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Phytochemistry and Free Radical Scavenging Activity of *Asparagus laricinus*

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Abstract: This study reports on the phytochemistry and radical scavenging activity of *Asparagus laricinus* Burch (Asparagaceae) in order to explain the anti-cancer properties it possesses. The root extracts of *Asparagus laricinus* were qualitatively screened for antioxidant properties using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), on silica gel GF_{254} TLC plates. Quantitative Radical scavenging activity using DPPH was also evaluated. The extracts were tested for the presence of alkaloids, saponins, tannins, terpenoids and steroids using standard phytochemical screening methods. Gas chromatography/mass spectrometry (GC/MS) was used to further confirm the phytoconstituents. Three spots that exhibited antioxidant activity were resolved on the TLC plates. The free radical scavenging activity of *A. laricinus* extract was comparable with that of Trolox® and was dependent on the concentration of the extract. The aqueous extract of *A. laricinus* contained alkaloids, saponins, tannins and terpenoids while the ethanol and dichloromethane extracts produced negative results. The phytoconstituents confirmed through the use of GC-MS were indole-3-carbinol, α-sitosterol and ferulic acid. The phytochemicals present and the antioxidant activity demonstrated by extracts of *A. laricinus* explain its *in vitro* anti-cancer properties as well as its continued use in traditional medicine for the treatment of cancer and other ailments.

Key words: *Asparagus laricinus*, antioxidant, free radical scavenging, phytochemical screening

INTRODUCTION

The use of medicinal plants in all cultures is well documented. Plants have been the main component of traditional pharmacopoeias for generations and continue to supply new remedies for the treatment of various maladies. For instance, an estimated 80% of the world's population mainly uses plant medicines for primary health care. Traditional healers in South Africa attend to approximately 60% of South Africans. The bulk of traditional medicine in the country is based on nearly 3000 plant species (Taylor *et al*., 2001; Masoko *et al*., 2010).

Modern pharmaceutics also have benefited from medicinal plants. For example, only 39% of the 877 molecules used in drug development between 1981 and 2002 were truly synthetic in origin. The rest were naturally inspired or derived. Furthermore, more than half of the drugs used in cancer treatment are of natural origin (Gurib-Fakim, 2006; Newman and Cragg, 2007) with the therapeutic alkaloids (vinblastine and vincristine) epipodophyllotoxins, taxanes and camphothecins as examples of plant-derived anti-cancer compounds that are currently in clinical use (Balunas and Kinghorn, 2005). Plants therefore are potent sources of new drugs and drug leads (Balunas and Kinghorn, 2005; Newman and Cragg, 2007; Harvey, 2008; Shyur and Yang, 2008).

The therapeutic properties of plants are attributed to the broad spectrum of secondary metabolites (Pieters and Vlietinck, 2005; Kaur *et al*., 2005) including polyphenols, alkaloids and flavonoids. These phytochemicals are produced to protect the plant against herbivory and microbial attacks. Secondary metabolites are also manufactured by plants to attract pollinators and symbionts, as well as to respond to abiotic stresses (McRae *et al*., 2007). These ecological functions form the basis of the bioactivity exhibited by the compounds and has resulted in antimicrobial medicines (Briskin, 2000), anti cnacer drugs and plant-based antioxidants. The medicinal potency of a plant may result from a single compound or the synergistic or additive action of several constituents in the plant (Shyur and Yang, 2008; Van Vuuren, 2008; Eloff *et al*., 2008). Therefore, the phytochemical screening of plant extracts in the process of drug discovery is very important.

This Study reports on the phytochemical screening of *Asparagus laricinus* Burch (Asparagaceae). *A. laricinus* is part of traditional medicine in many

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communities in South Africa. The leaves and stem are used medicinally in South West Gauteng (Dzerefos and Witkowski, 2001). The use of the roots of *A. laricinus* as a diuretic and to treat tuberculosis is reported in Khoi-San and Cape Dutch medical ethnobotany (Van Wyk, 2008). The roots also have ethnoveterinary use for the treatment of sores, redwater, urine infections, umbilical cord inflammation and general ailments among the Setswana people of the North West Province of South Africa (Van der Merwe *et al*., 2001). *A. laricinus* is also used in the treatment of cancer (Mashele and Kolesnikova, 2010).

Previous studies have indicated that alcoholic and aqueous extracts of *A. laricinus* have active anti-cancer properties in-vitro against three human cancer cell lines (Mashele and Kolesnikova, 2010). The aqueous extract of the plant also showed antimutagenic effects (Mashele and Fuku, 2011) using the Ames test, whilst the mutagenicity tests were negative. Traditional medicinal use of plants in South Africa is strongly related to physiological and pharmacological activity of active plant ingredients. The phytochemical characterization of the plant may validate its use in traditional medicine for the treatment of cancer. Oxidative stress is involved in the development of diseases such as cancer due to the overproduction of free radicals (Adewusi and Afolayan, 2009; Aremu *et al*., 2011). *A. laricinus* was also screened for antioxidant (radical scavenging) properties in an effort to further evaluate its potential as a comprehensive anti-cancer agent.

METHODS

Collection and validation of samples: *Asparagus laricinus* was collected from traditional healers in Pretoria, South Africa in July 2011. The plant was cross-identified by its vernacular names and later validated at the National Botanical Gardens in Pretoria, South Africa (Voucher specimen: Mash 002).

Preparation of extracts: Two hundred and fifty gram roots of *Asparagus laricinus* were cleaned with tap water to eliminate dust and soil, then air dried under shade. The dried material was sliced into small fragments and extracted at room temperature thrice with ethanol, distilled water and dichloromethane for 72 h. Finally, the extracts were concentrated using a rotary-evaporator (R215 Buchi Instrument, Switzerland) at a reduced pressure and at <40°C. The recovered weight of the plant material obtained was 20% of the dried material (Al-Azzawi and Al-Juboori, 2012).

Phytochemical analysis: The presence of phytochemicals in the three extracts, such as alkaloids, saponins, tannins (5% ferric chloride), terpenoids (2, 4-dintrophenyl hydrazine) and steroids (Liebermann-Burchard test) were evaluated according to the methods described by Edeoga *et al*. (2005).

Thin layer chromatography analysis of antioxidant constituents: Qualitative screening for antioxidant activity was done using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to Tomohiro *et al*. (1994). Thin layer chromatography (TLC) of extracts was developed with methanol: Chloroform: Hexane (70:20:10%) on silica gel GF_{254} (Fluka, 20×20 cm^2) as the stationary phase. The plates were air dried and sprayed with 0.05% DPPH in methanol. Antioxidant activity was detected on the chromatogram by the appearance of yellow spots produced by bleaching of DPPH (Bors *et al*., 1992). All detected active antioxidant constituents were noted according to their Rf values. Gallic acid was used as positive control.

Free radical scavenging activity: The free radical scavenging activity of the *Asparagus laricinus* extracts was analyzed by using 2,2-diphenyl-2-picrilhydrazyl (DPPH) assay (Von Gadow *et al*., 1997; Fuhrman *et al*., 2001). Different concentrations (between $10^{-1}\times10^{-04}$ mg mL^{-1}) of the extract were prepared. Aliquots (100 μL) of the extract or standard solution (Trolox® and ascorbic acid) were mixed with 2 mL of 0.1 mmol L^{-1} methanolic solution of DPPH radical. The tubes were mixed and allowed to stand for 60 min in the dark. Absorbance was read against a blank at 517 nm using a spectrophotometer. Methanol was used to blank the spectrophotometer, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) and Ascorbic Acid were used as positive controls. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the extracts was calculated according to the equation used in Turner *et al*. (2011):

$$\text{Inhibition (\%)} = [1 - (A_s/A_0)]\times100$$

where, A_s is absorbance of sample (i.e., extracts or standard) and A_0 is the absorbance of the DPPH solution, the EC_{50}, defined as the concentration of the sample leading to 50% of the reduction of the initial DPPH concentration, was determined at 95% confidence interval, using GraphPad Prism® software. The data on regression graphs represents Mean+/-SEM (Standard error of means). The experiment was done in triplicate.

Gas chromatography/mass spectrometry (GC/MS): GC-MS analysis was carried out on an Shimadzu 2010 QB gas chromatograph with a MSD detector equipped with an HP-5 fused silica capillary Column (30m×0.25mm×25m film thickness). The aqueous plant extract was injected via., an all-glass injector working in split mode with Helium as the carrier gas at a flow rate of 1 mL min^{-1}. Temperature program: Injector temperature 200°C, Ion source 200°C, Interphase 200°C. Column temperature was raised to 45°C (3 min hold at 45°C, 4°C min), then gradually increased to 150°C (3 min hold at 150°C, 4°C min) then raised to 250°C and a 15 min hold. A split ratio of 1:5 was used (Ajayi *et al*., 2011). Compound identification was accomplished by comparing the retention times with those of authentic compounds or the spectral data obtained from the Wiley Library and National Institute of Standards and Technologies Library, as well as with data published in the literature.

RESULTS

Thin layer chromatography analysis of antioxidant constituents : Figure 1 shows the TLC chromatogram for the qualitative screening for antioxidants. Three spots with Retention Factors (Rf) of 0.87, 0.38 and 0.35 exhibited antioxidant activity when sprayed with DPPH. This indicated differences in the polarity of the separated antioxidant molecules.

Free radical scavenging activity: The addition of the *A. laricinus* extract to the DPPH solution induced a rapid decrease in absorbance at 517 nm indicating free radical scavenging activity of the extract (Fig. 2). The EC_{50} value of the extract was recorded to be between 0.9205 to 1.188 mg mL^{-1} and was higher than that of Trolox® (Fig. 2d). The radical scavenging activity of the extract was concentration-dependent and similar to that of known antioxidants (Fig 2a, b and c). *A. laricinus* showed a concentration-dependant free radical scavenging activity at concentrations above 1 mg mL^{-1} while ascorbic acid no longer showed increasing activity above the same concentration.

Phytochemical analysis: Phytochemical screening of the aqueous extract was positive for the presence of alkaloids, saponins, tannins and terpenoids. However, the ethanolic and dichloromethane extracts had negative results for all the chemical tests that were carried out, as shown in Table 1.

GC-MS study: The GC-MS chromatogram gave rise to three peaks suggesting the existence of three secondary metabolites and percentage values of composition of these phytochemicals present in the aqueous root extract of *Asparagus laricinus*. The results suggested the presence of indole-3-carbinol, α-sitosterol and ferulic acid, as shown in Fig. 3.

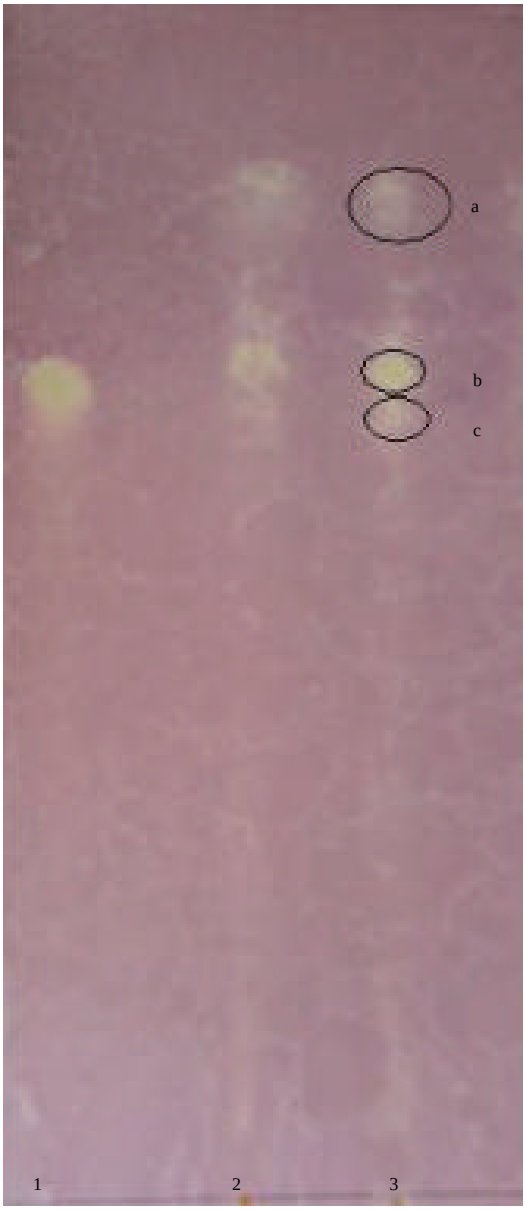

Fig. 1: Chromatogram of gallic acid (1) and *A. laricinus* aqueous extract (2 and 3), separated with methanol:chloroform: hexane (70:20:10%) and sprayed with DPPPH. The yellow spots indicated antioxidant activity (RF values: a = 0.87; b = 0.38; c = 0.35)

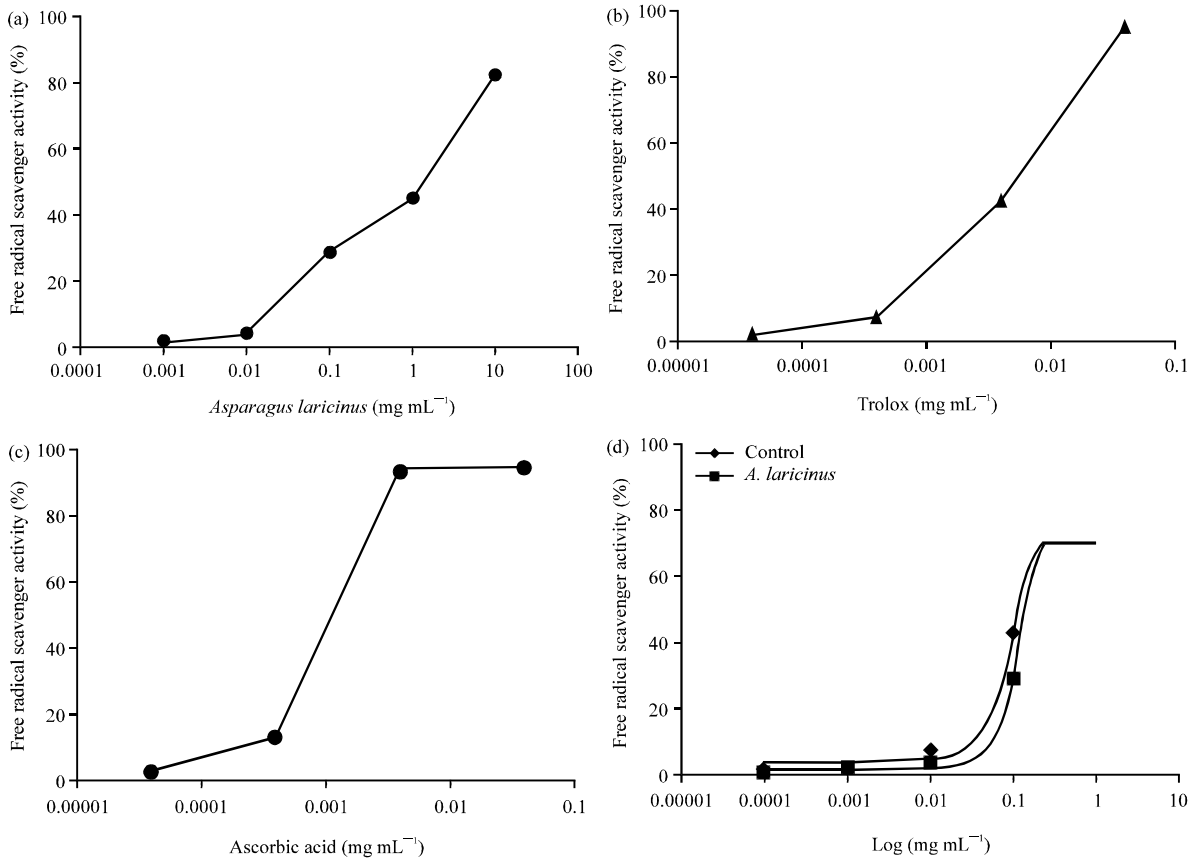

Fig. 2(a-d): Scavenging activity of *A. laricinus* aqueous extract on the free radical DPPH, (2a) *A. laricinus* extract, (2b) Trolox®, (2c) and ascorbic acid, (2d) EC_{50} shift graph of *A. laricinus* extract with Trolox® as the control

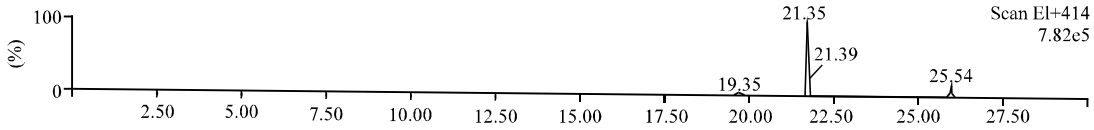

Fig. 3: GC-MS chromatogram of the aqueous extracts of *A. laricinus* roots

Table 1: Phytochemical screening of ethanol, aqueous and dichloromethane extracts of the roots of *A. laricinus*

Constituents	Aqueous	Ethanolic	Dichloromethane
Alkaloids			
Dragendorff's test	+	-	-
Steroids			
Libarman-Burchard's test	+	-	-
Terpenes			
Salkowski test	+	-	-
Tannins			
FeCl3 test	+	+	-
Gelatin test	-	-	-
Saponins			
Frothing test	+	-	-

Key: -: Negative (absent): +: Positive (present)

DISCUSSION

The antioxidant capacity of crude drugs is widely used as a parameter for evaluating medicinal bioactive components. The present study has demonstrated the antiradical activity of *A. laricinus*. We observed three spots that exhibited antioxidant properties on the TLC chromatograms. The characteristics of the three different

antioxidants observed in the *Asparagus laricinus* extracts are yet to be elucidated. The antioxidant in the plant extract may largely be due to polyphenols (Thabrew *et al*., 1998). Phenolics are the largest group of phytochemicals and most of the antioxidant activity of plants or plant products is attributed to them. Many studies have shown that natural antioxidants are able to reduce DNA damage, mutagenesis and carcinogenesis. These events are often associated with the termination of free radical propagation in biological systems (Covacci *et al*., 2001; Zhu *et al*., 2002). The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability or radical scavenging ability (Baumann *et al*., 1979). Considering the fact that the *A. laricinus* extract used was crude and the comparison was not on the basis of molar concentration, the above results are promising, despite having a higher EC50 value than Trolox®.

Phytochemical screening of indigenous plants is essential to evaluate their medical value and their potential use in the treatment of various diseases. In the current study of the *A. laricinus* extract, three secondary metabolites were found in the aqueous extract. The extract showed the presence of ferulic acid, a phenolic acid derivative that has antioxidant activity and several therapeutic benefits in the treatment of cancer. Ferulic acid is a plant constituent that arises from the metabolism of phenylalamine and tyrosine. It occurs primarily in seeds and leaves both in its free form and covalently linked to lignin and other biopolymers. Due to its phenolic nucleus and an extended side chain conjugation it readily forms a resonance stabilized phenoxy radical which accounts for its potent antioxidant potential (Imaida *et al*., 1990; Srinivasan *et al*., 2007). β-Sitosterol has a number of therapeutic and chemo preventive uses in the medical field (Zak *et al*., 1990; Baskar *et al*., 2010). Indole-3-carbinol is used in prostate cancer (Garikapaty *et al*., 2005). The phytoconstituents detected in the plant materials could be responsible for the cytotoxic activity, though their exact mode of action is poorly understood at present.

CONCLUSION

The aqueous extract of *Asparagus laricinus* roots showed significant activity as an antioxidant. It showed free radical scavenging activity comparable to Trolox and Ascorbic acid. The free radical scavenging activity of the extract may be attributed to Ferulic acid, β-Sitosterol and/or indole-3-carbinol. The results of this study further justify the use of the plant in traditional medicine and highlight its potential for use in drug development.

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