GC–MS Profiling, HPTLC Fingerprinting, and *In Vitro* Antioxidant Evaluation of Hydroalcoholic Leaf Extract of *Acer saccharum* Marshall

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Abstract

Background: *Acer saccharum* Marshall (Sugar maple) is a medicinally important species known for its rich phytochemical composition and diverse biological properties. However, comprehensive phytochemical characterization and antioxidant evaluation of its leaf extract remain limited. The present study aimed to investigate the phytochemical profile, establish chromatographic fingerprints, and evaluate the in vitro antioxidant potential of the hydroalcoholic leaf extract of *A. saccharum* (HEAS). Methods: Fresh leaves were extracted with 70% ethanol using the Soxhlet method. The extract was subjected to preliminary phytochemical screening, determination of total phenolic content (TPC) and total flavonoid content (TFC), Fourier transform infrared (FTIR) spectroscopy, high-performance thin-layer chromatography (HPTLC), and gas chromatography–mass spectrometry (GC–MS) analyses. The antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays. IC_{50} values were calculated for the free radical scavenging assays. Results: HEAS exhibited a percentage yield of 19.45% (w/w) and contained alkaloids, phenolics, flavonoids, tannins, saponins, and triterpenoids. The extract possessed high TPC (169 ± 1.45 mg GAE/g extract) and TFC (193 ± 2.06 mg QE/g extract). FTIR analysis confirmed the presence of hydroxyl, aromatic, and oxygen-containing functional groups. HPTLC fingerprinting generated characteristic chromatographic profiles with five distinct bands at both 254 and 366 nm, supporting the presence of multiple phytoconstituents. GC–MS analysis tentatively identified quinic acid, caffeine, 9,12-octadecadienoic acid (linoleic acid), 9,12-octadecadienoyl chloride, and a lipid-like bicyclic ester derivative. HEAS exhibited concentration-dependent antioxidant activity in DPPH and ABTS assays with IC_{50} values of 181.1 µg/mL and 104.9 µg/mL, respectively, and demonstrated appreciable ferric reducing antioxidant power. Conclusion: The hydroalcoholic leaf extract of *Acer saccharum* is a rich source of phenolic and flavonoid compounds with significant antioxidant potential. The combined phytochemical, spectroscopic, chromatographic, and antioxidant findings provide valuable baseline data for the quality standardization of *A. saccharum* leaves and support their future development as a natural source of antioxidant agents for pharmaceutical and nutraceutical applications.

Keywords: *Acer saccharum*, Hydroalcoholic leaf extract, Antioxidant activity, HPTLC fingerprinting, GC–MS profiling

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INTRODUCTION

Acer saccharum Marshall (Sugar maple), belonging to the family Sapindaceae, is a deciduous hardwood tree native to the temperate forests of North America and is recognized worldwide for its ecological, nutritional, and economic importance. Besides being the principal source of maple syrup and maple sugar, *A. saccharum* has emerged as a promising medicinal plant owing to its rich phytochemical composition and diverse biological activities. Phytochemical investigations of the genus *Acer* have revealed the presence of numerous secondary metabolites, including phenolic acids, flavonoids, tannins, lignans, diarylheptanoids, coumarins, terpenoids, alkaloids, and phenylpropanoid derivatives, many of which possess significant antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, antimicrobial, and anticancer properties. These phytoconstituents contribute substantially to the pharmacological potential of the genus and support its traditional medicinal use in several regions of the world.

Oxidative stress, resulting from excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), plays a pivotal role in the pathogenesis of numerous chronic diseases, including diabetes mellitus, cardiovascular disorders, neurodegenerative diseases, cancer, and inflammatory conditions. Natural antioxidants derived from medicinal plants have attracted considerable attention because they can scavenge free radicals, inhibit lipid peroxidation, and enhance endogenous antioxidant defense systems with comparatively fewer adverse effects than synthetic antioxidants. Phenolic compounds and flavonoids are among the most effective plant-derived antioxidants because of their ability to donate hydrogen atoms or electrons, thereby neutralizing reactive free radicals and preventing oxidative cellular damage. Consequently, evaluation of the antioxidant potential of medicinal plant extracts has become an important component in the discovery of novel natural therapeutic agents.

Although considerable attention has been devoted to the phytochemistry of maple sap, maple syrup, bark, and other parts of *Acer* species, comparatively fewer studies have investigated the phytochemical composition of *Acer saccharum* leaves. Previous phytochemical investigations have reported the isolation of several triterpenoids and phytosterols from the leaves of *A. saccharum*, suggesting that the leaves constitute an important reservoir of biologically active metabolites deserving further exploration. Moreover, comprehensive chemical characterization of the leaf extract is essential for understanding its phytochemical diversity and correlating its chemical composition with biological activities.

Reliable phytochemical characterization and quality standardization are indispensable prerequisites for the development of botanical preparations intended for pharmaceutical and nutraceutical applications. High-performance thin-layer chromatography (HPTLC) has become one of the most widely accepted analytical techniques for herbal standardization because it provides rapid, reproducible, and cost-effective chromatographic fingerprints for complex plant extracts. HPTLC enables simultaneous separation and visualization of multiple phytoconstituents, thereby facilitating authentication, quality assessment, and batch-to-batch consistency of herbal materials. Likewise, gas chromatography–mass spectrometry (GC–MS) is a highly sensitive analytical technique extensively employed for the identification of volatile and semi-volatile phytochemicals based on their chromatographic behavior and mass spectral fragmentation patterns. The complementary application of HPTLC and GC–MS offers a comprehensive chemical fingerprint that is valuable for phytochemical profiling, quality control, and the identification of bioactive constituents responsible for the biological activity of medicinal plants.

Despite the recognized medicinal importance of the genus *Acer*, studies integrating in vitro antioxidant evaluation, HPTLC fingerprinting, and GC–MS profiling of hydroalcoholic leaf extracts of *Acer saccharum* remain scarce. Most published investigations have focused primarily on maple sap, syrup, bark, or isolated phytochemicals, whereas systematic phytochemical profiling and chromatographic standardization of the leaves have received limited attention. Therefore, the present study was undertaken to evaluate the in vitro antioxidant activity of the hydroalcoholic leaf extract of *Acer saccharum* Marshall (HEAS) and to establish its chemical fingerprint using HPTLC and GC–MS analyses. The findings of this study are expected to provide valuable baseline data for the phytochemical standardization of *A. saccharum* leaves and support future pharmacological investigations aimed at exploring their therapeutic potential as a source of natural antioxidant compounds.

MATERIALS AND METHODS

Plant material

Fresh mature leaves of *Acer saccharum* Marshall (Sugar Maple) were collected and authenticated by Prof. T. Vijaya, Department of Botany, S.V. College of Sciences, Sri Venkateswara University, Tirupati, Andhra Pradesh, India, based on their morphological and taxonomical characteristics. The authenticated specimen was deposited in the Herbarium of Sri Venkateswara University, Tirupati, under Herbarium No. 17231, for future reference. Following authentication, the collected leaves were thoroughly washed with distilled water to remove adhering dust and extraneous matter, shade-dried at ambient temperature (25–30 °C) for approximately two weeks until a constant weight was attained, and pulverized using a mechanical grinder. The powdered material was passed through a 60-mesh sieve and stored in airtight containers protected from moisture and light until further use (Harborne, 1998; Evans, 2009).

Preparation of hydroalcoholic extract, percentage yield and preliminary phytochemical screening

The shade-dried and coarsely powdered leaves of *Acer saccharum* Marshall (500 g) were extracted with 70% ethanol (ethanol:water, 70:30, v/v) using a Soxhlet extraction apparatus. The extraction was continued for 8–10 h until complete exhaustion of the plant material. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary

vacuum evaporator maintained below 45 °C. The concentrated extract was further dried in a vacuum desiccator to obtain a semisolid mass, designated as the hydroalcoholic extract of *Acer saccharum* (HEAS). The percentage yield of the extract was determined gravimetrically using the following equation: Percentage Yield (%) = Weight of dried extract/ Weight of powdered plant material×100. The dried extract was transferred into airtight amber-coloured containers and stored at 4°C until further phytochemical and antioxidant analyses. The obtained hydroalcoholic extract was subjected to preliminary phytochemical screening using standard qualitative methods to detect the presence of major classes of secondary metabolites. Alkaloids were identified using Mayer's and Wagner's reagents, flavonoids by the alkaline reagent test, phenolic compounds and tannins by ferric chloride and lead acetate tests, saponins by the foam test, steroids and terpenoids by Liebermann–Burchard and Salkowski tests, glycosides by Keller–Killiani test, carbohydrates by Molisch's test, proteins by the Biuret test, and fixed oils by the spot test (Harborne, 1998; Evans, 2009; Kokate, 2021). The formation of characteristic colour changes or precipitates was considered indicative of the presence of the respective phytochemical constituents.

Determination of total phenolic content

The total phenolic content (TPC) of HEAS was determined by the Folin–Ciocalteu colorimetric method using gallic acid as the reference standard. Briefly, 0.5 mL of appropriately diluted extract was mixed with 2.5 mL of ten-fold diluted Folin–Ciocalteu reagent and allowed to react for 5 min. Subsequently, 2.0 mL of 7.5% sodium carbonate solution was added, and the reaction mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 765 nm using a UV–Visible spectrophotometer. A calibration curve was constructed using gallic acid, and the results were expressed as milligrams of gallic acid equivalents (mg GAE/g extract). All determinations were performed in triplicate (Singleton, 1999).

Determination of total flavonoid content

The total flavonoid content (TFC) was estimated using the aluminium chloride colorimetric method with quercetin as the reference standard. One millilitre of the extract solution was mixed with an equal volume of 2% aluminium chloride solution prepared in methanol and incubated at room temperature for 30 min. The absorbance was measured at 415 nm against a reagent blank (Chang et al., 2002). The flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents (mg QE/g extract). All measurements were carried out in triplicate.

***In vitro* antioxidant activity**

The antioxidant potential of HEAS was evaluated using DPPH and ABTS radical scavenging assays. For the DPPH assay, a freshly prepared 0.1 mM DPPH solution in methanol was used. Equal volumes (1 mL each) of DPPH solution and HEAS at different concentrations (25–800 µg/mL) were mixed and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer, and ascorbic acid was used as the reference antioxidant (Blios, 1958). The percentage radical scavenging activity was calculated using the standard equation, and IC_{50} values were determined from the concentration-response curve.

For the ABTS assay, the ABTS radical cation was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating the mixture in the dark for 12–16 h. The radical solution was diluted to obtain an absorbance of 0.70 ± 0.02 at 734 nm. One millilitre of diluted ABTS solution was mixed with 1 mL of extract at different concentrations and incubated for 6 min before measuring the absorbance at 734 nm (Re et al., 1999). Percentage inhibition and IC_{50} values were calculated using the standard formula.

Fourier transform infrared (FTIR) analysis

FTIR spectroscopy was performed to identify the major functional groups present in HEAS. Approximately 2 mg of dried extract was mixed with spectroscopic-grade potassium bromide (KBr), finely ground, and compressed into a transparent pellet. The spectrum was recorded over the range of 4000–400 cm^{-1} using an FTIR spectrophotometer with a spectral resolution of 4 cm^{-1}. The characteristic absorption bands were interpreted by comparing the observed wavenumbers with published reference spectra (Coates, 2000).

HPTLC fingerprinting

HPTLC fingerprinting of HEAS was carried out on silica gel 60 F_{254} precoated aluminium plates. The extract was dissolved in hydroalcoholic solvent, and 10 µL of the sample solution was applied as a band using an automated applicator. Chromatographic separation was achieved using ethyl acetate:methanol:formic acid (7:3:0.1, v/v/v) as the mobile phase. The chromatographic chamber was saturated with the mobile phase for 30 min before development, and the plates were developed to a migration distance of approximately 8 cm. After development, the plates were air dried and visualized under ultraviolet light at 254 and 366 nm. Densitometric analysis was performed using JustTLC software (Version 4.6.1), and chromatographic

fingerprints were recorded in terms of Rf values, peak area, and relative peak intensity. The experimental conditions followed those documented in your HPTLC report (Wagner, 1996; Lakshminarayanan et al., 2017).

GC–MS analysis

GC–MS analysis of HEAS was performed using a gas chromatograph coupled with a mass spectrometer. A 1.0 µL aliquot of the extract was injected under split mode, and chromatographic separation was carried out on a capillary column using helium as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was programmed according to the optimized instrument conditions. Mass spectra were acquired over an appropriate mass range, and the detected compounds were tentatively identified by comparing their spectra with those available in the National Institute of Standards and Technology (NIST) mass spectral library. The retention time, molecular formula, molecular weight, and relative peak area percentage of each identified compound were recorded (Adams, 2007; Lakshminarayanan et al., 2017).

RESULTS

Percentage yield and preliminary phytochemical screening

Hydroalcoholic extraction of *Acer saccharum* Marshall leaves using 70% ethanol yielded 19.45% (w/w) of dried extract (HEAS). The extract was dark green in colour with a semisolid consistency. Preliminary phytochemical screening revealed the presence of several biologically important secondary metabolites, including alkaloids, phenolic compounds, flavonoids, tannins, saponins, and triterpenoids, whereas glycosides, carbohydrates, and fats were absent. The qualitative screening indicated that the hydroalcoholic solvent efficiently extracted polyphenolic and other bioactive constituents from the plant material.

Total phenolic and total flavonoid contents

The hydroalcoholic extract exhibited appreciable quantities of phenolic and flavonoid compounds. The total phenolic content (TPC) was determined to be 169 ± 1.45 mg GAE/g extract, whereas the total flavonoid content (TFC) was 193 ± 2.06 mg QE/g extract (Table 1). The results demonstrated that HEAS is a rich source of polyphenolic constituents.

Table 1. Total phenolic and total flavonoid contents of HEAS.

Sl. No	Phytochompounds	Quantity
1.	total phenolic content (TPC	169 ± 1.45 mg GAE/g extract
2.	total flavonoid content (TFC)	193 ± 2.06 mg QE/g extract

In vitro antioxidant activity

HEAS exhibited concentration-dependent antioxidant activity in all the in vitro free radical scavenging assays evaluated. The percentage radical scavenging activity increased progressively with increasing extract concentration (25–800 µg/mL). In the DPPH assay, HEAS exhibited radical scavenging activity ranging from 17.86 ± 1.12% at 25 µg/mL to 82.57 ± 1.31% at 800 µg/mL, while the standard ascorbic acid showed inhibition values ranging from 34.86 ± 1.45% to 96.43 ± 0.89% over the same concentration range. The calculated IC_{50} value of HEAS was 181.1 µg/mL, compared with 52.7 µg/mL for ascorbic acid, indicating moderate DPPH radical scavenging activity (Table 3).

Similarly, the ABTS radical scavenging assay demonstrated a concentration-dependent increase in antioxidant activity. HEAS produced inhibition values ranging from 21.43 ± 1.21% at 25 µg/mL to 88.29 ± 1.15% at 800 µg/mL. The IC_{50} value of HEAS was 104.9 µg/mL, whereas the corresponding value for ascorbic acid was 37.5 µg/mL, indicating comparatively stronger activity in the ABTS assay than in the DPPH assay (Table 4).

Table 3. DPPH Radical Scavenging Activity of HEAS

Concentration (µg/mL)	HEAS absorbance	HEAS inhibition (%)	Ascorbic acid inhibition (%)
25	0.575 ± 0.008	17.86 ± 1.12	34.86 ± 1.45
50	0.522 ± 0.007	25.43 ± 1.35	49.14 ± 1.62
100	0.436 ± 0.006	37.71 ± 1.48	65.29 ± 1.75
200	0.330 ± 0.005	52.86 ± 1.64	80.71 ± 1.38
400	0.219 ± 0.004	68.71 ± 1.57	91.14 ± 1.12
800	0.122 ± 0.003	82.57 ± 1.31	96.43 ± 0.89

Table 4. ABTS Radical Scavenging Activity of HEAS

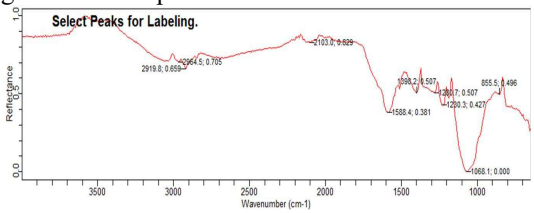
Concentration (µg/mL)	HEAS absorbance	HEAS inhibition (%)	Ascorbic acid inhibition (%)
25	0.550 ±	21.43 ±	42.00 ±

	0.007	1.21	1.52
50	0.458 ± 0.006	34.57 ± 1.43	58.00 ± 1.46
100	0.355 ± 0.005	49.29 ± 1.38	74.00 ± 1.33
200	0.254 ± 0.004	63.71 ± 1.56	86.00 ± 1.18
400	0.161 ± 0.003	77.00 ± 1.42	94.00 ± 0.92
800	0.082 ± 0.002	88.29 ± 1.15	98.00 ± 0.64

FTIR analysis

The FTIR spectrum of HEAS revealed several characteristic absorption bands corresponding to different functional groups present in the extract (Figure 1). The absorption bands at 2964.5 cm^{-1} and 2919.8 cm^{-1} corresponded to aliphatic C–H stretching vibrations of methyl and methylene groups. A weak band was observed at 2103.0 cm^{-1}, while a distinct absorption band at 1588.4 cm^{-1} represented aromatic C=C stretching vibrations. Additional absorption bands at 1398.2 cm^{-1}, 1280.7 cm^{-1}, and 1230.3 cm^{-1} were attributed to C–O stretching and O–H bending vibrations. The intense absorption band at 1068.1 cm^{-1} indicated C–O stretching of alcohols, ethers, and related oxygenated compounds, whereas the absorption band at 855.5 cm^{-1} corresponded to aromatic C–H out-of-plane bending vibrations. Collectively, the FTIR spectrum demonstrated the presence of hydroxyl, aromatic, aliphatic, and oxygen-containing functional groups within the extract (Figure 1).

Figure 1. FTIR spectrum of HEAS.



HPTLC fingerprint analysis

HPTLC fingerprinting of HEAS generated well-resolved chromatographic profiles at both 254 nm and 366 nm, demonstrating the presence of multiple UV-active and fluorescent phytoconstituents. At 254 nm, five distinct chromatographic bands were detected with Rf values of 0.725, 0.520, 0.100, 0.053, and 0.015 (Table 9). The band at Rf 0.725 exhibited the highest densitometric response with a displayed volume of 888.63, accounting for 60.64% of the total chromatographic response. The second major constituent was detected at Rf 0.520, contributing 26.55% of the total response, whereas the remaining bands represented relatively minor constituents (Figure 2). The chromatographic profile recorded at 366 nm also revealed five bands with Rf values of 0.759, 0.645, 0.516, 0.331, and 0.012 (Table 10). Among these, the fluorescent band at Rf 0.645 was the predominant constituent, contributing 36.59% of the total chromatographic response, followed by the bands at Rf 0.331 (28.87%) and Rf 0.516 (26.59%). The chromatographic fingerprints obtained at both wavelengths demonstrated reproducible separation of the phytochemical constituents present in HEAS (Figure 3).

Figure 2. HPTLC chromatogram of HEAS at 254 nm.

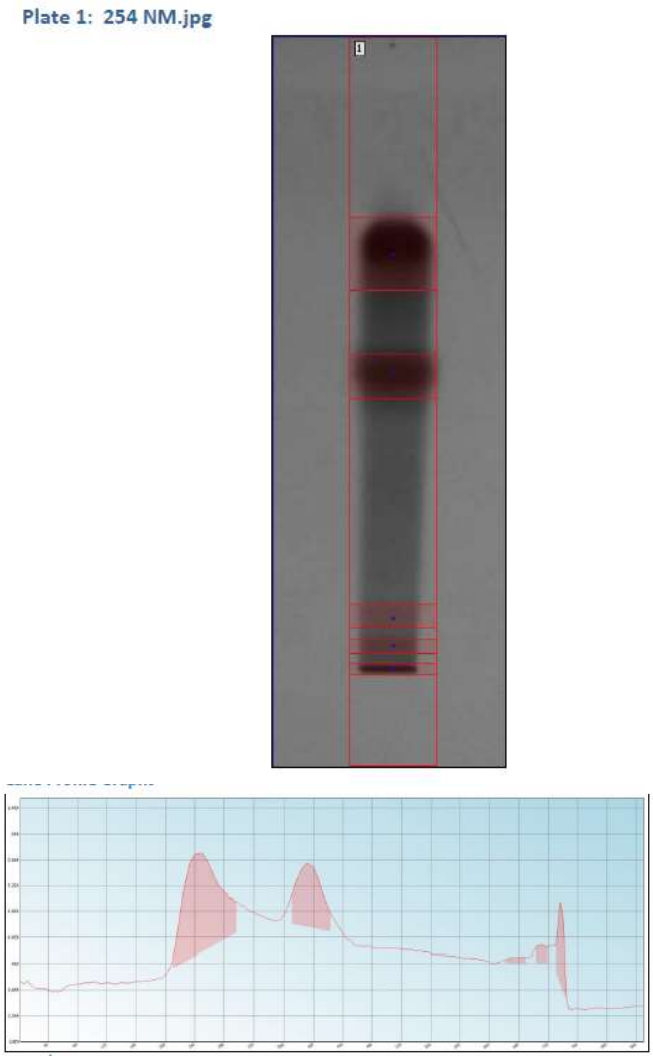


Figure 3. HPTLC chromatogram of HEAS at 366 nm.

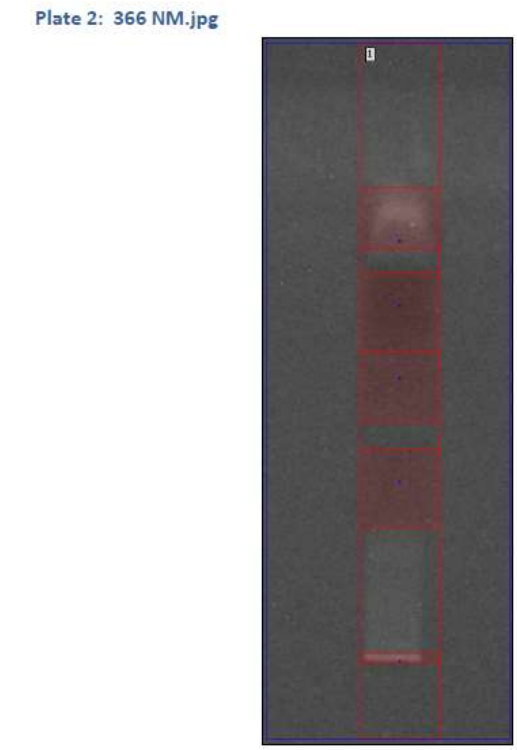


Table 9. HPTLC fingerprint profile of HEAS at 254 nm.

Band No.	Rf value	Area	Displayed volume	Relative contribution (%)	Probable phytochemical nature
1	0.725	8600	888.63	60.64	Moderately non-polar UV-active phenolic/flavonoid-like compound
2	0.520	5300	389.04	26.55	Phenolic/flavonoid derivative
3	0.100	2700	21.89	1.49	Polar phenolic/glycosidic compound
4	0.053	1600	41.75	2.85	Highly polar polyphenolic/tannin-like compound
5	0.015	1400	124.00	8.46	Strongly polar glycoside/polyphenol-type compound

Table 10. HPTLC fingerprint profile of HEAS at 366 nm.

Band No.	Rf value	Area	Displayed volume	Relative contribution (%)	Probable phytochemical nature
1	0.759	6789	147.03	7.14	Less polar fluorescent compound
2	0.645	8742	753.79	36.59	Major flavonoid/coumarin-like fluorescent constituent
3	0.516	7626	547.89	26.59	Flavonoid or phenolic derivative
4	0.331	8742	594.82	28.87	Phenolic glycoside/flavonoid glycoside
5	0.012	1023	16.70	0.81	Highly polar glycosidic/polyphenolic compound

GC–MS phytochemical profiling

GC–MS analysis of HEAS identified five major phytochemical constituents, which were tentatively assigned by comparison of their mass spectra with the NIST spectral library (Figure 4). The identified compounds included quinic acid, caffeine, 9,12-octadecadienoyl chloride (Z,Z), 9,12-octadecadienoic acid (Z,Z), and 4-hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene (Table 11). Among the detected compounds, caffeine was the predominant constituent, eluting at a retention time of 17.793 min with an area percentage of 86.76%. The second most abundant compound was 9,12-octadecadienoic acid (linoleic acid), detected at 19.838 min, contributing 7.05% of the total chromatographic area. Quinic acid was identified at 16.743 min with an area percentage of 3.07%, whereas 9,12-octadecadienoyl chloride and 4-hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene accounted for 1.13% and 1.99%, respectively.

The chromatographic profile demonstrated the presence of organic acids, alkaloid-type compounds, unsaturated fatty acid derivatives, and lipid-like constituents in HEAS.

Figure 4. GC–MS total ion chromatogram of HEAS.

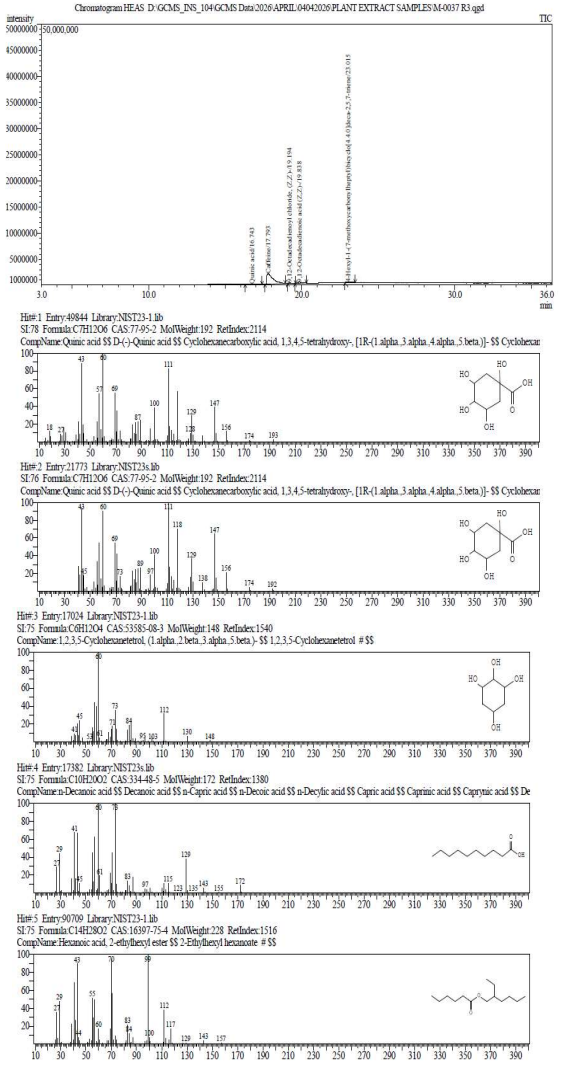


Table 11. GC–MS identified phytochemical constituents of HEAS.

Peak No.	Retention time (min)	Compound identified	Formula	Molecular weight	Area %	Compound class
1	16.743	Quinic acid	$C_7H_{12}O_6$	192	3.07	Polyhydroxy organic acid
2	17.793	Caffeine	$C_8H_{10}N_4O_2$	194	6.76	Xanthine alkaloid
3	19.194	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	1.13	Unsaturated fatty acyl derivative
4	19.838	9,12-Octadecadienoic acid, (Z,Z)-	$C_{18}H_{32}O_2$	280	7.05	Polyunsaturated fatty acid
5	23.015	4-Hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene	$C_{25}H_{40}O_2$	372	1.99	Lipid-like cyclic ester/terpenoid-like compound

DISCUSSION

The extraction efficiency of medicinal plants is largely influenced by solvent polarity, extraction technique, and the chemical nature of phytoconstituents. In the present investigation, hydroalcoholic extraction of *Acer saccharum* leaves produced a 19.45% (w/w) yield, indicating effective recovery of polar and moderately polar secondary metabolites. Hydroalcoholic solvents are widely recognized for extracting diverse phytochemical classes, particularly phenolics, flavonoids, tannins, alkaloids, and triterpenoids, because the presence of water enhances swelling of plant tissues while ethanol improves the solubility of less polar constituents. Consequently, hydroalcoholic extraction has become the preferred method for phytochemical investigations of medicinal plants.

Qualitative phytochemical screening demonstrated the presence of alkaloids, phenolic compounds, flavonoids, tannins, saponins, and triterpenoids, whereas glycosides, carbohydrates, and fats were not detected. These findings agree with previous reports describing members of the genus *Acer* as rich sources of phenolic compounds, flavonoids, tannins, terpenoids, and other specialized metabolites responsible for a broad spectrum of pharmacological activities. More than 330 phytochemicals have been identified from different *Acer* species, with flavonoids and tannins representing two of the predominant classes

contributing to antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic properties.

The abundance of phenolic compounds observed in HEAS was further confirmed quantitatively. The extract contained 169 ± 1.45 mg GAE/g total phenolics and 193 ± 2.06 mg QE/g total flavonoids, demonstrating that *A. saccharum* leaves are a rich source of polyphenolic constituents. Phenolic compounds are recognized as one of the most important groups of natural antioxidants because their hydroxyl groups readily donate hydrogen atoms or electrons to neutralize reactive oxygen species while simultaneously stabilizing the resulting phenoxyl radicals through resonance. Similarly, flavonoids possess multiple hydroxyl substitutions and conjugated aromatic structures that facilitate efficient scavenging of free radicals and inhibition of oxidative chain reactions. Consequently, extracts possessing high TPC and TFC generally exhibit stronger antioxidant activity than extracts containing lower concentrations of these compounds.

The present findings are also consistent with phytochemical investigations conducted on other *Acer* species. Previous studies have demonstrated that *Acer rubrum*, *Acer pictum*, *Acer ginnala*, *Acer tegmentosum*, and *Acer saccharum* contain abundant flavonoids, phenolic acids, tannins, coumarins, and lignan derivatives possessing potent antioxidant properties. Yoshikawa and co-workers isolated several aromatic phenolic constituents, including cleomiscosin derivatives and scopoletin, from *A. saccharum* wood and demonstrated significant antioxidant activity in superoxide dismutase-like assays. These observations support the hypothesis that polyphenolic constituents represent the principal antioxidant components of *Acer* species.

The antioxidant potential of HEAS was evaluated using multiple in vitro models that represent different mechanisms of free radical scavenging. Employing several complementary assays provides a more comprehensive assessment because individual antioxidant tests measure different reaction mechanisms, including hydrogen atom transfer, single electron transfer, metal ion reduction, and scavenging of distinct reactive oxygen and nitrogen species. Therefore, no single assay can fully characterize the antioxidant capacity of a complex plant extract. In the DPPH assay, HEAS exhibited concentration-dependent radical scavenging activity, increasing from 17.86% at 25 µg/mL to 82.57% at 800 µg/mL, with an IC_{50} value of 181.1 µg/mL. Although the activity was lower than that of ascorbic acid, the progressive increase in inhibition clearly demonstrates the ability of the extract to donate hydrogen atoms or electrons for neutralization of DPPH radicals. The relatively low IC_{50} value further indicates moderate antioxidant potency. Since DPPH is a stable nitrogen-centred free radical, compounds possessing phenolic hydroxyl groups readily reduce DPPH to its non-radical form. Therefore, the substantial DPPH scavenging activity observed in HEAS is likely attributable to its high phenolic and flavonoid contents together with other antioxidant phytochemicals detected during phytochemical screening. Similar relationships between phenolic content and DPPH scavenging activity have been reported for numerous *Acer* species.

Among all assays performed, HEAS exhibited its strongest activity against ABTS radicals, with an IC_{50} value of 104.9 µg/mL, substantially lower than that obtained in the DPPH assay. The ABTS assay is capable of evaluating both hydrophilic and lipophilic antioxidants and is therefore particularly suitable for complex botanical extracts containing chemically diverse constituents. The superior performance of HEAS in the ABTS assay suggests that the extract contains multiple antioxidants capable of efficient electron transfer reactions in both aqueous and organic environments. Previous investigations have similarly reported potent ABTS radical scavenging activities for phenolic-rich *Acer* extracts, emphasizing the important contribution of flavonoids, tannins, and related polyphenols to their antioxidant properties.

In the present study, the FTIR spectrum of the hydroalcoholic extract of *Acer saccharum* (HEAS) exhibited several characteristic absorption bands corresponding to hydroxyl, aromatic, aliphatic, and oxygen-containing functional groups. These spectral features corroborate the results obtained from preliminary phytochemical screening and quantitative estimation of phenolics and flavonoids. The broad absorption band observed around 3400 cm^{-1} is characteristic of O–H stretching vibrations of phenolic compounds and alcohols. Hydroxyl groups are the principal structural features responsible for the antioxidant properties of polyphenols because they readily donate hydrogen atoms to neutralize reactive oxygen species. Similarly, the absorption bands observed at 2964.5 cm^{-1} and 2919.8 cm^{-1} represent aliphatic C–H stretching vibrations of methyl and methylene groups commonly associated with terpenoids, fatty acids, and lipid-derived phytochemicals. The aromatic C=C stretching band at 1588.4 cm^{-1}, together with the C–O stretching vibrations between 1280 and 1068 cm^{-1}, further confirms the presence of aromatic phenolics, flavonoids, and oxygenated secondary metabolites. Similar FTIR profiles have been reported for phenolic-rich sugar maple extracts, in which hydroxyl and aromatic functional groups were strongly associated with antioxidant activity.

In the present study, HEAS generated well-resolved chromatographic fingerprints at both 254 nm and 366 nm, indicating the presence of multiple UV-active and fluorescent phytoconstituents. At 254 nm, the predominance of the band at Rf 0.725 (60.64%), followed by Rf 0.520 (26.55%), suggests that moderately polar aromatic compounds constitute the major UV-absorbing constituents of the extract. These compounds are likely to represent phenolic acids, flavonoids, or related aromatic metabolites because these classes typically exhibit strong UV absorption owing to their conjugated benzene ring systems. At 366 nm, three prominent fluorescent bands were observed at Rf 0.645, 0.516, and 0.331, indicating the presence of several fluorescent phytoconstituents. Flavonoids, coumarins, and certain phenolic glycosides are well known to exhibit fluorescence under long-wave ultraviolet light owing to their conjugated π-electron systems. Therefore, the chromatographic pattern obtained at 366 nm further supports the presence of flavonoid-rich constituents in HEAS.

The occurrence of multiple chromatographic bands at both wavelengths demonstrates that the extract contains chemically diverse constituents rather than a single dominant compound. Such characteristic fingerprints are valuable for authentication, quality assurance, batch-to-batch consistency, and detection of adulteration in herbal preparations. Modern pharmacopoeias increasingly recommend chromatographic fingerprinting as an essential component of herbal standardization because biological activity generally results from the synergistic interaction of numerous phytochemicals rather than one isolated constituent.

GC–MS analysis enabled tentative identification of five major volatile or semi-volatile constituents present in HEAS. The identified compounds included quinic acid, caffeine, 9,12-octadecadienoic acid (linoleic acid), 9,12-octadecadienoyl chloride, and 4-hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene. These constituents belong to chemically diverse classes including organic acids, alkaloids, unsaturated fatty acids, and lipid-derived compounds. Among the identified compounds, quinic acid represents an important naturally occurring cyclitol that serves as a biosynthetic precursor for chlorogenic acids and other hydroxycinnamate derivatives. Quinic acid derivatives have attracted considerable attention because of their antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and neuroprotective activities. They are also reported to contribute to the overall antioxidant capacity of plant extracts by scavenging free radicals and inhibiting oxidative damage. Consequently, the presence of quinic acid may partially explain the antioxidant activity observed in HEAS.

The second notable compound identified was 9,12-octadecadienoic acid (linoleic acid). Linoleic acid is an essential polyunsaturated fatty acid that has been reported to possess antioxidant, anti-inflammatory, wound-healing, antimicrobial, cardioprotective, and metabolic regulatory properties. Experimental studies have demonstrated that linoleic acid contributes to membrane stability and modulates inflammatory signalling pathways while reducing oxidative stress under physiological conditions. The detection of this fatty acid is therefore consistent with the observed radical scavenging and reducing activities of HEAS.

The GC–MS chromatogram indicated caffeine as the predominant library-matched compound, accounting for approximately 86.76% of the total chromatographic area. Caffeine is a methylxanthine alkaloid with well-documented antioxidant and neurostimulant properties. Nevertheless, because compound identification in the present study relied exclusively on NIST spectral library matching, the assignment should be regarded as tentative until confirmed using an authentic reference standard or complementary analytical techniques such as LC–MS/MS or HPLC with reference standards. Library matching occasionally produces false-positive identifications when structurally related compounds possess similar fragmentation patterns. Therefore, confirmation of caffeine should be considered in future studies before drawing chemotaxonomic conclusions.

The remaining minor constituents, including fatty acid derivatives and lipid-like cyclic compounds, may also contribute to the biological activity of the extract through synergistic interactions. Numerous reports have demonstrated that the biological effects of medicinal plants arise from the combined activity of multiple metabolites rather than individual purified compounds. Consequently, the antioxidant potential of HEAS is likely attributable to the collective action of phenolic compounds, flavonoids, quinic acid derivatives, unsaturated fatty acids, tannins, triterpenoids, and other unidentified phytochemicals detected by chromatographic analyses.

CONCLUSION

The present study demonstrated that the hydroalcoholic leaf extract of *Acer saccharum* is a rich source of bioactive phytochemicals with significant antioxidant potential. The extract showed a satisfactory yield (19.45%) and contained alkaloids, phenolics, flavonoids, tannins, saponins, and triterpenoids. High total phenolic (169 ± 1.45 mg GAE/g extract) and flavonoid (193 ± 2.06 mg QE/g

extract) contents were consistent with its concentration-dependent antioxidant activity in DPPH and ABTS assays. FTIR analysis confirmed the presence of characteristic functional groups associated with polyphenolic compounds, while HPTLC fingerprinting provided a reproducible phytochemical profile for quality assessment. GC–MS analysis tentatively identified several bioactive constituents, including quinic acid and linoleic acid, which may contribute to the observed antioxidant activity. Overall, these findings indicate that *Acer saccharum* leaves are a promising natural source of antioxidant phytochemicals and provide a scientific basis for their further investigation in nutraceutical and pharmaceutical applications. Future studies should focus on the isolation and structural characterization of the active compounds and validation of their biological activities through in vivo and mechanistic studies.

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