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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Evaluation of Free Radical Scavenging Property and Cytotoxicity Potential of Various Extracts of *Aegle Marmelos* Leaves

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Abstract : Free radicals are chemicals which possess one or more unpaired electrons that make them highly unstable and these molecules extract electrons from other molecule in order to become stable, which damages macromolecule. Human body too produces these free radicals continuously because of physiological and metabolic function. Thus produced free radicals damages biomolecules, such as protein, lipid, amino acids etc and induces cell injury which leads to numerous diseases. Antioxidant can delay effect of these free radicals and lowering the risk for various diseases. Plants are considered as good source of natural antioxidants and safer than synthetic antioxidants. In the present study, free radical scavenging property of five different solvent (Ethyl acetate, petroleum ether, acetone, chloroform and ethanol) extract of *A.marmelos* leaves was studied using DPPH scavenging activity. Further, cytotoxicity of the extracts were studied using *Artemia salina* as a model of study. IC50 value for antioxidant activity ranged between 30.11±0.75 to 54.13±1.8 which indicate good activity. LD50 for cytotoxicity ranged between 198.42 µg and 340.12 µg which indicate presence of potential phytochemicals.
Keyword : Antioxidant, cytotoxicity, *Aegle marmoles* , free radical scavengering, DPPH.

Introduction

Aegle marmelos (L.) Correa belongs to family Rutaceae with nativity of Southeast Asian countries[1]. Almost all of its parts has been used for ayurvedic medicine preparation among different part fruit posses most medicinal value and been used for various preparations. Oxidative stress induced free radicals damage biological macromolecules like DNA, Proteins and Lipids and leads to causes of various diseases[2]. As chemical derived antioxidants have got many side effects, natural derived compound is best option. Identifying natural antioxidants derived from plant source is the greater call of the hour. It has been reported that different phenolic compound present naturally in plant show antioxidant properties, which delays or restricts oxidative reactions attributed by free radical[3]. These natural derived compounds scavenge free radicals and it is choice to treat free radical associated disorders[4,5]. It was thought worthwhile to evaluate antioxidant activity of *A. marmelos* leaves to confirm its traditional medicinal value. In the present investigation, antioxidant activity of various extracts of *A. marmelos* leaves was assessed and its cytotoxicity range were analysed.

Experimental Work

Preparation of crude plant extract

Test plants were collected locally and dried. The dried plant materials were powdered using a mixer grinder. The cold extraction was done by soaking ground material in various solvents in the ratio (1:10), viz petroleum ether, acetone, chloroform, ethyl acetate and ethanol. The extracts were filtered using Whatman filter paper No.1 (Whatman Ltd., England). Thus obtained filtrates were evaporated and stored at 4^0C for further use. The stock solution of crude extracts (1 mg/ml) was prepared by dissolving a known amount of dry extract in methanol.

DPPH Radical Scavenging Assay:

The antioxidant activity of the extracts was measured on the using 1, 1- diphenyl 2-picrylhyorazyl (DPPH) method of Brand-Williams et al[6] with slight modifications. 1ml of plant extract solution of was diluted serial to attain following concentrations (35.25,62.5 125, 250,500, and1000µg/ml) which was added with 1 ml of 0.1mM DPPH solution and made up with 1ml of in methanol . Corresponding blank sample were prepared and L-Ascorbic acid was used as reference standard. 1ml of each methanol and DPPH solution without any extract was used as control. The reaction was carried out in triplicate and the absorbance was read using UV-Vis spectrophotometer (Cary) at 517nm after 30 minutes of incubation in dark. The inhibition % was calculated using the following formula.

Inhibition % = Ac-As/Ac×100

Ac - absorbance of the control, As - absorbance of the sample[7]

IC50 value

Inhibition Concentration (IC50) was determined[6] from the percentage of sample's antioxidant property plotted against the concentration of extract. It is denoted as the amount of sample required for reduction of the DPPH by 50 %.

Table 1 Lc 50 Values for Antioxidant Potential of Various Extracts 0f *A. Marmelos* Leaves

SL.No	EXTRACT	IC50(µg/ml)
1	Petroleum ether	35.89±0.31
2	Chloroform	38.8±0.41
3	Ethyl Acetate	30.11±0.75
4	Acetone	54.13±11.81
5	Ethanol	39.45±1.19

Bioassay of *A. Salina*

Samples were dissolved in 50 µl DMSO and made to 1mg/ml concentration in sea water. Serial dilution was done with sea water in the wells of 96-well microplates[8], triplicates were maintained for every concentration. Control wells with DMSO were included in each experiment. A suspension of *Artemia salina* containing 10 organisms/100µl sea water was added to each well and incubated at room temperature for 24 hours. Plates were observed and counted for number of dead/non-motile *Artemia salina* under microscope. LC_{50} values were then calculated.

Results And Discussions

In our present study, antioxidant properties of *A.marmelos* extracts (petroleum ether, acetone, chloroform, ethyl acetate and ethanol) were carried out using DPPH method. The antioxidant activities shown by various extract were represented in the Fig. 1. IC 50 value for antioxidant activity ranged between 30.11±0.75 µg/ml and 54.13±1.8 µg/ml, which indicates good activity. Among the crude extracts, acetone extract showed a maximum activity with IC 50 as 30.11±0.75 µg/ml. The antioxidant activity of *Aegle marmelos* might be due to prevention of free radicals complex which would have been a combined effect of phytochemical such as flavonoids and other polyphenolic compounds. The hydroxyls groups present in flavonoids are considered to possess free radical scavenging activity in plant[9,10,11].

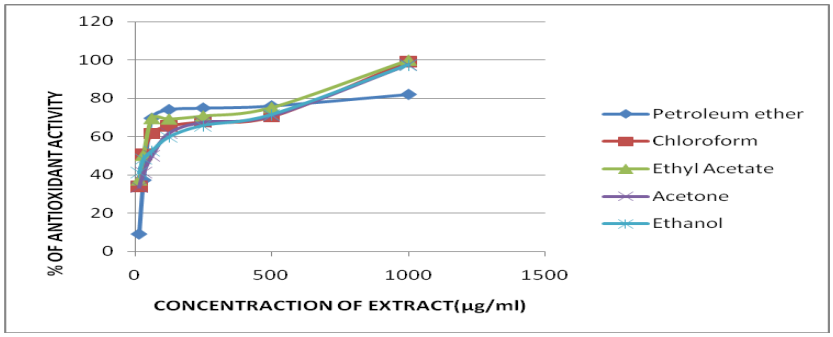

Fig. 1 Antioxidant Potential Of Various Extracts Of *A. marmelos*

Table 2 Lc 50 Values for Cytotoxicity of Various Extracts of *A. Marmelos* Leaves

SL.No	EXTRACT	IC50(µg/ml)
1	Petroleum ether	316.52
2	Chloroform	198.42
3	Ethyl Acetate	247.60
4	Acetone	340.12
5	Ethanol	240.69

The cytotoxic activity of various extracts of *A.marmelos* leaves were investigated against brine shrimp (*Artemia salina*) in vitro. The analysis using *Artemia salina* are quantitative, reproducible in respective of cytotoxicity analysis[12]. The cytotoxity potential for all the five was plotted against different concentration of extract are shown in Fig. 2. LD 50 was ranged between 198.42 µg and 340.12 µg concentrations (Table 2) which indicate presence of potential phytochemicals. All the crude extracts of *A.marmelos* leaves whose LC50 values is less than 200 µg/ml were considered as active against brine shrimp and these are needed to be further studied. Similarly, the cytotoxicity properties of extracts of *Aegle marmelos* leaves was reported using brine shrimp lethality assay[13,14].

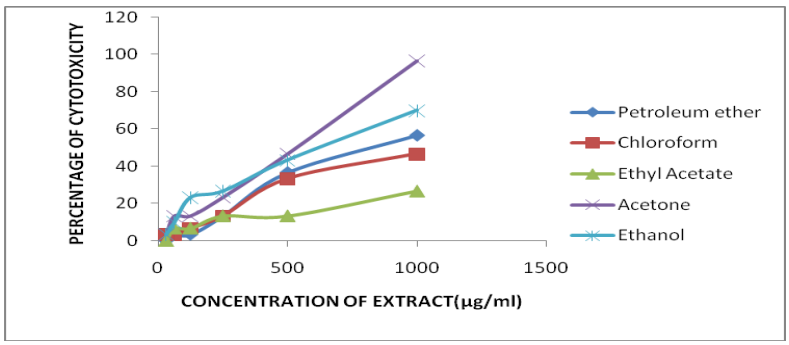

Fig 2 Cytotoxicity Potential Of Various Extracts Of *A. marmelos*

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