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Preliminary Phytochemical Screening, Identification of Some Bioactive Constituent and Isolation of Unknown Compound from *Murraya paniculata* Cultivated in Iraq

Zainab Ali Qasim[*,1] and Amjed Haseeb Khamees [2]

[1]College of Pharmacy, University of Dhi Qar, Dhi Qar, Iraq.

[2]Department of Pharmacognosy and medicinal plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

***Corresponding author**

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Abstract

Murraya paniculata, sometimes known as orange Jessamine, is a significant decorative plant found in tropical and subtropical regions. It belongs to the Rutaceae family. The distribution of this species extends from South Asia to Australia. This plant possesses a multitude of applications in traditional medicine for the treatment of several ailments. The plant demonstrates an extensive array of pharmacological actions, involving antinociceptive, antioxidant, anti-diabetic, antibacterial, and analgesic effects**.** This study aims to conduct preliminary phytochemical screening, phytochemical investigation of some bioactive constituents, and isolate an unknown compound from *Murraya paniculata* cultivated in Iraq. It is worth noting that no previous phytochemical survey has been conducted on this plant in Iraq. The entire plant underwent extraction using the hot technique (Soxhlet) in 85% methanol. Subsequently, the extract was fractionated employing solvents with varying polarity, such as petroleum ether, chloroform, and ethyl acetate. A preliminary phytochemical screening was conducted using established procedures. The petroleum ether, chloroform, and ethyl acetate fractions underwent high-performance liquid chromatography (HPLC) analysis using authenticated standards. Preparative layer chromatography was employed to isolate unknown chemical from the chloroform fraction. The isolated compound was identified using FT-IR, ^{1}HNMR, and LC-MS. The initial tests suggest the existence of alkaloids, flavonoids, steroids, coumarins, and other secondary metabolites. The chromatographic and spectroscopic analyses reveal the occurrence of various phytochemicals in different fractions of *Murraya paniculata* as follows: the petroleum ether fraction contains stigmasterol; the chloroform fraction contains aegeline and severifoline; and the ethyl acetate fraction contains apigenin, luteolin, and 5,7,3',4'-tetramethoxy flavanone. These findings will be of great value to phytochemists and pharmacologists in their future efforts to develop new active compounds from this plant, considering its notable pharmacological activity.

Keywords: FT-IR, HNMR, HPLC, *Murraya paniculata,* Preliminary Phytochemical Screening.

Introduction

Herbal therapy has a long history of being utilized for preventing and treating ailments, promoting health, and improving our lifespan and well-being with negligible adverse effects [1, 2]. Phytochemical research is a crucial scientific approach. This technique determines the primary components present in all plant parts, including bark, leaves, stem, root, and fruits. While the exact number of plant species utilized for therapeutic purposes worldwide is unknown, both herbal and Western medicine recognize the value of these plants[3]. *Murraya paniculata*, often known as orange jasmine, is a plant in the Rutaceae family. This plant is an attractive and economically valuable agricultural crops found in Cuba and the Andaman Islands, up to an elevation of 1500 meters.

The species is indigenous to tropical Asia, encompassing areas ranging from India and Sri Lanka to Myanmar, southern China, Taiwan, and Thailand. Furthermore, it expands in an eastern direction, encompassing the Malesia region and reaching as far as northeastern Australia and Caledonia. The arboreal has a humble appearance, featuring a broad canopy and a small, often twisted trunk; it has a somewhat corky texture and emits a pleasant fragrance. The leaves are arranged alternately and are not divided into leaflets, measuring 10-17 cm in length. The leaflets are typically 3-5 in number, mainly 3-7 cm long, and have an oblong, elliptic-lanceolate, or rhomboid shape. They are glossy and darker on the upper surface, with glands present, and have a cuneate or rounded base[4-6].*Murraya paniculata* is traditionally utilized for treating gastrointestinal, respiratory, and

cardiovascular conditions [7]. The plant displays pharmacological properties that combat several ailments such as diabetes, obesity, bacterial infections, implantation issues, oxidative stress, cancer, diarrhea, depression, and anxiety[8,9]. *Murraya paniculata* extract contains a variety of biologically active compounds like alkaloids, flavonoids, triterpenoids, phenols, steroids, cardiac glycosides, and carbohydrates[10]. The assessment of a plant's medicinal value relies on its physiologically active components and their quantities. The process of extracting and isolating these physiologically active components from plants has been carried out since ancient times [11]. The extraction process primarily relies on crucial input parameters, knowledge of the plant matrix's nature, the chemistry of bioactive substances, and scientific experience[12].

The aqueous extract of this plant is a rich source of antioxidant and phenolic chemicals, which are highly beneficial for therapeutic purposes [13]. Various alkaloids have been extracted from different parts of the *Murraya paniculata* plant. These include paniculidines D, E, F, A, B, and C, as well as tanakine and yuehchukene from the plant's roots, and alanditrypinone, alantryphenone, alantrypinene, and alantryleunone from its leaves[14,15]. In this study, a preliminary phytochemical test was conducted to detect various secondary metabolites in different fractions of plant extracts (petroleum ether fraction, chloroform fraction, and ethyl acetate fraction). High-performance liquid chromatography was then utilized to identify these phytochemicals in the plant extract fractions. Subsequently, an unknown alkaloid was isolated from the chloroform fraction using preparative layer chromatography in a specific quantity sufficient for identifying the molecular structure of this alkaloid through FT-IR, ^{1}HNMR, and LC-MS.

Figure 1. Photos of the whole plant of *Murraya paniculata* that cultivated in Iraq in Al-Hilla nurseries.

Materials and Methods

Plant material collection and authentication

The entire plant of *Murraya paniculata*, cultivated in Iraq, was gathered from a farm near Alhilla during April 2023. The plant underwent examination and validation by Dr. Israa Abdel Razzaq Al Majeed, an expert from the Department of Biology at the College of Sciences, University of Baghdad. The herb underwent a thorough washing process, followed by a month-long period of drying in a shady area. The material was initially crushed manually and subsequently refined using a mechanical grinder and weighed.

Plant extraction

Extraction is a crucial first step in the study of medicinal herbs since it allows for the isolation of the specific chemical constituents that may later be separated and identified [16]. A quantity of 200 grams of dried powder of the whole plant is then dried & placed in a thimble for extraction using the hot method with a soxhlet apparatus. In a round flask, 1500 milliliters of a solvent consisting of 85% methanol and water is added and connected to the thimble chamber. Boiling chips are added to the round flask, and the mixture is heated on a heating mantle approximately for 22 hours. The methanolic extract was filtered and evaporated using a rotary

evaporator to yield approximately 20 g, which was then suspended in 250 ml of distilled water to produce a crude fraction designated as (E1).

Fractionation of extract

The active constituents were fractionated through the utilization of solvents of different polarity in a separatory funnel such as petroleum ether, chloroform, and ethyl acetate. These fractions were designated as (E2, E3, and E4) respectively. The solvents were combined with the crude extract, which had been suspended in 250ml of purified water. The procedure was carried out for three consecutive days, using 250ml of solvent each day. The total volumes of each solvent were concentrated using a rotary evaporator.

Initial phytochemical analysis

A general phytochemical screening was conducted on the methanolic extract and various fractions (petroleum ether, chloroform, and ethyl acetate) to detect several phytochemical derivatives such as carbohydrates, cardiac glycosides, alkaloids, flavonoids, tannin, phenolics, steroids, coumarins, and saponins, using the method outlined by Harborne [17]. The presence of phytoconstituents is presented in the Table (1).

Identification of compounds by HPLC:

An HPLC analysis was performed using an HPLC device (CECIL company in Britain, at the Scientific Center for Chemical and Industrial Analysis in Al-Jamea District, Baghdad) to qualitatively identify active components in different fractions of the entire *Murraya paniculata* plant (petroleum ether, chloroform, and ethyl acetate). The retention periods of the specimens examined were compared to conventional materials under the same conditions.

- **Identification of petroleum ether (E2) fraction by HPLC**

High-Performance Liquid Chromatography was performed using isocratic elution on a C18 column (5 µm particle size, 250 x 4.6) with a flow rate of 1.5 mL/min. Revealing was done at λ = 210 nm and the injection volume was set at 20 µL. The mobile phases are composed of 95% acetonitrile and 5% water.

- **Identification of chloroform fraction (E3) by HPL**

High-Performance Liquid Chromatography was performed using isocratic elution on a C18 column (5 µm particle size, 250 x 4.6) with a flow rate of 1.5 mL/min. Detection was done at λ = 220 nm, and the injection volume was set at 20 µL for both the E3 fraction and aegeline authenticated standard. The mobile phases are composed of 95% acetonitrile and 5% water.

- **Identification of ethyl acetate (E4) fraction by HPLC**

For E4 fraction luteolin, apigenin, and 5,7,3, 4,-tetramethoxyflavonone, authenticated standards: The High-Performance Liquid Chromatography was performed using isocratic elution on a C18 column with a stationary phase (5 µm particle size, 250 x 4.6). The flow rate was 0.75 mL/min, the detection wavelength was set at 254 nm, and the injection volume was 20 µL. The mobile phases consist of methanol, water (1:1) and formic acid 5%

Isolation and structural determination of unknown alkaloid

Unknown alkaloid isolation was conducted by PLC. The chloroform fraction of the entire plant was dissolved in chloroform and applied a few mL using a capillary tube in a row of concentrated spots in the form of a streak on each plate, with each spot being applied five times. The spots must dry before further application. Preparative layer plates, 0.5 mm thick, made of silica gel GF_{254}nm (20x20cm) from Taiyang, China, were used. The plates were heated at 110°C for 30 minutes. For the mobile phase, 100 ml was used, with the following proportions: ethyl acetate, toluene, methanol, and formic acid, adjusted to a volume ratio of 110:67:20:3 V/V [18]. The retardation factor (R_f) value of this alkaloid was 0.7. Detection was performed by applying Dragendorff's spraying reagent on one side of the plate and utilizing UV light at wavelengths of 254 nm and 366nm. An orange-colored band is observed on one side of PLC plates when sprayed with Dragendorff's reagent. This band is then scraped off and transferred into a beaker. Chloroform: methanol (95:5) is added to the beaker with agitation and then allowed for an hour before being filtered. Upon solvent evaporation, the resulting filtrate yields an off-white powder. Structural identification was performed using FT-IR (analyzed at the BPC analysis lab in Baghdad Adhamiya near Al-Nu'man teaching hospital using a SHIMADZU instrument), ^{1}HNMR (analyzed at the University of Basra, College of Education for Pure Sciences, Department of Chemistry used Bruker AVANCE-NEW device) and LC-mass (analyzed in Jordan University of science and technology, Irbid, Jordan using LC/MS-8040 series system Shimadzu/ Japan, coupled to a Shimadzu mass spectrometer with an electrospray interphase (ESI).

Results

Extraction and fractionation

Table (1) displays the weight and percentage output from every component obtained from 200g after the procedure for defatting (E1) fraction and from fractionated raw extract (E2, E3, and E4) fractions.

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Table 1. The weight and percentile yield of each fraction were obtained from the *Murraya paniculata* plant.

The fraction	The Percent in w/w% in the whole plant	The Weight of fraction in gram
E1	10%	20
E2	2.5%	5g
E3	1.5%	3g
E4	0.75%	1.5g

The preliminary qualitative result:

The initial qualitative phytochemical study of the crude extract and various fractions using Chemical assays is presented in Table (2).

Table 2. The result of a phytochemical test for the crude extract and various fractions of the whole plant of *Murraya paniculata*.

Constituent	Methanolic extract	Different fractions of *murraya paniculata*		
		Petroleum ether	Chloroform	Ethyl acetate
Alkaloids	+	-	+	-
Flavonoids	+	-	-	+
Coumarins	+	-	+	+
Steroids	+	+	-	-
Terpenoids	+	+	-	-
Saponins	+	+	+	+
Tannins	+	-	+	+
Anthraquinone glycosides	-	-	-	-
Cardiac glycoside	-	-	-	-

Identification of different compound in different fraction of extract by HPLC

Petroleum ether fraction: The HPLC chromatogram results showed that one of the estimated E2 fraction retention times(2:28.4 min: sec) matched the validated reference retention time for stigmasterol(2:19.4 min: sec), as shown in Figure (2) and (3).

Chloroform fraction: The HPLC chromatogram results indicated that the retention time of a compound in the E3 portion(2:16 min: sec) matched the retention time of the verified aegeline norm (2:17.3 min: sec), as shown in Figure (4) and (5).

Ethyl acetate fraction: The HPLC chromatogram observations indicated that the retention time of one compound in the E4 fraction matched the retention time of the verified references (luteolin, apigenin, and 5,7,3',4'-tetramethoxy flavanone) as shown in Figure(6),(7),(8),(9) and table(3).

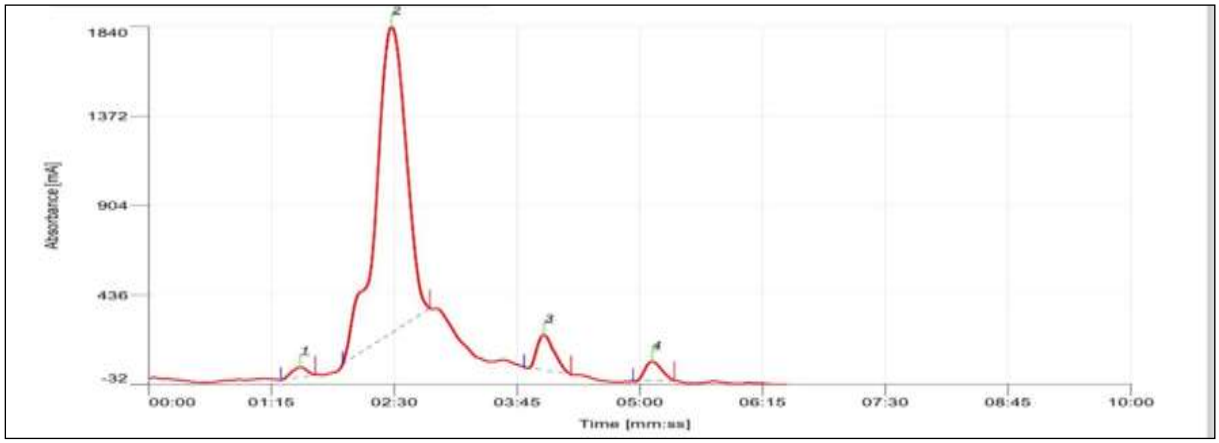

Figure 2. HPLC chromatogram of petroleum ether employing a mobile phase consisting of 0.01M acetonitrile (95%) and water (5%).

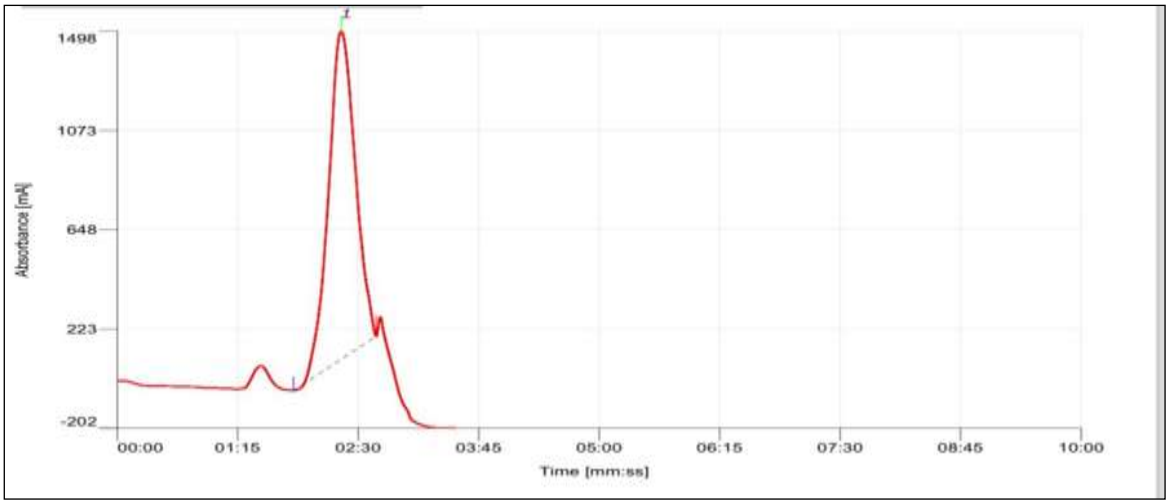

Figure 3. HPLC chromatogram of the stigmasterol norm, employing a mobile phase consisting of 95% acetonitrile and 5% water.

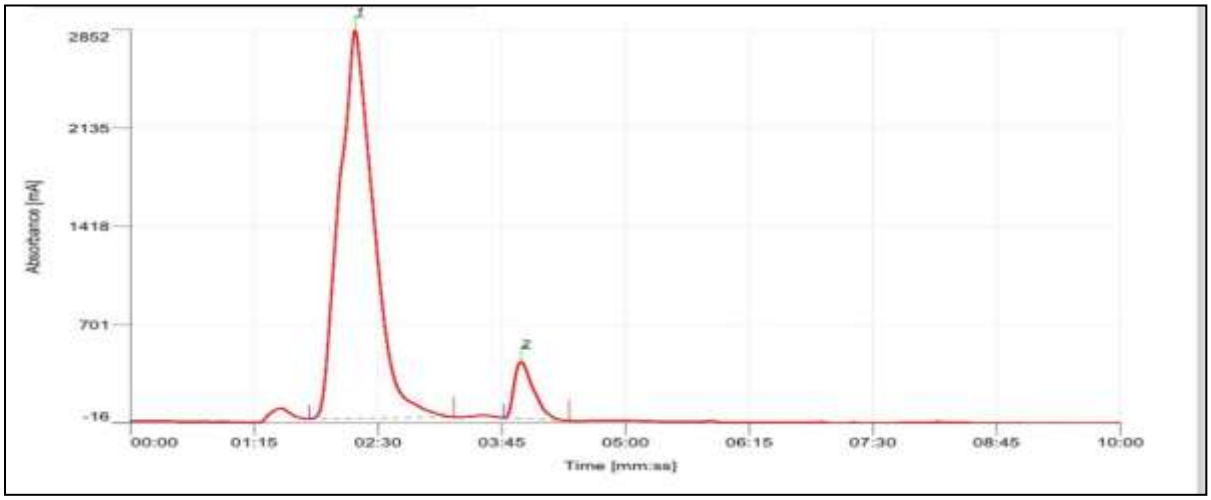

Figure 4. HPLC chromatogram of chloroform, utilizing a mobile phase consisting of 95% acetonitrile and 5% water.

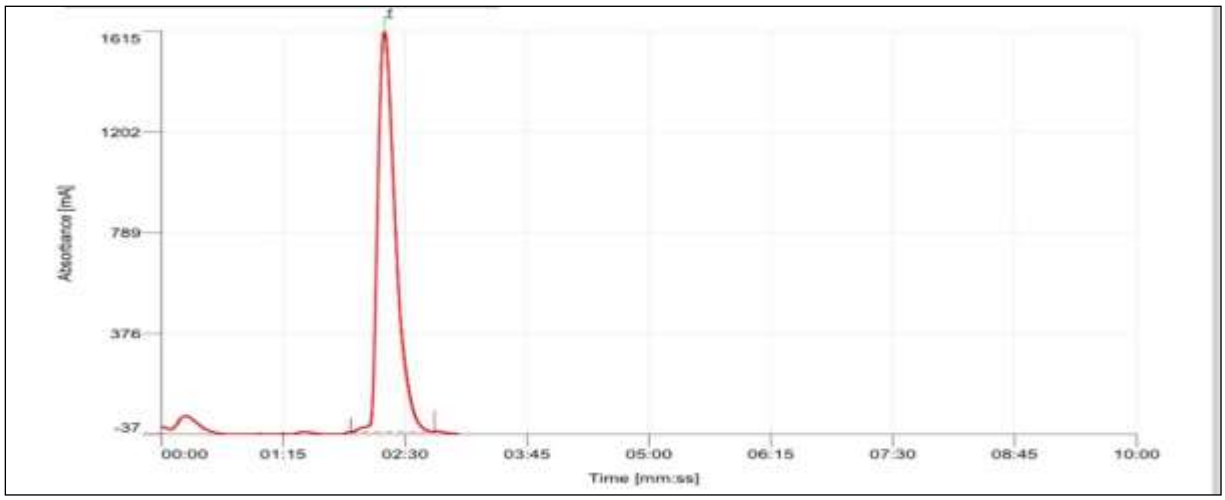

Figure 5. HPLC chromatogram of aegelline standard, employing a mobile phase consisting of 95% acetonitrile and 5% water.

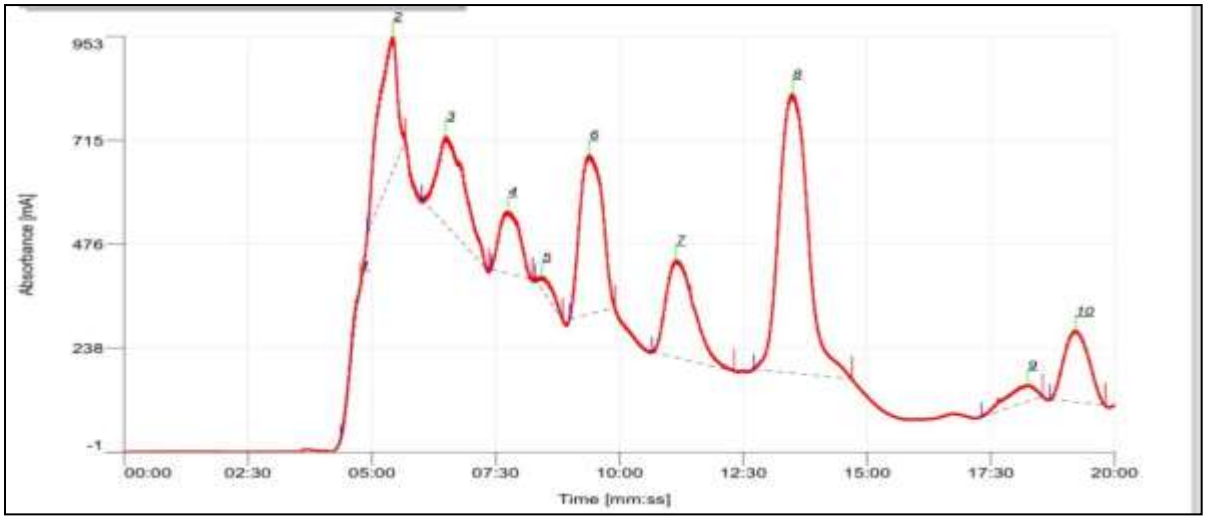

Figure 6. HPLC chromatogram of the ethyl acetate fraction. The mobile phase employed consists of a mixture of methanol and water in a 1:1 ratio, with 5% formic acid.

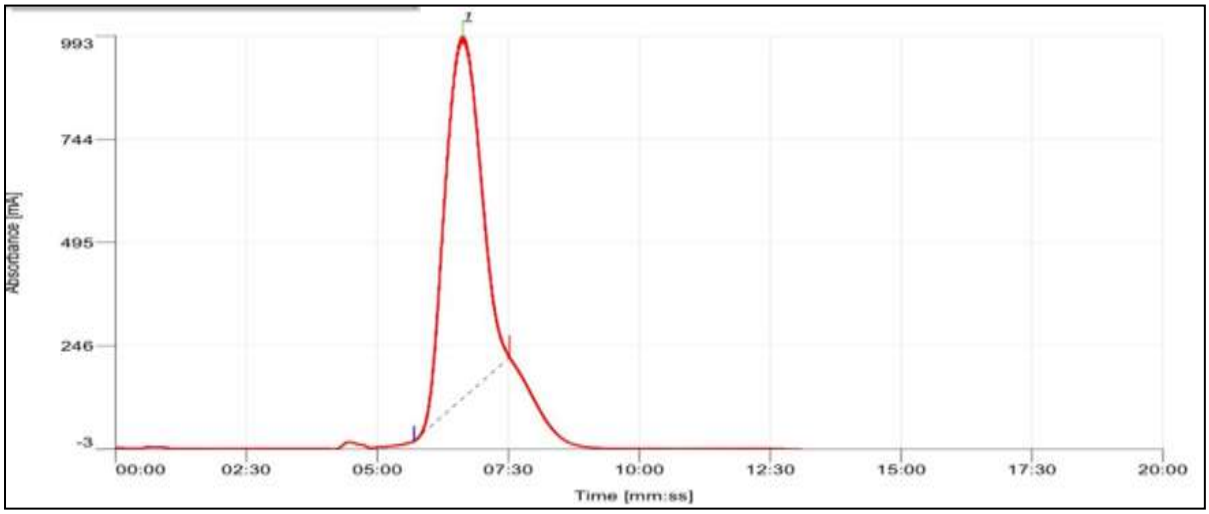

Figure 7. HPLC chromatogram of the luteolin norm. The mobile phase employed was a mixture of methanol and water in a 1:1 ratio, with 5% formic acid added.

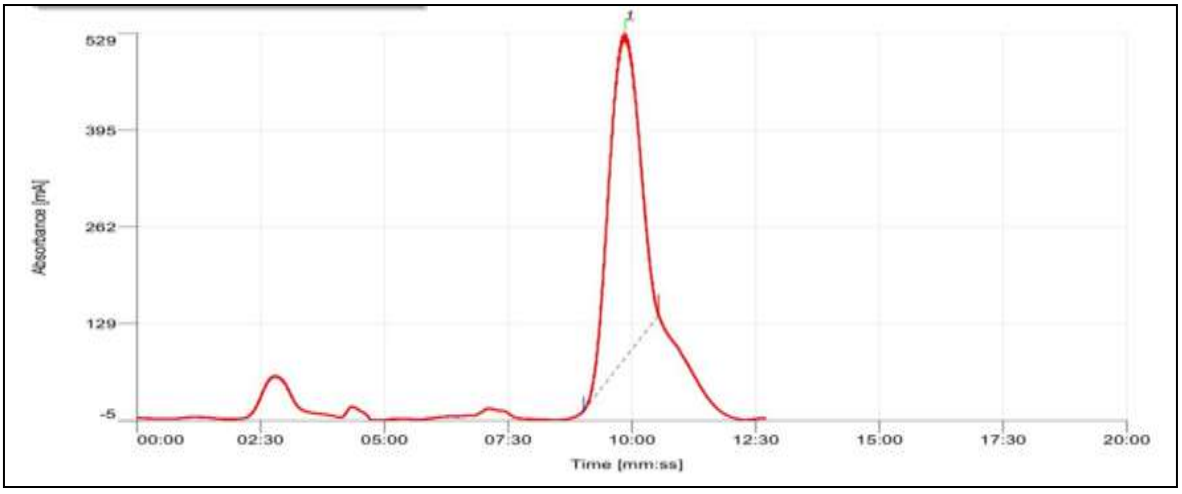

Figure 8. HPLC chromatogram of the apigenin norm. The mobile phase employed was a mixture of methanol and water in a 1:1 ratio, with 5% formic acid.

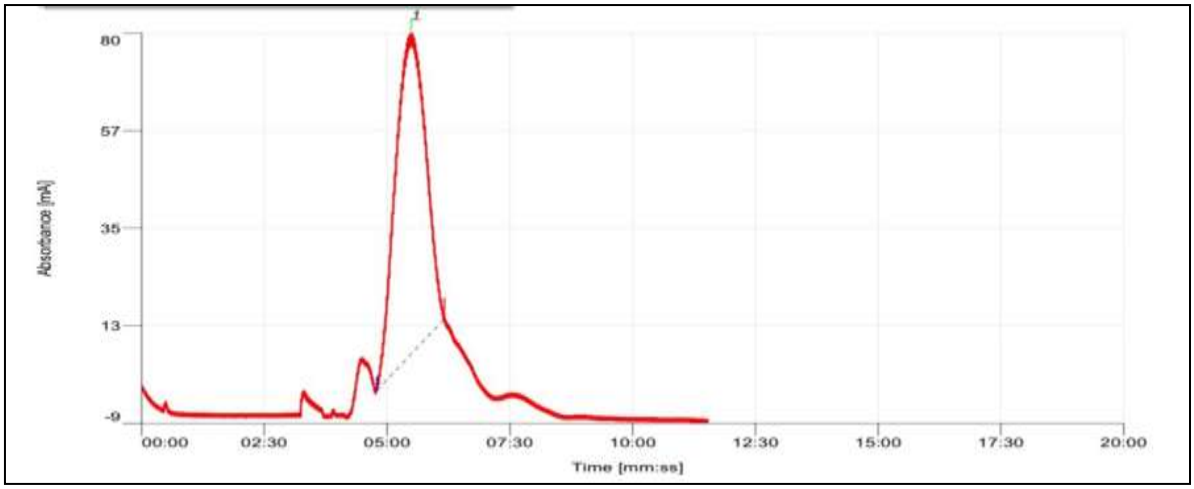

Figure 9. HPLC chromatogram of luteolin tetramethyl ether standard using methanol: water(1:1) and formic acid 5%as mobile phase.

Table 3.HPLC retention time for various component in ethyl acetate fraction and their corresponding standard retention time in minute: second.

NO.	Component	retention time in ethyl acetate fraction
1	Luteolin	6:29.4
2	Apigenin	9:23.4
3	5,7,3',4'-tetramethoxy flavanone	5:25.4

Isolation of unknown alkaloid by PLC

The bands separated from the E3 fraction on the plate are displayed in Figures (10) and (11). They were extracted from the sorbent using chloroform and methanol, filtered, then evaporated to dryness and recrystallized. An off-white powder was acquired.

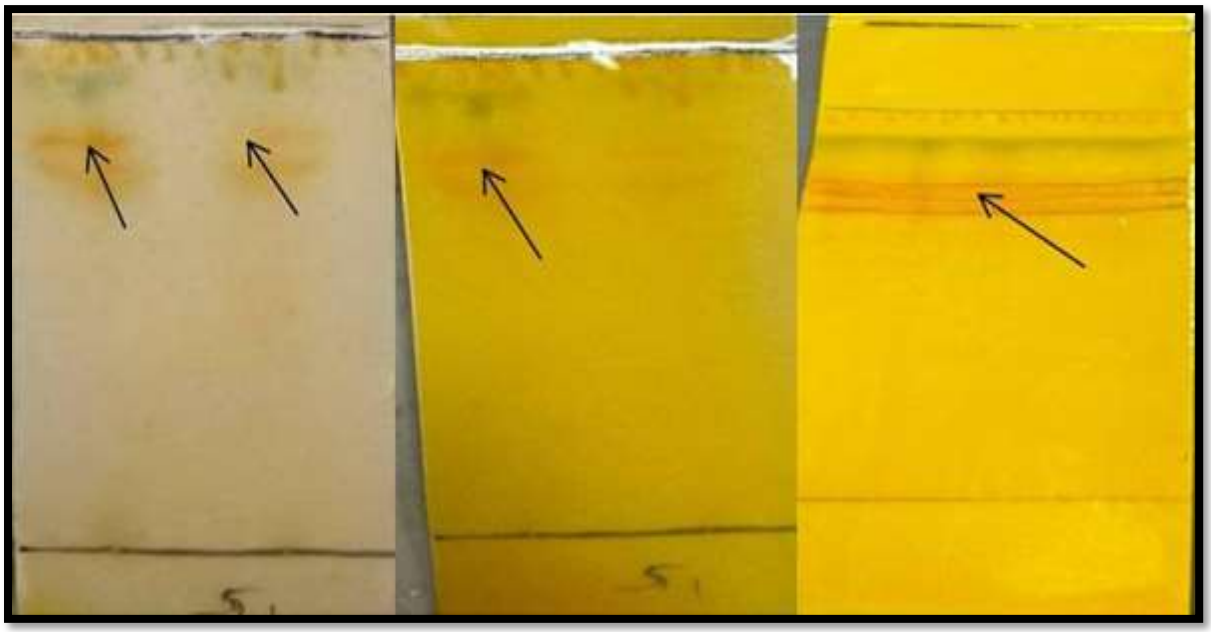

Figure 10. TLC chromatogram of unknown alkaloid in *Murraya paniculata* plant using silica gel GF_{254}nm as adsorbent and [ethyl acetate: toluene: methanol: formic acid (110:67:20:3)] as a mobile phase. Detecting by spraying with dragendorrf's reagent

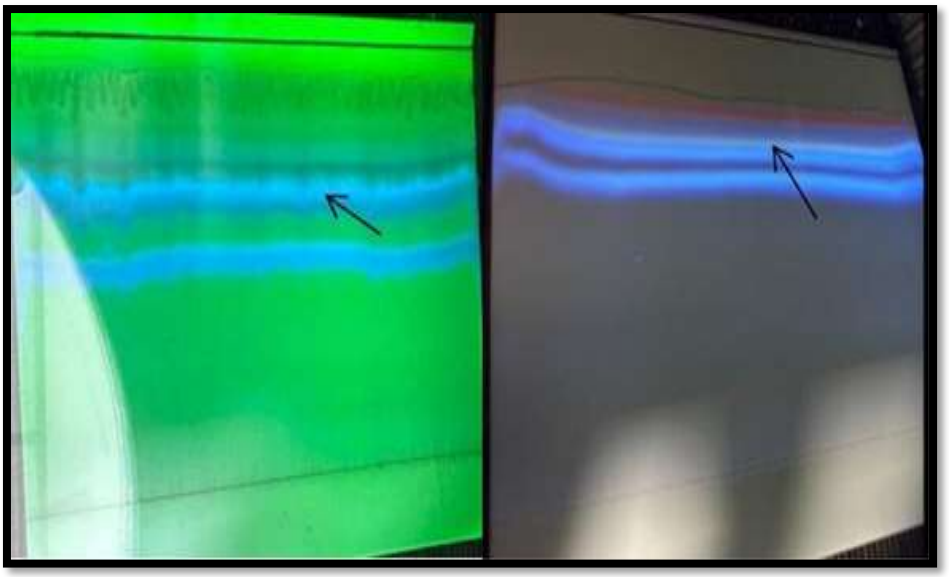

Figure 11. Preparative layer chromatography plate of unknown alkaloid used [ethyl acetate: toluene: methanol: formic acid (110:67:20:3)] as a mobile phase and was observed at 254 nm and 365 nm.

structural identification of unknown alkaloid :

1-FT-IR

In phytochemical research, Fourier transform infrared spectroscopy (FT-IR spectroscopy) is used as a fingerprinting instrument since it deals with the interaction of molecules and radiation in the infrared portion of the spectrum (IR region = 4000 - 400 cm $^{-1}$) [19]. Figures (12) display the IR spectra of an unknown alkaloid isolated from the chloroform fraction of the entire plant of *Murraya paniculata*. The unique infrared absorption spectra of the unknown alkaloid are explained in Table (4).

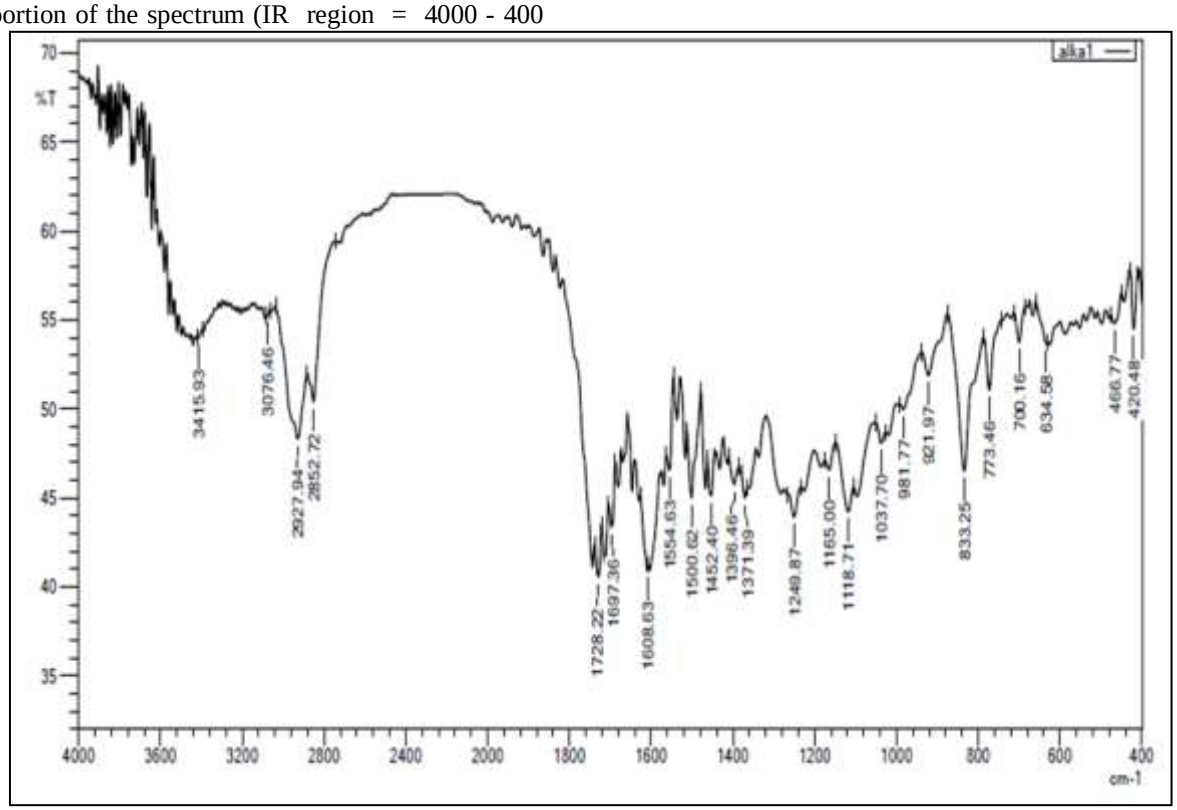

Figure 12. FT-IR of unknown alkaloid isolated from chloroform fraction of *Murraya paniculata.*

Table 4. characteristic IR absorption bands of unknown alkaloid isolated from chloroform fraction of *Murraya paniculata*

Functional group	Group frequency wave number in cm^{-1}	Assignment
N-H	3500-3300	N-H stretch, single weak band indicate secondary amine
C=C-H	3076	C-H stretch of aromatic ring
C-H	2930-2850	Aliphatic C-H stretch
C=O	1670	C=O stretch
C=C	1640	C=C stretch of aliphatic
C-H	1452	C – H bending of CH2.
C=C	1608	C = C stretching aromatic ring
C-O-C	1249	C-O-C stretching of ether
C-H	1037	C – H bending of aromatic (in plane)
C-H	921,833,773	Aromatic C-H bending (out of plane)

2-^{1}HNMR

The isolated unknown alkaloid from the chloroform fraction of the whole plant of *Murraya paniculata* exhibited unique chemical shifts in the ^{1}HNMR spectrum. The chemical shifts are explained in table (5) and figure (13). The putative unknown compound's structure is depicted in Figure (14), with the chemical shift for each carbon atom indicated by red letters.

Table 5. Assignment of ^{1}H-NMR spectral data of unknown alkaloid isolated from chloroform fraction of *Murraya paniculata.*

Carbon atom	Ppm experimental	Integration	Multiplicity	Assignment
a	1.21-1.29	6H	Multiple	CH3
b	1.72	3H	Singlet	CH3
c	1.81	3H	Singlet	CH3
d	3.26	2H	Doublet	CH2
e	3.93	1H	Triplet	CH
f	4.97	1H	Doublet	CH
g	6.17	1H	Doublet	CH
h	6.79	1H	Singlet	NH
i	6.83	1H	Triplet	CH
j	7.34	1H	Triplet	CH
k	7.43	1H	Doublet	CH
l	7.57	1H	Doublet	CH
m	7.59	1H	Singlet	OH

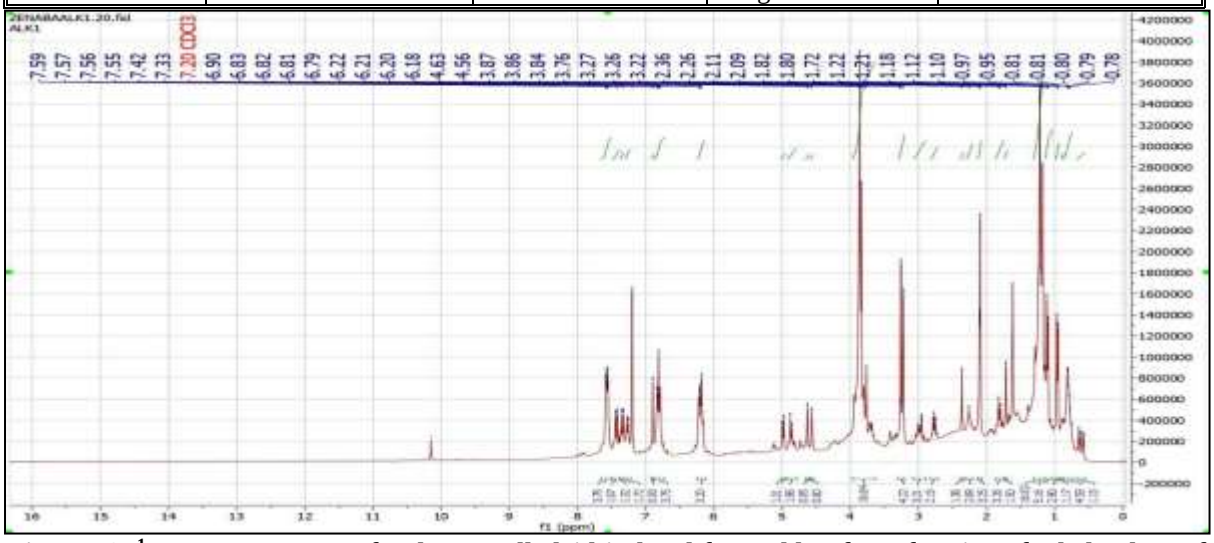

Figure 13. ^{1}HNMR spectrum of unknown alkaloid isolated from chloroform fraction of whole plant of *Murraya paniculata*.

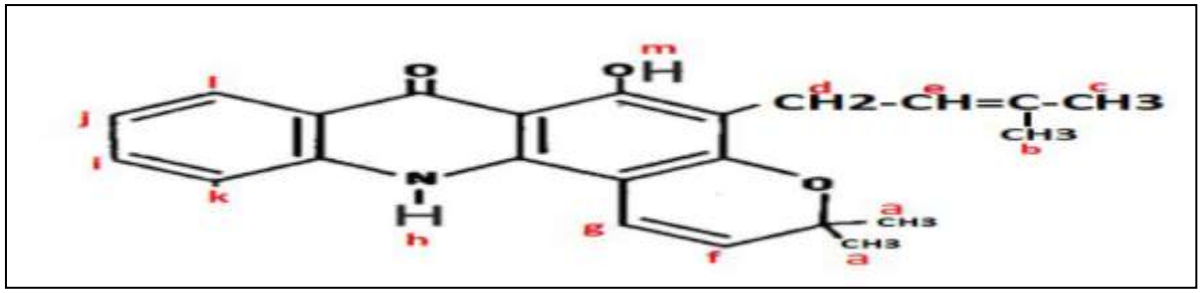

Figure 14. The putative unknown compound's structure (severifoline)with red letters indicate the chemical shift for each carbon atom .

3-LC-MS

Liquid chromatography-mass spectrometry (LC-MS) is a very efficient method of analysis. Used to determine the specific chemical components found in natural products. By doing a comparative analysis of the mass spectra of an unknown chemical with those of known compounds stored in databases[20]. LC–MS result showed that the molecular ion for the unknown compound was m/z 361 as in figure (15).The main fragments seen were as follows: at m/z 345 [M-HO•] +, 291 [M-C5H9•] +, 219 [M-C5H8•-C3H7O2•-H2O2•] +, 207 [M-C5H9•-C5H8O•] +, as shown in Figures(16) .

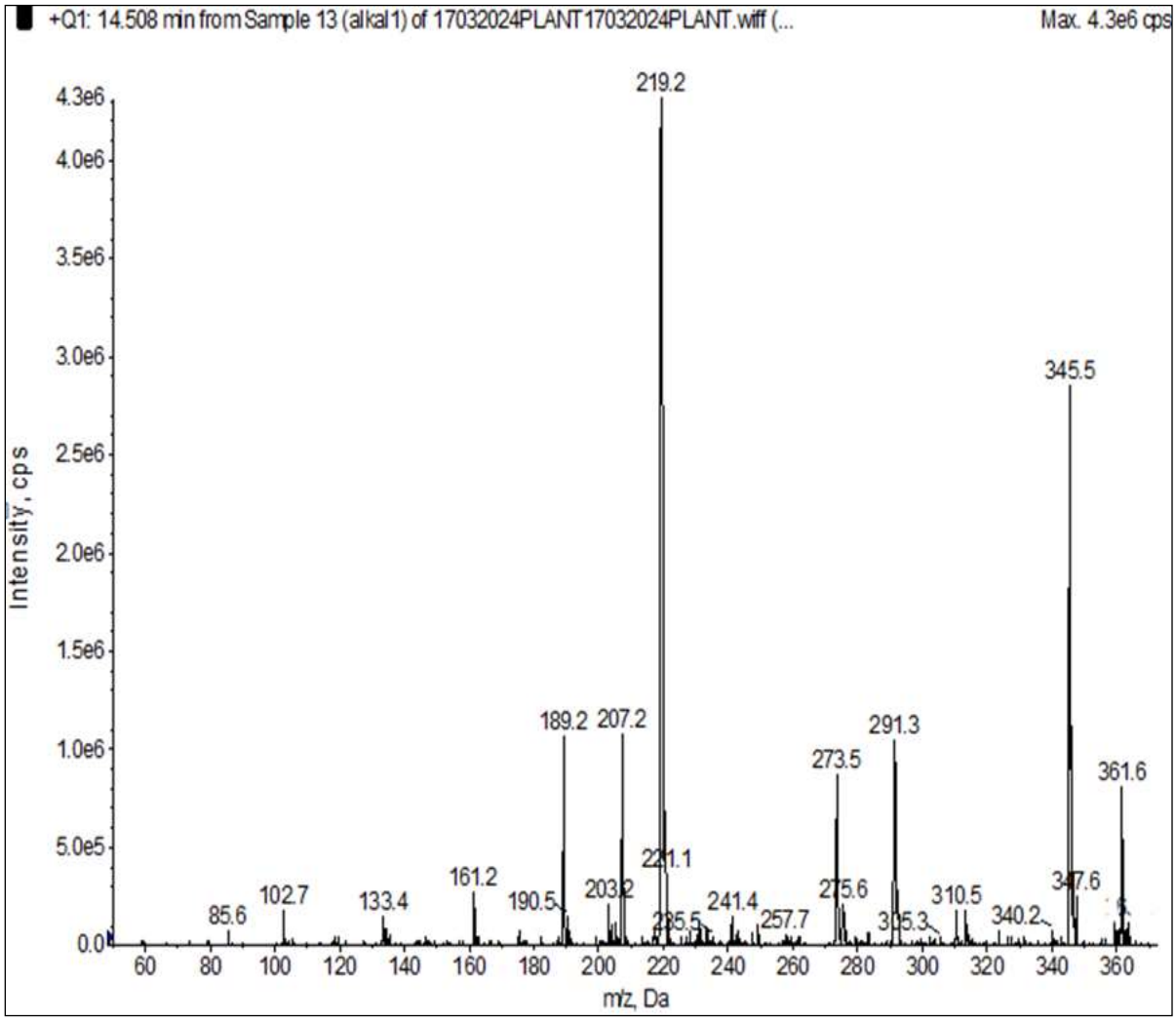

Figure 15 . The complete scan result of ion mass fragmentation spectra of isolated unknown alkaloid from chloroform fraction.

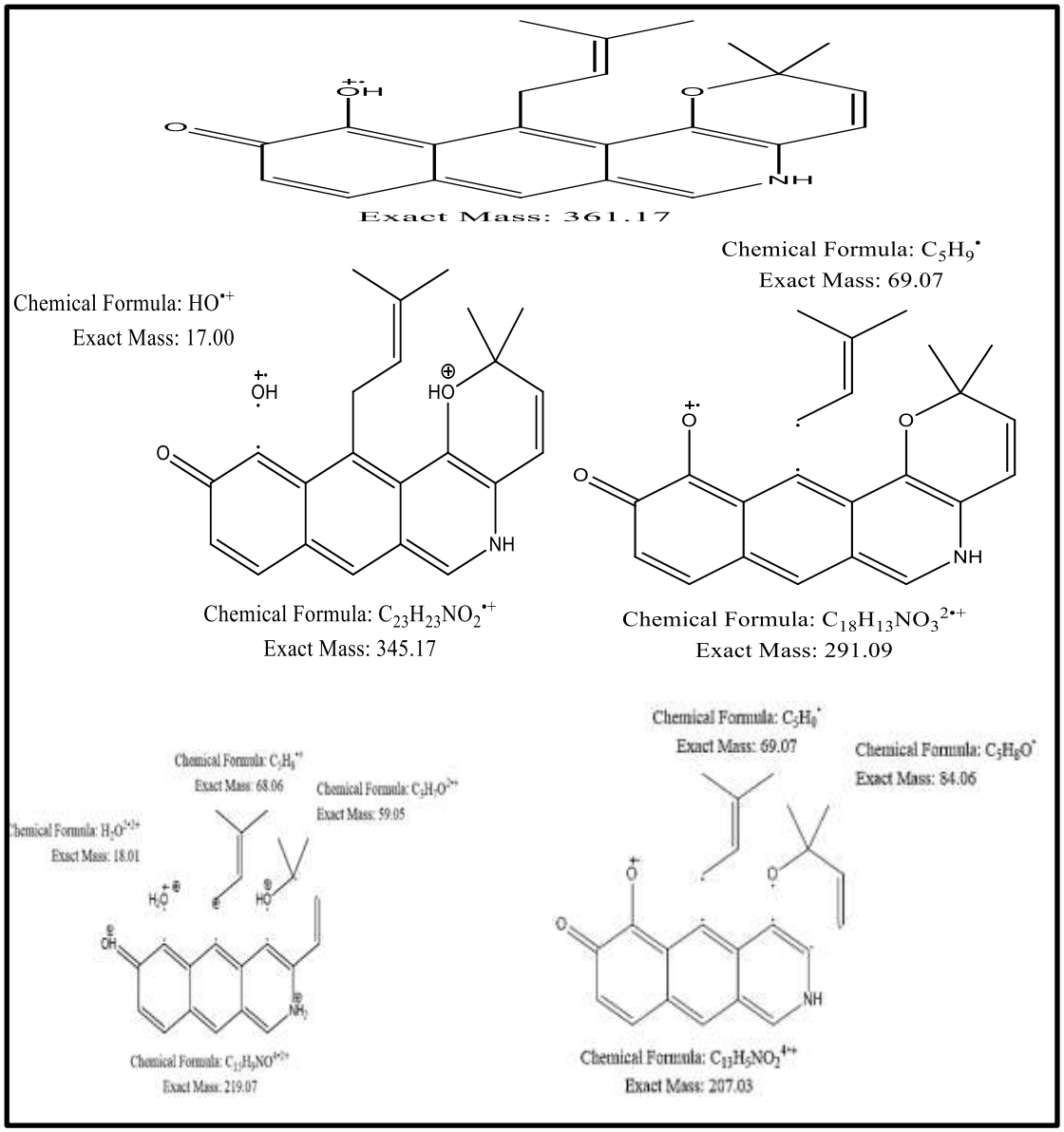

Figure 16 . Fragmentation pattern of isolated unknown alkaloid from chloroform fraction of whole plant of *Murraya paniculata*.

Discussion

This work is regarded as the initial effort to identify the physiologically active components of *Murraya paniculata* in Iraq. The components and their concentration in the plant vary depending on the plant part, extraction process and, many other environmental and biological factors[21] The majority of significant secondary metabolites were identified in the crude methanolic extract of the *Murraya paniculata* plant using preliminary test and HPLC analysis. Using petroleum ether, chloroform, and ethyl acetate, the crude plant extract was fractionated to detect phytochemicals based on their polarity. The petroleum ether and chloroform fractions contained alkaloids, coumarins, steroids, and terpenoids, but did not have flavonoids and phenols. The ethyl acetate fractions contained coumarins, flavonoids, phenols, and terpenoids, but did not have alkaloids and steroids[22]. HPLC is a very sophisticated version of TLC, effective for both qualitative and quantitative analysis [23]. Qualitative HPLC exposed the presence of stigmasterol in the petroleum ether fraction, aegeline alkaloid in the chloroform fraction and the ethyl acetate fraction contains apigenin, luteolin, and 5,7,3',4'-tetramethoxy flavanone. Plant-based compounds are commonly used in drug development as they are rich in valuable lead compounds. Nuclear magnetic resonance (NMR) is utilized to thoroughly identify the components of these compounds for various purposes including screening, drug discovery, and quality control for products like phytomedicine[24]. chloroform fraction gives distinct and recognized orange spots when spraying by using Dragendroff's reagent on the TLC plate making the study interesingt when isolated and identified this

unknown compound. The outcomes of diverse chromatographic methods and multiple spectral analyses like FT-IR, HNMR, and LC-MS demonstrated that the unknown alkaloid is probably severifoline. This acridone alkaloid is revealed in the root bark of the Chinese *Severinia buxifolia* [25,26].

Conclusion

The investigation findings revealed the presence of steroids, such as stigmasterol, in the petroleum ether, while the chloroform fraction included aegeline and unknown alkaloid separated using preparative layer chromatography and identified using FT-IR,^{1}HNMR, and LC-MS. Severifoline is Probably a new alkaloid found in the Iraqi-cultivated *Murraya paniculata* plant. The ethyl acetate fraction of the entire plant includes apigenin, luteolin, and 5,7,3',4'-tetramethoxy flavanone. The discoveries will be highly beneficial for phytochemists and pharmacologists in their future endeavors to create new active chemicals from this plant, given its remarkable pharmacological properties.

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Conflict of interest

The writers assert that they have no conflict of interest.

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Ethics Statements

This is an in-vitro study does not required ethical approval.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design; data collection; analysis and interpretation of results by Zainab Ali Qasim and Amjed Haseeb Khamees. All authors reviewed the results and approved the final version of the manuscript.

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فحص كيميائي نباتي أولي وتحديد بعض المكونات النشطة بيولوجيا وعزل مركب مجهول من نبات زهرة المورايا المزروع في العراق

زينب علي قاسم[1] و امجد حسيب خميس[2]

[1]كلية الصيدلة، جامعة ذي قار، ذي قار، العراق.
[2]فرع العقاقير والنباتات الطبية، كلية الصيدلة، جامعة بغداد، بغداد، العراق.

الخلاصة

نبات زهرة المورايا المعروف باسم الياسمين البرتقالي. يوجد هذا النبات في المناطق الاستوائية و شبة استوائية. وينتمي الى عائلة السماري ويمتد توزيع هذا النوع من جنوب آسيا إلى أستراليا. و يمتلك هذا النبات العديد من التطبيقات في الطب التقليدي لعلاج العديد من الأمراض. يظهر النبات مجموعة واسعة من الفعاليات الدوائية بما في ذلك الفعالية المضادة للألم، مضادة للأكسدة، مضادة للسكري، مضادة للبكتريا، ومسكنة للألم .

تهدف هذه الدراسة الى إجراء فحص كيميائي نباتي أولي وتحديد بعض المكونات النشطة بيولوجيا و عزل مركب جديد من نبات زهرة المورايا المزروع في العراق. ويجدر بالذكر أنه لم يتم إجراء أي فحص كيميائي سابق على هذا النبات في العراق. تم اجراء الاستخلاص باستخدام ٨٥ % من الميثانول باستخدام الطريقة الساخنة, ثم تجزئه المستخلص باستخدام مذيبات مختلفة (البترواليم ايثر كلوروفورم, اسيتات أيثيل) .تم اجراء الفحص الاولي للنبات باستخدام الفحوصات المعتمدة وتم التعرف على مختلف المواد بمختلف الاجزاء للمستخلص النباتي(البترواليم ايثر كلوروفورم, اسيتات أيثيل) باستخدام تقنية الكروماتوغرافيا السائلة عالية الأداء. وتم عزل مركب جديد من مستخلص الكلوروفورم باستخدام كروماتوارافيا الطبقة التميليرية. تم التعرف على المركب المعزول باستخدام جهاز "الفوربيه" لتحويل طيف الأشعة تحت الحمراء وجهاز مطيافية الرنين المغناطيسي النووي الهيدروجيني. تكشف الاختبارات الأولية إلى وجود قلويدات، وفلا فونويدات، وستيرويدات، وكومارينات، ومركبات ثانوية أخرى. بينما كشفت التحاليل الكروماتوغرافيه والطيفية الى وجود مختلف المواد الكيمونباتية كالتالي: يحتوي البترواليم ايثر على ستيجماستيرول؛ يحتوي الكلوروفورم على أيجلين و سيفريفولين؛ ويحتوي الأسيتات الإيثيلي على أبيجينين ، ولوتيولين، و٣،٧،٥،٤'-ثترامثوكسي فلافانون. ستكون هذه النتائج ذات قيمة كبيرة للفيتوكيميائيين والصيادلة في جهودهم المستقبلية لتطوير مركبات نشطة جديدة من هذا النبات لنشاطه الدوائي الملحوظ .

الكلمات المفتاحية: طيف الأشعة تحت الحمراء, الكروماتوغرافيا السائلة عالية الاداء, طيف الرنين المغناطيسي النووي الهيدروجيني, نبات زهرة المورايا.