Research Article

ANTIOXIDANT AND PHYTOCHEMICAL STUDY OF *AEGLE MARMELOS* FRUITS AND ROOT

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ABSTRACT

The use of herbal heritage has become a part of general health care by the tribes since time immemorial .The use of modern medicine of synthetic origin is imparting dramatic results in a short span in the therapeutic field. Traditional medicines, chiefly obtained from plants have played a vital role in sustaining disease free human existence on this planet. The herbal drugs those show the antioxidant activity are very safe for use in the treatment of various diseases because they do not produce toxic effect in the human body but the allopathic drugs which use as antioxidant produce some toxic effects which may be very harmful for human beings. The aim of present research article is to perform antioxidant activity of one of the herbal drug *Aegle Marmelos,* commonly called as Bael fruits, which are used in traditional medicine and as a food throughout its range. In this research article, attempt is also done to perform the preliminary phytochemical study of *Aegle Marmelos.*

Keywords: *Aegle Marmelos*, Bael, Bengal quince, Phytochemical Screening, DPPH, Antioxidants.

INTRODUCTION

The herbal drugs those show the antioxidant activity are very safe for use in the treatment of various diseases because they do not produce toxic effect in the human body but the allopathic drugs which use as antioxidant produce some toxic effects which may be very harmful for human beings[1, 2]. The present work is based on selection of herbal drug *Aegle Marmelos* (Bael) for antioxidant activity. The main objective of this study was to focus on the antioxidant activity of *A. marmelos.*

- Carrying out preliminary phytochemical screening.
- Checking the antioxidant activity of plant by in vitro & in vivo method.

Introduction about Bael (*Aegle Marmelos*)[3, 4]

Morphology

It is a very beautiful medium sized tree (average 8.5m tall) with spines on the branches and very aromatic. It matures in about 60 years. Leaves are pulp green, trifoliate. Flowers are greenish white, sweetly scented. Fruits are 2.5 to 3.25 inches in diameter, globular or ovoid in shape, color greyish brown, outside surface hard and nearly smooth. Rind about 1/8 inches thick and adherent to a light red pulp, in which are ten to fifteen cells, each containing several woody seeds. It has a faint aromatic odor and mucilaginous taste.

Distribution

Aegle marmelos is a fruit-bearing tree indigenous to dry forest on hills and plains of central and southern India, Silence, Myanmar, Pakistan, Bangladesh, Nepal, Vietnam, Laos, Cambodia and Thailand. It belongs to the family Rutaceae, related to citrus. Aegle marmelos has been used as an herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India, Bangladesh and Sri Lanka.

Pharmacological Studies

The different pharmacological studies of Aegle marmelos were done and it reveals antibacterial[13], antidiarroheal, antifungal, antiulcer, anticancer, antiviral, anti-inflammatory, analgesic, antipyretic, antifertility as well as antihyperglycemic[12] effect. Some more pharmacological studies on Aegle marmelos also reveal antidyslipidemic, antithyroid as well as antioxidant effects.

MATERIALS AND METHODS

Collection of the plant material

The fruit and root of plants of *A.marmelos* (family – Rutaceae) was collected from Botanical Garden of N. B.R. I. (National Botanical Research Institute), Lucknow, India in month of august 2009.

Authentication of plant material

The plant materials were authenticated by Dr. Ch.V. Rao, Scientist N.B.R.I. (National Botanical Research Institute) Lucknow, (U.P.) India.

Chemicals used

Gallic acid, TBA, TCA, DPPH, Deoxyribose and Ascorbic acid.

Chemicals were purchased from Sigma and Aldrich (St. Louis, MO) and other chemicals and solvent were purchased from Merck chemicals Mumbai.

Animals used

Sprague-Dawely rats **(**140-180 g) of ten were purchased from the Central Drug Research Institute, Lucknow. They were kept in departmental animal house in well crossed ventilated room at 27 ± 2^0 C, and relative humidity 44-56%.

Methods

Preparation of 50% EtOH Extract of A. marmelos

Fruit (200 g) were washed with distil H_2o to remove dirt and soil, and were properly dried in shade for 4-7 days then dried in tray drier, and the Root (500 g) were also washed dried in shade for 7-10 days then dried in tray drier, maintained at 40^0 C. After drying, the plant materials were crushed and milled to powder and passed through the sieve (mesh size 40). The powdered materials were mixed in 50% ethanol solution (EtoH) for two days. The extract was separated by filtration and concentrated on rota vapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure to obtain solid residue (fruit yield- 3.2% and root yield- 1.6%w/w).

Methods for antioxidant activity of Aegle Marmelos (fruit and root) Extract[6, 7, 8, 11]

Basic principle of antioxidant activity

Various environmental, physical and chemical stresses on cells may induce either an overproduction of ROS (Reactive Oxygen Species) or a deficiency of antioxidant enzymes. ROS are responsible for various cellular anomalies like protein damage, deactivation of enzymes, alteration of DNA and lipid peroxidation which in turn leads to pathological conditions like carcinogenesis, reperfusion injury, rheumatoid arthritis, diabetes etc. The regular intake of antioxidants seems to limit or prevent the dangerous effects caused by ROS. Thus, to maintain cellular health, it is important to have a specific and effective antioxidant that scavenges multiple types of free radicals so that it can be used in multiple diseases.

In vitro antioxidant activity of A. marmelos (fruits and root) [9]

Estimation of Total Phenolic content (TPC)[10]

TPC was analyzed the Folin-Ciocalteu colorimetric method using Gallic acid as standard developed by Ragazzi & Veronese (1973) with modification and expressed as mg/ g gallic acid equivalent (GAE) on dry weight basis. The 25 mg plant extract was dissolved in 10 ml of 50% MeOH: H_2o (1:1) at room temperature and in its 1.0 ml. 1.0 ml of folin's reagent (1N) and 2.0 ml of sodium carbonate (20%) were added subsequently. The test mixture was mixed properly on cyclomixer, left at room temperature for 30 minute and maintained to 25 ml with distilled water. The absorbance of text mixture was measured at 725 nm. The reported TPC were expressed as Gallic acid equivalent (GAE) mg/g.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

Principle

DPPH reduction can be measured directly in the reaction medium by a continuous spectrophotometry assay. DPPH assay give reliable information concerning the antioxidant ability of the tested compound.

Procedure

Aliquots of 20-100 µl (5 mg/10 ml) was added to 2.9 ml of freshly prepared solution of DPPH (6×10^{-5}M in MeOH). After incubation at 37^0 C for 1 hour the absorbance was recorded at λmax 517 nm using spectrometer.

Formula

% inhibition or Scavenging activity of DPPH = (1- B/A) %

Where, B = Absorbance taken by control solution

A = Absorbance taken by different concentration of solution

Hydroxyl radical scavenging activity

Principle and methods

The hydroxyl radical scavenging activity[5] of test compounds were determined using the Fenton reaction. This reaction was started by mixing Ferrous ion (Fe) with hydrogen peroxide (H_2O_2) in solution. The generated hydroxyl radical was measured as a spin adduct of PBN and as CLA-dependent chemiluminescence (CL), respectively. Scavenging activities were expressed as 50% inhibition concentration (IC).

Reaction mixture

Ascorbic acid	– 50 µM
Ferric chloride	– 20 µM
EDTA	- 2 µM
Hydrogen per oxide	- 1.42 mM
Deoxyribose	- 2.8 mM

Above chemicals dissolved in 100 µl distilled water.

Procedure

With different concentration of *A.Marmelos* (fruits & roots) extract in a final volume of 1 ml in potassium phosphate buffer (10 Mm, pH-7.4). It was incubated at 37°C for 1 hour and 1 ml of 2.8%TCA & 1% TBA was added. Mixture was heated in a boiling water bath for 15 minute, after cooling absorbance was taken at λ_{max} 532nm.

Scavenging of Hydrogen peroxide (H_2O_2)

Principle

Hydrogen peroxide assay- The assay for hydrogen peroxide in fruit & root extracts of *A.marmelos* was

carried out following procedures. The antioxidant capacity of fruit extract against H_2O_2 value was expressed as mmole of ascorbate equivalent per kg fresh weight.

Procedure

0.6 ml of 4 mM hydrogen per oxide (in phosphate buffer solution) was added to 4 ml solution of fruits & roots of *A.marmelos* and incubated for 10 minute. The absorbances of the solutions were measured at λ_{max} 230 nm against a blank solution containing hydrogen per oxide without extract.

Measurement of lipid peroxidation

The degree of lipid peroxidation was assay by estimating the TBA (thiobarbituric acid) reactive substances.

Principle

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage.

This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lies methylene -CH_2- groups that possess especially reactive hydrogen's. As with any radical reaction the reaction consists of three major steps: initiation, propagation and termination.

Procedure

Different concentration of extract (50-200 µg/ml) was added to 500 µl liver homogenate (2 g liver).Lipid peroxidation was initiated by adding 50 µl of 15mM ferrous sulphate solution to liver homogenate. After 30 minute, 100 µl of this reaction mixture was taken in a tube containing 1.5 ml of 10% TCA. After 10 minutes tube will be centrifuged and supernatant was separated and mix with 1.5 ml of 0.67%TBA in 50% acetic acid (pH-3.5). The mixture was heated in a hot water bath at 85°C for 30 minute. The intensity of colored complex formed was measured 532 nm. The percentage of inhibition of lipid peroxidation was calculated by comparing the results of test with those of control not treated with extracts.

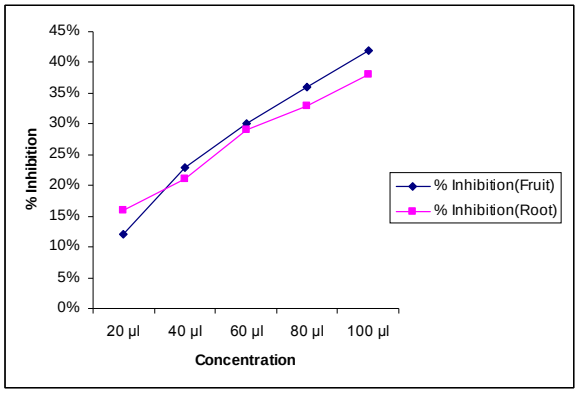

Figure 1: % inhibition of DPPH by *A.marmelos* (fruit & Root)

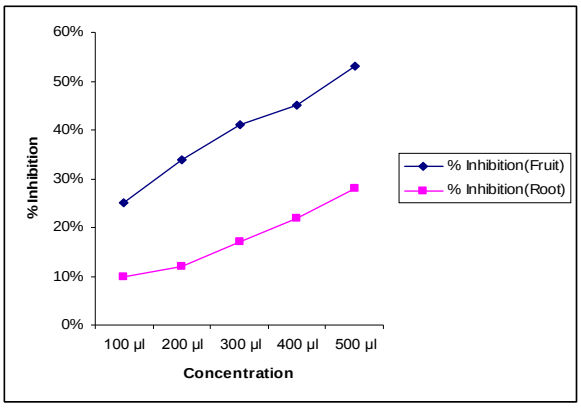

Figure 2: % inhibition of hydroxyl radical by *A.marmelos* (fruit & Root)

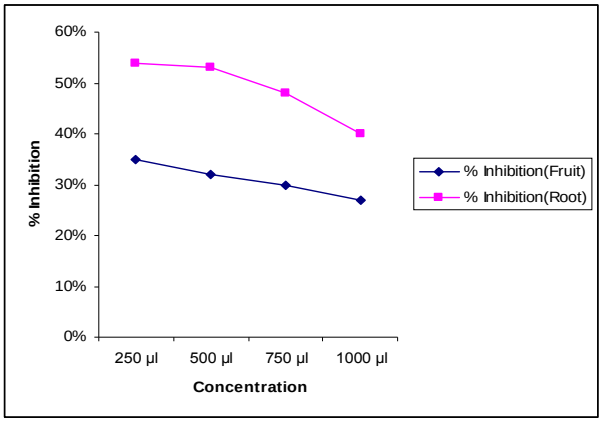

Figure 3: % inhibition of free radical production by H_2O_2 of A.marmelos (fruit & Root)

High Performance Thin Layer Chromatography (HPTLC) Analysis

Method of Chromatographic Fingerprint

Sample preparation: 50 mg extract of fruits & Roots were dissolved in the 1ml ethanol separately. These solutions were used for HPTLC profile.

Standard solution of umbelliferone: Dissolve 10 µg of umbelliferone in 10 µl of ethanol.

HPTLC Fingerprint of Crude Samples and Formulations
Stationary Phase: HPTLC plate (10 cm×10 cm; Merck; batch number 0B255473 and catalogue number is 1.05554.0007).

Relative Humidity: 55 ± 5% (pre-equilibrate twin-trough chamber with the mobile phase for 30 min prior to analysis).

Mobile Phase: Toluene– ethyl acetate – Formic acid (8:2:0.01 *v/v/v*).

Detection: The photographs were taken by Camag reprostar 3 video documentation unit under UV 254nm and UV 366 nm light.

Densitometric Scanning: The densitometry was performed on Camag TLC scanner III with Wincats 3.2.1 software in absorbance mode at 320 nm for umbeliferone. The source of light was deuterium beam.

In vivo antioxidant activity of A. marmelos (fruits & roots)

The animals (Sprague-Dawely rats 140-180 g) were divided into five groups of four animals each.

Group-1 Control animals received 1% carboxy methyl cellulose in distilled water (10ml/kg body weight) orally and this served as solvent control for 14 days.

Group-2 Animal received carbon tetra chloride (CCl_4) 2 ml/kg with olive oil (1:1) ratio alternate day.

Group-3 Animal received carbon tetra chloride (CCl_4) and 50% EtOH fruit extract of *A.marmelos* (100 mg/kg) for 14 days.

Group-4 Animal received carbon tetra chloride (CCl_4) and 50% EtOH fruit extract of *A.marmelos* (200 mg/kg) for 14 days.

Group-5 Animal received carbon tetra chloride (CCl_4) and Gallic acid for 14 days.

After completion of the treatment, isolate the liver washed in saline solution, weighed and homogenized. The liver homogenate were used for the biochemical analysis.

Table 1: Effect *A. Marmelos* extract on lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) in CCl_4 induced Rat in 14 days.

Treatment/dose	LPO (MDA/g tissue/min)	SOD(U/mg)	CAT(U/mg)
Control	0.46 ± 0.06	115.46 ± 2.40	26.14 ± 2.12
CCl_4	6.54 ± 0.38	52.32 ± 3.50	8.06 ± 0.42
AME(100 mg/kg)	4.28 ± 0.32	64.64 ± 2.82	16.14 ± 1.03
AME(200 mg/kg)	2.50 ± 0.26	80.22 ± 4.20	18.29 ± 0.88
Gallic acid	0.82 ± 0.06	109 ± 2.88	24.32 ± 0.72

RESULTS AND DISCUSSION

Percentage yield of *A.marmelos* with 50% ethanol

Fruit raw material - 200 gram

Extract obtained - 6.4 gram

% Yield of Fruit - 3.2%

Root raw material - 500 gram

Extract obtained - 8 gram

% Yield of Root - 1.6%

Preliminary Phytochemical screening

The phytoconstituents were identified by chemical tests, which showed the presence of various phytoconstituents in 50% ethanolic extract of *A. Marmelos* presented in Table no1.

HPTLC analysis of *A.marmelos* fruits, roots

HPTLC: Profile of 50% extract *Aegle marmelos*

Solvent system: Toluene: ethyl acetate: formic acid (8:2:0.01)

Visualization: Observe the developed plate under UV light at 254 nm & 366 nm after spraying with test solution.

Scan wavelength: 254 nm and 366 nm

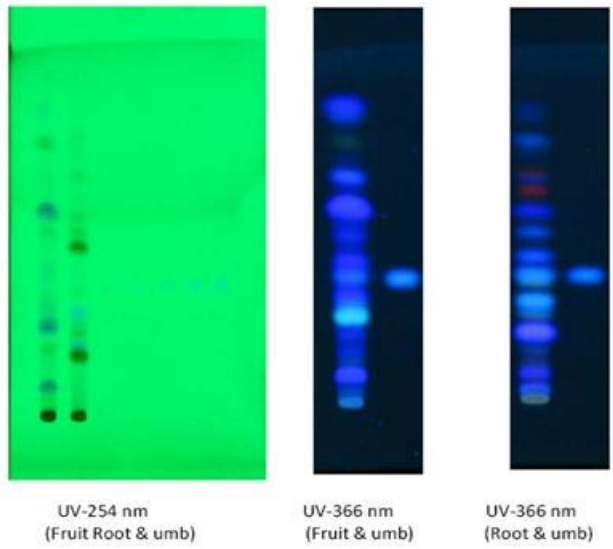

Figure 4: HPTLC Fingerprint profiling of fruit and Root extract of *A. Marmelos* along with the umbelliferone standard marker

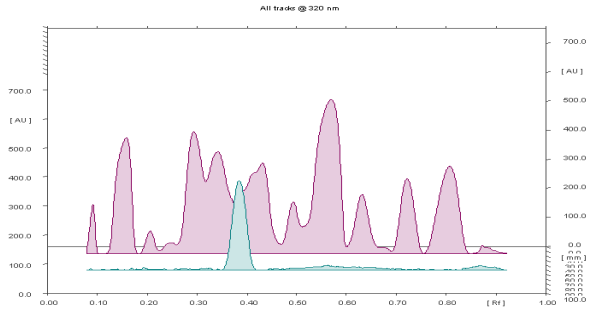

Figure 5: Graphical HPTLC finger print profile of Fruit extract of *A. Marmelos* and Umbelliferone

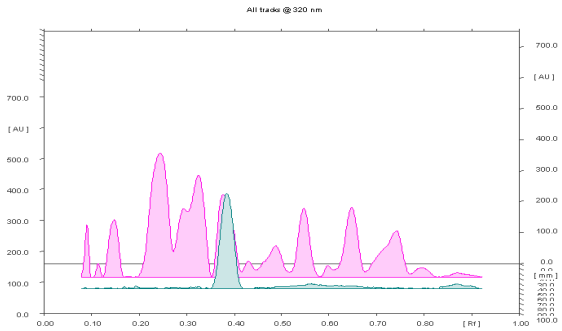

Figure 6: Graphical HPTLC finger print profile of Root extract of *A. Marmelos* and Umbelliferone

CONCLUSION

When CCl_4 & AME (100 mg/ kg & 200 mg/kg) received animals LPO level decreases but SOD and CAT enzyme activity increases. So overall result according to above table free radical production was less. AME has more scavenging activity.

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