Nutritional and Medicinal value of the edible stinkbug, *Encosternum delegorguei* Spinola consumed in the Limpopo Province of South Africa and its host plant *Dodonaea viscosa* Jacq. var. *angustifolia*

By

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PREFACE

I, Leah Snow Teffo, declare that the thesis hereby submitted to the Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria for the degree of Philosophiac Doctor has not been submitted by me for a degree at this or any other University. The results obtained from this study are my own work in design and execution and except where specifically acknowledged.

Signed: .....……

Dated: ……..

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Conference Proceedings and Publications

Conferences:

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1. Teffo LS, Toms RB and Eloff JN. *Medicinal and nutritional value of edible stink-bug Encosternum delegorguei consumed in Limpopo Province of South Africa*. Faculty Day, University of Pretoria, September 2004.

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1. Teffo LS, and Eloff JN. *Antibacterial and antioxidant activities of Dodonaea viscosa Jacq. var. angustifolia leaf extracts.* To be submitted to South African Journal of Botany.

2. Teffo LS, Toms RB and Eloff JN. *Nutritional composition of edible stink bug, Encosternum delegorguei Spinola consumed in Limpopo Province of South Africa*. Submitted to South African Journal of Science.

3. Teffo LS, Aderogba MA and Eloff JN. *Antibacterial and antioxidant activities of kaempferol methyl ethers from Dodonaea viscosa Jacq. var angustifolia leaf extracts.* To be submitted to Journal of Ethnophamacology.

Summary

In most rural areas, reliance on traditional medicine and food is high and this is attributed to both economic and cultural factors. The edible stink-bug (*Encosternum delegorguei* Spinola), local name "thongolifha" is consumed by the Venda tribe in Limpopo Province of South Africa. Thongolifha is important to the Venda tribe in terms of culture, nutrition and economical value. They eat it raw or cooked with porridge or as a snack. The edible stink-bug is sold at the Thoyandou open market and provides valuable income for the harvesters. Many insects have been reported to contain good source of proteins minerals and vitamins. In terms of medicinal value, insects have also been used in folk medicine in the past by various cultures to treat many ailments such as stroke, asthma, cold, etc. Some insects are also known to sequester compounds from their host plant and use them for its defensive mechanism. The host plant for thongolifha was identified as *Dodonaea viscosa* Jacq. var. *angustifolia* which has been reported to have antibacterial activity.

The aim of this study was to investigate the possible interaction between the thongolifha and its host plant *Dodonaea viscosa*. Since thongolifha is so important to the Venda tribe and its nutritional value was never analysed before it was also relevant to determine its nutritional composition. Nutritional components of thongolifha such as the proteins, fats, amino acids and carbohydrates contents were detected by using standardized methods. Thongolifha contained 35.2% protein, 50.6% fat and 7.63 g/100 g carbohydrate with an energy content of 2599 kJ/100 g. These results compare well to nutritional values of other edible insects such as termites (41.8% protein and 44.3% fat) and wasps (31% protein and 62% fat).The mopane worm has higher protein content (63.5%) and a lower fat content of (18%). Mineral content of thongolifha (1.2 g/100 g) was found to be at a comparable level to other bugs such as the *Acantocepphala declivis* (1.0 g/100g). Analysis of essential amino acids varied from 0.82 mg/100 g (threonine) and 1.32 mg/100 g (valine). The nutritional composition of thongolifha is acceptable and thongolifha is not just a traditional delicacy but also contributes as a diet supplement of the Venda tribe. Between 680 and 3400 of thongolifha will however have to be consumed to supply the daily nutritional needs for the essential amino acids phenylalanine and methionine.

Analysis for antibacterial activity of thongolifha was carried out after extracting with solvents of varying polarities, separation by thin layer chromatography (TLC) and bioautography against four nosocomial bacteria; Gram-negative; *Escherichia coli* and *Pseudomonas aeruginosa;* Gram positive; *Staphylococcus aureus* and *Enterococcus faecalis.* Bioautography results of the thongolifha extracts revealed some activity against *S. aureus* and *E. faecalis.* Evaluation of the minimal inhibitory concentration (MIC) by using a serial dilution microplate method indicated low antibacterial activity (MIC > 2.5 mg/ml). Thongolifha therefore does not contain antibacterial compounds but there may be present in a low concentration or the serial dilution microplate method does not work well with insects extracts. Some insects contain peptides as antibacterial compounds. Spraying thongolifha extracts chromatograms with several spraying reagents indicated that these antibacterial compounds were not peptides

Methanol crude leaf extracts of *D. viscosa* was reported in the literature to have antibacterial activity against *S. aureus* however the results were obtained by using the agar diffusion method which does not provide convincing quantitative results. In our study we extracted components from *D. viscosa* using solvents of varied polarities to evaluate its antibacterial and antioxidant activities. Dichloromethane and acetone extracted more compounds with good antibacterial activity against *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*. The average MIC values varied from 0.04 to 0.28 mg/ml, indicating excellent to good antibacterial activity. The antioxidant activity using the DPPH Spectrophotometric assay gave the EC_{50} of the extracts of 212 ± 4 to 469 ± 9 μg/ml. As expected, the more polar crude extracts, ethyl acetate and acetone demonstrated higher activity compared to other extracts. This was supported by the TLC qualitative assay showing more yellow bands in these extract on TLC chromatograms after spraying with 0.2% DPPH in methanol.

To determine whether thongolifha has sequestered compounds from the host plant, *D. viscosa*, a serial exhaustive extraction was performed in order to extract components from the crude extracts of both insect and the host plant by using various solvents and separating the extracts on TLC plates by using various eluant systems. After the TLC chromatograms were sprayed with vanillin-sulphuric acid to reveal separated components, there were few similarities of components from both insect and host plant. This could mean that either a sequestered plant compounds are present in low quantities in the insect extract, that insect may have metabolized the sequestered compounds or alternatively that the insect might not have sequestered the compounds from the host plant at all. It should be kept in mind that the stink-bugs feed by sucking sap from the host plant.

By using bioassay-guided fractionation based mainly on silica gel chromatography, four compounds (5-hydroxy-3,74'-trimethoxyflavone (1); 5,7-dihydroxy-3,6,4'-trimethoxyflavone (2); 5,7,4'-trihydroxy-3,6-dimethoxyflavone (3); and 4'-O-methylkaempferol (4) where isolated from dichloromethane fraction of a crude *D. viscosa* leaf extract, acetone crude leaf extract yielded only one compound, Kaempferol (5). Compounds 1, 2, 3 and 5 where previously isolated from *D. viscosa* whereas compound 4 was isolated for the first time from *D. viscosa*. Compounds 4 is not a novel compound because it was previously isolated from other plants species such as the *Lens culinaris.* Compounds 3, 4 and 5 have good antibacterial activity against *E. coli* and *E. faecalis* at MIC values of 15.63 μg/ml and 31.25 μg/ml respectively. In addition, the DPPH quantitative assay has demonstrated that Compounds 4 and 5 have strong antioxidant activities (EC_{50} = 35.1 ± 0.85 and 75.5 ± 1.76 μM respectively) but lower than L-ascorbic acid (EC_{50} = 13.5 ± 0.28 μM) used as a standard antioxidant agent. The antibacterial and antioxidant activity of most of the isolated compounds were not known previously. Investigation of structure antibacterial-activity relationship in the isolated compounds did not yield a clear correlation.

When investigating the possible interaction between the insect and the host plant, bioautography assay has shown compounds with antibacterial activity against *S. aureus* (Rf 0.79) *E. faecalis* (Rf 0.66) when separated with CEF

eluent system. Some of the isolated compounds from *D. viscosa* leaf extracts had an Rf of 0.79 (5, 7-dihydroxy-3, 6, 4'-trimethoxyflavone) and Rf 0.61 (5, 7, 4'-trimethoxxyflavone) when separated with CEF eluent system. These findings indicate that thongolifha may have sequestered the two compounds from *D. viscosa* leaves, but this possibility has to be confirmed.

This study has indicated that thongolifha is good source of protein, vitamins, minerals and amino acids; however we have not found evidence of significant antibacterial activity of thongolifha. If fresh material of thongolifha can be analysed in future work the probability of finding medicinal properties may be greater. On the other hand, *D. viscosa* crude leaf extracts have demonstrated strong antibacterial and antioxidant activities; although isolated compounds have shown moderate level of activities. Because practically all antibacterial compounds based on bioautography have been isolated, it is clear that in the crude dichloromethane extract some synergism must have taken place because the antibacterial activity on a mass basis was nearly as good as the isolated compound without removing >90% of non active compounds.

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GLOSSARY

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BEA- benzene/ethanol/ammonia (18:2:0.2)

CE- chloroform/ethyl acetate (8:2)

CEF- chloroform/ethyl acetate/formic acid (10:8:2)

^{13}C NMR- Carbon 13 Nuclear Magnetic Resonance

CSIR- Centre for Scientific and Industrial Research

DCM- Dichloromethane

EMW- ethyl acetate/methanol/water (10:1.35:1)

EtOAc- Ethyl Acetate

EtOH- Ethanol

DPPH- 2, 2 Diphenyl-1-picrylhydrazyl

Hex- Hexane

H NMR- Proton Nuclear Magnetic Resonance

INT- *p*-iodonitrotetrazolium violet

IR- Infra Red

LD_{50}- the concentration that would kill 50% of the organism

M^+- Molecular ion

MEDUNSA- Medical University of South Africa

MIC- Minimum Inhibitory Concentartion

MH- Mueller-Hinton broth

MS- Mass Spectrometry

M/Z- Mass to charge ratio

NMR- Nuclear Magnetic Resonance

RAU- Rand Afrikaans Universiteit

SANAS- South African National Accreditation System

TA- Total Activity value

TEAC- Trolox Equevalent Antioxidant Capacity

TLC- Thin Layer Chromatography

UV- Ultra violet

VLC- Vacuum Liquid Chromatography

Codes for Isolated Compounds

T1055= 5-hydroxy -3, 7, 4'-trimethoxyflavone (Compound 1)

C= 5, 7-dihydroxy-3, 6, 4'-trimethoxyflavone (Compound 2)

E= 5, 7, 4'-trihydroxy-3, 6-dimethoxyflavone (Compound 3)

Cpd2= 4'-O-methylkaempferol (Compound 4)

AB= kaempferol (Compound 5)

D. viscosa is used to indicate *Dodonaea viscosa* Jacq. var. *angustifolia*