Original Article

Evaluation of Total Phenolics, Flavonoids, and Antioxidant and Cytotoxic Potential of *Ailanthus altissima* (Mill.) Swingle Leaves

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Abstract

Context: People all over the world are suffering from cancer. Liver cancer is considered the second most common malignancy among Egyptian men and the sixth most common malignancy among Egyptian women. Plant-derived antioxidants are believed to prevent or delay the occurrence of many chronic diseases such as cancer. *Ailanthus altissima* has been used in many traditional prescriptions. **Aims:** The current study aimed at investigating the phytochemical profile of *A. altissima* leaves' extract and its derived fractions, determining their content of phenolics and flavonoids as well as assessing their antioxidant and cytotoxic potential. **Materials and Methods:** The phytochemical screening was carried out using standard methods. The total phenolic, flavonoid, and flavonol contents were determined using Folin-Ciocalteu, aluminum chloride, and aluminum chloride/ sodium acetate assays, respectively. The antioxidant activity was evaluated using different *in vitro* methods: DPPH˙, total antioxidant capacity, hydroxyl (˙OH), nitric oxide (NO˙) radical scavenging activities, and permanganate-reducing antioxidant capacity (PRAC). The antiproliferative potential against HepG2 cells was evaluated using sulforhodamine-B assay (SRB). **Results:** The results showed that the ethyl acetate fraction had the highest content of phenolics, flavonoids, and flavonols (551.72 ± 1.81 mg GAE/g ext., 371.24 ± 4.36 mg RE/g ext., and 100.47 ± 1.30 mg QE/g ext., respectively). It also had the most potent reducing power (DPPH˙ SC_{50} = 7.19 ± 0.05 µg/mL, TAC= 369.88 ± 1.51 mg AAE/g ext., ˙OH SA = 95.46 ± 0.14%, NO˙ SA = 40.65 ± 0.91%, and PRAC = 77.19 ± 0.27%). The *n*-butanol fraction exhibited the most potent cytotoxic potential against HepG2 cells (IC_{50} = 16.70 µg/mL). **Conclusion:** *A. altissima* leaves could be considered potent antioxidant and cytotoxic alternatives.

Keywords: *Ailanthus altissima, antioxidant activity, cytotoxic activity, phytochemical screening, total flavonols, total phenolics*

Key Message: *Ailanthus altissima* leaves are characterized by significant antioxidant activity, which could be due to their being rich with phenolic compounds. Also, they exhibit potent cytotoxic potential against HepG2 cells. *A. altissima* could be used as natural antioxidant and cytotoxic agents.

Introduction

Reactive oxygen and nitrogen species (RONS) are intracellular oxidants produced as a byproduct of normal metabolic processes or as a result of exposure to different pollutants.[1] When their concentration exceeds the ability of the body to eliminate them, they accumulate, causing severe damage to cellular biomolecules; a condition named "oxidative stress" arises, which is the main reason of the pathogenesis of many diseases such as atherosclerosis, neurological diseases, cancer, diabetes, etc.[2]

The liver, one of the most vital organs within the human body, is the most threatened organ to develop cancer among Egyptian citizens. Also, the proportion of liver cancer or hepatocellular carcinoma (HCC) is predicted to triplicate by 2050.[3,4] Hepatic viruses (HCV and HBV), intake of aflatoxins, pollutants such as chlorinated byproducts, insecticides, and pesticides, in addition to alcohol abuse, obesity, and diabetes mellitus, are considered the most common risk factors of HCC development.[5]

Nevertheless, the human body is provided with a defense line against free radical-induced diseases represented in enzymatic and nonenzymatic antioxidants.[6] These antioxidants are insufficient to provide the body with the required protection. Synthetic antioxidants such as butylated hydroxy toluene

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(BHT) and butylated hydroxy anisole (BHA) have been reported to exert toxic side effects on animal cell models and can cause carcinogenesis,[7,8] which favors the use of natural antioxidants; dietary antioxidants, and plant-derived antioxidants. The search for powerful and naturally occurring antioxidants, especially of plant origin, has been intensified recently to find equipotent, nontoxic, and effective antioxidative alternatives to synthetic antioxidants, especially in the commercial field. Medicinal plants can act as a potential alternative for developing new and more effective antioxidant agents for future therapy,[9] due to their being rich with different classes of phytochemicals.

Ailanthus altissima (Mill) Swingle [syn. *Ailanthus glandulosa* (Desf), *Toxicodendron altissimum* Mill] belongs to the family Simarobaceae. It is commonly known as the tree of heaven.[10] Since ancient times, *A. altissima* has been used in many cultures as a remedy for gastric and intestinal diseases, cough, gonorrhea, anemia, hemorrhage, and hemorrhoids.[11] Many studies demonstrated the richness of the plant leaves with different bioactive phytoconstituents such as quassinoids,[12] diverse phenolics,[13] lipids and fatty acids,[14] as well as indole alkaloids[15] and volatile compounds.[16] The current study was designed to determine the phytochemical profile of the methanolic extract of *A. altissima* leaves as well as its fractions; assess their *in vitro* antioxidant and cytotoxic activities; and quantify their phenolic, flavonoid, and flavonol contents.

Materials and Methods

Chemicals and reagents

DPPH (1,1-diphenyl-2-picryl hydrazyl radical), SRB, trichloroacetic acid (TCA), and Folin Ciocalteu reagent were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium persulfate and disodium hydrogen phosphate were purchased from Bio Basic Inc. (Canada). Ammoniummolybdate, sodium carbonate, sodium nitrite, sodium hydroxide, sodium nitroprusside, sulfanilic acid, α-napthylamine, and aluminum chloride were purchased from Merck (Darmstadt, Germany). Salicylic acid, ferrous sulfate, and potassium permanganate were purchased from El Nasr Pharmaceutical chemicals company (Kaliobeya, Egypt). Rutin, gallic acid, and ascorbic acid (AA) were purchased from Sigma–Aldrich (St. Louis, USA). All the used solvents were of high analytical grade.

Collection and preparation of plant material

Leaves of *A. altissima* were collected, in September 2017, from Orman Garden, Giza, Egypt after being identified by Mrs. Rehab Mohamed Eid, a botanist at Orman Garden Herbarium; further confirmation was made by Mrs. Treaze Labib, a consultant of plant taxonomy at the Agriculture Ministry and the ex-director of Orman Garden. A voucher specimen (No. 198 AC) was deposited in Orman Garden Herbarium. The plant leaves were cut into small fragments; allowed to dry in a clean, well-ventilated, and dry place away from the sunlight; and finally ground into fine powder using an electric mixer.

Extraction and fractionation

Overall, 2 kg of plant leaves dry powder was soaked in 85% aqueous methanol and then filtered; the excess solvent was removed at reduced pressure via a rotary evaporator (BUCHI, Germany). This process was repeated several times, affording the crude extract (400 g), which was successively fractionated using different organic solvents: petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol. The fractions were evaporated under vacuum till complete dryness, weighted, and kept in a dry and dark place for further biological and chemical investigations.

Phytochemical screening

Preliminary phytochemical screening of the plant crude extract and its fractions were carried out based on standard procedures of analyses described by Banu and Cathrine[17] and Tiwari *et al*.[18].

Determination of bioactive phytoconstituents

Total phenolics

The total phenolic content (TPC) was measured according to the Folin-Ciocalteu technique reported by Takao *et al*.[19] In brief, 2.5 mL of Folin-Ciocalteu reagent (10-fold pre-diluted with distilled water) was mixed with 0.5 mL of plant samples (200 µg/mL) and standard gallic acid solutions (10–100 µg/mL) followed by the addition of 2 mL Na_2CO_3 (7.5%, w/v). The tubes were kept in the dark for 0.5 h with intermittent shaking. The absorption of the developed blue color was measured at 760 nm against blank. The TPC was calculated from the gallic acid calibration curve and expressed as mean ± SD (n = 3) milligrams of gallic acid equivalents (GAE)/g of dry extract.

Total flavonoids

The total flavonoid content (TFC) was measured according to the aluminum chloride colorimetric assay described by Bhandari and Rajbhandari.[20] Overall, 75 µL of $NaNO_2$ (5%, w/v) was added to 250 µL of plant extract (500 µg/mL) and rutin standard solutions (10–250 µg/mL) and then diluted with 1.3 mL of distilled water. After 5 min, 150 µL of $AlCl_3$ (10%, w/v) was added. After 6 min, 500 µL of NaOH (4%, w/v) and 275 µL of distilled water were finally added. After 15 min, the intensity of the pink-colored complex was measured at 510 nm against blank. The TFC was calculated from the rutin standard curve and expressed as the average of three analyses in milligrams of rutin equivalents (RE)/g of dry extract.

Total flavonols

The total flavonol content (TF_{OL}) was determined using the method reported by Abdel-Hameed.[21] To 1 mL plant extract (500 µg/mL), 1 mL of $AlCl_3$ (2%, w/v) was added followed by the addition of 3 mL of CH_3COONa (5%, w/v). The reaction mixtures were mixed well and incubated at room temperature for 2.5 h. After the incubation period, the absorption of the developed colored complex was measured at 440 nm against

blank. A standard curve of quercetin (5–200 µg/mL) was established under the same circumstances. TF_{OL} was expressed as milligrams of quercetin equivalents (QE)/gram dry extract.

Determination of *in vitro* antioxidant potential

DPPH radical scavenging activity

Different concentrations of the plant samples were mixed with freshly prepared DPPH• (1,1-diphenyl-2-picryl hydrazyl radical) solution (0.09 mM) in a ratio of 1:1 according to the method described by El-sayed *et al*.[22] The reaction mixtures were allowed to stand for 30min in the dark; then, the absorption decrease was measured at 517 nm against blank. AA was used as a positive control. DPPH• scavenging activity% = ($A_{control}$ − A_{sample}/$A_{control}$) × 100, where $A_{control}$ is the absorbance of the control solution containing methanol instead of test sample and A_{sample} is the absorbance of the plant sample. The determination was carried out in triplicate, and the results were expressed as $SC_{50,}$ which is the concentration of the plant sample required to reduce half of DPPH•. The results were also expressed as ascorbic acid equivalent antioxidant activity (AEAC) using the equation proposed by Uddin *et al*.[23] AEAC is the concentration of AA required to produce the same antioxidant capacity (same SC_{50}) as the test sample, and it is expressed as milligrams of ascorbic acid/ 100 g fresh weight.

$$\text{AEAC (mg AA/100 g FW)} = \frac{SC_{50(\text{ascorbic acid})}}{SC_{50(\text{plant sample})}} \times 10^5$$

Antioxidant activity index

A criterion named antioxidant activity index (AAI) was created to express the antioxidative power of the tested sample, which is the ratio of the final concentration of DPPH in control solution (in µg/mL) to the SC_{50} of the plant extract in µg/mL.[24] The plant exhibited poor antioxidant activity if the AAI was less than 0.05, moderate if it ranged between 0.05 and 1, strong if it was between 1 and 2, and very strong if it was higher than 2.

Total antioxidant capacity (TAC) via phosphomolybdenium method

Overall, 500 µL of plant extract (500 µg/mL) or standard solution of AA (20–350 µg/mL) was mixed with 5 mL of freshly prepared reagent composed of 0.6 M sulfuric acid, 28 mM disodium hydrogen phosphate, and 4 mM ammonium molybdate. The tubes containing the reaction mixtures were covered and incubated in a 95°C water bath for 1.5 h. After the incubation period, the tubes were allowed to cool at room temperature; then, the intensity of the developed color was measured at 695 nm against blank.[25] The TAC was expressed as the average of the three analyses in milligrams of ascorbic acid equivalents (AAE)/g dry extract.

Hydroxyl radical scavenging activity

According to the method reported by Geng *et al*.,[26] 500 µL of different plant samples or standard ascorbic acid (200 µg/mL) was mixed with 500 µL $FeSO_4.7H_2O$ (9.0 mM) and 500 $\mu L H_2O_2$ (9.0 mM), respectively. The reaction mixtures were then incubated at room temperature for 10min. Finally, 500 µL salicylic acid solution (9.0 mM/ MeOH) was added to initiate the reaction. The tubes were then incubated at 37°C for 1h. The absorbance was measured at 510 nm against water blank.

$$^{\bullet}\text{OH Scavenging ability \%} = 1 - \left(\frac{A_{sample} - A_{self} - A_{blank}}{A_{control}} \right) \times 100,$$

where $A_{control}$ is the absorbance of the control that contained all the reagents except the test sample, A_{blank} is the absorbance of the blank group that was devoid of both test sample and salicylic acid, and A_{self} is the absorbance of the self-group that contained all reagents except H_2O_2. The results were expressed as the mean percentage of scavenging potential for the three analyses.

Nitric oxide scavenging potential

At physiological pH, aqueous sodium nitroprusside spontaneously produces NO•, which is a short-lived free radical that rapidly oxidizes into nitrite ions under aerobic conditions. The formed nitrite ions could be estimated by Griess reagent based on their diazotization with sulfanilic acid and subsequent coupling with α-naphthylamine under acidic conditions. The ability of the plant extract to stabilize and form NO• could be assessed by measuring the amount of nitrite ions produced. According to the method described by Kumar and Sudha,[27] 2 mL sodium nitroprusside (10 mM) and 0.5 mL phosphate buffer saline (0.1 M, pH 7.4) were added to 0.5mL of plant extract or AA standard solution (500 µg/mL). The tubes were then incubated at 25°C for 150 min. Then, 0.5 mL of the reaction mixtures was taken and mixed with 1 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid). After 5min, 1 mL of α-naphthylamine (5%, w/v) was added. Finally, the tubes were allowed to stand for 30min at 25°C. The absorbance was measured at 540 nm. Nitric oxide radical scavenging activity

$$\left(\text{NO}^{\bullet}\text{ SA}\right)\% = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100,$$

where $A_{control}$ is the absorbance of the control reaction containing methanol instead of the test sample and A_{sample} is the absorbance of the test sample.

Permanganate reducing antioxidant capacity (PRAC)

According to Eftimová *et al*.,[28] 1 mL of different plant extracts (200 µg/mL) was added to tubes containing 1 mL of freshly prepared potassium permanganate solution (0.35 mM) as well as 500 µL of 2 M H_2SO_4. The absorbance decline was measured directly after the addition of plant extracts at 530 nm against a distilled water blank.

$$\text{PRAC}(\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100$$

where $A_{control}$ is the absorbance of the control solution containing methanol instead of the test sample and A_{sample} is the absorbance of the plant sample.

Cytotoxic activity

Cell line and cell culture

The human liver cancer cell line (HepG2) was obtained from VACSERA (Cairo, Egypt). The cells were maintained and subcultured according to the recommendations of the American Type Culture Collection (ATCC). The cells were kept in RPMI-1640 media (phenol red free) supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, and 10% heat-inactivated fetal bovine serum at 37°C and 5% CO_2.

Cell viability assay

The effect of the different samples on the viability of HepG2 cells was evaluated using the SRB assay for three independent replicates.[29,30] Briefly, 100 µL cell suspensions were cultured in 96-well plates at 5 × 10^3 cells/well and incubated for 24 h in complete media. Overall, 100 µL media containing various concentrations of the tested extracts (0–100 µg/mL) were added to cells for 72 h; then, there was an addition of 150 µL, 10% TCA; and finally, incubation at 4°C for 1 h. Next, 70 µL, 0.4% SRB solution were added and the plates were incubated in the dark for 10 min. The plates were then washed with 1% acetic acid and allowed to air-dry overnight. Finally, 150 µL, 10 mM Tris–HCl were added to dissolve the protein-bound SRB stain. The absorbance was measured at 540 nm via a BMG LABTECH FLUOstar Omega microplate reader (Ortenberg, Germany). Doxorubicin was used as a reference. IC_{50}, which is the concentration of the extract required to decrease the percentage of viable cells to its half, was calculated from concentration–response curves constructed by Sigma Plot software (12.0) using an E-max model equation:

$$\text{cell survival}\,\% = 100 - R \times \left(\frac{1-[D]^m}{\left(K_d^m + [D]^m\right)} \right) + R,$$

where R is the residual unaffected fraction (the resistance fraction), D is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate, and m is a Hill-type coefficient.

Statistical analysis

The statistical analyses were carried out using Microsoft Excel 2010 and SPSS (16) software. The results were expressed as means ± standard deviation (SD) of three independent analyses.

Results and Discussion

Phytochemical screening

The preliminary phytochemical screening of the crude extract and the derived fractions revealed the presence of many different phytochemicals [Table 1]. Phenolics, flavonoids, steroids, and triterpenes represent the most abundant phytoconstituents, especially in the crude plant extract, ethyl acetate, and *n*-butanol-derived fractions. The petroleum ether-derived fraction represented the poorest fraction with phytonutrients.

Quantitative determination of plant phytoconstituents

Total phenolics, flavonoids, and flavonols

The defatted 85% methanol extract of the plant leaves possessed a larger amount of phenolics, flavonoids, and flavonols in comparison with the crude methanolic extract, which may be due to the ability of petroleum ether to extract oily and fatty substances [Table 2].[31] The ethyl acetate derived fraction exhibited the highest phenolic content, followed by the *n*-BuOH derived fraction, then the methylene chloride and the aqueous fractions; however, the petroleum ether fraction

Table 1: Preliminary phytochemical screening of the methanolic extract, defatted methanolic extract of *A. altissima* leaves, and the derived fractions of the crude leaf extract

Phytochemical	Test	MeOH ext.	Defatted MeOH ext.	MeOH extract derived fractions				
				Pet.	CH_2Cl_2	EtOAC	*n*-BuOH	H_2O
Flavonoids	Shinoda T.	+	+	–	+	+++	++	+
	Alkaline T	++	++	–	+	++++	+++	++
Alkaloids	Wagner's T.	–	–	–	–	–	–	–
	Dragendroff's T.	–	–	–	–	–	–	–
	Mayer's T.	–	–	–	–	–	–	–
Tannins	Lead acetate T.	+	+	–	+	+++	++	+
Phenols	Ferric chloride T.	++	++	–	+	++++	+++	+
Phytosterols	Salkowski's T.	++	++	+	+	+++	++	+
Steroids and triterpenes	Libermann Burchard's T.	++	++	+	+	+++	++	+
Diterpenes	Copper acetate T.	+++	+++	+	++	+++	++	+
Cardiac glycosides	Keller Killiani's T.	–	–	–	–	–	–	–
Anthranol glycosides	Brontroger's T.	–	–	–	–	–	–	–
	Modified Brontroger's T.	–	–	–	–	–	–	–

Pet. = petroleum ether fraction; MeOH ext. = methanolic extract; (−) = absence; (+) = little; (++) = moderate; (+++) = abundant

possessed the lowest phenolic content. The same behavior was observed in the total flavonoid and total flavonol contents of the understudied samples.

Much research revealed the antioxidant activity of medicinal plants toward their phenolic content. Phenolics are considered a large diverse group of natural products; they comprise flavonoids and flavonols, which are able to donate electrons or protons to the RONS, stabilizing them and terminating their harmful actions toward the biomolecules without losing their stability.[32]

Determination of *in vitro* antioxidant potential

DPPH radical scavenging activity

The DPPH assay is considered the simplest, the most accurate, and the most widely used *in vitro* antioxidant assay.[33] It is based on the ability of the tested sample to reduce dark purple DPPH$^{\bullet}$ to yellow non-radical hydrazine.[34] The maximum scavenging capacity is due to the ethyl acetate fraction, which was almost equal to that of AA [Table 3]. The *n*-BuOH fraction, the defatted methanol extract, and the crude methanol extract exhibited high DPPH$^{\bullet}$ scavenging potential. The AEAC ranged from 89.57 to 3.39 mg AA/100g FW for the ethyl acetate and the petroleum ether-derived fractions, respectively. According to AAI, the ethyl acetate derived fraction possessed very strong antioxidant activity similar to that of AA. The *n*-BuOH fraction, the defatted methanol extract, and the crude methanol extract had strong antioxidant activity whereas the other samples exhibited moderate activity.

Total antioxidant capacity (TAC)

Phosphomolybdenium method was used to prove that both fat-soluble and water-soluble antioxidants existed in the plant samples through their ability to reduce Mo (VI) to Mo (V) and the subsequent formation of the green phosphate Mo (V) complex.[35] The results in Table 3 showed that the ethyl acetate derived fraction possessed the most powerful antioxidant capacity followed by the *n*-BuOH fraction whereas the petroleum ether fraction showed the least potent antioxidant capacity.

Hydroxyl radical scavenging activity

Among all reactive oxygen species, hydroxyl radical ($^{\bullet}$OH) is considered the most harmful radical due to its high instability; thus, it tends to attack the cellular biomolecules, inducing oxidative stress-related diseases so that the ability to scavenge $^{\bullet}$OH attained high priority in recent research.[33] Hydroxyl radicals could be generated *in vitro* through Fenton's reaction. The $^{\bullet}$OH scavenging activity could be assessed spectrophotometrically by the following hydroxylated products of salicylic acid.[36] The highest $^{\bullet}$OH inhibition percentage

Table 2: Recovery yield, total phenolics, total flavonoids, total flavonols, and flavonoids to phenolics ratio of *A. altissima* leaves crude extract and its derived fractions

Extract/fraction	Yield %	TPC (mg GAE/g ext.)	TFC (mg RE/g ext.)	TFC/TPC (%)	TF_{OL} (mg QE/g ext.)
85% MeOH ext.	23.50	209.79 ± 1.55	169.33 ± 4.36	80.71	58.34 ± 0.14
Defatted MeOH ext.	23.38	232.13 ± 1.30	171.24 ± 4.36	73.77	60.70 ± 0.16
Petroleum fraction	0.121	64.77 ± 1.57	33.14 ± 2.86	51.17	14.45 ± 0.43
CH_2Cl_2 fraction	1.91	198.63 ± 3.11	153.14 ± 2.86	77.10	39.47 ± 0.37
EtOAc fraction	3.18	551.72 ± 1.81	371.24 ± 4.36	67.29	100.47 ± 1.30
n-BuOH fraction	8.10	266.67 ± 2.09	204.57 ± 2.86	76.71	56.12 ± 0.46
H_2O fraction	7.15	149.49 ± 2.18	74.10 ± 3.30	49.57	34.43 ± 0.44

The results are represented as mean of three analyses ± SD.
TPC = total phenolic content, GAE = gallic acid equivalents, TFC = total flavonoid content, RE = rutin equivalents, TF_{OL} = total flavonol content, QE = quercetin equivalents.

Table 3: Antioxidant activity of *A. altissima* leaves crude extract and its derived fractions

Extract/fraction	Antioxidant activity						
	DPPH (SC50 µg/mL)	AAI	AEAC (mg AA/100g FW)	TAC (mg AAE/g ext.)	PRAC (Inhibition %)	NO$^{\bullet}$ SA (max. inhibition %)	$^{\bullet}$OH SA (Inhibition %)
85%MeOH ext.	22.00 ± 0.28	1.61	29.27	226.15 ± 0.78	48.94 ± 0.47	33.06 ± 1.28	66.85 ± 0.12
Def.MeOH ext.	19.98 ± 0.07	1.78	32.23	226.65 ± 0.66	55.26 ± 0.37	37.30 ± 0.40	74.35 ± 0.14
Pet. fraction	189.83 ± 0.66	0.19	3.39	103.03 ± 3.43	11.76 ± 0.54	19.21 ± 0.30	62.10 ± 0.39
CH_2Cl_2 fraction	38.56 ± 0.56	0.92	16.7	184.55 ± 1.44	37.35 ± 0.54	27.65 ± 0.26	63.64 ± 0.15
EtOAc fraction	7.19 ± 0.05	4.94	89.57	369.88 ± 1.51	77.19 ± 0.27	40.65 ± 0.91	95.46 ± 0.14
n-BuOH fraction	18.91 ± 0.09	1.88	34.06	233.32 ± 1.51	57.98 ± 0.53	38.85 ± 0.40	91.35 ± 0.06
H_2O fraction	50.86 ± 0.31	0.7	12.66	177.89 ± 2.86	36.29 ± 0.27	20.81 ± 1.28	63.42 ± 0.15
Ascorbic acid	6.44 ± 0.03	5.51	—			41.65 ± 1.70	76.14 ± 0.17

The results are represented as the mean of three analyses ± SD.
AAI; antioxidant activity index, AEAC; ascorbic acid equivalent antioxidant capacity, TAC; total antioxidant capacity, AAE; ascorbic acid equivalent, PRAC; permanganate reducing antioxidant capacity, NO$^{\bullet}$ SA; nitric oxide scavenging activity, $^{\bullet}$OH SA; hydroxyl radical scavenging activity.

Table 4: Cytotoxic activity of *A. altissima* leaves crude extract and its derived fractions against HepG2 cells

Extract/fraction	Cytotoxic activity	
	IC_{50} (µg/mL)	R fraction (%)
85% MeOH ext.	>100	NA
EtOAc fraction	42.00	9.00
n-BuOH fraction	16.70	NA
Doxorubicin	3.00	3.80

Doxorubicin; a standard anticancer drug

was attained by the ethyl acetate and the *n*-BuOH fractions, which were higher than those of ascorbic acid at the same concentration. The other plant samples exhibited lower scavenging potential.

Nitric oxide scavenging potential

Nitric oxide is a reactive nitrogen species that is produced normally within the human body by phagocytes and endothelial cells; it plays a crucial role in the antimicrobial and anticancer potential, neuronal signaling, and vasodilatation.[37] It also plays a key role in inflammation-related diseases, as it could be further oxidized to peroxynitrite, which could be decomposed to a highly toxic hydroxyl radical.[38] The ethyl acetate fraction exhibited the highest maximum percentage of NO˙ inhibition, which was lower than that of AA [Table 3].

Permanganate reducing antioxidant capacity (PRAC)

PRAC is a simple, rapid, and sensitive spectrophotometric method that is used to estimate the antioxidant activity based on the reduction ability of potassium permanganate.[39] In Table 3, the PRAC ranged from 77.19 ± 0.27% to 11.76 ± 0.54% for the ethyl acetate and the petroleum ether fractions, respectively.

Correlation between antioxidant activity and the plant phytoconstituents

According to our current study, it was found that the plant extract and its derived fractions, especially the ethyl acetate and the *n*- BuOH fractions, are rich with phenolics. A strong positive correlation was found between TPC and AAI, TAC, and PRAC of tested samples where r^2 = 0.98, 0.96, and 0.82, respectively. A moderate correlation was observed between the total phenolics and NO˙, ˙OH scavenging activities, where r^2 = 0.62 and 0.72, respectively; this indicates that the plant antioxidative power mainly depends on its content of phenolics. Such result was in agreement with previously published reports, where the significant antioxidant activity of the plant leaves could be attributed to their content of phenolics and flavonoids.[40-42]

Cytotoxic activity

The ability of the different plant samples to eradicate cultured HepG2 cells was assessed via the SRB assay. The results show that the *n*-butanol fraction exhibited the most potent cytotoxic activity followed by the ethyl acetate fraction and the crude extract [Table 4]. HepG2 cells showed low resistance against the ethyl acetate fraction and doxorubicin. According to the U.S. National Cancer Institute (NCI) and Geran protocol, the extract is considered a potent cytotoxic agent if $IC_{50} \leq 20$ µg/mL, moderately active when 200 µg/mL ≤ $IC_{50} \geq 21$ µg/mL, and weak if 500 µg/mL ≤ $IC_{50} \geq 200$ µg/mL.[43] Based on the previously mentioned criterion, the *n*-butanol fraction exhibited potent cytotoxic potential whereas the other samples possessed moderate antiproliferative activity against the tested cell line.

Conclusion

The present study demonstrated that the ethyl acetate, the *n*-BuOH derived fractions, as well as the crude extract of *A. altissima* possessed potent antioxidant and cytotoxic activity, which may be attributed to their richness with phenolics. These data add valuable information to the existing knowledge on *A. altissima* as well as are useful in the formulation of supplementary food and the pharmaceutical industry. It is recommended to subject the ethyl acetate and the *n*-butanol fractions to further separation and identification of their constituents via advanced chromatographic and spectroscopic techniques.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

RONS: Reactive oxygen and nitrogen species;
HCC: hepatocellular carcinoma;
BHT: butylated hydroxy toluene;
BHA: butylated hydroxy anisole;
TPC: total phenolic content;
TFC: total flavonoid content;
TF_{OL}: total flavonol content;
DPPH˙: 1, 1diphenyl-2-picryl hydrazyl radical;
AAI: antioxidant activity index;
AEAC: ascorbic acid equivalent antioxidant capacity;
TAC: total antioxidant capacity;
AAE: ascorbic acid equivalent;
PRAC: permanganate-reducing antioxidant capacity;
NO˙ SA: nitric oxide scavenging activity;
˙OH SA: hydroxyl radical scavenging activity;
SRB: sulforhodamine-B assay.

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