Phytochemical profiling of aqueous methanol extract of *Terminalia bellirica* from Bokaro district of Jharkhand, India

ABSTRACT

Plants are a vital source of medicine for human being from ancient times. Various plant parts are highly medicinal due to the presence of different bioactive compounds and secondary metabolites. Many of these compounds present in medicinal plants are unknown to the scientific community. *T. bellirica* is distributed in tropical parts of the world and is a known ethnomedicinal plant that is the reservoir of various bioactive compounds. The present investigated the bioactive and secondary metabolite present in the fruits of *T. bellirica* using Gas chromatography-Mass spectrometry analysis.

The investigation found 55 compounds from the methanol extract of the fruit sample of *T. bellirica*. Among the recorded compounds, Pyrogallol is the major constituent with 85% area in the chromatogram, followed by 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)with 5% area, and the rest of the compounds are altogether 10% of the total.

In conclusion,the present study recorded and highlighted a total of 55 bioactive compounds from the dry fruits and pericarp, which is more than a double of the compounds earlier known from the plant species. Further, among the recorded biochemical compounds, the concentration of Pyrogallol was alone more than 85% of the total compounds found from the methanol extract of the fruit sample of *T. bellirica*.

Key-words: Phytochemical, Beheda, Bioactive compounds, Ethno-medicine, Bokaro

INTRODUCTION

Plants are a vital natural source of medicine for the treatment and cure of various health problems of human beings as well as of a large number of animals. These medicines are used and practiced as ethnomedicine by human beings for a long time. Ethnomedicine is very common for rural people across the world and is mainly dominating in areas inhabiting

indigenous communities. Among the ethnomedicines, Triphala is a famous ayurvedic formulation, commonly practiced and prescribed by healers/practitioners in India. Triphala is acombination of the plant parts of *Terminalia chebula* (blackmyrobalan), *Terminalia bellirica* (bastard myrobalan), and *Phyllantusemblica* (emblic myrobalan or Indian gooseberry).It has been used as a laxative in chronic constipation, colon cleansing, digestion problems, and poor food assimilation [1-2].

The study was conducted on the ethnobotany among the indigenous communities of the Bokaro district of Jharkhand, India, which is located in the Chhotanagpur plateau and is one among the 25 Biotic Provinces of India which is under the Deccan peninsula Biogeographic Zone. Jharkhand is a tribal-dominated landscape in India and more than 70% of the rural people use ethnomedicine for treating common health problems. Among the three plants used in the formulation of Triphala, *Terminaliachebula*and *Terminalia bellirica* are native and common plants of the Chhotanagpur parts of Jharkhand. *Terminalia bellirica*, commonly known as Beheda is a large deciduous tree belonging to the family Combretaceae. The fruit of *Terminalia bellirica* is used by local healers for curing various types of health problems.

T. bellirica is also widely used in Unani, Siddha, and Chinese systems of traditional medicine[3-5]. They have a range of pharmacological activitiessuch as laxative, antioxidant, antidiabetic, analgesic, antiulcer, antifungal, antibacterial,anti-helminthic, anti-pyritic properties and anti-hypertensive activity through in-vitro and in-vivo studies[4-15]. The dried ripe fruit of *T. bellirica*is used against various health problems like hepatitis, bronchitis, asthma, dyspepsia, piles, cough, diarrhea, dropsy, leprosy, eye disease, scorpion-sting and as a hair tonic[4,5,15-16]. The fruits of *T. bellirica* have several ethnomedicinal uses by people and have multiple medicinal properties identified by various workers from time to time[6, 9, 17-28].

The chemical composition of Triphala has been identified and described by various workers, while the chemical composition of *T. bellirica* has been investigatedby various workers. The chemical compound groups viz. phenol, carbohydrate, protein, Alkaloid, anthraquinone glycoside, saponins, flavonoids, polysaccharides, Steroids, and Tannin were derived from the fruit of *T. bellirica* which are recorded from various workers' time to time[19,29-31]. Further, various chemical compounds/secondary metabolites derived from *T. bellirica* by various workers[32-37].

However, a detailed account of the phytochemicals present in the fruit of *T. bellirica* has been lacking. Therefore, this study was conducted to understand the chemical constituents of the *T. bellirica* collected from the forests of Bokaro district, Jharkhand.

MATERIALS AND METHODS

Fruit collection and sample preparation

The dry fruits of *Terminalia bellirica*(Beheda) werecollected from the forests of Bokaro district, Jharkhand. From the dry fruit, seeds were removed and the peri-carp of the fruit was dried and crushed it to powder form using an electric blender. A total of 2.5 gmof dried powder of the fruit was steeped in 25ml sterile distilled water with occasional shaking for two days and then filtered. The filtrate was simply subject to a rotary evaporator and then left overnight for drying.

Sample Analysis

The sample was dissolved in Methanol and injected in a GC-MS QP2010 model (Shimadzu®), Column, GC, SH-I-5Sil MS Capillary, 30m x 0.25mm x 0.25um, injection mode: Split less. The operating conditions of the GC–MS set for the analysis were as follows:

oven temperature at 45 °C for 2 min then 140 °C at 5°C/ min and finally increased to 280 °C and held isothermally for 10 min. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample components was carried out at 70 eV. The running time of the GC was from 9.10 min – 52.0 min. NIST14.L library (2020) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST14.L)

RESULTS AND DISCUSSION

The GC-MS analysis revealed a total of 55 phytochemicals found from the fruit sample of *T. bellirica* belonging to various types of chemical compounds such as phenol, flavonoid,terpenoid, etc (Table 1 and Table 2). Among the phytochemicals recorded from the fruit sample of *T. bellirica*, the concentration of 1,2,3-Benzenetriol is the highest which is alone 85% area of the total, followed by 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.32%), n-Hexadecanoic acid (0.97%), Ethanamine, N-ethyl-N-nitroso-(0.64%), 5-Hydroxymethylfurfural (0.56%), while rest of the concentration of phytochemicals are of less than 0.5% (Table-1 and Figure1). 1,2,3-Benzenetriol is commonly known as Pyrogallol which is a plant metabolite.

The chromatogram shown in figure 1 showed the R. Time of each of the phytochemicals present in the sample in which the R. Time peak of 1,2,3-Benzenetriol was on 21.59 minutes and of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-on 14.68 minutes. Similarly, the highest area (%) and height (%) ratio were observed for1,2,3-Benzenetriol(29.28), followed by 2-Furancarboxaldehyde, 5-methyl- (9.65), 1-Deoxy-d-arabitol (8.07), Benzoic acid, 4-hydroxy-, pentyl ester (8.04) and Octadecanoic acid, ethenyl ester (7.45) (Table 1).

Table 1: GC-MS Analysis results of the *T. bellirica* fruit sample

Peak	R.Time	F.Time	Area	Area (%)	Height	Height (%)	A/H	Name
1	6.584	6.660	192496	0.05	57431	0.25	3.35	3-Furanmethanol
2	7.163	7.270	252085	0.07	60548	0.26	4.16	Ethanamine, 2-methoxy-N-(2-methoxyethyl)-N-methyl-
3	7.549	7.650	420259	0.12	75032	0.33	5.60	L-Lactic acid
4	7.706	7.855	384274	0.11	139726	0.61	2.75	Oxime-, methoxy-phenyl-_
5	7.902	8.025	213426	0.06	58831	0.26	3.63	N-(n-Butoxymethyl)acrylamide
6	8.127	8.205	137681	0.04	46086	0.20	2.99	2-Cyclohexen-1-ol
7	8.250	8.350	527102	0.15	189663	0.83	2.78	1,2-Cyclopentanedione
8	9.009	9.140	951588	0.27	98634	0.43	9.65	2-Furancarboxaldehyde, 5-methyl-
9	9.755	9.825	1467079	0.41	630805	2.76	2.33	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
10	9.862	9.945	205989	0.06	48393	0.21	4.26	Phenol
11	10.235	10.310	213278	0.06	45247	0.20	4.71	Triethylenediamine
12	10.338	10.425	119201	0.03	44420	0.19	2.68	[(2-Amino-3-hydroxypropanoyl)amino]acetic acid
13	10.915	10.975	121090	0.03	46653	0.20	2.60	2,5-Furandione, dihydro-3-methylene-
14	11.641	11.685	190762	0.05	78339	0.34	2.44	2-Thiazolamine, 4,5-dihydro-
15	11.741	11.885	788497	0.22	159581	0.70	4.94	[(2-Amino-3-hydroxypropanoyl)amino]acetic acid
16	12.197	12.250	797516	0.22	244787	1.07	3.26	Furaneol
17	12.519	12.570	131393	0.04	57688	0.25	2.28	Cyclotrisiloxane, hexamethyl-
18	12.768	12.800	353569	0.10	104572	0.46	3.38	3-Furancarboxylic acid
19	13.202	13.255	295852	0.08	39713	0.17	7.45	Octadecanoic acid, ethenyl ester
20	13.670	13.780	394856	0.11	67491	0.29	5.85	Maltol
21	14.032	14.095	356700	0.10	103771	0.45	3.44	4-Methylpentyl pentanoate #
22	14.335	14.490	2307845	0.64	485977	2.12	4.75	Ethanamine, N-ethyl-N-nitroso-
23	14.684	14.825	19083895	5.32	3513568	15.35	5.43	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
24	14.986	15.065	226648	0.06	79921	0.35	2.84	Dehydromevalonic lactone
25	15.428	15.485	500983	0.14	137006	0.60	3.66	Benzoic acid
26	15.821	15.940	922205	0.26	219904	0.96	4.19	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
27	15.984	16.090	227154	0.06	59336	0.26	3.83	5-(Hydroxymethyl)dihydrofuran-2(3H)-one
28	16.362	16.545	1689621	0.47	316877	1.38	5.33	Catechol
29	16.602	16.710	1158399	0.32	286484	1.25	4.04	Dianhydromannitol

Peak	R.Time	F.Time	Area	Area (%)	Height	Height (%)	A/H	Name
30	16.882	16.935	120140	0.03	39436	0.17	3.05	4-Vinylphenol
31	17.059	17.280	2012214	0.56	288301	1.26	6.98	5-Hydroxymethylfurfural
32	17.866	17.970	288057	0.08	85867	0.38	3.35	1,2-Benzenediol, 3-methoxy-
33	18.443	18.580	934062	0.26	115721	0.51	8.07	1-Deoxy-d-arabitol
34	18.632	18.730	357656	0.10	75666	0.33	4.73	Hydroquinone
35	18.784	18.930	702348	0.20	238830	1.04	2.94	Cyclotetrasiloxane, octamethyl-
36	19.381	19.495	1387676	0.39	429737	1.88	3.23	Cyclotetrasiloxane, octamethyl-
37	19.765	19.915	106389	0.03	25323	0.11	4.20	1-(Methylthio)-3-pentanone
38	19.955	20.070	337637	0.09	71910	0.31	4.70	1H-Pyrazole-3-carboxylic acid, 1-methyl-
39	20.156	20.235	880809	0.25	195111	0.85	4.51	Decanoic acid, 3-methyl-
40	20.339	20.450	256359	0.07	65223	0.28	3.93	Glutaric acid, 1-naphthyl tridecyl ester
41	21.589	22.320	306431498	85.38	10466161	45.73	29.28	1,2,3-Benzenetriol
42	22.721	22.790	155034	0.04	43760	0.19	3.54	2-Propenoic acid, 3-phenyl-
43	24.047	24.120	444917	0.12	107575	0.47	4.14	Cyclopentasiloxane, decamethyl-
44	25.541	25.710	1432424	0.40	178124	0.78	8.04	Benzoic acid, 4-hydroxy-, pentyl ester
45	30.552	30.655	379208	0.11	55827	0.24	6.79	Ethyl gallate
46	31.655	31.810	1181083	0.33	260146	1.14	4.54	Nonylamine, N,N-di(allyl)-
47	31.859	31.930	195838	0.05	87536	0.38	2.24	Tetradecanoic acid
48	32.776	32.865	356091	0.10	102892	0.45	3.46	Succinic acid, 3-methylbut-2-en-1-yl 3-methoxyphenyl ester
49	34.546	34.650	3475355	0.97	1517	016	6.63	n-Hexadecanoic acid
50	36.051	36.135	303806	0.08	68814	0.30	4.41	3-Methoxy-5-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulen-2-yl acetate
51	37.120	37.160	164249	0.05	54504	0.24	3.01	9,12-Octadecadienoic acid (Z,Z)-
52	37.227	37.310	304319	0.08	83987	0.37	3.62	cis-9-Hexadecenal
53	37.691	37.805	1040363	0.29	309416	1.35	3.36	Octadecanoic acid
54	42.805	42.850	357346	0.10	176635	0.77	2.02	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
55	44.739	44.805	195322	0.05	69290	0.30	2.82	Octadecanoic acid, 2,3-dihydroxypropyl ester
56	45.389	45.460	277271	0.08	104765	0.46	2.65	13-Docosenamide, (Z)-
57	45.767	45.815	190679	0.05	75117	0.33	2.54	Squalene
			358899593	**100.00**	**22889174**	**100.00**		

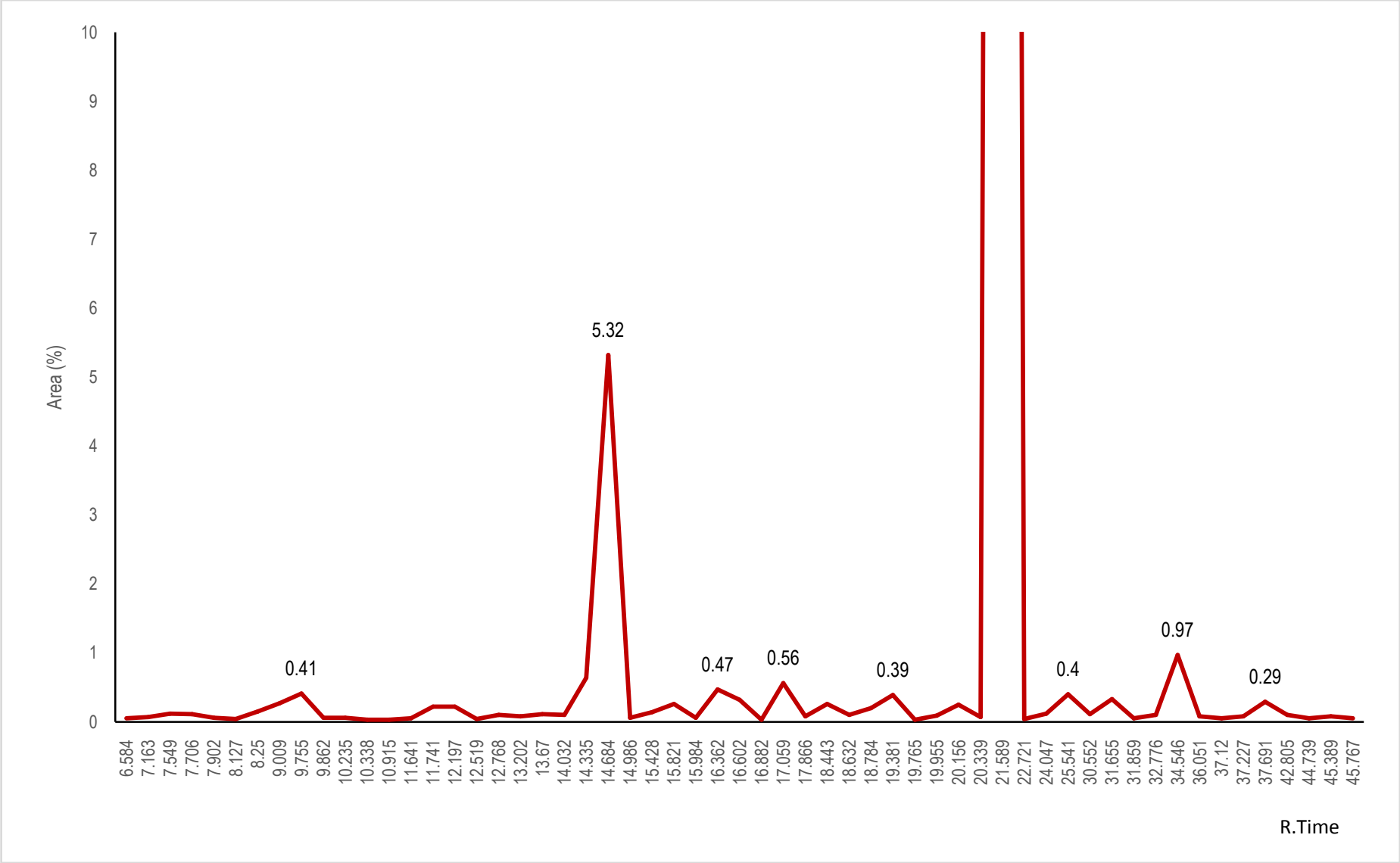
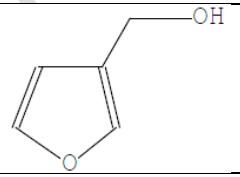
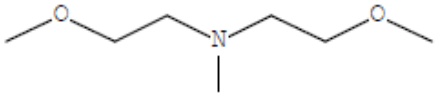
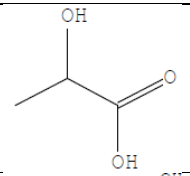
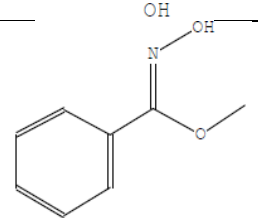
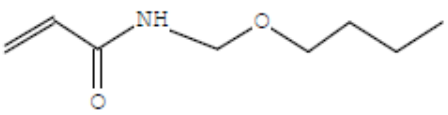
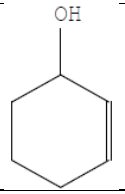
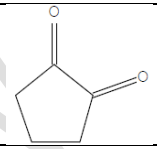
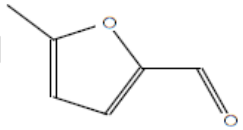
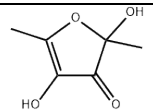
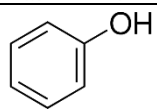
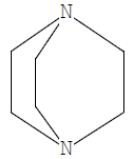
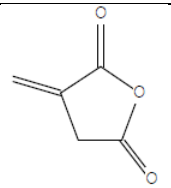
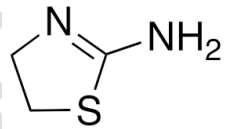
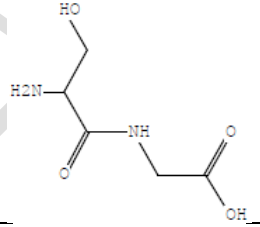
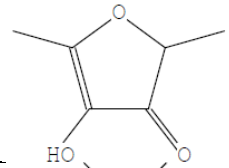
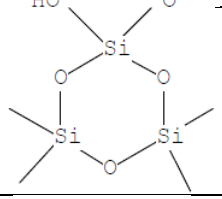
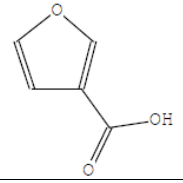
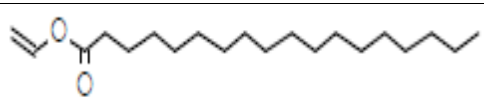
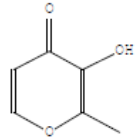


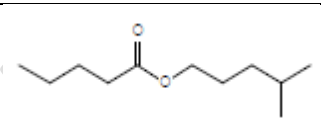
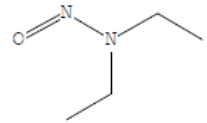
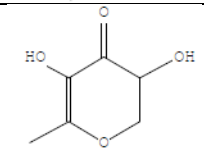
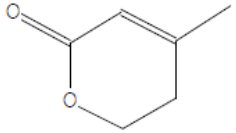
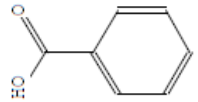
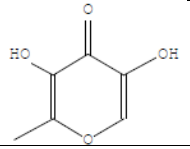
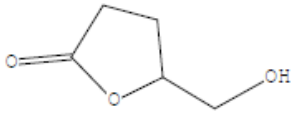
Figure 1: GC-MS Chromatogram of fruits extract of *Terminalia Billerica*

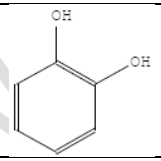
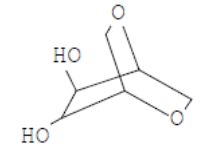
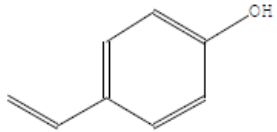
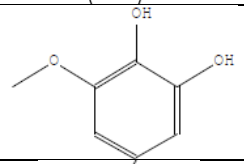
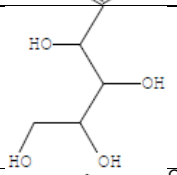
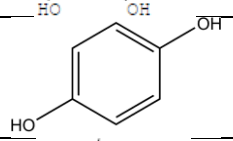
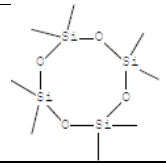
Table 2: Chemical compound and their composition recorded from the *T. bellirica* sample using GC-MS analysis

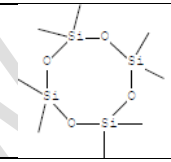
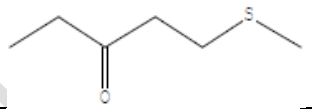
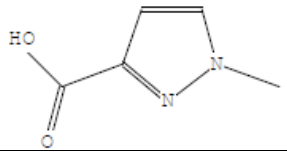
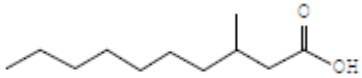
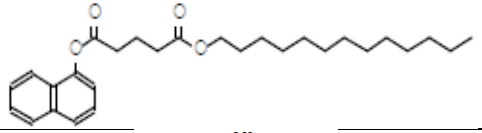
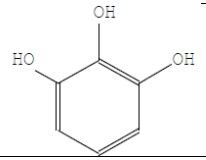
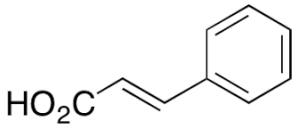
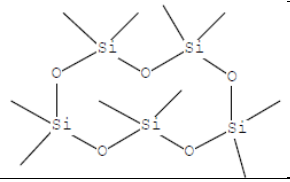
Peak	R.Time	Area%	Height%	Name of the compound	Chemical formula	Molecular structure
1	6.584	0.05	0.25	3-Furanmethanol	C5H6O2	
2	7.163	0.07	0.26	Ethanamine, 2-methoxy-N-(2-methoxyethyl)-N-methyl-	C7H17NO2	
3	7.549	0.12	0.33	L-Lactic acid	C3H6O3	
4	7.706	0.11	0.61	Oxime-, methoxy-phenyl-_	C8H9NO2	
5	7.902	0.06	0.26	N-(n-Butoxymethyl) acrylamide	C8H15NO2	
6	8.127	0.04	0.20	2-Cyclohexen-1-ol	C6H10O	

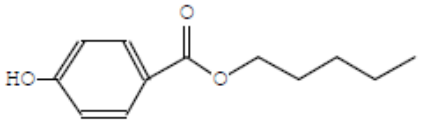
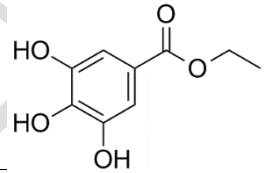
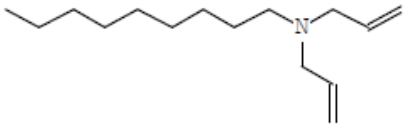
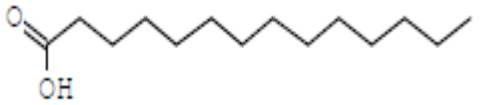
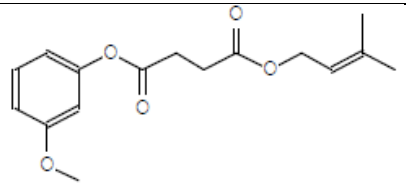
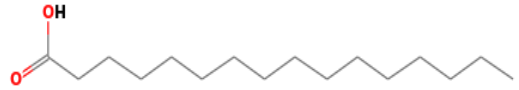
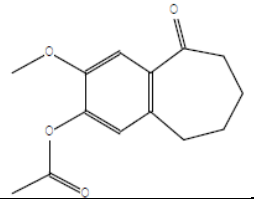
Peak	R.Time	Area%	Height%	Name of the compound	Chemical formula	Molecular structure
7	8.250	0.15	0.83	1,2-Cyclopentanedione	C5H6O2	
8	9.009	0.27	0.43	2-Furancarboxaldehyde, 5-methyl-	C6H6O2	
9	9.755	0.41	2.76	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C6H8O4	
10	9.862	0.06	0.21	Phenol	C6H6O	
11	10.235	0.06	0.20	Triethylenediamine	C6H12N2	
12	10.338	0.03	0.19	[(2-Amino-3-hydroxypropanoyl) amino] acetic acid	C5H10N2O4	
13	10.915	0.03	0.20	2,5-Furandione, dihydro-3-methylene-	C5H4O3	

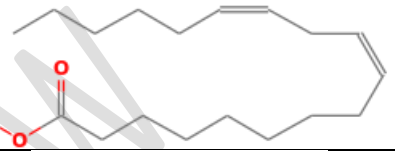
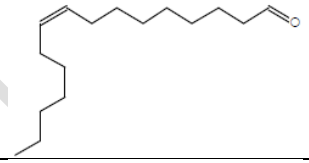
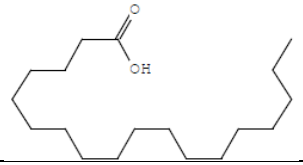
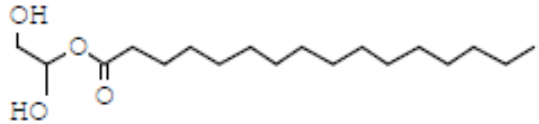
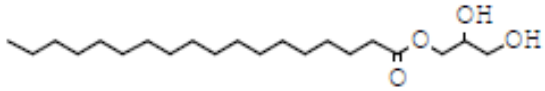
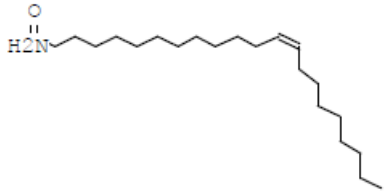
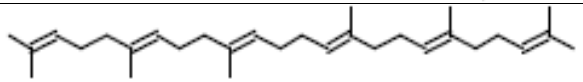
Peak	R.Time	Area%	Height%	Name of the compound	Chemical formula	Molecular structure
14	11.641	0.05	0.34	2-Thiazolamine, 4,5-dihydro-	C3H6N2S	
15	11.741	0.22	0.70	[(2-Amino-3-hydroxypropanoyl) amino] acetic acid	C5H10N2O4	
16	12.197	0.22	1.07	Furaneol	C6H8O3	
17	12.519	0.04	0.25	Cyclotrisiloxane, hexamethyl-	C6H18O3Si3	
18	12.768	0.10	0.46	3-Furancarboxylic acid	C5H4O3	
19	13.202	0.08	0.17	Octadecanoic acid, ethenyl ester	C20H38O2	
20	13.670	0.11	0.29	Maltol	C6H6O3	

Peak	R.Time	Area%	Height%	Name of the compound	Chemical formula	Molecular structure
21	14.032	0.10	0.45	4-Methylpentyl pentanoate	C11H22O2	
22	14.335	0.64	2.12	Ethanamine, N-ethyl-N-nitroso-	C4H10N2O	
23	14.684	5.32	15.35	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	
24	14.986	0.06	0.35	Dehydromevalonic lactone	C6H8O2	
25	15.428	0.14	0.60	Benzoic acid	C6H7O2	
26	15.821	0.26	0.96	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	C6H6O4	
27	15.984	0.06	0.26	5-(Hydroxymethyl) dihydrofuran-2(3H)-one	C5H8O3	

Peak	R.Time	Area%	Height%	Name of the compound	Chemical formula	Molecular structure
28	16.362	0.47	1.38	Catechol	C6H6O2	
29	16.602	0.32	1.25	Dianhydromannitol	C6H10O4	
30	16.882	0.03	0.17	4-Vinylphenol	C8H8O	
31	17.059	0.56	1.26	5-Hydroxymethylfurfural	C6H6O3	
32	17.866	0.08	0.38	1,2-Benzenediol, 3-methoxy-	C7H8O3	
33	18.443	0.26	0.51	1-Deoxy-d-arabitol	C5H12O4	
34	18.632	0.10	0.33	Hydroquinone	C6H6O2	
35	18.784	0.20	1.04	Cyclotetrasiloxane, octamethyl-	C8H24O4Si4	

Peak	R.Time	Area%	Height%	Name of the compound	Chemical formula	Molecular structure
36	19.381	0.39	1.88	Cyclotetrasiloxane, octamethyl-	C8H24O4Si4	
37	19.765	0.03	0.11	1-(Methylthio)-3-pentanone	C6H12OS	
38	19.955	0.09	0.31	1H-Pyrazole-3-carboxylic acid, 1-methyl-	C5H6N2O2	
39	20.156	0.25	0.85	Decanoic acid, 3-methyl-	C11H22O2	
40	20.339	0.07	0.28	Glutaric acid, 1-naphthyl tridecyl ester	C28H40O4	
41	21.589	85.38	45.73	1,2,3-Benzenetriol	C6H6O3	
42	22.721	0.04	0.19	2-Propenoic acid, 3-phenyl-	C9H8O2	
43	24.047	0.12	0.47	Cyclopentasiloxane, decamethyl-	C10H30O5Si5	

Peak	R.Time	Area%	Height%	Name of the compound	Chemical formula	Molecular structure
44	25.541	0.40	0.78	Benzoic acid, 4-hydroxy-, pentyl ester	C12H16O3	
45	30.552	0.11	0.24	Ethyl gallate	C9H10O5	
46	31.655	0.33	1.14	Nonylamine, N,N-di(allyl)-	C15H29N	
47	31.859	0.05	0.38	Tetradecanoic acid	C14H28O2	
48	32.776	0.10	0.45	Succinic acid, 3-methylbut-2-en-1-yl 3-methoxyphenyl ester	C16H20O5	
49	34.546	0.97	016	n-Hexadecanoic acid	C16H32O2	
50	36.051	0.08	0.30	3-Methoxy-5-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulen-2-yl acetate	C14H16O4	

Peak	R.Time	Area%	Height%	Name of the compound	Chemical formula	Molecular structure
51	37.120	0.05	0.24	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	
52	37.227	0.08	0.37	cis-9-Hexadecenal	C16H30O	
53	37.691	0.29	1.35	Octadecanoic acid	C18H36O2	
54	42.805	0.10	0.77	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C9H38O4	
55	44.739	0.05	0.30	Octadecanoic acid, 2,3-dihydroxypropyl ester	C21H42O4	
56	45.389	0.08	0.46	13-Docosenamide, (Z)-	C22H43NO	
57	45.767	0.05	0.33	Squalene	C30H50	
		100.00	100.00			

DISCUSSION

Triphala contains 177 bioactive compounds including 114 from *E. officinalis*, 25 from *T. bellirica* and rest of 63 from *T. chebula* respectively as per Universal Natural Product Database (UNPD). Bioactive such as chebulanin, ellagic acid, gallussaeure, 1,6-digalloyl-beta-d-glucopiranoside, methyl gallate, and tannic acid are common among the three plants[38].

A total of 50 compoundswere recorded from the leaf extract of *T. bellirica*[39]. The present study revealed a total of 55 phytochemicals derived from the fruit sample of *T. bellirica*. The Duke phytochemicals and ethnobotanical database showed a total of 27 phytochemicals observed by various authors[34, 40-42],while UNPD enlisted 25 bioactive compounds from the fruit and pericarp of *T. bellirica*. Among the recorded bioactive compounds and secondary metabolites from the methanol extract of fruits of *T. bellirica*, Pyrogallol was recorded in the highest concentration. It has a highly cytotoxic effect which was recorded on human lung cancer cell lines and less effect on human bronchial epithelium cell lines[43].

The other major phytochemical recorded from the sample was 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- known as DDMP, has received much attention for its antifungal activity toward wood-degrading fungi of rubberwood[44]. Strong antioxidant activity of DDMP was also reported by many researchers[45-46].

CONCLUSION

T. bellirica is an important medicinal plant for the rural people and is one of the three constituents of Triphala. It is known for its use in the treatment of various health problems. Various bioactive or secondary metabolites present in the fruits of *T. bellirica* play important role in curing various health problems. Though Triphala contains 177 bioactive compounds in which only 25 bioactive compounds were recorded from the fruits of *T. bellirica*, the present study found 55 bioactive compounds from the dry fruits and pericarp, which is more than double of the compounds known from the plant species. Among the recorded compounds from the methanol extract of the fruit sample,the concentration of Pyrogallol was alone more than 85% of the total compounds found through GC-MS analysis.

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