IN VITRO ANTIOXIDANT ACTIVITY OF GALLS OF *PISTACIA INTEGERRIMA*

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Summary

Free radicals are implicated for several diseases including diabetes mellitus, arthritis, cancer, ageing, liver disorder etc. In treatment of theses diseases, antioxidant therapy has gained an utmost importance. Current research is now directed towards finding naturally occurring antioxidant of plant origin. *Pistacia integerrima* (Anacardiaceae) showed presence of phenolics, flavonoids, carbohydrates and volatile oils in preliminary phytochemical screening. The Co-TLC of methanol extract and ethyl acetate fraction of methanol extract confirmed presence of Gallic acid, and Quercetin which are known to possess antioxidant activity. Methanol extract and the ethyl acetate fraction of methanol extract of *P. integerrima* were subjected to determination of antioxidant activity by DPPH free radical activity, reducing power assay, scavenging of hydroxyl radicals etc.

Key words: Pistacia, Antoxidant, Anacardiaceae, Gallic acid

Introduction

Reactive oxygen species (ROS) are various forms of activated oxygen, which include free radicals such as superoxide ions, and hydroxyl radicals, as well as non free-radical species such as hydrogen peroxide (1, 2). In living organisms various ROSs can be formed in different ways, including normal aerobic respiration, stimulated olymorphonuclear leukocytes and macrophages, and peroxisomes. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (3, 4, 5). Reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart diseases, stroke, atherosclerosis, diabetes and cancer. When produced in excess, ROS can cause tissue injury. Plants and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (6).Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities(7).

Pistacia integerrima (Anacardiacea) leaf galls, commonly known as Kakadshingi are one of the appendages of plant. Its medicinal uses are diverse and used in cough, asthma, fever and respiratory disorders. It is reported to be useful in liver disorders. (8). Galls are used as tonic and stimulant. Galls are used in form of decoction or lotion as gargles to suppress haemorrhage from gums. It is also used to suppress bleeding from nose. Hakims consider galls useful in pulmonary infections, diarrhea and vomiting (9). *Pistacia integerrima* showed the presence of Gallic acid, Quercetin, leuteolin and chebulenic acid (10, 11). The Galls are used in some of the ayurvedic formulations like '*Chvyanprash avaleha'*, '*KumariAsava',* '*KumariKalp'* (12) etc. prescribed in weakness as rejuvenating agent and tonic. The use of leaf galls of *P. integerrima* as rejuvenator may be attributed to antioxidant property due to presence of phenolics and flavonoids.

Materials and methods

Plant material

Pistacia integerrima leaf galls were purchased from local market of Pune, India and authenticated at Regional Research Institute (Ayurveda), Pune.

Chemicals

Nitroblue tetrazolium (NBT), EDTA, sodium nitropruside, thiobarbituric acid, L ascorbic acid were purchased from Qualigens, India. DPPH was purchased from Sigma Aldrich, US. All the chemicals and solvents used were of analytical grade. Quercetin and Gallic acid was purchased from Hi media.

Preparation of extract

Course powder (500 g) of the leaf galls was macerated for overnight in methanol (1.5 L). The extract so obtained was concentrated in a rotary vacuum evaporator and then placed in a desiccator. The Yield was 21.5% w/w of methanol extract (ME). Methanol extract was further fractionated using ethyl acetate (EAFME) by subjecting methanol extract to column chromatography (Silica gel G 60-120) and eluting with ethyl acetate. The yield was 8.45%.

Estimation of total phenolic and flavonoid content

The phenolic and flavonoid content of ME and EAFME of *P. integerrima* was determined by the reported methods (13, 14).

In vitro antioxidant activity

DPPH free radical scavenging activity (15)

Free radical scavenging potential of ME and EAFME was tested against a methanolic solution of DPPH. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3 ml of different concentrations (5-400 µg/ml) of ME and EAFME in methanol. It was incubated at room temperature for 30 min and absorbance was measured at 517 nm against the corresponding blank solution. Ascorbic acid was taken as reference. Inhibition of DPPH free radical was calculated by following equation

% scavenging activity = [(Ac- As)/ Ac] x 100

Ac is absorbance of control reaction, As is the sample. The antioxidant activity is expressed as IC_{50}.

Reducing power assay (15)
The different concentration of ME and EAFME (5-60 µg/ml) in 1 ml deionized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5ml). The mixture was incubated at 50°C for 20 min. Trichloro acetic acid (2.5 ml, 10%) was added . it was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%)and absorbance was measured at 700 nm. Ascorbic acid was taken as standard.

Scavenging of hydrogen peroxide (16)
A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by recording the absorbance at 230 nm. Different concentrations of ME and EAFME (5-100 µg/ml) in distilled water were added to hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance was measured at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The % scavenging was calculated by
% scavenging activity = [(Ac- As)/ Ac] x 100
Ac is absorbance of control reaction, As is absorbance of sample.

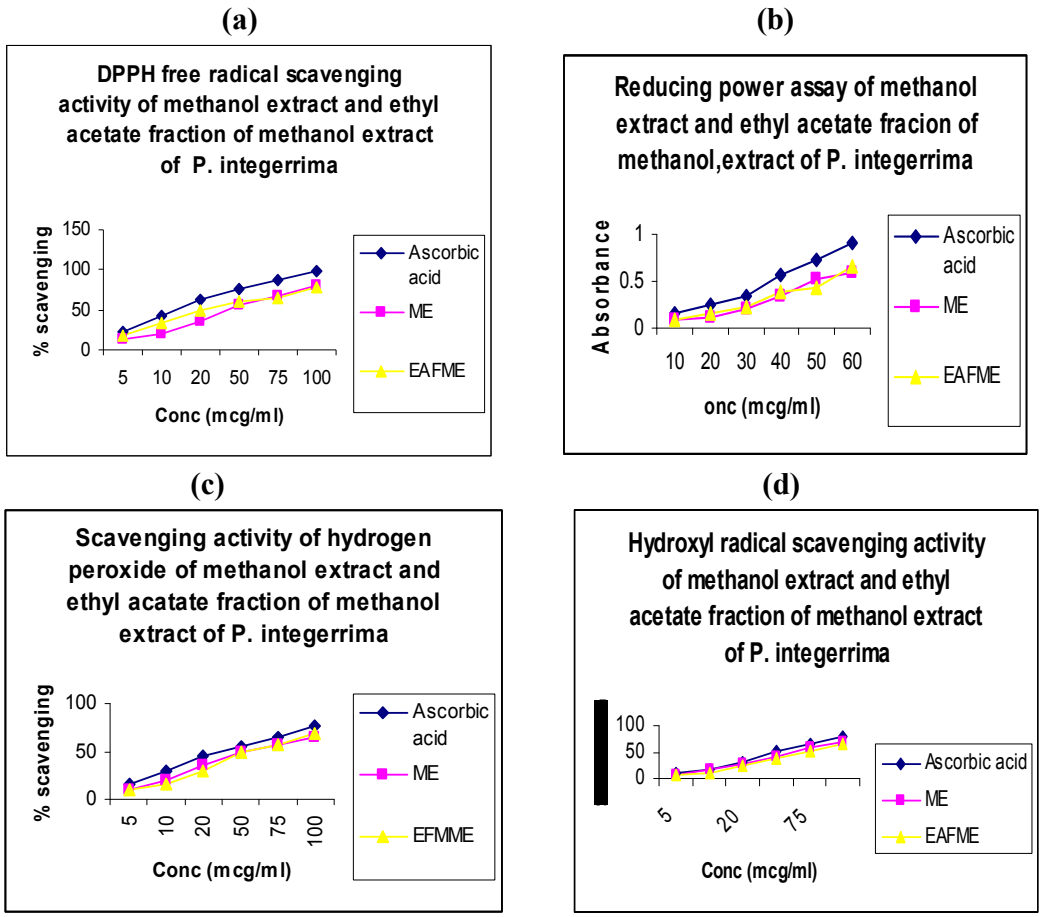
Hydroxyl radical scavenging activity (17)
The assay was performed by adding 0.1 ml EDTA, 0.1ml hydrogen peroxide, 0.36 ml deoxyribose, 1 ml test solution of ME and EAFME (10-100µg/ml) dissolved in distilled water, 0.33 ml, of phosphate buffer (50mM, pH 7.4) and 0.1 ml ascorbic acid in sequence. The mixture was incubated at 37° C for 1hr. A 1 ml portion of the incubated mixture was mixed with 1 ml of 10% trichloroacetic acid and 1.0 ml of 0.5% thiobarbituric acid to develop pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity was reported as % inhibition of deoxyribose degradation and calculated as
% scavenging activity = [(Ac- As)/ Ac] x 100
Ac is absorbance of control reaction, As is absorbance of sample.

Results and Discussion

The leaf galls of *P. integerrima* were subjected to successive solvent extraction using solvents of different polarity. The different successive extracts showed presence of volatile oil, carbohydrate, phenolics and flavonoids when subjected to preliminary phytochemical screening. The methanol extract of *P. integerrima* was found to be rich in phenolics and flavonoids. The presence of Gallic acid and Quercetin was confirmed using co-TLC with authentic samples.

Folin Ciocalteu method was used to determine the total phenolic content of ME and EAFME of *P. integerrima*. ME showed presence of 14.29 % phenolics and EAFME was found to content 6.82 % of phenolics. ME showed 12.85% of flavonoid when determined by aluminum chloride method. EAFME was found to content 3.15 % of flavonoid. ME showed 7.82% of flavonoid when determined by DNPH method. EAFME was found to content 3.32 % of flavonoids.

Figure 1: Antioxidant activity of ME and EAFME of *P. integerrima*.



DPPH free radical scavenging activity is a quick and reliable parameter for assessing the in-vitro antioxidant activity of crude plant extracts. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron on hydrogen radical to become a stable diamagnetic molecule.

The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecule and radical progresses, results in the scavenging of the radical by hydrogen donation. The absorption maximum of a stable DPPH radical in methanol was at 517 nm (18). It was observed that scavenging of free radicals was comparable to that of ascorbic acid. The results are indicated in Figure 1(a). There was a decrease in the concentration of DPPH radical due to scavenging ability of methanol extract and ethyl acetate fraction of *P. integerrima.*

The results revealed that the ME and EAFME inhibit or scavenge free radical acting as antioxidants. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidant has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (19). Figure 1 (b) depicts the reductive effect of *P. integerrima* similar to the antioxidant activity, the reducing power of *P. integerrima* increased with increasing dosage.

All the doses showed activity comparable to that of control which indicates that *P. integerrima* consists of hydrophilic polyphenolic compounds that cause the greater reducing power. Hydrogen peroxide itself is not very reactive but it may sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems (20). Scavenging of H_2O_2 by antioxidants may be due to donation of electrons to H_2O_2, thus neutralizing it to water. Figure 1(c) illustrates a significant decrease in the concentration of H_2O_2 radical due to the scavenging ability of both extracts and ascorbic acid.

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The effect of ME and EAFME on the inhibition of free radical-mediated deoxyribose damage was assessed by means of the Iron (II)-dependent DNA damage assay. The Fenton reaction generates hydroxyl radicals (OH) which degrade DNA deoxyribose, using Fe^{++} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. Both ME and EAFME were found to be effective when compared to Ascorbic acid (21).

Conclusion

The results showed that methanol extract (ME) and ethyl acetate fraction of methanol extract (EAFME) of *P. integerrima* was rich in phenolic and flavonoid content. The results are encouraging to pursue further studies on isolation and characterization of probable bio active molecules and their bioactivity.

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