

Phytochemical Analysis and Antimicrobial Activity of *Aegle Marmelos* (L.) Corr.

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Abstract

The present study was carried out in physico-chemical analysis of ethnolic extracts from leaves of *Aegle marmelos* variants. It is commonly called *Vilvam* in Tamil. The leaves of *Aegle marmelos* variant-I and variant-II of the plant were collected and investigated for their phyto-chemical. The leaves of variants showed distinct variations. Anti microbial activity of ethanolic extracts both the variants of *A. marmelos* showed positive result against tested organism in a concentration dependent manner.

Key words : *Aegle marmelos*, Phyto-chemicals, Anti-microbial activity.

Introduction

Medicinal plants form the backbone of Traditional Systems of medicine in India. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti *et al.*, 2008).

Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients in Traditional Systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008).

Aegle marmelos (L.) (Tamil Name: Vilvam) is important medicinal plant available in TamilNadu, India and are reported to have various medicinal property in Traditional medical systems.

Aegle marmelos is common medicinal plant available in South India and is used as medicine in Siddha and Ayurveda. The plants are distributed throughout India, cultivated as well as grow in wild. In TamilNadu it is located in the river belt of Vattaru and Cauvery and it is also present in most of the Shiva temple of TamilNadu.

The unripe and ripe fruits of *A. marmelos* are bitter, acrid, sour, astringent, digestive and stomachic and are useful in diarrhoea, dysentery and stomachalgia (Warrier *et al.*, 1998). Stem bark is used in fever (Kurup *et al.*, 1979).

Ethanolic of extract of *A. marmelos* shows positive result against tested fungal organisms (Jain *et al.*, 1998).

Material and Methods

Preparation of Extracts

Cold Extraction Technique

The air-dried material was coarsely powdered to aid the extraction. They were soaked in alcohol and kept for 48 hours. The extract thus obtained was decanted and filtered. The clear extract was subsequently concentrated using rotary vacuum evaporator. This method was done to save the active principles if any that would have been otherwise inactivated by a heating process that usually involved in any concentration process. Pilot biological studies conducted using this extract and when compared its effects with the heated extracts, the active principles withstood the heating up to 65°C for least 24 hours. So subsequently the extracts were concentrated over a boiling water bath on glass Petri-dishes by free evaporation.

Preparation of Extract

Ethanolic (50%) extract was prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1996). The ethanolic extract was subjected to pharmacological studies. For pharmacological purpose the 50% ethanolic extract was eluted with n-Butanol – Water in 1:1 ratio.

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Qualitative Phytochemical Analysis

Qualitative phytochemical analyses were done using the procedures of Kokate (1994). Alkaloids, carbohydrates, tannins and phenols, flavonoids, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Alkaloids

The extracts were dissolved in dil. H₂SO₄ and filtered. The filtrate was treated with Mayer's, Dragendorff's, Hager's and Wagner's reagents separately. Appearance of cream, orange brown, yellow and reddish brown precipitates in response to the above reagents respectively indicate the presence of alkaloids.

Carbohydrates

300mg of 50% alcoholic extracts were dissolved in water and filtered. The filtrate was treated with con. H₂SO₄ and then with Molisch's reagent. Appearance of pink or violet colour indicates the presence of carbohydrates. The filtrate was boiled with Fehling's and with Benedict solution. Formation of brick red precipitate in Fehling's and Benedict's solution is the positive result for reducing sugars and non-reducing sugars respectively.

Tannins and phenols

Small quantity of 50% alcoholic extract was dissolved in water and 5% ferric chloride solution or 1% Gelatin solution or 10% lead acetate solution was added. Appearance of blue colour with ferric chloride or precipitation with other reagent indicates the presence of tannins and phenols.

Flavonoids

The extract mixed with few ml of alcohol was heated with magnesium and then con. HCl was added under cooling. Appearance of pink colour indicates the presence of flavonoids. The extract was treated with few ml of aqueous NaOH. Appearance of yellow and change to colorless with HCl indicate the presence of flavonoids.

Gum and mucilage

About 10ml of the extract was slowly added to 25ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gum and mucilage

Fixed oils and fats

A drop of concentrated extract was pressed in between two filter papers and kept undisturbed. Oil stain on the paper indicates the presence of oils and fats.

Saponins

About 1ml of the extract was dissolved in 20ml of water and shake in a graduated cylinder for 15 minutes. Formations of one cm layer of foam indicate the presence of saponins.

Phytosterol

The extract was treated with Liebermann Burchard under suitable conditions. Appearance of blue-emerald green indicates the presence of phytosterol and terpenes.

Anti-Microbial Activity

Well Diffusion Assay Method (Bauer *et al.*, 1996)

The ethanol extract of leaves of *Aegle marmelos* was tested for their antibacterial and antifungal studies.

The microbial strains tested against *Aegle marmelos* extracts were *Escherichia coli*, *Streptococcus pyogenes*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Candida albicans*, *Trichoderma viride* and *Fusarium* spp.

Test against standard controls

The commercially available antibiotics disc was used as standard controls for all the test micro-organism. The sensitivity patterns were recorded and the readings were interpreted according to the critical diameter given by National Committee for Clinical Standards (NCCLS, 1997).

The microbes were obtained from the Microbiology Laboratory, Sea Horse Hospital Pvt., Tiruchirapalli. The test bacterial strains were seeded over the Muller Hinton agar plates and Sabouraud's dextrose agar plates were prepared for fungi aseptically. Wells were made on the agar surface with 5mm cork borer. The test drugs (0.5ml) were injected into the well using a micropipette for all concentration (5, 10 and 20%) separately and it was compared with the standard drugs Amoxicillin and Clotrimazole for bacterial and fungal strains respectively. The plates were incubated at 37 ± 2°C for 48 to 72 hrs under microaerophilic condition. The plates were observed for the elevating zone around the well. The zone of inhibition was calculated by measuring the diameter of their inhibition zone around the well (in mm) including the well diameter. Readings were taken in three different fixed directions in all three replicates and the average values were calculated.

Results

Qualitative Phytochemical Analysis

Successive solvent extracts of leaf of *Aegle marmelos* Variant-I and Variant-III were subjected to qualitative phytochemical screening and the values are given in Table- 1.

It was observed that presences of alkaloids, carbohydrates, tannins & phenols, gums & mucilage, fixed oils & fats and saponins was noted in the leaf extracts of both variants. Appreciable and moderate amount of carbohydrate were noticed in the alcohol and water extracts, of variant III. More amount of alkaloid was noticed in chloroform, alcohol and water extracts of variant I, while it was present in small amount in

S.No	Compound Tested	Test applied/ Reagent used	Variant I					Variant II				
			Pet. Ether	Benzene	Chloroform	Alcohol	Water	Pet. Ether	Benzene	Chloroform	Alcohol	Water
1.	Carbohydrate	Fehilings	-	-	-	+	+	-	-	-	+	+
		Benedicts	-	-	-	+	+	-	-	-	-	+
2.	Alkaloids	Mayer's	-	-	-	-	+	-	-	-	-	+
		Wagner's	-	-	+	+	+	-	-	+	+	+
		Hager's	-	-	+	+	+	-	-	+	+	+
		Dragendroff's	-	-	+	+	+	-	-	-	-	-
3.	Phytosterols	L.B. Test	+	+	+	+	+	+	+	+	+	+
4.	Tannins & phenols	10% lead Acetate	-	-	-	+	+	-	-	-	+	+
5.	Fixed oil & fats	Spot test	+	+	-	-	-	+	+	-	-	-
6.	Saponins	Foam test	-	-	-	+	+	-	-	-	+	+
7.	Gum & mucilage	Alcoholic Precipitation	-	-	-	-	+	-	-	-	-	+

constituents: + = present - = Absent

Table - 1. Qualitative Phytochemical screening of leaf powder of *Aegle marmelos* Var. I and Var. III

variant III. Tannins and phenols were present in appreciable and moderate amount in alcohol and water extract of both the variants. Moderate and small amount of fixed oils and fats were present in pet. ether and benzene extracts of variant I compared to that of variant III. Small amount of Saponins was noticed in alcohol and water of extracts of both variants. In water extract of leaf of *Aegle marmelos*, moderate amount of gums and mucilage were present in both variants.

Anti-Microbial Activity

Anti-microbial activity of ethanol extracts of leaf of *Aegle marmelos* at different concentration (10, 20, 30 mg %) against *Escherichia coli*, *Streptococcus pyogenes*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Candida albicans*, *Trichoderma viride* and *Fusarium* spp. were given in Table 2.

Variant-I

Ethanol extract of leaf of *Aegle marmelos* var. I showed antimicrobial activity in a concentration dependent manner. The drug gave positive result against *Helicobacter pylori*, *Pseudomonas aeruginosa*, and *Trichoderma viride*. No zone of inhibition was found in *Streptococcus pyogenes*, *Aspergillus niger*, *Candida albicans*, and *Fusarium* spp.

Variant-III

Ethanol extract leaf of *Aegle marmelos* var. III also showed antimicrobial activity in a concentration dependent manner. The drug gave positive result against *Streptococcus pyogenes*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Trichoderma viride* and *Fusarium* spp. No zone of inhibition was found in *Candida albicans* and *Escherichia coli*.

Among the leaf extracts of *Aegle marmelos* Variant-I and Variant-III showed the broad spectrum of antimicrobial activity in tested organism in well diffusion assay method.

Discussion

Qualitative phytochemical analysis of various extracts of both the variants of *A. marmelos* showed distinguished values and can be used as diagnostic values.

Anti microbial activity of ethanolic extracts of *A. marmelos* showed positive result against tested organism in a concentration dependent manner. Phenolic, aldehyde, ester, flavonoid and alkaloid compounds are known to be antimicrobial in action.

Previous work also reported antimicrobial activity of fruits and leaves of *A. marmelos* (Valsraj *et al.*, 1997). Rind extracts of *A. marmelos* showed significant antimicrobial activity (Kalkar *et al.*, 2005). Anti fungal activity was also observed in the leaves of *A. marmelos* (Kaushik *et al.*, 2004). Ethanol extracts also showed antifungal activity (Jain *et al.*, 1998).

Conclusion

Phytochemical studies on the leaf of *Aegle marmelos* were carried out with a view to standardize and distinguish the characters of leaf of two variants, variant I and variant III. Qualitative and anti-microbial studies were undertaken on the leaf of the two variants.

Qualitative phytochemical values showed variation among the two variants. By the present work, it is possible to identify the leaf of the two variants.

Name of the Organism	Zone of inhibition (mm)						Amoxicillin/ Clotrimazole
	Variant I			Variant II			
	10mg %	20mg %	20mg %	10mg %	20mg %	20mg %	
<i>Escherichia coli</i>	-	-	-	-	-	-	09
<i>Streptococcus pyogenes</i>	-	-	-	15	18	21	12
<i>Helicobacter pylori</i>	7.1	9.2	10.1	8.2	10.8	12.5	06
<i>Pseudomonas aeruginosa</i>	6.1	8.2	9.2	7.4	8.7	9.9	25
<i>Aspergillus niger</i>	-	-	-	9.2	10.1	14.2	23
<i>Candida albicans</i>	-	-	-	-	-	-	20
<i>Trichoderma viride</i>	3.1	7.1	9.4	5.2	7.3	10.1	24
<i>Fussarium spp.</i>	-	-	-	9.1	9.4	13.1	12

Table - 2 Anti Microbial activity of *Aegle marmelos* variant I and variant III

The antimicrobial property of the leaf of *A. marmelos* established their efficacy as climbed in Siddha literatures and previous ethnobotanical studies. It is suggested that *A. marmelos* could be a potential natural antimicrobial drug. It can also be used for further clinical studies for various chronic diseases.

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