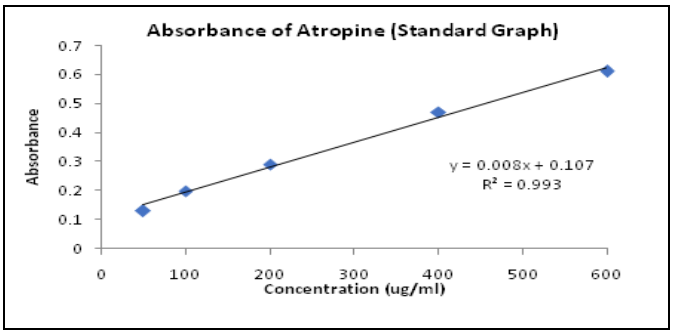

FIG. 3: STANDARD CURVE OF ATROPINE

TABLE 4: TOTAL ALKALOID CONTENT OF EXTRACTS OF MEDICINAL PLANTS

Plant extracts	Aqueous (mg AE/gm extract)	Ethanolic (mg AE/gm extract)
Tinospora cordifolia	2.1 ± 0.034	3.3 ± 0.073
Vitex negundo	2.0 ± 0.014	5.2 ± 0.04
Berberis aristata	1.6 ± 0.052	4.7 ± 0.081

Values are means ± SD (n=3). Within rows, mean values are significantly different (P<0.01; Student's t-test)

H_2O_2 Scavenging Assay: The ability of aqueous and ethanolic extracts of four medicinal plants to scavenge hydrogen peroxide is summarized in **Table 5**. The ethanolic and aqueous extract of *Tinospora cordifolia* and *Cedrus deodara* exhibited maximum inhibition of 56.1 ± 0.9% and 55.7 ± 1.058 %, respectively, against H_2O_2, indicating a significant level of antioxidant compounds in them. The results were compared using 100% inhibition of ascorbic acid (60 ug/ml) against H_2O_2 as control, and percentage inhibition of different concentrations of ascorbic acid is shown in **Fig. 4**.

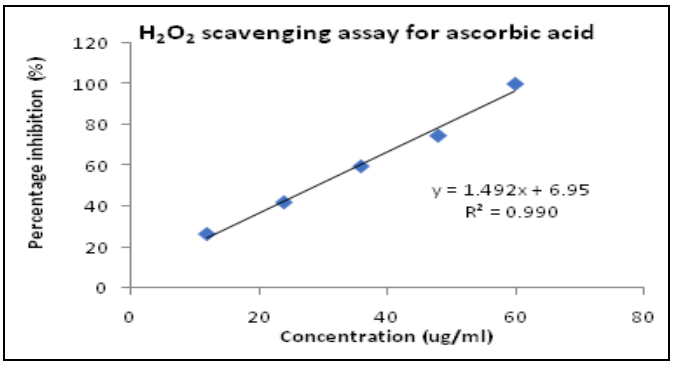

FIG. 4: HYDROGEN PEROXIDE SCAVENGING ASSAY OF ASCORBIC ACID

TABLE 5: H_2O_2 SCAVENGING ASSAY OF EXTRACTS OF MEDICINAL PLANTS

Plant	% Inhibition	
	Aqueous	Ethanolic
Tinospora cordifolia	52.1.±0.586	56.1±0.9
Vitex negundo	48.4±0.603	45.7±0.58
Berberis aristata	53.4±0.495	23.5±1.212
Cedrus deodara	55.7±1.058	46.2±0.458

Values are means ± SD (n=3). Within rows, mean values are significantly different (P< 0.01; Student's t-test).

Ferric Reducing Potential Assay (FRPA): This assay measures reducing potency of plant extract against the oxidative effects of reactive oxygen species. It is based on the reduction of ferric ions via the addition of hydrogen removed from phenolic antioxidant compounds. The higher absorbance indicates the higher reducing potency of the sample. The mean values of FRPA presented in **Table 6** illustrated that ethanolic extract of *Tinospora cordifolia* revealed maximum FRPA potential as 6.8 ± 0.306 mg AAE/g followed by aqueous extract of *Vitex negundo* as 5.7 ± 0.452 mg AAE/g. A lower FRPA value was observed for aqueous extract of *Cedrus deodara* as 1.0 ± 0.227 mg AAE/gm. Ascorbic acid at the concentration of 100 ug/ml was used as the reference antioxidant for comparison.

TABLE 6: FERRIC REDUCING POTENTIAL ASSAY OF PLANT EXTRACTS

Plant	Antioxidant content (mg/gm) equivalent to Ascorbic acid	
	Aqueous	Ethanolic
Tinospora cordifolia	4.2 ± 0.427	6.8 ± 0.306
Vitex negundo	5.7 ± 0.452	4.7 ± 0.132
Berberis aristata	4.8 ± 0.229	3.9 ± 0.350
Cedrus deodara	1.0 ± 0.227	5.0 ± 0.169

Values are means ± SD (n=3). Within rows, mean values are significantly different (P< 0.01; Student's t-test)

Total Antioxidant Capacity (TAC): This method evaluates both water-soluble and fat-soluble antioxidants. The results indicate higher TAC of the ethanolic extract of medicinal plants as compared to their aqueous extract. The mean values regarding the antioxidant potential capacity of both the extracts of plants are shown in **Table 7.** Among them, ethanolic extract of *Vitex negundo* demonstrated the highest antioxidant potential (28.9 ± 0.915 mg AAE/gm) followed by *Cedrus deoara* (27.4 ± 0.965), and the lowest were in aqueous extract of *Berberis aristata* (0.9 ± 0.359). Ascorbic acid at the concentration of 100 ug/ml was used as the reference anti-oxidant for comparison.

TABLE 7: TOTAL ANTIOXIDANT CAPACITY OF MEDICINAL PLANTS

Plant	Antioxidant content (mg/gm) equivalent to ascorbic acid	
	Aqueous	Ethanolic
Tinospora cordiofolia	10.2 ± 1.422	11.7 ± 1.377
Vitex negundo	3.6 ± 0.802	28.9 ± 0.915
Berberis aristata	0.9 ± 0.359	2.9 ± 0.913
Cedrus deodara	1.422 ± 0.607	27.4 ± 0.965

Values are means ± SD (n=3). Within rows, mean values are significantly different(P< 0.01; Student's t-test)

TABLE 8: *IN-VITRO* ANTI-HEMOLYTIC ASSAY FOR MEDICINAL PLANTS

Plant	Percentage Haemolytic Inhibition	
	Aqueous	Ethanolic
Tinospora cordiofolia	40.4 ± 0.708	49.3 ± 0.440
Vitex negundo	37.9 ± 0.373	57.7 ± 0.411
Berberis aristata	40.8 ± 0.519	47.1 ± 2.485
Cedrus deodara	38.9 ± 0.546	82.8 ± 0.231

Values are means ± SD (n=3). Within rows, mean values are significantly different (P< 0.01; Student's t-test)

Anti-Hemolytic Assay: Selected plants with antioxidant potential were further evaluated for *in vitro* determination of antioxidant activity on H_2O_2 induced erythrocyte haemolysis.

Their ability under present investigation to inhibit H_2O_2 induced erythrocyte haemolysis is shown in **Table 8**. The concurrent addition of H_2O_2 along with aqueous plant extracts of all four plants to RBC suspension significantly reduced the H_2O_2 induced haemolysis as compared to ethanolic plant extracts, as shown in the table below. Maximum inhibition of haemolysis was observed for ethanolic fraction of *Cedrus deodara* (82.8 ± 0.231%), which is statistically highly significant. The aqueous fractions exhibited a comparatively lower inhibitory (37 - 40%) activity than ethanolic (47-82.8%) ones.

DISCUSSION: Phyto-metabolites have attracted the interest of research and development over many years. These compounds largely function as antioxidants and therefore are useful in the treatment of many diseases, such as cancer, cardiovascular diseases, and inflammatory diseases. One possible way of increasing the capability of a person to fight diseases is to supplement the diet with antioxidant compounds that are obtained from plant sources.

These natural plant antioxidants can thus serve as a type of preventive medicine. The aim of the present study is to evaluate and compare the *in-vitro* antioxidant and anti-hemolytic activities, total phenolic, flavonoid, alkaloid contents of crude extract of four medicinal plants *viz, Tinospora cordifolia*, *Berberis aristata*, *Vitex negundo*, and *Cedrus deodara*, which are traditionally used to treat many diseases.

Information on antioxidant potentials of these plants collectively could be relevant in the treatment of oxidative stress-induced diseases that have not been investigated so far. The phenolic substances and flavonoids are associated with antioxidant activity due to their redox and metal chelating properties [23, 24]. The present study entailed that both aqueous and ethanolic extract of plants showed the presence of abundant phytochemicals. All extracts except the aqueous extract of *C. deodara* exhibit a good amount of total phenol content. The maximum values for TPC were recorded for aqueous extract of *V. negundo* and *B. aristata* (1.6 ± 0.002 and 1.2 ± 0.002 mg GAE /gm, respectively). Only the ethanolic extract of *C. deodara* showed the highest total phenol content (1.1 ± 0.002 mg GAE /gm) amongst other plants. The significant level of flavonoid content in the extract of the four medicinal plants also contributes to their antioxidant property. In our experiment, aqueous extract has a significant amount of flavonoids as compared to ethanolic extract *C. deodara* is poor in flavonoid content as the minimum amount of flavonoids was obtained in its water and ethanolic extract (0.1 ± 0.003 and 0.2 ± 0.011 mg quercetin/ gm repectively).

The following hierarchy: *Vitex negundo* > *Tinospora cordifolia* > *Berberis arsistata* (aqueous) and *Tinospora cordifolia* > *Berberis aristata* > *Vitex negundo* > *Cedrus deodara* (ethanolic) was obtained for flavonoid content **Table 9**. Water has higher polarity than ethanol therefore, it can extract

out a greater number of phenolic compounds, including flavonoids. Our result indicates most of the phenolic and flavonoid compounds in *T. cordifolia*, *B. aristata*, and *V. negundo* are hydrophilic and hydrophobic in *C. deodara*. Antioxidant properties of phenolic compounds have directly been linked to their structure, whereas low redox potential and thermodynamic stability of flavonoids make them potent antioxidants. Alkaloids have a wide range of biological activities, including anticancer and antibacterial activities, and hence responsible for many healing properties in natural medicine based on their antioxidant activity [25]. The ethanolic extract of all the four plants showed greater alkaloid content as compared to aqueous extract indicating their hydrophobic nature. The ethanolic extract of *C. deodara* (7.2 ± 0.125 mg atropine/ gm is rich in alkaloid **Table 9** which can be effectively used as potential medical candidate.

The antioxidant potential of aqueous and ethanolic extract of medicinal plants was evaluated using H_2O_2 scavenging assay, FRPA, and TAC. The quantitative difference in antioxidant activity was observed due to difference in the concentration of the phytochemicals **Table 9**. Several studies demonstrated a linear relationship between antioxidant activity, phenolic and alkaloid content of plant extracts [26]. Our study also confirms that ethanolic extract of the *C. deodara* and aqueous extract of *V. negundo* are very rich in antioxidant compounds, particularly phenolics, which contribute to their high antioxidant activity. It was consistent with one report, which regarded phenolic compounds as the primary active phytochemical in *C. deodara* [27]. Till date this is the first study which reports that the high antioxidant potency of ethanolic extract of *C. deodara* could *be* due to its significant alkaloid content. Our findings reveal that both the aqueous and ethanolic extracts of *T. cordifolia* are equally potent in their antioxidant potential. This is primarily due to presence of the pretty good amount of phenolics, flavonoids and alkaloids in giloy. Oxidative damage to erythrocytes is manifested as increased haemolysis due to lipid peroxidation. It has been reported that phytochemicals can protect erythrocytes from oxidative damage caused by H_2O_2 [28]. In our present study, we found that the ethanolic extract of the medicinal plants could reduce the effects of oxidative stress induced by exposure to H_2O_2 more than aqueous one as manifested by an increase in percentage haemolytic inhibition at the concentration of 3.3 ug/ml. The ethanolic extract of *C. deodara* demonstrated maximum 82.8% inhibition followed by *V. negundo* (57.7%). The results show a strong connection between total phenolic, alkaloid content and anti-hemolytic assay.

TABLE 9: ANTIOXIDANT POTENTIAL OF AQUEOUS AND ETHANOLIC EXTRACTS OF MEDICINAL PLANTS

Plant Extracts	Total Phenol Content, mg/gm GAE	Total Flavonoid Content, mg/gm QE	Total alkaloid content, mg/gm AE	H_2O_2 Savenging Assay, % Inhibition	FRAP, mg/gm AAE	TAC, mg/gm AAE	Anti-haemolytic Assay, % Inhibition
Water							
T. cordifolia	0.8±0.002	0 0.5±0.017	2.1±0.034	52.1.±0.586	4.2±0.427	10.2±1.422	40.4±0.708
V. negundo	1.6±0.002	00.7±0.015	2.0±0.014	48.4±0.603	5.7±0.452	3.6±0.802	37.9±0.373
B. aristata	1.2±0.003	0 0.1±0.003	1.6±0.052	53.4±0.495	4.8±0.229	0.9±0.359	40.8±0.519
C. deodara	0.2±0.003	00.3±0.038	1.2±0.049	55.7±1.058	1.0±0.227	1.422±0.607	38.9±0.546
Ethanol							
T. cordifolia	0.0.9±0.002	0.5±0.003	3.3±0.073	56.1±0.9	6.8±0.306	11.7±1.377	49.3±0.440
V. negundo	1.1.0±0.003	0.3±0.027	5.2±0.04	45.7±0.58	4.7±0.132	28.9±0.915	57.7±0.411
B. aristata	0.0.5±0.002	0.2±0.011	4.7±0.081	23.5±1.212	3.9±0.350	2.9±0.913	47.1±2.485
C. deodara	1.1.3±0.002	0.4±0.025	7.2±0.125	46.2±0.458	5.0±0.169	27.4±0.965	82.8±0.231

CONCLUSION: Diversity of active and therapeutically useful compounds from plants is provided by the traditional Indian medical systems. Four such plants were studied, and their total phenolic, flavonoid, and alkaloid content were evaluated for antioxidant property by different standard assays. The overall results of the present study certainly provide positive baseline information to ascertain the potency of the crude extracts obtained from various parts of *Tinospora cordifolia*, *Berberis aristata*, *Vitex negundo* and *Cedrus deodara*. Our findings reveal that the

ethanolic extract of the *C. deodara* and aqueous extract of *V. negundo* is very rich in antioxidant compounds as demonstrated by their high antioxidant activity and percentage hemolytic inhibition. The results showed that alkaloids and polyphenols contribute to the antioxidant activity of ethanolic extract of *C. deodara*, of which anti-oxidant effect of alkaloids seemed to be higher than phenols. The aqueous extract of *V. negundo* is highly abundant in polyphenols and flavonoids, as demonstrated by their high percentage. *Tinospor acordifolia* is the only plant that exerts its significant antioxidant potential in both aqueous and ethanolic fractions. However, further investigations are suggested to isolate and characterize the efficacy of the individual components present in the crude extracts. We suggest combined *in-vitro* and *in-vivo* antioxidant activities to attribute more precise therapeutic and safety value to the individual or combined plant antioxidant entities in the future. Such studies would be useful in establishing their applications in the pharmaceutical and food industries to cure and prevent various emerging diseases.

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