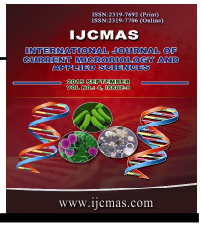
Int.J.Curr.Microbiol.App.Sci (2015) 4(9): 826-830

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 9 (2015) pp. 826-830**
http://www.ijcmas.com



Original Research Article

Phytochemical Analysis and Antioxidant property of *Aegle marmelos* Extracts

Anjay Kumar Gupta*, Sumeet Verma and Nitin Doshi

Dr. C.V. Raman University, Kota, Bilaspur, Chhattisgarh, India
**Corresponding author*

K e y w o r d s

Aegle marmelos, Antioxidants, Phytochemical, Petroleum benzene, Ethanolic Extract

A B S T R A C T

The present work deals with the phytochemical screening of *A. marmelos* (L.) and assay of antioxidant property of the plant. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce degenerative disease risk *Aegle marmelos* (L.) (Common name: Belpatra) was the medicinal plant taken for the current study. Chemical tests were performed for phytochemical screening and DPPH is used for antioxidant assay. Flavnoids, Alkaloids and Terpenoids phytochemicals were found in *Aegle marmelos* extract. Alcoholic extract of leaves of *A. marmelos* (L.) presented the highest scavenging activity at all concentrations.

Introduction

Plant's secondary metabolites have been of interest to man for a long time due to their pharmacological relevance (Arora, Kaur & Kaur, 2003).

Oxidation is a natural process in organisms for the production of energy to fuel biological cycles. Conversely, the uninhibited production of oxygen-derived free radicals, this is involved in the onset of many diseases such as arthritis, atherosclerosis, rheumatoid and cancer as well as in many degenerative diseases related with aging (Halliwell *et al.*, 1984). Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources.

These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. The unripe and ripe fruits of *A. marmelos* (L.) are bitter, acrid, sour, astringent, digestive and stomachic and are useful in diarrhea, dysentery and stomachalgia (Khanna *et al*., 1991). Stem bark is used in fever.

Materials and Methods

The leaf part of *A. marmelos* (L.) was collected and dried under shade (Harborne, 1973). This dried material was mechanically powdered, sheaved and stored at a dry place. This powdered material was used for further phytochemical, antimicrobial and antioxidant analysis.

Preparation of Fat sample and Plant Extract (Successive solvent extraction)

Collected leaves of *A. marmelos* (L.) were weighed prior to drying. The dried sample was then crushed and defatted with petroleum benzene using Soxhlet apparatus till the sample becomes colourless. Defatting was carried out at 40°C and Whatman filter paper No. 1 was used for carrying the sample. Petroleum benzene and fat solution is removed from the flask and petroleum benzene was finally evaporated at 40^0 C. The fat sample obtained was weighed and stored in airtight container in refrigerator. The defatted leaves' sample was then soxhleted with ethanol at 50°C. The liquid ethanolic extract of *A. marmelos* (L.) was then evaporated at 40°C till ethanol gets evaporated completely. The extract sample was also weighed and stored in airtight container in refrigerator for future use.

Qualitative phytochemical analysis

The fat and plant extract samples were analyzed for the presence of alkaloids, terpenoids, tannins, flavonoids, saponins, and steroids (Adetuyi *et al*., 2001)

Antioxidant activities

1, 1-Diphenyl-2-picrylhydrazyl

DPPH is used for antioxidant assay. It was obtained from HiMedia Lab. Pvt. Ltd., India. LOT no. 67714.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The scavenging activity of *A. marmelos* (L.) leaf extracts on DPPH was determined using the method described by [Choi, C.W.; Kim *et al*]. This method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine. The determination of the disappearance of free radicals was done using spectrophotometer [Abdel-Hameed *et al*]. The remaining DPPH which showed maximum absorption at 518 nm was measured. Each plant extract sample's stock solution (1.0 mg/mL) was diluted to final concentrations of (0.5, 0.4, 0.3, 0.2, and 0.1) mg/mL, in ethanol.

One mL of a 0.3 mM DPPH ethanol solution was added to 2.5 mL of sample solution of different concentrations. These are test solutions. One ml of ethanol was added to 2.5 mL of sample solution of different concentration.

These are blank solutions. One mL DPPH solution plus 2.5 mL of ethanol was used as a negative control. The blank for this solution is ethanol. As DPPH is sensitive to light, it is exposed to the minimum possible light.

These solutions were allowed to react at room temperature for 30 minutes. The absorbance values were measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

Scavenging capacity (%) = 100 – [(absorbance of sample – absorbance of blank) × 100/absorbance of control]

Same procedure was applied with Ascorbic acid and data was compared through plotting a graph.

Results and Discussion

The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution loses color stoichiometrically depending on the number of electrons taken up. DPPH was used to determine the proton radical scavenging action of ethanolic extracts of the leaves of *A. marmelos* (L.), because it possesses a proton free radical and shows a characteristic absorbance at 517 nm. The alcoholic extract of the leaves of *A. marmelos* (L.) reduces the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principles. A decrease in the concentration of DPPH radical was observed due to the scavenging ability of the soluble constituents in the ethanolic extract of the leaves of *A. marmelos* (L.) and the standard ascorbic acid is taken as a reference compound. Alcoholic extract of leaves of *A. marmelos* (L.) presented the highest scavenging activity at all concentrations. The highest activity was found to be 95.3% at 0.5mg/ml concentration of alcoholic extract of the leaves of *A. marmelos* (L.), whereas 93.7% of scavenging activity was shown by ascorbic acid at the same concentration.

Table.1 Observation table for phytochemical screening

Phytochemical	Fat sample	Defatted sample
Alkaloids	+	+
Flavonoids	+	+
Tannins	-	-
Saponins	-	-
Terpenoids	+	-
Cardiac glycoside	-	-

Table.2 Observation table for antioxidant activity of ascorbic acid

S. No.	Concentration (mg/ml)	O.D. (Blank - Test)	% Scavenging capacity
1	0.1	0.05	68.75
2	0.2	0.04	75.00
3	0.3	0.03	81.25
4	0.4	0.02	87.50
5	0.5	0.01	93.75
6	Control	0.16	0

Table.3 Observation table for antioxidant activity of plant extract

S. No.	Concentration (mg/ml)	O.D. (Blank - Test)	% Scavenging capacity
1	0.1	0.17	73.43
2	0.2	0.13	79.68
3	0.3	0.11	82.81
4	0.4	0.06	90.62
5	0.5	0.03	95.31
6	Control	0.64	0

Graph.1 Comparative DPPH scavenging activity

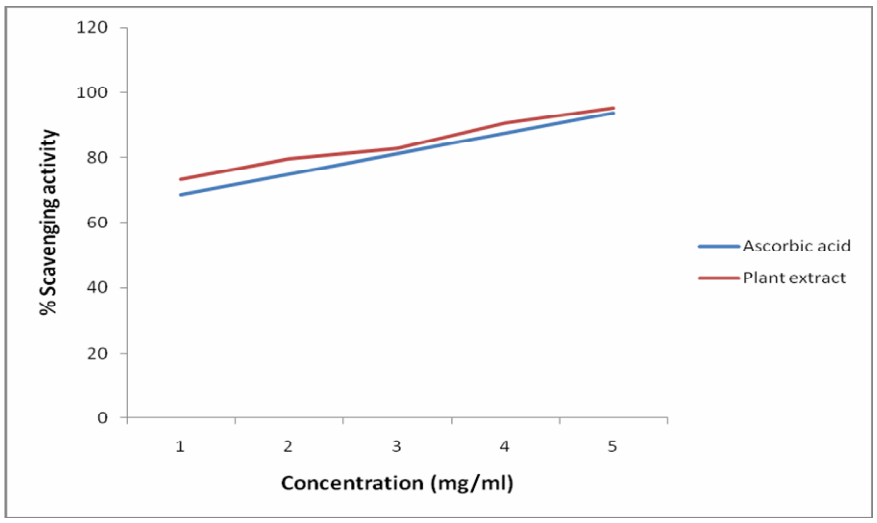


Fig.1 Defatted extract showing positive result for Flavonoids

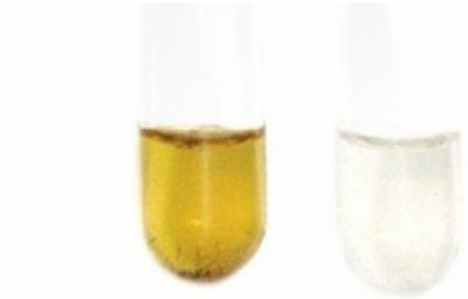


Fig.2 Fat extract showing positive result for Flavonoids

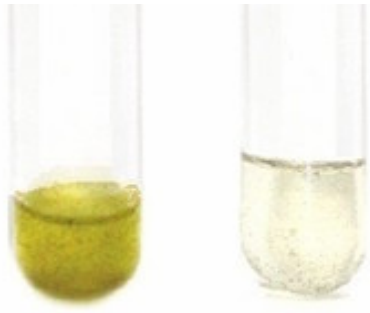


Fig.3 Comparative defatted & fat extraxt Sample showing negative & positive test Respectively for Terpenoids

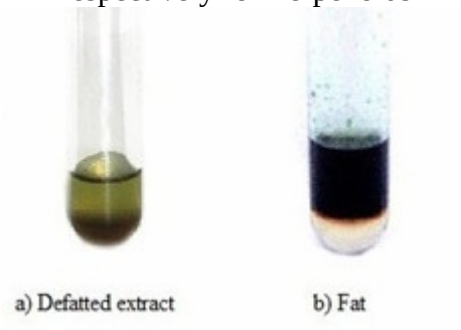


Fig.4 Comparative defatted extract & Fat sample showing positive test for Alkaloids (Mayer's test)

Abbreviations

DPPH - 1, 1-Diphenyl-2-picrylhydrazyl

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