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Extraction, Isolation, Purification and Identification of Caffeine in Zamioculcas Zamiifolia L. Leaves Cultivated in Iraq

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Abstract

Background: Phytochemical analysis of *Zamioculcas zamiifolia* has revealed the presence of alkaloids, phenols, flavonoids, endogenous metabolites, vitamins, carotenoids, and tannins. phytochemical profile of *Zamioculcas zamiifolia* holds promise for discovering new therapeutic agents and natural products. Caffeine is a purine alkaloid, its trimethyl xanthine compound that has stimulant effect on central nervous system.

Materials and Methods: the fresh leaves of plant were extracted by harborne extraction method in reflux apparatus by using ethyl acetate, chloroform, chloroform- methanol, and distilled water and then determine compounds preliminary by using specific tests through phytochemical assay and TLC. Further phytochemical investigation of compounds performed by using GC-MS, FT-IR, melting point, HPLC, and Mass spectrometer.

Results: the results exhibit different fractions as ethyl acetate, chloroform, chloroform: methanol, chloroform and distilled water weighed 0.92, 0.19,1.51, and 28.65 gram respectively, also the presence of alkaloids specifically purine alkaloids, terpenoids, and phenolic compounds which show in leaves extract, in addition to the isolation of pure caffeine from chloroform-methanol fraction and this study was the first one that isolate caffeine from *Zamioculcas zamiifolia* in the world.

Conclusion: The phytochemical analyses results exhibit the extract fractions of *Zamioculcas zamiifolia* leaves indicate the plant considered as an important source of active compounds that might feeding the modern medicine with drugs especially caffeine discovering in plant. Therefore, additional researches are required to confirm the antimicrobial, anthelminthic, anti-hyperglycemic, and anti-inflammatory activities. As well as, compounds isolation, purification and even characterization are essential to make the plant has a novel important study.

عزل وتنقية وتشخيص الكافيين في نبات الزاميا القلقاسية المستزرع في العراق

استبرق حسين ناصر

الخلاصة

المقدمة: كشف التحليل الكيميائي النباتي لنبات الزاميا القلقاسية عن وجود قلويدات وفينولات وفلافونويدات ومستقلبات داخلية وفيتامينات وكاروتينات وعفص. يحمل الملف الكيميائي النباتي لنبات الزاميا القلقاسية وعدًا باكتشاف عوامل علاجية ومنتجات طبيعية جديدة. الكافيين هو قلويد البيورين، ومركبه ثلاثي ميثيل الزانثين له تأثير منبه على الجهاز العصبي المركزي.

المواد والطرق: تم استخلاص أوراق النبات الطازجة بطريقة الاستخلاص هاربورن في جهاز الارتجاع باستخدام أسيتات الإيثيل، والكلوروفورم، والكلوروفورم-الميثانول، والماء المقطر ومن ثم تحديد المركبات الأولية باستخدام اختبارات محددة من خلال التحليل الكيميائي النباتي والتصوير المقطعي بالبلورات السائلة، ثم تم إجراء مزيد من التحقيق الكيميائي النباتي للمركبات باستخدام GC-MS وFT-IR ودرجة الانصهار وHPLC وMass spectrometer.

النتائج: أظهرت النتائج وجود اجزاء مختلفة من خلات الإيثيل، الكلوروفورم، الكلوروفورم، الميثانول، الكلوروفورم والماء المقطر بوزن 0.92، 0.19، 1.51، و28.65 جرام على التوالي، كما أظهرت وجود قلويدات وخاصة قلويدات البيورين، التربينويدات، والمركبات الفينولية والتي تظهر في مستخلص الأوراق، بالإضافة إلى عزل الكافيين النقي من جزء الكلوروفورم-الميثانول وهذه الدراسة هي الأولى من نوعها لعزل الكافيين من نبات الزاميا القلقاسية في العالم

الاستنتاج: أظهرت نتائج التحليلات الكيميائية النباتية أن مستخلصات أوراق نبات الزاميا تشير إلى أن النبات يعتبر مصدرًا مهمًا للمركبات النشطة التي قد تغذي الطب الحديث بالأدوية خصوصا اكتشاف وجود الكافيين في النبات. لذلك هناك حاجة إلى أبحاث إضافية لتأكيد الأنشطة المضادة للميكروبات ومضادات الديدان ومضادات ارتفاع السكر في الدم ومضادات الالتهابات. كما أن عزل المركبات وتنقيتها وحتى توصيفها أمر ضروري لجعل النبات موضوعًا لدراسة جديدة مهمة.

1. Introduction

Zamioculcas zamiifolia is a monocotyledonous perennial flowering and ornamental plant that belongs to family Araceae (Badizadegan et al., 2023; Croat and Ortiz, 2020; Krömer et al., 2019). Phytochemical analysis of *Z. zamiifolia* has revealed the presence of alkaloids, phenols, flavonoids, endogenous metabolites, vitamins, carotenoids, and tannins (Belakhdar et al., 2015). The traditional use of *Z. zamiifolia* in folk medicine for treating various disease such as external use of leaves by people of Malawia to treat children's earache, for the moment roots are used by people of Sukuma to treat gastric problems in Tanzania (dos Santos et al., 2022). The health beneficial properties of medicinal plants are relatively attributed to antioxidant actions of their phytochemical components (Shang et al., 2022; Xu et al., 2017).

Iraq, with its unique ecological diversity and rich botanical heritage, offers an intriguing setting for the study of Z. *zamiifolia* and its phytochemical constituents. However, despite its widespread cultivation in the region, there remains a paucity of scientific literature regarding the phytochemical composition and pharmacological potential of *Z. zamiifolia* specimens cultivated in Iraq (Sapiun et al., 2020; Zuecco et al., 2022). This study focusses to bridge this knowledge gap by conducting a comprehensive phytochemical investigation of *Z. zamiifolia* plants grown in Iraqi soil. By employing advanced analytical techniques such as chromatography and spectroscopy (El-Emary, 2021; Türkyılmaz, 2022).

Taxonomy of *Zamioculcas zamiifolia* (Pourhassan et al., 2023)

- **Kingdom**: Plantae
- **Division**: Angiosperms (flowering plant)
- **Class**: Liliopsida (monocotyledonous)
- **Order**: Arales
- **Family**: Araceae
- **Genus**: *Zamioculcas*
- **Species**: *Z. zamiifolia*

Phytochemical investigation of *Z zamiifolia's* leaves and petioles extract exhibits seven compounds, the major constituents of leaves is apigenin 6-C-(6″-(3-hydroxy-3-methyl-glutaroyl)-ß-glucopyranoside), as in Fig.1 (Price, 1985). *Zamioculcas zamiifolia* has antibacterial activity and the plant considered as a potential source of antibiotic compounds that significant to attack the antibiotic-resistant bacteria (Sasidharan et al., 2011; Seneviratne et al., 2020). Hence, this study seeks to extract, isolate, purify, identify and characterize the bioactive compounds present in *Z. zamiifolia* extracts, elucidate their chemical structures through a systematic analysis of the phytochemical composition of *Z. zamiifolia* cultivated in Iraq.

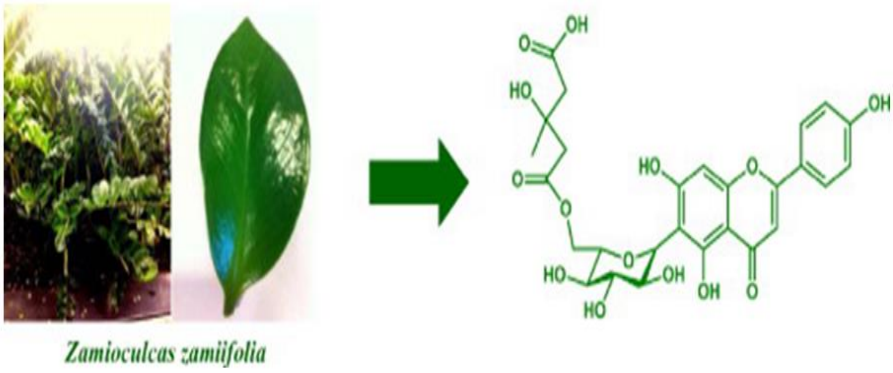

Figure 1: Describes Apigenin 6-C-(6″-(3-Hydroxy-3-Methyl-Glutaroyl)-ß-Glucopyranoside) Structure in *Zamioculcas Zamiifolia* Leaves (Price, 1985)

2. Materials, Patients and Methods

2.1. Experimental Work

The fresh leaves from *Zamioculcas zamiifolia* L. plant were collected from the garden during October, November in 2023. The plant leaves were washed thoroughly with tap water and then with distilled water and used as raw materials for the extraction of phytochemical compounds from the plant.

2.2. Preparation of Plant Extract

The extract was prepared by using 200 g of fresh leaves that homogenized in methanol: water (4:1) for 24 hrs. and extracted according to Harborne (Paterson, 1999; Richardson and Harborne, 1990) by using reflux apparatus, as in Fig.2. Preliminary phytochemical investigations of active compounds detected according to alkaloids, terpenoids, purine alkaloids, and phenolic compounds tests in the crude extract (Naser and Kathem, 2023). Chemical identification performed by GC-MS, FT-IR, melting point, TLC, and HPLC.

2.3. Chemical Identification by GC-MS

The investigation by GC-MS was performed by GC-MS Perkin Elmer Clarus 500 apparatus. Capillary column (30.0 m × 0.32 mm × 1.80 µm). the carrier gas was helium (99.9995% purity) at a constant flow rate of 1.61 mL/min with injection volume of 2 µL was employed in a split mode (El Hafidi et al., 2023). The temperature of injector was preserved at 280°C, and the column temperature was automated to 60°C (isothermal for 2 min) with an increase in temperature from 10°C/min to 280°C (isothermal for 6 min). 200°C for ion source temperature and 280°C for interface temperature were sustained. The mass spectra were gained through ionization energy of 70 eV in the EI mode. about 30 min was needed to run GC-MS. The compounds were recognized by comparison of their mass spectra with national libraries (NIST - 11) (Amudha et al., 2018; Munda et al., 2019; Sasikala and Chandra Mohan, 2014).

2.4. FT-IR (Fourier Transform Infrared Spectroscopy)

The FT-IR spectrum of isolated component was determined at pharmaceutics department in pharmacy College/ Kerbala University by a SHIMADZU apparatus. The structural duties have been connected for

characteristic bands of different chemical groups, many functional groups can be determined by their characteristic vibration frequency this makes the spectrums obtained from IR the simplest and often the mostreliable method of assigning a chemical substance to its class (Abd-Elhafeez et al., 2024; Bonfilio et al., 2010; Mudigiri and Jorige, 2023; Siddiqui et al., 2017; Zagade et al., 2020).

2.5. Melting Point Measurement

Melting point of an isolated compound was measured and matched with the reference standard, using electro–thermal melting point apparatus (Stuart / UK) at University of Kerbala/ College of Pharmacy.

2.6. Qualitative and Quantitative Identification of Analysed Fraction by High Performance Liquid Chromatography

Qualitative estimations occur using LC800-0101, USA. Diode array detector 2.1L, and C18 (150X4.6) 5 µm particles size from water corporation, USA. The mobile phase used was water: methanol (60:40) both are in HPLC grade water, standard compound was caffeine, flow rate was 1 ml / min, and the HPLC chromatogram was detected using a photo diode array UV detector at (275 nm) (Braz et al., 2012) at AL- Ameed university in Kerbala. Identification was made by comparing retention times obtained at identical chromatographic condition of analyzed sample and authentic standard (Gupta and Garg, 2014).

2.7. Mass Spectrometer

Isolated caffeine mass was measured at Al-Zahrawi University College, Karbala, Iraq by using Advion's expression® Compact mass spectrometer apparatus, USA. The measured mass then matched with standard caffeine mass by interpreted the fragmentation peaks.

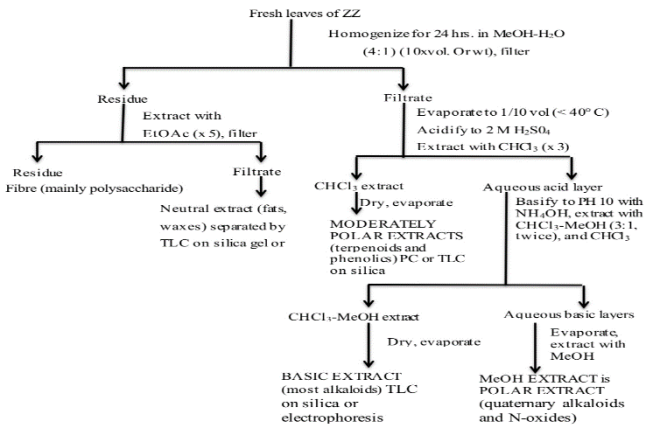

Figure 2: Extraction procedure of *Zamioculcas zamiifolia plant* leaves

3. Results

3.1. The extraction of *Zamioculcas zamiifolia* leaves yield four different fractions weighed as in Table 1 and undergoing phytochemical tests as in Table 2.

Table 1: Weight Yield of Each Fraction Obtained from Extraction Method

Extraction method	Weight of crude extract	Yield%
Ethylacetate fraction	0.92 g	0.46
$CHCl_3$ fraction	0.19 g	0.1
$CHCl_3$:MeOH fraction	1.51 g	0.76
Aqueous fraction	28.65 g	14.33

Table 2: Phytochemical Tests of Alkaloids, Purines Alkaloids, Terpenoids, And Phenolic Compounds of *Zamioculcas Zamiifolia* Leaves

Test Name in Plant Fraction	Result
Alkaloids test in aqueous fraction	+
Purines alkaloids test in CHCl3- MeOH fraction	+
Terpenoids test in Ethylacetate fraction	+
Phenolic compounds in $CHCl_3$ (1) fraction	+
(Absent –, Presence of compound +)	

3.2. GC.MS Of Phytochemical Constituents of *Z. Zamiifolia*

The analysis of ethylacetate fraction carried out by GC-MS apparatus, exhibited about nine different compounds in *Z. zamiifolia* leaves fractions based on the extraction method as shown in Fig.3. and recorded in Table 3.

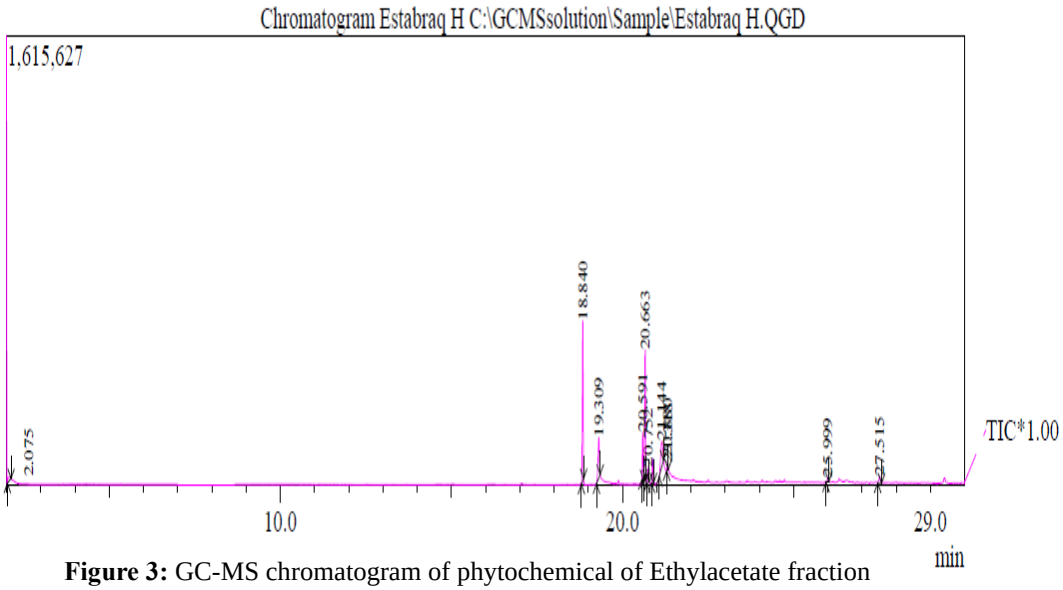

Figure 3: GC-MS chromatogram of phytochemical of Ethylacetate fraction

Table 3: Chemical components in leaves of *Z. zamiifolia detected* by GC.MS

NO.	Compound name	Chemical formula	SI	RT
1	Peroxide, bis(1-methylpropyl)	C8H18O2	81	2.075
2	Methyl 4-hydroxybutanoate	C5H10O3	62	18.842
3	1-Butanol, 3-methyl-, formate	C6H12O2	68	19.308
4	2,4-Pentadien-1-ol, 3-ethyl-, (2Z)-	C7H12O	75	20.667
5	Oxalic acid, isobutyl pentyl ester	C11H20O4	84	20.750
6	9-Oxabicyclo[6.1.0]nonane, cis-	C8H14O	74	21.142
7	1-Pentanol	C5H12O	61	21.317
8	Furost-5-en-3-ol, 22,26-epithio-, (3.beta.,22.alpha.,25R)-	C27H42O2S	77	26.000
9	1,3,5,7,9-Pentaethylbicyclo[5.3.1]pentasiloxane	C10H28O6Si5	51	27.517

3.3. Fourier Transforms Infrared (FT-IR) Spectra

The FT-IR of the isolated caffeine constituent showed identical spectrum to that of standard caffeine as reported in literature (Bansode et al., 2016; Mudigiri and Jorige, 2023) and shown in Fig.4. The characteristic IR absorption bands of isolated caffeine constituent are recorded in Table 4.

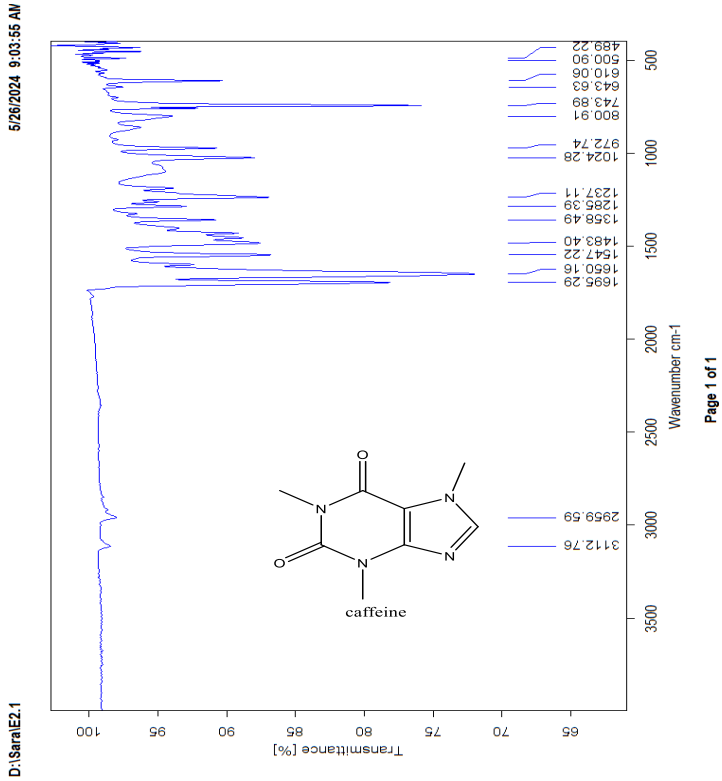

Figure 4: FT-IR spectra of isolated caffeine compound

Table 4: Characteristic FTIR Absorption Bands (Cm^{-1}) of the Isolated Caffeine Constituent

Functional group	Group frequency wave number (in cm^{-1})		Main attributed
	Isolated caffeine	Standard caffeine	
N–H	3112.76	3111	N-H stretching
C—H	2959.59	2953	C-H stretching
C=O	1695	1703	C=O stretching (conjugation and H- bonding)
C—N	1547.22	1546	C-N stretching
C-H	1358.49	1361	C-H bending of CH2
C–C	972.74	974	C-C stretching

3.4. Melting Point Measurement

The melting point of isolated caffeine was 235-238°C, compared to standard caffeine 238°C.

3.5. Qualitative and Quantitative Analysis of Caffeine by High Performance Liquid Chromatography (HPLC)

The qualitative identification of the active constituent was carried out by contrasting the retention periods of analysed sample and authentic standard (caffeine) obtained at identical chromatographic conditions, as in Fig.5 and Fig.6. The quantitative identification carried out by calculating Area under the curve (AUC) versus five concentration levels of caffeine sample and its standard was used to plot the calibration curve for quantification studies. The concentration of the analyte was determined using a straight-line equation, as illustrated in Fig.7.

3.6. TLC

In analytical TLC, the isolated caffeine constituent appeared as a single spot with the same colour and R_f value as that of standard caffeine (R_f = 0.41) after devolved in Ethylacetate: Methanol: water (100:13.5:10) (Lederer, 1985; Makin, 1985; Thorburn Burns, 1986; Wagner and Bladt, 1996)and detected at UV 254nm as shown in Fig.8.

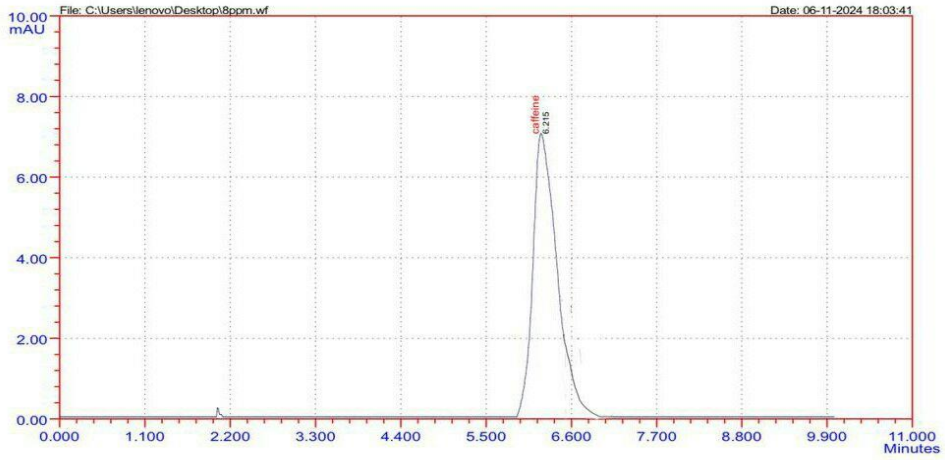

Figure 5: HPLC Chromatogram of Standard Caffeine

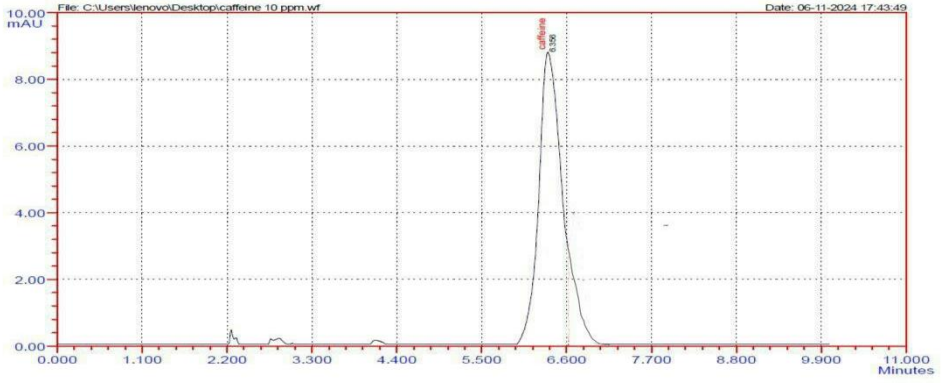

Figure 6: HPLC Chromatogram of Isolated Caffeine

concentration stander (ppm)	Area stander	conce sample ppm
0	0	y = 17.653x + 6.5953
2	45.4	45.56057502
5	98.7	
8	157.2	
10	173	

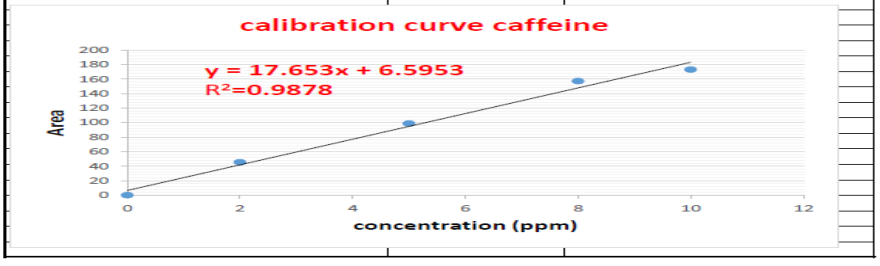

Figure 7: Calibration Curve of Caffeine

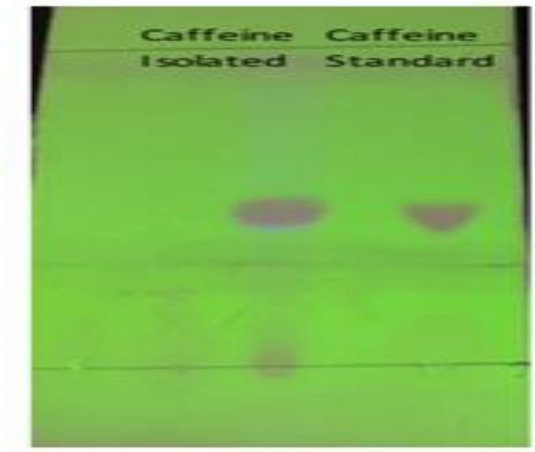

Figure 8: TLC Chromatogram of Isolated and Standard Caffeine at 254 nm.

3.7. Mass Spectrometer

The measured mass of isolated caffeine was 195.2 m/z that matched with standard caffeine mass by interpreted the fragmentation peaks according to literature (Dubale et al., 2023; Muharini et al., 2018; Salim et al., 2024) as in Fig.9.

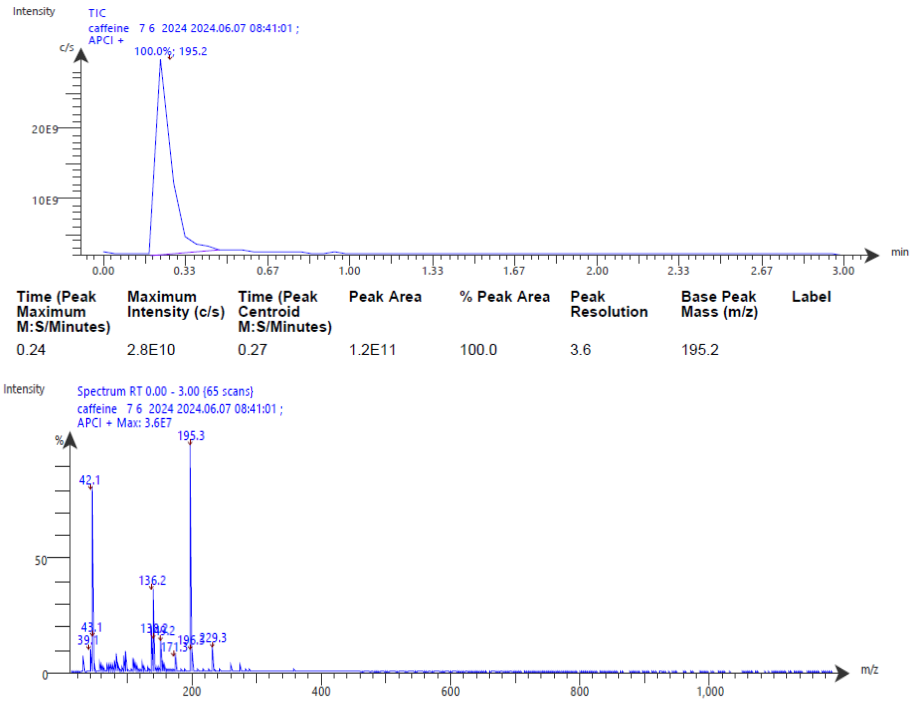

Figure 9: Representative Full Scan Product Ion Mass Fragmentation Spectra of Isolated Caffeine

4. Discussion

Zamioculcas zamiifolia extracted with solvents (chloroform, Ethylacetate, methanol-chloroform, and distilled water) according to differences in polarity, from Ethylacetate which is semi polar to extract fat and waxes or nonpolar substances and residue extract by solvent with increase polarity gradually, chloroform to extract moderately polar substances, that don't extracted with Ethylacetate like terpenoids and phenolic compounds, increase polarity as by using chloroform- methanol to extract basic compounds like purine alkaloids, increase polarity to distilled water mixed with methanol solvent to extract quaternary alkaloids and N-oxide. Fractions of crude extract then tested by using chemical test to identifying substances that present in extract solutions like alkaloids, terpenoids, phenolic compounds, and purine alkaloids as in literature (Gupta and Maurya, 2023; Koina et al., 2023). The result showed that the percent of active compounds by these solvents were almost different in the weight of final crude extract, Ethylacetate fraction was 0.92 g, chloroform was 0.19, chloroform- methanol was 1.51 g, and distilled water mixed with methanol fraction was 28.65 g, these different weights explain that the plant mostly contain polar compounds like quaternary alkaloids and N-oxide. The chemical components in Ethylacetate fraction of the leaves of *Z zamiifolia* which identified by GC-MS as tabulated in Fig.4. exhibit nine compounds including Peroxide, bis(1-methylpropyl); Methyl 4-hydroxybutanoate; 1-Butanol, 3-methyl-, formate; 2,4-Pentadien-1-ol, 3-ethyl-, (2Z)-; Oxalic acid, isobutyl pentyl ester; 9-Oxabicyclo[6.1.0]nonane, cis-; 1-Pentanol; Furost-5-en-3-ol, 22,26-epithio-, (3.beta.,22.alpha.,25R)-; 1,3,5,7,9-Pentaethylbicyclo[5.3.1]pentasiloxane. The predominant constituents were Methyl 4-hydroxybutanoate 28.99%; 1-Butanol, 3-methyl-, formate 12.45%; Oxalic acid, isobutyl pentyl ester 24.03%; and 9-Oxabicyclo[6.1.0]nonane, cis- 11.85%. The differences in these percent explained by the rule of like dissolve like. The chloroform- methanol fraction show the presence of pure caffeine when compared with standard one on TLC plate, melting point, FT-IR, HPLC, and mass spectrometer and this is the first study that isolate caffeine from *Z. zamiifolia* plant fresh leaves. The R_f value of isolated and standard caffeine was the same 0.41, as in Fig.8. The melting point of isolated caffeine was 235-238 as in literature (Dyulgerov et al., 2023). FT-IR spectrum peaks were the same spectrum when matched with standard caffeine as in literature (Arimurti et al., 2020; Nugrahani et al., 2019), HPLC results showed the same peak retention time of isolated caffeine that appear at 6.356 min. while the standard one appear at 6.215min., the calibration curve of different concentrations of standard caffeine (0,2,5,8,10) give straight line according to equation Y= a X +b, Where Y: is the response factor (AUC), a: is the slope of the curve (slop=y/x), x :is the concentration in part per million (ppm) or in (mg/ml), and b: is the y-intercept (Mohammed and Al-Bayati, 2009; Priyadi and Saifudin, 2023). Mass of isolated caffeine was matched with the standard one 195.2 m/z as in literature (Bianco et al., 2009).

5. Conclusion

Zamioculcas zamiifolia is considered as very interesting plant of secondary metabolites that may provide a new source of drugs to medicine. The plant biological activities such as antimicrobial, anthelminthic, anti-hyperglycemic, and anti-inflammatory should be studied. As well as, compounds isolation, purification and even characterization are essential to make the plant has a novel important study. Additional studies are needed to uncover other compounds in this plant that have yet to be discovered utilizing different portions of the plant. Other pharmacological studies are required to elucidate other reported and or unreported pharmacological activities.

6. Acknowledgements

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