



**COMPARATIVE EVALUATION OF PHYTOCOMPONENTS PRESENT IN THE METHANOLIC EXTRACT OF *TERMINALIA CHEBULA* RETZ., *TERMINALIA BELLIRICA* ROXB., AND *PHYLLANTHUS EMBLICA* L., FRUIT EXTRACTS USING GC-MS ANALYSIS**

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**ABSTRACT**

*Terminalia chebula*, *Terminalia bellirica* and *Phyllanthus emblica* are known as Haritaki, Bibhitaki and Amlaki and involve in the holistic system of medicine, used by natural healers. The main aim of the study was the identification of phytochemicals present in the methanol fruit extracts and to evaluate their major compounds present and its nature and activities with the aid of GC-MS technique which may provide an insight to the researchers. Powdered fruit sample was extracted with Methanol as solvent using Soxhlet apparatus. GC-MS analysis of fruits extract were performed using Perkin-Elmer GC Clarus 500 system and gas chromatograph interfaced to Mass Spectrometer equipped with a Elite-5MS (30mx250µm) composed of 5% Phenyl 95% dimethylpolysiloxane. Chromatogram showing total of 24, 26 & 27 compounds was identified from mesocarp and pericarp fruits. Phytochemicals had the universal properties like 1,2,3 Benzenetriol, 2-Furancarboxaldehyde, D-Allose, Furfural, n-Hexadecanoic acid, 13-Octadecenal, (Z)-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Levoglucosenone, Phenol, Octadecanoic acid, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 1,3,5-Triazine-2,4,6-triamine, 1,6,3,4-Dianhydro-2-deoxy-a-d-lyxohexopyranose, 1H-1,3-Diazepine, 4,5,6,7-tetrahydro-2-methyl, Butanedioic acid, hydroxy-(S)-, 5-Oxotetrahydrofuran-2-carboxylic acid, methyl ester, 2,4(1H,3H)-Pyrimidinedione, 6-(hydroxymethyl)-1,3-dimethyl and 1,6-Anhydro-a-D-glucofuranose. This study possesses several bioactive compounds have been evaluated and justifies their uses for various ailments by traditional practitioners and logical approach for drug formulation, discovery and it may open a door to the society.

**KEY WORDS:** GC-MS, 1,2,3 Benzenetriol, Phytocomponents, *Terminalia chebula*, *Terminalia bellirica* and *Phyllanthus emblica*.



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## INTRODUCTION

Plants are used as medicine in many countries and also act as a source for many potent drugs<sup>1</sup>. Natural remedies from medicinal plants proved as safe and effective. Many plant species have been used in folklore medicine to treat various ailments<sup>2</sup>. Phytocompounds from medicinal plants are important in pharmaceutical industry for drug development and preparation of therapeutic agents<sup>3</sup>. The development of pharmaceuticals begins with the identification of active principles, detailed biological assays and dosage formulations followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drug<sup>4</sup>. Plants and fruits are considered as one of the main sources of biologically active compounds. An estimate of the World Health Organization (WHO) states that around 85-90% of the world's population consumes traditional herbal medicines<sup>5</sup>. Triphala is a herbal formulation used in the ancient science of Ayurveda. The word Triphala is translated as three fruits. These fruits are also known as myrobalan plums are Amalaki, Bibhitaki and Haritaki<sup>6</sup>. There is a folk saying in India which says, "No Mother? Do not worry so long as have Triphala". This is an allusion to the belief that Triphala cares for internal organs, much in the same way a mother takes care of her children and among laxative herbs, triphala is the safest and escalated quality. Plants are rich source of secondary metabolites with interesting biological activities. Distinguished examples of these compounds include flavanoids, phenols, saponins and cyanogenic glycosides<sup>7</sup>. GC-MS is a valuable tool for reliable identification of bioactive compounds<sup>8</sup>. According to the Ayurveda Encyclopedia, amalaki works on all tissues, and the "Circulatory, digestive and excretory" systems, it further lists the actions of amalaki as "Aphrodisiac, astringent, hemostatic, laxative, nutritive tonic, refrigerant, rejuvenative and stomachic"<sup>9</sup>. Bibhitaki is a herb that works on the "plasma, muscle, bone" tissues and the "digestive, excretory, nervous and respiratory" systems. Further, the actions of Bibhitaki are "Anthelminthic, antiseptic, astringent, expectorant, laxative, lithotriptic, rejuvenative and tonic"<sup>10</sup>. Haritaki will "eliminate the doshas", stimulating digestion and promotes longevity<sup>11</sup>. Actions are "rejuvenative, tonic,

astringent, laxative, nervine, expectorant and anthelmintic". *Terminalia bellirica* and *Phyllanthus emblica* have more phytochemical, nutraceutical, pharmaceutical, antimicrobial and antioxidant properties. Methanol extract of *Terminalia bellirica* and *Phyllanthus emblica* have more antibacterial activity and antifungal activity which was proved in disc diffusion method and Food poisoning technique<sup>12</sup>. *Terminalia chebula* has antifungal, antibacterial, antidiabetic, antiproliferative, antiarthritic, anticaries and has multiprotective effects such as hepato-protective, pulmonary-protective, renal-protective, radio-Protective, cyto-protective and cardio-Protective effects<sup>13</sup>. The present communication deals with the GC-MS analysis of phytochemicals present in the methanolic fruit extracts.

## MATERIALS AND METHODS

### (i) Sample Collection and authentication

*Terminalia chebula* Retz., *Terminalia bellirica* Roxb. and *Phyllanthus emblica* L. were collected from hill areas, Atthipattu (Thiruvannamalai), Therambattu (Vellore) and Sirumalai (Dindugal). The same was identified and authenticated by Dr. John Britto, Rapinet Herbarium, St. Joseph's College Trichy, Tamilnadu, India and given the Voucher Specimen No. VEA/001/2013, VEA/002/2013 and VEA/003/2003 respectively.

### (ii) Preparation of extracts

Fruits of *Terminalia chebula*, *Terminalia bellirica* and *Phyllanthus emblica* were shade dried, pericarp and mesocarp of fruits were pulverized into fine powder in 1:1:1 ratio using a stainless steel blender. Extracts were prepared by using Soxhlet extractor and 95% Methanol filtrates were individually pooled and each solvent removed at 40°C under reduced pressure by rotary evaporator<sup>14</sup>. The methanol, acetone and aqueous extracts were subjected to preliminary screening of various plant constituents.

### (iii) GC-MS Analysis

GC-MS technique was used in this study to identify the phytochemicals present in the extract; it was carried out in SASTRA University, Thanjavur, Tamilnadu. GC-MS

analysis of this extract was performed using Perkin Elmer Clarus 500 and gas chromatograph interfaced with a mass spectrometer equipped with capillary column Elite-5MS (30MX250 $\mu$ M) composed of 5% Phenyl 95% dimethylpolysiloxane. For GC-MS detection, an electron ionization energy system with ionization energy of 70eV (Electro Volt) was used. Helium gas was used as the carrier gas at a constant flow rate of 1ml/min and injection volume 1.4 $\mu$ l was employed split ratio of 10:1. Injector temperature was 280°C, ion source temperature 200°C, 150°C for 20 minutes. Total GC running time was 20 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatogram was a Turbo Mass Ver.5.2.0. These compounds were detected in the range of 40-600amu (Atomic Mass Unit) by matching with NIST library 2005.

#### (iv)Compound identification

Interpretation of mass spectrum GC-MS was conducted using the data base of National Institute Standard and Technique (NIST) having more than 62000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library, Compound Name, Retention Time, Molecular Weight, Percentage, Compound Nature and their activities were ascertained.

## RESULTS

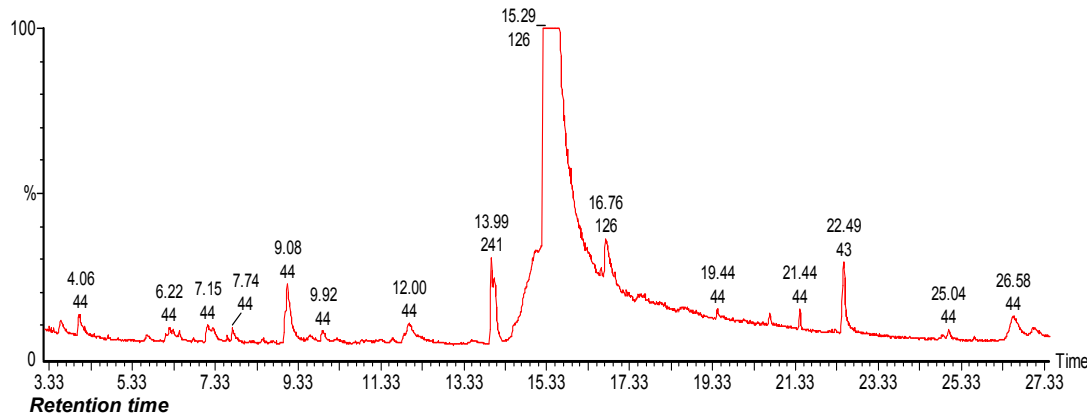
GC-MS is one of the paramount techniques to identify the constituents of volatile matter, long and branched chain hydrocarbons, alcoholic acids and esters etc. Traditional knowledge of medicinal plants has always guided the search for new drugs. Authentication of medicinal plants in genetic and chemical level is a critical step in the use of these botanical materials for both research purposes and commercial preparations<sup>2</sup>.The result pertaining GC-MS analysis leads to a credential number of compounds from GC fractions of the methanolic extract *Terminalia chebula*, *Terminalia bellirica* and *Phyllanthus emblica*. These compounds were identified through mass spectrometer attached with GC-MS. *Terminalia chebula* revealed that Isoglutamine,

Furfural, 2-Cyclopenten-1-one, 2-hydroxy-, Ethanone, 1-(3-ethyloxiranyl)-, 1,2-Cyclohexanedione, Phenol, 2(1H)-Pyridinone, 6-hydroxy-, 3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl-, 5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-, Hydouracil, 1-methyl-, Levoglucosenone, 5-Formyl-3-methyluracil, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 1,4:3,6-Dianhydro- $\alpha$ -d-glucopyranose, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 1,2,3-Benzenetriol, Tetradecanoic acid, Tridecanoic acid, methyl ester, n-Hexadecanoic acid, D-Allose, 9-Octadecenoic acid (Z)-, methyl ester, Octadecanoic acid, methyl ester, 13-Octadecenal, (Z)- and Octadecanoic acid were analyzed experimentally. The detected compounds of *Terminalia bellirica* were 3-Amino-2-oxazolidinone, Furfural, 2-Furanmethanol, 2-Cyclopentene-1,4-dione, 1,2-Cyclopentanedione, 2-Furancarboxaldehyde, 5-methyl-, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Pentanoic acid, 4-oxo-, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 2,4,5-Trihydroxypyrimidine, 1,3,5-Triazine-2,4,6-triamine, 4H-Pyran-4-one, 5-hydroxy-2-methyl-, 4H-Pyran-4-one, 5-hydroxy-2-methyl-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-, 2-Furanmethanol, tetrahydro-5-methyl-, trans-, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, Phenol, 2-methoxy-4-(1-propenyl)-, DL-Proline, 5-oxo-, methyl ester, 1,2,3-Benzenetriol, D-Allose, 1,6-Anhydro- $\alpha$ -d-galactofuranose, Tetradecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 13-Octadecenal, (Z)- and Octadecanoic acid phytochemicals were investigated respectively. *Phyllanthus emblica* showed Butanimidamide, Furfural, 2-Cyclopenten-1-one, 2-hydroxy-, 2,5-Furandione, 3-methyl-, 2-Furancarboxaldehyde, 5-methyl-, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 1H-1,3-Diazepine, 4,5,6,7-tetrahydro-2-methyl-, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, Acrylic acid, 3-amino-3-cyano-, methyl ester, 5-Oxotetrahydrofuran-2-carboxylic acid, methyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, D-Allose, 1,6;3,4-Dianhydro-2-deoxy- $\alpha$ -d-lyxo-hexopyranose, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, Butanedioic acid, hydroxy-, (S)-, Undecanol-5, 2,4(1H,3H)-Pyrimidinedione, 6-

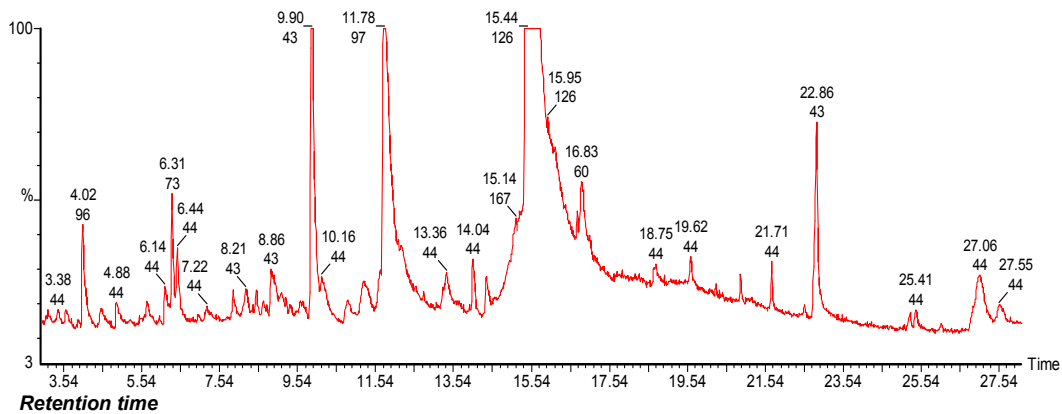
(hydroxymethyl)-1,3-dimethyl-,  
 Benzenetriol, Pyrogallol, 1,6-Anhydro- $\alpha$ -D-  
 glucofuranose, Gluconic acid, Hexadecanoic  
 acid, methyl ester, n-Hexadecanoic acid, 9,12-  
 Octadecadienoic acid, methyl ester, 7-

Hexadecenoic acid, methyl ester, (Z)-,  
 Octadecanoic acid, methyl ester, 13-  
 Octadecenal, (Z)- and Octadecanoic acid were  
 discovered using research tools.

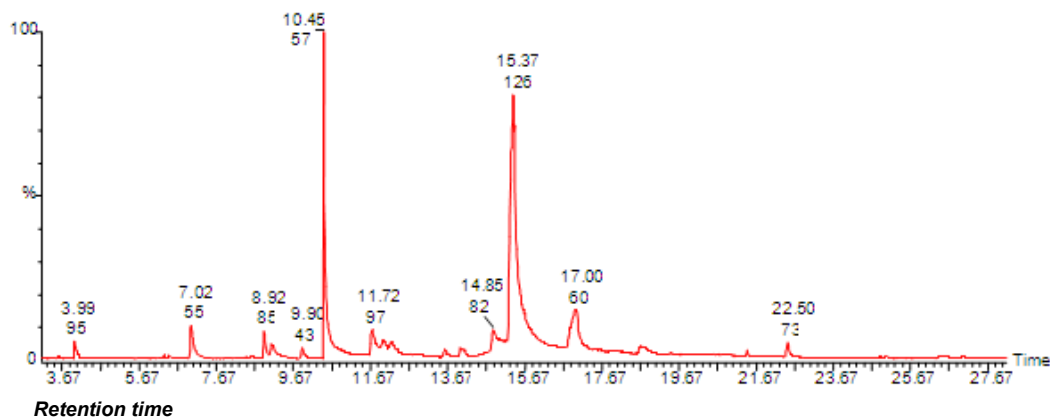
**Figure 1**  
**Analysis of Compounds present in Methanolic Terminalia chebula fruit by GC-MS**



**Figure 2**  
**Analysis of Compounds present in Methanolic Terminalia bellirica fruit by GC-MS**



**Figure 3**  
**Analysis of Compounds present in Methanolic Phyllanthus emblica fruit by GC-MS**



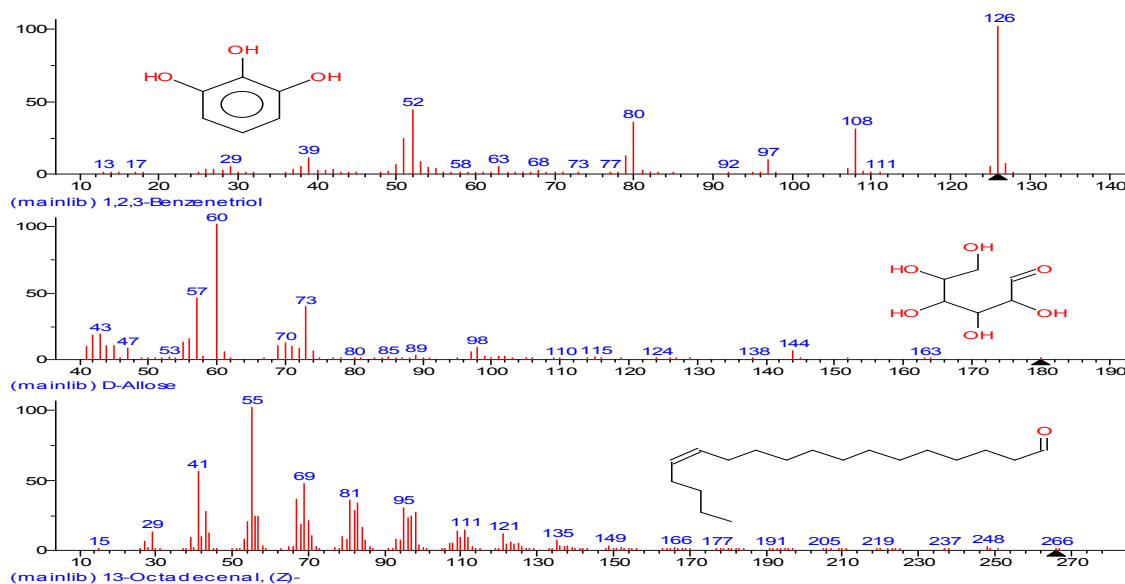
**Table 1**  
**Compounds Identified in Terminalia chebula, Terminalia bellirica and Phyllanthus emblica by GC-MS with their biological activities.**

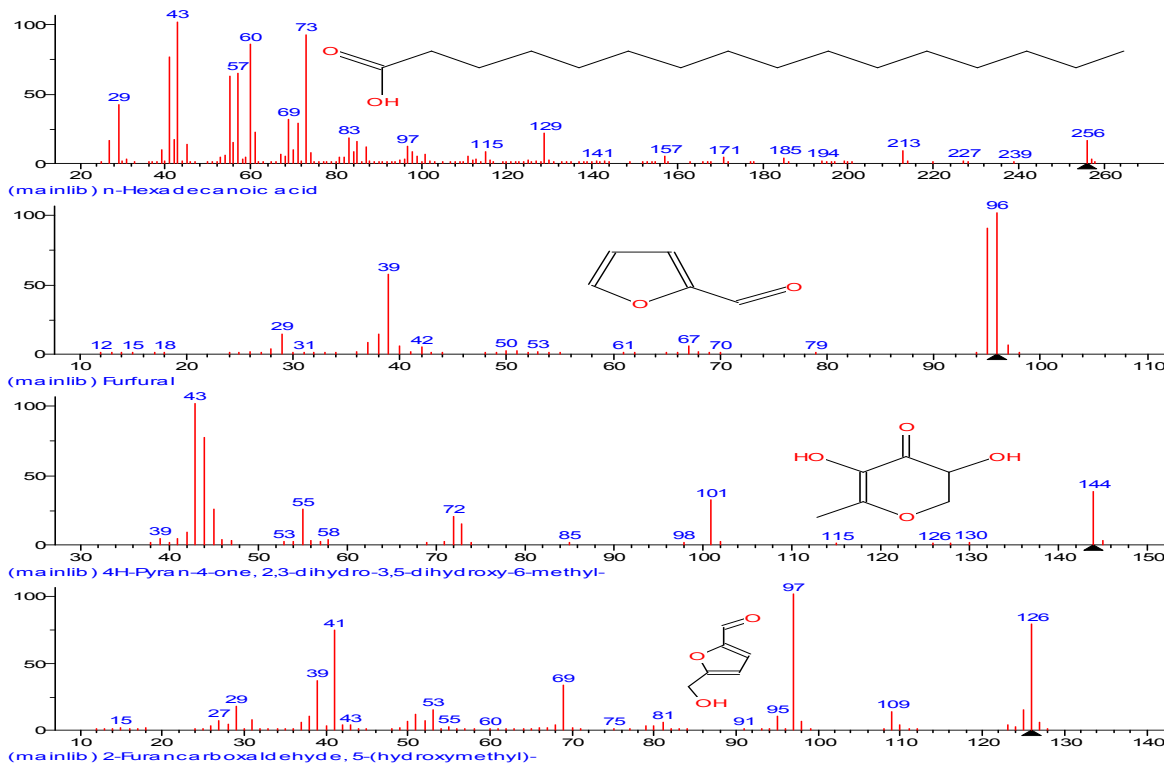
Compound Name	<i>T.chebula</i>			<i>T.bellirica</i>			<i>P.emblica</i>			Mean %	Compound Nature	Activity	
	R.T	M.Wt	%	R.T	M.Wt	%	R.T	M.Wt	%				
1,2,3-Benzenetriol	15.23	126	85.03	15.44	126	52.75	15.37	126	54.73	64.2	Aromatic Alcohol	Antiseptic, Antioxidant, Antidermatitic, Fungicide, Insecticide, Candidicide	
D-Allose	16.74	180	1.34	16.83	180	1.2	17	180	9.93	4.16	Carbohydrate	Antisecretory, Preservative	
13-Octadecenal, (Z)	26.54	266	1.94	27.04	266	3.75	26.5	266	0.83	2.19	Alcohol	Antimicrobial	
n-Hexadecanoic acid	22.48	256	2.29	22.84	256	4.14	22.5	256	1.39	1.6	Fatty Acid Ester	Antioxidant, Hypercholesterolemic, Cancer Preventive, Hepato Protective, Nematicide, Insecticide, Anticarcinory, Antiandrogenic, Hemolytic	
Furfura	4.06	96	0.74	4.02	96	2.38	3.99	96	1.62	1.62	Aldehyde	Antiseptic, Flavour, Fungicide, Pesticide, Insecticide, Irritant	
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	9.92	144	0.45	10.84	144	0.83	9.9	144	1.24	0.83	Flavonoid	Antimicrobial, Anti-inflammatory, Antiproliferable	
2-Furancarboxaldehyde, 5-(hydroxymethyl)	12	126	0.56	6.14	110	0.98	6.14	110	0.03	0.53	Aldehyde	Antimicrobial, Preservative	
Octadecanoic acid	27.07	0.5091	0.53				25.62	298	0.12	0.31	Fatty Acid	Hypercholesterolemic, Antiarthritic, Anti-inflammatory, Hepato Protective, Nematicide, Antimicrobial	
1,6,3,4-Dianhydro-2-deoxy- $\alpha$ -D-lyxohexopyranose	11.32	144	0.15				10.45	128	7.51	3.82	Glucose Moiety	Preservative	
Levogluconone	9.08	126	3.24							3.24	Glucose Moiety	Preservative	
Phenol	7.15	94	0.58							0.58	Alcohol	Antibacterial, Antioxidant, Antiseptic, Antiviral, Fungicide, Cancer Preventive	
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one				6.31	144	2.07					2.07		Not known
1,3,5-Triazine-2,4,6-triamine				8.86	126	1.75					1.75	Organic Base	Cleaning agent
1H-1,3-Diazepine, 4,5,6,7-tetrahydro-2-methyl							7.02	112	4.64	4.65	Carboxylic Acid	Antihypertensive	
Butanedioic acid, hydroxy-(5)							12.22	134	2.81	2.81	Organic Acid	Antiarthritic, Antioxidant, Additive, Cancer Preventive	
5-Oxotetrahydrofuran-2-carboxylic acid, methyl ester							8.92	144	2.58	2.58		Not known	
2,4(1H,3H)-Pyrimidinedione, 5-(hydroxymethyl)-1,3-dimethyl							14.85	170	2.22	2.21	Pyrimidine	Antimicrobial	
1,6-Anhydro- $\alpha$ -D-glucofuranose							18.66	162	2.14	2.16	Sugar Moiety	Preservative	

RT-Retention Time, Mol. Wt-Molecular Weight

Source: Dr. Duke's Phytochemical and ethnobotanical database (Online database)

**Figure 4**  
**Structure and Mass Spectrum of Universal Phytochemicals identified by GC-MS in Methanol Extracts**





## DISCUSSION

There was a growing awareness in correlating the phytochemical compounds and their biological activities<sup>16</sup>. Phytochemicals are natural bioactive compounds found in plants such as vegetables, fruits, medicinal plants, leaves and roots that work with the fibers to act as a defense system against diseases of more accurately, to protect against diseases<sup>17</sup>. The more precise information in qualitative analysis can be obtained by Gas Chromatography coupled with Mass Spectrometry<sup>18</sup>. For quantitative determination, Gas chromatography with flame ionization detector and GC-MS are preferred<sup>19</sup>. GC-MS chromatogram of the methanolic fruit extracts had collective and combined properties of bioactive compounds namely 1,2,3 Benzenetriol, 2-Furancarboxaldehyde, D-Allose, Furfural, n-Hexadecanoic acid, 13-Octadecenal, (Z)-, 4H-Pyran-4-one and 2,3-dihydro-3,5-dihydroxy-6-methyl- which has synergistic effect collectively. These chemicals were suggested as an aromatic alcohol, aldehyde, carbohydrate, palmitic acid and flavanoid compound recommended to be an antiseptic, antioxidant, antidermatitic, fungicide, insecticide, candidicide, antimicrobial, preservative, antisecretory, flavor, pesticide, irritant, nematicide, hepato protective,

anticarony, antiandrogenic, hemolytic and hyper-cholesterolemic respectively. Table-1, Figure 1,2,3 were shown the presence of various compounds retention time, molecular weight and percentage. Among that 1,2,3 Benzenetriol holds the uppermost percentage (85%, 53%, 55%) of the aromatic alcohol compounds recommended as antiseptic, antioxidant, antidermatitic, fungicide, insecticide and candidicide. Pyrogallol is a polyphenol known for its fungicidal and fungistatic properties<sup>20</sup>. Mass Spectrum and structure of Universal Phytochemicals identified by GC-MS in Methanol Extracts were presented in Figure-4. *Terminalia chebula* has the special properties of Levoglucosane the glucose moiety and Phenol an alcoholic compound prescribed as preservative, antibacterial, antioxidant, antiseptic, antiviral, fungicide and cancer preventive correspondingly. Octadecanoic acid the fatty acid compound was present in the methanolic *Terminalia chebula* and *Terminalia bellirica* had been used for hypercholesterolemic, antiarthritic, anti-inflammatory, antimicrobial, hepatoprotective and nematicide. *Phyllanthus emblica* has unique properties like Butanedioic acid, 2,4(1H,3H)-Pyrimidinedione, 6-(hydroxymethyl)-1,3-dimethyl, 1,6-Anhydro- $\alpha$ -D-glucofuranose were independently employed as antiarthritic, antimicrobial and preservative.

Previous study depicted major bioactive components were present in methanolic extract of *Triphala* further screened by GC-MS analysis and revealed the presence of 10 bioactive compounds<sup>21</sup>. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acids and its compounds etc<sup>22</sup>. Gas Chromatography and Mass Spectrometry are highly compatible technique in separation of volatile compound and identifying characteristics of the compound<sup>23</sup>. Presence of various bioactive compounds justifies the use of whole plant for various plants for various ailments by traditional practitioners<sup>24</sup>. The results of the present comparative study gives a bird's eye view on biological and pharmacological properties of methanolic extracts and isolated phytoconstituents of *Terminalia chebula*, *Terminalia bellirica* and *Phyllanthus emblica* to enrich our knowledge through GC-MS analysis.

## CONCLUSION

The results of the present study comparatively indicated the presence of various compounds in *Terminalia chebula*, *Terminalia bellirica* and *Phyllanthus emblica* fruit methanol extract. Discovered 1,2,3 Benzenetriol which was predominantly present and it was detected through GC-MS compound analysis and proved by the literature survey. According to that *Terminalia chebula* holds remarkable quantity 85%, *Terminalia bellirica* possess moderately 53% and *Phyllanthus emblica* acquire low amount 55%, which have reasonably antimicrobial, antioxidant, antiseptic, antidermatitic, fungicide, insecticide and candidicide properties. GC-MS studies concretely illustrated various many other phytochemicals and their pharmacological activities and it is the hallmark to phytochemical, pharmacognostical and biomedical fields to carry out drug formulations and research activities.

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