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Phytochemical screening, antibacterial and anti-oxidant activities of *Asparagus laricinus* leaf and stem extracts

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Abstract

The aim of this study was to investigate anti-oxidant activities, antibacterial activities and a phytochemical constituent of *Asparagus laricinus* stem and leaf extracts. Determination of antibacterial activity of extracts was assessed by agar dilution method and anti-oxidant properties by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. The minimum inhibitory concentration (MIC) of the leaf was at a concentration of 0.125 mg/mL against *S. saprophyticus* and *E. cloacae*, and at a concentration of 1 mg/mL against *S. aureus* and *B. subtilis*. There was no MIC of the stem extract at any concentration. The leaf extract showed effective free radical scavenging activity (72.1%), while stem extract had low activity. Qualitative phytochemical analysis of these plant extracts revealed the presence of tannins, saponins, flavonoids and phlobatannins. The leaf extract further confirmed the presence of glycosides, steroids, ternoids and carbohydrates. Our results indicate that, *A. laricinus* leaf extracts have potential antimicrobial and anti-oxidant activities.

Introduction

Phytochemicals or secondary metabolites are chemical compounds formed during the plants normal metabolic processes and plants use them to protect themselves (Ning et al., 2009). The resistance that pathogens build against antibiotics and the oxidative stress caused by free radicals, has sparked interest in the search for new antibacterial and anti-oxidant compounds also from nature (Berrino et al., 2009; Pervival, 1997; Auroma, 1998; Michael et al., 2006). Natural crude drug extracts isolated from plant species can be prolific resources for such new drugs.

The genus Asparagus comprises approximately 100 species and consists of herbs, shrubs and vines. *Asparagus laricinus* is a monogeneric family (previously included within the Liliaceae family), belonging to Asparagaceae family (Brummitt, 1992). *A. laricinus* known as *lesitwane* in Setswana is reported to have indications for sores, tuberculosis, redwater, uterine infection, general aliments and umbilical cord inflammation (van der Merwe et al., 2001). *A. laricinus* polyphenol root extract exhibited a dose dependent antimutagenic ability (Mashele et al., 2011). The anticancer activity of *A. laricinus* root extracts was sensitive against three cell lines (Mashele et al., 2010). The leaf and stem of this plant have not yet been investigated for anti-oxidant and antibacterial activity. Therefore in the present study, *A. laricinus* stem and leaf were collected to study their antibacterial activities, anti-oxidant activities and to investigate their active compounds in order to discover resources for new lead structures.

Materials and Methods

Plant

The plant materials were authenticated by scientists at the National Botanical Gardens in Bloemfontein South Africa (MASH002). The collected materials were dried at room temperature and pulverized by mechanical mills and weighed. It was then stored in a cool place until analysis.

Extraction methods

Plant material (10 g of the dried leaf and stem) was weighed and soaked with purified water for 72 hours with occasional stirring. The extracts were filtered and lyophilized.

Microorganisms

Clinical isolates of *Staphylococcus aureus* (ATCC29213), *Staphylococcus saprophyticus* (ATCC 15305), *Escherichia cloacae* (ATCC13047) and *Bacillus subtilis* (ATCC11774) were used in this study.

Antibacterial activity test (MIC determination)

Antibac-terial activity was assessed using the agar-dilution method as described elsewhere (Madamombe and Afolayan, 2003). *Staphylococcus aureus* (ATCC 29213), *Staphylococcus saprophyticus* (ATCC15305), *Escherichia cloacae* (ATCC13047) and *Bacillus subtilis* (ATCC11774) were cultured and maintained on nutrient broth. Different volumes of extracts (51.9; 25.9; 12.9; 6.4; 3.2 µL) were added to nutrient agar respectively, mixed to obtain different concentrations (2; 1; 0.5; 0.25; 0.125 mg/mL), and dishes were allowed to set. Using a sterile swab, microorganisms were streaked on diluted nutrient agars. Dilutions of chloramphenicol served as a positive control while broth without plant extract was used as a negative control. Plates were covered and incubated for 24 hours at 37°C. Bacterial growth was determined by visual looking at the plates and results were recorded as growth or no growth. Since the activity of the antibacterial depends frequently on its concentration, the minimum inhibitory concentration of the *A. laricinus* extracts were determined.

Scavenging ability towards 2, 2-diphenyl-1-picry-lhydrazyl (DPPH) radical

The (DPPH) assay was performed as described by Shirwaikar et al. (2006). 100 µL of various concentrations of each sample was added to 2 mL solution of 0.1 mM DPPH. 100 µL of methanol and 2 mL DPPH served as control. After 60 min of incubation at 25°C in the dark, the absorbance was recorded at 517 nm. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated according to the following equation:

% DPPH radical scavenging activity = 1 - [Asample/ Acontrol] x 100

where Asample and Acontrol are absorbance of sample and control respectively. The SC_{50} (concentration of sam -ple required to scavenge 50% of DPPH radicals) values were determined. The decrease of absorbance of DPPH solution indicates an increase of the DPPH radical scavenging activity. The anti-oxidant activity was expre -ssed as the number of equivalents of ascorbic acid.

Phytochemical screening of the plant extract

A small portion of the dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods with little modifications (Trease and Evans, 1989; Harborne, 1998).

Total phenolic content

Total phenolic content in the obtained extracts were estimated by a colorimetric assay based on procedures described elsewhere (Singleton and Rossi, 1965) with some modifications. Briefly, 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve. The results were expressed as mg of gallic acid equivalents/g of extract (GAEs).

Statistical analysis

Tests were carried out in triplicates. MIC of test samples was compared with that of positive and negative control. The mean values were calculated from the triplicate values. Values were expressed as the Mean ± SD (n = 3) and differences between groups were considered to be statistically significant if $p<0.05$. Data from the test groups were compared with SC_{50} value of the standard which is the concentration of sample, required to scavenge 50% of DPPH free radicals.

Results

The MIC is the lowest concentration of the agent that completely inhibits visible growth, disregarding a single colony or a thin haze within the area of the inoculated spot. *A. laricinus* stem and leaf extracts were tested with agar-dilution assay. The stem extract of *A. laricinus* showed absence of susceptibility when compar -ed with a chloramphenicol (positive control) therefore all organisms grew at different extract concentration. Results showed (Table I) that only leaf extracts possess antibacterial activities with MIC of 0.125 mg/mL against *S. saprophyticus* and *E. cloacae* and 1 mg/mL against *S. aureus* and *B. subtilis*. This part of the plant can be further investigated for toxicity and may be used to develop new antibacterial medical drugs against *S. aureus*, *S. saprophyticus*, *E. cloacae* and *B. subtilis* at >1 mg/mL concentration.

The DPPH radical scavenging activity of *A. laricinus* stem and leaf extracts was done in comparison with that of ascorbic acid (Table II). Ascorbic acid showed a high activity with SC_{50} from a concentration of 1.25 mg/mL. The radical scavenging activity of the aqueous extract of Asparagus laricinus leaf on DPPH showed high activity with SC_{50} <2.5 mg/mL. The anti-oxidant activity of leaf extract was significantly higher than stem extract ($p< 0.05$), and the anti-oxidant activity of ascorbic acid was higher than leaf and stem extracts. Values of leaf extracts were statistically similar to

Table I

Antibacterial activity of Asparagus laricinus leaf and stem

Bacterial species	Minimum Inhibition Concentrations (0.125 to 2 mg/mL)			
	Stem	Leaf	Chloramphenicol (positive control)	Water (negative control)
S. aureus	-	1 mg/mL	0.125 mg/mL	-
S. saprophyticus	-	0.125 mg/mL	0.125 mg/mL	-
E. cloacae	-	0.125 mg/mL	0.125 mg/mL	-
B. subtilis	-	1 mg/mL	0.125 mg/mL	-
(–) Bacteria grew	(–) Bacteria grew	(–) Bacteria grew	(–) Bacteria grew	(–) Bacteria grew

Table II

DPPH scavenging activity of ascorbic acid, *Asparagus laricinus* leaf and stem

Concentration (mg/mL)	Leaf		Stem		Ascorbic acid	
	% Scavenging	Δ Absorbance @517	% Scavenging	Δ Absorbance @517	% Scavenging	Δ Absorbance @517
2.500	72.1	0.209 ± 0.028	6.3	1.407 ± 0.004	95.3	0.024 ± 0.002
1.250	42.3	0.433 ± 0.002	2.2	1.468 ± 0.016	89.5	0.053 ± 0.003
0.625	16.9	0.623 ± 0.001	1.6	1.500 ± 0.013	23.1	0.389 ± 0.009
0.313	3.9	0.721 ± 0.007	0.13	1.499 ± 0.014	-12.5	0.569 ± 0.011
0.078	-7.6	0.807 ± 0.006	-0.9	1.516 ± 0.016	-29.8	0.657 ± 0.004

Results are represented as mean ± standard deviation, n = 3

Table III

Phytochemical screening of *Asparagus laricinus* leaf and stem

Test	Stem	Leaf
Glycosides	-	++
Steroids	-	++
Alkaloids	-	-
Flavonoids	+	++
Saponins	+++	++
Tannis	++	++
Phlobatannins	+	+
Terpenoids	-	+
Carbohydrates		
Reducing sugars	-	++
Non-reducing sugars	-	-

+ = present, - = absent

Ascorbic acid at 2.5 mg/mL. Total phenolic content of asparagus leaf (0.572 ± 0.063 mg/GAE) showed the strong correlation with the determined anti-oxidant activity.

The qualitative phytochemical screening of the leaf extract revealed the presence of tannins, saponins, flavonoids, phlobatannins, glycosides, steroids, ternoids and carbohydrates while that of the stem extract showed the presence of tannins, saponins, flavonoids and phlobatannins.

Phytochemical analysis of *A. laricinus* leaf and stem extracts demonstrated in Table III revealed the presence of tannins, saponins, flavonoids and phlobatannins. The leaf extract further confirmed the presence of glycosides, steroids, ternoids and carbohydrates.

The total phenol content of *A. laricinus* leaf and stem extracts which were analysed at 1 mg/mL against gallic acid. Leaf extract showed more polyphenols than stem extract. The gallic acid equivalents of the estimated phenolic concentrations ranged from 0.277 ± 0.010 to 0.572 ± 0.063 mg/GAE.

Discussion

Free radicals are highly reactive oxygen species produced through oxidative process within the mammalian body. In normal conditions, the human body possesses many defense mechanisms against oxi-dative stress, including anti-oxidant enzymes and non-enzymatic compounds but also under some circumstan-ces including exposure to some environmental pollutants, e.g. cigarette smoke, pesticides, smog, UV radiation, etc. The natural anti-oxidant mammalian mechanism become insufficient and then the excess of free radicals can damage both the structure and function of a cell membrane in a chain reaction leading to many degene-

rative diseases (Atta-ur-Rahman and Choudhary, 2001). Many anti-oxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Mosquera et al., 2007). Recently, interest has increased considerably in finding naturally occurring anti-oxidants to replace synthetic anti-oxidants, which are being restricted due to their carcinogenicity (Sasaki et al., 2002).

Free radicals are important mediators that provoke inflammatory processes and are neutralized by antioxidant which exerts anti-inflammatory effect (Filomena et al., 2008). Free radical scavenging molecules such as flavonoids, tannins, alkaloids, quinones, amines, vitamins, and other metabolites possess anti-inflammatory, anti-carcinogenic, antibacterial and antiviral activities (Sala et al., 2002). The aqueous extracts of *A. laricinus* stem and leaf were investigated for their anti-oxidant properties using rapid and stable *in vitro* methods. Evaluation of the anti-oxidant activity of *A. laricinus* was done in comparison with that of ascorbic acid in Table II. Ascorbic acid showed a high activity with SC_{50} from a concentration of 1.25 mg/mL. The radical scavenging activity of the aqueous extract of *A. laricinus* leaf on DPPH showed high activity with SC_{50} <2.5 mg/mL. The *A. laricinus* stem extracts did not show any scavenging activity even at the concentration of 2.5 mg/mL when compared to ascorbic acid.

The data presented in Table I shows that only *A. laricinus* leaf totally inhibited growth of all microorganisms tested against at a concentration of 1 mg/mL. *A. laricinus* leaf extract had high antibacterial activity against *E. cloacae* and *S. saprophyticus* with the minimum inhibition concentration of <0.125 mg/mL. The stem extracts of *A. laricinus* did not inhibit the growth of any organism even at the high concentration of 2 mg/mL as compared to the growth inhibition by chloramphenicol which was used as a positive control. In these screenings, the Gram-positive bacteria were found to be more susceptible to plant extracts than the Gram-negative ones (Kelmanson et al., 2000; Massika and Afolayane 2002; Fennell et al., 2004). Indeed, Gram-positive bacteria have only an outer peptidoglycan layer which is not an effective barrier (Scherrer and Gerhardt, 1971). The Gram-negative bacteria have an outer phospholipid membrane that makes the cell wall impermeable to lipo-philic solutes, while the porines constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600Da (Nikaido and Vaara, 1985). Thus this might be the reason why the Gram-positive bacteria were more inhibited by *A. laricinus* leave extracts.

Phytochemicals or secondary metabolites are chemical compounds formed during the plants normal metabolic processes and plants use them to protect themselves (Alison et al., 2001; Ning et al., 2009). Plants with anti-oxidants properties are used for minimizing the severity of the inflammation related diseases and a health-promoting effect of anti-oxidants from plants is thought to arise from their protective effects by counteracting ROS (Wong et al., 2006).

The flavonoids in these plants extracts may contribute to their effects as antibacterial and anti-oxidant agents. Flavonoids have anion radicals and inhibit membrane-bound enzymes (Li et al., 2003), this may explain the mechanisms of antioxidative action of *A. laricinus* leaf extract. The leaf extract was also positive for steroids which are very important compounds especially due to their relationship with compounds such as sex hormone. Both plant extracts were revealed to contain saponins, which are known to produce inhibitory effect on inflammation (Just et al., 1998) and this tend to justify the use of *A. laricinus* in traditional medicine. Tannins are found in almost every plant part: bark, wood, leaf, fruits, and roots and can be toxic to filamentous fungi, yeasts, and bacteria (Scalbert, 1991). Alkaloids were not detected in this study plant and studies on Asparagus species showed no evidence of alkaloids in the Asparagaceae family.

Phenols are known to be synthesized by plants in response to microbial infection (Dixon et al., 1983) it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. The mechanism behind phenolic toxicity to microorganisms includes enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987). Their presence correlates with the antibacterial and anti-oxidant activities of the leaf extract. These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments.

Conclusion

The leaf extracts of the *A. laricinus* exhibited a significant free radical scavenging effect of DPPH in a concentration dependent manner and antibacterial activity at the minimum inhibition concentration of <1 mg/mL.

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Conflict of Interest

Authors declare no conflict of interest

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