

INHIBITORY EFFECT OF LIPOXYGENASE AND DPPH RADICAL SCAVENING ACTIVITY OF *FRAXINUS RHYNCHOPHYLLA*

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ABSTRACT

The purpose of the present study is to evaluate plant extracts as sources of natural antioxidants and to examine whether *Fraxinus rhynchophylla* having significant 1- diphenyl 2-picrylhyorazyl (DPPH) activity and Lipoxygenase (LOX) inhibitory activity. The plants of *F. rhynchophylla* were divided into three parts: leaves, outer bark, and endodermis. The antioxidant activity of the *F. rhynchophylla* extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical. DPPH scavenging activity of outer bark extracts of *F. rhynchophylla* was evaluated at 4.0 mg/ml was 75.6% and that of leaves was 70.8% at same concentration. The outer cortex of *F. rhynchophylla* showed maximum inhibition of DPPH activity ($IC_{50} = 70.5$ ug/ml). The highest LOX inhibition was recorded in the outer cortex extract among three vegetative parts. The outer bark of *F. rhynchophylla* showed maximum inhibition of LOX activity ($IC_{50} = 62.6$ ug/ml). Although the degree of inhibition of lipoxygenase by *F. rhynchophylla* were different among leaves, outer cortex, and endodermis at different concentrations, there were not show a statistically significant difference ($p < 0.05$). Strong inhibition of LOX enzymes by extract from *F. rhynchophylla* makes this pharmacopeial plant material an interesting topic for further biological and phytochemical examination.

Keywords: 1- diphenyl 2-picrylhyorazyl (DPPH), *Fraxinus rhynchophylla*, lipoxygenase.

INTRODUCTION

Oxidative is the condition as elevated levels of free radicals or other oxygen species which can direct either direct or indirect damage to the body (Chen, 2009). Free radicals have been implicated as playing a role in the etiology of cardiovascular disease, cancer, Alzheimer's disease and Parkinson's disease (Enujiugha, 2012). The antioxidant capacity of most plant food sources is usually associated with their phenolic contents (Kedare and Singh, 2011). The 1, 1- diphenyl 2-picrylhyorazyl (DPPH) is a well-known radical and a trap (scavenger) for other radicals (Brand-Williams et al., 1995). Many research works have also been done for antioxidant activity of leafy vegetables (Dasgupta and De, 2007; Sahu et al., 2013).

Lipoxygenases (LOXs; EC1.13.11.12) are nonheme iron-containing dioxygenases widely distributed in plants and animals. LOX enzyme catalyzes the hydroperoxidation of polyunsaturated fatty acids containing a cis, cis-1, 4-pentadiene system (Meriles et al., 2000). LOX converts the arachidonic, linoleic and other polyunsaturated fatty acid into biologically active metabolites involved in the inflammatory and immune responses (Azila and Don, 2012). In plants, linolenic and linoleic acids are the most common substrates for LOX (Siedow, 1991). The LOX enzymes are classed into several subcategories including 5-, 12- and 15-lipoxygenases. 15-Lipoxygenases have been found in plants as well as in animal tissue. Commercially obtainable 15-lipoxygenase is isolated from soybeans as the type 1 isoenzyme. There is a good correlation between inhibitory activity towards the mammalian and the soybean-derived enzymes (Mansuy et al., 1998; Nuhn et al., 1991).

Fraxinus is a genus of flowering plants in the olive and lilac family, Oleaceae. The genus is widespread across much of Europe, Asia and North America. *Fraxinus rhynchophylla* Hancei (Oleaceae) is a tree plant growing to 25.0 m tall. The species has been known for its bright, cutting tone and sustaining quality. Thus it is often used as material for acoustic guitar bodies. This species grows in moist rich soils on hillsides and in river valleys over a wide range which extends from western China to Korea and the Russian Far East. The plant is used in the traditional medicine of Korea. The bark is analgesic, anti-inflammatory, antitussive, astringent, diuretic, expectorant and stomachic (Duke and Ayensu, 1984). The purpose of the present study is to evaluate plant extracts as sources of natural antioxidants for DPPH and to examine whether the herbal medicine (*F. rhynchophylla*) having significant LOX inhibitory activity.

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MATERIALS AND METHODS

Sample extract

The plants of *F. rhynchophylla* were divided into three parts: leaves, outer bark, and endodermis. Each sample (100 g) of *F. rhynchophylla* was ground with pestles and liquid nitrogen at -70°C and homogenized prior to beginning extraction experiments. The ground powders were dissolved in 1000 ml ethanol and treated with ultrasound at room temperature for a given duration. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was further stirred with a magnetic bar at 65°C for 12 hours. Extracted sample was filtered. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber.

DPPH free radical

The antioxidant activity of the *F. rhynchophylla* extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams et al. (1995) with slight modifications. 1 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of plant extract solution of various concentrations (0.1, 1.0, 2.0 and 4.0 mg/ml). To determine the IC₅₀ value of the active component, the technique using 96-well microplates was employed (Lee et al., 1998). DPPH was added to the solutions prepared with plant extracts and standard antioxidant substances and stirred. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 µl of ethanol DPPH solution (final concentration 300 µM) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. After 30 min, the optical density (OD) of the solution was read using the UVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 517 nm. Absorbance changes are measured at 517 nm. Corresponding blank sample was prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard (positive control). The inhibition % was calculated using the following formula. Percentage inhibition was calculated using the following formula:

% Inhibition = $[1 - \text{OD (DPPH + sample)} / \text{OD (DPPH)}] \times 100\%$.

A dose response curve was plotted to determine the 50% inhibition (IC_{50}) values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.

Lipoxygenase activity

Lipoxygenase (LOX) inhibitor Screening Assay Kit (Abnova, CA, USA) was used and measured the hydroperoxides produced in the lipoxygenation using a purified LOX. Stock solutions of the tested samples 15-lipoxygenase standard (Abnova, CA, USA) were prepared by dissolving the extracts in ethanol or methanol. The reaction was initiated by the addition of aliquots (90 μl) soybean LOX solutions (prepared in potassium phosphate buffer, pH 9.0) in a sufficient concentration to give an early measurable initial rate of reaction to 10 μl of arachidonic acid in phosphate buffer. The enzymatic reaction was performed in presence or absence of inhibitor and their kinetics were compared. Quercetin was used as positive control. Nordihydroguaiaretic acid (NDGA) and Rutin used as negative control. LOX inhibition activity was determined using a spectrophotometric method at 490 nm.

The concentration that gave 50% inhibition (IC_{50}) was calculated from the outline of the inhibition percentages as a function of the inhibitor concentration (Akular and Odhav, 2008). Aqueous extracts ($\text{IC}_{50} \geq 100 \mu\text{g/ml}$) were not taken in this study.

Statistical analysis

All the analysis were carried out in triplicate and the results were expressed as the mean \pm SD. Correlation co-efficient (R) to determine the relationship between two or more variables among Radical Scavenging activity tests were calculated using the SPSS software (Release 21.0). Regression analysis was used to calculate IC_{50} , defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction. The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

$$\text{Inhibition \%} = (\text{IA} - \text{As}) / \text{IA} \times 100$$

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

RESULTS

Table 1 was shown the antioxidant activities of the *F. rhynchophylla*. Various concentrations of outer bark extracts were higher than those of leaves and endodermis. The maximum high antioxidant activity found on outer bark extracts. DPPH scavenging activity of outer bark extracts of *F. rhynchophylla* was evaluated at 4.0 mg/ml was 75.6% and that of leaves was 70.8% at same concentration. DPPH scavenging activity of endodermis extracts of *F. rhynchophylla* was evaluated at 4.0 mg/ml was only 50.2% at same concentration. The inhibitory activity of outer bark ($\text{IC}_{50} = 62.6 \mu\text{g/ml}$) was at the same levels as that of L-ascorbic acid ($\text{IC}_{50} 23.5 \mu\text{g/ml}$) (Fig. 1). The all young and matured groups for leaves, stems, and roots did not show a statistically significant difference ($p < 0.05$).

Table 2 was shown the LOX activity of *F. rhynchophylla* extracts. The highest LOX inhibition was recorded in the outer bark extract among three vegetative parts. LOX inhibition of matured leaves was 47.3% at 4.0 mg/ml and endodermis and leaves were 39.4% and 32.9% at same concentration, respectively. Although the values of LOX inhibition of outer bark was higher than those of endodermis and leaves, there were not show a statistically

significant difference ($p < 0.05$). When the NDGA used as a negative control, extract for outer barks of *F. rhynchophylla* was 38.1% inhibitory effects on the activation of LOX and that of endodermis and leaves were 31.6% and 26.4% (Fig. 2). The outer bark of *F. rhynchophylla* showed maximum inhibition of LOX activity ($IC_{50} = 62.6 \text{ ug/ml}$) (Fig. 3).

Table 1. Free radical scavenging effects of *Fraxinus rhynchophylla* at different concentrations

Concentration (mg/ml)	Leaf	Outer bark	Endodermis
0.1	16.91±3.03	18.91±2.12	6.67±2.47
0.5	27.50±3.42	30.94±3.59	14.65±3.86
1.0	36.76±4.39	50.59±5.31	24.73±1.45
2.0	56.52±5.77	61.74±3.27	39.03±6.34
4.0	70.80±5.43	75.60±4.76	50.16±4.06
F-test		1.314, $p < 0.05$	

Data represent the mean \pm SD from three replicates.

Table 2. Percent inhibition of lipoxygenase by *Fraxinus rhynchophylla* at different concentrations

Concentration (mg/ml)	Leaf	Outer bark	Endodermis
0.1	10.10±2.03	16.76±3.93	13.83±3.33
0.5	14.65±3.14	24.25±5.13	18.40±5.72
1.0	18.20±2.82	27.99±3.80	24.35±4.42
2.0	24.57±2.73	36.60±1.74	29.52±4.80
4.0	32.85±2.15	47.43±4.70	39.41±5.83
F-test		1.275, $p < 0.05$	

Data represent the mean \pm SD from three replicates.

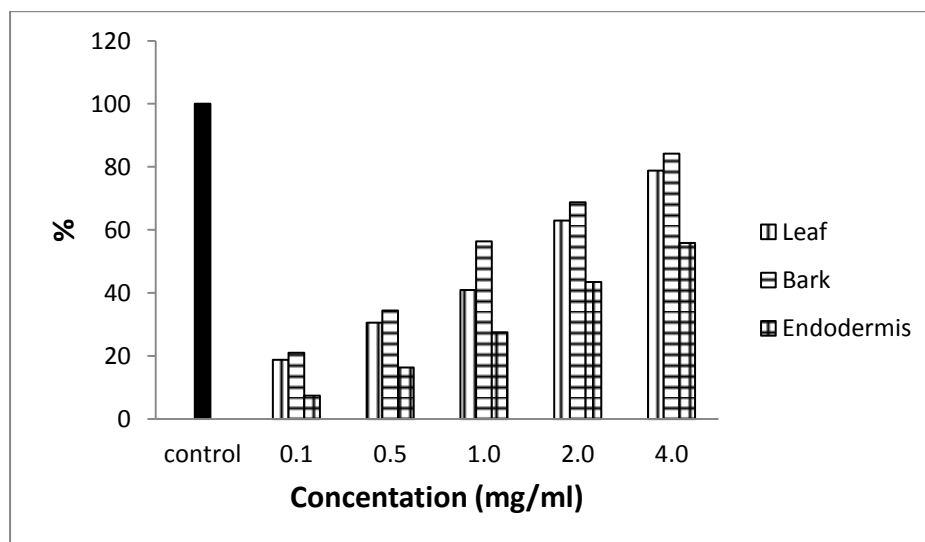


Fig. 1. Relative antioxidant values of the *Fraxinus rhynchophylla* extracts for control group (L-Ascorbic acid).

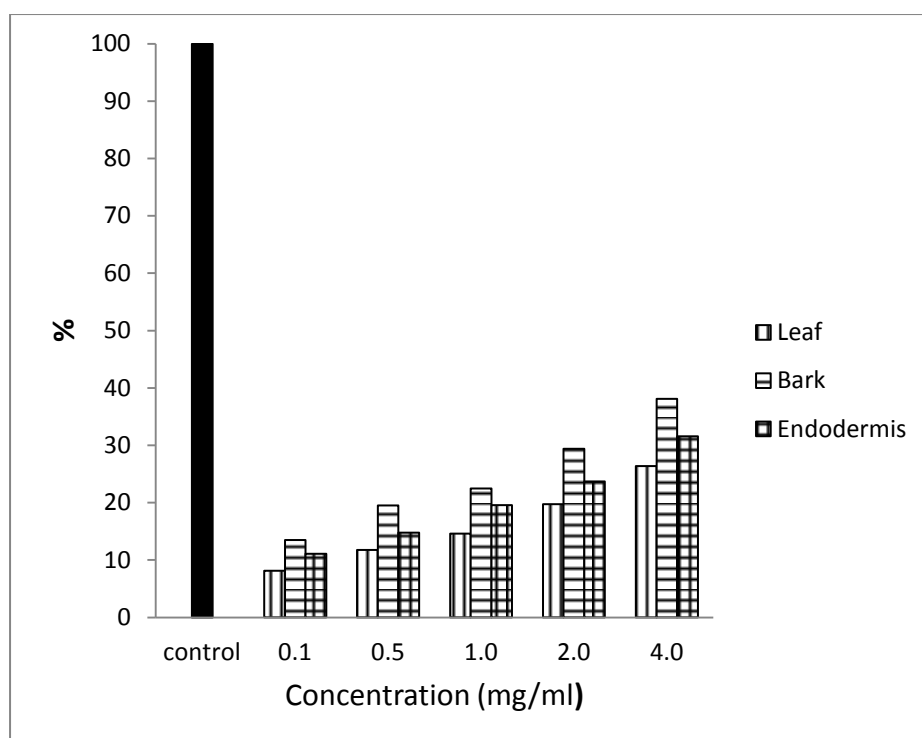


Fig. 2. The rate of lipoxygenase inhibitory of nordihydroguaiaretic acid (negative control) and relative inhibitory rate of *Fraxinus rhynchophylla*.

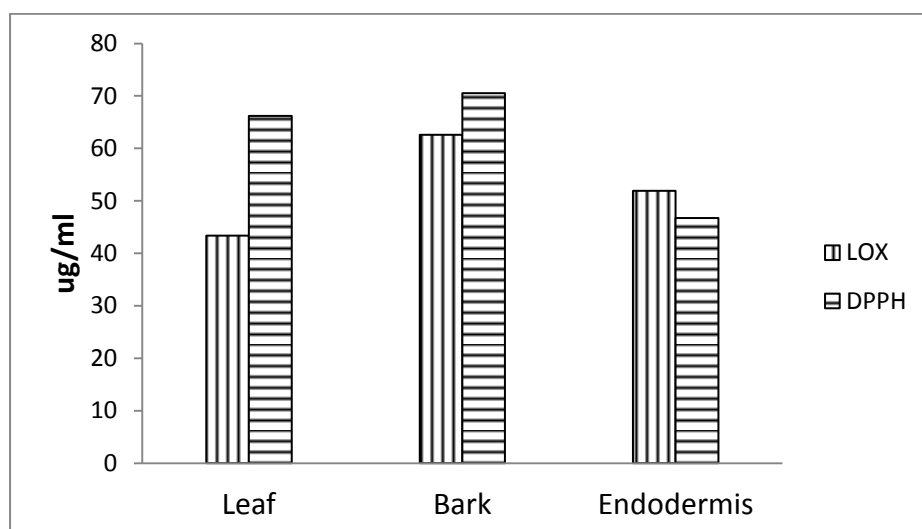


Fig. 3. 50% inhibition {IC₅₀ (mg/ml)} values on DPPH and lipoxygenase by *Fraxinus rhynchophylla* at 4.0 mg/ml concentration.

DISCUSSION

Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities. About 60 to 85% of the populations of every country of the developing world rely on herbal or indigenous forms of medicine (Oyetayo, 2008). World health organization (WHO) notes that 74% of the plant derived medicines are used in modern medicine, in a way that their modern application directly correlates with their traditional use as herbal medicines by native cultures (Kumar and Parmar, 2003). The herbal plant is a common element of ayurvedic, homeopathic, and naturopathic medicine (Amit et al., 2013).

The many traditional herbal species in Korea exhibited DPPH-free radical scavenging activity (Choi et al., 2003). Out of 40 species, four species (*Emcommia cortex*, *Moutan radices*, *Paeonine radix*, and *Rubus coreanus*) were shown above 90% inhibition of DPPH radical scavenging activity (Choi et al., 2003). Sahu et al. (2013) reported that *Leucas aspera* in India showed relatively high antioxidant activities (96.2%). Akular and Odhav (2008) reported antioxidants from 18 species in South Africa. *Portulaca oleracea* was high radical scavenging activity (96.5%) (Akular and Odhav, 2008). In this study, DPPH values of *F. rhynchophylla* were also relatively high (Table 1).

Camellia sinensis, *Rhodiola rosea*, and *Koelreuteria henryi* had notable significant inhibitory activities towards lipoxygenase (Chen et al., 2009). These results show that these plants have some phytochemical constituents which may be active against the lipoxygenase enzyme. We have shown that 4.0 mg/ml weight of ethanol *F. rhynchophylla* extract has inhibitory effect of lipoxygenase and antioxidants for DPPH. Anti-lipoxygenase activity of chosen antioxidant-rich plant materials can support their traditional use in folk medicine (Wichtl, 1994). Strong inhibition of LOX enzymes by extract from *F. rhynchophylla* makes this pharmacopeial plant material an interesting topic for further biological and phytochemical examination (Oyetayo, 2008).

The EtOAc fraction of the stem barks of *F. rhynchophylla* showed significant inhibitory activity on adipocyte differentiation as assessed by measuring fat accumulation (Shin et al., 2010). *F. rhynchophylla* is also used in the treatment of apoplexy, liver diseases, diarrhea, dysentery, eye diseases such as cataracts, cough and asthma (Natural Products Research Institute, 1998). The bark contains aesculin, this has anti-inflammatory, anticoagulant and analgesic actions (Yeung, 1985). The bark also contains fraxetin. This has an inhibitory effect on the central nervous system, is a stronger and safer anodyne than aspirin and has some antibacterial activity (Yeung, 1985). Although, *F. rhynchophylla* is classified as a medicine species, many studies will be required for the purification of active chemical groups from the crude extracts and to ascertain the mechanisms of action of these crude extracts (Park et al., 2004).

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