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RESEARCH ARTICLE

Phytochemical screening and chromatographic (HPLC and GC) analysis of leaves of *Cycas revoluta* Thunb cultivated in Iraq

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Abstract

Cycas revoluta Thunb is considered the pervasive species of the genus *Cycas* is known for its diverse phytochemicals. This study employed Gas Chromatography-Mass Spectrometry (GC-MS) and High Performance Liquid Chromatography (HPLC) to characterize the phytochemical composition of the plant's leaves. Leaves had been dried and subsequently extracted with solvents of n-hexane and ethyl acetate and the so-obtained fractions were subjected to qualitative screening of their phytochemicals. These two extracts were screened for the presence or absence of compounds by utilizing the tests for these compounds. These tests unveiled the presence of compounds in ethyl acetate extract, which are sterol, phenolic acid, terpenoid, tannin and flavonoid, whereas only terpenoid and sterol compounds have been detected as positive in n-hexane fraction. It's worth mentioning that the absence of alkaloids in both leaf extracts contrasts with some previous reports on *Cycas revoluta*, which showed positive results when Wagner's reagent was added. GC-MS and HPLC analyses confirmed the presence of the various phytochemicals' classes in the extract. The chloroform and n-butanol fractions were not studied in this research, so it's necessary to analyze these two samples by chromatographic techniques in the future. This work highlights the importance of *C. revoluta* as a source of bioactive compounds for further research.

Keywords: *Cycas revoluta*; ethyl acetate extract; GC-MS; HPLC; n-hexane extract; phytochemicals

Introduction

The *Cycadaceae* family is considered one of the largest among cycads. It comprises 120 species under the only genus *Cycas* (1). *Cycas* is the solitary extant genus of the family *Cycadaceae,* order *Cycadales*. *Cycas revoluta* is the foremost prevalent species of the genus *Cycas*, often referred to as king sago palm or sago cycas (2). *Cycas revoluta* is a gymnosperm found in the tropical and subtropical countries that takes a long time to grow, as well as in xerophytic environments such as exposed slopes with limited water availability.

It is prevalent in Polynesia, India, Australia, Myanmar, Malaysia, Japan, China and the Ryukyu Islands. In certain places, it is cultivated as an attractive plant. Furthermore, it thrives in ultramafic, volcanic, sandy or waterlogged soils within forest and grassland ecosystems, flourishing in full sun, partial shade or shaded environments (3).

The seeds of *C. revoluta* are utilized in China as a tonic, an expectorant and anti-rheumatic. The stalks are used as astringent and diuretic. The young leaves are beneficial for alleviating flatulence and vomiting. It used to relieve dizziness, sore throat and headaches (4). In addition, the plant is used to address ailments such as cancer, hepatoma, diarrhea, hemorrhoids, wounds and dysentery (5).

It has been shown that the extract of *C. revoluta* demonstrated a recognized antioxidant effect (4). Furthermore, another study found that the extract of the plant has effectively suppressed the growth of gastric cancer and increased anti-cancer effect of 5-Fu (6).

About 68 compounds have been extracted from various parts (seeds, male cones, pollen grains and leaves) of *C. revoluta*, comprising 15 non-protein amino acids, 9 glucosides, 8 fatty acids, 3 benzenoids, 2 terpenes, 1 amino acid, 1 diterpenoid, 1 triterpenoid, 1 sterol, 1 ester and 1 steroid, 25 flavonoids and female cones (3).

This study aims to analyze the phytochemicals of *C. revoluta* Thunb. leaves by using GC-MS and HPLC to find bioactive compounds in both n-hexane and ethyl acetate fractions.

Materials and methods

Collect and extract the leaves of *Cycas revoluta* plant

C. revoluta Thunb from the Cycadaceae family is authenticated by Associate Professor Zainab Abd-oun Ali, Biology Department/ College of Sciences/Baghdad University, after which the entire plant was collected from a nursery in Al Najaf City in September 2021 (Fig. 1), at room temperature, the leaves dried for 21 days in shade (Fig. 2) and then ground into the powder in order to the extraction of phytochemical constituents.

Fig. 1. Picture of Iraqi *C. revoluta* Thunb plant.

Fig. 2. Picture of Iraqi *C. revoluta* dry leaves.

After that, 85 g of this dried powder was macerated to defat the ground with n-hexane (Sisco Research Laboratories/India) for 48 hr. Then, by using a rotary evaporator (HAHNSHIN s&t co. ltd/Korea), the n-hexane solvent was evaporated to obtain the n-hexane extract for GC-MS analysis. Then, dry the powder to get rid of any remaining n-hexane solvent. Following that, the dried ground leaves were extracted with 250 mL of 85 % ethanol by (MERCH KGaA/GERMANY) by Soxhlet (Borosil Glass Works Ltd.) for 14 hr. Then, collect the extract and filter it with Whatman filter paper (No.1). By utilizing a rotary evaporator, the filtrate that have been extracted was evaporated (7).

Fractionation procedures were performed since each solvent may dissolve the compounds that resemble or match its polarity; this ability results from the principle of (like dissolves like) and was used in this procedure to fractionate and separate the active components according to their polarity (8). The aqueous layer was partitioned using chloroform (Central Drug House (P) Ltd., India; 100 mL × 2) and ethyl acetate (Thomas Baker, India 100 mL × 2) and n -butanol (Scharlab S.L., Spain 100 mL × 2) in that order. The fractions were then filtered, evaporated in the filtrate's solvent using a rotary evaporator (Hahnshin S&T Co. Ltd., Korea) and finally weighed and labeled individually based on the solvent employed (9).

Screening of compounds of n-hexane and ethyl acetate extracts

Screen the extract for the presence or absence of the secondary metabolites by utilizing the tests that depend on precipitate or coloration formation to qualitatively assess the extract and identify which classes of these secondary metabolites are present (alkaloids, flavonoids, terpenes, etc.) (10,11).

Test for alkaloids

In a test tube, add 3 drops of the Wagner's reagent to 1 mL of both extracts to form a brown precipitate. This result indicate the presence of alkaloids (10).

The positive result of alkaloid identification in Dragendorrf test was identified as orange precipitate. The precipitate come from complex compound of potassium alkaloid in Dragendorff reagent. Into the alkaloid identification nitrogen acted to form covalent coordination bond with potassium ion (K^+) (metal ion). Orange color precipitate with the reagent of Dragendorff reagent (12).

Test for flavonoids

NaOH test: The extract (5 mL) was treated with aqueous NaOH, looking for the formation of a yellow orange color after add HCL the color disappears (13).

Steroids tests

Test of Liebermann-Burchard: chloroform, acetic anhydride and drops of sulphuric acid (Hangzhou Hyper Chemicals, China) were added to the 3 mL of the extract. The formation of bluish green show that the extract contain steroid compound (14).

Tannins and phenolic compound test

Add 3 mL of ferric chloride [$FeCl_3$ solution (5 % w/v)] to 3 mL of the filtrate that comes from 10 mg of the plant in 10 mL of distilled water. when the blue black of dark green precipitate form, this result mean that the extract contain tannins and phenolic compounds (15).

Test for terpenoids

2 mL of the extract of the sample was solubilized with 2 mL of chloroform solvent, then evaporated to dryness. The next step is the addition of 2 mL of sulphuric acid and heating for 2 min. When the reddish brown color appeared, this is an indication that the extract contains terpenoid (16).

Test for cardiac glycoside

Test of Keller-kiliani: About 1 mL of glacial acetic acid (BDH Ltd., England), concentrated sulfuric acid and ferric chloride was added to 2 mL of alcoholic extract. The end result is to form ring junction that have green blue color (17).

Saponin test (foam test)

Add distilled water to the extract of the plant then the suspension that will be formed was shaken for about 15 min in graduated cylinder. When the foam (about 1 cm) appear, this indicate that the sample contain saponins (17).

GC-MS of n-hexane fraction

For the identification of compounds present in the n-hexane leaf extract, which is a non-polar oily mixture, Gas Chromatography–Mass Spectrometry (GC–MS) was selected as the most suitable technique. The test of the sample with GC-MS was done at Ministry Industry and Minerals/Center of Ibn Al-Baitar Research. The instrument conditions settled in the following regimen:

Gas chromatograph: USA Agilent (7820A) GC analytical column and mass spectrometer, Agilent HP-5ms Ultra inert (30 m length x 250 µm inner diameter x 0.25 µm thickness of film).

Volume that injected 1 µL, Pressure 11.933 psi, Inlet line of GC at heat: 250 °C, Aux heaters at heat 300 °C, Helium 99.99 % was used as carrier gas, heat at the Injector site about 250 °C, range of the scan: m/z 25-1000, type of the injection: splitless, program of the oven: heat, Ramp1: 60 °C hold to 3 min. Ramp2: 60-180 °C: 7 °C/min.

Ramp3: 180-280 °C: 8 °C/min. Ramp4: 280 °C that held for 3 min.

Examination of HPLC

This technique is utilized for the definitive identification and quantification of the likely steroidal substances since it is a very sensitive, selective and effective qualitative/quantitative analytical approach. Peaks from the chemicals in the ethyl acetate extract and n-hexane extract were measured, evaluated and their retention durations were compared with standards under identical HPLC conditions. Since this study focused only on the qualitative identification of phytochemical constituents, without any quantitative measurements or comparative experiments, statistical analysis was not applicable and was therefore not carried out.

The HPLC examination was done at the Ministry Science and Technology/Environmental and the Department of Water Research.

Model of the HPLC system utilized was (SYKAM)/Germany, with quaternary model of the gradient pump (S2100), auto-sampler (S5200), UV detector type (S3240) and model of oven of the column (S4115).

For n-hexane that was extracted by the maceration method

Dissolve 10 mg of the n-hexane extract with grade methanol of HPLC (injected about 0.1 mL), the separation of the compound that found in the extract was performed by isocratic elution of the mobile phase (acetonitrile 60:distilled water 25:acetic acid 5) and a flow rate of 1 mL/min for 20 min, using a C18 column (25 cm × 4.6 mm), detector UV 280 nm, heat set on 52 °C and compounds injection was achieved by auto sampler.

The qualitative detection process involved comparing sample peak retention time peak and standard peak on the absorption spectrum (0.1 mL of the standards were used and they were examined at a concentration of 5 parts per million (ppm) under the same conditions as the fraction sample) (18,19).

For *Cycas revoluta* ethyl acetate leaves extract

Gradient elution method followed, with a mobile phase composed of eluent methanol and eluent 1 % formic acid in the water (v/v).

Volume injected of samples and standard 100 µL and 100 mg of sample of ethyl acetate extract dissolved with 3 mL of methanol. It is worth mentioning that the compounds injection was performed automatically by utilizing an auto-sampler and the UV at 280 nm (20).

Results

Preliminary tests

The phytochemical test is a very important step to evaluate the possible medicinal ingredients which are responsible for the known and unknown biological activities exhibited by the plants. Furthermore, it underlines targeted isolation of compounds and initiates precise investigations whether a defined group of compounds as a qualification process, is present or not (11). The preliminary screening of the phytochemicals results from the crude and the fractions listed in Table 1 and 2, respectively.

HPLC examination of n-hexane fraction

HPLC represents the best method to identify the presence of stigmasterol or betasterol or presence of both of them and by this method, the misleading of R_f value is eliminated. Moreover, HPLC is a very sensitive technique and gives reliable results even very low concentrations of the sample and standards.

Table 1. Preliminary assay of *C. revoluta* n-hexane leaves fractions

Phytochemical group	Result
Alkaloid	-
Sterol	+
Phenolic acid	-
Terpenoid	+
Saponin	-
Tannin	-

(+) represent the presence phytochemicals while (-) represents the phytochemical absence

Table 2. Preliminary assay of *C. revoluta* ethyl acetate leaves fractions

Phytochemical group	Result
Alkaloid	-
Sterol	+
Phenolic acid	+
Terpenoid	+
Saponin	-
Tannin	+
Flavonoid	+

(+,-) represent the presence and absence of phytochemicals, respectively

All the sample and standard analyses are in the same condition after that the retention time compared between each other. Both the fraction and standards undergo the same conditions when analyzed so the retention times compare between standards and the fraction to know if there are any similarities between each other. Fig. 3 shows the HPLC chromatogram of the n-hexane sample, whereas Fig. 4 and 5 present the HPLC chromatograms of the standards. Furthermore, the retention times of both standards and the n-hexane extract sample are listed in Table 3.

Examination of ethyl acetate extract by HPLC method

Examination of ethyl acetate fraction by HPLC technique shows six retention times/peaks which means the availability of six compounds within the ethyl acetate fraction. Fig. 6 shows the spectrum of HPLC of ethyl acetate sample, while Fig. 7–10 show the spectra of the standards. In addition, the retention times of the standards and ethyl acetate sample are listed in Table 4.

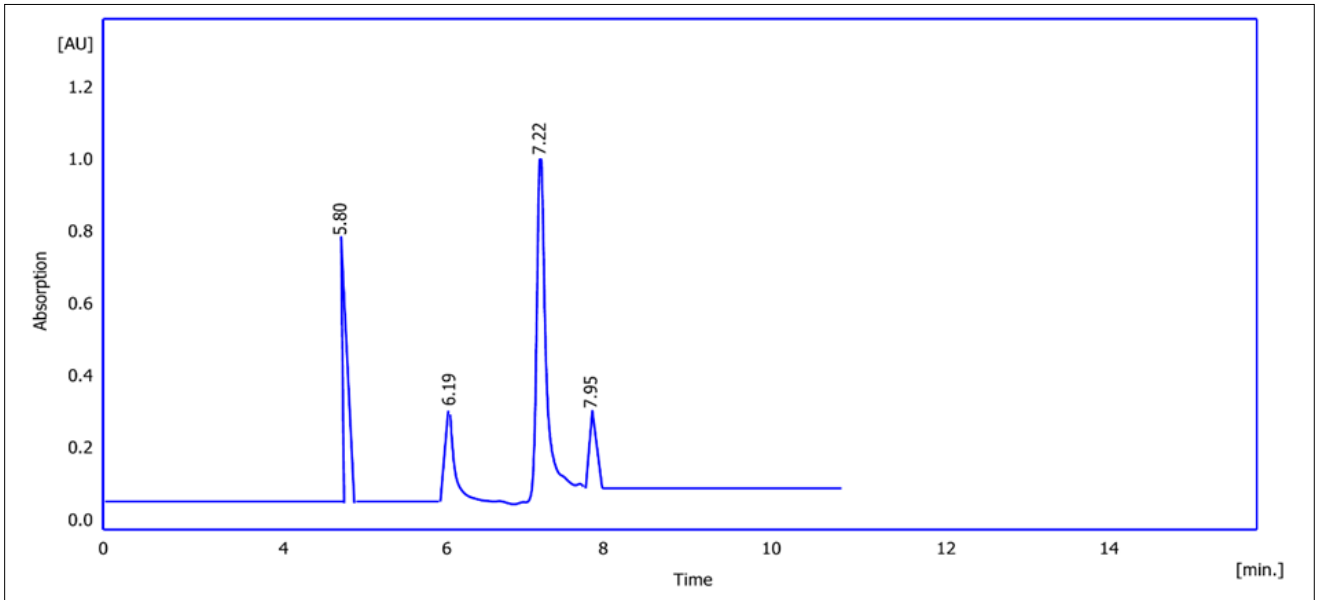
Identification of n-hexane fraction chemical components utilize GC-MS test

Gas chromatography of n-hexane extract demonstrates several compounds' content, as shown in Fig. 11. Table 5 illustrates the most important substance and their retention time.

Discussion

The phytochemical tests of ethyl acetate and n-hexane fractions of *C. revolute* Thunb leaves revealed that both extracts contain many natural compounds that may play a part in the medical properties of the plant. These results support the previous studies of the species of *Cycas*, which have shown a lot of phytochemicals such as terpenoids, sterols, flavonoids and fatty acids (21–23). The absence of alkaloids in both leaf extracts contrasts with some previous reports on *Cycas revoluta*, which gave positive results when the sample was tested with Wagner's reagent (24).

The positive Liebermann-Burchard test for steroids in both fractions suggests that phytosterols are present. This was documented by HPLC analysis in n-hexane fraction. However, The saponin foam test didn't show any results. The HPLC test on the n-

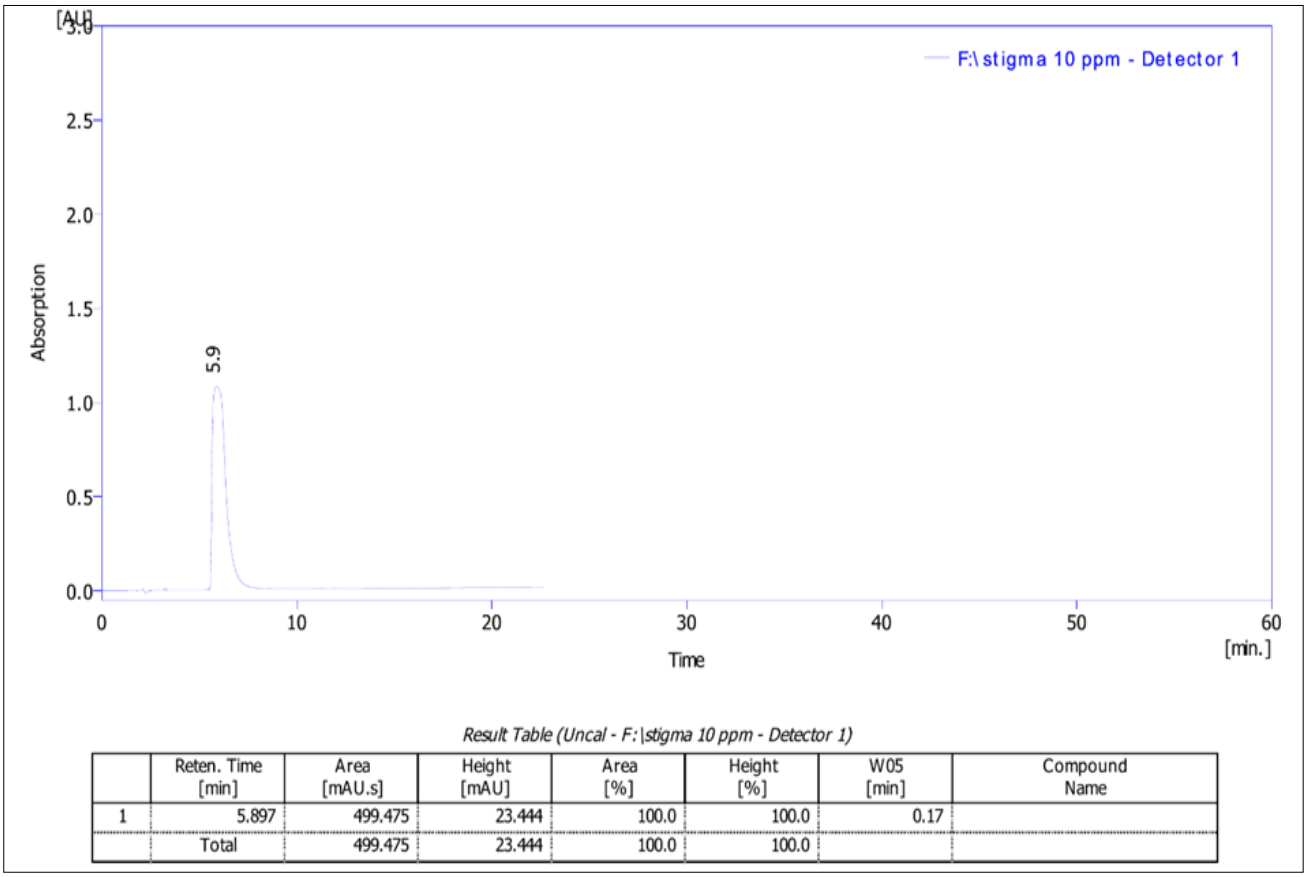


Result Table (Uncal - F:\ hexane fraction (fronds)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.80	4897.58	746.25	32.56	32.89	0.20	
2	6.19	659.22	325.65	19.25	19.24	0.14	
3	7.22	8142.02	1597.44	36.45	39.22	0.36	
4	7.95	459.58	95.44	7.48	6.00	0.04	
	Total	14158.40	2764.78	100.00	100.00		

Fig. 3. HPLC of *Cycas revoluta* n-hexane leaves extract.

By using the isocratic elution mobile phase which represented by distilled water 25: acetic acid 5 and acetonitrile 60 with 1 mL/min for 20 min flow rate, the separation of compounds that found in the sample was performed, utilizing column C18 column (25 cm x 4.6 mm), UV detector at 280 nm, heat at 52 °C and the injection of sample achieved by auto- sampler

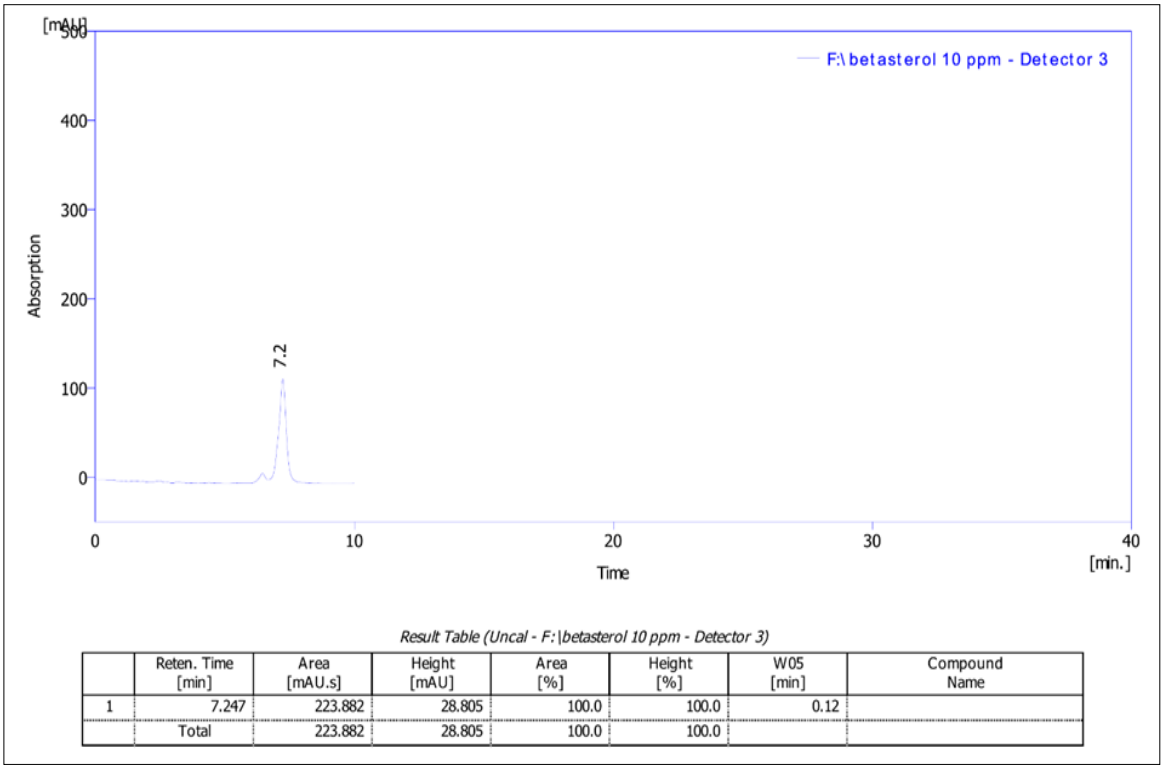


Result Table (Uncal - F:\stigma 10 ppm - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.897	499.475	23.444	100.0	100.0	0.17	
	Total	499.475	23.444	100.0	100.0		

Fig. 4. HPLC of stigmasterol standard.

By using the isocratic elution mobile phase which represented by distilled water 25: acetic acid 5 and acetonitrile 60 with 1 mL/min for 20 min flow rate, the separation of compounds that found in the sample was performed, utilizing column C18 column (25cm x 4.6 mm), UV detector at 280 nm, heat at 52 °C and the injection of sample achieved by auto- sampler



Result Table (Uncal - F:\betasterol 10 ppm - Detector 3)

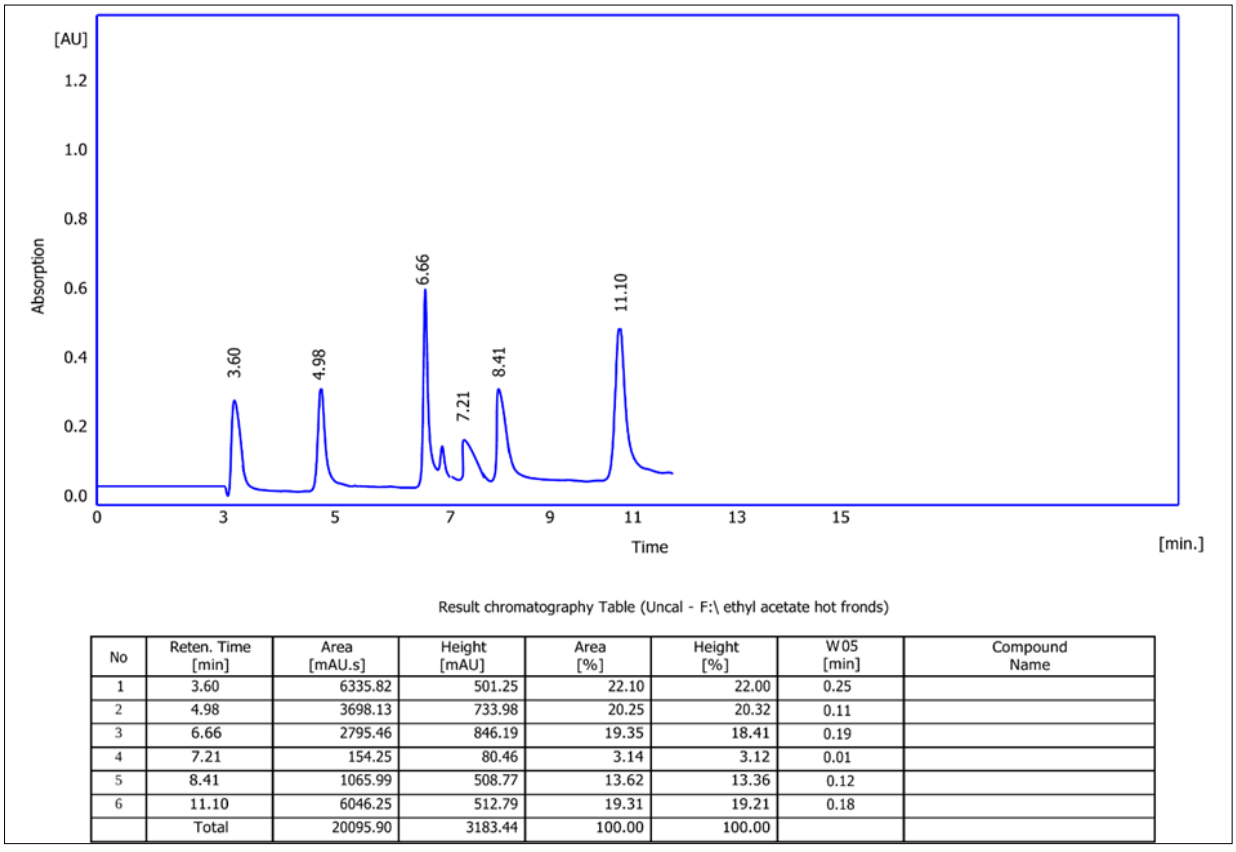
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.247	223.882	28.805	100.0	100.0	0.12	
	Total	223.882	28.805	100.0	100.0		

Fig. 5. HPLC of betasitosterol standard.

By using the isocratic elution mobile phase which represented by distilled water 25: acetic acid 5 and acetonitrile 60 with 1 mL/min for twenty min flow rate, the separation of compounds that found in the sample was performed, utilizing column C18 column (25 cm x 4.6 mm), UV detector at280 nm, heat at 52 °C and the injection of sample achieved by auto- sampler

Table 3. HPLC retention times of standard and n-hexane extract sample compounds

Compounds	n-hexane extract retention time	Standard retention time
Stigmasterol	5.8	5.897
Betasitosterol	7.22	7.247

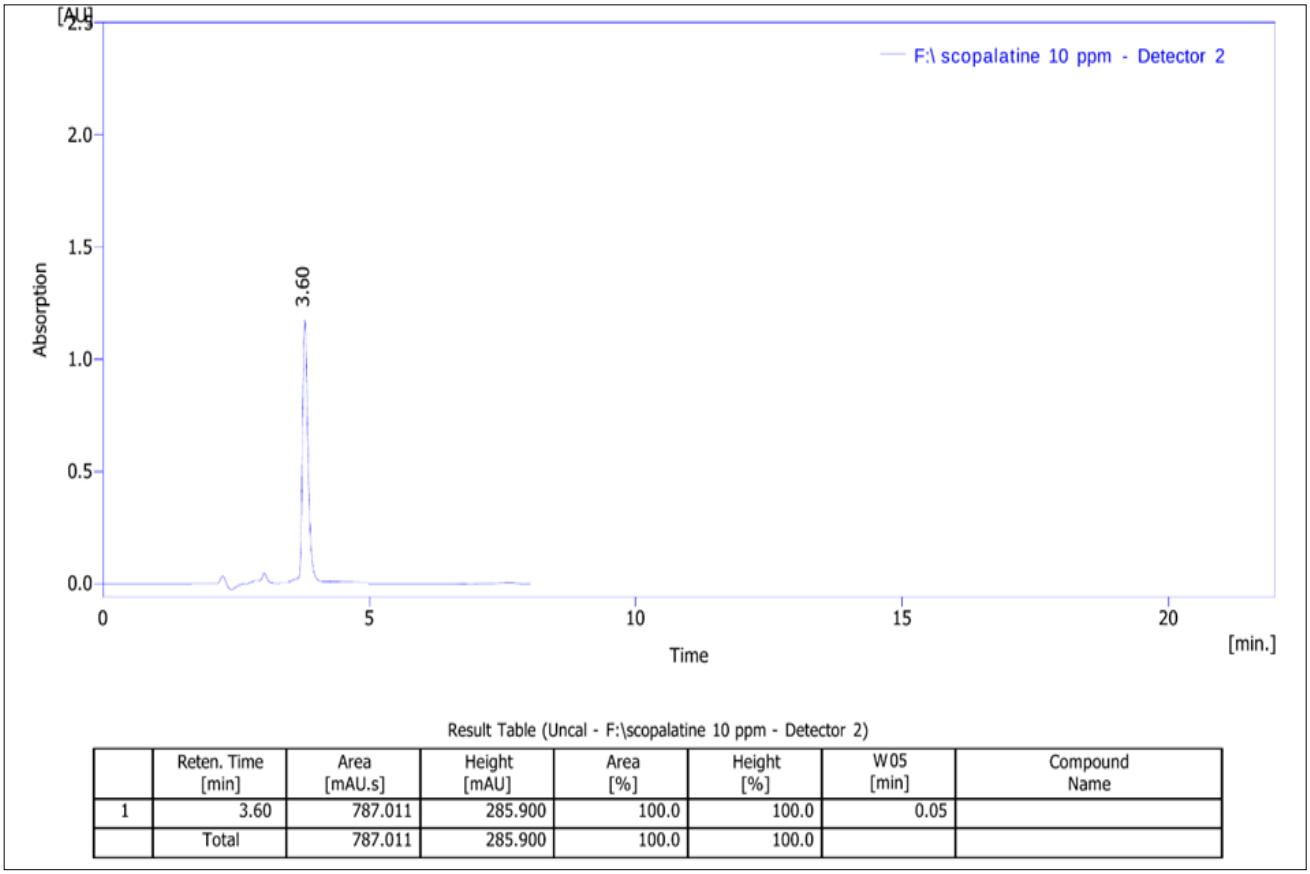


Result chromatography Table (Uncal - F:\ ethyl acetate hot fronds)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.60	6335.82	501.25	22.10	22.00	0.25	
2	4.98	3698.13	733.98	20.25	20.32	0.11	
3	6.66	2795.46	846.19	19.35	18.41	0.19	
4	7.21	154.25	80.46	3.14	3.12	0.01	
5	8.41	1065.99	508.77	13.62	13.36	0.12	
6	11.10	6046.25	512.79	19.31	19.21	0.18	
	Total	20095.90	3183.44	100.00	100.00		

Fig. 6. HPLC for sample ethyl acetate leaves extract.

By using C18OSD (25cm, 4.6mm) column, gradient elution with a mobile phase composed of eluent methanol and eluent 1 % formic acid in the water (v/v), the rate flow 0.7 mL/min

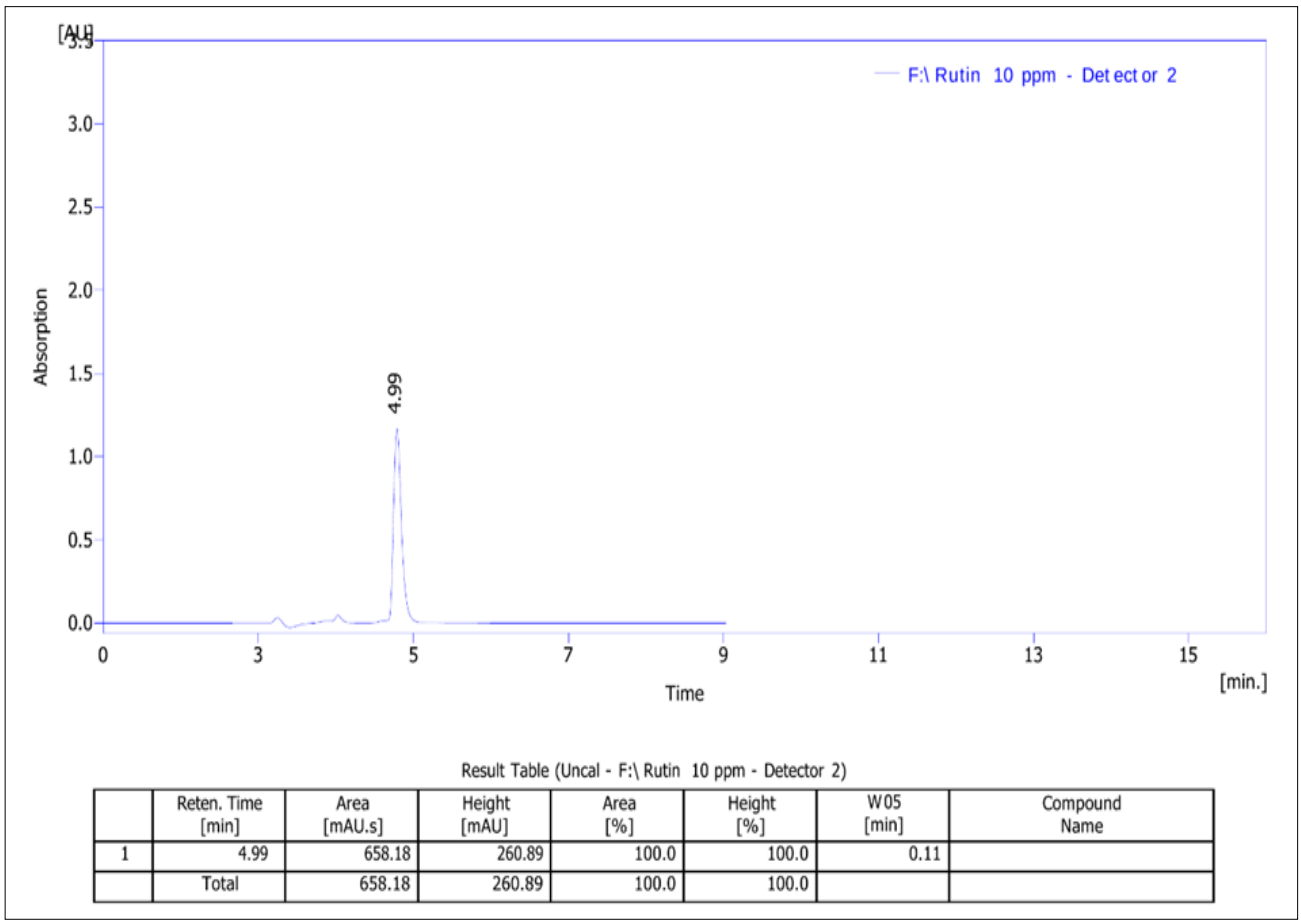


Result Table (Uncal - F:\scopalatine 10 ppm - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.60	787.011	285.900	100.0	100.0	0.05	
	Total	787.011	285.900	100.0	100.0		

Fig. 7. HPLC of scopalatine standard.

By using C18OSD (25 cm, 4.6 mm) column, gradient elution with a mobile phase composed of eluent methanol and eluent 1 % formic acid in the water (v/v), the rate flow 0.7mL/min

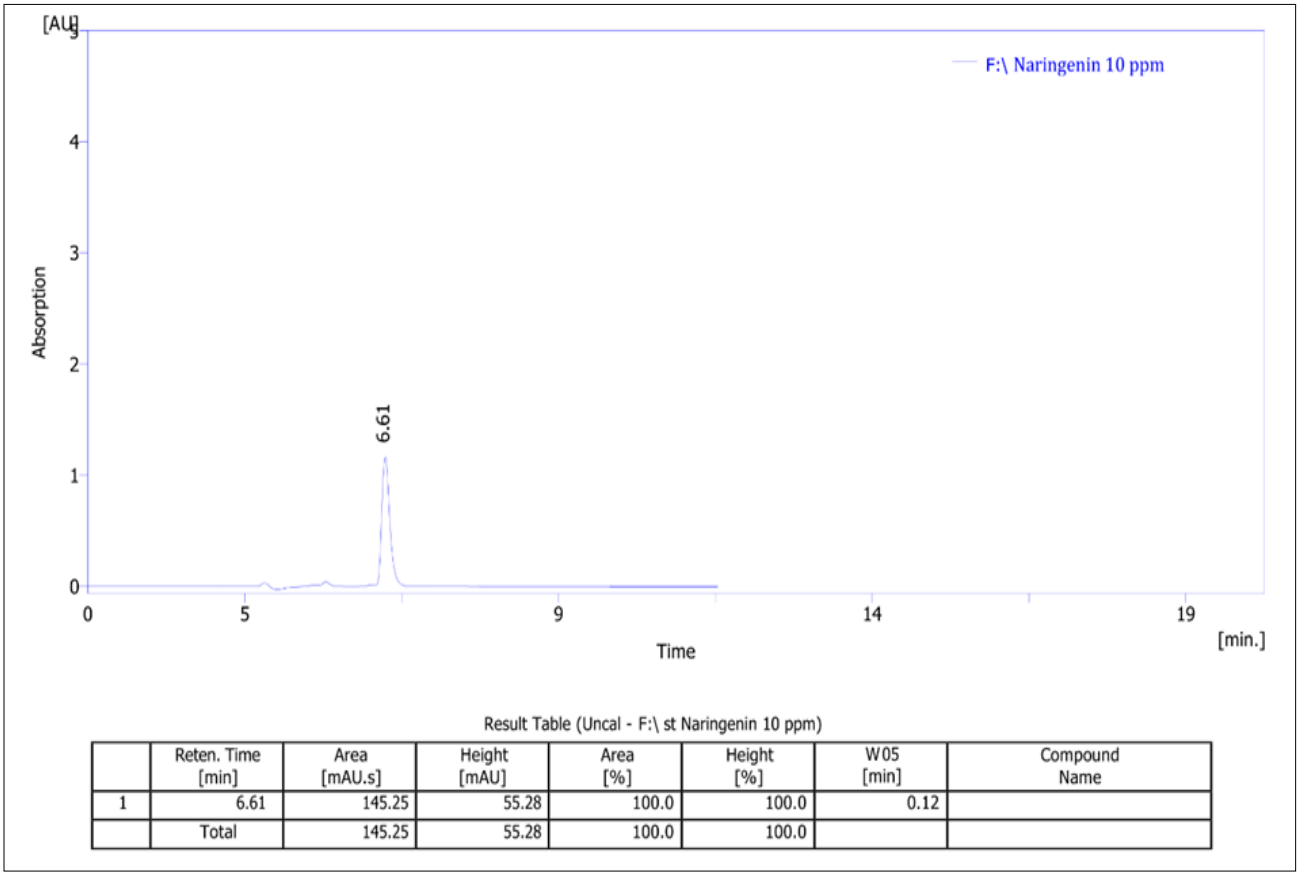


Result Table (Uncal - F:\ Rutin 10 ppm - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.99	658.18	260.89	100.0	100.0	0.11	
	Total	658.18	260.89	100.0	100.0		

Fig. 8. HPLC of rutin standard.

By using C18OSD (25 cm, 4.6 mm) column, gradient elution with a mobile phase composed of eluent methanol and eluent 1 % formic acid in the water (v/v), the rate flow 0.7 mL/min, eluent methanol and eluent 1 % formic acid in water (v/v), the rate flow 0.7 mL/min

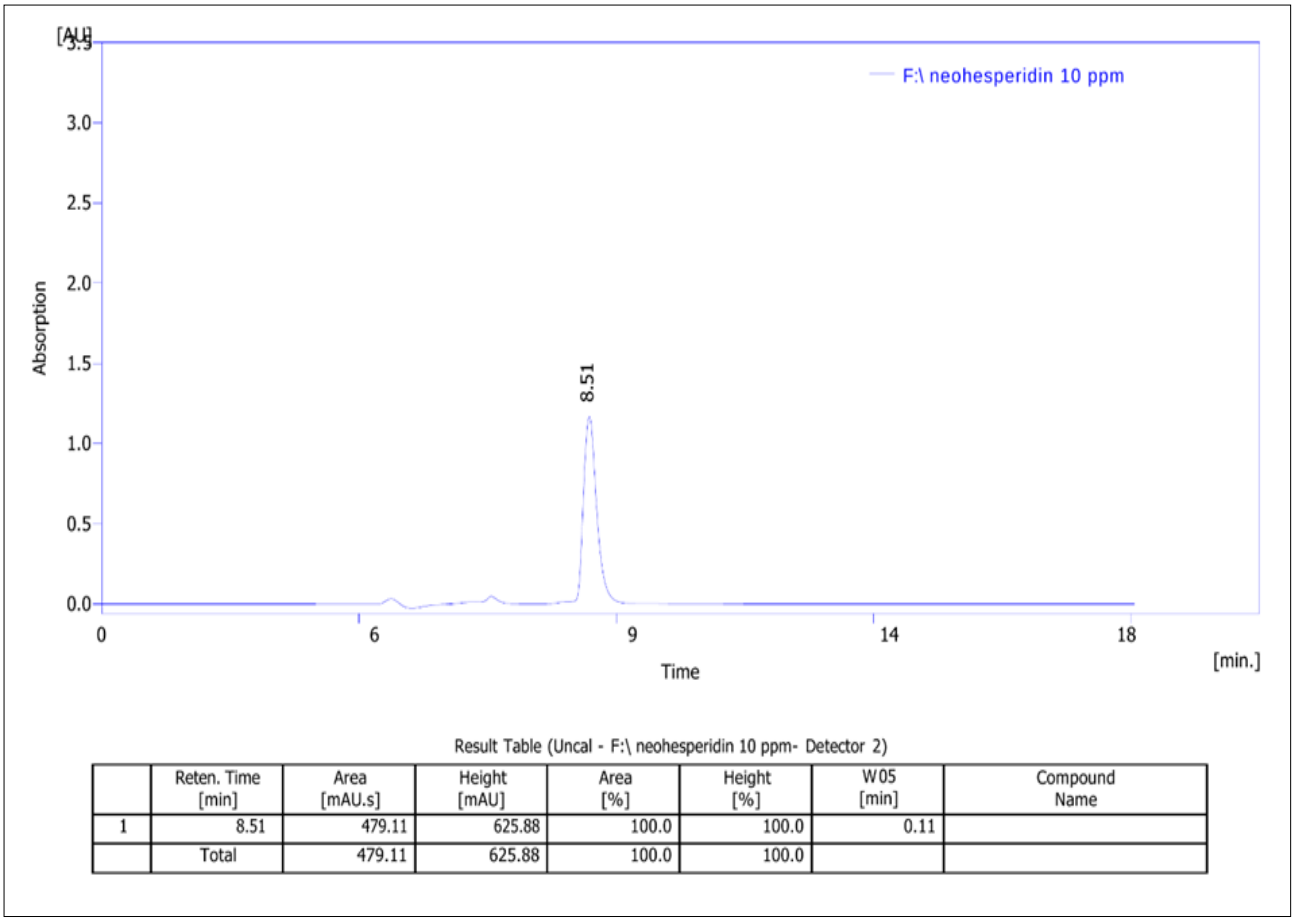


Result Table (Uncal - F:\ st Naringenin 10 ppm)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.61	145.25	55.28	100.0	100.0	0.12	
	Total	145.25	55.28	100.0	100.0		

Fig. 9. HPLC of naringenin standard.

By using C18OSD (25 cm, 4.6 mm) column, gradient elution with a mobile phase composed of eluent methanol and eluent 1 % formic acid in the water (v/v), the rate flow 0.7 mL/min



Result Table (Uncal - F:\ neohesperidin 10 ppm- Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8.51	479.11	625.88	100.0	100.0	0.11	
	Total	479.11	625.88	100.0	100.0		

Fig. 10. HPLC of neohesperidin standard.

By using C18OSD (25 cm, 4.6 mm) column, gradient elution with a mobile phase composed of eluent methanol and eluent 1 % formic acid in the water (v/v), the rate flow 0.7 mL/min

Table 4. HPLC retention times of standard and ethyl acetate extract sample compounds

Compounds	Ethyl acetate extract retention time	Standard retention time
Scopoletin	3.60	3.60
Rutin	4.98	4.99
Narengenin	6.66	6.61
Neohesperidin	8.41	8.51

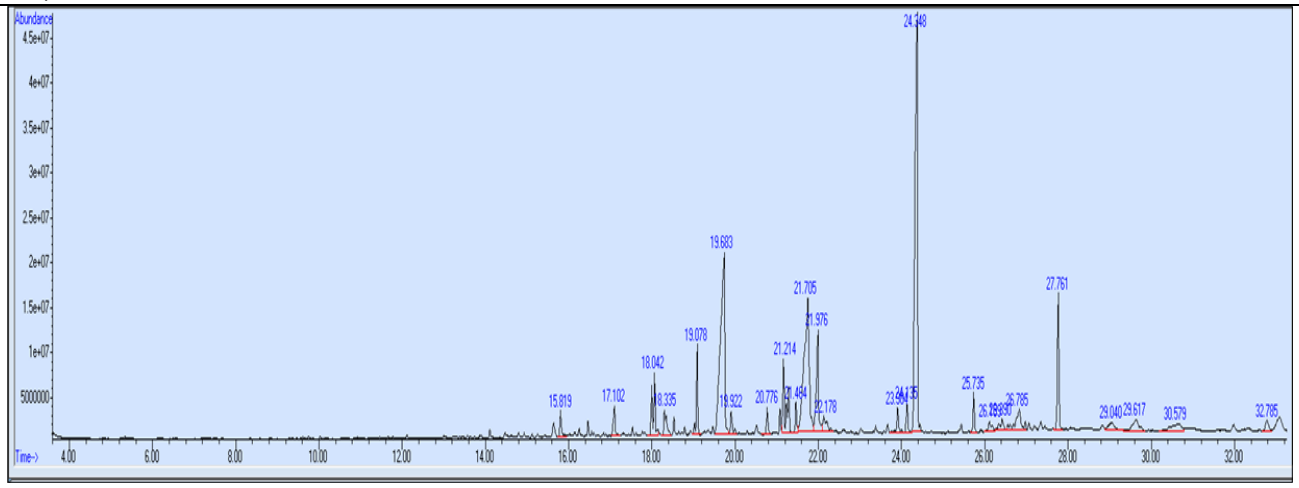


Fig. 11. Typical gas chromatogram of n-hexane leaves extract.

Table 5. The compounds detected in the n-hexane leaves extract by GC-MS

S.no	Compound	R/time	Composition (100 %)
1	alpha.-Cadinol	15.819	0.994 %
2	Tetradecanoic acid	17.102	1.257 %
3	2-pentadecanone,6,10,14trimethyl	18.042	3.376 %
4	Pentadecanoic acid	18.335	1.569 %
5	Hexadecanoic acid, methyl-ester	19.078	2.663 %
6	Methyl-Z,Z-3,13-octadecadienol	21.705	15.317 %
7	Octadecanoic acid	21.976	5.092 %
8	4,8,12,16-Tetramethylheptadecan-4-olide	23.904	1.134 %
9	Eicosanoic acid	24.135	1.146 %
10	Hexanedioic acid,bis (2-ethylhexyl) ester	24.348	25.040 %

hexane fraction found stigmasterol and β-sitosterol and the retention times were very similar to those of the reference standards (Table 3). It has been shown that these phytosterols have anticancer activity and reduce inflammation (25–27). The presence of β-sitosterol (the retention time of the peak in the sample in Fig. 3 matched that of the β-sitosterol standard in Fig. 5 for HPLC analysis) in *C. revoluta* is consistent with findings of a previous study which identified this compound in the leaves of *C. revoluta* cultivated in Egypt through GC–MS analysis (28).

Furthermore, literature indicates β-sitosterol suppresses inflammatory reactions of macrophages through activation of the negative regulator of the NF-κB and STAT1 signaling pathways, the phosphatase SHP-1. In murine J774A.1 macrophage cells, β-sitosterol treatment increased SHP-1, inhibited STAT1 phosphorylation and inhibited NF-κB nuclear localisation, leading to inhibited production of inflammatory cytokines (e.g., TNF-α) and chemokines and increased production of the anti-inflammatory cytokine IL-10 (29).

Compounds such as scopoletin, rutin, naringenin and neohesperidin were found in the ethyl acetate fraction (Table 4). There is a lot of evidence that these chemicals may fight cancer, inflammation and free radicals (30–33). The fact that these flavonoids are present suggests that *C. revoluta* might be valuable sources of antioxidants (4). The presence of these flavonoids aligns with earlier phytochemical reports on *C. revoluta* and related *Cycas* species, where flavonoids had been identified in the leaves of the plant (34). Flavonoids are widely recognized for their broad range of biological activities, particularly their antioxidant and radical-scavenging properties. Since reactive oxygen species are implicated in the development of various diseases, including atherosclerosis and certain types of cancer, these compounds may play an important protective role (35).

C. revoluta n-hexane leaf extract GC-MS study identified many pharmacologically significant components, including fatty acids and terpenoids, consistent with its traditional therapeutic use. Hexadecanoic acid is noteworthy due to its antibacterial, anti-inflammatory and wound-healing effects (36). Finding α-cadinol, a sesquiterpene alcohol, adds to the plant's therapeutic potential since this kind of sesquiterpenoid may kill fungi (37). This discovery is very important for the development of phytopharmaceuticals since sesquiterpenes are being explored more and more for their ability to fight cancer and parasites (38–42).

This research only tests both fractions, which are n-hexane and ethyl acetate fractions whereas n-butanol and chloroform fractions are not tested by HPLC and GC-MS. Therefore, further study is needed to interpret the whole phytochemical profile of the *C. revoluta* plant. In addition, this research detects compounds including α-cadinol, stigmasterol and rutin. However, these compounds did not evaluate their real cytotoxic, antibacterial or antioxidant activities *in vitro* or *in vivo*. This is the reason why it's highly recommended to perform *in vitro* and *in vivo* investigations.

Importantly, it is worth noting that the present investigation was limited to the qualitative identification of phytochemical

constituents in the n-hexane and ethyl acetate fractions. No quantitative determination of the compounds detected was performed. This represents a limitation, as without precise concentration data, it is not possible to accurately assess how the abundance of each phytochemical may contribute to the plant's overall biological potential. Although HPLC was employed in this study for qualitative purposes, future investigations should apply quantitative analytical approaches using HPLC or LC-MS with appropriate calibration curves to accurately measure the concentration of each compound and to establish meaningful correlations between these concentrations and specific biological activities. Lastly, it is strongly recommended to study other parts of plants, such as seeds and roots.

Conclusion

This study shows that *C. revoluta* leaves are a rich source of important phytochemicals, including sterols and flavonoids. The n-hexane extract contained amounts of stigmasterol, β-sitosterol, while the ethyl acetate extract was rich in scopoletin, rutin, naringenin and neohesperidin. Many of these compounds have been reported in earlier research for their antioxidant, anti-inflammatory and anticancer potential. While our results agree with previous findings, we did not perform quantitative measurements or biological activity tests. Future work should focus on precise quantification, experimental evaluation of pharmacological effects and exploring other plant parts to fully realize the therapeutic potential of *C. revoluta*.

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Authors' contributions

MRK collected the sample of the *Cycas revoluta* plant, dried the plant in the shade, extracted the plant with both solvents (n-hexane and ethyl acetate), performed the test of the leaves fractions, contributed to the design of the methodology of the study, drafted the manuscript, analysed the results of GC and HPLC. EJK helped in planning the study, reviewing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors have no conflict of interest to declare.

Ethical issues: None

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