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Phytochemical profiling, GC-MS analysis and vitamin C estimation in *Emblica officinalis* fruit

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ABSTRACT

Background: Over the past decade, herbal feed additives have gained attention from scientists as valuable resources for enhancing the health of humans and animals. Among these herbs, *Emblica officinalis* is one of the most effective and readily available herbal feed supplements in India. It is well-known for its numerous therapeutic properties due to its high vitamin C content and the presence of certain phytochemicals. **Objective:** The present study was conducted to evaluate the phytochemical composition of *E. officinalis* through qualitative, quantitative and GC-MS analyses and to estimate the vitamin C content. **Materials and Methods:** In the present study, *E. officinalis* fruits were chopped, shade-dried, powdered and subjected to alcohol maceration technique. The qualitative and quantitative phytochemical analysis of the extract was performed to identify specific phytoconstituents such as carbohydrates, tannins, alkaloids, flavonoids, phenols, terpenoids, glycosides, phytosterols, saponins and proteins. Additionally, GC-MS analysis of the extract was conducted to identify bioactive compounds based on retention time. Vitamin C content of fruit powder was estimated by the iodometric titration method. **Results:** Qualitative analysis confirmed the presence of various phytochemicals including carbohydrates, tannins, alkaloids, flavonoids, phenols, terpenoids, phytosterols and saponins. GC-MS analysis revealed the presence of eleven major compounds. Vitamin C estimation showed comparable results in both sun-dried and shade-dried *E. officinalis* powder. **Conclusion:** The study demonstrates the diverse bioactive compounds and rich vitamin C content in *E. officinalis*. These findings support its traditional use and provide a scientific basis for its application in improving antioxidant defence and mitigating oxidative stress.

Keywords: *E. officinalis*, Phytochemicals, Vitamin C, GC-MS analysis.

INTRODUCTION

Many herbs of therapeutic importance are gifted to mankind, among which *E. officinalis*, commonly known as Indian gooseberry or amla, belonging to the family Euphorbiaceae, is one of the common medicinal herbs available in India. It is recognized as a panacea in the Indian system of medicine [1]. The renowned significance of *E. officinalis* is well documented in Ayurvedic medicine, where all the famous ancient texts have explored its preventive and curative properties and acclaimed its incredible medicinal efficacy [2]. This herb is extensively grown in tropical and subtropical countries such as India, Indonesia, Sri Lanka, China, and Southeast Asia [3]. It is a medium-sized deciduous plant with a height ranging between 8-18 meters, featuring a crooked trunk and spreading branches. Its leaves are linear and feathery; flowers are yellowish green; fruits are hard, spherical and pale yellow [4].

The amla fruit contains various phytoconstituents, including carbohydrates, phenols, alkaloids, tannins, flavonoids, glycosides, terpenoids, amino acids, phytosterols and saponins [5,6,1]. *E. officinalis* is well known for its antioxidant, analgesic, anti-inflammatory, and antipyretic effects. It also possesses hepatoprotective, cardioprotective, anti-diabetic, and anti-carcinogenic properties [7,8,1]. It is also a natural source of vitamin C, which makes it useful in treating scurvy and diabetes. Due to its wide medicinal applicability, a comprehensive phytochemical profiling is essential. Therefore, the present study was undertaken to analyse the phytoconstituents present in the amla fruit.

MATERIALS AND METHODS

Collection of materials

Fresh amla fruits were procured from the local market, Namakkal. The chemicals and reagents used in this study were of analytical grade and high purity, obtained from M/s Hi-Media.

Processing of Amla

The amla fruits were crushed into small pieces and then shade dried for about one week. The dried piece

pieces were ground into a fine powder and stored in an air-tight container.

Preparation of extract

Extract is prepared via the alcohol maceration technique, where amla powder and 70% ethanol were mixed in a 1:10 ratio and placed on a rotary shaker for about 72 hrs and then filtered. The filtrate was collected in a tray and kept in a hot air oven for 48 hrs and then the extract was scraped, which is slightly gummy in texture [9].

Qualitative phytochemical analysis

Extract was mixed in a solvent (distilled water) and analysis was carried out [10].

Detection of Carbohydrates: 1 ml of *E. officinalis* extract was taken into a test tube and 1 ml of Fehling's solution A and B was added and kept in a water bath at 55 ºC. The Presence of carbohydrates results in the formation of red precipitate.

Detection of Tannins: 1 ml of *E. officinalis* extract was taken into a test tube and a few drops of ferric chloride were added. The presence of tannins results in the formation of a blue-green colour.

Detection of Flavonoids: 2 ml of *E. officinalis* extract was taken into a test tube and a drop of 10% sodium hydroxide solution was added. The presence of flavonoids results in the formation of a yellow colour, which becomes colourless on the addition of dilute HCl along the sides of the test tube.

Detection of Alkaloids: 2 ml of *E. officinalis* extract was taken into a test tube and 2 ml of 2% picric acid was added (Hager's reagent). The presence of alkaloids results in the formation of an orange colour.

Detection of Phenols: 2 ml of *E. officinalis extract* was taken into a test tube and 2 ml of distilled water, followed by 3 ml of 10% lead acetate, were added. The presence of phenols results in the formation of white precipitate.

Detection of Terpenoids: 2 ml of *E. officinalis* extract was taken into a test tube and 2 ml of chloroform was added. Concentrated H_2SO_4 was then added along the sides of the test tube. The presence of terpenoids results in the formation of a brown colour ring at the junction of two liquids.

Detection of Glycosides: 2 ml of *E. officinalis* extract was taken into a test tube and 2 ml of glacial acetic acid and 1 drop of 10% ferric chloride, followed by 2 ml of concentrated H_2SO_4 were added. The presence of glycosides results in the formation of three different layers.

Detection of Phytosterols: 2 ml of *E. officinalis* extract was taken into a test and 2 ml of chloroform was added. Concentrated H_2SO_4 was then added along the sides of the test tube. The presence of phytosterols results in the formation of a reddish-brown colour.

Detection of Saponins: 2 ml of *E. officinalis* extract was taken into a test tube and 5 ml of distilled water was added and shaken for 15 min. The presence of saponins results in the formation of foam.

Detection of Proteins: 2 ml of *E. officinalis* extract was taken into a test tube and 1ml of 0.2% ninhydrin solution was added and boiled in a water bath for 5-10 min. The presence of proteins results in the formation of a purple colour.

Quantitative phytochemical analysis

Total phenol content: Total phenol content was estimated via spectrophotometric method [11]. One ml of *E. Officinalis* extract @ 0.1 mg/ml was taken into a test tube and 0.5 ml of Folin-Ciocalteu phenol reagent and 4 ml of 7.5% sodium carbonate solution were added. The mixture is subjected to vortexing and kept undisturbed for 40 min. The absorbance was measured at the wavelength of 765 nm using a UV-VIS spectrophotometer. Total phenolic content was calculated from a standard curve, which was obtained using different concentrations of gallic acid in distilled water ranging from 2.5 to 50 µg/ml. The total phenolic content was expressed in terms of micrograms of gallic acid equivalent per ml of extract.

Total Flavonoid Content: Total flavonoid content was estimated via spectrophotometric method [12]. One ml of *E. officinalis extract* @ 10 mg/ml was taken into a test tube and 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water were added. The mixture was undisturbed for 30 min and absorbance was measured at the wavelength of 415 nm using a UV-VIS spectrophotometer. Total flavonoid content was calculated from a standard curve, which was obtained using different concentrations of quercetin in distilled water ranging from 10 to 100 µg/ml. The total flavonoid content was expressed in terms of micrograms of quercetin equivalent per ml of extract.

Total alkaloid content: The quantity of alkaloids was estimated via gravimetric method [13]. To one gm of *E. officinalis* extract in a test tube, 40.0 ml of 10 % acetic acid in ethanol was added and undisturbed for 4 hrs. It was filtered and then concentrated to one fourth of its original volume by boiling in water bath. Concentrated ammonium hydroxide was added drop by drop to the filtrate until the precipitation was complete. The whole solution was filtered again and the precipitate was collected in a small flask. It was washed with dilute ammonium hydroxide, filtered and dried. The residue was weighed and total alkaloid content was expressed in per cent.

GC-MS analysis

The *E. officinalis extract* was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis, which was carried out using PerkinElmer Clarus 500 GC-MS in the Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA, Deemed University, Thanjavur. The setup was equipped with an Elite-5 MS non-polar capillary column of 30 m length, 0.25 mm inner diameter and 0.25 m film thickness. Helium was used as the carrier gas with a constant flow rate of 1 ml/min and the injector temperature was maintained at 280 ºC. The oven temperature was initially held at 60 ºC and gradually increased to 150 ºC @ 6 ºC/min for 2 min and held for 10 min and then raised to 280 ºC. Fragments from mass spectra were collected at 70 eV and resultant compounds were identified based on the National Institute of Standards and Technology library database [14].

Estimation of Vitamin C

Analysis of Vitamin C content in *E. officinalis* powder was conducted at the Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA, Deemed University, Thanjavur. *E. officinalis* powder of 0.1 g was dissolved in 100 ml of freshly boiled and cooled water. 25 ml of 1 M sulphuric acid is added, which is then titrated with 0.05 M I_2 using starch as indicator until a persistent blue-violet colour is formed (1 ml of 0.05 M Iodine is equivalent to 0.008806 g of ascorbic acid) [15].

RESULTS

The descriptive macroscopic profile of amla fruit, powder and extract was recorded and summarized in Table 1.

Qualitative phytochemical analysis

The preliminary phytochemical analysis of the ethanolic extract of *E. officinalis* revealed the presence of various phytoconstituents. The presence or absence of phytoconstituents in the tested sample, along with their tests, were tabulated in Table 2.

Quantitative phytochemical analysis

The concentrations of major phytoconstituents in the ethanolic extract of *E. officinalis* were quantified according to standard procedures. Total phenol content and total flavonoid content were estimated using gallic acid and quercetin as standards, respectively. The regression equation obtained from absorbances of different concentrations of gallic acid to calculate phenol content is y = 0.0118x + 0.0243, while the regression equation obtained from absorbances of different concentrations of quercetin to calculate flavonoid content is y = 0.0062x - 0.0055. The results of total phenol content, total flavonoid content and total alkaloid content were presented in Table 3.

GC-MS analysis

Eleven different compounds were determined by GC-MS analysis in the tested sample based on their molecular weight, retention time, area and peaks. The identified compounds are tabulated in Table 4 along with their classes. The GC-MS chromatogram with well-separated peaks of different compounds was depicted in Figure 1.

Vitamin C Estimation

Amla is commonly preserved in its dry form for year-round use. Therefore, it is relevant to assess the retention of vitamin C in its dried state. Accordingly, it was evaluated in both sun-dried and shade-dried fruit. The vitamin C content of *E. officinalis* fruit powder was calculated using the Iodometric titration method. The analysis revealed that vitamin C concentration was found to be 1.503 %w/w and 1.562%w/w in sun-dried and shade-dried powder, respectively. This indicates that 1.503 grams and 1.562 grams of vitamin C is present in 100 grams of sun-dried and shade-dried fruit powder, respectively.

Table 1: Macroscopic description of amla fruit, powder and its ethanolic extract

Fruit shape	:	Globular
Fruit size	:	4 cm on average
Fruit colour	:	Greenish yellow
Fruit weight	:	42- 48g
Odour	:	Sour and Astringent
Taste	:	Characteristic
Dried powder	:	Lighter, brownish
Ethanolic extract of fruit	:	Dark brown, slightly gummy texture

Table 2: Qualitative phytochemical analysis of ethanolic extract of *E. officinalis*

S. No	Phytochemical compound	Test	Ethanolic extract
1	Carbohydrates	Fehling's test	+
2	Tannins	Ferric chloride test	+
3	Flavonoids	Alkaline reagent test	+
4	Alkaloids	Hager's test	+
5	Phenols	Lead acetate test	+
6	Terpenoids	Salkowski's test	+
7	Glycosides	Keller-killiani test	-
8	Phytosterols	Liebermann- Burchard test	+
9	Saponins	Foam test	+
10	Proteins	Ninhydrin test	-

Table 3: Quantitative phytochemical analysis of ethanolic extract of *E. officinalis*

S. No	Phytochemical compound	Ethanolic extract
1	Total phenol content	33.44 mcg GAE/ml
2	Total flavonoid content	62.41 mcg QE/ml
3	Total alkaloid content	23.5 mg/g

Table 4: Identification of phytoconstituents in the ethanolic extract of *E. officinalis* via GC-MS analysis

Peak	Compound	Retention time	Area %	Class
1	4-Morpholinecarbonitrile	7.575	6.81	Heterocyclic compound
2	2-Furancarboxylic acid	9.763	2.65	Heterocyclic compound
3	Decane	10.667	1.28	Alkanes
4	Levoglucosenone	10.886	0.35	Carbohydrates
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	11.823	0.93	Heterocyclic compound

6	4-Methyl-1h-imidazole-5-carbaldehyde, N-methyl	14.163	24.53	Imidazoles
7	1,2,3-Benzenetriol	18.143	48.50	Polyphenol
8	1-Tridecene	18.760	0.86	Alkenes
9	1,2,3,4-Cyclopentanetetrol, (1. alpha., 2. beta., 3. beta., 4. alpha.)-	21.256	10.29	Cyclitol
10	9-octadecanoic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester	23.700	1.23	Fluorinated aliphatic compound
11	Z, Z-6,28-Heptatriactontadien-2-one	25.467	2.58	Aliphatic compound

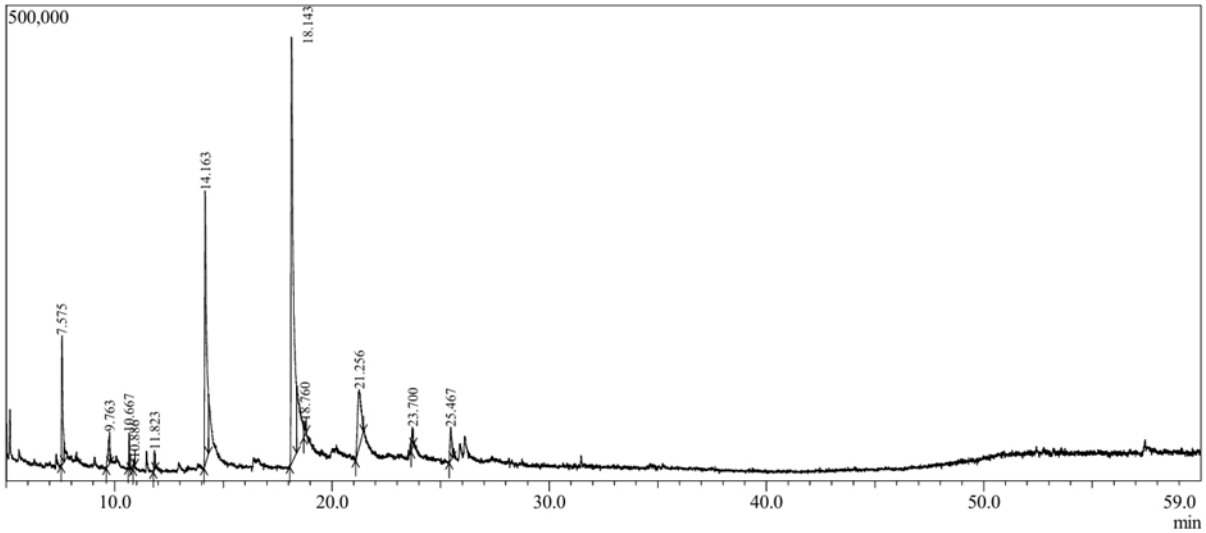

Figure 1: GC-MS chromatogram of phytoconstituents in the ethanolic extract of *E. officinalis*

DISCUSSION

All these phytochemicals contribute to the significant medicinal value of *E. officinalis*. Similar presence of phytochemicals was reported in earlier studies highlighting their anti-inflammatory, anti-microbial and anti-oxidant effects [16,6]. The appreciable results are responsible for their ability to scavenge free radicals and combat free radicals, thereby contributing pharmacological action to the fruit [17].

Two compounds- Levoglucosenone and 1,2,3-Benzenetriol (pyrogallol), belonging to the classes Carbohydrates and polyphenols particularly significant due to their well-documented pharmacological properties. Levoglucosenone presence revealed the partial thermal breakdown of sugar compounds during the drying process and is responsible for anticancer, anti-inflammatory and antimicrobial activities [18]. Another significant compound identified is pyrogallol, which is attributed to the potent antioxidant efficacy of amla [19].

Vitamin C is one of the most important phytonutrients which is sensitive to high temperatures, light and oxygen that making it easily degradable [20]. Despite this, the present study found comparable results of vitamin C in both sun-dried and shade-dried amla fruit powders. Similar findings have been reported by previous author [21] who obtained 213 and 237mg of vitamin C in 100 g of sun-dried and shade-dried amla fruit powders by the indophenol-xylene method. This suggests that there are negligible differences in vitamin C concentration if the process is performed under controlled environmental conditions, like moderate sunlight exposure and shade drying. One more reason is the presence of tannins in the fruit, which is present as a bound structure to ascorbic acid, which may prevent the oxidation during the drying process [22].

CONCLUSION

Although every part of *E. officinalis* plant has certain medicinal activity, the fruits are more commonly employed in rasayana formulations, either alone or in combination with other traditional herbs for the treatment of various diseases in humans and animals due to its appreciable phytochemical profile and rich source of vitamin C. The detailed study into the active constituents of *E. officinalis* fruit is not only relevant but essential for exploring its potential in modern medicine. Therefore, further studies were essential to validate its therapeutic claims and uncover the underlying mechanisms of its bioactive compounds.

Conflict of interest

The authors declared no conflict of interest.

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