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Original Article

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF THE INDONESIAN FERNS, *NEPHROLEPIS FALCATA AND PYRROSIA LANCEOLATA*

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ABSTRACT

Objective: The aim of this research is to evaluate antioxidant and anti-inflammatory activity of extracts of the Indonesian ferns *Nephrolepis falcata and Pyrrosia lanceolata.*

Methods: Antioxidant activity was measured by using the 1,1-diphenil-2-picrylhydrazyl (DPPH) method and anti-inflammatory activity was evaluated by using anti-denaturation of heat bovine serum albumin (BSA) method. Phytochemical screening was carried out by using the standard of analysis.

Results: Ethanol (EtOH) extracts of the Indonesian ferns *N. falcata and P. lanceolata* showed very strong and moderate antioxidant activity with antioxidant activity index (AAI) values 3.8±0.5 and 1.4±0.0, respectively. Ethyl acetate (EtOAc) and methanol (MeOH) extracts of *P. lanceolata* was considered to have anti-inflammatory property (percentage anti-denaturation>20%) at concentration of 1 and 10 µg/ml and becoming not active at concentration of 100 µg/ml. Only MeOH extract of *N. falcata* showed significant anti-inflammatory at concentration of 1 and 10 µg/ml and also becoming not active at concentration of 100 µg/ml. Phytochemical screening indicates that EtOAc extract of *N. falcata* contains flavonoids and terpenoids, while MeOH extract contains flavonoids, phenols and saponins. Meanwhile, terpenoids, flavonoids and phenolic type of secondary metabolites were detected from both EtOAc and MeOH extracts of *P. lanceolata*

Conclusion: Both Indonesian ferns *N. falcata and P. lanceolata* have antioxidant activity and also consider having anti-inflammatory property. These activities are predicted due to the presences of flavonoid and phenolic secondary metabolites in these ferns.

Keywords: Antioxidant, Anti-inflammatory, *Nephrolepis falcata*, *Pyrosia lanceolata.*

INTRODUCTION

Antioxidant can be defined as a substance that can delay or inhibit the oxidation of a substrate when present in low concentration. Oxidative stress is referring to the situation of a serious imbalance between the productions of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and antioxidant defense. Oxidative stress can cause damage to all types of biomolecule, including DNA, protein, and lipids [1].

Inflammation is a pervasive form of defense that is defined as a nonspecific response to tissue malfunction and is employed by both innate and adaptive immune systems to combat pathogenic intruders [2]. Among complicated factors that are involved in the inflammation process, in a certain type of inflammation, tissue injury was proposed to be mediated by reactive oxygen metabolites (ROMs) directly or indirectly [3].

The commonly drug that can be used to reduce inflammation conditions are nonsteroidal anti-inflammatory drugs (NSAIDs) which have some adverse effects, especially can cause gastric irritation. Activities of NSAIDs are well known due to their ability in inhibiting endogenous production prostaglandins. The activity of NSAIDs on a certain inflammatory condition such as rheumatoid arthritis is proposed not only due to inhibition of prostaglandin, but also act by protecting endogenous protein against denaturation [4, 5].

Previously we have a concern in searching new therapeutically compounds from lower plants, especially liverwort (bryophyte) [6-8]. In this research, we continue to search potential drugs derived from other lower plant, ferns (pteridophyte). For preliminary screening, we collected some ferns that were found in the area of Syarif Hidayatullah State Islamic University, Indonesia and then tested for their antioxidant activity. The results of our screening indicated that EtOH extracts of two ferns *Nephrolepis falcata* (Lomaripsidaceae) and *Pyrrosia lanceolata* (Polyppodiceae) were found to be active as an antioxidant as the data will be presented in this paper. As EtOH extract of these ferns were found to be active as antioxidant, hence we continue to determine their anti-inflammatory activity. In Indonesia, these ferns are easily found as a wild plant (*P. lanceolata*) and ornamental plant (*N. falcata*). In South Africa decoction of *P. lanceolata* is used for curing colds and sore throat and in Mexico a tea prepared from the fronds is used as itch guard [9]. So far, there is no scientific investigation of these ferns on antioxidant and anti-inflammatory activity. In this research, antioxidant activity was carried out by using the DPPH method and anti-inflammatory activity was determined by using anti-denaturation of the heat BSA method.

MATERIALS AND METHODS

Plant materials

Ferns *Nephrolepis falcata* and *Pyrrosia lanceolata* were collected at Syarif Hidayatullah State Islamic University, Indonesia in September 2012 (for the screening of antioxidant activity) and December 2013 (for the screening of anti-inflammatory activity). These ferns are identified in Bogoriense Herbarium, Bogor, Indonesia.

Preparation of crude extracts

For screening of antioxidant activity, each dried and powdered of *N. falcata* (39 g) and *P. lanceolata* (22 g) was extracted by using EtOH to give 6.5 and 3.6 g of EtOH crude extract, respectively. As an ethanol extract of these ferns was found to be active as an antioxidant, screening for their activity as anti-inflammatory was continued. Recollected dried and powdered of *N. falcata* (340 g) and *P. lanceolata* (161 g) were extracted separately using a solvent of increasing polarity, beginning with EtOAc followed by MeOH.

Removal of solvents under reduced pressure by using a rotary evaporator, gave 5.3 g (EtOAc crude extract), 20.0 g (MeOH crude extract) of *N. falcata* and 3.9 g (EtOAc crude extract) and 16.3 g (MeOH crude extract) of *P. lanceolata*.

DPPH free radical scavenging assay

EtOH extracts of *N. falcata* and *P. lanceolata* at various concentrations (200, 100, 50, 25, 12,5, 6,25 µg/ml) in 4 ml MeOH were added 1 ml of a methanolic solution containing a final DPPH radical concentration of 0.25 mM (4.9 mg of DPPH was dissolved in 50 ml MeOH). The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm using a Hitachi U-2910 spectrophotometer. Vitamin C (Sigma-Aldrich) was used as a standard. Radical scavenging activity was obtained from the following equation [10]:

$$\text{I (\%)} = \frac{(\text{absorbance control} - \text{absorbance sample})}{\text{absorbance control}} \text{ x } 100\%$$

The IC_{50} (concentration providing 50% inhibition) was calculated graphically using a calibration curve in the linear range by plotting the extract concentration vs the corresponding scavenging effect. The antioxidant activity was expressed as the antioxidant activity index (AAI), calculated as follows as [11]:

$$\text{AAI} = \frac{\text{final concentration of DPPH (µg/ml)}}{\text{IC}_{50}\text{ (µg/ml)}}$$

Anti-denaturation of heat BSA assay

A stock solution of 0.2% (w/v) BSA, fraction V of 96% purity (Sigma Aldrich) was prepared in a mixture of 0.05 M tris-buffer saline, which was adjusted pH to 6.3 using glacial acetic acid. The following stock solution of 10.000, 1000 and 100 µg/ml of extracts were prepared in MeOH (Merck). From each of stock solutions, 50 µl was added to 5.0 ml of 0.2 % (w/v) stock BSA in tris-buffer saline to give concentrations of 100, 10 and 1 µg/ml. The control consisted of 50 µl MeOH in 5 ml stock 2% (w/v) BSA. Each sample was heated for 5 min at 72 °C in a test tube placed in a water bath, then cooled for 25 min under laboratory conditions and its turbidity measured at 660 nm using Hitachi U-2910 spectrophotometer. Na diclofenac (Dipharma) was used as a standard. The degree of inhibition of denaturation or precipitation of the BSA from the solution by each extract was calculated by using following equation [12]:

Percentage of Inhibition (%)

$$= \frac{(\text{absorbance control} - \text{absorbance sample})}{\text{absorbance control}} \text{ x } 100\%$$

In this assay, extracts that able to inhibit denaturation greater than 20% over the range of concentrations were considered as having anti-inflammatory properties and could be of value for drug development [12]

Phytochemistry screening

Extracts of EtOAc and MeOH were used for phytochemical screening using standard procedures of analysis [13, 14]

RESULTS

Antioxidant activity

The antioxidant activity of EtOH extracts of ferns and the standard was determined by way of the radical scavenging activity method using radical DPPH. The IC_{50} and AAI values were used to express their antioxidant activity (table 1).

Table 1: Antioxidant activity of EtOH extracts of *N. falcata* and *P. lanceolata*

Plant	Extract	IC_{50} (µg/ml)	AAI
N. falcata	EtOH	25.8±3.5	3.8±0.5
P. lanceolata	EtOH	70.3±0.2	1.4±0.0
Vitamin C (standard)		2.9±0.2	33.5±2.3

Each value in the table is represented as mean±SD (n=3)

Table 2: Percentage of inhibition denaturation of extracts of *N. falcata* and *P. lanceolata*

Plant	Extract	Concentration (µg/ml)	Percentage of inhibition denaturation (%)
N. falcata	EtOAc	1	18.9±1.5
		10	18.2±1.4
		100	-3.0±0.5
	MeOH	1	24.9±0.6
		10	49.5±0.2
		100	-1.7±0.1
P. lanceolata	EtOAc	1	27.5±0.6
		10	33.0±1.6
		100	-20.2±1.4
	MeOH	1	9.7±0.2
		10	52.6±0.6
		100	-20.1±0.0
Na Diclofenac		10	28.5±3.8
		100	95.4±0.2

Percentage of inhibition denaturation values are represented as mean±SD (n=3)

Table 3: Phytochemical screening of extracts of *N. falcata* and *P. lanceolata*

Plant	Extract	Secondary metabolites					
		Alkaloid	Flavonoid	Triterpenoid	Steroid	Phenol	Saponin
N. falcata	EtOAc	-	+	+	-	-	-
	MeOH	-	+	-	-	+	+
P. lanceolata	EtOAc	-	+	-	-	+	-
	MeOH	-	+	-	-	+	-

(+): presence, (-): absence

Anti-inflammatory activity

The anti-inflammatory activity was measured by using anti denaturation of BSA assay. EtOAc and MeOH extracts of ferns were evaluated for their ability in stabilizing heat BSA. Each extract and standard was divided into three concentrations of 1 μg/ml, 10 μg/ml and 100 μg/ml. The degree of inhibition of denaturation was expressed as percentage inhibition. Results of the assay are summarized in table 2.

Phytochemical screening

Phytochemical screening was conducted to determine the type of secondary metabolites contain in extracts of *N. falcata* and *P. lanceolata*. Phytochemical screening is carried by using standard procedures of analysis. Results of screening are summarized in table 3.

DISCUSSION

Some theories suggested that an antioxidant might be to have anti-inflammatory activity due to certain inflammation can be mediated by ROMs [1, 3]. Antioxidants play a role in the inflammatory process by protecting cells and tissue against the continuous production of ROMs [3]. Protein denaturation has been known to be associated with inflammation, which is confirmed by observation of good anti-denaturation activity of some NSAIDs [4, 5, 15]. It also has been reported that denatured albumins generally increase in conditions of oxidative stress [16].

Antioxidant activity was measured by using the DPPH method. As IC_{50} and AAI values shown in table 1, it can be concluded that the EtOH extract of *N. falcata* has very strong antioxidant activity (AAI value 3.8±0.5) and *P. lanceolata* has moderate antioxidant activity (AAI value 1.4±0.0) [11].

Anti-inflammatory activity was tested by using anti-denaturation of BSA assay. This assay was developed by Williams *et al.* [12] based on studied by researchers for many years [4, 5, 15]. There is a phenomenon that non-steroidal anti-inflammatory molecules will stabilize BSA when exposed to a few degrees rises in temperatures. Williams suggested that extracts which can inhibit denaturation greater than 20% over the range concentration were considered as having anti-inflammatory property [12]. As a result of the assay that was tabulated in table 2, it shows that all of the extracts can protect BSA from denaturation (>20 % anti-denaturation), except EtOAc extract of *N. falcata* which only show about 18% anti-denaturation activity. MeOH extracts of both *N. falcata* and *P. lanceolata* showed significant anti-inflammatory activity in the concentration of 10 μg/ml, which showed higher activity than Na diclofenac on the same concentration. Interestingly, all extracts of these ferns are consistently becoming inactive at a concentration of 100 μg/ml. This phenomenon occurs can be predicted due to at high concentration (100 μg/ml), these extracts are no longer capable of inhibiting the denaturation process, but has a tendency to trigger the occurrence of denaturation. In the paper that is published by Williams *et al.* [12], it is suggested to use concentration range from 50.0 μg/ml to 0.0035 μg/ml in order to evaluate anti-denaturation activity. They stated that the anti-denaturation action of the biologically (therapeutic) interesting compounds and extracts is greatest when the concentrations are lower.

The result of screening on the phytochemical contents of samples is tabulated in table 3. It is important to note that there is no previous publication described chemical components of *N. falcata.* Previous phytochemical study of *N. exaltata* and *N. occidentalis* reported that both *Nephrolepis* species contain C-glycosyl xanthones, mangiferin and iso-mangiferin [17]. A Drimanes-type sesquiterpenoid was reported to be isolated from *N. Biserrata* [18]. In this research, we identified the presence of terpenoid and flavonoid in the EtOAc extract; flavonoid, saponin and phenolic in the MeOH extract. In similarly, there is no any publication that reported about chemical components *of P. lanceolata*. However, previous studies on the genus *Pyrossia* indicated that this genus is rich in phenolic and flavonoid compounds [19-23]. As a result of phytochemistry screening (table 3), it found that EtOAc and MeOH extracts of *P. lanceolata* contain flavonoid and phenolic compounds too. Based on the screening on the phytochemical content of both ferns *N. falcata* and *P. lanceolata*, we can be suggested that flavonoid and phenolic compounds are responsible for antioxidant and anti-inflammatory activity of these ferns. The therapeutic applications of flavonoid and phenolic compounds on antioxidant and anti-inflammation have previously been reported [24, 25].

CONCLUSION

The present study investigated antioxidant and anti-inflammatory activity of the Indonesian Ferns *N. falcata* and *P. lanceolata*. Both Ferns are found to be active as an antioxidant and anti-inflammatory agent. These activities were predicted due to the presence of phenolic and flavonoid compounds in the ferns. This research further supports that antioxidant has a relationship with an anti-inflammatory.

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CONFLICT OF INTERESTS

Declare None

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