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Full Length Research Paper

Free radical scavenging activity and lipoxygenase inhibition of *Woodfordia fructicosa* Kurz and *Betula utilis* Wall.

M. V. Kumaraswamy and S. Satish*

Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore 570 006, India.

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Woodfordia fructicosa **and** ***Betula utilis*** **were extracted with solvents of different polarities. 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 lipoxygenase inhibition assay. Ascorbic acid, gallic acid and indomethacin were used as reference standards for DPPH, ABTS and lipoxygenase assays. In both DPPH and ABTS scavenging activities, methanol and water extract of** ***W. fructicosa*** **had showed significant scavenging activity (4.96, 5.08 µg/ml IC_{50} for DPPH, and 6.4, 7.15 µg /ml IC_{50} for ABTS assay). In addition to antioxidant activity methanol and water extract of** ***W. fructicosa*** **showed lipoxygenase inhibition activity (45.22 and 74.24% inhibition at 1.0 mg/ml). The methanol and water extract of** ***B. utilis*** **was showed DPPH and ABTS scavenging activity (8.4, 35.08 µg/ml IC_{50} for DPPH, and 83.18, 37.14 µg/ml IC_{50} for ABTS assay) but very mild activity against lipoxygenase inhibition activity (18.74 and 28.78% inhibition at 1.0 mg/ml). The results obtained in the present study indicate that** ***W. fructicosa*** **can be a potential source of anti-inflammatory and antioxidant agents.**

Key words: *Woodfordia fructicosa* Kurz., *Betula utilis* Wall., lipoxygenase, DPPH, ABTS.

INTRODUCTION

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. About three quarter of the world's population relies on plants and plant extracts for their healthcare. India represented by rich culture, traditions and natural biodiversity, offers a unique opportunity for drug discovery researchers (Jachak and Saklani, 2007). Ayurveda, the traditional medicinal systems in India, describes certain plants that reduces inflammation and pain. Flowers of *Woodfordia fructicosa* Kurz. (Family, Lythraceae) are acrid, astringent, styptic, depurative, uterine sedative, antibacterial (Nadakarni,1976; Parekh and Chanda, 2007) and possess immunomodulatory activity (Labadie et al., 1989; Das et al., 2007). Infusion of *Betula utilis* Wall. bark (Family, Betulaceae) is antiseptic and carminative (Nadakarni, 1976).

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 (Sircar et al., 1983; Bhattacharjee, 2007).

Reactive oxygen species (ROS), which include free radicals such as anion radicals (O_2^-), hydroxyl radical (OH.) and non free radical species such as H_2O_2 and singlet oxygen ($^1O_2$), are various forms of activated oxygen. By their ability to react with and damage many structures in the body, ROS are involved in various related physiological processes and diseases such as aging, cancer and atherosclerosis (Rackova et al., 2007; Fikel and Holbrook, 2000; Senthil et al., 2004).

*Corresponding author. E-mail: umaraswamymv@gmail.com. Tel: + 91821 2419734.

Table 1. *In vitro* lipoxygenase inhibition activity of *Woodfordia fructicosa* and *Betula utilis extracts.*

Plant	Test extract	% Inhibition
Woodfordia fructicosa	Petroleum ether	6.27±0.64
	Chloroform	5.37±1.62
	Methanol	45.22±3.53
	Water	74.24±4.83
Betula utilis	Petroleum ether	9.16±1.39
	Chloroform	10.60±2.87
	Methanol	18.74±2.47
	Water	28.78±3.96
Reference standard	Indomethacin	52.20±4.48

The values are mean of three replicates ± standard error. Extracts tested at 1.0 mg/ml. Reference standard-Indomethacin tested at 60 µg/ml.

No report on the anti-lipoxygenase and antioxidant activities of *W. fructicosa* and *B.utilis* are presently available. In this work, we reported that their successive extraction displayed anti-lipoxygenase and antioxidant activities in *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging activity and lipoxygenase inhibition assays.

MATERIALS AND METHODS

Plant collection and extraction

W. fructicosa flowers and *B. utilis* leaves free from diseases were collected from Bangalore (India), washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried, powdered and used for extraction. The plants were identified and authenticated by Prof. Balakrishnagouda, GKVK, Bangalore (India). The dried powder material of the flowers of *W. fructicosa* and *B. utilis* leaves were extracted successively with petroleum ether, chloroform, methanol and water in the increasing order of their polarity (Kokate, 1999). The solvent was removed under pressure to obtain a total extracts. Yield for *W. fructicosa* flowers were 0.6, 0.7, 17.3 and 13.4% in petroleum ether, chloroform, methanol and water, respectively. Yield for the *B. utilis* leaves were 4.4, 9.4, 14 and 13% in petroleum ether, chloroform, methanol and water, respectively.

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.

Lipoxygenase inhibition activity

Lipoxygenase inhibition assay was studied using linoleic acid as substrate and lipoxidase as enzyme (Shinde et al., 1999). Test solution was dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000 U/ml) and incubated for 5 min at 25°C. After which, 1.0 ml of linoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. 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.

Free radical scavenging activity

Antioxidant scavenging activity was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) (Blois, 1958). Various concentration of test solution in 0.1 ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Methanol only (0.1 ml) was used as experimental control. After 30 min of incubation at room temperature, the reduction in the number of free radical was measured by reading the absorbance at 517 nm. Ascorbic acid was used as reference standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The percent inhibition was calculated from the following equation:

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

ABTS radical scavenging assay

ABTS radical cations are produced by reacting ABTS (7 mM) and potassium persulfate (2.45 mM) and incubating the mixture at room temperature in the dark for 16 h. The solution thus obtained was further diluted with PBS to give an absorbance of 1.000. Different concentrations of the test sample in 50 µl were added to 950 µl of ABTS working solution to give a final volume of 1 ml. The absorbance was recorded immediately at 734 nm (Auddy et al., 2003). 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.

Statistical analysis

The Results are given as mean ± standard deviation. The IC_{50} value was calculated using linear regression analysis of the percent inhibition obtained using different concentrations. The regression equation was obtained and the concentration required to produce 50% effect (IC_{50}) was calculated.

RESULTS

Successive petroleum ether, chloroform, methanol and water extracts of *W. fructicosa* and *B. utilis* were assayed at 1.0 mg/ml. Successive extracts of *W. fructicosa* showed 6.27, 5.37, 45.22 and 74.24% lipoxygenase inhibition for petroleum ether, chloroform, methanol and water extracts, respectively, and the values in *B. utilis* extracts were 9.16, 10.60, 18.74 and 28.78%, respectively. Reference standard indomethacin showed a 52.20% inhibition at a conc. of 60 µg/ml (Table 1).

Effect of extracts on DPPH free radical scavenging activities of petroleum ether, chloroform, methanol and water extracts of *W. fructicosa* has been checked at various concentrations from 2.5, 5, 10, 25, 50 and 100 µg/ml. DPPH is used as a free radical to evaluate anti-

Table 2. *In vitro* antioxidant activity (DPPH free radical scavenging activity) of *Woodfordia fructicosa* and *Betula utilis* extracts.

Plant	Test extract	IC_{50} (µg/ml)
Woodfordia fructicosa	Petroleum ether	At 100µg/ml 17.97±3.64% inhibition
	Chloroform	62.93±5.9
	Methanol	4.96±0.38
	Water	5.08±0.51
Betula utilis	Petroleum ether	At 100µg/ml 25.32±1.82% inhibition
	Chloroform	25.37±1.16
	Methanol	8.4±0.42
	Water	35.08±2.06
Reference standard	Ascorbic acid	2.9±0.27

The values are mean of three replicates ± standard error. Extracts tested at 2.5, 5, 10, 25, 50 and 100 µg/ml. Reference standard-Ascorbic acid tested at 1, 2, 4, 8 and 16 µg/ml.

Table 3. *In vitro* antioxidant activity (ABTS free radical scavenging activity) of *Woodfordia fructicosa* and *Betula utilis* extracts.

Plant	Test extract	IC_{50} (µg/ml)
Woodfordia fructicosa	Petroleum ether	82.60±4.58
	Chloroform	66.96±4.27
	Methanol	6.4±0.95
	water	7.15±0.33
Betula utilis	Petroleum ether	At 100µg/ml 9.58±1.46% inhibition
	Chloroform	At 50µg/ml 9.22±1.61% inhibition
	Methanol	83.18±7.96
	water	37.14±6.59
Reference standard	Gallic acid	1.72±0.03

The values are mean of three replicates ± standard error. Extracts tested at 2.5, 5, 10, 25, 50 and 100 µg/ml. Reference standard (gallic acid) tested at 1, 2, 4, 8 and 16 µg/ml.

oxidant 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 in methanol and water extract (4.96 and 5.08 µg/ml IC_{50}) and mild scavenging activity for petroleum ether and chloroform extracts (17.97% inhibition at 100 µg/ml, 62.93 µg/ml IC_{50}). Only methanol extract of *B. utilis* showed a potent DPPH scavenging activity (8.4 µg/ml IC_{50}) (Table 2).

Effect of extracts on ABTS free radical scavenging activities of petroleum ether, chloroform, methanol and water extracts of *B. utilis* was assayed at various concentrations; from 2.5, 5, 10, 25, 50 and 100 µg/ml. ABTS is used as a free radical to evaluate antioxidant activity of extract. The method is 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 methanol and water extracts (6.4 and 7.15 µg/ml IC_{50}) and mild scavenging activity for petroleum ether and chloroform extracts of *W. fructicosa* (82.60 and 66.96 µg/ml IC_{50}). Methanol and water extracts of *B. utilis* exhibited a better scavenging activity (83.18, 37.14 µg/ml IC_{50}), while petroleum ether and chloroform extracts have 9.58 and 9.22% scavenging activity at 100 µg/ml (Table 3).

DISCUSSION

This study elucidates the possible contribution of the radical scavenging effect on the lipoxygenase inhibitory mechanism of *W. fructicosa* and *B. utilis*. 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 of *W. fructicosa* have

shown potential anti-inflammatory and antioxidant activity. The study was carried out according to Shinde et al. (1999) using soyabean lipoxidase as enzyme and linoleic acid as substrate. It was found that 1.0 mg/ml of water extract of *W. fructicosa* showed significant inhibition of enzyme lipoxidase.

The present study demonstrates that the successive methanol and water extracts of *W. fructicosa* and *B. utilis* has DPPH and ABTS free radical scavenging activities. These results showed the ability to reduce free radicals which may stop the free radical initiation or retard free radical chain reaction in the propagation of the oxidation mechanism. In addition *W. fructicosa* inhibited the lipoxygenase enzyme activity also. This indicates that the plants are more useful in the treatment of inflammation and in various related physiological processes and diseases such as aging, cancer and atherosclerosis. Though the inhibition of lipoxygenase enzyme by *B. utilis* is lesser, it may act on free radical to reduce the inflammation.

Conclusion

W. fructicosa has shown strong antioxidant and lipoxygenase inhibition activities in successive methanol and water extraction. This indicates that the direct scavenging of free radicals cannot be ruled out in the mechanism of lipoxygenase inhibition by the *W. fructicosa* extracts. In addition, *W. fructicosa* has been evaluated for antibacterial activity and found positive for several bacterial strains of Enterobacteriaceae (Parekh and Chanda, 2007). Though *B. utilis* having free radical scavenging activity, it has to be evaluated for other pharmacological properties. The results obtained confirm the therapeutic potency of *W. fruticosa and B. utilis* used in traditional medicine. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation. The present study support the folkloric usage of the studied plant and suggest that the plant extract possesses certain constituents with antioxidant, anti-inflammatory properties that can be used for the diseases such as aging, cancer and atherosclerosis.

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