



LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS AND PHYTOCHEMICAL SCREENING OF *TRIMAD*: AN AYURVEDIC POLYHERBAL FORMULATION

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ABSTRACT

Trimad is a polyherbal formulation mentioned in Ayurvedic literature. The formulation is a composite mixture of three plant parts including rhizomes of Nagarmotha (*Cyperus rotandus* L.), fruits of Vidang (*Embelia ribes* Burm.) and root of Chitrak (*Plumbago zylenica* L.). The present study was undertaken to evaluate phytochemical constituents of *Trimad* polyherbal formulation through Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. Chromatographic conditions for separation are as follows: The mobile phase was a combination of solvents: water (0.1% formic acid) (A) and Acetonitrile (B). The flow rate was 0.35mL/min. The gradient begins at 2% B and reached 98% B by 12 min. Consequently, the mixture returned to 2% B for a 3 min re-equilibration period. The components were subjected to the mass analyzer through electrospray ionization probe operating in positive and negative mode with the following conditions: capillary voltage for negative and positive, 2500, 4000V; fragmentor voltage, 150V; source temperature, 325°C; sheath gas temperature, 295°C; nebulizer pressure, 45 PSI; and gas flow, 8 L/min. Total twenty putative compounds were identified from methanolic extract of *Trimad*. Negative and Positive ionization mode detected around 17 and 12 compounds respectively. Out of which, nine compounds are detected in both ionization mode. In negative ionization mode, β -glucopyranosyl plumbagic acid; xanthyletin; embelin; sugeonyl acetate; 1-tetradecene; cyclopentadecane; tetracosanoic acid methyl ester and tricosanoic acid methyl ester compounds were detected. In positive ionization mode, rotundines; γ – muurolene and ethyl p methoxycinnamate were identified. Nine compounds such as cyperol; β -asarone; oleic acid; camphoraldehyde; thymol; 1-epineo-isoshinanolone; α -cyperone; phenol 2,4- bis(1,1-dimethylethyl) and n-hexadecanoic acid were detected in both ionization modes. Probably, this is the first report of high-throughput screening of *Trimad* phytoconstituents through LC-MS analysis. Further studies can examine the mechanisms of action of this formulation.

KEYWORDS: *Trimad polyherbal formulation, LC-MS, α -Cyperone, β -Glucopyranosyl plumbagic acid and Embelin*



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INTRODUCTION

Plants have served as a basis of traditional medicinal systems for thousands of years¹. Herbal medicines are sources of natural constituents which help to eliminate the root cause of a disease². Herbal medicines have been widely used for thousands of years mainly in Indian, Chinese, Greek, and Egyptian medicine for various therapies². Similarly, polyherbal formulations are known to show high effectiveness due to the presence of multiple phytoconstituents³. In the preparation of effective polyherbal formulation, scientific evidence and clinical validation are required with multiple approaches, including biological assays, compound standardization, preclinical and clinical trials^{4,5}. The quality assessment of herbal medicine is a necessary task for implementation in efficacy, safety and their acceptability in modern system of medicine⁵. These phytoconstituents could be assessed through various techniques. The establishment of quality control parameters is difficult because of their complex nature and variability in the profile of constituents. However, modern analytical techniques overcome these problems⁵⁻⁷. These include various chromatographic and spectroscopic techniques such as HPLC, UPLC, GS-MS and LC-MS. Out of these, combined approach of both techniques, especially liquid chromatography-mass spectroscopy (LC-MS) is widely used for the analysis of abundant chemical compounds present in medicinal plants and their formulations⁸⁻¹². The advances made in the separation techniques of chromatography lead to make qualitative as well as quantitative estimation of compounds from complex mixture¹³. The Indian traditional literature (Ayurveda) describes numerous Ayurvedic formulations which can be used in the treatment of various diseases. One such formulations is *Trimad*¹⁴. *Trimad* is an Ayurvedic polyherbal formulation of three ingredients namely Vidang (*Embelia ribes* Burm., Myrsinaceae), Nagarmotha (*Cyperus rotundus*

L.,Cyperaceae) and Chitrak (*Plumbago zeylanica* L., Plumbaginaceae). It has been recommended for management of "Meda" in in Ayurvedic literature¹⁴. Though the therapy or treatment is effective, it lacks any scientific validation. Nagarmotha (*Cyperus rotundus* L.) contains cyperene, cyperone, cyperotundone, patchoulone, sugeonol, selinene, kobusone compounds and exhibits several pharmacological activity including antidiabetic^{15,16}, anti-inflammatory¹⁷, antidiarrhoeal¹⁸, antimalarial¹⁹, Wound healing²⁰, hepatoprotective²¹ and anti-cancer^{22,23} activities. Numerous phytoconstituents isolated from Chitrak (*Plumbago zeylanica* L.) include plumbagin, maritinone, chitranone, isoshinanolone, elliptinone, seselin, suberosin, xanthyletin, various derivatives of plumbagic acid etc²⁴⁻²⁶. The pharmacological activity of *Plumbago zeylanica* L. against hyperglycemia, lipid abnormalities, atherosclerosis as well as inflammatory, antimicrobial and antifungal activities have been reported²⁷⁻³². Vidang (*Embelia ribes* Burm.) is known to contain embelin, christembine, quercitol, vilangin, resinoid etc^{33,34}. The plant showed anti-hyperglycemic, anti-hyperlipidemic, anti-obesity and anti-diabetic activity and is a rich source of antioxidants³⁵⁻³⁹. With this background, the present study evaluated the phytoconstituents of *Trimad*, an Ayurvedic polyherbal formulation, by Liquid chromatography-mass spectroscopy (LC-MS) analysis.

MATERIALS AND METHODS

Trimad collection and extract preparation

The *Trimad* polyherbal formulation was procured from a local Ayurvedic pharmacy in Pune, Maharashtra, India. The composition of *Trimad* is depicted in Table 1. The powdered sample of *Trimad* (100g) was soaked in 500ml methanol and incubated in a shaker at 100 rpm for 24h at room temperature. The extract was filtered through filter paper (Whatman No. 1) and then concentrated by using a rotary evaporator.

Table 1
Composition of *Trimad* polyherbal formulation

Ingredients	Ayurvedic Name	Part Used	Quantity (Parts)
Nagarmotha	(<i>Cyperus rotundus</i> L.)	Rhizomes	1 Part
Chitrak	(<i>Plumbago zeylanica</i> L.)	Roots	1Part
Vidang	(<i>Embelia ribes</i> Burm.)	Fruits	1 Part

Liquid Chromatography -Mass Spectrometry Conditions

Trimad polyherbal formulation was analyzed by liquid chromatography-mass spectrometry (LC-MS) using an Agilent (Agilent Technologies) Binary LC 1260 coupled with Agilent 6540 UHD QTOF mass spectrometer. Samples (2µl) were injected onto a column Zorbax Eclipse Plus C18, 2.1x50mm 1.8µM (Agilent) at 40°C. Chromatographic conditions for separation are as follows: The mobile phase was a combination of two solvents: water + 0.1% formic acid (Solvent A) and Acetonitrile (LC-MS grade, J.T. Baker) (Solvent B). The flow rate was 0.350 mL/min. The gradient began at 2% B, reached 98% B by 12 min. Consequently, the mixture returned to 2% B for a 3-min re-equilibration period. The components were subjected to the mass analyzer through electrospray ionization probe operating in positive and negative mode with the following

conditions: capillary voltage for negative and positive, 2500, 4000V; fragmentor voltage, 150V; source temperature, 325°C; Sheath Gas Temperature, 295°C; nebulizer pressure, 45 PSI; and gas flow, 08 L/min. Masshunter workstation software v. B.05.01 was used for this analysis.

RESULTS AND DISCUSSION

In the present study *Trimad*, an Ayurvedic polyherbal formulation, was evaluated for its phytochemical constituents. LC-MS analysis was performed by both negative and positive ionization mode. A total of 20 different compounds were detected in the prepared *Trimad* methanolic extract. The detection is based on the mass measurement and mass error (ppm), retention time, molecular formula, target score, abundance, mass

spectrometry fragmentation of ions and comparison of data with compound databases library.

Compounds detected in negative mode of ionization

The detected compounds in negative mode of ionization are listed in Table 2. Seventeen putative compounds were identified from methanolic extract of *Trimad* in negative ionization mode and these are represented in

Figure 1-5. Those compounds are β -glucopyranosyl plumbagic acid; 1-epineo-isoshinanolone; xanthyletin; phenol 2,4- bis(1,1-dimethylethyl); campholaldehyde; β -asarone; thymol; α -cyperone; cyperol; embelin; sugeonyl acetate; 1-tetradecene; cyclopentadecane; n-hexadecanoic acid; oleic acid; tetracosanoic acid methyl ester and tricosanoic acid methyl ester.

Table 2
Putative identification of compounds from methanolic extract of *Trimad*, polyherbal formulation by negative ionization mode in LC-MS

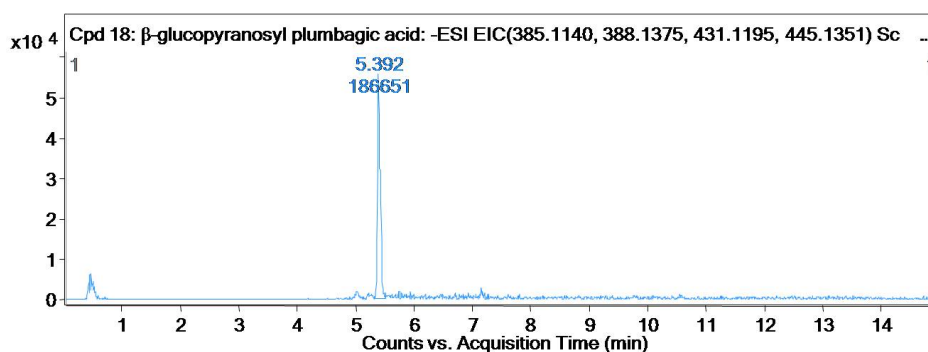
S. No	Name of Compounds	R _T	Target Score	Mass Error (ppm)	Formula	Ref. Mass	Obs. Mass	MS Fragment -ions		Abundance	Ion/Isotope
								(Obs. m/z)			
1	β -Glucopyranosyl plumbagic acid	5.392	98.68	-1.59	C ₁₇ H ₂₂ O ₁₀	386.13	386.12	385, 386, 387, 388, 445	32004, 6033, 1196, 216, 260	(M-H) ⁻ , (M-H) ⁻ , (M-H) ⁻ , (M-H) ⁻ , (M+CH ₃ COO) ⁻	
2	1-Epineo-isoshinanolone	5.882	94.96	-2.27	C ₁₁ H ₁₂ O ₃	192.08	192.09	191, 192, 193, 237, 238, 251	5783, 872, 119, 249, 23, 122	(M-H) ⁻ , (M-H) ⁻ , (M-H) ⁻ , (M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻	
3	Xanthyletin	6.506	80.76	-3.1	C ₁₄ H ₁₂ O ₃	228.08	228.08	227, 228, 273, 274, 287, 288	527, 80, 283, 46, 126, 27	(M-H) ⁻ , (M-H) ⁻ , (M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻	
4	Phenol, 2,4- bis(1,1-dimethylethyl)	7.308	84.99	-3.54	C ₁₄ H ₂₂ O	206.17	206.17	251, 252, 265, 266	580, 86, 239, 32	(M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻ , (M+CH ₃ COO) ⁻	
5	Campholaldehyde	7.764	98.47	-4.63	C ₁₀ H ₁₆ O ₂	168.16	168.11	167, 170, 213, 214, 215, 227, 228, 229	184, 433, 2426, 190, 56, 2929, 417, 34	(M-H) ⁻ , (M+H ₂) ⁻ , (M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻ , (M+CH ₃ COO) ⁻ , (M+CH ₃ COO) ⁻	
6	β - Asarone	7.832	83.47	-4.58	C ₁₂ H ₁₆ O ₃	208.11	208.11	207, 208, 210, 253, 267	1336, 210, 250, 77, 95	(M-H) ⁻ , (M-H) ⁻ , (M+H ₂) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻	
7	Thymol	7.84	83.96	-4.68	C ₁₀ H ₁₄ O	150.10	150.10	195, 209, 210	130, 1535, 240	(M+HCOO) ⁻ , (M+CH ₃ COO) ⁻ , (M+CH ₃ COO) ⁻	
8	α -Cyperone	9.005	82.92	-3.15	C ₁₅ H ₂₂ O	218.17	218.17	263, 264, 277	1655, 332, 51	(M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻	
9	Cyperol	10.305	81.02	-4.96	C ₁₅ H ₂₄ O	220.18	220.18	219, 265, 266, 279	107, 2014, 353, 227	(M-H) ⁻ , (M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻	
10	Embelin (2, 5- dihydroxy-3- undecyl-1, 4- benzoquinone)	11.09	99.33	-1.17	C ₁₇ H ₂₆ O ₄	294.18	294.18	293, 294, 295, 296, 339	49088, 9248, 1386, 154, 116	(M-H) ⁻ , (M-H) ⁻ , (M-H) ⁻ , (M-H) ⁻ , (M+HCOO) ⁻	
11	Sugeonyl acetate	11.841	84.72	-4.07	C ₁₇ H ₂₄ O ₃	276.17	276.17	321, 322, 323, 335, 336	698, 116, 25, 579, 161	(M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻ , (M+CH ₃ COO) ⁻	
12	1-Tetradecene	12.415	98.13	-2.12	C ₁₄ H ₂₈	196.22	196.22	241, 242, 255, 256, 257	1102, 105, 8225, 1378, 244	(M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻ , (M+CH ₃ COO) ⁻ , (M+CH ₃ COO) ⁻	
13	Cyclopentadecane	12.415	98.15	-2.05	C ₁₅ H ₃₀	210.23	210.23	255, 256, 257, 269, 270	8129, 1360, 240, 1017, 112	(M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+HCOO) ⁻ , (M+CH ₃ COO) ⁻	

												(M+CH3COO)-
14	n-Hexadecanoic acid	12.415	98.39	-1.48	C ₁₆ H ₃₂ O ₂	256.24	256.24	255, 256, 257, 301, 315, 316, 317	256, 301, 316, 20	8129, 240, 1383, 256	1360, 139, 256	(M-H)-, (M-H)-, (M-H)-, (M+HCOO)-, (M+CH3COO)-, (M+CH3COO)-, (M+CH3COO)-
15	Oleic acid	12.533	85.82	-2.24	C ₁₈ H ₃₄ O ₂	282.26	282.26	281, 282, 284, 285, 341, 342	282, 3587, 765, 88, 778, 235	18366, 240, 1383, 256		(M-H)-, (M-H)-, (M+H2)-, (M+H2)-, (M+CH3COO)-, (M+CH3COO)-
16	Tetracosanoic acid methyl ester	13.09	86.88	-3.28	C ₂₅ H ₅₀ O ₂	382.38	382.38	427, 428, 429	428, 84	1140, 293		(M+HCOO)-, (M+HCOO)-, (M+HCOO)-
17	Tricosanoic acid methyl ester	13.09	84.52	-3.9	C ₂₄ H ₄₈ O ₂	368.37	368.36	427, 428, 429	428, 87	1052, 286		(M+CH3COO)-, (M+CH3COO)-, (M+CH3COO)-

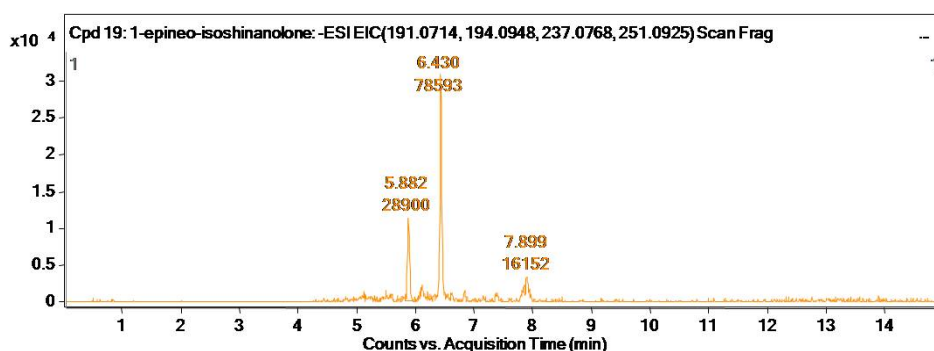
#R_T: retention time.; PPM: parts per million; m/z: mass-to-charge ratio

The chromatogram is depicted in the Figure 1 to 5. All the above mentioned compounds showed a distinct peak at different retention times. Medicinal plants are rigorously used for the treatment of various diseases⁴⁰. Detected compounds, which include cyperene, cyperone, cyperotundone, patchoulenone, sugeonol, selinene, kobusone compounds exhibits antidiabetic^{15,16} anti-inflammatory¹⁷ wound healing²⁰, hepatoprotective²¹ anti-cancer^{22,23} activities. The fruits of *E. ribes* contain embelin, christembine, quercitol, vilangin and resinoid

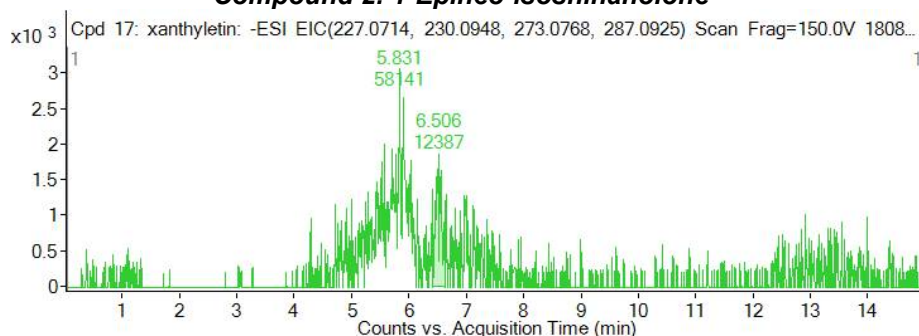
as principle compounds⁴¹ etc. According to Ayurveda, *E. ribes* is used for the treatment of fever, inflammatory diseases, and gastrointestinal ailments¹⁷. There are several studies reported that fruits of *E. ribes* showed anti-hyperglycemic, anti-hyperlipidemic, anti-obesity, wound-healing anti-tumor, cardioprotective, hepatoprotective activity and also have high antioxidant potential which can be correlated to its phytochemical content⁴²⁻⁴⁵.



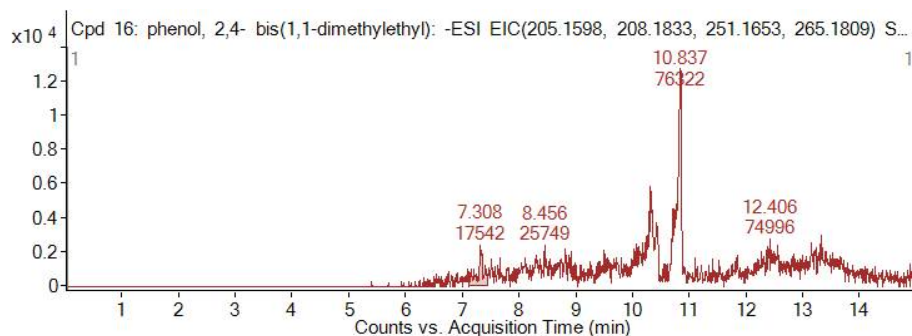
Compound 1: β-Glucopyranosyl plumbagic acid



Compound 2: 1-Epineo-isoshinanolone

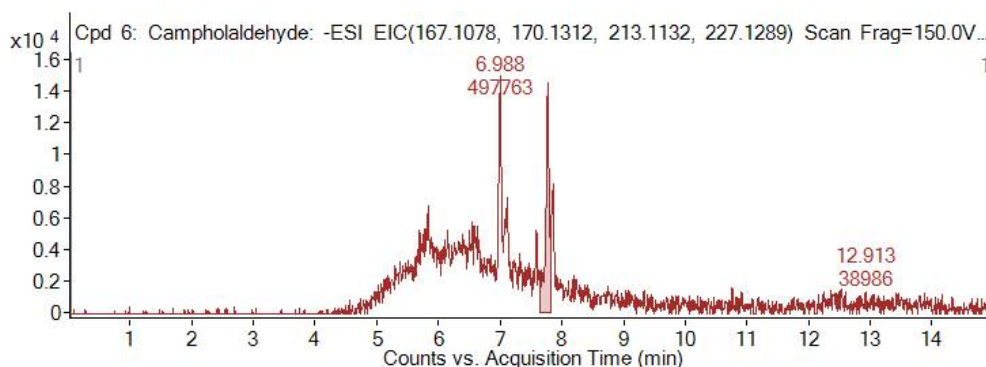


Compound 3: Xanthyletin

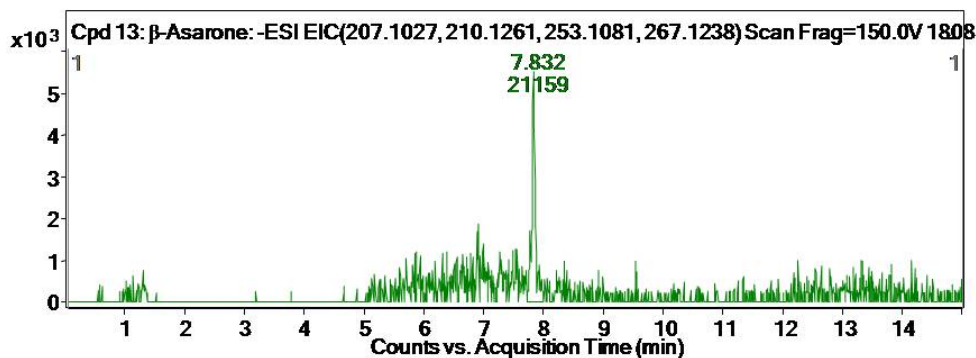


Compound 4: Phenol, 2,4- bis(1,1-dimethylethyl)
 LC-MS chromatogram of methanolic extract of Trimad, Ayurvedic polyherbal formulation

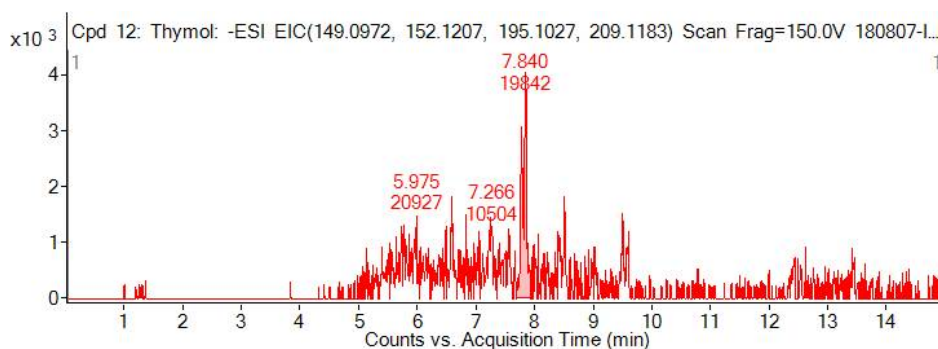
Figure 1
 LC-MS chromatogram of compound 1 to 4 at negative mode of ionization



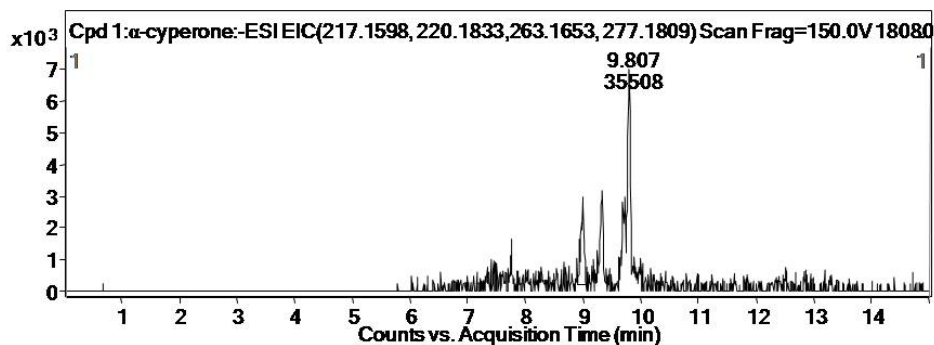
Compound 5: Camphoraldehyde



Compound 6: β - Asarone



Compound 7: Thymol

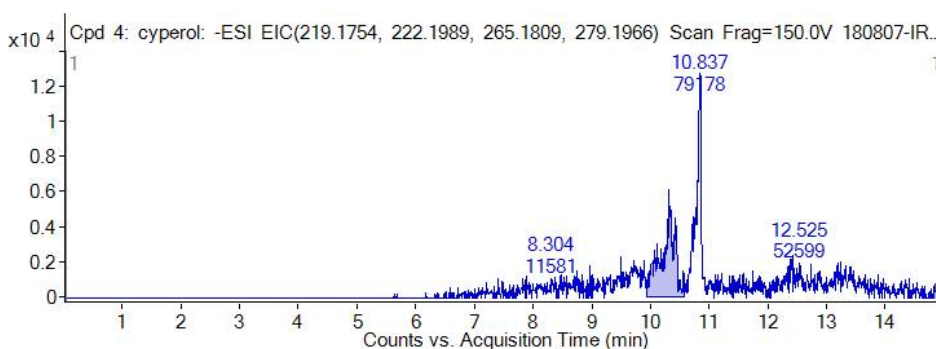


Compound 8: α -Cyperone

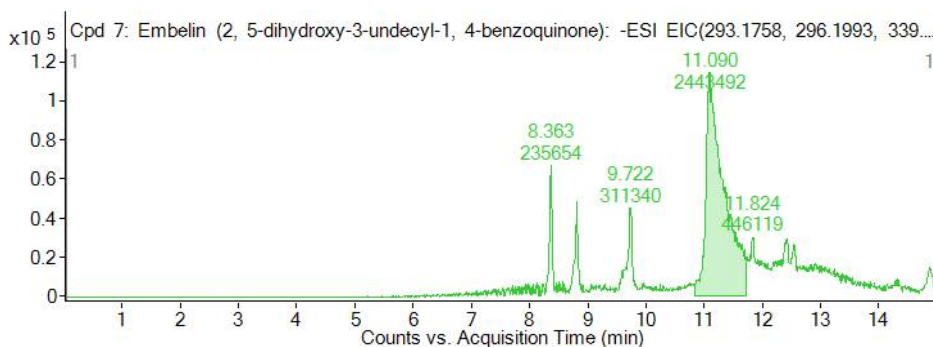
LC-MS chromatogram of methanolic extract of Trimad, Ayurvedic polyherbal formulation

Figure 2

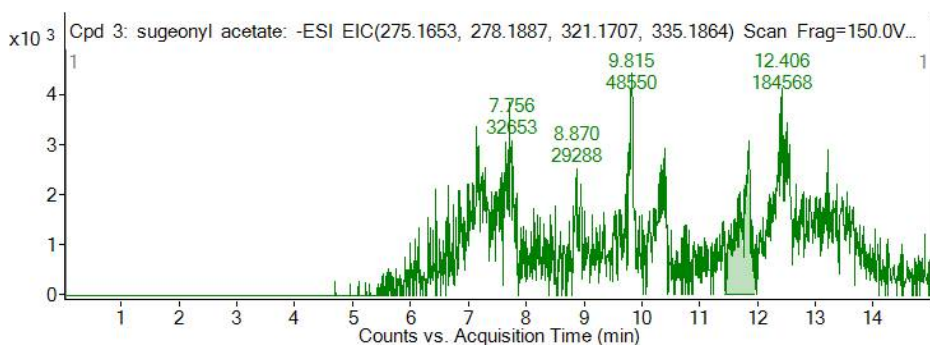
LC-MS chromatogram of compound 5 to 8 at negative mode of ionization



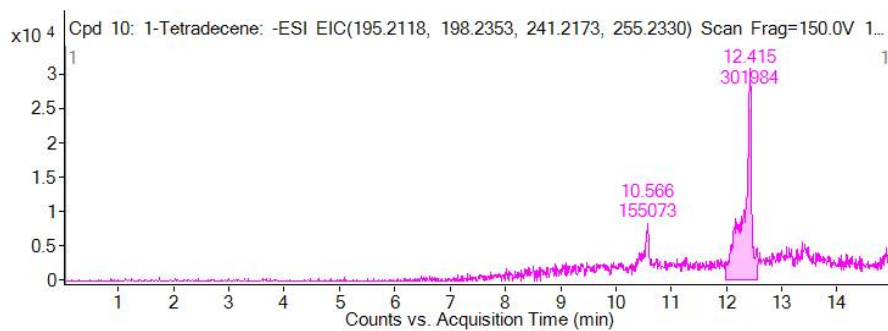
Compound 9: Cyperol



Compound 10: Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone)



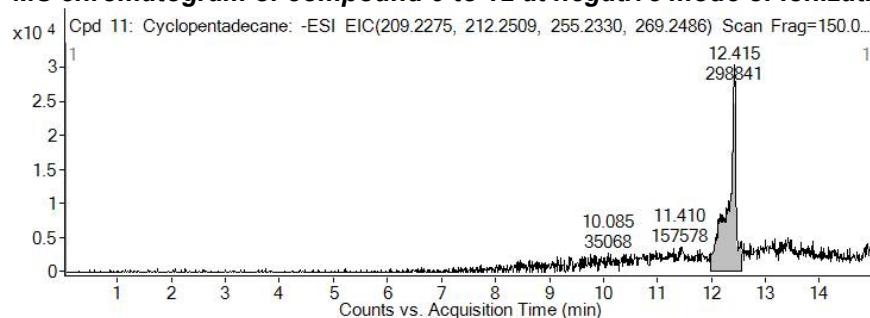
Compound 11: Sugeonyl acetate



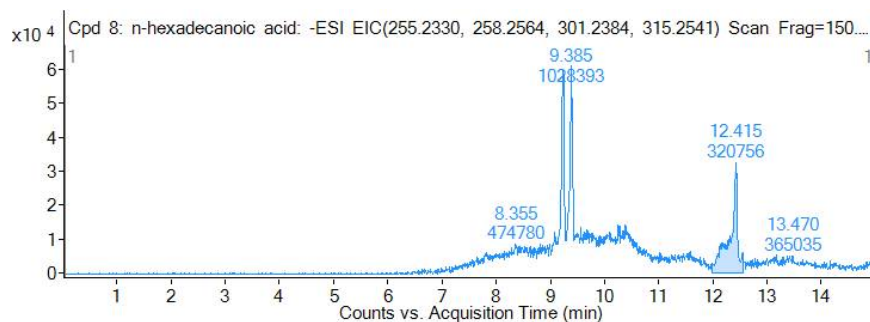
Compound 12: 1-Tetradecene
 LC-MS chromatogram of methanolic extract of Trimad, Ayurvedic polyherbal formulation

Figure 3

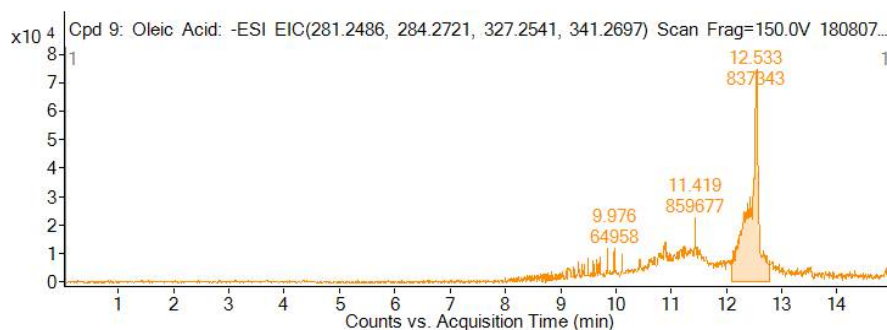
LC-MS chromatogram of compound 9 to 12 at negative mode of ionization



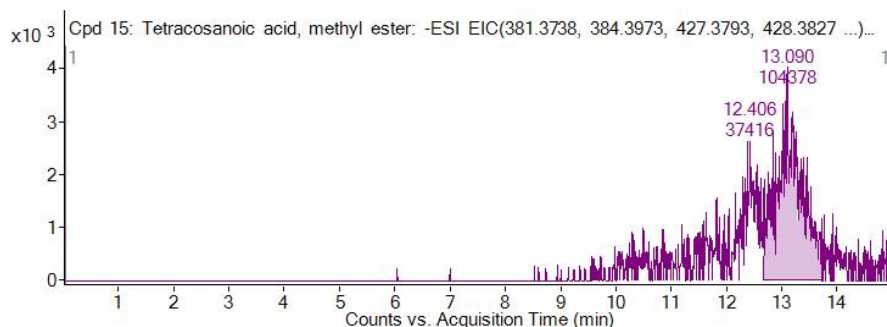
Compound 13: Cyclopentadecane



Compound 14: n-Hexadecanoic acid



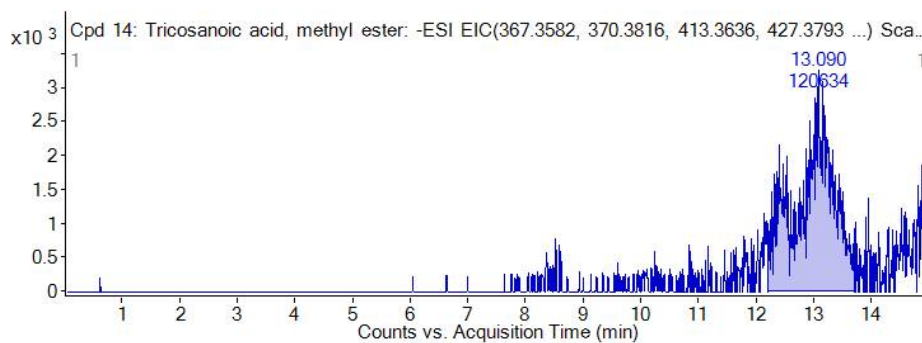
Compound 15: Oleic acid



Compound 16: Tetracosanoic acid methyl ester
 LC-MS chromatogram of methanolic extract of Trimad, Ayurvedic polyherbal formulation

Figure 4

LC-MS chromatogram of compound 13 to 16 at negative mode of ionization



Compound 16: Tricosanoic acid methyl ester
 LC-MS chromatogram of methanolic extract of Trimad Ayurvedic polyherbal formulation

Figure 5

LC-MS chromatogram of compound 17 at negative mode of ionization

Compounds detected in positive mode of ionization

Table 3 represents detected putative compounds from methanolic extract of *Trimad* polyherbal formulation in positive ionization mode. Twelve compounds were detected in positive mode of ionization and showed in Figure 6. These compounds are cyperol; β -asarone; oleic acid; camphoraldehyde; thymol; 1-epineo-

isoshinanolone; α -cyperone; phenol 2,4-bis(1,1-dimethylethyl); n-hexadecanoic acid; rotundines, γ -muurolene; ethyl p methoxycinnamate. About nine of these compounds were also detected in negative mode of ionization while, three different compounds were detected in positive mode of ionization (rotundines, γ -muurolene and ethyl p methoxycinnamate).

Table 3
 Putative identification of compounds from methanolic extract of *Trimad*, polyherbal formulation by positive ionization mode in LC-MS

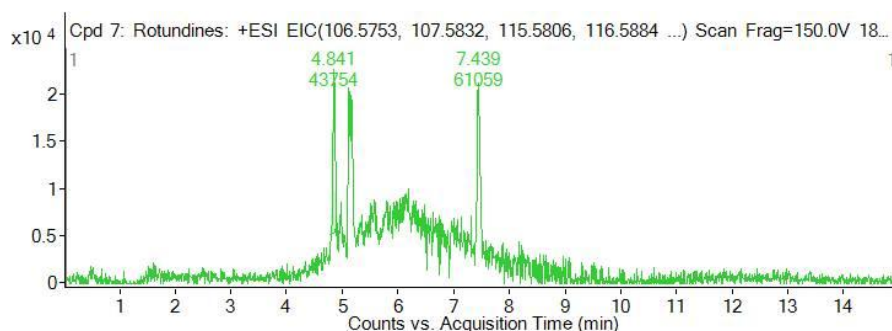
S. No	Name of Compounds	R _T	Target Score	Mass Error (ppm)	Formula	Ref. Mass	Obs. Mass	MS Fragment -ions (Obs. m/z)	Abundance	Ion/Isotope
1	Cyperol	0.062	85.06	2.31	C ₁₅ H ₂₄ O	220.18	220.18	203, 221, 222	143, 341, 65	(M+H)+[-H ₂ O], (M+H)+, (M+H)+
2	Rotundines	4.841	81.14	-6.31	C ₁₅ H ₂₁ N O	231.16	231.16	213, 214, 232, 254, 255, 256	1466, 254, 123, 13678, 3256, 110	M+[-H ₂ O], M+[-H ₂ O], (M+H)+, (M+Na)+, (M+Na)+, (M+Na)+
3	β -Asarone	5.432	92.06	-0.66	C ₁₂ H ₁₆ O ₃	208.11	208.11	191, 192, 209, 210, 211	1893, 413, 5512, 899, 131	(M+H)+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+, (M+H)+, (M+H)+
4	Oleic acid	5.59	99.72	0.53	C ₁₈ H ₃₄ O ₂	282.26	282.26	264, 265, 266, 283, 284, 285, 305, 306	89, 962, 174, 20227, 4129, 559, 196, 36	M+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+, (M+H)+, (M+H)+, (M+Na)+, (M+Na)+
5	Camphoraldehyde	5.857	90.31	-2.41	C ₁₀ H ₁₆ O ₂	168.16	168.11	151, 152, 168, 169, 169, 170, 171, 191, 192	6535, 838, 737, 388, 4285, 528, 97, 1745, 410	(M+H)+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+[-H ₂ O], M+, (M+H)+, (M+H)+, (M+H)+, (M+Na)+, (M+Na)+
6	Thymol	6.34	87.28	0.71	C ₁₀ H ₁₄ O	150.10	150.10	132, 133, 133, 134	186, 34, 4551, 485	M+[-H ₂ O], M+[-H ₂ O],

								151, 152, 3925, 45,	(M+H)+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+, (M+H)+, (M+Na)+[-H ₂ O]	
7	1-Epineo-isoshinanolone	6.631	86.07	2.04	C ₁₁ H ₁₂ O ₃	192.08	192.08	174, 175, 176, 192, 193, 194, 197, 215	93, 4374, 622, 78, 4639, 578, 50, 53	M+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+, (M+H)+, (M+Na)+[-H ₂ O], (M+Na)+
8	α-Cyperone	7.264	86.58	0.45	C ₁₅ H ₂₂ O	218.17	218.17	200, 201, 218, 219, 220, 241, 242	54, 1085, 64, 10072, 1793, 197, 125	M+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+, (M+H)+, (M+Na)+, (M+Na)+
9	Phenol, 2,4-bis (1,1-dimethylethyl)	7.788	87.18	6.67	C ₁₄ H ₂₂ O	206.17	206.17	188, 189, 190, 207, 208, 229, 230	97, 1493, 213, 1721, 352, 1251, 197	M+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+, (M+H)+, (M+Na)+, (M+Na)+
10	γ-Murolene	8.08	86.85	-3.11	C ₁₅ H ₂₄	204.19	204.19	204, 205, 206	237, 1316, 215	M+, (M+H)+, (M+H)+
11	Ethyl p-methoxycinnamate	11.39	83.39	-3.22	C ₁₂ H ₁₄ O ₃	206.09	206.09	188, 189, 207, 208	127, 81, 365, 48	M+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+, (M+H)+
12	n-Hexadecanoic acid	12.32	86.41	7.24	C ₁₆ H ₃₂ O ₂	256.24	256.24	238, 239, 240, 256, 257, 258, 261, 279, 280	500, 4926, 922, 703, 334, 91, 862, 10078, 2080	M+[-H ₂ O], (M+H)+[-H ₂ O], (M+H)+, (M+H)+, (M+Na)+[-H ₂ O], (M+Na)+, (M+Na)+

*R_T: retention time;; PPM: parts per million; m/z: mass-to-charge ratio

The obtained chromatogram was depicted in Figure 6. All the above mentioned compounds showed a distinct peak at different retention time. Various compounds were present in *P. zeylanica* like plumbagin, isoshinanolone and elliptinone, seselin, xanthyletin, xanthoxyletin, 3'-O-β-glucopyranosylplumbagic acid and

its methyl ester and 1-epineo-isoshinanolone²⁴⁻²⁶ and these compounds have been reported to have anti-hyperglycemic, hypolipidaemic, anti-atherosclerotic, anti-inflammatory, anti-microbial activities and also a rich antioxidants²⁷⁻³² pharmacological activities.



Compound 1: Rotundines

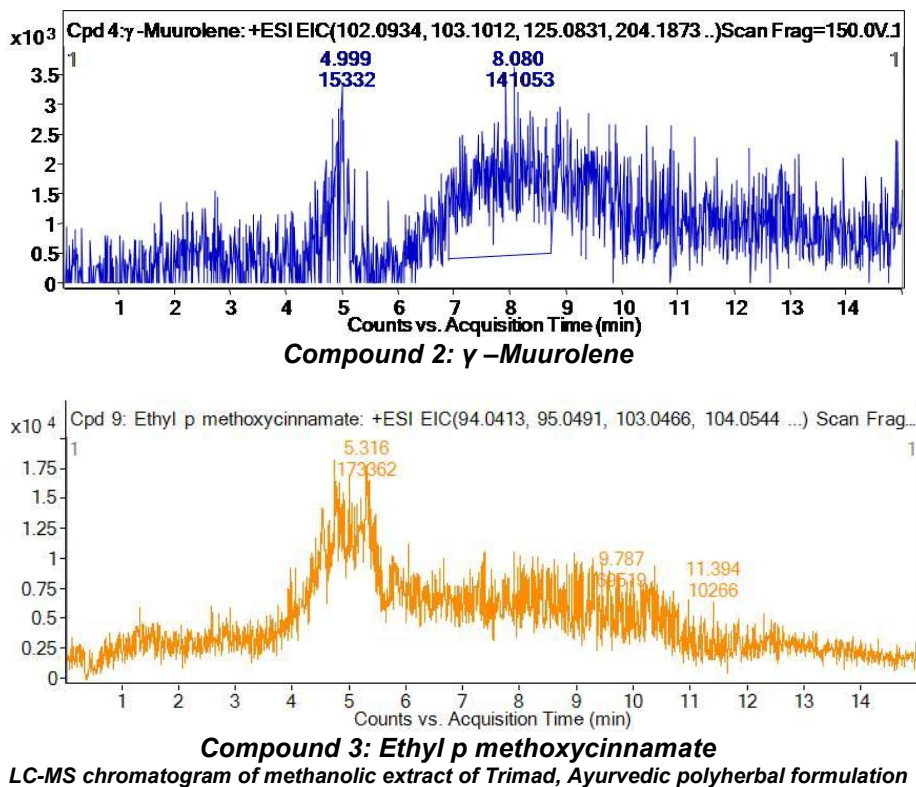


Figure 6
LC-MS chromatogram of positive mode of ionization

CONCLUSION

In the present study, we have examined phytoconstituents of *Triamad*, an Ayurvedic polyherbal formulation, through LC-MS analysis. Around twenty different principle compounds are detected through negative and positive ionization mode. To the best of our knowledge, this is the first report of LC-MS analysis of *Triamad* polyherbal formulation. This formulation further can be subjected for preclinical study to know its different pharmacological activities against hyperglycemia, dyslipidemia etc.

AUTHOR CONTRIBUTION STATEMENT

Suresh P Khadke performed the experiment, acquisition

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of data, interpretation and drafted the manuscript; Aniket A Kuvalekar conception of the study and design of experiments, interpretation of results, revising and editing the manuscript. All authors reviewed the manuscript.

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CONFLICT OF INTEREST

Conflicts of interest declared none.

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