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RESEARCH ARTICLE

Evaluation of the Antimicrobial, Antioxidant, and Cytotoxicity Against MCF-7 Breast Cancer Cell Lines of *Phyllanthus emblica* L. Bark Extract

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Abstract

Background: The euphorbiaceous plant family includes *Phyllanthus emblica*, which demonstrates a variety of pharmacological qualities. **Aim:** This study aims to investigate any potential medical benefits that the *Phyllanthus emblica* tree's bark may have. **Methods:** An aqueous extract was made with sterile water, and alcohol-based extracts were obtained by macerating with 100% ethanol. Different extraction parameters were changed to evaluate their impact on polyphenol extraction, such as the concentrations of ethanol and aqueous extracts. The phosphomolybdate method was used to assess antioxidant activity and Total Phenol Content (TPC). Both ethanolic and aqueous extracts were found to have antibacterial activity against clinical isolates of *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli*. Thin Layer Chromatography (TLC) was used to compare the extracts' metabolite profiles. The MTT assay was employed to detect cell growth and survival, and the ethanolic bark extract showed potential cytotoxic action against the MCF 7 cancer cell line. **Result:** The results of the aforementioned tests showed that the ethanolic bark extract had greater antibacterial activity than the aqueous extract. Furthermore, the MTT assay showed that the ethanolic bark extract may have cytotoxic effects on the cancer cell line MCF 7. **Conclusion:** In summary, more research is necessary to completely understand the antibacterial and chemotherapeutic effects of the active ingredients, opening the door for their possible development as effective anticancer treatments.

Keywords: Antibacterial, Antioxidant, Total Phenol Content, Cytotoxicity Assay

1. Introduction

One well-known member of the Euphorbiaceous family, *Phyllanthus emblica* is mostly distributed throughout many tropical areas and subtropical region countries[1]. In quickly-dried bark, we can find more tannin than slowly-dried bark. The plant bark contains proanthocyanidins, tannins, and leucodelphinidin. *Phyllanthus emblica* is native to tropical Southeast Asia and can be found in Nepal, Central and Southern India, and nearby Islands[2]. This plant is found to be in the Euphorbiaceous family[3]. Pharmacological studies reveal that it has antioxidant[4], cytoprotective[5], antiaging[6], anticancer[7], immunomodulatory[8], anti-viral[9], anti-jaundice, anti-dyslipidemia[10], anti-apoptotic[11], anti-inflammatory[12], nephroprotective[13] and anti-diabetic[14]. *P. emblica* is present with a higher content of natural vitamin C. Leaves were crudely extracted, and their potential anti-inflammatory, antimicrobial, anticancer, antidiabetic and antioxidant properties were investigated[15]. As per research bark is rich in antioxidants, antimicrobial activity and

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anticancer factors. Its fruit is taken in India to treat disorders which include cancer, diabetes, liver and heart disorders[16]. The village residents are known to employ *P. emblica* for anti-inflammatory treatment. Because of its anticancer properties, and it aids in the treatment which includes several conditions involving heart disease, antidiabetic[17].

2. Materials and Methods

2.1 Source of Plants

Bark of *P. emblica* was bought from the botanical garden, Centre of Bioscience and Nanoscience Research (CBNR) Eachanari post, Coimbatore - 641021, Tamil Nadu, India and dried under sunlight for 48 hours.

2.2 Lab Glasswares and Utilities

Conical flask, beaker, screw cap, TLC sheet, iodine chamber, Petri plate, T-flask, well plate and test tube.

2.3 Chemical

20% Sodium carbonate, anti-oxidant reaction mixture, methanol, ethyl acetate, N-butanol, acetic acid, MCF 7 cell line, MTT dye, DMEM, trypsin, DMSO.

2.4 Instrumentation

Shaker, UV visible spectrophotometer, incubator, CO_2 incubator and laminar airflow.

2.5 Extraction

The bark was peeled from the tree and dried in the sun. It was then mashed into a fine powder. The dried bark powder (2g) was extracted into two screw-cap conical flasks, macerated separately with 20 mL of 100% ethanol and another set completely with distilled water. The materials were macerated for 24 hours at 37°C after being well mixed. After a whole day, the material was filtered with Whatman filter paper and kept in screw-cap tubes[18].

2.6 Quantification of Total Phenolic Content

Plant phenolics act as a main antioxidant or free radical scavenger. Total phenolic content was analyzed by Folin-Ciocalteau's method[19]. 10% Folin-Ciocalteu reagent was added to 0.2 mL of extract, shaken, and allowed to stand at room temperature for five minutes. Afterwards, 1 mL of 20% sodium carbonate was added, and distilled water was added to make up to 3 mL of the reaction mixture. Finally, the mixture was incubated for 45 minutes at 45°C. The sample's absorbance at the wavelength of 765 nm was measured using a UV-visible spectrophotometer (Labtronics LT29, Microprocessor). The extract's phenolic content was calculated using the gallic acid equivalent (GAE)/g measure, created in methanol at concentrations of 20, 40, 60, 80, and 100 µg/mL of extract. Similar studies have been reported, suggesting that the ethanolic bark extract of *P. emblica* possesses considerable total phenolic content when compared to fruit and leaf extract illustrating its antioxidant properties[20].

2.7 Phosphomolybdate Assay

Herbs contain antioxidants, flavonoids, and phenolic compounds that are essential for combating free radicals, which are the primary cause of many undesirable skin alterations[21]. Free radicals can be scavenged by antioxidants, which can halt or even postpone cellular damage. So it is possible to attribute the pharmaceutical activities of *P. emblica* for its active phytoconstituents like rutin, gallic acid, caffeic acid and kaempferol[22]. Phatakand, Hendre and Priet *et al.,* verified the analysis of bark extracts using the phosphomolybdate method. A reaction mixture comprising 28M sodium phosphate, 0.6M sulfuric acid and 4M ammonium molybdate was supplemented with 0.5 ml of the sample. Along with the blank, solution was incubated at 50 degree celsius for 60 minutes. After the incubation, the tube was adjusted to the ambient temperature and around 695 nm, absorbance was measured using a UV visible spectrophotometer (LT 29 labtronics microprocessor) and total antioxidant was analyzed. Using the following formula, the antioxidant capacity was calculated:

$$Antioxidant\ effect\ \% = \frac{\left(Absorbance\ Control\ -\ Absorbance\ of\ Sample\right)}{Absorbance\ Control} x\ 100$$ [23]

2.8 Thin Layer Chromatography

Thin layer chromatography, aids in the identification of mixtures by separating the substances in the mixture[24]. Thin-layer chromatography was used to purify the crude pigment. Silica gel coated

chromatography sheets (50 x 20 cm size) have been purchased commercially. The solvent mixture was composed of water, acetic acid, N-butanol, methanol, and ethyl acetate in the following proportions 2:2:2:1:1. The crude extract was dissolved in the above solvent mixture. The sample was spotted at the bottom of the silica gel-coated sheet by use of the capillary tube and later, placed at the developing beaker having a mobile phase, which was covered with a watch glass to avoid solvent evaporation. The retention factor value of the separated spot on the TLC plate was determined as shown in Figure 1.

RF value = solute movement from the origin/ solvent front.

2.9 Preparative TLC

The TLC plate was made by evenly spreading the silica gel slurry onto the plate. For 15 minutes, the plate has been activated at 100°C. The chromatogram was done with the solvent system water, acetic acid, N-butanol, methanol, and ethyl acetate in the following proportions: 2:2:2:1:1 and the crude extract spotted on the plate. After drying, the pigment spot was scrapped and blended with the same mobile phase solvent mixture and it is centrifuged at 3000 rpm for 15 minutes. The supernatant was bought from a pre-weighed vial and allowed to evaporate. Then the purified extract from preparative TLC was evaluated for antibacterial efficacy against selected bacterial pathogens using the well diffusion method[25].

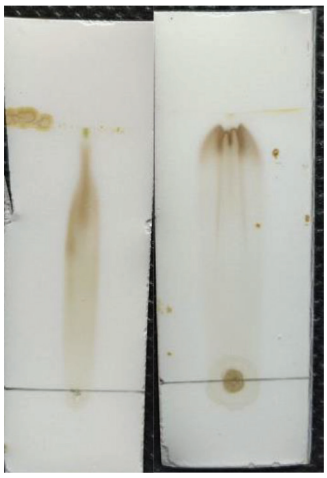

Figure 1. Aqueous extract (left) and ethanolic extract (right).

2.10 Antibacterial Efficacy

P. emblica medicines have antibacterial qualities that make them effective and safe at the same time[26,27]. Using the well diffusion method, the antibacterial activity of ethanolic and aqueous extracts of the crude sample as well as the partially purified compound obtained from preparative TLC was evaluated. After preparing 2.28 grams of Muller Hinton Agar media (Hi media) in 60 mL of distilled water,and it was autoclaved at 121°C for 15 minutes. 80 µL of the bacterial pathogen culture was swabbed with a cotton swab and then the wells were cut with a cork borer before the sample (50µL) was added. 24 hours were spent incubating the plate at 37°C with an antibiotic disc (ampicillin 10 mg) serving as a positive control.

2.11 Cytotoxicity Effect of the Bark Extract

The ethanolic bark extract's cytotoxicity activity was investigated on the MCF 7 Breast Cancer cell line. The lineage known as MCF 7 was obtained the National Centre for Cell Science in Pune, India, and is maintained at the Centre for Bioscience and Nanoscience Research Laboratory, Eachanari in Coimbatore, Tamil Nadu, India. Following collection, the MCF 7 Breast Cancer cell line have been sub-cultured in DMEM media supplemented with glucose, sodium carbonate, and 10% BSA. After adding the media to the T flask, 10µl of the cell line was inoculated into the medium and incubated in a CO_2 incubator for 24-72 hours with optimal conditions of pH 7 at 37°C, with humidity between 70-80%. After incubation, the cell line's proliferation was validated using an inverted microscope and was employed for future research. 96-well plates were used to seed cells for the MTT experiment, and they were allowed to adhere for a whole day at 37°C, 80% humidity and with 5% CO_2. The cell line underwent 24 hours incubation with different concentrations of sample. DMSO as a blank, and a cell line without extract as the control. Following incubation, 50µL DMSO and trypsin were used to detach the cells. Each well-received 20µL of MTT dye after washing. Following mixing, the plate was placed in a CO_2 incubator as well as incubated for 24 hours at 37°C. After the reaction mixture was carefully taken out, 100µL of Ethanol was added to each well and thoroughly mixed to solubilize formazan crystals. After 24 hours, the optical density

of the purple colour was measured at 570 nm using a 96 well plate ELISA reader (Robonik, India) and the proportion of cell death and viability were determined by the formula given below.

$$\% \ of \ cell \ viability \ = \frac{COD \ - TOD}{COD} \ X \ 100$$

3 Results and Discussion

3.1 Quantification of Total Phenol

The phytochemical screening of *P. emblica* indicates the presence of phenolic compounds[28]. The sample with aqueous and ethanol was recorded using UV spectrophotometry and concentration was determined using a standard curve as given in Figure 2. The total phenol concentration in aqueous and ethanol extract was 30.1µg/mL and 51.1µg/mL respectively. Thus, the ethanolic extract has the highest phenolic content than the aqueous extract in the *P. emblica* bark sample.

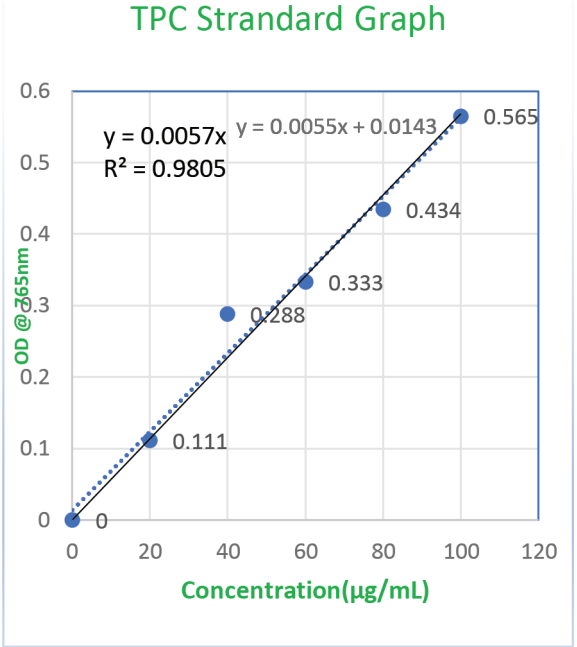

Figure 2. Standard curve for determining Total Phenolic Concentration (TPC).

3.2 Quantification of Total Antioxidant Capacity

Compounds known as antioxidants work to mitigate the harmful effects of free radicals. It has been discovered that antioxidants are chemical compounds with the ability to halt oxidation reactions at their source by giving free radicals one or more electrons to defeat high levels of free radicals in the body. The antioxidant assay was performed using the phosphomolybdate method and the optical density of sample aqueous and ethanol was recorded using UV spectrophotoscopy. The total antioxidant capacity estimated in aqueous extract was 75.10% and ethanol extract content was 86.06%. Hence it is evident that the bark consists of high antioxidant content in the ethanolic sample, the *P. emblica* extract in rats showed a dose-dependent decrease in levels of oxidative damage[29,30].

3.3 Thin Layer Chromatography

The method of Thin-Layer Chromatography (TLC) was used for separating compounds in non-volatile mixtures. The RF values of the phytochemicals offer crucial details regarding their polarity as well as crucial cues for the separation of the phytochemicals during the separation procedure. When using more solvent systems for TLC testing, different RF values of the molecule also reveal information about their polarity, which may be important in choosing the right solvent system[31]. Saturated iodine chamber was used in the development of colour bands. In the TLC examination of the *P. emblica* bark extract, two spots were visible; the ethanol extract of the first spot was light brown, while that of second spot was brown. The ethanol extract's retention factor values were 0.302 and 0.488, respectively. The solvent mixture was made up of water, acetic acid, N-butanol, methanol, and ethyl acetate in the following ratios 2:2:2:1:1. The watery extract had a single spot of brown colour with a retention factor value of 0.68 was found in the TLC analysis. Hence the plant extracts containing antimicrobial compounds must have their constituent parts separated to evaluate microorganisms' reactions to them and determine whether other compounds are inhibiting the growth of the germs[32].

3.4 Antibacterial Efficacy

The different solvent bark extract of *P. emblica* showed antibacterial activity adverse to all the organisms as shown in Table 1. The sample possesses antibacterial activity adverse to bacterial pathogens. Crude bark extract and partially purified bark extract from

Table 1. Zone of inhibition against *E. coli, P. aeruginosa* and *S. aureus* (all diameters are measured in millimetres)

Name of organism	Aqueous crude plant extract	Ethanolic crude plant extract	Partially purified aqueous extract from preparative TLC	Partially purified ethanolic extract from preparative TLC	Control ampicillin disc.
E. coli	Nil	10	Nil	11	20
S. aureus	Nil	20	18	23	23
P. aeruginosa	17	23	Nil	25	20

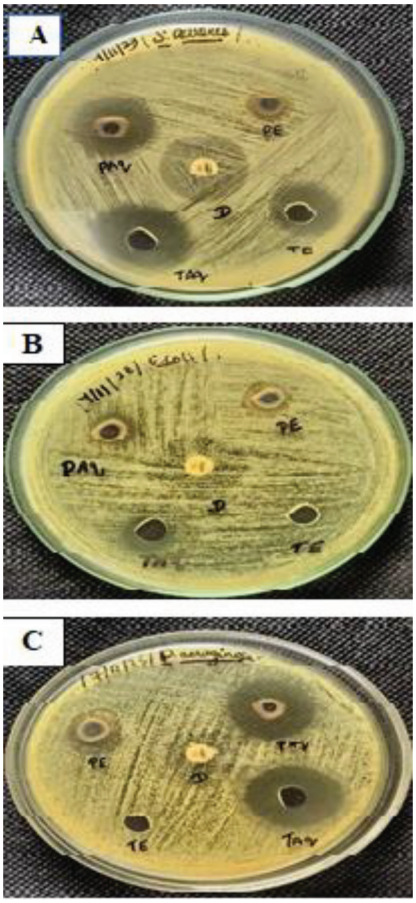

Figure 3. Agar well diffusion antimicrobial assays against – **(A).** *S. aureus,* **(B).** *E. coli* and **(C).** *P. aeruginosa.*

preparative TLC were used to calculate the zone of inhibition against the bacteria as shown in Figure 3.

3.5 Assessment of Cytotoxicity of Purified Ethanolic Extract

Phyllanthus emblica bark ethanolic extract was selected and examined for anticancer activity utilizing human cancer cell lines. Specifically, MCF-7 mammary gland breast cancer cell lines. An ELISA plate analyzer set at 570 nm was used to record the anticancer property. The result shows promising potential to influence cell

Table 2. Effects of *P. emblica* ethanolic bark extract on MCF-7 cell viability

Concentration of sample used (µL)	% of cell viability
0 (control)	100
5	93.81
10	88.92
15	77.74
20	73.58
25	64.76

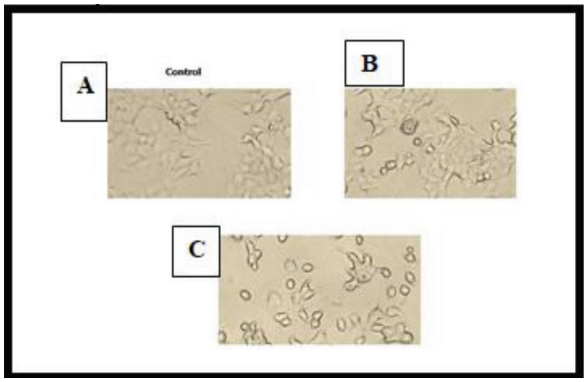

Figure 4. Microscopic image of MCF cells treated with *P. emblica* bark ethanolic extract in the range of 5µl (B), 25µl (C) and control (A).

viability. As the concentration of ethanolic bark extract was varied from 5 to 25µL, as shown in Figure 4; it shows a concentration-dependent cytotoxicity effect. The intensification of cytotoxic was evident at 25µL of extract where it leads to cell viability of 64.76%. The finding illustrates the potential of ethanolic bark extract for curbing the proliferation of MCF-7 cancer cells. Table 2 provides a summary of the obtained data. Previous reports have indicated that the chlorophyllin that was extracted from the leaves of *P. emblica* exhibited cytotoxic effects on MCF Breast Cancer cell lines[33]. Consequently, our analysis of *P. emblica* bark demonstrates a noteworthy cytotoxic effect.

4. Conclusion

Bark from medicinal trees has been used to treat a wide range of illnesses since prehistoric times. In summary, the examination of diverse facet of the investigated sample's phenolic content, chromatography (TLC) study, and possible cytotoxicity and antibacterial characteristics has yielded significant insights into the potential of *P. emblica* bark extract. The overall outcome of this study revealed that the ethanolic bark extract has more bioactive constituents than the aqueous bark extract which contributed the capacity to scavenge free radicals, which are essential to prevent oxidative stress. Using the MCF 7 Breast Cancer cell line, the examination of the cytotoxicity properties showed encouraging findings of cell viability with 64.76%. Furthermore, the antimicrobial tests demonstrated how well the compounds worked against the growth of selected pathogenic bacteria.

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6. References

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