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Emblica officinalis seed coat: Extraction and their preliminary phytochemical analysis

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Abstract

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in amla and subsequently may lead to drug discovery and development. In the present study, chief phytoconstituents of the amla were identified to relate their presence with the bioactivities of the plants. The preliminary phytochemical of amla leaf and bark were analyzed by researchers and its efficacy of amla fruit is widely proved. This paper also shows the presence of phytochemical series of compounds effectively.

1. Introduction

Emblica officinals (Amla) belong to the plant family Euphorbiaceae. It is a good dietary source of vitamin C, minerals, and amino acids (Haung *et al*., 2001). Vitamin C is found to be highly stable due to the presence of tannins and polyphenols. This fruit can be used as a major constituent in Ayurveda preparations (Bansal *et al*., 2014). The plant leaves have anti-platelet, anti-neurophilic, anti-viral, anti-mutagenic, anti-allergic, and antibacterial activities. Amla is widely used for the treatment of diarrhea, inflammatory disease, jaundice and acts as a glucose-lowering agent in Type II diabetes. Thus all parts of plants including fruit, seed, seed coat, leaves, roots, bark, and flowers are used in herbal preparation (Mishra *et al*., 2011). Leaves of amla are also used as an anti-inflammatory and antipyretic activity, more common in the Asian population. The nutritional benefits of amla can be used as a beverage, candy powder, sauce, etc. The present study was to extract the biologically active compounds in different solvents from seeds of amla and check its antibacterial activity.

2. Geographical distribution

E. officinalis is widely distributed in most tropical and subtropical countries including India, China, Indonesia, Burma, and the Malay Peninsula. It is native to tropical southeastern Asia, particularly

in central and southern India, Nepal, Pakistan, Bangladesh, Bhutan, Srilanka, and the Mascarene Islands. It is abundant in the deciduous forests of Madhya Pradesh (Rajeshkumar *et al*., 2001). It additionally grows in Pakistan, Sri Lanka, Uzbekistan, Malaysia, Ceylon, Indonesia, and some other countries over the world. Amla is found throughout the seacoast districts and on hill slopes up to 200 meters and is also cultivated in plains. It is a potential crop that grows in the marginal soils and various kinds of degraded lands such as salt-affected soils, salines, and dry and semi-dry regions. With orchard cultivation, about 200 trees can also be accommodated per acre (Scartezzini *et al*., 2006).

3. Experimental

E. officinalis seed coat was purchased from the local market of Ujjain (Madhya Pradesh). The purchased raw material was rinsed thoroughly and dried under the shed. This dried material was mechanically powdered and stored in an airtight container for further phytochemical and physiochemical analysis (Poonam *et al.*, 2009).

4. Methods of extraction

4.1. Serial Exhaustive Extraction

It is another common method of extraction that involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compounds could be extracted. Some researchers employ soxhlet extraction of dried plant material using an organic solvent. This method cannot be used for thermolabile compounds as prolonged heating may lead to the degradation of compounds (Das *et al*., 2010).

4.2. Soxhlet Extraction

Soxhlet extraction is only required where the desired compound has limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermo-labile compounds as prolonged heating may lead to the degradation of compounds (Nikhal *et al*., 2010).

4.3. Maceration

In maceration (for fluid extract), whole or coarsely powdered plant- the drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until the soluble matter is dissolved. This method is best suitable for use in case of the thermo-labile drugs (Ncube *et al*., 2008).

4.4. Decoction

This method is used for the extraction of the water-soluble and heats stable constituents from the crude drug by boiling it in water for 15 minutes, cooling, straining, and passing sufficient cold water through the drug to produce the required volume (Remington *et al*., 2006).

4.5. Infusion

It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period with either cold or boiling water (Remington *et al*., 2006)

4.6. Digestion

This is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when the moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is increased thereby (Remington *et al*., 2006).

4.7. Percolation

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstrum and allowed to stand for approximately 4 h in a well-closed container, after which the mass is packed and the top of the percolator is closed. Additional menstrum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstrum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstrum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting (Handa *et al*., 2008).

4.8. Sonication

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of a root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through the formation of free radicals and consequently undesirable changes in the drug molecules (Handa *et al*., 2008).

4.9 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) uses supercritical fluid (SF) as the extraction solvent. SF has similar solubility to liquid and similar diffusivity to gas and can dissolve a wide variety of natural products. Their solvating properties dramatically changed near their critical points due to small pressure and temperature changes. Supercritical carbon dioxide (S-CO_2) was widely used in SFE because of its attractive merits such as low critical temperature (31 °C), selectivity, inertness, low cost, non-toxicity, and capability to extract thermally labile compounds. The low polarity of S-CO_2 makes it ideal for the extraction of non-polar natural products such as lipid and volatile oil. A modifier may be added to S-CO_2 to enhance its solvating properties significantly (Conde *et al*., 2017).

4.10 Ultrasound-assisted extraction (UAE)

Ultrasonic-assisted extraction (UAE), also called ultrasonic extraction or sonication, uses ultrasonic wave energy in the extraction. Ultrasound in the solvent producing cavitation accelerates the dissolution and diffusion of the solute as well as the heat transfer, which improves the extraction efficiency. The other advantage of UAE includes low solvent and energy consumption, and the

reduction of extraction temperature and time. UAE is applicable for the extraction of thermolabile and unstable compounds. UAE is commonly employed in the extraction of many types of natural products (Falcao *et al*., 2017; Barba *et al*., 2016)

4.11 Microwave-assisted extraction (MAE)

Microwaves generate heat by interacting with polar compounds such as water and some organic components in the plant matrix following the ionic conduction and dipole rotation mechanisms. The transfers of heat and mass are in the same direction in MAE, which generates a synergistic effect to accelerate extraction and improve extraction yield. The application of MAE provides many advantages, such as increasing the extract yield, decreasing the thermal degradation, and selective heating of vegetal material. MAE is also regarded as a green technology because it reduces the usage of organic solvent. There are two types of MAE methods: solvent-free extraction (usually for volatile compounds) and solvent extraction (usually for non-volatile compounds) (Chemat *et al*., 2012; Vinatoru *et al*., 2017).

5. Qualitative and quantitative analysis of phytochemicals

5.1. Preliminary Qualitative Analysis

Screening of the *E. officinalis* seed coat for various phytochemical constituents was carried out using standard methods (Banu *et al*., 2015; Yadav *et al*., 2011).

1. Test for Alkaloids (Hager's Test)

a. Mayer's test

To a few ml of plant sample extract, two drops of Mayer's reagent (1.36 gram of mercuric chloride + 5 gram of potassium iodide in 100 ml of distilled water) are added along the sides of the test tube. The appearance of a white creamy precipitate indicates the presence of alkaloids.

b. Wagner's test

A few drops of Wagner's reagent (2.5 gram of iodine is dissolved in 12.5 gram of potassium iodide in 250 ml distilled water) is added to a few ml of plant extract along the sides of the test tube. A reddish-brown precipitate confirms the test as positive

2. Test for Amino acids

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to test for Amino acids.

a. Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. The appearance of purple color indicates the presence of amino acids.

3. Test for Carbohydrates (Molisch's Test)

a. Molish' s test

To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid are added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

b. Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated in a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.

4. Test for Fixed oils and Fats

a. Spot test

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

b. Saponification test

A few drops of 0.5 N alcoholic potassium hydroxide solution are added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated in a water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

5. Test for Glycosides

For 50 mg of the extract is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests.

a. Borntrager's test

To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. The pink color indicates the presence of glycosides.

b. Legal's test

50 mg of the extract is dissolved in pyridine; sodium nitroprusside solution is added and made alkaline using 10% NaOH. The presence of glycoside is indicated by pink color.

6. Test for Phenolic compounds and Tannins (Braymer's Test)

a. Ferric Chloride test

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5%, ferric chloride solution are added. The dark green color indicates the presence of a phenolic compound.

b. Gelatin test

The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

c. Lead acetate test

The extract (50 mg) is dissolved in distilled water and to this 3 ml of 10% lead, acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

d. Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

e. Magnesium and Hydrochloric acid reduction

The extract (50 mg) is dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (dropwise) are added. If any pink to crimson color develops, the presence of flavonols glucoside is inferred.

7. Test for phytosterols

a. Libermann-Burchard's test

The extract (50 mg) is dissolved in 2 ml acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of color changes shows the presence of phytosterols.

8. Test for Flavonoids

The extract (50 mg) is added 1ml of (l0%) of lead acetate solution. The presence of yellow-colored precipitate confirms the presence of flavonoids.

9. Test for Terpenoids

50mg extract is mixed with 2ml $(CH_3CO)_2O$ and add 2-3 drops conc. H_2SO_4. The deep red coloration is indicated the presence of terpenoids.

10. Test for Coumarins

10% sodium hydroxide solution is added to 50 mg of extract. The presence of yellow precipitate confirms the coumarins.

11. Test for Steroids (Salkowski Test)

A fixed amount of extract is taken; add 2ml of chloroform, and then 2ml H_2SO_4 added dropwise. It appeared a reddish-brown ring at the junction confirms the presence of steroids.

6. Results and Discussion

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, antimalarial, anti-cholinergic, anti-leprosy activities, etc. Amla seeds coat contain a lot of nutritive and anti-nutritive compounds. Saponins, tannins, flavonoids ascorbic acid, cardiac glycosides, and terpenoids were found to be higher in ethanolic extract than other solvent extract. The alkaloids, flavonoids, saponins, carbohydrates were present in the seed coat extract in polar solvent more efficiently than other solvents. The less amount of biologically active compound was extracted by other solvents also (Table-1).

Table-1 Results of phytochemical analyses of *E. officinalis* seed coat

Phyto chemical tests	Hexane Extract	Benzene Extract	Acetone Extract	Butanol Extract	EtOH Extract	Aq. Extract	Ethyl acetate Extract	Chloroform Extract	MeOH Extract
Alkaloids	-	-	+	+	+	+	+	+	+
Flavonoids	-	+	+	+	+	+	+	+	+
Phenols	-	-	-	-	+	+	-	+	+
Tannins	-	-	+	-	+	+	-	-	+
Quinones	-	-	+	+	-	-	+	+	-
Saponins	-	+	+	+	+	+	-	-	+
Amino acid	-	-	-	-	+	+	-	-	+
Carbohydrate	+	+	+	+	-	-	+	+	+
Sterols	-	+	-	+	-	-	-	-	-
Glycosides	-	-	-	-	+	-	-	-	-
Steroids	+	+	-	-	-	-	-	-	-
Terpenoids	-	-	+	+	+	-	+	+	+
Coumarins	-	-	+	-	+	+	-	-	+

Conclusion

The present study shows that the seed coat of Indian gooseberry contains phytochemicals. The bioactive compounds were extracted through different solvents. The polyphenols and ascorbic acids are lead to medicinal and antioxidant activity. This therapeutic nature of seed coat also supports the treatment of diseases and the discovery of new drugs.

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