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Original Article

An *in vitro* Study of the Antioxidant and Antiproliferative Properties of *Artemisia absinthium*- A Potent Medicinal Plant

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ABSTRACT

Background: *Artemisia absinthium* is a valuable medicinal plant which has vast ethnopharmacological significance. Traditionally, it has been used to cure various ailments like fever, jaundice and other gastrointestinal problems in humans of ancient periods. **Objective:** The aim of the present study was to evaluate antioxidant and anti-proliferative properties of this medicinal plant. **Methods**: Total phenolics as well as flavonoid content in different extracts were estimated. Antioxidant activity was evaluated by metal (copper and iron) chelating ability and scavenging of free radicals (DPPH, ABTS, Nitric oxide, Hydroxyl, etc.). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay was done for evaluation of the antiproliferative ability of the extracts. The Fourier Transform Infrared (FTIR) analysis was performed to identify the functional groups present in the metabolites in the different extracts. **Results:** Among all the extracts, Phenolic and flavonoid content was highest in the hydroalcoholic extract. Different extracts showed variability in total antioxidant activity and free radical scavenging activity. Hexane and methanolic extracts were shown to possess significant antiproliferative activity in a concentration-wise manner. FTIR results revealed the presence of amines, carboxylic acids, aldehydes, ethers, hydroxyl groups and amides which indicate the large variability of metabolites present in the extracts apart from phenolics and flavonoids. **Conclusion:** The present study validates that *A. absinthium* is abundant in phytochemicals with natural antioxidants and anticancer activity. It can be a valuable source of anticancer drugs as well as a useful ingredient of pharmaceutical preparations for the treatment of diseases caused by oxidative stress.

Keywords: Free radicals, Peroxidation, Cytotoxic, FTIR, Chelation.

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INTRODUCTION

Reactive free radical species like oxygen and nitrogen under a controlled regulation by the human body mediate certain essential roles, but an overproduction or an unregulated release is manifested in the form of various life threatening disorders like atherosclerosis, inflammatory diseases and cancer.[1,2] There are some evidences that free radicals are associated with particular pathways involved in the pathophysiology of many chronic health disorders including inflammation, hypertension, and cancer, etc.[3] Compounds (natural or synthetic), which have the power to quench or scavenge these free radicals provide a great scope for correcting such oxidative imbalances.[4] Earlier synthetic antioxidants like Butylated Hydroxy Toluene (BHT) and Butylated Hydroxyanisole (BHA) were used for mitigating oxidative stress related pathologies, however, the serious concerns emerging out of their extensive use like toxicity and carcinogenicity necessitated the endeavour to search for better alternatives.[5] Recently, there has been upsurge of curiosity in the natural antioxidants especially of plant origin since they are useful therapeutic agents for free radical induced pathologies and disease prevention.[6] Natural antioxidants apart from being safe are known to be more affordable as well as more efficient than their synthetic counterparts.[7] Medicinal plants have been recognized for their tremendous therapeutic potential owing to the abundance of phytochemicals like phenols and flavonoids which exhibit myriad biological properties.[8] Medicinal plants have a long history of being used to alleviate human diseases and improve health across different times and civilizations. Due to the ever increasing interest in the anti-inflammatory and anticancer properties of the medicinal plants by the pharmaceutical companies and researchers around the globe, the discovery of novel chemical entities from them could potentially emerge as novel leads in the production of drugs for treating these chronic disorders.[9,10] Not only a plethora of drugs are of plant origin but also the whole plant extracts in some cases could be used as promising therapeutic modalities taking into consideration their bioactivity as well as target specificity. There is increasing evidence which suggests that plant-derived products inhibit tumorogenesis and associated inflammatory processes at various stages emphasiz-

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ing the importance of these products in chemotherapy. Cancer has evolved different mechanisms to evade controlled growth regulations and avoid apoptosis. Thus, the usage of cell extracts, which contain a variety of components having different possible intracellular targets, may provide an advantage over using one isolated compound as drugs.[11] *Artemisia absinthium* L. (Wormwood), a member of the family Asteraceae is a bitter herb distributed widely in many regions like Europe, North Africa, parts of Asia, North and South America.[12,13] In India, the plant grows throughout the Kashmir valley and is locally known as "Tethwan".[14] Traditionally, the plant has been of prominent ethnopharmacological significance as the dried leaves and flowering tops are used as antihelminthic, antiseptic, antispasmodic, carminative, sedative, stimulant and tonic. Folk remedies have also documented wormwood extracts as being useful against cold, fever and jaundice.[12] Earlier reports suggested that *A. absinthium* possesses antibacterial and anti-diabetic properties.[15-17] The essential oil from this plant has tremendous medicinal value and its constituents include 1,8-cineole, myrcene, myrtenol, α and β-thujone, trans-sabinyl acetate, β-pinene, camphor, chrysanthenyl acetate, cis-epoxyocimene, sabinene, bornyl acetate, Artemisia ketone, linalool, sesquiterpene lactones, hydrocarbon monoterpenes etc.[18] The aim of the study was to evaluate the plant for its antioxidant and antiproliferative properties using a series of cancer cell lines.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals used in the study were purchased from Hi-Media and Merck (India). Standard chemicals and drugs were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, USA). Cell culture media, RPMI-1640, DMEM, Fetal bovine serum (FBS) and antibiotics from Gibco (Invitrogen, USA). The different solvents used mostly were of analytical grade.

Plant material and extract preparation

The plant material of *A. absinthium* was collected in the month of June 2014 from the open areas of District Pulwama, Jammu & Kashmir, India. The plant was identified and authenticated in the Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir (Voucher Specimen No.2031-KASH). The above ground plant material was shade dried and macerated with a blender. Powdered material (100 g) was soaked in n-hexane for 48 h at room temperature with constant shaking. The extract was filtered through whatman No. 1 filter paper. The marc left was again soaked with n-hexane, and the whole process was repeated twice. The pooled filtrate was then evaporated to dryness under reduced pressure using rotary evaporator. The extract was designated as AAH. Similarly, the procedure was followed with methanol followed by hydro alcoholic solution and extracts were designated as AAM and AAE respectively. The extraction yield was calculated, and the crude extracts were kept at 4°C until used for the experiments.

Phytochemical analysis

Total phenolics (TP) and total flavonoid (TF) contents were measured using the Folin-Ciocalteu assay and aluminium chloride method respectively, as described earlier.[19] TP content was standardized against Gallic acid and expressed as mg g^{-1} of Gallic acid equivalents (GAE). Similarly, TF was expressed as Quercetin equivalents mg g^{-1} (QE) of dry weight.

In vitro antioxidant assays

Phosphomolybdate assay

The total antioxidant capability of the extracts was determined by phosphomolybdenum method taking ascorbic acid as a standard.[20] An aliquot of 0.10 mL of extracts solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in tubes. The tubes were capped and incubated in a water bath at 90 °C for 1 h 30 min. The absorbance of the sample mixtures was measured at λ 695 nm with the aid of UV-Vis spectrophotometer (Shimadzu, Japan) against a blank after the temperature of samples was cooled down. The total antioxidant capacity of the extracts was calculated and expressed as milligrams of ascorbic acid equivalents (mg AAE/g) of dry weight.

Chelating activity of Cu^{2+} ions

The Cu^{2+}-chelating activity of extracts was monitored according to the described method with certain changes.[21,22] Precisely, 60 µL of 20 mM $CuSO_4$ aqueous solution was mixed with hexamine HCl buffer (30 mM; pH 5.3) containing 30 mM KCl and 0.20 mM murexide. After incubation of this mixture at room temperature conditions for 1 min, an addition of 10 µL sample solutions was made to the mixture. Finally, the volume was made to 1.5 mL with methanol. Further, the mixture was vortexed vigorously and incubated at room temperature again for 10 min. Finally, the absorbance of the solutions was measured with a UV-visible spectrophotometer at λ 485 nm and λ 520 nm. The absorbance ratio (A_{485}/A_{520}) indicated the free Cu^{2+} content. Hence, the percentage of cupric chelating effect was calculated by the following formula:

$$\text{Relative chelating effect (\%)} = (A_{485}/A_{520})_{max} - (A_{485}/A_{520})_{sample}/(A_{485}/A_{520})_{max} - (A_{485}/A_{520})_{min} \times 100$$

Where the $(A_{485}/A_{520})_{sample}$ is the absorbance ratio of the mixtures with the sample, $(A_{485}/A_{520})_{max}$ is the maximum absorbance ratio without sample and $(A_{485}/A_{520})_{min}$ is the minimum absorbance ratio of the mixture without $CuSO_4$ aqueous solution and sample in the test.

Chelating activity of Fe^{2+} ions

The extracts were evaluated for their ability to compete with ferrozine for binding iron (II) ions in a free solution as per the method reported.[23] Extracts (20-200 µg/mL) were dissolved in a solution containing 0.1 mL of 2 mM $FeCl_2.4H_2O$. The reaction was triggered by adding 0.2 mL of 5 mM ferrozine, and the reaction mixture was shaken briskly. After that, the solution was left standing at room temperature for 10 min to allow the solution to reach the equilibrium. The absorbance of the solution was then taken at λ 562 nm against the blank prepared in a similar manner with $FeCl_2$ and water. EDTA at the concentration range of 1-25 µg/mL taken as the positive control. The percentage of inhibition of the formation of the ferrozine-Fe^{2+} complex was then calculated using the given equation:

$$\text{Chelating activity (\%)} = (A_{Control}\text{-}A_{Sample}/A_{Control}) \times 100$$

Free radical scavenging assays

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) scavenging assay

The antioxidant activity of plant extracts and the reference compound was assessed by the radical scavenging effect of stable DPPH free radical as described earlier.[20]

2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) scavenging assay

Antioxidant capability of the extracts of *A. absinthium* was measured by 2, 2′-azinobis [3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) assay.[24] An aqueous solution of 7 mM ABTS solution was made and then mixed with a 2.45 mM potassium persulphate solution to yield a radical cation ($ABTS^+$). After that, the reaction mixture was kept in the dark conditions at room temperature for 12–16 h for color development. Freshly prepared ABTS solution was diluted with ethanol to attain an absorbance of 0.700±0.02 at λ 734 nm wavelength. In a 96 well plate, 10 µl of each

extract or standard was added to each well containing 190 µL of $ABTS^+$ solution. The reaction mixture was incubated for 15 min under dark conditions after shaking properly. The absorbance was measured spectrophotometrically at λ 734 nm using microplate reader (InfinintePro, Tecan, Switzerland).

ABTS radical scavenging activity (%) = $[Abs_{control} - Abs_{sample}]/Abs_{control} \times 100$.

Hydroxyl radical scavenging activity

The scavenging effect of *A. absinthium* extracts on hydroxyl radicals was assayed by using the Fenton reaction method.[20] The amount of pink chromogen generated was taken as control and its intensity measured spectrophotometrically at λ 532 nm. The protective effect of the extracts on the oxidation of D-ribose has been conducted by pre-incubation with the *A. absinthium* extracts in increasing concentrations and decrease in the pink color reflected the antioxidant ability of these extracts which was compared to the standard ascorbic acid.

Nitric oxide scavenging activity

The nitric oxide scavenging activity was studied by using Griess reagent method as described earlier. [19] The percentage inhibition of nitric oxide generation was measured by comparison of the absorbance values of control and samples, and accordingly, IC_{50} values were calculated.

Lipid peroxidation inhibition activity

The lipid peroxidation inhibition effect of extracts *in vitro* was determined in goat liver homogenate purchased from local market by TBARS method with minor modifications.[25] Goat liver tissue (2.0 g) was sliced into pieces and homogenized with 10 mM KCl–Tris-HCl buffer (pH 7.4) followed by centrifugation of homogenate at 800 *g* for 15 min at 4 °C. The extracts at different concentrations (40–400 µg/mL) were mixed with this liver supernatant and the mixtures were incubated in absence and presence of Fenton's reagent (50 µL of 10 mM $FeCl_3$; 10 µL of 2.5 mM H_2O_2) in phosphate buffer (0.2 M, pH 7.4) and the total volume was adjusted to 1 mL. After an incubation period of 37 °C for 30 min, 2 mL of icecold HCl (0.25 N) containing 15% trichloroacetic acid, 0.5% thiobarbituric acid, and 0.5% butylated hydroxytoluene (BHT) was added to the tubes containing the reaction mixture. Subsequently, the mixtures were heated at 100 °C for about 10 min. The reaction mixture was cooled in an ice bath for 5 min. The mixture was centrifuged at 1000 *g* for 10 min and the degree of lipid peroxidation was observed by the formation pink chromogen spectrophotometrically at λ 532 nm. A decrease in the formation of pink color in the treated reactions was suggestive of the inhibition of lipid peroxidation.

Evaluation of antiproliferative activity

Cell culture

The cell lines HeLa, HepG2, C6, K562, MCF-7 and THP-1 were obtained from National Centre for Cell Science (NCCS), Pune, India These cell lines were cultured in RPMI 1640 culture medium and DMEM, supplemented with 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 µg/mL). The culturing of the cells was performed at 37 °C in a humidified 5% CO_2 incubator.

Antiproliferative assay

The extracts of *A. absinthium* were tested for cytotoxicity test *in vitro* using cancer cell lines of varied origin by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[20] Briefly, cell suspensions (200 µL) at optimized concentrations of 1×10^5 cells/mL were dispensed in triplicate in 96-well culture plates. After incubation at 37 °C for 24 h, the culture medium was removed from the wells, and 200 µL fresh medium containing the extracts at increasing concentration (5, 25,50, 100, 200,250 µg/mL) was added to each well and further incubated for another 48 h. At the end of the incubation period, the medium in each well was taken out carefully and replaced with fresh media along with 20 µL of 5 mg/mL MTT working solution (made in PBS). The cells were incubated at 37 °C for 4 h, and then the medium was removed and replaced with 100 µL Dimethyl Sulfoxide (DMSO) to dissolve the formazan crystals formed. The absorbance of each well was measured at λ 540 nm in a microplate reader after shaking for 1 min, and the percentage inhibition was calculated manually using the formula:

% Inhibition = [1−(OD in sample well/OD in control well) × 100].

FTIR spectral analysis

The FTIR spectra of the dried lyophilized extracts were read at room temperature conditions using attenuated total reflectance (ATR) and internal reflection element composed of diamond using ATR instrument (Bruker, Germany). The spectral region ranged from 4000-500 cm^{-1} at a resolution 4 cm^{-1}. The ATR crystal was cleaned properly between the measurements using alcohol and acetone.

Calculations and statistical analysis

The results were expressed as mean ± standard deviation (SD) values average from independent experiments performed in triplicate. The statistical calculations and analysis were performed using GraphPad Prism Version 6. Statistical differences between the samples were evaluated using appropriate statistical tests (one-way ANOVA, Turkey's and Students-t-tests). A *p*-value of ≤ 0.05 was considered significant.

RESULT

Phytochemical analysis

The total phenolics content in different extracts varied from 0.43±0.07 to 9.29±0.51 mg of Gallic acid equivalents per gram dry weight. AAE extract showed maximum and AAH showed the minimum phenolic content among the selected extracts. Another part of this study showed that the total flavonoid contents in the extracts also varied widely between 3.02±0.05 to 19.08±0.12 mg of Quercetin equivalents per gram dry weight. The highest flavonoid content was found in AAE (19.08±0.12 mg QE/g), although the lowest flavonoid content was observed in AAH (3.02±0.30) (Table 1).

In vitro antioxidant assays

Phosphomolybdate assay

The total antioxidant potential of the different extracts of *A. absinthium* was presented as ascorbic acid equivalents (AAE) per gram of dry weight plant material. The investigated extracts significantly exhibited a reduction of Mo (VI) to Mo (V). The maximum total antioxidant capacity was observed for AAE with a value of 3.57±0.17 mg AAE/g dw. The AAH showed least antioxidant capacity (2.42±0.04) (Table 1).

Chelating activity of Cu^{2+} ions

The Cu^{2+} ions chelating ability of the extracts and the sodium citrate (SC) followed a concentration-dependent pattern from 40-400 µg/mL as presented in the Figure 1A. Although a Cu^{2+} chelating activity of the positive control was higher than the extracts, AAE and AAM also chelated 68.37±0.62% and 63.37±3.08 of Cu^{2+} ions respectively at 400 µg/mL. AAE, however, displayed highest Cu^{2+} ion chelation at 320 µg/ml after that there was saturation of the activity. AAH possessed the least cu^{2+} chelation potential and chelated only 24.04±0.98% of ions at 400 µg/mL. (Figure 1A, Table 2).

Table 1: Phyto-constituents and antioxidant power of *A. absinthium* extracts

Name of extract	Phenolic Content (mg GAE/g dw)	Flavonoid Content (mg QE/g dw)	Total antioxidant activity (mg AAE/g dw)
AAH	$0.43\pm 0.07_{a}$	3.02±0.09	2.42±0.04
AAM	$3.55\pm0.39_{b}$	6.40±0.0.39	$3.16\pm0.02_{a}$
AAE	$9.29\pm0.51_{a}$	$19.08\pm0.12_{a}$	$3.57\pm0.17_{a}$

Each value in the table is represented as mean ± SD (n = 3). a- Highest value and significantly different (p<0.05) from other values (ab and b) of each row; Ab- significantly different (p<0.05) from b only.

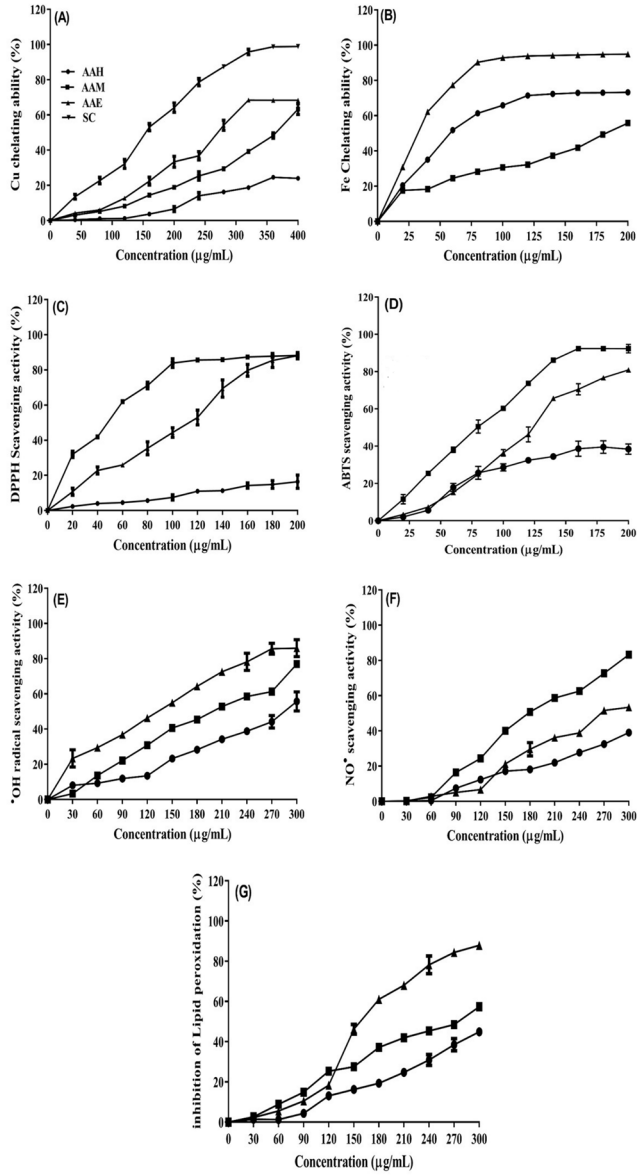

Figure 1: Antioxidant activities of different extracts of *A. absinthium* by seven different methods. (A): Chelation of Cu^{2+} ions (B): Chelation of Fe^{2+} ions(C): DDPH radical scavenging (D) ABTS radical Scavenging (E): Hydroxyl radical Scavenging (F): Nitric oxide radical scavenging (G) Lipid peroxidation inhibition.

Table 2: IC_{50} (µg/mL) of *A. absinthium* extracts on tested metal ions and free radicals

Scavenging/ Chelating assay	Type of Extract			
	AAH	AAM	AAE	Reference Compound
DPPH·	612.33±7.33	112.53±2.41	47.72±1.21	3.61+0.07#
ABTS·	261.08±9.47	80.65±3.72	130.23±10.75	3.33±0.08#
·OH	335.51±3.91	270.92±8.09	154.90±7.56	56.90±2.89#
NO·	384.30±5.39	177.26±1.72	261.56±7.39	15.62±0.01#
LPO	333.48±6.89	278.06±8.60	162.30±5.09	62.46±11.02#
Fe^{2+}	57.87±1.07	182.56±7.45	32.13±1.50	5.04±0.08≠
Cu^{2+}	843.30±21.79	373.95±9.72	260.52±5.80	208.09±17.09†

Each value in the table is represented as mean ± SD (n = 3) and expressed in µg/ml of extract. #Ascorbic *acid;* ≠EDTA; † Sodium citrate

Chelating activity of Fe^{2+} ions

The Fe^{2+} ion chelating activities of AAH, AAM, and AAE are shown in Figure 1B. EDTA was used positive metal chelator in this assay and inhibited 95.66±3.92% of Fe^{2+} ions at the highest concentration of 25 µg/mL. As depicted, the extracts also hindered the development of ferrous and ferrozine complex indicating that they have metal chelating activity and are also able to chelate ferrous ions before ferrozine in a concentration wise pattern. The Ferrous ion binding effect of the samples decreased in the order of AAE>AAH>AAM (Figure 1B, Table 2).

Free radical scavenging assays

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) scavenging assay

In this assay, AAE showed the most powerful free radical scavenging activity exhibiting an IC_{50} value of 47.72±1.21 µg/mL while the AAH was least potent among the extracts on scavenging of DPPH free radicals (Figure 1C; Table 2).

2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) scavenging assay

The scavenging of ABTS cations generated by the reaction of ABTS and potassium persulphate provides an indication of hydrogen/electron donating or reducing capabilities of the extracts. There are prominent differences in the scavenging of ABTS radicals among the extracts as can be seen in Figure 1D. As shown in Table 2, the IC_{50} values are in the order of AAM<AAE<AAH. AAH is the least potent scavenger among the extracts whereas AAM showed an inhibition of 92.32±2.31% at the highest concentration of 200 µg/mL. However, there is a saturation of inhibition potential before this concentration, since the maximum inhibition of 92.41±1.42% was observed at a lower concentration of 160 µg/mL.

Hydroxyl radical scavenging activity

OH, scavenging activity is directly related to the antioxidant activity of any extract. In the case of all the extracts, there was a concentration-dependent increment in scavenging of •OH radicals from 30-300 µg/ mL. AAE with the highest •OH radical scavenging ability, exhibiting minimal IC_{50} values of 129.42±2.90 µg/ mL scavenged 85.96±4.79% at the maximal concentration used. AAH was found to be least potent radical scavenger among the counterparts and inhibited only 55.73±5.45% of •OH radicals at this concentration. The IC_{50} values decreased in the order AAH>AAM>AAE (Figure 1E, Table 2).

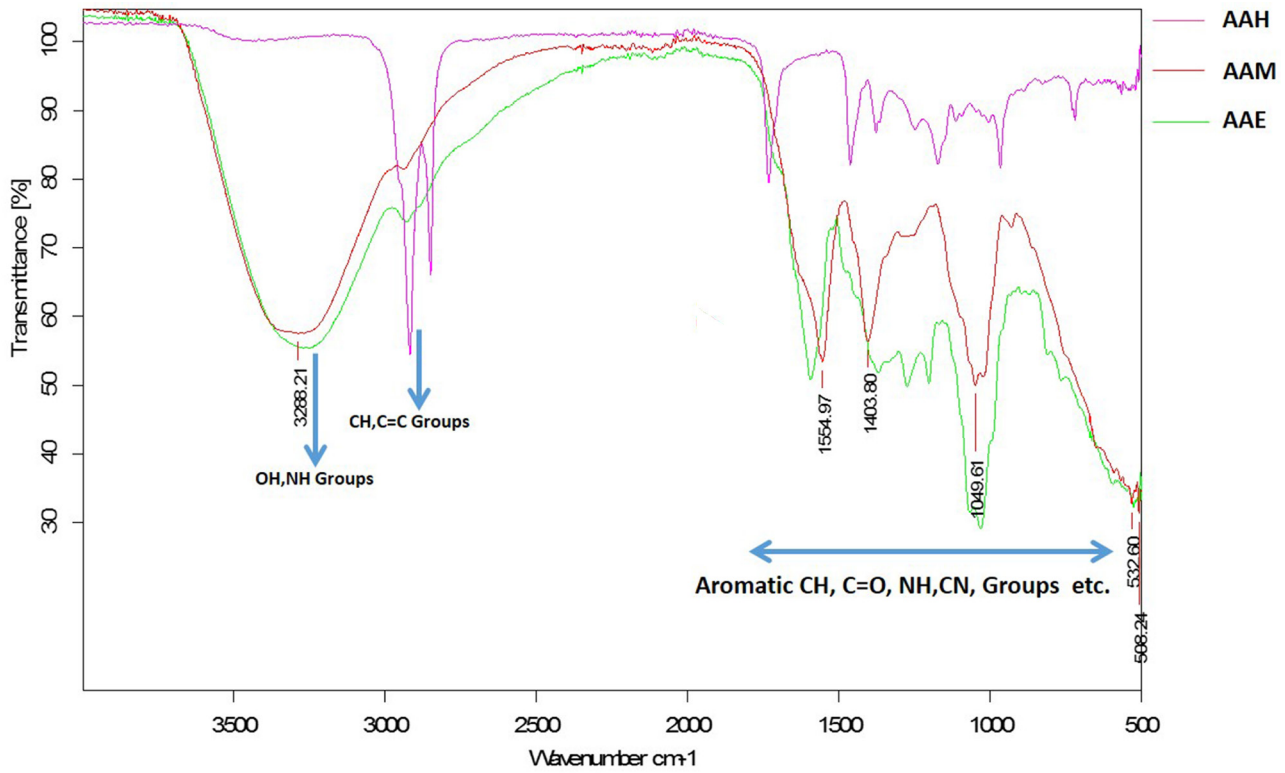

Figure 2: FTIR spectra of different extracts of *Artemisia absinthium*. Lyophilised extracts were put on diamond crystal pedestal of the ATR and readings were taken from 4000-500 cm^{-1}.

Nitric oxide scavenging activity

The extracts displayed significant concentration wise NO• radical scavenging effect generated by Griess reagent at the highest concentration of 300 µg/ mL. AAM exhibited the highest scavenging potential and inhibited 83.24±0.33% of NO• radicals. The inhibition power was followed by AAE and AAH which showed an inhibition of 53.41±0.77% and 39.04±0.54% when concentration reached to 300 µg/ mL. The corresponding IC_{50} values follow the order of AAM<AAE<AAH (Figure 1F, Table 2).

Lipid peroxidation inhibition activity

To examine whether the extracts of *A. absinthium* were capable of mitigating oxidative stress *in vitro*, lipid peroxidation assay that results in the production of malondialdehyde and many other related compounds in the liver homogenates was carried out. Thiobarbituric acid reactive substances (TBARS) are produced as by-products of lipid peroxidation. Our results indicated that the extracts were able to quench the extent of lipid peroxidation in liver homogenates. The lipid peroxidation was inhibited proficiently by AAE with 87.96±0.12% at the highest concentration of 300 µg/mL. In contrast, AAH inhibited only 44.93±1.02% of lipid peroxidation at the highest concentration used and thus was a least efficient inhibitor. The IC_{50} values for inhibition of lipid peroxidation follow the order as AAE <AAM<AAH (Figure1G, Table 2).

Anti-proliferative assay

A specificity of anti-proliferative action was observed in the extracts on cell type. It was observed that the anti-proliferative activity was more pronounced by AAH and AAM extracts in four cell lines among all the six cell lines used in this study. However, the growth of He La and HepG2 cells were least affected by treatments of any of the extracts. The highest decline in cells viability reached by 250 µg/ mL of the extracts after an incubation of 48 h when compared to the control group of untreated cells. Based on the IC_{50}values, the results in Table 3 showed that extracts had low activity against HeLa and HepG2 cells in comparison to their activity against the other cancer cells suggesting that these extracts are less toxic against HeLa and HepG2 cells (Table 3).

FTIR spectral analysis

The FTIR analysis was done to identify the functional groups present in the extracts of *A. absinthium*. These studies revealed the existence of various functional groups in the whole plant extracts. Various peaks which indicated different functional groups present in the metabolites of the extracts are depicted in Figure 2. It is pertinent to note that AAM and AAE exhibited a similar IR profile probably due to the polar nature of molecules present in them.

DISCUSSION

There is no doubt that plants have been consumed as medicine through ages and even continue to provide a vast array of drugs and formulations to counter certain human ailments and diseases even today. According to the estimates of WHO, around 80% of the world population mostly comprising of rural folk in developing countries depend upon herbal health care system in one way or the other. In the developed countries also, there has been a recent upsurge in the mining of bioactive compounds from natural sources like medicinal herbs barring the side effects and toxicity of the chemotherapeutic drugs.[26] The study of any plant useful in traditional and folk system for any bioactivity becomes necessary for two valid reasons; (i) that plant based formulations and crude extracts have also gone a long way in improving human health in a plethora of cases, (ii) the purified compounds obtained from a medicinal plants have paved the way for new lead structures to formulate novel and readily available drugs. Medicinal plants have a tremendous potential to

Table 3: *Artemisia absinthium* extracts exhibiting *in vitro* cytotoxicity in six selected cancer cell lines. Values expressed in µg/ml of extract or positive control.

Cell Line	Extract type	IC_{50} (µg/mL)	% inhibition at (250 µg/mL)
THP1	AAH	215.33±4.75	58.05±2.78
	AAM	45.64±1.09	90.77±2.85
	AAE	299.04±11.93	41.80±1.32
	Doxorubicin	1.47±0.05	92.78±3.87
C6	AAH	67.74±2.89	96.30±0.53
	AAM	36.25±3.95	97.41±0.12
	AAE	198.29±8.42	70.24±0.34
	Doxorubicin	1.02±0.03	85.04±2.13
K562	AAH	43.10±2.03	96.06±0.34
	AAM	44.31±1.99	95.98±1.22
	AAE	201.69±7.09	54.67±2.89
	Curcumin	15.78±1.04	96.79±4.10
MCF-7	AAH	54.98±6.80	89.08±0.58
	AAM	53.44±5.65	93.99±0.31
	AAE	259.98±12.71	48.08±1.92
	Doxorubicin	0.89±0.05	90.43±2.69
HepG2	AAH	943.65±11.20	13.53±0.16
	AAM	615.90±12.90	20.38±3.37
	AAE	1639.34±16.91	7.37±0.64
	Doxorubicin	0.78±0.06	89.78±3.08
HeLa	AAH	307.71±11.65	40.33±1.88
	AAM	191.38±3.70	54.24±1. 35
	AAE	722.52±11.06	17.78±1.11
	Doxorubicin	1.98±0.25	88.09±3.09

Significant p value ($p<0.05$) was obtained by Student's t test analysis. Composite treatments were compared using one-way analysis of variances (ANOVA) and probability values were found to be equal to or less than 0.05 for all the six cell lines.

diminish or entirely inhibit the disturbances caused by the free radical onslaught. There are various reports of preliminary screening of different medicinal herbs for evaluation of their antioxidant potential, and it was concluded that some specific extracts could be used in hindering the free radical chain initiation and progression.[27] Moreover, the bioactivity guided fractionation and isolation of pure compounds from the bioactive crude extracts is one of the integrated approaches for novel drug discovery. Thus an initial screening for bioactivity of any medicinal plant becomes indispensable. We aimed to study *A. absinthium*, an ethano-pharmacologically important medicinal herb, for its antioxidant and cytotoxic properties against different cancer cell lines.

In this study, the quantitative estimation proved that the polar extracts of methanol and hydro-alcoholic are rich in phenolic and flavonoid content. Recent investigations have demonstrated that polyphenols and flavonoids contribute in a great way to the antioxidant activity of medicinal herbs.[28] The complex nature of phytochemicals necessitates a multiple assay approach for evaluating the antioxidant activity of the extracts and that is what we have followed in this study. DPPH, ABTS, $NO^{\bullet}$, $^{\bullet}OH$, and lipid peroxidation radicals were scavenged by the extracts in order of the increasing polarity. The differential scavenging activities observed by different extracts against various systems may be because of the unique and varied mechanisms of the free radical antioxidant reactions taking place in the different assays.[20] However, these effects could also be attributed to the intrinsic reducing capabilities of extracts using delocalization of the single electron of the radical and metal ion chelation activity.[29,30] Therefore, the results obtained from this *in vitro* study suggested that the extracts of *A. absinthium* might reduce oxidative damage in the human body and provide health protection.

Plants contain almost large capacity to generate compounds that fascinate researchers throughout the globe in the pursuit for new and novel chemotherapeutics.[31] The consistent search for new anticancer compounds in plant medicines is a rational and promising approach to its prevention.[32,33] Tremendous advancement has been made in cancer chemotherapy, a substantial portion of which may surely be accredited to plant-derived drugs.[34,35] Although many compounds isolated from plants are being tested across the world for their anticancer properties, the beneficial effects of plant extracts as a complex cocktail of the composite mixture of compounds cannot be at all ignored. Keeping this fact in view, we undertook the evaluation of extracts of *A. absinthium in vitro* for anti-proliferative properties. Cell-specific antiproliferative activity was noticed in plant extracts. The results obtained by MTT assay showed hexane and methanolic extracts have inhibitory effects on the growth of cancer cell panel in a concentration-dependent manner. However, they were diverse in the pattern of inhibition levels on different cells shown by these extracts and the inhibition levels of each extract were significantly different ($p<0.05$). It is known that different cell lines exhibit different sensitivity towards an anticancer compound(s), so the use of a series of cell lines were, therefore, considered compulsory in the evaluation of anti-cancer

effects. This action specificity of plant extracts is probably due to the presence of varied classes of compounds in the extracts.[36,37] Cytotoxic actions of a drug is believed to be provided by upsetting the fundamental mechanisms associated with cell growth, mitosis, cell differentiation, development, and function.[38] The cytotoxic activity attributed to these extracts may be due to one of these fundamental mechanisms.

FTIR spectroscopy is an advanced and widely used technique that could be utilized for analysis of pharmacologically active compounds of natural origin. The FTIR spectra of compounds is unique as it emerges from molecular properties and acts as a chemical fingerprint.[39] The more intense and bands present at different frequencies are indicative of the presence of N–H/N-H, C–H, aldehyde and ketonic skeletal vibrations which denote the presence of various classes of metabolites in any plant material. However, results obtained from FTIR spectra alone are not enough to prove the existence of compound classes particularly in complex plant extract mixtures.[40]

CONCLUSION

A. absinthium extracts showed tremendous antioxidant and free radical scavenging properties *in vitro*. Furthermore, the results indicated the extracts are promisingly cytotoxic and they might possess antitumor activity against different malignancies. Overall the findings suggested that *A. absinthium* extracts possessed potent pharmacologically active compounds, which if appropriately and systematically studied, could prove to be chemically remarkable and novel active drug candidates in the future, including some with potential antiproliferative properties. However, additional studies need to be undertaken to characterize the bioactive molecules and to carry out the *in vivo* pharmacological experimentation of this plant.

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CONFLICT OF INTEREST

The authors have declared that they have no conflict of interest.

ABBREVIATION USED

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; **BHA:** Butylated Hydroxyanisole; **BHT:** Butylated Hydroxy Toluene; **DMEM:** Dulbecco's Modified Eagle's Medium; **DMSO:** Dimethyl Sulfoxide; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **FBS:** Fetal Bovine Serum; **FTIR:** Fourier transform infrared spectroscopy; **IC_{50}:** Half maximal inhibitory concentration; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **RPMI:** Roswell Park Memorial Institute; **TBARS:** Thiobarbituric acid reactive substances.

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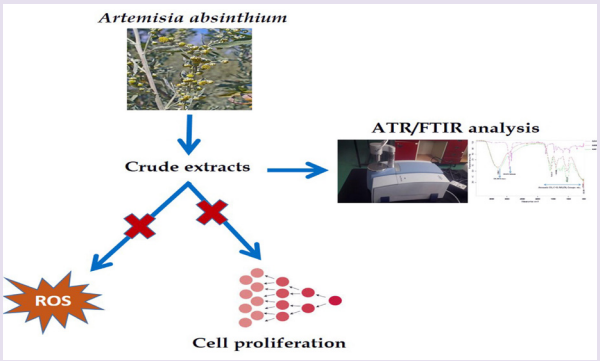
GRAPHICAL ABSTRACT



SUMMARY

- The present study quantified the phenolic and flavonoid content in different extracts of *Artemisia absinthium*.
- The extracts possess tremendous ability to scavenge toxic free radicals *in vitro* and exhibit prominent metal chelating ability.
- The extracts also show antiproliferative ability towards six cancer cell lines.
- This study encourages further research with this plant for finding novel molecular entities against oxidative stress related ailments and cancer.

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