

## PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *AEGLE MARMELLOS* (L.) CORR.

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The present study was carried out to screen and evaluate the antimicrobial activities of fruit and leaves extract from *Aegle marmelos*. Aqueous, ether, acetone and methanol extracts were tested against two gram negative bacteria i.e. *E. coli* and *V. cholerae*. Result indicated that phytochemical extracts of *A. marmelos* exhibited significant anti bacterial activity at all dosages tested (1mg/disc and 5mg/disc). However, the inhibitory activity was found to be dose dependent. Methanol extract was found to be more active towards the organisms tested than the any other extracts and can be used as a potential source of novel antimicrobial agent.

**Keywords :** *Aegle marmelos*; Antimicrobial; Constituents; Phytochemical.

### Introduction

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<sup>1</sup>. Phytochemicals from the medicinal plants serve as a source of lead compounds in drug discovery and design<sup>2,3</sup>.

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infections. This situation, coupled with the undesirable side-effects of certain antibiotics and the emergence of previously uncommon infections is a serious medical problem<sup>4,5</sup>. This situation has forced scientists to search for new antimicrobial substances from various plants<sup>6</sup>. According to Mathekg and Mayer<sup>7</sup>, *in vitro* screening methods have provided the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.

*Aegle marmelos* is popularly known as Bael, Bilwa, Bengal quince etc. It is a tree, native to northern India, but is found widely through out the Indian peninsula. The Bael tree grows upto 18 m. and bears thorns and fragrant white flowers. It has a woody-skinned, smooth fruit 5-15 cm in diameter. It has numerous seeds, which are densely covered with fibrous hairs and are embedded in a thick, gluey, aromatic pulp.

*A. marmelos* is a very popular medicinal plant

and has a very long history as a multipurpose folk remedy. It exhibits antidiabetic, antihyperlipidemic, antidiarrhoeal, antidysentric and antioxidant properties<sup>8</sup>. All the parts of the tree *viz.* root, leaf, fruit are used in traditional system of medicine. Fruits are used in dysentery, dyspepsia, chronic diarrhoea with malabsorption or in the intestinal dysfunction. The dried roots are used in the disorder of nervous system, vomiting and rheumatism and the leaves are used in the treatment of jaundice, cholera, asthma and ophthalmia etc.

### Material and Methods

Fruits and leaves of *Aegle marmelos* were collected from the cantonment area of Jabalpur district. The flora of Jabalpur and the flora of Bhopal were used for identification and authentication of plants. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grounded into powder<sup>9</sup>.

**Preparation of phytochemical extracts:** Eight gms of fresh fruits and leaves of *A. marmelos* were grinded with 15ml of solvent in mortar and pestle and filtered. The filtrates and crude extracts were used for phytochemical screening. Different extracts of fruits and leaves were prepared in acetone, ether, methanol and water for phytochemical screening.

**Test organisms:** Pure cultures of bacterial isolates were collected from the Aeroallergen and Immunology Lab, Department of Biological Sciences, Rani Durgawati Vishwavidyalaya, Jabalpur. The stock cultures were sub-cultured in nutrient broth for 24 hours and maintained at 37° C and also the bacterial organisms were maintained

Table 1. Results of phytochemical analysis of *Aegle marmelos* fruit.

S.No.	Test	Aqueous	Ether	Acetone	Methanol
1.	<i>ALKALOIDS</i>				
	a. Mayer's test	+	+	+	+
	b. Dragendoff's test	+	-	-	+
2.	<i>CARBOHYDRATE</i>				
	a. Benedict test	+	-	+	+
	b. Fehling test	+	+	+	+
3.	<i>SAPONINS</i>				
	a. Foam test	+	-	-	-
	<i>PROTEINS</i>				
4.	a. Xanthoprotein test	+	-	-	+
	b. Biuret test	+	-	-	-
5.	<i>FLAVANOIDS</i>				
	a. Flavanoid test	+	+	+	+
6.	<i>TANNINS</i>				
	a. Ferric chloride test	+	+	+	+
7.	<i>RESIN</i>				
	a. Resin test	-	-	-	-
8.	<i>STEROL</i>				
	a. Salkowaski test	+	-	+	+
9.	<i>LIPID</i>				
	a. Glycerol test	+	-	-	+

on nutrient agar media (NAM).

**Phytochemical screening:** Phytochemical analysis of the extract was carried out as described by Harbone<sup>10</sup>. By this analysis, the presence of several phytochemicals like sugar, protein, alkaloids, flavonoids, saponins, tannins, cardiac glycoside, terpenoids and lipids were tested (Table 1 and 2).

**Antimicrobial Activity test:** The disc-diffusion assay was used to determine the antimicrobial activity of the investigated extracts. The sterile broth was inoculated with microbial cell (200 µl of microbial cell suspension in 20 ml agar medium) and poured into sterile petri dishes. Sterile filter paper disc of 6 mm diameter were impregnated with 20 µl of the extract solution. The paper disc were allowed to evaporate and after that placed on the surface of the inoculated agar plate. Plates were kept for 2 hour in refrigerator to enable pre diffusion of the extract into the agar. Then, the plates were incubated over night (18 hr) at 37° C. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zone<sup>11</sup>.

### Results and Discussion

Present investigation includes the phytochemical screening

of fruit and leaves of *Aegle marmelos* in various solvents i.e. aqueous, ether, acetone and methanol extract. The Table 1 and 2 show the qualitative determination of phytochemical constitution of leaves and fruit of *A. marmelos*, respectively. The extracts obtained by successive extraction were directly subjected to preliminary phytochemical screening for the detection of various metabolites such as carbohydrates, proteins, lipid, flavanoids, alkaloids, tannins, resins, sterols and saponin.

All the extracts screened gave positive test for alkaloid, carbohydrate, tannin and flavanoid while protein and lipids were detected in aqueous and methanol medium. Saponin showed positive results only in the aqueous medium not in other three media. Resin showed negative results in all the four solvents.

The Antibacterial effect of *A. marmelos* fruits and leaves extract in two different solvents i.e. in aqueous and methanol against gram negative bacteria *E. coli* and *V. cholerae* is shown in Table 3 and 4.

Antibacterial activity of fruit extract against *Escherichia coli* and *Vibrio cholerae* gave better results in methanol extract as compared in both the cases. The crude extract of fruit for *E. coli* gave 8.4 mm zone of

Table 2. Results of phytochemical analysis of *Aegle marmelos* leaves.

S.No	Test	Aqueous	Ether	Acetone	Methanol
1.	<b>ALKALOIDS</b>				
a.	Mayer's test.	+	-	+	+
b.	Dragendoff's test	+	-	+	-
c.	Wagner's test	+	+	+	+
2.	<b>CARBOHYDRATE</b>				
a.	Benedict test	+	+	+	+
b.	Fehling test	+	-	+	+
c.	Tollens test	+	+	-	-
3.	<b>SAPONINS</b>				
a.	Foam test	+	-	+	+
4.	<b>PROTEINS</b>				
a.	Xanthoprotein test	-	-	+	-
b.	Biuret test	+	-	+	-
5.	<b>FLAVANOIDS</b>				
a.	Flavanoid test	+	+	+	+
6.	<b>TANNINS</b>				
a.	Ferric chloride test	+	+	+	+
b.	Lead acetate test	+	+	-	+
7.	<b>RESIN</b>				
a.	Resin test	-	-	+	-
8.	<b>STEROL</b>				
a.	Salkowaski test	+	-	+	+
9.	<b>LIPID</b>				
a.	Glyserol test	+	-	+	-

inhibition where as in methanol extract it was found to be 9.6 mm. Zone of inhibition in crude extract was found to be 9.3 mm for *V. cholerae* while in case of methanol extract it was recorded as 12.1 mm.

In case of leaf extract, the antimicrobial activity against *E. coli* was recorded greater than methanol extract. Zone of inhibition in crude extract for *E. coli* was found to be 9 mm whereas in case of methanol extract it was recorded as 8.2 mm.

The leaf extract in methanol solvent gave better antimicrobial results for *V. cholerae* than in the crude solvent. Zone of inhibition was found to be 10.2 mm in methanol extract while it was 7.9 mm in crude extract.

According to the results, the diameter of zone of inhibition in the plates inoculated with both gram negative bacteria i.e. *E. coli* and *V. cholerae* showed the antimicrobial activity. The ripe fruit pulp and leaves of *A. marmelos* have pronounced bactericidal activity against enteropathogen. *E. coli* and *V. cholerae* are more sensitive towards methanolic extracts as compared to the crude extract. This result is similar to the work enumerated by Sudharameshwari<sup>12</sup>, which showed strong activities of *A. marmelos* in the methanol extract due to the presence of active chemical constituents in the plant like carbohydrate,

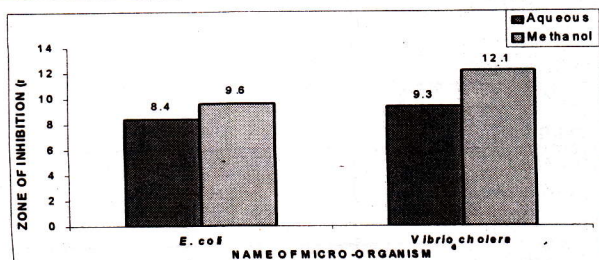
alkaloid, flavanoid, tanins, and saponin etc. and other secondary metabolites which are reported to be antimicrobial<sup>13</sup>. The extracts of fruit and leaves of *A. marmelos* were found to be mostly active against the strain of *V. cholerae* followed by *E. coli*.

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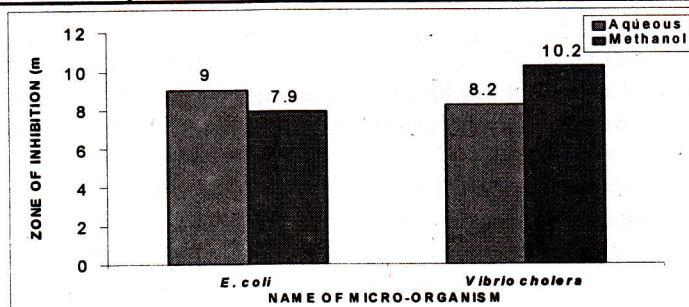
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**Table 3.** Antimicrobial activity of *Aegle marmelos* (Fruit).

S.No.	Microbial agent	Sample	Zone of inhibition (Diameter in mm)			Average
1.	<i>E. coli</i>	Aqueous	8.0	8.8	8.6	8.4
2.		Methanol	9.8	9.4	9.7	
3.	<i>Vibrio cholerae</i>	Aqueous	9.5	9.2	9.4	9.3
4.		Methanol	12.4	11.9	12.2	

**Fig.1.** Antimicrobial activity of *Aegle marmelos* (Fruit)**Table 4.** Antimicrobial activity of *Aegle marmelos* (Leaves).

S.No.	Microbial agent	Sample	Zone of inhibition (Diameter in mm)			Average
1.	<i>E. coli</i>	Aqueous	9.0	8.9	9.2	9.0
2.		Methanol	8.2	7.8	8.5	
3.	<i>Vibrio cholerae</i>	Aqueous	7.8	8.0	7.9	7.9
4.		Methanol	10.2	9.8	10.3	

**Fig.2.** Antimicrobial activity of *Aegle marmelos* (Leaves)

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