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Cellular Antioxidant Potential and Inhibition of the Enzymatic Activities Mediated through *Achyranthes aspera* Phytocomponents

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Various medicines of plant origin have been widely used, by many individuals, showing effective and safe results in combating diseases. Similarly, *Achyranthes aspera* is among these plants which have many potentials. Different concentrations of solvents (water and ethanol) were used to prepare the extracts *A. aspera* seeds. The phytochemical analysis of these extracted was conducted to evaluate the phenolic, flavonoid and condensed tannin contents. However, 25% hydroalcoholic extract of *A. aspera* (25% HA-Aca) exhibited the highest antioxidant potential by DPPH (IC_{50} 4.17 ± 1.73 µg/mL), ABTS (IC_{50} 117.33 ± 3.02 µg/mL), lipase (IC_{50} 114.4 µg/mL) and α-amylase (IC_{50} 105.4 µg/mL) inhibition activity using *in vitro* assays. Moreover, a cellular antioxidant assay was also conducted in 3T3-L1 cells revealed that the cells pre-treated with various *A. aspera* extracts were effective against the oxidative stress. Also, 25% HA-Aca displayed highest free radicals scavenging capability in the cellular antioxidant assay as compared to the other extracts. Stigmasterol, dolichol phosphate and 9′-hydroxy-9′-apo-epsilon-caroten-3-one are the major compounds present in the 25% HA-Aca extract as identified by LC-MS method. These findings suggest that *A. aspera* phytocomponents can modulate the redox status and regulate the lipid and carbohydrate metabolism, which might help in obesity.

Keywords: Phytochemicals, Antioxidant activity, Phenolic compounds, Lipase, α-Amylase, LC-MS method.

INTRODUCTION

As per the world health organization, approximately 2.1 million of different plant species are capable to function as medicinal herbs to treat a number of diseases [1]. A vast majority of these drugs have been derived from plants because of their safety and effectiveness against various diseases. A thorough investigation of these traditional plants based medicines has been used by pharmaceutical sector to develop these new drugs. Among these traditional plants *Achyranthes aspera*, a plant naturally found in the tropical and subtropical regions across the globe, also known as the prickly chaff flower/chirchita/latjira and a member of the *Amaranthaceous* family [2] has been widely acknowledged for its diverse range of medicinal properties. *A. aspera* has been harvested to treat the malarial fever, dysentery, hypertension and especially for wound healing by the traditional healers [3].

Traditionally all the parts *i.e.* root, shoots and seeds are used in traditional system of medicines and it was also believed and claimed by the traditional healers that by addition of *A. aspera* in any other drug would also enhance the efficacy of that drug [4]. The phytochemical constituents of *A. aspera* have been extensively studied, revealing a vast array of bioactive compounds that contributes to its medicinal prowess. More-

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over, more lately, studies on the adaptability of the plant have highlighted possible uses in the pharmaceutical sector. It has also been found that the presence of these phytochemicals in *A. aspera* is believed to be considered as underlying source responsible for its observed pharmacological activities and properties including antioxidant, antidiabetic, antimicrobial and anti-inflammatory properties [5,6].

The chemical profile of *A. aspera* is characterized by the presence of a diverse array of phytochemicals, including alkaloids, flavonoids, glycosides, saponins, steroids and tannins [7]. These diverse secondary metabolites have been associated with a multitude of therapeutic activities, making *A. aspera* a valuable resource in the realm of natural and alternative medicines. These compounds work synergistically to enhance the general effectiveness of the plant and offer a complete method of treatment for certain health disorders. This study created aqueous, hydroalcholic (25%), 50% and 75% ethanolic extracts from the *A. aspera* seeds. The antioxidant capacity, lipase and α-amylase inhibition potential of these extracts were evaluated. The antioxidant potential of *A. aspera* was also examined by utilizing a cell-based system. Although, the plant has been thoroughly investigated for a number of its therapeutic properties, there is no record available in literature reports on compounds identification of *A. aspera* seeds by LC-MS. *A. aspera* extract (25% HA-Aca) with the highest antioxidant potential and lipase and α-amylase inhibitory capability was utilized by LC-MS for the identification of the compounds.

EXPERIMENTAL

Gallic acid, quercetin, ascorbic acid, potassium persulfate, hydrogen peroxide, 2,2′-diphenyl-1-picrylhydrazyl, dimethyl sulfoxide, rutin, Dulbecco's modified eagle medium, sodium hydroxide, 2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid, penicillin/streptomycin, (Hi-Media), 4-nitrophenyl butyrate, porcine pancreatic lipase (Sigma), fetal bovine serum, sodium carbonate, aluminium chloride, Folin-ciocalteu's reagent, starch, sodium nitrite, ethanol, dinitrosalicylic acid, phosphate buffer saline (pH-7.4), orlistat, acetonitrile and ammonium hydroxide, all chemicals employed in the current study were of analytical-grade and used without any further purification.

Plant collection: *Achyranthes aspera* plant was collected from Maharishi Markandeshwar (Deemed to be University) campus and plant material was identified by Forest Research Institute (FRI, Dehradun) issuing letter no. 2086/Dis./2018/Syst.Bot./Rev.Gen./4-5). After the collection of the plant, it was washed thoroughly and dried in the shade. After drying, the seeds were collected and transformed into a fine powder *via* grinding.

Extraction of *A. aspera*: A sample weighing 25 g of powdered *A. aspera* seeds was dissolved in 250 mL of solvent and kept for 5 days for maceration [8] with occasional stirring at 25 ºC. The different combinations of the solvents used for the plant extraction are provided in Table-1. After 5 days of maceration solution was filtered and stored and residues were again mixed with 150 mL of solvent for 2nd maceration and the same procedure was repeated. After the incubation of 5 days residues were again added in 100 mL of solvent for final 3rd maceration to collect the filtered solution for extract preparation. Collected solutions of different solvents were subjected to 10 min of centrifugation at 12000 rpm. Then, the supernatant was subsequently collected to freeze and then lyophilized at -40 ºC using lyophilizer to obtain the extracts.

TABLE-1
DIFFERENT COMBINATIONS OF SOLVENTS USED FOR PREPARATION OF THE *A. aspera* EXTRACTS

Extracts of *Achyranthes aspera* (Aca)	H_2O (%)	Ethanol (%)	Total volume
Aqueous (A-Aca)	100	0	100
25% hydroalcoholic (25% HA-Aca)	75	25	100
50% hydroalcoholic (50% HA-Aca)	50	50	100
75% hydroalcoholic (75% HA-Aca)	25	75	100
Ethanolic (E-Aca)	0	100	100

Evaluation of phytochemicals in dry powdered sample of *A. aspera*

Alkaloid content determination: For the determination of alkaloid content, powdered sample of *A. aspera* weighing 1 g was combined with 40 mL of an ethanol having 10% acetic acid solution and incubated for 4 h [9]. The incubated mixture was then filtered and the resulting filtrate subsequently concentrated to one-quarter (25%) of its initial volume. The solution of concentrated NH_4OH was carefully introduced drop by drop until the formation of precipitates. A diluted NH_4OH was added to wash these precipitates, which were filtered and then dried. The dried precipitates were weighed and measured in milligrams per gram (mg/g) of dry weight of the initial powdered material.

Saponin content determination: The saponin content of *A. aspera* was determined by a mixture of 2 g dried powder sample in 50 mL of 20% ethanolic solution. The mixture was maintained at consistent temperature of 40 ºC for 4 h with continuous agitation. After filtration, the remaining residue was subjected to another extraction in 30 mL of 20% ethanol by repeating same procedure. The collected filtered solution was then concentrated at 90 ºC to a volume of approximately 10 mL. Following this, 20 mL of diethyl ether was mixed with this solution and shaken vigorously to facilitate its separation into two distinct layers. The lower aqueous layer containing saponins was carefully separated. The, 30 mL of *n*-butanol was introduced to the isolated aqueous layer and subjected to 10 mL of 5% NaCl twice for washing. Then it was dried to express the saponin content in mg/g of the initial dry weight of powdered plant [10].

Evaluation of phytochemicals in *A. aspera* extracts

Total phenolics content (TPC): The plant extracts (1 mg/mL) was diluted with 5 mL of distilled water followed by addition of 5% Folin-Ciocalteu reagent (0.5 mL) and incubation in dark, at room temperature for 5 min. Then 20% Na_2CO_3 (1.5 mL) was introduced and the reaction mixture was further diluted with distilled water (final volume 10 mL) and kept for 30-40 min. The absorbance was taken at 750 nm using Shimadzu UV-double beam spectrophotometer [11]. The phenolic content was evaluated by the linear equation ($y = 0.0089x - 0.033$, $R^2 = 0.9992$) of the reference compound (gallic acid) and the data was expressed in mg GAE/g dry wt. of the sample.

Total flavonoids content (TFC): Extracts (1 mL, 1 mg/mL) was mixed with 5% sodium nitrite (0.3 mL). After 5 min, incubation at room temperature and 10% $AlCl_3$ (0.3 mL) was introduced and mixed with 2 mL of 1 M NaOH. By adding distilled water, the reaction volume was made upto 10 mL and absorbance was measured at 510 nm using Shimadzu UV-double beam spectrophotometer [12]. Then the flavonoid content was evaluated by the linear equation ($y = 0.0048x + 0.0509$, $R^2 = 0.9995$) of the reference compound (quercetin) obtained from the standard curve and the data was reported as mg QE/g dry wt. of the sample.

Total tannin content (TTC): Rutin was utilized as standard to analyze the tannin content. The plant extracts (1 mg/mL) was mixed with 4% vanillin (3 mL) in methanol and conc. HCl (1.5 mL) and incubated for 15 min. The absorbance readings were taken at 500 nm by using Shimadzu UV-double beam spectrophotometer [13]. The condensed tannin content was evaluated by the linear equation ($y = 0.0006x + 0.1833$, $R^2 = 0.9901$) obtained from the standard curve and the data was reported as mg RE/g dry wt. of sample.

Analysis of antioxidant potential of *A. aspera* extracts

DPPH assay: The antioxidant potential of *A. aspera* extracts was analyzed by DPPH assay [14]. Plant extracts at various concentrations ranging from 10-100 µg/mL were added in the DPPH solution (0.5 mM) for 1.5 h to incubate in dark. The absorbance readings at 517 nm were recorded on spectramax iD3 (Molecular Devices). Ascorbic acid was utilized as a standard compound. The following formula was utilized to evaluate the scavenging activity.

$$\text{Scavenging activity } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where the absorbance of control and reaction mixture was presented by A_0 and A_1, respectively. The IC_{50} values were also calculated by using Graph pad prism version 5.01.

ABTS assay: A stock solution of $ABTS^{\bullet+}$ was formulated by mixing the equal amount of 7 mM ABTS solution with 2.45 mM potassium persulfate solution and left for incubation in the dark at room temperature for 12-16 h [15]. A working solution with the absorbance of 0.70 ± 0.02 at 734 nm was obtained by diluting the $ABTS^{\bullet+}$ stock solution with methanol. For analysis 2 mL of working $ABTS^{\bullet+}$ solution and 1 mL of standard/samples at various concentrations (10- 100 µg/mL) were mixed and then incubated for 5 min in dark at room temperature. The readings of absorbance were recorded at 734 nm using spectramax iD3. Ascorbic acid was used as a positive control.

Cellular antioxidant assay: A cell-based assay with slight modifications was performed [16] to measure the antioxidant capacity of the extracts on 3T3-L1 cells. The cells were sourced through the National Centre for Cell Science (NCCS), Pune, India. These cells were cultured and maintained at 37 ºC with 5% CO_2 in a humidifier. The DMEM media was supplemented with penicillin, streptomycin and 10% FBS to maintain the cells. In 96-well microtiter plates 5,000 cells of 3T3-L1 cells were cultured in all the wells for 24 h and then exposed to 500 µM of H_2O_2 treatment for 1 h at 37 ºC in CO_2 incubator. Then the cells were rinsed by using PBS and subjected to a range of varying concentrations (25-200 µg/mL) of *A. aspera* extracts to incubate for 1 h. After that PBS was again used to rinse the cells and administrated with 20 µM of DCFDA. Then the fluorescence was determined at 538 nm for emission and 485 nm for excitation by spectramax iD3. The scavenging percentage of each extract was calculated by using following formula:

$$\text{Scavenging } (\%) = \left(1 - \frac{F_{sample}}{F_{control}}\right) \times 100$$

where $F_{control}$ is the fluorescence of cells without extracts and F_{sample} is the fluorescence of cells incubated with extracts.

Enzyme inhibition potential of hydroalcoholic extracts of *A. aspera*

Pancreatic lipase inhibition assay: Orlistat was utilized as a positive control in lipase inhibition assay. In 100 µL of lipase (1 mg/mL), 200 µL of extracts (50-400 µg/mL) were added and mixed with 700 µL of buffer (potassium phosphate with pH adjusted to 6.8) and incubated for 1 h at 25-30 ºC. After the incubation, 100 µL of 4-nitrophenylbutyrate (1 mg/mL) in acetonitrile was introduced. The absorbance at 410 nm was taken after 30 min. The following formula was employed to evaluate the % inhibition of lipase:

$$I\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 and A_1 are the optical densities of control and tested samples/standard, respectively [17].

α-Amylase inhibition assay: A 1 mL solution of α-amylase (1 mg/mL) was treated with 1 mL of samples/control (50-400 µg/mL) for 20 min at 37 ºC. Then, 1% starch (1 mL) in buffer (potassium phosphate with pH adjusted to 6.8) was introduced. After the 1 h of incubation at 37 ºC, 2 mL of dinitrosalicylic acid (DNSA) was mixed followed by boiling for 10 min and measurement of absorbance at 540 nm against blank (buffer and DNSA). The following formula was used to evaluate the% inhibition of α-amylase:

$$I\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 and A_1 are the optical densities of control and tested samples/standard, respectively [18].

Identification of components by LC-MS: The 25% HA-Aca extract showed highest antioxidant potential and lipase and α-amylase inhibitory activity. Therefore, LC-MS of the 25% HA-Aca was carried out at Central Facility of Panjab University, Chandigarh, India. Spectra for all the peaks were taken and detected for its area% and *m/z* ratio. All compounds were identified by various literature reports and comparing spectral data.

Statistical analysis: All the results derived from the experiments are presented in the form of mean ± SEM. All measurements were conducted in triplicates. The significant differences of the data was analyzed at $p \leq 0.05$* *via* ANOVA (one-way analysis of variance) and Dunnett test by operating GraphPad Prism 5.01 statistical software. Linear regression analysis was also employed to derive the IC_{50} values.

RESULTS AND DISCUSSION

The polarity of solvent and the method used for extraction play a critical role in composition of the extract and its productivity [19]. Therefore, the extracts of *Achyranthes aspera* were prepared by varying the amount of water and ethanol as given in Table-1.

Alkaloid and saponin contents: Alkaloids are the nitrogenous compounds that protect from pathogens and used in narcotics and pharmaceutical industries. While the saponins are surface active in nature and used as a drug delivery agent in pharmaceutical industries. It also possess many biological activities like anti-inflammatory, hypoglycemic, antioxidant and antitumor [20]. The contents consisting of alkaloid (301.7 ± 3.4 mg/g dry powder) and saponin (15.6 ± 2.9 mg/g dry powder) in *A. aspera* seed powder were measured. Earlier, *A. aspera linn* leaves have been shown to contain alkaloids as determined by HPLC [21]. Similarly, the ethanolic extract of *A. aspera* seeds was reported to contain saponins (147.7mg/g of extract) as analytically determined through HPLC [22,23].

Total phytochemical content of phenolics (TPC), flavonoids (TFC) and tannins (TTC): Among the aqueous, hydroalcholic (25%, 50% and 75%) and ethanolic extracts of *A. aspera* seeds the maximum amount of TPC, TFC and TTC were found in 25% HA-Aca extract (Table-2). Phenolics and polyphenolics (polymeric phenolics) can help in treating degenerative diseases and physical ailments, such as cancer and cardiovascular disease [24]. In addition, the content of phenolics found in extracts uplift and enhance the antioxidant activity because these compounds have hydroxyl groups, which act as hydrogen donors [25]. Flavonoids, a diverse class of naturally occurring secondary metabolites that serve as major antioxidant agents [26]. Research also showed that tannins play a vital role by contributing in plant defence mechanism against pathogens [27] and also regulates the nutrients and induces the antioxidant effect. Previouly, researhers have found appreciable amounts of phenolic (0.34 mg/g GAE) and flavonoids (0.30 mg/g QE) in the ethanolic extract of *A. aspera* seeds [22]. Also, tannins were shown to be present in the methanolic extract of *A. aspera* seeds [28].

TABLE-2
TOTAL PHENOLICS, FLAVONOIDS AND TANNIN CONTENT OF DIFFERENT EXTRACTS OF *A. aspera* SEEDS

A. aspera seeds extracts	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg RE/g)
A-Aca	96.4 ± 2.4	145.9 ± 2.2	477.1 ± 1.6
25% HA-Aca	159.6 ± 0.5	194.7 ± 0.4	661.2 ± 1.9
50% HA-Aca	63.9 ± 0.7	117.8 ± 0.9	357.5 ± 1.6
75% HA-Aca	30.3 ± 0.1	31.0 ± 0.2	270.9 ± 1.5
E-Aca	22.4 ± 0.3	18.5 ± 0.5	170.9 ± 1.4

Note: All the experiments were conducted in triplicates. Results are presented as Mean ± SEM and considered significant as p value was < 0.05.

Free radical scavenging potential of *A. aspera* extracts: The antioxidant capacity of *A. aspera* extracts were analyzed with DPPH and ABTS assays. The values of the data are expressed as % inhibition with their IC_{50} value (Table-3), DPPH is a deep purple organic nitrogen radical with a long half-life [29]. The colour of the corresponding hydrazine changed into yellow from purple when the solution of DPPH radical was combined with an antioxidant/reducing compound. Furthermore, ABTS assay also determine the antioxidant potential of both the hydrophilic and lipophilic compounds over a wide range of pH [30]. The DPPH assay data depicted that 25% HA-Aca extract (IC_{50} 4.17 ± 1.73 µg/mL) showed highest activity to scavenge free radicals followed by 75% HA-Aca extract (IC_{50} 10.67 ± 1.52 µg/mL) (Table-3). Similarly, the ABTS assay also showed that 25% HA-Aca extract (IC_{50} 117.33 ± 3.02 µg/mL) exhibited highest free radicle scavenging potential as compare to the other extracts (Fig. 1). The remarkable antioxidant potential of 25% HA-Aca extract could be attributed to the rich and elevated amount of phenolics, flavonoids and tannins found in this extract surpassing the other extracts of *A. aspera* (Table-2).

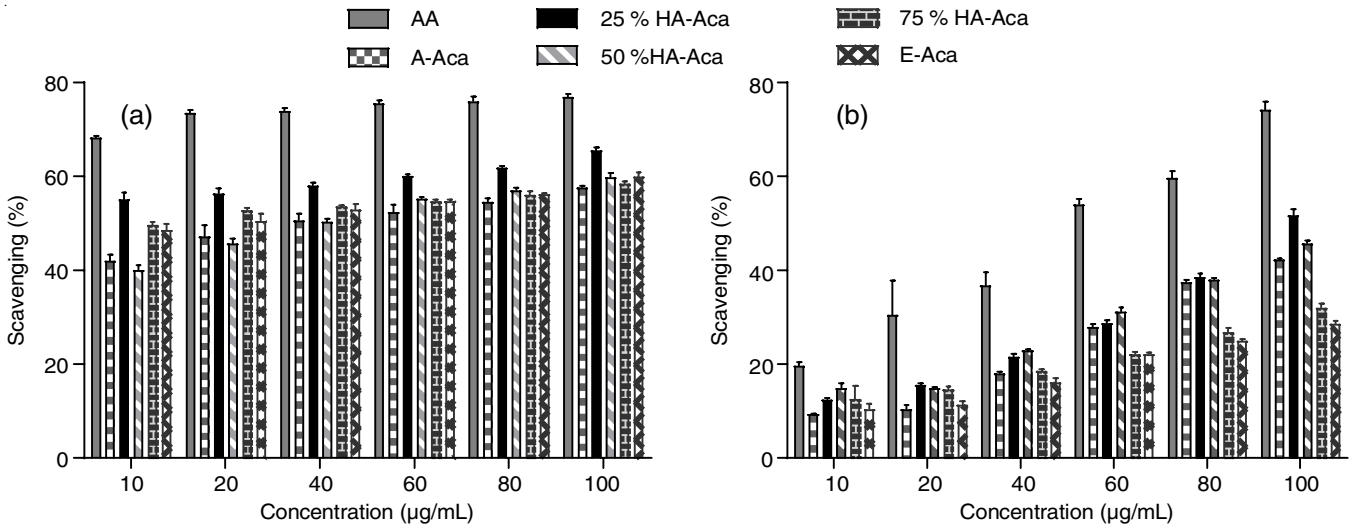

Fig. 1. Free radical scavenging activity of *A. aspera* extracts was evaluated at various concentrations by (a) DPPH assay and (b) ABTS assay. All the experiments were conducted in triplicates. Results were presented as Mean ± SEM. Data is significant as $p < 0.05$ was observed

TABLE-3
IC_{50} (µg/mL) VALUES OF *A. aspera* EXTRACTS SHOWING ANTIOXIDANT CAPACITIES

Samples (control and extracts)	IC_{50} (µg/mL) values		
	DPPH assay	ABTS assay	CAA assay
AA	0.1 ± 0.1	49.7 ± 1.8	–
A-Aca	35.3 ± 4.8	134.6 ± 1.5	70.63 ± 5.8
25% HA-Aca	4.2 ± 1.7	117.3 ± 3.0	10.73 ± 3.4
50% HA-Aca	33.5 ± 2.7	142.3 ± 6.9	27.04 ± 2.2
75% HA-Aca	10.7 ± 1.5	707.7± 354.8	101.59 ± 5.7
E-Aca	15.9 ± 4.4	459.8 ± 38.2	118.57 ± 20.9

Note: All the experiments were conducted in triplicates. Results were depicted as Mean ± SEM and were significant as $p < 0.05$ was observed. AA = ascorbic acid.

Cellular antioxidant assay: The intracellular antioxidant capability of *A. aspera* seeds extracts were examined in 3T3-L1 (murine preadipocytes) cells *via* DCF assay to determine the H_2O_2-induced intracellular ROS levels in cells. The analysis of data depicts that in a concentration-dependent manner the *A. aspera* seeds extracts can inhibit the intracellular ROS (Table-3). According to the cellular antioxidant assay highest antioxidant capacity was displayed by the 25% HA-Aca extract (IC_{50} 10.73 ± 3.39 µg/mL) capacity as compared to the other extracts (Fig. 2). Since cell-based assays serves as biologically relevant model to evaluate the antioxidant potential, therefore, these results confirmed that *A. aspera* seeds phytocomponents can overcome intracellular oxidative stress. The cellular antioxidant potential of *A. aspera* seeds extracts has not been reported earlier. However, the saponins from *A. aspera* have been shown to improve serum antioxidant status of the high cholesterol diet fed rats [23].

Inhibition of pancreatic lipase and α-amylase: The enzyme inhibition assays showed that *A. aspera* seeds extracts were capable of inhibiting the activities of lipase and α-amylase (Fig. 3). As shown earlier, 25% HA-Aca extract can inhibit both lipase (IC_{50} 87.34 ± 1.68 µg/mL) and α-amylase (IC_{50} 105.26 ± 2.75 µg/mL) activities rather efficiently by surpassing the other extracts of *A. aspera* (Table-4). Both lipase and α-amylase enzymes are integral part in the process of digestion of food [31]. By inhibiting these fundamental enzymes it can also reduce the prevalence of various metabolic disorders like obesity and type 2 diabetes [32]. Lipase inhibition lowers the blood lipid

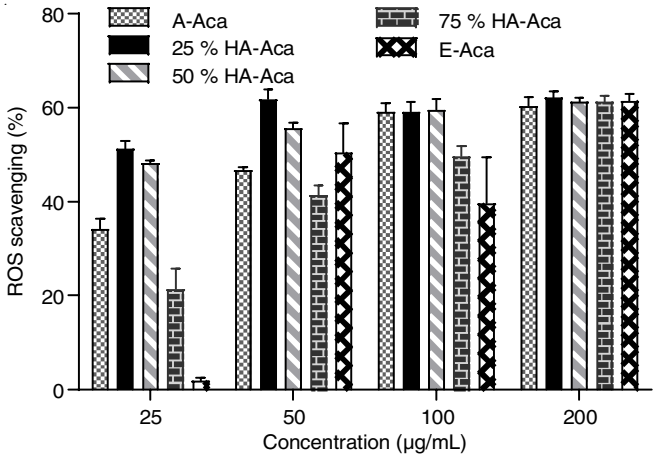

Fig. 2. ROS scavenging activity of *A. aspera* seed extracts was evaluated at various concentrations by cellular antioxidant assay on 3T3-L1 cells. Results were presented as Mean ± SEM (n=3) and data was significant as $p < 0.05$ was observed

TABLE-4
LIPASE AND α-AMYLASE ENZYMES INHIBITION ACTIVITY OF *A. aspera* EXTRACTS DEPICTED THROUGH IC_{50} (µg/mL) VALUES

Samples	IC_{50} (µg/mL)] values for enzyme inhibition assays	
	Lipase	α-Amylase
Positive control	24.2 ± 2.4	43.7 ± 3.2
A-Aca	880.0 ± 41.4	129.8 ± 8.0
25% HA-Aca	87.3 ± 1.7	105.3 ± 2.7
50% HA-Aca	524.3 ± 11.9	165.6 ± 6.2
75% HA-Aca	1748.7 ± 106.8	395.8 ± 13.7
E-Aca	2914.3 ± 44.8	1076.0 ± 57.1

Note: Results (n = 3) were depicted as Mean ± SEM and were significant as p value < 0.05 was observed. Orlistat and rutin were used as positive controls for lipase and α-amylase inhibition assays, respectively.

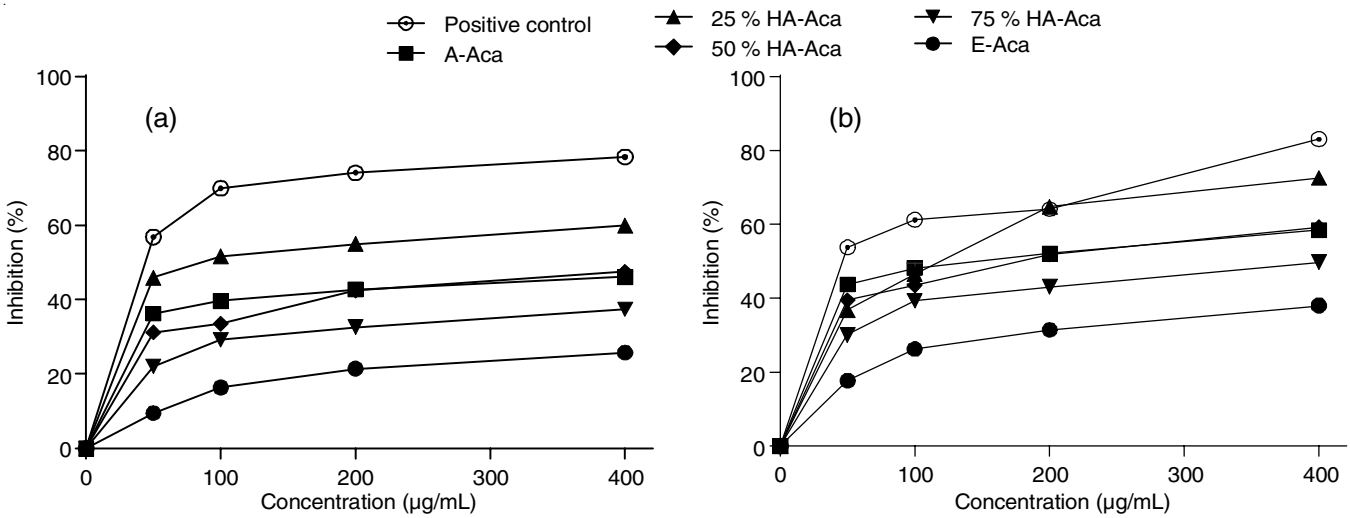

Fig. 3. Lipase (a) and α-amylase (b) inhibition by *A. aspera* extracts at various concentrations (50-400 µg/mL). Where, orlistat (for lipase) and rutin (for α-amylase) were utilizd as positive controls. The experiment was conducted in triplicates. Results were presented as Mean ± SEM and considered significant as $p < 0.05$ was observed

levels and prevents the breakdown and absorption of dietary fats which leads to the weight loss [33]. While inhibiting the amylase enzyme post-meal blood sugar spikes can be controlled due to the slow rate of carbohydrate breakdown into glucose thus helping the diabetic patients [34]. The ethanolic extract of *A. aspera* seeds have been shown to inhibit lipase and α-amylase activities (IC_{50} values; 2.34 mg/mL and 3.83 mg/mL, respectively) [22].

LC-MS of 25% HA-Aca extract: Since 25% HA-Aca extract displayed highest antioxidant and enzymes inhibitory activities. Therefore, LC-MS of 25% HA-Aca was carried out. The total ion chromatograms of UPLC-MS of 25% HA-Aca are shown in Fig. 4. The observed *m/z* values of the compounds are matched to the previously reported *m/z* values for their identification. All identified 25 compounds were summarized in Table-5. The major compounds found in 25% HA-Aca extract was stigmasterol. Stigmasterol is a type of plant sterol found earlier in *A. aspera* seeds [35] and many other plants. It is known for improving the low-density lipoprotein cholesterol and besides it also exhibits antidiabetic effects by improving glucose transporter type 4 translocation [36]. Other compounds like dolichol phosphate and 9′-hydroxy-9′-apo-epsilon-caroten-3-one are also present in appreciable amounts in 25% HA-Aca extract. Dolichol phosphate acts as a lipid carrier that helps in

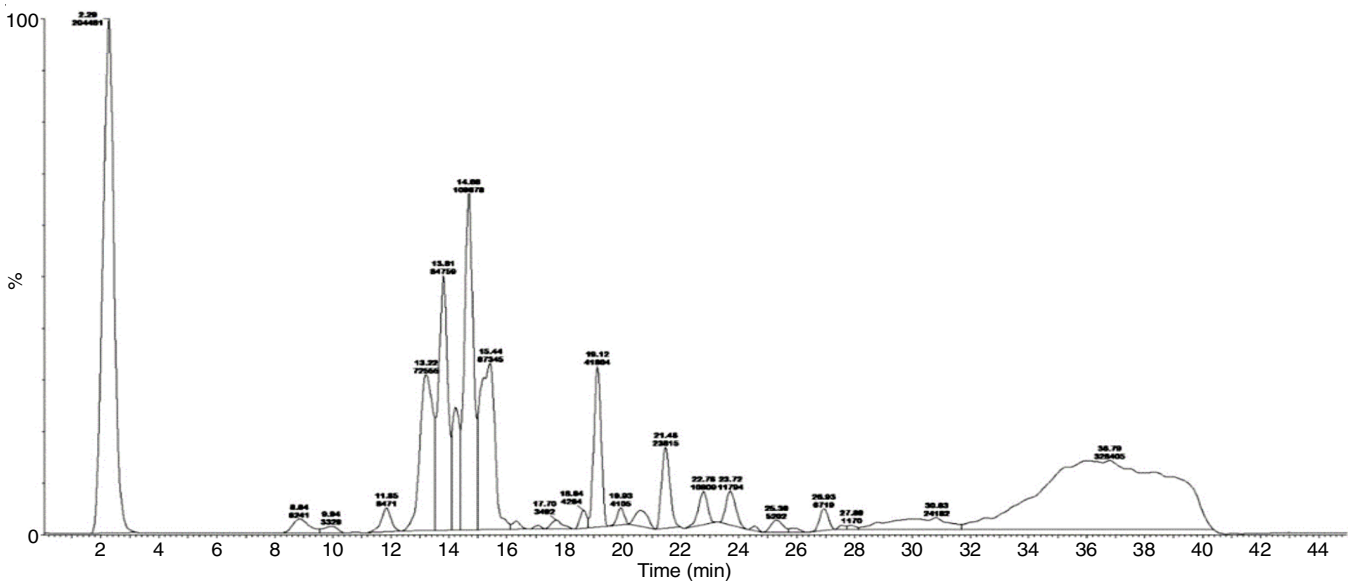

Fig. 4. Total ion chromatogram of 25% HA-Aca extract as depicted by UPLC-MS

TABLE-5
LIST OF COMPOUNDS DETERMINED BY LC-MS ANALYSIS OF 25% HYDROALCHOLIC EXTRACT OF *A. aspera* SEEDS

RT	*m/z* ratio	Compound	Area%	Formula
2.29	1225.947	Dolichol phosphate	18.77	$C_{80}H_{131}O_4P$
8.84	98.98782	(2Z)-2-Fluoro-2-butenedioic acid	0.76	$C_4H_3FO_4$
9.94	652.2201	Neohesperidin	0.31	$C_{28}H_{34}O_{15}$
11.85	98.98637	(2Z)-2-Fluoro-2-butenedioic acid	0.78	$C_4H_3FO_4$
13.22	1170.411	3-Oxotetracosanoyl-CoA	6.66	$C_{45}H_{80}N_7O_{18}P_3S$
13.81	182.1642	*sym*-Homospermidine	7.78	$C_8H_{21}N_3$
14.23	1170.41	3-Oxotetracosanoyl-CoA	2.85	$C_{45}H_{80}N_7O_{18}P_3S$
14.68	232.1311	9′-Hydroxy-9′-apo-epsilon-caroten-3-one	10.07	$C_{29}H_{38}O_2$
15.44	283.0978	7,8-Dimethoxyflavone	8.02	$C_{17}H_{14}O_4$
16.32	329.1816	2-Hydroxyastaxanthin	0.21	$C_{40}H_{52}O_5$
17.08	423.237	Ginsenoside R	0.07	$C_{42}H_{72}O_{14}$
17.70	175.1063	L-Theanine	0.32	$C_7H_{14}N_2O_3$
18.64	290.1966	Farnesylcysteine	0.39	$C_{18}H_{31}NO_2S$
19.12	1070.347	18-Hydroxyoleoyl-CoA	3.84	$C_{39}H_{68}N_7O_{18}P_3S$
19.93	233.1118	2-Amino-2,3,7-trideoxy-D-lyxo-hept-6-ulosonic acid	0.38	$C_7H_{13}NO_5$
20.61	1078.353	8,11,14-Icosatrienoyl-CoA	0.67	$C_{41}H_{68}N_7O_{17}P_3S$
21.48	148.0123	2-Aminoethylphosphonic acid	2.19	$C_2H_8NO_3P$
22.78	895.3729	Paclitaxel	0.99	$C_{47}H_{51}NO_{14}$
24.54	203.1774	Curcumene	0.10	$C_{15}H_{22}$
25.30	339.2746	9,10-Epoxystearate	0.48	$C_{18}H_{33}O_3^-$
25.86	323.1939	Cycloheximide	0.13	$C_{15}H_{23}NO_4$
27.55	503.1202	4′-Methylepicatechin-3′-glucuronide	0.09	$C_{22}H_{24}O_{12}$
27.89	339.179	Geranyl glucoside	0.11	$C_{16}H_{28}O_6$
30.83	743.538	1-18:2-2-18:2-Monogalactosyldiacylglycerol	2.22	$C_{45}H_{78}O_{10}$
36.79	393.3503	Stigmasterol	30.14	$C_{29}H_{48}O_2$

glycosylation [37]. While 9′-hydroxy-9′-apo-epsilon-caroten-3-one belongs to the family of apocartenoids act as an antioxidant to protect against reactive oxygen species [38]. Various compounds present in the 25% HA-Aca extract like 7,8-dimethoxyflavone, gensenoside, curcumene, 9,10-epoxystearate, geranyl glucoside, neohesperidin and farnesylcystein, 2-hydroxyastaxanthin and 1-18:2-2-18:2-monogalactosyldiacyl glycerol have displayed antioxidant and anti-inflammatory properties. 2-Aminoethylphosphonic acid was found to be lipase inhibitor thus helps in combating the obesity [39].

Conclusion

The current study described the phytochemical content of *A. aspera* by analyzing the total alkaloids, saponins, phenolics, flavonoids and tannin contents. Antioxidant capacity of *A. aspera* extracts was analyzed by DPPH assay, ABTS assay and cell-based antioxidant assay (on 3T3-L1 cells). The results clearly showed that 25% hydroalcoholic extract of *A. aspera* is more potent in comparison to the other extracts which can be attributed to the high content of the phenolics, flavonoids and tannins. This data suggests that *A. aspera* phytoconstituents can combat free radicals and manage cellular oxidative stress. In addition, *A. aspera* extracts can act as inhibitors for pancreatic lipase and α-amylase. The LC-MS data of 25% hydroalcoholic extract of *A. aspera* revealed presence of many compounds with anti-oxidant, anti-obesity and antidiabetic effects. Further research work is required so that *A. aspera* extracts can be utilized as supplements for managing oxidative stress related human disorders.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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