Full Length Research Paper

Antioxidative and antibacterial activity of the methanol extract of *Artemisia anomala* S. Moore

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The antibacterial and antioxidative activities of the methanol extract of *Artemisia anomala* S. Moore were investigated. The methanol extract was subjected to screening for their possible antioxidative activities by using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching methods. In the former case, the methanol extract was found to have better antioxidative activity than synthetic antioxidant butylated hydroxytoluene (BHT) with IC_{50} value of 16.8 and 19.4 µg/ml, respectively. The extract seemed to inhibit the oxidation of linoleic acid with an 89% inhibition in the latter case. The methanol extract from *A. anomala* had great antibacterial activity against all five test bacteria and most activity against Gram-negative ones. The minimum inhibitory concentration (MIC) against *Proteus vulgaris*, *Escherichia coli* and *Salmonella typhi* were 1.5, 4.5, 3.9 mg/ml, respectively.

Key words: *Artemisia anomala*, antioxidative activity, antibacterial activity, methanol extract.

INTRODUCTION

Recently, the extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of foods from the toxic effects of oxidants. Particularly, the antioxidative and antimicrobial activities of plant extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997). Because of the possible toxicities of the synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), increasing attention has been directed toward natural antioxidants (Naimiki, 1990). The trend to use essential oils or extracts containing plants in foods which may act as natural antimicrobials or antioxidant preservatives, may also influence health of consumers as well as prolong the shelf-life of relevant food products (Burt, 2004; Dorman et al., 2000; Svoboda et al., 2006).

Artemisia anomala S. Moore belongs to the family Compositae, and is a fragrant shrub that grows widely in the Zhejiang region of China. Phytochemical analysis of *A. anomala* shows that it is a rich source of phenolic compounds, such as flavonoids including eupatilin and arteanoflarone. The presence of phenolic compounds (phenolic acids, polyphenols and flavonoids) in herbs and spices, along with the essential oils, is gaining increasing attention because of their various functions, such as antioxidant activity and flavouring properties (Issa et al., 2006; Sacchetti et al., 2005; Lagouri et al., 1993; Tsimidou and Boskou, 1994; Lagouri and Boskou, 1995). Consumption of food containing natural essential oils or aromatic plant extracts is expected to prevent the risk of many free radical-mediated diseases (Young and Woodside, 2001; Milan, 2006). The plants under study have not generally received much attention as antioxidants and flavouring sources; because of their low yield they have little commercial use (Zrira et al., 2003; Bradesi et al., 1997; Soong and Barlow, 2004).

There are few literatures about reporting the antimicrobial and/or antioxidant activities of *A. anomala*. Only a few literatures about the bioactivities of essential oils from *Artemisia judaica L.* (Liu et al., 2004; Khaled et al., 2002)

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and *Artemisia annua* (Fabien et al., 2002) have been reported.

In the present study, the antibacterial and antioxidative activities of the methanol extract of *A. anomala* are investigated. The antioxidant activities are determined by using inhibition of 2, 2'-diphenylpicryl-1-hydrazyl (DPPH) radical and β-Carotene/linoleic acid assay. The antimicrobial activities are determined by using Disc-diffusion assay and the minimal inhibitory concentration (MIC) values.

MATERIALS AND METHOD

Plant material

The herbal parts of *A. anomala* were collected from the Tianmushan Mountain, Zhejiang Province, P.R.China on August 2006.

Preparation of the methanolic extracts

The air-dried and finely ground samples were extracted with 85% methanol using a Soxhlet apparatus (Flavil, Switzerland) for about 12 h at $50^{\circ}C$. The extracts were concentrated in a rotary vacuum evaporator Buchi (Flavil, Switzerland) at temperature not higher than $50^{\circ}C$. Then the concentrated samples were dried at $50^{\circ}C$ in a lyophiliser (yield 8.7%, w/w) and kept in the dark at $4^{\circ}C$ until used.

Antioxidative activity

DPPH assay

The hydrogen atom- or electron-donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 2,2'-diphenylpicryl-1-hydrazyl (DPPH). This spectrophotometric assay used stable radical DPPH as a reagent (Burits and Bucar, 2000; Cuendet et al., 1997). 50 ml of various concentrations of the extracts in methanol were added to 5 ml of a 4 mg/100 ml methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC$_{50}$) was calculated using the graph by plotting inhibition percentage against extract concentration. Ascorbic acid (AA) and synthetic antioxidant reagent butylated hyroxytoluene (BHT) were used as positive controls and all tests were carried on triplicates.

β-Carotene–linoleic acid assay

In this assay antioxidative capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene/linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 μl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen was added with vigorous shaking (30 min, 100 rpm); 2500 μl of this reaction mixture was dispersed to test tubes and 350 μl portions of the extracts prepared in ethanol at 2 g/L concentrations were added and the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with the positive controls AA, BHT and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Antioxidative capacities of the extracts were compared with those of AA, BHT at the same concentration and blank consisting of only 350 μl ethanol.

Microbial strains

The methanol extracts were tested against a panel of microorganisms including *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi* and *Proteus vulgaris*. Bacterial strains were cultured overnight at $37^{\circ}C$ in Mueller-Hinton agar (MHA).

Disc-diffusion assay

The methanol extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 30 mg/ml and sterilized by filtration by 0.45 μm Millipore filters. Antibacterial tests were then carried out by disc diffusion method (Murray et al., 1995), using 100 μl of suspension containing 10^8 CFU/ml of bacteria, spread on nutrient agar. The discs (6 mm in diameter) were impregnated with the 30 mg/ml extracts (300 μg/disc) and placed on the inoculated agar. Negative controls were prepared using DMSO. Gentamicin (30 μg/disc) was used as positive reference standards to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at $37^{\circ}C$ for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms.

MIC assay

The minimal inhibitory concentration (MIC) values were studied for the bacterial strains, being sensitive to the extracts in the disc diffusion assay. The bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts were first dissolved in 10 ml/100 ml DMSO and then diluted to the highest concentration (500 μg/ml) to be tested, and then serial two fold dilutions were made in a concentration range from 7.8 to 500 μg/ml in 10 ml sterile test tubes containing nutrient broth. MIC values of the extracts against bacterial strains were determined based on a micro-well dilution method as previously described (Şahin et al., 2004).

In brief, the 96-well plates were prepared by dispensing into each well 95 μl of nutrient broth and 5 μl of the inocula. A 100 μl of aliquot from the stock solutions of the extracts initially prepared at the concentration of 500 μg/ml was added into the first wells. Then, 100 μl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 μl of nutrient broth without compound and 5 μl of the inocula on each strip was used as negative control. The final volume in each well was 200 μl. Maxipime at the concentration range of 5 – 500 μg/ml was prepared in nutrient broth and used as standard drug for positive control. The plate was covered with a sterile plate sealer. Contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Bacterial growth was determined by absorbance at 600 nm using the Model 680 series microplate reader (Bio-rad Laboratories, Inc., USA) and confirmed by plating 5 μl samples from clear wells on nutrient agar

Table 1. Effects of methanol extract from *Artemisia anomala* S. Moore and positive controls (BHT and ascorbic acid) on the *in vitro* free radical (DPPH) scavenging.

Sample	DPPH, IC$_{50}$ (µg/ml)*
Methanol extract	16.8 ± 1.21
BHT	19.4 ± 1.42
Ascorbic acid	7.8 ± 0.93

medium. The extract tested in this study was screened three times against each organism. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity of methanol extracts of *A. anomala* was examined by comparing it to the activity of known antioxidants such as ascorbic acid and BHT with inhibition of DPPH radical. In addition, the effect on inhibition of lipid peroxidation of the extract was assayed using β-carotene bleaching induced linoleic acid peroxidation methods. Free radicals involved in the process of lipid peroxidation play a primary role in nume-rous chronic diseases and are implicated in the aging process. Phytochemicals recognized as possessing potent antioxidative activity are also strong scavengers of DPPH (Dapkevicius et al., 2002). Substances capable of donating electrons/hydrogen atoms are able to convert DPPH into their non-radical form 1,1-diphenyl-2-picrylhydrazine, a reaction which can be followed spec-trophotometrically. On the other hand, the cell walls contain unsaturated fatty acids such as linoleic and arachidonic acids. The cell permeability is changed after the oxidation of these fatty acids then chronic diseases may occur. The extension of shelf-life and control of deterioration of fatty foods can be achieved via the protection of these acids (Braca et al., 2003; Liyana-Pathirana and Shahidi, 2006). For this reason, the free radical scavenging activity and β-carotene/linoleic acid co-oxidation activity of the extract were investigated and the results are given in Table 1 and Figure 1. BHT and ascorbic acid were used as positive controls in both test systems. Free radical scavenging activity of the extracts is concentration dependent and lower IC$_{50}$ value reflects better protective action. The methanol extract was able to reduce the stable free radical DPPH to the yellow-colored diphenylpicrylhydrazine with an IC$_{50}$ of 16.8 ± 1.21 µg/ml, exhibiting better activity than the synthetic antioxidant agent BHT (19.4 ±1.42 µg/ml), but lower activity than ascorbic acid (7.8 ± 0.93 µg/ml). On the other hand, the inhibition values of the methanol extract was estimated as 89.7% in the β-carotene/linoleic acid assay (Figure 1), exhibiting a little below than ascorbic acid (96.8%) and
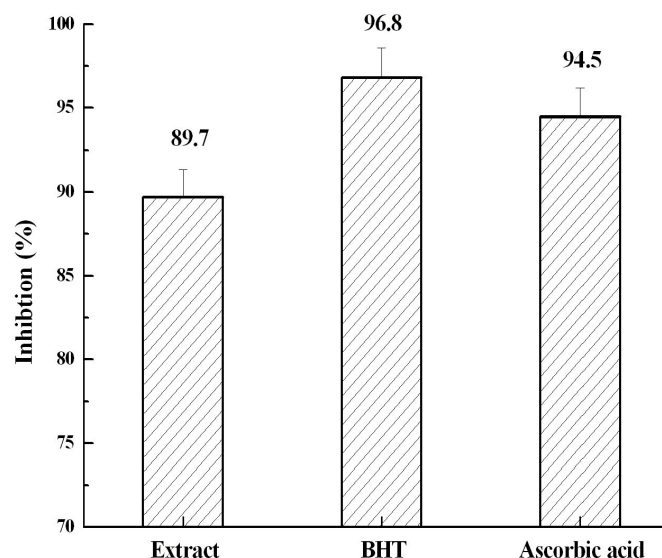
Figure 1. Effects of methanol extract from *Artemisia anomala* S. Moore and positive controls (BHT and ascorbic acid) on inhibition of β-carotene/linoleic acid. Values are means of three replications.

BHT (94.5%). The antioxidative activity of the methanol extract could be attributed to high contents of polar flavonoids, which could be extracted by methanol/water solution. The crude flavonoids were about 5.4 mg/g dry mass in the test samples.

Antibacterial activity

According to the results given in Table 2, the methanol extract of *A. anomala* had great antibacterial activity against all five test bacteria and the diameter of inhibition zone from 9 to 13 mm. The growth inhibitions of test microorganisms ranged from 1.5 mg/ml (w/v) to 78.0 mg/ml (w/v) with the lower MIC value against *P. vulgaris*, *E. coli* and *S. typhi* at 1.5, 4.5, 3.9 mg/ml, respectively. It meant that the methanol extract had most activity against Gram-negative ones.

Conclusion

Antioxidants and antibacterial properties of the essential oils and various extracts from many plants have recently been of great interest in both research and the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants with natural ones. Owing to strong anti-bacterial and protective features exhibited in antioxidant activity tests, the methanol extracts of *A. anomala* could be considered a natural herbal source that can be freely used in the food and pharmaceutical industries. *A. anomala* is widely planted in southeast and southwest of China. In order to develop this resource, further studies

Table 2. Antibacterial activity of methanol extract from *Artemisia anomala* S. Moore against the bacterial strains tested.

Microorganisms	Diameter of inhibition zone (mm)*			MIC (µg/ml)**
	Methanol Extract	Gentamicin	Control	
S. aureus	13 ± 0.8	16 ± 0.6	0	78
E. coli	9 ± 0.9	10 ± 0.5	0	4.5
B. subtilis	11 ± 1.1	18 ± 0.9	0	67
S. typhi	10 ± 0.7	8 ± 0.4	0	3.9
P. vulgaris	10 ± 0.9	12 ± 0.7	0	1.5

* Diameter of inhibition zone including disc diameter of 6 (mm). Values are means of three replications ± SD.
**MIC, minimum inhibitory concentration (as mg/ml).

are needed to do. One of these is to obtain more information regarding the practical effectiveness of the extracts in animal models.

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