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QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL CONTENT AND GC-MS PROFILING OF *PHYLLANTHUS EMBLICA* (AMLA)

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Abstract

The family Phyllanthaceae is distributed in tropical regions consisting of herbs, shrubs, and trees. or *Phyllanthus emblica* L. (Syn.: *Emblica officinalis Gaertn*), known as amla or Indian gooseberry, has been traditionally used to treat fever, diarrhea, dysentery, hemorrhage, and inflammatory conditions. Recently, our research group also found *P. emblica* was efficient in the cure of hepatitis C and reduces the viral load. Based on the medicinal properties, the current study was designed to evaluate the phytochemical profile of the fruit, seeds and leaves of *P. emblica* grown in district Mansehra, KP, Pakistan. The total phenolic contents were determined using Folin Calteau assay, total flavonoids by aluminum chloride method while total alkaloids were eluted by bromocresol green (BCG) approach and compared in different parts. The volatile compounds in different fractions (methanolic, n-hexane, ethyl acetate, n-butanol, and dichloromethane) of leaves and fruits were analyzed using Gas Chromatography Mass Spectrometry (GC-MS). The antioxidant potentials of the extracts of different parts were also compared. Fruit exhibited higher contents of alkaloid (720±3.25 µg CAF/g) whereas the leaves showed the least contents (170±3.12 µg CAF/g). Total phenolic contents also showed the same pattern but in contrast the total flavonoids were highest in leaves and lowest in fruits. The seeds were in between fruits and leaves in terms of alkaloids, phenolics and flavonoids. The volatile compounds were detected in crude methanolic extract and in different fractions. Overall, thirty five compounds were detected in leaves while thirty six distinct volatile compounds were identified in fruits. Only seven compounds were found common in both leaves and fruits in all extracts. The most abundant compounds which were unique in leaves were 2-furancarboxaldehyde,5-(hydroxymethyl), squalene and 1,2,3-benzentriol while fruits 1,2,3-benzentriol, butanoic acid and 1,2,3 benzenetriol pyrogallol. Further purification, identification, and elucidation of these compounds are necessary to understand their significance in drugs development.

Keywords: *Phyllanthus emblica*; Antioxidant; Phytochemicals; Bioactive compounds; GC-MS

Introduction

The global population relies heavily on plants for medicine, food, and fuel. Since ancient times, medicinal plants and herbs have been utilized for their healing potential and exploration the favors the revolution of novel drug candidates for curing diverse diseases and disorders (Yao *et al.*, 2024). These drugs derived from plants are more convenient and powerful for curing various diseases as they are less cytotoxic and less expensive compared to other allopathic drugs. Scientists have identified distinct secondary metabolites extracted from plants and have been used in drugs development worldwide. Traditional medicines are still used by nearly 80% of the global population for curing various health issues (Uzma *et al.*, 2024; Wulandari *et al.*, 2024). The progress in developing novel drugs from medicinal plants and herbs has garnered significant attention in recent years. Members of family phyllanthacae has gained special attention due to their versatile properties. Among them, *P. emblica* has been reported for its significant potential in hair growth and amla oil is used worldwide. Studies have revealed the antioxidant (Bhattacharya *et al.*, 2002), antipyretic and analgesic (Sdayria *et al.*, 2018) immune modulatory (Medeiros *et al.*, 2024), gastro protective (Farruggia *et al.*, 2024), anti-inflammatory, antitussive (Rajizadeh *et al.*, 2024; Sun *et al.*, 2024), anti-ulcer (Ram *et al.*, 2002), and cytoprotective potential of *P. emblica* (Gazwi *et al.*, 2024). Additionally, it has been reviewed for their strong memory-enhancing effect and cholesterol-lowering capabilities (Vasudevan *et al.*, 2007), applications in ophthalmic disorders (Zhao *et al.*, 2024), antimicrobial potential (Talebi *et al.*, 2024) and snake venom neutralization (Kusar *et al.*, 2024).

The fruits of *P. emblica* have been reported for its rich nutritional contents such as vitamin C, amino acids, and minerals (Srivasuki, 2012). Furthermore, fruits have also been reported for other biochemical constituents

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such as alkaloids, tannins, phenols and flavonoids *(*Abu-Lafi *et al.,* 2024*)*. Some of the important polyphenols such as ellagic acid, gallic acid, quercetin, chebulagic acid, and kaempferol-3-O-α-L-(6"methyl)-rhamnoside, kaempferol-3-O-α-L-(6"ethyl)-rhamnopy-ranoside acid hase been identified from the fruit of this plant (Habib-ur-Rehman *et al.*, 2007). The fruits were widely used and evaluated due to extensive medicinal importance. However, there was a limited study reporting the detailed biochemical constituents in the leaves and seeds of *P. emblica.* The focus of the current study was to evaluate the phytochemical constituents and biological potentials of leaves and fruits of *P. emblica*.

Material and Methods

Collection of plants: Fresh and healthy leaves and fruits of *P. emblica* were collected in mid-November from Mansehra, KPK, Pakistan. Identification of plant was carried out at the Botany Department of Hazara University, Mansehra, Pakistan. The leaves and fruits were rinsed with distilled water to remove any unwanted material. The leaves were separated from branches while fruits were separated and cut into small pieces and shade dried at room temperature (20-25°C). The dried samples were pulverized using pestle and mortar and stored in airtight bottles for further processing.

Extraction and fractionation: The Soxhlet extractor was employed, methanol was selected as the primary solvent for Soxhlet extraction due to its high polarity, efficiency in extracting a wide range of phytochemicals including alkaloid, phenolics and flavonoids. 20g of powder and 250 mL of methanol was added in distillation flask and placed in a cellulose thimble and heated at 64°C for 6 h, resulting in approximately 25-35 cycles. The solvent was removed using a rotary evaporator, kept the water bath at 40°C, and a vacuum pressure of 337 mbar, with the process lasting 30 min, and then the crude methanolic extract was obtained. Crude extract was then fractionated sequentially with solvents increasing polarity, such as n-hexane, ethyl acetate, dichloromethane, and n-butanol solvent. The fractions were concentrated using rotary evaporator and stored at 4°C for further analysis.

Total alkaloid contents: The total alkaloids contents were determined using Bromocresol Green dye. The presence of alkaloid was qualitatively tested using Dragendroff's approach. The alkaloid presence was confirmed by the brown color produced when Dragendroff's reagent was applied. Quantitative estimation of alkaloid was done using UV-spectrophotometer (Salamah & Ningsih, 2017). This approach is dependent on the interactions amid alkaloid and bromocresol green (BCG) dye. Soxhlet extract of 925 mg was dissolved in 2 N HCl and then filtered. 1 mL of filtrate was added into separatory funnel then rinsed with chloroform in three separate intervals. The pH was adjusted to neutral using 0.1 N NaOH. Then, 5 mL of bromocresol green (BCG) and 5 mL of phosphate buffer was combined, the mixture was agitated and further extracted with chloroform through vigorous shaking. The mixture was then transferred into a volumetric flask and diluted with chloroform. The absorbance was measured at 470 nm. Caffeic acid was used as a standard to quantify total alkaloids contents.

Determination of total phenolic contents: The total phenolic contents in fruit and leaves of the *P. emblica* was determined using the Folin-Ciocalteu (FC) assay with slight modification (Rehman *et al.*, 2019). In this procedure, 0.2 mL of the sample or standard was mixed with 0.5 mL of FC reagent, 4 mL of 1 M sodium carbonate, and 10 mL of distilled water. The mixture was allowed to equilibrate for 30 min at room temperature, after which the absorbance was measured at 760 nm using a UVD-3200 spectrophotometer (Labomed Inc., USA). The TPC was expressed in terms of milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g).

Determination of total flavonoid content (TFC): The total flavonoid contents (TFC) of fruit and leaves of *P. emblica* was assessed through a colorimetric method involving aluminum chloride (Singh *et al.*, 2019). In this process, 300 µL of the sample was combined with 150 µL of a 0.3 M $AlCl_3$, 150 µL of 0.5 M $NaNO_2$, and 3 mL of 30% aqueous methanol in a test tube. Subsequently, 1 mL of 1 M sodium hydroxide (NaOH) was added. The absorbance was then measured at 506 nm using a UVD-3200 spectrophotometer (Labomed Inc., USA). The TFC was calculated and expressed as milligrams of rutin equivalent per gram of dried extract (mg RE/g).

Gas chromatography-mass spectroscopy (GC-MS) analysis: The crude extract of methanol of the leaves and fruits fractions of *P. emblica* were analyzed employing GC-MS instrument (GC utilizing the Agilent 7890A and MS from the Agilent 5975C). Hp-5-MS standard capillary nonpolar column was employed, with dimensions of 30 m x 0.25 mm and a film thickness of 0.25 µm. The temperature was initially set at 60°C for 2 min and then increased to 240°C at a rate of 4°C per min. The temperature of both injector and detector were maintained at 240°C, helium (99.99 %) was used as the carrier gas at a flow rate of 1 mL/min, employing a split/spitless injector for the mass spectrometer (240°C, split ratio 1:10) was used, and 1 µL sample was introduced for analysis of volatile bioactive constituents in the plant samples. The identification of compounds was done using NIST library.

DPPH radical scavenging activity assay: For the evaluation of antioxidant potential DPPH assay was used (Herald *et al.*, 2012). For assay mixture, 10 µL extract of each botanical of *P. emblica* was added in the well of 96-well microplate. Then, 90 µL of deionized water and 7.8% of DPPH solutions were added subsequently in the same well. The incubation was done in dark for 30 min and absorbance was measured at 517 nm using UVD-3200 spectrophotometer (Labomed Inc., USA). The % activity was calculated using the formula below.

$$\% \text{ Activity} = \left(1 - \frac{Abs\ (sample)}{Abs\ (control)}\right) \times 100$$

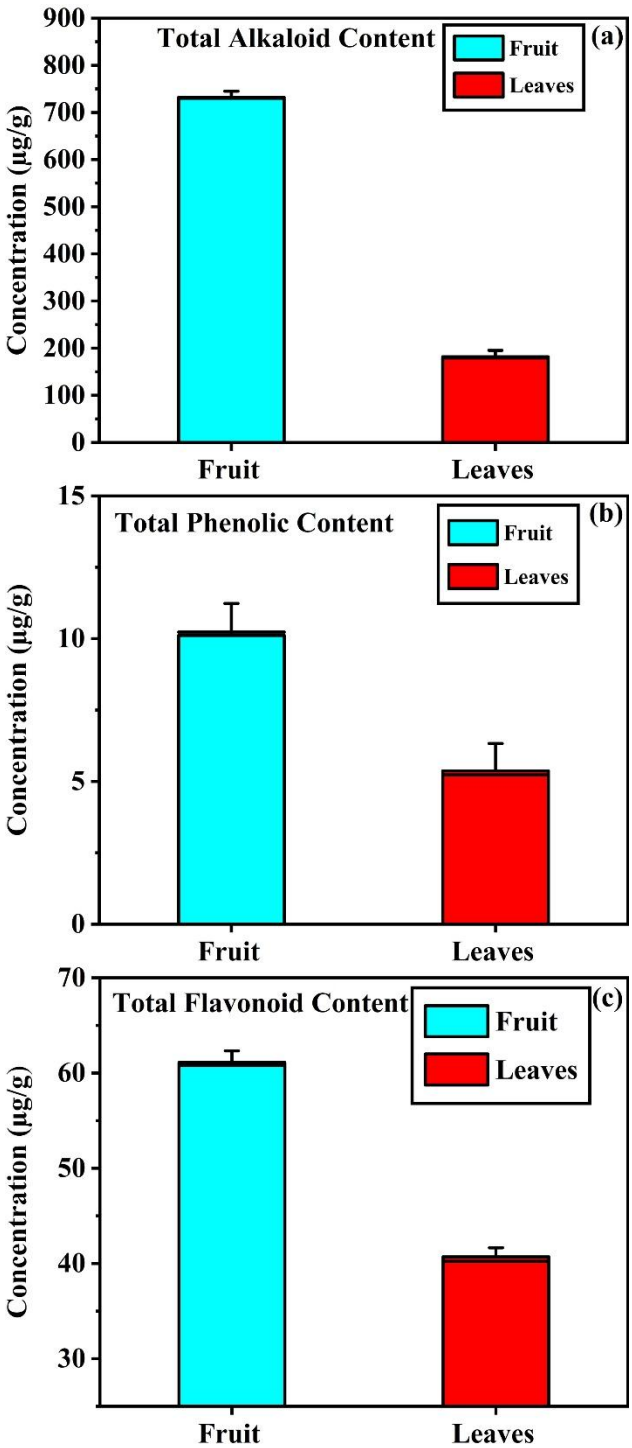

Fig. 1. Phytochemical profiling: a, TAC; b, TPC; c, TFC of fruits and leaves of *P. emblica*.

Results and Discussion

In this study, a comprehensive phytochemical analysis of *P. emblica* was conducted to evaluate its bioactive constituents. The investigation focused on quantifying the total alkaloid, phenolic and flavonoid contents of the plant, which are key indicators of its potential therapeutic properties. In addition to the quantitative analysis of these phytochemicals, the study also employed Gas Chromatography-Mass Spectrometry (GC-MS) to identify and characterize the volatile bioactive compounds present in the *P. emblica* samples.

Total phenolics, flavonoids and alkaloids contents: In this study, comparative analysis was carried out on leaves and fruit methanolic extracts of *P. emblica* to assess their relative concentrations and potential therapeutic value. Many studies reported the secondary metabolites of *P. emblica* fruits and few reported leaves (Liu *et al*., 2008; Yang & Liu, 2014; Hasan *et al*., 2016), but limited literature was available on seeds and comparative analysis of all botanicals, most specifically from district Mansehra. In the current study the *P. emblica* growing in the climate of district Mansehra was evaluated for total alkaloids, flavonoids and phenols in different botanicals.

The total alkaloid contents were higher in fruit extract (720±3.25 µg CAF/g) compared to leaves extract (170±3.12 µg CAF/g) as depicted in Fig. 1(a). The higher concentration of alkaloids in fruits extract suggests possessing of greater pharmacological activity associated with alkaloids such as anti-inflammatory, analgesic, antimicrobial potentials. It has been reported that alkaloids are the prime center of investigation which targets the development of new drug in medicinal chemistry due to broad pharmacological potentials (Rajput *et al.*, 2022). The total phenolics and flavonoid contents showed a similar pattern of concentration as in alkaloids (Fruits>leaves) as shown in Fig. 1(b & c). This substantial difference suggests that fruit may offer a more concentrated source of phenolic and flavonoids content of *P. emblica* can offer more medicinal potential compared to leaves. Phenolics and flavonoids have been reported as potent antioxidants and their role in protecting against oxidative stress and associated degenerative diseases. The current study aligned with previously reported study where fruits were considered as one of the best sources for antioxidant in medicinal and food chemistry (Nambiar *et al.*, 2015).

GC-MS (gas chromatography-mass spectrometry) analysis: Crude methanolic extracts and different fractions of leaves and fruit were subjected to GC-MS analysis for identification of volatile bioactive compounds. In GC, the volatile compounds are separated based on their retention time (RT). The separated compounds are then identified in the mass spectrometry (MS) component based on their mass to charge ratio (m/z). Identification was done by comparing the MS data against the NIST 5 standard library, revealing the compounds presence and their percentage composition in the extract.

Methanolic leaves and fruit extract *P. officinalis*: In methanolic crude dried extract fifteen compounds were detected in leaves, while nine compounds were identified in the methanolic dried extract of fruit. The leaves and fruits shared 3 common compounds that were 3, 5-dihydroxy-6-methyl 2, 3-dihydro-4H-pyran-4-one, 1, 2, 3-benzenetriol, and 2-furancarboxaldehyde, 5-(hydroxymethyl). Further. 2H-pyran-2,6(3H)-dione and 13-heptadecyn-1-ol were found in fruit methanolic extract at higher concentrations while in the leaves ethanone, 1-[4-methoxy-3-(4-methylphenoxyy) phenyl] and levoglucosenone were recorded (Table 1).

Table 1. Chemical identified compounds in methanolic extract of leaves and bark of *P. officinalis* by GC-MS.

RT (Min)	Compounds	Molecular Formula	M.WT (g)	Leaves (%)	Fruit (%)
5.46	2-cyclohexen-1-one, 4-hydroxy	$C_6H_8O_2$	112	1.41	N.A.
5.47	2H-pyran-2,6(3H)-dione	$C_5H_8O_3$	112	N.A.	8.72
5.86	2-cyclopropylcarbonyloxydodecane	$C_{16}H_{30}O_2$	254	2.78	N.A.
6.51	6-hydroxy-9-oxabicyclo[3.3.1]nonan-2-one	$C_8H_{12}O_3$	156	2.98	N.A.
6.89	Levoglucosenone	$C_6H_6O_3$	126	7.92	N.A.
6.91	(S)-(+)-2',3'-dideoxyribonolactone	$C_5H_8O_3$	116	N.A.	1.89
7.57	3,5-dihydroxy-6-methyl 2,3-dihydro-4H-pyran-4-one,	$C_6H_8O_4$	144	0.91	1.27
8.97	2-furancarboxaldehyde,5-(hydroxymethyl)	$C_6H_6O_3$	126	1.55	10.42
10.84	Ethanone,1-[4-methoxy-3-(4-methylphenoxyy)phenyl]	$C_{16}H_{16}O_3$	256	16.60	N.A.
10.85	Phenol,4,4'-(1-methylethylidene)bis[2-methyl	$C_{17}H_{20}O_2$	256	N.A.	6.09
11.76	1,2,3-benzentriol	$C_6H_6O_3$	126	45.24	78.25
15.58	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.90	N.A.
16.02	13-heptadecyn-1-ol	$C_{17}H_{32}O$	252	1.16	0.84
16.47	Pentadecanoic acid, 14 –methyl-, methyl ester	$C_{17}H_{34}O_2$	270	0.79	N.A.
17.05	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	N.A.	0.84
18.30	Phytol	$C_{20}H_{40}O$	296	0.75	N.A.
18.69	9,12-octadecadienoic acid (Z, Z)	$C_{18}H_{32}O_2$	280	N.A.	0.33
21.92	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	1.15	N.A.
23.92	Squalene	$C_{30}H_{50}$	410	3.39	N.A.
26.11	Vitamin E	$C_{29}H_{50}O_2$	430	1.54	N.A.

Note: N.A. denotes "not available"

Table 2. Chemical identified compounds in n-hexane extract of leaves and bark of *P. officinalis* by GC-MS.

RT (Min)	Compounds	Molecular Formula	M.WT (g)	Leaves (%)	Fruit (%)
15.58	3-Eicosyne	$C_{20}H_{38}$	278	10.52	N.A.
16.03	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl) methy], methyl ester	$C_{21}H_{38}O_2$	322	4.75	N.A.
16.47	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	N.A.	10.74
16.51	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	8.67	N.A.
17.17	Palmitic acid	$C_{16}H_{32}O_2$	256	N.A.	8.32
18.11	Linoleic acid	$C_{19}H_{34}O_2$	294	N.A.	7.60
18.16	11-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	N.A.	7.70
18.21	7,10,13-hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264	9.43	N.A.
18.36	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	N.A.	3.38
18.39	Heptadecanoic acid, 9-methyl, methyl ester	$C_{19}H_{38}O_2$	298	11.52	N.A.
18.80	9,12-octadecadienoic acid (Z, Z)	$C_{18}H_{32}O_2$	280	N.A.	4.61
21.93	Phthalic acid	$C_{16}H_{22}O_4$	278	13.96	12.74
23.91	Squalene	$C_{30}H_{50}$	410	32.27	
25.80	Ergosta-5,7,22-ttrien-27-ol,3-methoxymethoxy	$C_{30}H_{48}O_3$	456	N.A.	2.49
26.11	Vitamin E	$C_{29}H_{50}O_2$	430	8.83	N.A.
27.68	Gamma, sitosterol	$C_{29}H_{50}O$	414	N.A.	21.10
27.79	Olean-12-en-3-one #	$C_{30}H_{48}O$	424	N.A.	16.48
28.17	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	N.A.	2.56
28.43	Lupeol	$C_{30}H_{50}O$	426	N.A.	2.26

Note: N.A. denotes "not available"

Table 3. Chemical identified compounds in n-butanol extract of leaves and bark of *P. officinalis* by GC-MS.

RT (Min)	Compounds	Molecular Formula	M.WT (g)	Leaves (%)	Fruit (%)
4.17	1-butanol,2,methyl	C_5H_{120}	88	24.08	N.A.
4.88	Pentane,2,3-dimethyl	C_7H_{16}	100	5.06	N.A.
4.97	Butanoic acid,2-methylpropyl ester	$C_8H_{16}O_2$	144	N.A.	7.11
5.02	4-heptanone,3-methyl	$C_8H_{16}O$	128	17.45	N.A.
5.38	Butanoic acid,butyl ester	$C_8H_{16}O_2$	144	37.75	74.13
7.06	Butanoic acid,3-oxo,2-methylpropyl ester	$C_8H_{14}O_3$	158	10.33	N.A.
12.01	Butane,1,1-dibutoxy	$C_{12}H_{26}O_2$	202	N.A.	4.00
18.02	(2-methyl[1, 3]dioxolan-2-yl)thio acetic acid, S-[3-(2-methyl[1, 3]dioxolan-2-yl)-2-oxopropyl] ethanthioate	$C_{13}H_{20}O_6S$	304	5.30	N.A.

Note: N.A. denotes "not available"

Table 4. Chemical identified compounds in ethyl acetate extract of leaves and bark of *P. officinalis* by GC-MS.

RT (Min)	Compounds	Molecular Formula	M.WT (g)	Leaves (%)	Fruit (%)
5.82	2H-pyran-2,6(3H)-dione	$C_5H_4O_3$	112	N.A.	5.05
9.34	2-furancarboxaaldehyde,5-(hydroxymethyl)	$C_6H_6O_3$	126	N.A.	5.47
11.71	1,2,3-benzenetriol pyrogallol	$C_6H_6O_3$	126	N.A.	49.77
15.60	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	26.22	N.A.
16.06	Cyclohexanepropanol,2,2-dimethyl-6-methylene	$C_{12}H_{22}O$	182	12.62	N.A.
17.07	n-hexadeacanoic acid	$C_{16}H_{32}O_2$	256	N.A.	20.49
18.86	Octadecanoic acid	$C_{18}H_{36}O_2$	284	N.A.	19.21
21.95	1,2-benzenedicarboxylic acid, mono92-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	30.10	N.A.
23.92	Squalene	$C_{30}H_{50}$	410	20.98	N.A.
24.36	3-hydroxy spirost-8-en-11-one	$C_{27}H_{40}O_4$	428	10.05	N.A.

Note: N.A. denotes "not available"

Table 5. Chemical identified compounds in dichloromethane extract of leaves and bark of *P. officinalis* by GC-MS.

RT (Min)	Compounds	Molecular Formula	M.WT (g)	Leaves (%)	Fruit (%)
5.57	2H-pyran-2,6(3H)-dione	$C_5H_4O_3$	112	N.A.	8.91
5.71	2-cyclohexen-1-one,4-hydroxy	$C_6H_8O_2$	112	4.57	N.A.
6.53	Furaneol	$C_6H_8O_3$	128	5.85	N.A.
7.08	2-furancarboxylic acid	$C_5H_4O_3$	112	N.A.	2.37
7.53	2,3-dihydro-3,5-dihydroxy-6-methyl,4H-pyran-4-one,	$C_6H_8O_4$	144	18.89	2.64
8.10	Butanoic acid, 3-ox0,2-hydroxyethyl ester	$C_6H_{10}O_4$	146	N.A.	4.72
8.95	2-furancarboxaldehyde,5-(hydroxymethyl)	$C_6H_6O_3$	126	60.36	7.30
10.40	Goitrin	C_5H_7NOS	129	N.A.	1.84
10.83	Phenol,4,4'-(1-methylethylidene)bis[2-methyl	$C_{17}H_{20}O_2$	256	10.31	N.A.
10.83	(2S,13S)-12,13-dihydroxy-1,4,7,10, tetraoxacyclotetradecane	$C_{10}H_{20}O_6$	236	N.A.	4.16
10.84	Ethanone,1-(1,2,3,5,6,7-he	$C_{18}H_{24}O$	256	N.A.	4.67
11.70	1,2,3-benzenetriol	C_6H_6O3	126	N.A.	45.69
17.04	n-hexadecaoic acid	$C_{16}H_{32}O_2$	256	N.A.	8.88
18.00	1,3-dioxolane-2-propanol,2-methyl.acetate	$C_9H_{16}O_4$	188	N.A.	3.55
18.88	Stearic acid	$C_{18}H_{36}O_2$	284	N.A.	9.89

Note: N.A. denotes "not available"

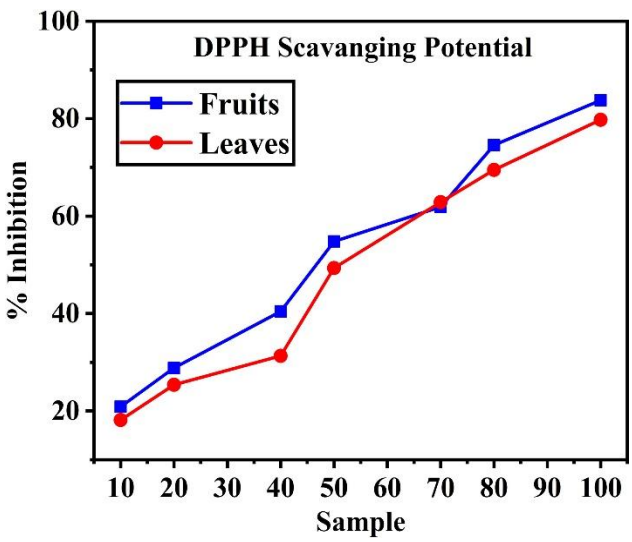

Fig. 2. DPPH scavenging potential of fruits and leaves of *P. emblica*.

Identified compounds in n-hexane fractions: In n-hexane fraction 8 distinct compounds were identified from leaves, whereas 12 different compounds from fruit fraction. Phthalic acid was commonly detected in both leaves and fruit of n-hexane fractions. γ sitosterol, and palmitic acid were the two abundant compounds found only in fruit fraction while squalene and heptadecanoic acid, 9-methyl, methyl ester were the compounds of higher concentration found in the leaves of n-hexane fraction (Table 2).

Identified compounds in butanol fractions: In the n-butanol fraction, 3 distinct compounds were found in the leaves, while 6 compounds were identified in the fruit. Butanoic acid, butyl ester was the only common compound occurring in both n-butanol fractions of leaves and fruit. Butane,1,1-dibutoxy and butanoic acid, 2-methylpropyl ester were found in fruit fraction while 1-butanol, 2-methyl, 4-heptanone, 3-methyl and butanoic acid, 3-oxo, 2-methyl-propyl ester were identified in leaves of butanol fraction (Table 3).

Identified compounds in ethyl acetate fractions: Five chemical compounds were identified in ethyl acetate fraction of leaves and fruit. Each fraction contained different compounds, with no overlap between the leaves and fruits fraction of ethyl acetate. 1, 2, 3-benzenetriol pyrogallol, octadecanoic acid and n-hexadeacanoic acid were the foremost constituents of fruit fraction while 1, 2-benzenedicarboxylic acid, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and squalene were the main bioactive constituents reported in ethyl acetate fraction of leaves (Table 4).

Identified compounds in dichloromethane fractions: The dichloromethane fraction of leaves exhibited five different compounds while fruit fraction showed 12 distinct compounds. A common compound that was identified in both fractions was 2-furancarboxaldehyde, 5-(hydroxymethyl). Fruit fraction of dichloromethane revealed the presence of 1, 2, 3-benzenetriol, octadecanoic acid, n-hexadecaoic acid, and stearic acid while the leaves exhibited the higher concentration of 2-furancarboxaldehyde, 5-(hydroxymethyl), 2, 3-dihydro-3, 5-dihydroxy-6-methyl, 4H-pyran-4-one, and phenol,4,4'-(1-methylethylidene)bis[2-methyl] as presented in Table 5.

Antioxidant potential of *P. emblica*: The antioxidant activity of *P. emblica* was assessed using the DPPH free radical-scavenging assay, which measures the ability of plant extracts to donate hydrogen atoms or electrons to neutralize free radicals. Among the tested samples, the fruit extract demonstrated the highest DPPH radical scavenging activity, with a value of 83.9%, while the leave extract showed slightly lower activity at 81.6%, as depicted in Fig. 2. This strong radical-scavenging capacity indicates a high potential for neutralizing oxidative stress, which is a key contributor to various degenerative diseases.

Discussion

Currently, the attention of researchers is gained towards the identification of biochemical constituents from plants (phytochemicals) to develop novel drug due to ineffectiveness and side effects of synthetic drugs. The integration of gas chromatography and mass spectrometry (GC-MS) is an effective approach for the separation and identification of both volatile and semi-volatile compounds (Baravalia *et al.*, 2011). In the current study, total alkaloid, phenolics and flavonoids contents were compared among different botanicals of *P. emblica*. Furthermore, the profiling of volatile bioactive was eluted in crude methanolic extracts of leaves and fruit of *P. emblica*.

The results revealed that the total alkaloids, phenolics and flavonoid contents showed the same pattern among the botanicals of *P. emblica*. The quantity of phytochemical constituents in term of alkaloid, phenolic and flavonoid were observed high in fruit extracts while leaves revealed lower concentration of phytochemicals. Interestingly, the antioxidant activity also showed a similar pattern of phenolic compounds which have also been reported for their substantial antioxidant potential. The study aligned to previously reported data on the fruit of Indian gooseberry possessed many biological important phytochemical constituents and significant biological potentials such as antioxidant and anticancerous (Pareek, 2012; Balasubramanian *et al.*, 2014). The literature review suggested the fruit of Indian *P. emblica* could be a valuable addition in functional food based on their significant nutritional, biochemical and biological properties (Avinash *et al.*, 2024). Previously many studies reported the TAC, TPC and TFC contents in *P. emblica* particularly in fruit extracts (Laulloo *et al.*, 2018; Halim *et al.*, 2022) but there was no study reported the comparison of different botanicals. Furthermore, the focus of the study was on secondary metabolites which are always to environmental conditions and varies from region to region. Moreover, different growing stages can cause variations in secondary metabolites where they increased at one stage and decreased at other (Koseki *et al.*, 2002; Samarth *et al.*, 2008).

Phenolic compounds are well-documented for their antioxidant activity, which plays a crucial role in neutralizing free radicals and reducing oxidative stress, a key factor in the prevention of chronic diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions. The higher phenolic content in the fruit suggests that it could be more effective in these protective roles, making it a valuable component for dietary supplements and functional foods aimed at enhancing antioxidant intake. In addition to phenolic content, the flavonoid levels in both the fruit and leaves were significantly higher than their phenolic counterparts, Flavonoids are another class of potent antioxidants that contribute to a wide range of biological activities, including anti-inflammatory, anti-carcinogenic, and cardioprotective effects. The substantial amount of flavonoid content, particularly in the leaves, underscores the potential of fruit and leaves as a rich source of these compounds, which could be harnessed for various therapeutic applications (Badshah *et al*., 2025).

The analysis of GC-MS revealed the presence of biologically active compounds in the different solvent, among them the most abundant and biologically important compounds belonging to different classes of metabolites such as alkanes, benzoid, fatty acyls, ketone, esters and phenol. The most abundant compounds were 1, 2, 3-benzenetriol, 2-furancarboxaldehyde,5-(hydroxymethyl), ethanone, 1-[4-methoxy-3-(4-methylphenoxy) phenyl], and levoglucosenone and pyrogallol as shown in Fig 3. These constituents are believed to play a significant role in the plant's medicinal properties. One notable polyphenol component, pyrogallol, is recognized for its antifungal and fungistatic effects (Shukla *et al.*, 2009). This compound is an effective antimicrobial agent, with its toxicity attributed to the presence of three hydroxy group in its molecular structure (Mahady, 2005; Kocaçalışkan *et al.*, 2006). Furthermore, wide range of other biochemical constituents were identified for significant number of biological potentials elucidated its importance in medical and pharmaceutical sciences (Mohamed *et al.*, 2017; Shin *et al.*, 2019; Tian *et al*., 2023).

Overall, the current study showed that a wide range of compounds were detected in different solvent fractions of fruit and leaves of *P. emblica*. Previously many studies reported on the biological constituents of fruits of *P. emblica* from different regions of the world (Akhtar *et al.*, 2011, Alkandari *et al.*, 2019). Interestingly, some of these compounds were commonly found in many studies but large numbers were unique indicated that climatic conditions were major factors influencing phytochemistry (Acharya, 2016). This is the first study that reported the comprehensive analysis of phytochemicals of different botanicals of *P. emblica* fractions in different solvents. Butyl butyrate was as a major constituent, having pleasant odor and used in essence industry to generate fruity sweet extracts while 2-methyl-1-butanol, an intermediate used in the production of various chemicals, a component of commercially available amyl alcohol mixtures. The study suggested that the extracts of *P.emblica* possessed wide range of biologically important constituents which could be significant addition in pharmaceuticals.

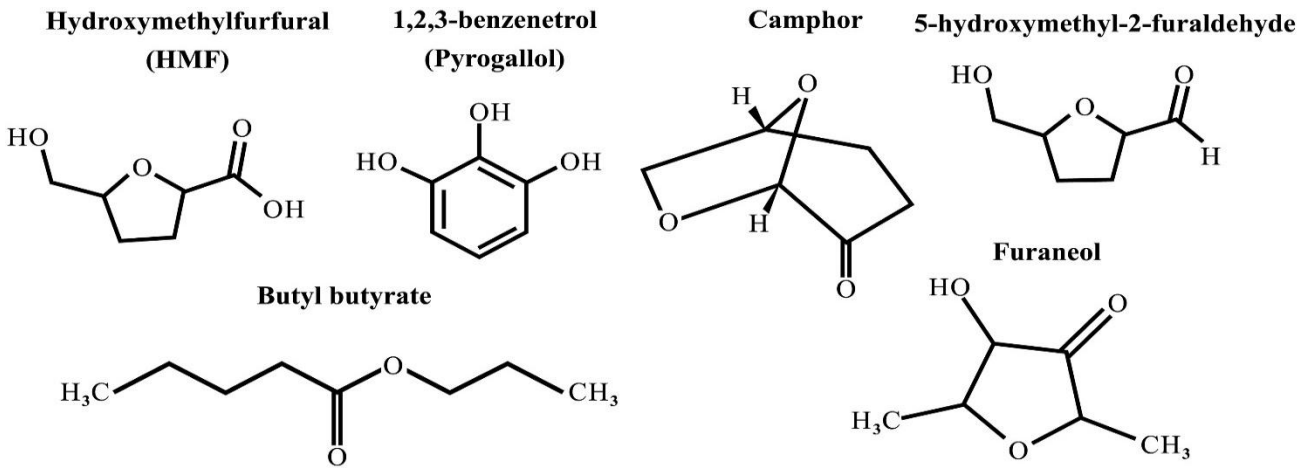

Fig. 3. Chemical structures of major identified bioactive compounds.

Conclusion

In conclusion, the current study provides a comprehensive analysis of the volatile bioactive constituents and alkaloid contents in the leaves and fruits of *P. emblica*. The findings reveals a higher alkaloid, concentration in the fruit extracts compared to the leaves, indicating a potentially greater pharmacological activity of fruits as compared to leaves. The GC-MS analysis identified several key compounds, which possess a significant biological potential based on previous literature. Interestingly, some of the compounds were found in fruits while absent in leaves and others were unique to leaves. The study underscores the significant therapeutic potential of *P. emblica* and highlights the need for further purification, identification, and elucidation of these bioactive compounds to fully understand their efficacy against different diseases. The current study contributes valuable insights into the pharmacological properties of *P. emblica*, supporting its traditional use in herbal medicine and encouraging the development of novel plant-based therapeutics.

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