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Research Article

Phytochemical Profiling of *Phyllanthus emblica* Leaf Extract

Shashi Kumar Dubey[1] *and Deepak Mishra*[1*]

[1]*Department of Biotechnology, AKS University, Satna (MP) - India*

[*]*Corresponding Author E-mail: deepakrewabiotech@gmail.com*

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Abstract

Phyllanthus emblica is one of the most important plants in the traditional Indian system of medicine with wide range of pharmacological and nutritional applications even in present day therapeutics. In the present investigation identification of different bioactive components present in *Phyllanthus emblica* has done by performing various phytochemical test. For this the methanolic extract of leaves of *Phyllanthus emblica* has been prepared by Soxhlet extraction method. The presence of different bioactive component i.e., carbohydrate, protein, alkaloid, Triterpenoids and Steroids, Saponins, etc. has determined by performing different qualitative test i.e., Mayer, Molish, Ninhydrin foam test etc. The positive result of different qualitative test indicates the presence of alkaloids, carbohydrate, reducing sugar and steroids whereas negative results indicate the absence of saponin and proteins.

Keywords: *Phyllanthus emblica, phytochemical analysis, leaf extract*

Introduction

Phyllanthus emblica is a medicinally important fruit crop species. It is one of the important plant variety of the family Phyllanthaceae (earlier, Euphorbiaceae) and widely distributed in South Eastern Asia (Dassanayake and Fosberg, 1988). All parts of the tree are utilized in indigenous medicine, yet drupe plays the central role as a remedy to a number of ailments (Krishnaveni and Mirunalini, 2011). *P. emblica* has a high economic value because of its medicinal and nutritive richness. Therefore, it is used to produce cosmetics, commercial beverages, confectionaries and medicinally important value-added products. Fruit itself has a high demand as fresh fruit in the market. Thus, the mature drupe size and the bitterness of *P. emblica* are the most important traits for commercial exploitation. In present day period where there is a lift in 'worldwide natural market', *P. emblica* has a high potential as a money crop in Sri Lanka. In India alone 10,000 tons of *P. emblica* drupes are industrially used per annum (Sharma et al., 2008a). Traditional system of therapeutics and medicine is a valuable asset for any country as it plays a vital role in sustainable development of society. There is little documentation of conventional information on natural medication in classified frameworks like Ayurveda, around 1500 plant species are known yet in society custom in excess of 4500 species are utilized. The solid connection among people and nature can gives probability of finding new uses for restorative plants and to find new home-grown medications. Restorative plants accordingly have significant commitment in the essential medical services practices of ancestral networks. The World Health Organization (WHO) estimates that up to

80% of the world's population in developing countries depends on locally available plant resources for their primary healthcare, since western pharmaceuticals are often expensive (Arora, 1997).

The main objective of the research work was to analyze the phytochemical profile of Amala leaf.

Materials and Methods

Sampling Sites & Sample Collection

The plant material was selected from different localities of Vindhya region and identified on the basis of morphometric parameters (Table 1). Various considerations involved in the plant selection especially for its chemoprofilling activity. Leaves of *Phyllanthus emblica* were subjected for extract preparation in by using Soxhlet Extraction method, where Methanolic solvent system is used.

Phytochemical Screening of Sample

The Methanolic extract of *Phyllanthus emblica* leaves were subjected for phyto-chemical profiling to confirm the presence of different bioactive components i.e., Carbohydrate, Alkaloid, Saponins, Flavonoids, Triterpenoids and Steroids, Tannin and Phenolic Compounds, Glycoside etc. via performing specific qualitative test as methods suggested by Trease & Evans (1989).

Test for Carbohydrates

Molisch's Test

To 1 ml of aqueous solution of the extract mixed with few drops of Molish reagent (α-Naphthol) followed by addition of conc. H2SO4 by the wall of the test tube. was added along the wall of the tube. Formation of purple colored ring at junction indicated the presence of carbohydrates.

Fehling's Test

Equal volume of Fehling A and Fehling B solution were mixed (1ml each) and 2ml of aqueous solution of extract was added followed by boiling for 5-10 minutes on water bath. Formation of reddish-brown colored precipitate due to formation of cuprous oxide indicated presence of reducing sugar.

Benedict's Test

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presences of reducing sugar.

Barfoed's Test

To the aqueous solution of extract, 1 ml of Benedict solution was added and heated almost to boiling. Red colour due to formation of cupric oxide indicates the presence of monosaccharides.

Tests for Alkaloids

Dragendorff's Test

To 1 ml of extract dissolved in alcohol was shaken well with a few drops of acetic acid and Dragendroff's reagent. Presence of alkaloid confirmed via formation of orange red precipitate.

Wagner's Test

To 1 ml of extract dissolved in acetic acid, a few drops of Wagner's reagent were added. A reddish-brown precipitate formed indicated the presence of alkaloids.

Mayer's Test

To 1 ml of extract dissolved in acetic acid, a few drops of Mayer's reagent were added. A dull white precipitate formed which indicated the presence of alkaloids.

Hager's Test

To 1-2 ml of extract dissolved in acetic acid, 3 mL of Hager's reagent was added; the formation of yellow precipitate indicated the presence of alkaloids.

Test for Saponins

Froth Test

To 1ml of extract, distilled water was added and shaken. Stable froth formation indicated the presence of saponin.

Test for Triterpenoids and Steroids

Libermann-Burchard Test

The extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. 1-2 drops of concentrated sulfuric acid were added at the edges of the test tube. Bluish green color formed which indicates the presence of steroids.

Salkowski Test

The extract was dissolved in chloroform and equal volume of concentrated sulphuric acid was added. Presence of the steroid has been confirmed through formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer.

Test for Tannin and Phenolic Compounds

Ferric Chloride Test

Take 2ml of extract and add few drops of dilute solution of ferric chloride. Formation of dark blue color indicates the presence of tannins.

Gelatine Test

Some quantity of extract was dissolved in distilled water. Add 2ml of 1% gelatin solution containing 10% sodium chloride was added. A white coloured precipitate formed which indicates presence of phenolic compounds.

Lead Acetate Test

Some amount of extract dissolved in distilled water; few drops of lead acetate solution were added. A white colored precipitate formed which confirms presence of phenolic compounds.

Ferric Chloride Test

Take 2ml of extract and add few drops of dilute solution of ferric chloride. Formation of dark blue color indicates the presence of tannins.

Test for Flavonoids

Shinoda's Test

To the 1 ml of extract in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and heated on a water bath. A development of red to pink tone showed the presence of flavonoids.

Test for Glycosides

Borntragers Test

Take 3 ml of test solution and add dilute sulfuric acid, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. development of pink to red tone in ammonical layer shows presence of anthraquinone glycosides.

Keller Killiani Test

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add 0.5 ml of conc. H2SO4 by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presences of Cardiac glycosides

Results and Discussion

Five individual populations of *Phyllanthus emblica* were collected from different areas of Vindhya region. Phytochemical screening of leaves of *Phyllanthus emblica* was done to confirm the presence or absence of various bioactive constituents using standard tests (Kokate et al., 2004). The results of qualitative phytochemical analysis of the species of the *Phyllanthus emblica* are shown in Table 2. The leaves sample of species S1 shows the presence of saponins, flavonoids, glycosides, proteins and amino acids, but absences of alkaloids, tannins, phenolic compounds and some carbohydrates. The leaves sample of species S2 shows the presence of Tannin, Phenolic compounds flavonoids, proteins and amino acids; but absences of glycosides, saponins, steroids, triterpenoids and some carbohydrates, alkaloids. The leaves sample of species S3 shows the presence of carbohydrates, glycoside, saponins, flavonoids, proteins and amino acids. The leaves sample of species S4 shows the presence of carbohydrates, alkaloids, saponins, glycosides and some proteins. The leaves sample of species S5 shows the presence of glycosides, saponins, steroids, alkaloids and carbohydrates, as protein and amino acids are present. Medicinal plants are the source of numerous chemical compounds which are synthesized endogenously by plants. Phytochemical analysis is an important tool to recognize the presence of bioactive constituents. The Phytochemical investigation produced useful

knowledge regarding the different bioactive constituents present in the extracts, which allows the potential investigations on the selection of the specific extract for the usage and isolation of the active principle compound (Mishra et al., 2010). Similarly, Jhaumeer Laulloo et al, (2018) reported the presence of phenols, flavonoids, non-flavonoid, tannins, alkaloids, saponins, and phytosterols in various extracts of dried fruits of *Phyllanthus emblica* (Amla). Also, Gaire et al (2014) proved that *P. emblica* contains phytochemicals compounds i.e., fixed oils, phosphatides, amino acids, fatty acids, minerals, vitamins, essential oils, tannins, glycosides, etc. They also reported pharmaceutical potential of *P. emblica* as it has antimicrobial, analgesic and antipyretic, anti-inflammatory, antioxidant, hepatoprotective, adaptogenic, antitumor and antiulcerogenic activities.

Table No. 1. Sampling Sites of *Phyllanthus emblica* with their Geographical Locations

Sample Code	Sampling Site	Geographical location
S1	Sirmour area (Wardha ghat), Rewa	24.85°N 81.38°E
S2	Semariya, Rewa	24°47'42"N 81°9'8"E
S3	Rampur Naikin, Sidhi	24°20'23"N 81°28'29"E
S4	Hanumana, Rewa	24°46'30"N 82°5'24"E
S5	Mukundpur, Rewa	24°42'18" N, 81°24'36"E

Table No. 2. Phytochemical evaluations of different samples of *Phyllanthus emblica*

S. No.	Experiment	Result of species				
		S1	S2	S3	S4	S5
Test for Carbohydrates						
	Molisch's Test	+ve	+ve	+ve	+ve	+ve
	Fehling's Test	-ve	-ve	+ve	+ve	+ve
	Benedict's Test	-ve	-ve	+ve	+ve	+ve
	Barfoed's Test	+ve	+ve	+ve	-ve	+ve
Test for Alkaloids						
	Dragendorff's Test	-ve	+ve	-ve	+ve	+ve
	Wagner's Test	-ve	-ve	+ve	+ve	-ve
	Mayer's Test	-ve	+ve	+ve	+ve	+ve
	Hager's Test	-ve	+ve	-ve	+ve	+ve
Test for Triterpenoids and Steroids						
	Libermann-Burchard Test	+ve	-ve	+ve	-ve	+ve
	Salkowski Test:	-ve	-ve	+ve	-ve	-ve
Test for Saponins						
	Froth Test	+ve	-ve	+ve	+ve	+ve
Test for Tannin and Phenolic Compounds						
	Ferric Chloride Test	-ve	+ve	+ve	-ve	-ve
	Gelatin Test	-ve	+ve	-ve	-ve	-ve
	Lead Acetate Test	-ve	+ve	+ve	-ve	-ve
Test for Flavonoids						
	Shinoda's Test	+ve	+ve	+ve	-ve	-ve
Test for Glycosides						
	Borntragers Test	+ve	-ve	+ve	+ve	+ve
	Keller Killiani Test	+ve	-ve	+ve	+ve	+ve
Test for Protein & Amino acids						
	Biuret's Test	+ve	+ve	+ve	-ve	+ve
	Ninhydrin Test	+ve	+ve	+ve	+ve	+ve

+ve = Present; -ve = Absent

CONCLUSION

From the above studies we concluded that, the leaves extract of *Phyllanthus emblica* contains broad range of pharmacologically active compound classes namely alkaloid, glycoside, tannins, saponins, phenolic compounds, resins and reducing sugars. This data can be employed for the detailed pharmacological and nutritional evaluation of *Phyllanthus emblica* leaves for its application in human and veterinary health care settings.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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