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Phytochemical and Biological Characterization of *Phyllanthus emblica* Seed Extract

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Abstract:

➢ ***Background:***

Plant-derived compounds play a vital role in the development of several therapeutic agents. *Phyllanthus emblica*, Indian gooseberry has both dietary and medicinal uses. Its fruit is health beneficial, and the seeds are eliminated during consumption which may have biological activity hence the seed of *P. emblica* has to be investigated. Our study aims to evaluate the antioxidant, antibacterial activity, *in vitro* cytotoxic potential and to determine the presence of various bioactive compounds of *P. emblica* seed extract. The ethanolic *P. emblica* seed extract was subjected to phytochemical screening and antibacterial activity was evaluated using the well diffusion method against *Streptococcus mutans* and *Enterococcus faecalis*. Antioxidant ability was determined by DPPH, ABTS and phosphomolybdenum reduction assay. Further, Gas chromatography-mass spectrometry (GC-MS) was used for the identification of bioactive compounds.

➢ ***Results:***

The phytochemical screening identified the presence of secondary metabolites such as terpenoids, phytosterols, phenols, flavonoids, tannins, glycosides, carbohydrates, saponins, and quinines in *P. emblica* seed extract. The total tannin and flavonoid contents were 514.24 mg TAE/g and 861.9 mg QE/g respectively. The seed extract exhibited antibacterial activity against *S. mutans* and *E. faecalis*. The ethanolic seed extract showed high scavenging activity in DPPH (96.38%), ABTS (98.88%) assay and had a potent reductive ability (81.92%) in phosphomolybdate assay. The MTT assay revealed the extract exhibited maximum cytotoxicity against SCC25 cells at a concentration of 200 µg/ml. The GC-MS analysis of the *P. emblica* seed extract showed the presence of 17 bioactive compounds and the major compounds were cyclotrisiloxane, hexamethyl, di-n-octyl phthalate, 1,2-dihydro-1,4-dimethoxy-2-oxoquinazoline, calycotomine, methyl, phenol, 2,4-bis (1,1-dimethyl ethyl) and 1-dodecanol, 3,7,11-trimethyl. Hence, the presence of these compounds in seed extract may be responsible for therapeutic intervention.

➢ ***Conclusions:***

The study analysed the biological activity of the seed, and the identified bioactive phytochemicals could be selected in the pharmaceutical industry for drug discovery.

Keywords: *Phyllanthus emblica, Antioxidant, Antibacterial, Cytotoxicity, GC-MS, Bioactive Compounds.*

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I. INTRODUCTION

➢ *Background*

Phyllanthus emblica Linn, Indian gooseberry, belongs to the Euphorbiaceae family is found in subtropical and tropical areas of India, China, Indonesia and Malaysia [1]. *P. emblica* nutrient-rich plant with a complex phytochemical composition, encompassing vitamins, carotenoids, minerals, and bioactive acids such as phyllembelic acid and emblicol. *P. emblica* contains a wide array of polyphenols, flavones, tannins and other bioactive compounds [2]. Various active components such as quercetin, kaempferol, gallic acid, ellagic acid, 1-O-galloyl-D glucose, chebulagic acid, mucic acid and 1,4-lactone 3-O-gallate are present in the aqueous extract of Amla [3]. *P. emblica* has been traditionally used to mediate diabetes, diarrhoea, diuretics, laxatives, fever and gonorrhoea. According to several reports its bioactive components have antimicrobial [4], antioxidant[5][6], anti-

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inflammatory[7], analgesic and antipyretic[8][9], adaptogenic[10], hepatoprotective[11], antitumor [12]and antiulcerogenic activities[13].

All components of *P. emblica* have been extensively used in different traditional therapies including Ayurveda, Chinese Traditional Medicine, Tibetan Medicine, and Greek Arabic Medicine. *P. emblica* is referred to as "the best among rejuvenators" and "the best among the sour fruits" in the two primary classical works on Ayurveda; Charaka Samhita and Sushruta Samhita [14]. Although the functional characteristics of *P. emblica* have been defined, there has been limited exploration of the seed and seed coat regarding their functional properties and compositional analyses. According to Suttijit Sriwatcharakul, seed extract exhibits potential antioxidant activity as well as cytotoxicity against MCF-7 cell lines. In this study, we examined the phytochemical contents, antioxidant potential, antimicrobial activity, *in vitro* cytotoxic potential and Gas chromatography-mass spectrometry **(**GC-MS) analysis of *Phyllanthus emblica* seed extracts to explore their biological activity and the role of its active compounds in therapeutic purposes.

II. METHODS

➢ *Chemicals*

All the chemicals and reagents employed in this study were of high analytical standard.

➢ *Collection of the Plant Samples and Preparation of the Plant Extract*

Phyllanthus emblica was collected in January 2023 from the local market of Chennai, Tamilnadu, India. The collected *P. emblica* were thoroughly washed, and the seed was separated from the fruit. All the fruit bits were removed and washed. The excess water on the seeds was dried with a towel and crushed using a mortar and pestle. The crushed seeds were placed in a permeable bag or thimble and extracted with 99% ethanol using a Soxhlet apparatus for 24 hours at a temperature below 60°C. The *P. emblica* seed extract was then subjected to phytochemical screening.

➢ *Phytochemical Screening*

Several chemical analyses were performed to characterize the phytochemical composition of the *P. emblica* seed extract and to identify the various phytochemical constituents present. The preliminary phytochemical screening of the ethanol extract of *P. emblica* seeds utilized standard protocols [15] and chromophore reagents.

➢ *Test for Alkaloids*

The plant extract (50 mg) were treated with concentrated hydrochloric acid and shaken well. Addition of Dragendorff's reagent produced an orange-brown coloration, confirming the presence of alkaloids.

➢ *Test for Terpenoids*

The plant extract (50 mg) was dissolved in chloroform and concentrated sulfuric acid were added along the test tube wall. The appearance of a red ring at the interface indicated the presence of terpenoids.

➢ *Test for Phytosterols*

The presence of phytosterols in the extract was indicated by various colour changes observed after mixing with 1 mL of $C_4H_6O_3$ and concentrated H2SO4.

➢ *Test for Phenols*

Addition of 5% neutral ferric chloride solution to the extract (500 µL, aqueous), a dark green or violet color appeared, indicating the presence of phenolic constituents.

➢ *Test for Flavonoids*

The extract was added to a diluted sodium hydroxide solution, which resulted in the formation of a vibrant yellow coloration, indicating the presence of flavonoids.

➢ *Test for Tannins*

The addition of a 10% lead acetate solution to the dissolved extract, resulting in a bulky white precipitate confirmed the presence of tannins.

➢ *Test for Glycosides*

The extract was treated with pyridine and sodium nitroprusside solution in a test tube, followed by concentrated H2SO4 to see the appearance of a pink color indicated glycosides.

➢ *Test for Carbohydrates*

Two drops of alcoholic α-naphthol solution were added to the extract and shake thoroughly. Subsequently, a few drops of concentrated H2SO4 were introduced along the edges of the test tubes, resulting in the formation of a violet ring.

➢ *Test for Saponins*

500 µL extract was mixed with 2 mL of distilled water. The mixture was shaken vigorously for 1 minute, resulting in the formation of a layer of foam reveals the presence of saponins.

➢ *Test for Protein*

To determine the presence of proteins, 500 µL of the extract was treated with concentrated nitric acid and then gently heated, resulting in the formation of a yellow precipitate.

➢ *Test for Quinones*

The 500 µL extract was treated with concentrated sulfuric acid, resulting in the observation of a red coloration, which indicated the presence of tannins.

➢ *Quantitative Estimations*

- *Determination of Total Phenol*

Modified Folin-Ciocalteau reagent method was used to determine the total phenolic compounds in the ethanol extract of *P. emblica* seed [16]. 100 µL of the extract was combined with 900 µL of methanol and then combined with 1 mL of Folin-Ciocalteau reagent and 1mL Na2CO3 (7%

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w/v). The mixture was left at room temperature (RT) for 30 minutes and then the absorbance was recorded at 765nm using a spectrophotometer. Theresults were expressed as micrograms of gallic acid equivalent (µg GAE/mg extract) using gallic acid as the reference standard.

- *Determination of total flavonoids*

The ethanol extract of *P. emblica* seed was tested for total flavonoid content using the aluminium chloride colourimetric method [17]. A mixture was made by combining 500 µL of 1mg/mL extract with 500µL of CH_3OH and then adding 1 mL each of 5% sodium nitrite and 10% aluminium chloride solutions. After the addition of 100µL of 1 M NaOH, thoroughly mixed, and left to incubate at RT for 30 minutes. The absorbance was recorded at 510nm, and the findings were reported as quercetin equivalent (µg QE/mg of extract).

- *Estimation of Total Tannins*

Tannin content was analyzed using the Folin-Ciocalteu method [18] . The extract was dissolved in methanol and reacted with Folin-Ciocalteu reagent (1:10 ratio) followed by 1mL of 35% sodium carbonate solution. The mixture was shaken well and incubated at RT for 30 minutes before measuring absorbance at 700 nm using a spectrophotometer. Reference standard solutions containing tannic acid at concentrations ranging from 20 to 100µg/mL were also prepared following the same procedure for comparison. The tannin content was quantified in terms of µg of tannic acid equivalents/mg.

➢ *Determination of Antioxidant Activity*

- *DPPH˙ Radical Scavenging Activity*

The antioxidant potential of the ethanol extract from *P. emblica* seeds was evaluated by testing its ability to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals [19]. Different concentrations (20-120 µg/mL) of seed extract were mixed with 1 mL of 0.1 mM DPPH solution in methanol and left to stand for a 30-minute dark incubation period. Ascorbic acid served as the positive control. Absorbance readings were recorded at 517 nm, and the percentage inhibition of DPPH radicals was calculated using the equation:

$$\%\ \text{of DPPH}^{\cdot}\ \text{radical inhibition} = \frac{(\text{control absorbance} - \text{sample absorbance})}{(\text{control absorbance})} \times 100$$

- *ABTS Radicle Cation Scavenging Assay*

The ability of the ethanol extract of *P. emblica* to eliminate the ABTS cation was examined [20]. To generate the ABTS•+ cation radical, a reaction between 7 mM ABTS (2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)) and 2.45 mM potassium persulfate (1:1) was carried out in water and then incubated in dark for 12-16 hours at RT before use. The resulting ABTS•+ solution was diluted with methanol and added to the extract at varying concentrations from 20 to 120 µg/mL. The reaction mixtures were then incubated for 30 minutes. The absorbance of the coloured complex was measured at 734 nm. Ascorbic acid was used as the reference standard. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{of ABTS radical inhibition} = \frac{(\text{control absorbance} - \text{sample absorbance})}{(\text{control asbsorbance})} \times 100$$

- *Phosphomolybdenum Reduction Assay*

The antioxidant capacity of the ethanol extract of *P. emblica seed* was evaluated by the phosphomolybdenum reduction assay method [21] with ascorbic acid acting as the standard reference. The reaction mixture contained the extract (20–120 µg/mL) and reagent solution composed of 4 mM ammonium molybdate, 28 mM sodium phosphate, and 600 mM sulfuric acid. The reaction mixtures in the test tubes were sealed and incubated in a water bath at 90°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm. The formula below was utilised to calculate the percentage of inhibition:

$$\%\ \text{of phosphomolybdenum reduction} = \frac{(\text{Sample absorbance} - \text{Control absorbance})}{\text{Sample absorbance}} \times 100$$

- *Antibacterial Activity*

The antibacterial efficacy of *P. emblica* seed extract was evaluated using agar well diffusion method with Gram-positive microbial strains such as *Enterococcus faecalis* and *Streptococcus mutans* [22]. The nutrient agar plates surface was swabbed with microbial inoculum using sterilized cotton swabs and five wells were created in each plate using a sterile well-borer with an 8 mm diameter. Subsequently, the wells were filled with *P. emblica* seed extract at concentrations of 250, 500, and 1000 µg/mL respectively. The plates were then incubated for 24 h at 37ºC and the antibacterial activity was evaluated by measuring the zone of inhibition. Tetracycline (25 µg) served as the positive control.

➢ *In vitro cytotoxicity study*

- *Cytotoxicity Test*

The cytotoxicity of the *P. emblica* seed extract was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay, which measures cellular metabolic activity by evaluating the conversion of the colorless tetrazolium compound into the purple formazan product. Approximately 5 × 10^4 SCC25 cells/ml were seeded per well and incubated for 24 hours. The cells

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were then treated with varying concentrations of seed extract (10, 50, 100, 200, 500, 1000 ug/ml), along with a control group without any extract. Following treatment, the cells were exposed to MTT solution (5 mg/mL) and maintained at 37 °C for 3 hours to allow formazan formation. The resulting crystals were solubilized in DMSO, and absorbance was measured at 570nm. The concentration required to inhibit 50% of cell growth (IC_{50}) was calculated from the dose–response curve. Cell viability (%) was calculated as follows:

$$\text{Cell viability (\%)} = \frac{[\text{Absorbance of control} - \text{Absorbance of treated cells}] \times 100}{\text{Absorbance of control}}$$

- *Gas Chromatography-Mass Spectrometry (GC-MS) Analysis*

The *P. emblica* seed extract was analyzed using a GC–MS system (Agilent 7890B GC coupled with 5977A MS). The separation of compounds was carried out using an HP-5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness) with helium serving as the carrier gas at a constant flow of 1 mL per minute. The injector temperature was maintained at 200 °C, while the oven temperature was gradually increased from 50 °C to 280 °C at a rate of 10 °C per minute and held at the final temperature for 10 minutes. The MS conditions included an ionization voltage of 70 eV, ion source and interface temperatures set at 250°C, and mass range between 50-600 mass units. Analysing the average peak area of each component in relation to the total peak areas enabled us to determine the proportional percentage of each component. The mass spectra obtained were interpreted using the The National Institute of Standard and Technology (NIST) library for identification of phytoconstituents based on their molecular weight, formula, and structural similarity.

III. RESULTS

➢ *Preliminary Phytochemical Screening.*

The initial analysis of the phytochemical profile indicated that the extract contained a variety of important phytochemicals such as phenolic compounds, tannins, flavonoids, and fixed oils and fats, as depicted in Table 1.

Table 1 Estimation of Phytochemicals in *P. emblica* Seed Extract

Phytochemical group	Test	Inference
Alkaloids	Dragendorffs Test	-
Terpenoids	Salkowski test	+
phytosterols	Libermann-Burchard's test	+
Phenolic compounds	Ferric chloride test	+
Flavonoids	Alkaline reagent test	+
Tannins	Lead acetate test	+
Glycosides	Legal's test	+
Carbohydrates	Molisch test	+
Saponins	Foam test	+
Protein	Xanthoproteic test	-
Quinones	H2SO4 test	+

➢ *Determination of the Total Phenolic, Flavonoid and Tannins*

P. emblica seed extract exhibited a higher concentration of tannin and flavonoid, with total contents of 514.24 mg TAE/g and 861.9 mg QE/g respectively. The calibration curve for tannin estimation followed the regression equation $y = 0.1088x + 0.0628$, whereas that for total flavonoids was $y = 0.0276x + 0.0614$. The total phenol, tannin and flavonoid content in *P. emblica* seed are shown in Table 2.

Table 2 Total Phenol, Tannin and Flavonoid in *P. emblica* Seed Extract.

Parameters	Ethanolic extract of *P. emblica* seed
Total phenolic content (mg GAE/g extract)	50.22
Total Tannin content (mg TAE/g extract)	514.424
Total Flavonoid content (mg QE/g extract)	861.9

➢ *Antioxidant Assay*

The antioxidant activities of P. emblica seed extract were determined using the DPPH assay, ABTS radicle cation scavenging assay, and Phosphomolybdenom reduction assay with ascorbic acid as standard reference. The percentage of inhibition of the three antioxidant assays at different concentration is given in Table 3.

Table 3 Percentage of Inhibition of Ethanolic Extract of *P. emblica* Seeds

Concentration (µg/ml)	DPPH (%)	ABTS (%)	Phosphomolybdenom reduction (%)
5	74.693	96.085	14.42
10	80.101	97.886	54.987

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15	80.824	98.259	64.759
20	83.537	98.321	68.539
25	86.442	98.446	77.739
30	96.383	98.881	81.92

➢ *DPPH Assay*

The ethanolic seed extract exhibited the DPPH radical scavenging activity with an inhibition percentage of 96.38 ± 1.12. Antioxidant efficiency increased proportionally with concentration (5–30 µg/mL), as presented in Fig 1(A).

➢ *ABTS Assay*

The ABTS radical scavenging assay generated a blue/green ABTS+ chromophore via reacting ABTS with potassium persulfate. The maximum inhibition of ABTS radical at the concentration of 30 μg/mL was 98.88% which was effective than that of standard (92.98%). The scavenging effect of *P. emblica* seeed extract on ABTS radical was observed to be concentration dependent (5 - 30 µg/ml), as shown in Fig 1 (B). The extract possessed strong ABTS scavenging activity.

➢ *Phosphomolybdenum Reduction Assay*

The phosphomolybdate method is quantitative approach that measures the total antioxidant capacity (TAC) in terms ascorbic acid equivalents [23]. It determines the antioxidant capacity of fractions by spectophotometrically measuring the reduction of Mo (VI) to Mo (V), leading to the formation of greeb phosphate /Mo(V) compounds with maximum absorption at 765nm [24]. Results showed dose-dependent antioxidant activity at concentrations of 5 to 30 µg/ml, with the seed extract exhibiting a % reduction in antioxidant capacity of 81.92 ± 1.5 in terms ascorbic acid equivalents. The findings suggest that the seed extract has a strong antioxidant effect which increases with higher concentrations. Fig 1(C) depicts the antioxidant capacity of the seed extract with ascorbic acid equivalents.

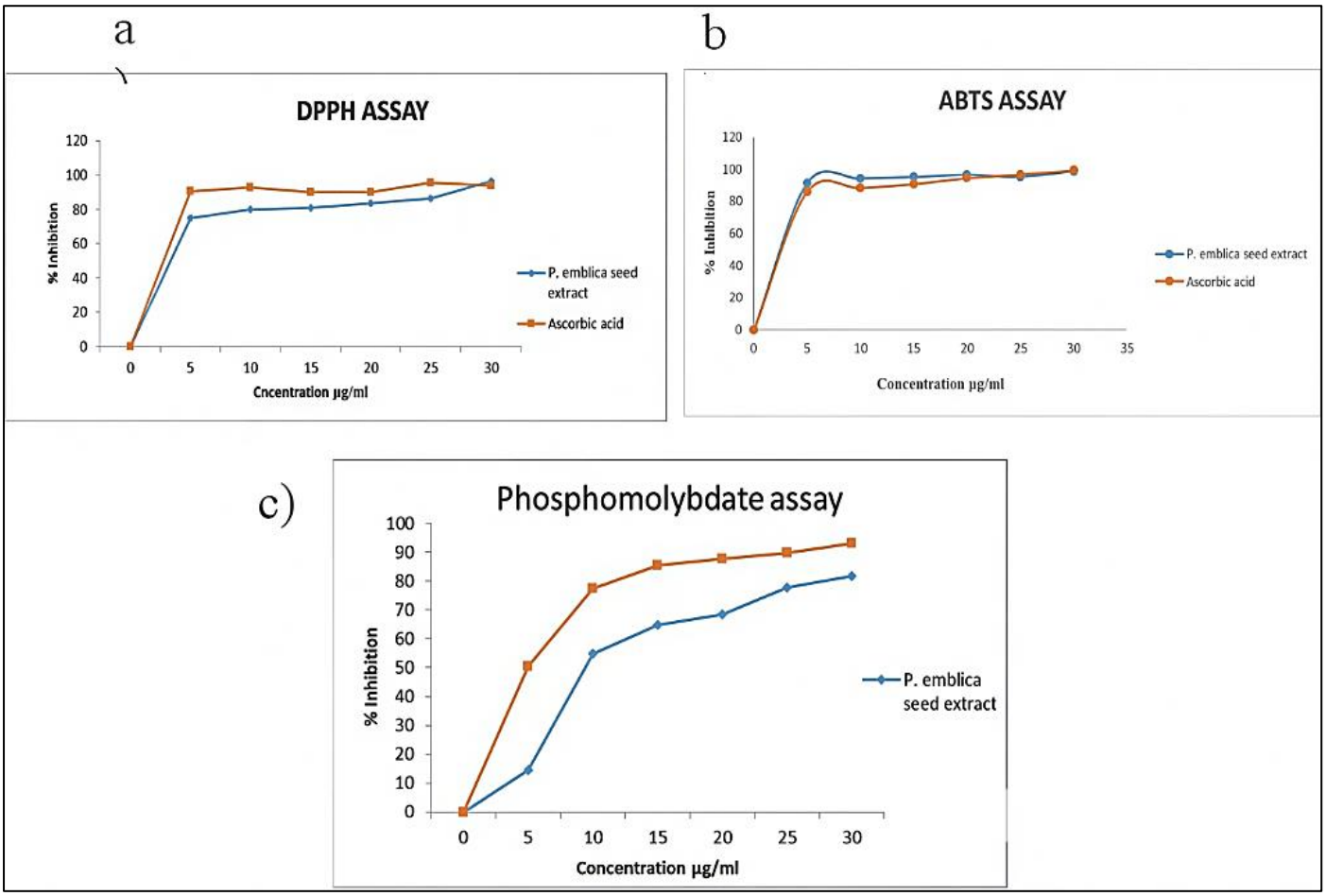


Fig 1 Antioxidant Activity of *Phylanthus emblica* Seed at Various Concentrations. (A) DPPH Radical Scavenging Activity, (B) ABTS Radical Scavenging Activity, (C) Phosphomolybdenum Reduction Assay

➢ *Antibacterial Activity*

The antibacterial activity of *P. emblica seed extract* against gram-positive strains such as *Enterococcus faecalis* and *Streptococcus mutans* was examined by agar well diffusion assay. Antibacterial activity was recorded as inhibition zone diameter measured in millimetre (mm) as shown in Fig 2. Maximum inhibitory activity was recorded against *S. mutans* with a 5mm inhibition zone and *E. faecalis* was also found to be equally sensitive with a zone of inhibition 3mm.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25oct623

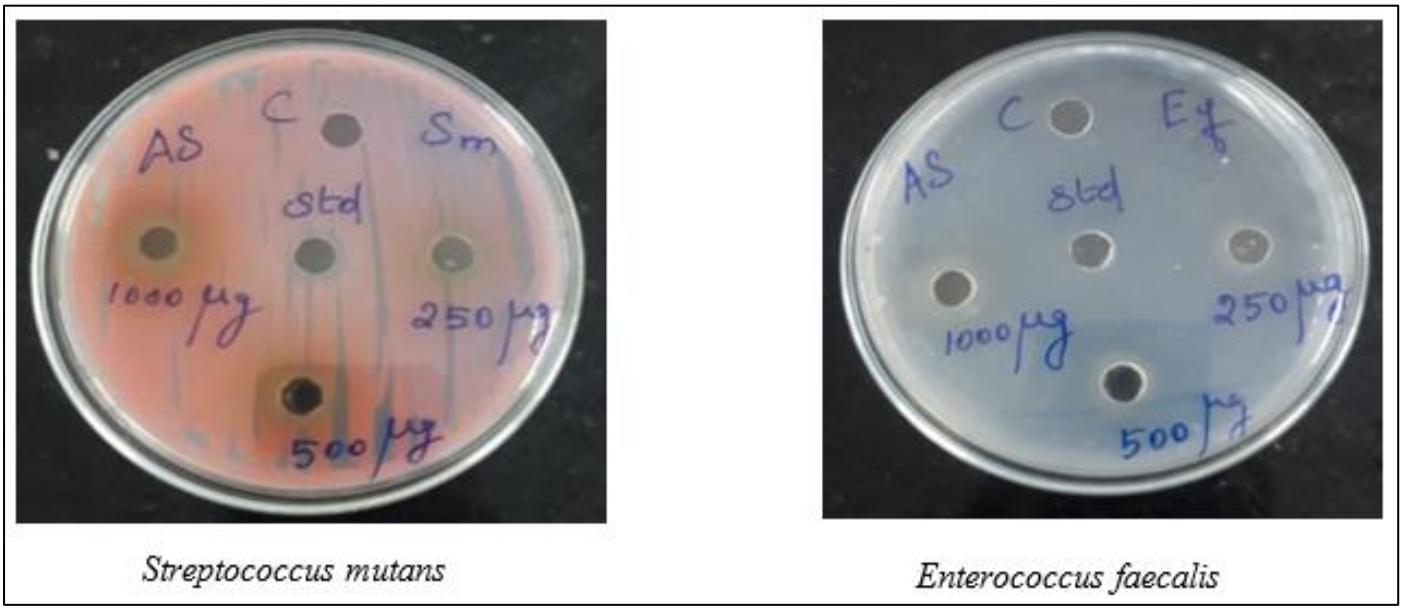


Fig 2 Antibacterial Activity of *P. emblica* Seed Extract

➢ *In Vitro Cytotoxicity*

The cytotoxicity of *P. emblica* seed extract against SCC25 cell lines was investigated using the MTT assay at 24, 48 and 72 hours. The results are presented in Table 4 (Fig 3). The seed extract showed strong concentration – dependent cytotoxic activity against SCC25 cells. After 24 hours, moderate reduction in viability was observed at 100 µg/mL, with a significant decrease at 1000 µg/mL. This effect intensified at 48 and 72 hours, with cell viability dropping to 10**%** at 1000 µg/mL. The estimated CC_{50} value at 72 hours was approximately 110–130 µg/mL. These results showed that *P. emblica* seed extract exhibits promising anticancer potential against SCC25 cells.

Table 4 Cytotoxic Response of SCC25 Cells to *P. emblica* Seed Extract.

Time Point	Control	10 µg	50 µg	100 µg	200 µg	500 µg	1000 µg
24 h	100.00 ± 0.00	94.00 ± 1.00	88.00 ± 1.00	84.00 ± 1.00	41.00 ± 1.00	23.33 ± 1.00	17.00 ± 1.00
48 h	100.00 ± 0.00	98.00 ± 1.00	93.00 ± 1.00	82.00 ± 1.00	28.00 ± 1.00	16.33 ± 0.58	10.00 ± 1.00
72 h	100.00 ± 0.00	69.00 ± 1.00	65.00 ± 1.00	56.67 ± 0.58	17.00 ± 1.00	16.00 ± 1.00	10.33 ± 0.58

*Values are Expressed as Mean ± SD from Triplicate Experiments

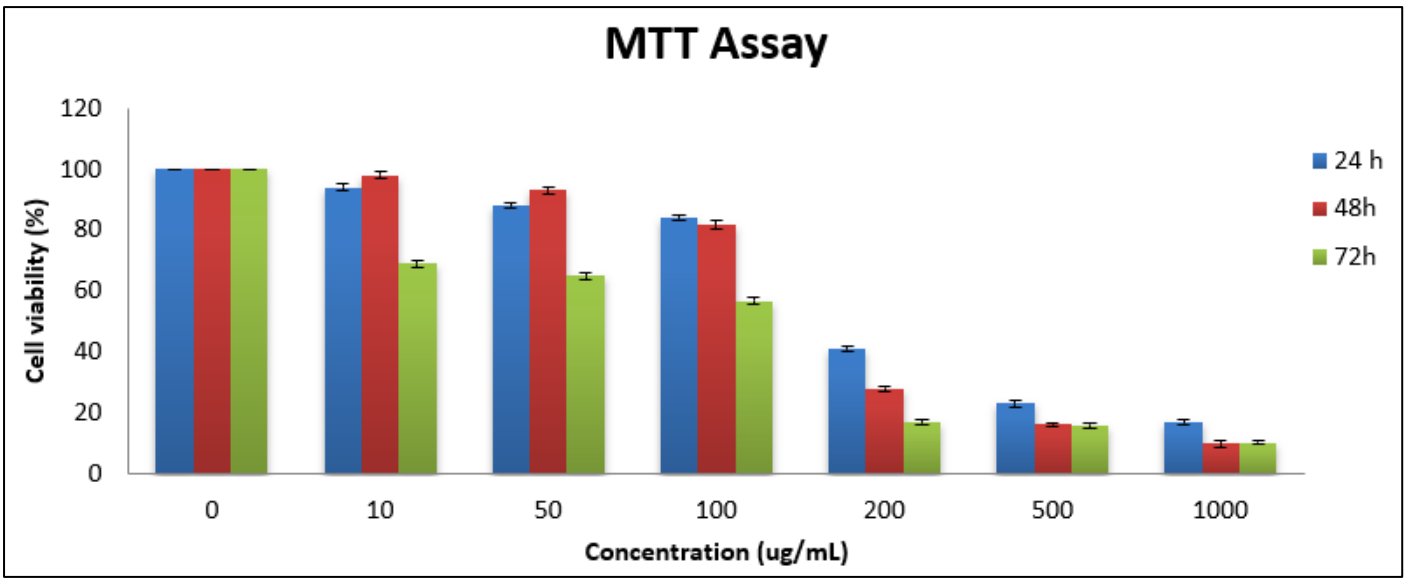


Fig 3 Cytotoxic Effects of *P. emblica* Seed Extract on Cells SCC25 Cells.

➢ *Phytocomponent Identification by GC-MS*

The volatile compounds of ethanolic extracts of *P. emblica* seed was measured by the GC–MS technique. The GC-MS profile of various components with different retention times is shown in Fig 4.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25oct623

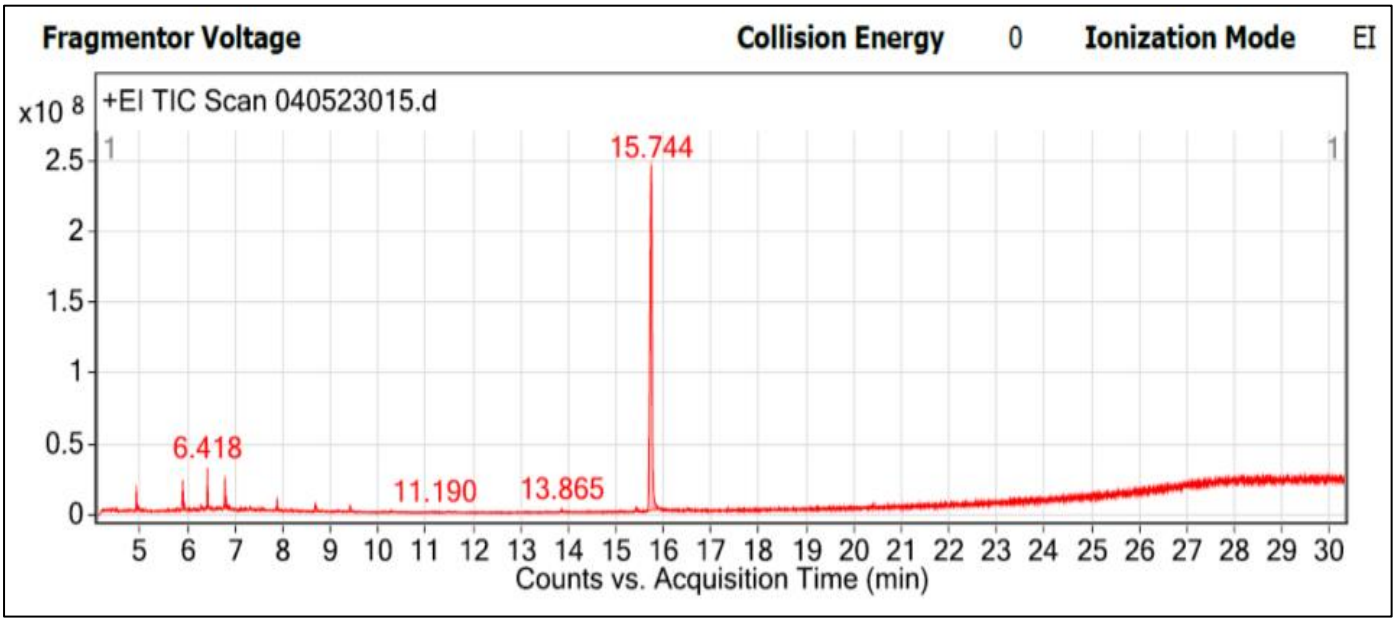
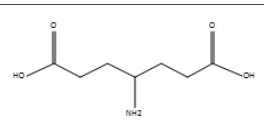
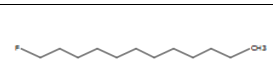
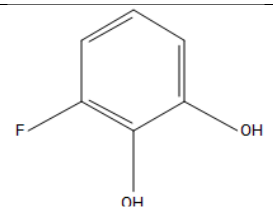
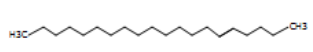
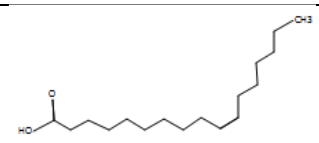


Fig 4 GC-MS of *P. emblica* Seed Ethanol Extract

The GC-MS analysis identified seventeen compounds from the ethanol extract of *P. emblica* seed based on parameters such as retention time, peak area %, molecular formula, and compound structure, which are presented in Table 4. The following bioactive compounds were present in the GC-MS analysis carried on ethanol fraction of P. emblica seed: 4-Amino-1,5-pentandioic acid, Dodecane, 1-fluoro-, 1,2-Benzenediol, 3-fluoro-, 5-Eicosene, (E)-, cis-10-Heptadecenoic acid, Phenol, 2,4-bis(1,1-dimethylethyl)-, Octanal, (2,4-dinitrophenyl) hydrazine, 5-Eicosene, (E)-, Diethyl Phthalate, Icosapent, 1-Dodecanol, 3,7,11-trimethyl-, Benzenemethanol, .alpha.-[1-(ethylmethylamino) ethyl]-, Hexadecanoic acid, ethyl ester, Calycotomine, N-methyl-, 1,2-Dihydro-1,4-dimethoxy-2-oxoquinazoline, Di-n-octyl phthalate and Cyclotrisiloxane, hexamethyl. The compound Cyclotrisiloxane, hexamethyl- contributed the most significant peak area among the detected bioactive components.

Table 5 Bioactive Compounds Found in Ethanolic Extracts of P. emblica Seed

No.	RT	Peak area (%)	Name of the compound	Molecular Formulae	Structure
1.	4.498	0.26	4-Amino-1,5-pentandioic acid	C7H13NO4	
2.	4.922	1.57	Dodecane, 1-fluoro-	C12H25F	
3.	5.022	0.21	1,2-Benzenediol, 3-fluoro-	C6H5FO2	
4.	5.897	2.99	5-Eicosene, (E)-	C20H40	
5.	6.022	0.62	cis-10-Heptadecenoic acid	C17H32O2	

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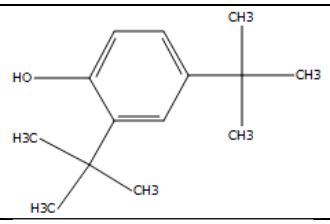
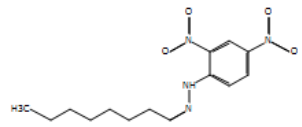
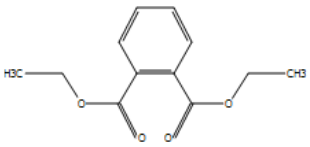
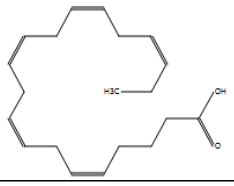
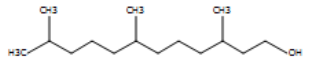
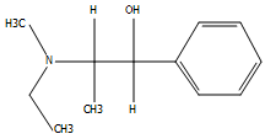
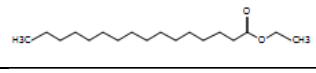
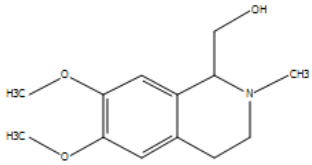
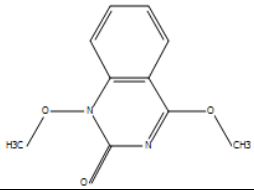
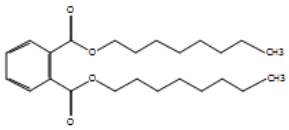
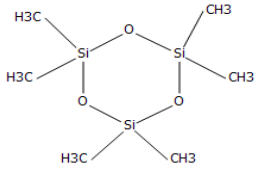
6.	6.418	0.28	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	
7.	6.675	0.38	Octanal, (2,4-dinitrophenyl) hydrazone	C14H20N4O4	
8.	6.78	2.82	5-Eicosene, (E)-	C20H40	
9.	6.809	0.24	Diethyl Phthalate	C12H14O4	
10.	7.122	2.34	Icosapent	C20H30O2	
11.	7.881	1.08	1-Dodecanol, 3,7,11-trimethyl-	C15H32O	
12.	8.685	0.3	Benzenemethanol, .alpha.-[1-(ethylmethylamino) ethyl]-	C12H19NO	
13.	9.418	1.03	Hexadecanoic acid, ethyl ester	C18H36O2	
14.	11.19	1.56	Calycotomine, N-methyl-	C13H19NO3	
15.	15.429	0.91	1,2-Dihydro-1,4-dimethoxy-2-oxoquinazoline	C10H10N2O3	
16.	15.744	0.28	Di-n-octyl phthalate	C24H38O4	
17.	16.513	0.61	Cyclotrisiloxane, hexamethyl-	C6H18O3Si3	

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IV. DISCUSSION

Medicinal plants are a rich source of potential bioactive compounds for new cancer treatments and have used in traditional and modern medicine to treat various diseases and manage different pathogens. The increasing demand for natural alternatives to conventional drugs has encouraged extensive studies on plant-derived molecules. [25]. The phytochemical characterization process involves the qualitative and quantitative assessment of the chemical compound classes present in medicinal plant species. The application of solvent extraction techniques coupled with GC-MS analysis has been extensively employed to identify phytochemicals with clinical relevance. The present study examined the ethanol extract of *P. emblica* seeds, revealing the presence of a diverse array of phytochemical constituents, including flavonoids, carbohydrates, glycosides, tannins, phenols, quinones, terpenoids, phytosterols, and saponins. These compounds are classified as secondary metabolites that naturally occur within plant systems. These secondary metabolites contribute significantly towards the biological activities of the seed such as antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, antiproliferative, antipyretic activities etc. The *P. emblica* seed extract showed higher levels of total flavonoids and tannins. Total Flavonoid compounds have an important role in anticancer [26], antimicrobial, antiviral, antiangiogenic [27], antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative agents [28]. Sumalatha documented the presence of significant phytochemicals in *P. emblica* fruit extract, including flavonoids, alkaloids, protein, saponin and tannis [29]. Studies have demonstrated that flavonoids are potent scavengers of various oxidizing molecules such as singlet oxygen and free radicals associated with numerous diseases [18] Their remarkable bioavailability allows for consistent intake to achieve pharmacologically relevant plasma concentrations in humans [30]. Moreover, multiple studies have suggested potential cardioprotective effects of flavonoids during ischemia-reperfusion [31]. Saponins have the ability to stimulate protective factors in mucous membranes, while tannins can reduce mucosal permeability to chemical irritation leading to decreased inflammation and exerting astringent actions on stomach lining as well as reducing excess acidity [32]. Free radicals are crucial in the development of different pathological conditions. Antioxidants help to neutralize reactive oxygen species and maintain antioxidant defence mechanisms, thus protecting against a range of diseases [33].

The electron-donating capacity of natural compounds can be assessed by using a purple-coloured solution DPPH [34] which assesses the ability of antioxidants to interact with and decolourize the DPPH solution. The degree of colour change is directly proportional to the concentration and effectiveness of the antioxidants. The results suggest that the *P. emblica* seed extract contains phytochemical constituents that can donate hydrogen to free radicals to scavenge potential damage. Recent research indicates that numerous flavonoids and associated polyphenols play a key role in the phosphomolybdate scavenging activity of medicinal plants [35]. The seed extract demonstrated a greater overall antioxidant capacity, with noticeably superior outcomes across all the antioxidant assays conducted in the present analysis. Plant-derived compounds represent a valuable source of potent antibacterial agents capable of combating a range of infectious diseases caused by virulent microorganisms. In this study, the antibacterial efficacy of *P. emblica* seed extract was evaluated against the bacterial pathogens Streptococcus mutans and Enterococcus faecalis.

In our study, in vitro screening using the MTT assay was applied to the ethanol extract of *P. emblica* seed against oral cancer cell line. The results demonstrated that the seed extract are able with variable potency to inhibit the cellular proliferation of SCC25 cells over time and with icreasing concentration. The strongest cytotoxic effect was observed at 72 hours, where cell viability dropped below 20% at concentrations of 200 µg/mL and above. This suggests that the extract may disrupt essential functions like mitochondrial activity and viability pathways in cancer cells. The gradual decline in viability over time also indicates that longer exposure enhances its anticancer impact. The CC_{50} value, estimated to range between 110 and 130 µg/mL at 72 hours, highlights the extract's strong potential as a plant-derived anticancer agent. Suttijit Sriwatcharakul reported that *P. emblica* seed showed notable antioxidant properties and demonstrated cytotoxic effect against MCF-7 cell lines [2]. The ethanolic extract of *P. emblica* fruit exhibited comparatively stronger cytotoxic activity toward HT-29 cell lines [29]. Purified phenolics from *P. emblica* fruit effected the survival of MCF-7 cells [36]. This study offers promising preliminary evidence for the potential application of the extract in oral cancer therapy.

The mass spectrometer examined the eluted compounds at various time points to determine their characteristics and arrangement. The fragmentation of larger compounds into smaller fragments resulted in the emergence of peaks with diverse m/z ratios. These distinct mass spectra serve as unique identifiers for each compound that can be matched against a data library for identification purposes. The GC-MS analysis of *P. emblica* seeds identified the presence of seventeen bioactive compounds, which may contribute to the medicinal potential of this plant species. Among these, the compound cyclotrisiloxane, hexamethyl- was detected with the highest percent peak area. The compound cyclotrisiloxane, hexamethyl has found to be a prominent constituent in many medicinal plant species, including *Glochidion candolleanum*, *Olea europaea*, and *Bauhinia acuminata* which demonstrated potent antimicrobial properties against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [25]. Other major compouds found include di-n-octyl phthalate, 1,2-dihydro-1,4-dimethoxy-2-oxoquinazoline, calycotomine, methyl, phenol, 2,4-bis(1,1-dimethyl ethyl) and 1-dodecanol, 3,7,11-trimethyl. These compounds possess a broad range of biological properties, including anti-bacterial, anti-fungal, anti-cancer, anti-inflammatory, and analgesic potencies. The *Phyllanthus emblica* seeds are rich source of pharmacologically active

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constituents and therapeutic phytochemicals that exhibit a diverse range of medicinal benefits. These include antimicrobial, antioxidant, anti-inflammatory, antidiabetic, analgesic, anti-aging, anticancer, hepatoprotective, antihistaminic, antiandrogenic, antifibrinolytic, diuretic, and anti-asthmatic properties, as well as preservative effects. Additional investigations are required to better understand and characterize the underlying biological activities and understand the medicinal significance of these innovative biomolecules present in the *P. emblica* seeds. This study represents the first report on the GC-MS analysis of *P. emblica* seed ethanolic extract.

V. CONCLUSION

In conclusion, the analysis of *P. emblica* seed extract demonstrates its potential as it possesses various secondary metabolites with pharmacological properties such as antioxidant activity and high levels of tannin and flavonoid content. The study analysed the biological activity of the seed and identified a wide array of bioactive phytochemicals with diverse properties. These findings suggest that the identified bioactive phytochemicals hold promise for selection in pharmaceutical industry for drug discovery.

DECLARATIONS

- *Data Availability*

All data generated or analysed during this study are included in this published article.

- *Competing Interests*

The authors declare that they have no competing interests.

- *Funding*

No funding was received for conducting this study.

- *Authors' Contributions*

AA: Conceptualization, Methodology, Data analysis, interpretation of results, Writing- Original draft preparation. US and AA: Editing and review. All authors read and approved the final manuscript.

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