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COMPARATIVE ASSESSMENT OF ANTIOXIDANT ACTIVITIES OF TWO WILD COLLECTED PLANTS AND ITS COMMERCIAL PREPARATIONS

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ABSTRACT: Objective: This study was to screen antioxidant nature of whole wild collected plant material (*Vernonea cinerea* and *Cardiospermum helicacabum*) and compared it with commercially available plant powder. Methods: Six different methods like DPPH, Reducing power assay, Nitric oxide scavenging activity, ABTS radical scavenging power, Superoxide radical scavenging activity and H2O2 Scavenging activity using standard operating procedure. Results: *Vernonea cinerea* and *Cardiospermum helicacabum* exhibited good antioxidant potentials in the various assays conducted with good IC_{50}.Conclusion: Wild freshly collected plant extracts showed better antioxidant activity when compared to commercial plant powder.

KEYWORDS: *Vernonea cinerea*, *Cardiospermum helicacabum*, Fruit rind, Antioxidants, Phytochemicals.

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1.INTRODUCTION

Main players of today's medicine are antioxidants. During 1990s microbial infections are the common threats to human society. Now a days, metabolic disorders creates a threat to human society in the name of diabetes mellitus, cancer, coronary disorders, kidney disorders etc.,[1, 2] . Metabolic disorders are due to cell bombardment by free radicals generated during our general oxidation process. This is because of an imbalance between formation and neutralization of pro oxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with

biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid per oxidation [3,4]. One of the major concepts of traditional system of medicine like siddha are food is medicine; medicine is food. Our traditional food is enriched with lots of medicine from nature. Now it is replaced with synthetic purified chemicals. These chemicals itself able to cause metabolic stress to cell. Phytochemicals present in medicinal plants are the rich source of free radical scavengers like phenolic compounds, tannins and flavonoids [5, 6]. These chemicals scavenge free radicals and protect human from metabolic disorders. In this context, in the present study two medicinal plants were selected and collected as wild as well as from commercial sources & assessed for antioxidant activity or free radical scavenging power [7,8]. Traditionally *Vernonea cinerea* and *Cardiospermum helicacabum* are used for the treatment of inflammatory disorders like arthritis, neuritis etc., Free radicals are one of the good reason for inflammatory disorders. *Vernonea cinerea* is an annual plant belonging to the family Asteraceae. It is commonly called as Mukuthipundu in Tamil, Sahadevi in Sanscrit, Puuvankirutala in Malayalam and little iron weed in English [9]. The whole plant is edible and used for the treatment of inflammation, bleeding, swelling, asthma, wound healing [10,11,12]. *Cardiospermum helicacabum* is an annual climber belonging to the family Sapindaceae [13]. This plant has been used for the treatment of rheumatism [14]. It also shows an analgesic, antipyretic and antifilarial activity [15]. It is commonly called as heartwine in English and Mudakaruthaan in Tamil [16]. Both these plants are used for the treatment of inflammatory disorders like arthritis, bronchitis etc as an anti-inflammatory agent. Having known the importance of these plants, the present study was undertaken to screen antioxidant nature of whole wild collected plant material using standard method and compared it with commercially available plant powder.

2. MATERIALS AND METHODS

Plant material

Whole plant of *Vernonea cinerea* and *Cardiospermum helicacabum* were collected as wild from Keezha Puthalam Village, Kanyakumari district and stored market powder of these plants were procured from local siddha medicinal store, Nagercoil, Kanyakumari. The wild collected plants were washed thoroughly with water and shade dried. After drying, the plant materials were ground into powder and then sieved using a sieve. Five hundred grams of powdered plant was transferred to airtight containers and stored at room temperature. Plant materials after extraction were coded as VCWEE (*Vernonea cinerea* Wild ethanolic extract), CHWEE (*Cardiospermum helicacabum* wild ethanolic extract), VCCEE (*Vernonea cinerea* Commercial ethanolic extract) and CHCEE (*Cardiospermum helicacabum* Commercial ethanolic extract). The plant material was identified by Dr. John Britto, Professor, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamilnadu, India and specimen was deposited in PG and research department of Microbiology, M. R. Government Arts College, Mannargudi, Tamilnadu, India.

Preparation of extracts

The powdered plant material (150gm) was extracted with alcohol using cold maceration method. The extracts were filtered with a muselin cloth and the filterate was concentrated in vacuum evaporator. Dried extracts were used for further studies[17].

***Invitro* antioxidant assay**

DPPH assay: (2, 2diphenyl1picrylhydrazyl)

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as

$$(DPPH) + (H\text{-}A) \rightarrow DPPH\text{-}H + (A)$$

(Purple) (Yellow)

Antioxidants react with DPPH, a stable free radical which was reduced to DPPH-H and as a consequence, the absorbance was decreased from the DPPH radical to the DPPH-H form. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

DPPH radical scavenging activity (Spectrophotometer)

The free radical scavenging capacity of the *Vernonea cinerea* and *Cardiospermum helicacabum* alcoholic extract was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. Extracts of *Vernonea cinerea* and *Cardiospermum helicacabum* were mixed with 95% methanol to prepare the stock solution (10mg/100mL). The concentration of extract solution was 10mg/100mL or 100µg/mL. From stock solution 2mL, 4mL, 6mL, 8mL and 10mL of the solution were taken in five test tubes and serially diluted, final volume of each test tube was made up to 10mL whose concentration was then 20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL and 100µg/mL respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing extracts (20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL, 100µg/mL) and after 10 minutes, the absorbance was taken at 517nm using a spectrophotometer (Systronics UV-Visible Spectrophotometer 119, INDIA). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10mg/100mL or 100µg/mL) of extracts. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank. % scavenging of the DPPH free radical was measured using the following equation (Acontrol-ATest)/Acontrol X 100. where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts. The IC50 value was defined as the concentration (in µg/mL) of extracts that produced 50% antioxidant effect[18].

Reducing power assay

Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form Potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm. This experiment was carried out as described

previously[19]. One mL of plant extract solution (final concentration 100-500mg/L) was mixed with 2.5mL phosphate buffer (0.2M, pH 6.6) and 2.5mL potassium ferricyanide [K3Fe (CN6)] (10g/L), then the mixture was incubated at 500C for 20 minutes. To this 2.5mL of trichloroacetic acid (100g/L) was added, and centrifuged at 3000rpm for 10 minutes. Finally, 2.5mL of the supernatant solution was mixed with 2.5mL of distilled water and 0.5mL Fecl3 (1g/L) and the absorbance was measured at 700nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 119, India). Ascorbic acid was used as standard and phosphate buffer as blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power. A test is the absorbance of test solution; A blank is absorbance of blank. The antioxidant activity of the rind extract was expressed as IC_{50} and compared with standard.

Nitric oxide scavenging activity [20]

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. Nitric oxide was generated from sodium nitroprusside, which at physiological pH liberates nitric acid. This nitric acid gets converted to nitrous acid and further forms nitrite ions (NO^{2-}) which diazotize with sulphanilic acid and couple with naphthylethylenediamine (Griess reagent), producing pink colour, which can be measured at 546nm. Sodium nitroprusside (10mM, 2mL) in phosphate buffer saline was incubated with the test compounds in different concentrations at room temperature for 30 minutes. After 30 minutes, 0.5mL of the incubated solution was added with 1mL of Griess reagent and the absorbance was measured at 546nm. The nitric oxide radicals scavenging activity was calculated according to the following equation (Acontrol-ATest)/Acontrol X 100.

Superoxide radical scavenging activity (PMSNADH System)

Superoxide anions were generated using PMS / NADH system. The superoxide anions are subsequently made to reduce nitroblue tetrazolium, which yields a chromogenic product, which is measured at 560nm. Phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system was used for the generation of superoxide anion. It was assayed by the reduction of nitroblue tetrazolium (NBT). About 1mL of nitro blue tetrazolium (156µM), 1mL NADH (468µM) in 100mM phosphate buffer of pH 7.8 and 0.1mL of sample solution of different concentrations were mixed. The reaction started by adding 100µl PMS (60µM). The reaction mixture was incubated at 25°C for 5 minutes and absorbance of the mixture was measured at 560nm against blank samples. The percentage inhibition was determined by comparing the results of control and test samples. (Acontrol-ATest)/Acontrol X 100 is a formula adopted to measure % of free radical scavenging activity[21].

ABTS radical scavenging assay

ABTS (2, 2'-azinobis-3-ethylbenzothiozoline- 6-sulphonic acid) assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process that is associated with a change in absorption. The relatively stable ABTS radical was green and it was quantified spectrophotometrically at 734nm. ABTS radical cations were produced by reacting ABTS and APS. The ABTS scavenging capacity of the extract was compared with that of BHT and ascorbic acid and percentage inhibition was calculated. The stock solutions included 7mM ABTS solution and 2.4mM potassium per sulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowed them to react for 14 hrs at room temperature in the dark. The solution was then diluted by mixing 1mL of ABTS solution with 60mL methanol to obtain an absorbance of 0.706 ± 0.01 units at 734nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1mL) were allowed to react with 1mL of the ABTS solution and the absorbance was taken at 734nm after 7 minutes using a spectrophotometer. (Acontrol-ATest)/Acontrol X 100 formula is used to assess ABTS radical scavenging activity where A control is the absorbance of ABTS radical in methanol. All determinations were performed in triplicate (n = 3) [22].

H_2O_2 scavenging activity

H_2O_2 scavenging ability was determined according to the method of Ali *et al*.,[23]. A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). The aqueous and alcoholic extracts at the 30µg/mL concentration in 3.4mL phosphate buffer were added to a H_2O_2 solution (0.6mL, 40mM). The absorbance value of the reaction mixture was recorded at 230nm. Blank solution was containing the phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging of aqueous and alcoholic extracts and standard compounds were calculated using the formula:[(AControl-ASample) /AControl] × 100 Where AControl is the absorbance of the control, and ASample is the absorbance in the presence of the sample of aqueous and alcoholic extracts.

3. RESULTS AND DISCUSSION

DPPH Assay

Spectrophotometric assay of DPPH mediated free radical scavenging activities of alcoholic extracts of wild and commercial plant materials of *Vernonea cinerea* and *Cardiospermum helicacabum* and standard were presented in table1. Result revealed that VCWEE at 100 µg/ml concentration showed 60.3±1.5 free radical scavenging power with 82.9 µg/ml of IC_{50}. IC_{50} of CHCEE was found to be lower when compared to other extracts (112.1µg/ml).

Table 1: *In-Vitro* Free Radical scavenging effect of *Vernonea cinerea* and *Cardiospermum helicacabum* by DPPH method

Test	Percentage Scavenging (mean±SD) of Triplicates					
	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC_{50}µg/ml
VCWEE	15.3±2.5*	20.3±1.5*	36.6±4.1*	41.6±3.7*	60.3±1.5	82.9
CHWEE	17.3±0.6*	23.6±3.2	34.6±1.5**	42.0±3.0**	56.3±3.8**	88.8
VCCEE	11.3±1.5*	19.0±1.0	29.3±3.0	37.0±3.61	57.3±2.5	87.2
CHCEE	15.6±0.5	19.0±1.0	28.6±2.3	34.0±1.0	44.6±4.1	112.1
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	5.3±0.6***	14.4±0.9*	24.9±0.6**	37.9±1.7*	52.6±0.5*	64.7

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

Reducing Power Assay

Antioxidant plays a very important role in human health. This method is used for measuring the Fe^{3+} to Fe^{2+} reducing ability of extracts. This method was a significant indicator, which showed antioxidant power of the study material. The conventional antioxidants have been shown to improve the animal performance by increased tissue oxidant levels such as stress injury and also infections. Table 2 showed the reducing power of *Vernonea cinerea* and *Cardiospermum helicacabum*alcoholic extracts. Here yellow coloured test solution changes to various shades of green and blue depends on the available reducing power components. Reducing components converts Fe^{3+} to Fe^{2+}, which was analyzed spectrophotometrically. CHWEE showed better ferric reducing power (97.0 ± 1.0%) with 51.5µg/mL IC_{50}. Similarly VCWEE also produced good ferric reducing activity with 54.5µg/mL IC_{50}. IC_{50} for ascorbic acid was found to be 19.5µg/mL.

Table 2: *In-Vitro* Free Radical scavenging effect of *Vernonea cinerea* and *Cardiospermumhelicacabum* by Reducing power assay

Test	Percentage Scavenging (mean±SD) of Triplicates					
	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC50µg/ml
VCWEE	25.0±3.0*	32.0±2.0	41.0±2.6	74.0±3.5	91.6±6.1	54.5
CHWEE	68.3±2.9*	68.0±3.6*	76.0±5.2*	89.6±5.2*	97.0±1.0*	51.5
VCCEE	26.3±4.0*	44.0±6.0*	65.6±2.5*	47.0±2.6*	85.3±0.6*	58.6
CHCEE	22.3±4.5*	33.3±4.1*	47.6±6.8*	65.3±2.5*	85.0±5.0*	58.8
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	28.2±3.6**	76.8±1.9*	83.8±2.7	86.6±4.8	96.3±1.1*	19.5

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

Nitric Oxide Scavenging Assay

NO is a very stable species that react with oxygen and produce nitrite and nitrate. NO is a potent plessiotrophic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and cell mediated toxicity regulation [24]. More or less similar nitric oxide scavenging activity was exhibited by VCWEE, CHWEE and VCCEE (Table 3). The percentage of radical scavenging power (% inhibition) increased with the increased concentration of extracts. VCWEE, CHWEE, VCCEE and CHCEE and standard produced 57.9, 50.1, 60.9, 80.33 and 52.0µg/mL IC_{50} respectively.

Table 3: *In-Vitro* Free Radical scavenging effect of *Vernonea cinerea* and *Cardiospermumhelicacabum*nitric oxide scavenging assay

Test	Percentage Scavenging (mean±SD) of Triplicates					
	20µg/ml	**40µg/ml**	**60µg/ml**	**80µg/ml**	**100µg/ml**	**IC_{50}µg/ml**
VCWEE	24.6±2.9**	54.0±3.6*	65.3±2.5**	70.6±1.5*	86.3±5.1**	57.9
CHWEE	36.6±2.1**	52.7±2.5*	71.0±4.4*	73.0±1.0*	87.6±1.5*	50.1
VCCEE	18.0±1.0**	25.3±2.1**	47.3±1.5**	67.0±2.7*	82.0±6.1**	60.9
CHCEE	18.3±0.6**	24.3±2.1**	39.6±6.5**	59.3±3.8*	62.3±3.2**	80.3
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	13.4±0.7**	21.5±1.4**	33.2±2.2**	31.8±0.0*	36.4±4.6**	52.0

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

Superoxide Radical Scavenging Assay

Superoxide anion radical is generated by four electron reduction process and converted molecular oxygen into water. It is a highly reactive free radical resulting in cell death and tissue damage. Superoxide radical is considered to be very harmful to cellular component responsible for tissue damage, which causes cancer emphysema, cirrhosis, arthritis, atherosclerosis and various kinds of infections. In the present study, CHWEE produced 94.6±2.5 % superoxide radical scavenging activity followed by 75.0±6.1% for VCWEE, 64.3±2.1% for VCCEE and 58.6±0.5 for CHCEE (Table 4).

Table 4: *In-Vitro* Free Radical scavenging effect of *Vernonea cinerea* and *Cardiospermum helicacabum* by Superoxide radical scavenging assay method

Test	Percentage Scavenging (mean±SD) of Triplicates					
	20µg/ml	**40µg/ml**	**60µg/ml**	**80µg/ml**	**100µg/ml**	**IC_{50}µg/ml**
VCWEE	26.3±1.5**	37.3±2.1*	43.6±1.5**	66.0±2.6*	75.0±6.1**	66.6
CHWEE	30.6±1.2***	51.3±6.1*	56.0±3.6*	85.3±3.5**	94.6±2.5**	52.8
VCCEE	23.1±2.7**	31.3±0.6**	50.3±4.5**	57.6±2.5**	64.3±2.1**	77.7
CHCEE	21.6±2.5***	27.6±1.2**	34.6±1.1**	44.6±1.2**	58.6±0.5**	85.3
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	4.6±1.5***	11.0±1.1**	35.5±4.9**	41.8±1.1*	50.7±1.2*	67.8

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

ABTS Radical Scavenging Activity

The ABTS (2, 2-azinobis-3-ethylbenzothioline 6- sulfonic acid) is a decolouorizing assay employing a specific absorbance at a wave length of 734 nm. It is a short reaction experiment and accessed within a quick time. It is widely applied for lipophilic and hydrophilic processes. The 2, 2-azinobis-3-ethylbenzothiazoline 6-sulfonic acid (ABTS) activity of the extracts were comparable to standard ascorbic acid. Ascorbic acid showed good antioxidant power (IC_{50} =18.2µg/mL) when compared to extracts in ABTS radical scavenging assay (Table 5). VCWEE produced 94.3±1.5% radical scavenging power.

Table 5: *In-Vitro* Free Radical scavenging effect of *Vernonea cinerea* and *Cardiospermum helicacabum* ABTS radical scavenging assay method

	Percentage Scavenging (mean±SD) of Triplicates					
	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC_{50}µg/ml
VCWEE	18.6±2.0**	38.3±5.7*	62.6±2.1*	85.6±3.2*	94.3±1.5*	53.02
CHWEE	22.3±0.5**	32.0±4.4**	49.1±2.6*	63.6±2.1*	79.6±2.0*	62.8
VCCEE	11.3±0.5**	24.3±6.7**	31.6±1.5*	56.5±2.0*	80.3±1.1**	62.3
CHCEE	16.0±9.5**	25.3±2.5**	38.6±3.2*	62.3±2.0*	79.6±1.5**	62.8
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	55.5±7.64*	71.1±7.5*	81.7±3.0*	86.7±0.3*	92.79±2.2*	18.2

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

H_2O_2 Method

The OH radicals are extremely reactive in biological systems and they are capable of damaging to the biomolecules of living cell. This radical combines with the nucleotide of DNA molecule and causes the strand breakage mechanism leading to cancer, mutation and also cell cytotoxicity [25]. Good H_2O_2 conversion was noted in wild collected plant VCEWW and CHWEE with 89.0±2.6 and 86.3±3.7% radical scavenging power (Table 6). Results of antioxidant nature of both extracts and standard were significantly different among various concentrations ($p<0.001/0.01/0.05$).

Table 6: In-Vitro Free Radical scavenging effect of Vernonea cinerea and Cardiospermum helicacabum H2O2 method

Test	Percentage scavenging (mean±SD) of triplicates					
	20µg/ml	**40µg/ml**	**60µg/ml**	**80µg/ml**	**100µg/ml**	**IC_{50}µg/ml**
VCWEE	31.3±2.0**	62.6±4.0**	77.6±1.9*	76.6±1.1*	89.0±2.6*	56.2
CHWEE	15.3±3.5**	38.0±4.3**	56.3±1.5**	63.3±1.5*	86.3±3.7*	57.9
VCCEE	12.6±3.1**	51.3±1.5**	47.6±2.0**	56.3±4.6*	75.0±2.6*	66.6
CHCEE	14.3±4.9**	51.0±3.5**	39.6±3.5**	45.3±2.0*	70.6±2.0*	70.8
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	28.6±5.1**	41.5±2.3*	50.4±2.5*	58.2±8.3*	74.6±0.5*	27.8

* Significant at $p<0.001$ level ** Significant at $p<0.01$ level *** Significant at $p<0.05$ level

DISCUSSION

Free Radicals and ROS are responsible for multiple numbers of metabolic stress related disorders [26]. Oxidation generates singlet oxygen, which bombard our cellular system and cause chronic diseases[27]. Phytoconstituents of medicinal plants may reduce the risk of metabolic disorders like cancer; cardiovascular diseases and diabetes. Antioxidant is one of the most essential ingredient of today's menu/therapy because the antioxidative system protects the animal against reactive oxygen species (H_2O_2, superoxide, OH, singlet oxygen & nitrogen species) induced oxidative damage. Alcoholic extracts of *Vernonea cinerea* and *Cardiospermum helicacabum* wild collected and commercially available plant powder has been studied for its antioxidant properties using different in vitro antioxidant methods. Flavonoids, phenolic acids, tannins, steroids are found in the extracts of *Vernonea cinerea* and *Cardiospermum helicacabum* [28]. In this respect, poly phenolic compounds commonly found in plants have been reported to have multiple biological effects like Anticancer[29], Antiproliferative[30], Antimicrobial[31], wound healing [32] and Antibacterial [33] activities including Antioxidant activity [34,35]. The efficiency of each extract differed against various free radicals depending on the specific assay methodology, which reflects the complexity of the mechanisms and diversity of the chemical nature of the plant material. Many scientists have reported the potent antioxidant capacity of *Vernonea cinerea* and *Cardiospermum helicacabum* [36,

37]. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating activity[38]. Alcoholic extracts of *Vernonea cinerea* and *Cardiospermum helicacabum* had significant radical scavenging effect on the DPPH radical. Hydrogen donor in the extract may responsible for DPPH radical scavenging power of the extracts which increased with the increased concentration of the extract. Antioxidants present in the extracts reduce $Fe^{3+}$to$Fe^{2+}$by donating electrons. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging [39]. The data in table 2 indicated the superior antioxidant power of extracts i.e., more than 90% at 100µg/ml concentrations. Antioxidants compete with oxygen and reduce nitric oxide production which in turn reduces the burden of nitric oxide. Nitric oxide plays a vital role in various inflammatory processes. Higher levels of these radical are toxic to tissue and contribute to the vascular collapse. Hyper level expression of nitric oxide radical is associated with various carcinoma and ulcerative colitis. The toxicity of nitric oxide increases when it reacts with superoxide radical forming highly reactive peroxy nitrate anion. Reactive oxygen species, nitric oxide is implicated in inflammation, cancer and other pathological conditions [40]. The results of the present study suggested that the extracts of *Vernonea cinerea* and *Cardiospermum helicacabum* is a more potent scavenger of superoxide radical with <66.6µg/ml of IC_{50} value (Table 4). Superoxide anions are highly toxic to cellular components. Rupesh Kumar *et al.,* [39] indicated the effect of flavones from *Cardiospermum helicacabum* as an effective antioxidant thereby it protects liver as an hepatoprotective agent. Extracts inhibited the formation of blue formazan in superoxide radical scavenging system which is evidenced in table 4. Kumaran and Joal Karunakaran [40] also illustrated that extracts of *Cardiospermum helicacabum* showed a good NO reducer. Chromatophore ABTS+ was formed by the reaction between ABTS and potassium persulphate and reduced to ABTS by the action of antioxidants available in the extracts. In the present study, IC_{50} value for the extracts on ABTS scavenging process were 53.024µg/ml for VCWEE, 62.8 µg/ml for CHCEE, 62.3 µg/ml for VCCEE and 62.8 µg/ml for CHCEE.Hydrogen peroxide inactivates a few enzymes directly, usually by oxidation of essential thiol group. It can cross membranes and reacts with Fe^{2+} and Cu^{2+} ions to form hydroxy radical and are responsible for various toxic effects.Asha and Annie [41] stated that antioxidant compounds react with H_2O_2 and convert it into H_2O, which is involved in normal metabolic activities of the cell[39]. Quantitative phytochemical analysis indicated that the plant contains significant amounts of phenolic compounds such as Total phenolic acids, tannin and flavonoids. These classes of compounds were responsible for antioxidant and free radical scavenging effect of plant material [39, 40, 41]. Furthermore all of our results were significantly different among various concentrations as well as different extracts and standard ($p<0.05$).

4. CONCLUSION

The present study clearly revealed that the two medicinally important plants *Vernonea cinerea* and *Cardiospermum helicacabum exhibited good antioxidant potentials in the various assays conducted.* However the extracts of the two plants collected of various diseases and rejuvenate the whole metabolic system of animals and human beings. These two medicinally important plants can be used as a potential antioxidant agent after testing ADME properties of the plant extract.

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CONFLICT OF INTEREST

The authors declared that there are no conflicts of interest.

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