



RESEARCH ARTICLE

Quantitative estimation of quercetin through HPTLC and GC-MS profiling of selected wild edible plants

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Abstract

Quercetin is a naturally occurring polyphenol. It is present in many edible plants, including various fruits and leafy vegetables. Quercetin's wide-ranging pharmacological relevance is very significant. It exhibits anti-inflammatory, antidiabetic and anti-asthmatic properties and supports cardiovascular health. The present study was conducted in August to evaluate the quercetin content and overall phytochemical composition of four wild edible species: *Amaranthus spinosus* L., *Thuja occidentalis* L., *Euphorbia hirta* L. and *Nyctanthes arbor-tristis* L., collected from the district Tonk, Rajasthan, India. Gas chromatography-mass spectrometry (GC-MS) and high-performance thin-layer chromatography (HPTLC) were used in the experiments. The nitric oxide scavenging assay (NOSA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to assess the antioxidant activity. In comparison to the other plants under study, the methanolic extracts of the leaves of *E. hirta* and *N. arbor-tristis* (1809.7, 1065.9 and 982.02 mg QE/g, respectively) showed higher flavonoid contents and improved antioxidant capacity (42.34 and 49.21 µg/mL for DPPH and 60.6 and 44.15 µg/mL for NOSA, respectively) in comparison to other plants. According to the validated HPTLC method, which has been improved for accuracy, linearity and repeatability, *E. hirta* (98.08 ng/mg) had the highest quercetin concentration, whereas *N. arbor-tristis* (28.04 ng/mg) had the lowest. The GC-MS analysis showed that carvacrol, thymol, eicosapentaenoic acid, pterin-6-carboxylic acid and panaxydol are the main components in these plant methanolic extracts. The study found that the wild edible plants tested are rich in flavonoids, phenolics and other bioactive compounds, which help explain their strong antioxidant activity. *Euphorbia hirta* had the highest quercetin content, suggesting it could be a valuable natural source of these compounds. These findings highlight the therapeutic potential of these plants and support further research into their bioactive components for managing oxidative stress-related conditions.

Keywords: antioxidants; leafy vegetables; phytochemicals; secondary metabolites; traditional medicine

Introduction

In developing countries, medicinal plants are used for their therapeutic benefits and have been used in traditional medicine for thousands of years. Between 70 and 95 % of people in developing nations use traditional therapies (1). As essential therapeutic agents and important raw materials for a variety of contemporary and traditional medicines, medicinal plants are an essential component of natural resources (2). Now, the plants are a major source of bioactive compounds that can be used as therapies or vital molecules to create cutting-edge medications with distinctive mechanisms of action. Phytochemicals found in plants include tannins, phenols, alkaloids, amino acids and carboxylic acids (3).

Including natural products with antioxidant properties in the diet is thought to be the most effective way to lower the prevalence of many illnesses. As a result, there is increasing interest in unknown species or wild plants with bioactive compounds that may be beneficial to human health (4). Numerous scientific investigations have identified the chemical makeup of plant extracts and emphasised their detrimental effects, whereas traditional medicine has long recognised the

healing properties of wild plants. Traditional medicine frequently uses old extraction techniques such as decoction, maceration, percolation and infusion (5). Most wild plants with medicinal properties are frequently used as food. For a balanced diet, wild edible plants (WEPs) may be a vital source of vitamins, minerals and antioxidant-rich phytochemicals. Typically, all components of the plant-leaves, bulbs, sensitive stems, seeds and roots are consumed. Many of these plants have therapeutic uses (6). Four of these plants, *Nyctanthes arbor-tristis* L., *Euphorbia hirta* L., *Thuja occidentalis* L. and *Amaranthus spinosus* L. are chosen for this study because they have a variety of therapeutic uses.

Euphorbia hirta, a leafy vegetable, is a member of the genus *Euphorbia* and family Euphorbiaceae. The hotter regions of Australia and India are home to *E. hirta*, which is frequently found in roadside waste areas (Fig. 1A). It is an annual, hairy, slender-stemmed plant that grows up to 40 cm in height and has several branches from base to top (7). It is reddish or purplish in colour. Opposite, elliptic, lanceolate, oblong to oblong, dark green above, acute or subacute, pale below, 1-2.5 cm long, with a purple splotch in the centre and teeth at the margin. Diarrhoea, intestinal parasitosis, dysentery and other gastrointestinal conditions are treated with *E. hirta* (8).



Fig. 1. (A) *Euphorbia hirta*; (B) *Nyctanthes arbor-tristis*; (C) *Thuja occidentalis*; (D) *Amaranthus spinosus*.

Nyctanthes arbor-tristis, also known as Harsingar in Unani, belongs to the Oleaceae family. This plant's leaves have antispasmodic, anthelmintic, antibacterial, cholagogue, bitter tonic and febrifuge properties (Fig. 1B). Additionally, it is utilised as a respiratory stimulant, hypotensive and anti-allergenic. Glycosides, beta-amyrin, mannitol, hentriacontane, beta sitosterol, anthocyanin, oleanolic acid and other compounds are found in this plant's leaves. Joint pain and other inflammation-related problems may be alleviated by drinking Parijat leaf juice (9).

Thuja occidentalis (Cupressaceae), commonly known as “tree of life” or “white cedar,” is widely cultivated as an ornamental species in Brazil and Europe. It is native to eastern North America (Fig. 1C) (10). The leaves are evergreen, with green abaxial surfaces bearing resin ducts. The seeds are enclosed in small green to brown woody cones, measuring approximately 1–2 cm in length. The aromatic leafy branchlets, which are reported to contain vitamin C, are traditionally used as a substitute for tea (11).

Amaranthus spinosus, the weedy amaranth, is most likely native to the lowlands of Central and South America (Fig. 1D). It is currently widely distributed over subtropical and tropical regions, including temperate Europe, Southeast Asia and tropical Africa. It is used as boiled, steamed, or fried vegetables. It is used in traditional medicine to alleviate fever and pain due to its analgesic and antipyretic properties (12).

Nowadays, many plant-derived phytochemicals are used as essential drugs worldwide and may even help prevent disease. Plant extracts are used as a potential source of novel bioactive compounds. The evaluation of phytochemicals present in plants is the main phase of plant research (13). Plant biological behaviours can be ascertained through phytochemical analysis. Plant phytochemical components are determined and estimated using various methods. The most widely utilised methods for this are chromatographic and spectroscopic analysis. The spectroscopic technique is a useful analytical tool for both qualitative and quantitative research of plant extracts (14).

Quercetin stands out among numerous significant bioactive compounds in these plants because it is known to deliver the intended results. Numerous health benefits have been demonstrated for quercetin, a bioflavonoid. These include qualities that enhance mental and physical function, as well as proven antihyperglycemic, anti-inflammatory and antineoplastic effects (15). High-performance thin-layer chromatography (HPTLC), is an extremely automated, sophisticated and successful advancement of conventional thin-layer chromatography, characterised by improved separation efficiency and lower detection limits. The HPTLC is significantly faster, easier and more flexible than other chromatographic techniques (16).

The most popular technique for identifying and quantifying novel phytochemical substances is gas chromatography–mass spectrometry (GC-MS). By comparing GC-MS spectra with reference spectra, this approach can be used to identify unknown chemical substances. Despite the recognised therapeutic potential and rich phytochemical content of these plants, limited comparative data exist regarding the presence and concentrations of specific bioactive compounds such as quercetin. Furthermore, systematic analytical validation using advanced techniques, such as HPTLC, remains underexplored. For the quality control of specific wild edible plants utilised for medicinal purposes, phytochemical, antioxidant, HPTLC and GC-MS studies are helpful (17). Thus, this study's objectives are: (i) perform a phytochemical screening and evaluate the levels of total flavonoids and phenols; and (ii) assess the radical scavenging activities of the various plant extracts, (iii) to identify the substances present in the methanolic extract (ME) using GC-MS analysis and, lastly, (iv) uncover the levels of quercetin in methanolic extracts from selected wild edible plants through HPTLC. By scientifically validating the traditional uses of these plants and exploring their promise as sources of bioactive compounds, the research hopes to reveal measurable differences in quercetin content. These discoveries could shine a light on the pharmacological value of these edible plants.

Materials and Methods

Chemical products

All chemicals and reagents used in the present study were of analytical grade. Methanol, chloroform and other solvents were procured from HiMedia Laboratories Pvt. Ltd. and SRL Pvt. Ltd. (India). Folin–Ciocalteu reagent, sodium carbonate, sodium hydroxide, aluminium chloride, sodium nitrate, ascorbic acid, quercetin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (USA). Sodium nitroprusside (SNP), sulfanilamide, phosphoric acid and N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) used in the nitric oxide scavenging assay were also purchased from Sigma-Aldrich (USA). Standard compounds, including gallic acid ($\geq 98\%$ purity) and quercetin ($\geq 95\%$ purity), were used for the estimation of total phenolic and flavonoid contents.

Plant material

Fresh plant materials (leaves) of *N. arbor-tristis*, *E. hirta*, *A. spinosus* and *T. occidentalis* were collected from the campus of Banasthali Vidyapith, Rajasthan, in August. Voucher specimens were prepared, labelled and deposited in the Banasthali University Herbarium (BURI) for future reference under voucher numbers: *N. arbor-tristis* L. (BURI-5917/2025), *E. hirta*. (BURI-5916/2025), *A. spinosus* (BURI-5918/2025) and *T. occidentalis* (BURI-5919/2025).

Extract preparation

The leaf samples (500 g) were sanitised and allowed to desiccate in the shade for one week before extraction. Next, a grinder was used to grind each dried sample into a powder. The dried samples (100 g) were weighed and placed in the Soxhlet thimble. This process yielded a fresh-to-dry ratio of approximately 5 : 1. The sample was then sequentially extracted with solvents of increasing polarity. First, the powdered sample was extracted with 250 mL of chloroform in a Soxhlet apparatus for 72 hr. After this step, the residue was air-dried and extracted again with 250 mL of methanol under the same conditions for another 72 hr. Finally, for the aqueous extraction, the remaining residue was soaked in 250 mL of distilled water at room temperature for 24 hr, with occasional shaking. The solvent was evaporated using a rotary evaporator under reduced pressure at a bath temperature of 65 °C, which corresponds to a lower effective evaporation temperature due to the vacuum, thereby minimising thermal degradation of the bioactive compounds. Then, the water bath was used to dry the extracted materials. For further examination, the dried extracts were placed in 20 mL sterile culture tubes and refrigerated at 2–8 °C (18).

Qualitative phytochemical analysis

Alkaloids, cardiac glycosides, sugars, tannins, steroids, flavonoids, phenols, saponins, fixed oils and lipids were detected by phytochemical analysis following the usual protocol, as shown in Table 1 (19).

Quantitative analysis

Preparation of the standard solution

Gallic acid and quercetin, both measured approximately 10 mg, were precisely weighed into dry, clean volumetric flasks. They were then dissolved in methanol and a 1 mg/mL solution was obtained by diluting to 10 mL with the same solvent. A total of 1 mg of pure atropine was dissolved in 10 mL of distilled water to create the standard atropine solution.

Preparation of the sample

A 10 mg of dehydrated methanolic extract was diluted in 10 mL of methanol to produce a 1 mg/mL stock solution.

Determination of total flavonoid content

A slightly modified version of Ahmed's technique was used to estimate the total flavonoid content (TFC) in methanol extracts from all samples (20). Each plant extract was dissolved in methanol to create a 1 mg/mL extract solution. Next, 300 μ L of extract was placed in a test tube with 3.4 mL of aqueous methanol (30 % v/v). Subsequently, each test tube was filled with 150 μ L of a 0.5 M aqueous NaNO₂ solution and 150 μ L of a 0.3 M aluminium chloride solution. After 5 min, about 1 mL of 1 M NaOH solution was added and the mixture was thoroughly mixed. Then, the absorbance at 415 nm was measured with a UV-visible spectrophotometer against a blank. The control was established similarly, but methanol was utilised in place of the plant extract in equal measure. A quercetin standard curve (Fig. 2B) was established using identical methods to determine the TFC, quantified as milligrams of quercetin equivalents (QE) per gram of extract. The total flavonoids in the extracts and standards were quantified three times, as given in equation 1 (21).

$$\text{TFC (mg QE/g DW)} = C \times V/m \quad (\text{Eq. 1})$$

Where C = concentration from calibration curve (mg/mL, as gallic acid equivalent), V = volume of extract (mL), m = mass of plant sample (g).

Determination of total phenol content

The most common method for determination of total phenol content (TPC) is spectrophotometric analysis. The total phenol concentration in the selected plant leaf extract was measured spectrophotometrically using the FC (Folin–Ciocalteu) colorimetric

Table 1. Qualitative tests for phytochemical screening (19)

Phytochemicals	Test	Procedure	Observations
Alkaloids	Dragendorff's/ Kraut's test	Few mL filtrate + 1–2 mL Dragendorff's reagents	Reddish-brown precipitate
Sterols	Libermann-Burchard's test	50 g extract is dissolved in 2 mL acetic anhydride + 1–2 drops of conc. H ₂ SO ₄ (along the side of the test tube)	An array of colour changes
Cardiac glycosides	Keller-Killani test	1mL filtrate + 1.5 mL glacial acetic acid +1 drop of 5 % ferric chloride + conc. H ₂ SO ₄ (along the side of the test tube)	Blue coloured solution (in acetic acid layer)
Phenols	Ferric chloride test	Extract the aqueous solution with a few drops of 5 % ferric chloride solution.	Dark green/bluish black colour
Tannins	10 % NaOH test	0.4 mL plant extract + 4 mL 10 % NaOH + shaken well	Emulsion formation
Flavonoids	Pew's test	A few mL aqueous extract solution + 0.1 g metallic zinc +8mL conc. H ₂ SO ₄	Red colour precipitate
Saponin	Foam test	0.5 g plant extract + 2 mL water (vigorously shaken)	Persistent foam for 10 min

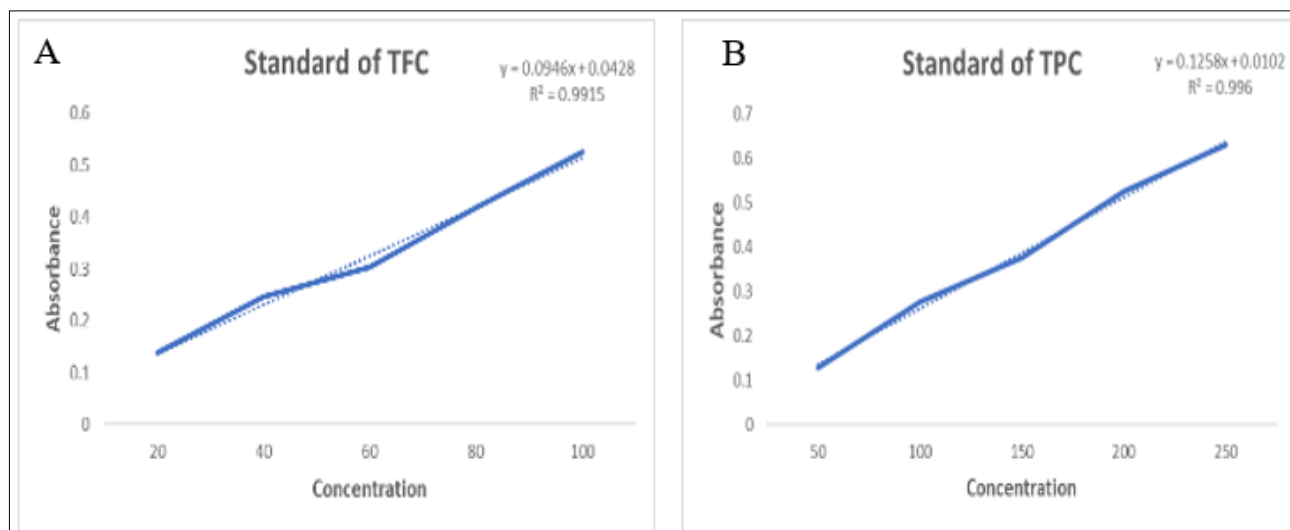


Fig. 2. Standard curve of (A) quercetin; (B) gallic acid.

method. Gallic acid was used as the standard and the results were expressed as gallic acid equivalent (GAE) per gram of material (Fig. 2 A). Gallic acid was prepared in various quantities (0.01–0.1 mg/mL) using methanol. Next, aliquots of 0.5 mL of the test sample (1 mg/mL) and each standard solution were mixed with 2 mL of FC reagent (1 : 10 in deionised water) and 4 mL of a saturated Na_2CO_3 solution (7.5 % w/v). Silver foil was placed over the tubes and they were left at room temperature for half an hour, with periodic shaking. The absorbance at 765 nm was measured using methanol as a blank. Each sample underwent 3 replications of analysis. The concentration was calculated using the formulae given in equation 2 (22).

$$\text{TPC (mg GAE/g DW)} = C \times V / m \quad (\text{Eq. 2})$$

Where C = concentration from calibration curve (mg/mL, as gallic acid equivalent), V = volume of extract (mL), m = mass of plant sample (g).

Antioxidant assay

Samples for the DPPH radical scavenging assay and nitric oxide radical scavenging assay tests were suitably diluted using 50 % (v/v) methanol. Rutin and ascorbic acid were utilised as standard antioxidants.

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The decrease of DPPH, a stable free radical, is the foundation of the DPPH test procedure. It is a stable free radical that interacts with antioxidants when a hydrogen donor is present; it then becomes paired off and is reduced to DPPH, causing the absorbance of DPPH to drop. Greater decolourisation results in increased reduction power, which causes the violet colour to fade. However, the picryl group should still be present and give off a slight yellow colour. The standard approach was used to evaluate the plant extract's ability to scavenge DPPH radicals. In a typical experiment, 2 mL of a 0.1 mM DPPH solution in methanol was mixed with approximately 2 mL of a plant extract solution. Afterwards, the mixture was incubated in the dark at 37 °C for 30 min. The absorbance at 517 nm was measured using methanol as a blank. The DPPH radical scavenging activity percentage was determined as follows in equation 3:

$$\text{Percentage (\%) of DPPH radical scavenging assay} = \frac{A_0 - A_1}{A_0} \times 100 \quad (\text{Eqn.3})$$

Where: A0 was the absorbance of the control and A1 was the absorbance of the treated sample (23).

Nitric oxide radical scavenging assay

The process relies on the idea that, in an aqueous solution at physiological pH, sodium nitroprusside (SNP) spontaneously produces nitric oxide (NO). Griess reagent can be used to measure the nitrite ions produced when this oxide reacts with oxygen. Then 50 μL of each concentration of the plant extracts that had been previously dissolved in Dimethylsulfoxide (DMSO) and ascorbic acid (the reference component) were added to separate tubes. The volume was then evenly increased to 150 μL with methanol. A 2.0 mL solution of 10 mM SNP in PBS (phosphate-buffered saline) was added to each tube. The solutions were incubated for 150 min at room temperature. A similar procedure was repeated using methanol as the control blank. Each tube, including the control, got 5 mL of Griess reagent (1 % sulphanilamide in 5 % H_3PO_4 (phosphoric acid) and 0.1 % NEDD (naphthyl ethylene diamine dihydrochloride) after the incubation period. Using a UV-visible spectrometer, the absorbance of the purple azo dye chromophore, which was created when nitrite ions were diazotised with sulphanilamide and then coupled with NEDD, was measured at 546 nm. To calculate the quantity of nitrite generated in the presence or absence of the plant extract, a standard curve based on NaNO_2 solutions with known values was used. Every experiment was conducted at least three times and the results were reported as the mean of three separate assessments (24). The IC_{50} value for each test compound and the standard preparation was determined as follows in equation 4:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \quad (\text{Eq. 4})$$

Gas chromatography-mass spectrometry profiling analysis of the methanolic extract of the edible plant leaves

A GC-MS analysis was carried out to determine which bioactive substances were contained in the ME of *N. arbor-tristis*, *E. hirta*, *A. spinosus* and *T. occidentalis*. With a split ratio of 10 : 1, an injection volume of 1 μL and a constant flow rate of 1 mL/min, helium was used as the carrier gas. The mass scan range was set to 50–650 m/z. A Thermo Scientific Triple Quadrupole GC-MS system (Trace 1300 gas chromatography paired with a TSQ 8000 triple quadrupole mass spectrometry) fitted with a TG-5 MS column (30 m $\times$ 0.25 mm, 0.25 μm) was used for the analysis. The injector and mass spectrometry (MS) transfer line temperatures were maintained at 280 °C. The oven temperature was initially set to 50 °C for 3 min, then increased at 5 °C

per min to 280 °C and held at 280 °C for 5 min. The chemicals were identified and quantified using total ion chromatography (TIC) after characterisation by retention indices (RI) and retention times (RT). The collected spectra were matched to previously published publications and the National Institute of Standards and Technology library for additional identification (25).

High-performance thin-layer chromatography profiling analysis

Instrumentation and chromatographic conditions

Silica gel 60 F254 (E. Merck, Darmstadt, Germany) coated aluminium HPTLC plates with a 250 µm layer were used for the experiment. The standard stock solutions were made by dissolving 2 mg of quercetin in methanol in a 10 mL volumetric flask and the final volume was adjusted with methanol. The pre-coated thin-layer chromatography (TLC) plates were spotted with solutions ranging from 1 to 8 µL for the quercetin calibration curve. The TLC plate was also coated with 12 µL of a solution prepared by dissolving dried extracts of *N. arbor-tristis*, *E. hirta*, *A. spinosus* and *T. occidentalis* in 10 mL of methanol. The plates were activated by heating them at 50 °C for 3 min and pre-washed with methanol before chromatography. A 100 µL Hamilton syringe (Bonaduz, Switzerland) and a CAMAG (Muttentz, Switzerland) Linomat V sample applicator were used to apply the sample under a constant nitrogen flow, producing 6-mm-wide bands. A 150 nL/s was the constant application rate. We examined mobile phases containing varying amounts of dichloromethane, toluene, methanol, chloroform, acetone, formic acid, ethyl acetate and glacial acetic acid. The mixture of toluene : ethyl acetate: formic acid (6 : 4 : 0.3, V/V) showed good separation of quercetin. After that, the plates had been placed in the mobility phase. After plate development, they were allowed to air-dry before densitometric scanning at 366 nm using a CAMAG TLC Scanner III. WinCATS software (Version 1.4, CAMAG) was used to control the scanner, which ran in reflectance-absorbance mode. The slit was 20 × 10 cm and the scanning speed was set at 20 mm/s. A deuterium lamp that generates light in the UV range (200–400 nm) served as the radiation source. The intensity of the light diffusely reflected was used to calculate the quantities of the compounds chromatographed. The linear regression of peak regions served as the basis for the assessment.

High-performance thin-layer chromatography method validation

All measurements were performed in triplicate and the method validation adhered to the ICH (2005) standards. Plotting peak area against concentration was used to create calibration curves for quercetin and least-squares linear regression was used to evaluate the intercept, slope and correlation coefficient. The limits of detection (LOD) and limits of quantification (LOQ), which were established using the standard deviation method in compliance with ICH recommendations, were used to evaluate the method's sensitivity. Six samples at the same concentration were applied to the plate to assess repeatability; the results were expressed as the

percentage relative standard deviation (% RSD) and standard error (SE). Recovery trials were utilised to evaluate precision. To ensure that the samples remained within the linear range after standard addition, a predefined volume of the standard drug solution at three distinct concentrations (50 %, 100 % and 150 %) was mixed with a pre-analysed sample solution (100 ng/band). Three separate analyses of each concentration level were conducted. Robustness was examined by spotting 100 ng of each standard six times and making slight adjustments to the chromatographic conditions, including variations in mobile phase volume (10 ± 2 mL), saturation time (20 ± 5 min) and scanning wavelength (366 nm ± 2 nm).

Statistical analysis

Statistical analysis was performed using SPSS software. Data, expressed as mean ± standard deviation from triplicate samples (n = 3), were subjected to one-way ANOVA. Treatment means were compared using Tukey's post hoc test, with results considered statistically significant at $p < 0.05$.

Results and Discussion

Phytochemical analysis

The local use of *N. arbor-tristis*, *E. hirta*, *A. spinosus* and *T. occidentalis* to treat a variety of ailments is justified by the presence of phytochemicals in their leaves, as shown in Table 2. Depending on the solvent used, extracts from this plant showed varying levels of phytochemicals (26, 27). Alkaloids, saponins and a significant number of sterols are abundant in the leaves. The most efficient of the three solvents for the quantitative and qualitative extraction of primary and secondary metabolites was found to be methanol. It was found that distilled water was less effective at extracting the phytochemicals under investigation. The methanol extract with the highest metabolite content may reflect the polarity of the methanol solvent (28, 29). The phytochemical profile of this plant suggests it may exhibit a variety of biological activities. Thus, all these plants are pharmacologically exploited to produce novel chemicals beneficial to human health.

Quantitative analysis

Total polyphenols

The TPC varied significantly ($p < 0.05$) among the extracts tested (898–3546.23 mg GAE/g DW), indicating that both the plant species and the extraction solvent strongly affect phenolic recovery (Fig. 3; Table 3). *Euphorbia hirta* had the highest TPC in its methanolic extract (3546.23 ± 0.01 mg GAE/g DW), which suggests methanol is effective for extracting its phenolic compounds. This is probably because methanol's intermediate polarity helps dissolve many phenolic compounds, including low-molecular-weight acids like gallic acid.

Table 2. Phytochemical screening of *Euphorbia hirta* (EH), *Nyctanthes arbor-tristis* (NA), *Thuja occidentalis* (TO) and *Amaranthus spinosus* (AS) obtained by successive methanolic, chloroform and aqueous extractions

Phytochemicals	Methanolic extract				Chloroform extract				Aqueous extract			
	EH	NA	TO	AS	EH	NA	TO	AS	EH	NA	TO	AS
Alkaloids	+	+	++	++	++	+	++	++	+	++	++	++
Sterols	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac Glycosides	++	-	-	+	+	-	-	+	+	-	-	+
Phenols	+++	++	+++	++	+++	++	++	++	++	++	+++	++
Tannins	++	+	++	++	++	+	++	+	+	+	+	+
Flavonoids	++	++	+++	++	+++	++	+++	+++	++	++	++	++
Saponin	+	-	+	+	+	-	-	-	+	+	-	-

+++; highly present; ++; moderately present; +; present; -: absent.

Table 3. Total polyphenol content of *Euphorbia hirta* (EH), *Nyctanthes arbor-tristis* (NA), *Thuja occidentalis* (TO) and *Amaranthus spinosus* (AS) was obtained by successive methanolic, chloroform and aqueous extractions

S. No.	Plants	Phenol content of edible plants (mg GAE/g DW extract)			Flavonoid content of edible plants (mg QE equivalent/g DW extract)		
		ME	CE	AE	ME	CE	AE
1.	<i>N. arbor-tristis</i>	1008.74 ± 0.01 ^d	1869.3 ± 0.09 ^c	1686.24 ± 0.03 ^d	1065.9 ± 0.01 ^b	1126.84 ± 0.01 ^a	741.72 ± 0.01 ^d
2.	<i>E. hirta</i>	3546.23 ± 0.01 ^a	3265.32 ± 0.01 ^a	2878.74 ± 0.03 ^a	1809.7 ± 0.02 ^a	250.5 ± 0.01 ^d	1552.3 ± 0.08 ^b
3.	<i>T. occidentalis</i>	1392 ± 0.07 ^c	898 ± 0.01 ^d	2073 ± 0.02 ^b	982.02 ± 0.01 ^c	879 ± 0.01 ^b	1589 ± 0.01 ^a
4.	<i>A. spinosus</i>	3193.9 ± 0.01 ^b	2918.12 ± 0.03 ^b	1934.02 ± 0.02 ^c	972.8 ± 0.01 ^c	784.3 ± 0.01 ^c	1010.2 ± 0.01 ^c

TFC: total flavonoid content (mg QE/g); TPC: total phenolic content (mg GAE/g); ME: methanolic extract; CE: chloroform extract; and AE: aqueous extract. Values are mean ± SD of three replicates. Post hoc comparisons were conducted using Tukey's HSD test. Distinct letters indicate statistically significant differences among plant samples within each solvent group ($p < 0.05$).

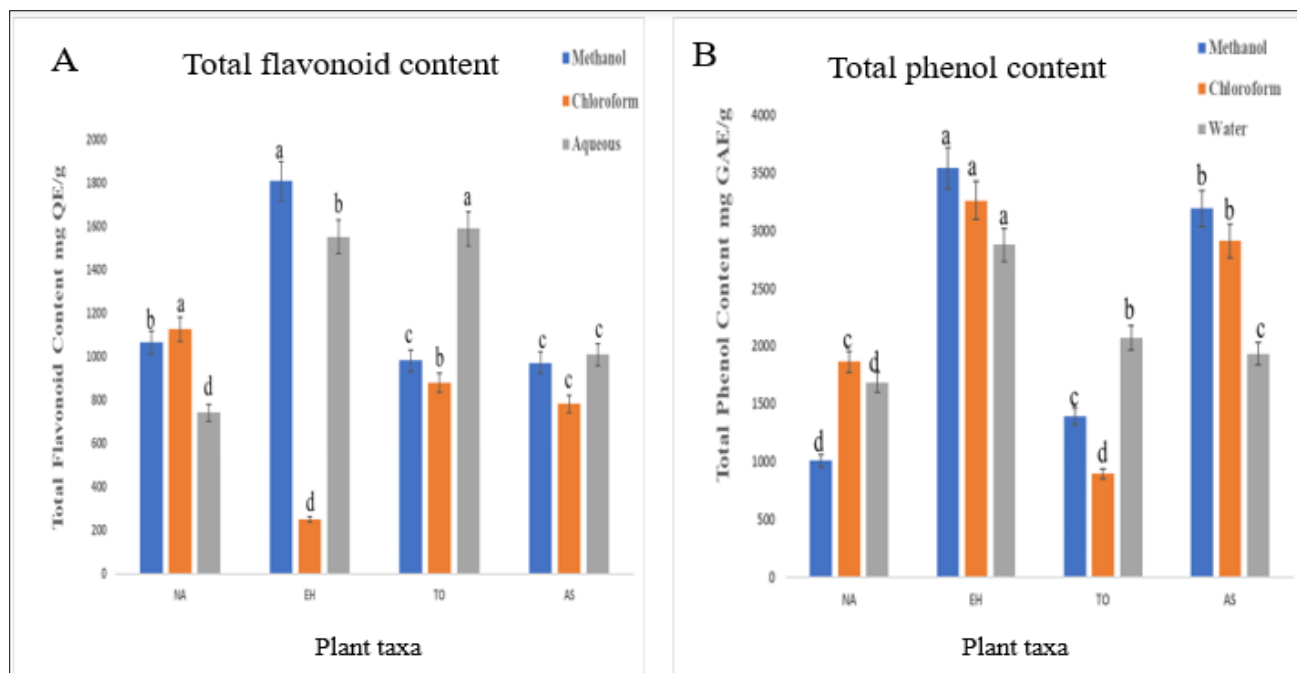


Fig. 3. Comparison of (A) total flavonoid content (TFC); (B) total phenolic content (TPC) of *Euphorbia hirta* (EH), *Nyctanthes arbor-tristis* (NA), *Thuja occidentalis* (TO) and *Amaranthus spinosus* (AS) obtained using methanolic, chloroform and aqueous solvents. TPC was expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW), whereas TFC was expressed as mg quercetin equivalents per gram dry weight (mg QE/g DW). Values are presented as mean ± standard deviation (SD) of three independent experiments ($n = 3$). Post hoc comparisons were conducted using Tukey's HSD test. Distinct letters indicate statistically significant differences among plant samples within each solvent group ($p < 0.05$).

In contrast, *A. spinosus* showed a higher phenolic content in the chloroform extract (2918.1 ± 0.03 mg GAE/g DW), which suggests many of its phenolics are less polar or linked to non-polar substances. This result shows that no single solvent works best for all cases; the best extraction method depends on the type of phenolics in each plant. Other studies have also found that the choice of solvent affects phenolic extraction, with methanol and mixed-polarity solvents often working better than water, while non-polar solvents are better at extracting bound or fat-soluble phenolics (30). The large differences between species in this study suggest they have different abilities to make and store phenolic compounds. These differences probably relate to how each plant adapts to its environment, copes with stress, or its genetic makeup, all of which affect how it produces secondary metabolites.

Total flavonoids

There is a considerable variation in the total flavonoid concentration (TFC) between different plant extracts ($p < 0.05$). *E. hirta* is rich in flavonoids, especially derivatives of quercetin; its methanolic extract had the highest flavonoid concentration (1809.7 ± 0.02 mg QE/g DW), while its aqueous extract had the second-highest (1552.3 ± 0.08 mg QE/g DW) (Fig. 3; Table 3).

Amaranthus spinosus and *N. arbor-tristis* both had modest flavonoid levels; their aqueous and chloroform extracts showed the highest quantities, respectively. On the other hand, *T. occidentalis* showed a significant amount of flavonoids in the aqueous extract (1589 ± 0.01 mg QE/g DW).

The higher total TFC in methanolic and aqueous extracts than in chloroform extracts indicates that flavonoids in these species are predominantly polar, consistent with their chemical structures, which contain many hydroxyl groups. Previous studies also show that solvent polarity strongly affects the extraction efficiency of phytochemicals, with polar solvents such as methanol and water typically yielding higher yields of bioactive compounds (31). *Euphorbia hirta* and *T. occidentalis* had higher TFC among the species studied, which suggests they have more bioactive flavonoids that could explain their antioxidant and anti-inflammatory effects. On the other hand, *N. arbor-tristis* and *A. spinosus* had lower TFC, so other types of phytochemicals might be responsible for their biological activity. These results show that, while flavonoids are important antioxidants, their extraction and measured levels depend heavily on the solvent used and do not fully account for differences in biological activity among extracts (30).

Antioxidant activity

According to DPPH and nitric oxide scavenging assays, the antioxidant activity of the edible plants under investigation differed significantly ($p < 0.05$) between species and solvent extracts, reflecting their varied phytochemical profiles (Table 4). Higher radical scavenging effectiveness is indicated by lower IC_{50} values (30). With IC_{50} values of 42.34 ± 0.01 and 49.21 ± 0.05 $\mu\text{g/mL}$, respectively, *E. hirta* and *N. arbor-tristis* extracts in methanol demonstrated better DPPH scavenging capabilities, as shown in Fig. 4A. The antioxidant activity of these plants is largely correlated with their phenolic and flavonoid content. However, antioxidant potential did not always match up with TPC or TFC. This suggests that other non-phenolic compounds may also play a big role in radical scavenging. For *A. spinosus*, the strong DPPH activity of the aqueous extract, despite only moderate TPC and TFC, suggests the presence of water-soluble antioxidants such as ascorbic acid, reducing sugars, or other polar compounds. It may also be due to the combined effects of different phytochemicals. Previous researchers found similar results, showing that aqueous and methanolic extracts can exhibit high antioxidant activity even when their total phenolic and flavonoid levels are low (32). This highlights the importance of the extraction solvent and how compounds interact.

The nitric oxide scavenging activity (NOSA) assay showed a similar pattern, indicating that the extracts also effectively scavenge reactive nitrogen species (Fig. 4B). This suggests their antioxidant effects go beyond just neutralising free radicals and may also help reduce nitric oxide-related oxidative stress. These plants exhibit antioxidant activity, with IC_{50} values ranging from 44.15 to 74.37 $\mu\text{g/mL}$.

mL across 3 different solvents. The selected plants are varied significantly at $p < 0.05$ (Table 4). *N. arbor-tristis* show a higher scavenging activity than other plants in all solvents. *Euphorbia hirta* also showed a moderate scavenging activity in this assay.

The observed differences between DPPH and nitric oxide scavenging activities in the studied plants indicate that antioxidant effects are mechanism-dependent rather than uniform across all assays. The DPPH assay evaluates the capacity to donate hydrogen atoms or electrons, whereas the nitric oxide scavenging assay assesses the ability of compounds to inhibit reactive nitrogen intermediates (28). These variations in activity suggest that specific plant compounds may exhibit greater efficacy against types of radicals. Consequently, reliance on a single assay may not provide a comprehensive assessment of antioxidant potential, as different compounds demonstrate variable reactivity depending on the radical species involved. The results from both assays show that *E. hirta* and *N. arbor-tristis* are rich natural sources of antioxidant compounds; the substantial free radical-scavenging capabilities of *A. spinosus* and *T. occidentalis* support their traditional therapeutic and nutritional significance. These results lend credence to their potential use in developing natural antioxidant compositions for medicine and nutraceuticals.

Gas chromatography-mass spectrometry profiling analysis of the methanolic extract of the edible plant leaves

Through GC-MS analysis, numerous therapeutically significant chemicals were found in the methanol extract of the leaves of *N. arbor-tristis*, *E. hirta*, *A. spinosus* and *T. occidentalis*. Chemical compounds with molecular weight and formula at varied

Table 4. IC_{50} values of the selected edible plants' extracts in different solvents using the DPPH and nitric oxide scavenging activity (NOSA) method

Sl. No.	Plants	DPPH IC_{50} ($\mu\text{g/mL}$)			NOSA IC_{50} ($\mu\text{g/mL}$)		
		ME	CE	AE	ME	CE	AE
1.	<i>N. arbor-tristis</i>	49.21 ± 0.05^b	68.51 ± 0.12^a	53.07 ± 0.06^c	44.15 ± 0.2^c	52.3 ± 0.05^d	58.08 ± 0.06^d
2.	<i>E. hirta</i>	42.34 ± 0.01^c	63.67 ± 0.05^c	87.24 ± 0.2^a	60.6 ± 0.02^a	69.45 ± 0.02^a	64.34 ± 0.05^b
3.	<i>T. occidentalis</i>	50.01 ± 0.02^b	64.84 ± 0.06^b	66.69 ± 0.02^b	50.7 ± 0.02^b	57.95 ± 0.01^c	62.87 ± 0.14^c
4.	<i>A. spinosus</i>	68.91 ± 0.011^a	59.53 ± 0.22^d	11.68 ± 0.06^d	60.78 ± 0.08^a	65.84 ± 0.02^b	74.37 ± 0.03^a

Results are expressed as mean $\pm$ SD ($n = 3$); DPPH: 2,2-diphenyl-1-picrylhydrazyl; NOSA: nitric oxide scavenging assay expressed in $\mu\text{g/mL}$; IC_{50} : inhibitory concentration; ME: methanolic extract; CE: chloroform extract; and AE: aqueous extract. Post hoc comparisons were conducted using Tukey's HSD test. Distinct letters indicate statistically significant differences among plant samples within each solvent group ($p < 0.05$).

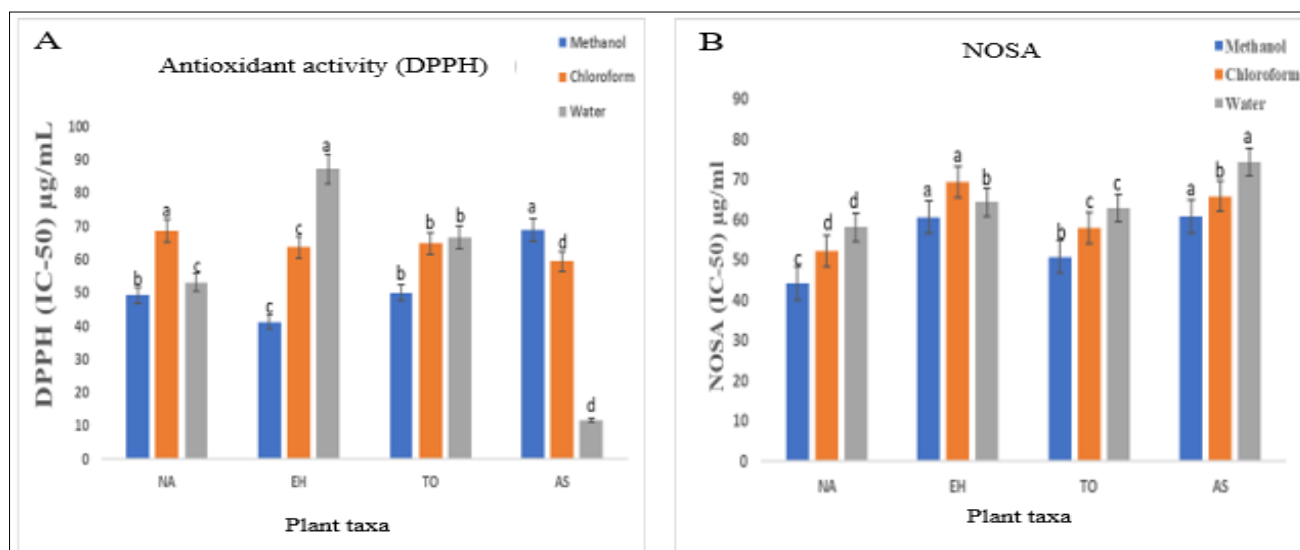


Fig. 4. Comparison of antioxidant activity of selected edible plant extracts using (A) DPPH and (B) nitric oxide scavenging assays (NOSA). The plant species included *Euphorbia hirta* (EH), *Nyctanthes arbor-tristis* (NA), *Thuja occidentalis* (TO) and *Amaranthus spinosus* (AS). Extracts were prepared using methanol, chloroform and aqueous solvents. Antioxidant activity was expressed as IC_{50} values. Values are presented as mean $\pm$ standard deviation (SD) of three independent experiments ($n = 3$). Post hoc comparisons were conducted using Tukey's HSD test. Different letters indicate statistically significant differences among plant samples within each solvent group ($p < 0.05$).

retention times of four distinct plants were listed in Table 6–9. A GC-MS analysis of the methanolic extract and antioxidant activity revealed bioactive substances with notable antioxidant potential. The GC-MS analysis revealed that each species has a distinctive phytochemical fingerprint, shedding light on why their antioxidant activities differ. In *E. hirta*, the presence of 1,25-dihydroxyvitamin D₃, doconexent, 2,5-octadecadiynoic acid methyl ester and thymol derivatives highlight a rich tapestry of bioactive compounds, spanning phenolic and terpenoid-like molecules. Renowned for their roles in modulating redox reactions and neutralising free radicals, these compounds likely work in concert to deliver the heightened flavonoid-driven antioxidant power observed in this species (33).

In *N. arbor-tristis*, the detection of 3,5-dimethoxycinnamic acid, panaxydol and benzaldehyde derivatives reveals a collection of phenylpropanoid and aromatic compounds celebrated for their antioxidant and anti-inflammatory properties. These molecules excel at stabilising free radicals through resonance and electron donation, which may underpin the plant's antioxidant activity, even though they occur at lower concentrations than in other species (34). *Thuja occidentalis* was found to contain eicosapentaenoic acid, 2,5-octadecadiynoic acid methyl ester and thymol derivatives, showing a mix of unsaturated fatty acids and terpenoid compounds. These substances are known to boost antioxidant activity by directly scavenging radicals and modulating oxidative pathways, which could explain their consistent results across various antioxidant tests. *Amaranthus spinosus* stood out with abundant bioactive compounds such as eicosapentaenoic acid, 4-methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione and carvacrol derivatives, each renowned for potent antioxidant and radical-quenching abilities (35). This diverse chemical arsenal likely drives its impressive DPPH activity, despite only moderate TPC and TFC values, hinting that non-phenolic compounds and their interactions play a crucial role in its antioxidant strength.

In summary, these findings reveal that the antioxidant power of these plants is shaped more by the diversity of their phytochemicals than by sheer amounts of phenolics or flavonoids. This underscores the value of GC-MS profiling in unravelling the unique antioxidant signatures revealed by different assays.

High-performance thin-layer chromatography analysis of the methanolic extract of the edible plant leaves

Densitometric quantification of quercetin

The HPTLC analysis was used to fingerprint and separate the phytochemical components of the methanolic extracts of *N. arbor-tristis*, *E. hirta*, *A. spinosus* and *T. occidentalis*, as well as standard reference chemicals such as quercetin. The R_f values of phytochemicals vary depending on the solvent system. The polarity of phytochemicals can be determined and a suitable solvent system can be chosen for efficient separation and isolation of pure bioactive compounds based on differences in R_f values. The distance travelled by the solute divided by the distance travelled by the solvent yields the R_f value, which shows the mobility of compounds on the plate. Various solvent mixtures were tested through trial and error and in this study, the chosen mobile phase was a mixture of toluene, ethyl acetate and formic acid in a 6:4:0.3 (V/V) ratio.

Quercetin was successfully and efficiently separated using this mobile phase. The quercetin peak was identified by comparing the standard with R_f = 0.56 (Fig. 5A and B). Accuracy was assessed

using recovery studies. The linearity range of the curve was 20–120 ng/spot. The linearity of the calibration plot ($R^2 = 0.994$), indicating a strong correlation between quercetin concentration and its corresponding peak area, demonstrates the procedure's accuracy and reliability. The traditional quercetin linear regression data are presented in Fig. 5C. The results demonstrated exceptional precision and reproducibility with an RSD percentage of 0.47 %. It was determined that the LOQ was 96.10 ng/spot and the LOD was 31.7 ng/spot. It indicates that the method's sensitivity was sufficient to identify and measure quercetin at trace levels. Table 5 displays the validation parameters for the developed HPTLC method. At three concentration levels (50 %, 100 % and 150 %), the average percentage recovery ranged from 96.84 % to 99.26 %. The recovery values further demonstrate the reliability of the accepted technique for quantifying quercetin in plant extracts. The improved method provides better separation, resolution, accuracy and repeatability, as well as faster analysis. Additionally, its simple sample preparation, cost-effectiveness, minimal solvent requirement and capability to analyse multiple herbal samples simultaneously make HPTLC a preferred method.

In the present study, quercetin content was quantified in the methanolic extract of some plants. The chromatogram demonstrating the separation of quercetin using methanol extracts of four plant species is shown in Fig. 5. It shows that *E. hirta* contains quercetin 98.08 ng/mg of methanolic extract (Fig. 5D), 21.57 ng/mg present in *N. arbor-tristis* (Fig. 5E), 24.86 ng/mg of methanolic extract present in *T. occidentalis* (Fig. 5F) and *A. spinosus* contains quercetin 28.04 ng/mg of methanolic extract (Fig. 5G). These results contribute to a better understanding of quercetin's distribution among these four plants and its potential health benefits associated with different plant components. Humans eat a wide range of plant leaves across the country. To help evaluate health benefits and counsel consumers on dietary choices, the current study presents the quantitative distribution of quercetin, a major flavonoid, across different components (5).

Table 5. Validation parameters for quercetin estimation

Parameters	Results
	Quercetin
Linearity range (ng)	20–120 ng/band
Correlation coefficient (r ²)	0.99
Precision (%RSD)	0.47
LOD (ng/spot)	31.7
LOQ (ng/spot)	96.10
Accuracy (%Recovery)	96.84–99.26
Robustness (%RSD)	
Mobile Phase (10 ± 2 mL)	0.12
Saturation time (20 ± 5 min)	0.157
Specificity	Specific (R _f match, no overlapping peaks)

Conclusion

This study compares the phytochemical composition and antioxidant potential of several wild edible plants, revealing distinct interspecies differences. *Nyctanthes arbor-tristis* and *Euphorbia hirta* exhibited significantly higher concentrations of phenolics and flavonoids, which correlated with their pronounced antioxidant activity. *Amaranthus spinosus* and *N. arbor-tristis* demonstrated the lowest IC₅₀ values, indicating the greatest efficacy in neutralising free radicals. Gas chromatography–mass spectrometry (GC-MS) analysis identified key bioactive compounds, while high-performance thin-layer chromatography (HPTLC) profiling established that *E. hirta* contained the highest quercetin content, designating it as the

Table 6. Secondary metabolites found in the methanolic extract of *Euphorbia hirta* leaves were analysed using gas chromatography–mass spectrometry (GC-MS)

Sl. No.	RT	Compound names	Peak area (%)	Molecular formula
1.	6.73	2,5-Octadecadiynoic acid, methylester	2.56	C ₁₉ H ₃₀ O ₂
2.	6.73	Doconexent	2.56	C ₂₂ H ₃₂ O ₂
3.	6.73	Methyl 10,12-octadecadiynoate	2.56	C ₁₉ H ₃₀ O ₂
4.	19.62	Pterin-6-carboxylic acid	3.52	C ₇ H ₅ N ₅ O ₃
5.	19.62	à-d-Glucofuranosyl benzenesulfonate	3.52	C ₁₂ H ₁₆ O ₈ S
6.	19.62	1-Fluoro-2-methyl-1-(N-methyl-N-phenylamino)-1,3-butadiene	3.52	C ₁₂ H ₁₄ FN
7.	20.10	1,25-Dihydroxyvitamin D3, TMS derivative	3.30	C ₃₀ H ₅₂ O ₃ Si
8.	20.48	5,7-Dodecadiyn-1,12-diol	3.09	C ₁₂ H ₁₈ O ₂
9.	22.83	Malonic acid, mononitrile, monothioamide, 2-(3-methyl-2-thienylmethylene)	3.35	C ₉ H ₈ N ₂ S ₂
10.	22.83	Thymol, TBDMS derivative	3.35	C ₁₆ H ₂₈ OSi
11.	22.83	Melezitose	3.35	C ₁₈ H ₃₂ O ₁₆
12.	23.00	4-Methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione	3.69	C ₁₀ H ₇ NO ₆
13.	23.12	Silane, triethyl(2-phenylethoxy)-	2.48	C ₁₄ H ₂₄ OSi
14.	23.12	Acetic acid, 2-[[3-cyano-4-(methoxymethyl)-6-methyl-2-pyridinyl] thio]-	2.48	C ₁₁ H ₁₂ N ₂ O ₃ S
15.	25.01	Cyclotrisiloxane, hexamethyl-	2.99	C ₆ H ₁₈ O ₃ Si ₃
16.	25.01	1-(2-Acetoxyethyl)-3,6-diazahomoadamantan-9-one oxime	2.99	C ₁₃ H ₂₁ N ₃ O ₃
17.	25.07	1,4-Bis(trimethylsilyl)benzene	2.96	C ₁₂ H ₂₂ Si ₂
18.	25.41	Cyclopentene	5.37	C ₁₆ H ₃₂ O ₂ Si ₂

RT: retention time.

Table 7. Secondary metabolites found in methanolic extract of *Thuja occidentalis* leaves were analysed using gas chromatography–mass spectrometry (GC-MS)

Sl. No.	RT	Compound names	Peak area (%)	Molecular formula
1.	5.59	8,11-Octadecadiynoic acid, methylester	2.80	C ₁₉ H ₃₀ O ₂
2.	5.59	Pterin-6-carboxylic acid	2.80	C ₇ H ₅ N ₅ O ₃
3.	5.59	Methyl 10,12-octadecadiynoate	2.80	C ₁₈ H ₂₈ O ₂
4.	9.12	cis-5,8,11,14,17-Eicosapentaenoic acid	2.51	C ₂₀ H ₃₀ O ₂
5.	9.12	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	2.51	C ₁₈ H ₃₀ O ₂
6.	14.64	Cyclopropaneacetic acid, 2-hexyl-	2.95	C ₁₁ H ₂₀ O ₂
7.	14.64	d-Glycero-l-gluco-heptose	2.95	C ₇ H ₁₄ O ₇
8.	16.96	Methyl 5,7-hexadecadiynoate	2.59	C ₁₇ H ₂₆ O ₂
9.	16.36	à-D-Glucopyranose, 1-thio-, 1-(N-hydroxybenzenepropanimidate)	3.08	C ₁₅ H ₂₁ NO ₆ S
10.	17.49	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	2.92	C ₂₅ H ₃₆ O ₂
11.	17.49	Benzenamine, 3-azido-N,N-dimethyl-4-nitro-	2.92	C ₈ H ₉ N ₅ O ₂
12.	18.03	5,7-Dodecadiyn-1,12-diol	2.69	C ₁₂ H ₁₈ O ₂
13.	18.37	2-Bromotetradecanoic acid	4.29	C ₁₄ H ₂₇ BrO ₂
14.	18.37	à-d-Glucofuranosyl benzenesulfonate	4.29	C ₁₂ H ₁₆ O ₈ S
15.	21.61	Cyclobarbitol	2.81	C ₁₂ H ₁₆ N ₂ O ₃
16.	22.91	Thymol, TBDMS derivative	3.08	C ₁₆ H ₂₈ OSi
17.	22.91	d-Mannitol, 1-decylsulfonyl	3.08	C ₁₆ H ₃₄ O ₇ S
18.	26.26	1,4-Bis(trimethylsilyl)benzene	4.09	C ₁₂ H ₂₂ Si ₂
19.	25.36	Cyclotrisiloxane, hexamethyl	4.26	C ₆ H ₁₈ O ₃ Si ₃

RT: retention time.

Table 8. Secondary metabolites found in methanolic extract of *Nyctanthes arbor-tristis* leaves were analysed using gas chromatography–mass spectrometry (GC-MS)

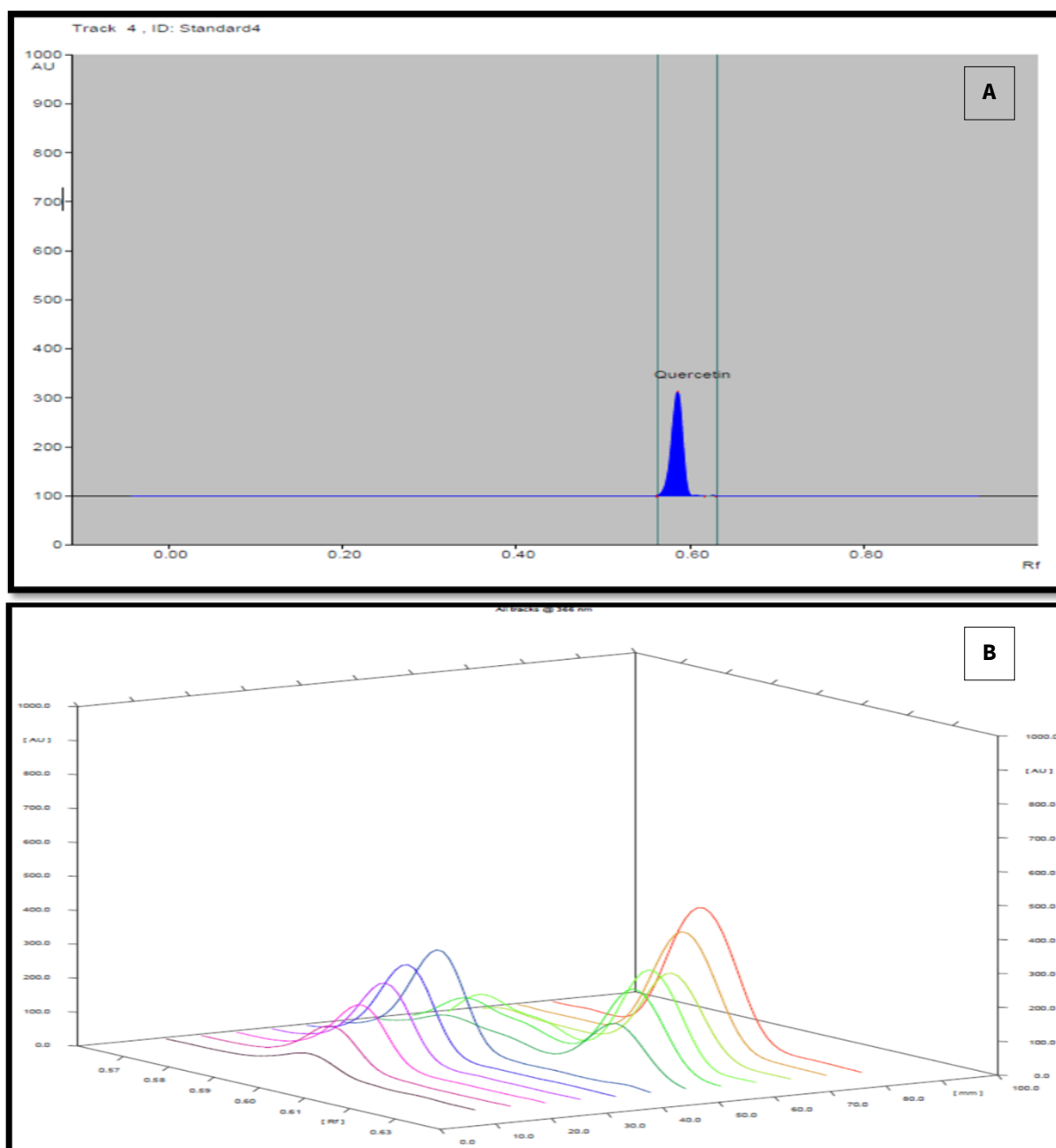
Sl. No.	RT	Name of the compound	Peak area (%)	Molecular formula
1.	3.21	à-N-Normethadol	3.07	C ₂₀ H ₂₇ NO
2.	5.71	cis-5,8,11,14,17-Eicosapentaenoic acid	2.62	C ₂₀ H ₃₀ O ₂
3.	7.11	Panaxydol	2.61	C ₁₇ H ₂₄ O ₂
4.	16.98	1-Octanol, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluor	2.95	C ₈ H ₃ F ₁₅ O
5.	16.98	Geranyl vinyl ether	2.95	C ₁₂ H ₂₀ O
6.	18.37	2H-Pyran, 2-(7-heptadecyloxy) tetrahydro-	2.77	C ₂₂ H ₄₀ O ₂
7.	24.14	Thymol, TBDMS derivative	2.58	C ₁₆ H ₂₈ OSi
8.	24.14	Carvacrol, TBDMS derivative	2.58	C ₁₆ H ₂₈ OSi
9.	24.14	1,2-Benzisothiazol-3-amine, TBDMS derivative	2.58	C ₁₃ H ₂₀ N ₂ SSi
10.	24.66	Silane, triethyl(2-phenylethoxy)-	2.67	C ₁₄ H ₂₄ OSi
11.	24.82	Cyclotrisiloxane, hexamethyl-	2.63	C ₆ H ₁₈ O ₃ Si ₃
12.	25.19	Benzaldehyde, 2-nitro-4-trimethylsilyl-	2.62	C ₁₀ H ₁₃ NO ₃ Si
13.	25.19	3,5-Dimethoxycinnamic acid	2.62	C ₁₁ H ₁₂ O ₄
14.	28.84	1-(2-Acetoxyethyl)-3,6-diazahomoadamantan-9-one oxime	3.32	C ₁₃ H ₂₁ N ₃ O ₃
15.	26.63	Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl-	2.56	C ₈ H ₂₂ O ₃ Si ₂

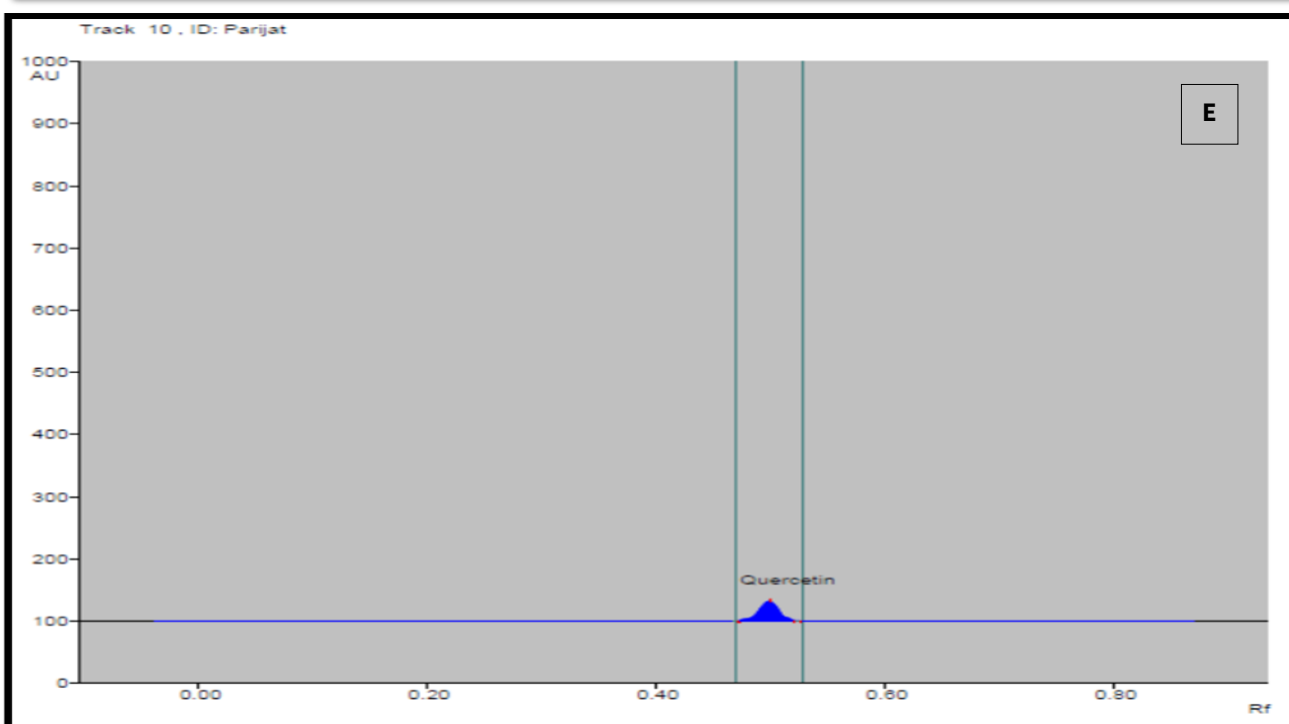
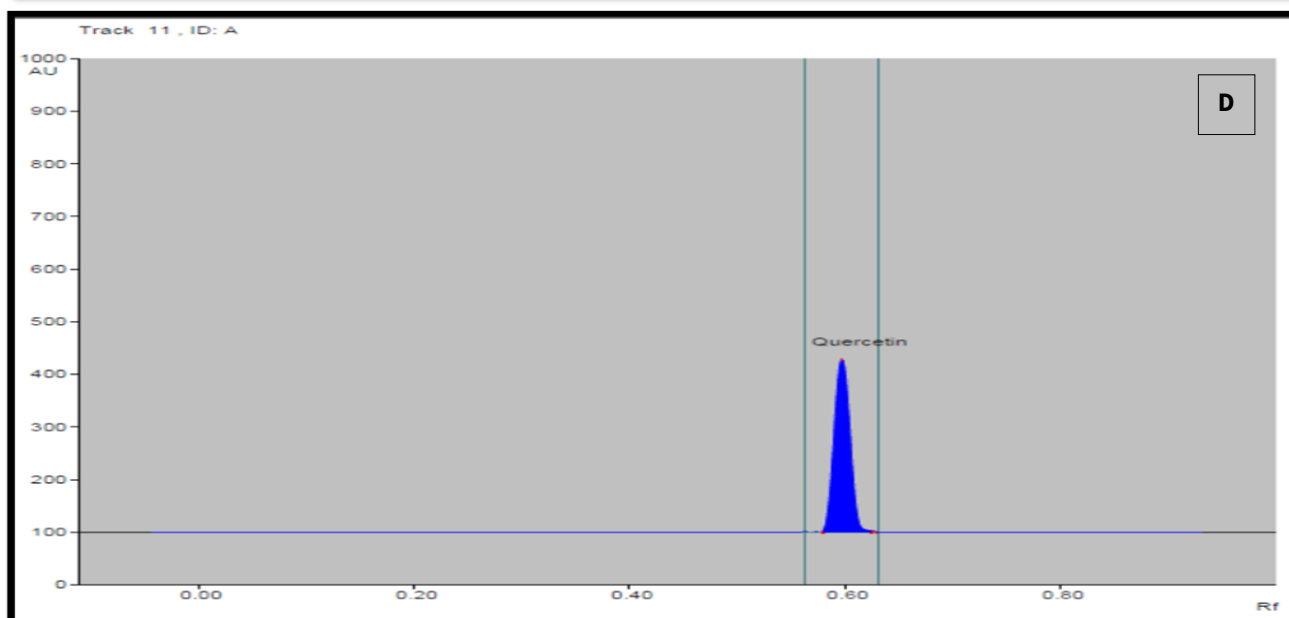
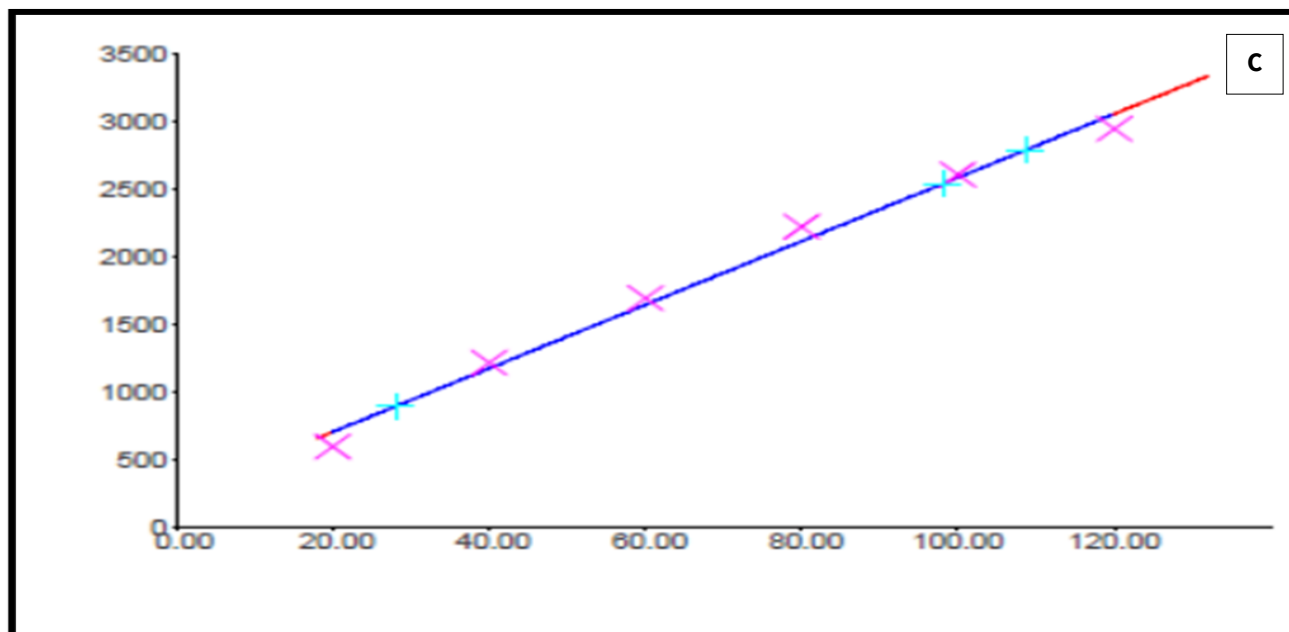
RT: retention time.

Table 9. Secondary metabolites found in methanolic extract of *Amaranthu spinosus* leaves were analysed using gas chromatography-mass spectrometry (GC-MS)

Sl. No.	RT	Compound names	Peak area (%)	Molecular formula
1.	3.14	Z,Z,Z-4,6,9-Nonadecatriene	2.89	C ₁₉ H ₃₄
2.	3.76	8,11-Octadecadiynoic acid, methylester	2.78	C ₁₉ H ₃₀ O ₂
3.	11.33	Methyl 3,5-tetradecadiynoate	2.27	C ₁₅ H ₂₂ O ₂
4.	16.36	1-Fluoro-2-methyl-1-(N-methyl-N-phenylamino)-1,3-butadiene	2.80	C ₁₂ H ₁₄ FN
5.	16.87	Bicyclo[3.1.1] hept-3-ene-spiro-2,4'-(1',3'-dioxane), 7,7-dimethyl-	2.66	C ₁₂ H ₁₈ O ₂
6.	16.87	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3 α ,5Z,7E)	2.66	C ₂₇ H ₄₄ O ₃
7.	20.09	2-Bromotetradecanoic acid	2.31	C ₁₄ H ₂₇ BrO ₂
8.	24.34	4-Methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione	4.54	C ₁₀ H ₇ NO ₆
9.	24.34	Carvacrol, TBDMS derivative	4.54	C ₁₆ H ₂₈ OSi
10.	24.92	Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl-	2.36	C ₈ H ₂₆ O ₃ Si ₄
11.	24.59	Cyclotrisiloxane, hexamethyl	2.62	C ₆ H ₁₈ O ₃ Si ₃
12.	24.34	2,2,4,4-Tetramethyl-6-(1-oxo-3-phenylprop-2-enyl)-cyclohexane-1,3,5-trione	4.54	C ₁₉ H ₂₀ O ₄
13.	29.25	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	4.50	C ₁₃ H ₂₂ OSi ₂
14.	24.92	Carvacrol, TMS derivative	2.36	C ₁₃ H ₂₂ OSi
15.	25.60	Tris (tert-butyldimethylsilyloxy)arsane	2.63	C ₁₈ H ₄₅ AsO ₃ Si ₃

RT: retention time.





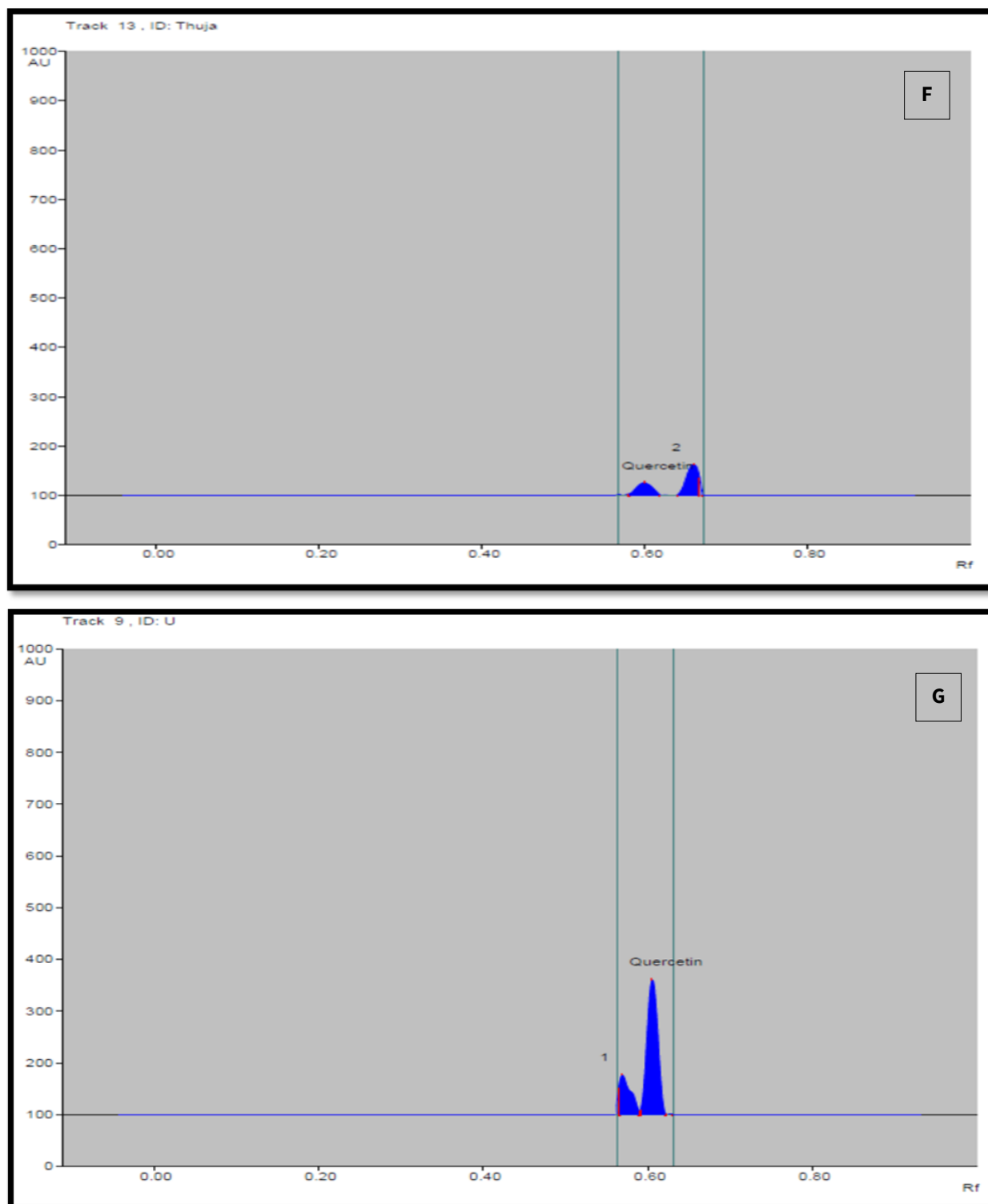


Fig. 5. HPTLC analysis of quercetin in selected plant extracts: (A) densitometric chromatogram of quercetin scanned at 366 nm; (B) 3D spectra of quercetin and *Euphorbia hirta* extract; (C) linearity plot of quercetin; (D–G) densitograms of quercetin in methanolic extracts of *Euphorbia hirta* (EH), *Nyctanthes arbor-tristis* (NA), *Thuja occidentalis* (TO) and *Amaranthus spinosus* (AS), respectively.

most potent species in this group. These findings indicate that these underutilised wild edible plants represent promising natural sources of antioxidants with potential applications in nutraceutical and pharmaceutical products. However, as these results are based solely on *in vitro* assays, further research is required to isolate individual compounds, assess their bioavailability and evaluate their effects *in vivo*.

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Authors' contributions

PB and AA conceived and designed the research. PB and AA collected, assembled, interpreted and analysed the data. PB and AA drafted the manuscript, while AA critically revised the manuscript for important intellectual content. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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