

Phytochemical Evaluation of different Solvent Extracts of *Aegle marmelos* fruit at different Stages of its Ripening

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Abstract

Phytochemicals from medicinal plants serve as lead compounds for drug discovery and design. *Aegle marmelos* (L) Correa belonging to family *Rutaceae* is one of the most useful medicinal plants of India. Its fruit is packed with enormous medicinal advantages that are used in diarrhea, dysentery and gastrointestinal disorders. It also possesses anti-viral, anti-helminthic and anti-inflammatory virtues. To identify and understand the bioactive chemical compounds dry powders of ripe and unripe fruits were subjected to different solvents such as methanol, ethanol and acetone sequentially in a soxhlet apparatus. An aqueous decoction representing the nearest form of traditional preparations was also prepared separately. Although all the three solvents and decoction exhibited promising phytochemicals, yet maximum phytochemicals was observed in decoction followed by ethanol in ripe fruit. In unripe fruit, decoction and ethanol observed same number of phytoconstituents. The methanol extract showed the presence of tannins, flavonoids and alkaloids as major bioactive compound. Acetone extract of both the ripe and unripe fruit showed only few secondary metabolites that confirms the presence of less phytoconstituents. Thus the current study shows that ethanol extract and decoction of ripe and unripe fruits of Bael have both polar and non-polar phytoconstituents, therefore they can be considered as a suitable solvent for further pharmacological research.

Key words: *Aegle marmelos*, phytochemical, fruit, ethanol extract, decoction

1. Introduction

Aegle marmelos (L) Correa belonging to family *Rutaceae* and commonly known as Bael has been used as a folklore medicine since ancient time to cure various human diseases. This plant is indigenous to India and is abundantly found in Himalayan tract, Bengal, Central and South India. Almost every part of this tree viz. root, stem, bark, leaf, flowers and fruit at all stages of maturity have medicinal virtues as in (Maity *et al.* 2009). In fact as per Charaka (1500 BC) no drug has been longer or better known or appreciated by the inhabitants of India than the Bael as in (Pandeya 1983). Among the other parts of the tree fruit is reported to be valuable Ayurvedic medicine for chronic diarrhea, tonic for heart and brain, anti-viral activity, hypoglycemic activity, antibacterial activity, antiproliferative activity and against parasites as in (Sunita *et al.* 2011). The ripe fruit is aromatic, cooling, alternative, laxative and nutritive. When taken fresh, it is useful in habitual constipation, chronic dysentery and dyspepsia. It also relieves flatulent colic in patients suffering from a condition of chronic gastrointestinal catarrh. Ripe fruit marmalade is used as prevention during cholera epidemics. Powder of the dried fruit pulp is used as febrifuge, antiscorbutic, nauseant, stimulant and antipyretic as in (Patkar *et al.* 2012). Unripe fruit powder is found to be effective against intestinal parasite *Entamoeba histolytica* and *Ascaris lumbricoides*. Brijesh *et al.* (2009) in their studies have found the decoction of unripe fruit to be an astringent that is useful in diarrhea and chronic dysentery.

The rural population of India is more disposed to traditional ways of treatment because of easy availability and cheaper cost. Plant are the richest resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs as in (Hammer *et al.* 1999). The medicinal value of plants lies in some chemical active substances that produce definite physiological action on human body as in (Aiyelaaghe & Osamudiamen 2009). Moreover pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds. These bioactive substances are normally accumulated as secondary metabolites in all plant cells but then their concentration varies according to the plant part, season, climate and particular growth phase as in (Maji *et al.* 2010). The aim of the present paper is to determine the bioactive substances contributing to the medicinal value of the ripe and unripe fruit of *Aegle marmelos* using different solvent systems.

2. Material and Methods

2.1 Plant study materials

Aegle marmelos fruit were collected from Sambha district of Jammu, the northern part of India. The unripe fruits were collected in the month of February and the ripe fruits in the month of June. The fruits were washed, shells were broken and pulp was scooped out and dried in shade. The dried fruit pulp were homogenized to powder and stored in airtight bottles for further analysis.

2.2 Preparation of Extract

2.2.1 Decoction: One gram powdered dry fruit was boiled in 16ml of double distilled water till the volume was reduced to 4 ml. The decoction was filtered and used for further analysis.

2.2.2 Soxhlet Extract: Crude plant extract were prepared by soxhlet extraction method. About 50gms of powdered fruit material was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvent used were ethanol, methanol and acetone. The process of extraction continued till the solvent in siphon tube of an extractor became colorless. After that the extract was taken in a beaker and kept on the hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in the refrigerator at 4°C for their future use in phytochemical analysis.

2.3 Determination of plant yield

The percentage yield was obtained using the formula $W_2 - W_1 / W_0 \times 100$. Where W_2 is the weight of the container and extract, W_1 the weight of the container alone and W_0 the weight of the initial dried sample as in (Anokwuru *et al.* 2011).

2.4 Qualitative Phytochemical analysis of Plant Extract

The extract and decoction were tested for the presence of bioactive compounds by using the following standard methods given as in (Harbrone 1973),(Trease & Evans 1989).

2.4.1 Test for Tannins: Crude extract was mixed with 2ml of neutral $FeCl_3$. A dark green coloration indicated the presence of tannins.

2.4.2 Test for Phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

2.4.3 Test for Phenols: Crude extract was mixed with few drops of 10% solution of lead acetate. White precipitate indicated the presence of phenols.

2.4.4 Test for Carbohydrates: Crude extract was mixed with few drops of α naphthol solution in alcohol and concentrated H_2SO_4 was added from the side of the test tube. Violet ring formed at the junction of two liquids showed the presence of carbohydrate.

2.4.5 Test for Proteins: Crude extract was mixed with 2ml of Biuret reagent. Violet color indicated the presence of proteins.

2.4.6 Test for Flavonoids: Crude extract was mixed with 5ml of dilute ammonia followed by the addition of concentrated H_2SO_4 . A yellow coloration observed in the extract indicated the presence of flavonoids. The yellow coloration disappears on standing.

2.4.7 Test for Saponins: Crude extract was mixed with 5ml of distilled water in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

2.4.8 Test for Alkaloids: Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagent were added to the mixture. Appearance of cream color precipitates with Mayer's reagent and appearance of reddish brown precipitates with Wagner's reagent indicates the presence of alkaloids.

2.4.9 Test of Steroids: Crude extract was mixed with 1ml of chloroform, few drops of acetic anhydride and two drops of concentrated H_2SO_4 . The development of a greenish coloration indicated the presence of steroids.

2.4.10 Test for Terpenoids (Salkowski test): Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sideways. A reddish brown coloration at the interface indicates the presence of terpenoids.

2.4.11 Test for Triterpenoids (Liebermann-Burchard): Crude extract was mixed with 1ml of chloroform, few drops of acetic anhydride and concentrated H_2SO_4 was added along the side of the test tube. Appearance of reddish brown color indicated the presence of triterpenoids.

2.4.12 Test for Cardiac glycosides (Keller-Killani test): Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4 . A brown ring at the interphase indicated the presence of cardiac glycosides.

3. Result and Discussion

The result of the percentage yield in Table 1 shows that ethanol was a better solvent for the extraction of unripe fruit and ripe fruit followed by methanol. Difference in percentage yield of extracts products might be due to variation in the solubility of various ingredients in different solvents used, methods and type of extraction used as

in (Perucci *et al.* 1995).

Table 1. Yield of ripe and unripe fruit extract of *Aegle marmelos*

Stage	Aqueous	Methanol	Ethanol	Acetone
Ripe fruit	18.94%	26.30%	28.80%	2.39%
Unripe fruit	20.67%	29.59%	34.48%	3.03%

Traditionally used medicinal plants produce a variety of known therapeutic properties that can be attributed to its secondary metabolites. Plants are natural source of producing these compounds in a most efficient way with precise selectivity. Phytochemical screening of various solvent extract and decoction of *Aegle marmelos* ripe and unripe fruit showed the presence of most important phytoconstituents. The medicinal virtues of the fruit can be ascribed due to the presence of various bioactive chemical compounds as shown in Table 2 and 3.

Table 2. Phytochemical compounds of unripe fruit of *Aegle marmelos* in different solvents

S.NO.	Phytochemical compounds	Aqueous	Ethanol	Methanol	Acetone
1	Tannin	-	+	+	-
2	Phlobatannin	-	-	-	-
3	Phenol	+	+	-	+
4	Carbohydrates	+	+	+	-
5	Proteins	+	+	+	-
6	Flavonoids	+	+	+	-
7	Saponins	+	-	-	-
8	Alkaloids	-	+	+	+
9	Steroids	+	+	-	-
10	Terpenoids	+	+	-	-
11	Triterpenoids	+	+	-	-
12	Cardiac glycoside	+	-	-	-

Decoction (aqueous extract) of unripe fruit showed the presence of phenols, protein, carbohydrate, flavonoids, steroids, terpenoids, triterpenoids, saponins and cardiac glycoside and showed the absence of tannins and alkaloids. Ripe fruit decoction showed the presence of tannins, phlobatannins, phenols, protein, carbohydrates, alkaloids, steroids, terpenoids, saponins and absence of flavonoids and triterpenoids.

Table 3. Phytochemical compounds of ripe fruit of *Aegle marmelos* in different solvents

S.NO.	Phytochemical compounds	Aqueous	Ethanol	Methanol	Acetone
1	Tannin	+	+	+	-
2	Phlobatannin	+	-	+	-
3	Phenol	+	+	+	-
4	Carbohydrates	+	+	+	-
5	Proteins	+	+	+	-
6	Flavonoids	-	+	+	-
7	Saponins	+	+	-	+
8	Alkaloids	+	-	+	+
9	Steroids	+	+	-	-
10	Terpenoids	+	-	-	-
11	Triterpenoids	-	-	-	+
12	Cardiac glycoside	+	-	-	-

Ethanol extract of unripe fruit showed the presence of tannins, phenols, protein, carbohydrates, flavonoids, alkaloids, steroids, terpenoids, triterpenoids. Ripe fruit showed the presence of saponins, cardiac glycoside apart from the above compounds and absence of alkaloid, terpenoids and triterpenoids. Unripe ethanol extract showed the absence of phlobatannin, saponins and cardiac glycoside. According to the study of Sujata *et al.* (2011) aqueous and alcoholic extract of fruit has revealed the presence of most of the phytoconstituents. Moreover the presence of these phytochemical compounds in the ethanolic extract has showed maximum antibacterial activity in several studies as in (Joshi *et al.* 2009).

Methanol extract of the ripe fruit showed the presence of tannins, phlobatanins, phenols, protein carbohydrates, flavonoids, alkaloids and absence of steroids, terpenoids, tripterpenoids, saponins, cardiac glycoside. The unripe fruit methanol extract showed the presence of only tannins, protein, carbohydrates and flavonoids. Acetone extract of unripe fruit showed the presence of only phenols and alkaloids. Ripe fruit acetone extract showed the presence of alkaloids, triterpenoids and saponins.

The phytochemical screening and quantitative estimation of the percentage of crude yields the most promising secondary metabolites such as alkaloids, flavonoids, phenols, proteins, tannin and carbohydrates. They are known to show medicinal activity as well as physiological activity as in (Edoga *et al.* 2005). Tannin has been found to react with proline-rich protein to form irreversible complexes resulting in the inhibition of the cell protein synthesis. Fruits that have tannins as their major components are astringent in nature. They are used in treating intestinal disorders such as diarrhea and dysentery as in (Chrinius *et al.* 2011),(Nisha *et al.* 2011). The presence of saponins lends credence to the use of this fruit in managing inflammation. Flavonoids have also exhibited wide range of biological activities; such as antimicrobial, antioxidant, anti-inflammatory, anti-allergic and cytostatic properties (Tsuchiya *et al.* 1996). Tannins and flavonoids in general have been reported to have antidiarrhoeal activity through inhibition of intestinal motility and antisecretory effects as in (Brijesh *et al.* 2009). Steroids and triterpenoids are reported to have antibacterial properties; triterpenoids are known to weaken the membranous tissues, which results in dissolving cell wall of microorganisms. Alkaloids have shown to be analgesic, antispasmodic and antibacterial as in (Rao *et al.* 2003),(Okwu & Okwu 2004).

4. Conclusion

In the present study the ethanol extract and decoction of ripe and unripe fruit confirmed the presence of most of the polar and non polar components followed by the methanol extract. Least compounds were extracted in acetone. Hence decoction and ethanol fruit extract can be considered as suitable solvent for further pharmacological investigation.

Secondly the presence of bioactive compounds like tannin, phenols, flavonoids, alkaloids and steroids in both ripe and unripe fruits of *Aegle marmelos* supports the traditional use of this fruit by the rural population of India. Thus this fruit may provide novel or lead compounds that could become the starting material for the synthesis of a new cheap drug.

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