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Phytochemical analysis and assessment of *in vitro* antioxidant properties of different plants

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Abstract
The Indian system of medicine such as Ayurveda, Siddha, Unani and Homeopathy rely on plant based crude materials and their formulations. Plants produce wide array of bioactive principles and constitute a rich source of medicines. To investigate the phytochemical analysis and antioxidant properties of *Brassica oleracea* (flouresece), *Terminalia chebula* (fruits), *Terminalia belerica* (fruits), *Phyllanthus emblica* (fruits) *Abutilon indicum* (stems and leaves) and *Swertia chirata* (stems). The aqueous as well as ethanolic extracts of these selected plants materials were studied qualitatively for the presence of different phytochemicals i.e. Alkaloids, Phenolic compound, Protein, Saponins, carbohydrate and glycosides. These extracts were also studied for antioxidant property with Ferric Reducing Antioxidant Power Assay, Ascorbate - Iron (III) - Catalyzed Phospholipid Peroxidation method, Total flavonoid content, Total phenol content and 1, 1-diphenyl-2-picrylhydrazyl assay. *In vitro* qualitative and quantitative phytochemical analysis of these selected plant materials revealed potent antioxidant activity in ethanolic extracts of both *Terminalia belerica* and *Terminalia chebula* among all 14 extracts screened. The study recommends strongly using more than one method due to difference between the tests systems investigated.

Keywords: Antioxidant, *In- vitro,* Flavonoid, Phenol and Phytochemical

Introduction
The Indian system of medicine such as Ayurveda, Siddha, Unani and Homeopathy rely on plant based crude materials and their formulations [1]. Ayurveda is a time tested medicinal system developed by Dhanvantris of ancient India who have continuously screened the plants of India for their therapeutic effects in an empherical manner. The present multinational companies are also screening these same plants but in scientific manner to obtain active molecules for certain target receptors. About 80 % of the world populations rely on traditional medicine which is predominantly based on plant materials [2]. Around 35,000-70,000 species of plants have been used in medicine, neutraceuticals and/or cosmetic purpose [3]. In Asian countries, plants have been traditionally used for human and veterinary health care. In India, about 7,500 plants are used in health care, mostly in rural and tribal villages. Out of these, the real medicinal value of over 4000 plants is either little known or unknown to the mainstream population [4].

Many diseases are due an imbalance between formation and detoxification of pro-oxidants i.e. "oxidative stress". It plays an important role in the pathogenesis of various diseases *viz.* atherosclerosis, alcoholic liver cirrhosis, cancer etc. Oxidative stress is initiated by reactive oxygen species (ROS), such as superoxide anion (O-2), perhydroxy radical (HOO-) and hydroxyl radical (HO·), as a byproduct of electron transport in mitochondria [5]. The increase in ROS or decreased antioxidant availability can result in a net increase in intracellular ROS and hence cause extensive tissue damage [6] leading to various degenerative diseases. The mechanism behind these diseases is lipid peroxidation of the membrane lipids, damage of DNA, lipoprotein, protein, etc. [7] by excess ROS. Antioxidants defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS [8]. Most living species have efficient defense systems to prevent themselves against oxidative stress [9]. Innate defence system of the body some time may not be sufficient for curing the damage caused by continued oxidative stress. Thus, there is a need to supply the antioxidants exogenously to balance their levels in the body. External supplementation of antioxidants is recommended in such cases to protect cells from the deleterious effects of oxidative stress. Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction. The mechanisms behind the use of any compound which scavenge free radicals and can protect against oxidative damage; have adverse side effects [10].

All over world, there is an increased interest in plant extracts, and this is due to several reasons, specifically, synthetic medicine can be inefficient, abusive and or incorrect use of these drugs results in deleterious side effects whereas, drugs obtained from natural plant origin are non-narcotic, having no or fewer side effects and are cost effective [1,11]. Keeping in mind the adverse effects of synthetic antioxidants, researchers have channeled their interest in isolating natural antioxidants [12] which are very effective to control the oxidative stress and hence prevent the initiation of disease propagation. Therefore, an alternative is the consumption of natural antioxidants from various food supplements and traditional medicines [13]. Naturally occurring antioxidants such as ascorbic acid, vitamins E and phenolic compounds, possess the ability to reduce the oxidative damage associated with many diseases [14]. The present study was undertaken to assess the status of different bioactive compounds in the aqueous as well as ethanolic extracts of *Brassica oleracea* (fluoresce), *Terminalia chebula* (fruits), *Terminalia belerica* (fruits), *Phyllanthus emblica* (fruits) *Abutilon indicum* (stems and leaves) and *Swertia chirata* (stems).

Materials and methods

Collection of samples

Plant materials *viz.*, *Brassica oleracea* (flouresece), *Terminalia chebula* (fruits), *Terminalia belerica* (fruits), *Phyllanthus emblica* (fruits) *Abutilon indicum* (stems and leaves) and *Swertia chirata* (stems) (fig 1) were collected and air dried and grinded to mesh for preparation of plant extracts.

Solvent extraction of samples

About 100 gm powder samples were placed in coloumner soxhlet extraction system and extracted by using two different solvent *viz.*, aqueous and absolute ethanol. Collected extracts were dried on hot plate till the solvent got evaporated and dried powder was stored in refrigerator at 4^0C for further analysis.

Qualitative phytochemical analysis

Alkaloids

Solvent free extract, 50 mg is stirred with 1 ml of dilute hydrochloric acid and filtered.

Mayer's test: To a 1 ml of filtrate, two drop of Mayer's reagent was added by side of the test tube. A white or creamy precipate indicated the test as positive [15].

Wagner's test: To a 1 ml of filtrate, two drop of Wagner's reagent was added by side of the test tube. A reddish-brown precipate indicated the test as positive [15].

Hager's test: To a 1 ml of filtrate, 2 ml of Hager's reagent (saturated aqueous solution of picric acid) was added by side of the test tube. A prominent yellow precipate indicated the test as positive [15].

***Abutilon indicum* (flouresece)** | ***Terminalia chebula* (fruits)**

***Terminalia belerica* (fruits)** | ***Phyllanthus emblica* (fruits)**

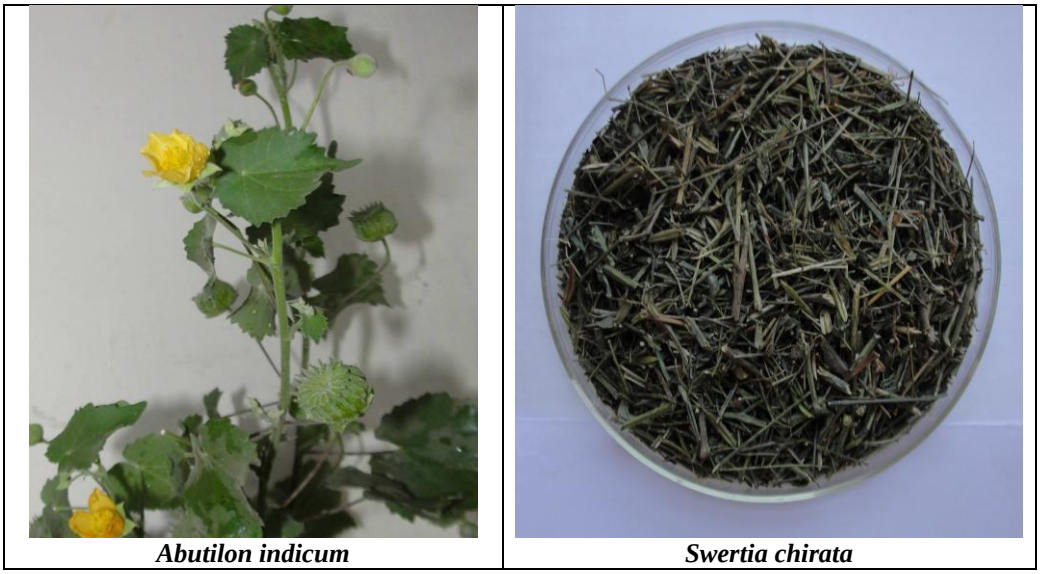

Fig 1: Plant materials used for *in vitro* study

Phenolic compound

Lead acetate test: The extract (50 mg) was dissolved in distilled water and to this, 3 ml of 10% lead acetate solution was added. A bulky white previpitate indicated the presence of phenolic compounds.

Ferric chloride test: The extract (50 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5 % ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds [16].

Protein

Biuret test: The extract (100 mg) was dissolved in 10 ml of distilled water and filtered. An aliquot of 2 ml of filtrate was treated with one drop of 2 % copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicated the presence of protein [17].

Test for carbohydrate and glycosides

The extract (100 mg) was dissolved in 5 ml of distilled water and filtered.

Fehling's test: One ml of filtrate was boiled on water bath with 1 ml each of fehling solution A and solution B. A red colour indicated the presence of sugar [18].

Fehling solution A: copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 ml using distilled water.

Fehling solution B: Potassium sodium tartarate (173 g) and sodium hydroxide (50 g) was dissolved in distilled water and made up to 500 ml using distilled water.

Benedict's test: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characterisric colourd precipitate indicated the presence of sugar [18].

Saponins

Foam test: The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins [19].

Quantitative phytochemical analysis

Ferric Reducing Antioxidant Power Assay (FRAP): The antioxidant activity of selected plants was determined on the basis of ability to reduce ferric (III) form of iron to ferrous (II) form. The FRAP assay was done as per the method described by Sahgal *et al.* (2009) [20]. The FRAP reagent was prepared by mixing acetate buffer (25 ml, 300 mmol/L, pH 3.6), 10 mmol/L TPTZ solution (2.5 ml) in 40 mmol/L HCl and 20 mmol/L $FeCl_3$ solution (2.5 ml) in proportion of 10:1:1 (v/v), respectively and warmed to 37ºC in a water bath prior to use. FRAP reagent (4.5 ml) was added to extract sample (150µl) and the absorbance of the reaction mixture was recorded at 593 nm after 4 min. The assay was carried out in triplicates for accuracy. The standard curve was constructed using FeSO4 solution (0.5-10 mg/mL). The results were expressed as mmol Fe (II)/gm dry weight of plant extracts. L-ascorbic acid was also used as a comparative model for this assay.

Ascorbate - Iron (III) - Catalyzed Phospholipid Peroxidation (AICPP): The ability of the extracts to scavenge hydroxyl radicals was determined as per Aruoma *et al.* (1997) [21] with suitable modifications. Goat liver was mixed (1:10) with 10 mM PBS (pH 7.4) and sonicated in an ice bath for preparation of the homogenate liposomes. The liposomes (0.2 ml) were added with 0.5 ml of PBS buffer, 0.1 ml of 1 mM $FeCl_3$ and various volumes (100µl and 200µl) of plant extracts and subsequently 0.1 ml of 1 mM ascorbic acid was added. After incubation at 37^0C for 60 min, 1 ml of 10% trichloroacetic acid (TCA) was added and centrifuged at 2000 rpm for 10 min at room temperature. Finally, one ml of 0.67% 2-thiobarbituric acid (TBA) in 0.05 M NaOH was added to the supernatant, vortexed and heated in a water bath at 100°C for 20 min. After cooling, 1 ml of distilled water was added and absorbance was recorded at 532 nm. Control containing all reagents except the extracts was kept. The assay was carried out in triplicate.

The percentage inhibition activity was calculated as: [(Abs. of control – Abs. of sample)/Abs. of control] × 100%.

Total flavonoid content: The total flavonoid content of the extracts was determined as per the method described by Nabavi *et al.* (2008) [22]. The sample solution (0.5 ml) was mixed with distilled water (2 ml) and subsequently with 5% $NaNO_2$ solution (0.15 ml). After 6 min of incubation, 10% $AlCl_3$ solution (0.15 ml) was added and then allowed to stand

for 6 min, followed by addition of 4% NaOH solution (2 ml) to the mixture. Consequently, distilled water was added to the sample to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture's absorbance was determined at 510 nm. The total flavonoid content was expressed in mg of catechin per gram of extract.

Total phenol content: The total phenolic content in the extracts was measured using Folin-Ciocalteu reagent method [23]. The samples (0.4 mL) (1mg/mL extracts) were transferred into test tubes. To this solution, distilled water (1.0 mL) and Folin-Ciocalteu reagent (1.0 mL) were added, and the tubes shaken thoroughly. After 1 min, Na_2CO_3 solution (1.6 mL, 7.5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. A linear dose response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm. The total phenolic compounds concentration in the extract and potencies was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g). The content of phenolic compounds in the extract was calculated using this formula: C = A/B; where C is expressed as mg GAE/g dry weight of the extract; A is the equivalent concentration of gallic acid established from calibration curve (mg); and B is the dry weight of the extract (g).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay: DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al*. (2006) [24]. The different concentrations of each of the extracts were prepared in ethanol and were added to 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in dark. Absorbances of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. The assay was performed in triplicates for accuracy. Ascorbic acid was used as the standard. The percentage radical scavenging activity was calculated using the following formula, % inhibition (DPPH) = (A0 - A1/ A0) X100

Where, A0 is the absorbance of the control (without test samples), A1 is the absorbance of test samples.

Statistical analysis

The obtained results were expressed as Mean ± SE. Data were evaluated statistically with computerized SPSS package program (SPSS 9.00 software) using one-way analysis of variance (ANOVA). Significant differences among means were estimated at $P < 0.05$ according to Snedecor and Cochran (1994) [25].

Results

Qualitative phytochemical analysis

The result of qualitative tests for different phytochemical found in aqueous and ethanolic extracts of selected plants is presented in table 1. Qualitative analysis of aqueous and ethanolic extracts of the selected plant samples revealed the presence of different phytochemicals such as alkaloids, phenolic compound**,** protein, saponins, carbohydrates and glycosides. Both aqueous

Table 1: *In vitro* qualitative analysis of different plant extracts

Plant extracts	Alkaloids			Phenol		carbohydrate and glycosides		Protein	Saponins
	Mayer's test	Wagner's test	Hager's test	Lead acetate test	Ferric chloride test	Fehling's test	Benedict's test	Biuret test	Foam test
Brassica oleracea (aqueous)	+	++	+	++	-	+	+	-	+
Brassica oleracea (ethanolic))	+	++	++	++	-	+	++	-	-
Terminalia chebula fruit (aqueous)	-	-	-	++	++	+	+	-	-
Terminalia chebula fruit (ethanolic))	-	-	-	++	++	++	+	+	++
Terminalia belerica fruit (aqueous)	-	-	-	++	++	-	-	-	+
Terminalia belerica fruit (ethanolic))	-	-	-	++	++	+	-	+	++
Phyllanthus emblica fruit (aqueous)	+	-	-	++	++	+	+	-	++
Phyllanthus emblica fruit (ethanolic))	+	+	+	++	++	+	+	+	++
Abutilon indicum stem (aqueous)	+	+	+	+	-	-	-	-	+
Abutilon indicum stem (ethanolic))	+	+	-	-	-	+	+	-	-
Abutilon indicum leaves (aqueous)	+	+	+	++	-	-	-	-	+
Abutilon indicum leaves (ethanolic)	+	+	-	-	-	++	++	-	-
Swertia chirata stem (aqueous)	+	+	+	-	-	-	-	-	++
Swertia chirata stem (ethanolic)	+	+	+	-	-	++	++	-	-

Negative (-), Mild Positive (+), highly positive (++)

and ethanolic extract of *Brassica oleracea* showed presence of alkaloids, phenol, carbohydrate and glycosides whereas, absence of protein. The aqueous extract of *Brassica oleracea* was found positive for saponins, on the other hand its ethanolic extract showed absence of saponins. Aqueous extract of *Terminalia chebula* showed presence of phenol, carbohydrate and glycosides whereas, negative for alkaloids, protein and saponin. Its ethanolic extract showed presence of phenol, protein, saponin, carbohydrate and glycosides and absence of alkaloids. Aqueous extract of *Terminalia belerica* showed presence of phenol and saponin and absence of alkaloids, carbohydrate, glycosides and protein whereas, its ethanolic extract was found positive for phenol, protein and saponin, and negative for alkaloid. On the contrary, in the ethanolic extract of *Terminalia belerica*, carbohydrates and glycosides were found positive by Fehling's test and negative by Benedict's test. Aqueous extract of *Phyllanthus emblica* was found positive for phenol, carbohydrates and glycosides

and saponins; and alkaloids by Mayer's test and negative for protein; and alkaloid by Wagner's and Hager's test. The ethanolic extract of *Phyllanthus emblica* showed positive for alkaloids, protein, phenol, carbohydrates, glycosides and saponin. Aqueous extract of stems as well as leaves of *Abutilon indicum* found positive for alkaloids, saponins and phenolic compound by lead acetate test whereas, negative for protein, carbohydrates and glycosides; and phenolic compound by Ferric chloride test. Ethanolic extract of both showed presence of carbohydrates and glycosides; and alkaloids by Mayer's and Wagner's test, absence of protein, phenol, saponin and alkaloids by Hager's test. Aqueous extract of *Swertia chirata* stems was found positive for alkaloids, saponins and negative for protein, phenol, carbohydrates and glycosides. On the other hand, ethanolic extract revealed presence of alkaloids, carbohydrates and glycosides and absence of protein, phenol and saponins.

Quantitative phytochemical analysis for antioxidant potential

The antioxidant capacity expressed as the FRAP, AICPP, DPPH of selected plants as well as the amount of total flavonoid and phenols of these plants in rank order are shown in table 2. Using FRAP assay, the antioxidant activity of these plant extracts was determined based on the ability to reduce ferric (III) form of iron to ferrous (II) form. The standard curve (fig 2) was generated in the range of 0.1mg/ml, 0.2 mg/ml, 0.4mg/ml, 0.6 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1.0 mg/ml of ferrous sulphate and the results were expressed as mmol ferrous ion equivalent per gram (mmmol Fe^{2+}/ gm) of sample dry weight (y= 0.728x + 0.422, R^2 = 0.991). By this assay, we obtained a ladder of antioxidant capacity ranging from 0.66± 0.61 to 0.04±0.01 mmol Fe (II)/gm of dry weight. The highest antioxidant function was found in the ethanolic extract of *Terminalia chebula* followed by ethanolic extract *Terminalia belirica*. On the other hand, the most widely used aqueous extract of *Phyllanthus emblica* fruits showed the lowest antioxidant property.

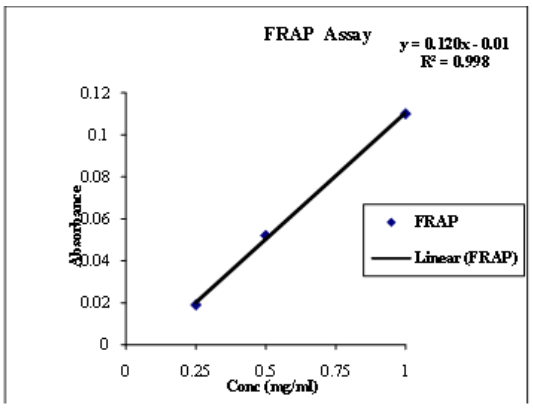

Fig 2: Regression curve constructed using different concentration of FeSO4 solution

The ability of extracts to scavenge hydroxyl radicals generated by ascorbic-iron III to inhibit the formation of 2-thiobarbituric acid reactive species (TBARS) tested. The degree of inhibition of different plant extracts ranged from 28.51±1.60 to 3.21±0.40. Percent inhibition of phospholipid peroxidation was significantly (P<0.05) highest in ethanolic extract of *Terminalia belirica* and *Terminalia chebula* and was significantly (P<0.05) lower in ethanolic extract of *Swertia chirata* stem followed by aqueous extract of *Abutilon indicum* leaves.

The percentage radical scavenging activity of selected plants was measured using DPPH assay test and it ranged from 22.25±0.60 to 2.61±1.18. It was significantly highest in ethanolic extract of *Terminalia belirica* followed by *Terminalia chebula* and was significantly lower in extract of *Abutilon indicum* stems.

The total flavonoid content of the extracts was expressed in mg of catechin equivalent per gm (mg CE/gm) of extract, by reference to standard curve (y = 0.0089x + 0.0165, R^2 = 0.9276) (fig 3). The total flavonoid content in the different extracts analyzed was in range of 1.28± 0.42 to 0.29±0.13mg CE/gm of extract. Significantly (P<0.05) highest concentration of total flavanoid content was observed in ethanolic extract of *Terminalia bellirica* and *Terminalia chebula* followed by aqueous extract of *Terminalia belirica* whereas, significantly (P<0.05) lower in aqueous extract of *Swertia chirata* stems.

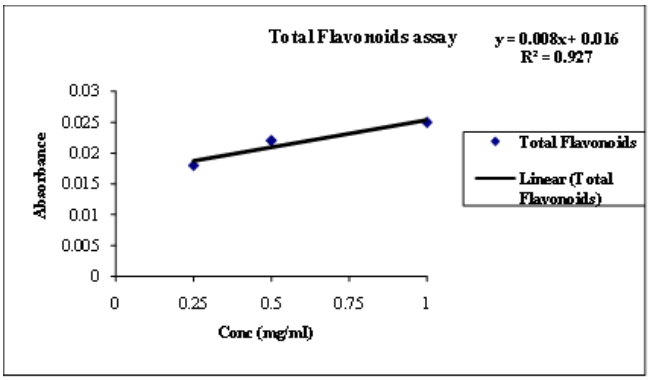

Fig. 3: Regression curve for estimation of total flavonoids

The total phenol content in selected plants extracts analyzed was in range of 4.69±2.16 to 1.30±0.37 mg GAE/g of dry weight by reference to standard curve (y = 0.0451x - 0.006, R^2 = 0.983) (fig 4). The significantly (P<0.05) highest total phenolic content was found in ethanolic extract of *Terminalia belirica* followed by *Terminalia chebula* and significantly lowest in *Swertia chirata* stems.

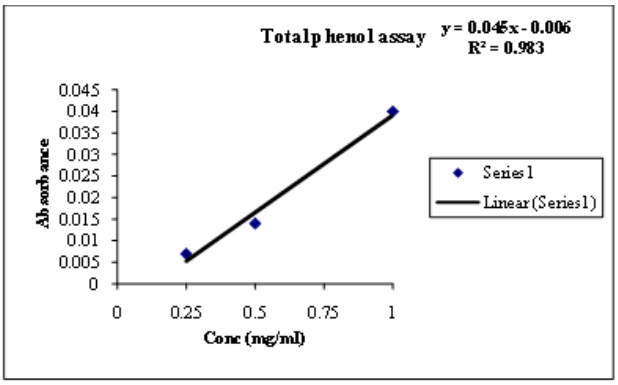

Fig 2: Regression curve for estimation of total Phenol

Table 2: *In vitro* quantitative phytochemical analysis of different plant extracts (Mean ±SE)

Plant extracts	TPC (GAE/g)	Rank	TFC (mg catechin/g)	Rank	DPPH assay	Rank	FRAP (mmol Fe/ g)	Rank	AICPP (% inhibition)	Rank
Brassica oleracea (aqueous)	3.83±1.03	5	0.83± 0.35^{b}	7	11.69±0.76^{b}	8	0.09±0.03	11	10.44±0.40^{cd}	6
Brassica oleracea (ethanolic)	2.82±1.06	10	0.66± 0.19^{ab}	9	12.30±1.08^{b}	7	0.21±0.03	5	10.04±2.00^{cd}	7
Terminalia chebula (aqueous)	3.45±1.01	7	0.51± 0.25^{ab}	11	15.70±1.92^{bc}	6	0.08±0.02	12	16.06±0.80^{ef}	4
Terminalia chebula (ethanolic)	4.57±2.03	2	1.23±0.42^{de}	2	21.55±2.16^{d}	2	0.66±0.61	1	24.89±1.60^{g}	2
Terminalia belerica (aqueous)	3.92±1.07	4	1.19± 0.42^{d}	3	18.93±1.14^{cd}	4	0.17±0.02	7	17.26±0.80^{f}	3
Terminalia belerica (ethanolic)	4.69±2.16	1	1.28± 0.42^{de}	1	22.25±0.60^{d}	1	0.60±0.17	2	28.51±1.60^{g}	1
Phyllanthus emblica (aqueous)	4.24±1.76	3	1.09± 0.35^{cd}	4	18.15±0.61^{cd}	5	0.04±0.01	14	9.23±0.40^{bcd}	8
Phyllanthus emblica (ethanolic)	2.74±0.90	11	0.66± 0.16^{ab}	10	19.45±0.74^{a}	3	0.16±0.02	8	11.24± 0.80^{de}	5
Abutilon indicum stems (aqueous)	3.56±0.75	6	0.71± 0.10^{b}	8	2.61±1.18^{a}	14	0.15±0.03	9	6.76±6.20^{abc}	11
Abutilon indicum stems (ethanolic)	3.12±0.14	8	0.87±0.36^{bc}	6	2.12±0.03^{a}	13	0.31±0.08	3	7.63±0.40^{abcd}	9
Abutilon indicum leaves (aqueous)	2.87±0.46	9	0.93± 0.39^{bc}	5	3.40±0.30^{a}	12	0.22±0.07	4	4.41±0.40^{ab}	12
Abutilon indicum leaves (ethanolic)	1.91±0.71	12	0.44± 0.11^{a}	13	3.83±0.17^{a}	10	0.06±0.02	13	9.23±0.40^{bcd}	8
Swertia chirata stems (aqueous)	1.30±0.37	13	0.29± 0.13^{a}	14	4.27±0.53^{a}	9	0.20±0.03	6	6.82±0.40^{abcd}	10
Swertia chirata stems (ethanolic)	1.82±0.56	14	0.45± 0.16^{a}	12	3.57±0.61^{a}	11	0.14±0.02	10	3.21±0.40^{a}	13
Standard	----------				58.43±1.45^{a}		0.58±0.07		39.45±0.46	

Values bearing different superscript in the same column vary significantly (P<0.05)

Discussion

Popularity of herbal drugs is increasing day by day globally because of their lesser side effects as compared to synthetic drugs [26]. The herbal medicines are cheap, and have natural origin with higher safety of margins and lesser or no side effects [27]. Medicinal plants are part of human society from the dawn of civilization to combat diseases, as it valuable and cheap source of unique phytoconstituents which are used extensively in the development of drugs against various diseases [28, 29, 30]. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80% [31]. Thus, the economic importance of medicinal plants is much more in countries like India than in rest of the world. In the last few decades, the field of herbal medicine is getting popularized in both developed and developing countries [31].

The acetone, aqueous, chloroform as well as ether extracts of *Brassica oleracea* contains alkaloids, phenols and saponins whereas, negative for amino acids [32]. The qualitative phytochemical screening of the polyphenolic extract of *Terminalia chebula* fruits revealed the presence of tannins, saponins, flavonoids and alkaloids [33] and *Terminalia belerica* fruits extract in petroleum ether, chloroform and aqueous solvent showed presence of phenols but absence of amino acids, carbohydrates, glycosides, saponins; and alkaloids by Hager's and Wagner's tests [34]. Phytochemical analysis of *Phyllanthus emblica* fruits showed presence of alkaloids and tannins in ether, chloroform and alcoholic extracts and carbohydrates in alcoholic extract [35]. The *Abutilon indicum* plant contains saponins, flavonoids, alkaloids, hexoses, nalkane mixtures (C22-34), alkanol as main classes of compounds [36]. Analysis of *S. chirata* showed the presence of tannins, alkaloids, glycosides and flavonoids in its methanol extract and only tannins and glycosides in the aqueous extract [37].

Oxidative stress from increased amounts of reactive oxygen species (ROS) can cause extensive damage to cellular and extracellular macromolecules, such as proteins, lipids, and nucleic acids [6, 38] and is considered a major factor in the pathogenesis of the chronic diseases [39]. It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status [40]. Regular consumption of fruits and vegetables minimizes some of these harmful effects, which has been somewhat accredited to the presence of compounds possessing antioxidant properties [41]. The major antioxidants present in fruits and vegetables are: vitamin C, vitamin E, carotenoids and polyphenols, especially flavonoids, which all provide protection against free radicals [42]. The quality and quantity of these antioxidant components are major attributes to the health benefits of fruits and vegetables that are associated with reduced risk of chronic disease [39]. Antioxidants are vital substances which possess the ability to protect body from damage by free radical induced oxidative stress [43]. The characteristic of antioxidant depends on the solubility of specific antioxidant compounds present in plant material as per their polarity in different solvent system.

In the present study, *in vitro* qualitative phytochemical analysis of different plant materials extracts revealed highest rank of ethanolic extract of *Terminalia belirica* followed by ethanolic extract of *Terminalia chebula*, ethanolic extract of *Phyllanthus emblica* and aqueous extract of *Phyllanthus emblica.* Many researchers have attempted to rank the antioxidant properties of different plant materials using different methods [44, 45]. The leaves, bark and fruits of *T. chebula* exhibits *in vitro* antioxidant and free redical-scavenging activities [46] and phenolics were found to be responsible for this activity [47]. Aqueous extract of *T. chebula* inhibit xanthine/xanthine oxidase activity and is also an excellent scavenger of DPPH radicals [31]. Alam *et al.* (2011) [48] postulated that the crude methanolic extract of the fruits of *Terminalia belerica* along with its various organic fractions elicited both *in vitro* and *in vivo* antioxidant activity.

Natural antioxidants are usually phenolic and polyphenolic compounds [49]. Phenolic compounds had shown significant antioxidant activity [50, 51]. Polyphenol are the major compounds and are commonly found in both edible and non-edible common plants and animal products. It has multiple biological effects, including antioxidant activity [52]. Their antioxidant activity is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [53]. In the present study total phenolic content was highest in ethanolic extract of *Terminalia belirica* followed by ethanolic extract of *Terminalia chebula,* aqueous extract of *Phyllanthus emblica* and aqueous extract of *Terminalia belirica.* Phenolic contents are very important plant constituents because of their scavenging ability due to

their hydroxyl groups [54].

Flavonols (such as quercetin, myricetin kaempherol) and flavones (e.g. apigenin, luteolin) in plant materials are closely associated with their antioxidant function mainly due to their redox properties exerted by various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity [55, 56]. Highest concentration of total flavanoid content was observed in ethanolic extract of *Terminalia belirica* followed by ethanolic extract of *Terminalia chebula*, aqueous extract of *Terminalia belirica* and aqueous extract of *Phyllanthus emblica*. It has been acknowledged that flavanoids show significant antioxidant action on human and animal health and fitness. They act through scavenging or chelating process [54, 57].

Studies on the antioxidant properties of the plant materials, viz., *T. chebula* [46, 58] and *Terminalia belerica* [59] have been reported. However, this study provides a definitive report about the free radical scavenging capacity of *Terminalia chebula* and *Terminalia belerica* since the antioxidant activity of a drug may depend on the free radical scavenging activity [60]. The results indicate that the fruit extracts of *Terminalia belerica* contain significantly higher amount of flavonoids and phenolic content than that of *Terminalia chebula.* Nevertheless in the work reported here, the data unequivocally show that TRAP, DPPH and AICCP values are strictly dependent on polyphenol content. These data are in accordance with that of other authors, who have shown that a high total polyphenol content increases antioxidant function and that there is a linear correlation between polyphenol content and antioxidant function [61].

Conclusion

By this work using more than one antioxidant assay is strongly recommended as a single method will provide basic information about antioxidant properties, but a combination of methods describes the antioxidant properties of the sample in more detail. *In vitro* qualitative and quantitative phytochemical analysis of different plant extracts revealed potent antioxidant activity in ethanolic extracts of both *Terminalia belerica* and *Terminalia chebula* among all 14 extracts screened.

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