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Phytochemical analysis on leaf extract of *Aegle marmelos* Correa

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Abstract

Aegle marmelos is a subtropical plant which can grow up to an altitude of 1200 m from the sea level. It grows well in the dry forests of hilly and plain areas. *A. marmelos* can adapt a wide range of habitat and can be cultivated worldwide. It is native to India and has its origin from Eastern Ghats and central India. This tree is mentioned in the pre-historic writings dating back to 800 B.C. The Chinese Buddhist pilgrim, Hiuen Tsiang, when came to India (1629 A.D.), noticed the presence of this tree in India. 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. During present study the qualitative phytochemical analysis of *Aegle marmelos* leaf extract was carried out and the results were discussed.

Keywords: Phytochemical, Leaf, *Aegle marmelos*.

1. Introduction

Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin, *et al.* 1998) ^[1]. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases (Perumal and Ignacimuthu, 2000) ^[2].

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 (Devi and Manoharan, 2011) ^[3] 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 (Fakim, 2006) ^[4].

Aegle marmelos Correa is commonly called as Bael in Hindi, 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 of its fruits (Ariharan and Prasad, 2013) ^[5].

The leaves are used as astringent, laxative, febrifuge and expectorant. The leaves are useful in ophthalmia, inflammations, catarrh, diabetic and asthmatic complaints (Chakraborty, 2012) ^[6]. The leaves are used for the heart and brain disorders.

2. Material and Methods

The leaves of *Aegle marmelos* were collected from, Near Shiva temple Kothi Compound Rewa (M.P.). The collected leaves were cleaned and dried under shade. The powdered leaves was extracted in chloroform by using Soxhlet apparatus for 24 hours and the extract was used for the phytochemical screening. The whole experiments were conducted in Environmental Biology Dept. A.P.S. University, Rewa (M.P.)

Phytochemical screening

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

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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) Dragendroff's Test: Filtrates were treated with Dragendroff'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 nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Diterpenes

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^[7] and Audu, *et al.*, 2007^[8]).

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 Bortrager'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 Leuanthocyanin

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) Libermann Burchard'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.

3. 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 Tridosha namely Jaundice, Asthma and Inflammation. And more over the *Aegle marmelos* possess the bioactive chemicals in the leaves which are 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	+

4. 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 drug. 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*.

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