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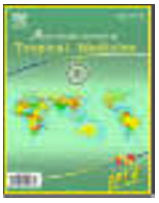

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Antioxidant activity and identification of bioactive compounds from leaves of *Anthocephalus cadamba* by ultra-performance liquid chromatography/electrospray ionization quadrupole time of flight mass spectrometry

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ABSTRACT

Objective: To evaluate the antioxidant potential of different extract/fractions of *Anthocephalus cadamba* (*A. cadamba*) (Roxb.) Miq. (Rubiaceae) and study the tentative identification of their active constituents. **Methods:** The extract/fractions were screened for antioxidant activity using various *in vitro* assays viz. DPPH assay, ABTS assay, superoxide anion radical scavenging assay, reducing power assay and plasmid DNA nicking assay. Total phenolic content of extract/fractions was determined by colorimetric method. An ultra-performance LC-electrospray-quadrupole-time of flight mass spectrometry method was used to analyse the active constituents of extract/fractions of *A. cadamba*. **Results:** The ethyl acetate fraction was found to be most active fraction in all the assays as compared to other extract/fractions. The IC_{50} value of ethyl acetate fraction (ETAC fraction) was 21.24 μg/mL, 1.12 μg/mL, 9.68 μg/mL and 57.81 μg/mL in DPPH assay, ABTS assay, reducing power assay and superoxide scavenging assay respectively. All the extract/fractions also showed the potential to protect the plasmid DNA (pBR322) against the attack of hydroxyl radicals generated by Fenton's reagent. The bioactive compounds were identified by UPLC-ESI-QTOF-MS, by comparing the mass and λ_{max} with literature values. **Conclusions:** The potential of the extract/fractions to scavenge different free radicals in different systems indicated that they may be useful therapeutic agents for treating radical-related pathologic damage.

1. Introduction

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Natural plant products are frequently reported as efficient chemopreventive agents. Antigenotoxic and antioxidative mechanisms are considered crucial for the prevention of some degenerative diseases such as cancer[1-3]. In food industry and medicinal research, antioxidant research is an important topic. The importance of reactive oxygen species (ROS) and free radicals in cellular damage and the ageing process has attracted increasing attention over the past 20 years[4,5]. ROS, such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•) and other free radicals are by-products of biological metabolism. Within biological systems, there are enzymatic systems and chemical scavengers: dietary antioxidants (α-tocopherol, β-carotene, ascorbic acid, glutathione and uric acid), some hormones (estrogen, angiotensin) and endogenous enzymes (superoxide dismutase, glutathione peroxidase and catalase), all of them are able to remove free radicals formed in cells and thus protect against oxidative damage[6,9]. The oxidation induced

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by ROS can result in cell membrane breakdown, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease[10-12]. The antioxidant phytochemicals from plants, particularly flavonoids and other polyphenols, have been reported to inhibit the propagation of free radical reactions, to protect the human body from disease and longer life expectancy[13-17]. Interest has increased in naturally occurring antioxidants for use in foods or medicinal material as compared to the synthetic antioxidants because of their non-side effects nature[18]. *Anthocephalus cadamba (A. cadamba)* (Roxb.) Miq. (Rubiaceae) is known as wild cinchona and popular in India as "Kadamb". Its bitter and pungent bark is used in ayurvedic medicine for uterine complaints, blood diseases, leprosy and dysentery. A decoction of the leaves is recommended as a gargle in cases of stomatitis[19]. The crude extracts from *A. cadamba* have been shown to possess biological activities viz. anti-inflammatory[20], antihepatotoxic activities[21], analgesic activitiy[22], hypoglycemic activity[23], antimicrobial and anthelmintic activities[24]. The chemical constituents of *A. cadamba* have been investigated and so far, terpenoids and alkaloids have been identified[25-30]. However, not many reports are available regarding the bioactive phytochemicals from this plant. Keeping this in mind, the present study was planned to evaluate the antioxidant potential of extract/fractions from leaves of *A. cadamba* and identification of active constituents using UPLC-ESI-QTOF-MS.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric chloride, L-Ascorbic acid, NADH (Nicotinamide Adenine Dinucleotide), PMS (Phenazine Methosulphate), NBT (Nitroblue Tetrazolium Chloride) were obtained from HiMedia Pvt. Limited Mumbai, India. Potassium persulfate, ABTS [2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt], Gallic acid, from Sigma (St. Louis, MO, USA). Plasmid pBR322 was purchased from Genei Pvt. Ltd., Banglore. All other reagents were of analytical grade (AR).

2.2. Collection of plant material

The leaves of *A. cadamba* were collected from the trees growing in the campus of Guru Nanak Dev University, Amritsar.

2.3. Extraction and fractionation

The leaves were washed with running water to remove any dust impurities and dried at 40 ℃. The material was finely powdered and extracted with 95% ethanol under reflux conditions to obtain ethanol extract (ETAC) and was concentrated under reduced pressure using rotary evaporator. ETAC was made aqueous with distilled water in a separating funnel and further fractionated with organic solvents in order of increasing polarity viz. ethyl acetate and *n*-butanol to obtain the fractions, viz., Ethyl acetate fraction (EAAC), butanol fraction (NBAC) and remaining water fraction (WAC) (Figure 1).

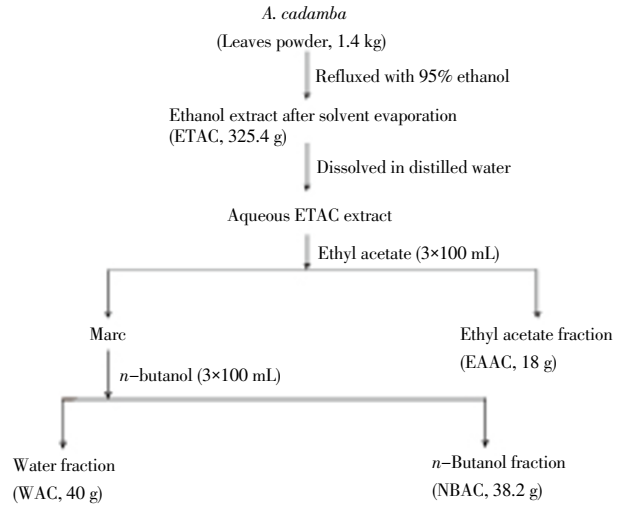

Figure 1. Isolation of various extract/fractions from leaves of *A. cadamba*.

2.4. Phytochemical analysis

2.4.1. Determination of total phenolic content

The total phenolic content of extract/fractions was determined using Folin-Ciocalteu method[31] and gallic acid was used as standard. 100 μL of extract/fractions was added to 900 μL of double distilled water followed by the addition of 500 μL of FC reagent. This was followed by the addition of 1.5 mL of 20% sodium carbonate. The volume of mixture was made up to 10 mL with distilled water and allowed to stand for 2 h. Finally absorbance was taken at 765 nm. The phenolic content was calculated as gallic acid (mg/g) equivalents on the basis of standard curve of gallic acid.

2.5. Antioxidant activity

2.5.1. DPPH-radical scavenging assay

The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. It was carried out by

the method of Blois (1958)[32] with modifications. Different concentrations (20-400 μg/mL) of extract/fractions of *A. cadamba* leaves were dissolved in methanol and taken in test tubes in triplicates. Then 2 mL of 0.1 mM methanol solution of DPPH was added to each of the test tubes and were shaken vigorously. After 30 min absorbance was taken at 517 nm using UV-VIS spectrophotometer.

%Radical scavenging activity (%) = [Abs (control) − Abs (sample) /Abs (control)] ×100.

where, Abs (control): Absorbance of DPPH radical + solvent alone,

Abs (sample): Absorbance of DPPH radical + extract/fractions.

2.5.2. ABTS radical scavenging assay

The spectrophotometric analysis of $ABTS^{\cdot+}$ scavenging activity was determined according to the protocol given by Re *et al*[33]. ABTS radical cations were produced by reacting ABTS stock solution (7 mM) and potassium persulfate (2.45 mM) solution in the ratio of 1: 0.5 and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of (0.70±0.02) at 734 nm. 300 μL of different concentrations (20-400 μg/mL) of the extract/fractions were added to the diluted ABTS radical cation solution and absorbance was taken up to 5 min.

$$\% \text{ Radical scavenging activity } (\%) = \frac{[\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}]} \times 100$$

2.5.3. Reducing power assay

Reducing potential of the extract/fractions was determined using the method of Oyaizu[34]. Different concentrations of extract/fractions (200-1 000 μg/mL) of *A. cadamba* leaves were dissolved in methanol and taken in test tubes in triplicates. To the test tubes phosphate buffer (pH 6.6, 0.2 M) and 1% Potassium ferricyanide solution was added. These contents were mixed well and were incubated at 50 ℃ for 20 min. After incubation 10% TCA was added and were kept for centrifugation at 3 000 rpm for 10 min. After centrifugation 2.5 mL of supernatant was taken and mixed with double distilled water (2.5 mL) and ferric chloride (0.1%). The O.D (absorbance) was measured spectrophotometrically at 700 nm. Increase in absorbance of reaction mixture was interpreted as increase in reducing ability of the sample and rutin was used as a standard.

2.5.4. Superoxide anion radical scavenging assay

The measurement of superoxide anion scavenging activity of extract/fractions was performed according to the method of Nishikimi *et al* (1972) [35] with slight modifications. About 1 mL of nitroblue tetrazolium (NBT) solution (156 μM prepared in phosphate buffer, pH 7.4), 1 mL of NADH solution (468 μM prepared in phosphate buffer pH 7.4) and 1 mL of various concentrations of the fractions (100, 200, 300, 400 and 500 μg/mL) and the reference compound rutin were mixed and the reaction was started by adding 100 μL of phenazine methosulphate (PMS) solution (60 μM in phosphate buffer, pH 7.4). The reaction mixture was incubated at 25 ℃ for 5 min and the absorbance was measured at 560 nm. The percentage inhibition was calculated by the formula:

Superoxide anion radical scavenging activity (%) = [Abs (control) − Abs (sample) /Abs (control)] ×100.

Where, Abs (control): Absorbance of control,

Abs (sample): Absorbance of sample.

2.5.5. Plasmid DNA protection assay

To measure the hydroxyl radical scavenging effect of extract/fractions, DNA nicking experiment was performed according to the protocol of Lee *et al*[36]. Plasmid DNA was incubated with Fenton's reagent containing extract/fractions and finally the volume of the mixture was raised up to 20 μL. The mixture was then incubated for 30 min at 37 ℃ followed by addition of loading dye. Electrophoresis was carried out in TAE (Tris Acetic acid EDTA) buffer and DNA was analyzed followed by ethidium bromide staining.

2.6. UPLC-electrospray ionization-quadrupole time-of-flight mass spectrometry

2.6.1. Sample preparation

For UPLC-ESI-MS analysis, samples were prepared in a mixture of acetonitrile/water (80/20; v/v) and filtered through a 0.22 lm MILLEX GV syringe filter (Millipore, MA, USA) prior to inject into the UPLC system.

2.6.2 UPLC instruments and chromatographic conditions

All analysis were performed on Waters Acquity UPLC system (Waters, MA, USA), including binary solvent manager, sample manager, column compartment and photo diode array (PDA) detector, connected with Waters Mass Lynx software. An Acquity UPLC BEH C18 column (100 mm × 2.1 mm i.d, 1.7 μm) also from Waters was used. The column temperature was maintained at 30 ℃. Samples were separated using a gradient mobile phase consisting of 0.05% formic acid in water (A) and acetonitrile (B). The gradient elution is: 0-0.2 min, 82% A; 0.2-1.4 min, 75% A; 1.4-2.0 min, 65% A; 2.2-3.5 min, 65% A, and finally reconditioning the column with 82% A for 1 min. The flow rate was set 0.28 $mL \cdot min^{-1}$ and the injection volume was 1 μL.

2.7. Statistical analysis

The results are presented as the mean ± standard error of three experiments. Regression analysis was carried out by best fit method and IC_{50} values were calculated using regression equation. The significance of results was checked at $P \leqslant 0.05$.

3. Results

3.1. Total phenolic content (TPC)

The results of the total phenolic content evaluated using Folin-Ciocalteu method, are shown in Table 1. TPCs of the extract/fractions was found in the order, ethyl acetate fraction (EAAC) > *n*-butanol fraction (NBAC) > ethanol extract (ETAC) > water fraction (WAC). The TPC of the EAAC fraction (194.07 ± 4.08 mg GAE/g extract) was higher than those of all other extract/fractions.

Table 1
Total phenolic content (TPC) of extract/fractions from leaves of *A. cadamba*.

Extracts/fractions	Phenolic content (mg GAE/g of extract)
Ethanol extract	149.87±4.40
Ethyl acetate fraction	194.07±4.08
n-butanol fraction	182.45±4.09
Water fraction	107.63±8.40

3.2. DPPH assay

The scavenging effect of crude extract and various fractions based on their IC_{50} values was in the order of EAAC (21.24 μg/mL) > NBAC (36.41 μg/mL) > ETAC (63.94 μg/mL) > WAC (138.66 μg/mL). The % inhibition values of ETAC, EAAC, NBAC and WAC were 88.06%, 90.03%, 89.24% and 88.63% at highest tested concentration (400 μg/mL) respectively (Figure 2). The results were compared with standard compound rutin (IC_{50}=54.05 μg/mL).

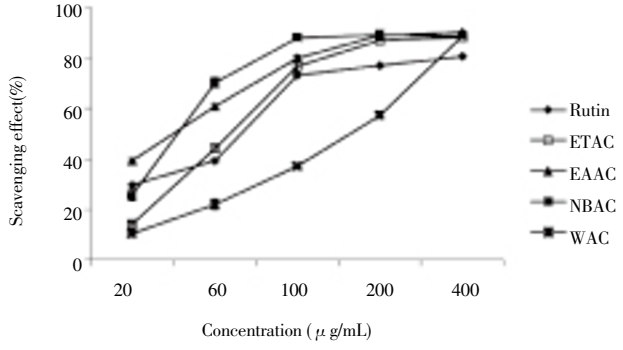

Figure 2. Scavenging effect of extract/fractions from leaves of *A. cadamba* on DPPH radicals. *$P<0.05$.

3.3. ABTS radical scavenging assay

All the extract/fractions showed good potential to scavenge ABTS radical cations. The scavenging effect was 95.21%, 95.70%, 94.72% and 81.13% in ETAC, EAAC, NBAC and WAC respectively, at highest tested concentration (Figure 3).

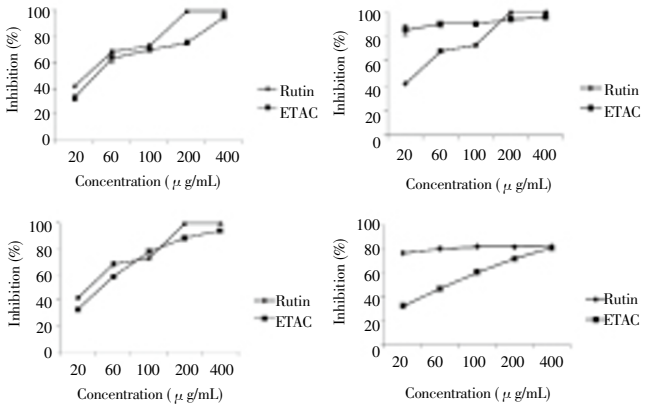

Figure 3. Scavenging effect of extract/fractions from leaves of *A. cadamba* on ABTS radicals. *$P<0.05$.

3.4. Reducing power assay

Substances with reducing potential reacts with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) which then reacts with ferric chloride to form ferric ferrous complex that has absorption maximum at 700 nm. The increased absorbance of the reaction mixture indicates increased reducing power. The reducing potential of crude extract and various fractions was in the order of EAAC > NBAC > ETAC > WAC (Figure 4). The results were compared with the standard compound rutin.

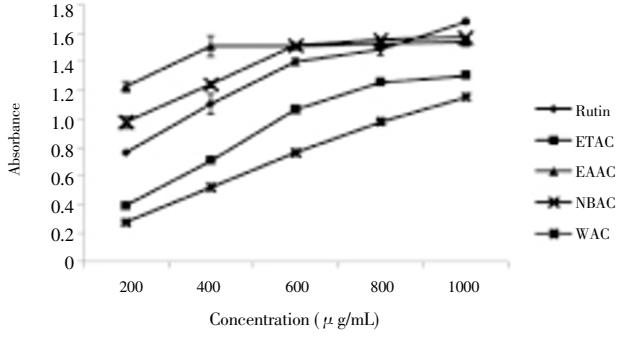

Figure 4. Reducing potential of extract/fractions from leaves of *A. cadamba*. *$P<0.05$.

3.5. Superoxide scavenging assay

Superoxide anion is implicated as harmful ROS. It has detrimental effect on the cellular components in a biological system[37,38]. Although they can not directly initiate lipid oxidation, they are potential precursors of highly reactive species such as hydroxyl radical and thus the study of scavenging of this radical is important. Different extract/fractions showed superoxide scavenging activity of 55.18% (ETAC), 72.31% (EAAC), 71.28% (NBAC) and 45.61% (WAC) at highest tested concentration (500 μg/mL) (Figure 5).

Rutin was used as standard (IC_{50}=58.75 μg/mL).

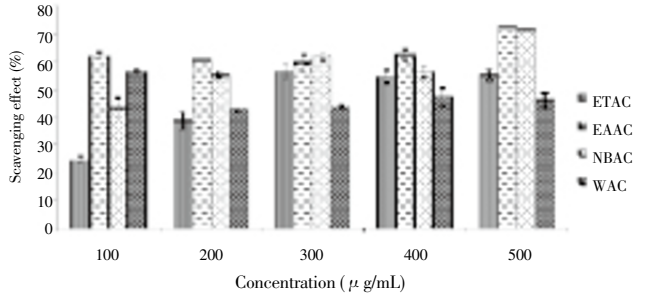

Figure 5. Superoxide scavenging potential of extract/fractions from leaves of *A. cadamba*.

3.6. Plasmid DNA nicking assay

DNA protective activity was assessed by measuring the degree of protection on DNA scission that was induced by the attack of hydroxyl radicals generated by Fenton's reagent. Hydroxyl radical is one of the ROS formed in biological systems, causing DNA strand breakage, which brings about carcinogenesis, mutagenesis and cytotoxicity[39,40]. The protection against the damage caused by hydroxyl radicals was shown by the agarose gel electrophoresis pattern (Figure 6). It is clear from the results that extract/fractions protected the pBR322 plasmid DNA against the DNA damaging effect of hydroxyl radicals.

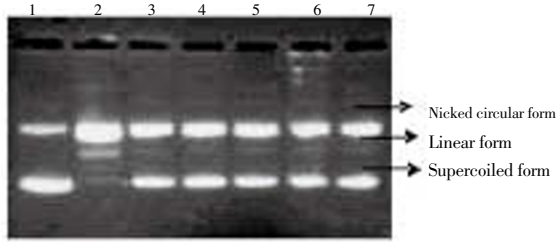

Figure 6. Effect of different extracts/fraction of leaves of *A. cadamba* in DNA protection assay.
Lane 1: Negative Control (DW+DNA); Lane 2: Fenton's reagent (FR) + DNA; Lane 3: pBR322 plasmid DNA+FR +gallic acid (200 μg/mL); Lane 4: pBR322 plasmid DNA+FR +ETAC (200 μg/mL); Lane 5: pBR322 plasmid DNA +FR+EAAC (200 μg/mL); Lane 6: pBR322 plasmid DNA +FR+ NBAC (200 μg/mL); Lane 7: pBR322 plasmid DNA +FR+WAC (200 μg/mL).

3.7. Correlation between total phenolic content and antioxidant activity

Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups[41,42]. In the present study a linear correlation have been observed between total phenolic content and antioxidant activity (Figure 7). Ethyl acetate fraction (EAAC) of *A. cadamba* showed highest scavenging activity in all the assays. The phenolic content was also high in EAAC fraction.

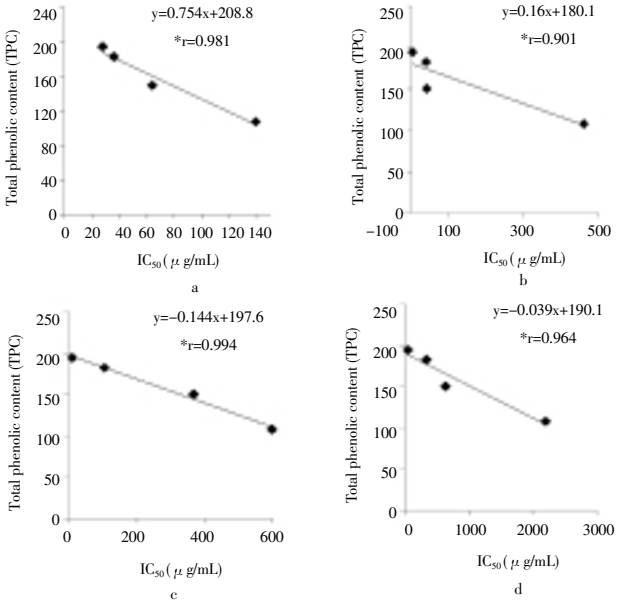

Figure 7. Correlation between total phenolic content (GAE mg/g of extract) of *Anthocephalus cadamba* extract/fractions and their antioxidant capacity as determined by (a): DPPH assay; (b): ABTS assay; (c): reducing power assay; (d): superoxide scavenging assay. *P <0.05.

3.8. Identification of bioactive compounds by UPLC

Representative UPLC chromatograms are shown in Figure 8. The retention times (tR), wavelengths of maximum absorbance (λ_{max}), and m/z are listed in Table 2. Peak identification was based on comparision of mass spectra and λ_{max} from literature. UPLC-ESI-QTOF-MS study of ETAC extract from leaves of *A. cadamba* showed major peaks at tR 1.21, 2.73 and 3.33 which exhibited the typical UV spectra of chlorogenic acid (m/z=355), dihydrocadambine (m/z=547) and β-sitosterol (m/z=411) respectively. The occurrence of peaks with low intensities at t_R 1.96 and 2.20 have absorption in the UV region at 347 nm (m/z=611) and 280, 340 nm (m/z=449) respectively, therefore indicating the identity as rutin and kaempferol 3-O-glucoside (Figure 8a). EAAC fraction showed the high intensity of the peak corresponding to chlorogenic acid. Other minor peaks were of catechin/epicatechin (m/z=291, λ_{max}=279), feruloyquinic acid (m/z=369, λ_{max}=286, 326) and cadambine (m/z=545, λ_{max}=283) (Figure 8b). NBAC and WAC showed the presence of compounds which were qualitatively similar, only the difference in relative abundance of each compound have been observed. NBAC showed one major peak of dihydrocadambine at retention time 2.73 (Figure 8c). The peaks with high intensity corresponding to chlorogenic acid, rutin, dihydrocadambine and β-sitosterol have been observed in WAC fraction (Figure 8d).

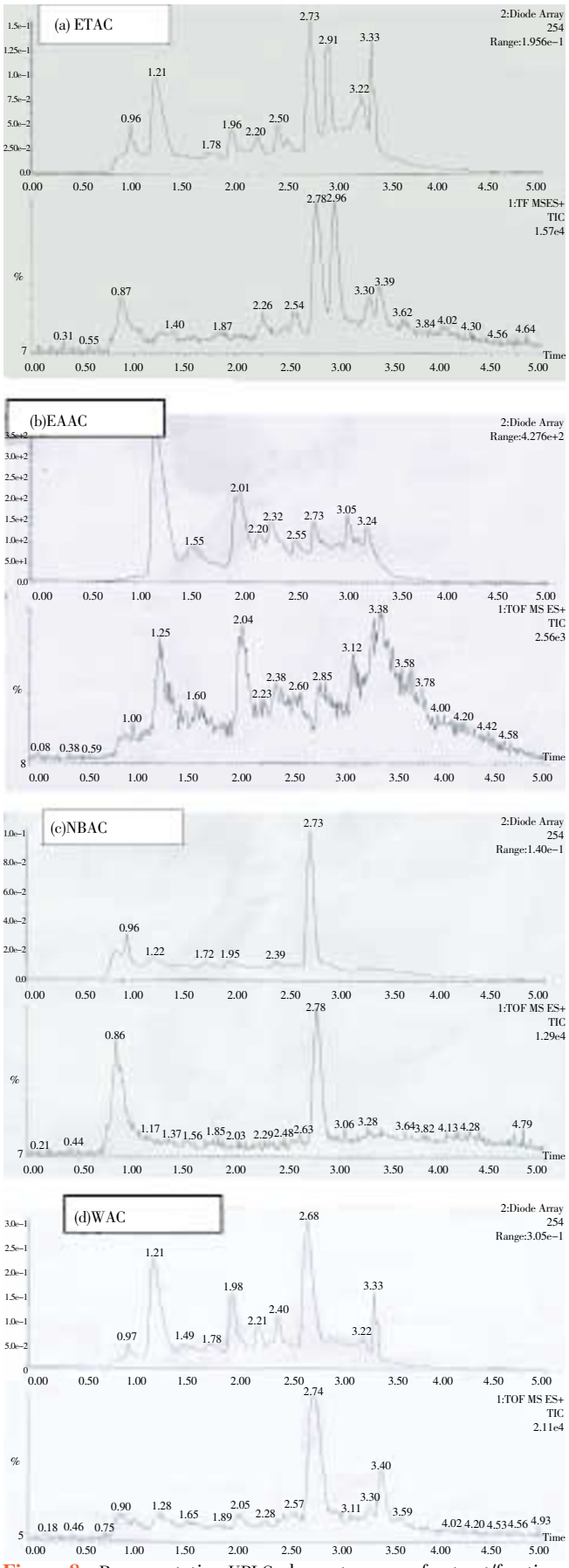

Figure 8. Representative UPLC chromatograms of extract/fractions from leaves of *A. cadamba*: (a) ETAC; (b) EAAC; (c) NBAC; (d) WAC.

4. Discussion

DPPH assay has been widely used to determine the free radical-scavenging activity of various plant extracts and pure compounds[43-48]. DPPH is a stable free radical which dissolves in methanol, and its purple colour shows a characteristic absorption at 517 nm. An antioxidant when scavenges the free radical by hydrogen donation, the colour changes from purple to light yellow. In the present study, ethyl acetate fraction was found to be most effective scavenger of DPPH radical among all the fractions and crude ethanol extract. Zhenbao *et al*[49] evaluated the antioxidant potential of fractions from *Cassia tora* and reported ethyl acetate fraction the most effective among all the fractions. Sun *et al* [50] evaluated the antioxidant activity of total flavonoids extract from Persimmon (*Diospyros kaki* L.) leaves and attributed the potent antioxidant activity on the DPPH radical to a direct role in trapping free radicals by donating a hydrogen atom. Since the DPPH assay was used as a preliminary free radical-scavenging evaluation, we subsequently performed analysis on ABTS radical scavenging, reducing power assay, superoxide scavenging and DNA nicking assay to confirm the free radical-scavenging effect from the leaves extract/fractions of *A. cadamba*. $ABTS^{\bullet+}$, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals[51]. However, among all the fractions EAAC fraction was most effective based on the IC_{50} value (1.12 μg/mL) which was lower than the standard compound rutin (IC_{50}=24.78 μg/mL). Similar results were reported by Guo *et al*[52] where they evaluated the antioxidant activity of extract/fractions from *Tuber indicum* where ethyl acetate and *n*-butanol fractions were found to be most effective among all the extract/fractions and correlated with content of total phenolic and total flavonoid which was high in both the fractions. In the present study, it has been observed that the scavenging of $ABTS^{\bullet+}$ by the extract/fractions was higher than that of $DPPH^{\bullet}$. This may be due to high reactivity of $ABTS^{+\bullet}$ radicals than $DPPH^{\bullet}$ radicals, and unlike the reactions with DPPH radicals which involve HAT (hydrogen atom transfer), the reactions with $ABTS^{+\bullet}$ radicals involve both HAT and SET (single electron transfer)[53]. Several factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals[54]. Gramza *et al*[55] evaluated the ABTS radical scavenging activity of tea extracts and attributed the scavenging potential to the presence of bioactive compounds catechins and tannins. Ferric ion (Fe^{3+}) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action[56,57]. The existence of

Table 2
Identified compounds from leaves of *A. cadamba*.

S. No.	t_R	UV λ_{max}	m/z [M+H]+	Tentative identification	References
1	1.21	217, 245, 325	355	Chlorogenic acid	Broils et al., 1998; Zhao et al., 2011
2	1.55	279	291	Catechin, epicatechin	Aaby et al., 2007; Zhao et al., 2011 Abad-Garcia et al., 2009
3	1.96	347	611	Rutin	Zhao et al., 2011
4	2.01	286, 326	369	Feruloyquinic acid	Dartora et al., 2011
5	2.20	280, 340	449	Kaempferol 3-O-glucoside	Zhao et al., 2011; Engels et al., 2011
6	2.32	226, 280	741	Glycosides of flavonoids (on the basis of mass fragmentation patteren only)	Zhao et al., 2011
7	2.55	283	545	Cadambine (indole alkaloid)	Handa et al., 1983
8	2.73	285, 326	547	Dihydrocadambine(indole alkaloid) (on the basis of mass fragmentation patteren only)	Brown and Fraser, 1974
9	3.33	220, 327	411 [(M+H)+-4H+]	β -sitosterol	Huang et al., 2007

reductones is the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom[58-67]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation[56,68]. Wang *et al*[60] evaluated the reducing ability of tea (*Camellia sinensis* L.) fruit peel extracts using FRAP assay and found a significant positive correlation between FRAP values and total flavonoids and concluded that fractions enriched with flavonoids and phenolics, act like ferric-reducing agents and have a potential to protect humans from oxidative stress induced by excessive ferric ion. Prasad *et al*[70] evaluated antioxidant activities of different species of *Cinnamomum* and *C. zeylanica* exhibited a stronger reducing power and attributed the reducing power to the hydroxyl group in phenolic compounds which might act as electron donors. The antioxidant activity has been reported to have a direct, positive correlation with the reducing power[71]. All the extract/fractions showed good scavenging potency towards superoxide radicals. Hu *et al*[72] reported that flavonoid molecule with polyhydroxylated substitution on ring B and a free 3-hydroxyl substitution could present the superoxide scavenging activity. Further the extract/fractions showed the potential to protect the pBR322 plasmid DNA from the damage caused by hydroxyl radicals. Rajkumar *et al*[73] evaluated the effect of methanolic and aqueous extracts of *Bergenia ciliata* rhizome at 50 μg concentration on the protection of DNA against •OH radicals generated by photolysis of H_2O_2. Bibi *et al*[74] reported the high DNA protection activity against the H_2O_2 induced damage by the extracts of *Aster thomsonii* and attributed the DNA protection activity to the high antioxidant activity. In all the *in vitro* antioxidant assays a significant correlation was observed between total phenolic content and antioxidant activity. Our results are in agreement with the reports of Sghaier *et al*[75] where they studied the antioxidant and antigenotoxic properties of different extracts of *Teucrium ramosissimum* and observed a distinct correlation between total phenolic content, total flavonoid content, antioxidant activity and antigenotoxic activities. Report by Shukla *et al*[76] showed a direct correlation between phenolic contents and antioxidant activity of aqueous leaf extract of *Stevia rebaudiana*. In the present study the antioxidant potential of various extract/fractions from leaves of *A. cadamba* may be attributed to the presence of compounds viz. Chlorogenic acid, Catechin/epicatechin, Rutin, Feruloyquinic acid, Kaempferol 3-O-glucoside, Glycosides of flavonoids, indole alkaloids and β -sitosterol which were identified by UPLC-ESI-QTOF.

In all the assay systems, it was found that EAAC fraction showed a higher antioxidant activity followed by NBAC fraction and WAC fraction. The bioactive compounds viz. catechins/epicatechins, feruloyquinic acid and cadambine which were exclusively present in EAAC fraction might contributing the higher antioxidant activity of this fraction. The total phenolic content analysis also showed the EAAC fraction with high phenolic content in terms of gallic acid equivalents/g of the fraction. The potential of the extract/fractions to scavenge different free radicals in different assay systems indicated that they may serve as useful therapeutic agents for treating radical-related pathologic damage. Further studies are in progress to isolate the antioxidant principles from the extract/fractions using different spectroscopic techniques.

Conflict of interest statement

We declare that we have no conflict of interest.

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