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Antibacterial screening of stem, fruit and leaves of *A. marmelos*

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ABSTRACT

The principal objective of the present research work was to determine the antibacterial potential of A. marmelos against four standard pathogenic bacterial strains. To evaluate antibacterial activity the agar-well diffusion assay was used. All the four extracts showed the highest and significant antibacterial activity against both Gram negative and Gram-positive bacteria. It is the strain Bacillus subtilis that is almost resistant to the four extracts of A. marmelos.

Keywords: Antibacterial activity, Standard pathogenic bacteria, *A. marmelos*, antibacterial screening **.**

INTRODUCTION

Use of traditional medicine is one of the common practices in India due to their wide pharmacological activities. Developed and developing countries use traditional medicine at the primary health care level. Many currently used drugs are expensive or not readily available and a major setback to their continued usage is the development of resistance. This situation urgently forced scientists for searching new, inexpensive drugs that will be able to act for longer periods before resistance sets in. There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones [19]. Medicinal plants have been used to cure a number of diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms [21].

Antibacterial properties of various plants parts like root stem leaves, seeds, flowers, fruits have been well documented for some of the medicinal plants for the past two decades [16]. Medicinal and aromatic plants and essences are rich in antibacterial compounds could be an alternate way to combat against bacterial diseases [1][17]. Since the 1940's, but many bacteria are now becoming resistant to them. According to Braunter and Grein (1994) natural plant products may offer a new source of antibacterial agents. In recent years antimicrobial properties of Indian medicinal plants have been increasingly reported [2][3]. The traditional treatment approach is of much significance, especially in India due to the endemic presence of infective gastro intestinal diseases which are the major causes of infant and adult mortality [18].

A. marmelos is belongs to the family Rutaceae, commonly called as Bael (English), Vilvam (Tamil) and is found throughout India . Bael is a medium sized decidous tree bearing strong axillary thorns. Leaves with 3 or 5 leaflets. Bael leaves are extremely useful for treating diabetes, jaundice, cholera and asthma. Bael leaves are made into a poultice and used in the treatments of ophthalmic. Bael leaf poultice is applied to inflammations-with black pepper for edema, constipation, and jaundice.

MATERIALS AND METHODS

Plant preparation : The present project work was carried out in the Department of Zoology, E.S.A. College Of Science , Vasai Road ,Dist-Thane, with the object of screening the antibacterial activity of medicinal plant such as *A. marmelos*. Their botanical identities were determined and authenticated in botany department, St. Xavevior College. The plant materials chosen based on therapeutic properties and their availability in our campus. The selected plant was thoroughly washed and then dried under shade at 25 ± 2 degree Celsius for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The plant powder was stored in air sealed polythene bags at room temperature before extraction.

Extraction procedures : 25g of dried plant powder was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 200ml of solvent. Solvents used for extraction were Chloroform (61 o C), Ethanol (78.5 o C) ,Acetone (560c)and distilled water (*Fong, 1973*) and the extracts were dried. The dried extracts were stored in a refrigerator at 4 oC.

Phytochemical screening : The extracts were subjected to Preliminary phytochemical Screening methodology were adapted from Kemp (1986) and Sofowara (1982) method. The test for alkaloids was carried out by subjecting 0.5g aqueous extract in 5ml 1% HCl, boiled , filtered and Mayer's reagent was added [22][23]. The presence of flavanoides was determined using one ml of extract was added with a few drops of neutral ferric chloride solution. The presence of carboxylic acid was determined by one ml of each extracts was separately treated with a few ml saturated solution of sodium bicarbonate. One ml of various extracts were dissolved in 5ml of alcohol was treated separately with a few drops of neutral ferric chloride solution to find the presence of phenols. The test for steroids, phytosterols was carried out by 1ml each of concentrated Sulphuric acid was added to the extract and allowed to stand for 5 minutes

Table1: Phyto-chemical screening of some medicinal plants

Sr No	Phyto-chemical compound	Tests	Leaf				Fruit				Stem			
			1	2	3	4	1	2	3	4	1	2	3	4
1	Alkaloid	Mayer' Test Wanger's. Test Dragonho's Test	+ + -	- +-	+++	+++	++ -	++-	- - -	++ +	+ + -	- + -	++-	+ + +
2	Phytosterol	Salkowaski Test	-	-	+	-	-	+	-	-	-	+	-	-
3	Saponin	Froth, Foam	-	-	-	+	-	-	-	-	-	-	-	+
4	Resins	Acetone.	-	+	+	-	+	+	+	+	-	+	+	-
5	Phenol	Fecl Test	-	+	+	+	+	-	+	+	-	-	+	+
6	Tannins	Gelatine Test	-	-	-	-	-	-	-	-	-	-	-	-
7	Protein and amino acid	Xanthoproteic Test	+	-	-	+	-	+	-	+	-	+	+	+
8	Diterpene	Copper Acetate	+	-	+	+	+	-	+	-	-	-	+	+
9	Flavonoids	Lead Acetate Test	-	-	+	-	-	-	-	-	-	-	-	-
10	Glycoside	Legal's Test	-	-	-	-	-	-	-	-	-	-	-	-

Solvents: 1. Ethanol; 2.Chloroform; 3. Distilled Water; 4.Acetone, Keys: + : Positive ; - : Negative

Bacterial susceptibility testing: The Agar plate well–diffusion method was used as described by *Desta (2005).* A standardized inoculum 1-2 × 10 7 cfu/ml 0.5 MC Farland standards was introduced onto the surface of sterile agar plate, and evenly distributed by using a sterile glass spreader. Simultaneously, 8 mm wells were cut from the plate using a sterile cork borer. 70 µl of extract at a concentration of 50 mcg/ml was introduced into each well. The agar plates were incubated aerobically at 37 oC. After 24hr, the inhibition zones were measured with a ruler and compared with the control well containing only acetone. 30 mcg/ml of ampicillin served as control.

Determination of MIC: MICs of the extracts were determined as described by *Kabir et al. (2005).* MICs of the extracts were determined by diluting them to various concentrations ranging from 10 to 200 mcg/ml. Each volume

of each extract and nutrient broth were mixed in a test tube and 0.1 ml of standard inoculum (1-2 × 107 cfu/ml) was added to each tube. Control tubes were maintained simultaneously. The tubes were incubated aerobically at 37 0C for 24 hrs.

The lowest concentration of extract that produced no visible bacterial growth (no turbidity) when compared with control tube was regarded as MIC.

RESULTS AND DISCUSSION

The extracts were subjected to preliminary phytochemical screening and the results were tabulated in Table 1.

Ethanol extracts of leaf showed only diterpene where as fruit extract showed resins and phenolic compounds, where as stem extract showed only alkaloids. Chloroform extracts of leaf, fruit and stem showed only resins. Aqueous extract of leaf showed alkaloids, resins, diterpene and flavonoids, where as fruit extract showed resins, phenols and diterpene, where as stem extract showed alkaloids, resins, phenols and diterpene. Acetone extract of leaf, fruit, stem showed alkolids, saponins, phenols, diterpene. Ethanol and acetone extract of leaf,fruit,stem showed the presence of alkaloids. Alkoloids also present in chloroform extract of leaf and stem. Phytosterols are present in chloroform extract of fruit and stem, phytosterols are also present in aqueous extract of leaf. Saponions are only present in acetone extract of fruit and stem. Resins and phenols are intermediately present .Both are absent in ethanol extract of leaf and stem. Diterpene are consistent acetone extract of leaf and stem but absent in fruit. Diterpene are also present in ethanol extract of leaf and fruit, it also consistent in aqueous extract of leaf, fruit and stem.

Table 2: Antibacterial activity of ethanol, chloroform, distilled water and acetone extract of fruit and standard antibiotics .

SOLVENT	*ZONE OF INHIBITION (In mm)*						
	1	**2**	**3**	**4**	**5**	**6**	**7**
E.coli	22	-	27	23	28	31	-
B.subtilis	22	-	-	28	25	29	-
P.aeruginosa	21	-	-	24	26	30	-
S.aureus	25	-	-	25	16	20	-

Keys:- 1-Ethanol; 2-Chloroform; 3-Distilled Water; 4-Acetone; 5-Streptomycin; 6-Spectinomycin; 7-Control.

Table 2 showed that the antibacterial activity of fruit extract of all four solvents showed maximum zone of inhibition (2.8cm) against *Bacillus* subtilis. The ethanol extract showed maximum zone of inhibition (2.5cm) against *S.aureus*.The ethanol extract showed equal zone of inhibition(2.2cm) with *E.coli* and *B*.subtilis. *P.aeriginosa* showed lower zone of inhibition (2.1cm). The maximum antibacterial efficiency was found to be present in acetone extract against *B*.subtilis. Acetone extract showed significant results against *E*.coli, *P. aerugenosa* and *S. aureus* with zone of inhibition of 2.3cm,2.4cm and 2.5cm.Chloroform extract has showed no activity against any of the pathogen. Aqueous extract showed efficacy against only *E.coli* and there is no zone of inhibition against other pathogens. Acetone extract showed higher zone of inhibition against *B.subtilis* and *S.aureus* compared to streptomycin and spectiomycin

Table 3: Antibacterial activity of ethanol, chloroform, distilled water and acetone extract of stem and standard antibiotics

SOLVENT	*ZONE OF INHIBITION (In mm)*						
	1	**2**	**3**	**4**	**5**	**6**	**7**
E.coli	-	12	18	20	28	31	-
B.subtilis	-	-	21	22	25	29	-
P.aeruginosa	-	-	-	21	26	30	-
S.aureus	-	20	-	16	16	20	-

Keys:- 1-Ethanol; 2-Chloroform; 3-Distilled Water; 4-Acetone; 5-Streptomycin; 6-Spectinomycin; 7-Control.

Table 3 showed that the antibacterial activity of acetone extract of stem showed maximum zone of inhibition (2.2 cm). The ethanol extract showed no inhibitory action against four pathogens. Chloroform extract exhibit activity only against *E.coli* and *S.aureus* with 1.2cm and 2.0cm zone of inhibition repectively. Acetone extract showed zone of inhibition against all the four pathogens in which *B.subtilis* and *P.aerugenosa* showed maximum zone of inhibion of 2.2cm and 2.1cm respectively. No activity was found to be against *P.aeruginosa* and *S.aureus* in chloroform extract of *A. marmelos*. Similar result was obtained from the antibacterial activity of *A. marmelos (Prema, 2004).*

Table 4: Antibacterial activity of ethanol, chloroform, distilled water and acetone extract of leaves and standard antibiotics.

SOLVENT		*ZONE OF INHIBITION (In mm)*					
	1	**2**	**3**	**4**	**5**	**6**	**7**
E.coli	-	10	14	12	28	31	-
B.subtilis	-	08	15	14	25	29	-
P.aeruginosa	14	-	-	14	26	30	-
S.aureus	10	-	-	11	16	20	-

Keys:- 1-Ethanol; 2-Chloroform; 3-Distilled Water; 4-Acetone; 5-Streptomycin; 6-Spectinomycin; 7-Control .

Table 4 showed that the antibacterial activity of leaves extract showed maximum zone of inhibition (1.5cm) against *B. subtilis*. Acetone extract showed positive results against all 4 pathagens. Similar result was obtained from the ethanol extract of *A. marmelos* showed antibacterial activity against *P.aeruginosa* and *S.aureus* (*Prema*, 2004).

CONCLUSIONS

From the results of antibacterial screening of four solvents (ethanol, chloroform, distilled water and acetone) used in this study, acetone was exhibited best antibacterial activity. Among the three parts of the plant used in the study fruit was considered as the most effective. Because it exhibited maximum zone of inhibition against all pathogens,may be due to the presence of alkaloids, phenols, flavanoids, Phytosterol and Diterpene. The inability of extracts of some selected parts to demonstrate any visible activity against some bacteria may probably be due to the low concentration of the extracts.

In this endeavor, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serves further global needs.The drugs derived from herbs may have the possibility of using in medicine because of its good antibacterial activity. Further research in this pursuit, focusing on the isolation of individual compounds and finally subjecting to clinical trails promises to open new avenues in the use of plants for therapeutic purpose.

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