Preliminary Phytochemical Analysis of Emblica Officinalis Seed

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ABSTRACT

Phytochemical investigation of n-butanol extract of Emblica Officinalis Seed. This research is to check the phytochemical agent determination by various methods. Study is done to check the test for carbohydrate and protein, saponin, terpenoid, tannins, glycosides, alkaloid by the procedure performed to find the chemical observed in Emblica officinalis seed. The investing the phytochemical present in n-butanol extract of Emblica Officinalis seed by using in vitro methods to check the phytochemical agent present or absent in plant.

Keywords: Phytochemical, n-butanol, Carbohydrate, Alkaloids, Tannins.

INTRODUCTION

Plants have long been recognized for their therapeutic properties. For centuries, indigenous cultures around the world have used traditional herbal medicine to treat a myriad of maladies [1]. Emblica officinalis (Amla) are widely used in the Indian system of medicine and believed to increase defense against diseases. This article discuses and summarizes important medicinal values of Emblica officinalis (EO) [2,3]. In this communication, we reviewed the EO in cancer, diabetis, liver treatment, heart disease, ulcer, anemia and various other diseases [4,5,6,7].The use of EO as antioxidant, immunomodulatory, antifungal activity, antipyretic, analgesic, cytoprotective, antitussive and gastro protective are also reviewed [8,9]. Further for the phytochemical investigation Extraction is the first step to separate the desired natural products from the raw materials [10,11,12]. The extraction of natural products progresses through the following stages: the solvent penetrates into the solid matrix; the solute dissolves in the solvents; the solute is diffused out of the solid matrix; the extracted solutes are collected.

MATERIAL AND METHODS

Collection of the Plant sample

Emblica officinalis stem (P. Emblica L.), leaves and seeds were collected from Department of Pharmacognosy, College of Pharmacy, Chopda (Jalgaon, Maharashtra) and identified authenticated by Dr. C R. Jadhav, Botanist at Botanical Survey of India, Pune, M.H.

Preparation of Plant Extract [13]

Collected plant parts were air dried under shade and then ground to a coarse powder using a grinder. Extraction and fractionation technique was referred from standard textbboks with suitable solvents. Powdered seed material was extracted first with petroleum ether for defatting and then

extracted with methanol in soxhlet apparatus. Further, the methanolic extract of amla seed powder was polarity based fractionated using a seperating funnel by chloroform, ethyl acetate and n-butanol. Extracts and fractions were filtered and the solvent was evaporated to dryness under natural conditions. The final extract was stored in well closed containers under refrigeration (2-4ºC) until used for the biological testing. Petroleum ether, chloroform, ethyl acetate and n-butanol fractions were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, flavonoids, steroids, saponins, amino acids, carbohydrates, triterpenoids and tannins by using following procedures.

Test for Alkaloids [14]

Mayer's test: Fractions were treated with Mayer's reagent (Potassium mercuric iodide solution) and was analyzed for cream coloured precipitate for the presence of alkaloids.

Dragendorffs test: Fractions were treated with Dragendorff's reagent (Potassium bismuth iodide solution) and was analyzed for reddish brown precipitate for the presence of alkaloids.

Wagner's test: Fractions were treated with Wagner's reagent (Solution of iodine in potassium iodide) and was analyzed for reddish brown precipitate for the presence of alkaloids.

Hager's test: Fractions were treated with Hager's reagent [saturated solution of Picric acid] and was analyzed for yellow precipitate for the presence of alkaloids.

Tannic acid test: Fractions were treated with 10% Tannic acid solution and was analysed for buff colored precipitate for the presence of alkaloids.

Test for Glycosides [14]

Chemical tests for Specific Glycoside

Test for Saponin Glycosides

Foam Test: Placed 1mL solution of drug in water in a test tube shaken well and noted for the stable foam.

Test for Anthraquinone Glycosides

Borntrager's test: Fractions were boiled in 1mL of dilute sulphuric acid for 5min (anthracene glycosides are hydrolyze to aglycone and sugars by boiling with acids) centrifuged or filtered while hot (if centrifuged hot, the plant material can be removed while anthracene aglycones are still sufficiently soluble in hot water, they are however insoluble in cold water), pipetted out the supernatant or filtrate, cooled and shake with an equal volume of dichloromethane (the aglycones dissolve preferably in dichloromethane) separated the lower dichloromethane layer and shaken with half its volume with dilute ammonia. A rose pink to red color is produced in the ammonical layer (aglycones based on anthraquinones give red color in the presence of alkali) if anthraquinone is present.

Modified Borntrager's test: Fractions were boiled with 2 mL of dilute sulphuric acid, 2 mL of 5% aqueous ferric chloride solution for 5 min and continued the test as above. As some plant contains anthracene aglycone in a reduced form, if ferric chloride is used during the extraction, oxidation to anthraquinones takes place, which shows response to the Borntrager's test if anthraquinone is present.

Test for Cardiac Glycosides

Kedde's test: To the fraction, added one drop of 90% alcohol and 2 drops of 2% 3,5-dinitro benzoic acid (3,5-dinitro benzene carboxylic acid-Kedde's reagent) in 90% alcohol. Make alkaline with 20% sodium hydroxide solution. If purple color is produced it shows presence of cardiac glycosides.

Keller-killiani test [test for Deoxy sugars]: Fractions were added with 0.4mL of glacial acetic acid and small amount of ferric chloride. Followed by the addition of 0.5mL of concentrated sulphuric acid by the sides of the test tube, and analysed for the blue color in the acetic acid layer for the presence of cardiac glycosides.

Raymond's test: To the fractions, added a few ml of 50% ethanol and 0.1 ml of 1 % solution of m- dinitrobenzene in ethanol. To this solution, added 2-3 drops of 20% sodium hydroxide solution. Violet colour appeared, indicated presence of cardiac glycosides.

Legal test: To the fractions, added few ml of pyridine and 2drops of nitroprusside and a drop of 20% sodium hydroxide solution. Production of deep red colour indicated the presence of cardiac glycosides.

Test for Flavonoids

Shinoda test (Magnesium Hydrochloride reduction test): Few fragments of magnesium ribbon and conc. hydrochloric acid drop were added to the fractions and were observed for the appearance of pink scarlet, crimson red or occasionally green to blue color on standing which indicated presence of flavonoids.

Alkaline reagent test: Fractions were added with few drops of sodium hydroxide solution which lead to the formation of an intense yellow color and turns colorless on addition of few drops of dil. acid, indicating the presence of flavonoids

Test for Tannins & Phenolic Compounds [15,16]

Ferric chloride test: Fractions were analyzed for blue green color with ferric chloride for the presence of phenols. Vanillin Hydrochloride test: Fractions were analyzed for the purple color which forms after the addition of vanillin hydrochloride in the presence of phenolics and tannins Fractions when treated with heavy metals, tannins precipitate out.

Gelatin test: To fractions, aqueous solution of gelatin and sodium chloride were added. White buff coloured precipitates were formed for positive inference.

Match stick test (Catechin test): A match stick was dipped in aqueous plant fractions, dried near burner and moistened with concentrated hydrochloric acid. On warming near flame, the matchstick wood turned pink or red due to formation of phloroglucinol.

Test for Proteins & Amino Acids [15,16]

Millons test: Fractions were treated with 2mL of Millons reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), gave white precipitate which turns red upon gentle heating indicating the presence of proteins and amino acids.

Ninhydrin test: Fractions were boiled with 0.2% solution of Ninhydrin (Indane 1,2,3 trione hydrate) and formation of violet color indicated presence of proteins and amino acids.

Test for Sterols & Triterpenoids [17]

Liebermann-Burchard test: Fractions were treated with few drops of acetic anhydride and then boiled and cooled; concentrated sulphuric acid was then added from the sides of the test tube, which showed brown ring at the junction of two layers. If upper layer turned green that indicated the presence of Steroids and if deep red color then that indicated the presence of triterpenoids.

Test for Carbohydrates [18]

Molisch's test: Fractions were treated with few drops of alcoholic alpha naphthol and 0.2 mL of conc. sulfuric acid slowly through the sides of the test tube.Formation of purple to violet color ring at the junction indicated the presence of carbohydrates.

Benedict's test: Fractions were boiled with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) on water bath, formation of reddish brown precipitate indicated presence of reducing sugars.

Barfoed's test: This test is for presence of Monosaccharide. Test tube containing fraction and 1mL of barfoed reagent was heated in a beaker of boiling water. Formation of red cuprous oxide indicated presence of monosaccharides.

Fehling's test: Into the few drops of fractions, equal volume of Fehling's A (Copper sulfate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents were added, this mixture on boiling leads to the formation of brick red precipitate of cuprous oxide indicating about the reducing sugars.

RESULT

Preliminary Phytochemical Screening

n-butanol extract was subjected for preliminary phytochemical screening to access the presence of different plant secondary metabolites using qualitative tests summarized in table 1.

Table 1: Preliminary Phytochemical Screening

Sr. No.	Chemical tests	Result
1	**Alkaloids**	
	Hager's reagent	+
	Wagner's reagent	+
	Mayer's reagent	+
2	**Carbohydrates and Glycosides**	
	Molisch's reagent	+
	Fehling solution	+
	Benedict's reagent	+
	Iodine test	+
	Libermann-Burchard's test	+
	Legal's test	+
3	**Fixed oils and fats**	
	Spot test	-
	Saponification test	-
4	**Saponins**	
	Foam test	-
5	**Tannins**	
	Ferric chloride solution	+
	Lead acetate solution	+
6	**Proteins**	
	Millon's reagent	-
	Ninhydrin reagent	-

Phytochemical Study

Plant materials were defatted using petroleum ether (PE) and further extracted with ethanol. The ethanolic extracts of all the plants were fractioned into four different fractions using successive solvents chloroform, ethyl acetate and n- butanol. The color, consistency and yield of all the fractions obtained are given in Table 2.

Table 2: Details of Color, Consistency and Yield of Plant Fractions

Sr. No.	Extract	Color	Consistency	Percentage Yield
1	Petroleum ether	Yellowish	Waxy	25%w/w
2	Chloroform	Reddish	Hard	14%w/w
3	Ethyl Acetate	Greenish	Sticky	13.5%w/w
4	n-butanol	Colourless	Loose	15.4%w/w

CONCLUSION

The preliminary phytochemical screening showed various aspects in the extract. Alkaloids, Carbohydrates & Tannins were present whereas Saponins & Proteins were absent.

CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

ACKNOWLEDGEMENTS

The authors are thankful to the Management and Principal, Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Maharashtra, India for providing necessary facilities for research work.

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