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Original Article

STUDIES ON ELLAGIC ACID AND 4-HYDROXYISOPHTHALIC ACID ISOLATED FROM SWALLOW ROOT (DECALEPIS HAMILTONII)

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ABSTRACT

Objective: The aim of the study was to elucidate the free radical scavenging activity of purified major phenolic acids from swallow root (*Decalepis hamiltonii*) extract against 2, 2'-azobis-(2-amidinopropane) dihydrochloride (AAPH) induced oxidation in rat erythrocyte membrane and tissues.

Methods: Major phenolic acids, ellagic acid (EA) and 4-hydroxyisophthalic acid (4-HIA) were isolated from aqueous root extract of *Decalepis hamiltonii* by column chromatography and identified using HPLC, LC-MS, NMR and FT-IR. Free radical scavenging activities of the isolated compounds were estimated. Marker of lipid peroxidation was determined in erythrocyte membrane, liver and brain.

Results: The EA showed higher 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical scavenging activities, while 4-HIA exhibited potent hydroxyl radical scavenging activity when compared to standard antioxidants. Malondialdehyde (MDA) levels were increased with AAPH treatment in erythrocytes, liver and brain tissues and the treatment with phenolic acids inhibited the formation of MDA. 4-HIA was found to be more profound in attenuating the lipid peroxidation which may be attributed to its high hydroxyl radical scavenging activity.

Conclusions: Our study demonstrated the antioxidant properties of the major phenolic acids isolated from *Decalepis hamiltonii* and further suggests the therapeutic potential of these compounds in treating the oxidative stress disorders.

Keywords: AAPH, Brain, *Decalepis hamiltonii*, Erythrocytes, Liver, MDA

INTRODUCTION

Plants are the rich sources of various natural compounds including phenolics, flavonoids, terpenoids, alkaloids, etc. Among these, the phenolic compounds are the prime secondary metabolites found in fruits, vegetables and medicinally important plants and considered as antioxidants [1-3]. Biomedical research have revealed that, the dietary phenolics gained much importance due to their health-promoting activities, majorly involved in reduction of cancer, atherosclerosis, age-related degeneration and certain chronic diseases [4-7]. In general, phenolic compounds have chemically different characteristics and exhibits bitterness, astringency, color, flavor and odour [8]. Plant phenolics scavenge the free radicals through hydrogen transfer mechanism involved in the single electron transfer process. They also chelate the transition metal ions through their carbonyl moiety and multiple hydroxyl groups [9]. The beneficial aspects of dietary polyphenols on the improvement of cognition and memory by modulation of neurogenesis in the hippocampal regions of rat brain have been reported by Dias *et al.* [10]. The metabolized products of dietary polyphenols are also involved in xeno-metabolism in hepatic tissues of rats [11]. Studies have reported that, the plant phenolics are involved in inhibition of lipid peroxidation [12, 13]. The formation of lipid hydroperoxides, the primary product of lipid peroxidation leads to cytotoxicity and impairs various cellular functions [14].

Oxidative stress plays an important role in many physiological processes. Erythrocytes have been used as a model to investigate oxidative damage in biomembrane because of their high vulnerability to peroxidation [15]. Oxidative damage alters membrane fluidity leading to cell injury and death [16]. Furthermore, post-mitotic tissues such as brain may be more vulnerable to oxidative stress because they have lesser ability to up-regulate the antioxidant defenses than the tissues with greater proliferation capacity such as liver [17]. Studies have demonstrated that, the oxidative stress experienced by erythrocytes, brain and liver can be suppressed by the intervention of dietary antioxidants [18, 19].

AAPH is a hydrophilic azo compound; generate peroxyl radicals rapidly via interaction with carbon-centered radicals and molecular oxygen, eventually causing the lipid peroxidation [20]. Therefore, we made an attempt to examine the influence of antioxidants from plant extract on oxidative stress induced by AAPH in erythrocytes, brain and liver.

Decalepis hamiltonii (Wight and Arn.) (Swallow root) is a climbing shrub, belonging to the Asclepediaceae family found in peninsular India. The roots have been used as a folk medicine, ayurvedic preparations [21] and consumed as oriental food and health drink. The swallow root is rich in various bioactive compounds possessing various health-promoting properties [22-25].

Although studies have been reported on plant based extracts, reports are limited on the bioactivities of root extracts. However, isolation and elucidation of rich fractions of polyphenolic compounds with their bioactive principles is yet to be explored in detail. Based on the above facts, the present study was designed to isolate the major phenolic acids from the root extract and to investigate their influence against peroxyl radical induced oxidation in erythrocyte membrane and tissues of adult rats *in vitro*. Further, the synergetic influence of isolated phenolic acids with standard antioxidants were determined and correlated.

MATERIALS AND METHODS

Materials

Standards EA, 4-HIA, gallic acid, thiobarbituric acid (TBA), 1,1,3,3 tetra-methoxypropane (TMP), phenazinemethosulphate (PMS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were procured from Sigma (St. Louis, MO). HPLC grade acetic acid, acetonitrile, orthophosphoric acid, methanol were purchased from SRL Chemicals (Mumbai, India). Silicic acid and silica Gel 60 (70-230, mesh size), 2, 2'-azobis-(2-amidinopropane) dihydrochloride (AAPH) were procured from Himedia Chemicals (Mumbai, India). All other chemicals and solvents were of analytical grade.

Preparation of aqueous extract of swallow root

Decalepis hamiltonii roots were collected from Savanadurga forest at Magadi (Bengaluru, India) and was identified and authenticated at the department of Botany, Bangalore University. A voucher specimen was deposited in the herbarium (BUB, No. 2223). The aqueous extract was prepared according to the method of Srivastava *et al.* with slight modifications [26]. Briefly, the tuberous roots were washed thoroughly with water. The fleshy part was separated from the woody core by using a scalpel and dried at room temperature (RT). The outer fleshy layer was finely powdered using grinder. A known (500 g) quantity of powdered sample was soaked in warm water at 50 °C and kept on a magnetic stirrer overnight (Remi, India) and filtered through Whatmann No.1 filter paper (150 mm). The filtrate was lyophilized (Cleanvac 8 lyophilizer, Biotron) and weighed.

Estimation of total phenolics

Total phenolics were estimated according to the procedure of Singleton *et al.* [27]. Briefly, 0.5 ml of aqueous extract was added to 1.5 ml of Folin-Ciocalteu reagent and incubated in dark for 90 min with intermittent shaking. The reaction was neutralized by the addition of 2.0 ml of 7.5% of sodium carbonate and the absorbance was read at 725 nm in a spectrophotometer (Shimadzu-1800, Japan). Gallic acid was used as a reference standard to obtain a standard curve and total phenolics was expressed as gallic acid equivalents (mg/g).

Column chromatography

The lyophilized extract (35 g) was dissolved in methanol (50 ml), immobilized on silicic acid (20 g) and lyophilized. The lyophilized sample was subjected for the purification of major phenolics using column chromatography (Silica gel 60, Mesh size: 70-230 µm, Himedia, Mumbai) (Dimension, 38 x 4.5 cm glass column). The column was initially saturated with hexane and fractionation was conducted by successive applications of 250 ml of each system: hexane, dichloromethane (DCM), and methanol (1, 2, 5, 10, 20, 30, 50 and 100%) in DCM. All the above fractions were collected (250 ml) separately and the solvent was removed by rotary evaporation (Heidolph, Germany) in vacuo at 35 °C. The dried residue of the each fraction was dissolved in 5.0 ml of methanol and aliquoted for HPLC analysis [28, 29].

HPLC analysis and purification

Analytical HPLC (Agilent 1200 series, Germany) was conducted on visible wavelength detector (VWD) fitted with C18 reversed phase column (Agilent Zorbax 4.6 x 250 mm, 5 µm)according to the procedure of DaSilva *et al.* with minor modifications [29]. The open column chromatography fractions were dissolved in methanol (5.0 ml) and, when necessary, were further diluted prior to injection (20 µl) into the HPLC. The mobile phase consisted of 2% acetonitrile (solvent-A) and orthophosphoric acid in water (solvent B) with following gradient: 80% A for 0 min, 20% A for 5 min, 0% A for 10 min, 0% A for 14 min, 80% A for 14.1 min, 80% for 15 min until completion of run and the flow rate was 0.8 ml/min. The phenolic compounds in eluant were detected at 254 nm. Quantification of EA and 4-HIA in the extract and the column chromatography fractions was performed against their respective standard curves.

HPLC–ESI-MS

The EA and 4-HIA rich fractions were collected, concentrated and detected by HPLC-ESI-MS analysis (Agilent 6410 Triple quad MS, Germany) according to the procedure of DaSilva *et al.* [29]. The chromatographic conditions remained same as mentioned in the HPLC analysis. Instrument was conditioned by using following parameters: capillary voltage set at 3500 V with a charging voltage of 1000 V, source temperature 150 °C, probe temperature 350 °C, nitrogen gas flow 10 ml/min and sheath gas flow 1 ml/min monitored. Fragmentation energy and collision energy in the second quadropole were 135 V and 30 V respectively with a dwell time of 15 milliseconds. The fractions rich in EA and 4-HIA were subjected to semi preparative HPLC and the conditions were similar to analytical HPLC. The EA and 4-HIA peaks were isolated and lyophilized and stored at 4 °C until further use.

FT-IR and NMR spectroscopy

FT-IR spectra of EA and 4-HIA were analyzed using KBr on a Perkin Elmer (89230) spectrometer at υ 400-400 cm^{-1}. ^{1}H and ^{13}CNMR spectra were recorded in the DMSO-d6 solvent on a Bruker (AMX 400 FT-NMR) spectrometer, operating at 9.4 T, observing ^{1}H at 400.13 MHz and ^{13}C at 100.61 MHz.^{1}H and ^{13}C chemical shifts are reported in ppm relative to tetramethyl silane with an internal reference in mixed solvents or indirectly via pure solvent signals. All reagents and solvents used were of spectroscopy grade.

Determination of free radical scavenging activities

DPPH assay

DPPH radical scavenging assay was carried out as per the method of Shon *et al.* with slight modifications [30]. Briefly, variable concentrations of aliquots were prepared in 1 ml of methanol and added to 1 ml of 0.1 mM DPPH solution and incubated for 30 min at RT in dark. After the incubation period, the sample was read at 517 nm in a UV spectrophotometer. The percentage of scavenging activity was calculated by following formula: Scavenging effect (%) = {Absorbance of control (517 nm)–Absorbance of sample (517 nm)/Absorbance of control (517 nm)} x 100.

Superoxide radical scavenging assay

Superoxide radical scavenging activity was measured according to the method of Nishikimi *et al.* [31]. Briefly, 1 ml of nitroblue tetrazolium and 1 ml of nicotinamide adenine dinucleotide, reduced prepared in phosphate buffer (pH 7.4) were added to different concentrations of samples. Finally, the reaction mixture was accelerated by the addition of 100 µl PMS solution for the superoxide radical production. The reaction mixture was incubated at 25 °C for 5 min, and absorbance was measured at 560 nm. Methanol was used as a control. Percentage inhibition was calculated similar to the DPPH assay.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay was carried out by the method of Halliwell and Gutteridge [32]. Briefly, different concentrations of sample (20 µl) were added to a reaction mixture containing 120 µl of 20 mM deoxyribose, 400 µl of 0.1 M phosphate buffer, 40 µl of 20 mM H_2O_2, 40 µl of 500 µM $FeSO_4$ and the final volume was made up to 800 µl with double distilled water. The reaction mixture was incubated at 37 °C for 30 min and 0.5 ml of 2.8% TCA (trichloroacetic acid) was added to stop the reaction, followed by the addition of 0.4 ml of 0.6% TBA solution. The reaction mixtures were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in the spectrophotometer. The hydroxyl radical scavenging activity (%) was calculated using the following equation: (Absorbance of blank-Absorbance of sample)/Absorbance of blank x 100.

Animal study and preparation of erythrocyte ghosts and tissue (Liver and Brain) homogenate

Animal care and maintenance

Animal experiments were approved by the Institutional Animal Ethics Committee (IAEC), Bangalore University, Bengaluru, India (BUB/Zoo/IAEC/Anim/2011-15). The experiments were carried out in accordance with the guidelines set by CPCSEA (Committee for the purpose of control and supervision of experiments on animals), India. Adult male *Wistar* albino rats weighing 180±12 g (n=5) were housed in polypropylene fitted steel mesh-bottom cages and maintained in an animal house at temperature of 28±1 °C, relative humidity of 77±1% under a daily 12-h light and 12-h dark cycle. Animals had a free access to feed (Amruth Feed, Bengaluru) and tap water *ad libtum* for 7 d for acclimatization.

Blood sampling and preparation of erythrocyte ghosts

Animals were sacrificed using diethyl ether anesthesia. The blood sample was drawn directly from the heart by cardiac puncture into EDTA-coated tubes. Erythrocytes were isolated by the method of Dodge *et al.* [33]. In brief, the blood sample was centrifuged for 20 min at 1000xg (Plastocrafts, Superspin-RV/FM). The plasma and

buffy coat were removed by aspiration. The cells were washed three times with isotonic phosphate buffer, pH 7.4, and finally suspended in an equal volume of the same buffer. This constituted the erythrocyte suspension. This suspension was hemolysed by the addition of hypotonic buffer (pH 7.4). The contents were mixed by gentle swirling and then centrifuged at 20,000xg for 40 min. The supernatant was decanted carefully and the erythrocyte ghost was suspended by adding the same strength buffer to reconstitute the original volume. Ghosts were washed three times subsequent to hemolysis. The membrane pellet was resuspended in an isotonic buffer for the assay.

Preparation of tissue homogenates

The tissue homogenates were prepared according to the previous protocol [34]. The brain and liver tissues were excised, weighed and homogenized with ice cold 50 mM phosphate buffer (pH 7.0). The obtained homogenates were used for the estimation of MDA content.

Induction of oxidative stress in erythrocyte membrane and tissue homogenates

The erythrocyte membrane and tissue homogenates were pretreated with various concentrations of EA and 4-HIA prior to the induction of oxidative stress. Oxidative stress was induced by the addition of 50 mM AAPH for 60 min at 37 °C.

Lipid peroxidation

MDA, a product of lipid peroxidation was measured according to the procedure of Ohkawa *et al.* [35]. In brief, 100 µl of membrane/tissue homogenate sample was added to 8.1% SDS, vortexed, and incubated for 10 min. This was followed by the addition of 20% acetic acid and 0.6% TBA, and placed in a boiling water bath for 60 min. The samples were allowed to cool and 5 ml of butanol: pyridine (15:1) was added and centrifuged at 640xg for 5 min. Absorbance of the colored layer was measured at 532 nm using TMP as standard. MDA concentration was expressed as nmol/mg protein.

Estimation of protein content

Total protein content in the erythrocyte membrane and tissue homogenates were estimated by Lowry's method [36].

Statistical analysis

Results are represented as mean±SE. Values between the groups were analyzed by one-way ANOVA. Significant F ratios between the group differences were further analyzed by Tukey's test using SPSS 20 software package for Windows. Probability values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Isolation, characterization and quantification of fractions rich phenolic acids (EA and 4-HIA)

The beneficial effects of polyphenols are mainly attributed to their antioxidant properties [37]. The isolation of bioactive compounds from plant materials is the key step in the preparation and evaluation of bioactive potentials for dietary supplements or nutraceuticals, food ingredients, pharmaceuticals [38]. In this study, we have found a complex mixture of polyphenols in the lyophilized sample of swallow root extract. The typical HPLC profile of aqueous root extract of *Decalepis hamiltonii* is shown in fig.1a.

The peaks 5 and 7 were identified and confirmed as EA and 4-HIA based on their retention time and UV spectra against their reference standards. The EA and 4-HIA peaks were eluted at 5.6 and 5.9 min respectively [fig 1b and 1c]. The EA rich fraction (83%) was eluted with 20% methanol in DCM and 4-HIA (64%) in 50% methanol in DCM through open column chromatography. Further, these fractions were subjected to HPLC purification to obtain purified EA (97%) and 4-HIA (98%). These purified compounds were further characterized and confirmed by mass spectra and spectroscopy analysis.

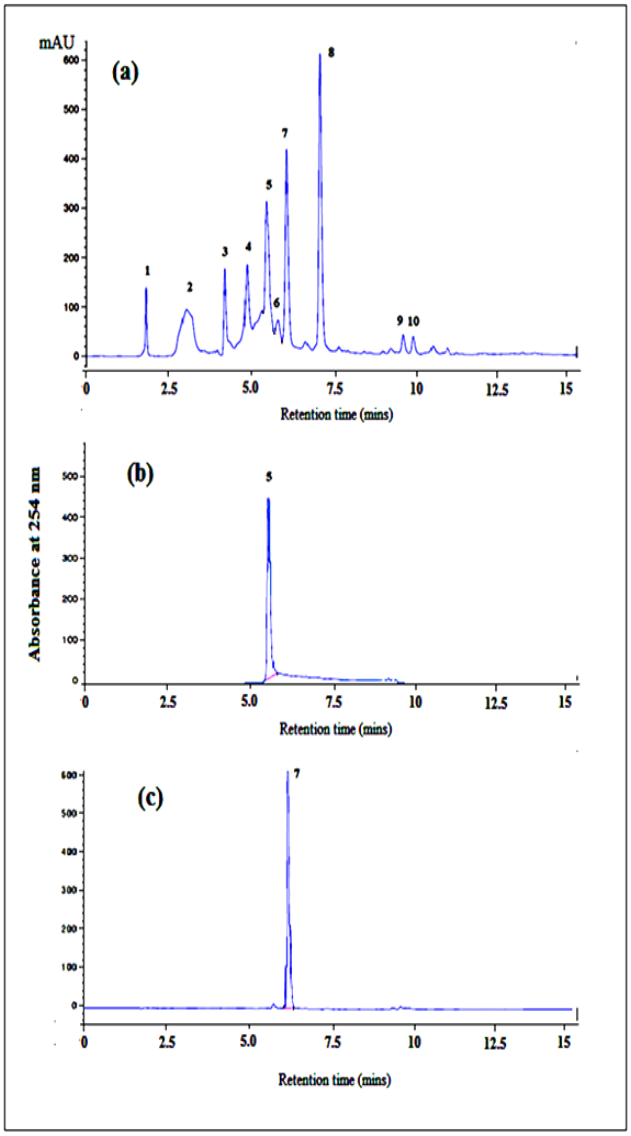

Fig. 1: Representative RP-HPLC (C-18) chromatograms of crude extract (a), purified EA (b) and 4-HIA (c) from swallow root extract

The typical ESI^{-ve} mass spectra profile of EA and 4-HIA showed signals at *m/z* 301.1 and *m/z* 181.1 respectively and were comparable with their respective standards.

In addition, NMR and FT-IR analysis for EA and 4-HIA were performed to confirm and elucidate the structure of the compounds. FT-IR was used for identification of functional groups of isolated phenolic acids. EA showed carbonyl stretch vibration detected at υ1698.5 cm^{-1}, the hydroxyl stretches were identified at υ 3476 and 3557.5 cm^{-1}.

In case of 4-HIA, the carbonyl stretch vibration was detected at υ 1654 cm^{-1}, CH vibration at 2529.3 cm^{-1}, C=C vibration at 1584.1 cm^{-1}and hydroxyl vibration at 3424 cm^{-1}(fig.2). ^{13}CNMR spectrum (fig. 3a) of EA showed 7 carbon signals: 159.09 (C-7,7'), 148.09 (C-4,4'), 139.54 (C-3,3'), 136.37 (C-2,2'), 112.29 (C-1,1'), 110.24(C-5,5'), 107.64 (C-6,6'). ^{1}HNMR showed two aromatic protons at δ 7.47 (s), and 4 OH signals at 10.7 (s). Our carbon and proton assignments compared with the relative shifts are similar to the earlier reports [28]. ^{13}C NMR spectrum (fig. 3b) of 4-HIA exhibited 8 signals 173.16(C1), 169.03 (C2), 167.04 (C3), 137.83 (C4), 134.32 (C5), 123.09 (C6), 118.63 (C7) and 113.98 (C8). ^{1}HNMR showed OH signals at δ 4.89 (s) and protons at 8.56 (s), 8.12(s) and 7(s). The chemical shifts were comparable to standards.

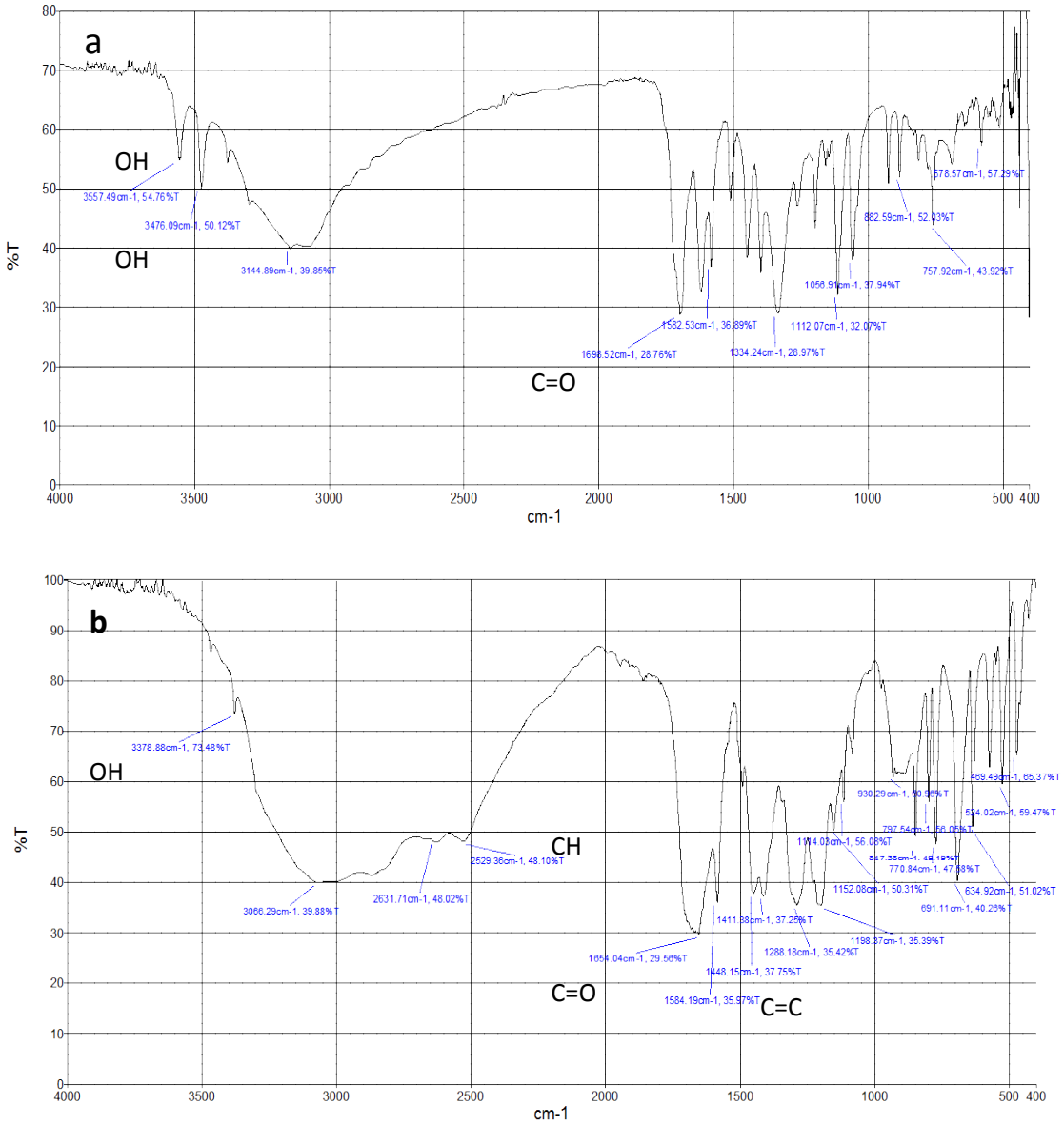

Fig. 2: Representative FT-IR spectra of EA (a) and 4-HIA (b)

Based on HPLC peak area of the standard curves, the contents of isolated phenolics were quantified. EA was found to be 2.98±0.58 and 4-HIA, 5.05±0.41 mg/g lyophilized root extract. The total phenolic content of *Decalepis hamiltonii* root extract was found to be 14.5±1.6 mg/g lyophilized extract, wherein, EA comprised 21 and 4-HIA 35% in the total phenolic content. In our study, the total phenolic content was found to be higher by 12.96 and 4.8 % compared to previous studies of Samydurai and Thangapandian [39] and Srivastava *et al.* [40], respectively. The EA and 4-HIA yield was also higher by 24 and 8 % compared with the reports of Srivastava *et al.* [40, 41]. These variations may be attributed due to size of samples, geographical locations and other climatic factors which may be responsible for the significant differences in the terms of composition, contents of phenolics and bioactive potentials [42].

Determination of Antioxidant activities

Table 1 indicates the IC_{50} values of DPPH, superoxide radical and hydroxyl radical scavenging activities of the phenolic compounds in comparison with same concentration of Vitamin C (Vit C), Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA). In this study, the antioxidant capacity was determined by the DPPH radical scavenging activity. This method was chosen due to the fact that DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelating and enzyme inhibition as reported by Amessis-Ouchemoukh *et al.* [43]. The EA, 4-HIA and the standard compounds showed significant dose-dependent activity. Among them, EA was most efficient with the lowest IC_{50} value of 11.6 n moles/ml compared to 4-HIA and the standard antioxidants.

The compounds might have neutralized the free radical character of DPPH by transferring either electrons or hydrogen atoms to DPPH radical [44]. The antiradical activity of phenolic compounds depends on their molecular structure [fig. 4], that is, on the availability of phenolic hydrogens and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation [45].

Superoxide anion, derived from dissolved oxygen by PMS-NADH coupling reaction. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion. EA (IC_{50}-13.6±1.7) and 4-HIA (IC_{50}-16.4±1.5) exhibited higher superoxide radical scavenging activity than vit C and BHT. The scavenging ability of these compounds is due to their free hydroxyl groups, which provide active hydrogen to scavenge the superoxide radicals.

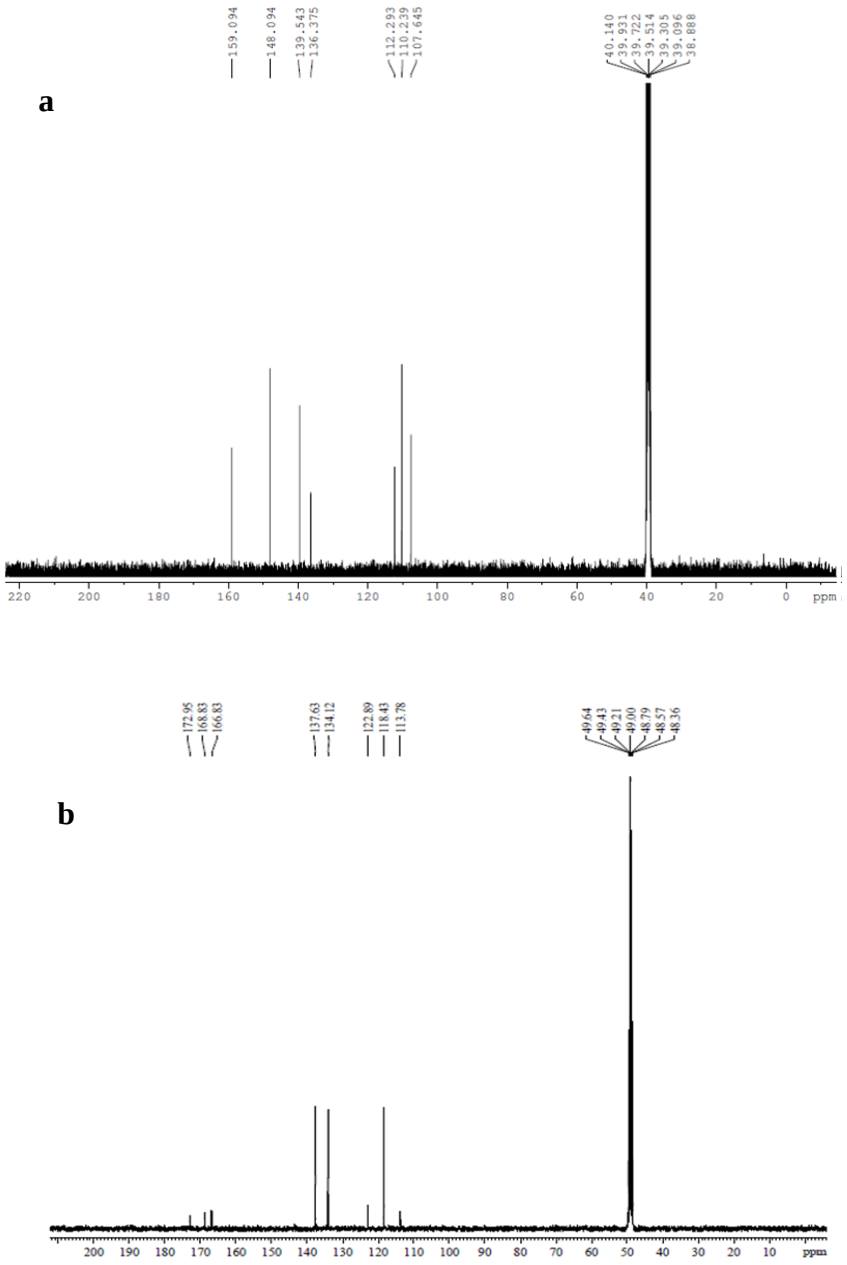

Fig. 3: Representative ^{13}C NMR spectra of isolated EA (a) and 4-HIA (b)

Table 1: Effect EA and 4-HIA on free radicals scavenging activities

Compounds	Free radical scavenging activity (IC_{50}, nmoles/ml)		
	DPPH	$O_2^{\cdot-}$	$^{\cdot}OH$
Ellagic acid	11.6±2.2^{b}	13.6±1.7^{b}	186.5±8.8^{b}
4-hydroxyisophthalic acid	13.2±2.4^{b}	16.4±1.5^{b}	23.4±2.2^{c}
Vit C	21.6±1.1^{b}	36.9±3.0^{a}	63.4±3.2^{c}
Butylatedhydroxytoulene	11.9±1.0^{b}	25.2±4.1^{ab}	217.0±4.6^{b}
Butylatedhydroxyanisole	40.8±5.6^{a}	0.0	876.7±32.8^{a}

Values are means±SEM of five samples. Values not sharing a common superscript within a column are statistically significant at $p<0.05$.

The hydroxyl radical is the most reactive radical known to initiate lipid peroxidation and damage of biomolecules [46]. The effect of phenolic acids on OH radical generated by Fe^{2+} ions was measured by determining the degree of deoxyribose degradation. The phenolic acids scavenged the OH radicals on concentration-dependent manner. A noticeable feature in the hydroxyl radical scavenging activity was that 4-HIA was found to be a powerful quencher of OH radicals than EA, thereby preventing the propagation of lipid peroxidation (IC_{50}-23.4±2.2) compared to well-known standard antioxidant vit C (IC_{50}-63.4±3.2). The higher scavenging activity of 4-HIA could be due to its efficiency in acting as chelators of the Fe^{2+} ions in the system, thereby preventing them from complexing with the deoxyribose, or simply donating hydrogen atoms and accelerating the conversion of H_2O_2 to H_2O [47].

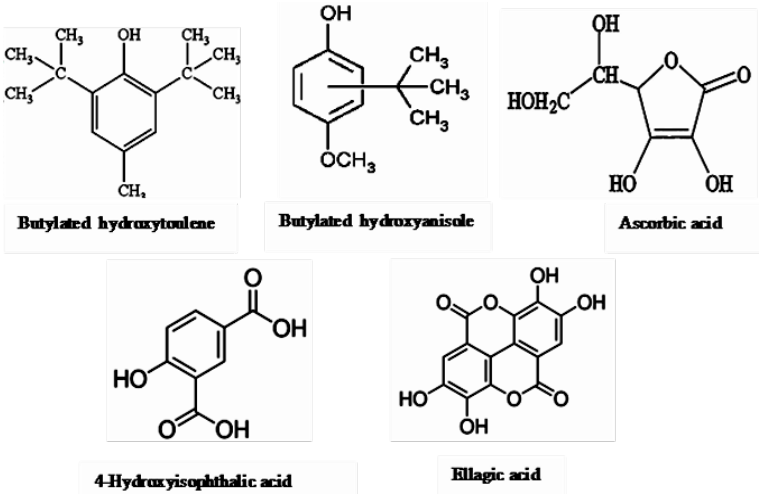

Fig. 4: Molecular structures of reference standard antioxidants (BHT, BHA and vit C) and isolated phenolic compounds (EA and 4-HIA)

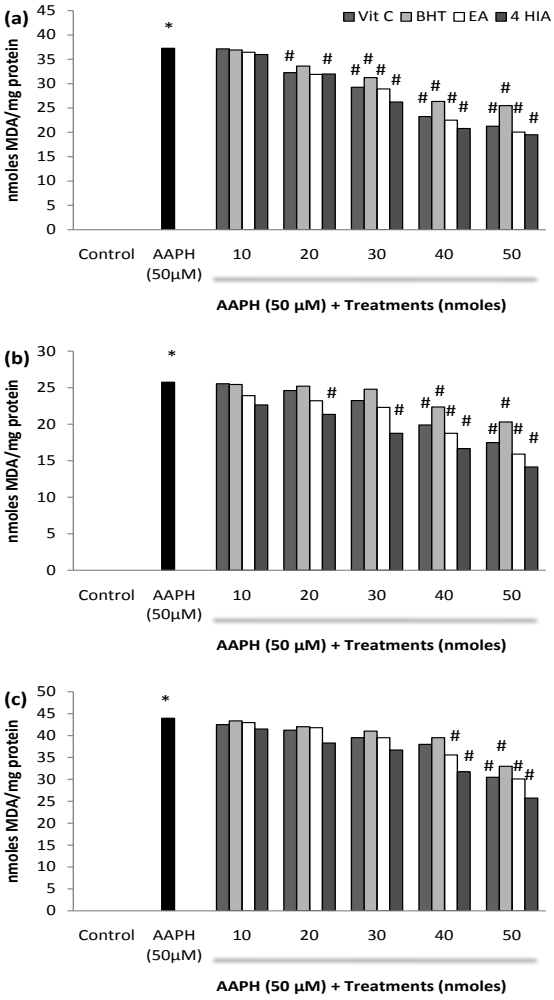

Fig. 5: Effect of phenolic acids and standard antioxidants on AAPH induced oxidative stress in erythrocyte membrane (a), liver (b) and brain (c)

Values represent mean±S. E of five experiments. * depicts the comparison between control and AAPH, # depicts in comparison of different treatments of antioxidants with AAPH. considered. Statistical analysis was done by one-way ANOVA followed by Tukey's test.*# $p<0.05$ was considered statistically significant.

Lipid peroxidation

Lipid peroxidation involves the formation and propagation of lipid radicals with numerous deleterious effects, including destruction of membrane lipids and production of MDA is a hallmark of this process. Inhibition of lipid peroxidation was assessed by the amount of MDA produced. The MDA content was elevated by 60%, 54% and 48% in 50 mM AAPH treated erythrocyte membrane, liver and brain tissues over their respective controls [fig. 5]. Experiments were conducted to test the potential of EA, 4-HIA and the standard antioxidants, vit C and BHT for the reduction of oxidative stress induced by AAPH. We pre-incubated the membrane and the tissues with these antioxidants with different concentrations before incubating with AAPH. EA and 4-HIA reduced the MDA content in erythrocyte membrane by 46% and 48% over AAPH treated ones, respectively. While, in the liver and brain tissues, significant reductions of 38 % and 32% (EA) and 45% and 41% (4-HIA) in MDA content were evident when compared to AAPH treated tissues.

The 4-HIA prevented lipid peroxidation more efficiently compared to EA, vit C and BHT which may be due to the presence of OH and carboxyl groups in para and ortho position to COOH groups in the aromatic ring that influences the antiradical activity of the compound [48]. Pawlikowska–Pawlega *et al.* [49] and Alvarez-Suarez *et al.* [16] have demonstrated that the polyphenols compartmentalized into the cell membranes, seems to exert a significant inhibition of lipid peroxidation and enhance the membrane integrity against several chemical and physical stress. Previous studies reported that phenolic acids inhibit the lipid peroxidation in liver and brain tissues [50, 51].

CONCLUSION

In conclusion, our studies explore the antiradical activities of phenolic compounds isolated from *Decalepis hamiltonii*. The phenolic acids, EA and 4-HIA efficiently protect erythrocytes, liver and brain against AAPH induced oxidative injury. However, further studies have to be carried out *in vivo* to explore the therapeutic potential of these compounds in combating oxidative stress-related diseases.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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