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Phytochemical profiling and evaluation of antioxidant potential of *Achyranthes aspera* (prickly chaff flower) seed extract

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ABSTRACT

The present study investigated the phytochemical profile and antioxidant potential of *Achyranthes aspera* (prickly chaff flower) seed extract.Freshly mature dried seeds were collected from around Bilaspur, Chhattisgarh, India, and an ethanolic extract was prepared using Soxhlet extraction.Qualitative phytochemical screening revealed the presence of multiple compounds, including alkaloids, carbohydrates, proteins, amino acids, glycosides, steroids, saponins, tannins, flavonoids, and triterpenoids. Antioxidant capacity was examined using three distinct radical scavenging assays: DPPH, ABTS, and H_2O_2. The ethanolic extract demonstrated potent antioxidant effects, with IC_{50} values of 26.586, 28.2641, and 15.7931 µg/mL.Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified key bioactive compounds, such as n-hexadecanoic acid, oleic acid, and saponins, contributing to the plant's antioxidant, antimicrobial, anti-inflammatory, and anti-diabetic properties.High-Performance Liquid Chromatography (HPLC) was used to quantify the presence of triterpenoid compounds in the extract.These findings suggest that *A. aspera* seed extract is a rich source of bioactive compounds with significant antioxidant potential, supporting its traditional use in herbal medicine, and indicating its potential for pharmaceutical development.

Figures : 07 References : 33 Table : 05
KEY WORDS : *Achyranthes aspera,* Antioxidant, GC-HR-MS, HPLC, Phytochemical

Introduction

Achyranthes aspera (*A. aspera*), commonly known as prickly chaff flower, Apamarga, Latjeera (in Hindi), and Nayuruvi (in Tamil), is a herbaceous plant widely recognized for its medicinal properties.This plant commonly thrives as a weed on roadsides, on field borders, and in neglected areas.*A. aspera* seeds are a cornerstone of traditional medicine systems, such as Ayurveda, Unani, and Siddha.Known for its anti-inflammatory and analgesic effects. Studies have

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TABLE-1 : Qualitative analysis of *A. aspera* seed extract

S. No	Phytochemical analysis	Result
1	**Alkaloids** Mayer's test Drangondroff test	++
2	**Protein and Amino acids** Ninhydrin test Millon's test	++
3	**Carbohydrates** Felling's test Benedict's test	++
4	**Flavonoids** Alkaline reagent test $FeCl_3$ test	++
5	**Glycosides** Boruetrager's test Keller-Kilani test	--
6	**Phenolic compounds and tannins** Lead Acetate test $FeCl_3$ test	++
7	**Phytosterol** Liberman Burchard's test Salkowskis test	--
8	**Saponins** Foam test Forth test	++
9	**Steroids** Libermann's test Salkowskis test	++
10	**Terpenoids** Libermann's test Salkowskis test	++

demonstrated its efficacy in reducing inflammation and alleviating pain, making it particularly beneficial in conditions like arthritis[18].Different parts of *A. aspera* exhibit significant antimicrobial properties owing to their bioactive compounds, which are effective against bacteria, fungi, and viruses.Traditionally, they have been used to treat skin infections and wounds[23].Moreover, *A. aspera* serves as a natural diuretic that stimulates urine formation and contributes to the alleviation of urinary issues, renal calculi, and fluid retention.[15].In traditional practice, *A. aspera* plants are commonly used to stimulate appetite, support digestion, and relieve constipation.The qualitative analysis of *A. aspera* indicated the presence of various secondary metabolites[31].

Many studies have emphasized that *A. aspera* extract helps regulate blood sugar levels, making it valuable in diabetes management[2].Multiple phytochemicals have potential therapeutic or physiological effects on the human body, making the leaves and stems of *A. aspera* a promising source of essential drugs[27].Most preliminary studies of *A. aspera* have shown its potential to inhibit the growth of certain cancer cells[30].*A. aspera* has been documented to exhibit cancer chemopreventive activity and possess antitumor properties[9].The non-alkaloid fractions of plants have been identified as significant antitumor-promoting agents[17].Moreover, its extracts have shown potential in improving heart function and circulation, with mild hypotensive effects that may benefit cardiovascular health[28]. Lastly, *A. aspera* is traditionally regarded as a general tonic for boosting immunity and enhancing resistance to infections[13].

In recent years, *A. aspera* has garnered significant attention because of its wide range of medicinal properties. It has the potential to treat cardiovascular diseases[33], wound-healing[11],antioxidants, hemolytic[20],anti-inflammatories[19], antibacterial[3],and antifungal activities[12].Advanced analytical techniques, such as GC-HR-MS and HPLC, have been employed to profile the phytochemical constituents of *A. aspera* seed extracts, revealing the presence of phenolic compounds, flavonoids, alkaloids, steroids, and terpenoids.The antioxidant properties and potential to suppress crucial enzymes implicated in Alzheimer's disease, type 2 diabetes, and skin hyperpigmentation disorders are characteristic of these compounds.[29].Moreover, *A. aspera* exhibited potent antimicrobial properties, demonstrating its effectiveness against various bacterial and fungal pathogens. Its extracts have also shown promise in combating multidrug-resistant bacteria[1].*A. aspera* seed holds numerous promising natural compounds for obesity treatment that remain largely investigated, presenting a potentially effective and safe alternative for developing anti-obesity drugs[6].The present study aimed to further investigate the baseline effects of *A. aspera* seed extract through qualitative and quantitative phytochemical profiling and antioxidant

TABLE-2 : DPPH radical scavenging activity

S. No	Sample (µL)	Concentration of Sample (µg/mL)	DPPH reagent (ML)	Incubation time	Absorbance at 517 nm	% of Inhibition	IC 50 value
1	10	100	3	Incubate	0.140	68.87	
2	20	200	3	in the	0.235	47.17	
3	30	300	3	dark	0.328	28.61	26.586
4	40	400	3	chamber for	0.438	4.60	
5	50	500	3	15 min	0.421	4.60	

analysis for use as therapeutic targets for treating obesity and diabetes mellitus in animal models.

Materials and Methods

Collection of plant material : Freshly mature dried *A. aspera* seeds were collected from the Bilaspur region of Chhattisgarh, India,and identified with the assistance of Flora's Presidency of Madras.The plants and seeds were deposited for verification and authentication in the Department of Botany, School of Life Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India.(Fig.1).The department retained a voucher specimen(No. Bot/GGV/2022/05).

Preparation of plant extract : To increase the extraction efficiency, *A. aspera* seeds were initially dehydrated and pulverized into a fine powder to maximize the available surface area.Powdered seeds (100 g) were placed into the thimble of a Soxhlet extractor.The Soxhlet apparatus was then assembled and 90% ethanol was used as the solvent.Ethanol was added to the round-bottom flask to ensure that the solvent level was below that of the siphon tube.The system was heated, allowing ethanol to vaporize, condense, and wash the plant material in the extractor.This process was repeated for 72 h, during which the ethanol continuously extracted the compounds from the seed powder.After the extraction procedure, the solvent was removed by evaporation using a rotary evaporator operating under reduced pressure.The percentage yield of the brown chocolate extract was 7.5%.A final crude extract was obtained and processed for further analysis(Fig. 2).

Qualitative analysis : Qualitative phytochemical screening involves a series of tests to identify bioactive compounds in the ethanolic extract of *A. aspera* (EEAA) seeds.Ethanol was used to dissolve the extract, and the resulting solution was filtered to eliminate insoluble particles.The samples were analyzed using a standard protocol to detect various phytochemical constituents, including alkaloids, carbohydrates, proteins, amino acids, glycosides, phytosterols, steroids, triterpenoids, flavonoids, saponins, and tannins.

Antioxidant analysis

DPPH radical scavenging activity : The DPPH radical scavenging assay is a commonly employed technique for assessing the antioxidant potential of various substances and plant extracts. This method was used to evaluate the antioxidant properties of samples. The procedure involved preparing a DPPH stock solution by combining 0.004 g DPPH with 100 mL ethanol. The assay was performed by mixing 3 mL of DPPH solution with an equal volume of the test sample or standard at different concentrations. The resulting mixture was left to react in the dark at ambient temperature for 30 min. The absorbance was recorded at 517 nm using a UV-Vis spectrophotometer, with ethanol as the blank. Ascorbic acid was used as the positive control. The DPPH radical scavenging activity was determined using a specific formula.

$$\% \text{ Inhibition} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

Ascorbic acid (AA) was used as a positive control. The results are reported as IC50 values, denoting the

TABLE-3 : ABTS radical scavenging activity

S. No	Sample (µL)	Concentration of Sample (µg/mL)	ABTS reagent (ML)	Incubation time	Absorbance at 734 nm	% of Inhibition	IC 50 value
1	20	20	3	Incubate	0.391	14.40	
2	40	40	3	in the	0.383	9.83	
3	60	60	3	dark	0.317	32.21	28.2641
4	80	80	3	chamber for	0.423	39.20	
5	100	100	3	15 min	0.345	32.20	

sample concentration needed to neutralize half of the DPPH radicals. This method provides a reliable and simple technique for assessing the antioxidant potential of a given sample[7].

ABTS radical scavenging activity : Evaluation of ABTS radical scavenging activity typically involves measuring the effectiveness of a substance to neutralize the ABTS + radical cation. This cation is generated when ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) reacts with a potent oxidizing agent, commonly potassium persulfate. The process began with creating an ABTS + solution and introducing the sample or antioxidant. The interaction between the sample and ABTS + radical was observed by monitoring the decrease in absorbance at a specific wavelength, usually 734 nm. A reduction in absorbance signified a scavenging effect, indicating that the ABTS + radical cation was neutralized. The extent of inhibition was quantified using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Control Absorbance - Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

Quantification of scavenging activity typically involves comparing the ability of the sample to neutralize the ABTS + radical against a standard antioxidant, such as vitamin C or Trolox.This comparison facilitated the calculation of the IC50 value or percentage of radical scavenging activity[22].

H_2O_2 radical scavenging activity : Hydrogen peroxide (H_2O_2) radical scavenging assay is a prevalent technique employed to evaluate the antioxidant capabilities of various compounds or extracts.This technique generally involves combining a H_2O_2 solution (typically 40 mM) in a phosphate buffer (pH 7.4) with varying concentrations of the test sample. The mixture was allowed to react for 10–30 minutes at room temperature. Following incubation, the remaining H_2O_2 was quantified using chromogenic agents, such as potassium titanium oxalate or a mixture of phenol red and horseradish peroxidase. Spectrophotometric measurements were performed at 504 nm to determine the absorbance, and a specific formula was employed to calculate the percentage of H_2O_2 scavenged by the sample.

$$\% \text{ Inhibition} = \frac{\text{Control Absorbance - Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

The absorbance of the control (lacking sample) and test samples was evaluated. This technique relies on the capacity to counteract H_2O_2, thus preventing oxidative damage[24]. Nonetheless, it is essential to incorporate suitable controls and duplicate measurements to ensure precision.

GC-HR-MS analysis : The phytochemical constituents present in the *A. aspera* seed extract were identified by gas chromatography-high-resolution-mass-spectrometry (GC-HR-MS) using the facility from SAIF at IIT Bombay, Mumbai, India. The GC-MS analysis involves several key steps: Initially, *A. aspera* extract samples were prepared by dissolving them in suitable ethanol solvent, typically requiring 5-10 mg for solid samples or 0.2-1 ml for liquid samples. A 1 µL portion of the prepared sample was introduced into the GC-MS apparatus, consisting of an Agilent 7890 B gas chromatograph linked to a 5977A mass selective detector (MSD) spectrometer. The gas chromatograph featured a capillary column (HP-5 MS UI) and used helium as the carrier gas at a flow rate of 1 ml/min. The

TABLE-4 : H_2O_2 radical scavenging activity

S. No	Sample (µL)	Concentration of Sample (µg/mL)	H_2O_2 reagent (ML)	Incubation time	Absorbance at 504 nm	% of Inhibition	IC 50 value
1	100	100	3	Incubate	0.152	55.94	15.7931
2	200	200	3	in the	0.237	31.30	
3	300	300	3	dark	0.276	20.00	
4	400	400	3	chamber	0.284	17.68	
5	500	500	3	for 15 min	0.294	14.78	

temperature program for the oven began at 35°C and was maintained for 3 min, followed by an increase of 8°C per minute until the temperature reached 280°C. The mass spectrometer was operated in electron impact ionization mode at 70 eV, scanning an appropriate mass range for the target analytes. Software such as OpenLab CDS facilitated data acquisition and analysis, whereas compound identification was accomplished by comparing the acquired spectra with the NIST MS 2.0 structural library.

Quantitative analysis by HPLC : Quantitative analysis of saponins (triterpenoids), flavonoids, and phenolic compounds from *the A. aspera* seed extract was performed using HPLC.A Shimadzu HPLC system was used for chromatographic analysis.The set up included an LC-10ATVP pump, a diode array detector (SPD-M10AVP), and a Supelco C18 (RP) column (25 cm × 4.6 mm; 5 µm) maintained at 30°C.Shimadzu Class VP Series software was used for data acquisition and peak integration, and the results were compared to standard references at 278 nm UV detection.The mobile phase consisted of acetic acid (0.1% v/v) and methanol, with the following gradient program: 0-15 min with 5% methanol, 15-40 min with 80% methanol, 40-42 min with 5% methanol, and 42-50 min with 5% methanol. The flow rate was maintained at 1.0 mL/min, with an injection volume of 20 µL. These chromatographic conditions were slightly modified[26] using ascorbic acid and gallic acid as marker compounds to analyze the *A. aspera* seed extract.The quantity of the triterpenoid (saponin) compounds in the extract was quantified using a specific formula.Amount = Sample area standard area × standard dilution × dilution sample amount × mean weight of the sample.

Results

Phytochemical investigation : The ethanol extract of *A. aspera* seeds was subjected to qualitative phytochemical analysis, which indicated the presence of various compounds. These included alkaloids, carbohydrates, proteins, amino acids, steroids, triterpenoids, flavonoids, saponins, and tannins (Table-1).

DPPH radical scavenging : The *A. aspera* seed extract demonstrated notable antioxidant properties through its DPPH radical scavenging activity. The extract proved effective in neutralizing DPPH radicals, which are commonly employed as a stable free radical to evaluate antioxidant capacity. The percentage of IC_{50} value of 26.586 µg/mL typically increased with the extract concentration, indicating a dose-dependent response (Table-2, Fig. 3).The presence of bioactive compounds such as flavonoids, polyphenols, and saponins likely contributes to their ability to donate electrons and quench free radicals. The extract demonstrated potential as a promising component in natural antioxidant formulations, as it may possess moderate to strong scavenging capabilities when evaluated against typical antioxidants such as ascorbic acid. The extract results underscore its potential for therapeutic applications in oxidative stress-related conditions.

ABTS radical scavenging : The ABTS radical scavenging capability of *A. aspera* seed extract was significantly increased, suggesting its strong potential as an effective antioxidant compound.The extract percentage with an IC_{50} value of 28.2641 µg/mL inhibited ABTS radicals in a dose-dependent manner, with higher concentrations showing increased scavenging capacity (Table-3, Fig. 4).These findings indicate that the bioactive

TABLE-5 : GC-HR-MS analysis of *A. aspera* seed extract

S. No	RT	Name of the compound	Molecular formula	*Biological Activity
1	5.66	Carbamic acid	$C_{20}H_{23}N_3O_3$	Anti-bacterial, Anti-tubercular activity
2	14.66	Ethanone, 2-(5,7-bisethylamino-	$C_{17}H_{21}N_7O_2S$	Anti-tumour, Anti-malignant activity
3	17.83	Demeclocycline	$C_{21}H_{21}ClN_2O_8$	Anti-bacterial activity
4	18.24	(5â) Pregnane-3,20â-diol	$C_{28}H_{43}NO_6$	Anti-microbial activity
5	18.86	2,4 (1H)-Cyclo-3, 4-secoakuammilanium	$C_{22}H_{29}N_2O_4$	Anti-microbial activity
6	19.20	Benzeneethanamine, 2,5-difluoro-â, 3,4 trihydroxy	$C_9H_{11}F_2NO_3$	Drugs against various ailments
7	21.50	Scilliroside	$C_{32}H_{44}O_{12}$	Analgesic activity
8	21.92	3-Pyridine carboxylicacid	$C_{32}H_{39}NO_{10}$	Anti-inflammatory activity
9	24.60	Imidazole, 2-amino-5-((2-carboxy)vinyl)	$C_6H_7N_3O_2$	Antimicrobial and Anti-inflammatory activity
10	26.14	Tetraacetyl-d-xylonic	$C_{14}H_{17}NO_9$	Anti-tumour,Antioxidant activity
11	26.40	Nor-diazepam, 3-((N-hydroxymethyl)-	$C_{18}H_{16}ClN_3O_4$	Antioxidant, Antimicrobial activity
12	27.31	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Hypocholesterolemic activity
13	27.58	Hexadecanoic acid	$C_{18}H_{36}O_2$	Antioxidant activity
14	29.23	9- Hexadecenoic acid	$C_{16}H_{30}O_2$	lipogenesis, â-Oxidation activity
15	30.57	Dodecanoic acid	$C_{12}H_{24}O_3$	Acne treatment, high-density lipoprotein
16	32.15	9-Octadecenoic acid (Z)-, hexyl ester	$C_{24}H_{46}O_2$	Anti-Inflammatory activity
17	32.44	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	Anti-bacterial, Anti-fungal activity

S. No	RT	Name of the compound	Molecular formula	*Biological Activity
18	32.76	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	Anti-microbial, Anti-inflammatory activity
19	33.75	Pentadecanoic acid	$C_{15}H_{30}O_2$	Anti-tumour activity
20	37.08	Hexadecanoic acid	$C_{35}H_{68}O_5$	Antioxidant activity
21	37.59	Oleic acid	$C_{18}H_{34}O_2$	Antihypertensive activity
22	38.13	7-Methyl-Z-tetradecen-1-ol	$C_{17}H_{32}O_2$	Hepatoprotective activity
23	39.68	1,3-Dipalmitin trimethylsilyl ether	$C_{38}H_{76}O_5Si$	Anti-microbial and Cytotoxic activity
24	42.82	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	Antioxidant activity

*Source: Dr. Duke's phytochemical and Ethnobotanical databases (USDA Online database).

components present in the extract play a role in its capacity to scavenge free radicals, thus providing a defense against damage associated with oxidative stress.The effective radical-scavenging ability of the ethanolic extract suggests that it is a promising natural antioxidant source for therapeutic applications.

H_2O_2radical scavenging : The H_2O_2 radical scavenging activity showed that *A. aspera* seed extract effectively neutralized hydrogen peroxide (H_2O_2), showing its potential as an antioxidant.The scavenging activity increased with an IC_{50} value of 15.7931 µg/mL at the extract concentration, indicating a dose-dependent response (Table-4, Fig. 5).These results suggest that the extract's active compounds might contribute to reducing oxidative stress by lowering peroxide levels.This mechanism could have potential therapeutic uses in alleviating the oxidative damage associated with various health disorders.These observations support the traditional use of *A. aspera* seed extracts in treatments aimed at combating oxidative stress.

GC-HR-MS analysis : Ethanolic extract of*A. aspera*seed have shown many phytochemicals that contribute to the medicinal activity and pharmaceutically important detected through GC-HR-MS analysis are: Carbamicacid,Ethanone,2-(5,7-bisethylamino-, Demeclocycline, (5â) Pregnane-3,20â-diol, 2,4 (1H)-Cyclo-3,4-secoakuammilanium, Benzeneethanamine, 2,5-difluoro-â,3,4 trihydroxy, Scilliroside, 3-Pyridine carboxylic acid, Imidazole, 2-amino-5-((2-carboxy)vinyl), Tetraacetyl-d-xylonic, Nor-diazepam, 3-((N-hydroxymethyl)-, n-Hexadecanoic acid, Hexadecanoic acid, 9- Hexadecenoic acid, Dodecanoic acid, 3-hydroxy,

Fig. 1 : *Achyranthes aspera* plant and collected seed

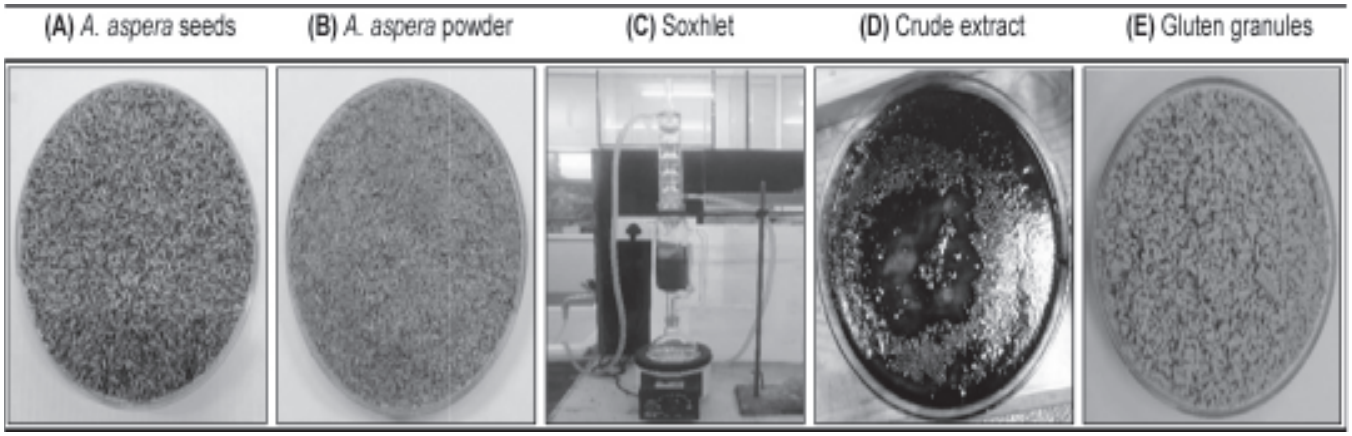

Fig. 2 : Soxhlet extraction process of *A. aspera* seed

9-Octadecenoic acid (Z)-, hexyl ester, Z-8-Methyl-9-tetradecenoic acid, Ethyl iso-allocholate, Pentadecanoic acid, Hexadecanoic acid, Oleic acid, 7-Methyl-Z-tetradecen-1-ol, 1,3-Dipalmitin trimethylsilyl ether, and Octadecanal, 2-bromo-. The identified compounds possess a range of biological properties, including antimicrobial, antifungal, antioxidant, anti-inflammatory, anti-obesity, and anti-diabetic properties. A comprehensive list of these compounds, detailing their retention times (RT), molecular formulas, molecular weights, and concentrations (measured as peak area %), is provided in (Table 5). (Fig. 6) showcases the GC-MS chromatogram and chemical structures of all twenty-four identified compounds.

HPLC analysis : *A. aspera* seed extracts were standardized and subjected to quantitative analysis to identify saponin as a marker compound. HPLC analysis employed a mobile phase with a gradient elution system combining methanol and 0.1% (v/v) acetic acid in HPLC-grade water.A Supelco C18 reverse-phase column was used, and the flow rate was set at 1.0 mL/min.The chromatograms of the phytochemical compounds were obtained, as illustrated in (Fig. 7). HPLC analysis of the ethanolic extract of *A. aspera* seed produced a high peak with retention times of 11.44 and 11.63 with areas of 32.89 and 15.58, respectively, indicating a high concentration of the two bioactive compounds. The first peak, showing greater than 90% area coverage, was indicative of the presence of the highest concentration in the extract.

Discussion

The ethanolic extract of *A. aspera* seeds can yield a broader range of secondary metabolites than a single solvent system when using sequential extraction with solvents of increasing polarity index. Consequently, this

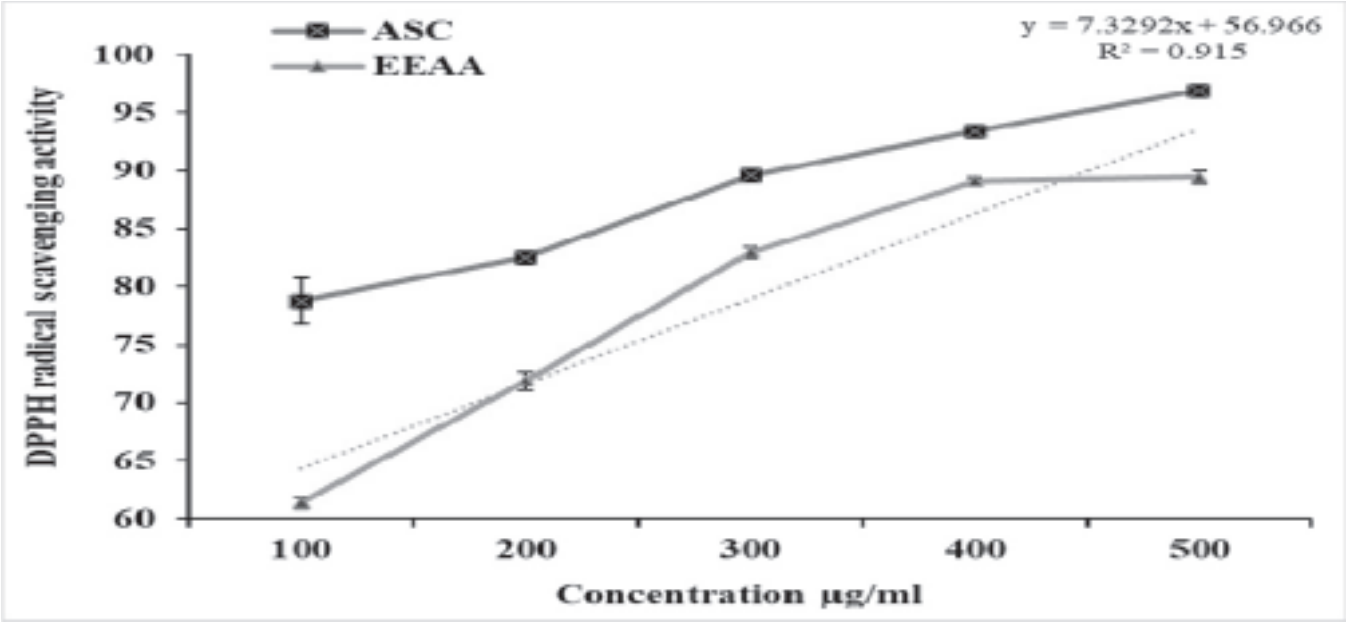

Fig. 3 : DPPH activity of *A. aspera* seed extract

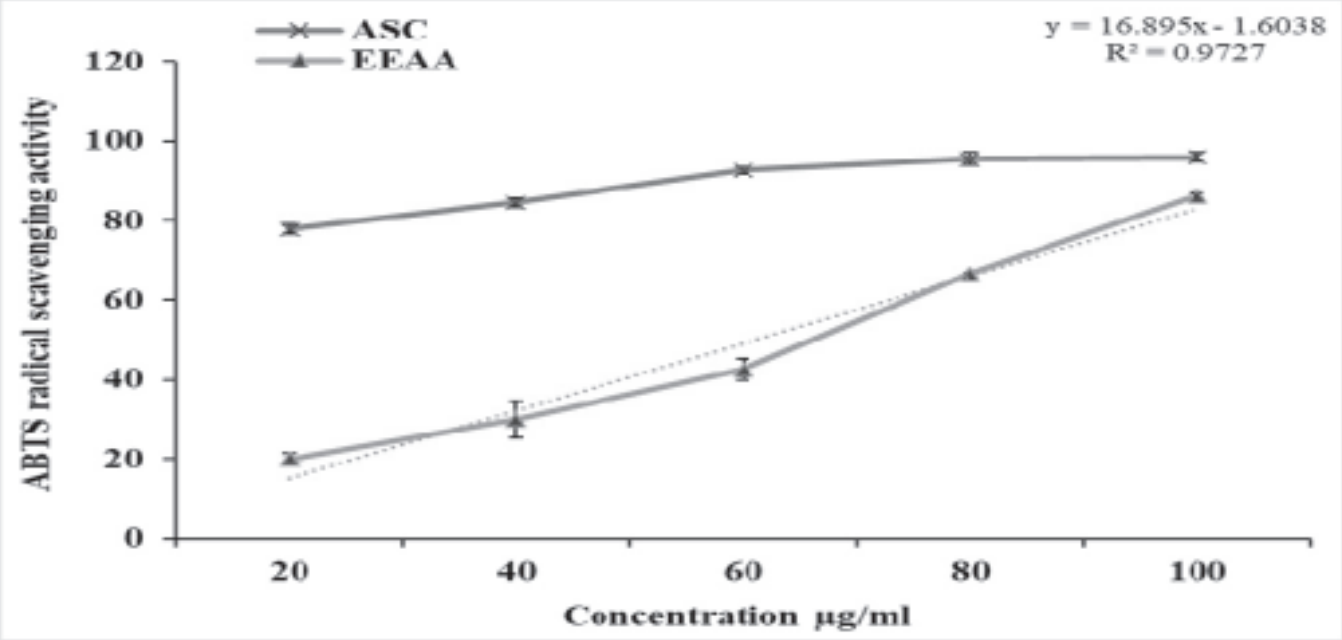

Fig. 4 : ABTS activity of *A. aspera* seed extract

method was employed in this study. These findings indicated that ethanol was the most effective solvent for extracting phytochemicals from *A. aspera* seeds. Previous research has suggested that the high antioxidant activity observed in plant extracts could be attributed to the presence of elevated levels of phenolic and flavonoid compounds, which exhibit polarity in the 50% methanol extract[4].These findings highlight the strong antioxidant activity of DPPH, ABTS, and H_2O_2 in evaluating the free radical scavenging potential of *A. aspera* seed extract, demonstrating the superior effectiveness of L-ascorbic acid as a reference antioxidant.

Gas chromatography-mass spectrometry (GC-MS) investigations have revealed principal compounds with substantial biological promise for pharmaceutical advancements in the future. n-Hexadecanoic acid (5.98%) was the predominant compound, followed by the alcoholic compounds hexadecanoic acid, 1-(hydroxymethyl) (4.03%), and 1,3-Dipalmitin trimethylsilyl

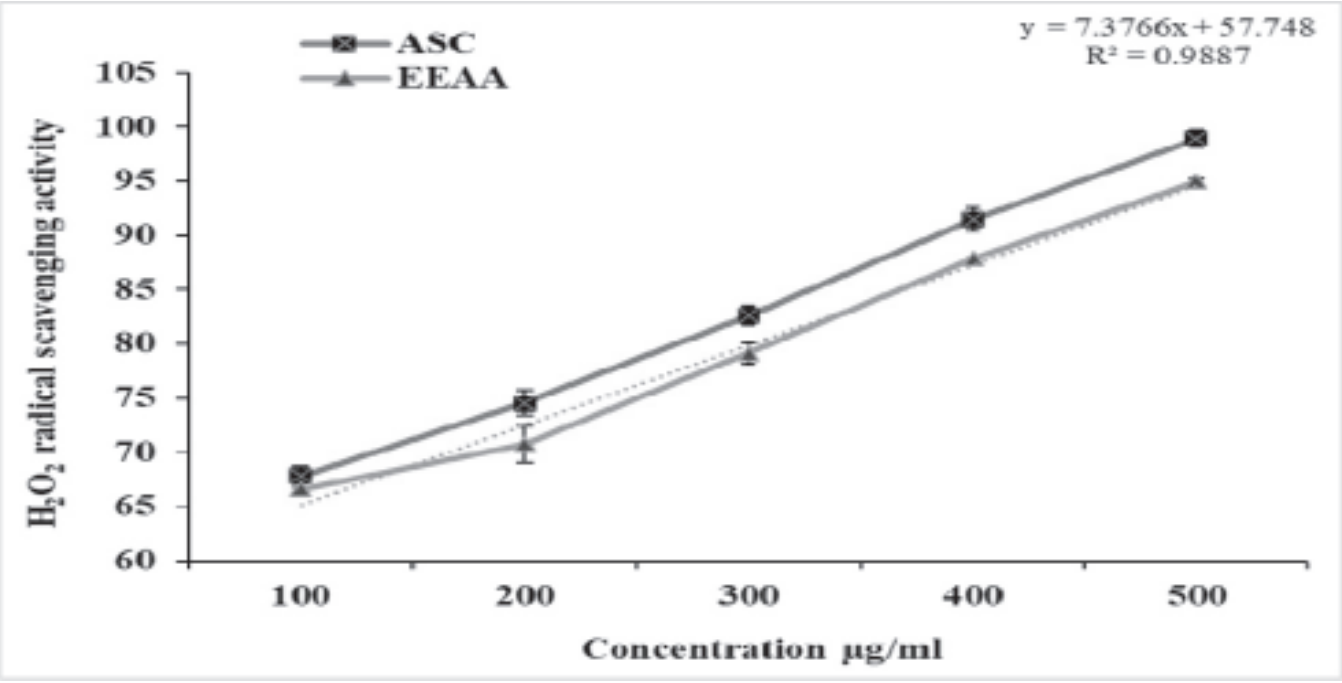

Fig. 5 : H_2O_2 activity of *A. aspera* seed extract

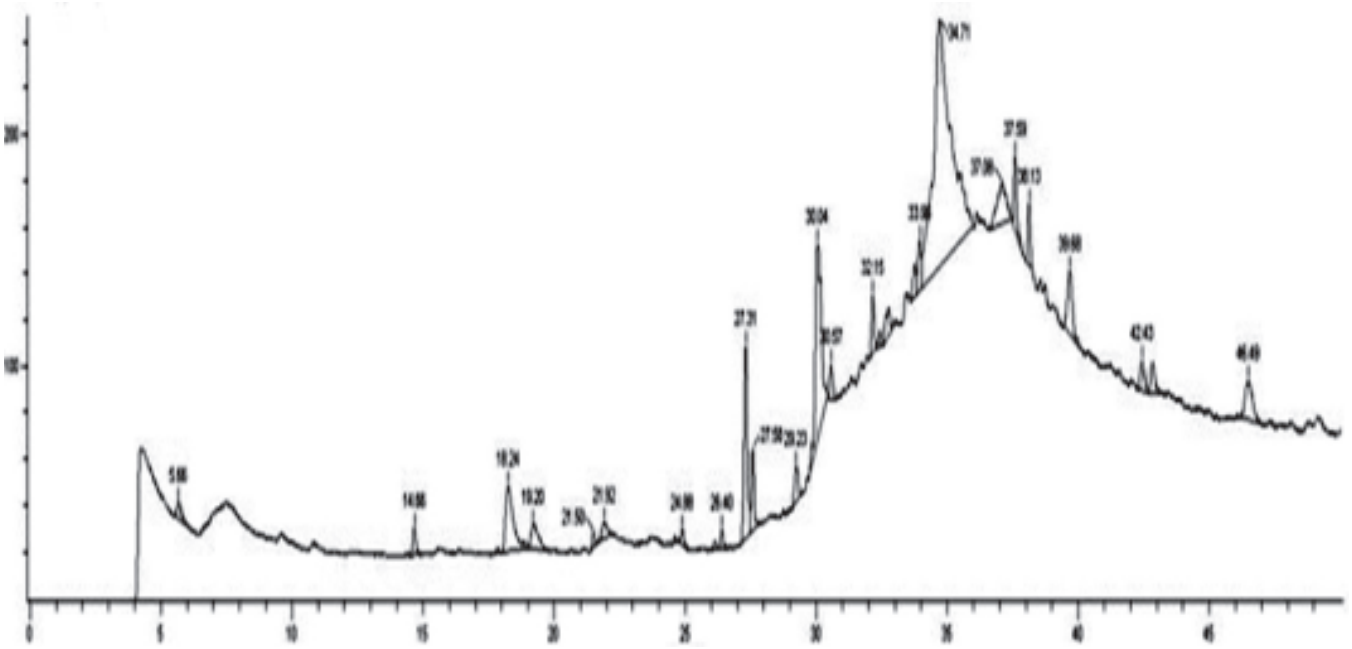

Fig. 6 : GC-MS analysis of *A. aspera* seed extract

ether (3.32%).The compound n-hexadecanoic acid, also known as palmitic acid with the highest % peak area (5.98), has been reported to exhibit anti-inflammatory properties by modulating immune responses.It is involved in the regulation of inflammatory mediators through certain pathways.This study confirms the effectiveness of using medicated oils rich in n-hexadecanoic acid for managing rheumatic symptoms within the traditional Indian medical system, Ayurveda[14].Palmitic acid (PA), the predominant saturated fatty acid in human physiology, accounts for 20–30% of the total fatty acids in both membrane phospholipids and adipose triacylglycerols. This ubiquitous compound can be derived from dietary sources or is endogenously generated through the metabolism of other fatty acids, carbohydrates, or amino acids[8]. Hexadecanoic acid, ethyl ester decreases hypocholesterolemic activity and ethyl palmitate has shown anti-inflammatory effects in some studies. It is thought to modulate inflammatory pathways, making it potentially useful in treating conditions associated with chronic inflammation[16].Ethyl palmitate may influence lipid metabolism and may have applications in managing metabolic disorders, although more research is needed in this area[32].The topical application of ethyl palmitate significantly reduced ear swelling caused by croton oil in rats. In the same model, it also decreased neutrophil infiltration, as evidenced by lower myeloperoxidase (MPO) activity. These findings highlight the anti-inflammatory potential of ethyl palmitate in various experimental models[25].

Oleic acid is an unsaturated fatty acid commonly present in many edible oils. Many studies have demonstrated that oleic acid and its derivatives possess a range of biological properties, including antimicrobial and anticancer effects[5]. The 7-Methyl-Z-tetradecen-1-ol acetate (1.62%) A study analyzing bioactive compounds in certain plant species reported that 7-methyl-Z-tetradecen-1-ol acetate acts as an anticancer and anti-inflammatory agent. 9-Octadecenoic acid (Z)-hexyl ester is a fatty acid, especially unsaturated acids such as oleic acid (the major constituent of (Z)-9-octadecenoic acid), and is known for its antioxidant effects.The compound 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-diyl ester exhibited antibacterial properties that varied based on duration and concentration when tested against the three bacterial strains[21].Ethyl isoallocholate derivatives have been shown to affect cholesterol metabolism and bile acid synthesis.Bile acids play a significant role in the digestion and absorption of dietary fat.By modulating bile acid synthesis, ethyl iso-allocholate could be a potential therapeutic agent for managing cholesterol levels and cardiovascular diseases[10].The previously mentioned compounds isolated from the *A. aspera* seed ethanol extract exhibited biological activity.Future research on these phytochemicals may demonstrate their medicinal significance.

Conclusion

Phytochemical compounds are key for medicinal uses in plants.Plant extracts contain various compounds with potential biological activity.Alkaloids may play a role in plant metabolic processes, whereas triterpenoids may exhibit cytotoxic effects on a broad spectrum of

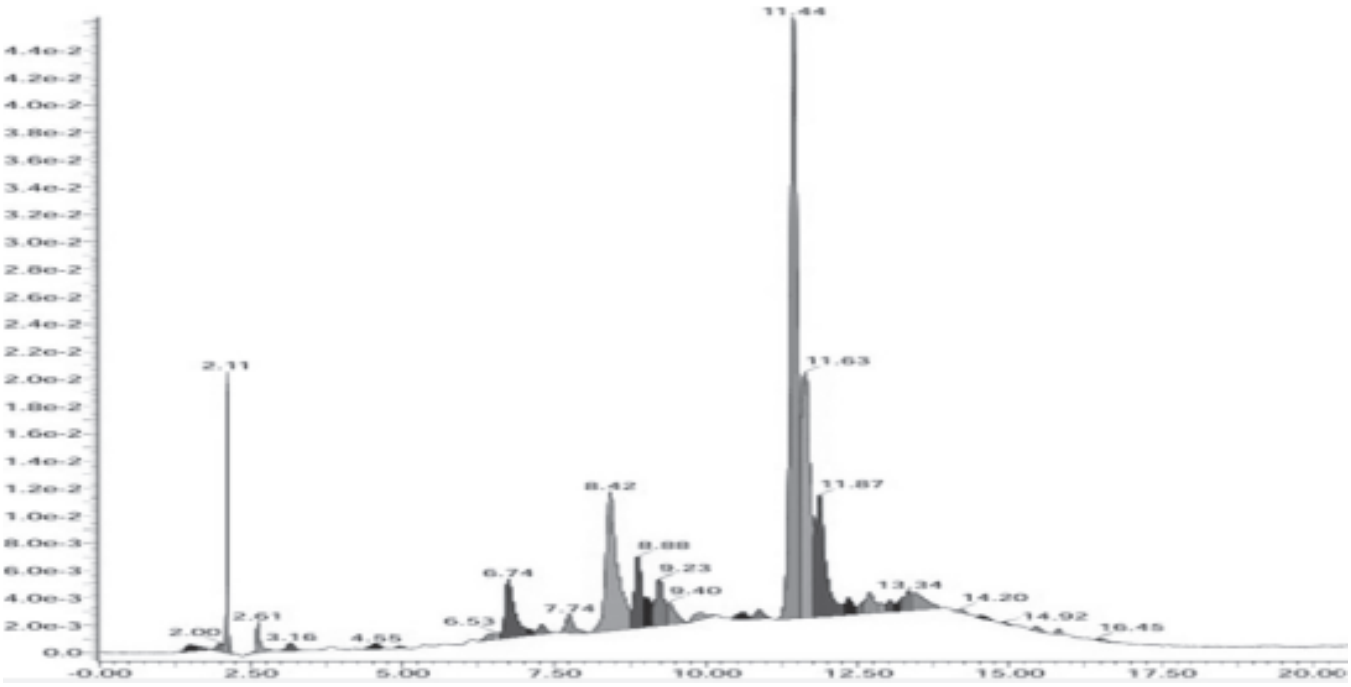

Fig. 7 : HPLC analysis of *A. aspera* seed extract

organisms, including bacteria and fungi.As glycosides of triterpenes and steroids, saponins have potential applications as anti-infective agents in traditional medicine. The presence of flavonoids and tannins in plants is thought to contribute to their ability to neutralize free radicals. These compounds belong to the phenolic family, a major group of plant-derived substances that function as primary antioxidants and free radical scavengers.In the present study, *A. aspera* seed extracts were recognized as a rich source of phytochemicals and are widely used in herbal medicine.In addition, *A. aspera* is valued for its diverse therapeutic properties, including antimicrobial, larvicidal, anti-fertility, immunostimulatory, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, and diuretic activities.The phytochemicals contained in these compounds demonstrate significant antioxidant properties, rendering them potential alternatives to synthetic medications for the management of obesity and diabetes.

Conflicts of Interest

The authors declare no conflict of interest pertinent to this study.

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