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PHYTOCHEMICAL SCREENING OF *AEGLE MARMELOS* (L.) CORREA FRUIT PULP: A POTENTIAL SOURCE OF ETHNOMEDICINE

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ABSTRACT

Medicinal plants have played a significant role in ancient traditional systems of medication in many countries. They are rich source of bioactive compounds and thus serve as important raw materials for drug production. *Aegle marmelos* is known for various medicinal properties in traditional medicinal system and use to cure a variety of diseases. In last few decades, *Aegle marmelos* is extensively studied for its medicinal properties by advanced scientific techniques and a variety of bioactive compounds have been isolated from the different parts of the plant and were analysed pharmacologically. In our present investigation phytochemical analysis of fruit pulp of *Aegle marmelos* has been evaluated for the presence of bioactive compounds using various polarity solvents including petroleum ether, chloroform, benzene, ethanol and water. The study revealed the presence of alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides and tannins. The results suggest that *Aegle marmelos* has promising therapeutic potential and can be used as a base for the development of novel potent drug in ethnomedicine.

Key words: Medicinal plants, *Aegle marmelos*, Phytochemical Screening, Bioactive Compounds, Ethnomedicine.

INTRODUCTION

Knowledge of herbs has been handed down from generation to generation for thousands of years [1]. Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need and are easily accessible and inexpensive [2]. There is a widespread belief that the green medicines are healthier and more harmless or safer than synthetic ones [3]. Plant derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

Aegle marmelos (L.) Correa, commonly known as bael (or bel), belonging to the family Rutaceae, is a moderate-sized, slender and aromatic tree. It is indigenous to India and is abundantly found in the Himalayan tract, Bengal, Central and South India. This plant has long been used as an ancient and modern traditional medicine to relieve dysentery, cholera, constipation [4] and diabetes mellitus [5]. *Aegle marmelos* has several pharmacological activities such as anti-inflammatory, antipyretic, analgesic [6], antioxidant [7], and antidiabetes [8]. All the parts of this tree including stem, bark, root, leaves and fruit at all stages of maturity have medicinal virtues and have been used in the Indian traditional medicines from time immemorial. Chemical investigation on the different parts of the plant has resulted in the isolation of a large number of novel and interesting metabolites.

The medicinal value of the plants lies in some active chemical substances called phytochemicals that produce a definite physiological action on the human body. Phytochemicals are divided into two groups, which are primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, aminoacids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, flavonoids, tannins, phenolic compounds [9]. It is necessary to focus and develop these compounds to be more effective drugs. In view of its medicinal value, the present study is aimed to screen the pharmaceutically important bioactive substances from *Aegle marmelos* fruit pulp that greatly contribute the ethnomedicinal properties.

MATERIALS AND METHODS

Collection of plant material

The fruit pulp of *Aegle marmelos* were collected from Herbal Garden, Sree Narayana Guru College and identified by Dr.S.N.Suresh, Head, Department of Biotechnology, Sree Narayana Guru College, Coimbatore and the specimen was later shade dried, powdered and stored in an air-tight container for further use. The powdered material was used for pharmacological investigation, while for phytochemical screening the powder was extracted with different solvents in their increasing order of polarity such as petroleum ether, chloroform, benzene, ethanol and water on orbital shaker. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator and stored at 4ºC. The crude extracts were collected in amber coloured sample bottles and stored. All chemicals and reagents used including the solvents were of analytical grade.

Pharmacological Studies

Physicochemical parameters

Ash Values

The determination of various physicochemical parameters such total ash, water-soluble ash, alkalinity of water soluble and acid insoluble ash values of the powdered material was determined as per the Indian Pharmacopoeia [10].

Extractive Values

Extract of the powdered fruit pulp were prepared with different solvents for the study of extractive value [11].

Fluorescence Analysis

A small quantity of dried and finely powdered fruit pulp was placed on a clean grease free microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed gently by tilting the slide and waited for 1-2 minutes. Then the slide was viewed in day light and (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded [12].

Phytochemical Screening

Phytochemical screening of all extracts were carried out by following standard procedures [13, 14, 15].

Test for alkaloids

Dragendroff's test

To 5 ml of the extract few drops of Dragendroff's reagent was added for the formation of orange coloured precipitate.

Mayer's test

To 5 ml of the extract few drops of Mayer's reagent was added for the formation of cream coloured precipitate.

Wagner's test

To 5 ml of the extract few drops of Wagner's reagent was added for the formation of reddish brown coloured precipitate.

Hager's test

To 3 ml of the extract few drops of Hager's reagent was added for the formation of prominent yellow precipitate.

Test for flavonoids

To 3 ml of the extract few magnesium ribbons are dipped and conc. HCl was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoids.

Test for proteins

Biuret test

To 3 ml of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

Millon's test

To 3 ml of the extract few drops of Millon's reagent was added for the formation of red colour.

Test for carbohydrates

Molisch's test

To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to

stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

Fehling's test

The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath. The formation of yellow or red colour precipitate indicates the presence of reducing sugar.

Test for tannins

A fraction of the extract was dissolved in water and then it was subjected to water bath at 37^0 C for 1 h and treated with ferric chloride solution and observed for the formation of dark green colour.

Test for sterols

Liebermann-Burchard test

To a small amount of the extract few drops of chloroform, acetic anhydride and H_2SO_4 was added along the sides of the test tube to observe the formation of dark red or pink colour.

Test for glycosides

Baljet's Test

To 5 ml of the extract few drops of sodium picrate was added to observe yellow to orange colour.

Keller-Killiani test

To 5 ml of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides.

Test for phenols

Ferric chloride test

A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

Test for saponins

Foam test

To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam was observed.

Test for terpenoids

Chloroform test

To 5 ml of the extract few drops of chloroform and conc. H_2SO_4 was added carefully along the sides of the test tube to form a layer and observed for the presence of reddish brown colour.

RESULTS AND DISCUSSION

Ash value

The powdered material was evaluated for its physico-chemical parameters like Ash values, Water soluble ash, Acid Insoluble ash and the results are shown in Table 1.

Table 1. Physico-chemical studies of *Aegle marmelos* fruit pulp.

Types of Ash value	Observation (% w/w)
Total ash	6.32
Water soluble ash	1.21
Acid insoluble ash	2.41

Extractive values

Extractive values of the successive extracts of *Aegle marmelos* fruit pulp are shown in Table 2.

Table 2. Percentage of successive extracts of *Aegle marmelos* fruit pulp.

Solvents	Extract values (% w/w)
Petroleum ether	2.3
Chloroform	5.24
Acetone	6.34
Ethanol	8.3
Water	9.7

Fluorescence Analysis

The powder was subjected to fluorescence analysis as per the standard procedure and the results are shown in Table 3.

Table 3. Fluorescence analysis of *Aegle marmelos* fruit pulp.

Plant sample	Day light	UV light (365nm)
Powder	Green	Dark green
Powder + Distilled water	Light green	Black
Powder + NaOH	Dark red	Brown
Powder + H_2SO_4	Magenta	Red
Powder + HCl	Red	Greenish black
Powder + HNO_3	Light brown	Dark violet
Powder + Ammonia	Light green	Greenish yellow
Powder + $CHCl_3$	Orange	Dark green
Powder + $FeCl_3$	Green	Greenish brown

Phytochemical Screening

Powdered fruit pulp of *Aegle marmelos* were subjected to various qualitative tests for the identification of phytochemical constituents includes tests for alkaloids (Dragendroff's test, Mayer's test, Hager's test, Wagner's test), saponins, glycosides (Baljet's test, Kellar-Killiani test), carbohydrates (Molisch's test, Fehling's test), proteins (Biuret test, Xanthoprotein test, Millon's test), tests for tannins, flavonoids, steroids (Liebermann-burchard test), phenols, terpenoids were performed using specific reagents. Preliminary phytochemical screening of *Aegle marmelos* fruit pulp revealed the presence of bioactive compounds such as alkaloids, tannins, phenols, terpenoids, flavonoids, glycosides, carbohydrates and saponins in different extracts (Table 4).

Table 4. Phytochemical analysis of extracts *Aegle marmelos* fruit pulp.

Phytoconstituents	Petroleum ether	Chloroform	Benzene	Ethanol	Water
Alkaloids	-	-	+	+	+
Flavonoids	+	-	+	+	+
Proteins	-	+	+	+	+
Carbohydrates	+	+	-	+	+
Tannins	+	+	+	+	-
Sterols	+	-	-	-	+
Glycosides	-	+	-	+	+
Phenols	-	-	-	+	+
Saponins	+	-	+	+	-
Terpenoids	-	-	-	+	+

"+" present, "-" absent

CONCLUSION

Plants have been utilized as a natural source of medicinal compounds since thousands of years. A number of traditional herbal medicines are used for the management of various diseases. Historically the plant has been used for the number of ethnomedical purposes. *Aegle marmelos* fruit pulp contains a number of phytoconstituents, which are the key factors in the medicinal value of this plant. As the pharmacologists are looking forward to develop new drugs from natural sources, development of modern drugs from *Aegle marmelos* can be emphasized for the control of various diseases. A systemic research and development work should be undertaken for the development of products for their better economic and therapeutic utilization.

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REFERENCES

1. Bown D. Encyclopedia of Herbs. The Royal Horticulture Society, London; Dorling Kindersley Ltd: 1995.

2. Mukherjee PK. Quality control of herbal drugs. Business Horizon Pharmaceutical Publishers: 2008.
3. Parvath S, Brindha R. Ethnobotanical medicines of Animalai union. Ancient Sci Life, 2003; 22: 14.
4. Manandhar MD, Shoeb A, Kapil RS, Popli SP. New Alkaloid from *Aegle marmelos*. Phytochemistry, 1978; 17: 1814-15.
5. Ohashi K, Watanabe H, Okumura Y, Uji T, Kitagawa I. Indonesian Medicinal Plants. XII. Four isomeric lignan-glucosides from the bark of *Aegle marmelos* (Rutaceae). Chem Pharm Bull, 1994; 42(9): 1924-26.
6. Arul V, Miyazaki S, Dhananjayan R. Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos* Corr. J Ethnopharmacol, 2005; 96(1-2): 159-63.
7. Sabu MC, Kuttan R. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. Indian J Physiol Pharmacol, 2004; 48(1): 81-88.
8. Upadhya S, Shanbhag KK, Suneetha G, Balachandra, Naidu M, Upadhya S. A study of hypoglycemic and antioxidant activity of *Aegle marmelos* in alloxan induced diabetic rats. Indian J Physiol Pharmacol, 2004; 48(4): 476-480.
9. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine - A move towards nature. Biotechnol Mol Biol Rev, 2007; 1(4): 97-104.
10. Anonymous. The wealth of India: A dictionary of Indian raw materials and industrial products. CSIR, New Delhi, 1998; 2: 116-18.
11. Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence of powdered vegetable drugs in ultra-violet radiation. J Am Pharm Assoc, 1958; 47: 715-17.
12. Pratt RT, Chase ER. Fluorescence powder vegetable drugs in particular to development system of identification. J Am Pharm Assoc, 1949; 38: 324-31.
13. Raaman N. Phytochemical Techniques, New India Publishing Agency: 2008.
14. Harborne JB. Phytochemical Methods, Chapman and Hall Ltd., London: 1999.

Trease GE, Evans WC. Textbook of pharmacognosy. 12th edn., Balliere-Tindal, London: 1979; 343.