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Estimation of total phenolic content and antioxidant activity of digera muricata extracts by various in vitro assays

S. Saini*

Department of Botany, Janta Vidya Mandir Ganpat Rai Rasiwasia College, Charkhi Dadri-127306, Haryana, India

***Address for Correspondence: Sushila Saini**
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ABSTRACT

The existing study was planned to evaluate the free radical scavenging activity of *Digera muricata* solvent extracts by different in vitro assays and compare it with total phenolic content. *Digera muricata* is a wild edible herb used traditionally for treating diabetes, urinary discharges, as laxative and to increase lactation after child birth. Different solvent extracts (petroleum ether, benzene, chloroform, methanol and aqueous) were assessed for their ability to scavenge free radicals such as DPPH, superoxide, and hydroxyl radicals, as well as their metal chelating capacity, reducing power along with quantification of phenolic content. All the solvents exhibited substantial free radical scavenging activity in a concentration dependent manner. Among the five extracts, the methanolic extract showed the highest free radical scavenging activity and yielded 32.38 mg/g gallic acid equivalents (GAE) of phenolic content. The study found a significant positive correlation between the total phenolic content and the antioxidant assays, indicating that phenolic compounds played a role in the antioxidant activity of *D. muricata* extracts. These findings suggest that the methanolic extract of *D. muricata* contains a significant amount of phenolic compounds, which could be accountable for its antioxidant activity. These results highlight the potential of *D. muricata* as a source of natural antioxidants and support its traditional use for various health conditions.

Keywords: Free radicals, *Digera muricata*, antioxidant, plant extracts, phenolics, DPPH

INTRODUCTION

Generation of free radicals is unavoidable for biological systems due to aerobic respiration (endogenous factors), stress, ionizing radiations, environmental pollution (exogenous factors). Their generation is associated with various pathological conditions such as atherosclerosis, neurodegenerative disorders, diabetes, cardiovascular diseases and cancer. Antioxidants by their electron donating ability can prevent the deleterious effects of oxidative stress. During normal conditions, a complex antioxidant defence mechanism present in the body consisting of enzymatic and nonenzymatic pathways keeps a check on the over generation of free radicals. However during disease state these systems can become compromised, resulting in build-up of reactive oxygen species causing oxidative damage to cellular machinery [1,2,3]. Generally oxidative stress is managed by use of various synthetic antioxidants such as BHA, BHT and PG, but their use is associated with mutagenesis, hepatotoxicity and tissue damage along with various drawbacks such as high cost, inaccessibility thus limiting their utilization. Therefore there is always a search for novel antioxidants that are economical and without any side effects. Medicinal plants, which produce a wide range of secondary metabolites, can serve as alternative antioxidants by acting as electron donors or serving as metal chelators to prevent the generation of free radicals. These antioxidant systems help the plant to cope with the abiotic and biotic stress and when consumed by human beings can provide similar effects [4,5].

False Amaranth also called *Digera muricata* L. (Amaranthaceae) is a herbaceous plant widespread throughout the plains of India as a weed in cultivated fields during rainy season. The whole plant has medicinal properties and is also used for making vegetables. Due to the presence

of several nutrients such as calcium, potassium, iron, magnesium and ascorbic acid it is recognised as famine food. In folklore medicine it is documented for the management of urinary discharges, renal stones, bowel complaints, constipation and considered as cooling, mild astringent and expectorant. Its extracts were reported to possess noticeable antidiabetic, antidiarrheal, antimicrobial, antidepressant, analgesic, neuroprotective and hepatoprotective activities. Various secondary metabolites such as flavonoids, alkaloids, terpenoids, glycosides, tannins, saponins, coumarins and cardiac glycosides have been isolated from different parts of *D. muricata* [6,7,8]. Considering the growing interest in finding natural antioxidants from plant sources, the current work focuses on investigating in vitro free radical scavenging activity of various solvent extracts obtained from the stem of *Digera muricata*.

MATERIAL AND METHODS

Preparation of plant extracts

For the present study disease free plants of *Digera muricata* were collected from nearby areas. Plant specimen was identified with the help of flora of Janaki Ammal Herbarium, Jammu with the accession number 8014. Stem of *D. muricata* were shade dried, grounded in a mechanical grinder and petroleum ether, benzene, chloroform, methanol and aqueous extracts were prepared by cold percolation method. The extracts were then analyzed for total phenolic content and antioxidant activity at varying concentrations ranging from 0.1 mg/ml to 0.5 mg/ml.

Assessment of total phenolics and free radical scavenging activity

Total phenolics were estimated using a modified method based on Singleton and Rossi [8] and the concentration of phenolic content was calculated by constructing a standard gallic acid calibration curve. The free radical scavenging activity of the plant extracts was evaluated by measuring the reduction of DPPH radical using the method described by Lee et al [9]. The decrease in absorbance at 517 nm indicated an increase in free radical scavenging activity, and the results were compared with a standard (ascorbic acid, 10 µg-50 µg/ml). Generation of superoxide anion in the PMS-NADH system was analyzed by NBT reduction method stated by Liu et al [10]. Hydroxyl radical scavenging activity of extract was measured by TBA (Thiobarbituric acid) reaction as recommended by Kunchandy and Rao [11]. Metal ion chelation by various extracts of *D. muricata* was done according to Dinis et al [12] and compared with the standard EDTA. Reducing capacity of solvents were evaluated by analysing their capacity to reduce ferric cyanide complex to ferrous form according to Yen and Duh [13], and an increase in absorbance indicated an increase in reducing ability.

Statistical analysis

Results were expressed as mean ± standard deviation and percentage scavenging activity was estimated as:

Scavenging (%) = (Acontrol - Asample)/Acontrol x 100

Data was statistically analysed by one-way ANOVA, and significance of differences between concentration was estimated by DMRT test ($p<0.05$). Pearson correlation coefficient was used to estimate correlation between various assays and phenolic content.

RESULT AND DISCUSSION

Total phenolic content (TPC)

Plant phenolics possess various beneficial properties, including their capacity to quench radicals, exhibit reductive ability, chelate transition metals, prevent radical chain initiation, and decompose peroxides. These properties make phenolic compounds effective in preventing the progression of chronic diseases caused by oxidative damage [14]. Therefore, assessing the antioxidant activity of an extract requires the quantification of phenolic compounds present. Figure 1 shows the phenolic content in different extracts at various concentrations. Among the extracts tested, the methanolic extract exhibited the highest total phenolic content, with a value of 32.38 mg/g GAE (gallic acid equivalents). The chloroform extract had the second highest phenolic content, followed by the aqueous, petroleum ether, and benzene extracts, in descending order. The higher phenolic content in the methanolic extract suggests that it may have greater potential for scavenging free radicals and providing antioxidant benefits compared to the other extracts.

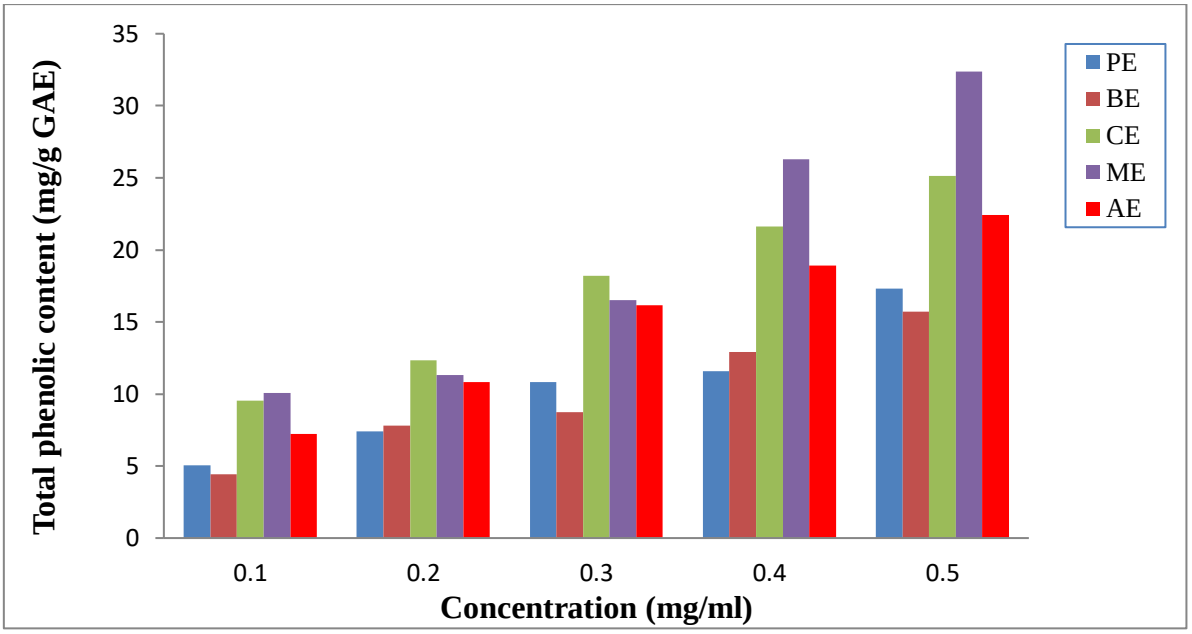

Fig 1: Determination of Total phenolic content (TPC) of *D. muricata* stem extracts; PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract

DPPH radical scavenging activity

It is convenient, rapid and successfully utilized assay for evaluating hydrogen donating capability of cereals, vegetables, fruits and oils. As DPPH is a stable radical and does not require to be generated, it can be used to estimate antioxidants in aqueous as well as nonpolar organic solvents. In this assay, the degree of discoloration indicates the antioxidant activity of an extract in terms of its ability to donate hydrogen. When DPPH accepts an electron it changes to stable diamagnetic molecule resulting in decrease in absorption and change of color of solution to yellow. Numerous reports have described the properties of plant extracts to scavenge DPPH radical in vitro [15,16]. In the case of *Digera muricata*, different extracts demonstrated concentration dependent scavenging of DPPH radicals, with the methanolic extract exhibiting the highest percentage inhibition, followed by the chloroform, aqueous, benzene, and petroleum ether extracts (Table 1). It is worth noting that the IC_{50} value of ascorbic acid, used as a reference compound, was much lower than those of all the extracts. This indicates that the plant extracts had weaker antioxidant activity compared to the reference compound, as they required higher concentrations to achieve the same level of DPPH radical scavenging.

Table 1: Determination of DPPH free radical scavenging activity (%) of *Digera muricata* stem extracts

	Stem extract (% scavenging activity)					Ascorbic acid (% scavenging activity)	
Conc. (mg/ml)	**PE**	**BE**	**CE**	**ME**	**AE**	**Conc. (µg/ml)**	**AS**
0.1	14.08±0.20^{e}	14.33±0.66^{e}	12.99±0.77^{e}	30.00±0.49^{e}	20.85±0.19^{e}	10	20.84±0.62^{e}
0.2	18.03±0.57^{d}	18.82±0.64^{d}	27.20±0.45^{d}	40.20±0.86^{d}	26.98±0.55^{d}	20	38.63±0.40^{d}
0.3	22.17±0.29^{c}	23.95±0.51^{c}	33.47±0.54^{c}	57.56±0.37^{c}	31.74±1.05^{c}	30	75.17±0.60^{c}
0.4	25.56±0.34^{b}	30.47±0.30^{b}	43.28±0.73^{b}	62.59±0.12^{b}	37.10±0.66^{b}	40	80.28±0.12^{b}
0.5	28.60±0.32^{a}	36.05±0.72^{a}	51.08±0.47^{a}	66.77±0.23^{a}	43.30±0.37^{a}	50	84.80±0.66^{a}

Columns having different letters (a-e) are significantly different at p<0.05 by DMRT; PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; AS- ascorbic acid

Superoxide radical scavenging activity

In biological system production of superoxide radical is common occurrence. These superoxide anions directly initiate lipid peroxidation or indirectly they damage biomolecules by generation of highly reactive hydroxyl radical in the presence of iron [17]. Superoxide radical scavenging of solvents and reference BHT increased with increasing concentration (Figure 2). The results indicate that the methanolic extract exhibited the highest inhibition of superoxide radicals, with a percentage inhibition of 52.54% at a concentration of 0.5 mg/ml. This was followed by the aqueous, chloroform, benzene, and petroleum ether extracts in decreasing order of inhibitory effect. The higher inhibitory effect of the methanolic extract on superoxide radical formation suggests that this solvent has promising antioxidant activity. The IC_{50} value of the methanolic extract, which represents the concentration required to inhibit 50% of the superoxide radicals, was found to be 0.45 mg/ml. In comparison, the IC_{50} value of BHT, the reference compound, was 0.18 mg/ml indicating that the methanolic extract has a weaker inhibitory effect on superoxide radicals compared to BHT.

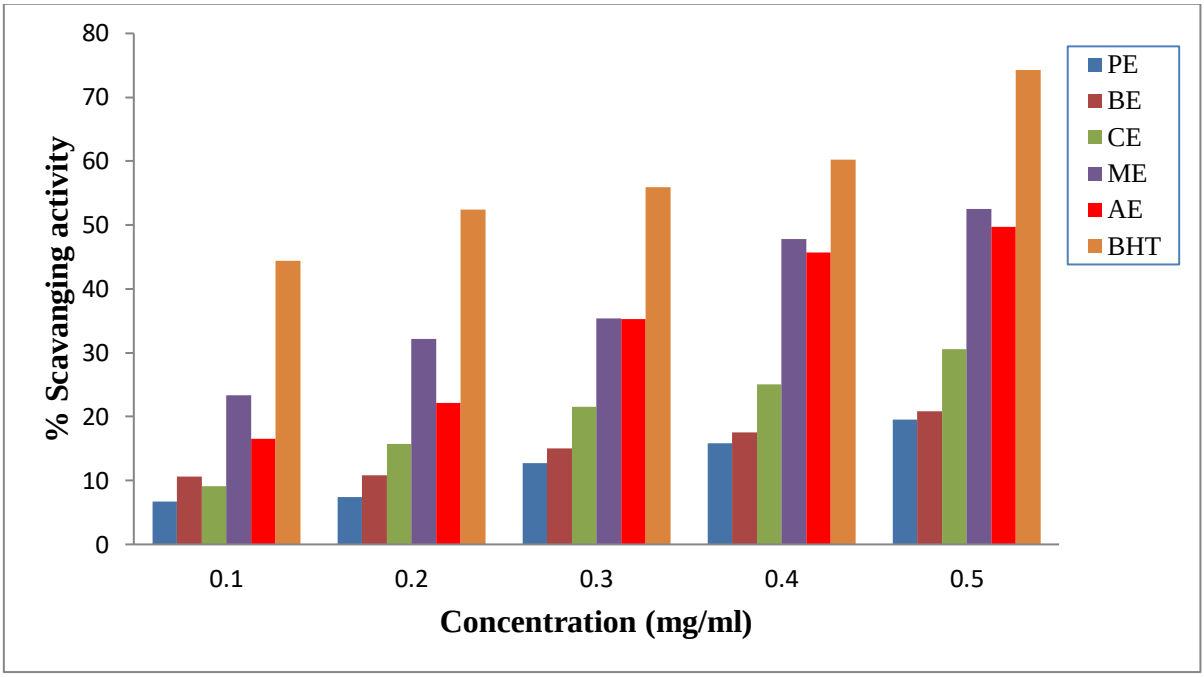

Fig 2: Determination of Superoxide radical scavenging activity (%) of *D. muricata* stem extracts ; PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; BHT- Butylated hydroxytoluene

Hydroxyl radical scavenging activity

Hydroxyl radical is the most deleterious free radical and it by direct reaction with DNA causes DNA breakage resulting in mutagenesis and cancer. Hydroxyl radicals generated in the solution degrades 2-deoxyribose to form malondialdehyde (MDH) that reacts with TBA to form pink coloured chromagen. Plant extracts by quenching hydroxyl radicals prevents the formation of chromagen [18]. The results show that the extracts of *Digera muricata* exhibit significant hydroxyl radical scavenging activity in a dose-dependent manner, as depicted in Figure 3. The methanolic extract demonstrated the highest hydroxyl radical scavenging activity, trailed by the aqueous, chloroform, petroleum ether, and benzene extracts. The IC_{50} value was found to be 0.52 mg/ml for the methanolic extract and 0.09 mg/ml for ascorbic acid. This indicates that the methanolic extract is a weaker antioxidant compared to ascorbic acid, as it requires a higher concentration to achieve the same level of hydroxyl radical scavenging activity.

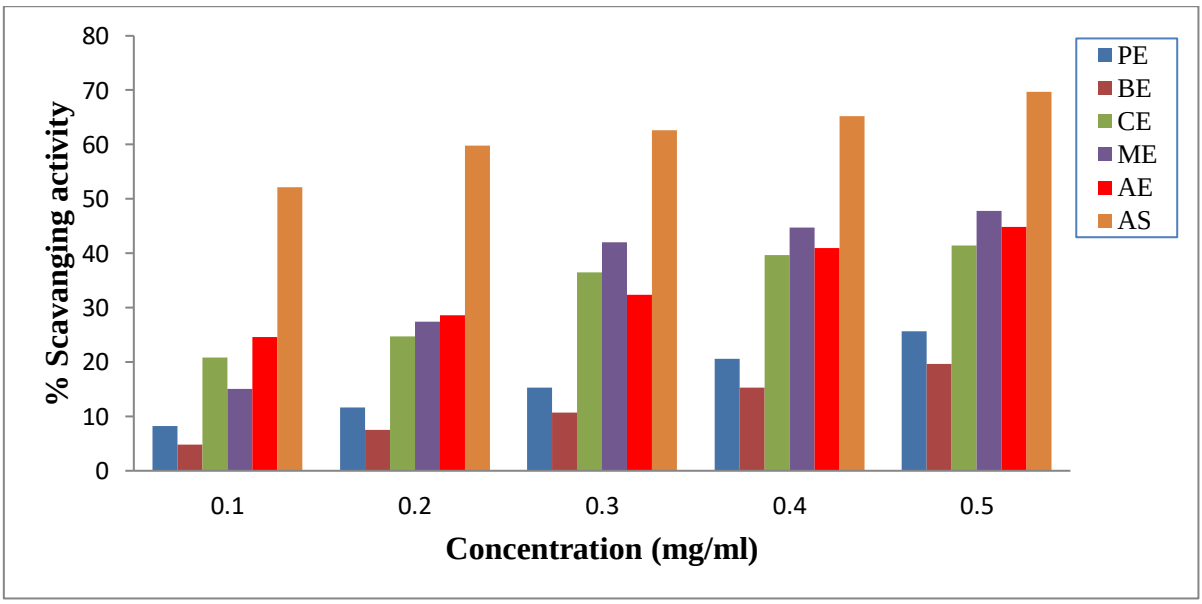

Fig 3: Determination of Hydroxyl radical scavenging activity (%) of *D. muricata* stem extracts; PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; AS-Ascorbic acid

Metal chelating ability

Ferrous ions by causing generation of free radicals through Fenton reaction can lead to lipid peroxidation and DNA damage. Iron chelators by forming stable, soluble complexes with iron mobilize them leading to their excretion in urine or faces. Antioxidants having the ability to chelate Fe^{2+} will reduce its concentration and inhibit its ability to catalyze free radicals generation thus protecting against oxidative damage related diseases [19,20]. Ferrous ion chelating ability was measured by inhibition of Fe^{2+} Ferrozine complex by test samples. Extracts of *D. muricata* chelate metal ions and inhibit Fe^{2+}ferrozine complex formation as depicted by decreased absorbance in dose dependent manner. Among the extracts, the methanolic extract exhibited the highest metal-chelating ability, followed by the chloroform, aqueous, benzene, and petroleum ether extracts (Figure 4).The IC_{50} value of the methanolic extract for ferrous ion binding was found to be 0.51 mg/ml, which was higher than that of the standard EDTA (0.21 mg/ml). This suggests that the plant extract is not as potent of a chelator as the standard EDTA. However, the extract still displayed significant chelating activity, as indicated by the concentration-dependent decrease in absorbance. It is important to note that while EDTA is a commonly used chelator in laboratory settings, the plant extract may possess its own unique chelating properties and mechanisms.

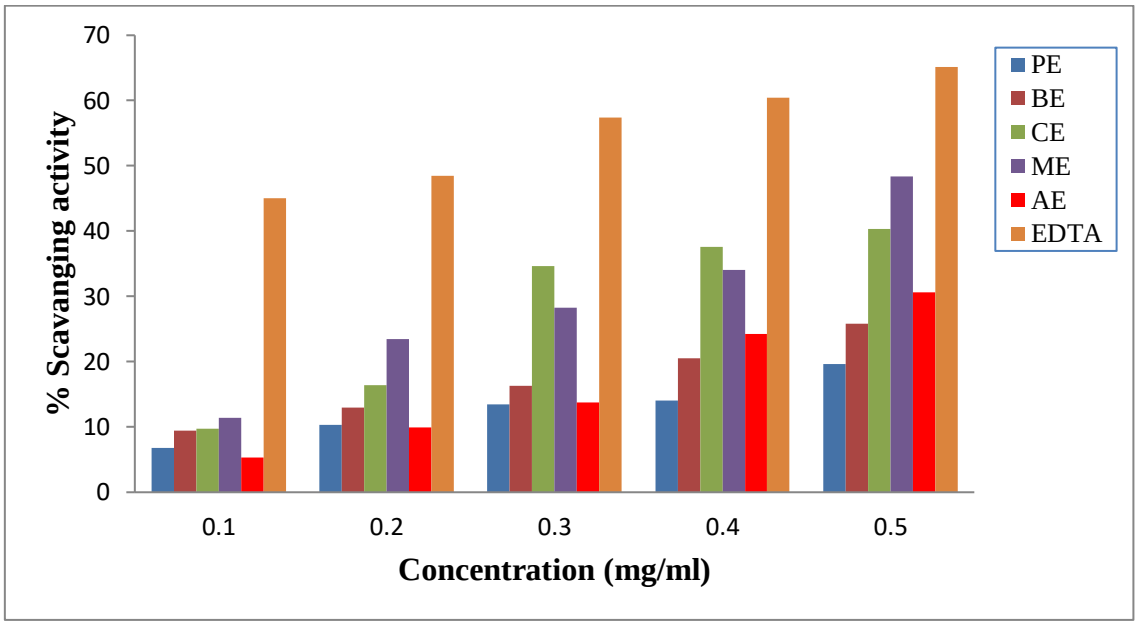

Fig 4: Determination of Metal chelating activity (%) of *D. muricata* stem extracts; PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; EDTA- Ethylenediamine tetra acetic acid

Reducing power assay

The reducing power assay is used to evaluate the ability of an antioxidant to donate an electron. The presence of reductones in the extract leads to the reduction of ferricyanide to ferrocyanide, resulting in a color change of the solution to various shades of blue, which is directly proportional to the reductive potential of the antioxidant [21]. Figure 5 demonstrates the reducing power of different solvent extracts of *Digera muricata* compared to the standard ascorbic acid. As the concentration of the extracts increased, the reducing power also increased. The order of the extracts' ability to reduce Fe^{3+} to Fe^{2+} was as follows: methanol > benzene > aqueous > petroleum ether > chloroform. The methanolic extract exhibited the highest reductive ability among the extracts, indicating that it is a stronger antioxidant compared to the other extracts due to its higher reductive potential. This finding suggests that the methanolic extract of *Digera muricata* possesses a significant capacity to donate electrons and effectively reduce ferricyanide, indicating its strong antioxidant activity. The reducing power assay provides valuable insights into the antioxidant potential of the plant extract and its ability to counteract oxidative stress.

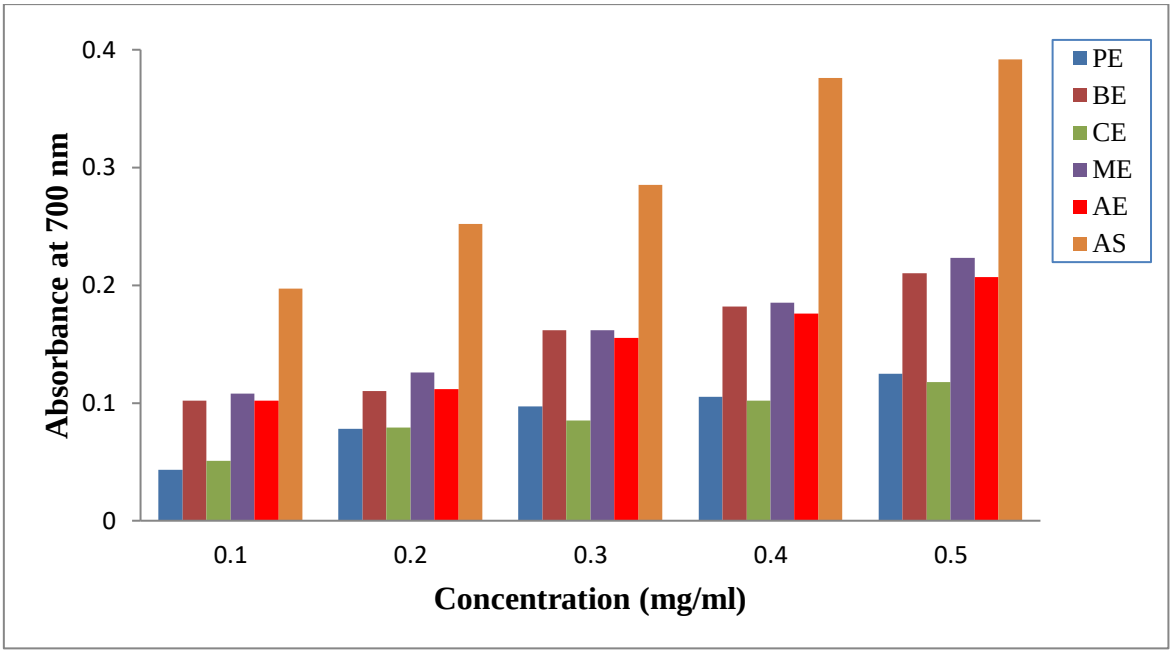

Fig 5: Determination of Reducing power assay of *D. muricata* stem extracts; PE-Petroleum ether extract; BE-Benzene extract; CE-Chloroform extract; ME- Methanol extract; AE- Aqueous extract; As- Ascorbic acid

Correlation of total phenolic content and antioxidant activity

The antioxidant property of herbal plants is primarily attributed to the presence of polyphenolic compounds, which include phenolic acids, flavonoids, anthocyanins, and tannins. The reactivity of phenolics is due to the aromatic ring in their structure having hydroxyl groups that can act as scavengers of free radicals. Previous studies also showed substantial relationship between plant phenolics and antioxidant activity indicating the potential of phenolics in quenching free radicals [22,23,24]. In the case of *D. muricata* stem methanolic extract, a significant positive correlation was observed between various antioxidant assays and the total phenolic content. The value of correlation coefficients were calculated as 0.902895, 0.972504, 0.87159, and 0.94107 for DPPH, superoxide, hydroxyl radicals, and metal chelating assay, respectively at 0.5 mg/ml concentration (Table 2). These high correlation values confirm the important role of phenolic compounds in contributing to the antioxidant activity of the extract. Previous studies reported the presence of bioactive compounds such as alkaloids, flavonoids, terpenoids, saponins, coumarins, tannins and cardiac glycosides from different fractions of *D. muricata* [25]. From chloroform extract of *D. muricata* flavonoid quercetin and sesquiterpene **β**-caryophyllene were isolated [26]. Additionally, phenolic compounds such as quercetin, sinapic acid, and ferulic acid, alkaloids including cystine and berberine, and a terpenoid called limonene were detected in the roots and shoots of *D. muricata* [27]. The presence of these phenolic compounds in *D. muricata* contributes to its antioxidant activity and underscores the therapeutic potential of this plant.

Table 2: Determination of correlation between total phenolics and antioxidant activity

Antioxidant activity	Total phenolic content	
	r	R^2
DPPH assay	0.902895*	0.815*
Superoxide radical scavenging assay	0.972504*	0.945*
Hydroxyl radical scavenging assay	0.87159*	0.759*
Metal chelating assay	0.94107*	0.885*

**significance at 95% confidence level*

CONCLUSION

The data obtained from the present investigation clearly demonstrate the significant free radical scavenging activity of the methanolic extract of *D. muricata* stem. The observed antioxidant activity can be attributed to the presence of phytochemical compounds present in the extracts, particularly the phenolic compounds discussed earlier. These findings highlight the potential of *D. muricata* stem as a valuable source of natural antioxidants. Further research and exploration of the specific bioactive compounds within *D. muricata* could provide valuable insights into its mechanism of action and its potential applications in the field of medicine and healthcare.

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