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Article

In vitro free radical scavenging potential of acetone extract and sub-fractions of *Albizia amara* (Roxb.) Boiv. stem bark

Kandhasamy Sowndhararajan[1], Sun-Chul Kang[1*]
[1]Department of Biotechnology, Daegu University, Gyeongsan, Gyeongbuk 712-714, Republic of Korea.

Abstract

In the present study, acetone extract and sub-fractions of *Albizia amara* stem bark were evaluated for their free radical potential. The results showed that the crude extract and all the fractions exhibited antioxidant and free radical scavenging activities under different *in vitro* assays. Among the different fractions, the ethyl acetate fraction exhibited higher DPPH and ABTS radical scavenging activities than the standard quercetin. *A. amara* stem bark might be valuable source of natural antioxidants that could be used for medicinal and food applications.

Keywords : *Albizia amara,* Antioxidant, DPPH, Phenolics, Xanthine oxidase, ABTS

Introduction

Reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and hydrogen peroxide are known to play important roles in oxidative damage. These ROS are considered to be important causative factors in the development of diseases such as cardiovascular diseases, cancer, liver disease, inflammatory diseases and the aging process (Prior & Cao 2000). Several studies have demonstrated that natural antioxidants, such as phenolics, flavonoids, carotenoids and tocopherols, can effectively prevent and cure oxidative stress related diseases (Vitaglione *et al.* 2004). Plants are rich source of natural antioxidants and have been found to possess a variety of biological activities including antioxidant potential.

The genus *Albizia* belongs to sub family Mimosoideae of family Leguminosae and is highly valued multipurpose tree legume. *Albizia amara* (Roxb.) Boiv. grows throughout southern India and in some parts of Madhya Pradesh (Akilandeswari *et al.* 2009). *A. amara* is a small to moderate-sized, much-branched deciduous tree. It resembles the acacias but lacks thorns. The leaves and flowers have been applied to boils, eruptions, and swellings, also regarded as an emetic and as a remedy for coughs, ulcer, dandruff and malaria (Mar *et al.* 1991). The *Albizia* species contain tannin and the bark is used as an astringent in diarrhoea and dysentery and internally to check uterine bleeding and the discharge in gonorrhoea as well as topically in ophthalmia and as wound dressing. Several researchers reported that the leaves and flowers of *A. amara* possess strong antioxidant and free radical scavenging properties (Muchuweti *et al.* 2006; Suresh Kumar *et al.* 2008; Rajkumar *et al.* 2012).

Based on the above knowledge, the present study was designed to investigate the antioxidant properties of acetone extract and sub-fractions of *A. amara* stem bark.

Materials and Methods

Sample collection and extraction

The stem barks of *A. amara* were collected from Gobichettipalayam, Erode District, Tamil Nadu, India. The plant was authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. The sample was cleaned thoroughly, shade dried at room temperature and powdered. The powdered sample was macerated thrice with acetone at room temperature. The combined acetone extract was concentrated by low-pressure evaporation (<40℃) and then dried in lyophilizer. The aqueous solution was successively partitioned with hexane (AHF), ethyl acetate (AEF) and n-butanol (ABF). The obtained extracts, in addition to the aqueous solution (AWF) remained after the extraction were filtered and dried under vacuum. The crude extract and fractions were used for the assessment of free radical scavenging activity through various *in vitro* assays.

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*Corresponding Author: Sun-Chul Kang, Tel. 82-53-850-6553, Fax. 82-53-850-6559, Email. sckang@daegu.ac.kr
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Determination of total phenolic content

The total phenolic content of acetone extract and sub-fractions of *A. amara* stem bark was determined by Folin-Ciocalteu method. The amount of total phenolics was calculated as the gallic acid equivalents (GAE) (Siddhuraju & Becker 2003).

Antioxidant activity assays

The free radical scavenging activity of the crude acetone extract and fractions of *A. amara* was carried out by using ferric reducing antioxidant power (FRAP) according to the method described previously by Pulido *et al.* (2000), phosphomolybdenum reduction assay was evaluated according to method of Prieto *et al.* (1999).

The free radical scavenging activity of the samples was measured using the DPPH radical method described by Liyana-Pathirana and Shahidi (2005). Radical scavenging activity of *A. amara* stem bark was assessed spectrophotometrically by $ABTS^{\bullet+}$ cation decolorization and the absorbances were taken at 734 nm (Re *et al.* 1999). Nitric oxide (NO) scavenging activity was carried out based on the method of Sreejayan and Rao (1997). The xanthine oxidase (XO) inhibitory activity of acetone extract and sub-fractions of *A. amara* was evaluated using the method described by Havlik *et al.* (2010).

The data on different parameters were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range tests using Statistica (Statsoft Inc., Tulsa, OK, USA).

Results and discussion

Natural chemical compounds from plants, especially phenolics are suggested to be the major bioactive compounds for health benefits. In this work, the total phenolic content of the extract and its fractions was expressed as gallic acid equivalents. The acetone extract of *A. amara* stem bark contains higher level of total phenolics (487.2 mg GAE/g extract). Acetone extract was further separated to hexane, ethyl acetate, n-butanol and water fractions. Ethyl acetate fraction registered higher amount of total phenolics (568.5 mg GAE/ g extract) when compared with other fractions and crude extract (Table 1). Phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. Several studies have reported on the close relationship between antioxidant activity and the amount of total phenolics or total flavonoids (Negro *et al.* 2003; Ramandeep & Geoffrey 2005).

The FRAP assay is commonly used in routine analysis for evaluation of antioxidant capacity. The reducing capacity of a compound might serve as a significant indicator of its potential antioxidant capacity. The antioxidant capacity of acetone extract and its fractions using FRAP assay, is shown in Table 1. In FRAP assay, acetone extract and its fractions were registered strong ferric reducing activity except hexane fraction. The reducing power of acetone extract and its fractions were found between 864.2 and 2209.8 μmol Fe(II)/mg extract. A strong significant correlation ($p < 0.01$) was observed between total phenolic content and ferric reducing capacity of the tested extracts ($r = 0.990$).

The phosphomolybdenum assay is based on the reduction of Mo(V) to Mo(IV) by the antioxidant substance in the sample

Table 1. Total phenolics content, FRAP and phosphomolybdenum reduction activity of crude extract and different fractions of *A. amara*

Sample	Total phenolics (mg GAE/g extract)	FRAP μmol Fe(II)/mg extract)	AEAC (mg AAE/g extract)
ACE	487.2 ± 21[b]	1864.3 ± 76.9[c]	678.1 ± 39.8[c]
AHF	84.3 ± 19[e]	864.2 ± 29.8[f]	107.5 ± 12.4[f]
AEF	568.5 ± 38[a]	2209.8 ± 102.7[b]	718.4 ± 59.7[b]
ABF	224.2 ± 29[c]	1383.2 ± 47.3[d]	453.6 ± 31.4[d]
AWF	144.7 ± 12[d]	1093.7 ± 29.1[e]	315.3 ± 21.5[e]
Quercetin		14529 ± 182.2[a]	857.6 ± 98.2[a]

Values are means of three replicate determinations (n = 3) ± standard deviation.
ACE - *A. amara* crude extract; AHF - *A. amara* hexane fraction; AEF - *A. amara* ethyl acetate fraction; ABF - *A. amara* butanol fraction; AWF - *A. amara* water fraction. Total phenolics are expressed as gallic acid equivalent (GAE).
FRAP - Ferric reducing antioxidant power (Concentration of substance having ferric-TPTZ reducing ability as equivalent to 1 μmol Fe(II); AEAC-Ascorbic acid equivalent antioxidant capacity (g equivalent of ascorbic acid per 100 gram extract) through the formation of phosphomolybdenum complex.
Mean values followed by different superscript in a column are significantly different ($p < 0.05$).

and subsequent formation of a green phosphate/Mo(V) complex at acidic pH with an absorbance maximum at 695 nm. Increase of the absorbance indicated the increase of the total antioxidant capacity. The total antioxidant capacity of the acetone extract and its fractions was found to be in the order AEF > ACE > ABF > AWF > AHF (Table 1). Among the different fractions, the ethyl acetate fraction (718.4 mg AAE/g extract) exhibited higher phosphomolybdenum reduction activity. Kumaran and Karunakaran (2007) reported that the total antioxidant activity of some plant extracts ranged between 245 and 376 mg ascorbic acid/g. The differences in their antioxidant activity of the crude extract and fractions might be the effect of solvents used for extraction.

The DPPH free radical scavenging assay has been widely used to evaluate antioxidant capacities. The DPPH free radical scavenging capacities of acetone extract and its fractions are presented in Table 2. The extracts were capable of scavenging DPPH radicals in a concentration dependent manner. IC_{50} of DPPH radical scavenging activity ranged from 9.8 to 187.3 μg/mL with the highest activity being found in ethyl acetate fraction and the least in hexane fraction. Ethyl acetate fraction (IC_{50} of 9.8 μg/mL) exhibited higher DPPH radical scavenging activity than the standards ascorbic acid, butylated hydroxytoluene (BHT) and quercetin (Borneo *et al.* 2009). Several authors reported that the radical-scavenging activity of extract and fractions could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability (Singh *et al.* 2007).

Another antioxidant activity screening method, ABTS radical cation decolorization assay, showed results similar to those obtained by the DPPH assay. The ABTS radical scavenging activity of *A. amara* stem bark extracted from acetone and its fractions are shown in Table 2. All the extracts possessed free radical scavenging activity but at different levels. The highest activity was obtained from the ethyl acetate fraction with IC_{50} of 4.1 μg/mL, followed by the crude extract and butanol fraction with the IC_{50} of 4.5 and 7.4 μg/mL, respectively. It was considered that the crude extract and its fractions of *A. amara* stem bark was a good source of potent natural antioxidant activity. Its higher scavenging activity indicated that the mechanism of antioxidant action of this fraction was as a hydrogen donor and it could terminate the oxidation process by converting free radicals to the stable forms.

Nitric oxide (NO) is an essential bioregulatory molecule required for several physiological processes in living system. However, production of excess nitric oxide (NO) during infection or inflammation has been implicated in the pathogenesis of several diseases, such as chronic inflammation, autoimmune diseases and cancer (Szekanecz & Koch 2007). The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH, producing NO. Under aerobic conditions, NO reacts with oxygen to produce the stable products nitrate and nitrite, the quantities of which can be determined using Griess reagent. The acetone extract and its sub-fractions (except hexane fraction) were effectively inhibited the production of NO. The NO scavenging capacity increased significantly in a concentration dependent manner, with the IC_{50} values ranged between 95.8 and 457.9 (Table 2).

The influence of the extracts on xanthine oxidase (XO) activity evaluated by uric acid formation as the final product was evaluated by *in vitro*. The IC_{50} values of the tested extracts for the inhibition of XO are given in Table 2. Inhibition of XO involves a decrease in production of uric acid which can be followed spectrophotometrically. Inhibitory activity of

Table 2. IC_{50} values of crude extract and its sub-fractions of *A. amara* on free radicals scavenging and xanthine oxidase inhibitory activities

Sample	IC_{50} (μg/mL)			
	DPPH	ABTS	Nitric oxide	Xanthine oxidase
ACE	10.6 ± 0.7^{b}	4.5 ± 0.2^{b}	112.6 ± 6.2^{c}	89.5 ± 5.3^{b}
AHF	187.3 ± 4.2^{e}	70.3 ± 1.7^{e}	457.9 ± 14.6^{f}	357.6 ± 12.5^{e}
AEF	9.8 ± 1.0^{a}	4.1 ± 0.1^{a}	95.8 ± 2.2^{b}	86.7 ± 3.2^{b}
ABF	12.5 ± 0.8^{c}	7.4 ± 0.5^{c}	129.6 ± 4.1^{d}	94.6 ± 2.7^{c}
AWF	24.9 ± 1.7^{d}	12.5 ± 1.1^{d}	150.4 ± 6.4^{e}	135.9 ± 8.9^{d}
Quercetin	12.7 ± 0.3^{c}	4.6 ± 0.4^{b}	23.8 ± 1.2^{a}	10.2 ± 0.6^{a}

Values are means of three replicate determinations (n = 3) ± standard deviation.
ACE - *A. amara* crude extract; AHF - *A. amara* hexane fraction; AEF - *A. amara* ethyl acetate fraction; ABF - *A. amara* butanol fraction; AWF - *A. amara* water fraction.
Mean values followed by different superscript in a column are significantly different ($p < 0.05$).

acetone extract and its sub-fractions on XO is decreasing in the order of Quercetin > AEF > ACE > ABF > AWF > AHE. It was concluded that the acetone extract and its sub-fractions *A amara* were effective inhibitors of XO. Ethyl acetate fraction is the most potent XO inhibitor and at the same time it was the most enriched in polyphenolic compounds. Whereas, hexane fraction showed the weakest XO inhibitory effect. Lespade and Bercion (2010) reported that the benefit of XO inhibitors is a possible antioxidant action, as the XO generates hydrogen peroxide and might be therefore an initiator of tissue damage in a range of pathological states.

Conclusion

The results of present investigation revealed that the higher free radical scavenging activity was observed in the ethyl acetate fraction of *A amara*. The difference in free radical scavenging activity of the acetone extract and different fractions may be attributed to the difference in the phenolic content. Further studies are needed on the isolation and characterization of individual phenolic compounds in the extract, especially in the ethyl acetate fraction of acetone extract and to elucidate their different antioxidant mechanisms.

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