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Phytochemical profile for *Cestrum nocturnum* leaves ethanolic extract and isolation of a rare flavonoid using different chromatographic and spectroscopic techniques

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Abstract
In this study, flavonoids and phenolic acids were detected in the alcoholic extract of *Cestrum nocturnum* leaves using chromatographic technique. Phytochemical survey was also conducted for the different parts of the plant using more than one experiment for each component we wanted to search for. A quantitative estimate was also made for the content of plant leaves from secondary metabolites, specifically alkaloids, flavonoids, saponins, tannins and phenolic acids. All experiments showed that the leaves of the *Cestrum nocturnum* plant are rich in many phenolic compounds and flavonoids, which prompted us to separate one of these compounds by traditional separation methods. The results showed that the leaves of the *Cestrum nocturnum* plant are rich in secondary metabolites in different fractions and Phytochemical profiles using HPLC (high-performance liquid chromatography) explain, leaves of the plant are expensive with lots of chemical compounds, like cinnamic acid vanillin, gallic acid, luteolin, kaempferol, caffeic acid, quercetin, propyl gallate, isovetixin, rutin, narinjirnin, coumaric acid, cinnamic acid, apigenin, chlorogenic acid, and hispertin. Quantitative estimation of total phytochemicals main groups for different plant parts exhibits that all parts of the plant contain the main groups of secondary metabolites (flavonoids, alkaloids, saponin, and tannins) in good quantities, especially the leaves. The propyl gallate compound recovered from 96 percent ethanolic leaves extract using different chromatographic methods was then identified using Rf-values, UV, 1H-NMR spectrum studies, and mass spectroscopy.

Keywords: *Cestrum nocturnum*, phytochemical composition, flavonides, phenolic extract. chromatography

1. Introduction
Plants are a vital resource for combating the world's most serious diseases. [1]. According to the World Health Organization (WHO), more than 80% of the world's population relies on plant-based treatments for their primary health care needs [2, 3]. Plants are the source of novel medications, the bulk of which have yet to be discovered. Several percentages of the 25 000 000 to 50 000 000 plant species are examined for phytochemical and biological screening. [4] Plants have provided humanity with essential requirements such as food, clothing, and shelter for generations, all of which were created or constructed from plant matrices (leaves, woods, and fibers) and storage parts (fruits and tubers). Plants have also been used to treat a variety of ailments. Secondary metabolites (e.g., carbohydrates, amino acids, and lipids) are derived biosynthetically from primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in plant growth, development, or reproduction. Flavonoids, alkaloids, terpenoids, and phenolics are examples of secondary metabolites that can be categorized into numerous groups based on their chemical classifications [5]. Plants have an almost infinite ability to produce aromatic compounds, most of which are secondary metabolites, of which at least 12,000 have been isolated, accounting for less than 10% of the total. In many situations, these compounds act as plant defense molecules, protecting plants against microbes, insects, and herbivores. Furthermore, some of them may be involved in odor (terpenoids), pigmentation (tannins and flavonoids), and flavone (tannins and flavonoids) in plants (capsaicin). Several of these compounds, however, have therapeutic characteristics [6]. Phytochemical components serve as the foundation for several pharmaceutical enterprises. The chemical elements of the plant have an important role in determining the identity of crude medications.

Phytochemical screening is critical for discovering new sources of medicinal and industrially relevant substances such as alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, and terpenoids, among others [7]. Saponins contain anticarcinogenic capabilities, immunological modulating activities, and cell proliferation regulation, as well as health benefits like cancer cell inhibition and cholesterol-lowering function [8]. Because of the large number of separated products and their pharmacological efficacy, alkaloids are one of the most important families of secondary metabolites. They have a limited range and are easily influenced by the location of plant development and climatic conditions [9]. In both industrialized and developing countries, cancer is a major public health concern. Globally, it is projected that 10.9 million new cases, 6.7 million deaths, and 24.6 million people are living with cancer [10]. In the United States, cancer is the second greatest cause of mortality, accounting for one out of every four fatalities [11]. The National Cancer Institute has analyzed roughly 114,000 extracts for anticancer potential after collecting 35,000 plant samples from 20 nations [12]. Several species of the genus Cestrum (Solanaceae) have been studied for their antitumor properties, based in part on information about plants that have traditionally been used to treat a variety of human ailments [13]. It has been shown to have a potent anti-tumor effect against sarcoma 180 ascites, leukemia in animals, and cytotoxic activity against some cancer cell lines [14]. Egypt is recognized for its thousands of species with medicinal properties and the usage of various plant components to treat certain alignments [15]. *Cestrum nocturnum* is a Solanaceae plant. Raat rani, lady of the night, or night Jessamine [16] are some of its common names. Simple glossy leaves, vine-like stalks, and greenish-creamy white tubular blooms make up this plant. The term 'nocturnum' alludes to a plant that opens it's little, strongly perfumed flowers at night. [17-18]. Hemant Kumar Nagar *et al*. reported wound healing [19], antidiabetic [20], and antibacterial [21-22] properties of *Cestrum nocturnum* (L.) ointment.

2. Materials and Methods

2.1 Plant material

During the flowering season (March 2020), different parts (stem, leaves, and flowers) of *Cestrum nocturnum* were harvested from a greenhouse in South Giza, Egypt. After collecting the various plant parts, they were washed in water, dried in a dry place, placed on clean paper in the open air, dried in a 50 °C oven, and ground to a fine powder. The powder was kept in a dark, room-temperature condition for future research. The Desert Research Center's ecological team confirmed the taxonomic identification of plant components.

2.2 Preparation of different extracts

Using ethanol 96 percent, 100 grams of dry powder of each portion were extracted. The waste from the crude material was first washed with benzol to remove plant colours, then dissolved in chemicals such as cyclohexane, distilled water, pet. Ether, trichloromethane, acetic acid ethyl acetate, methyl alcohol 70%, and finally pet. Ether.

2.3 Phytochemical analysis

Including phytochemical screening and aromatic oil isolation [23]. Test for aglycon compounds and/or glycosides, resins [24], saponins [25]., tannins [26]., flavonoids [27]., phytosterols and terpenes [28] and test for alkaloids [29].

2.4 Quantitative estimation of phytochemicals

Estimation of phenolics in *Cestrum nocturnum*, were determined spectrophotometrically and calculated as gallic acid [30]. Estimation of flavonoids in *Cestrum nocturnum*, was determined spectrophotometrically and calculated as quercetin [31]. Assessment of tannins using cupric acetate method according to [32]. Estimation of total saponins depending on [33]. and assessment of alkaloids were determined depending on the method in a way [33].

2.5 Isolation of phenolic acids and flavonoids

Using Paper and column chromatography, polyamide column, and Sephadex LH-20 column chromatography [34] are used to determine the number of phenolic acids and flavonoids in *Cestrum nocturnum*. Chemical analysis techniques for identifying flavonoid molecules. UV, nuclear magnetic resonance (1H-NMR measurement utilizing a Joel Ex500 spectroscopy; 500MHertz (1NMR), 125 Mertz (13C-NMR), or Joel JNMEX 270 spectroscopy; 270 Mertz (1H-NMR) [35].

2.6 Extraction and identification of phenolics by HPLC

The Ben-Hammouda method [36] will be used to separate the phenolic and flavonoids components from the ethanolic extract of the leaves. Three hundred grams of dried plant leaves were placed in 500 millilitres of sterile water and shaken for twenty-four hours at 200 revolutions per minute on a rotary shaker. The combination produced by these procedures is filtered through a three-millimetre-thick sheet of filter paper from Whatman Company under vacuum, then centrifuged at twelve and a half revolutions per minute for thirty minutes at eighteen degrees Celsius. The mixture is then diluted with phosphoric acid until it reaches a pH of 2.5. The diethyl ether was then eliminated on a left evening by evaporation at thirty degrees Celsius after being sacked three times in a separate funnel with a 1:1 ratio of diethyl ether. Before HPLC analysis, the residue was redissolved in three millilitres of high-grade HPLC methanol and filtered through a 0.2-micron filter-sterilized membrane [37]. Individual phenolic compounds in plant samples were identified using a hypersil C18 reversed-phase column (250 x 4.6mm) with a particle size of 5m at Hewlett-Packard (Model 1100). The injection was done with a Rheodyne injection valve (Model 7125) and a 50-l fixed loop. Sigma (St. Louis, USA) chemical companies provided the eighteen standard phenolic compounds listed in table 3.

2.7 Separation, purification, and identification of flavonoids

Flavonoids are chemicals found in abundance in the *Cestrum nocturnum* plant. They stay for a preliminary chromatographic examination before being examined under a UV lamp. According to the results of phytochemical screening investigations, one of these flavonoids has been isolated from the plant's leaves. The component was isolated from the ethanolic 96 percent extract, and multiple identification methods were used to identify it. When employing a thin layer and paper chromatography to explore the dissociation and purification of phenolic compounds, it was discovered that the ethanol 96 percent extract from the *Cestrum nocturnum* plant has a large number of active chemicals.

2.8 Removing impurities from ethanol 96% fragment

This mixture is treated at the head of the chromatography

column in numerous solvents, in essence with butane, then with butane/chloroform to improve polarity until chloroform only. It was followed by chloroform/ethyl acetate until it was only ethyl acetate, then ethyl acetate/methanol until it was only methanol, where one primary fragment was picked up. When they used butyl alcohol: ethanoic acid: distilled water in percentage (4: 1:5) plus AcOH-15 percent as a solvent capable of carrying components contained in the extract in paper chromatography in two ways, one spot was picked up. When they used butyl alcohol as a mobile phase in preparative paper chromatography, they used the following solvent: ethanoic acid: distilled water (4: 1:5) for 24 hours, providing one band, which was delicately sliced and packed with 96 percent ethanol.

3. Results and Discussion

Table 1: Phenolics, flavonoids, tannins, saponins, and alkaloids contents in stem, leaves, and flowers of *Cestrum nocturnum*.

Item	Plant parts		
	Mean ± SE		
	Stem	Leaves	Flowers
Total phenolics (mg/gm GAE)	3.01±0.09	3.17±0.07	3.74±0.1
Total flavonoids (mg/gm QE)	1.25±0.02	1.97±0.12	2.19±0.1
Total tannins (%)	3.21±0.17	3.44±0.15	3.04±0.12
Total saponins (%)	3.07±0.12	3.02±0.16	2.03±0.02
Total alkaloids (%)	1.31±0.06	1.75±0.07	1.28±0.09

GAE: Gallic Acid Equivalent and QE: Quercetin Equivalent.

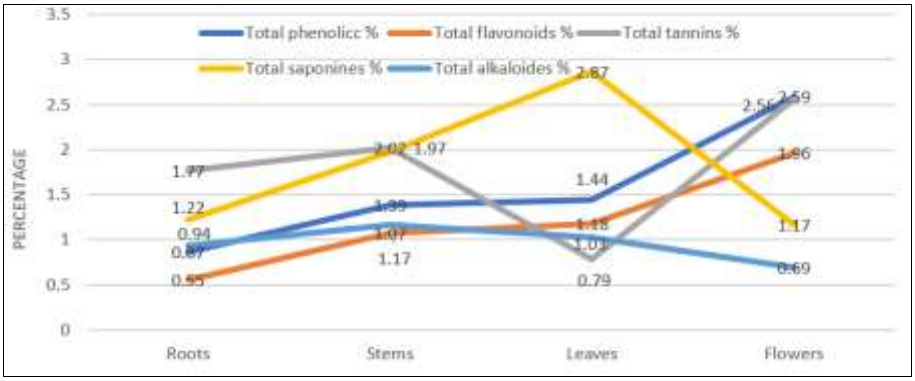


Fig 1: Phenolics, flavonoids, tannins, saponins, and alkaloids contents in stem, leaves, and flowers of *Cestrum nocturnum*.

From table 1 and figure 1: stem and Flowers extract exhibit higher concentration in phenolic contents rather than stem extract. The absorbance of the ethanolic (96%). stem, leaves, and flowers were 0.1569, 0.1896, and 0.1497, which correspond to 3.01, 3.17and 3.74 mg GAE/g respectively. Determination of the total flavonoid contents of both leaves and flowers extracts are rich with flavonoid contents while stem the ethanolic (96 percent) extracts of *Cestrum nocturnum* stem, leaves, and flowers had the lowest value (Figure 1), with 0.1025,0.2031, and 0.1430, respectively. Which correspond to 1.25, 1.97, and 2.19 mg QE/g respectively. The percentage of total tannins and alkaloids that have maximum value in stem and leaves were recorded at 3.07 and 1.75 and minimum values in flowers were recorded at 2.03 and 1.28 respectively

Table 2: The preliminary phytochemical screening of *Cestrum nocturnum*. Stem, leaves, and flowers.

Bioactive constituents	Plant parts								
	Stem			Leaves					Flowers
	Hexane	CH Cl $_3$	EtOH 96%	Hexane	CH Cl $_3$	EtOH 96%	Hexane	CH Cl $_3$	EtOH 96%
Glycosides and/or carbohydrates	+	+	-	+	-	-	+	-	+
Alkaloids	-	-	+	+	-	+	+	+	-
Flavonoids	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Sterols and/or terpenes	-	–	+	-	+	-	–	+	–
Coumarins	-	+	-	-	+	+	+	-	-
Anthraquinones	+	+	+	–	–	-	-	-	-
Volatile oils	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	–	+	–	+	+	+	–	+
Phenolics	+	+	+	+	+	+	+	+	+

+: presence –: absence

From table 2: The phytochemical survey of both the stem, leaves, and flowers of the *Cestrum nocturnum* plant is used by three solvents, the hexane, chloroform, and ethanol 96%. Phytochemical constituents-based results are as shown in table 2. The ethanolic extract was the song for all the plant parts and the ethanolic 96% fraction for leaves was the bunny of all the effective materials.

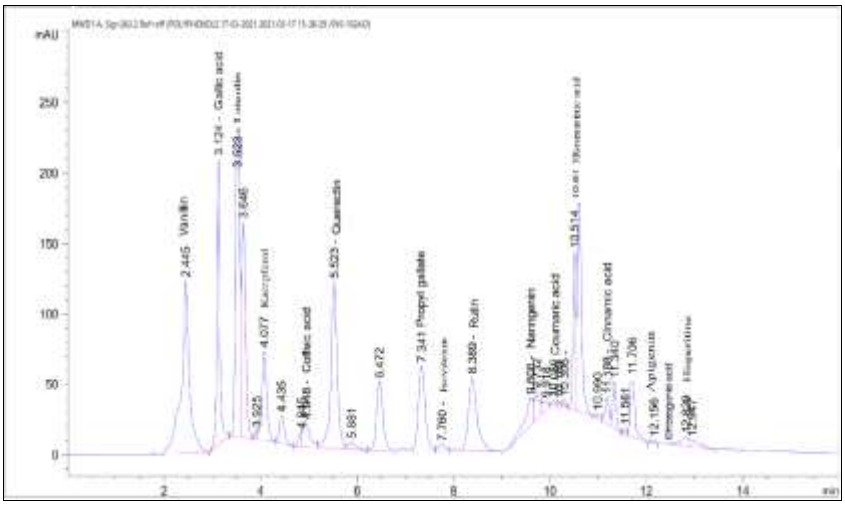


Fig 2: Identification of phenolic and flavonoid compounds using HPLC.

Table 3: Identification of phenolic and flavonoid compounds using HPLC.

Peak	Retention Time [min]	Type	Width [min]	Area [Mau*s]	Area (%)	Name
1	2.445	BB	0.1232	1003.70201	2.9429	Vanillin
2	3.124	BB	0.1328	90.20594	4.3605	Gallic acid
3	4.435	BB	0.1006	1367.32061	3.3408	?
4	3.646	VB	0.1596	706.41010	0.2441	?
5	3.825		0.0000	0.00000	0.00000	Luteolin
6	3.925	BV	0.0637	34.70713	0.2353	Kamferol
7	4.077	VB	0.7026	329.29071	3.5955	?
8	4.435	BB	0.3596	113.50312	1.3008	?
9	4.845	BV	0.4368	63.98642	0.4910	?
10	4.948	VV	0.1068	110.18240	1.4070	Coffeic acid
11	5.288		0.0000	0.00000	0.0000	?
12	5.523	VV	0.2316	1068.04246	7.5143	Quericetin
13	5.881	VB	0.2695	74.09390	0.4420	?
14	6.472	BB	0.2016	140.51004	3.0066	?
15	6.747	VV	0.4563	0.252364	3.2062	
16	7.341	BV	0.1298	483.31102	4.3057	Propyl gallate
17	7.760	VV	0.1049	46.25605	0.4222	Isovetixen
18	8.389	BB	0.1736	615.32922	4.0514	Rutin
19	8.876	VB	0.2036	236.32520	1.2091	?
20	9.606	BV	0.1329	136.77971	1.1530	Narinjirnin
21	9.732	VV	0.0263	226.91063	1.1548	?
22	9.918	VB	0.2365	63.32630	0.4150	?
23	10.089	BV	0.1235	14.20609	0.1093	?
24	10.189	VB	0.1026	7.11709	0.0836	Coumaric acid
25	10.306	BB	0.2365	13.07249	0.1503	?
26	10.514	BV	0.7326	265.21322	0.2313	?
27	10.612	VB	0.2365	572.23250	0.1353	?
28	10.990	BV	0.3021	19.73687	2.2313	?
29	11.188	VV	0.1235	103.78630	0.1093	Cinamic acid
30	11.340		0.0000	0.00000	0.00000	
31	11.561	BV	0.3265	16.54208	0.2313	?
32	11.706	VB	0.3589	309.20739	4.9334	?
33	12.156	BB	0.2356	3.93147	0.1777	Apeginin
34	12.829	BV	0.2103	71.50942	1.5031	Chlorogenic acid
35	12.947	VB	0.1365	54.12609	0.1545	Hispertine
36	13.022	BV	0.1968	26.5007	0.2537	?
37	13.159		0.00000	0.00000	0.0000	?
Totals:			1.2974611			

From table 3 and fig 2 The flavonoid and phenolic components in ethanolic extract of leaves were assessed qualitatively and quantitatively using high-performance liquid chromatography (HPLC), where each compound was identified, separated using an authentic pattern, and its concentration measured. Vanillin, gallic acid, luteolin, kaempferol, coffeic acid, quercetin, propyl gallate, isovetixin, rutin, narinjirnin, coumaric acid, cinnamic acid, apigenin, chlorogenic acid, and hispertin were among the chemicals found and isolated. The phytochemical screening revealed that this plant is rich in various chemical compounds with medicinal, industrial, and nutritional significance in a study. The chemicals isolated in this study could be used to classify plants belonging to the Solanaceae family regardless of their internal chemical makeup, implying that these taxa are members of the Solanaceae family without further

investigation. These isolated chemicals have been shown in research to have antibacterial, antioxidant, and anti-cancer properties, and they are employed to preserve food in many companies. According to a lot of studies, it's possible to use the isolated compounds that have been discovered to build a variety of pharmaceuticals that can help treat a variety of ailments, including cancer [38]. The economic values of separated flavonoids and phenolic components from *Cestrum nocturnum* leaves extract are particularly rich in phenolic and flavonoids compounds, according to previous studies. These chemicals are high in nutritional value and have the ability to suppress DNA damage, as well as possess antioxidant and anti-tumor properties. These chemicals can also help to lower blood pressure. Antimutagenic properties have been shown in various phenolic and flavonoid compounds [38]. Several studies have revealed that certain of the chemicals found in the leaves of the *Cestrum nocturnum* plant, for example, have significant medical value. Anticancer properties of 5, 7 dihydroxy isoflavone [39]. as well as anti-freedom zealots. Naringenin is a phytoestrogen that enters the structure of sex hormones and aids in their normal function at optimal expression. It also enters the creation of estrogen receptors. Anti-nociceptive properties can be found in 3, 4', 7 trihydroxy flavones. [40] Kaempferol has a cytotoxic impact. Hesperidin is involved in the synthesis of vitamin B, which is necessary for the absorption of vitamin C from the diet. It also helps to lower blood pressure by reducing capillary fragility, which protects us from heart disease. Gallic acid has five times the potential of vitamin C to neutralize free radicals, which are responsible for cell damage in our bodies. Ferulic acid and gallic acid inhibit Fusarium mycelial development via reducing damping off. As a result, mint could be considered a medicinal plant. Tannins are waste products from the metabolic process. The leather was typically cured with tannins extracted from a variety of plants, and tannins were also utilized to treat liver ailments through regular consumption. Even when tannins are extensively dispersed throughout the plant, the amounts vary substantially from one organ to the next. As a result, we assessed *Cestrum nocturnum's* plant tannin content to determine the plant's economic value. [40]. The ethanolic 96 percent leaf extract showed strong activity on various tumour cell lines in this investigation. The presence of phenolic acids, hydrolysable tannins, and flavonoids (aglycone and glycosides) in the ethyl alcohol 96 percent extracts may explain these findings [41].

Identification of propyl gallate compound

Table 1 shows the Rf-values and colour reactions of the purified compound, which was produced as an amorphous powder soluble in methanol (3).

Table 4: Rf-values and color reaction for propyl gallate compound.

Solvents	Rf values	Colour		
		Visible	UV	UV + ammonia
BAW	0.39	-	yellow	pale yellow
AcOH-15%	0.18	-	yellow	Pale yellow

The chemical appeared to be an aglycone based on Rf-values and colour response.

UV spectral data, λ_{max}, nm of propyl gallate table 5 and fig. 3, 4 & 5) was

Table 5: UV spectral data of propyl gallate compound using different ionizing and complexing reagents

	MeOH	NaOMe	NaOAc	NaOAc+H_3BO_3	$AlCl_3$	$AlCl_3$ + HCl
Propyl gallate compound	198	203	221	196	233	236
	293	268	252	244	294	289
	302	307	307	296	322	367
		359	399	401	417	403

^{1}H-NMR data of propyl gallate compound in $CDCl_3$ (Fig. 6)

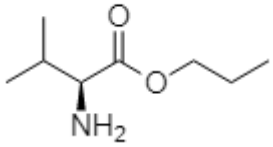
The compound's spectra in DMSO revealed signals at ppm 3.02 and 2.84 (5H, d, J= 3Hertz, H-3, and Hertz-3), 7.12 (4H, m, J= 4Hertz, H-3, 4, 5, 6, 7, and 7), and 4.42 (H, signals, H-3), as well as a singlet for H-5 at 5.16 ppm (indicating flavonoid) [31]. The existence of hydroxylation at C-4, C-3 was indicated coupling between H-5 and H-4 based on data from the 1H-NMR spectrum of this molecule. As a result, at 3.14 and 2.41, respectively, H-and H-3 appeared as doublets.

Mass spectrum (fig. 7):

The compound's mass spectrum (Fig. 7) revealed the existence of a molecular ion peak (M+, 17%) at m/z 289, as well as additional significant ions at m/z 306, 258 (Propyl gallate 100 percent), 263, 281, and 156.

The isolated component was recognized as propyl gallate based on previously collected data.

Table (6) scheme 1 for Propyle gallate compound

Compound No.	Name	Structure
1	Propyle gallate	 Scheme (1)

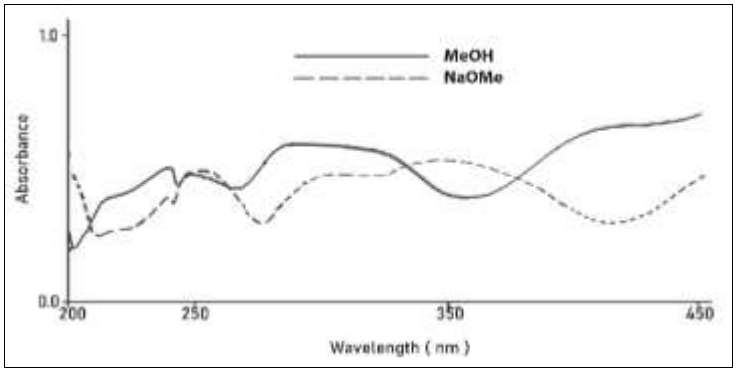


Fig 3: UV spectrum

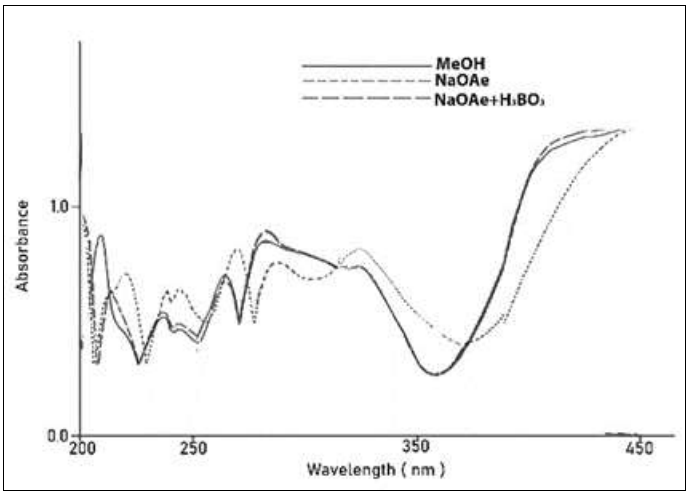


Fig 4: UV spectrum

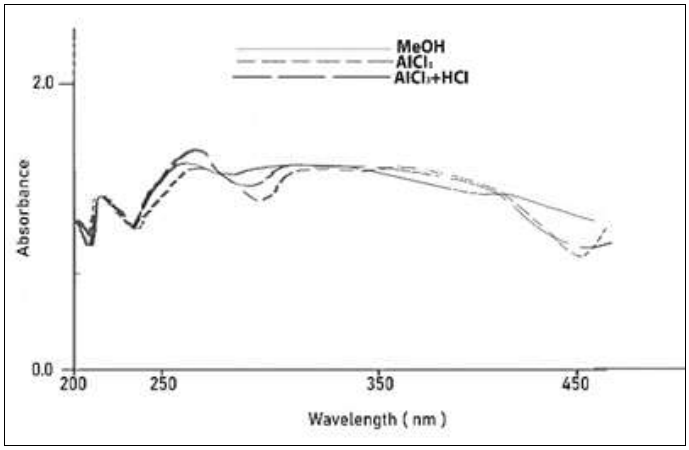


Fig 5: UV spectrum

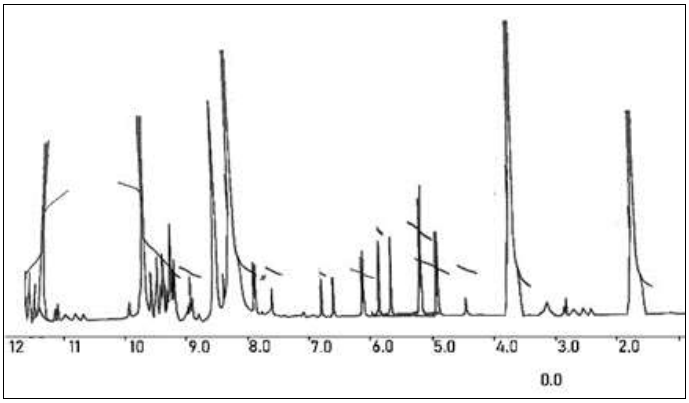


Fig 6: ^{1}H-NMR data of propyl gallate compound in $CDCl_3$

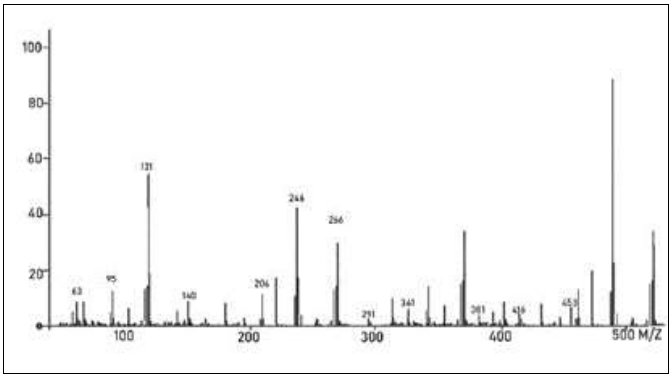


Fig 7: The compound's mass spectrum

4. Conclusions

This study found that the *Cestrum nocturnum* plant contains many secondary metabolites in various parts of the plant, especially its leaves, and also those significant quantities of main groups of phytochemical materials are present and when his leaves were also examined using HPLC, several compounds, including flavonoids and phenolic acid, are present, giving rise to the isolation and identification of Propyl gallate compound using traditional methods.

Conflicts of interest: There are no conflicts interested

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