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. Ethanol extract of leaves, fruit rind and fruit pulp were used for the bioactivity study. Among the various ethanol extracts, ethanol extract of fruit pulp showed a good antibacterial activity. Fruit rind ethanol extract showed noticeable DPPH scavenging activity with IC$_{50}$ value of 159.37µg /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 value$^1$. 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 (digoxin), 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 drugs$^7$. 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 disorders$^8$. Worldwide market potential for drugs derived from herbal source was estimated to be around US$40 billion$^9$.

*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 etc$^{10}$. 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.
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 technique\(^1\). 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 al\(^1\). 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.

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\(^1\) 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 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 assay\(^1\).

![Fig. 1. Antioxidant activity of ethanol extract of A.marmelos Leaves, Fruit rind and pulp](image1.png)

![Fig. 2. Antiproliferative activity of ethanol extract of A.marmelos leaves against vero cell](image2.png)
activity of various medicinal plants have been reported earlier\textsuperscript{16,17}. \textit{A.marmelos} is also known to possess antioxidant activity which has been studied by TLC Bioautography\textsuperscript{18}. 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 oxygens\textsuperscript{19}. The presence of various other bioactive metabolites such as tannins, saponins etc might also be the reason\textsuperscript{18}.

Anti-proliferative activity of the ethanol extracts of three different parts of \textit{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 (Fig 2,3).

Secondary metabolites like tannins in the extracts play a major role in cancer prevention and anticancer activity\textsuperscript{20}. In multistage carcinogenesis, reactive oxygen species takes a major part as it damages DNA and progresses the cancer stage\textsuperscript{21}. \textit{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 \textit{Aegle marmelos} showed a good antimicrobial, antioxidant and anticancer activity.

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