

Screening of Phytochemicals and Antioxidant of *Phyllanthus Emblica* (Amla)

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Abstract: Some plants have good medicinal and therapeutic importance for healthy lifestyle. One among them is *Phyllanthus emblica* (Linn) belongs to Euphorbeaceae family, commonly known as Amla which has superior value in traditional system of medicine. It has several pharmacological properties, mainly antioxidant activity and anti-inflammatory activity. The study is focused on phytochemical investigation and analysis of antioxidant activities of extracts derived from three different extracts of *Phyllanthus emblica* prepared by the Soxhlet extraction method. The extracts were used to detect the presence of alkaloids, phenols, flavonoids, terpenoids, steroids and other phytochemicals. Antioxidant assay was performed as DPPH radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl). DPPH reacts with the free radicals and change its colour.

Keywords: *Phyllanthus emblica*, phytochemical, DPPH, antioxidant.

I. INTRODUCTION

Phyllanthus emblica (Amla) is widely distributed in subtropical and tropical areas of India, China, Indonesia and Malaysia.[1] Amla as major constituent in several Ayurvedic treatment for health and longevity[2]. Amla is known for good source of polyphenols, flavonoids, tannins and other bioactive compounds. These substances being strong antioxidants might contribute to the health effects of Amla. Several active compounds like gallic acid, ellagic acid, 1-O-galloyl-D glucose, chebulinic acid, quercetin, chebulagic acid, kaempferol, mucic acid 1,4-lactone 3-O-gallate, isocorilagin, chebulanin, mallotusin and acylated apigenin glucoside compounds have been isolated from the aqueous extract of Amla[3]. These bioactive components have anticancer, hypolipidemic, expectorant, purgative, spasmolytic, antibacterial, hypoglycaemic[4,5] hepatoprotective, hypolipidemic activities and also can attenuate dyslipidaemia.[6] The World Health Organization have been estimated that 80% of the population believes in traditional medicine for their basic health care requirements[7].

The biological antioxidants are compounds that protects organic structures in opposition to the doubtlessly harmful consequences of approaches or reactions that causes excessive oxidation. These days the oxidative strain is one of the severe issues of the present-day society. Results in Oxidative stress of cells, results in diverse. Diseases like dermatitis, melanomas or photo ageing of the skin, cancer, heart sickness, irritation, arthritis, immune suppression, brain disorder and cataracts and so on. The consumption of plants greens has been located to be associated with lowering of those illnesses as they comprise a big amount of phenolic compounds, antioxidants and flavonoids. In diverse research it's been observed that antioxidants can inhibit or put off the oxidation of an oxidisable substrate in a series reaction hence antioxidants appear to be very essential in prevention of those diseases (8,9,10).

Phyllanthus emblica (Linn). (Euphorbeaceae), commonly known as amla which has superior value in traditional system of medicine. The general techniques of extraction includes maceration, infusion, percolation, digestion, decoction, hot continuous extraction (soxhlet), Aqueous-alcoholic extraction by way of fermentation, counter modern extraction, microwave assisted Extraction, ultrasound extraction (sonication), Supercritical fluid extraction (SFE), phytonic extraction (with hydro-flouro-carbon solvents), and so forth. There are styles of hydrodistillation strategies (water distillation, steam Distillation, steam and water distillation), hydrolytic maceration accompanied by using distillation method, expression technique and enfleurage technique (cold Fat extraction) can be used.(11)

The aim of this study is to find out the antioxidant potential of amla, conducted to study is conducted for the phytochemical analysis, antioxidant activity using three different solvent extracts.

II. MATERIALS AND METHODS

CHEMICALS

Good Quality and analytical grade chemicals and reagents such as DPPH, methanol, ascorbic acid, sulfuric Acid, millon's reagent, sodium nitrite, mayer's reagent, benedict's reagent, molisch's, glacial acetic acid, iodine, ferric chloride, Sodium hydroxide, ninhydrin, and chloroform were purchased from registered chemical suppliers in ECR, Chennai, Tamil Nadu, India.

SAMPLE FRUIT COLLECTION

The good quality fertile *Phyllanthus emblica* fruits were purchased in local market Poothamallee, Chennai, Tamil Nadu, India.

EXTRACT PREPARATION

Freshly purchased *Phyllanthus emblica* fruits were washed with tap water 2–3 times, and dried under the shade at room temperature, and then blended to powder using an electric blender. Powdered sieved and stored in a sterile airtight container for further use. Three types of extracts such as aqueous, ethanol, methanol were prepared by adopting stand procedure. Distilled water, 100% ethanol and 100% methanol were used, to each 10 g of dry powder in 3 separate conical flask (250 ml capacity) 100 ml of different solvents like water, ethanol and methanol were added separately. Flasks were tightly sealed with parafilm and allowed to be shaken vigorously on a magnetic stirrer for 48 h. Extracts were filtered using Whatman No.1 filter paper. The filtrates were then stored in an air tight bottle and kept in refrigerator at 4°C until required.

PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS

The presence of phytochemical substances are determined based on standard qualitative test procedures [12]. The aqueous, ethanol and methanol extracts of *P.emblica* was used for the following phytochemical assays.

- 1. Test for Acids-Millon's Test:** To 1.0 ml extract, five drops Millon's reagent was added, heated on a water bath for 5 min. and allowed to cool followed by addition of 1% sodium nitrite solution. Then observed for the formation of red colour, which indicates the presence of acids.
- 2. Test for Alkaloids-Mayer's Test:** To 2.0 ml extract, 2.0 ml concentrated hydrochloric acid followed by few drops Mayer's reagent were added and observed for the formation of green colour or white precipitate, which indicates the presence of alkaloids.
- 3. Test for Carbohydrates -Molisch's Test:** To 2.0 ml extract, 1.0 ml Molisch's and few drops of concentrated sulphuric acid were added and observed for the formation of purple or reddish ring, which indicates the presence of carbohydrates.
- 4. Test for Cardiac Glycosides-Ferric Chloride Test:** To 0.5 ml extract, 2.0 ml glacial acetic acid and few drops 5% ferric chloride were added. This was under layered with 1.0 ml concentrated sodium hydroxide. Formation of the brown ring at the interface was observed, which indicates presence of cardiac glycosides.
- 5. Test for Flavonoids-Sulphuric Acid Test:** 1.0 ml extract was treated with few drops of concentrated sulphuric acid and observed for the formation of orange colour.
- 6. Test for Glycosides-Sulphuric Acid Test:** To 2.0 ml extract, 1.0 ml glacial acetic acid, 5% ferric chloride and few drops concentrated sulphuric acid were added and observed for the formation of greenish blue colour, which indicates the presence of glycosides.
- 7. Test for Phenols-Ferric Chloride Test:** To 1.0 ml extract, 2.0 ml distilled water, followed by few drops of 10% ferric chloride were added. Formation of blue or green colour was observed, which indicates presence of phenols.
- 8. Test for Proteins-Ninhydrin Test:** To 2.0 ml extract, few drops of 0.2% ninhydrin was added and heated for 5 min. and observed for the formation of blue colour. This indicates the presence of proteins.
- 9. Test for Quinones-Sulphuric Acid Test:** To 1.0 ml extract, 1.0 ml concentrated sodium hydroxide was added and observed for the formation of red colour, which indicates the presence of quinones.
- 10. Test for Saponins-Foam Test:** To 1.0 ml extract, 5.0 ml distilled water was added and shaken well in a graduated cylinder for 15 min. lengthwise. Formation of 1.0 cm layer of foam was observed, which indicates the presence of saponins.
- 11. Test for Starch-Iodine Test:** To 2.0 ml extract, few drops of iodine solution was added and observed for the formation of blue-purple colour, which indicates the formation of starch.
- 12. Test for Steroids-Salkowski Test:** To 5.0 ml extract, 2.0 ml of chloroform and few drops concentrated sulphuric acid were added and observed for the formation of red colour, which indicates the presence of steroids.
- 13. Test for Tannins-Ferric Chloride Test:** To 1.0 ml extract, 2.0 ml 5% ferric chloride was added and observed for the formation of dark blue or greenish black colour, which indicates the presence of tannins.

14. Test for Terpenoids-Sulphuric Acid Test: To 0.5 ml extract, 2.0 ml chloroform was added and to this, concentrated sodium hydroxide was added carefully. Formation of red brown colour at the interface was observed, which indicates presence of terpenoids.

ANTIOXIDANT ACTIVITY:

Antioxidant activity is screened by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The Antioxidant activity of extracts of *Phyllanthus emblica* and the standard were assessed based on the following the procedure [12,13]. Five test tubes were taken and to three test tubes 1ml of each extract (sample) was taken and 3.7 ml of methanol was added next 200µl DPPH reagent was added and mixed, in the fourth tube DPPH reagent was added with 3.8 ml methanol and kept as blank, in the fifth tube 200µl of DPPH was added to 100µl ascorbic acid and kept as standard. Incubate all test tubes at room temperature in dark conditions for 30 minutes. The absorbance was read at 517nm in a spectrophotometer. Percentage of antioxidant activity was calculated using the formula given below.

CALCULATION

$$\% \text{ of Antioxidant activity} = \frac{(\text{Absorbance at blank}) - (\text{Absorbance at test})}{(\text{Absorbance at blank})} \times 100$$

STATISTICAL ANALYSIS

Results were treated statistically and expressed as Mean and Standard Deviation.

III. RESULTS & DISCUSSION

TABLE: 1 – Qualitative phytochemical screening of *Phyllanthus emblica* extracts.

S.No.	Test	Method	Aqueous	Ethanol	Methanol
1.	Amino Acids	Million`s Test	-	-	-
2.	Alkaloids	Mayer`s Test	-	+	+
3.	Carbohydrates	Molisch`s Test	+	+	+
4.	Cardiac Glycosides	Ferric Chloride Test	+	-	+
5.	Flavonoids	Sulphuric Acid Test	+	+	+
6.	Glycosides	Sulphuric Acid Test	-	-	+
7.	Phenols	Ferric Chloride Test	+	+	+
8.	Proteins	Ninhydrin Test	-	-	-
9.	Quinones	Sulphuric Acid Test	-	-	+
10.	Saponins	Foam Test	-	+	+
11.	Starch	Iodine Test	-	-	-
12.	Steroids	Salkowski Test	+	+	+
13.	Tannins	Ferric Chloride Test	+	+	+
14.	Terpenoids	Sulphuric Acid Test	+	+	+

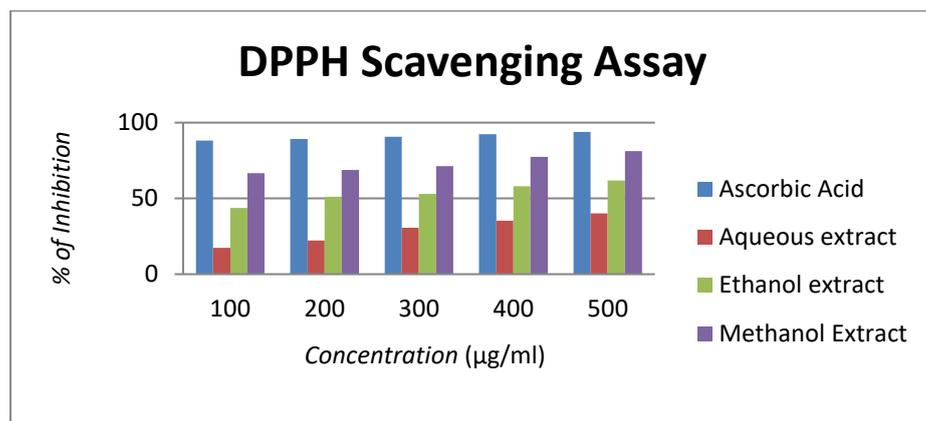
+Presence -Absence

TABLE: 2- Antioxidant activity of *Phyllanthus emblica* extracts

DPPH % of inhibition of <i>Phyllanthus emblica</i> extracts				
Concentrations of the sample (µg/ml)	Standard Ascorbic Acid	Aqueous extract	Ethanol extract	Methanol extract
100	88.21±0.2	17.34±1.27	43.77±0.29	66.66±0.50
200	89.22±0.29	22.22±0.50	51.01±0.50	68.68±0.50
300	90.74±0.58	30.63±1.05	53.03±0.50	71.38±0.77
400	92.42±0.50	35.35±0.50	58.08±0.50	77.44±1.27
500	93.93±0.50	40.06±0.29	61.78±0.77	81.31±0.50

Mean ± Standard deviation (SD) for analysis in six replicates

Figure 1. Total antioxidant activity of aqueous, ethanol and methanol extract of *Phyllanthus emblica* and standard Ascorbic acid.



Qualitative analysis of phytochemical screening of the 3 one kinds of extracts of *Phyllanthus emblica* included the presence of various chemical substance gatherings. [Table 1]. The results of Phytochemical Screening and antioxidant activity of *Phyllanthus emblica* fruit extracts are presented in Table 1 & 2. Qualitative Phytochemical screening of aqueous, ethanolic & methanolic extracts showed the presence of Amino acids, alkaloids, carbohydrates, cardiac glycosides, flavonoids, glycosides, phenols, proteins, quinones, saponins, starch, steroids, tannins and terpenoids. Among the three solvents used methanolic extract showed maximum number of Phytochemical compounds than ethanolic and aqueous extracts. Carbohydrates, flavonoids, steroids tannins and terpenoids are present in all three extracts, whereas cardiac glycosides, glycosides and quinones are available in the methanolic extract alone, not found in aqueous and ethanolic extracts. Alkaloid is the main phytochemical compound and significantly showing the antibacterial activity (Pokhrel & Karki, 2021). Earlier in the year 2010 itself Shilaseedee and his coworkers found the antibacterial activity of methanolic extract of *Kernet Eugenia jambolana* and of coral extract of *cassia auriculata*. Flavonoids are substances synthesized by the plants act against the microbial infection. The antimicrobial activity of the phytochemical compounds of methanolic extracts against pathogenic bacterial strains were confirmed by Shihabudeen et al. (2010). From the results obtained it is confirmed that methanol has the best efficacy to obtain maximum phytochemical compounds.

In the biological systems free radicals generated are very harmful to the host and includes immunosuppression which leads to dislodge development. The antioxidant interest of *Phyllanthus emblica* is the usage of the DPPH radical scavenging assay is based on the capacity of antioxidants, to de-colourize DPPH. Every of the evaluated concentrates of *Phyllanthus emblica* had the choice to decrease the solid, red-coloured extremist DPPH to the yellow-shaded DPPH. The Methanol extract of *Phyllanthus emblica* revealed the very best percentage of inhibitory activity in (81.31 ± 0.05%), and the Ethanol extract of *Phyllanthus emblica* found the best percentage of inhibitory pastime in (61.78 ± 0.77%) The Aqueous extract of *Phyllanthus emblica* found out the best per cent of inhibitory activity in (40.06 ± 0.29%) whilst compared with trendy ascorbic acid it found out the best per cent of inhibitory interest in (93.93 ± 0.50%) at 500 µg/ml attention. The least per cent of inhibition in Aqueous (17.34 ± 1.27%), Ethanol (43.77 ± 0.29 %), and Methanol (66.66 ± 0.50%) and compared with trendy ascorbic acid (88.21 ± 0.29%) was recorded at a hundred µg/ml concentration. [Table 2 & Figure 1]. From the above take a look at, *Phyllanthus emblica* famous excellent effects. An antioxidant or scavengers of free radicals were found in the extracts carica (Pokhrel & Karki, 2021) similar results were obtained in our study also.

IV. CONCLUSIONS

In this current above study, it very well may be pronounced that the shown Aqueous, Ethanol and Methanol extract of *Phyllanthus emblica* extract have good Antioxidant Properties. The methanol extract has very good properties than the Ethanol and Aqueous Extract. *Phyllanthus emblica* contains the functional groups that can be implemented in pharmaceutical industries to develop drugs to cure many diseases.

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