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QUANTITATIVE AND *IN VITRO* ANTIOXIDANT POTENTIAL OF VARIOUS FRACTIONS OF *HELICTERES ISORA* ROOTS

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ABSTRACT

Objects: The main object of this study is to explore the antioxidant and phyto-chemistry of various sequential (petroleum ether, benzene, chloroform, ethyl acetate, ethanol and aqueous) extracts of *H. isora* roots which is a medicinal plant. **Methods**: *In vitro* Phytochemical (phenol, flavonoid, proanthocynidins, tannins and saponin) of *H. isora* roots were estimated and various antioxidant (DPPH, FRAP, total antioxidant activity, metal chelating, hydroxyl radical, superoxide anion, nitric-oxide radical scavenging activity and reducing power assay) assays were performed. **Results:** The aqueous extract of *H. isora* exerted significant ($P<0.05$ and $P<0.001$) phytochemicals, and was found to have good radical scavenging activity as compared to the others extracts as well as synthetic antioxidants. These results were also supported by IC_{50} values. **Conclusions:** From the results of the present investigation, it could be concluded that *H. isora* roots extracts can be explored as a potential source for the isolation of natural antioxidant compounds.

KEYWORDS: *Helicteres isora*; Reducing power; Antioxidant activity; Flavonoids; IC_{50}.

INTRODUCTION

Antioxidants are naturally present in human body for the detoxification of ROS (Reactive oxygen species) and for protection against stress conditions. But overproduction of ROS is responsible for the various problems like oxidative damage of DNA, proteins, tissue and cells. Oxidative damage is a major mechanism that is involved in the development of chronic disease such as diabetes, aging, heart problems, cancer etc.[1-3] So, in this condition external

antioxidants are provided to the patient. Many synthetic antioxidants e.g. BHT, BHA etc. are available in the market. Use of these synthetic antioxidants is prohibited in some areas due to their less specificity, side effects and high cost. So, synthetic antioxidants are substituted with naturally present antioxidants i.e. herbal antioxidants. Medicinal plants are the good source of natural antioxidants. *Helicteres isora* a medicinal plant is one of them which is selected for the present study.

Helicteres isora belongs to Sterculiaceae family. It is commonly known as "Marorphali" in Hindi and "East India Screw Tree" in English is a medium sized tree, abundantly found in hills and forests, well known for its use in traditional medicinal values.[4] The roots and bark are expectorant, demulcent and are useful in colic, scabies, gastropathy, diabetes, diarrhea and dysentery.[5]

Helicteres isora showed antidiabetic[6], hypolipidaemic[7], anti-nociceptic[8], hepatoprotective[9] and cardiotonic[10] activities. Root juice of *H. isora* was claimed to be useful in fever, cough, asthma, stomach infections, intestinal infections[11] and was also useful for cuts, wounds[12] and scabies. From the roots betulic acid, daucosterol, sitosterol, isorin[13], cucurbitacin B and isocucurbitacin B were isolated.[14] Bark showed a significant hypoglycemic effect[15] and lowering effect of hepatic enzymes[16] Fruits were used in alleviating griping and flatulence[17] and also showed antispasmodic effects.[18]

Despite the extensive work on *H. isora*, the root part of this plant has not been studied for quantitative and antioxidant activities. Hence keeping these perspectives and the above mentioned medicinal properties of *Helicteres isora* in mind, the present study is designed to elucidate the antioxidant and quantitative screening of this plant part *In vitro*.

MATERIALS AND METHODS

All chemicals used in this study are of analytical grade and were purchased from reliable firms (SRL, MERCK, HIMEDIA and SIGMA).

Experimental plant

The experimental plant *Helicteres isora* was collected from Shiwalik range of Uttar Pradesh. The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra, Banasthali Vidyapith, Banasthali, Tonk district. The work was conducted on roots of *H. isora*.

Preparation of various fraction of *Helicteres isora*

The roots of *Helicteres isora* were washed, shade dried. For preparation of different type extract, shade dried plant parts (50 g) were subjected to size reduction to a coarse powder using electric grinder. The powder so obtained was packed into soxhlet apparatus and extracted with different solvent ranging from non polar to polar (petroleum ether, benzene, chloroform, ethyl acetate, and ethanol) and then aqueous extract was prepared via maceration method. These extracts were concentrated using rotary evaporator and stored at 4ºC in airtight containers for further experimental studies.

Quantitative assay

Total phenolic content

The principle of total phenolic content was given by Singleton and Ross, 1965. The content of the phenols in plant extracts is determined by Folin Ciocalteu method.[19] Phenolic compounds are potential antioxidants and free radical scavengers. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. Different concentrations of extracts and standard-gallic acid were prepared. Sample (1 ml) was mixed with 1ml of Folin-Ciocalteu´s reagent (1:20). After 5 minutes, 4 ml of Na_2CO_3 (7%) and 4 ml of distilled water were added. Incubation for 90 minutes at room temperature (dark) was provided and centrifuged at 10,000 rpm for 2-3 minutes. Absorbance of blue color supernatant was observed at 750 nm.

Total flavonoid content

Total flavanoid content in various extracts was estimated by method of Zhisen *et al.*, 1999[20] with slight modification, is also known as aluminum chloride colorimetric method in which aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl groups of flavonols and flavones. Different concentration of extracts and standard (rutin) were prepared. Sample (0.5 ml) was mixed with 3.5 ml distilled water and 0.3 ml of $NaNO_2$, after 5 minutes 0.3 ml of $AlCl_3$ solution was mixed and allowed to stand for 5 minutes. Then 2 ml of NaOH and 2.4 ml of distilled water was mixed, allowed to stand for 15 minute and absorbance was taken at 420 nm.

Total proanthocynidins contents

Total proanthocynidins content of plant extract was determined by Heirmann *et al.*, 1986.[21] Different concentration of extracts and standard (rutin) were prepared. Sample (10 ml) was mixed with 10 ml ethanol (70% v/v), 15 ml of HCl (25%) and 10 ml of distilled water. This

mixture was heated at 80-90ºC for 80 minutes. After evaporation up to 3 ml, cool and 15 ml of n-butanol was mixed. Absorbance was observed at 545 nm.

Total tannins contents

Total tannin content of plant extracts was determined by Folin-Ciocalteu procedure of Hagerman *et al.*, 2000.[22] Different concentrations of extracts and standard (tannic aid) were prepared. Sample (1ml) was mixed with 8 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent (1:20) and 1.5 ml of Na_2CO_3 (20%). Absorbance was observed at 775 nm against blank.

Total saponin content

Total saponin content of plant extracts were determined by modified method of Madland, 2013.[23] Different concentration of sample and standard (saponin) were prepared. Evaporated samples (100 µl) were mixed with 400 µl of vanillin-acetic acid (5%), 1.6 ml of perchloric acid and kept on water bath at 70-75ºC for 15 minutes. After cooling on ice for 2 minutes 2.5 ml of glacial acetic acid was added, mixed and absorbance was observed at 550 nm against blank.

Calculation for phytoconstituents in plant extracts

All results were calculated using following formula

$C = c. V/ m$

Where: C- Total content, mg/g plant extract (SE), c- The concentration of sample established from the calibration curve of standard (mg/ml), V- The volume of extract (ml), m- The weight of pure plant extract (g).

Antioxidant activities

DPPH-(1,1-diphenly-2-picrylhdrazyl) free radical scavenging activity

This is the most widely reported method for screening of antioxidant activity of many plant systems.[24] DPPH has the advantage of being unaffected by certain side reactions, such as metal chelation and enzyme inhibition. The free radical DPPH is reduced to the corresponding hydrazine when it reacts with the hydrogen donors. Different concentrations of extracts and standard-tocopherol were prepared. Samples (0.1 ml) were mixed with 3 ml of 0.004% DPPH solution and 30 minutes incubation at 37ºC was provided. Absorbance was measured at 517 nm against blank.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging capacity was measured using modified method of Halliwell *et al.*, 1987.[26] Different concentrations of extracts and standard- Quercetin were prepared. Samples (0.5 ml) were mixed with 0.2 ml of $FeSO_4.7H_2O$ (10 mM), 0.2 ml of EDTA (10 mM), 0.2 ml of deoxyribose (10 mM) and 0.7 ml of phosphate buffer (0.1M, pH= 7.4) to make volume up to 1.8 ml of whole mixture. This mixture was mixed with 0.2 ml of H_2O_2 (10 mM) and incubated at room temperature for 60 minutes. Then 0.5 ml of TBA and 1 ml of ice cold TCA (2.8% in 25 mM NaOH) were added to the mixture and again incubated for 30 minutes at 80ºC. Readings of the colored product (pink chromogen) was measured at 532 nm.

Super oxide scavenging activity

The super oxide radical scavenging capacity of various plant extracts were measured using method of Shivkumar *et al.*, 2006.[27] Different concentrations of extract and standard- ascorbic acid were prepared. Reaction mixture of 1ml NBT (144 µM in 100 mM phosphate buffer of 7.4 pH), 1ml of NADH (677 144 µM in phosphate buffer), 0.5 ml of sample or standard and 0.1 ml of PMS (60 µM in phosphate buffer) was prepared and after the 5 minutes incubation at 25ºC absorbance were observed at 560 nm.

Nitric oxide radical inhibition activity

The nitric oxide radical scavenging capability of the plant extracts were quantified by the method of Garrat, 1964.[29] Different concentrations of samples and standard (rutin) were prepared. Reaction mixture of sample or rutin (0.5 ml), 0.5 ml of phosphate buffer (pH = 7.4), 2 ml of sodium nitroprusside solution (10 mM) was prepared and this reaction mixture was incubated for 2.5 h at 25ºC. From above reaction mixture 0.5 ml was taken, mixed with 1 ml of sulphanilic acid (0.33% in 20% GAA) and allowed to stand for 5 minutes for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 minutes at 25°C. A pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm.

Metal chelating

The metal chelating activity of the plant extract was quantified by the method of Yamaguchi *et al.*, 2000.[31] Different concentrations of sample and standard (EDTA) were prepared. Sample (0.5 ml) was mixed with 0.5 ml of $FeSO_4$ (1mM), 1 ml tris HCl buffer, 0.5 ml of bi-

pyridyl solution (0.1%), 0.4 ml of 10% hydroxylamine HCl and 2 ml of ethanol. At 522 nm absorbance was measured.

Calculation of percentage scavenging potential and IC_{50} (50% Inhibition Concentration) values

The free radical scavenging and chelating activity of the extracts were calculated according to the following equation.

Radical scavenging activity (%) = (1- As/ Ac) x 100.

Where Ac was the absorbance of the control (without extract) and As was the absorbance of the sample.

IC_{50} values for above mention all parameters were calculated by regression equation (y = xa ± b) that was obtained from the standard graph and IC_{50} is the 50% inhibition concentration for particular sample. IC_{50} value is inversely proportional to the antioxidant activity.

Reducing power assay

The reductive capability of the plant extract was quantified by the method of Oyaizu, 1986.[30] Different concentrations of samples and standard (rutin) were prepared. Sample (1ml) was mixed with 2.5 ml of 0.2 M phosphate buffer and 2.5 ml of 1% potassium ferricyanide. After the 20 minutes incubation at 50ºC, 2.5 ml of 10% TCA was added for the reaction termination. The upper layer solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. At 700 nm absorbance was measured. Increased absorbance of the reaction mixture indicates greater reducing power.

FRAP (Ferric Reducing Antioxidant Power) assay

A modified method of Benzie and Strain, 1996[25] was used for this assay. The stock solution of FRAP includes 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6 and 20 mM $FeCl_3.6H_2O$ solution. The fresh working FRAP solution was prepared by mixing 2.5 ml TPTZ, 25 ml acetate buffer and 2.5 ml $FeCl_3.6H_2O$. Different concentrations of extract and standard-$FeSO_4$ were prepared. Working solution of FRAP (2.85 ml) was mixed with 0.15 ml sample and incubated for 30 minutes in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Results were expressed in µM Fe (II)/g dry mass.

Total Antioxidant Capacity (TAC)

It is a spectroscopic method for the quantitative determination of antioxidant capacity of samples through the formation of phosphomolybdenum complex. This assay is based on the reduction of Mo (VI) to Mo (V); by the sample constituents and subsequent formation of green phosphate Mo (V) complex at acidic pH.[28] Different concentration of extracts and standard-gallic acid were prepared. Reagent mixture of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate (1ml) was mixed with samples (1 ml). Tubes were capped and incubated in water bath at 95^0C for 90 minutes. Cool at room temperature and absorbance was recorded at 695 nm against blank. Total antioxidant capacity in plant extract in gallic acid equivalent (GAE) was calculated by the following formula: C = c. V/ m

Where: C- Total antioxidant capacity, mg/g plant extract (GAE), c- The concentration of gallic acid established from the calibration curve (mg/ml), V- The volume of extract (ml), m- The weight of pure plant extract (g).

Statistical Analysis

The experimental results obtained are expressed as mean ± standard deviation (SD). The data is subjected to one way analysis of variance (ANOVA) and difference between samples is determined by Bonferroni's multiple comparison test using the SPSS 16.0 (Statistical Program for Social Sciences) program. Results with $p<0.05$ are regarded as satisfactory and considered $p<0.001$ as very significant. For IC_{50} values calculation linear regression analysis was used.

RESULTS AND DISCUSSION

Quantitative Phytochemical assays

Secondary metabolites of *H. isora* root such as phenol, flavonoid, proanthocynidin, tannin and saponin in various extracts were analyzed and summarized in table 1. Total content in different sequential fraction of *H. isora* roots was calculated as gallic acid equivalent (GAE) or rutin equivalent (RE) using regression equations obtained from standard graph. Regression equations used for estimation of total phenol ($y=0.165x+0.073$, $R^2=0.988$), flavonoids ($y=0.970x+0.004$, $R^2=0.990$), proanthocynidin ($y=0.095x-0.002$, $R^2=0.949$), tannin ($y=0.05x+0.093$, $R^2=0.955$) and saponin ($y=0.076x+0.031$, $R^2=0.984$). Aqueous extract of *H. isora* was identified to possess the highest content of all five phyto-constituents (phenol, flavonoid, proanthocynidins, tannin and saponin). So, aqueous extract is rich in these components in comparison to other five extracts. Table 1 showed that petroleum ether and

ethyl acetate extract does not contain total flavonoid content. Total proanthocynidin content was absent in all extracts, but present in aqueous extract. Petroleum ether extract of *H. isora* possess very less amount of all components in comparison to the other extracts. According to the observation polar fraction possess more amounts of phytochemicals necessary for the free radical scavenging activity and antioxidant activity then the non-polar fraction. This might be the reason for the aqueous extract to possess more phenol, flavonoid and other phytochemicals in comparison to the other all extracts. Phenols have free radical scavenging ability due to the presence of hydroxyl groups. These are very important for the treatment of various diseases like cardiovascular diseases because phenols inhibit auto-oxidation of unsaturated lipids. The presence of phenols and flavonoids in the *H. isora* indicates that this plant might be used for antimicrobial, anti-inflammatory, anti clotting, immune enhancer, hormone modulators, antioxidant activity,[32] antipyretic, analgesic, spasmolytic and antiinflammation activities.[33-35] These finding suggested that *H. isora* is important medicinal plant for the treatment of various ailments. *H. isora* also contain saponin that bind to cholesterol and form insoluble complex that is excreted through the bile, so it prevents the reabsorption of cholesterol. Thus saponins are potentially useful for the cure of hypercholesterolemia.[36] *H. isora* is also helpful in wound healing and this is due to the

Table 1: Quantitative analysis of various bioactive compounds in different fractions of *H. isora*

EXTRACTS	Total Phenol (mg in gallic acid equivalent /g of dried plant extract)	Total Flavonoid (mg in rutin equivalent /g of dried plant extract)	Total Proanthocynidins (mg in rutin equivalent /g of dried plant extract)	Total Tannin (mg in tannin acid equivalent /g of dried plant extract)	Total Saponin (mg in saponin equivalent /g of dried plant extract)
PEHI	196.2±0.009*[a]	-	-	223.43±0.001* [a]	93.88±0.001* [a]
BEHI	230.97±0.013	190.84±0.015* [a]	-	228.4±0.007* [a]	108.27±0.001* [a]
CEHI	224.33±0.017*	177.84±0.008* [a]	-	229.23±0.001* [a]	100.89±0.002* [a]
EAEHI	208.71±0.004*[a]	-	-	228.76±0.002* [a]	113.85±0.007* [a]
EEHI	223.94±0.017*	264.31±0.005* [a]	-	230.06±0.002* [a]	99.46±0.003* [a]
AEHI	228.63±0.002*	769.396±0.031* [a]	10.78±0.001* [a]	231.95±0.001* [a]	91.54±0.002* [a]

Results are expressed in mean±SD of triplicates measurements, *P>0.05 compared with standard; aP>0.001 compared with standard, **PEHI-** Petroleum ether extract of *Helicteres isora*; **BEHI-** Benzene extract of *Helicteres isora*; **CEHI-** Chloroform extract of *Helicteres isora*; **EAEHI-** Ethyl acetate extract of *Helicteres isora*; **EEHI-** Ethanol extract of *Helicteres isora*; **AEHI-** Aqueous extract of *Helicteres isora*.

Presence of tannin content which plays an important role in wound healing.[37] The presence of tannin in *H. isora* strongly supports their use in treatment of frostbite, hemorrhoids, wounds, varicose ulcer and burns in herbal medicine.[38-39] Thus all these constituents are known to exhibit medicinal as well as physiological activities.

Antioxidant assays

DPPH radical scavenging activity

DPPH is a stable free radical even at room temperature and it is used to test the potential of compound as free radical scavenger of hydrogen donor and to investigate the antioxidant activity of *H. isora* root extracts. DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by extract either by transfer of hydrogen or of an electron.[40] All six extract of *H. isora* revealed a good DPPH radical scavenging activity, are shown in figure 1. DPPH scavenging activities of various fractions of *H. isora* depends on the concentration of the extracts. Maximum DPPH scavenging activity and minimum IC_{50} value were shown by aqueous extract after the standard (Tocopherol), which contained the highest amount of phenol, flavonoid, tannin and saponin. Petroleum ether extract showed minimum scavenging activity and maximum IC_{50} value as well as less amount of phenolic content. The ability to scavenge 50% of DPPH was found to be IC_{50} 0.4±0.12 mg/ml for aqueous > ethanol (0.66±0.23 mg/ml) > benzene (0.73±0.32 mg/ml)> ethyl acetate (1.73±0.91 mg/ml)> chloroform (3.28±1.01 mg/ml)> petroleum ether (7.01±1.24 mg/ml). According to these results, each extract of *H. isora* showed a phenol concentration dependent antioxidant activity. It has been found that ascorbic acid, flavonoid, tannin and aromatic amines, reduce and decolourise DPPH by their hydrogen donating ability.[41-42] Results also prove this because aqueous extract showed maximum amount of phenol, flavonoid and tannin and also showed maximum DPPH scavenging activity.

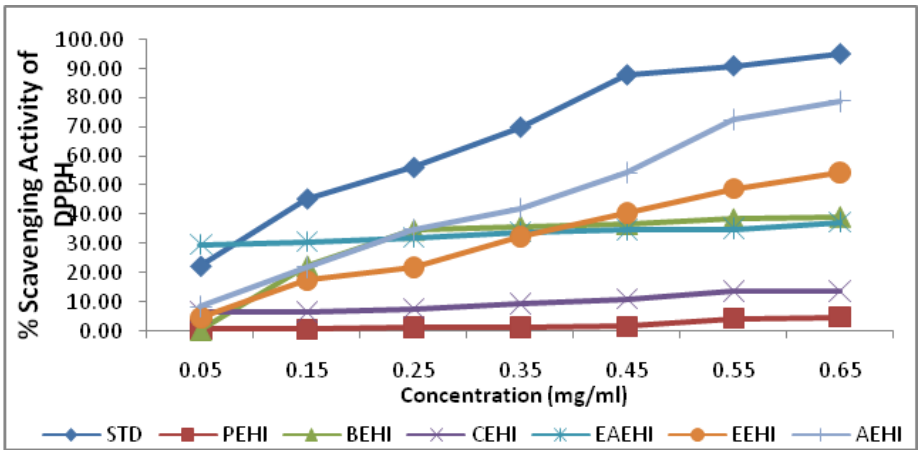

Figure 1: DPPH scavenging potential of various sequential fraction of *H. isora*.

Hydroxyl radical scavenging activity

Figure 2 shows percentage inhibition of the hydroxyl radical scavenging activity of different fractions of *H. isora* in the dose dependent manner. All extracts were found to exhibit strong antioxidant activity against hydroxyl radical with the IC_{50} values from 0.81 to 1.37 mg/ml compared to rutin. Ethanol extract was found to have highest antioxidant activity with an IC_{50} value of 0.81 mg/ml after the standard rutin. Benzene extract exhibit low level of antioxidant activity in comparison to the other extracts. The ability to scavenge 50% of hydroxial radical was found to be IC_{50} 0.81±0.10 mg/ml for ethanol > petroleum ether (1.13±0.33 mg/ml) > chloroform (1. 37±0.42 mg/ml)> ethyl acetate (1.62±0.91 mg/ml)> aqueous (3.04±0.81 mg/ml)> benzene (3.09±1.04 mg/ml). Hydroxyl radical is most reactive and induces severe damage to adjacent bio-molecules by abstracting hydrogen atom from membrane lipids and brings about peroxidation of lipids.[45] It is capable of damaging almost every molecule found in living cells[46] and induces carcinogenesis and mutagenesis.[47] The radical scavenging power of the extract can be accounted by the presence of phenol, tannins, flavonoid and saponin.

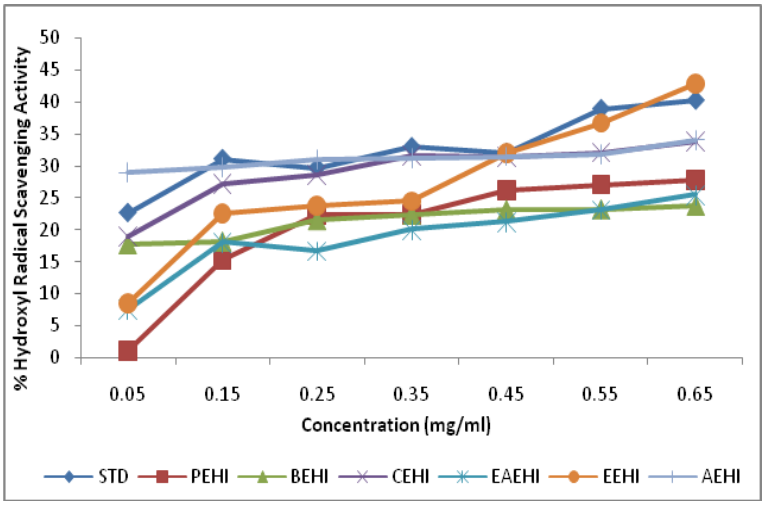

Figure 2: Hydroxyl radical scavenging capacity of the *H. isora* extracts.

Superoxide anion radical scavenging activity

Figure 3 shows percentage inhibition of the superoxide anion radical scavenging activity of different fractions of *H. isora* in the dose dependent manner. All extracts were found to exhibit strong antioxidant activity against superoxide anion radical with the IC_{50} values from 0.4 to 1.72 mg/ml compared to rutin. Aqueous extract was found to have highest antioxidant activity with an IC_{50} value of 0.4 mg/ml in comparison to standard and other extracts. Benzene extract exhibit low level of antioxidant activity in comparison to the other extracts.

The ability to scavenge 50% of superoxide anion was found to be IC_{50} 0.04±0.02 mg/ml for aqueous > ethanol (0.22±0.03 mg/ml) > petroleum ether (0.52±0.12 mg/ml)> chloroform (0.88±0.19 mg/ml)> ethyl acetate (0.91±0.11 mg/ml)> benzene (1.72±0.24 mg/ml). Superoxide is biologically important as it can form singlet oxygen and hydroxial radical[48], but when overproduction occur then these are known to be a very harmful species to cellular components as a precursor of more reactive oxygen species.[49] So it could be presumed that the aqueous extracts of *H. isora* act as a potential therapeutic agent for treatment of oxidative damage and prevent the effects of excessive superoxide anion generation in the human body.

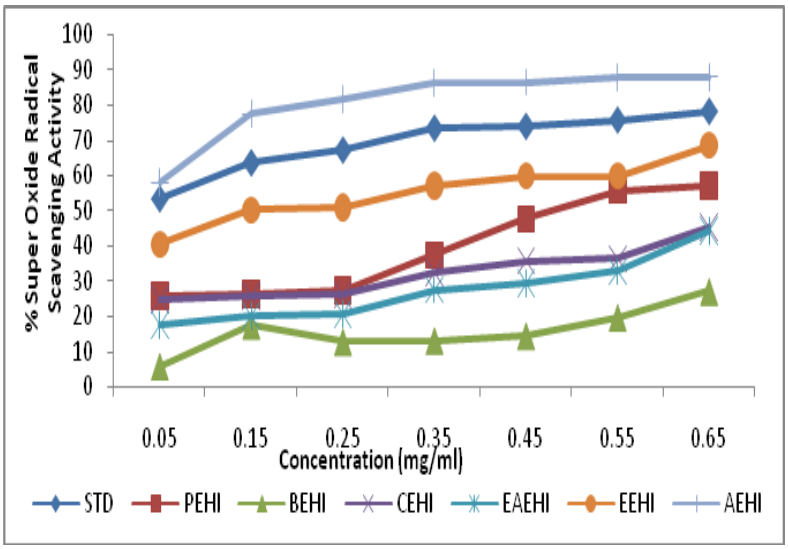

Figure 3: Superoxide anion radical scavenging activity of the *H. isora* extracts.

Nitric oxide radical scavenging activity

Figure 4 shows percentage inhibition of the nitric oxide radical scavenging activity of different fractions of *H. isora* in the dose dependent manner. All extracts were found to exhibit strong antioxidant activity against nitric oxide radical with the IC_{50} values from 0.03 to 0.5 compared to rutin. Aqueous extract was found to have highest antioxidant activity with an IC_{50} value of 0.03 mg/ml, after the standard rutin. But petroleum extract exhibit low level of antioxidant activity in comparison to the other extracts. The ability to scavenge 50% of nitric oxide was found to be IC_{50} 0.03±0.01 mg/ml for aqueous > ethanol (0.04±0.02 mg/ml) > ethyl acetate (0.05±0.02 mg/ml)> chloroform (0.27±1.01 mg/ml)> benzene (0.34±0.01 mg/ml)> petroleum ether (0.50±0.24 mg/ml). Nitric oxide is involved in a variety of biological functions, like vascular homeostasis, antimicrobial and antitumor activities,[50] but it can react with superoxide to form the peroxynitrite anion, that is a potential oxidant, which can decompose to produce OH^- and $NO^{\cdot}$,[51] it may lead to tissue damage, inflammation,

cancer and other biological problems. So it could be presumed that the extracts of *H. isora* act as a potential therapeutic agent for treatment of oxidative damage and prevent the effects of excessive nitric oxide radical generation in the human body.

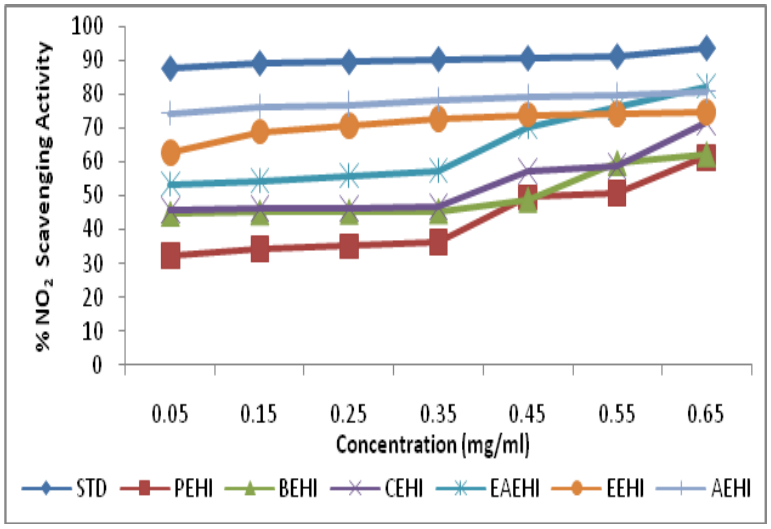

Figure 4: Nitric oxide Scavenging Capacity of the *H. isora* extracts.

Metal Chelating Activity

Metal chelation is the mechanism of antioxidation, which prevents catalysis of Fenton type reaction and hydrogen peroxide decomposition.[43] Figure 5 showed metal chelating activity of different fractions of *H. isora* in the dose dependent manner. All extracts were found to exhibit strong metal chelating activity with the IC_{50} values from 0.36 to 1.08 mg/ml compared to EDTA (0.3 mg/ml). Aqueous extract was found to have highest antioxidant activity with an IC_{50} value of 0.36 mg/ml after the standard EDTA. But benzene extract exhibit no antioxidant activity. The ability to chelate 50% of metal was found to be IC_{50} 0.36±0.11 mg/ml for aqueous > ethanol (0.42±0.13 mg/ml) > petroleum ether (0.77±0.24 mg/ml)> chloroform (0.79±0.11 mg/ml)> ethyl acetate (1.08±0.71 mg/ml). The metal chelating activity of the plant extract is of great significance, because metal ions contribute to the oxidative damage in neuro-degenerative diseases, like Parkinson's and Alzheimer's diseases.[44] Therefore, *H. isora* root extract displays a chelating activity then it can be of remedial potential in the cure of various diseases.

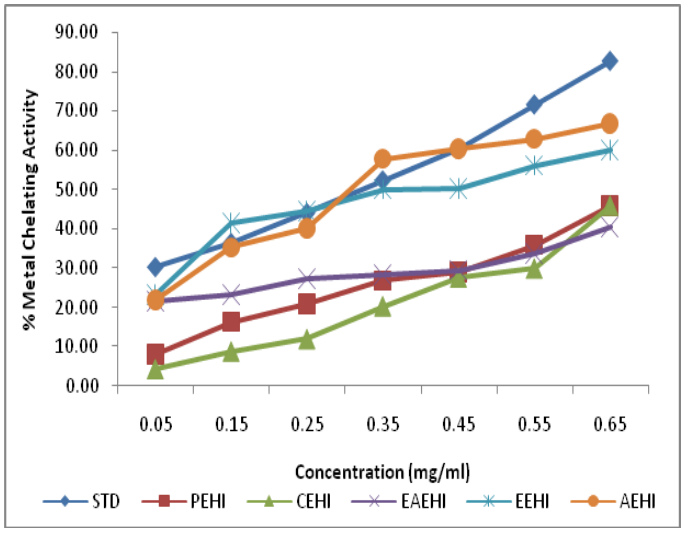

Figure 5: Metal chelating capacity of the *H. isora* extracts.

Reducing power assay

Reducing power assay estimate the electron donating capacity of plant extract.[52] Presence of reducers in the plant extract causes the conversion of Fe^{3+}/ferricyanide complex to the ferrous, which serve as indicator of potent antioxidant capacity.[53] Reducing power of the various extracts of *H. isora* was increased with an increase in its concentration (figure 6). According to the results aqueous extract showed maximum reducing power in comparison to the other extracts. Petroleum ether extract showed minimum reducing power. Reducing capacity of plant may serve as a significant indicator of its potential antioxidant activity. So plants reducing capacity and antioxidant activity are directly correlated to each other. *H. isora* root extract showed high reducing capacity as well as high antioxidant activity.

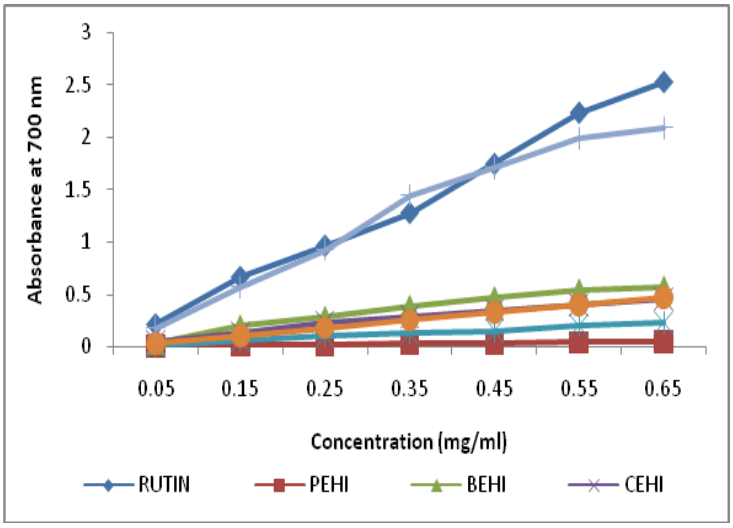

Figure 6: Reducing power of various fractions of *H. isora*.

FRAP (Ferric Reducing Antioxidant Power) assay

FRAP determine the ability of the plant extract to donate electron to Fe (III). All sequential extracts of *H. isora* were found to exhibit FRAP activity (table 2). Aqueous extract demonstrated a higher FRAP activity than other extracts; while petroleum ether demonstrated minimum FRAP activity. The higher value of FRAP indicate the greater antioxidant activity of the *H. isora*. Based on the above results, the aqueous extract of *H. isora* was found to most effective in comparison to the other extracts.

Total antioxidant capacity

Total antioxidant capacity of different sequential fraction of *H. isora* roots was calculated using regression equations obtained from standard graph. All extracts of *H. isora* showed good total antioxidant capacity (table 2). Aqueous extract of *H. isora* (AEHI) possess maximum total antioxidant capacity in comparison to the other extracts. Total antioxidant capacity (mg/g of extract) of various sequential extracts of *H. isora* was increased in the order of: BEHI < EEHI < PEHI < EAEHI < CEHI < AEHI.

Table 2: Total antioxidant capacity and FRAP activity of *H. isora*.

Extracts	TAC (mg in gallic acid equivalent/g dry extract)	FRAP (µM Fe (II)/g dry extract)
PEHI	960.40±0.002 a*	3.36±0.006 a*
BEHI	848.87±0.003 a*	12.78±0.014 a*
CEHI	1280.95±0.001 a*	5.51±0.008 a*
EAEHI	1228.90±0.001 a*	8.45±0.003 a*
EEHI	844.74±0.003 a*	8.45±0.033 a*
AEHI	1432.14±0.005 a*	41.13±0.005 a*

Results are expressed in mean±SD of triplicates measurements, *P>0.05 compared with standard; aP>0.001 compared with standard. **PEHI-** Petroleum ether extract of *Helicteres isora*; **BEHI-** Benzene extract of *Helicteres isora*; **CEHI-** Chloroform extract of *Helicteres isora*; **EAEHI-** Ethyl acetate extract of *Helicteres isora*; **EEHI-** Ethanol extract of *Helicteres isora*; **AEHI-** Aqueous extract of *Helicteres isora*.

CONCLUSIONS

The finding of the present study showed that all extracts of *H. isora* roots are rich in phenol, flavonoid, tannin and saponin constituents. Aqueous extract of *H. isora* roots was possess maximum phytoconstituents as well as antioxidant and chelating potential in comparison to the all other extracts. It could be concluded that *H. isora* roots can be explored as a potential

source for the isolation and characterization of natural antioxidant compounds. *H. isora* could be a good source of natural antioxidants.

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