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UPLC-ESI-QTOF-MS profiling of Prasarinyadi kashayam

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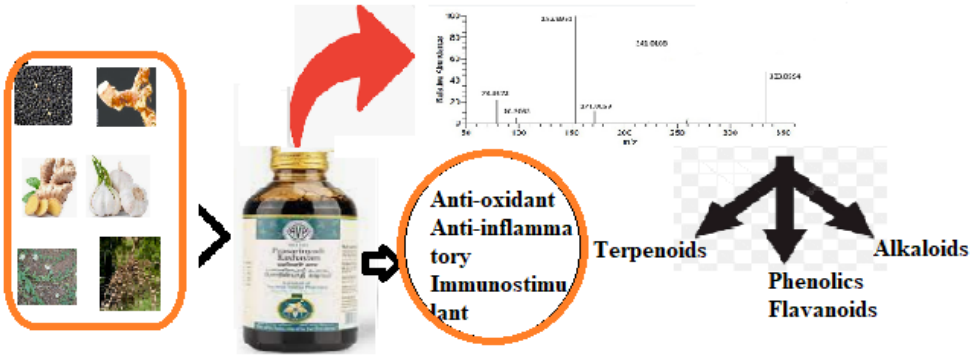
Article

ABSTRACT



Present study was the identification of active phytochemicals from Prasarinyadi kashayam using UPLC-ESI-QTOF-MS. During the preparation of kashayam phytochemicals present in plant parts may undergo different types of reactions such as condensation, decomposition, hydrolysis, dimerization, substitution, polymerization, and metal complex formation. So, the kashayam is the complex mixture of various compounds or phytochemicals with different or the same polarities and molecular mass. Their separation still remains a big challenge for the process of identification and characterization. The identification of such a complex mixture can be done easily and accurately by LCMS analysis. UPLC-ESI-QTOF-MS analysis identified 19 phyto-constituents. Identified compounds included secondary metabolites like terpenoids, phenolic compounds, flavonoids, and alkaloids. In detailed literature, the identified compounds are found to have properties such as antioxidant, immunostimulant, and anti-inflammatory.

Keywords: Prasarinyadi kashayam, anti-inflammatory, antioxidant, immunostimulant, UPLC-ESI -QTOF-MS

INTRODUCTION

Prasarinyadi Kashayam is a Ayurvedic polyherbal formulation mainly used for shoulder-related problems. This formulation is prepared as per Sahasrayogam, the Ayurveda Text, using 6 medicinal plants. Allium sativum (Bulb), Alpinia calcarata (Rhizome), Merremia tridentata (Whole plant), Sida rhombifolia (Root), Vigna mungo (Seed), and Zingiber officinale (Rhizome) are the plants used in the preparation of Prasarinyadi Kashayam.

When synergism is considered, polyherbal formulations offer some benefits not available with single herb preparations. A better therapeutic effect can be reached with multi-constituent formulation. The lower dose of the polyherbal formulation needed to achieve desirable pharmacological action, thus reducing the risk of harmful side effects. These benefits have resulted in Polyherbal formulations (PHF)'s popularity in the market compared to single herbal formulations.

The realization is that most chronic diseases are multi-genic and hence, a multi-targeted approach is required to ameliorate/cure the condition. So, in most cases, a combination of medicinal plants is recommended for treatment, a type of multitargeted or combinatorial approach. In the Allopathic system which uses mainly synthetic chemicals designed for specific target receptors that primarily give symptomatic relief. Since the Ayurvedic medicines are prepared from multiple plants, Ayurvedic medicines are rich in phytochemicals and are capable of eliminating the root cause of diseases by restoring balance.

The phytochemicals present in the polyherbal formulation leads to the desired healing effect. During the preparation of kashayam phytochemicals present in plant parts may undergo different types of reactions such as condensation, decomposition, hydrolysis, dimerization, substitution, polymerization, and metal complex formation. So, the kashayam is the complex mixture of various compounds or phytochemicals with different or the same polarities and molecular mass. Their separation remains a big challenge for the process of identification and characterization. The identification of such a complex mixture can be done easily and accurately by LCMS analysis.

LCMS is a hyphenated technique combining the separation techniques (chromatography)and analysis techniques (Mass spectroscopy). LCMS provides identification of unknown compounds through efficient separation capabilities of HPLC and exact structural characterization by mass spectrometer. In the

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present study UPLC QTOF MS is used for the identification of active components from the kashayam.

Ultra-HPLC (UPLC) coupled with time-of-flight mass spectrometry (TOF-MS) with electrospray ionization (ESI) provides higher throughput analysis and improved chromatographic resolution, high accuracy and sensitivity due to the high-frequency sampling of all ions simultaneously across the full mass range. The ESI usually produces [M+H] + or [M-H] + ions from which the molecular weight can be directly inferred.[1]

The ESI -MS fragmentation reactions of natural products can be divided into two main groups: charge retention fragmentations (CRF) and charge migration fragmentations (CMF).[2]

Charge retention fragmentations (CRF) are composed of a class of reactions that result in fragment ions with the charge located at the site identical to its precursor ion. Because CRF typically occurs at a location that is physically remote from the location of the charge and without its direct participation in the mechanism. Normally, CRF reactions proceed through concerted mechanisms and may occur even at low collision energies, depending on the chemical structure, ionization method, mass collision target, and other equipment parameters. CRF can be classified into nine main reaction mechanisms: remote hydrogen rearrangements, Retro-Diels–Alder (RDA) reactions, retro-ene reactions, retro hetero ene reactions, charge remote fragmentations, aromatic eliminations, other pericyclic processes, carbon monoxide eliminations from cyclic carbonyl compounds and radical eliminations. The CMF are fragmentation reactions in which the charge is displaced from the precursor ion. In general, CRF gas-phase reactions of negative ions are similar to those of positive ions because they occur in positions remote from the charge site. However, CMF in negative ions significantly differs from those of positive ions. In positive ions, CMF reactions usually eliminate a leaving group for which the charge site was initially the location of a neutral molecule. However, the neutral loss from negative ions typically occurs at the part of the ion structure that was initially charged.

RESULT AND DISCUSSION

Identification of the compound present in the supernatant of the kashayam obtained from centrifugation has been done with the UPLC-ESI-QTOF-MS analysis. Identified compounds are given in Table 1. 19 compounds have been identified. Out of the 19 compounds include 4 terpenes, 5 alkaloids, and 10 phenolic compounds. The fragmentation reactions of fourteen of the identified compounds have been elucidated and described in the schemes below.

Table 1 List of identified compounds

SI No.	Retention time	Compound name & Molecular formula	Molecular mass	Mass fragments
1.	3.561	Phenethylamine $C_8H_{11}N$	121.18	121.0293 (M^+), 77.0397
2.	3.655	Vasicinolone $C_{11}H_{10}N_2O_3$	218.21	218.2120(100%) (M^+),200.2010(31%), 174.1853(5%), 156.1750(2%)
3.	3.902	Marmesin $C_{14}H_{14}O_4$	246.26	246.2433(100%) (M^+),202.2164, 184.2064
4.	4.003	4-O-methyl syringic acid $C_{13}H_{12}N_2O$	212.25	212.0216(12%) (M^+),167.9952(100%), 194.0108(42%), 136.0230(8%)
5.	4.581	4-Methyl-6-gingerol $C_{18}H_{30}O_4$	310.43	311.1859(100%) ($M+H^+$), 293.1753(48%), 219.1034
6.	4.657	7-Hydroxy hinokinin $C_{20}H_{18}O_8$	386.35	386.8377(100%) (M^+), 343.0959, 283.2594
7.	4.892	Berberine $C_{20}H_{18}NO_4$	336.36	337.1630(100%) ($M+H^+$),320.0945 (2%), 279.16153
8.	4.573	6-Gingerol $C_{17}H_{26}O_4$	294.38	293($M-H^+$), 193.0484, 136
9.	5.015	Quercetin $C_{15}H_{10}O_7$	302.24	302.3064(100%) (M^+) , 284.2957(21%), 137.0082
10.	5.493	Trans-feruloylctopamine $C_{18}H_{19}NO_5$	329.35	330.3372(100%) ($M+H^+$), 312.3258(20%), 286.3114(2%)
11.	5.511	Tetrahydrocurcumin $C_{21}H_{24}O_6$	372.41	372.8219(100%) (M^+),357.0864, 217.9250(2%)
12.	6.076	8-Hydroxytinosporide $C_{20}H_{22}O_8$	390.38	389.8125(100%) ($M-H^+$), 262.9072(16%), 234.9126(28%)
13.	7.660	Asaraldehyde $C_{10}H_{12}O_4$	196.20	196.8947(2%) , 178.8843(100%)
14.	7.660	Tectochrysin-5-β-glc $C_{22}H_{22}O_9$	430.40	429.1953(32%) ($M-H^+$), 172.9535(100%), 339.1648(11%)
15.	3.274	Kaempferol-8C-glc-3, 5-glc, glc $C_{33}H_{43}O_{21}$	775.68	775.4722(100%) (M^+)757.4591(8%), 677.4946(10%), 581.3900(1%)
16.	4.336	Sanguinine $C_{16}H_{19}NO_3$	273.33	274.2751(100%) ($M+H^+$), 256.2643(28%), 230.2484(3%), 212.2374, 106.0869(28%), 88.0764(40%)70.0656(12%)
17.	3.553	Syringaresinol $C_{22}H_{26}O_8$	418.44	419.0219(76%) ($M+H^+$), 375.0328(50%), 298.9830(6%)
18.	3.264	Ecdysterone $C_{27}H_{44}O_7$	480.63	497.3337 (4%)(M+17), 488.4002, 462.6576, 451.3288(100%), 433.3113, 415.8143, 398.226
19.	4.990	Curzerenone	230.30	230.2486(100%)(M^+), 212.2381(62%)

The compound eluted with the retention time 3.561 produces a molecular ion(M^+) at m/z 121.0293 and fragments at m/z 77. 0397.The fragment at m/z 77.0397 formed by the removal of C_2H_6 N from the side chain. The elemental analysis and fragments formed identified the compound as Phenethylamine or 2-phenylethanamine with the molecular formula $C_8H_{11}N$.It is the constituent of Sida rhombifolia used in the kashayam.[3]

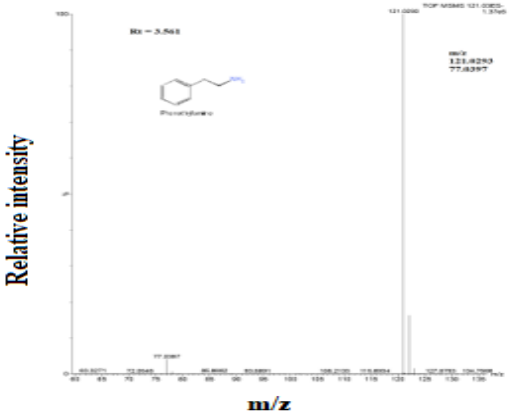
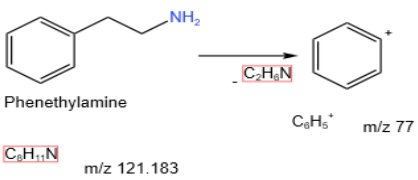


Figure 1. UPLC-ESI-QTOF-Mass Spectrum of Phenethylamine



Scheme 1. Mass fragments of Phenethylamine

The compound eluted with the retention time 3.655, forms a molecular ion, M^+ peak at m/z 218.2120(100%). Secondary fragments formed are at m/z 200.2010 (31%), 174.1853 (5%) and 156.1750(2%). The peak at m/z 200.2010 formed by the loss of water molecules from the molecular ion and m/z at 174.1853 formed by the removal of CO molecules from the ring containing carbonyl group. The fragment at m/z 156.1750 formed from 174.1853 by the removal of water molecules. Alternatively, the product ion also could be formed by a lone pair induced inductive cleavage that leads to the elimination of a molecule of ammonia (NH_3). Fragments formed are similar to that reported by Liu et al [4]. Its molecular mass and its fragmentation pattern confirmed the presence of Vasicinolone, 3, 7-dihydroxy-2, 3-dihydro-1*H*-pyrrolo [2, 1-b] quinazolin-9-one, with the molecular formula $C_{11}H_{10}N_2O_3$. Jam and Sharma [5] have reported the oxidation of Vasicinol to Vasicinolone. Vasicinol has been identified in the plant Sida rhombifolia[6] used in the preparation of kashayam**.** The compound eluted with retention time 3.902 produces a molecular ion (M+) peak at m/z 246.2433 (100%). Secondary fragments formed at m/z 288 (42%) and 202.2164 and 184. 2064.The fragment at m/z 202.2164 formed by the removal of carbon dioxide molecules by the collisional activation of the molecular ion. This ion loses a molecule of water from the 2-hydroxypropan

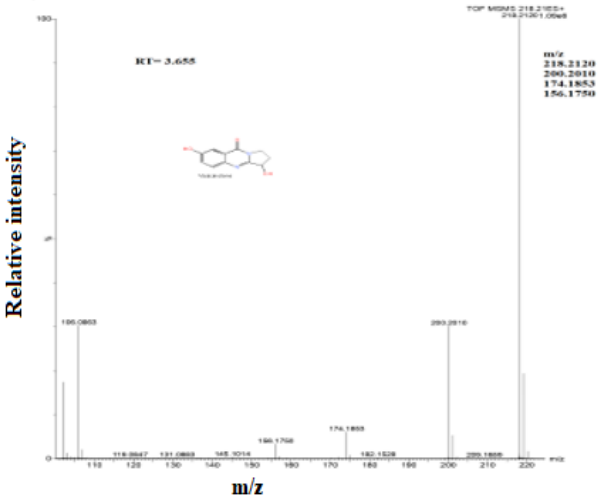
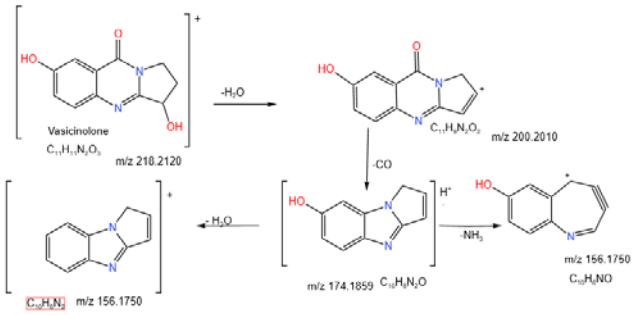


Figure 2. UPLC-ESI-QTOF-Mass Spectrum of Vasicinolone



Scheme 2. Fragmentation reactions of Vasicinolone

moiety and forms an ion with m/z 184.2064. Alternatively, the precursor ion first loses a molecule of water to form a product ion at m/z 228 which subsequently eliminates CO2 and forms the mass peak at m/z 184.2064. The reaction sequence is depicted in the scheme below. The elemental analysis and the information given above confirmed this compound as Marmesine, (2S)-2-(2-hydroxypropan-2-yl)-2, 3-dihydrofuro [3, 2-g] chromen-7-one, with the molecular formula $C_{14}H_{14}O_4$.

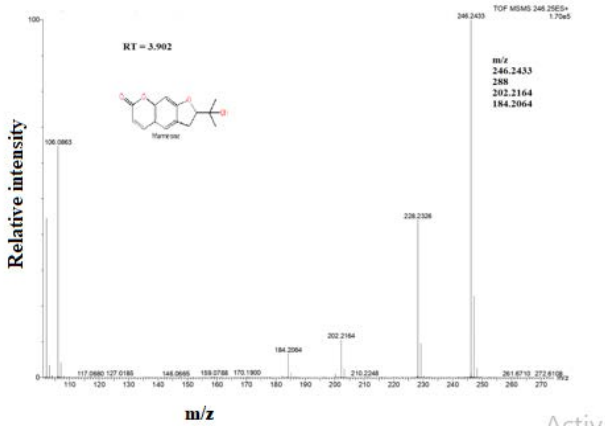
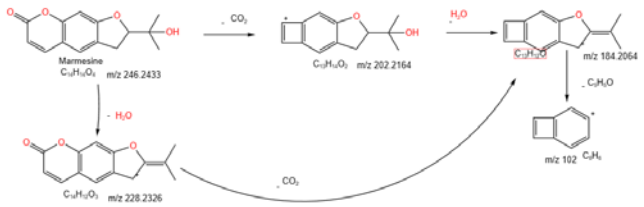


Figure 3. UPLC-ESI-QTOF-Mass Spectrum of Marmesine



Scheme 3. Mass fragments of Marmesine

The compound eluted with retention time 4.003 forms a molecular ion, M+ peak at m/z 212.0216 (12%) and base peak at m/z 167.9952 (100%). Other fragment peaks are at m/z 194.0108 and 136. 0230.The peak at m/z 194 is the result of loss of -OH of the acid functional group by the addition of an H^+. The product ion at m/z 167.9952 is due to the elimination of -CO which subsequently eliminates a -OCH_3 group to give the product ion at m/z 136. The molecule is identified as 4-O-Methyl syringic acid.

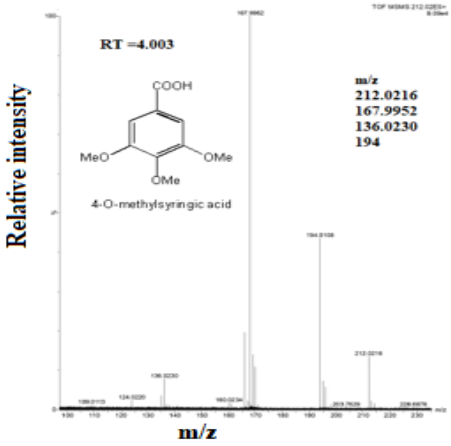
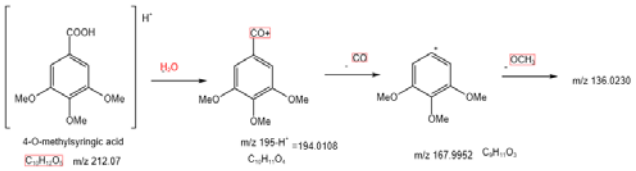


Figure 4. UPLC-ESI-QTOF-Mass Spectrum of 4-O- methyl syringic acid



Scheme 4. Mass fragments of 4-O- methyl syringic acid

The compound eluted with retention time 4.581 produces a molecular ion M+H+ peak at m/z 311.1859 (100%). Secondary fragments are at m/z 293.1753(48%) and 219. 1034.The peak at m/z 293.1753 formed by the loss of the 5'-OH as water molecule from molecular ion. This gives rise to an oxetane ring as shown in the scheme below, which results in the product ion at m/z 219.1034 by the elimination of C_5H_{12}. The elemental analysis and the information given above confirmed this compound as 4-Methyl-6-gingerdiol with the molecular formula $C_{18}H_{30}O_4$. 4-Methyl-6-gingerdiol is a phenolic compound identified from the plant Zingiber officinale[7] used in the kashayam.

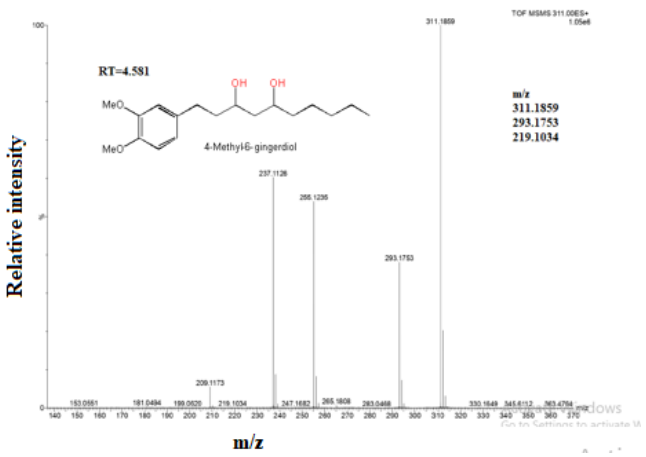
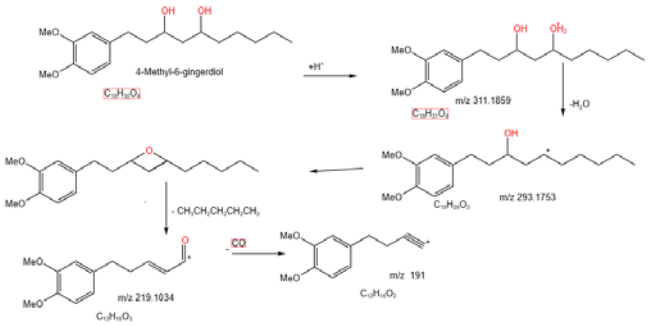


Figure 5. UPLC-ESI-QTOF-Mass Spectrum of 4-Methyl-6-gingerdiol



Scheme 5. Mass fragments of 4-Methyl-6-gingerdiol

The compound eluted with retention time 4.657, produces a molecular ion(M+) peak at m/z 386.8377 (100%). The characteristic ions are formed at m/z 343.0959 and 283.2594. The elimination of CO_2 from the lactone ring produces a product ion at m/z 343.0959. The elemental analysis and the mass fragments given above confirmed this compound as 7-Hydroxyhinokinin with the molecular formula $C_{20}H_{18}O_8$.

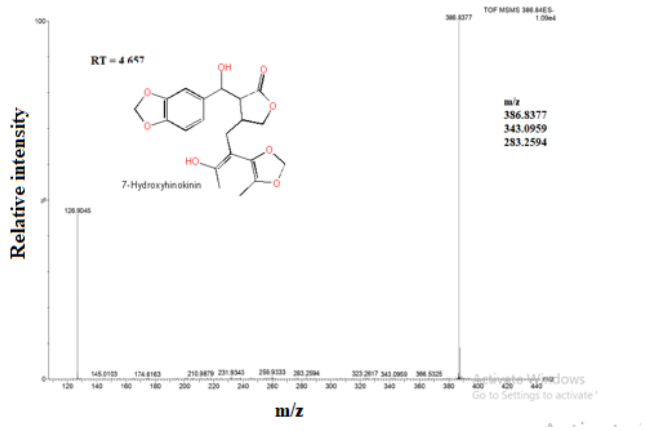
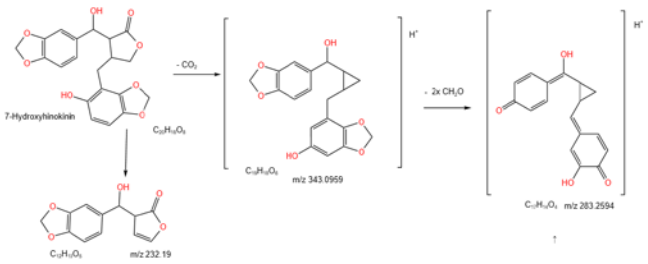


Figure 6. UPLC-ESI-QTOF-Mass Spectrum of 7-Hydroxyhinokinin



Scheme 6 Mass fragments of 7-Hydroxyhinokinin

The compound eluted with retention time 4.892 forms a molecular ion peak M+ H+ at m/z 337.1630 (100%) and fragments at m/z 320.0945(1%), 279.1615 and 164.8739 (8%). The peak at m/z 164.8739 formed due to the RDA reaction in the C-ring. The fragment at m/z 320.0945 by the removal of methyl molecule from molecular ion by abstracting an H (charge remote fragmentation) from the C-ring and the -CH_3 of the methoxyl at ring-D which by the elimination of –CH_3 and -CO forms the peak at m/z 279.1615 (-CO removal from cyclic carbonyls are a common reaction in the ESI MS of Cyclic carbonyl compounds). Formation of other peaks are explained in the scheme below. The elemental analysis and the mass fragments obtained leads the identification of the compound as Berberine with the molecular formula $C_{20}H_{18}NO_4$.Berberine is a quaternary ammonium salt ,9,10-Dimethoxy-7,8,13,13a-tetrahydro2'H-[1,3] dioxolo [4',5':2,3]berbin-7-ium.This molecule is a constituent of Alpinia calcarata.[8]

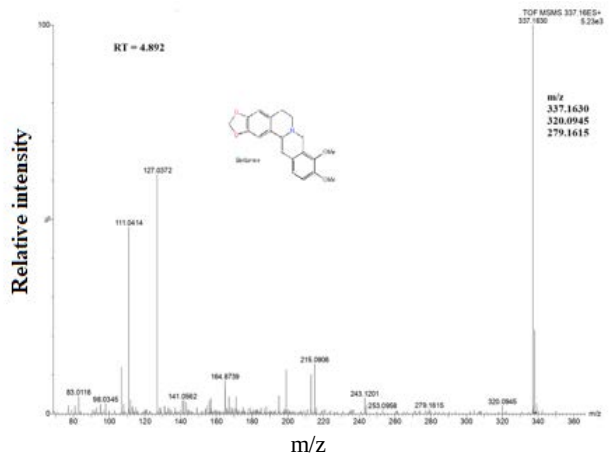
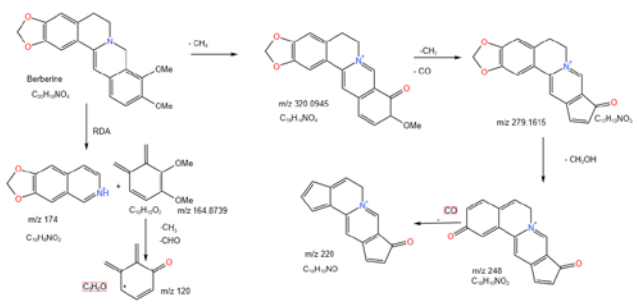


Figure 7. UPLC-ESI-QTOF-Mass Spectrum of Berberine



Scheme 7. Mass fragments of Berberine

The compound eluted with retention time 4.573, produces a molecular ion (M-H+) peak at m/z 293. Secondary fragments at m/z 193.0484 probably corresponds to the elimination of hexanal. The peak at m/z 262 formed by the removal of CH_3OH from the mass 294. The elemental analysis and the information given below confirmed this compound as 6-Gingerol with the molecular formula $C_{17}H_{26}O_4$.6-Gingerol, (5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one, is a phenolic compound identified from the plant Zingiber officinale.[7]

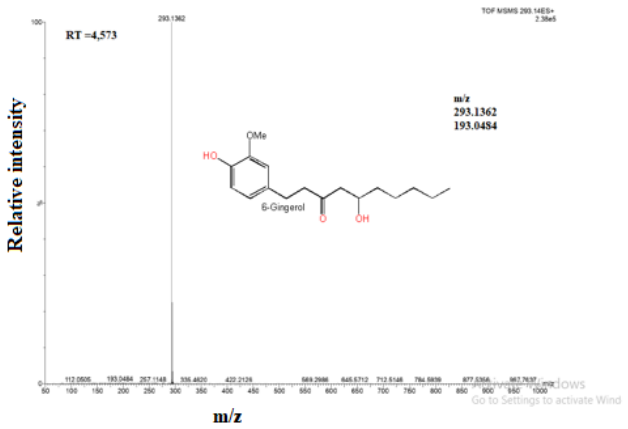
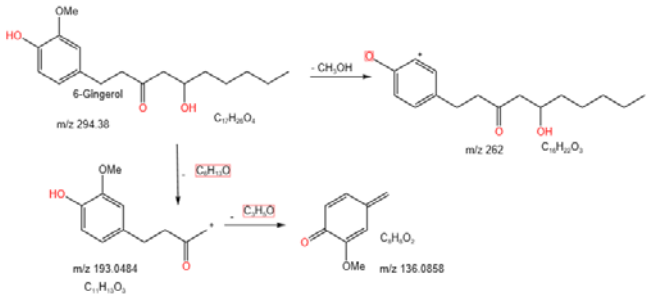


Figure 8. UPLC-ESI-QTOF- Mass Spectrum of 6-Gingerol



Scheme 8 Mass fragments of 6-Gingerol

The compound eluted with retention time 5.015, produces a molecular ion (M+) peak at m/z 302.3064 (100%). Secondary fragments formed are at m/z 284.2996 (21%), 258, 240 and 137.0082. The elimination of OH group from the ring C results in the fragment at m/z 284. 2996. The cycloreversion of the ring C leads to the mass peak at 153 and 137.Detailed mass fragmentation patterns are given in the scheme. Fragments obtained are similar to that of quercetin reported by Dimitrios et al 9. The elemental analysis and the mass fragments given above confirmed this compound as Quercetin with the molecular formula $C_{15}H_{10}O_7$. Quercetin, 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxychromen-4-one, is a flavonoid identified from Alpinia calcarata.[10]

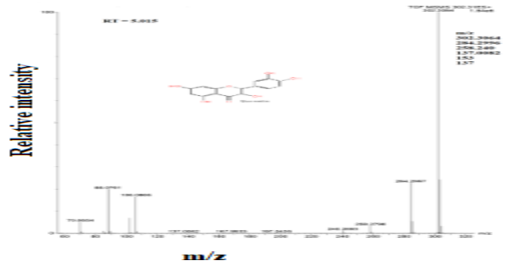
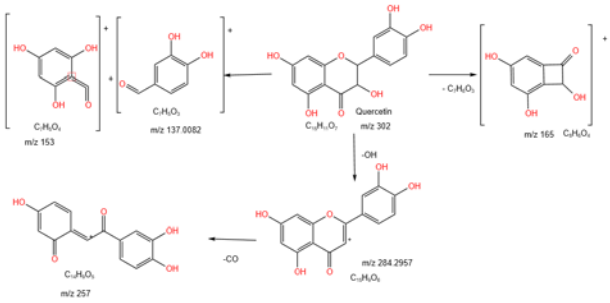


Figure 9. UPLC-ESI-QTOF-Mass Spectrum of Quercetin



Scheme 9. Mass fragments of Quercetin

The compound eluted with retention time 5.493, forms a molecular ion M+H+ at m/z 330.3372(100%) and other fragments at m/z 312.3258 (20%) and 286.3114(2%). The peak at m/z 312.3258 due to the removal of water molecules from precursor ions. The loss of -CO moiety from this produced the fragment at m/z 286.3114. The α- cleavage of the carbonyl of the amide linkage gave the fragment ion at m/z 178 and further a molecule of -CH_3OH is lost by remote hydrogen capture of the methoxyl function gave the mass peak at m/z 146.Based on the reaction pathway given in the scheme the presence of Trans feruloyl octopamine, (E)-N-[2-hydroxy-2-(4-hydroxyphenyl) ethyl]-3-(4-hydroxy-3-methoxyphenyl) prop-2-enamide, with the molecular formula $C_{18}H_{19}NO_5$ is identified. This molecule is a constituent of Allium sativum used in the kashayam.[11]

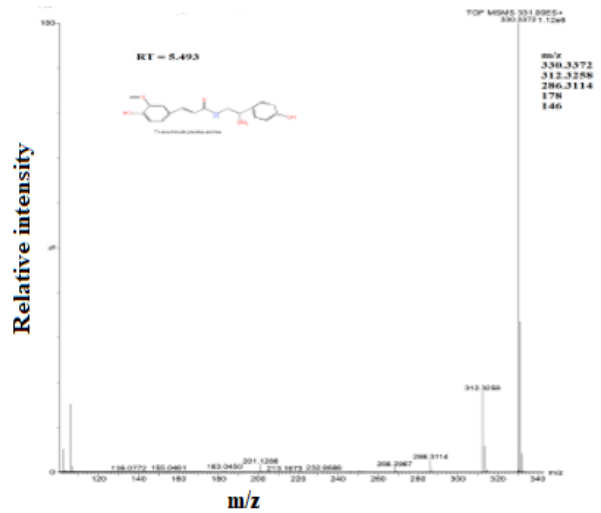
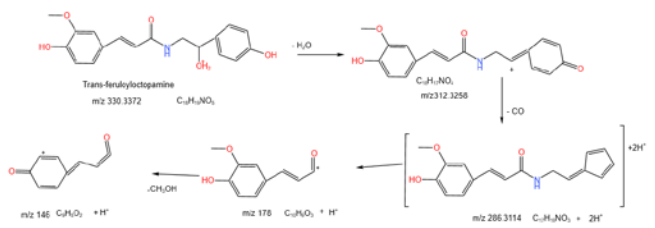


Figure 10. UPLC-ESI-QTOF-Mass Spectrum of Trans feruloyl octopamine



Scheme 10 Mass fragments of Trans feruloylctopamine

The compound eluted with retention time 5.511, forms a molecular ion M+ peak at m/z 372.8219(100%) and the most prominent fragments at m/z 357.0864 and 217.9250(2%). The fragment at m/z 357.0864 formed by the removal of methyl group from the molecular ion. The fragment at m/z 235 formed by the cleavage of C1-C2 bond, that is alpha cleavage of ketone functional group. Then elimination of hydroxyl function, as water molecule, results the ion peak at m/z 217.9250. This fragmentation pattern confirms the compound as Tetrahydrocurcumin with the molecular formula $C_{21}H_{24}O_6$. Tetrahydrocurcumin is a beta-diketone present in Zingiber officinale.[7]

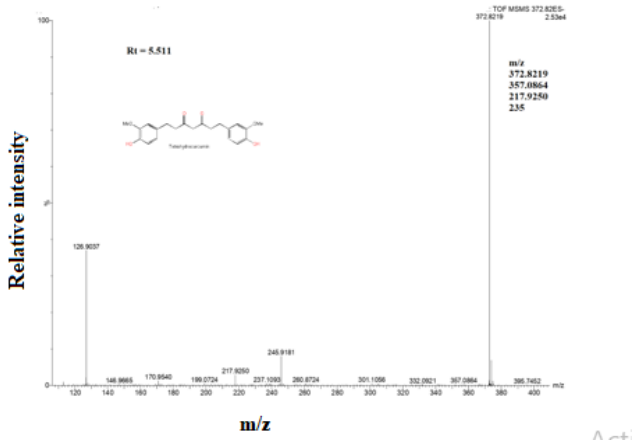
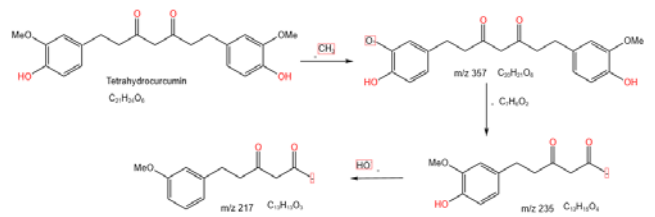


Figure 11. UPLC-ESI-QTOF- Mass Spectrum of Tetrahydrocurcumin



Scheme 11 Mass fragments of Tetrahydrocurcumin

The compound eluted with retention time 6.076, produces a molecular ion peak at m/z 389.8125, M-H+(100%). Other fragments are at m/z 262.9072(16%) and m/z 234.9126 (28%). The fragment at m/z 262.9072 produced by the loss of CH3OH and subsequent RDA reaction in the C ring. The fragment at m/z 234.9126 formed from 262.9072 as the result of removal of carbon monoxide molecule. The mass fragmentation pattern and its elemental analysis confirmed the compound as 8-hydroxy

tinosporide, (2S,4aR,6aR,7S,7aS,8aS,9S,9aS,9bS)-2-(3-Furanyl) dodecahydro-7-hydroxy-6a,9b-dimethyl-9,7-(epoxymethano)-4H-oxireno)[6,7]naphtho[2,1-c]pyran-4,11-dione, with the molecular formula $C_{20}H_{22}O_8$.

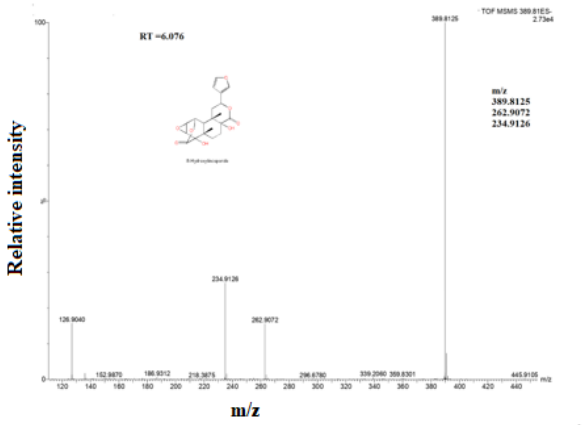
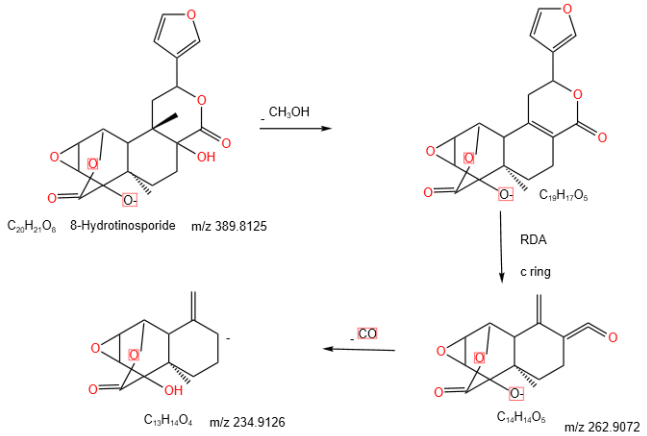


Figure 12. UPLC-ESI-QTOF-Mass Spectrum of 8-Hydroxy tinosporide.



Scheme 12. Mass fragments of 8-Hydroxytinosporide

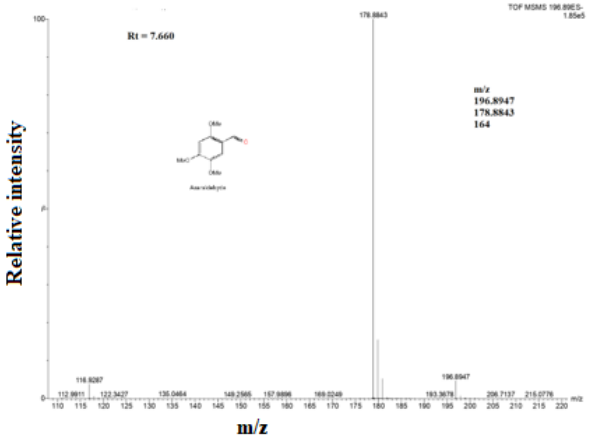
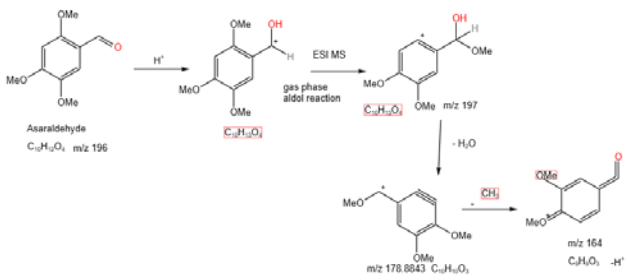


Figure 13. UPLC-ESI-QTOF-Mass Spectrum of Asaraldehyde

The compound eluted with retention time 7.660, forms a molecular ion peak at m/z 196.8947 (2%) and base peak at m/z 178.8843(100%). The base peak formed by the removal of water molecules from the molecular ion. The peak at m/z 164 is due to the loss of methyl molecules from m/z 178. From the elemental analysis the molecular formula of the compound was found to be $C_{10}H_{12}O_4$The mass fragmentation patterns confirmed the compound as Asaraldehyde or 2, 4, 5-trimethoxy benzaldehyde. Asaraldehyde is the constituent of Zingiber officinale.[7]



Scheme 13 Mass fragments of Asaraldehyde

The compound eluted with retention time 7.660, produces a molecular ion peak at m/z 429.1953 (32%) and base peak at m/z 172.9535(100%). Secondary fragments at m/z 339.1648 (11%). The fragment at m/z 339.1648 formed by the breaking of glucose molecules. The base peak is due to the removal of ring B in the molecule by the fragmentation depicted in the scheme below. The elemental analysis and the information given above confirmed this compound as Techtochrysin- 5-β-glucoside with the molecular formula $C_{22}H_{22}O_9$.Techtochryin- 5-β-glucoside is a flavonoid reported in the plant Alpinia calcarata.[10]

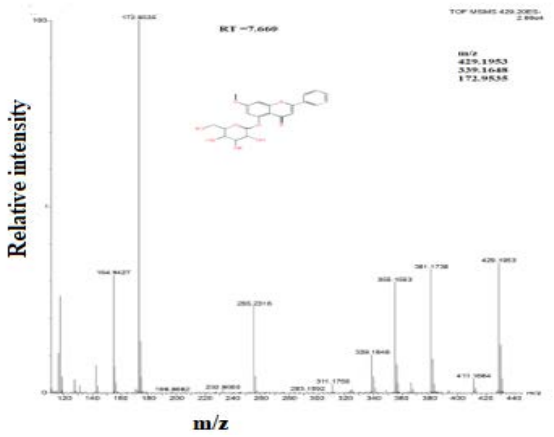
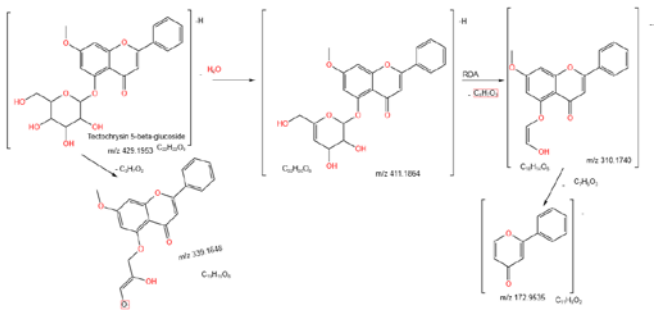


Figure 14. UPLC-ESI-QTOF-Mass Spectrum of Techtochrysin -5-β-glc



Scheme 14 Mass fragments of Techtochrysin-5-glucoside

The compound eluted with the retention time 3.274, forms a molecular ion peak at m/z 775.4722(100%) M + and other fragments at m/z 757.4591(8%), 677.4946(10%), 581.3900(1%). The fragmentation pattern given above confirm this compound as Kaempferol-8C-glc-3, 5-glc, glc with the molecular formula $C_{33}H_{43}O_{21}$. It has been reported from Vigna mungo.[12]

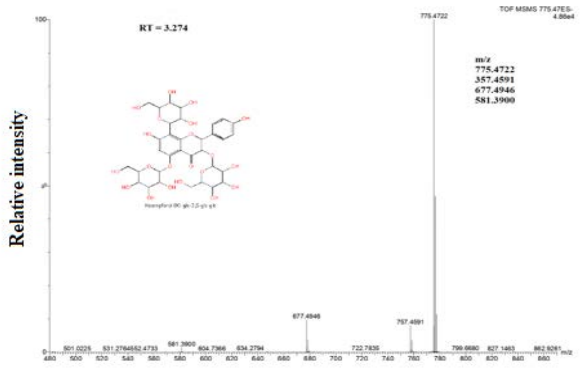


Figure 15. UPLC-ESI-QTOF-Spectrum of keampferol 8C-glc-3, 5-glc-glc

The compound eluted with the retention time 3.553, produce a molecular ion M+ peak at m/z 274.2751(100) and noticeable fragments at m/z 256.2643 (28%), 230.2484 (3%), 212.2374, 106.08 69 (28%), 88.0764 (40%) and 70.0656(12%). From the fragmentation patterns given above this compound is identified as Sanguinine with the molecular formula $C_{16}H_{19}NO_3$. Sanguinine, (1*S*, 12*S*, 14*R*)-4-methyl-11-oxa-4-azatetracyclo [8.6.1.01, 12.06, 17] heptadeca-6(17), 7, 9, 15-tetraene-9, 14-diol, has been identified in the plant Sida rhombifolia [13] used in the kashayam.

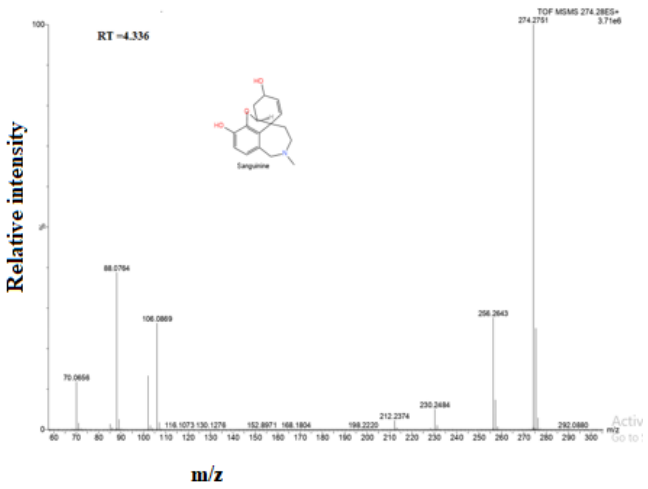


Figure 16. UPLC-ESI-QTOF-Spectrum of Sanguinine

The compound eluted with the retention time 3.553, forms a protonated molecular ion peak at m/z 419.0219(76%) $M+H^+$ and other fragments at m/z 375.0328(50%), 298.9830(6%). From the molecular ion peak and its fragments, it is identified as Syringaresinol, $C_{22}H_{26}O_8$.

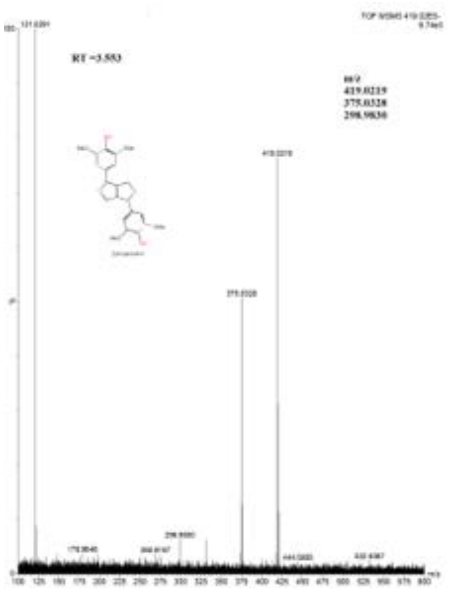


Figure 17. UPLC-ESI-QTOF-Spectrum of Syringaresinol

The compound, with the retention time 3.553, produces a molecular ion peak at m/z 497.3337 (4%) M+17. The fragments formed are 488.4002, 462.6576, 451.3288(100%), 433.3113, 415.8143, 398. 2262.From the fragments and the elemental analysis it is identified as Ecdysterone, $C_{27}H_{44}O_7$. It has been reported from Sida rhombifolia.[6]

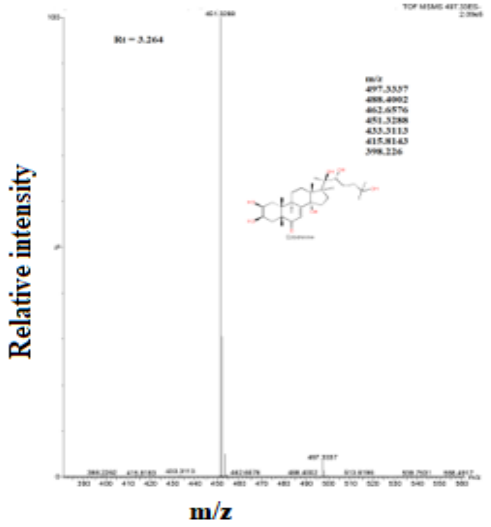


Figure 18. UPLC-ESI-QTOF-Spectrum of Ecdysterone

The compound eluted with the retention time 4.990, forms a molecular ion peak at m/z 230.2486(100%) M+ and the fragment at m/z 212.2381(62%). From the molecular ion peak and its fragment, it is identified as Curzerenone. It is the constituent of Zingiber officinale.[7]

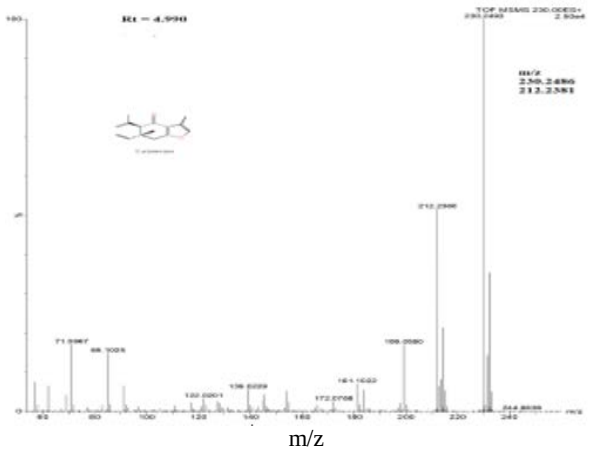


Figure 19. UPLC-ESI-QTOF-Spectrum of Curzerenone

In the detailed literature search, the identified compounds were found to be anti-oxidant, anti-inflammatory and immunostimulant. The reported activity of the identified compounds is given in Table 2.

Table 2. List of identified compounds showing antioxidant, anti-inflammatory and immunostimulant properties

SI No.	Compound name	Biological activity
1.	Vasicinolone	Anti-inflammatory effects[14]
2.	4-O-methyl syringic acid	Anti-inflammatory[15]
3.	4-Methyl-6-gingerol	Anti-inflammatory, antioxidant[16]
4.	Berberine	Anti-inflammatory, Antioxidant[17]
5.	6-Gingerol	Anti-inflammatory[18]
6.	Quercetin	Anti-inflammatory, Immunostimulant, Antioxidant[19,30]
7.	Tetrahydrocurcumin	Anti-inflammatory, Anti-oxidant, Immunomodulatory activity[16]
8.	Asaraldehyde	Anti-inflammatory[20]
9.	Kaempferol-8C-glc-3, 5-glc, glc	Anti-inflammatory[21]
10.	Syringaresinol	Anti-inflammatory[22]
11.	Ecdysterone	Anti-inflammatory, Antioxidant, Immunomodulatory medicine [23]
12.	Curzerenone	Anti-inflammatory effects[24]
13.	Phenethylamine	
14.	Marmesine	Angiogenesis inhibitor [25], Anti-tuberculosis, Anticancer [26]
15.	Sanguinine	Memory enhancer [27]
16.	Techtochrysin 5-β-glucoside	Anticancer[28]
17.	Trans fruloyloctapamine	Antiradical, Anticancer[29]
18.	8-Hydroxytinosporide	
19.	7- Hydroxyhinokinin	

Among the different biological activities that have been extensively investigated in medicinal plants and herbal drugs, anti-inflammatory and associated properties stand the highest. Alkaloids, flavonoids, polyphenols and terpenoids are the main classes of plant derived compounds that are potent to act as anti-inflammatory, immunostimulant and antioxidant.[30,31] Reported studies show that secondary metabolites such as polyphenols, alkaloids and terpenes can play a beneficial role in the prevention and the control of chronic diseases such as diabetes, obesity, neurodegeneration, cancers, and cardiovascular diseases, among other conditions. The phytochemicals identified in this study have been found to exhibit potent immunostimulant, antioxidant, anticancer and anti-inflammatory properties. Hence, this study forms a sort of scientific validation for the therapeutic use of this medicinal preparation.

EXPERIMENTAL

300 ml of the well shaken kashayam is centrifuged at 4000 rpm (3000g) in a REMI 8C centrifuge for 30 minutes. The Clear supernatant was carefully decanted (220ml). This was analyzed in QTOF MS MS.

UPLC-Q-TOF-MS analyses were carried out at MG University Kottayam. The chromatographic separation and detection of analytes was carried out with ultra-performance liquid chromatography coupled to quadrupole time of flight mass spectrometry (UPLC-Q-TOF-MS). The acquity UPLC system (Waters) consists of a TUV detector (JI2TUV750A), a column chamber (JI2 CHA730G), a quaternary solvent manager (HI2 QSM632A), and a sample manager FTN (KI2 SDI069G). A reversed -phase BEH C18 column (of dimension 50mm X 2.1mm X 1.7 µm) with a flow rate of 0.3mL min -1 was used for chromatographic separation(Waters). The mobile phase was a mixture of 0.1% Formic acid in water (A) and acetonitrile (B) in a gradient elution as follows initial 95% A, 0.1min 95%A, 6.00 min 5%A, 6.5 min 5%A, 9min 95%A and 10 min 95%A. The UPLC system was connected to the quadrupole time of flight mass spectrometer (Waters Xevo G2 QTOF) with electrospray ionization(ESI) interface working in positive and negative ionization modes. The injection volume was 10µL**.** The scanning m/z range was between 50 and 1000. The desolvation gas flow and the temperature were 900L/h and 350 0C, respectively. The mass spectra were obtained using collision energy ranging from 5 to 30eV.The instrument control and data acquisition was done using MassLynx software (v 4.1).

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