Research Article

Phytochemical Studies and Antioxidant Properties of Some Medicinal Plants

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Received: 20-05-2024; **Revised:** 26-08-2024; **Accepted:** 08-09-2024; **Published on:** 15-09-2024.

ABSTRACT

The objective of the present study is to compare the medicinal value of locally available different medicinal plants such as *Boerhavia diffusa, Calyptocarpus vialis, Turnera ulmifolia, Heliotropium indicum, Desmodium tortuosum*. The primary metabolites protein and carbohydrate highly present in *Desmodium tortuosum* (total carbohydrate content: 0.049 mg GAE/g and total protein content: 0.023 mg GAE/g) lowest amount of protein and carbohydrate present in *Heliotropium indicum* (total carbohydrate content: 0.002 mg GAE/g and total flavonoid content: 0.006 mg GAE/g). The results showed that *Desmodium tortuosum* (Fabaceae) is the richest source of phenolic and flavonoid content (total Phenolic content: 0.434 mg GAE/g and total flavonoid content: 0.396 mg GAE/g). The lowest phenolic and flavonoid content were noticed in *Heliotropium indicum* (0.144 mg GAE/g) and (0.185 mg GAE/g). The result of antioxidant activities such as DPPH and ABTS concluded that *Desmodium tortuosum* showed higher activity DPPH, ABTS and total antioxidant activity (0.576, 0.496 and 0.219µg/ml). The lowest activity was observed in *Heliotropium indicum* DPPH, ABTS and total antioxidant activity (0.167, 0.098 and 0.118 µg/ml).

Keywords: Phytochemical screening, primary metabolites, secondary metabolites, antioxidant activity.

INTRODUCTION

Medicinal plants have long been considered a rich source of therapeutic substances for the treatment and prevention of diseases[1]. According to the World Health Organization, up to 80% of the world's population still relies on traditional treatments such as herbs for their primary health care[2]. Plants and herbal medicines are primarily employed in the Ayurveda medicinal systems to treat many diseases[3]. Even though synthetic pharmaceuticals are readily available and very successful in curing numerous diseases in today's society, some people still prefer to use traditional folk medicines since they have fewer side effects[4]. *Desmodium tortuosum* is a large member of (Fabaceae) family. It included above 350 species mainly occur in tropical and subtropical regions of the worldwide plants[5]. The Desmodium plant extracts had a variety of pharmacological effects, including enhancing heart and cerebrovascular health, controlling the immune system, and exerting anti-inflammatory, cytotoxic, antiparasitic, antidiabetic, anti-nephrolithic, antibacterial, and nootropic effects[6]. *Boerhavia diffusa,* also known as red spiderling, is a member of the Nyctaginaceae family, also known as punarnava, which means that which renews or rejuvenates the body. It is used in a number of ayurveda and traditional ethnomedical treatments. *B. diffusa* has a long history of usage as a medicine in Ayurveda and Unani systems of medicine in India. Various plant parts are used as appetizers, alexiteric, eye tonics, to alleviate seminal weakness, to lower blood pressure, and to cleanse the kidneys[7]. *Boerhaavia diffusa* biology activity of qualities, including anti-inflammatory, diuretic, laxative, anti-urethritis, anticonvulsant, anti-nematodal, anti-fibrinolytic, antibacterial, and antihepatotoxic actions, according to pharmacological research, Ayurvedic medicine[8]. The little annual herb *Turnera ulmifolia L.* (Turneraceae), which is regarded as a weed there, can be found there. There is already evidence that *T. ulmifolia* L. has therapeutic significance because it is frequently used as an expectorant, an anti-inflammatory, and to treat a variety of ailments[9]. Traditional Indian medicine using *Heliotropium* is used as a folk remedy for a number of illnesses. There have been reports of antibacterial properties in *Heliotropium indicum*. A member of the Asteraceae family, *Calyptocarpus vialis* blooms. Alkaloids, phenolics, carotenoids, and other secondary plant metabolites have a key role in the treatment of a variety of diseases in traditional medicine and common practice. They provided critical ingredients for the creation of medicines used in Western medicine to treat conditions ranging from cancer to migraine[10].

MATERIALS AND METHODS

Phytochemical Qualitative Analysis

The collected medicinal plants were analysed for preliminary phytochemicals such as protein, carbohydrate, tannin, phenol, saponins, terpenoids, steroids, glycoside using standard methods.

Test for tannins (Ferric chloride test): Two millilitres (2 mL) of the aqueous solution of the extract were added to a few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue colour showed the presence of gallic tannins and a green-blackish colour indicated presence of catechol tannins.

Test for saponins (Frothing Test): Three millilitres (3 mL) of the aqueous solution of the extract were mixed with 10 mL of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 min, it was allowed to stand for 30 min and observed for honeycomb froth, which was indicative of the presence of saponins.

Tests for carbohydrate (Molisch's test): A few drops of Molischs solution were added to 2 mL of aqueous solution of the extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple colour as indicative of positive for carbohydrates.

Test for steroids (Salkowski's test): The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

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Proteins: White precipitate formation which turns yellow on boiling was only observed in the extract of showing thereby the presence of proteins and confirming thereby the absence of proteins in rest of the extracts.

Glycosides: Similarly, a color change from violet to blue to green confirming the presence of glycosides.

Test for Terpenoids 2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H_2SO_4 concentrated. A grey color formed which showed the entity of terpenoids.

Quantification Assay

1. Estimation of Total tannin: This was done using the method of [11]. Two (2 g) of each sample extract was weighed into a 250 mL flask followed by addition of 200 mL of 0.004 M $K_3Fe(CN)_6$ and 10 mL of 0.008 M $FeCl_3$ in 0.008 M HCl. The flask was allowed to stand for 20 min and stirred occasionally at 10 min interval and 1 mL aliquot was removed. To this aliquot, 2 mL of 0.008 M $FeCl_3$ was added in 0.008 M HCl and 10 mL of 0.0015 M $K_3Fe(CN)_6$. After adding the final reagent, the absorbance was read at 720 nm after 30 s against a blank.

2. Estimation of Flavonoids: Total flavonoid content was determined by aluminium chloride colorimetric assay with slight modification[12]. About 500 µL of methanol was added to10 mL flask containing 500 µL of the leaves' extracts. To this 50 µL, 10% $AlCl_3$ and 50 µL of 1 M CH_3COOK was added, respectively. The total volume was made up to 2500 µL with distilled water. The solution was then incubated at room temperature for 30 min. The absorbance was read against blank at 415 nm with spectrometer. The flavonoid was calculated using quercetin as standard. The same process was repeated for the ethanolic extracts.

3. Estimation of total phenol: The total phenol content (TPC) was determined by Folin–Ciocalteu assay[13] using gallic acid as standard. Fifty microliter of aqueous extract solution containing 0.5 mg of aqueous extract was dispensed into a test tube and also repeated for the ethanolic extract sample, 50 µL of distilled water and 500 µL of Folin–Ciocalteu reagent was added respectively into the test tube and shaken thoroughly, after 3 min, 400 µL of 7.5% sodium carbonate solution was added and the mixture was incubated at 45 °C in a water bath for 40 min. Absorbance was measured at 765 nm against blank. The same procedure was repeated to all standard gallic acid solution (0.1 mg/mL). The blank was a mixture of 100 µL of distilled water, 500 µL of Folin-Ciocalteu reagent and 400 µL of 7.5% sodium carbonate. The total phenolic content was expressed as gallic acid equivalent per gram of sample (mg of GAE/g sample) through the calibration curve of gallic acid and calculated

Antioxidant assay

1. DPPH Radical Scavenging Activity: The scavenging effect of the samples on 2, 2- diphenyl-1-picryhydrazyl (DPPH) free radical was measured according to the method of[14]. Appropriate dilution of the extracts (1 mL) was mixed with 1 mL of buffer (0.1 M sodium phosphate buffer, pH 7.0 containing 1% (w/v) Triton X-100). DPPH was dissolved in methanol to a final concentration of 100 µM. The sample (100 µl) which was mixed with 100 µL of the DPPH solution in the 96-well plate to a final assay concentration of 1 mg/mL and incubated at room temperature in the dark for 30 min. The absorbance values of the blank, Glutathione (GSH) (control) and samples were measured at 517 nm. The control is made up of sodium phosphate buffer in place of the extract samples while Glutathione (GSH) was used as the positive control. The percent DPPH radical scavenging activity of the samples was determined using the following equation.

2. ABTS Radical Scavenging Activity: The ABTS scavenging ability of the extracts was determined according to the method described by[15]. The ABTS was generated by reacting a (7 mM) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/L, final concentration) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. About 0.2 mL of the appropriate dilution of the extract was added to 2.0 mL of ABTS solution and the absorbance was read at 732 nm after 15 min. The ABTS scavenging activity was calculated.

Table 1: Phytochemical analysis of some selected traditional medicinal plants.

Plants name	Extract	Protein	Tannin	Phenol	Saponins	Terpenoids	Steroids	Glycoside
Boerhavia diffusa	HE	+	+	+	-	+	+	-
	PE	+	+	+	+	+	+	-
	ME	+	+	+	+	+	+	+
	WA	+	+	+	+	+	+	+
Calyptocarpus vialis	HE	+	-	+	-	-	-	+
	PE	+	-	+	+	-	-	+
	ME	+	+	+	+	+	+	+
	WA	+	+	+	+	+	+	+
Turnera ulmifolia	HE	+	+	+	-	-	+	+
	PE	+	+	+	+	+	+	+
	ME	+	+	+	+	+	+	+
	WA	+	+	+	+	+	+	+
Desmodium tortuosum	HE	-	-	+	+	+	+	+
	PE	+	+	+	+	+	+	+
	ME	+	+	+	+	+	+	+
	WA	+	+	+	+	+	+	+
Heliotropium indicum	HE	+	+	+	+	+	+	-
	PE	+	+	+	+	+	+	-
	ME	+	+	+	+	+	+	+
	WA	+	+	+	+	+	+	+

+ present of phytochemical, - absent of phytochemical.

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RESULTS

Phytochemical analysis of some selected traditional medicinal plant was screened for medicinally bioactive compounds and it revealed the presence of various active phytochemicals. Based on characteristic color intensity, the following results are tabulated in Table 1.

Biochemical parameters of some selected traditional medicinal plants.

The present study showed that carbohydrate and protein increased as the concentration of extract and fractions increased, with methanol fraction of medicinal plants exhibiting the highest levels of phenolics when compared with all other fractions, as shown in Table 2.

Quantification assays

The present study showed that TPC, TTC and TFC increased as the concentration of extract and fractions increased, with methanol fraction of medicinal plants exhibiting the highest levels of phenolics when compared with all other fractions, as shown in Table 2 (fig-1,2,3).

Table 2: Biochemical parameters of carbohydrate and protein some selected traditional medicinal plants.

S. No	Extracts	*D. tortuosum* mg/ml	*B. diffusa* mg/ml	*C. vialis* mg/ml	*T. ulmifolia* mg/ml	*H. indicum* mg/ml
Carbohydrate	HE	0.234±0.16	0.123±0.12	0.098±0.02	0.076±0.04	0.068±0.03
	PE	0.342±0.13	0.156±0.01	0.113±0.15	0.103±0.02	0.106±0.04
	ME	0.456±0.01	0.234±0.14	0.208±0.13	0.195±0.11	0.187±0.19
	WA	0.367±0.14	0.214±0.11	0.134±0.05	0.121±0.12	0.113±0.15
Protein	HE	0.213±0.09	0.134±0.09	0.112±0.06	0.098±0.08	0.078±0.06
	PE	0.342±0.01	0.195±0.08	0.123±0.11	0.117±0.03	0.102±0.08
	ME	0.378±0.03	0.345±0.12	0.233±0.01	0.213±0.09	0.211±0.11
	WA	0.356±0.08	0.277±0.07	0.221±0.02	0.218±0.18	0.198±0.03

Values were performed in triplicates determination (n=3) ± SD. HE: hexane PE: petroleum ether, ME: methanol, WA: water. mg GLUE/100 g extract, mg BSA/100 g extract, Mean values followed by different superscript in a column are significantly different (P<0.05).

Figure 1: Total phenolic content of some selected traditional medicinal plants

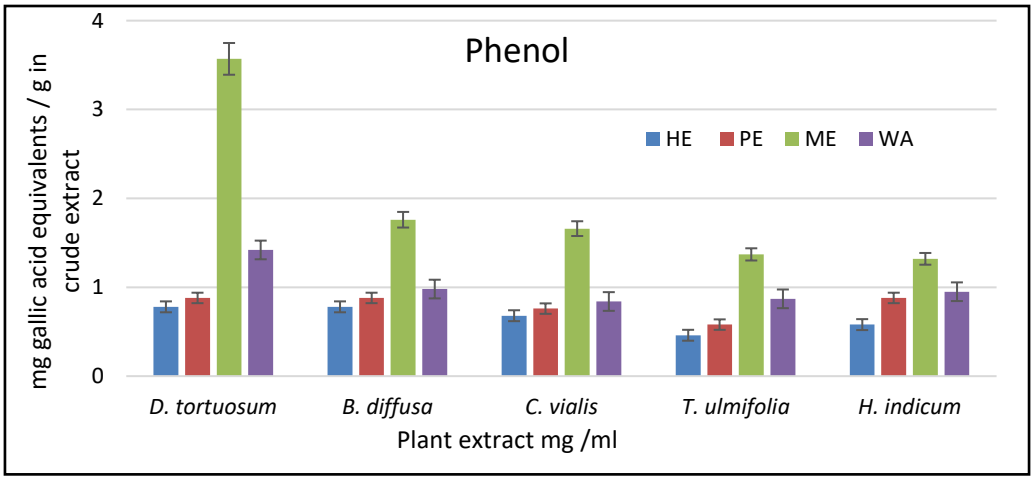

Figure 2: Total tannin content of some selected traditional medicinal plants

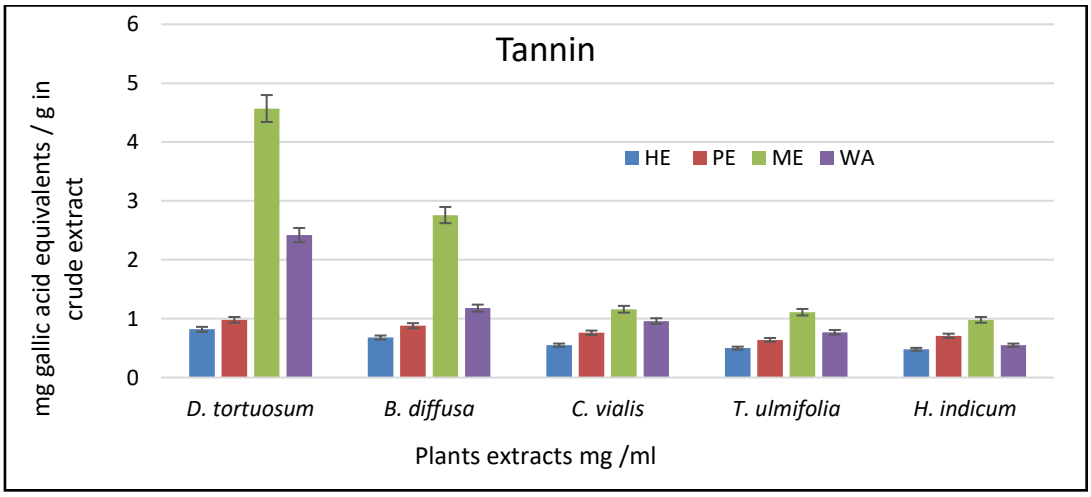

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Figure 3: Total flavonoids content of some selected traditional medicinal plants

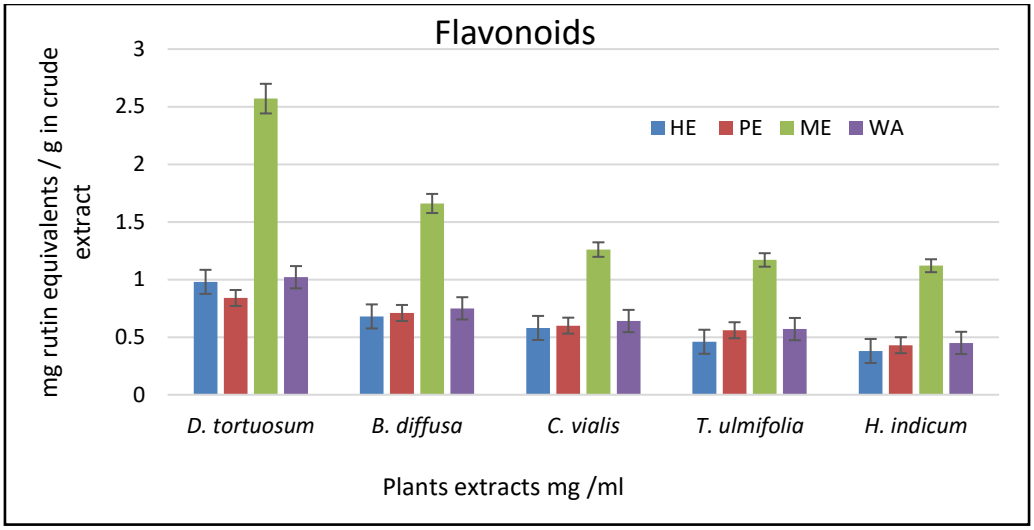


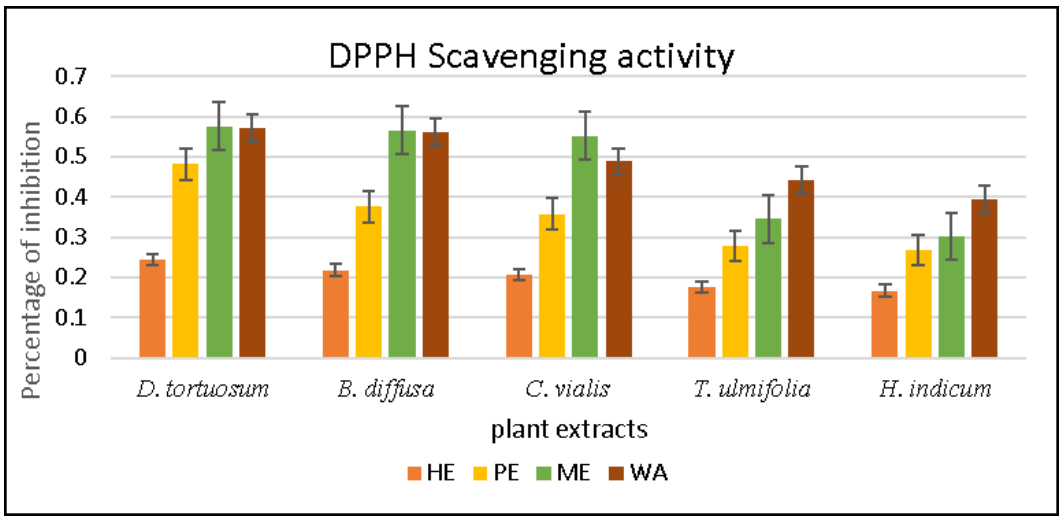
Table 3: Estimated Concentration of the Total Phenolic, Total Tannin and Flavonoid Content

Quantification	Extracts	*D. tortuosum* mg/ml	*B. diffusa* mg/ml	*C. vialis* mg/ml	*T. ulmifolia* mg/ml	*H. indicum* mg/ml
TPC (mg GAE/100g)	HE	0.76±0.07	0.78±0.02	0.68±0.09	0.46±0.02	0.67±0.08
	PE	0.78±0.01	0.82±0.01	0.76±0.01	0.58±0.01	0.68±0.01
	ME	3.57±0.03	1.76±0.03	1.66±0.03	1.37±0.03	1.32±0.03
	WA	1.42±0.09	0.98±0.09	0.84±0.09	0.88±0.09	0.95±0.09
TTC (mg GAE/100g)	HE	0.46±0.07	0.68±0.01	0.78±0.04	0.68±0.01	0.58±0.08
	PE	0.58±0.09	0.88±0.08	0.88±0.03	0.76±0.04	0.88±0.06
	ME	3.37±0.08	2.57±0.06	1.76±0.01	1.66±0.05	1.32±0.03
	WA	0.87±0.04	1.42±0.02	0.98±0.05	0.84±0.01	0.95±0.02
TFC (mg RE/100g)	HE	0.50±0.05	0.82±0.05	0.68±0.09	0.55±0.02	0.48±0.04
	PE	0.64±0.03	0.98±0.06	0.88±0.07	0.76±0.06	0.71±0.06
	ME	4.57±0.09	2.76±0.08	1.18±0.08	1.16±0.07	0.98±0.05
	WA	2.77±0.02	1.42±0.07	1.11±0.02	0.96±0.01	0.55±0.01

Values were performed in triplicates and represented as mean ± SD. HE: hexane PE: petroleum ether, ME: methanol, WA: water. mg GAE/100 g extract, mg RE/100 g extract, Mean values followed by different superscript in a column are significantly different (P<0.05)

Comparative *in vitro* Antioxidant activity studies on some traditional medicinal plants.

(a) DPPH Scavenging activity



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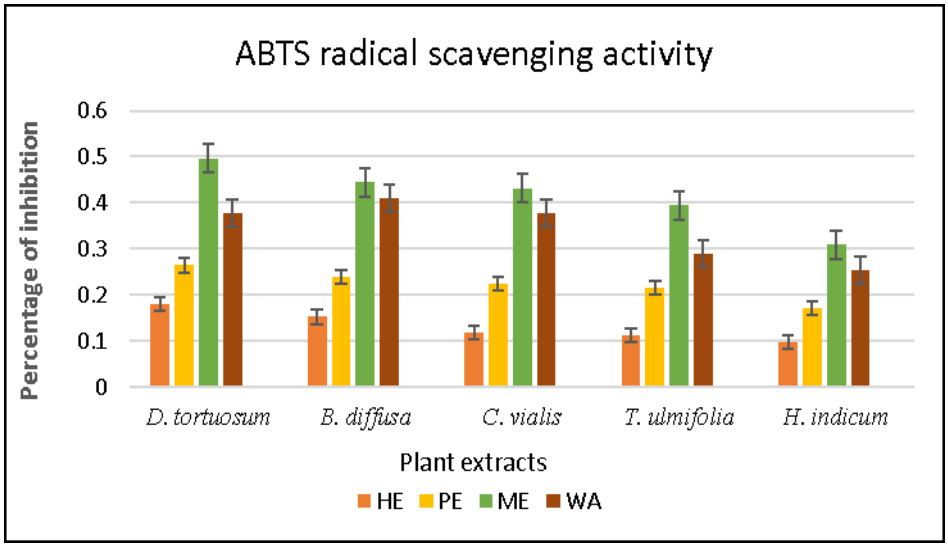
(b) ABTS radical scavenging activity



(c) Total antioxidant activity

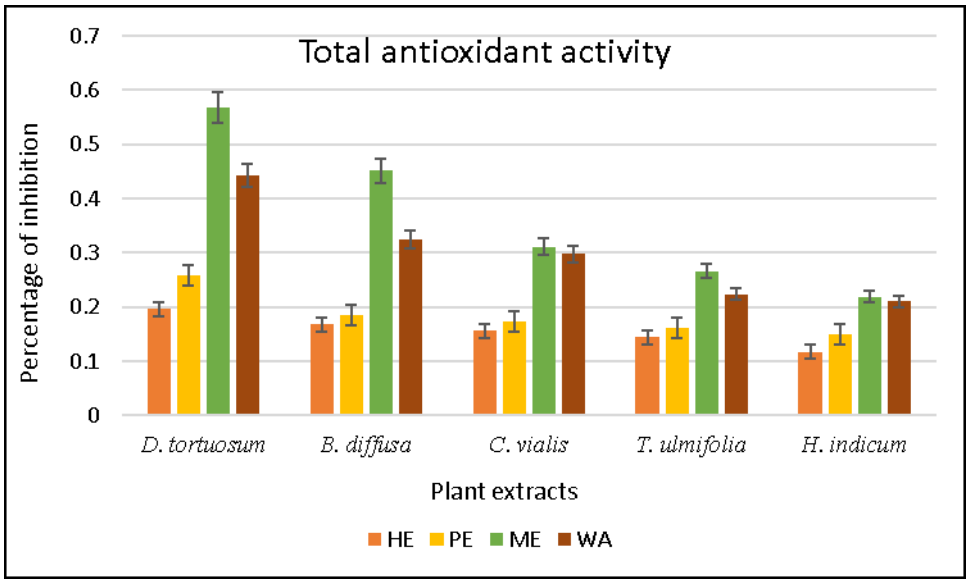


Table 4: DPPH, ABTS, Total antioxidant radical scavenging activity of some medicinal plants.

S. No	Extracts	*D. tortuosum*	*B. diffusa*	*C. vialis*	*T. ulmifolia*	*H. indicum*
DPPH (mg AAE/g)	HE	0.245±0.02	0.218±0.09	0.206±0.02	0.176±0.07	0.167±0.08
	PE	0.482±0.01	0.376±0.01	0.358±0.01	0.278±0.01	0.268±0.01
	ME	0.576±0.03	0.566±0.03	0.551±0.03	0.345±0.03	0.302±0.03
	WA	0.570±0.09	0.561±0.09	0.488±0.09	0.442±0.09	0.395±0.09
ABTS (mg QE/g)	HE	0.180±0.05	0.152±0.05	0.118±0.09	0.112 ±0.02	0.98±0.04
	PE	0.264±0.03	0.238±0.06	0.225±0.07	0.216±0.06	0.171±0.06
	ME	0.496±0.08	0.345±0.09	0.311±0.08	0.234±0.07	0.209±0.05
	WA	0.477±0.02	0.409±0.07	0.378±0.02	0.289±0.01	0.255±0.01
Total antioxidant (µM TE/gs)	HE	0.196±0.07	0.168±0.01	0.156±0.04	0.145±0.01	0.118±0.08
	PE	0.258±0.09	0.185±0.08	0.174±0.03	0.162±0.04	0.149±0.06
	ME	0.567±0.08	0.452±0.06	0.310±0.01	0.266±0.05	0.219±0.03
	WA	0.443±0.04	0.324±0.02	0.298±0.05	0.224±0.01	0.211±0.02

Values were performed in triplicates and represented as mean ± SD. HE: hexane PE: petroleum ether, ME: methanol, WA: water. Mean values followed by different superscript in a column are significantly different (P<0.05).

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DISCUSSION

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities[16]. Plant carbohydrates serve as substrates for energy metabolism, secondary metabolism, growth, development, and stress response. They are direct byproducts of photosynthetic CO2 fixation. Therefore, photosynthesis and glucose metabolism must be tightly controlled in order for plants to grow, develop, and respond to stress in a changing environment[17]. The proximate composition of carbohydrate and protein content in some selected traditional medicinal plants was determined and its results were expressed in terms of their standard in the total carbohydrate content in *D. tortuosum* whole plant methanolic extract was found to be 0.456±0.01mg/GAE/g, 0.234±0.14 mg/GAE/g, 0.208±0.13mg/GAE/g, 0.214±0.11mg/GAE/g, 0.195±0.11mg/GAE/g and 0.187±0.19mg/GAE/g, where its total protein content methanolic extract both plants was measure to be 0.378±0.03, 0.345±0.12, 0.233±0.01, 0.213±0.09 and0.211±0.11 mg/BSAE/g.

Phenolic compounds a large and diverse class of molecules known as includes a range of secondary aromatic metabolites found in plants. It has been reported to have antioxidant, anti-diabetic, and antibacterial effects, among other biological activities[18]. Total flavonoid content both whole plant extracts showed a strong presence of flavonoids when compared to standard rutin. The results exposed that the methanolic extracts of both plants the highest number of flavonoids was present in 4.57±0.09, 2.76±0.08, 1.18±0.08, 1.16±0.07 and 0.98±0.05 mg RE/100g extracts respectively.

The other extracts possess low flavonoid content at the concentration of 50µg/µl. We conclude that methanol extracts can be considered efficient solvents for better total phenolic and tannin content and flavonoids present[19]**.** Tannins are thought to have various properties, including analgesic, anti-diabetic, and anti-inflammatory properties. Tannins have the potential to be an efficient kidney relieving medication. Tannins are present in all selected plants, according to the results. These phytochemicals have a significant influence on hypoglycemic activity[20]. The total tannin content of both plant extracts showed strong tannin content presence of compared to the standard gallic acid[19]. Among the extract analysed, methanolic extracts both plant extracts showed better results and at a concentration of 50µg/µl found to be amounts of tannin content 3.37±0.03, 2.57±0.03, 1.66±0.03, 1.37±0.03 and 1.32±0.03 mg GAE/100 g extract respectively (Kurian *et al.,* 2010).

Desmodium triflorum methanol extract of whole plant was shown to possess a considerable scavenging antioxidant activity[21]. Among the crude extract, petroleum ether fraction, ethyl acetate fraction and n-butanol fraction of *Desmodium triflorum,* ethyl acetate fraction was the most active in scavenging DPPH and 1.25 mg of crude extract was similar to 61.2 ± 0.3 µg of ascorbic acid[22].

Boerhavia diffusa methanolic leaf extracts, respectively. However, this result was higher than 43% reported by another study for *Boerhavia diffusa* aqueous acetone extract. The differences observed in the various studies being reported might be due to differences in the experimental model, type of solvents used and plant species[23]. Significantly, free radical production and antioxidant defenses were out of balance, which led to oxidative stress. Free radicals are known to cause harm to a variety of molecular species, including proteins, lipids, and nucleic acids[24].

Reactive oxygen species production thus outpaced main endogenous antioxidants during infection and times of high stress in the body, which inevitably leads to health issues[25]. Therefore, the medicinal plant like the ones employed in this study are inexpensive, natural sources of a number of phytochemicals and polyphenols with these antioxidant properties that don't have any negative side effects. The results of the DPPH are commercial antioxidant assay were expressed in percentage inhibition value. The higher value of inhibition indicates a higher antioxidant activity**[26].** The following trend of inhibition values against DPPH radical in each phase was observed. The both plant extracts of methanol showed better radical to a yellowish-colored. The methanol extracts of *Desmodium triflorum* followed by *Boerhavia diffusa, Calyptocarpus vialis, Turnera ulmifolia,* and *Heliotropium indicum,* also exhibited potent antioxidant activities[27].

The results of the DPPH reaction and the ABTS radical cation decolorization assay were fairly similar. The radical produced when potassium persulfate oxidizes ABTS is a remarkable tool since it can disrupt chains and donate hydrogen[15]. Due to their higher phenolic contents, several plant extracts that have been considered as possible nutraceuticals have higher overall antioxidant activity[28]. The methanol extracts of *Desmodium triflorum* followed by *Boerhavia diffusa, Calyptocarpus vialis, Turnera ulmifolia,* and *Heliotropium indicum,* also exhibited potent antioxidant activities.

CONCLUSION

The present study analysis of qualitative, quantitative and antioxidant activities showed mainly in the medicinal plant of can be the potent source of natural antioxidants The present study revealed the phenolic and flavonoid spectrum of medicinally important plants. Among the extracts, methanolic extract has highest anti-oxidant property when compared to other extracts. In the present study it was found that *Desmodium tortuosum* can be regarded for natural plant sources of antioxidants with high potential value for drug preparation.

Acknowledgement

The work was financially supported by Ministry of Human Resource and Development, Department of Higher Education, Rashtriya Uchchatar Shiksha Abhiyan RUSA 2.0- BCTRC (Memorandom No. IQAC/ RUSA2.0/PA/2021/1 dated 08.01.2021) Government of India.

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

Funding Source: Rashtriya Uchchatar Shiksha Abhiyan RUSA 2.0-BCTRC

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