



## COMPARATIVE GC-MS ANALYSIS OF *PHYLLANTHUS EMBLICA* AND *TERMINALIA BELLIRICA* FRUITS EXTRACT TO IDENTIFY ACTIVE CHEMICAL PHYTO CONSTITUENTS

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### Abstract

**Background:** *Phyllanthus emblica* L. is a native of the family *Phyllanthaceae*, generally famous by Indian gooseberry or amla. It is used as a traditional medicine for the treatment of different diseases. *Terminalia bellirica* (Gaertn.) Roxb is commonly known as Baheda and widely beneficial in the traditional Indian medicinal system for centuries as a remedial source in various diseases.

**Methods:** In the present study, Phytochemical analysis of aqueous methanol fraction of *Phyllanthus emblica* L. (PEAM) and *Terminalia bellirica* (Gaertn.) Roxb (TBAM) fruits was done. Antibacterial activity was tested against *S. aureus* (MTCC 6908), *E. coli* (MTCC 1698), *P. aeruginosa* (MTCC 4306) and *K. pneumoniae* (MTCC 9544). To analyse chemical composition of PEAM and TBAM, GC-MS analysis has been performed.

**Results:** Overall 27 chemical components in PEAM and 38 components in fruits of TBAM were identified. The major components identified in the PEAM fruit extracts were 5-Hydroxymethylfurfural (30.61%), 1,2,3-Benzenetriol (24.78%), 9,12-Octadecadienoic acid (Z,Z) (9.52%) whereas other constituents were present in relatively small amounts. In TBAM fruit extracts the chief chemical components were 1,2,3-Benzenetriol (52.59), Oxacycloheptadec-8-en-2-one (20.66%), 5-Hydroxymethylfurfural (7.85%), respectively.

**Conclusion:** The phytochemical analysis of PEAM and TBAM showed presence of diverse range of phytochemicals and antibacterial assessment of these extracts showed efficacy against different pathogenic bacterial strains. The results of GC-MS analysis illustrated the existence of numerous chemical constituents in fruits of PEAM and TBAM and are recommended as potential drug candidates of pharmaceutical significance.

**Keywords:** Phytochemicals, GC-MS, Pharmaceutical, antibacterial, pathogenic

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## Background

*Phyllanthus emblica* L. (*Emblica officinalis*), is a deciduous tree, belongs to Euphorbiaceae family generally known as 'Aonla' or 'Amla' or 'Indian gooseberry'. According to the age-old Indian mythology 'Amla' is the very first tree is said to be originated on earth. The Amla fruit considered as pool of diverse nutraceuticals such as calcium, methionine, minerals, tryptophane, nicotinic acid, riboflavin, vitamin- C, lysine, phosphorus. Additionally, *P. emblica* is believed to have immunity enhancing capabilities beside various diseases, as well broadly useful in ayurvedic medicine, an Indian traditional medicine system (Bhagat, 2014). The unripe fruit of *P. emblica* is believed to have beneficial effects on health. It has various applications in the pharmaceutical, cosmetic division and food processing. *P. emblica* tree is very well adapted to develop in harsh (saline, wasteland soil) environment, as well, fruits persist in season for approximately a period of ten months. At the present time, *P. emblica* is considered as one of the ideal species for small industries in numerous countries, due to various nutraceutical factors in it. In addition to this, versatility of *P. emblica* in a variety of therapeutic products makes it a potent therapeutic candidate drug (Pathak *et al.*, 2003). India is the native place for *P. emblica*, as well grown in numerous tropical and sub-tropical countries like Malaysia, China, Bangladesh, Myanmar, Mascarene Islands, Sri Lanka, Uzbekistan and Pakistan. Generally, *P. emblica* can found in India at different altitude levels such as tropical, sub-tropical, some coastal regions and hilly areas (upto 4500ft) (Rai *et al.*, 2012; Thilaga *et al.*, 2013). *P. emblica* extracts and preparations possess therapeutic potential against various diseases in a beneficial way as compared to the normal medicines. That is due to its ethnobotanical, therapeutic importance. In addition to this, whole tree parts which include fruit, bark, root, seed, flower are extensively used in the Indian traditional medicine system of Ayurveda (Khan 2009; Kumar *et al.* 2012b). *Terminalia bellerica* (TB) Roxb belongs to Combretaceae family, is a huge deciduous tree and is generally known as beleric mycobalane. It is a traditional worthy herbal plant in ancient and Ayurvedic medicine structure for cure of large array of diseases having numerous therapeutic actions like anticancer, anti-inflammatory, immunomodulatory, hepatoprotective and antimicrobial activities (Saraphanchotiwiththaya and Ingkaninan, 2014). *T. bellirica* is an essential ingredient of traditional laxative formulation, one of the well known Triphala formulation, used since ancient era for

numerous ailments in Ayurvedic medicines (Rashed *et al.*, 2014). Fruits of TB are anthelmintic, analgesic, purgative and antipyretic as well beneficial in dyspepsia, asthma, bronchitis, diarrhea, piles and cough. *T. bellirica* leaf extracts exhibited promising free radical scavenging activity. It augments the immunity against several diseases and is used as potent herbal medicine.

## Methods

### Plant collection and identification

Plants were collected from Dehradun, Uttarakhand, India and crude extracts of fruits of PEAM and TBAM were prepared. Further, voucher specimens (BSI/NRC-114632) and (BSI/NRC-115221) has been preserved in the herbarium of Botanical Survey of India, Northern Regional Centre, Dehradun Uttarakhand, India.

### Preparation of Plant material

Fruit samples of both plants were thoroughly rinsed with running tap water, then shade dried and grinded to form fine powder. Powdered sample (500 g) was taken and extracted with methanol and water (80%) by using a Soxhlet apparatus. Further, obtained extracts were sieved and concentrated with a rotavapour (Strike-12, Steroglass, Italy) and finally formed aqueous methanolic extracts was used for future experiments (Phytochemical, antibacterial and GC-MS analysis).

### Preliminary phytochemical screening

Phytochemical profiling of aqueous methanolic fraction of PEAM and TBAM was done for qualitative analyses of major phytoconstituents likewise phenolics, Saponins, flavonoids, steroids, alkaloids, proteins, tannins, quinones, carbohydrates, coumarins and glycosides. Qualitative analyses of phytochemical of PEAM and TBAM were done according to Badoni *et al.*, 2016.

### Antibacterial activity

Antibacterial activity of PEAM and TBAM was assessed by agar well diffusion assay (Poonkothai *et al.*, 2014). The bacterial strains taken for experiment were *S. aureus* (MTCC-6908), *K. pneumoniae* (MTCC-9544), *E. coli* (MTCC-1698) and *P. aeruginosa* (MTCC-4306). An appropriate amount of bacterial suspension i.e. 100 µl was placed on Luria Bertani agar by using sterilized cotton swabs. Further, the plant extracts (20 µl) was added into wells (5 mm) and the petri plates were kept to permit the dispersion of the extract. In the current study, ampicillin was served as positive control and DMSO was used as negative control.

The width of zone of inhibition, was calculated to assess antibacterial potential by calculating mean of two replicates  $\pm$  SEM.

### GC-MS analysis

GC-MS analysis for both PEAM and TBAM was carried out at University Science Instrumentation Centre, Jawaharlal Nehru University (JNU), Delhi (India). The PEAM and TBAM extracts were analysed on a GC-MS (GCMS-QP2010 Plus, Shimadzu, Japan). An auto injector, a head space sampler, a mass selective detector, an ion source (220 °C), and an interface (260 °C) were all well-equipped for the GCMS. For GC-MS analyses, a Restek Company, Bellefonte, USA, Rtx-5 MS capillary column with dimensions of 30 m (length), 0.25 mm (diameter), and 0.25  $\mu$ m (film thickness) was utilised. With a threshold of 1000 ev, the mass range for analysis was employed in series between 40 and 650 m/z. The setup of injector was placed in the split injection mode which has temperature of 250 °C. The initial temperature was regulated to 80 °C (3 min), that subsequently augmented to 280 °C with a ramp rate of 10 °C/min. Helium was employed as a carrier gas of linear velocity (> 99.99%) with 40.5 cm/s. According to the established techniques, the system programme was set up with a total flow rate of 16.3 ml/min and a column flow rate of 1.21 ml/min. Gas chromatography was used to identify the

ingredients of the sample fruit extracts (PEAM and TBAM) based on retention time (RT), and the mass spectrum data was analysed by comparing the results.

### Results and discussion

#### Phytochemical Analysis

The preliminary phytochemical analyses determined that PEAM possess tannins, phenol, saponins, carbohydrates, Alkaloids, flavonoids and TBAM possess tannins, saponins, Quinones, phenols, carbohydrates, Alkaloids, flavonoids and Carbohydrates.

**Table 1:** Phytochemical constituents in PEAM and TBAM

Phytochemicals	PEAM	TBAM
Alkaloids	+	+
Tannins	+	+
Proteins	-	-
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Quinones	-	+
Coumarins	-	+
Sterols	-	-
Glycosides	-	-
Carbohydrates	+	+

PEAM- *P. emblica* aqueous methanolic extract, TBAM- *T. bellirica* aqueous methanolic extract (+) Present; (-) Absent.

**Table 2:** Antibacterial activity of PEAM and TBAM against Test bacteria

Plant Extracts	PEAM (1mg/ml) (ZOI in mm)	TBAM (1mg/ml) (ZOI in mm)	Positive Control (Ampicillin (1 mg/ml) (ZOI in mm)	Negative Control (DMSO)
<b>Bacterial Strains</b>				
<i>S. aureus</i>	11.5 $\pm$ 0.5	9.5 $\pm$ 0.5	24 $\pm$ 0	0
<i>K. pneumoniae</i>	12.5 $\pm$ 0.5	12 $\pm$ 1	23 $\pm$ 0	0
<i>E. coli</i>	10.5 $\pm$ 1	9 $\pm$ 1	25 $\pm$ 0	0
<i>P. aeruginosa</i>	12 $\pm$ 2	10.5 $\pm$ 0.5	22 $\pm$ 0	0

Antibacterial activity of aqueous methanolic extracts of *P. emblica* and *T. bellirica* fruits. Each value is expressed as the mean  $\pm$  standard error. There is a statistically significant difference ( $p < 0.001$ ). ZOI- Zone of inhibition. PEAM- *P. emblica* aqueous methanolic extract, TBAM- *T. bellirica* aqueous methanolic extract

### GC-MS analysis

The result of GC-MS analysis showed the presence of abundant chemical constituents in fruits of PEAM and TBAM.

**Table 3:** GC-MS analysis of PEAM

S. No.	Name of Compound	R. Time	Area %
1	2-Furancarboxaldehyde, 5-methyl-	3.088	0.14
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3.22	0.37
3	2H-PYRAN-2,6(3H)-DIONE	3.45	6.12
4	1,3-Dioxane-4-methanol, 4,5-dimethyl	3.978	0.56
5	3-FURANCARBOXYLIC ACID, ETHYL ESTER	4.433	4.82
6	5-OXO-TETRAHYDRO-FURAN-2-CARBOXYLIC ACID	4.723	0.2
7	2-METHYL-2H-PYRAN-3,4,5(6H)-TRIONE	5.901	0.7
8	5-Hydroxymethylfurfural	6.684	30.61
9	3,4-Anhydro-d-galactosan	7	1.1
10	2-HEPTANOL, ACETATE	8.11	1.21

11	1,2,3-BENZENETRIOL	8.982	24.78
12	trans-Cinnamic acid	9.365	0.92
13	D-Allose	10.762	7.83
14	1,5-Anhydro-d-mannitol	11.929	1.05
15	Hexadecanoic acid, methyl ester	14.626	0.07
16	PENTADECANOIC ACID	15.076	2.79
17	Methyl 10-trans,12-cis-octadecadienoate	16.302	0.08
18	Octadecanoic acid	16.97	0.71
19	10,12-HEXADECADIEN-1-OL	17.538	0.14
20	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	23.757	0.12
21	STIGMAST-5-EN-3-OL, OLEAT	30.681	0.09

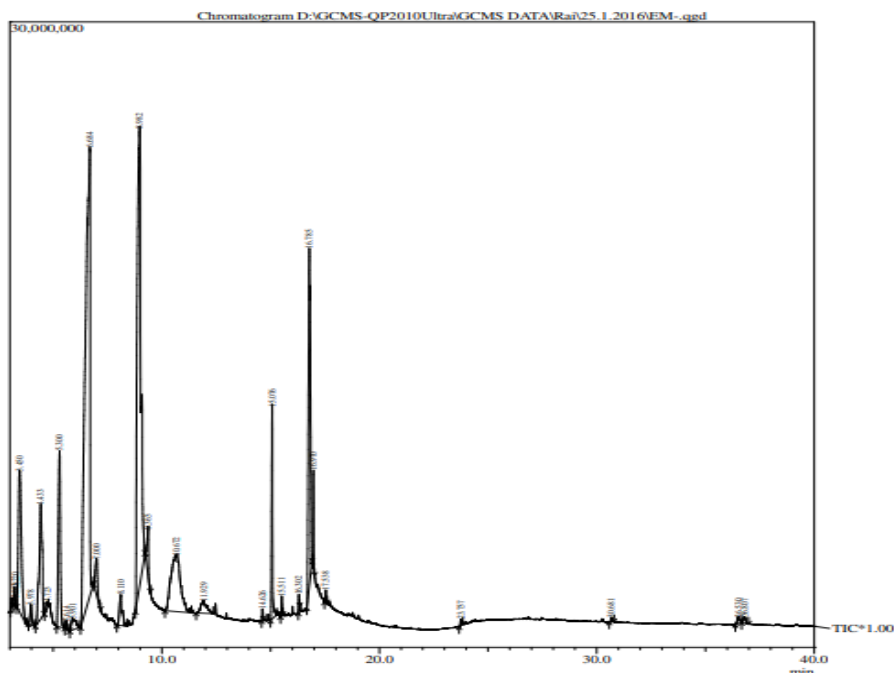


Figure 1: Chromatogram of GC-MS analysis

Table 4: GC-MS analysis of TBAM

S. No.	Name of Compound	R. Time	Area %
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3.204	0.89
2	1,3,5-TRIAZINE-2,4,6-TRIAMINE	4.339	0.47
3	Methyl 2-furoate	4.423	0.23
4	1,2,3-PROPANETRIOL	4.707	0.20
5	2-METHYL-2H-PYRAN-3,4,5(6H)-TRIONE	5.808	0.30
6	5-Hydroxymethylfurfural	6.444	7.85
7	3-Hydroxy-3-methylvaleric acid	6.626	0.19
8	5-Hydroxymethylfurfural	6.857	0.40
9	Methyl 3,4-ethylidene- $\alpha$ -D-galactopyranoside	7.465	0.05
10	CIS-DIMETHYL MORPHOLINE	7.612	0.17
11	2-HEPTANOL, ACETATE	7.992	0.12
12	CIS-DIMETHYL MORPHOLINE	8.116	0.11
13	1,2,3-BENZENETRIOL	8.948	52.59
14	D-Allose	10.345	0.73
15	Tetradecanoic acid	12.961	0.06
16	Hexadecanoic acid, methyl ester	14.627	0.11
17	cis-9-Hexadecenoic acid	14.876	0.08
18	PENTADECANOIC ACID	15.087	5.98
19	cis-Vaccenic acid	15.911	0.07
20	HEPTADECANOIC ACID	16.011	0.05
21	9-Octadecenoic acid (Z)-, methyl ester	16.352	0.09
22	Oxacycloheptadec-8-en-2-one, (8Z)	16.814	20.66
23	10,12-HEXADECADIEN-1-OL	17.553	0.27
24	9,12,15-OCTADECATRIEN-1-OL	18.248	0.10
25	Eicosanoic acid	19.04	0.15
26	STIGMAST-5-EN-3-OL, OLEAT	30.702	0.13
27	Vitamin E	31.434	0.11
28	Lup-20(29)-en-3-one	38.377	0.23





respectively. PEAM and TBAM showed the highest and lowest zone of inhibition (ZOI) against bacterial strains of *K. pneumoniae* and *E. coli*, respectively. Positive control ampicillin represents ZOI within a range of  $22 \pm 0$  to  $25 \pm 0$  mm for all mentioned bacterial strains, and negative control DMSO showed no zone of inhibition (Table 2). In the present study both PEAM and TBAM illustrated good antibacterial activity as compared to previous reports.

### Conclusion

The phytochemical analysis of PEAM and TBAM fruits demonstrate the diversity of bioactive components present in the extracts. Also, PEAM and TBAM showed antibacterial efficacy against various pathogenic microbes. GC-MS analysis of aqueous methanol fraction of PEAM and TBAM fruits illustrated the presence of diverse range of medicinally important bioactive components. In the present study, PEAM and TBAM possesses various secondary metabolites which contributes for many pharmacological properties. The GC-MS analysis revealed the presence of 21 phytochemical constituents in PEAM and 28 phytochemical constituents in TBAM. These phytochemicals support several biological processes, including antioxidant, anticancer, hypercholesterolemic, antimicrobial, anti-inflammatory, and other processes. Therefore, the therapeutic benefits of phytochemicals are due to their existence. In order to produce innovative medications using some of the bioactive chemicals discovered in PEAM and TBAM, more research is necessary. Hence, the present study concludes that PEAM and TBAM fruits possess diverse range of bioactive constituents and having therapeutic importance.

### Abbreviations

EOAM: Aqueous methanol fraction of *Phyllanthus emblica* L. fruits

PEAM: Aqueous methanol fraction of *Terminalia bellirica* (Gaertn.) Roxb fruits

Ampi: Ampicillin

DMSO: Dimethyl sulfoxide

GC-MS: Gas Chromatography- Mass spectrometry  
*E. coli*: *Escherichia coli*

*P. aeruginosa*: *Pseudomonas aeruginosa*

*K. pneumonia*: *Klebsiella pneumoniae*

*S. aureus*: *Staphylococcus aureus*

RT: Retention time

ZOI: Zone of inhibition

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