Research Article

In vitro antioxidant potential of acyclic isoprenoid isolated from *Semecarpus anacardium*

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ABSTRACT

The present study was aimed to examine the *in vitro* antioxidant potential of acyclic isoprenoid isolated from *Semecarpus anacardium* seeds by different *in vitro* methods. The antioxidant activity was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) activity, lipid peroxidation, nitric oxide, and hydroxyl radicals scavenging activity. Ascorbic acid was used as a standard antioxidant drug. The acyclic isoprenoid was used at 30, 60, 125.250, 500, and 1000 mg/ml concentrations and radical scavenging activity was determined in terms of inhibition percentage. Results revealed that acyclic isoprenoid inhibited the DPPH, lipid peroxide, nitric oxide, and hydroxyl radicals in a dose-dependent manner but pronounced radicals inhibitory effect was found at the concentration of 1000 µg/ml, and these inhibitory effects were less than that of ascorbic acid – used as a reference drug. This study revealed that the acyclic isoprenoid has significant antioxidant activity which might be helpful in preventing the progress of various oxidative stresses.

KEY WORDS: 1-Diphenyl-2-picrylhydrazyl, Acyclic isoprenoid, Antioxidant, Lipid peroxidation, Nitric oxide, *Semecarpus anacardium*

INTRODUCTION

Antioxidants are substances that significantly delay or inhibit the oxidation of an oxidizable substrate when present at low concentrations in comparison with those of the substrate.[1] The activities of free radicals have been implicated in aging, destruction of deoxyribonucleic acid (DNA), obstruction of arteries, cancer, strokes, cardiac, and central nervous system disorders which have led to an increase in the investigation of substances that can protect against these reactive oxygen species and thus may play a role in disease prevention.[2,3] Endogenous antioxidants are synthesized within the system of living organisms and repair free radical damage internally by initiating cell regeneration while exogenous antioxidants which are derived from sources outside the living systems such as diets[4] and stimulate cell repair externally.[5]

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The growing need to complement these endogenous antioxidants has led to an increased supplementation by exogenous sources. At present, there are keen interests and widespread researches on exogenous antioxidants from natural sources perhaps, due to the fact that they are less expensive, readily available and believed to have lesser side effects when compared to their synthetic counterparts.[6]

Semecarpus anacardium Linn. belongs to the family Anacardiaceae is distributed in the sub-Himalayan region, tropical and central parts of India. The nuts are commonly known as marking nut, and its vernacular name is "Ballataka" or Bhilwa. It has high priority and applicability in indigenous, Ayurvedic and Siddha system of medicine.[7] Chemical and phytochemical analyses of its nuts revealed the presence of catechol,[8] tetrahydroamentoflavone,[9] jeediflavanone,[10,11] semicarpoflavonone,[12] galluflavo none,[13] nallaflavonone,[14] semecarpetin,[15] and anacardioflavonone[16] which show various medicinal properties. Some extracts of *S. anacardium* nuts have

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been found to exhibit antioxidants, anti-inflammatory, antimicrobial, and bacterial activities. Recently, acyclic isoprenoid has been isolated from seeds of *S. anacardium* and reported to have antibacterial[17] and antihyperlipidemic activities.[18] However, to the best of our knowledge, there is no scientific evidence to prove the *in vitro* antioxidants potential of this drug. Therefore, the present study was designed to evaluate the antioxidant and free radical scavenging potential of acrylic isoprenoid in cell-free system.

MATERIALS AND METHODS

Sources of Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemicals Co. (St. Louis, USA). Deoxyribose, nitric acid, sulfanilamide, naphthyl ethylenediamine, thiobarbituric acid (TBA), hydrogen peroxide (H_2O_2), and ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade.

Plant Materials

S. anacardium seeds were purchased from Ramasamy Chettiyar, Traditional and Herbal Medicine shop, Parris, Chennai, Tamil Nadu, India. The identity of the plant was confirmed by Prof. Raman, plant taxonomist, Centre for Advanced Studies in Botany, University of Madras and voucher specimens (MUCASB-H105) were deposited in the Department of Herbarium.

Extraction, Isolation, and Characterization of Acyclic Isoprenoid

Acyclic isoprenoid was isolated from the seeds of *S. anacardium,* and its nuclear magnetic resonance, *Infrared* and mass spectral analysis were published previously by Purushothaman *et al*.[17] The structure of the compound is given in Figure 1.

Determination of *in vitro* Antioxidant Activity of a Cyclic Isoprenoid

DPPH radical scavenging activity

Different concentrations of the acrylic isoprenoid were measured for hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to a method of Koleva *et al*.[19] The reaction mixture containing 1.9 ml of a DPPH methanolic solution plus 0.1 ml of acrylic isoprenoid and ascorbic acid at different concentrations (30–1000 µg/ml). Methanol

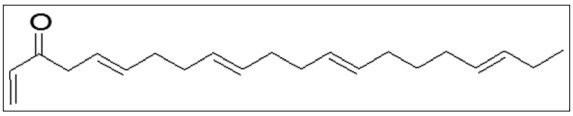

Figure 1: Acrylic isoprenoid. SOURCE: (IUPAC Name -5E, 9E, 13E, 18E)-Henicosa-1, 5, 9, 13, 18-pentaen-3-one

for the control was incubated at 37°C for 20 min and the absorbance was measured spectrophotometrically at 517 nm. The percentage of inhibition was calculated as follows:

$$\%\,\text{of Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Lipid Peroxidation (LPO) Inhibition Assay

LPO was estimated by the method of Ohkawa *et al*.[20] 10% liver homogenate was prepared using ice-cold KCl (0.15 M) in a Teflon tissue homogenizer, and the protein content was adjusted to 500 µg/ml. In the control systems, 1 ml of tissue homogenate, LPO was initiated by the addition of $FeSO_4$ (25 µM), ascorbate (100 µM), and KH_2PO_4 (10 mM) and the volume was made up to 3 ml with distilled water and incubated at 37°C for 30 min. In the test system, the homogenate was incubated with different concentration of acrylic isoprenoid and ascorbic acid (30–1000 µg/ml). The extent of inhibition of LPO was evaluated by the estimation of TBA reactive substances level by measuring the absorbance at 532 nm. The percentage inhibition of LPO was calculated by the formula:

$$\%\,\text{of Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Scavenging of Nitric Oxide (NO) Radical Activity

NO was assayed by the method of Green *et al*.[21] Aqueous sodium nitroprusside at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite, which can be estimated using Greiss reagent. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with 3 ml of different concentrations (30–1000 µg/ml) of the acrylic isoprenoid, and ascorbic acid was dissolved in methanol and incubated at 25°C for 150 min. The samples from the above were allowed to react with Greiss reagent. The absorbance of the chromophore formed during the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylenediamine was read at 546 nm. The experiments were repeated in triplicates. The percentage scavenging of NO radical activity was calculated by the following formula,

$$\%\,\text{of Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Determination of Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of the acyclic isoprenoid was assayed by the method of Halliwell and Gutteridge.[22] The reaction mixture contained 500 ml of 2-deoxyribose (2.8 mM) in potassium phosphate buffer (50 mM, pH 7.4), 200 ml of premixed ferric

chloride (100 mM) and ethylene diamine tetraacetic acid (EDTA,100 mM) solution (1:1; v/v), and 100 ml of hydrogen peroxide (H_2O_2) (200 mM) without or with the extract (100 mL) in different concentration (15–500 µg). The reaction was triggered by adding 100 ml of 300 mM ascorbate and incubated for 1 h at 37°C. A solution of TBA in 1 ml (1%; w/v) of 50 mM sodium hydroxide and 1 ml of 2.8% (w/v; aqueous solution) trichloroacetic acid was added. The mixtures were incubated for 15 min in a boiling water bath and then cooled. The absorbance was measured at 532 nm. The absorbance of the control was determined by replacing the sample with methanol. Vitamin C was used as a positive control. The scavenging activity on hydroxyl radical was calculated as follows

$$\%\,\text{of Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Statistical Analysis

The results are expressed as mean ± standard deviation. Differences between groups were assessed by ANOVA using the SPSS software package for Windows. *Post hoc* testing was performed for intergroup comparisons using the least significant difference $P < 0.05$ was considered as significantly altered.

RESULTS

In vitro Antioxidant Activity of Acyclic Isoprenoid

DPPH radical scavenging activity

DPPH scavenging activity of acyclic isoprenoid was found to increase with increasing concentration (from 30 µg to 1000 µg) which is represented in Figure 2. The maximum inhibition activity was found at the concentration of 1000 µg/ml. However, the scavenging activity of acyclic isoprenoid was lower than that of the ascorbic acid at the concentration of 1000 µg/ml.

LPO Inhibition Activity

LPO inhibition potential of acyclic isoprenoid is depicted in Figure 3. The maximum percent LPO inhibition activity was observed in acyclic isoprenoid at a concentration of 1000 µg/ml and those LPO inhibition activities were found to be lowered when compared to ascorbic acid – a standard antioxidant drug.

NO Scavenging Activity

The NO antiradical activity of acyclic isoprenoid was increased with increasing concentration which is represented in Figure 4. Acyclic isoprenoid exhibited more NO radical quenching activity at a concentration of 1000 µg/ml. However, the quenching activity was relatively lower than that of the ascorbic acid.

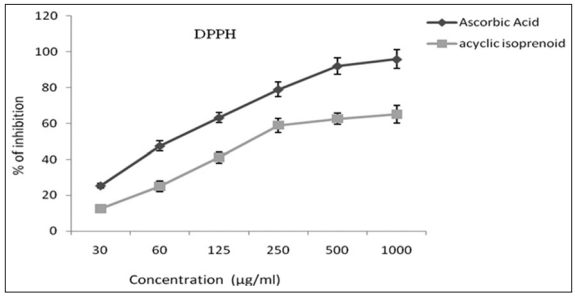

Figure 2: 1, 1-Diphenyl-2-picrylhydrazyl radical scavenging activity of isoprenoid and standard ascorbic acid. Data represent the percentage of inhibition. Each value are expressed as mean ± SD ($n = 3$)

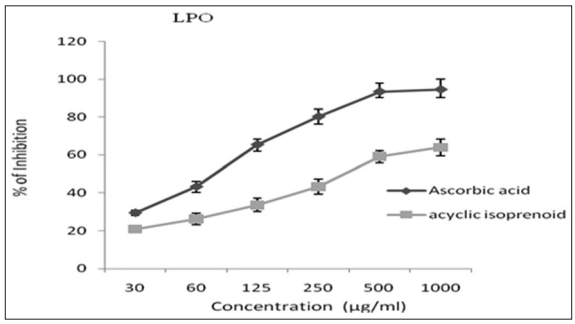

Figure 3: Lipid peroxide scavenging activity of isoprenoid and standard ascorbic acid. Data represent the percentage of inhibition. Each value are expressed as mean ± SD ($n = 3$)

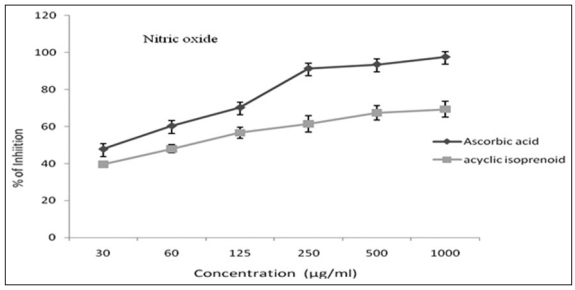

Figure 4: Nitric oxide radical scavenging activity of acrylic isoprenoid and standard ascorbic acid. Data represent the percentage of inhibition. Each value are expressed as mean ± SD ($n = 3$)

Hydroxyl Radical Scavenging Activity

Acyclic isoprenoid was found to exhibit a concentration-dependent hydroxyl radical scavenging activities which are represented in Figure 5. The highest percent inhibition of H_2O_2 was found at the concentration of 1000 µg/ml. Although, those inhibition activity was found to be lowered when compared to ascorbic acid.

DISCUSSION

The DPPH assay was used to preliminarily screen for antioxidant activity of the acyclic isoprenoid. Its solution appears deep violet and shows a strong absorbance at 517 nm. Substances which make its color

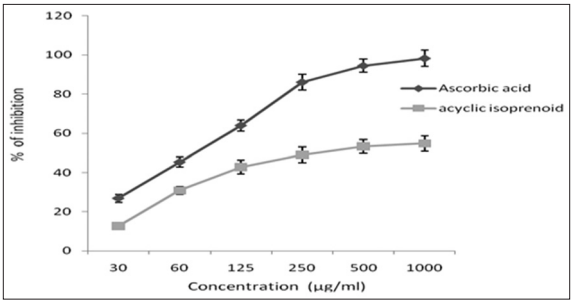

Figure 5: Hydroxyl radical scavenging activity of acrylic isoprenoid and standard ascorbic acid. Data represent the percentage of inhibition. Each value are expressed as mean ± SD ($n = 3$)

lighter and absorbance descendent can be considered as antioxidants and therefore radical scavengers.[23] In the present study, isoprenoid decolorized the DPPH in a dose-dependent manner. An appreciable free radical scavenging activity was observed at 1000 µg/ml which was weaker than ascorbic acid in the same concentrations. These results indicate that potent proton-donating ability of isoprenoid which is an important mechanism of antioxidants.

LPO was initiated from the process of the unsaturated fatty acids oxidation deterioration induced by free radicals, such as superoxide and hydroxyl radicals and other reactive oxygen species. Low concentrations of LPO are found in tissues in the normal physiologic conditions. However, under the oxidative stress conditions, LPO can harm the cells by inactivating the enzymes and receptors in the membrane, depolymerizing DNA, and proteins cross-link. Furthermore, several LPO by-products can damage other biomolecules.[24] The data obtained from this study showed that acrylic isoprenoid and standard drug-ascorbic acid increased their LPO inhibition activity with increasing concentration. However, a maximum lipid peroxide inhibition activity was observed at 1000 µg/ml which was weaker than the ascorbic acid at the same concentration. This result indicates that radical scavenging potential of acrylic isoprenoid on various free radicals which may contribute to its protective effect against LPO seen in rat liver homogenate.

NO is a reactive free radical generated from sodium nitroprusside in aqueous solution at physiological pH and reacts with oxygen to form nitrite. It is well known that NO plays an important role in various inflammatory processes such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis, and ulcerative colitis.[25] The scavenging activity of acrylic isoprenoid against NO was deducted by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of nitrogen in the reacting mixture. Acrylic isoprenoid showed an appreciable nitric oxide scavenging activity at the concentration of 1000 µg/ml which was lower than ascorbic acid in the same concentration.

Hydroxyl radical is highly important reactive oxygen species due to its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell by reacting with Fe^{2+} and possibly Cu^{2+} ions.[26] This assay shows the ability of acrylic isoprenoid to inhibit hydrogen peroxide in the reaction mixture in dose-dependent manner. The scavenging activity of acrylic isoprenoid was obviously lower than that of the ascorbic acid at the concentration of 1000 µg/ml. From these results, it can be concluded that acrylic isoprenoid has strong antioxidant activity in *in vitro* which will be valuable to examine the activity of the acrylic isoprenoid *in vivo*. Because, plant phytochemicals have strong protective effects against major disease risks including cancer, diabetes and cardiovascular diseases due to their antioxidant potential.[27]

CONCLUSION

The results obtained in the present study indicated that acyclic isoprenoid exhibited free radical scavenging activity against DPPH, LPO, NO, and hydroxyl radicals. The overall antioxidant activity of acyclic isoprenoid would have great importance as therapeutic agents in preventing or slowing the progress of reactive oxygen species and associated oxidative stress-related degenerative diseases.

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