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Gas Chromatography Tandem Mass Spectrometry for Quantitative Analysis of Pesticides in Sitopaladi Churna: Multi-Residue Method Development

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Abstract**—** *Pesticide residues are a consistent concern for consumers. A method validation study was conducted to analyze pesticide residues in Sitopaladi Churna using gas chromatography tandem mass spectrometry (GC-MS/MS) in a multiple reaction monitoring (MRM) mode of electron impact (EI) determination. The method employed the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach in compliance with European SANTE standards. Results fell within the specified criteria outlined in the validation guidelines. Validation parameters were assessed to confirm the method's suitability for the intended analysis. The optimized method was applied to detect residues of 55 pesticides in the herbal formulation 'Sitopaladi Churna,' with maximum residue limits (MRLs) defined in the Ayurvedic Pharmacopoeia of India (API). Notably, the method demonstrated high effectiveness, providing accurate results (70–120.0%) with precision (<20%) at three concentrations (10 µg/kg, 25 µg/kg, and 100 µg/kg). Limits of quantification (LOQs) were 5 µg/kg, and limits of detection (LODs) were 2 µg/kg. The analysis exhibited strong linearity with a regression coefficient exceeding 0.99 for all compounds. Consequently, this method can be employed for the determination of 55 pesticides in Sitopaladi Churna, as well as similar matrices, to analyze pesticide residues.*

Keywords**— Gas chromatography, Tandem mass spectrometry; Solid-phase extraction; Pesticide residues, Multiple reaction monitoring; Sitopaladi Churna**

I. INTRODUCTION

Ayurveda is a Sanskrit term, made up of the words "ayus" and "veda"meaning life and science; together translating to 'science of life'. A blend of several herbs and spices makes up the powdered mixture known as "churna". Depending on its intended use for medicinal, beauty, or culinary purpose, the recipe varies (Khalsa et al., 2008). Sitopaladi is in powder form and used for various respiratory ailments such as cough, cold, bronchitis, sinusitis, asthma, and chronic fever, loss of appetite, sore throat and

tuberculosis. It is taken with adjuvants such as ghee, honey and also as an ingredient of combination therapy with other Ayurvedic medicines. Sitopaladi churna is an inimitable mixture of Sitopala, Tugakheeree, Pippali, Ela and Twak. For the preparation of Sitopaladi churna, 16 parts, 8 parts, 4 parts, 2 parts and 1 part of Sitopala, Tugakheeree, Pippali, Ela and Twak are used, respectively (Sharmila et al., 2021).A research by the World Health Organization (WHO) states that over 80% of the global population uses traditional medicines, the majority of which are derived from plants. Therefore, the safety of these herbs needs to be considered just as much as that of other foods. While efforts are being made to set the fundamental guidelines to ensure their safety and quality, a significant obstacle is the absence of suitable techniques for identifying certain pollutants and residues in herbal formulations. There are reports about pesticides found in herbal formulations all around the world. (Tripathy et al., 2015).To ensure the quality of Sitopaladi Churna as well similar matrix, a large number of pesticides are used during cultivation, drying, and processing; consequently, massive pesticide exposure can lead to pesticide accumulation in the body during consumption, which harms human health and causes various diseases. Due to the fact that the growing demand for medicinal herbs necessitates higher agricultural output and the application of broad and ongoing agricultural procedures, substances, such as insecticides and fertilizers. The presence of several pesticide residues in medicinal herbs could also be the result of cross-contamination from pesticide-treated production locations, storage, or transit. To detect pesticide residues in Sitopaladi churna, gas chromatography–tandem mass spectrometry (GC–MS/MS) with MRM detection mode was utilized. Currently, QuEChERS (Chen et al., 2014; Yadav et al., 2017; Soltani et al., 2012) and solid-phase extraction (SPE) account for the majority of the pretreatment of spices, tea and other matrixes(Huo et al., 2014 & 2016). Although QuEChERS is simple and quick to operate, its clean-up effect is significantly inferior to solid-phase extraction, particularly when the matrix is herbal formulation. Moreover, solid-phase extraction has a very high recovery rate and can reduce instrument maintenance costs. As a result, it became imperative to create a uniform procedure for identifying multiclass pesticides in widely used medicinal herbs. In light of this, a modified QuEChERS based approach was investigated for the purpose of identifying 20 different classes of pesticides in the Sitopaladi Churna, with GC–MS/MS analysis as a follow-up. Until now, no reports or determinations using GC–MS/MS have been made regarding residue detection techniques for the simultaneous detection of 55 pesticides in Sitopaladi Churna.

This work designed and optimized a system for identifying a subset of 55 pesticide residues using GC-MS/MS in conjunction with SPE clean-up. This approach meets the criteria for identifying different pesticide residues in Sitopaladi Churna and provides technical assistance for developing guidelines for recently introduced pesticide residues.

II. EXPERIMENTAL

2.1 Chemicals and Materials

All the pesticide standards were of >98% purity and purchased from Sigma Aldrich, Germany. Chromatography grade solvents (Toluene, acetonitrile, Glacial acetic acid) and analytical grade reagents like anhydrous magnesium sulphate ($MgSO_4$), sodium sulfate and Sodium Chloride, Sodium acetatewere purchased from Merck India Ltd., Mumbai, India. Primary secondary amine (PSA, 40 µm, Bondesil) and C18 sorbent was purchased from Agilent Technologies, the USA anddde-ionized waterwas purchased from Thermo Fisher Scientific (Waltham, Massachusetts, US). Sitopaladi was used in this work and was purchased from a regular Ayurvedic dealer in Varanasi, Uttar Pradesh.

2.2 Standard Solutions and Calibration Curves

The stock solutions of the individual pesticide standards were prepared by accurately weighing 10 mg (±0.01 mg) of each analyte in volumetric flasks (certified 'A' class) and dissolving in10mL Toluene. These were stored in dark vials in a refrigerator at -20 °C (±1 C).An intermediate stock standard mixture of 10 mg/L was prepared by mixing the appropriate quantities of the individual stock solutions followed by requisite volume makeup and stored at -20 C (±1 °C). A working standard mixture of 1 mg/L was prepared by diluting the intermediate stock standard solution, from which the calibration standards within the range 1–100 µg/kg were prepared by serial dilution with Acetonitrile/toluene (1:1, v/v)

2.3 Extraction

Extraction was carried out according to a modified version of the QuEChERS method (Singh et al., 2020). To prepare sample extracts for GC-MS/MS analysis, in a polypropylene centrifuge tube weighed 5 grams of the homogenized sample. At this point, the recovery samples were prespiked with the mix pesticide standards in order to measure accuracy and precision. After spiking the standards, 15mL of Acetonitrile (with 1% acetic acid) was added and the mixture was vigorously shaken for a duration of one to two minutes. Emulsification is to be

used to separate the various aqueous layers; 6 gm of $MgSO_4$ and 1.5 gm of Sodium acetate, buffering salts, were added, and vigorous shaking was allowed to occur for a duration of one to two minutes. The solution was subjected to centrifugation at 8000 rpm for five minutes.2 ml of supernatant was taken, and 150 mg of$MgSO_4$ and 50 mg of PSA was added. It was followed by vertexing for a short duration and then centrifugation at 10,000 rpm for five minutes. Extreme care was made to ensure that the experimental procedures and tools were accurate and precise; spike samples were prepared at the Limit of Quantification (LOQ) level in accordance with the guidelines. In this study, six replicates were prepared at LOQ (5µg/kg), 25µg/kg and 100µg/kg concentration. The control sample was also extracted without any spiking for the preparation of the matrix-matched calibration curve in a range of 1 to 100µg/kg.

2.4 GC-MS/MS analysis

Sample acquisition for the detection and quantification of pesticide residues in the sample has been carried out by using the gas chromatography GC-MS/MS analysis was performed using an Agilent 7890A GC, coupled with a 7693 auto sampler, a 7000 triple quadruple MS, and a computer with Mass Hunter software (version B.05.00412) for data acquisition and processing (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated with DB-5 MS Ultra Inert capillary columns from Agilent (0.25 mm i.d. × 30 m, 0.25 µm film thickness)with the following operating conditions in Table 1 and triple quadruple mass spectrometer in electron impact (EI) ionization mode was operated with a 70 eV ionization voltage. The interface (transfer line to the tandem MS), ion source, and quadruple temperatures were maintained at 230 ◦C and 150 ◦C. Multiple reaction monitoring (MRM) mode was used for target detection and transition of each compounds are given in Table 2.

III. METHOD VALIDATION PARAMETERS

3.1 *Linear Range, Limit of Detection (LOD), and Limit of Quantification (LOQ)*

The performance of the analytical method was assessed as per SANTE validation guidelines. In accordance with the optimized experimental conditions, for the preparation of the matrix-matched calibration curve in a range of 1 to 100 µg/kg were prepared from the dilutions of the 55 pesticide standard solutions with blank Sitopaladi Churna sample extracts to calculate the standard curve. With a three-fold signal-to-noise ratio, the limits of detection (LODs) and limits of quantification (LOQs) of the 55 pesticides were calculated. The results indicated that the correlation coefficients between the concentrations of the 55 pesticide compounds and their peak areas in the range of 1 to 100 µg/kg were all greater than 0.99.The percent residual was in the range of ±20% (Figure1).Calibration curve for Dieldrin and Atrazin observed in matrix-matched linearity are shown in Figure 2. The limits of detection (LODs) and limits of quantification (LOQs) of the 55 pesticides in sample were 2 µg/kg and 5 µg/kg, respectively as shown in Table 3. As indicated in Ayurvedic Pharmacopoeia of India Standard Maximum Residue Limits for Pesticides in Herbal formulation (Ayurvedic Pharmacopoeia of India Part 1- Vol. 1) the LOQs for all 55 pesticides were significantly lower than the maximum residue levels specified, indicating that the method described in this paper meets the actual detection requirements. Finally, satisfactory recoveries (71.5 –114.6%) were achieved at three concentrations (10 µg/kg, 25 µg/kg, and 100 µg/kg).

3.2 Matrix Effect

The matrix effect refers to the presence of substances other than the target that appear to inhibit or boost the detection signal of the standard solution of the pure solvent. Sitopaladi Churna contains pigments, minerals, and other activities like tannins, saponins, alkaloids, glycosides, flavonoids and triterpenoids (Ekbote et al., 2019) that reduce the influence of endogenous substances on the precision of test results. The entire methodology has been performed to comply with the identification, confirmation, and performance parameters criteria as per the SANTE validation guidelines. The matrix effect was determined using the slope ratio of the matrix standard curve to the solvent standard curve (Steiner et al., 2020). More than±20 % matrix effect was observed for all 55 pesticide compounds according to the experimental results; therefore, a matrix-matched standard curve was utilized for quantification.

3.3 Spiked Recovery and Precision

Three mixed standard solutions of 10 µg/kg, 25 µg/kg, and 100 µg/kg were added to the blank matrix, and six parallel experiments were performed for each spiked level. The conditions were optimized for the determination. Based on the optimized work flow for these pesticides, Sitopaladi Churna from the market of Varanasi; were utilized to determine the pesticide residues in the same. As shown in Tables 3, the average percent recovery for six replicates has been observed in a range of 76.5 to 114.6 %; 78.2 to 109.8 % and 71.5 to 111.6 % for pre-spiked potato Sitopaladi Churna at a concentration level of 10 µg/kg, 25 µg/kg, and 100 µg/kg respectively (Figure 4). RSD analysis was performed for Sitopaladi Churna. The highest RSD of 10.9 % was observed for Parathion Ethyl, and a minimum of less than 0.5 % was observed for DDE-o, p' (Figure 5). Each molecule minimum of two ions was

selected with a signal-to-noise ratio > 3, and both ions were overlapped at the same retention time. The % RSD of six replicates was in the range of 0.6 to 10.2 %:0.9 to 10.9 % and 0.5 to 10.2 % for pre-spiked Sitopaladi Churna sample at a concentration level of 10 µg/kg, 25 µg/kg, and 100 µg/kg respectively. This workflow can effectively determine these 55 pesticide residues and thus, it is evident that the method offers excellent accuracy and precision and can be used to determine these 55 pesticide residues in Sitopaladi samples (SANTE/11312/2021).

3.4 Pesticide Residue Determination in Herbal Churna (Sitopaladi, Trikatu, Lavan Bhaskar Churna)

To evaluate the effectiveness and applicability of the developed method in measuring trace levels of the studied pesticides, the developed method was applied to the analysis of a total of 22 samples available for sale were used for pesticide residue determination. Traces of Chlorpyrifos Ethyl (an organophosphorus pesticide) residues were detected in one samples of Trikatu Churna purchased from Varanasi and in one sample of Lavan Bhaskar Churna obtained from Ghazipur Uttar Pradesh, but the level was below the Limit of Quantification (LOQ). None of the other samples were found to contain any other pesticide residues. The results obtained using the described preparation method for these real samples were accurate and precise. The proposed method was suitable for the analysis of the studied pesticides in herbal formulations as well related matrix (medicinal herb samples).

IV. RESULTS AND DISCUSSION

4.1 Optimization of GC-MS/MS Condition

GC-MS Solution version 4.45 and Microsoft Excel™ based files (MRM Optimization Tool and GC/MS/MS pesticide database version 1.01) were used to optimize the GC and MS parameters of all evaluated pesticides. All substances were examined in full scan mode between 45 and 650 m/z prior to MS-parameter tuning. After that, MS was run in MRM mode. The three strongest transitions and ideal collision energies (CE) for every pesticide were identified using the Agilent MRM Optimization Tool. The operating parameters of the MS, such as dwell periods, CE, MRM transitions, and retention times, are compiled in Table-1. Furthermore, the detector voltage (up to 0.5 kV) was tuned both absolute and in relation to the tuning result modes. The best sensitivity was found for chromatographic peak heights at a detector voltage of 2 kV. Using the optimized approach, mixtures of the 55 pesticide standards were found; the extracted ion chromatograms (EIC) are displayed in Figure 3.

4.2 Selection of the sample extraction method and optimization of clean up procedure

The challenge of determining the residual pesticides in herbal formulations is challenging due to the presence of diverse pigments and secondary metabolites. The widely used Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) strategy was utilized for sample preparation since it is a very versatile approach that provides numerous alternatives for analysis based on the variety of pesticides and matrices being examined. Following a thorough examination of the study's bibliography, we decided to employ acetonitrile due to its efficiency in removing both polar and non-polar pesticides from a variety of matrices, as well as its ability to yield high recoveries for a broad range of pesticide polarity (Anastassiades et al. 2003; Masˇtovska´ and Lehotay, 2004). Prior researchers have already thoroughly examined the physicochemical and practical benefits of acetonitrile over other solvents, such as ethyl acetate, in pesticide residue analyses (Anastassiades et al. 2003; Masˇtovska´ and Lehotay, 2004; Lehotay et al. 2010; Ramasubramanian et al. 2014; Tripathy et al., 2017). In order to allow the extraction solvent to more fully penetrate the dry plant tissues and guarantee full transfer of the analytes from naturally contaminated samples, we also reduced the sample amount (to 5 g) and added 15 mL of acetonitrile (with 1% acetic acid) prior to the extraction process. Thus, a modified QuEChERS-based methodology using acetonitrile as an extraction solvent was employed for additional research.Because of C18's strong affinity for fats and lipids, the QuEChERS technique inherently involves a dispersive-solid phase extraction (dispersive-SPE) step. In this step, sorbents such as primary secondary amine (PSA) are primarily used to remove organic acids and other polar matrix compounds. It was decided not to utilize graphitized carbon black (GCB) because it adsorbs pesticides with planar functionality, which causes many pesticides that are vulnerable to this adsorption to have inadequate recoveries (Tripathy et al., 2017). Consequently, PSA and C18 were chosen to clean up samples at all three spiked level 10 µg/kg, 25µg/kg, and 100 µg/kg. For every spiking level, six separate trials were carried out in parallel with respect to the blank matrix. For the determination, the conditions were optimized. To ascertain the pesticide residues in samples, the optimal methodology for these 55 pesticides was employed. This procedure can successfully identify these 55 pesticide residues, as Tables 3 demonstrate. It is clear from this that the approach can be used to determine these 55 pesticide residues in Sitopaladi Churna as well as related matrixes, and that it gives great accuracy and precision.

Table 1: GC-MS/MS analysis of phytochemical compounds in the hydro-alcoholic extract of Pistacia integerrima (PI).

S. No	Compound name	Molecular formula	RT	CAS number	Peak area
1	cis-5,8,11,14,17-Eicosapentaenoic acid	$C_{20}H_{30}O_2$	5.138	10417-94-4	0.33
2	Ethanone, 1-(1H-pyrrol-2-yl)-	C_6H_7NO	5.531	1072-83-9	0.54
3	Cholestan-22(26)-epoxy-3,16-dione	$C_{27}H_{42}O_3$	5.754	997857-38-1	0.27
4	Benzene, 1-methyl-3-(1-methylethenyl)-	$C_{10}H_{12}$	6.010	1124-20-5	0.07
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-	$C_6H_8O_4$	7.091	28564-83-2	0.65
6	Octanoic acid	$C_8H_{16}O_2$	7.488	124-07-2	5.60
7	Bicyclo[3.1.1]heptan-2-ol, 2,6,6-trimethyl-	$C_{10}H_{18}O$	7.529	473-54-1	0.71
8	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{18}O$	7.561	562-74-3	0.77
9	Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	7.620	106-32-1	2.86
10	1-Cyclopentene-1-methanol, 2-methyl-5-(1-	$C_{10}H_{18}O$	7.752	80113-82-2	0.30
11	(1S)-1,3,3-trimethylnorbornan-2-ol	$C_{10}H_{18}O$	7.830	470-08-6	1.99
12	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-,	$C_{10}H_{14}O$	7.958	1196-01-6	2.74
13	(2E)-3-(2-Hydroxyphenyl)-2-propenoic acid	$C_9H_8O_3$	8.149	614-60-8	0.78
14	Dihydrojasmone	$C_{11}H_{18}O$	8.678	1128-08-1	0.04
15	Butanedioic acid, hydroxy-, diethyl ester, (.+/-.)-	$C_8H_{14}O_5$	8.870	626-11-9	0.56
16	1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-one	$C_{10}H_{14}O$	9.171	22516-10-5	0.33
17	2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	$C_{10}H_{14}O$	9.509	503-93-5	0.09
18	3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene-2,5-dione	$C_{10}H_{12}O_2$	9.632	6617-34-1	0.19
19	3-Isopropyl-1-methyl-4-methylamino-pyrrole-2,5-	$C_9H_{14}N_2O_2$	9.805	997151-53-1	0.11
20	Methane, [(1-ethynylcyclohexyl)oxy]methoxy-	$C_{10}H_{15}DO_2$	10.047	5609-21-2	0.43
21	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	10.229	57156-91-9	0.45
22	Guanidine, N,N'-diphenyl-	$C_{13}H_{13}N_3$	10.453	102-06-7	0.42
23	Guanidine, N,N'-diphenyl-	$C_6H_6O_3$	10.617	87-66-1	0.40
24	Bisphenol C	$C_{17}H_{20}O_2$	10.850	79-97-0	0.85
25	Benzoic acid, 4-(1-methylethyl)-	$C_{10}H_{12}O_2$	11.096	536-66-3	0.16
26	Methanone, (4-bromo-5-methyl-2-nitro-3-	$C_{12}H_8BrNO_3S_2$	11.324	997735-67-2	0.35
27	cis-Pinonsaeure	$C_{10}H_{16}O_3$	11.393	473-72-3	0.22
28	2-Piperidinemethanol, .alpha.,.alpha.-diphenyl-	$C_{18}H_{21}NO$	11.479	467-60-7	0.26
29	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	11.575	56599-95-2	0.05
30	1H-Cyclopropa[a]naphthalene, 1a,2,6,7,7a,7b-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	$C_{15}H_{22}$	11.630	34143-96-9	0.30
31	4,7,10,13,16,19-Docosahexaenoic acid, methyl	$C_{23}H_{34}O_2$	11.671	2566-90-7	0.07
32	cis-2-phenyl-1, 3-dioxolane-4-methyl octadec-9, 12,	$C_{28}H_{40}O_4$	11.730	997894-27-7	0.05
33	4-Isopropyl-1,6-dimethyl-1,2,3,4-	$C_{15}H_{22}$	11.762	483-77-2	0.03
34	1,1,7-Trimethyl-4-methylene-1a,2,3,4,6,7,7a,7b-	$C_{15}H_{22}$	11.904	112362-74-0	0.05
35	Heneicosapentaenoic Acid methyl ester	$C_{22}H_{34}O_2$	12.182	65919-53-1	0.04

36	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	12.250	96-76-4	0.41
37	Bicyclo[4.1.0]heptan-2-ol, 1.beta.-(3-methyl-1,3-acetoxy-	$C_{16}H_{24}O_3$	12.342	997432-87-7	0.17
38	Benzoic acid, 3-hydroxy-, 2-methylpropyl ester	$C_{11}H_{14}O_3$	12.506	997187-49-7	0.04
39	Quinine	$C_{20}H_{24}N_2O_2$	12.574	130-95-0	0.10
40	Dodecanoic acid	$C_{12}H_{24}O_2$	12.926	143-07-7	0.12
41	fv	$C_{17}H_{28}O_2$	12.994	2306-78-7	0.65
42	4,4-Dimethyl-3-oxoandrost-5-en-17-yl acetate	$C_{23}H_{31}D_3O_3$	13.072	997740-43-1	0.16
43	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	13.341	106-33-2	0.07
44	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	$C_{15}H_{24}O$	13.386	6750-60-3	0.56
45	(-)-Globulol	$C_{15}H_{26}O$	13.514	489-41-8	1.62
46	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alp	$C_{15}H_{26}O$	13.642	552-02-3	0.29
47	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-(2.alpha.,4a.beta.,8.beta.)]-	$C_{15}H_{26}O$	13.765	63891-61-2	0.32
48	1,4-Diiodooctahydropentalene	$C_{15}H_{24}O$	13.874	74842-43-6	0.10
49	2-Naphthalenemethanol, decahydro-(2.alpha.,4a.alpha.,8a.beta.)]-	$C_{15}H_{26}O$	13.979	473-15-4	0.10
50	2-((2R,4aR,8aS)-4a-Methyl-8-	$C_{15}H_{24}O$	14.086	515-20-8	0.33
51	Sulfuric acid, 5,8,11-heptadecatrienyl methyl ester	$C_{18}H_{32}O_3S$	14.198	56554-67-7	0.46
52	1-S-[(1E)-2-(1H-Indol-3-yl)-N-	$C_{16}H_{19}N_2O_9S_2$	14.358	4356-52-9	0.34
53	2-Naphthalenemethanol, decahydro-[2R-(2.alpha.,4a.alpha.,8a.beta.)]-	$C_{15}H_{26}O$	14.404	51317-08-9	0.09
54	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	15.042	5129-60-2	0.10
55	cis-2-phenyl-1, 3-dioxolane-4-methyl octadec-9, 12,	$C_{28}H_{40}O_4$	15.125	997894-27-7	0.04
56	Tetradecanoic acid	$C_{14}H_{28}O_2$	15.677	544-63-8	0.07
57	Lactaropallidin	$C_{15}H_{24}O_3$	15.909	997389-81-8	5.16
58	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	16.133	124-06-1	0.13
59	Nonacosane	$C_{29}H_60$	16.247	630-03-5	4.88
60	1,11,11-Trimethyl-1,2,3,4-tetrahydro-1,4-	$C_{16}H_{18}N_2$	16.685	997341-19-0	0.10
61	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	16.749	542-44-9	0.11
62	Pentadecanoic acid	$C_{15}H_{30}O_2$	17.301	1002-84-2	0.28
63	Ethyl gallate	$C_9H_{10}O_5$	18.035	831-61-8	0.24
64	7-Hydroxy-4-(3,4,5-trimethoxyphenyl)chromen-2-	$C_{18}H_{16}O_6$	18.460	858002-39-8	1.04
65	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	19.167	2091-29-4	0.72
66	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	20.011	57-10-3	22.18
67	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	20.617	628-97-7	2.86
68	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	21.256	542-44-9	0.15
69	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	21.703	997888-97-8	0.06

70	12,24-Divinyl-1,13-dioxacyclotetracosane-2,14-	$C_{26}H_{44}O_4$	21.872	997866-77-6	0.07
71	Eicosanoic acid	$C_{20}H_{40}O_2$	22.287	506-30-9	0.47
72	Butyl 4,7,10,13,16,19-docosahexaenoate	$C_{26}H_{40}O_2$	22.944	997801-29-9	0.30
73	cis-Vaccenic acid	$C_{18}H_{34}O_2$	23.501	506-17-2	5.22
74	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	23.756	544-35-4	1.52
75	Octadecanoic acid	$C_{18}H_{36}O_2$	23.838	57-11-4	5.30
76	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	23.962	628-97-7	10.50
77	8-Chloro-1-octanol, benzyldimethylsilyl ether	$C_{17}H_{29}ClOSi$	24.007	997600-55-4	6.23
78	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	24.149	111-61-5	0.52
79	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	24.500	60-33-3	0.13
80	Flavonol 3',4',5,7-OH,3-O-Araglucoside	$C_{26}H_{30}O_{16}$	24.856	0-00-0	0.04
81	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	25.540	56599-95-2	0.04
82	Eicosanoic acid	$C_{20}H_{40}O_2$	25.759	506-30-9	0.57
83	Squalene	$C_{30}H_{50}$	26.015	111-02-4	0.88
84	(Z)-3-(Heptadec-10-en-1-yl)phenol	$C_{23}H_{38}O$	26.887	111047-33-7	0.09
85	Phenol, 3-pentadecyl-	$C_{21}H_{36}O$	27.083	501-24-6	0.25
86	1,2-Benzenedicarboxylic acid, 3-nitro-	$C_8H_5NO_6$	27.320	603-11-2	0.06
87	Sabinyl linoleate	$C_{28}H_{46}O_2$	27.421	997857-49-5	0.16
88	Heptacosane, 1-chloro-	$C_{27}H_{55}Cl$	29.127	62016-79-9	0.29

Table 2: GC-MS/MS analysis of phytochemical compounds in the aqueous extract of Pistacia integerrima (PI).

S. No	**Compound name**	**Molecular formula**	**RT**	**CAS number**	**Peak area**
1	Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	7.620	106-32-1	7.43
2	Dodecanoic acid	$C_{12}H_{24}O_2$	12.953	143-07-7	3.34
3	1H-Cycloprop[e]azulen-7-ol decahydro-1,1,7-trimethyl-4-methylene-, [1ar-ta.,7b.alpha.)]-(1a.alpha.,4a.alpha.,7.beta.,7a.be	$C_{15}H_{24}O$	13.377	6750-60-3	5.09
4	Tridecanoic acid, 12-methyl-,methyl ester	$C_{15}H_{30}O_2$	15.051	5129-58-8	1.41
5	7-Methyl-Z-tetradecen-1-olacetate	$C_{17}H_{32}O_2$	15.977	997448-21-4	3.30
6	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	16.160	124-06-1	12.46
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	19.855	57621	22.78
8	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	20.621	628-97-7	11.11
9	n-Propyl 5,8,11,14,17-eicosapentaenoate	$C_{23}H_{36}O_2$	22.920	997702-88-3	4.98
10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	23.377	60-33-3	1.87
11	cis-Vaccenic acid	$C_{18}H_{34}O_2$	23.463	506-17-2	3.07
12	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	23.746	544-35-4	3.39
13	Ethyl Oleate	$C_{20}H_{38}O_2$	23.828	111-62-6	3.26
14	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	24.134	111-61-5	2.31
15	1,3-Dioctanoin	$C_{19}H_{36}O_5$	25.904	1429-66-9	4.15

16	Phenol, 3-pentadecyl-	$C_{21}H_{36}O$	27.068	501-24-6	2.45
17	(Z)-3-(Heptadec-10-en-1-yl)phenol	$C_{23}H_{38}O$	29.067	111047-33-7	2.13
18	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	29.135	997764-68-7	5.46

Table 3: GC-MS/MS analysis of phytochemical compounds in the hydro-alcoholic extract of Quercus infectoria (QI).

S. No	Compound name	Molecular formula	RT	CAS number	Peak area
1	Hi-oleic safflower oil	$C_{21}H8_{22}O_{11}$	5.166	8001-23-8	1.08
2	Phenol, 2-methoxy-	C_7HO_2	5.941	90-05-1	0.55
3	3-Octanol, 3,7-dimethyl-	$C_{10}H_{22}O$	6.142	78-69-3	0.62
4	(+)-2-Bornanone	$C_{10}H_{16}O$	7.004	464-49-3	0.54
5	Glucosamine, N-acetyl-N-benzoyl-	$C_{15}H_{19}NO_7$	7.196	997642-31-4	1.53
6	Butanedioic acid, diethyl ester	$C_8H_{14}O_4$	7.314	123-25-1	1.33
7	Oxalic acid, isobutyl nonyl ester	$C_{15}H_{28}O_4$	7.706	997461-38-4	1.88
8	2-hydroxybutanedioic acid diethyl ester	$C_8H_{14}O_5$	8.745	626-11-9	2.75
9	1,3-Dioxocane, 2-pentadecyl-	$C_{21}H_{42}O_2$	8.888	41583-11-3	1.15
10	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	9.171	544-35-4	0.97
11	1-Octen-4-one	$C_8H_{14}O$	9.212	997033-68-1	0.41
12	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	$C_{18}H_{16}O_7$	9.271	6068-80-0	0.43
13	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	10.001	91-10-1	0.41
14	Tridecane	$C_{13}H_{28}$	10.745	629-50-5	0.54
15	Chlorothymol, trimethylsilyl ether	$C_{13}H_{21}ClOSi$	10.927	997402-91-6	15.10
16	Mefloquine	$C_{17}H_{16}F_6N_2O$	11.876	53230-10-7	0.31
17	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	12.264	96-76-4	1.24
18	3-Pyridinecarboxylic acid, 6-amino-	$C_6H_6N_2O_2$	12.565	3167-49-5	0.58
19	2-Hydroxy-5-methylbenzophenone,trimethylsilyl ether	$C_{17}H_{20}O_2Si$	12.688	997505-02-4	0.31
20	2-Hydroxy-5-methylbenzophenone,trimethylsilyl ether	$C_{17}H_{20}O_2Si$	12.816	997505-02-4	0.21
21	Dodecanoic acid	$C_{12}H_{24}O_2$	12.948	143-07-7	1.35
22	1,2-Benzenedicarboxylic acid,diethyl ester	$C_{12}H_{14}O_4$	13.386	84-66-2	1.58
23	Tetradecane	$C_{14}H_{30}$	13.441	629-59-4	0.81
24	2,2-Dideutero octadecanal	$C_{18}H_{34}D_2O$	14.157	56555-07-8	0.34
25	Nonacosane	$C_{29}H_{60}$	14.728	630-03-5	0.21
26	Tetradecanoic acid	$C_{14}H_{28}O_2$	15.576	544-63-8	1.80
27	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	16.119	41114-00-5	0.89
28	Nonacosane	$C_{29}H_{60}$	16.247	630-03-5	0.26
29	Benzene, 1,1'-ethylidenebis[3,4-dimethyl-	$C_{18}H_{22}$	16.735	1742-14-9	0.45
30	2l,4l-dihydroxyeicosane	$C_{20}H_{42}O_2$	17.009	997609-18-5	0.28

31	ethyl 3,5-dihydroxy-4-methoxybenzoate	$C_{10}H_{12}O_5$	17.063	997245-87-8	0.25
32	Ethyl gallate	$C_9H_{10}O_5$	18.081	831-61-8	3.28
33	Fluroxypyr	$C_7H_5Cl_2FN_2O_3$	19.240	69377-81-7	4.01
34	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	19.732	57-10-3	10.11
35	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	20.640	628-97-7	3.85
36	Ethyl cis-6-Hydroxy-7-oxobicyclo[4.3.0]nonanecarboxylate	$C_{12}H_{18}O_4$	20.727	997296-40-2	0.01
37	Ethyl cis-6-Hydroxy-7-oxobicyclo[4.3.0]nonanecarboxylate	$C_{12}H_{18}O_4$	20.837	997296-40-2	12.18
38	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methylester,cis-	$C_{28}H_{44}O_4$	22.292	56599-45-2	0.34
39	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	23.401	60-33-3	2.23
40	cis-Vaccenic acid	$C_{18}H_{34}O_2$	23.492	506-17-2	6.81
41	Octadecanoic acid	$C_{18}H_{36}O_2$	23.793	57-11-4	7.72
42	Ethyl Oleate	$C_{20}H_{38}O_2$	23.843	111-62-6	3.88
43	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	24.145	41114-00-5	0.88
44	(2R,4aS,5S,8aS)-2,5-Dipentyldecahydroquinoline	$C_{19}H_{37}N$	24.464	220024-74-8	0.29
45	FLAVONOL 3',4',5,7-OH,3-O-ARAGLUCOSIDE	$C_{26}H_{30}O_{16}$	25.746	0-00-0	0.33
46	Hexanedioic acid, mono(2-ethylhexyl)ester	$C_{14}H_{26}O_4$	25.975	4337-65-9	0.59
47	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	26.267	997888-97-8	0.41
48	1,2-Benzenedicarboxylic acid, 3-nitro-	$C_8H_5NO_6$	27.316	603-11-2	0.30

Table 4: GC-MS/MS analysis of phytochemical compounds in the aqueous extract of Quercus infectoria (QI).

S. No	Compound name	Molecular formula	RT	CAS number	Peak area
1	1,2,3-Benzenetriol	$C_6H_6O_3$	10.580	87-66-1	46.22
2	N-methyl-5-aminobicyclo[2.2.2]oct-2-ene	$C_9H_{15}N$	10.736	116907-45-0	14.79
3	Bisphenol C	$C_{17}H_{20}O_2$	10.859	79-97-0	28.72
4	Nonacosane	$C_{29}H_{60}$	25.854	630-03-5	2.17
5	Docosane, 11-decyl-	$C_{32}H_{66}$	28.620	55401-55-3	8.10

Table 5: GC-MS/MS analysis of phytochemical compounds in the hydro-alcoholic extract of *Terminalia chebula* (TC).

S. No	Compound name	Molecular formula	RT	CAS number	Peak area
1	Hi-oleic safflower oil	$C_{21}H_{22}O_{11}$	5.148	8001-23-8	1.11
2	Ethanone, 1-(1H-pyrrol-2-yl)-	C_6H_7NO	5.544	1072-83-9	1.00
3	1-(2-Furanyl)-2-hydroxyethanone	$C_6H_6O_3$	5.941	17678-19-2	2.54
4	3,6,6-trimethyl-1-cyclohex-2-enone	$C_9H_{14}O$	7.114	997050-80-5	0.81
5	Heneicosane	$C_{21}H_{44}$	7.711	629-94-7	0.41

6	Ethanone, 1-(2-furanyl)-	$C_6H_6O_2$	7.880	1192-62-7	0.44
7	2-Furancarboxaldehyde, 5-(ethoxymethyl)-	$C_8H_{10}O_3$	8.104	997080-80-1	1.32
8	5-Hydroxymethylfurfural	$C_6H_6O_3$	8.327	67-47-0	4.95
9	3-Methoxy-4-methyl-2-methylenepentanenitrile	$C_8H_{13}NO$	8.396	997052-52-5	0.07
10	2-hydroxybutanedioic acid diethylester	$C_8H_{14}O_5$	8.756	626-11-9	1.40
11	Cyclohexane, 1,4-dimethyl-2-octadecyl-	$C_{26}H_{52}$	9.221	55282-02-5	0.10
12	5-Acetoxymethyl-2-furaldehyde	$C_8H_8O_4$	9.353	10551-58-3	1.52
13	4-Hydroxy-2-methylacetophenone	$C_9H_{10}O_2$	9.504	875-59-2	0.12
14	Formic acid, 2,6-dimethoxyphenylester	$C_9H_{10}O_4$	10.006	997151-27-4	0.39
15	(4,6-dimethyl-2-methylsulfanyl-3-pyridyl)methanamine	$C_9H_{14}N_2S$	10.111	997151-58-2	0.37
16	1,2,3-Benzenetriol	$C_6H_6O_3$	10.635	87-66-1	15.72
17	Nonacosane	$C_{29}H_{60}$	10.736	630-03-5	1.86
18	Bisphenol C	$C_{17}H_{20}O_2$	10.832	79-97-0	9.50
19	2-Propenoic acid, 3-phenyl-	$C_9H_8O_2$	11.192	621-82-9	1.83
20	(E)-Ethyl cinnamate	$C_{11}H_{12}O_2$	11.771	103-36-6	0.30
21	N-[1-(3-methylphenyl)-2,3-dihydropyrrolo[2,3-b]quinolin-4-yl]-2-(1-piperidinyl)acetamide	$C_{25}H_{28}N_4O$	11.876	997832-76-4	0.17
22	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	11.967	55282-12-7	0.09
23	Ethyl 3-hydroxybenzoate	$C_9H_{10}O_3$	12.091	7781-98-8	1.36
24	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	12.250	96-76-4	0.60
25	4-[(2,3-dimethylquinoxalin-6-yl)carbamoyl]-3,3-dimethylbutanoic acid	$C_{17}H_{21}N_3O_3$	12.332	997611-15-6	0.12
26	Benzoic acid, 3-ethoxy-	$C_9H_{10}O_3$	12.492	621-51-2	0.47
27	Ingol 12-acetate	$C_{22}H_{32}O_7$	12.752	51906-01-5	0.06
28	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	12.889	542-44-9	0.18
29	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	13.336	997783-85-1	1.28
30	Docosane, 11-decyl-	$C_{32}H_{66}$	13.436	55401-55-3	0.26
31	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	14.029	487-11-6	0.20
32	8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3.beta.-methoxy-4,4-dimethyl-	$C_{24}H_{36}O_6$	14.244	997866-38-8	0.11
33	Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl]oxy]methyl]-1,2-ethanediyl ester	$C_{37}H_{74}NO_8P$	14.727	3026-45-7	0.21
34	Isochiapin B	$C_{19}H_{22}O_6$	15.348	997706-97-3	0.11
35	Tetradecanoic acid	$C_{14}H_{28}O_2$	15.540	544-63-8	0.47
36	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	16.133	2490-48-4	0.81

37	Nonacosane	$C_{29}H_{60}$	16.242	630-03-5	0.23
38	Dasycarpidan-1-methanol,acetate (ester)	$C_{20}H_{26}N_2O_2$	16.625	55724-48-6	0.26
39	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	16.990	56599-95-2	0.14
40	1,2-Benzenedicarboxylic acid,bis(2-methoxyethyl) ester	$C_{14}H_{18}O_6$	17.373	117-82-8	0.23
41	6-Fluorobenzofuroxane, 5-[4-(2-pyrimidyl)piperazin-1-yl]-	$C_{14}H_{13}FN_6O_2$	17.611	997613-06-7	0.31
42	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	$C_{18}H_{16}O_7$	18.427	6068-80-0	0.26
43	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	19.627	57-10-3	6.23
44	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	20.585	628-97-7	7.63
45	E-2-Methyl-3-tetradecen-1-olacetate	$C_{17}H_{32}O_2$	21.443	997448-18-4	0.19
46	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	22.821	54546-22-4	0.10
47	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	$C_{20}H_{38}O_2$	23.364	17367-08-7	0.63
48	cis-Vaccenic acid	$C_{18}H_{34}O_2$	23.446	506-17-2	1.98
49	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	23.742	544-35-4	5.67
50	Ethyl Oleate	$C_{20}H_{38}O_2$	23.824	111-62-6	8.84
51	Ethyl 13-methyl-tetradecanoate	$C_{17}H_{34}O_2$	24.130	997455-69-6	0.99
52	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	24.778	544-35-4	0.16
53	(5.beta.)Pregnane-3,20.beta.-diol,14.alpha.,18.alpha.-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{28}H_{43}NO_6$	26.005	997941-65-3	0.36
54	1,2-Benzenedicarboxylic acid,monononyl ester	$C_{17}H_{24}O_4$	27.315	24539-59-1	0.65
55	Licarin A	$C_{20}H_{22}O_4$	28.096	51020-86-1	0.36

Table 6: GC-MS/MS analysis of phytochemical compounds in the aqueous extract of Terminalia chebula (TC).

S. No	**Compound name**	**Molecular formula**	**RT**	**CAS number**	**Peak area**
1	1-(2-Furanyl)-2-hydroxyethanone	$C_6H_6O_3$	5.941	17678-19-2	1.84
2	1,2,4,5-Tetrazine	$C_2H_2N_4$	7.104	290-96-0	0.51
3	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	7.711	3386-33-2	0.38
4	2-Furancarboxaldehyde, 5-(ethoxymethyl)-	$C_8H_{10}O_3$	8.103	997080-80-1	0.62
5	5-Hydroxymethylfurfural	$C_6H_6O_3$	8.304	67-47-0	29.22
6	5-Acetoxymethyl-2-furaldehyde	$C_8H_8O_4$	9.353	10551-58-3	1.96
7	1,2,3-Benzenetriol	$C_6H_6O_3$	10.735	87-66-1	1.45
8	Ethanone, 1-[4-methoxy-3-(4-methylphenoxy)phenyl]-	$C_{16}H_{16}O_3$	10.822	116345-94-9	1.30
9	2-Phenylethyl hydrogencarbonate	$C_9H_{10}O_3$	12.077	997108-68-2	0.59

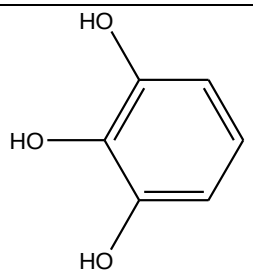
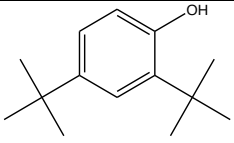
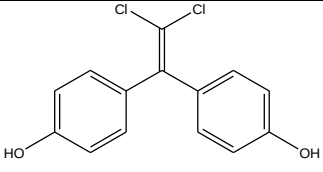
10	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	12.245	96-76-4	0.23
11	Hexadecane, 1,1-bis(dodecyloxy)-	$C_{40}H_{82}O_2$	13.345	56554-64-4	0.49
12	Tricosane	$C_{23}H_{48}$	13.436	638-67-5	0.37
13	Hexadecane, 1,1-bis(dodecyloxy)-	$C_{40}H_{82}O_2$	16.123	56554-64-4	0.49
14	Glycerol tricaprylate	$C_{27}H_{50}O_6$	26.675	538-23-8	21.88

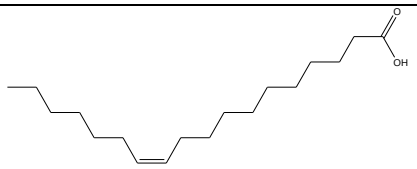
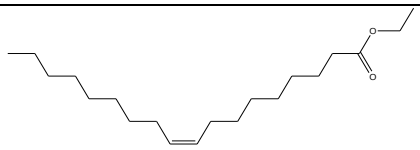
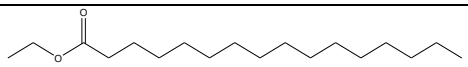
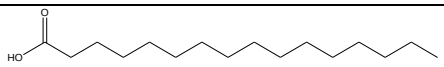
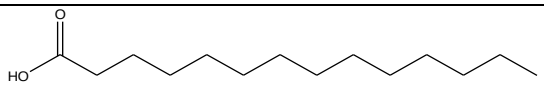
Table 7: List of common compounds present in different extracts of Pistacia integerrima (PI), Quercus infectoria (QI) and Terminalia chebula (TC)by GC-MS/MS.

S.No.	Compound name	Molecular formula	CAS number	PI-HA	PI-Aq	QI-HA	QI-Aq	TC-HA	TC-Aq
1	(Z)-3-(Heptadec-10-en-1-yl)phenol	$C_{23}H_{38}O$	111047-33-7	+	+	-	-	-	-
2	1-(2-Furanyl)-2-hydroxyethanone	$C_6H_6O_3$	17678-19-2	-	-	-	-	+	+
3	1,2,3-Benzenetriol	$C_6H_6O_3$	87-66-1	+	-	-	+	+	+
4	1,2-Benzenedicarboxylic acid, 3-nitro-	$C_8H_5NO_6$	603-11-2	+	-	+	-	-	-
5	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	$C_{15}H_{24}O$	6750-60-3	+	+	-	-	-	-
6	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	96-76-4	+	-	+	-	+	+
7	2-hydroxybutanedioic acid diethyl ester	$C_8H_{14}O_5$	626-11-9	-	-	+	-	+	-
8	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	$C_{18}H_{16}O_7$	6068-80-0	-	-	+	-	+	-
9	5-Acetoxymethyl-2-furaldehyde	$C_8H_8O_4$	10551-58-3	-	-	-	-	+	+
10	5-Hydroxymethylfurfural	$C_6H_6O_3$	67-47-0	-	-	-	-	+	+
11	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	60-33-3	+	+	+	-	-	-
12	Bisphenol C	$C_{17}H_{20}O_2$	79-97-0	+	-	-	+	+	-
13	cis-Vaccenic acid	$C_{18}H_{34}O_2$	506-17-2	+	+	+	-	+	-
14	Docosane, 11-decyl-	$C_{32}H_{66}$	55401-55-3	-	-	-	+	+	-
15	Dodecanoic acid	$C_{12}H_{24}O_2$	143-07-7	+	+	+	-	-	-
16	Ethanone, 1-(1H-pyrrol-2-yl)-	C_6H_7NO	1072-83-9	+	-	-	-	+	-
17	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	544-35-4	+	+	-	-	-	-
18	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	544-35-4	-	-	+	-	+	-
19	Ethyl gallate	$C_9H_{10}O_5$	831-61-8	+	-	+	-	-	-
20	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	997888-97-8	+	-	+	-	-	-

21	Ethyl Oleate	$C_{20}H_{38}O_2$	111-62-6	-	+	+	-	+	-
22	FLAVONOL 3',4',5,7-OH,3-O-ARAGLUCOSIDE	$C_{26}H_{30}O_{16}$	0-00-0	+	-	+	-	-	-
23	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	542-44-9	+	-	-	-	+	-
24	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	628-97-7	+	+	+	-	+	-
25	Hi-oleic safflower oil	$C_{21}H_{22}O_{11}$	8001-23-8	-	-	+	-	+	-
26	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	57621	+	+	+	-	+	-
27	Nonacosane	$C_{29}H_{60}$	630-03-5	+	-	+	+	+	-
28	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	56599-95-2	+	-	-	-	+	-
29	Octadecanoic acid	$C_{18}H_{36}O_2$	57-11-4	+	-	+	-	-	-
30	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	111-61-5	+	+	-	-	-	-
31	Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	106-32-1	+	+	-	-	-	-
32	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	5129-60-2	+	-	+	-	-	-
33	Phenol, 3-pentadecyl-	$C_{21}H_{36}O$	501-24-6	+	+	-	-	-	-
34	Tetradecanoic acid	$C_{14}H_{28}O_2$	544-63-8	+	-	+	-	+	-
35	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	124-06-1	+	+	-	-	-	-
36	Tricosane	$C_{23}H_{48}$	638-67-5	-	-	+	-	-	+

Table 8: List of compounds present in all species of Pistacia integerrima (PI), Quercus infectoria (QI) and Terminalia chebula (TC)by GC-MS/MS.

S. No.	Compound name	Molecular weight	Structure
1	1,2,3-Benzenetriol	126.11	
2	2,4-Di-tert-butylphenol	206.32	
3	Bisphenol C	281.1	

4	cis-Vaccenic acid	282.5	
5	Ethyl Oleate	310.5	
6	Hexadecanoic acid, ethyl ester	284.5	
7	n-Hexadecanoic acid	256.42	
8	Nonacosane	408.8	
9	Tetradecanoic acid	228.37	

V. CONCLUSION

The study was initiated to develop a GC-MS/MS method to analyze pesticide residues in herbal formulation. A rapid and sensitive quantitative method is always a major goal for analytical laboratories involved in pesticide analysis. The approach was proven to be linear, specific, recoverable, and repeatable with little time spent on sample preparation. This technique is useful for finding and verifying the presence of minute amounts of pesticides in challenging matrices, like herbal churnas. The technique may be able to identify trace amounts of chemicals at 5µg/kg of concentration. Finding out which pesticides are present in herbs is crucial for evaluating consumer safety and preventing long-term use-related chronic toxicity. Creating regulatory standards for the management of pesticide residues in herbal products would benefit greatly from the development of sensitive and straightforward analytical techniques. In light of this, a sensitive and quick method has been developed for the extraction of multiclass pesticides from various popular medicinal herbs in India. The method that has been standardized in this study will also be very helpful in monitoring market samples of herbal churna to guarantee food safety and quality for consumers across the globe.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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