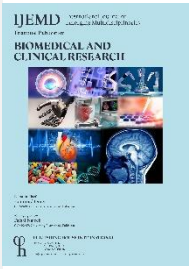
IJEMD-BMCR, 2 (1) (2024), 1 –21 https://doi.org/ 10.54938/ijemdbmcr.2024.02.1.272

International Journal of Emerging Multidisciplinaries:
Biomedical and Clinical Research

Research Paper
Journal Homepage: www.ojs.ijemd.com
ISSN (print): 2957-8620 ISSN (online): 2960-0731



Fractionation and Identification of Phenolic Compounds from Ethyl Acetate Fraction of *Ageratum Conyzoides* Methanolic Leaf Extract

Paul Chijioke Ozioko[1*], Aminu Ibrahim[2], Yusuf Yunusa Muhammad[2], Musa Bashir[3]

[1] *Biology Unit, Faculty of Science, Air Force Institute of Technology, Kaduna, Nigeria*
[2] *Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University, Kano, Nigeria*
[3] *Centre for Dryland Agriculture, Bayero University, Kano, Nigeria*
**Corresponding author*

Abstract

Natural phenols are significant functional substances that are commonly present in plants and provide advantages for human health. Phenolics can be utilized for a variety of therapeutic applications, such as antioxidant, anti-inflammatory, antidiabetic, and antibacterial activities. This work aimed to fractionate and identify the phenolic compounds present in ethyl acetate fractions of the methanolic leaf extracts of *A. Conyzoides*. Fractionation and purification of the ethyl acetate fraction using column chromatography and TLC, followed by FTIR and LC-MS for identification, were employed in this work. While the FTIR spectroscopy was used to identify the fractions with -OH functional group, LCMS was used to identify the phenolics. After fractionation and purification, six ethyl acetate samples were obtained, each with different Rf values, of which three showed a pronounced presence of OH functional groups in phenolics. Subsequently, four phenolic compounds were identified: furocoumaric acid, liquiritin, isorhamnetin, and syringin. This research has demonstrated that ethyl acetate fraction of *A. conyzoides* methanol leaf extract is a good source of the phenolic compounds: Furocoumaric acid, Liquiritin, Isorhamnetin, and syringin.

Keywords: *Ageratum conyzoides; Chromatography; Ethyl acetate; Fractionation; FTIR; LC-MS; Phenolics*

Email addresses: **paulcj82@gmail.com (P.C. Ozioko), aibrahim.bch@buk.edu.ng (A. Ibrahim), yymuhammad.bch@buk.edu.ng (Y.Y. Muhammad), mbashir.cda@buk.edu.ng (M. Bashir)**

1. Introduction

Phenolic substances are phytochemicals with one or more aromatic rings with hydroxyl groups connected inside the structure (phenolic acid or polyphenols). Plants contain them in both free and conjugated forms. The food and pharmaceutical industries are currently paying a lot of attention to phenolic chemicals for a number of reasons [1, 2]. Natural phenols are significant functional substances that are found in a wide variety of plants and offer advantages for enhancing human health. They are a significant class of phytochemicals and are further separated into polyphenols and phenolic acids [3]. Due to their numerous health benefits, phenols can be utilized medicinally for a variety of conditions. These benefits include antioxidant, anti-inflammatory, antidiabetic, antimicrobial, antiviral, anti-allergenic, antithrombotic, cardio protective, and vasodilatory action [4, 5]. Their structural variations or functional groupings may be responsible for their different therapeutic effects. For instance, phenolic compounds' capacity to scavenge free radicals and contribute hydrogen atoms, electrons, or chelated metal cations is thought to be the cause of their antioxidant activity [6, 7]. The ability of phenolic compounds to scavenge free radicals is conferred by their molecular structure, specifically the quantity and positioning of their hydroxyl groups and the type of substitution they undergo on their aromatic rings, which is known as the structure-activity relationship (SAR). These substances can also exist as derivatives, like esters and methyl esters, or in combination with mono- and polysaccharides linked to one or more phenolic groups. Of the numerous kinds of phenolics that have been found in *A. conyzoides*, phenolic acids, flavonoids, and tannins are thought to be the most significant dietary phenolic components.

Ageratum conyzoides, an annual plant of the family Asteraceae, has a long history of usage as a traditional medicine in numerous nations all over the world. The plant is often found in West Africa and is particularly prevalent during the wet season in southern and northwestern Nigeria. It can be found in Nigeria's marshes and savannah areas. It is frequently referred to as "horny goat weed." *A. conyzoides* is used in conventional medicine to treat a number of illnesses, including diabetes [8]. For instance, ethyl acetate extracts have been demonstrated to have anticancer and free radical scavenging activities, while aqueous extracts of the entire plant have been utilized as dermatological treatments [9]. In a study by Atawodi *et al*. [10], the high flavonoid content of the plant was shown to be responsible for the antihyperglycemic and lipid-lowering actions of methanol extracts of *A. conyzoides L* in normal and diabetic rats. In a study by Atawodi *et al*. [10], the high flavonoid content of the plant was shown to be responsible for the antihyperglycemic and lipid-lowering actions of methanol extracts of *A. conyzoides* L in normal and diabetic rats. Similar to this, Ozioko *et al*. [11] reported that *A. conyzoides* leaf extracts in methanol exhibited significant levels of total flavonoids and total phenolic content. A phytochemical screening showed that the roots of this plant extract contained various bioactive components such as flavonoids, saponins, tannins, terpenoids, alkaloids, coumarins, chromenes, benzofurans, sterols, terpenoids, and cardiac glycosides, but have been shown to contain no anthracene derivatives [11-14]. Additionally, antifungal substances such as precocene II and polymethoxy flavones have been found in *A. conyzoides* [8].

Due to reports of high levels of phenols and flavonoids in *A. conyzoides* leaf extracts and as a follow-up to work Ozioko *et al*. [11] (which claimed that the ethyl acetate fraction of the methanolic leaf extract of *A. conyzoides* has a high total flavonoid and phenol content as well as excellent antioxidant and antidiabetic effects), the goal of the current study was to fractionate and identify phenolics from the ethyl acetate fraction.

The aim of this research was to fractionate and identify phenolic compounds from the ethyl acetate fraction of *A. conyzoides* methanolic leaf extract.

2. Materials and Methods

This research started in 22nd February, 2022 and ended in March 2023. All reagents used were of analytical grade. They include silica gel (200-400 mesh size), column (2cm width x 40cm length), different solvents (methanol, ethyl acetate, and chloroforms), TLC Silica gel 60 F_{254}, Hand-held UV lamp (E-Series Corded; model number: 1497616; made in Japan), 10% sulphuric acid, chromatography jar, oven, and scissors.

Study Area: The primary place where the study took place was at the Research Laboratory of Department of Pharmaceutical and Medicinal Chemistry, Kaduna State University (KASU), Kaduna, Nigeria. This was where drying, extraction, and solvent-solvent fractionation were carried out as described by Ozioko *et al*. [11]. The CC and TLC were equally carried in this lab. However, FTIR and LCMS were respectively carried out at Ahmed Bello University (ABU) Zaria, Nigeria, Multipurpose Laboratory, and Bayero University Kano (BUK), Nigeria's Center for Dry Land Agriculture (CDA).

2.1 Column and Thin Layer Chromatographic Purification/Isolation of Ethyl Acetate Fraction

The weight of the ethyl acetate fraction used was 2.7g. Forty grams (40g) of silica gel (200–400 mesh size) were dissolved in ethyl acetate, poured on the column, and allowed to settle gently to make a slurry (the stationary phase). The mobile phase/eluent consists of ethyl acetate and methanol in a gradient ratio of 100:0, 98:2, 95:5, 90:10, 80:20, 70:30, etc. The sample was then dissolved in a small portion of ethyl acetate and introduced into the column. The mobile phase was added gently, and fractions were collected. Approximately 3 mL of the fractions were collected at intervals, but the first four fractions were 10 mL each. The fractions were labeled F1–F159.

Each of the fractions collected was spotted on the TLC plate to ascertain the number of prospective compound(s) or isolates present. It was then run in a solvent system containing ethyl acetate and methanol in a ratio of 5:1. This ratio was chosen after various TLCs were carried out on the ethyl acetate fraction with different solvent combinations. The chromatograms developed were visualized using a handheld UV lamp at 365nm and subsequently sprayed with 10% sulfuric acid and heated in an oven for 5 minutes for the development of spots. Fractions that have the same retention factor (Rf) values (similar spots) on the chromatogram were pooled together for further rechromatographic processing when two or more spots appeared.

2.2 Characterization and Compound Identification:

For the purpose of characterization and structural identification, the separated or purified fractions (samples) were sequentially subjected to FTIR spectroscopy (Agilent Technologies Cary 630 FTIR) and LC-MS spectroscopy (Agilent high-resolution liquid chromatography and mass spectrometry model-G6550A (0.01% mass resolution).

2.2.1 Fourier-Transform Infrared (FTIR) Spectroscopy

The FTIR spectroscopy was carried out using Agilent Technologies Cary 630 FTIR at Ahmadu Bello University (ABU) Zaria, Nigeria, Multipurpose Laboratory. About 10 mg of the dried ethyl acetate extract

powder was encapsulated in 100 mg of KBr pellet (FTIR grade) for preparation of translucent sample discs. The dried ethyl acetate samples fractions were respectively loaded into a FTIR spectroscope with a scan range of 650 to 4000 cm^{-1} and a resolution of 8 cm^{-1}.

2.2.2 Liquid Chromatography Mass Spectroscopy (LCMS)

The LCMS was conducted at the Bayero University Kano (BUK), Nigeria's Center for Dry Land Agriculture (CDA). This study employed the LCMS analysis procedure (generic approach), which makes use of the LC Waters e2695 separation module with W2998 PDA and is coupled to ACQ-QDA MS.
Liquid chromatography (LC) and a tandem mass spectrophotometer (MS), with some modifications from [15], were used to examine the materials. Reconstituted in methanol, the extracted samples were then run through a polytetrafluoroethylene (PTFE) membrane filter with a 0.45m size. The filtrate was filtered, and 10.0 µL of it was then put into the liquid chromatography system, where it was allowed to separate on a Sunfire C18 5.0 mm 4.6 mm x 150 mm column. A flow rate of 1.0 mL/min and a sample and column temperature of 25^{o}C were used for the run. With two separate gradient systems, the mobile phase is made up of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B).
From the A/B 95:5 ratios, this ratio was maintained for an additional minute, followed by periods of A/B 5:95 for 13 to 15 minutes, A/B 95:5 for 17 to 19 minutes, and then 20 minutes. With a resolution of 1.2 nm and a sampling rate of 10 points per second, the PDA detector was tuned to the 210–400 nm range. After making sure the settings were accurate, the mass spectra were obtained with a scan range of m/z 100-1250: Positive and negative electron spray ionization (ESI) sources; capillary voltages of 0.8 kV (positive) and 0.8 kV (negative); probe temperatures of 600^{o}C; flow rates of 10 mL/min; and 45 psi of nebulizer gas. MS is configured to operate in automated mode with a 125V fragmentation voltage. Software called Empower 3 was used to process the data. Elution order, retention time (Rt), fragmentation pattern, and base m/z data were all used to identify the compounds.

3 Results

3.1. Column Chromatography and TLC of Ethyl Acetate Fraction

At the end of first round of fractionation (Table 1), fractions with single spot as well as Rf value were set aside, while fractions with two or more spots or Rf values were rechromatographed (Table 2).

Table 1: First Column Chromatography of Ethyl acetate Fraction

Fractions	Rf Values	Designation
F1-F3	**No Spot**	
F4-F5	**0.93**	**E1***
F6-F20	**0.93; 0.88**	**E2**
F21-F38	**0.88; 0.81**	**E3**
F39-F40	**0.88**	**E4***
F41-F79	**0.87; 0.82**	**E5**

F80	**0.82**	**E6***
F81-F95	**0.82; 0.59**	**E7**
F96-F100	**0.59**	**E8***
F101-F120	**0.58; 0.68; 0.70**	**E9**
F121-F140	**0.48; 0.58**	**E10**
F141-F154	**0.48**	**E11***
F155-F159	**No Spot**	

*Fractions with single spot and Rf value.

Table 2: Rechromatographed Ethyl Acetate Fractions.

Fractions	Rf Values	Designation
E2 ($E2_{1-21}$)	**0.88**	**E2F1**
E2 ($E2_{22-49}$)	**0.93**	**E2F2**
E3 ($E3_{1-11}$)	**0.82**	**E3F1**
E3 ($E3_{12-25}$)	**0.88**	**E3F2**
E5 ($E5_{1-10}$)	**0.82**	**E5F1**
E5 ($E5_{11-18}$)	**0.88**	**E5F2**
E7 ($E7_{1-13}$)	**0.59**	**E7F1**
E7 ($E7_{14-31}$)	**0.81**	**E7F2**
E9 ($E9_{1-11}$)	**0.59**	**E9F1**
E9 ($E9_{12-28}$)	**0.71**	**E9F2**
E10 ($E10_{1-14}$)	**0.48**	**E10F1**
E10 ($E10_{15-25}$)	**0.59**	**E10F2**

Fractions in Table 1 and 2 with the same Rf values were pooled together. This gives six (6) samples labeled accordingly. The weight of each sample was equally indicated (Table 3).

Table 3: Samples from the ethyl acetate fraction of *A. conyzoides* methanol leaf extract

Pooled Fractions	Rf Values	Samples Designate	Sample Weight (mg)
E1+E2F2	**0.93**	**EtAcS1**	**107**
E4+E2F1+E3F2+E5F2	**0.88**	**EtAcS2**	**125**
E3F1+E5F1+E6F1+E7F2	**0.82**	**EtAcS3**	**118**
E7F1+E8F1+E9f1+E10F2	**0.57**	**EtAcS4**	**93**
E9F2	**0.71**	**EtAcS5**	**82**
E10F1+E11	**0.48**	**EtAcS6**	**76**

EtAc and S mean ethyl acetate and sample respectively.

3.2. Fourier-Transform Infrared (FTIR) Spectroscopic Analysis

In FTIR spectra each absorbance peak is due to the vibration of a particular functional group of the sample (compound). The summary of each relevant peak with its corresponding functional group was as outlined in Table 4.

Table 4: FTIR spectral peak values and functional groups obtained for each Ethyl acetate sample

Samples	Peak Values (cm^{-1})	Functional Grp	Inferences
1.	**2928.0 and 2855.1**	**C-H (alkanes)**	**No -OH group, so could not be phenolic.**
2.	**3337.2 (weak band)**	**-OH (Phenol)**	**Presence of -OH group, thus presence of**
	2937.1 and 2844.0	**C-H (alkanes)**	**phenolic compound(s).**
	1718.3	**C=O (ald/ket.)**	
	1632.6	**C=C (alkenes)**	
	3090.0	**=C-H (alkenes)**	
	2146.9	**Benzene ring**	

3. of	**3380.7 (Broad band)**	**-OH or Phenol**	**–OH group identified, thus presence**
	2922.2 and 2855.1	**C-H (alkanes)**	**phenolic compound(s).**
	1699.7	**C=O (ald/ket.)**	
	1610.2	**C=C (alkenes)**	
	2132.0	**Benzene ring**	
4.	**2937.1 and 2840.2**	**C-H (alkanes)**	**No -OH group, so might not contain**
	3000.5	**=C-H (alkenes)**	**phenolic compound.**
	2158.1	**Benzene ring**	
5.	**3254.0(Broad band)**	**-OH or Phenol**	**-OH group identified, thus presence of**
	2922.2 and 2855.1	**C-H (alkanes)**	**phenolic compound(s).**
	1707.1	**C=O (ald/ket.)**	
	1617.7	**C=C (alkenes)**	
	2113.4	**Benzene ring**	
6.	**3548.4 (weak band)**	**-OH or Phenol**	**Could contain a phenolic compound,**
	1729.5	**C=O (ald/ket.)**	**although there is no pronounced**
	2005.3		**–OH functional group**

From Table 4, Samples 2, 3 and 5 contain phenolic compounds since they all have pronounced hydroxyl (-OH) functional groups. Note that all the FTIR spectra were as listed in the supplementary data (Figures S1-S6).

3.3. Liquid Chromatography-Mass Spectroscopy (LC-MS)

The results of LCMS analysis of ethyl acetate fractions of *A. conyzoides* methanol leaf extract revealed the presence of many bioactive compounds like Quassin (Quassinoids), Furocoumaric acid (Phenolic Glycoside), Liquiritin (Polyphenol), and Syringin (phenolic Glycosides), among others, at positive and negative modes, which were subsequently identified using the database by comparing their molecular fragmentation patterns for all the ethyl acetate samples (1-6) as shown in Table 5.

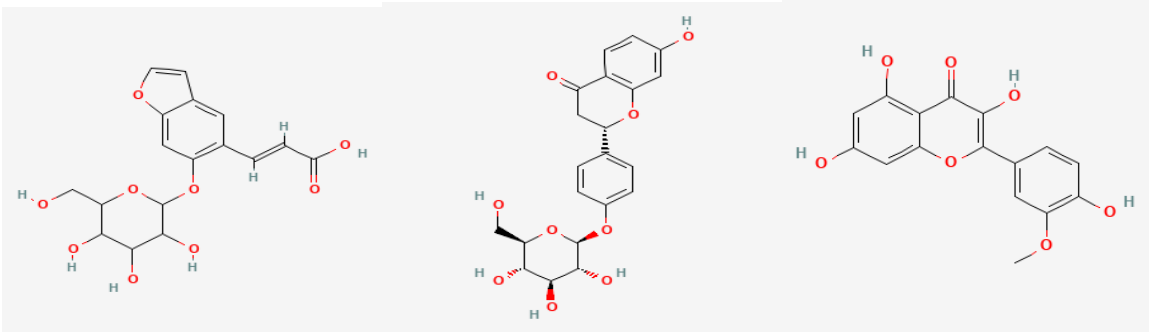
Table 5: LCMS Identified Compounds on Ethyl acetate Fractions of *A. conyzoides* Methanolic Leaf Extract

Samples	S/No	Rt(min)	Compounds	MW(g/mol)	LC-MS m/z	M. Formula
1	**1**	**2.43**	**Quassin**	**388.50**	**389.848 $[M+H]^+$**	**$C_{22}H_{28}O_6$**
	2	**11.28**	**Furocoumarinic acid**	**366.30**	**367.702 $[M+H]^+$**	**$C_{17}H_{18}O_9$**
2	**1**	**9.97**	**Quassin**	**388.50**	**389.378$[M+H]^+$**	**$C_{22}H_{28}O_6$**
	2	**10.17**	**Liquiritin**	**418.40**	**417.334$[M-H]^-$**	**$C_{21}H_{22}O_9$**
	3	**11.98**	**Furocoumarinic acid**	**366.30**	**367.565$[M+H]^+$**	**$C_{17}H_{18}O_9$**
3	**1**	**0.91**	**Blumenol A**	**224.30**	**225.912$[M+H]^+$**	**$C_{13}H_{20}O_3$**
	2	**2.15**	**Isorhamnetin**	**316.26**	**315.026$[M+H]^+$**	**$C_{16}H_{12}O_7$**
	3	**11.33**	**Furocoumarinic acid**	**366.30**	**367.573 $[M+H]^+$**	**$C_{17}H_{18}O_9$**
4	**1**	**11.13**	**Syringin**	**372.40**	**373.382$[M+H]^+$**	**$C_{17}H_{24}O_9$**
5	**1**	**11.27**	**Quassin**	**388.50**	**389.146$[M+H]^+$**	**$C_{22}H_{28}O_6$**
	2	**11.95**	**Furocoumarinic acid**	**366.30**	**367.551 $[M+H]^+$**	**$C_{17}H_{18}O_9$**
6	**1**	**11.92**	**Furocoumarinic acid**	**366.30**	**367.550$[M+H]^+$**	**$C_{17}H_{18}O_9$**

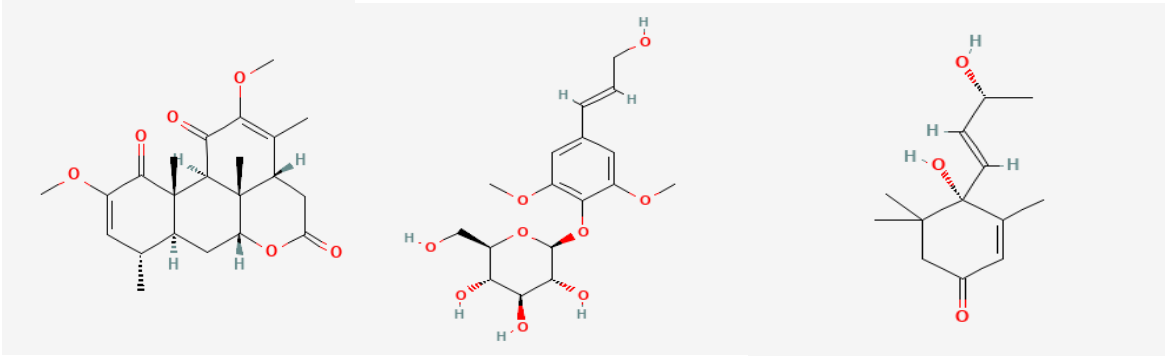
Keys: Rt= Retantion time; MW= Molecular weight; LCMS= Liquid chromatography- mass spectroscopy
From the LCMS analyses, furocoumarinic acid (phenolic glycoside) dominated in almost all six samples. Also, samples 2, 3, and 5 had higher concentrations (numbers) of phenolics identified. All the total ion chromatograms of the respective samples as well as the molecular fragments of the identified compounds were listed in the supplementary data (Figures S7-S21).

3.4. Chemical Structures of the Compounds Identified from LCMS as Obtained from PubChem Database

The 2-D structures of the compounds identified from ethyl acetate fraction of *A. conyzoides* methanolic leaf extract were as shown in Figure 1 below.



Furocoumaric acid Liquiritin Isorhamnetin



Quassin Syringin Blumol A

Figure 1: Structures of Compounds Identified from the LCMS Analyses

Quassin and blumol A are not phenolic compounds since there is no hydroxyl (-OH) group attached to benzene ring.

4. Discussion

The four phenolic compounds (furocoumaric acid, liquiritin, isorhamnetin, and syringin) identified in this present study (Table 5 and Figure 1) may have been found in *A. conyzoides* methanolic leaf extract. These substances may have been produced by the plant to protect itself from fungi and other toxic chemicals produced by microbes. It has been earlier reported by Adewale *et al*. [16] that *Staphylococcus aureus*, *Escherichia coli*, and *Clostridium perfringens* were all susceptible to the antibacterial and antifungal properties of the ethyl acetate extract of *A. conyzoides*. Thus, these phenolic compounds may have distinct biochemical and medicinal effects they confer on the plant.

Furocoumarinic acid (also known as furocoumarinic acid glucoside) is a class of chemical compound known as phenolic glycosides. It is a phenolic structure-attached aromatic heteropolycyclic molecule with a glycosyl moiety. According to a survey of the literature, few plants have been documented to contain furocoumarinic acid glucoside. For instance, the aqueous portion of *Combretum micranthum* leaf extract was used by Ibrahim and Bashir [17]to identify furocoumarinic acid. To the best of our knowledge, it hasn't been documented anywhere on the *A. conyzoides* plant, though.

Liquiritin, on the other hand, is a flavanone glycoside with a glycosidic link to a β-D-glucopyranosyl residue at position 4'. Monohydroxyflavanone is what it is. It is a sweet chemical with 200 times more sweetness than sucrose. As a plant metabolite and anti-inflammatory, it serves a purpose. One of the main components of *Glycyrrhiza* radix has been identified as liquiritin, which has a wide range of potential uses in medicine and pharmacology, including the treatment of allergic reactions, cancer, coughing, and

inflammation [18, 19]. Liquiritin is said to improve symptoms of rheumatoid arthritis (RA) by lowering pro-inflammatory cytokines (IL-6) and inhibiting MAPK (mitogen-activated protein kinase) signaling, according to Zhai *et al*. [20]. The primary flavonoid in licorice and *Glycyrrhiza* uralensi, liquiritin, has a number of other pharmacological properties, including anti-inflammatory, antitussive, antiasthmatic, analgesic, and neuroprotective actions [20].

Moreover, isorhamnetin is an o-methylated flavonol belonging to the flavonoid class. Pungent yellow or red onions are a typical food source of this 3'-methoxylated derivative of quercetin and its glucoside conjugates. Isorhamnetin is also abundant in pears, olive oil, wine, and tomato sauces [21]. Tyrosinase inhibitors, anticoagulants, and metabolites are all functions of this substance. Isorhamnetin may function as a powerful antioxidant that shields cells from harmful toxins (dangerous by-products of oxygen-related processes), much like flavonols do. It has been reported to have antioxidant and antiviral properties, so it is not only used as is but also comes in a variety of derivatized forms that can be turned into medications to treat conditions brought on by oxidative stress and cancer-causing viruses [22]. This compound has a wide range of biological effects, including anticancer effects [23, 24], neurological activity [4, 24], cardiovascular protection [25, 26], anti-inflammatory activity [27, 24], hepatoprotective activity [28], and antidiabetic effect [29]. Isorhamnetin's potential to scavenge free radicals, donate hydrogen atoms or electrons, or bind metal cations may be the cause of its myriad biological functions. Its structural characteristics, particularly the quantity and locations of hydroxyl groups and the types of substitutions on aromatic rings, may also contribute to its capacity to inhibit free radicals, defending cells and organs from degenerative illnesses like cancer, diabetes, and cardiovascular diseases.

Finally, Meillet first discovered the natural chemical component, syringin, in the lilac tree's (*Syringa vulgaris*) bark in 1841 [30]. Since then, syringin has been discovered that it is widely dispersed among a variety of plant species. *Eleutherococcus senticosus* (*Siberian ginseng*) contains eleutheroside B, commonly known as eleutheroside. In addition, syringin is a phenolic glycoside of a monosaccharide derivative where trans-sinapyl alcohol is glycosidically linked to a β-D-glucopyranosyl residue at position 1. According to Sundaram *et al*. [31], it might have anti-diabetic properties. It functions as a plant metabolite and hepatoprotective agent.

The furocoumaric acid, liquiritin, isorhamnetin or syringin identified could be responsible for the anti-diabetic and anti-oxidative effect of *A. conyzoides* methanolic leaf extract. Thus, the need to focus on these phenolics for the purpose of new drug discovery in the treatment of diabetes mellitus.

Recommendation:

It is recommended that the phenolic compounds identified in this research be isolated, and subsequent *in-vitro* and *in-vivo* experimentation to ascertain their anti-diabetic efficacy be carried out.

Limitations:

This research was limited to identification of phenolics from *A. conyzoides* methanolic leaf extract because of paucity of fund; if not we would have taking it further through NMR spectroscopy for isolation of the compounds.

5. Conclusion

This study has demonstrated that the phenolic compounds- furocoumaric acid, liquiritin, isorhamnetin, and syringin, are present in the ethyl acetate fraction of the methanol leaf extract of *A. conyzoides*. To the best of our knowledge, this is the first time these phenolics were identified in *A. conyzoides*. Since free radicals and oxidative species are known to exacerbate a number of diseases, including cardiovascular diseases, diabetes, malignancies, and neurological disorders, these compounds, when isolated from *A. conyzoides*, could be extensively used in the therapy and management of these conditions.

Significance Statement:
This study has discovered that ethyl acetate fraction of *A. conyzoides* methanolic leaf extract is a good source of the phenolics- furocoumaric acid, liquiritin, isorhamnetin, and syringin. As a result of their ability to protonate and donate electrons, these compounds when isolated from this plant could be a pathway to the discovery of new drug candidate(s) for the treatment and management of diabetes, cardiovascular diseases, cancer, and other radical aggravating illnesses. Also, being the first time these compounds were identified from *A. conyzoides* (to the best of our knowledge), this will open further research opportunities to carry out the *in-silico, in-vitro* and *in-vivo* experimentation on the pure fractions of this plant leaf extract.

6. References

[1] Dias, R., Oliveira, H., Fernandes, I., Simal-Gandara, J. & Perez, G. R. Recent advances in extracting phenolic compounds from food and their use in disease prevention and as cosmetics. *Critical Reviews in Food Science and Nutrition*, **61**(7), 1130–1151. https://10.1080/10408398.2020.1754162 (2021).
[2] Zeb, A. Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*, **44**(9), e13394. DOI: 10.1111/jfbc.13394 (2020).
[3] Arjeh, E. et al. Phenolic compounds of sugar beet (*Beta vulgaris* L.): Separation method, chemical characterization, and biological properties. *Food Science & Nutrition*, **10,** 4238–4246. https://doi.org/10.1002/fsn3.3017 (2022).
[4] Chen, M., Zhao, Z., Meng, H. & Yu, S. The antibiotic activity and mechanisms of sugar beet (*Beta vulgaris*) molasses polyphenols against selected food-borne pathogens. *LWT –Food Science and Technology*; **82,** 354-360. DOI: 10.1016/J.LWT.2017.04.063 (2017).
[5] Moura, A. C. et al. Anti-inflammatory and chronic toxicity study of the leaves of *Ageratum conyzoides* L. in rats. *Phytomedicine*; **12,** 138–142. https://doi.org/10.1016/j.phymed.2003.12.003 (2005).
[6] Huang, D. & Tocmo, R. Assays based on competitive measurement of the scavenging ability of reactive oxygen/nitrogen species. In F. Shahidi (Ed.), *Functional food science and technology*, 21–36. John Wiley and Sons Ltd. DOI: 10.1002/9781119135388.ch2 (2018).
[7] Ahmed, F.A. & Ali, R.F.M. Bioactive compounds and antioxidant activity of fresh and processed white cauliflower. *Biomedical Research International*, 1–9. DOI: 10.1155/2013/367819 (2013).
[8] Nyunaï, N., Abdennebi, E.H., Bickii, J. & Manguelle-Dicoum, M.A. Subacute antidiabetic properties of *Ageratum conyzoides* leaves in diabetic rats. *Intl J Pharm Sci Res*, **6**(4), 1378-1387. DOI: 10.13040/IJPSR.0975-8232.6(4).1378-87 (2015).

[9] Adebayo, A.H. et al. Biochemical, haematological and histopathological studies of extract of *Ageratum conyzoides* L. in Sprague Dawley rats. *J. Med. Plants Res*., **4**(21), 2264-2272. DOI: 10.5897/JMPR10.470 (2010).
[10] Atawodi, S.E., Adepoju, O.A. & Nzelibe, H.C. Antihyperglycaemic and hypolipidemic effect of methanol extracts of *Ageratum conyzoides* L (Asteraceae) in normal and diabetic rats.*Tropical Journal of Pharmaceutical Research*, **16**(5), 989-996. http://dx.doi.org/10.4314/tjpr.v16i5.4 (2017).
[11] Ozioko, P.C., Muhammed, Y.Y. & Ibrahim, A. *Ageratum conyzoides* Methanol Leaf Extract: Phytochemicals with Antidiabetic Potential via Antioxidant Activity. *Asian J. of Biochem*., **17**(1), 15-24. DOI: 10.3923/ajb.2022.15.24 (2022).
[12] Ojewale, A. O. et al. Hepatoprotective activities of ethanolic roots extract of *Ageratum conyzoides* on alloxaninduced hepatic damage in diabetic Wistar rats, *Journal of Morphological Sciences*, **36**(1), 039–045 (2019).
[13] Singh, S. B. et al. Ethnobotany, phytochemistry and pharmacology of *Ageratum conyzoides* Linn (Asteraceae). *Journal of Medicinal Plant Research*, **7**(8), 371–385. DOI:10.5897/JMPR012.897 (2013).
[14] Yadav, N., Ganie, S.A., Singh, B., Chhillar, A.K. & Yadav, S.S. Phytochemical constituents and ethnopharmacological properties of *Ageratum conyzoides* L. *Phytotherapy Research*, 1–16. https://doi.org/10.1002/ptr.6405 (2019).
[15] Piovesana, A., Rodrigues, E. & Norena, C.P.Z. Composition analysis of carotenoids and phenolic compounds and antioxidant activity from Hibiscus calyces (*Hibiscus sabdariffa* L) by HPLC-DAD-MS/MS. *Phytochemical Analysis*, **435,** 1-10. https://doi.org/10.1002/pca (2018).
[16] Adewale, A., Winston, A., Morgan, O. C. & Chimezie, F. Antibacterial activity and *in vitro* cytotoxicity of extracts and fractions of *Parkia bigbosa* (Jacq.) Benth. Stem bark and *Ageratum conyzoides* Linn. Leaves. *Environmental Toxicology and Pharmacology*, **34,** 478–483 (2012).
[17] Ibrahim, A. & Bashir, M. Liquid Chromatography Mass Spectrometer (Lc/Ms) Profile Revealed Flavonoids And Terpenoids With Antioxidant Potential In Aqueous Fraction of Combretum Micranthum Leaf Extract. *Dutse Journal of Pure and Applied Sciences (DUJOPAS)*, **7**(4b), 150-159.DOI: 10.4314/dujopas.v7i4b.16 (2021).
[18] Xie, R. et al. Liquiritin exerts synergistic effects against human gastric cancer cells and xenograft in nude mice through potentiating apoptosis and ROS generation. *Biomedicine pharmacotherapy*, **93,** 948–960. doi: 10.1016/j.biopha.2017.06.095 (2017).
[19] Li, X. Q. et al. Liquiritin suppresses UVB-induced skin injury through prevention of inflammation, oxidative stress and apoptosis through the TLR4/MyD88/NFkappaB and MAPK/caspase signaling pathways. *Int. J. Mol. Med*., **42**(3), 1445–1459. doi: 10.3892/ijmm.2018.3720 (2018).
[20] Zhai, K.F. et al. Liquiritin from *Glycyrrhiza uralensis* attenuating rheumatoid arthritis via reducing inflammation, suppressing angiogenesis, and inhibiting MAPK signaling pathway. Journal of Agricultural and Food Chemistry, **67**(10), 2856-2864. DOI: 10.1021/acs.jafc.9b00185 (2019).
[21] Holland, T.M. et al. Dietary flavonols and risk of Alzheimer dementia. *Neurology*, **94**(16), e1749–e1756. doi:10.1212/WNL.0000000000008981 (2020).
[22] Kandakumar, S. & Manju, V. Pharmacological Applications of Isorhamnetin: A Short Review. *International Journal of Trend in Scientific Research and Development*, **1**(4), 172-178. https://doi.org/10.31142/ijtsrd2202 (2017).

[23] Jin-Jing, Z., Jin-Qing, S., Shu-Yi, P. & Kai, W. Treatment with isorhamnetin protects the brain against ischemic injury in mice. *Neurochem Res*., **41**(8), 1939-1948 (2016).
[24] Tae, H.K., Sae-Kwang, K. & Jong-Sup, B. Anti-Inflammatory activities of isorhamnetin-3-O1galactoside against HMGB1-induced inflammatory responses in both HUVECs and CLP-induced septic mice. *Journal of Cellular Biochemistry*, **114,** 336-345. DOI: 10.1002/jcb.24361 (2013).
[25] Liqing, H. et al. Protective effects of isorhamnetin on cardiomyocytes against anoxia/reoxygenation1induced injury is mediated by SIRT1. *J Cardiovasc Pharmacol*., **67,** 526-537 (2016).
[26] Yun, L. et al. Isorhamnetin attenuates atherosclerosis by inhibiting macrophage apoptosis via PI3K/AKT activation and HO-1 induction. *PLoS ONE*, **10**(3), 0120259. https://doi.org/10.1371/journal.pone.0120259 (2015).
[27] Marilena, A., Janet, A.G., Carlos, M. & Sergio, O.S.S. Topical anti-inflammatory effects of isorhamnetin glycosides isolated from Opuntia ficus-indica. *BioMed Research International*, 10.1155/847320 (2015).
[28] Guang-Zhi, D. et al. AMPK activation by isorhamnetin protects hepatocytes against oxidative stress and mitochondrial dysfunction. *European Journal of Pharmacology*, **740,** 634-640. https://doi.org/10.1016/j.ejphar.2014.06.017 (2014).
[29] Yeon, S.S., Hye, S.B, Kazuo, O. & Kuk, H. Inhibitory effects of Isorhamnetin-3-O-b - D-glucoside from *Salicornia herbacea* on rat lens aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. *Biol. Pharm. Bull*, **28**(5), 916-918. DOI: 10.1248/bpb.28.916 (2005).
[30] Park, H., Jung, W., Basnet, P., Kadota, S. & Namba, T. Syringin 4-O-β-Glucoside, a New Phenylpropanoid Glycoside, and Costunolide, a Nitric Oxide Synthase Inhibitor, from the Stem Bark of Magnolia sieboldii. *Journal of Natural Products*, **59**(12), 1128–1130. doi:10.1021/np960452i (1996).
[31] Sundaram, C.K.S., Pillai, S.I. & Pillai, S.S. Isolation, characterization of syringin, phenylpropanoid glycoside from *Musa paradisiaca* tepal extract and evaluation of its antidiabetic effect in streptozotocin-induced diabetic rats. *Biomedicine & Preventive Nutrition*. **4**(2), 105–111. doi:10.1016/j.bionut.2013.12.009 (2014).

Supplementary Data:

1. FTIR Spectra of Samples 1-6

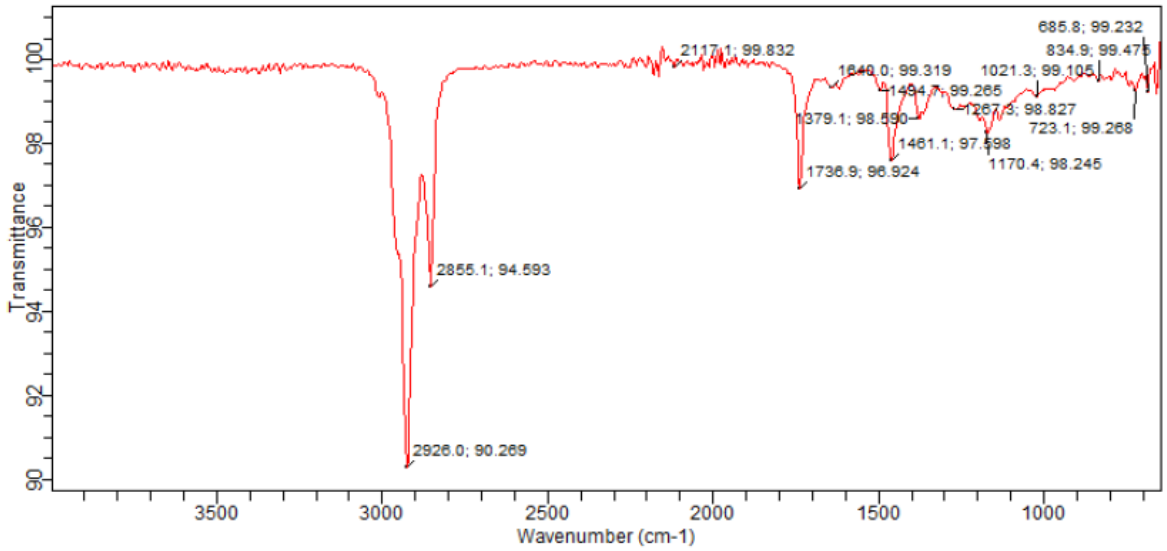


Fig. S1: The FTIR Spectrum of Ethylacetate Sample 1

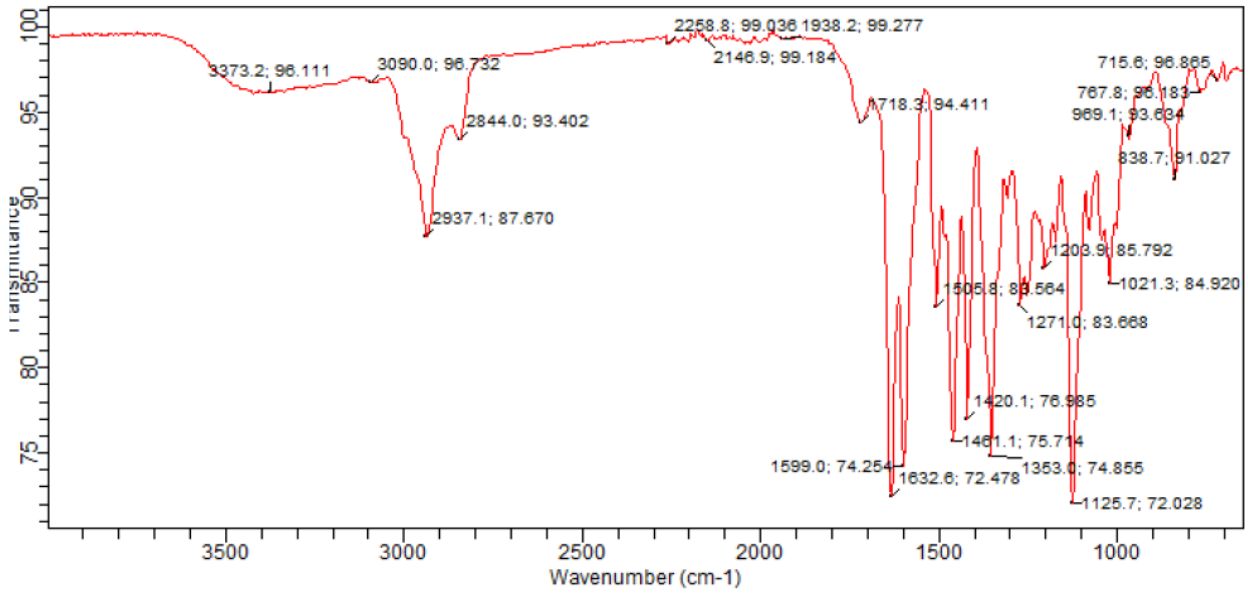


Fig. S2: The FTIR Spectrum of Ethylacetate Sample 2

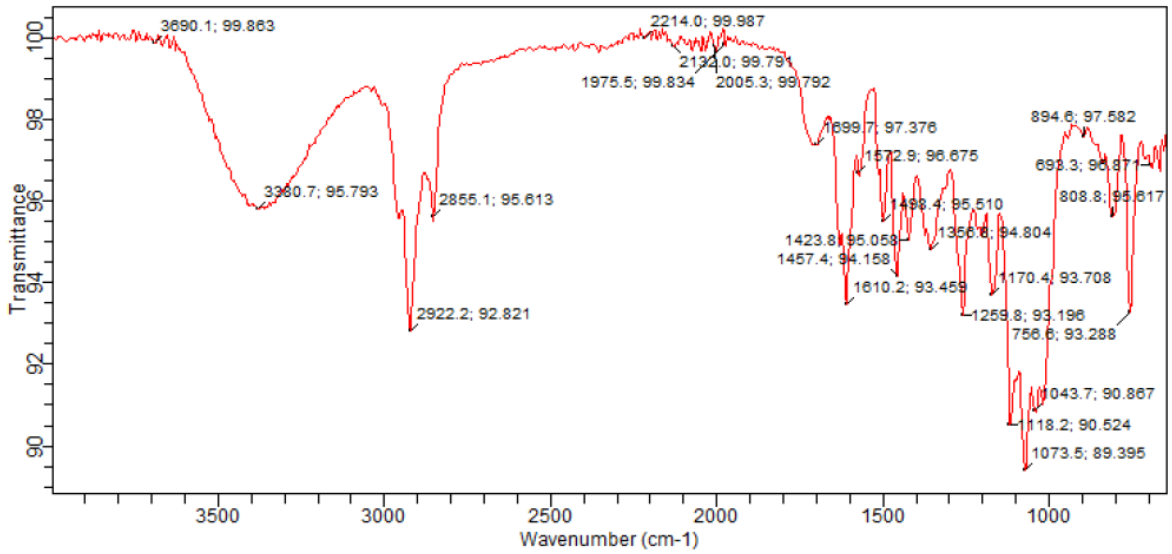


Fig. S3: The FTIR Spectrum of Ethylacetate Sample 3

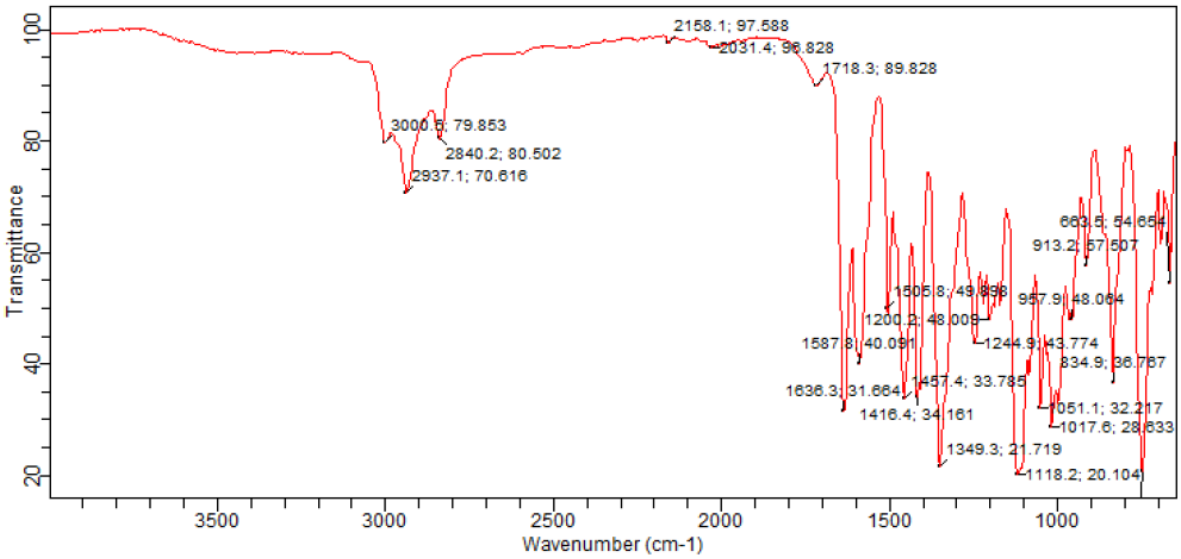


Fig. S4: The FTIR Spectrum of Ethylacetate Sample 4

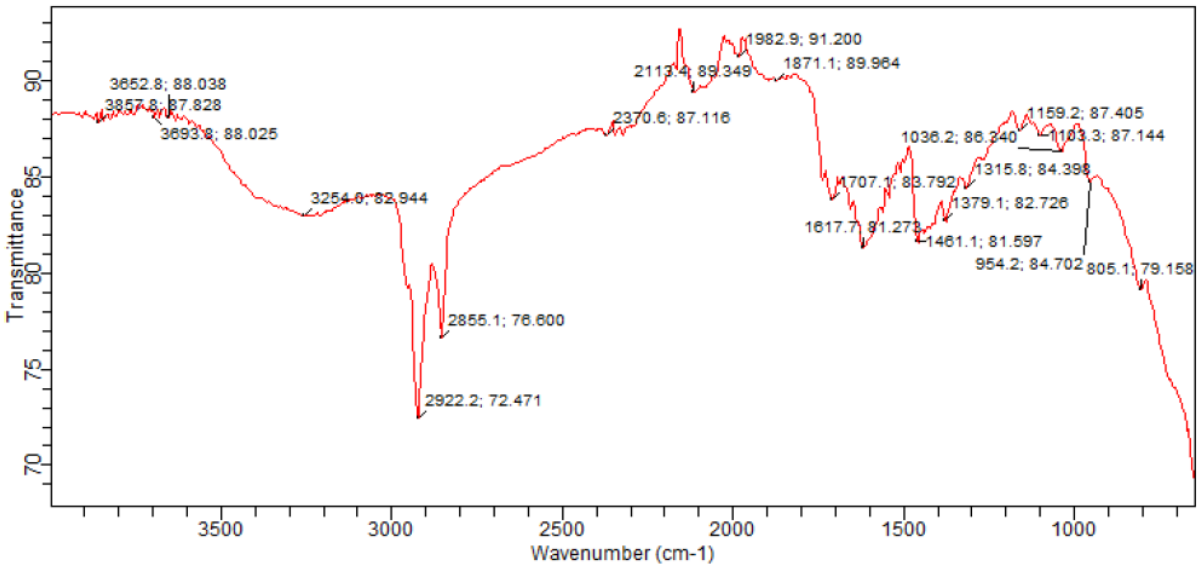


Fig. S5: The FTIR Spectrum of Ethylacetate Sample 5

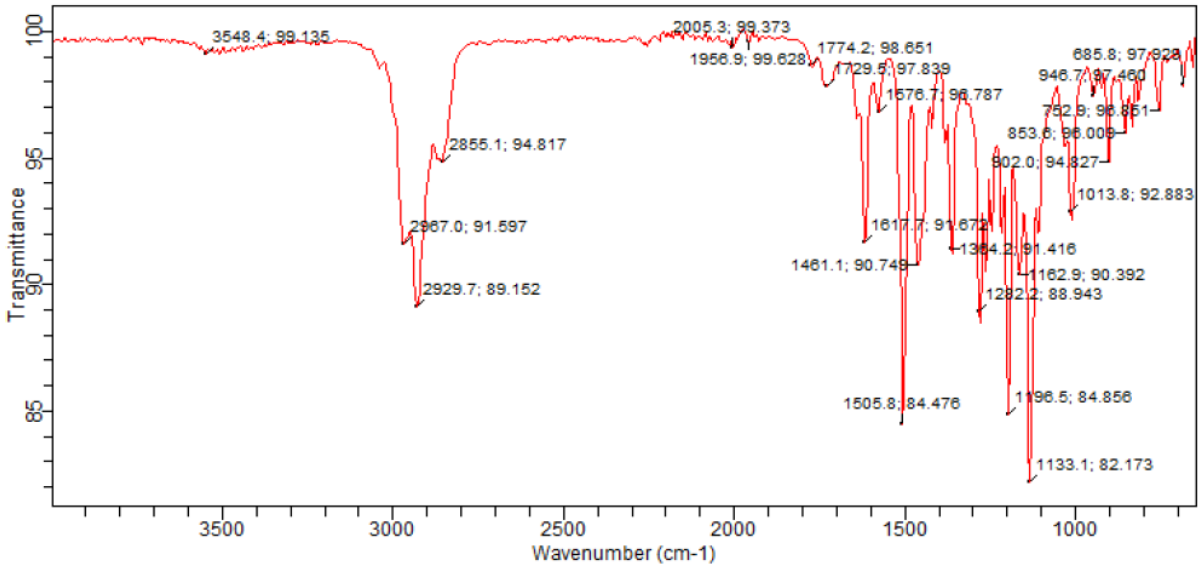


Fig. S6: The FTIR Spectrum of Ethylacetate Sample 6

2. LCSM Ion Chromatograms and Molecular Fragmentations of Samples 1-6

Sample1:

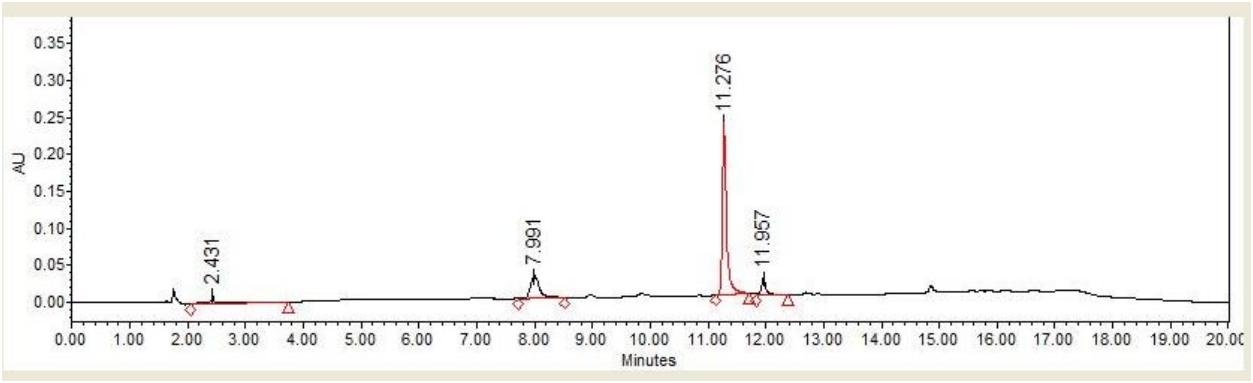
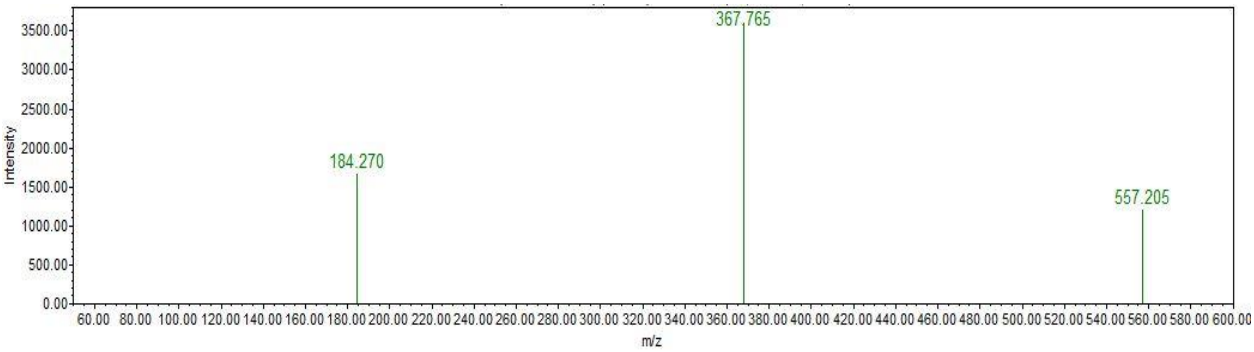


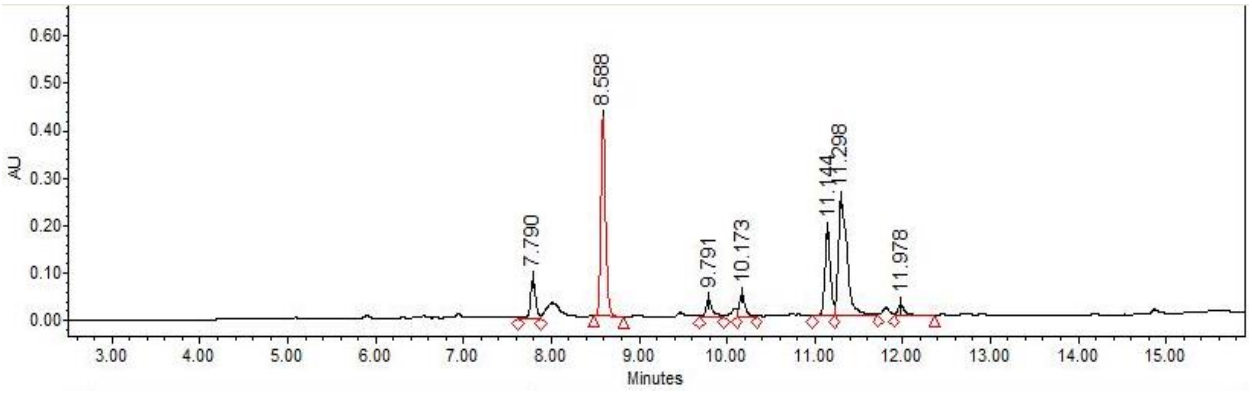
Fig. S7: Total Chromatogram of Sample 1



m/z: mass-to-charge ratio

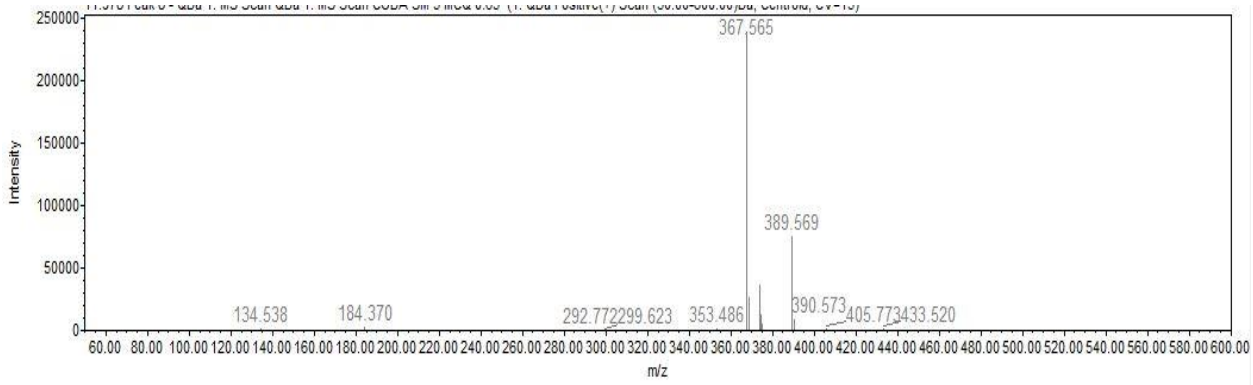
Fig. S8: Molecular fragmentation of Furocoumarinic acid 367.765 m/z

Sample 2:



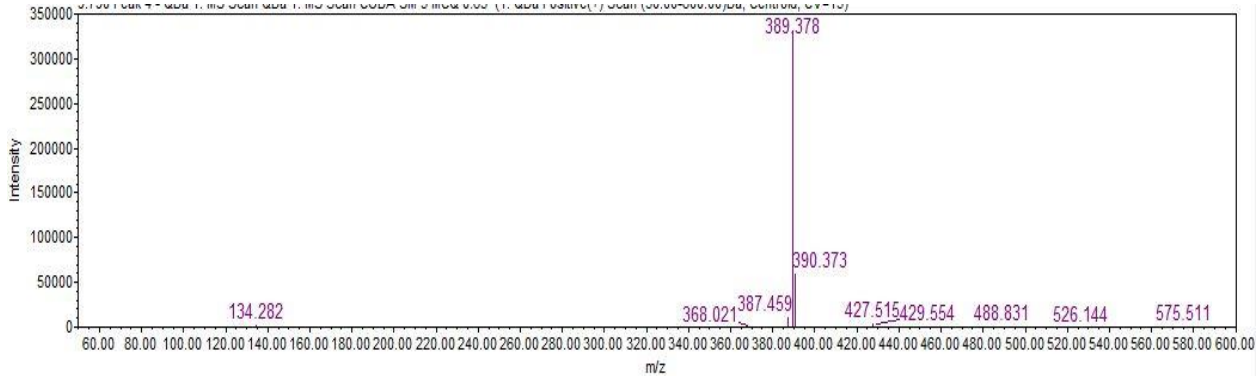
AU stands for absorbance unit

Fig. S9: Total Chromatogram of Sample 2



m/z: mass-to-charge ratio

Fig. S10: Molecular fragmentation of Furocoumarinic acid 367.565 m/z



m/z: mass-to-charge ratio

Fig. S11: Molecular fragmentation of Quassin 389.378 m/z

Sample 3:

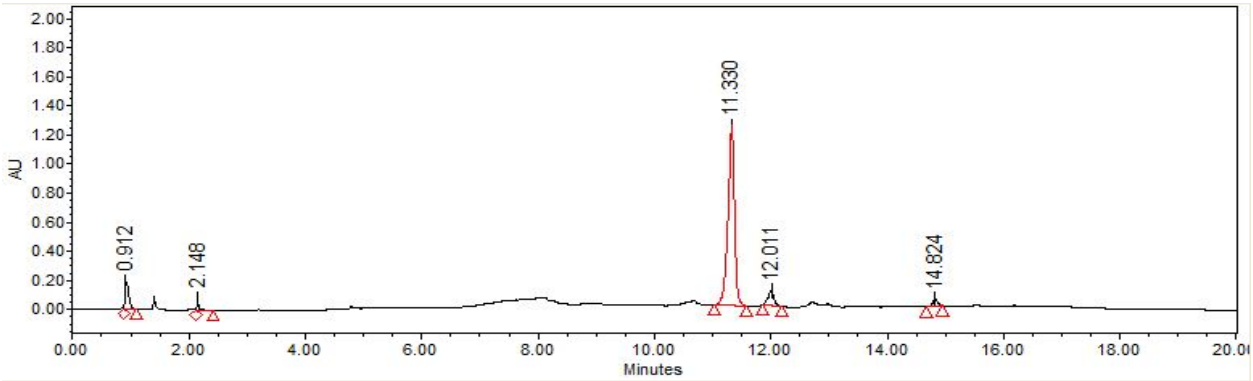
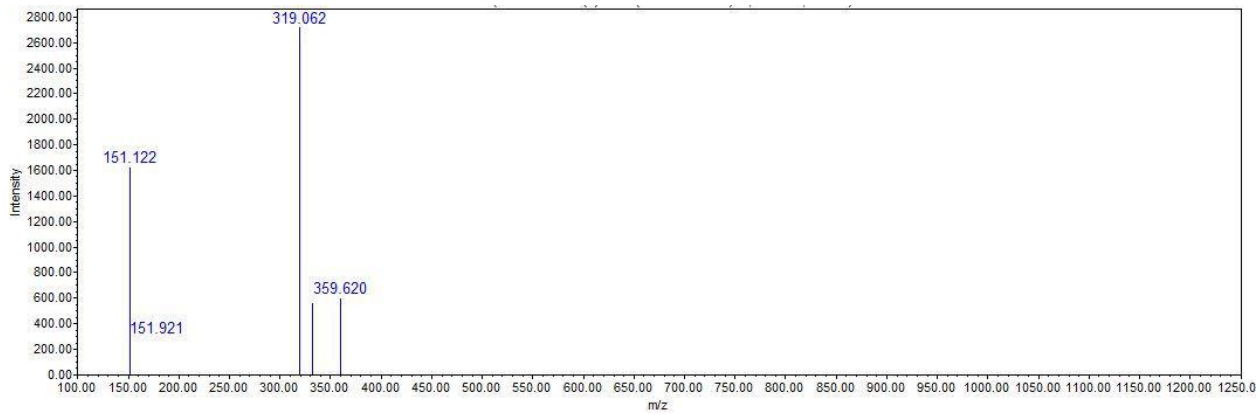


Fig. S12: Total Chromatogram of Sample 3

AU stands for absorbance unit



m/z: mass-to-charge ratio

Fig. S13: Molecular fragmentation of Isorhamnetin 319.062 m/z

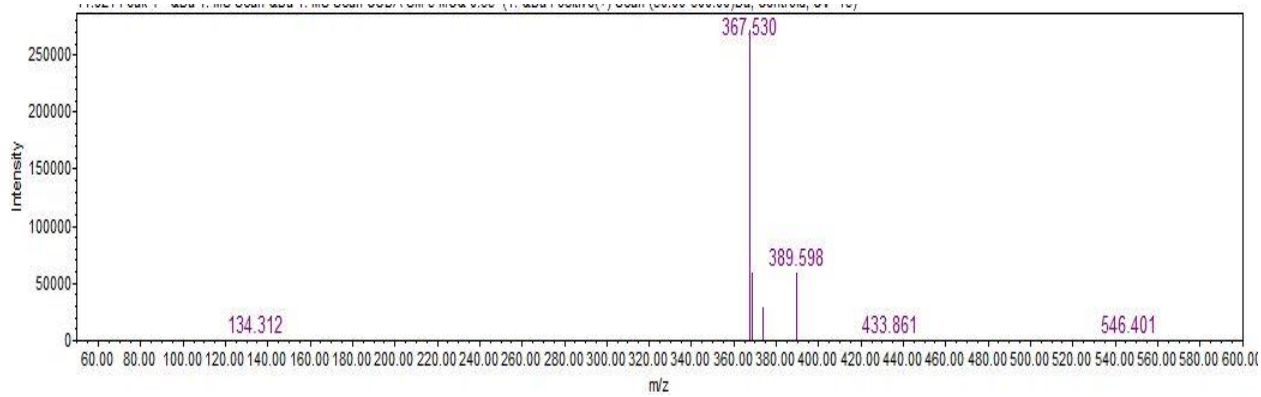


Fig. S14: Molecular fragmentation of Furocoumarinic acid 367.530 m/z

Sample 4:

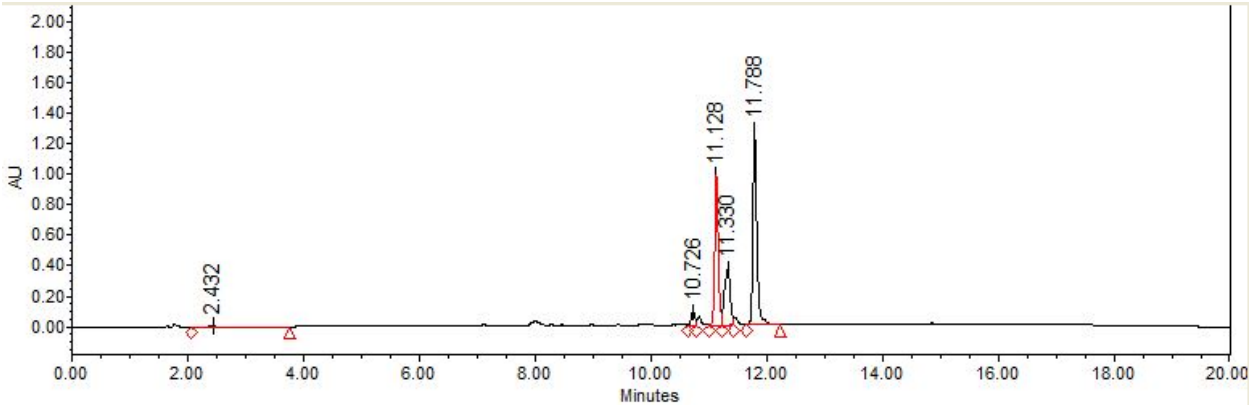
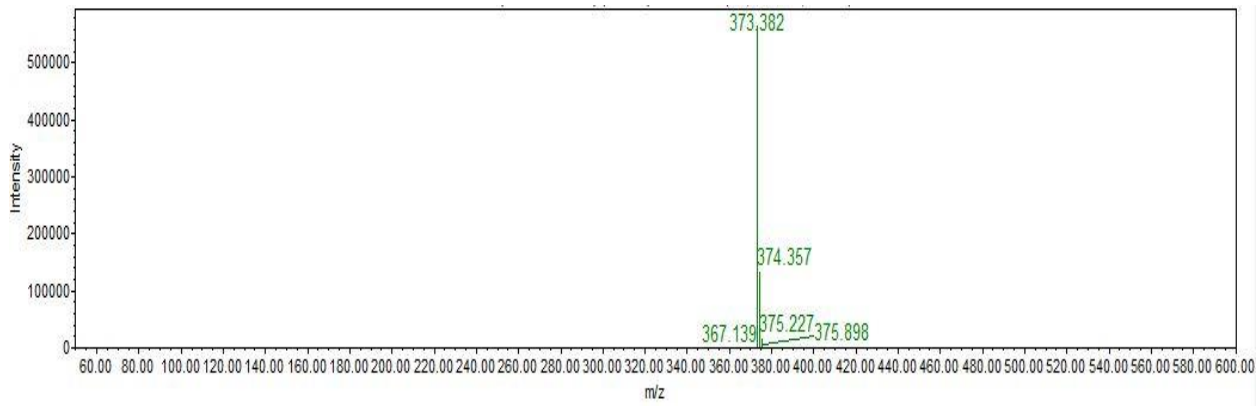


Fig. S15: Total Chromatogram of Sample 4



m/z: mass-to-charge ratio

Fig. S16: Molecular fragmentation of Syringin 373.382 m/z

Sample 5:

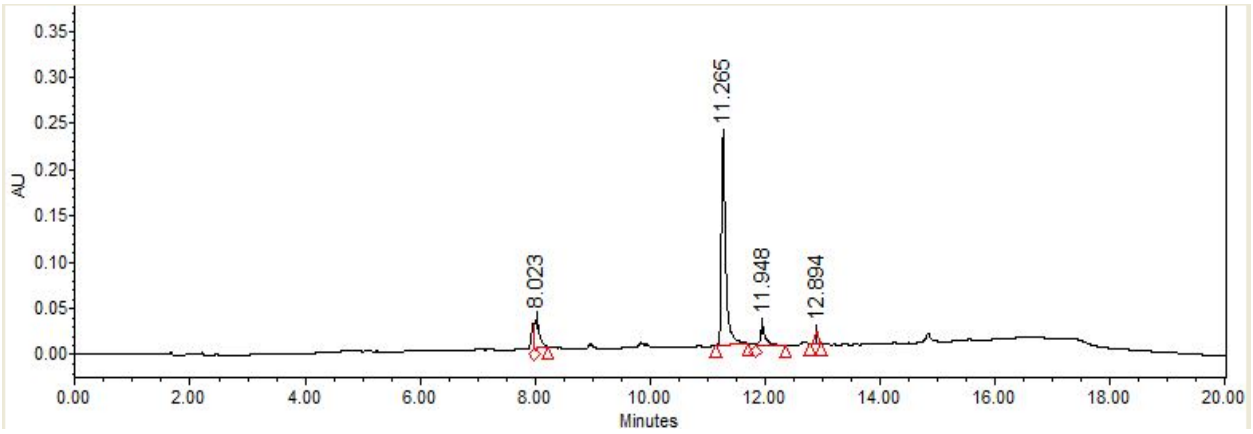
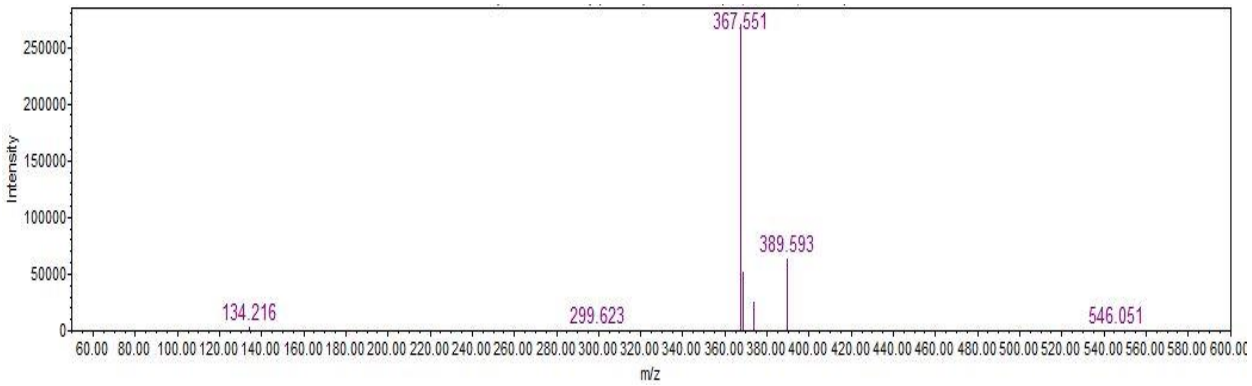
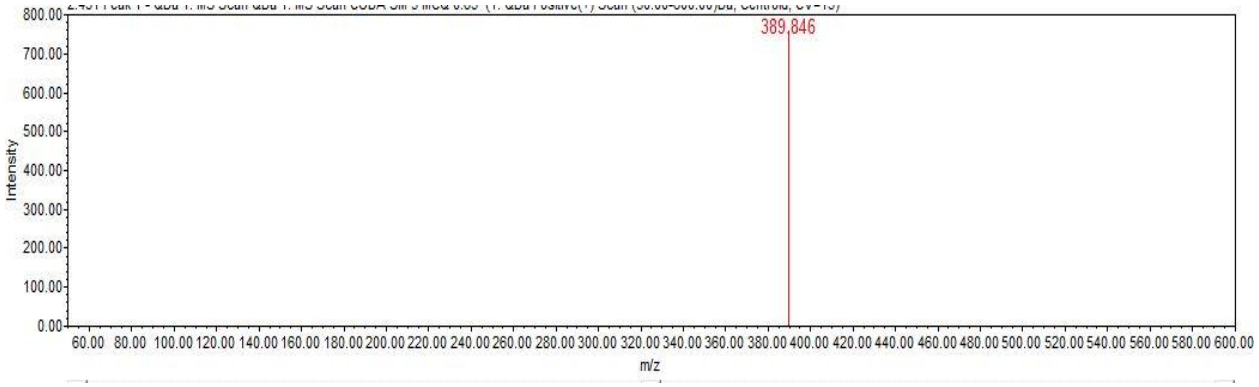


Fig. S17: Total Chromatogram of Sample 5



m/z: mass-to-charge ratio

Figure S18: Molecular fragmentation of Furocoumarinic acid 367.551 m/z



m/z: mass-to-charge ratio

Fig. S19: Molecular fragmentation of Quassin 389.846 m/z

Sample 6:

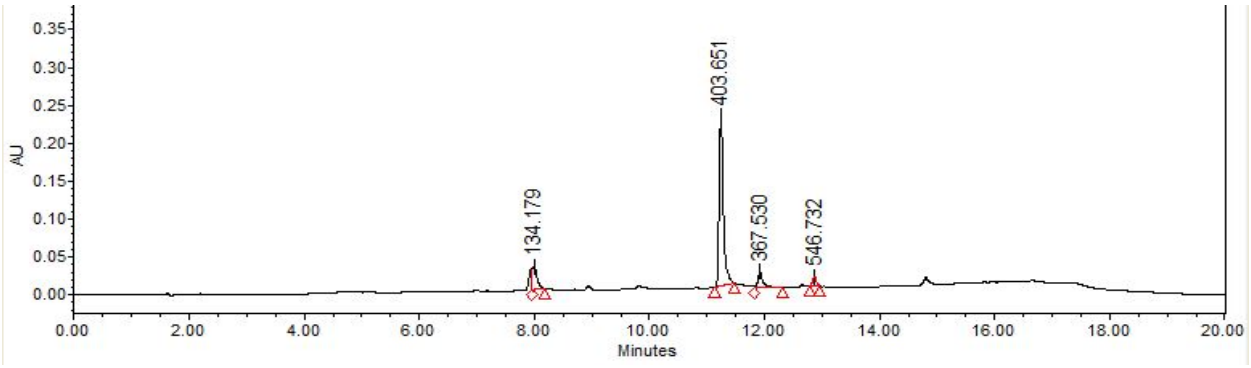
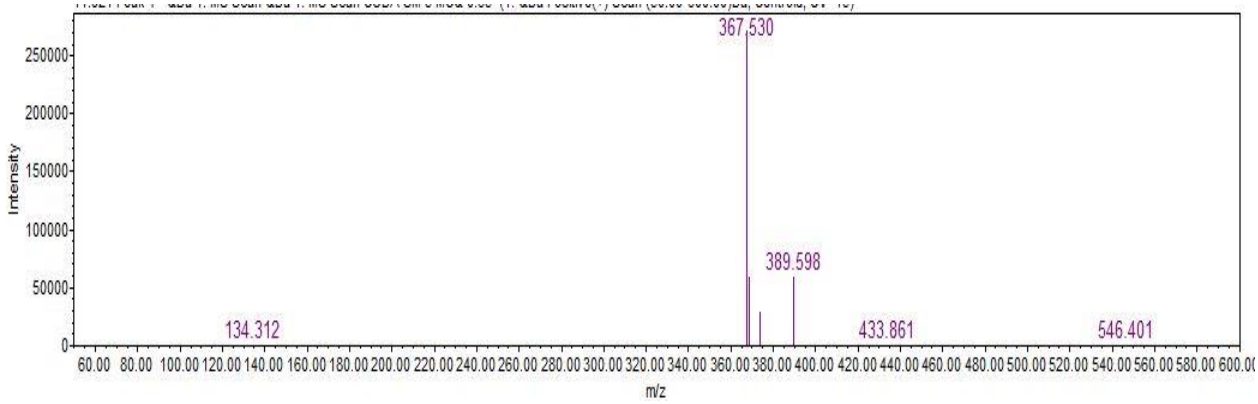


Fig. S20: Total Chromatogram of Sample 6



m/z: mass-to-charge ratio

Fig. S21: Molecular fragmentation of Furocoumarinic acid 367.550 m/z