RESEARCH ARTICLE

Phytochemical Investigation of *Euphorbia tirucalli* Cultivated in Iraq, and Isolation and Identification of Scopoletin and Quercitrin in the Plant Leaves Extract

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ABSTRACT

The plant *Euphorbia tirucalli* which belongs to the family Euphorbiaceae has different medicinal uses. It is used in folk medicine in East Africa, Malaysia, India and Brazil for the treatment of different diseases. It has many pharmacological activities such as anticancer activity, antioxidant, antimicrobial, antiviral, hepatoprotective and central nervous system (CNS) depressant. This project provides phytochemicals investigation and the methods of extraction and isolation from *E. tirucalli* cultivated in Iraq.

Objective: The study aims to investigate the chemical constituents of the leaves and the root of *E. tirucalli*. furthermore, isolation and identification of coumarins and flavonoids from the leaves extract.

Method: The leaves and the root of the plant were defatted separately in n. hexane for 24 hours. The defatted materials were extracted in 80% methanol by using the hot method (soxhlet) and the cold method (maceration), then the crude extracts were fractionated by using different solvents according to the increase in polarity petroleum ether, chloroform, ethyl acetate and n.butanol. The hexane fraction for the leaves and the root, chloroform fraction for the leaves and the ethyl acetate of the leaves were analyzed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Preparative layer chromatography (PLC) was used for the isolation of the phenols compounds from the chloroform and ethyl acetate fractions of the leaves. The isolated compounds were identified by using analytical TLC, analytical HPLC, high-performance thin-layer chromatography (HPTLC).

Results: The different chromatographic and spectroscopic results revealed the presence of scopoletin in the chloroform fraction while quercitrin in the ethyl acetate fraction of the leaves of the plant.

Keywords: *Euphorbia tirucalli,* Quercitrin, Scopoletin.

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INTRODUCTION

Because of the diversity of the chemical components that are found in *E. tirucalli* tissue, its medical history is tainted with a wide range of curative abilities. In east Africa, for example, latex is used against sexual impotence, warts, epilepsy, toothache, haemorrhoids, snake bites, extraction of ectoparasites, and cough while in Malaysia the roots or stems are applied to nose ulceration, haemorrhoids, and swellings. Also, root residue is mixed with coconut oil for stomach aches. In India, the plant is used for Spleen enlargement, asthma, dropsy, leprosy, biliousness, leucorrhoea, dyspepsia, jaundice, colic, tumours, and bladder stones while the latex is used as an emetic in large doses, it is a purgative in small doses helped against toothaches, earaches, rheumatism, warts, cough, neuralgia, and scorpion bites. Its branches are administered for colic and gastralgia while ashes are mordent for abscesses. In Brazil, despite there was no scientific proof to use the plant since the same one is known to be cocarcinogenic, it is used for cancer, epitheliomas, sarcomas, tumours, and warts. In India, latex is used as an emetic and antisyphilitic. In Indonesia, wood is applied against leprosy and hands and feet paralysis following childbirth.[1,2] While in Rajasthan, latex is used for asthma, cancer, cough, ear problems, snakebite, scorpion sting, toothache, intestinal parasite and skin problems. Leaves are used for skin problems, nose ulcers and haemorrhoids.

Stems are used for thorn extraction, swelling, leprosy, paralysis, colic and gastric problem. the root is used for Rheumatism.[3] Also as an ornamental plant, it is used in offices and homes for its facility of maintenance and wonderful over green branches which look like pencils.[4] Other uses for *E. tirucalli* such as Resources of rubber, glue and adhesives, a resource of energy, protection and agroforestry and pesticide.[5]

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Taxonomic Description

An unarmed shrub or small tree about 4–12 (15) m high with multiple cylindrical branches 7 mm thick contain white latex. Small linear-oblong leaves appear only at the tips of young branchlets.

Local names: Arabic (injil); English (finger euphorbia, Indian spurge tree, milk bush, pencil tree, rubber euphorbia); Filipino (bali bali).

The *E. tirucalli* flowers in October, it usually produces male flower while the female flower is less common. The flowers are very small with yellow, green, or pink colour in groups on the top of the branches. fruits from November-December which is a glabrescent capsule about (8–12) mm in width.[1,6,7] Seeds are oval and about (3.0–4.0) × (2.8–3.0) mm dimension, soft spotted with brown.[6]

Distribution

The *E. tirucalli* grows in any type of soil, altitude up to 2000 m.[6,7] The native countries are Angola, Eritrea, Ethiopia, Kenya, Malawi, Mauritius, Rwanda, Senegal, Sudan, Tanzania, Uganda, Zanzibar, also it was found in other countries such as Brazil, India, Indonesia, Malaysia, Philippines, Vietnam.[7] It is currently widely distributed in southern Europe, Asia, and the Americas.[1]

Phytochemical Constituents of *E. tirucalli*

Phenolic Compounds

It's known that the metabolism of plants consist of primary and secondary, the substances that are essential to cells maintenance such as (lipids, proteins, carbohydrates, and nucleic acids) are made during the primary metabolism while substances that are made by several biosynthetic pathways that are restricted to determined groups of organisms are results of secondary metabolite. Phenolic compounds are one of the biggest groups of secondary metabolites in most plants which include flavonoids and phenolic acids and their offshoots such as lignins and tannins.[8,9]

Phenolic Acids

The main phenolic acids that have been characterized in *E. tirucalli* are ferulic acid,[10,11] gallic acid.[11,12]

Ferulic acid: Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) and related compounds are ubiquitous components of the primary cell walls of several plants, occurring in their seeds and leaves in both its free form and esterified to mono- and disaccharides, plant cell wall polysaccharides, glycoproteins, polyamines, lignin and hydroxylated fatty acids.

Ferulic acid showed antioxidant activity by many mechanisms such as free radical scavenger, an inhibitor of enzymes that catalyze free radical generation and an enhancer of scavenger enzyme activity. It has a protective role for the main skin structures: keratinocytes, fibroblasts, collagen, and elastin. It prevents melanogenesis, promotes angiogenesis and wound healing as a result it is used in cosmetology and aesthetic dermatology as anti-ageing and antiapoptotic. This is due to its phenolic nucleus and an extended side chain conjugation, which resulted in a resonance stabilized free radical like phenoxyl radical, which accounts for its potent antioxidant potential.[13] Besides, ferulic acid has an anti-inflammatory effect, antidiabetic effect, anticancer effect, hepatoprotective effect, neuroprotective effect, pulmonary protective effect, radioprotective effect, hypotensive effect, anti-atherogenic effect.[14,15]

Finally, ferulic acid is used as a preclusion of food discolouration and growth-enhancing agent as well as a food preservative and a precursor of vanillin.[16]

Gallic Acid

Gallic acid (3, 4, 5-trihydroxy benzoic acid, GA) is found widely distributed in plants. It is found in the form of free acids, esters, catechin derivatives and hydrolysable tannins.[17]

GA has antioxidant, antitumor, anti-metastatic activities,[18] hepatoprotective potential, anti-microbial activity against methicillin-resistant *Staphylococcus aureus* and helicobacter pylori, anti-inflammatory activity, antidepressant, anti-parkinson, anti-diabetic, antimalarial, diuretic, cardioprotective, anti-viral, anti-fungal, wound healing, anthelmintic, anxiolytic and anticancer by arresting cell cycle via inducing apoptosis in tumoral cell lines and inhibit lymphocyte proliferation, inhibits ribonucleotide reductase and cyclooxygenases cause inactivating phosphorylation.[19]

Flavonoids

Flavonoids are classified according to the degree of hydroxylation and the presence of a C_2-C_3 double bond in the heterocyclic pyrone ring. They consist of 13 classes, the most important ones are flavonols, flavonols, flavones, isoflavones, anthocyanidins or anthocyanins and flavanones. They are one of the most important constituents in plants with different pharmacological activity such as antioxidant activity which is related to the presence of hydroxyl groups in positions 3' and 4' of the B ring a double bond between carbons C_2 and C_3 of the ring C together with the carbonyl group at the C_4 position .inaddition, free hydroxyl groups in position 3 of ring C and position 5 of ring A, together with the carbonyl group in position 4, are also important for the activity of these compounds as shown in Figures 1–6.[9,20] Other pharmacological activities like anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity, antiallergic activity, antioxidant activity and cytotoxic antitumour activity,[21,22] antidiabetics, antiulcer, vasorelaxant, hepatoprotective, antiatherosclerotic activity.[23] The antibacterial mechanisms of flavonoids are inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of the porin on the cell membrane, alteration of the membrane per- permeability, and attenuation of the pathogenicity.[24]

One of the most important flavonoid in *E. tirucalli* is Quercitrin.[12]

Quercitrin

Is the glycosylated form of quercetin (quercetin-3-O-rhamnoside) must reach the colon where it is hydrolyzed by rhamnosidase of the colonic microflora releasing the aglycone (quercetin) because this glycoside resists acid hydrolysis of the

Figure 1: *E. tirucalli*

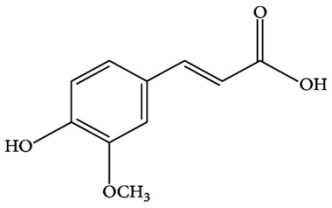


Figure 2: The chemical structure of ferulic acid

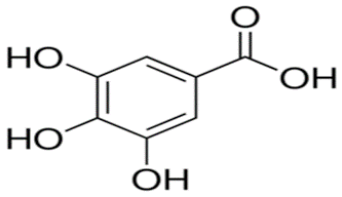


Figure 3: The chemical structure of gallic acid.

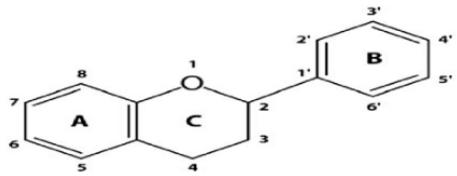


Figure 4: The basic chemical structure of flavonoids

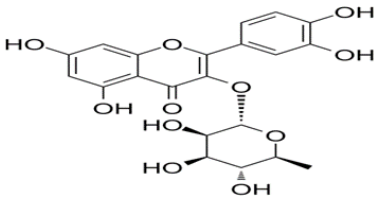


Figure 5: The chemical structure of quercitrin (3-rhamnosyl quercetin).

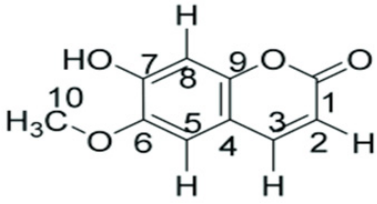


Figure 6: Chemical structure of scopoletin

stomach, quercetin is responsible for the anti-inflammatory effect, [25] anticancer activity, antiviral activity. [26]

In vitro studies, quercetin is tested for the anticancer activity in nine cancer cell lines: prostate adenocarcinoma LNCaP cells, colon carcinoma CT-26 cells, pheochromocytoma PC12 cells, human prostate PC3 cells, acute lymphoblastic leukaemia MOLT-4 T-cells, estrogen receptor-positive breast cancer MCF-7 cells, ovarian cancer CHO cells, human myeloma U266B1 cells and human lymphoid Raji cells; the results showed that quercetin can significantly induce apoptosis of every tested cell lines at $p < 0.001$ in comparison with control group while in vivo experiments showed in mouse models i.e., mice bearing MCF-7 tumours and mice bearing CT-26 tumours; the quercetin-treated group presented a significant decrease in tumour size and volume at $p < 0.001$ in comparison with the control group. The survival period of the quercetin-tested animals was also prolonged.[22]

Coumarins

Coumarins (2H-1-benzopyran-2-one) contain a large class of phenolic compounds found in plants and are made of fused benzene and a-pyrone rings. More than 1300 coumarins have been characterized as secondary metabolites from plants, bacteria, and fungi. coumarins have shown different pharmacological activities such as anti-inflammatory effect, anti-coagulant,anti-bacterial, anti-fungal, anti-viral, anti-cancer, [27] anti-hypertensive, antitubercular, anticonvulsant, for multiple sclerosis, anti-adipogenic, cytochrome p450 inhibiting activity, anti-hyperglycemic,anti-oxidant, neuroprotective activity and as phytoalexins which are oxygenated coumarin derivatives and they are made in plants in response to fungal infection, physical damage, chemical injury, or a pathogenic process. The vital role of phytoalexins is to prevent or destroy invading agents such as bacteria, insects, and viruses.[28,29]

One of the most coumarins that have been characterized in *E. tirucalli* is scopoletin.[12]

Scopoletin

Has shown anti-oxidant capacity, anti-inflammatory, anti-acetylcholine enzyme activity used medicinally in myasthenia gravis treatment to increase neuromuscular transmission and to treat Alzheimer's disease in which there is a deficiency in the production of acetylcholine.[30]

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of *E. tirucalli* was collected from Baghdad in April 2020. The plant was authenticated by Assist prof. Dr Khansaa R. Al-Joboury/Iraqi Natural History Museum Herbarium. The plant was cleaned, dried in shade at room

temperature, pulverized by electrical milled and then weighed.[31]

Extraction of Plant Material

Firstly, the pulverized plant material(about 120 gm of leaves and 20 gm of root were defatted separately with a sufficient amount of hexane solvent) for 24 hours to remove chlorophyll and the hexane soluble compounds like waxy material by filtration then evaporation.[32]

Cold Method (Maceration)

In this method, the dried defatted material (60 gm of leaves and 20 gm of the root) was soaked separately in 540 mL and 180 mL of 80% methanol respectively, with shaking at room temperature. After 9 days, the methanol soluble materials were filtered off. The filtrate was concentrated under reduced pressure using a rotary evaporator to about 15 mL then mixed with 50 mL distilled water,[34] which was designated as a crude fraction.[31,33]

Hot Method (Soxhlet Extraction):

The dried defatted material (only 60 gm of leaves) was packed in a thimble. The thimble is then placed in a soxhlet extractor. 540 mL of 80% methanol[35] was placed in the round flask. The sample was extracted until complete exhaustion for about 12 hours. The alcoholic extract was filtered by filter paper to remove the marc. The filtrate was concentrated under reduced pressure using a rotary evaporator to about 15 mL then mixed with 50 mL distilled water, which was designated as a crude fraction.[34,36]

Preliminary Qualitative Phytochemical Analysis for Crude Extract

Chemical tests were carried out using the methanolic extracts from plants to identify the active constituents.[32,37-39]

Alkaloid Detection (Mayer's Test)

Alcoholic extract (10 mL) was stirred with 5 mL of 1% HCL on a steam bath. Draggndroff reagent (0.8 gm Bismuth nitrate in 40 mL distilled water + 8 gm potassium iodide in 20 mL water + 10 mL glacial acetic acid, boiled for a few minutes) which is a general test for nitrogenous compounds and Mayer's (1.35 gm mercuric chloride in 60 mLwater + 5 gm potassium iodide in 10 mL water) were added, the appearance of a reddish-brown and white colour precipitate respectively indicates the presence of alkaloids.

Anthraquinone Detection (Borntrager's test)

2 mL 10% ammonia solution + 2 mL of methanolic extract + 2 mL chloroform (shaken vigorously for 30 seconds), a pink, violet, or red coloured solution indicates the presence of anthraquinones.

Coumarins Detection (NaOH test)

1 mL of methanolic extract + 0.5 mL of 10% NaOH + 1 mL chloroform, a yellow color solution indicates the presence of coumarins.

Cardiac Glycoside Detection (Killer kiliani Test)

1 mL filtrate + 1.5 mL glacial acetic acid +1 drop of 5% ferric chloride + 1 mL concentration H_2SO_4 (along the side of the test tube), a blue coloured solution (in the acetic acid layer) indicates the presence of cardiac glycoside or brown ring interface which indicates the presence of deoxy sugar.

Flavonoid Detection (Alkaline Reagent Test)

1-mL of methanolic extract + 2 mL of 2% NaOH solution + few drops of diluted HCl, an intense yellow colour, becomes colourless on the addition of dilute acid indicates the presence of flavonoids.

Tannin Detection (Braymer's test)

1 mL of methanolic extract + 3 drops 10% Ferric chloride solution, a blue-green colour indicates the presence of tannin.

Fractionation of Extract

The crude fraction of the plant (leaves and root) that obtained from the extraction methods (cold and hot methods) were suspended in about 50 mL of distilled water and subsequently fractionated by partitioning successively with petroleum ether (B.p. 40–60°C), chloroform ($CHCl_3$), ethyl acetate, and finally, n-butanol using the same volume of the suspended crude fraction which was about 65 mL × 3 for each solvent. The first three fractions dried over anhydrous sodium sulfate with except n.butanol fraction. Then they were filtered and evaporated to dryness under reduced pressure using a rotary evaporator.[33,36] Each fraction was weighted and assigned for further analysis.

Phytochemical Investigation of the Fractionated Extract

Chemical tests were performed for the fractions to identify the active constituents.

Thin-Layer Chromatography Examination for Chloroform and Ethyl Acetate Fractions

1 mg of chloroform and ethyl acetate fractions were suspended in about 1ml of chloroform, applied on a readymade analytical TLC plate precoated with silica gel GF_{254} and developed in different mobile phases to reach the best separation, the best ones were:

- S4: Toluene: acetone: chloroform(55:45:5).[40]
- S5: Petroleum ether: ethyl acetate (80:20).
- S6: Ethyl acetate: acetic acid: formic acid: water(50:3:3:4).[41]

After drying the plates, they were examined under UV and sprayed with Potassium hydroxide reagent (KOH) (10% ethanolic potassium hydroxide (Borntrager reaction)). The plate is sprayed with the reagent and evaluated in visible or in UV-365 nm, with or without warming.[38]

HPLC Analysis for Chloroform Fraction

HPLC conditions: Model = LC-2010A HT/Shimadzu.
Column = C18 – ODS (250 mm *4.6 mm)
Water: methanol (30:70) with UV- 366 nm.[42]
Flow rate = 1-mL/min
Elution = isocratic

HPLC Analysis for Ethyl Acetate Fraction

HPLC conditions: The same conditions as mentioned above but the mobile phase was water: acetonitrile (70:30) with UV-335 nm.[43]

Isolation of different constituents from chloroform and ethyl acetate fractions by preparative layer chromatography (PLC):

- Using ready-made preparative layer chromatography plates Thickness 0.25 mm, 0.5 mm and 1-mm silica gel GF_{254}, activated in the oven at 100°C for 20 min.
- *Mobile Phase:* the solvent system was introduced in a clean, dried, 20*20 cm jar. The jar was lined with filter paper, closed tightly, and left overnight.

The mobile phase used in chloroform fraction to isolate the coumarin is S4 while for ethyl acetate fraction to isolate the glycoside is S6 as mentioned above.

- *Application of Sample:* About 0.5 gm from the fraction was dissolved in 2 mL of ethanol, applied as a line carefully on the preparative layer using a capillary tube, left to dry and repeat for about 3 times, then introduced in the saturated jar, the jar is closed tightly and left for development.
- *Detection of the Separated Bands:* After development, the plates were taken out of the jar, left to dry and detected by UV 254 nm and 365 nm, the bands corresponding to each compound were scraped out and collected in a beaker, mixed with methanol, stirred and left aside for one hour, then filtered.[69] One band was separated from the chloroform fraction which is referred to by (D) and the ethyl acetate fraction which is referred to by (E).

Preliminary Qualitative Phytochemical Analysis for Crude Extract

Table 1: Phytochemical screening of crude extract of *E. tirucalli*

Chemical tests	*The crude extract (soxhlet) of leaves*	*The crude extract (maceration) of leaves*	*The crude extract (maceration) of root*
Alkaloid test	+	+	+
Anthraquinone test	+	+	+
Coumarin test	+	+	+
Cardiac glycoside test	+	+	+
Flavonoid test	+	+	+
Tannin test	+	+	+

Fractionation of Extracts

Table 2: The weight and the percentage of each fraction from leaves and root of *E. tirucalli*

Plant part	*Fraction (f.)*	*Weight in gram*
Leaves (hot method)	Petroleum ether f.	1.5 gm
	Chloroform f.	1.8 gm
	Ethyl acetate f.	1.2 gm
	N. butanol f.	0.6 gm
Leaves (maceration)	Petroleum ether f.	0.8 gm
	Chloroform f.	1 gm
	Ethyl acetate f.	0.5 gm
	N. butanol f.	0.2 gm
Root (maceration)	Chloroform f.	2 gm
	Ethyl acetate f.	1.6 gm
	N. butanol f.	0.5 gm

Identification of the Isolated Compound (D) from Chloroform Fraction

- By analytical TLC.
- By High-performance thin-layer chromatography (HPTLC).
- By Spiking in analytical HPLC with the standard.

Identification of the Isolated Compound (E) from Ethyl Acetate Fraction

- By Analytical TLC.
- By HPTLC.
- By Analytical HPLC with the standard.

RESULTS AND DISCUSSION

Methods of Extraction

The two methods of extraction (hot and cold methods) with 80% methanol were done to compare between them. It was found during the work that the amount of materials extracted from the hot method is more compared to the cold one, but the cold method is necessary for materials that cannot tolerate heat (thermolabile component) (Tables 1–3).

Thin-layer Chromatography for the Chloroform and Ethyl Acetate Fractions

By using different mobile phases and standard (scopoletin), the spot of this compound appeared in the fraction under UV- 365 nm fluorescent even after spraying with KOH reagent in both the leaves and the root of *E. tirucalli* (Figures 7).

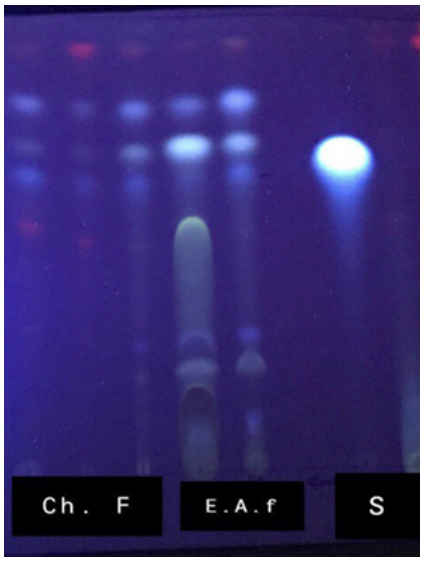


Figure 7: Thin layer chromatography for chloroform fraction (Ch. F) and ethyl acetate fraction (E. A. f) with scopoletin std. (S) developed in S4: Toluene: acetone: chloroform(55:45:5) under UV-365

Phytochemical Investigation of the Fractionated Extract

Table 3: Phytochemical screening of the fractionated extract.

The fraction	*Coumarin test*	*Flavonoid test*
Hexane f.	-	-
Chloroform f.	+	+
Ethyl acetate f.	+	+
N. butanol f.	-	+

Thin-layer Chromatography for the Ethyl Acetate Fraction

By using different mobile phases and standard quercitrin, the spot of the compound appeared in the fraction under UV-365 and 254 became more obvious after spraying with KOH reagent in the leaves only (The cold and the hot method) (Figures 8).

HPLC Analysis for Chloroform Fraction

Qualitative identification was done by using HPLC by comparing the retention time of each compound with the retention time of the standard in the same conditions.

From the Figures 9 and 10, the results showed that the retention time of scopoletin (3.2 min.) also appeared in the chloroform fraction of the leaves of *E. tirucalli*.

HPLC Analysis for Ethyl Acetate Fraction

Qualitative identification was done by using HPLC by comparing the retention time of each compound with the retention time of the standard in the same conditions.

From the Figures 11 and 12, the results showed that the retention time of quercitrin (2.2 min.) also appeared in the ethyl acetate fraction of the leaves of *E. tirucalli*.

Isolation and Purification of Active Constituent (D) from Chloroform Fraction

By preparative layer chromatography (PLC), compound D was isolated and purified by using S4: Toluene: acetone: chloroform (55:45:5) as a mobile phase. The band was identified under UV-365 nm (Figure 13).

Isolation and Purification of Active Constituent (E) from Ethyl Acetate Fraction

By PLC, the compound E was isolated and purified by using S6: Ethyl acetate: acetic acid: formic acid: water (50:3:3:4) as a mobile phase. The band was identified under UV-254 and 365 nm (Figure 14).

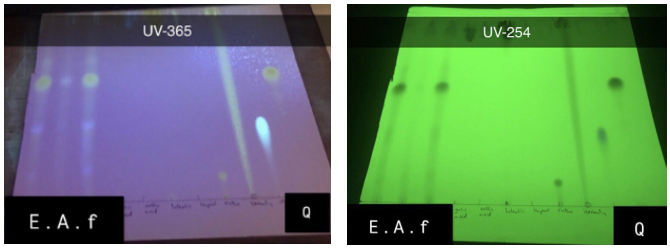


Figure 8: Thin later chromatography for ethyl acetate fraction (E.A.F) with quercitrin std. (Q) developed in S6: Ethyl acetate: acetic acid: formic acid: water (50:3:3:4) under UV-365 and 254 nm

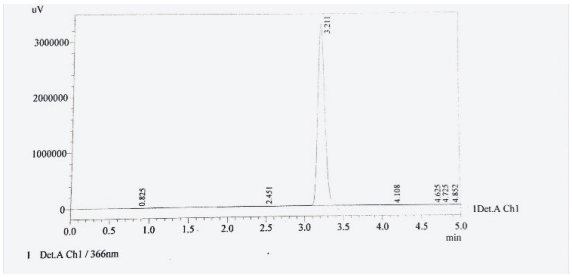
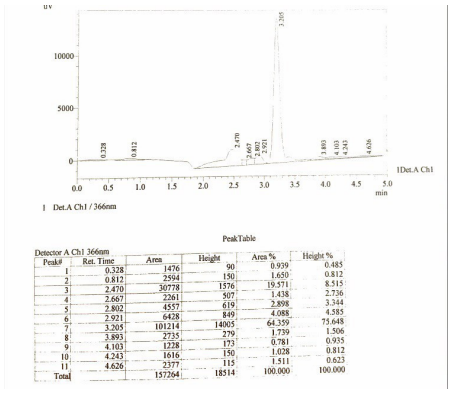


Figure 10: HPLC for scopoletin std. which showed the retention time at 3.211 minutes

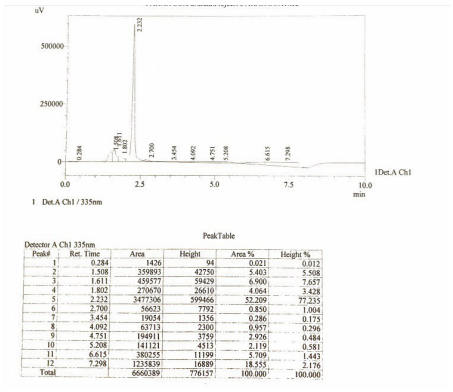


PeakTable

Detector A Ch1 366nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.328	1476	90	0.939	0.485
2	0.812	2594	150	1.650	0.812
3	2.470	30778	1576	19.571	8.515
4	2.667	2261	507	1.438	2.736
5	2.802	4557	619	2.898	3.344
6	2.921	6428	849	4.088	4.585
7	3.205	101214	14005	64.359	75.648
8	3.893	2735	279	1.739	1.506
9	4.103	1228	173	0.781	0.935
10	4.243	1616	150	1.028	0.812
11	4.626	2377	115	1.511	0.623
Total		157294	18514	100.000	100.000

Figure 9: HPLC analysis for chloroform fraction of the leaves of *E. tirucalli*



PeakTable

Detector A Ch1 335nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.284	1426	94	0.021	0.012
2	1.508	359893	42750	5.403	5.508
3	1.611	459577	59429	6.900	7.657
4	1.802	270670	26619	4.064	3.428
5	2.232	3477306	599466	52.209	77.235
6	2.700	56623	7792	0.850	1.004
7	3.454	19054	1356	0.286	0.175
8	4.092	63713	2300	0.957	0.296
9	4.751	194911	3759	2.926	0.484
10	5.208	141121	4513	2.119	0.581
11	6.615	380255	11199	5.709	1.443
12	7.298	1235839	16889	18.555	2.176
Total		6660389	776157	100.000	100.000

Figure 11: HPLC analysis for ethyl acetate fraction of the leaves of the *E. tirucalli*.

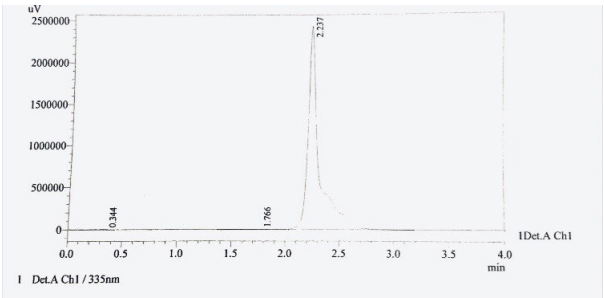


Figure 12: HPLC analysis for quercitrin std. which showed the retention time at 2.237 minutes

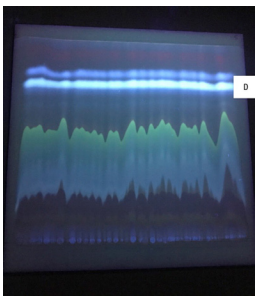


Figure 13: Preparative layer chromatography (PLC) for chloroform fraction of the leaves under UV-354 nm

Identification of the Isolated Compound D from Chloroform Fraction

By TLC

From Figure 15, the result showed that R_f value for the three spots (scopoletin std., isolated compound B+std., isolated compound B) was approximately the same (0.62 cm).

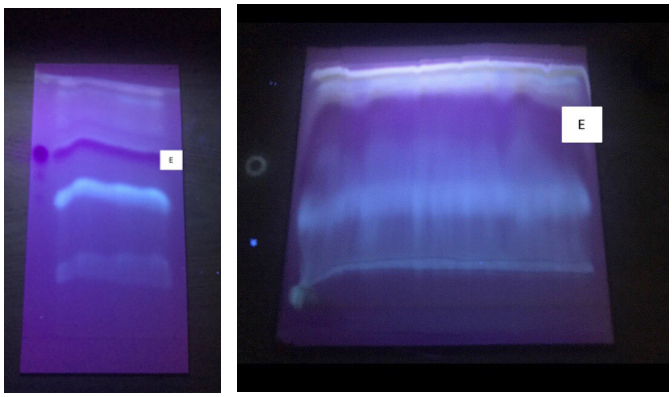


Figure 14: PLC for ethyl acetate fraction of the leaves under UV-354 nm

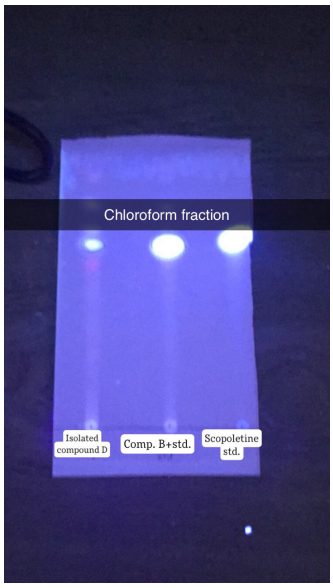


Figure 15: TLC analysis of the isolated compound D from chloroform fraction of the leaves with scopoletin std. under UV-356 nm.

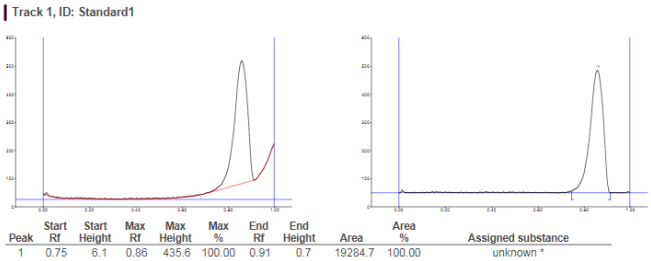


Figure 16:- HPTLC chromatogram for scopoletin std. in wavelength 310 nm

By HPTLC

By using S4: Toluene: acetone: chloroform (55:45:5) as a mobile phase at wavelength 254 nm.

By Spiking HPLC

From the Figures 16 to 19 above, the result showed an increase in the area% from 64% in the HPLC analysis for the isolated compound B only to 98% in the HPLC analysis for the mix of the latter compound with scopoletin std.

From all the above-mentioned results, we can conclude that the isolated compound (D) might be scopoletin.

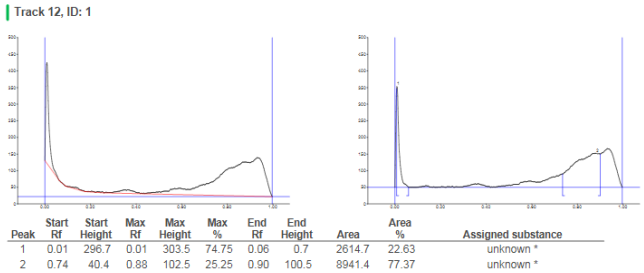


Figure 17: HPTLC chromatogram for the isolated compound D in wavelength 310 nm.

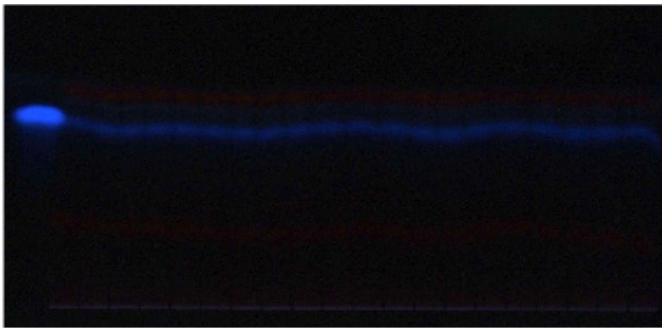
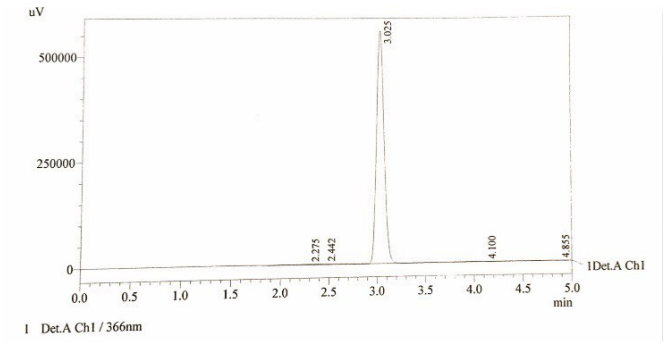


Figure 18: HPTLC for scopoletin std. and the isolated compound D in wavelength 366 nm



Peak Table

Detector A Chi 366 nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	2.275	16.387	943	0.525	0.167
2	2.442	26.277	1413	0.841	0.250
3	3.025	306.3234	98.077	99.511	
4	4.100	8984	274	0.288	0.049
5	4.865	8406	128	0.269	0.023
Total		312.3287	564357	100.000	

Figure. 20: HPLC analysis for the mix of scopoletin std. and the isolated D compound.

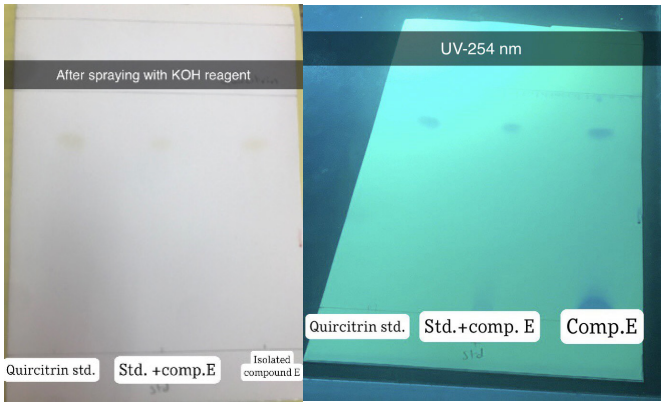
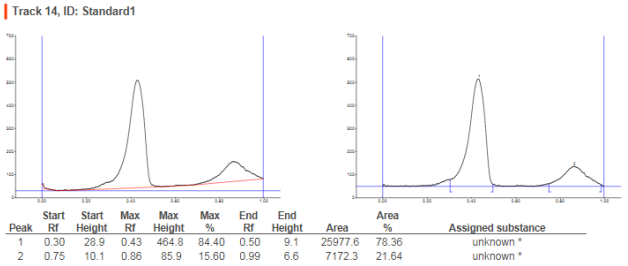
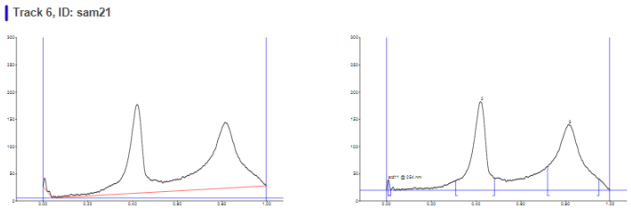


Figure 20: TLC analysis for the isolated compound (E) from ethyl acetate fraction of the leaves and quercitrin std



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.30	28.9	0.43	464.8	84.40	0.50	9.1	25977.6	78.36	unknown *
2	0.75	10.1	0.86	85.9	15.60	0.99	6.6	7172.3	21.64	unknown *

Figure 21: HPTLC chromatogram for quercitrin std. at wavelength 254 nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	19.0	0.01	19.0	6.29	0.02	1.1	92.2	0.51	std11
2	0.31	17.7	0.42	162.4	53.73	0.48	22.5	7738.5	42.67	unknown *
3	0.72	43.2	0.82	120.8	39.98	0.95	19.9	10306.6	56.83	unknown *

Figure 22: HPTLC chromatogram for the isolated compound (E) from ethyl acetate fraction of the leaves at wavelength 254 nm

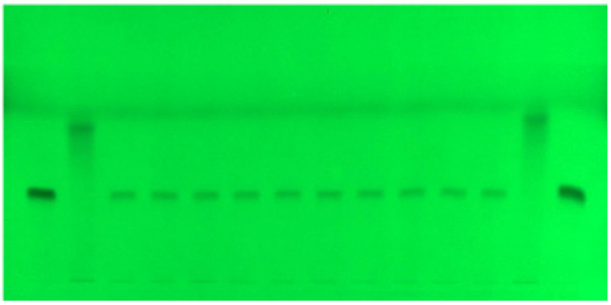
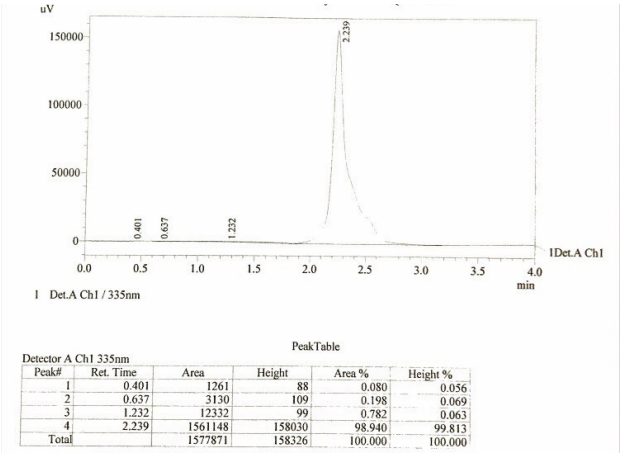


Figure 23: HPTLC analysis for quercitrin std. and the isolated compound (E) at wavelength 254 nm

Identification of the Isolated Compound E from Ethyl Acetate Fraction

By TLC

From the result above, the result showed the R_f value of the spots (quercitrin std., the mix of the isolated compound E and the std., the isolated compound (E)) was the same (0.67 cm) (Figure 19).

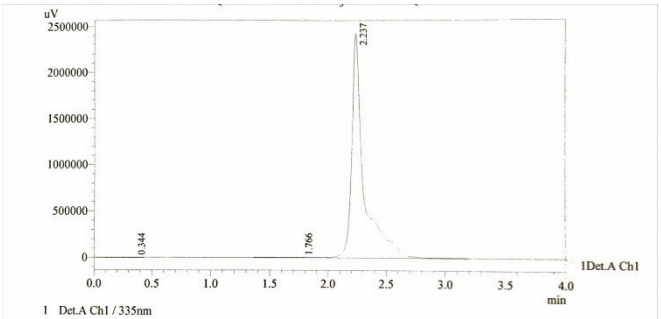


PeakTable

Detector A Ch1 335nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.401	1261	88	0.080	0.056
2	0.637	3130	109	0.198	0.069
3	1.232	12332	99	0.782	0.063
4	2.239	1561148	158030	98.940	99.813
Total		1577871	158326	100.000	100.000

Figure 24: HPLC analysis for the isolated compound (E) from ethyl acetate fraction of the leaves



PeakTable

Detector A Ch1 335nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.344	1185	69	0.007	0.003
2	1.766	14877	1087	0.084	0.045
3	2.237	17722644	2431602	99.909	99.952
Total		17738706	2432758	100.000	100.000

Figure 25: HPLC analysis for the quercitrin std

By HPTLC

By using S6: Ethyl acetate: acetic acid: formic acid: water as a mobile phase at wavelength 254 nm (Figure 20).

By HPLC Analysis

From the Figures 21 and 25, the result showed that the quercitrin std. and the isolated compound (E) had the same retention time (2.23 minutes).

From all the above-mentioned results, we can conclude that the isolated compound (E) might be quercitrin.

CONCLUSION

Two compounds were isolated, the first one is scopoletin from chloroform fraction and the second one is quercitrin from the ethyl acetate fraction.

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