Phytochemical characterization and the determination of antioxidant activities of *Asparagus nelsii* root extracts.

Simeon Ambuga [a], Lina Kweenani [a], and Marius Mutorwa [a]

[a] Faculty of Health, Natural Resources and Applied Sciences, Namibia University of Science and Technology, Namibia.

CONTACT

Dr Marius Mutorwa
Department of Natural and Applied Sciences
Email: mmutorwa@nust.na
Phone: +264 61 207 2839

www.nust.na

ABSTRACT

The phytochemicals present in water and methanol extracts of roots of *Asparagus nelsii* were determined. Antioxidant tests of the extracts and characterization of the compounds present in the extracts was conducted. This was done with crude extracts obtained by reflux extraction of dried roots. Different screening methods were employed to detect the phytochemicals present in the extracts. Characterization of the compounds was done using GCMS and the data was compared to the NIST library database for compound identification.
Antioxidant tests were conducted using the hydrogen peroxide scavenging essay at different concentrations of crude extracts. A total of 10 phytochemical classes were found to be present, including flavonoids, saponins, tannins and sterols. The water extract exhibited the highest antioxidant potential at a concentration of 110 µg/ml compared to ascorbic acid and the methanol extract. water extract also had high antioxidant capacity compared to methanol extract. The results of this study *A. nelsii* can be used as an easily accessible source of natural antioxidants.

INTRODUCTION

The contribution of oxidative stress in the mechanism of antibiotic-mediated cell death is still unclear and remains a subject of debate. It has been shown that under aerobic conditions bactericidal antibiotics with specific targets in bacterial cells stimulate the production of harmful ROS, which contribute to killing by these drugs. This phenomena points to the need for new therapeutic strategies in targeting anti-oxidant pathways together with new antibacterial agents able to fight chronic infections caused by multidrug resistant bacterial strains, and dual-target molecules from medicinal plants could give a successful answer. Studies on the genus *Asparagus* have been done, which include *Asparagus officinalis*, *Asparagus albus*, and *Asparagus racemosus*. indicate the vast use of these plants in the treatment of a variety of infections.

METHODS AND MATERIALS

Sampling method and analysis

Solvent extraction by reflux was used for the dried root samples as follows: to 50 ml of each solvent (methanol and water) was added 5 g of dried sample in a round bottom flask, the mixture was then refluxed for 3 hours each.

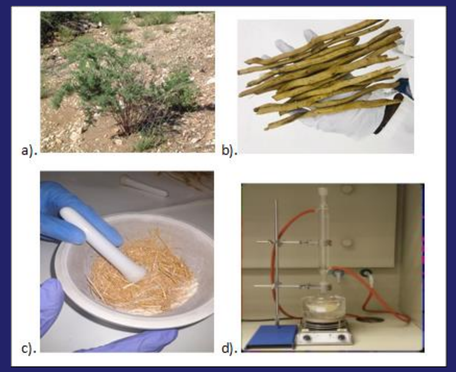

Figure 1:a)*Asparagus nelsii* plant at sampling location. *b)* roots of *asparagus nelsii* before crushing. c) Root samples being crushed using a mortar and pestle. d) reflux extraction setup.

RESULTS AND DISCUSSION

1. Phytochemical screening

Phytochemical	Water extract	Methanol extract
Alkaloids	-	-
Flavonoids	+	+
Tannins	+	+
Saponins	++	++
Terpenoids	+	+
Sterols	+	+
Phenols	+	+
Triterpenoids	+	+
Steroids	+	+
Quinone	+	+
Glycoside	+	+

Table 1. Presence of phytochemicals in extracts of different solvents

As seen in Table 1, the saponin content in both the extracts was high, the foam produced from the test lasted for more than 15 minutes and this is normally an indication of high amounts of saponins. No alkaloids were detected in both the water and methanol extracts, although some species of this genus were previously reported to contain alkaloids. The characteristics of the different species of Asparagus are reported to have diversity in their phytoconstituents..

RESULTS AND DISCUSSION

2. Antioxidant activities

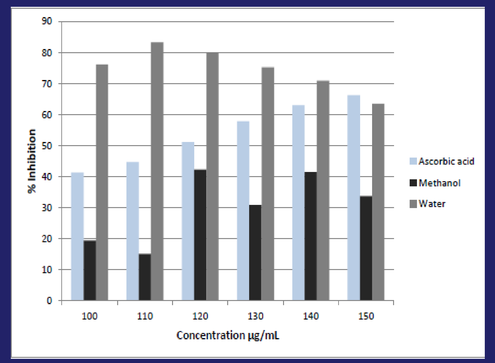

Figure 2: Scavenging activity of ascorbic acid, water and methanol extracts. All values are represented as means ± SD (n=3).

Figure 2 shows that the water extract demonstrated a higher antioxidant potential when compared to the methanol at all concentrations. Similarly, the water extract exhibited high antioxidant potential when compared to ascorbic acid at lower concentrations. Ascorbic acid was used as a positive control in this experiment.
A uniform decrease in the inhibition was observed from 80% to 63% with 110 to 150 µg/ml of the water extract. This trend in inhibition indicates that 110 µg/ml of the extract possessed the highest antioxidant activity in the range of concentrations studied for the water extract.

As the concentration of extract moves beyond 110 µg/ml (after the peroxide is neutralised), the inhibition begins to decrease. Methanol extracts scavenges up to approximately 43% at a concentration of 120 µg/mL; which is the highest inhibition observed for the methanol extract. There was no definite correlation between the increases in concentration of the extract with an increase in scavenging percentage, the data was not uniform. The lack of correlation between an increase in extract concentration with an increase in scavenged percentage could be because the reaction of H_2O_2 with the oxidant in the extract produces a compound that is unstable and breaks down to produce UV active components.

RESULTS AND DISCUSSION

3. Characterization - Thin Layer Chromatography and GC-MS

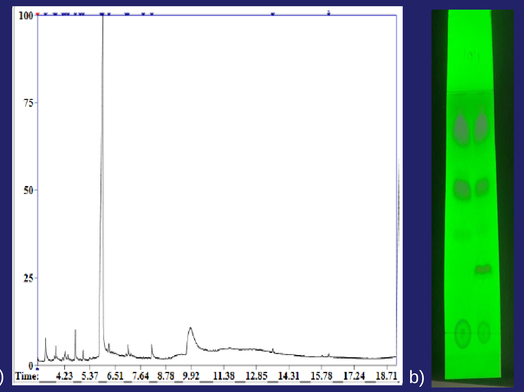

Figure 3. a) GC chromatogram of methanol extract cleaned using alumina. b) TLC of the methanol and water extracts in 1:1 hexane and ethyl acetate

Figure 3 a) revealed 5 spots in the methanol extract and 4 spots in the water extracts. This is an indication that there are organic compounds present in the extract. The mobile phase used was of medium polarity and the results showed that both the extracts contained compounds of different polarities. The methanol extract was subjected to GC-MS analysis after cleaning up of the extract with alumina.

The chromatogram of the cleaned methanol extract is shown in figure 3.a, There were 10 significant peaks identified in the chromatogram. This is an indication of the vast number of compounds present that could potentially be contribute to the extract's antioxidant effect.

SUMMARY

Percentage inhibition

Concentrations	Ascorbic acid inhibition %	Methanol extract inhibition %	Water inhibition %
100 µg/ml	41.8%	19.4%	73.1%
110 µg/ml	44.5%	15.1%	83.3%
120 µg/ml	51.2%	42.3%	80.0%
130 µg/ml	57.6%	30.9%	75.3%
140 µg/ml	63.7%	41.5%	70.9%
150 µg/ml	66.1%	33.7%	63.6%

CONCLUSION

Asparagus nelsii has a great potential to tackle problems associated with ROS species in the human body. The phytochemical screening provided a map of the types of phytochemicals that are present in the plant and their uses and potentials are known and well documented in previous studies. 10 different phytochemical classes were identified during screening. Proper characterization of the different compounds present is a key component in identifying the compounds responsible for the antioxidant activity that are demonstrated in the study. The results of this study show that the water and methanol extracts of *A. nelsii* exhibited antioxidant activity and can thus be used as easily accessible source of natural antioxidants, as a possible food supplement or used in the pharmaceutical industry. The water extract showed greater hydrogen peroxide scavenging activity when compared to the methanol extract as well as the standard i.e. ascorbic acid.