

INSIGHTS INTO THE THERAPEUTIC POTENTIAL OF *PHYLLANTHUS EMLICA*  
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**ABSTRACT**

Medicinal plants have been used in traditional medicine practices since prehistoric times. Amla (*Phyllanthus emlica*) which is commonly called as Indian gooseberry, belonging to the family Phyllanthaceae is one of the important medicinal plant gaining interest of researchers for many decades. Amla is a gift of nature to mankind. Qualitative phytochemicals analysis of different extracts of Amla seed coat was carried out according to different standard procedures used to analyze the various classes of secondary metabolites. Phytochemical screening revealed the presence of alkaloids, polyphenols, tannins, flavonoids and terpenes. Plant extract has been evaluated for antibacterial activity by agar well diffusion method against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus mutans*. Crude extract showed significant antibacterial activity. The antioxidant activity determined by free radical scavenging, DPPH assay using gallic acid as standard. Ethanol extract showed 80% scavenging activity at 500ug/ml whereas water extract showed 19.7% scavenging activity at the same concentration. This study revealed that the ethanol extract of the plant is a good source of antioxidant and antimicrobial agents.

**KEYWORDS:** Amla, phytochemicals, antibacterial, radical scavenging activity.**INTRODUCTION**

Amla fruit is one of the noncontroversial and extensively used herbs in Ayurvedic medicine. It belongs to family Phyllanthaceae. It is a glabrous, succulent, woody climbing tree native to India. It is also found in Burma and Sri Lanka. It thrives well in the tropical region, often attains a great height, and climbs up the trunks of large trees. The stem is gray or creamy white, deeply cleft spirally and longitudinally, with the space between spotted with large rosette-like lenticels.

The wood is white, soft, and porous, and the freshly cut surface quickly assumes a yellow tint when exposed to air. Leaves are simple, alternate, exstipulate, long petiolate, chordate in shape showing multicoated reticulate venation. Long threadlike aerial roots come up

from the branches. Flowers are small and Unisexual. Male flowers are in clusters female flower are solitary. Six sepals arranged in two whorls of three each. Six petals arranged in two whorls.<sup>[19-20]</sup>

**MATERIALS AND METHODS****Preparation of plant extract**

The fresh green Amla fruit (seed coat) were collected from Mysore region. The fruits were then washed thoroughly and dried under shade for 2 weeks and coarsely powdered. It was then kept at low temperature.



**Figure 1: coarsely powdered fruit extracts.**

Coarsely powdered fruit extracts were packed in Soxhlet apparatus and was continuously extracted individually using solvents such as ethanol and water.

#### **Phytochemical analysis of the extracts**

Phytochemical screening of the extracts of the plant was carried out in order to know the class of organic compounds present in the different extracts selected for the study. The extract of Amla fruit were subjected to standard chemical tests to determine the presence or absence of phytochemicals.<sup>[1,2,13]</sup>

#### **ANTIBACTERIAL ACTIVITY**

(Agar Well diffusion method to check the Minimum Inhibition Concentration (MIC))

The sample (Amla extract) was tested in duplicates for their MIC property against organisms (*Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus mutans*)

#### **Culture Media Preparation for Bacteria**

Luria Bertani (LB) broth (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000mL) 30mL was prepared in 4 Erlenmeyer flasks by adding Tryptone 0.3g, Sodium chloride 0.3g, Yeast extract 0.18g, Distilled water 30mL and autoclaved at 121°C for 15 minutes. Later, *Pseudomonas aeruginosa* strain (MTCC 2453), *Salmonella typhi* strain (MTCC735), *Staphylococcus aureus* (MTCC 96) and *Streptococcus mutans* strain (MTCC 497) was inoculated respectively in 30mL of sterilized LB broth and incubated at 37° C for 24hr.

#### **Plating for MIC against organisms**

Approximately 25mL of LB agar was poured into the sterilized petriplates and allowed it to solidify. 200µL prepared inoculums (*P.aeruginosa*, *S.typhi*, *S.aureus* and *S.mutans*) was poured in to the agar plates respectively and spread thoroughly using a plate spreader. Five wells measuring 0.6 cm was made in each plates using the borer and 50µL of prepared sample and control (Tetracycline) containing 1mg, 5mg, and 10 mg, were loaded into the respective wells.

The bacterial plates incubated at 37°C for 24h. Later, zone of inhibition was recorded in mm (Millimeter).<sup>[6,9,14]</sup>

#### **ANTIOXIDANT ACTIVITY**

##### **(DPPH RADICAL SCAVENGING ASSAY)**

Free radical scavenging capacities of the extracts from different samples were estimated using the stable DPPH radical. Different concentrations (0.1 – 0.5mg) of the samples were taken in the test tubes and the volume in each test tube was made up to 0.1mL with methanol. To all the tubes, 3mL of DPPH solution was added and incubated in dark condition for 15minutes. After incubation, the absorbance was read at 517nm spectrophotometrically with methanol as a blank. Gallic acid was used as reference compound. The percentage of inhibition of DPPH radical was calculated.<sup>[1,3,4,16,17]</sup>

#### **RESULTS AND DISCUSSIONS**

In order to establish the preliminary phytochemical profile of fruit extract the ethanol and water extract were subjected to various chemical tests.

**Table 1: Phytochemical screening of ethanol extracts.**

Sl. No	Phytochemicals tests	Inference
1	Alkaloids	+
2	Polyphenols	+
3	Tannins	+
4	Glycosides	-
5	Reducing sugars	-
6	Saponins	-
7	Flavonoids	+
8	Steroids	-
9	Terpenoids	+
10	Proteins and amino acids	+

(+) = presence & (-) = absence

Phytochemical screening revealed the presence of alkaloids, polyphenols, tannins, flavonoids, terpenes carbohydrates, and amino acids. However glycosides, steroids and saponins were found to be absent. Water extract revealed the presence of alkaloids, tannins, flavonoids and steroids.

#### ANTIBACTERIAL ACTIVITY

Antibacterial activity

Result: Crude Extracts has shown positive activity with (gram + and gram - cultures). Tetracycline was used as the standard.

**Table 2: Zone of inhibition by Plant fruit extracts.**

Extract(crude)	Zone of Inhibition In Millimeters			
	1mg/ml	5mg/ml	10mg/ml	Std 10ug/ml
Ethanol	00.00	9.5	11.5	35

**Figure 2: Petri dishes showing the zone of inhibition by crude extracts.**

The present study proposes that phytochemicals extracted from fruit extracts exhibit noteworthy antibacterial property.

#### ANTIOXIDANT ACTIVITY

**Table 3: DPPH Radical Scavenging Activity (Ethanol extracts).**

Sl no	Tested sample	Conc of sample in $\mu\text{g}$	Percentage Inhibition	IC50 ( $\mu\text{g/ml}$ )
1	Gallic acid	100	8.6	2.12
		200	18.5	
		300	30.5	
		400	51.8	
		500	70.2	
2	Amla Ethanol extract	100	48.6	112.2
		200	55.37	
		300	71.28	
		400	79.2	
		500	80.2	
3	Amla Water extract	100	6.8	1.58
		200	10	
		300	12.1	
		400	15.9	
		500	19.7	

The antioxidant activity determined by free radical scavenging, DPPH assay using gallic acid as standard. Ethanol extract showed 80% scavenging activity at 500ug/ml whereas water extract showed 19.7% scavenging activity at the same concentration.

### CONCLUSION

Herbal medicines have been the oldest forms of health care. In Ayurveda many indigenous plants have been mentioned and well established as antioxidant and antimicrobial potential. The present study can be concluded that ethanol extract of Amla fruit extracts possesses substantial amount of phytochemicals such as alkaloids, polyphenols, tannins, flavonoids, terpenes, carbohydrates, and amino acids. Crude ethanolic and water extracts showed desirable antimicrobial and antioxidant activities. Further studies on in vivo model to determine pharmacological action needs to be carried out.

### Conflict of Interest

None.

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