Advances in Biological Research 2 (3-4): 56-59, 2008
ISSN 1992-0067

Antioxidant and Anti-Lipoxygenase Activity of *Thespesia lampas* Dalz & Gibs

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Abstract: Antioxidant activities and Anti-inflammatory activity of the extracts were evaluated by a 1, 1-diphenyl, 2-picrylhydrazyl free radical (DPPH), 2, 2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and anti-lipoxygenase assay for the water extraction of *Thespesia lampas* Dalz & Gibs. Ascorbic acid, gallic acid and indomethacin were used as reference standards for DPPH, ABTS and anti-lipoxygenase assays. In both DPPH and ABTS scavenging activities extract showed significant scavenging activity (72.28 µg/ml and 103.4 µg/ml IC_{50} for DPPH and ABTS assay). In addition to the antioxidant activity water extract showed anti-lipoxygenase activity (IC_{50} value is 586.5 µg/ml). The results obtained in the present study indicate that *Thespesia lampas* can be a potential source of anti-inflammatory and antioxidant agents.

Key words: *Thespesia lampas* Dalz & Gibs. · Lipoxygenase · DPPH · ABTS

INTRODUCTION

Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species (ROS). Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radicals and hydrogen peroxide, are often generated as byproducts of biological reactions or from exogenous factors. *In vivo*, some of these ROS play positive roles in cell physiology; however, they may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity and DNA mutations leading to cancer, degenerative and other diseases [1-3].

Mammalian cells possess elaborate defense mechanisms for radical detoxification. Key metabolic steps are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which destroy toxic peroxides. In addition to antioxidant enzymes, nonenzymatic molecules, including thioredoxin, thiols and disulfide-bonding play important roles in antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as alpha-tocopherol, beta-carotene and ascorbic acid and such micronutrient elements as zinc and selenium. If cellular constituents do not effectively scavenge free radicals, they lead to disease conditions as described above [4].

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. 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. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge

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free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [5].

Lipoxygenases (LOXs) comprise a family of non-heme iron-containing dioxygenases, representing the key enzymes in the biosynthesis of leukotrienes that have been postulated to play an important role in the pathophysiology of several inflammatory and allergic diseases. The products of LOXs catalysed oxygenation [hydroperoxyeicosatetraenoic acids (HPETE), hydroxyeicosatetraenoic acids (HETE), leukotrienes and lipoxins] apparently are involved in the development of rheumatoid arthritis, psoriasis, asthmatic responses and glomerular nephritis [6,7]. *Thespesia lamapas* Dalz&Gibs belongs to the family Malvaceae. Roots and fruits are employed as remedy in gonorrhoea and syphilis [8].

Plant Material and Extraction: The roots were collected from the Thodupuzha, Idukki, Kerala, in the month of October 2008. The plant material after collection was dried in shade at room temperature for 10 days and coarsely powdered with the help of a hand-grinding mill. Powdered root weighing 200g was packed in soxhlet apparatus and then subjected aqueous extraction using 1.0 liters distilled water for 8 hrs. The aqueous extract was concentrated under vacuum at 40-45°C (yielding 30%).

Chemicals: 1, 1-diphenyl, 2-picrylhydrazyl radical (DPPH), 2, 2-Azinobis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS), Lipoxidase (E.C.1.13.12.12), indomethacin, ascorbic acid and gallic acid were purchased from Sigma, USA. Linoleic acid was purchased from Himedia.

Anti-Lipoxygenase Activity: Anti-lipoxygenase assay was studied using linoleic acid as substrate and lipoxidase as enzyme [9]. Test solution was dissolved in 0.25ml of 2M borate buffer pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000U/ml). And incubated for 5 min at 25°C. After which, 1.0 ml of linoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Indomethcin was used as reference standard. The percent inhibition was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x100

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Free Radical Scavenging Activity: Antioxidant scavenging activity was studied using 1, 1-diphenyl, 2-picrylhydrazyl free radical (DPPH) [10]. Various concentration of test solution in 0.1ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Methanol only (0.1ml) was used as experimental control. After 30 minute of incubation at room temperature, the reduction in the number of free radical was measured, reading the absorbance at 517nm. Ascorbic acid was used as reference standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. Ascorbic acid was used as reference standard. The percent inhibition was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x100

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

ABTS radical scavenging Assay: ABTS radical cations are produced by reacting ABTS (7mM) and potassium persulfate (2.45mM) on incubating the mixture at room temperature in dark for 16 hours. The solution thus obtained was further diluted with PBS to give an absorbance of 1.000. Different concentrations of the test sample in 50µl were added to 950 µl of ABTS working solution to give a final volume of 1ml. The absorbance was recorded immediately at [11]. Gallic acid was used as reference standard. The percent inhibition was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x100

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

RESULTS

Effect of Extract on Anti-lipoxygenase Activity: Water extraction of *Thespesia lampas* has been checked at 200, 400, 600 and 800 µg/ml, it showed 10.11, 40.17, 53.14 and 70.60% anti-lipoxygenase inhibition respectively

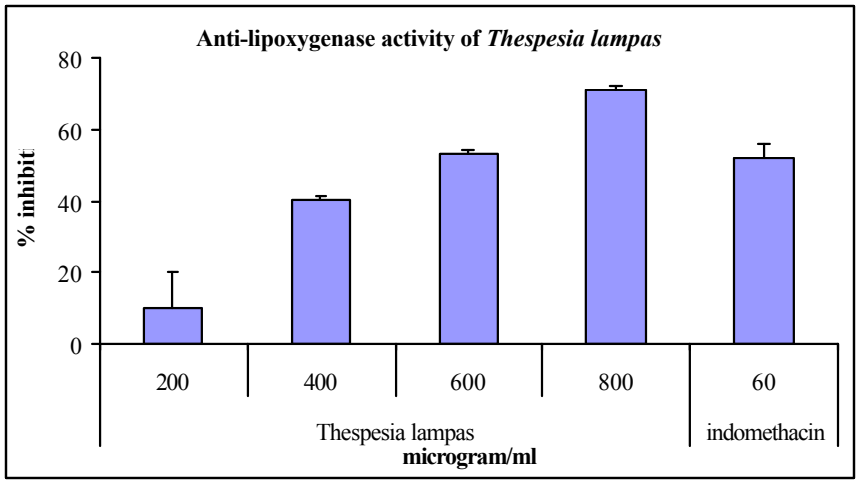

Fig. 1: Anti-lipoxygenase activity of *Thespesia lampas*

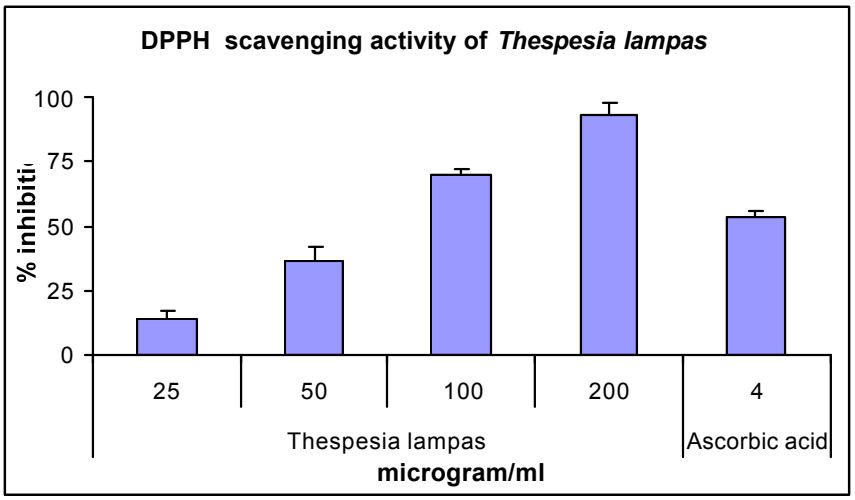

Fig. 2: DPPH scavenging activity of *Thespesia lampas*

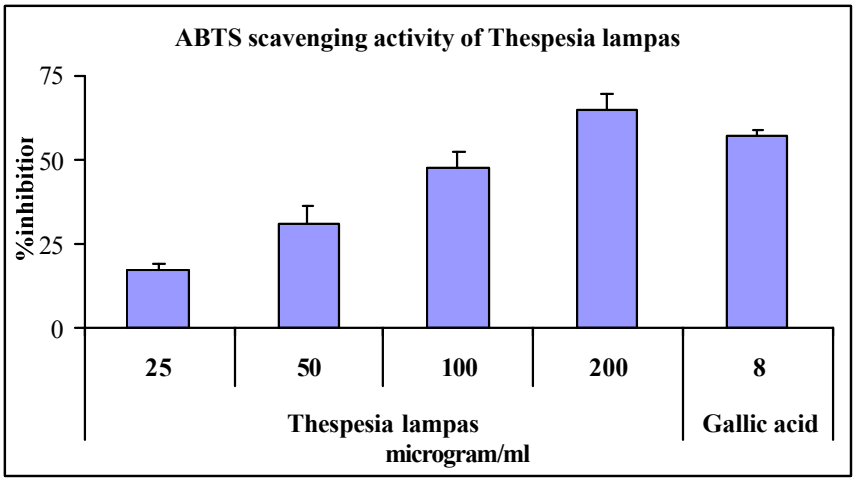

Fig. 3: ABTS scavenging activity of *Thespesia lampas*

(586.5 µg/ml IC_{50}). Reference standard indomethacin showed a 52.20% inhibition at a concentration of 60 µg/ml (Fig. 1).

Effect of extracts on DPPH free radical scavenging activity of water extraction of *Thespesia lampas* has been checked at various concentrations from 25, 50, 100 and 200 µg/ml. DPPH is used as a free radical to evaluate antioxidant activity of extract. The degree of its discoloration is attributed to hydrogen donating ability of test compounds. Significant DPPH free radical scavenging activity was evident water extract (72.28 µg/ml IC_{50}). Reference standard Ascorbic acid showed 53.7% inhibition at 4 µg/ml (Fig. 2).

Effect of extracts on ABTS free radical scavenging activities of water extraction of *Thespesia lampas* has been checked at various concentrations from 25, 50, 100 and 200 µg/ml. ABTS is used as a free radical to evaluate antioxidant activity of extract. The method, based on the ability of antioxidant molecules to quench the long-lived ABTS radical cation (ABTS+). Significant ABTS free radical scavenging activity was evident in water extract 103.4 µg/ml IC_{50}) Reference standard Gallic acid showed 57.08% inhibition at 8 µg/ml (Fig. 2&3).

DISCUSSION

This study elucidates the possible contribution of the radical scavenging effect on the lipoxygenase inhibitory mechanism of the *Thespesia lampas* used in the assays of the anti-radical and anti-lipoxygenase activity as standards. Lipoxygenases are the family of the key enzyme in the biosynthesis of leukotrienes which plays an important role in the pathophysiology of several inflammatory diseases. Lipoxygenases (LOX's) are sensitive to antioxidants and the most of their action may consist in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipidperoxy-radicals formed in course of enzymic peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX. The results obtained from the studies *T. lampas* has shown potential anti-inflammatory and antioxidant activity. The study was carried out according to Shinde *et al.* [9] using soyabean lipoxidase as enzyme and linoleic acid as substrate. Present study demonstrates, the water extract of *T. lampas* has showed DPPH and ABTS free radical scavenging activities. These results showed the ability to reduce free radicals of extracts which may stop the free radical initiation or retard free radical chain reaction in the propagation of the oxidation mechanism. In addition *T. lampas* inhibited the lipoxygenase enzyme activity also. This indicates that plants are more useful in studies of inflammation and in various related physiological processes and diseases such as aging, cancer and atherosclerosis.

ACKNOWLEDGEMENTS

The authors are thankful to DST, Govt of India, New Delhi, for providing financial support. They are also grateful to Prof. Balakrishnagouda, GKVK, Bangalore (India) for identification of plant species.

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