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ORIGINAL RESEARCH

Physico-chemical characterization and Phytochemical Analysis of Leaves of Rejuvnative Herb, *Emblica officinalis*

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Abstract: Towards authentication and quality assurance of medicinal herb, physicochemical and preliminary phytochemical studies of *Emblica officinalis* leaves were carried out. Physicochemical analysis revealed values for moisture contents, total ash, water soluble ash, acid insoluble ash, and water and alcohol extractives. Preliminary phytochemical screening revealed the presence of phytoconstituents viz., alkaloids, phenols, saponins, carbohydrates, proteins, in aqueous and hydroalcoholic extracts of *Emblica officinalis* leaves whereas flavonoids was present only in hydroalcoholic extract. Results showed that hydroalcoholic extract contain much more chemical constituents compare to aqueous extract. It was suggested that hydroalcoholic extract is the best solvent for extraction. Total ash (3.1%) was low implying that the plant leaves have low inorganic components. Water soluble ash and acid insoluble ash were found to be 1.7% and 1.3% respectively and water-soluble extractive value was low 28.8% compared to alcohol soluble extractive value 59.2%. Information obtained from present studies can be used in laying down identification and standardization of this plant as herbal remedy and also towards monograph development.

Keywords: Aqueous extract; Emblica officinalis; Physico-chemical; Phytochemical screening; Hydroalcoholic extract

1. Introduction

Mother nature has gifted many medicinal plants to encourage healthy life. Numerous medicinal plants are used for herbal preparations in Ayurveda. Phyllanthus emblica Linn. Or *Emblica officinalis* Gaertn. (Amla), commonly known as Indian gooseberry belongs to the family Phyllanthaceae widely distributed in tropical and subtropical area of India, China, Malaysia, Indonesia (Liu et al., 2008). Amla is an important horticulture crop of arid zone. Owing to its multiple health benefits, it may be called as "King of Rasayana" [rejuvenation]. All parts of the plant are used in various Ayurvedic herbal preparations, including the fruit, seed, leaves, root, bark and flower. The medicinal preparation from amla, have high potency in curing many diseases and promotion of health (Rajeshkumar et al., 2001) and used widely in combination with other two fruit-bearing plant species, chebulic and belleric as triphala. It is key ingredient in the Chyavanaprasha (ayurvedic recipe) (Singh et al., 2011). In India, fruits of *Emblica officinalis* are the most common ingredients of almost all Ayurvedic preparations like Lehya, Choorna etc. (Raghu et al., 2010). It contains polyphenols, flavonoids, tannins, pectin, gallic acid, emblicanin, punigluconin and other bioactive compounds (Zhang et al., 2003), (Prashanth et al., 2014). It has many therapeutic applications such as hepatoprotective, Immunostimulants, antimicrobial, antioxidants, anticancer, antipyretics, cytoprotective, analgesic, antitussive, anti-inflammatory, gastroprotective and used in eye disorder (Khan et al., 2009). It is found to be effective for the reversal of dyslipidemia and intima-media thickening and plaque formation in the aorta in hypercholesterolaemic rabbits (Antony et al., 2006). Antioxidants present in its juice in the form of polyphe-

nols and vitamin C help in maintaining healthy cholesterol level thus provide a cardio-protective effect (Pathak et al., 2003). The antibacterial activity of aqueous crude extracts of *Emblica officinalis* against human bacterial pathogens was reported (Rawat et al., 2015). Many chemical constituents present in its different parts like leaves-contain chebulic acid, gallic acid, chebulinic acid, ellagic acid, amlic acid, phyllantin, alkanoids, seeds-contain A phosphalides, fixed oil and a small quantity of essential oil barks-contain proanthocyanin, leukodelphinidin and tannin and roots-contain luprol and ellegic acid (Khan et al., 2009). A variety of phytochemicals such as tannins, flavonoids, terpenoids and alkaloids were reported to have several pharmacological properties such as antioxidant, anticancer, antitumor, antigenotoxic and anticarcinogenic effects. It was considered to be a safe herbal medicine without any adverse effects. Hence, the present study deals with the screening of phytochemical constituents present in the aqueous and hydroalcoholic extracts of *Emblica officinalis* leaves and their physicochemical characterization.

2. Materials and Methods

2.1. Plant material

The leaves of plant were collected from local area of Satna district and identification of the plant sample was done. The shade dried leaves were cut into small pieces and made into coarsely powder using mechanical grinder and preserved in air tight container (Harborne et al., 2007).

2.2. Plant Extracts Preparation

For preliminary phytochemical screening dried and coarsely powered leaves (50 g/250 mL) was extracted with solvents (water and hydroalcoholic) in a Soxhlet extractor by continuous hot percolation process. The extracts were evaporated for dryness in water bath and used for phytochemical determination (Harborne et al., 2007).

2.3. Phytochemical Screening

The extracts of *Emblica officinalis* leaves were analyzed for the presence of various phytoconstituents by procedure given in (Mukherjee et al., 2007), (Khandelwal et al., 2005), (Kokate, 1994).

2.4. Test for Alkaloids

Plant extracts were dissolved individually in dilute hydrochloric acid (dil. HCl) and filtered. Mayer's test: In this test procedure, filtrates were treated with Mayer's reagent (Potassium mercuric iodide) after few minutes yellow colored precipitate appeared in test tube indicates the presence of alkaloids in plant sample. Hager's test: In Hager's test, extract was treated with Hager's reagent (saturated picric acid solution) and presence of the alkaloids in sample confirmed by the formation of yellow colored precipitate in test tube.

2.5. Test for Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtrated. The filtrates were used to test for the presence of carbohydrates. In Fehling test, firstly plant extract was taken in test tube and hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solution, red color precipitate appeared in test tube indicates the presence of reducing sugar in plant sample.

2.6. Test for Saponins

For determination of saponins in plant sample, extracts were diluted with distilled water up to 20 ml in test tube and was shaken in a graduated cylinder for 15 minutes after some time 1 cm layer of foam form in test tube, indicates the presence of saponins in plant sample. In another foam test, 0.5gm of plant extract was shaken with 2 ml of distilled water. If foam produced and persists for 10 minutes, it indicates the presence of saponins in plant sample.

2.7. Test for Phenols

In this test, plant extracts were treated with 3 to 4 drops of ferric chloride solution; bluish black color form in the test tube confirmed the presence of phenols.

2.8. Test for Flavonoids

In alkaline reagent test, firstly, take 2 ml of test solution in test tube, add few drops of sodium hydroxide solution, intense yellow color was formed which turns to colorless on addition of few drops of dilute acid indicates presence of flavonoids. In lead acetate test, firstly, take 2 ml of plant extract solution subsequently, add few drops of lead acetate solution in test tube, yellow colour precipitate appeared in test tube indicates the presence of flavonoids in plant sample.

2.9. Test for Proteins

Xanthoproteic test: In this test procedure, 5 ml of test solution taken in test tube, and add 1 ml of concentrated nitric acid and boil, yellow color precipitate was formed in test tube. After cooling this test solution, add 40% sodium hydroxide solution, orange color precipitate formed confirmed presence of protein in plant sample.

2.10. Physico-Chemical Analysis

Physico-chemical analysis of plant sample was carried out according to the standard method of Indian Pharmacopoeia, (2010) and Tripathi and Sikarwar (2014).

2.11. Determination of Moisture (Loss of Drying)

Two gram of powdered plant material was weighted and dried in an oven at (100ºC) until two consecutive weighing do not differ by more than 2gm then, loss of weight of plant sample calculated in terms of percentage.

2.12. Ash value

About three gram of powdered plant sample was weighed into a tarred silica crucible and incinerated at 450°C in a muffle furnace until free from carbon. Subsequently, the crucible was cooled and weighed and percentage of total ash was calculated with compared to air dried sample.

2.13. Water Soluble Ash

1.5 gm ash obtained from the total ash was boiled with 25 ml of distilled water for (8 minutes) and filtered through an ash less filter paper and wash two times with hot water. The filter paper was transferred into a tarred silica crucible, incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed and percentage of water-soluble ash calculated with reference to air-dried substance.

2.14. Acid Insoluble Ash

1.5 gm ash obtained from the total ash was boiled with 25 ml of 2N HCl (for 8 minutes). Subsequently, filter through an ash less filter paper and wash filter paper two times with hot water. The filter paper was transferred into a tarred silica crucible, incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed and percentage of acid insoluble ash was calculated with reference to air-dried substance.

2.15. Determination of Alcohol and Water-Soluble Extractive

About 5 g of air-dried plant material was macerate with 100 ml of the solvents (ethanol and water) for 6 hours, shake frequently then allowed to stand for 18 hours in room temperature. Then, filtered rapidly and transferred 25 ml of the filtrate to a tarred flat-bottomed petri-dish and evaporate to dry on a water bath at 100ºC for 6 hours, cool in desiccators for 30 minutes and weighed and calculated as milligram per ml of extractive values in terms of percentage.

3. Results and Discussion

Medicinal plants are potential renewable natural resources and are generally considered to play a beneficial role in human health care. The medicinal value of this plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenols. The results of present study showed that *Emblica officinalis* is rich in phytochemical and physicochemical contents as highlighted in table 1 and 2. Multifunctional activities of the plants against various diseases may be attributed due to the presence of various chemical constituents in the plant. Preliminary phytochemical revealed the presence of phytoconstituents viz., alkaloids, phenols, saponins, carbohydrates, proteins in aqueous and hydroalcoholic extracts of leaves of *Emblica officinalis* whereas flavonoids was present only in hydroalcoholic extract. Similar observations were made by (Khopde et al., 2001) and (Patil et al., 2013). The presence of flavonoids in *Emblica officinalis* is likely to be responsible for the free radical scavenging effects. The plant also contains phenolics which are powerful antioxidants. The presence of saponins protects plant from microbial pathogen. It showed that hydroalcoholic extract contain much more phytochemical constituents than aqueous extract of leaves of this plant. Another study of Prashant, (2014) also revealed the presence of phytochemicals like flavonoid, alkaloids and phenols in Emblica officinalis leaves extract.

The determination of physicochemical parameter is an important in determination of adulterants and improper handling of drugs. The percentage of alcohol soluble extractive value and water-soluble extractive value were also determined and results are depicted in Table 2. The LOD results 10%, was an indication that the extract was least hygroscopic and the plant can be stored for a long period of time with less probability of microbial attack. Total ash of 3.1 % was low implying that the crude plant has low inorganic components. Water soluble ash and acid insoluble ash found to be 1.7% and 1.3% respectively. Acid insoluble ash is less than the water-soluble ash value. Water soluble extractive value is less 28.8% compared to alcohol soluble extractive value of 59.2% which shows that aqueous solvent not permeates the cells of leaves of *Emblica officinalis*. The results of percentage extractive yield for *Emblica officinalis* indicate that plant sample was highly soluble in alcohol than water solvents.

Table 1. Qualitative Phytochemical Screening of leaves extracts of *Emblica officinalis*

Chemical	Test/ Reagent	Results	
		Aqueous extract	Hydroalcoholic extract
Alkaloids	Hager's Test	+ ve	+ ve
	Wagner's Test	+ ve	+ ve
Carbohydrate	Fehling's Test	+ ve	+ ve
Phenols	Fecl3 Test	- ve	+ ve
Flavonoids	Alkaline Test	- ve	+ ve
	Lead Acetate Test	- ve	+ ve
Saponins	Foam Test	+ ve	+ ve
Proteins	Xanthoproteic	+ ve	+ ve
Diterpenes	Copper Acetate Test	- ve	- ve

(+) Ve: Indicates the presence of phytochemicals, **(-) Ve:** Indicates the absence of phytochemicals

4. CONCLUSION

For healthy look and fitness, present generation is using supplementary vitamin pills to sustain and boost their system according to medical consultation, they could even prove to be fatal for life if they don't choose the natures products. Despite various available allopathic formulations,

Table 2. Physico-Chemical characteristics of leaves of *Emblica officinalis*

Parameter	Results (%) W/W
Ash Value	
Total Ash	3.1%
Acid Insoluble Ash Value	1.3%
Water Soluble Ash Value	1.7%
Extractive Value	
Ethanol Soluble Extractive Value	59.2%
Water Soluble Extractive Value	28.8%
Loss on Drying	10%

the plant-based compounds are beneficial for various diseases, the present study was undertaken to reveal the physico-chemical and phytochemical characterization which could subsequently be exploited as cost-effective measures for health of well-being.

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6. CONFLICT OF INTEREST

The author declares no conflict of interest.

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