

Evaluation of Bioactive Potential of *Aegle marmelos*

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There are several novel medicines which have been developed from plants and used for treating various diseases after proper pharmacological evaluation. This present study was carried to evaluate the bioactivity viz antibacterial, antioxidant and anticancer potential of ethanolic extract of *Aegle marmelos* which has been considered to be sacred plant. Ethanolic extract of leaves, fruit rind and fruit pulp were used for the bioactivity study. Among the various ethanolic extracts, ethanolic extract of fruit pulp showed a good antibacterial activity. Fruit rind ethanolic extract showed noticeable DPPH scavenging activity with IC 50 value of 159.37 µg /ml and anti-proliferating activity with an IC 50 value of 234.28 µg /ml.

Key words: Inhibition Concentration50 - IC50, DPPH, Antioxidant, antibacterial, *A. marmeloes*.

Past four decades, more number of the bioactive potential compounds are isolated from plant and marine sources^{1,2}. From ancient time herbal drugs were used in the form of poultices, powders teas etc and even in form of formulations or pure compounds. Thousands of plants are estimated to have medicinal value³. Plants active metabolites or modified metabolites have been used for treating diseases. Humans make use of medicinal herbs as such or their extracts since time unknown for treating many kinds of ailments and valuable drugs such as analgesics (morphine), antitussives (codeine), antihypertensives (reserpine), cardiotonics (digioxin), antineoplastics (vinblastine and taxol) and antimalarials (quinine and artemisinin) are isolated from medicinal plants^{4,5,6}. These properties evoked the interest of pharmaceutical companies and also researchers to develop or discover novel drugs⁷. FDA has approved several new drugs which are derived

from natural sources, which are made available in market during the period 2000–2005. These drugs include medicine for cancer, neurological, cardiovascular, metabolic and immunological diseases, and genetic disorders⁸. Worldwide market potential for drugs derived from herbal source was estimated to be around US\$40 billion⁹.

Aegle marmelos (L) corr. of the family *Rutaceae* has been used in ayurveda, the phytochemicals which are isolated with different solvents will yield different compounds which are used as ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates, bioactive principle and lead compounds in synthetic drugs. *Aegle marmelos* was found to have various therapeutic use such as treatment for Asthma, wound, swollen joints, high pressure, jaundice, Diarrhoea etc¹⁰. In different system of medicine such as Ayurveda, Siddha and Unani Beal was used for treatment of *Diabetic mellitus*¹¹.

In this study *Aegle marmelos* was collected and subjected for cold extraction. Thus obtained extract was used for evaluating antibacterial, antioxidant and anticancer activity.

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MATERIALS AND METHODS

Extraction

Leaves, fruit pulp and fruit rind of *Aegle marmelos* were collected, washed and shade dried. After drying, leaves, fruit rind and fruit pulp were ground into powder and subjected for cold extractions using ethanol.

Microorganisms

Overnight bacterial culture of *E.coli*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Proteus* sp were used for studying antibacterial activity.

Screening of Antibacterial Activities

Antibacterial potential of the ethanol extract was done by the well diffusion assay technique¹². Using a sterile cotton swab overnight bacterial culture was swabbed on the solidified nutrient agar in a sterile petriplate. Evenly spaced six wells of 5mm diameter were bored on the solidified nutrient agar using gel puncture kit. In each well 5, 10, 15, 20 µg of crude extracts were added. Ethanol was used as negative and antibiotic was used as positive controls. The inoculated agar plates were left in refrigerator for one hour for proper diffusion then plates were incubated, at 37°C for the bacteria for 24 h. The zone of inhibition was measured using a ruler and recorded in millimeters.

DPPH Radical Scavenging Assay

DPPH assay was performed following modified method of Brand-Williams et al¹³. 1ml of plant extracts was diluted serial to attain following concentrations (37.5, 75, 150, 300 µg/ml) which was added with 1 ml of 0.1mM DPPH solution and made up to 3ml using methanol. DPPH solution and methanol without any extract was used as control.

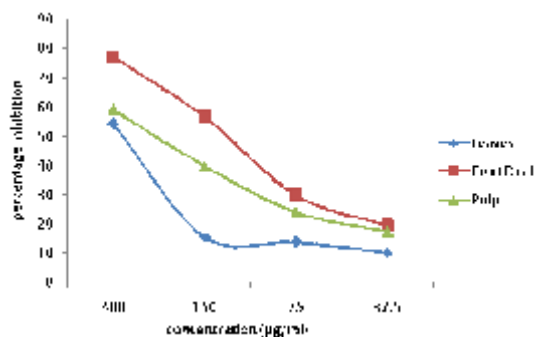


Fig.1. Antioxidant activity of ethanol extract of *A.marmelos* Leaves, Fruit rind and pulp

The reaction was carried out in triplicates and the absorbance was read using UV Vis spectrophotometer (Cary) at 517nm after 30 minutes of incubation in dark. The inhibition percentage was calculated using the following formula.

$$\text{Inhibition \%} = \frac{Ac-As}{Ac} \times 100$$

Ac - absorbance of the control, As - absorbance of the sample

The discoloration of sample was plotted against the sample concentration and the inhibition Concentration (IC₅₀) value was calculated.

Antiproliferative activity

Antiproliferative activity of crude extracts of *A. marmelos* against Vero (African green kidney cells) - normal cell line and Hep-2 (Liver cancer cell line) was determined by MTT assay¹⁴.

RESULTS AND DISCUSSION

The antimicrobial activity of Leaves, fruit pulp and fruit rind extracts of ethanol was assessed by agar plate diffusion technique against *B.subtilis*, *K.pneumoniae*, *E.coli*, and *Proteus* sp. All the extracts were exhibiting antibacterial activity, amongst all fruit rind extract showed greater activity against *Klebsiella pneumoniae*, *Bacillus subtilis* and *Proteus* sp (Table 1). Joshi *et al*¹⁵ also found that various extracts of Bael leaves, roots and fruits to have antibacterial activity against many bacterial strains.

Among all the three extracts used for analysis, fruit rind showed good antioxidant activity of 77.2% at 300µg/ml (Fig.1). The IC₅₀ value for all the extracts were calculated and found to be 292.69 µg/ml for leaf, 159.36 µg/ml for fruit rind and 234.15 µg/ml for pulp. DPPH scavenging

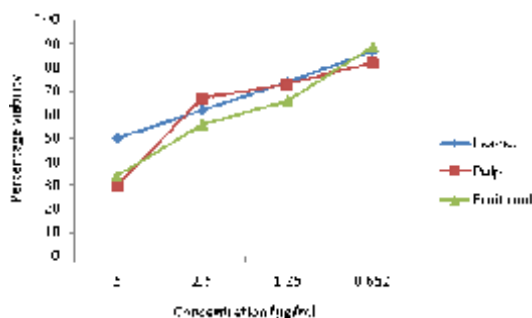


Fig. 2. Antiproliferative activity of ethanol extract of *A .marmelos* leaves against vero cell

activity of various medicinal plants have been reported earlier^{16,17} *A.marmelos* is also known to possess antioxidant activity which has been studied by TLC Bioautography¹⁸. The presence of phenolic compounds might be a major reason for its antioxidant activity, as phenolics compounds have got redox property and capable neutralizing free radicals like peroxides, singlet and triplet oxygens¹⁹. The presence of various other bioactive metabolites such as tannins, saponins etc might also be the reason¹⁸.

Anti -proliferative activity of the ethanol extracts of three different parts of *A.marmelos* plant was carried out and among the three ethanolic extract of fruit rind exhibited the highest cytotoxicity as compared to the other extracts used (Fig2,3).

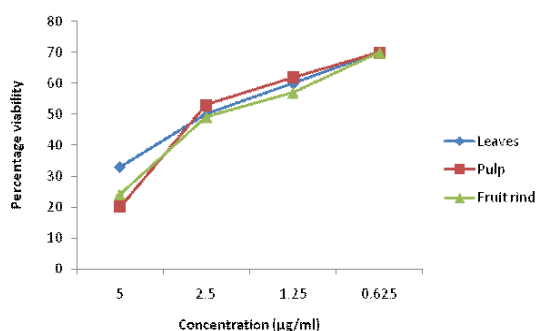


Fig. 3. Antiproliferative activity of ethanol extract of *A. marmelos* against Hep 2 cell line

Table 1. Antibacterial activity of ethanol extracts of Leaves, Fruit pulp and Fruit rind

Organism	Conc.	Fruit pulp	Fruit rind	Leaves
<i>Proteus</i> sp	20	0.9	0.7	0.4
	15	0.5	0.6	0.3
	10	0.5	0.4	0.3
	5	0	0.4	0.2
<i>Bacillus subtilis</i>	20	0.9	0.9	0.9
	15	0.7	0.8	0.8
	10	0.6	0.6	0.6
<i>Klebsiella pneumoniae</i>	5	0	0.4	0.4
	20	0.9	1.2	0.2
	15	0.6	0.8	0.2
	10	0.4	0.7	0.1
<i>E-coli</i>	5	0	0.5	0
	20	0.7	0	0
	15	0.6	0	0
	10	0.4	0	0
	5	0	0	0

Secondary metabolites like tannins in the extracts play a major role in cancer prevention and anticancer activity²⁰. In multistage carcinogenesis, reactive oxygen species takes a major part as it damages DNA and progresses the cancer stage²¹. *Aegle marmelos* possess both the antioxidant as well as anticancer activity, thus it would be a source for treating oxidative damage mediated carcinogenesis.

CONCLUSION

In the present study, ethanolic extract of Leaves, fruit rind and Fruit pulp of *Aegle marmelos* showed a good antimicrobial, antioxidant and anticancer activity.

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