J Pharm Biopharm Res, 2024, 6(1): 477-491
DOI: 10.25082/JPBR.2024.01.004

RESEARCH ARTICLE

Phyllanthus emblica: A boon or bane – Unlocking the phytopharmaceutical profile

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Received: June 19, 2024;
Accepted: September 21, 2024;
Published: September 27, 2024.

Citation: Tiwari R, Mahalpure GS, Mahalpure S, et al. *Phyllanthus emblica*: A boon or bane – Unlocking the phytopharmaceutical profile. *J Pharm Biopharm Res*, 2024, **6**(1): 477-491. https://doi.org/10.25082/JPBR.2024.01.004

Abstract: Herbal medicines have been utilised since ancient times to treat various diseases. Medicinal plants have played a significant role in global health, and despite the remarkable progress in modern medicine, plants continue to make a valuable contribution to health. Plants are abundant in tropical regions around the world. Recently, there has been a growing interest in drugs derived from higher plants, especially those used in phytotherapy. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants. In nature, herbs contain a variety of well-arranged medicinal properties. Their uniform medicinal compounds make herbal medicines more effective and of higher quality. *Phyllanthus emblica* has all the antidiabetic, antimicrobial, DPPH activity and chemical profiling for its potential under the Quality control assessment.

Keywords: *phyllanthus emblica,* vitamin C, chebulagic acid, medicinal plant, chebulinic acid

1 Introduction

The scientific community has long been intrigued by natural products, seeking to uncover new discoveries in the pharmaceutical and medical fields. Throughout history, humans have relied on plants for energy and medicinal purposes. Herbal medicines have maintained widespread popularity globally, with the international market for herbal drugs and products reaching approximately USD 166 billion in 2021 and projected to soar to about USD 348 billion by 2028. As the demand for herbal medicine continues to rise, so does the need for medicinal plants and their derivatives. An estimated 960 types of medicinal plants are traded worldwide, with 178 species having an annual consumption of over 100 metric tons. Notably, *Phyllanthus emblica*, commonly known as amla, is among the most widely used plant ingredients in herbal formulations. The global market for amla was valued at USD 37.70 billion in 2021, with an expected growth to USD 45.87 billion by 2026 and a compound annual growth rate (CAGR) of 7.53% during 2022-2029. Amla is renowned for its numerous phenolic compounds, particularly the potent antioxidant chebulagic acid, in addition to components such as ascorbic acid, gallic acid, emblicanin, chebulinic acid, and others [1]. *Chebulagic acid* is a benzopyran, categorised as a polyphenol and tannin, and is majorly found in *Terminalia chebula* and *Phyllanthus emblica*. The compound has been reported to exhibit diverse biological activities, such as antioxidant, antidiabetic, and antiproliferative; the compound chebulagic acid has been found to have various beneficial properties, such as being a potent inhibitor of certain enzymes responsible for inflammation, microbial infection, and other health issues. It has shown inhibitory effects on inflammation-related enzymes, microbial infection, and glucose metabolism. Research has demonstrated its ability to lower the production of specific molecules associated with inflammation and oxidative stress and inhibits the proliferation of particular cancer cells and viruses. Due to its high therapeutic value, chebulagic acid is in high demand and expensive [2].

The Indian Pharmacopoeia Commission (IPC) is actively working to maintain high standards for drugs and phytopharmaceutical ingredients and to ensure the quality of marketed products [3]. The study focuses on chebulagic acid in amla fresh fruit juice, marketed amla fruit juice, and amla powder, using advanced analytical techniques for detection and quantification. With the surge in demand for amla juice during the COVID-19 pandemic, concerns have arisen about manufacturers' potential compromise in product quality to meet market demands. The presence of chebulagic acid was analysed using HPLC, HPTLC, and HRMS techniques, providing valuable data for the study.

Phyllanthus emblica is utilized by the Unani, Tibetian, Srilanka, and Chinese systems. Amla is considered a strong rejuvenator. Amla helps in the degenerative and senescence process. It helps in various abdominal defects such as constipation, improve digestion, and longevity. It also helps in cough, fever, benefits of eyes, strength of the heart, courage hair growth, and hair pigmentation as per the Ayurveda system of medicine. Amla tree is of small and moderate size with greenish yellow color and greenish grey color bark formed in axillary clusters. Feathery leaves are linear-oblong with acute apex, subsessile, closely set along branchlet, light green pinnately leaves shown in Figure 1.

Figure 1 Flowering and leaves part of plant, Fruit of the plant, the root part of the plant, and powder form of plant fruit.

There is evidence that a lot of phytochemical constituents found in *Phyllanthus Emblica* e.g. flavonoids, tannins, phenolics, alkaloids, saponins exhibit various treatments such as anti-inflammatory, diabetes, gonorrhea, and cholesterol [4] (Table 1 and 2). Total phenolic compounds have a strong role in stabilizing oxidation and dealing with antioxidant activity. The bioactive compounds (total phenols, tannins, and flavonoids) contents found higher in seeds than in fruit of *phyllanthus emblica* [5].

Table 1 Phytochemical constituents in amla fruit

Corilagin	Emblicanin A
Mucic acid 2-o-gallate	Emblicanin B
Corilagin Chebulagic acid	Punigluconin
Elacocarpusin	Pedunculagin
Mucic acid	Methyl gallate
1-o-galloyl-β-D-glucose	Chlorogenic acid
Mucic acid 6-methyl ester 2-o-gallate	Ascorbic acid (Ascorbic acid)
Mucic acid 1	Ellagic acid
4-lactone 3-o-gallate,	Gallic acid

Table 2 The classes of chemical constituents reported in the amla fruit

Class	Compound
Alkaloids	Phyllantine, Phyllantidine, Phyllembein
Amino Acids	Glutamic acid, Proline, Aspartic acid, Alanine, Cystine, Lysine
Carbohydrates	Pectin
Flavonoids	Quercetin, Kaempferol
Hydrolysable Tannins	Emblicanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyran tannin), Corilagin (Ellagitannin), Geraniin (Dehydroellagitannin), Ellagotannin
Organic Acids	Citric Acid
Phenolic Compounds	Gallic acid, Methyl gallate, Ellagic acid, Trigallayl glucose
Vitamins	Ascorbic acid (Vitamin C)

2 Materials and methods

2.1 Chemicals and reagents

The solvents utilized for experimentation, including ethanol, methanol, ethyl acetate, dimethyl-sulfoxide (DMSO), and chloroform, were of analytical grade and were procured from Finar

Ltd. and Merck. Additionally, HPLC-grade solvents were acquired from Standards for HPTLC analysis. The compounds Rutin hydrate, Quercetin, Gallic acid, and Ellagic acid were obtained from Sigma Aldrich. Furthermore, the DNS reagent, Anisaldehyde reagent, Alpha-amylase enzyme, maltose, and starch were purchased from SRL Chemicals. Finally, the bacterial and fungal media, including nutrient broth, nutrient agar, and potato dextrose agar, were sourced from HiMedia Ltd.

2.2 Bacterial and fungal samples procurement

In order to determine their anti-microbial activity, we obtained two gram-positive bacteria, specifically Bacillus subtilis ATCC 6633 and Staphylococcus aureus ATCC 6535, two gram-negative bacteria, namely Escherichia coli ATCC 10536 and Salmonella typhi ATCC 14025, and one fungus (yeast) Candida albicans ATCC 10231 from the Microbiology division of the Indian Pharmacopoeia Commission in Ghaziabad.

2.3 Microscopical evaluation

The dried fruit pericarp of *Phyllanthus emblica* thin sections were cut using a sharp razor blade to obtain a very thin, almost transparent slice, placed on a glass slide and covered with a coverslip.

2.4 Determination of physicochemical parameters

Physicochemical standards such as extractive value, total ash, and loss on drying were determined in accordance with the procedures outlined in IP-2022 [3]. The methods used for the tests are described below.

2.4.1 Determination of loss on drying

5g of plant material was precisely weighed in a crucible and dried at 105°C for 3 hours in a hot air oven. The weight was recorded after drying to ascertain the loss. The sample was then intermittently dried at 1-hour intervals until a constant weight was achieved. Constant weight was attained when the difference between two consecutive weights was not more than 0.05mg. For weighing, the crucibles were cooled in a desiccator to prevent moisture absorption from the atmosphere during the cooling process. The percentage loss on drying was calculated using the following formula:

$$\% \text{ Loss on Drying} = (\text{Difference in the weight of air-dried sample and oven dried sample}) \times 100 \div (\text{weight of air-dried sample})$$

2.4.2 Determination of water-soluble extractive value

5 g of air-dried coarsely powdered neem were soaked in 100 mL of milli-Q-water in a closed flask for 24 hours. The mixture was shaken occasionally during the first 6 hours and then allowed to stand for 18 hours. After that, it was filtered through Whatman filter paper no.41. 25 mL of the filtered solution was evaporated to dryness in a preweighed flat-bottom petri dish, dried at 105°C to remove moisture, and then weighed. The % w/w water-soluble extractive value was calculated using the given formula.

$$\% \text{ Water soluble extractive} = [\{\text{weight of the dried 25 mL extractive/weight of sample (5 g)}\} \times \text{dilution factor} \times 100]$$

Weight of sample taken – 5 g; Dilution factor – 4.

Extractive weight can be calculated by subtracting the initial petri plate weight from the dried plate with extractive.

2.4.3 Determination of alcohol soluble extractive value

5 g of air-dried coarsely powdered neem was macerated with 100 mL of ethanol in a closed flask for 24 hours, shaken occasionally during the first 6 hours, and allowed to stand for 18 hours. The mixture was filtered through Whatman filter paper No.41, and 25 of the filtrate was evaporated in a preweighed flat-bottom petri dish dried at 105°C and weighed accordingly. The given formula calculated % w/w alcohol soluble extractive value:

$$\% \text{ Alcohol soluble extractive} = [\{\text{weight of the dried 25 mL extractive/weight of sample (5 g)}\} \times \text{dilution factor} \times 100]$$

Weight of sample taken – 5 g; Dilution factor – 4. Extractive weight can be calculated by subtracting the initial petri plate weight from the dried plate with extractive.

2.4.4 Determination of total ash

Approximately 2 g of air-dried drugs were precisely weighed and placed in a silica crucible that had been previously ignited and weighed to determine its empty weight. The sample was then ignited in a muffle furnace, with the heat gradually increased from 500 to 600±25°C until it turned into white ash over a period of 4 hours. Afterwards, the stove and crucibles were left to cool before being carefully removed from the stove using tongs and transferred to a desiccator to cool to room temperature. The percentage by weight of the total ash with respect to the air-dried material was then calculated using the provided formula.

% Total ash = {Total ash formed in the crucible/weight of sample (2 g)} × 100

Crucibles in the furnace should be handled with great care as it may cause severe burn.

2.4.5 Determination of acid insoluble ash

The ash produced in the previous experiment was treated with 25 mL of 2M HCl and boiled for 5 minutes. The resulting solution was filtered using ashless filter paper and transferred back into the same crucible. The crucibles with filter paper were then reheated at 600±25°C until they turned into white ash. After cooling down, the furnace and crucibles were carefully removed using tongs and placed in a desiccator until they reached room temperature. The percentage weight/weight of acid-insoluble ash based on the air-dried material was then calculated using the provided formula:

% Acid insoluble ash = {Total ash formed in the crucible with ashless filter paper/weight of the sample (2 g)} × 100

Crucibles in the furnace should be handled with great care as it may cause severe burn.

2.4.6 Extract preparation

Two types of extracts of *Phyllanthus emblica* leaves were prepared: methanol and hexane. Using a Soxhlet apparatus, 5 g of coarsely powdered leaves was refluxed with 100 mL each of hexane and methanol for 2 hours in separate round bottom flasks. The filtrates obtained were concentrated under a vacuum using a rotary evaporator. Yields were calculated as a percentage by weight using the following formula:

% Yield = (weight of dried extract/total weight of sample) X 100

2.5 Preliminary phytochemical screening

We conducted a preliminary phytochemical analysis of *Phyllanthus emblica* leaves to identify various compounds present in the plant. The tests carried out and their results are as follows:

Alkaloids: A few drops of Wagner's reagent were added to 2 mL of the leaf extract, forming a reddish-brown precipitate, indicating the presence of alkaloids.

Flavonoids: A yellow colour formed upon adding 2 mL of NaOH to 2 mL of the plant extract. Diluted acid was added, and the colour turned colourless, signifying the presence of flavonoids.

Tannins: Mixing 2 mL of plant extract with a few drops of ferric chloride produced a violet colour, indicating the presence of tannins.

Terpenoids: A mixture of 5 mL of the extract with 2 mL of chloroform and concentrated sulphuric acid formed a reddish-brown colour, indicating the presence of terpenoids.

Glycoside: Adding 2 mL of acetic acid and 2 mL of chloroform to the plant extract caused the sample to cool down. A few drops of H_2SO_4 resulted in the appearance of green colour, indicating the presence of glycosides.

Anthraquinone: Mixing 5 mL of extract with dilute H_2SO_4 and 1 mL of diluted ammonia caused the extract to appear pink, indicating the presence of anthraquinone.

Phytosterols: The addition of 2 mL of chloroform to the plant extract, followed by the addition of 2 mL of H_2SO_4, caused the chloroform layer to become red.

In addition to the phytochemical analysis, studied the therapeutic actions of the *Phyllanthus emblica* leaf extract by assessing its in-vitro anti-oxidant, anti-diabetic, and anti-microbial activities. For the anti-oxidant assay, I used the Phosphomolybdenum assay, also known as the reducing power assay. Prepared a 0.1 mM solution of Phosphomolybdenum reagent by mixing equal volumes of 0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Then, I added 1 mL of the prepared reagent to 1 mL of the extract solution in

methanol at different concentrations (0, 20, 40, 60, 80, and 100 μg/mL). Subsequently, I incubated the samples at 95°C in a water bath for 90 minutes and measured the absorbance at 517 nm.

Commercially available "amla swaras" and amla powder were bought from the general store of Raj Nagar, Ghaziabad. Fresh amla fruit was bought from a nearby vegetable market. Chebulagic acid was purchased from Sigma Aldrich. Solvents for HPLC, MS, and HPTLC were purchased from Merck of LC grade.

2.6 Sample preparation

Fresh amla juice: The amla fruit was cut into pieces, the seed removed, and weighed accurately. It was then macerated using a mortar and pestle. The macerated amla fruit was suspended in distilled water and sonicated for 2 hours at 50°C. The solution was filtered and concentrated using a rotary evaporator, and the percentage yield was measured according to the given formula.

Amla powder extract: 5 g of amla fruit powder was mixed with 100 mL of water and sonicated for 2 hours at 50°C. The solution was filtered and concentrated using a rotary evaporator, and the percentage yield was measured accordingly.

Commercial amla swaras: After proper shaking, 50 mL of amla swaras were taken and concentrated into paste form using a rotary evaporator. The total weight was noted for quantitation.

Percentage yield: $\{$Weight of extract/Weight of raw material taken$\} \times 100$

All three samples were reconstituted in water, and 5mg/mL stock solution was prepared for further experimentation.

2.6.1 HPTLC analysis

For standard and sample preparation, chebulagic acid was used as the standard for comparison. We dissolved 5mg of the compound in 20 mL of distilled water in a volumetric flask, resulting in a 0.25 mg/mL (250 ppm) solution. The test samples, stock solutions were then reconstituted to a 1mg/mL concentration as the working solution. For the sample application, we placed a 10×10 cm TLC plate on the CAMAG ATS4 sample applicator (Table 3). We applied six bands: two bands of chebulagic acid, two bands of amla swaras, and one band each of amla powder extract and fresh amla juice, all 8 mM in size.

Table 3 Sample application on TLC plate

Track	Sample	Application volume (μL)	Application position (mM)
1	Chebulagic acid	5	15
2	Amla swaras	5	29
3	Amla swaras	10	43
4	Chebulagic acid	10	57
5	Amla powder extract	10	71
6	Fresh amla juice	10	85

Mobile phase: 4 volumes of *glacial acetic acid*, 7 volumes of *chloroform*, 2 volumes of *methanol*, 1 volume of *formic acid*, and 0.5 volume of *water* were mixed in 20 mL of volumetric flask and sonicated for 5 minutes.

TLC plate was developed in the development chamber upto 8.0 cm and air dried. The plate was visualised at 254 nm, 366 nm, and daylight. After that, the plate was scanned at different wavelengths. The best wavelength was selected and scanned again. After the peak assignment of chebulagic acid on each track, all bands were scanned to obtain a UV-visible spectrum of chebulagic acid for comparison. Chebulagic acid content on each track was measured based on the obtained peak area by the following formula: -

% Chebulagic acid = (Sample peak area/Standard peak area) × (Standard amount applied on TLC plate/Sample amount applied on TLC plate) × 100

2.6.2 HPLC analysis

The working solutions of samples (1mg/mL) and standard (0.25mg/mL) were prepared and filtered using a syringe filter (0.45μm). The samples were analysed on C_{18} column, Orbit, 150 mM × 4.6 mM; 3.5μm on Agilent infinity 1260. The sample injection volume was 30 μL for the samples and 5 μL for the standard, while the mobile phase used was ACN: 0.1%

Orthophosphoric acid solution in MiliQ water (30:70) (Table 4). Elution was a gradient, and the run time was 30 minutes. Before sample injection, the column was washed for 45 minutes with the above-mentioned mobile phase. The peak detection was done at 254 nm.

Table 4 Gradient program of mobile phase for HPLC analysis

Time	Mobile phase A	Mobile phase B
0	30	70
2	0	100
5	20	80
12	55	45
15	70	30
18	80	20
20	60	40
25	30	70
28	20	80
30	20	80

Note: Total chebulagic content was measured by the given formula: Chebulagic acid concentration = (Sample area/Standard area) × (Standard concentration/Sample concentration) × (Standard purity) mg

2.6.3 Mass spectrometry

The samples and the standard were analysed using a direct sample analyser from Perkin Elmer. The system software analysed a drop of stock solution placed on the mesh. The peaks obtained were matched with the MS library.

2.6.4 DPPH radical scavenging assay

The antioxidant activity of *Phyllanthus emblica* fruit extract was evaluated by its ability to neutralise the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical [6]. The reduction in absorbance was recorded at 517 nm after 30 minutes using a spectrophotometer (Table 5). The percentage of inhibition was determined using the formula (extract stock solution concentration – 1 mg/mL): % of inhibition = (control – sample)/control × 100.

Table 5 DPPH radical scavenging assay

Extract volume (μL)	Water (mL)	Reagent	Incubate	Water
0	2.0	1 mL	Incubate for 90 minutes at 95°C	2 mL
20	1.8			
40	1.6			
60	1.4			
80	1.2			
100	1.0			

Note: The inhibition % was calculated using the following formula; % of inhibition = control - sample/control ×100

2.7 Antidiabetic activity

In vitro anti-diabetic assay, we examined the impact of the *Phyllanthus emblica* fruit extract on the alpha-amylase enzyme, which plays a crucial role in breaking down complex sugars without insulin. To conduct the assay, we utilised DNS (3, 5-dinitrosalicylic acid) to react with mono and disaccharide sugars and produce a colour for analysis [7]. We established a standard curve using maltose and prepared different concentrations of it. Subsequently, the DNS reagent was incubated for 10 minutes at 25°C in a water bath. Initially, we incubated the plant extract with the alpha-amylase enzyme for 10 minutes at 25°C. Following this, a 1% w/v starch solution was introduced and incubated for another 10 minutes in a phosphate buffer. After diluting the content with distilled water, we measured the absorbance of the standards and the extract at 540 nm. The concentrations of the stock solutions are - Metformin stock solution: 1 mg/mL; Alpha-amylase stock solution: 10 units/mL (2 mg/mL); Starch solution: 1% w/v (Table 6).

2.8 Anti-microbial activity

Alpha-amylase is a crucial enzyme that plays a key role in breaking down starch into maltose in both saliva and pancreatic secretions. Inhibitors are substances that can reduce glucose levels after a meal by slowing down alpha-amylase activity. One commonly used method to measure

Table 6 Dilution of Antidiabetic activity

Extract volume (μ)	Water/PBS (mL)	Enzyme (mL)	Incubate	Starch (mL)	DNS reagent	Incubate	Water (if needed)
0	2.0	0.5	Incubate at room temperature for 10 minutes	0.5	1 mL	Incubate for 15 minutes at boiling temperature	2 mL
20	1.8						
40	1.6						
60	1.4						
80	1.2						
100	1.0						

alpha-amylase activity involves DNS (3, 5-dinitrosalicylic acid) and a phosphate buffer [8]. The phosphate buffer is prepared by dissolving 10.9 g of anhydrous Sodium phosphate dibasic (Na2HPO4), 3.2 g of anhydrous sodium phosphate monobasic (NaH2PO4), and 90 g of sodium chloride (NaCl) in 1 liter of distilled water. The pH of the solution is adjusted to 7.4, and the volume is made up to 1 liter. Additionally, alpha-amylase inhibitors can have antimicrobial activity.

2.9 Antimicrobial susceptibility tests

The process of testing antibiotics through microbiological assays involves comparing the ability of the antibiotic being evaluated to inhibit the growth of microorganisms with that of a standard preparation. This method is used when the effectiveness of a drug substance cannot be ascertained through its chemical or physical properties. The approach is straightforward, precise, and cost-effective. Another method, known as disk diffusion, entails placing antimicrobial agents on disks and observing the zones where the growth of microorganisms is inhibited in the culture medium. The agar dilution method determines the minimum inhibitory concentration by introducing varying concentrations of the antimicrobial agent into an agar medium. On the other hand, the broth dilution method assesses the susceptibility of microorganisms to antibiotics in a liquid medium. The good diffusion method measures the zone where growth is inhibited from estimating the potency of the antibiotic. Culture media play a crucial role by providing the necessary nutrients to grow, select, or count microorganisms [9].

5 g of Amla dry fruit powder was added to 150 mL of MeOH in a round-bottom flask. Then, using a Soxhlet apparatus, it was refluxed at boiling temperature for 2 hours at 65°C. After refluxing, the mixture was cooled down for 20-25 minutes.

(1) The next step is filtering that mixture using filter paper and a funnel.

(2) The solid residue was stored for further work, and the liquid part was concentrated with the help of a rotary evaporator at 70ºC boiling temperature of hexane for 25-30 minutes at 120 rpm with 250 pressures to completely evaporate MeOH. Only the dry extract was taken.

(3) After evaporation of MeOH from the plant extract, weigh the total yield at the weight machine.

The total weight of plant extract in the bottle was 108.39 g, and the empty weight was 107.53 g, so subtracting the empty weight from the total weight, we got 0.25434 g, transferred the extract to a beaker for the DMSO extract preparation and added 10 mL DMSO to the total weight of the plant extract. We made two concentrations of our extract, such as 10% and 100%. After that, we stored that extract for further use.

2.9.1 Preparation of microbiological media

The media's primary objective was to support the rapid growth of indicator microorganisms being used in the bioassay. Soybean casein digest agar media were used for slant preparation for bacterial growth by dissolving 5 g in 250 mL distilled or Mili-Q. Nutrient agar was used to check the antimicrobial activity in this study [10]. NA media were dissolved 4 g in 100 mL the distilled or and the Media were sterilized in an autoclave at 121°C and 15 lbs. for 15 min.

2.9.2 Selection of test microbial strains

Different cultures from gram-positive and gram-negative microorganisms and fungi categories were used for antimicrobial activity. We used different gram-positive bacteria *Staphylococcus aureus* (ATCC-6538), *Bacillus subtilis* (ATCC-6633) and), *Kocuria rhizophila* (ATCC9341), *Micrococcus leuteus* (10240) and gram-negative bacteria *Escherichia coli* (ATCC-8739), *Salmonella typhi* (ATCC-14028) and one fungus *Candida albicans* (ATCC-10231). These microbial cultures were procured from the American Type Culture Collection (ATCC), National Collection of Type Cultures (NCTC), and Microbial Type Culture Collection (MTCC) [11].

2.9.3 Inoculum preparation and its standardization

A practical and fully characterized microbial strain is required for antimicrobial activity. Fresh microbial strains preserved on glycerol stock were revived and then subcultured on the slants of soybean casein digest agar media. The slants were incubated at 37°C for 24 hours for bacterial growth. Fresh culture slants were used throughout the study. About 5 mL of sterilized saline solution (0.9%) was used to wash the microorganisms from the agar slant and then to check the antimicrobial activity of the plant extract [12].

2.9.4 Preparation of sample dilutions

The extracts of *Phyllanthus emblica* were dissolved in DMSO solvents and then diluted to 100 mg/mL, and 10 mg/mL in sterile water to check the effectiveness of Amla against selected microorganisms (Patel et al., 2020).

(1) Sterilize the required glassware such as Petri plates, volumetric flasks, glass pipettes, borer, and test tubes in Hot Air Oven at 160°C for 2 hours.

(2) Prepare the required quantity of Nutrient Agar media as per instructions given on the label of the medium container, 0.9% NaCl, and distilled water for sample dilutions, and sterilize it in an autoclave at 121°C/15lbs for 20 minutes.

2.9.5 Preparation of inoculum suspension

(1) On one day before the experiment, take the working microbial culture slant and sub-culture it on the fresh slant of Soyabean casein digest agar medium and incubate at 30-35°C for 18-24 hours.

(2) Take the sub-cultured slant and, add 5 mL of normal saline; shake well.

(3) Transfer the required volume of culture suspension to the flask containing seed layer media and mix them properly.

2.9.6 Preparation of media plates

(1) Switch on the UV light of LAF for 30 minutes before starting the test.

(2) Sterilized Nutrient agar media was taken out of an autoclave and cooled down at 45-50ºC.

(3) Pour 25 mL of NA media into a sterilized Petri plate to form a base layer and allow it to solidify.

(4) Then, pour 4 mL of Nutrient Agar media seeded with diluted inoculum suspension into the Petri dish as per pharmacopoeia using a graduated measuring cylinder and allow them to solidify for 30 minutes at room temperature.

(5) Make wells of 6 mM diameter with the help of a borer.

(6) Transfer 100 μL of sample dilutions with the help of a micropipette into the wells labelled with different concentrations.

(7) Left the dishes or plates standing for 1 to 4 hours at room temperature.

(8) Then incubate the plates at 30-35°C for 18-24 hours.

(9) Observe the result and measure the zone of inhibition.

3 Results and discussion

Phyllanthus emblica dry fruit powder was found to have 5.65% moisture content as observed % LOD. The % water soluble extract was found to be 53.08%, while alcohol soluble extractive was 31.08%. It was observed that amla dry fruit contains 2.15% total ash, out of which only 0.66% ash in insoluble in acid (Table 7). The physicochemical results obtained were found to be correlated to that mentioned in Amalaki monograph of IP-2022 [13].

Table 7 Results of physicochemical parameters of *Phyllanthus emblica* dry fruit

Parameters	Results (%)	Limits given in IP 2022	Complies/ Not Complies
Loss on Drying	5.65	NMT 12.0%	Complies
Alcohol Soluble Extractive	31.08	NLT 30%	Complies
Water Soluble Extractive	53.08	NLT 40%	Complies
Total ash	2.15	NMT 5.0%	Complies
Acid insoluble ash	0.66	NMT 2.0%	Complies

3.1 HPTLC analysis

Fresh amla juice contained the highest amount of chebulagic acid as detected from the HPTLC analysis. Chebulagic acid band was observed at 0.28 Rf on track 1, 3, 4, 5, and 6. On

track 2, no chebulagic acid band was detected. As track 2 and 3 is commercial amla swaras at 5μL and 10μL respectively, it shows less presence of chebulagic acid. While, chebulagic acid band was clearly observed on track 5 and 6, indicating the presence of the compound in amla powder extract and fresh amla juice. The scanning of the band, after peak assignment, showed very similar pattern of UV spectrum in case of the standard and amla juice, while the patter was somewhat deviated in case of amla powder and no pattern was observed in commercial amla juice. From the peak area obtained at maximum wavelength (280nm) of chebulagic acid, the calculated content of the compound in amla swaras is only 0.14%, while fresh amla juice and amla powder contain 22.76% and 7.14% respectively. The result clearly shows the high chebulagic acid content in fresh amla juice (Figure 2 & 3, and Table 8).

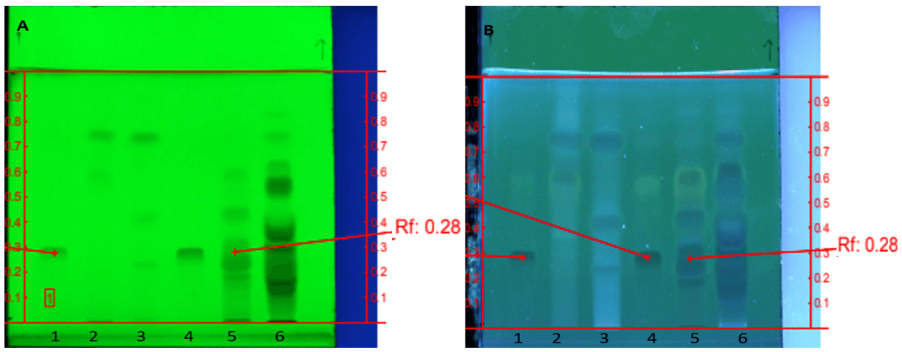

Figure 2 TLC plate visualization at 254 nm and 366 nm. Track 1 – Chebulagic acid (5 μL); Track 2 – Amla swaras (5 μL); Track 3 – Amla swaras (10 μL); Track 4 – Chebulagic acid (10 μL); Track 5 – Amla powder extract (10 μL); Track 6 – Fresh amla juice (10 μL).

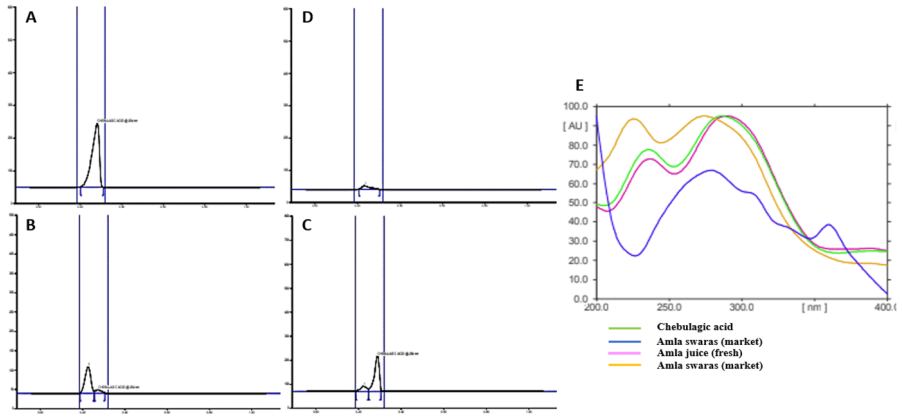

Figure 3 Assigned peak area of A. Standard (10 μL); B. Amla powder; C. Amla fresh juice; and D. Amla swaras. E. UV spectrum of assigned bands of standard (green); amla swaras (blue); amla powder (yellow); and amla fresh juice (pink).

Table 8 Total chebulagic acid content in different samples by HPTLC

Sample	Amount applied on plate (μg)	Peak area (366nm)	Chebulagic acid (%)
Chebulagic acid (standard)	1.25	9370	100.0%
Amla swaras (market)	10	58	0.147%
Amla powder extract	10	2817	7.14%
Amla fresh juice	10	8980	22.76%

3.2 HPLC analysis

The HPLC results resonate with the results obtained in the HPTLC analysis of the amla samples. For the HPLC analysis, it was observed that the marketed amla juice contains a minimal amount of chebulagic acid, which was calculated to be only 0.14%. While the chebulagic content in amla powder extract and the fresh amla juice was 10.72% and 24.05%, respectively. The standard was detected at 11.433 minutes. In the amla swaras, it was detected at 11.493 minutes,

while in amla powder and amla fresh juice; it was detected at 11.440 and 11.447 minutes, respectively. It is to be noted that the quantitative estimation of the compound through HPTLC and HPLC was almost identical (Figure 4 and Table 9).

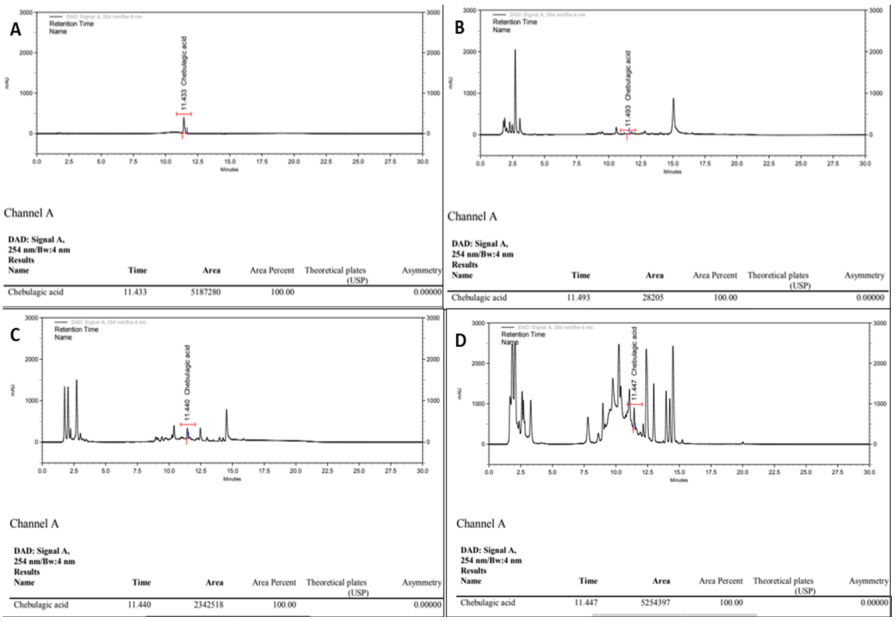

Figure 4 HPLC chromatograms of A. Standard, B. Amla swaras, C. Amla powder extract, and D. Fresh amla juice.

Table 9 Total calculated content of chebulagic acid in different amla samples by HPLC

Standard area	5187280	Standard purity	95%	
Sample	Sample concentration (mg/mL)	Standard concentration (mg/mL)	Sample area	Standard concentration
Amla swaras	1	0.25	28205	0.14%
Amla powder extract	1	0.25	2342518	10.72%
Fresh amla juice	1	0.25	5254397	24.05%

3.3 Mass spectrometry analysis

It was observed that chebulagic acid was breaking into several fragments, as the mass spectrum showed several peaks for the chebulagic acid standard. The highest detected peaks are 206.24, 207.24, 279.26, and 180.43. The mass spectrum of fresh amla juice was observed to have the highest peaks of – 206.24, 207.24, 279.26, 198.17, and 180.15. The mass spectrum of amla powder extract consisted of – 279.26, 288.22, 206.23, 207.24, 180.148, 198.17, and 391.43. Amla swaras showed - 198.17, 238.16, 210.14, 180.15, 263.17, 278.25, and 296.20 peaks.

From the above peak analysis, it was observed that the fragmentation pattern of chebulagic acid was similar in fresh juice, followed by amla powder extract. Hence, it was inferred that fresh amla juice contains the highest amount of chebulagic acid, followed by powder extract, and the lowest content of chebulagic acid was found in commercially available amla swaras (Figure 5).

3.4 In vitro screening

3.4.1 Anti-oxidant assay

The Extract and standard concentration with the absorbance on 695 nm the absorbance values are in Table 10 and Figure 6.

3.4.2 Reducing power assay of Amlaki extract

The reducing power of ascorbic acid is at 695 nm, as shown in Table 11.

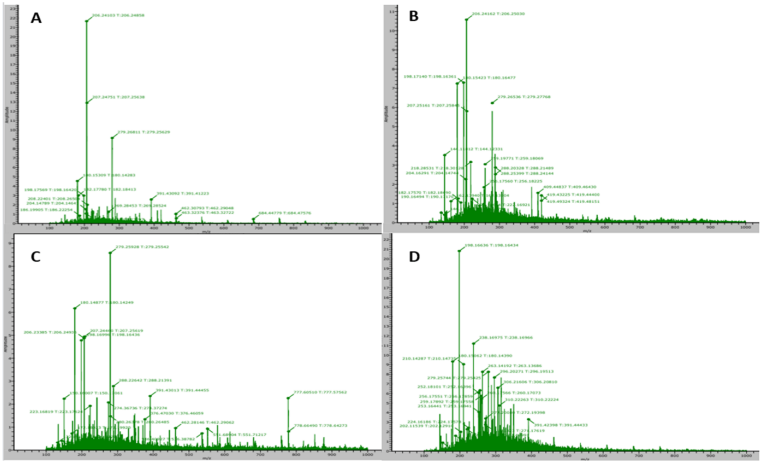

Figure 5 Mass Spectrometry analysis of A. Standard, B. Fresh amla juice, C. Amla powder extract, and D. Amla swaras.

Table 10 Anti-oxidant assay of ascorbic acid

Ascorbic acid Concentration	Absorbance at 695		Mean Absorbance	Standard Deviation
	Set 1	Set 2		
0	0	0	0	0
10	0.1196	0.1225	0.1211	0.002050610
20	0.1458	0.1727	0.1593	0.019021172
30	0.1743	0.2112	0.1928	0.026092240
40	0.2356	0.2629	0.2493	0.019304015
50	0.2865	0.3291	0.3078	0.030122749
60	0.3455	0.3169	0.3312	0.020223254
70	0.3987	0.3869	0.3928	0.008343860
80	0.4985	0.4922	0.4954	0.004454773
90	0.5112	0.5236	0.5174	0.008768124
100	0.5826	0.5830	0.5828	0.000282843

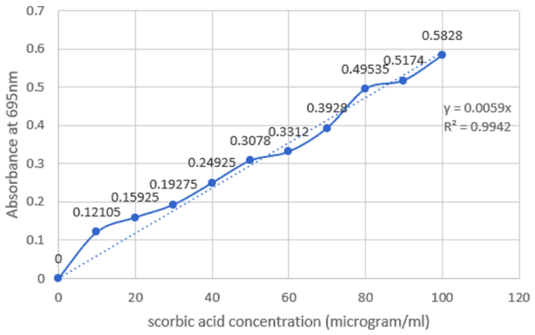

Figure 6 Ascorbic acid standard curve

Table 11 Reducing power assay of Amlaki extract

Sample concentration	OD at 695 nm	Reducing power AAE	Antioxidant/mg of extract
0	0	0	0.00
20	0.076	12.88	644.07
40	0.149	25.25	631.36
60	0.249	42.20	703.39
80	0.314	53.22	665.25
100	0.382	64.74	647.46
Mean			658.31
SD			27.96
RSD			4.25

The reducing power of the extracts was calculated by using the slope value of the ascorbic acid standard curve.

The formula of linear regression: y = mx + c, Where c is the y-intercept. In the case of the ascorbic acid curve, it is 0. y = 0.0059x, x = y/0.0059. Where x is the reducing power of extract equivalent to ascorbic acid; y is the Absorbance of extract at 695 nm.

3.4.3 Anti-diabetic assay

Extracts were first incubated with enzyme, and then the starch solution was added to the reaction mixture and incubated for 15 minutes. DNS reagent was added and incubated for 15 minutes at boiling temperature. Readings were noted at 517 nm after dilution. Metformin was used as a standard anti-diabetic drug (Table 12).

Table 12 Standard curve of Maltose

Maltose (mM)	Absorbance at 517 nm
0	0
10	0.024
20	0.063
30	0.098
40	0.114
50	0.162
60	0.186
70	0.228
80	0.279
90	0.314
100	0.329

Alpha–inhibition assay of Metformin is shown in Table 13 and Figure 7.

Table 13 Alpha – Inhibition assay of Metformin

Sample concentration	Absorbance	Maltose formation (mM)	Enzyme activity (Unit/mL)	Enzyme inhibition (%)	Enzyme activity/mg of extract
0	0.258	78.18	11.73	0.00	
20	0.074	22.42	3.36	71.32	331.82
40	0.041	12.42	1.86	84.11	203.41
60	0.026	7.88	1.18	89.92	146.97
80	0.025	7.58	1.14	90.31	110.80
100	0.002	0.61	0.09	99.22	99.09

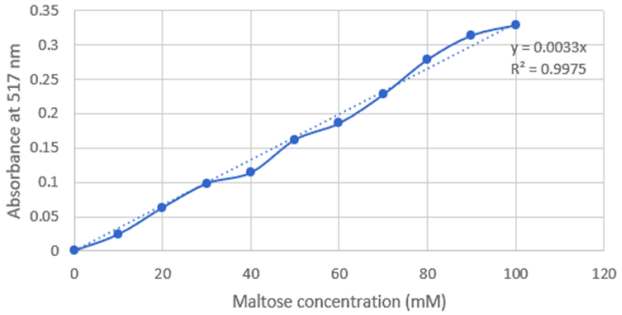

Figure 7 Maltose standard curve

Enzyme activity was assessed using the Maltose standard curve. Enzyme activity = (Maltose formation × Vol. of cuvette)/(No. of enzyme units). All extracts showed excellent anti-diabetic activity, comparable to Metformin. Meanwhile, the enzyme inhibition curves of amla and metformin were similar. At 100μg/mL conc., % a-amylase inhibition of amla and Metformin (equivalent activity) showed the highest anti-diabetic activity (Table 14 and 15, Figure 8).

Stock solution of 100 mg/mL of amla was prepared and that solution was diluted to 10% that is 10 mg/mL with diluent. The antimicrobial activity of extract and dilution are described below in Table 16 and Figure 9.

Table 14 Enzyme activity in the presence of extracts (% Enzyme Inhibition)

Sample Concentration	Metformin	Amlaki
20	71.32	80.09
40	84.11	90.91
60	89.92	90.04
80	90.31	94.81
100	99.22	95.24

Table 15 Enzyme activity in presence of extract

Sample Concentration	Metformin	Amlaki
0	11.73	10.50
20	3.36	2.09
40	1.86	0.95
60	1.18	1.05
80	1.14	0.55
100	0.09	0.50

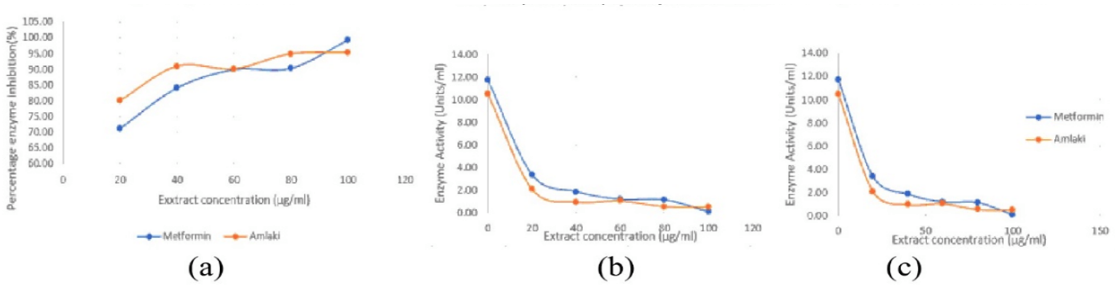

Figure 8 (a) Alpha amylase inhibition (b) & (c) Enzyme activity in the presence of extract Antimicrobial activity.

Table 16 Anti-Microbial activity

Type of Microbes		10 mg/mL	100 mg/mL	DMSO
Fungi	C.albicans 10231	10 mM	14.1 mM	10 mM
Gram–ve bacteria	E.coli 10536	10 mM	14.4 mM	10 mM
	S.typhirium14025	10 mM	10 mM	10 mM
Gram+ve bacteria	B.subtilus 6633	10 mM	13.0 mM	10 mM
	S.aureus 6535	10 mM	17 mM	10 mM
	k.rhizophila 9341	10 mM	14.8 mM	10 mM
	M.leuteus 10240	13.7 mM	18 mM	10 mM

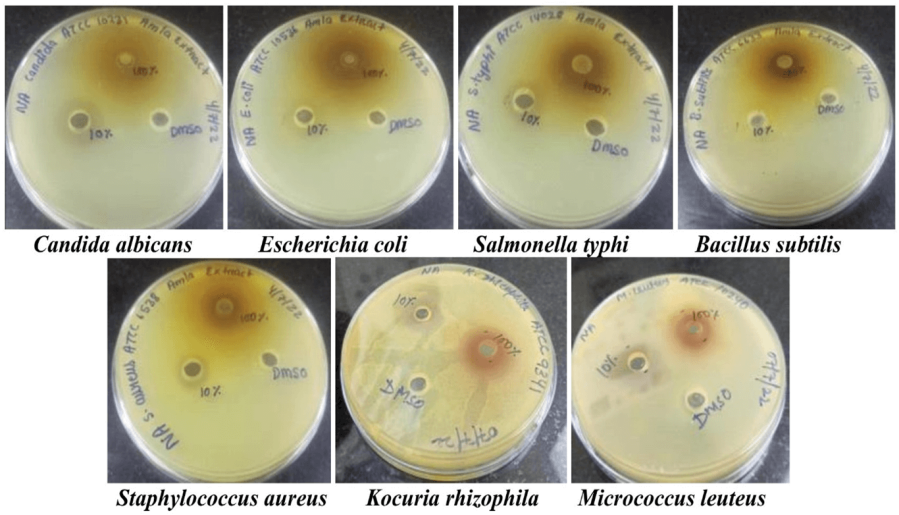

Figure 9 Fungi (*Candida albicans*), Gram '-ve' bacteria (*Escherichia coli, Salmonella typhi*), Gram '+ve' bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Kocuria rhizophila*, *Micrococcus leuteus*).

4 Discussion

Phyllanthus emblica is a commonly found plant in the Indian sub-continent. It is said to have several therapeutic activities, such as antioxidant, anti-diabetic, and anti-microbial activities. Amla powder is also used in several Ayurvedic formulations. However, there are very few evidence-based studies; no lead compound has been isolated for new drug design. Hence, this study evaluated and identified the bioactive compounds in *Phyllanthus emblica*. This study has also been conducted to prepare a Phytopharmaceutical Ingredient (PPI) monograph of *Phyllanthus emblica*. For this, physicochemical tests and microscopic analysis were performed to validate the raw material quality. From phytochemical screening, it was found that the plant majorly constitutes polyphenols along with tannins [4]. Major compounds were identified using various techniques such as UV, TLC, HPLC, and FTIR. The presence of bioactive compounds in the plant extract was confirmed by subsequent screening by U.V., TLC, HPLC, and FTIR. They were subsequently tested for their anti-oxidant, anti-diabetic and anti-microbial activity. The plant extracts were found to have excellent anti-oxidant and anti-diabetic properties. The extract was highly active against gram-positive bacteria (*Bacillus subtilis* 6633, *Staphylococcus aureus* 6535, *Kocuria rhizophila* 9341, and *Micrococcus leuteus* 10240) but not gram-negative bacteria. The extract also possesses anti-fungal activity, showing growth inhibition on *Candida albicans* 10231 [14].

5 Conclusion

Our study proves that the selected plant is rich in bioactive compounds responsible for anti-tubercular activity, which should be pursued further for anti-TB drug development. The study also directs the in-vitro and ex-vivo antimycobacterial potential of alkaloids in *Phyllanthus* and suggests their effectiveness more than vasicine, the principal alkaloid in the plant. The compounds of *M. tuberculosis* and the animal model need to be further analysed. *Phyllanthus emblica* is known for its nutraceutical value, but our study suggests that it possesses medicinal properties. Therefore, it should be pursued for discovering bioactive compounds. Our study, however small, is a valuable addition to proving the medicinal values of the plant. Our study also shows the presence of bioactive compounds such as Gallic acid, Ellagic acid, Ascorbic acid, and Chlorogenic acid. This study is an attempt to develop a PPI monograph of *Phyllanthus emblica*.

Acknowledgements

The authors acknowledge the Secretary-cum-Scientific Director, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare for the platform to develop the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Hassan SM, Mughal A, Farman M. Emblica officinalis (Amla): A prospective review on distinctive properties and therapeutic applications of Amla. Biomedicine and Nursing. 2020, 6(2): 22-30. https://doi.org/10.7537/marsbnj060220.03

[2] Prathiba S, Rajagopal P, Selvaraj Jayaraman MKP, et al. A Review on Therapeutic Perspectives of Anticancer Properties of Chebulagic Acid. Nveo-Natural Volatiles & Essential Oils Journal. 2021: 7598-7612.

[3] Indian Pharmacopoeia, 2022. The Indian Pharmacopoeia Commission (ninth edition), Ministry of Health & Family Welfare, Government of India.

[4] Unigwe R, Okey SN. Medicinal values of the genus Phyllanthus - A review. Journal of Current Biomedical Research. 2023, 3(4). https://doi.org/10.54117/jcbr.v3i4.5

[5] Sriwatcharakul S. Evaluation of bioactivities of Phyllanthus emblica seed. Energy Reports. 2020, 6: 442-447. https://doi.org/10.1016/j.egyr.2019.08.088

[6] Liu X, Cui C, Zhao M, et al. Identification of phenolics in the fruit of emblica (Phyllanthus emblica L.) and their antioxidant activities. Food Chemistry. 2008, 109(4): 909-915. https://doi.org/10.1016/j.foodchem.2008.01.071

[7] Başkan KS, Tütem E, Akyüz E, et al. Spectrophotometric total reducing sugars assay based on cupric reduction. Talanta. 2016, 147: 162-168. https://doi.org/10.1016/j.talanta.2015.09.049

[8] NOOR MOHAMAD ZIN NS, AZMI NAS, ANUAR NS, et al. A 96-Well-Plate–Based Method for the Estimation of Alpha-Amylase Activity Using Miniaturises 3,5-Dinitrosalicylic Acid (Dnsa) Colorimetric Method. Malaysian Applied Biology. 2022, 51(4): 95-102. https://doi.org/10.55230/mabjournal.v51i4.16

[9] Panda SK. Screening methods in the study of antimicrobial properties of medicinal plants. International Journal of Biotechnology and Research. 2012, 2(1): 1-35.

[10] Doughari JH. Antimicrobial activity of Tamarindus indica Linn[J]. Tropical Journal of Pharmaceutical Research. 2006, 5(2): 597-603. https://doi.org/10.4314/tjpr.v5i2.14637

[11] Al-Snai A E. Iraqi medicinal plants with antibacterial effect-A review. IOSR Journal of Pharmacy. 2019, 9(8): 22-103.

[12] Johnson DB, Shringi BN, Patidar DK, et al. Screening of Antimicrobial Activity of Alcoholic & Aqueous Extract of Some Indigenous Plants. Indo Global Journal of Pharmaceutical Sciences. 2011, 01(02): 186-193. https://doi.org/10.35652/igjps.2011.18

[13] Patel P, Joshi C, Kothari V. The Anti-infective Potential of Hydroalcoholic Extract of Phyllanthus emblica Seeds Against Selected Human-pathogenic Bacteria. Infectious Disorders - Drug Targets. 2020, 20(5): 672-692. https://doi.org/10.2174/1871526519666190821154926

[14] Hsu H, Sheth CC, Veses V. Herbal Extracts with Antifungal Activity against Candida albicans: A Systematic Review. Mini-Reviews in Medicinal Chemistry. 2021, 21(1): 90-117. https://doi.org/10.2174/1389557520666200628032116