



Quantitative phytochemical analysis on leaf extract of *Aegle marmelos*

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ABSTRACT

Maha Vilvam is a holly tree commonly found in the Sacred Groves from Kanyakumari to Kashmir in the foot hills and in plains which has many medicinal secrets. *Vilva* trees are the *Sthalavirusham* of the most of the Shiva temples in India. The Botanical Name of the *Vilvam* is *Aegle marmelos*(L.) Corr., which belongs to the Orange family Rutaceae. All the parts of the plant are used as an important medicine. Even though all the parts of the plants are useful, the leaves and fruits are mostly used as important drug in the ancient system of medicine to cure almost all the common ailments of the Human being. In this present study the qualitative phytochemical analysis of *Aegle marmelos* leaf extract was carried out and the results were discussed.

Keywords: *Aegle marmelos*, Phytochemistry, Tuberculosis, Phenols, Flavanoids

INTRODUCTION

Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments [1][2][3]. Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts of different kind of plants. Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives[4] and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries. In the past our ancestors have made new discoveries on the healing power of plants through trial and error. The medicinal plant therapy is based on the empirical findings of hundreds and thousands of years[5].

Aegle marmelos Linn. is commonly called as Bael in Hindi, *Vilvam* in Tamil and *Bilva* in Sanskrit. It belongs to the family Rutaceae. It is indigenous to India and is used in folk medicines. The Ayurvedic practitioners use almost all of their parts but the greatest medicinal value ascribed to its fruits[7]. *Vilvamis* a perennial tree, wild in the sub Himalaya tract, central and South India. *Aegle marmelos* is a medium sized armed deciduous tree grows up to a height of 9-10 meters with straight, sharp, axillary thorns and yellowish brown shallowly furrowed corky bark. The leaves are trifoliolate alternate, leaflets are ovate to lanceolate with pellucid – punctuate aromatic oil glands[8][9]. The lateral leaves are subsessile and the terminal one is long petioled. The flowers are greenish white sweet scented present in the axillary panicles. The fruits are Globus woody berry with yellowish ring, seeds numerous embedded in orange brown sweet gummy pulp. The leaves are used as astringent, laxative, febrifuge and expectorant. The leaves are useful in ophthalmia, inflammations, catarrh, diabetic and asthmatic complaints[10]. The leaves are used for the heart and brain disorders. The confection called *ilakam* is made of fruit is used to treat tuberculosis and loss of

appetite[11]. Stress is produced during normal metabolic process in the body as well as induced by a variety of environmental and chemical factors which cause generation of various reactive free radicals and subsequent damage to macromolecules like DNA, Proteins and Lipids could be cured by Vilvam. No specific scientific evaluation of antioxidant activity of *Aegle marmelos* fruit pulp has been reported so far. Therefore, it was thought worthwhile to evaluate antioxidant activity of *Aegle marmelos* fruit pulp to confirm its folk medicinal claim. Many naturally occurring products have been reported to contain large amount of antioxidant compounds other than vitamin C, E and carotenoid[12]. These antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radical. Antioxidant activity of medicinal plants might be due to the presence of phenolic compounds such as flavonoids, 3,4, Phenolic acids and phenolic diterpene[13]. Synthetic antioxidants like butylated hydroxy anisole (BHA) butylated hydroxy toluene (BHT), tertiary butylated hydroxy quinone and gallic acid esters have been suspected to be carcinogenic. Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants[14]. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity[15]. Hence, search for natural antioxidant has greatly been increased in the recent scenario. The *Aegle marmelos* contains more of natural Antioxidant in the leaves and the fruit pulp. Because of this specific property the Vilva is used in the treatment and preventive of all common ailment of mankind[7][16].

EXPERIMENTAL SECTION

The leaves of *Aegle marmelos* were collected from, Pechipparai region of South Western Ghats, Agasthiyamalai Biosphere reserve during September 2013. The collected leaves were cleaned and dried under shade. The powdered leaves were extracted in chloroform by using Soxhlet apparatus for 24 hours and the extract was used for the phytochemical screening

Phytochemical screening:

Phytochemical analysis were carried out for the chloroform extract as per the standard methods.

Alkaloids:

a) Mayer's Test: Filtrates were treated with Mayer's reagent (potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendorff's Test: Filtrates were treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Amino acids:

Ninhydrin test

To the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of blue colour indicates the presence of amino acid.

Anthocyanin

2 ml of aqueous extract is added to 2 ml of 2N HCl and NH₃, the appearance of pink red turns blue violet indicates presence of Anthocyanin.

Carbohydrates:

Molisch's Test: Filtrates were treated with 2 drops of alcoholic alpha-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

a) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

b) Fehling's Test: Filtrates were hydrolyzed with dil.HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Coumarin

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins.

Cardial Glycosides:

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Diterpenes:

a)Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes. (Roopashree et al, 2008; Obafsi et al, 2010; Audu et al, 2007).

Emodins

2 ml of NH₄OH and 3 ml of benzene was added to extract appearance of red colour indicates presence of emodins.

Flavonoids:

a)Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b)Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Fatty Acid

1g of sudan III is mixed with 5ml of distilled water and the and mixed with 1ml of extract. The appearance of dark red oil droplet in the upper layer indicates the presence of fatty acids.

Glycosides

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Leucoanthocyanin

5 ml of Isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in colour indicates the presence of Leucoanthocyanin

Phlobatannins

Deposition of red precipitate when aqueous extract of each plant sample is boiled with 1% Aqueous HCl was taken as evidence for presence of Phlobatannins.

Phytosterols:

a)Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc.sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of phytosterols.

b)LibermannBurchard's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Proteins:

Xanthoproteic test

Extract was treated with few drops of concentrated HNO₃ formation of yellow indicates the presence of proteins.

Phenols:

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Saponin

5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponin.

Steroid

1ml extract was dissolved in 10 ml of chloroform & equal volume of Conc. Sulphuric acid was added from the side of the test tube. The upper layer turns red and Sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of Steroid

Tannin

4ml extract was treated with 4 ml FeCl₃ formation of green colour indicates that presence of condensed tannin

Terpenoids

2ml of the extract was mixed with 2ml of chloroform and 3ml Conc. Sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate the presence of Terpenoids

RESULTS AND DISCUSSION

The results of qualitative phytochemical analysis of *Aegle marmelos* is shown in Table 1. The chloroform leaf extract of *Aegle marmelos* show the presence of Alkaloids, Amino acids, Anthocyanin, Carbohydrates, Cardial Glycosides, Coumarins, Diterpenes, Emodins, Fatty acids, Flavonoids, glycosides, Leucoanthocyanin, Phlobatannin, Phytosterol, Proteins, Phenols, Saponin, Steroids, Tannin, Terpenoids. Out of 20 phytochemical compound analyzed major 17 components are present. In the present study clearly indicates that the presence of many number of phytochemicals are present. Because of the presence of this bioactive chemicals in the leaves, it has the medicinal property to cure almost all common human ailments. So it evidence form this study that the leaves of *Aegle marmelos* can be used as a single drug to cure the Tridhosanamely Vadha, Pithaand Kabha. And more over the *Aegle marmelos* possess the bioactive chemicals in the leaves which is available throughout the year and also easy source for collection.

Table 1: Phytochemical Analysis of Chloroform Leaf Extract of *Aegle marmelos*

S. No	Phytochemicals	Presence (+) or Absence (-)
1.	Alkaloids	+
2.	Amino acids	+
3.	Anthocyanin	+
4.	Carbohydrates	+
5.	Cardial Glycosides	+
6.	Coumarins	+
7.	Diterpenes	+
8.	Emodins	+
9.	Fatty acids	+
10.	Flavonoids	+
11.	glycosides	+
12.	Leucoanthocyanin	-
13.	Phlobatannin	+
14.	Phytosterol	-
15.	Proteins	+
16.	Phenols	+
17.	Saponin	+
18.	Steroids	-
19.	Tannin	+
20.	Terpenoids	+

CONCLUSION

Even though various parts of *Aegle marmelos* are used for the treatment of human ailments, the leaves are predominately used to treat cardiac, neuro and digestive problems. Moreover it is also used as a immunomodulatory durg. Because of the potential use of the leaves, the qualitative phytochemical analysis work was carried out to find out the active principle present in the leaves. The present study clearly indicates that the compounds like alkaloids, flavonos, terpenoids and saponins are the active principles present in the leaves of *Aegle marmelos*.

Acknowledgements

The one of author Dr. P. Nagendra Prasad is thankful to the Department of Science and Technology Govt. of India (DST/TSG/AF/2012/01 dated 31 December 2012) for financial assistance to carry out this investigation. The authors are indebted to the Chancellor and the Management of Noorul Islam University, Kumaracoil for their valuable guidance, constant encouragement and providing basic facilities throughout the study.

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