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AEGELMARMELOS (L.) CORR.: EFFECTIVE DRUG YIELDING TREE AGAINST FUNGI

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ABSTRACT :

Aegelmarmelos (L.) Corr. a member of family rutaceae, Commonly called Bael(Bengal Quince) is a tall tree grows up to a height of 3 to 6 meters with long, stout thorns; young parts are pubescent. Leaves, fruits, bark and roots of the plants used in preparations of indigenous medicines. The parts of the plants are used variously as fruit pulp is used as a mild laxative,used to cure inflammation of the mucous membrane having a free discharge,recommended for the cure of asthma. Reduces or eliminates fever,promotes the removal of mucous secretions from the bronchial tubes for the abnormal accumulation of liquid in the cellular tissue accompanied with constipation and jaundice.

The juice of leaves is used orally to treat Jaundice, diabetesand eye disease. The poultice of leaves is used to treat eye diseases. Decoction of roots is anthelmintic and antipyretic. Powder of bark is used to control dysentery, diarrhea and dyspepsia. The fruits are most significant as the pulp or syrup of ripened fruit is gastro protective used in dyspepsia, debility and fever. It is also treats bleeding piles and constipation.Unripe fruits are astringent, stomachic and digestive. They are used to cure diarrhoea (Anonymous, 1948-76).Root extract is used against rabid dog bite, gastric trouble. Stem extract is used to cure dysentery and stomach disorder. Leaves are used for diabetes, jaundice, diarrhoea, dysentery and gastric troubles (Jain, 1991).Root bark extract, dried leaves are used to cure typhoid fever. Leaf extract is used as a remedy against night blindness, diabetes, male sterility, intestinal ulcer (Pawar and Patil, 2008).

In the present investigation the antimicrobial effect of***Aegelmarmelos***leaf andfruit extract was evaluated on bacterial strains like *Escherichia coli*, *Staphylococcus aureus*, *S. paratyphi, S. dysentry* and fungal strains like *Candida albicans*, *Fusariumstionifer, F. oxysporum*and*Aspergillusniger.* The solvent used for the extraction of plants were distilled water, methanol, alcohol, chloroform and acetone.

INTRODUCTION

Successful prediction of botanical compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent but present study showed that organic solvent and particularly methanol extracts of these plants were certainly much better and powerful. This may be due to better solubility of activity compound in organic solvent (de Boer *et. al.,* 2005).

MATERIAL AND METHOD:

a. Microbiological assay – To study efficacy of drug against micro flora related to human, the extracts of five plants were prepared in four different solvents of each using standard method (Chessbrough, 2000). The antibacterial assay was conducted by agar well diffusion method. (Perez, 1990). Antibacterial assay was performed using four different bacteria namely *Staphyllusaureus, Escherechia coli, Salmonella paratyphi, Shigella dysentery*. While antifungal assay was performed using four fungi namely *Aspergillusniger, Fusariumoxysporum, Candida albicans and Rhizopusstolonifer.*

b. Preparation of plant extracts - The plant materials were ground in a grinding machine in laboratory. 100 gm. of each paste was mixed with 1000 ml. of solvents like Chloroform, Methanol, Ethyl acetate and distilled water separately. The mixture kept for 24 h. in tightly sealed vessels at room temperature for maceration and stirred constantly on mechanical stirrer. The mixture was protected from sunlight to prevent loss of active components. This mixture was filtered through Whatmann No. 1 filter paper. The extracted liquid was subjected to evaporation in order to remove solvent. The semisolid extract produced was stored in an airtight container at 4^0C in refrigerator for further use. All the dried extracts were exposed U.V. rays (200-400 nm) for 24h and checked frequently for sterility on nutrient agar plates (Chessbrough, 2000).

c. Anti bacterial assay- The antibacterial assay was conducted by agar well diffusion method (Perez, 1990). Sterile molten Nutrient Medium was inoculated with 0.5 ml of inoculums. A well of 6 mm diameter were punched in Nutrient agar and filled with 50 μl solvent extracts. Plates were kept in refrigerator for 20 min for proper diffusion of extracts. Later on the plates were incubated at 37^0 C for 24 h. Microbial growth was determined by measuring diameter of zone of inhibition. For each bacterial strain control were maintained where pure solvents were used instead of the extract. The experiment was conducted three times and means values were considered. The results were compared with a standard antibiotic gentamycin (10 μg/m disc).

d. Antifungal assay – The assay was conducted by agar well diffusion method. The fungal strains grown of Potato Dextrose Agar (PDA) at 37^0 C for 24 h. were suspended in saline solution (0.85 % NaCl). Using Neubergh chamber microbial spores were adjusted to be uniform in each 10 ml of sample. The suspension was used to inoculate 90 mm diameter Petri dish containing 15 ml of PDA. Well of 6 mm diameter were punched in PDA agar and filled with 50 μl extracts. The control plates were also prepared with all solvents including distilled water. Plates were incubated at 37^0 C for 24 h. Antifungal activities were evaluated by measuring inhibition zone diameters. The experiment was conducted thrice.

RESULTS AND DISCUSSION:

Assay of antibacterial activity of plant extract:

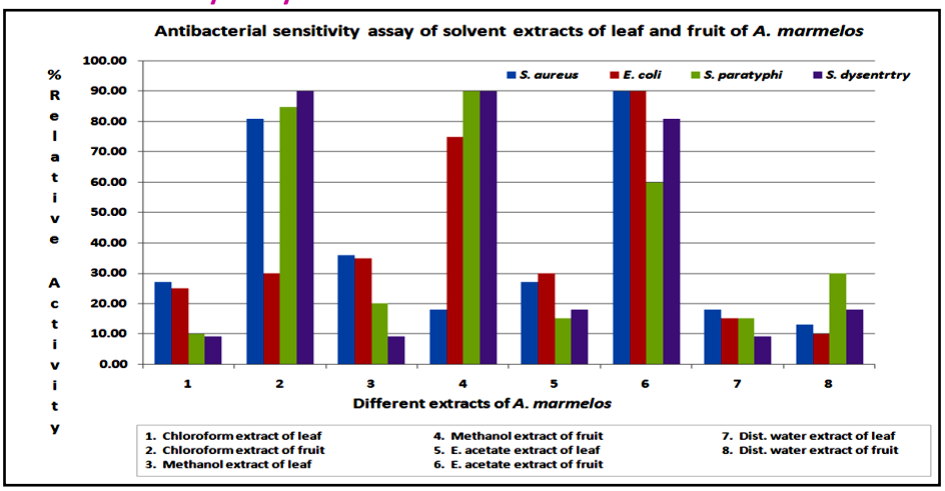
The antibacterial sensitivity assay of solvent extracts of leaf and fruit of *A. marmelos* against four pathogenic strains of bacteria showed following results.(Table 1 and Graph 1)

Table 1 - Antibacterial sensitivity assay of solvent extracts of leaf and fruit of *Amarmelos*

Sr. No.	Different extracts of *A. marmelos*	Dia. of Inhibitory zone (mm) / % inhibition			
		S. aureus	***E. coli***	***S. paratyphi***	***S. dysentery***
1	Chloroform extract of leaf	6 / 27	5 / 25	2 / 10	2 / 9
2	Chloroform extract of fruit	18 / 81	6 / 30	17 / 85	20 / 90
3	Methanol extract of leaf	8 / 36	7 / 35	4 / 20	2 / 9
4	Methanol extract of fruit	04 / 18	15 / 75	**18 / 90**	**20 / 90**
5	E. acetate extract of leaf	6 / 27	6 / 30	3 / 15	4 / 18
6	E. acetate extract of fruit	20 / 90	18 / 90	12 / 60	18 / 81
7	Dist. water extract of leaf	4 / 18	3 / 15	3 / 15	2 / 9
8	Dist. Water extract of fruit	3 / 13	2 / 10	6 / 30	4 / 18
9	Gentamycin	22	20	20	22

The methanolic extract of fruit exhibit maximum activity against *S. dysentery* (90%), moderate against *E. coli* (75%) and minimum against S. aureus and *S. paratyphi* (18%). The chloroform extract of fruit shows maximum activity against *S. dysentery* (90%), moderate against *S. aureus and S. paratyphi* (81, 85 %) and minimum against *E. coli* (30%). Ethyl acetate extract of fruit shows maximum activity against *S. aureus and E. coli* (90%) each, moderate against *S. dysentery* (81%) while lower activity against *S. paratyphi* (60%). Aqueous extracts of fruit showed however very less sensitivity towards all strains. It shows maximum activity against *S. paratyphi* (30%).

Graph: 1 Antibacterial sensitivity assay of solvent extracts of leaf and fruit of *A. marmelos*



DISUCSSION:

THE DIFFERENCE IN DEGREE OF ACTIVITY IN DIFFERENT TYPES OF SOLVENTS AGAINST THE SAME BACTERIAL STRAINS MAY BE DUE TO DIFFERENT SOLUBILITY OF ACTIVE SUBSTANCES PRESENT PLANTS. AS DIFFERENT SUBSTANCES OF PLANTS HAVE DIFFERENT ACTION, THE BACTERIAL STRAINS HAVE RESPONDED IN THE MANNER. THE CHLOROFORM EXTRACT OF LEAF SHOWED MAXIMUM INHIBITION OF GROWTH AGAINST *S. AUREUS AND E. COLI* (27,25 %), AND MINIMUM AGAINST *S. PARATYPHI AND S. DYSENTERY* (10,9 %). THE METHANOLIC EXTRACT OF LEAF SHOWED CONSIDERABLE LEVEL OF ACTIVITY AGAINST *E. COLI AND S. AUREUS* (35, 36 %), WHILE LESS EFFECTIVE AGAINST *S. PARATYPHI AND S. DYSENTERY* (18, 9 %). ETHYL ACETATE EXTRACT OF LEAF ALSO SHOWED MODERATE ACTIVITY AGAINST *S. AUREUS AND E. COLI* (27,30 %), AND MINIMUM ACTIVITY AGAINST *S. PARATYPHI* AND *S. DYSENTERY* (15,18 %). AQUEOUS EXTRACT OF LEAF SHOWED LESS ACTIVITY AGAINST *S. DYSENTERY* (9%), AND MODERATE AGAINST REMAINING THREE STRAINS OF BACTERIA, BETWEEN 15-18 %.

When the degree of activity was determined comparing with the activity of standard antibiotics. The fruit extracts of *A. marmelos*were found to be very effective as compared to leaf extracts and extracts of organic solvents were found to be more effective than aqueous extract. The fruit extract however, showed more or less equal activity against all forms of bacteria and found to be sensitive against all four strains, particularly against Gram-negative strains. (Photo plate 1)

Photographs of Antibacterial activity of *A. marmelos* *(Photo plate- 1)*

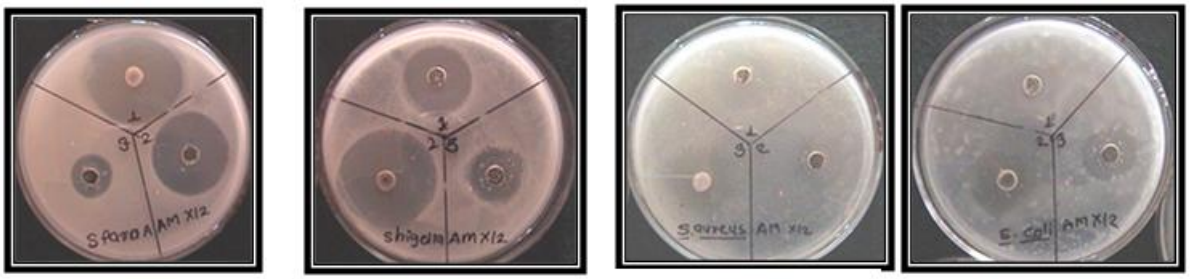


Table 2 Antifungal sensitivity assay of solvent extracts of flowers and seeds of *A. marmelos*

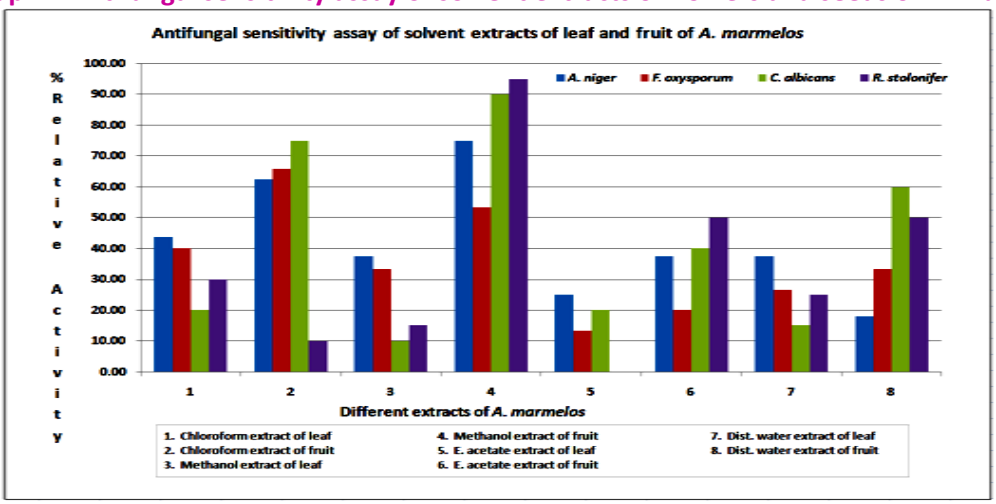
Sr. No	Different extracts of *A.marmelos*	Dia. of Inhibitory zone (mm)* / % inhibition			
		A.niger	***F.oxysporum***	***C.albicans***	***R. stolonifer***
1	Chloroform extract of leaf	14 / 43.75	12 / 40	04/ 20	06 / 30
2	Chloroform extractof fruit	14 / 62.5	20 / 66	15 / 75	18 / 10
3	Methanol extract of leaf	12 / 37.5	10/ 33.33	02 / 10	03 / 15
4	Methanol extract of fruit	18 / 75	16 / 53.3	18 / 90	19 / 95
5	E. acetate extract of leaf	08 / 25	04 / 13.3	04 / 20	00/00
6	E. acetate extract of fruit	12 / 37.5	06 / 20	08 / 40	10 / 50
7	Dist. water extract of leaf	12 / 37.5	08 / 26.6	03 / 15	05 / 25
8	Dist. water extract of fruit	09 / 18	10 / 33.3	12 / 60	10 / 50
9	Griseofulvin (5 mg/ml)	32	30	20	20

(Values are mean of three replicates; Diameter of cork borer is subtracted.)

The chloroform extract of fruit showed maximum inhibition against *R. stolonifer* (90%), moderate against *C. albicans* (75%) and minimum against *F. oxysporum* and *A. niger* (66, 62%). The methanolic extract of fruit showed most activity against *R. stolonifer* (95%), and *C. albicans* (90%), moderate against *A. niger* and least against *F. oxysporum* (53%). The fruit extract in ethyl acetate showed moderate inhibitory zones against *R. stolonifer* (60%), and less moderate against *C. albicans and A. niger* (40,37 %) and very less activity against *F. oxysporum* (20%). Aqueous extract of fruit seemed to be equally effective against *R. stolonifer and C. albicans* (60%), moderately active against *A. niger* and least active against *F. oxysporum* (33%).

In all methanolic extract of fruit was most effective as compared to other extracts and it was most sensitive for *R. stolonifer* and *C. albicans*. All fruit extracts were most effective against *R. stolonifer and C. albicans*while efficacy was less about *F. oxysporum and A. niger.*

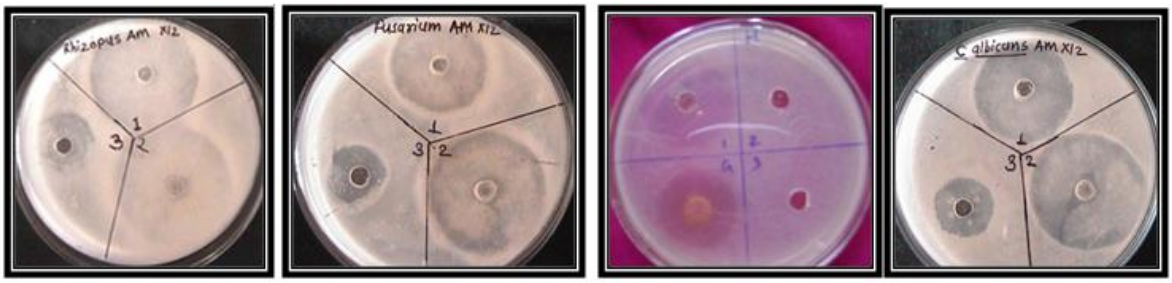
Graph 2:Antifungal sensitivity assay of solvent extracts of flowers and seeds of *A. marmelos*



The chloroform extract of leaf showed maximum inhibition against *A. niger* (43%), moderate in *F. oxysporum* (40%), and less in *C. albicans and R. stolo*nifer (30,40 %). The methanolic extract of leaf was also more or less effective against *A. niger and F. oxysporum* (37,33%), while it was least effective against *C. albicans and R. stolonifer* (10,15%). Ethyl acetate extract of leaf showed moderate inhibitory action against *A. niger* (25%), while its sensitivity was very less against *F. oxysporum* (13%), moderate against *C. albicans* (20%), and no effect against R. stolonifer. Aqueous extract of leaf however, showed moderate activity against *A. niger* (37%), *F. oxysporum* (26%), and *R. Stolonifer* (25%), and least active against *C albicans*

(15%). In all, the leaf extracts were sensitive against *A. niger and F. oxysporum*, while less sensitive to *R. stolonifer*. The fruit extracts of organic solvent showed marked antifungal activity. (Photo plate 2).

Photographs of Antifungal activity of *A.marmelos(Photo plate 2)*



CONCLUSION:

Phytochemical study of *A. marmelos* reveals presence of primary metabolites starch, proteins, fats, carbohydrates and secondary metabolites tannins saponnins, glycosides. Alkaloids, reducing sugars, in addition to inorganic constituents like calcium, sodium, potassium and iron. Due to presence of the secondary metabolites the fruits and leaf extracts in organic solvents found effective against pathogenic bacteria and fungi of this investigation.

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