

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:

Paper Presentations

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.
2. Teffo LS, Toms RB and Eloff JN. *Nutritional value of edible stink-bug Encosternum delegorguei consumed in Limpopo Province of South Africa*. IPUF Conference, Clanwilliams Town, Cape Town; June 2004.
3. Teffo LS, Toms RB and Eloff JN. *Medicinal value of edible stink-bug Encosternum delegorguei consumed in Limpopo Province of South Africa and its host plant Dodonaea viscosa*. IPUF Conference, Rhodes University, Grahamstown; July 2005.
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Papers Submitted for Publications

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 Ethnopharmacology.

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 *Acantocephala 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 EC₅₀ 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-3,7,4'-trimethoxyflavone (1); 5,7-dihydroxy-3,6,4'-trimethoxyflavone (2); 5,7,4'-trihydroxy-3,6-dimethoxyflavone (3); and 4'-O-methylkaempferol (4) were 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 were previously isolated from *D. viscosa* whereas compound 4 was isolated for the first time from *D. viscosa*. Compound 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 (EC₅₀ = 35.1 ± 0.85 and 75.5 ± 1.76 µM respectively) but lower than L-ascorbic acid (EC₅₀ = 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'-trimethoxyflavone) 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.

TABLE OF CONTENTS

	Page
Preface	i
Acknowledgements	ii
Conference Proceedings and Publications	iii
Abstract	vi-vii
List of Figures	viii
List of Table	x
Glossary of Abbreviations	xi
CHAPTER 1	
LITERATURE REVIEW	1
INTRODUCTION	1
1. Insects	1
1.1 Insects as human food	1-2
1.2 Chemical composition of insects	2-3
1.3 Why care for insects?	4
1.4 Economical importance of insects	4

1.5 Insects as animal feed	4-5
1.6 Insects as source of drugs	5-6
2. The plants	6
2.1 The use of plants extracts	6
2.2 The problem of drug resistance	6
2.3 Importance of antioxidants	7
2.3.1 Flavonoids	7-8
3. The host plants	8
3.1 <i>D. viscosa</i>	8
3.1.1 Taxonomy and description of <i>D. viscosa</i>	8-9
3.1.2 Biological activity of <i>D. viscosa</i>	9
3.1.3 Major chemical constituents of <i>D. viscosa</i>	10
Problem statement	10
Aim of the study	11
Objectives of the study	11
CHAPTER 2	
2. METHODOLOGY	12

2.1 Insect Experiment Procedures	12
2.1.1 Collection of insects	12
2.1.2 Traditional Knowledge of Insects Preparation	12-13
2.2.1 Nutritional analysis of insects	13
2.2.1.1 Determination of Macro Nutrients and Amino Acids	14
2.2.1.2 Determination of Minerals	14
2.2.1.3 Determination of Vitamins	14
2.2 Plant Experimental Procedures	14
2.2.1 Collection and Identification of the Host plant	14-15
2.2.2 Plant preparation	15
2.3 General Materials and Methods	15
2.3.1 Extraction Procedure	15
2.3.2 Thin Layer Chromatography Analysis	15
2.3.3 Bioassays	15-16
2.3.3.1 Bioautography assay	16
2.3.3.2 Microdilution assay	16
2.3.3.3 Antioxidant assays	17-18
2.3.4 Statistical analysis	18

CHAPTER 3

3. Nutritional value of <i>E. delegorguei</i>	19
3.1 Background	19
3.1.1 Morphology of thongolifha	19-20
3.2 Results and Discussion	20-24
3.3 Conclusion	24-25

CHAPTER 4

4. Medicinal values of insects	26
4.1 Background to use of insects	26
4.1.1 Use of insects in folk medicine	26-27
4.1.2 Insects as a source of new drugs	27
4.2 Results and Discussion	27
4.2.1 Thin Layer Chromatography assay	27-28
4.2.2 Bioautography assay	29
4.2.3. Microdilution assay	30
4.3 Conclusion	30

CHAPTER 5

5.1 Medicinal value of Host Plant	31
5.2 Results and Discussion	31
5.2.1 Thin Layer Chromatography Assay	31
5.2.2 Bioautography assay	32-33
5.2.3 Microdilution assay	33-35
5.2.4 Antioxidant assay	35-36
5.3 Conclusion	37

CHAPTER 6

6. Isolation of bioactive compounds from host plant (<i>D. viscosa</i>)	38
6.1 Spectroscopy	38
6.2 Methodology	38
6.2.1 Chromatographic purification of leaf extracts	38-39
6.3 Results and Discussion	39
6.3.1 Crude extract obtained by VLC	39
6.3.2 Chromatographic purification	40
6.3.1.1 Purification of DCM crude extracts	40-45

6.3.1.2 Purification of acetone crude extracts	45-46
6.3.2 Structure elucidation of pure compounds	46
6.3.2.1 Compound 1	46-48
6.3.2.2 Compound 2	49-51
6.3.2.3 Compound 3	52-54
6.3.2.4 Compound 4	55-57
6.3.2.5 Compound 5	58-59
6.4 Conclusion	60

CHAPTER 7

7. Biological activity of isolated compounds from *D. viscosa*

7.1 Biological assays of pure compounds	61
7.2 Results and discussion	61
7.2.1 Biological assays	61
7.2.1.1 Bioautography assays	61-63
7.2.1.2 Antioxidant assay	63-65
7.2.1.3 Microdilution assay- Structure activity relationship	65-66
7.3 Conclusion	67

CHAPTER 8

8. General Discussion and Conclusion	68
8.1 <i>E. delegorguei</i> extracts	68-69
8.2 <i>D. viscosa</i> extracts	69-70
8.3 Insect-Plant Interaction	70
BIBLIOGRAPHY	71-84
APPENDIX	
¹ H NMR Spectrum of Compound 4 (4'-O-methylkaempferol)	85
¹³ C NMR Spectrum of Compound 4 (4'-O-methylkaempferol)	86
Mass Spectrum of Compound 4 (4'-O-methylkaempferol)	87

LIST OF FIGURES

Figure:	Page
1.1: <i>Dodonaea viscosa</i> (Family Sapindaceae) (Picture by: Cathy) and its distribution map (Palgrave, 2002)	9
2.1 Steps that involved during traditional prepared of thongolifha before they are taken to the Venda market for sale. Step 1- insects are collected, dead ones are sorted out and discarded; step 2- Live ones are placed in a bowl; step 3- heads of dead bugs are removed and the thorax squeezed to exude translucent pale green gland; Step 4- live bugs are placed in warm water and stirred; Step 5- processed bugs are dried; Step 6- bugs are sold at Venda market. (Pictures by: Dr Rob Toms).	13
3.1: <i>E. delegorguei</i> settling on <i>D. viscosa</i> (Photograph- Dr R.Toms).	20
4.1: TLC chromatogram of plant and insect extracted with hexane, DCM, acetone and MeOH followed by spraying with vanillin in sulphuric acid.	28
4.2: Bioautography chromatograms of <i>E. delegorguei</i> extracts against <i>E. coli</i> , <i>S. aureus</i> and <i>E. faecalis</i> . The clear bands on the chromatograms show an area where the compound is active against the microorganism.	29
5.1: TLC chromatograms of <i>D. viscosa</i> crude leaf extracts developed in EMW, BEA, and CEF solvent systems. The solvents used for the extract acetone, EtOH, Hex, DCM and EtOAc.	31
5. 2: The extracts were separately extracted in Acetone, EtOH, Hex, DCM and EtOAc. The TLC chromatograms of <i>D. viscosa</i> extracts were developed in EMW solvent system. The extracts were tested against <i>S. aureus</i> , <i>E. faecalis</i> and <i>E. coli</i> and clear zones on the chromatograms indicated inhibition of growth.	32
5.3: The extracts of <i>D. viscosa</i> was separately extracted with Hex, DCM, EtOH, EtOAc, and Acetone. Bioautography chromatograms of <i>D. viscosa</i> extracts were developed in BEA eluent system and components tested for activity against <i>E. coli</i> . Clear zone indicate inhibition of growth.	33

- 5.4:** Shows components that have antioxidant activity. The crude extract of *D. viscosa* was separately extracted with Hex, DCM, EtOH, EtOAc and Acetone. The extracts were developed in EMW, BEA and CEF solvent systems. 36
- 6.1:** Schematic representation of isolation and purification of compounds from *D. viscosa* crude extract. 39
- 6.2:** TLC chromatograms separated by CEF eluent system: (a) shows the TLC profile of compounds separated by CEF solvent system followed by spraying with vanillin in sulphuric acid. (b) Indicates bioautography of extracts against *S. aureus*. 40
- 6.3:** TLC chromatogram (a) shows the TLC pattern of fractions obtained from DCM VLC and the extracts were separated by CEF solvent system followed by spraying with vanillin in sulphuric acid. TLC chromatogram, (b) indicates bioautography of VLC fractions against *S. aureus*, (c) Shows TLC chromatogram of antioxidant activity after spraying with 0.2% DPPH in methanol. 41
- 6.4:** TLC profiles of pooled fractions from column chromatography of fraction 3 eluted with CEF. Sample from test tube 3 formed a pure compound T1055 42
- 6.5:** TLC chromatogram obtained from fractions 4+5 column chromatography separated by CEF. Fractions were collected from test tube 1-30 (a), test tube 31-50(b), test tubes 51-66 (c), test tubes 67-78 (d). 43
- 6.6:** TLC chromatogram of fractions obtained from combined fractions 7+8+9 column chromatography separated by BEA eluent system. Fractions were collected from test tube 2-28 (a), test tube 3-48(b), test tubes 50-66 (c), test tubes 68-78 (d), test tubes 80-112 (e). 44
- 6.7:** TLC chromatograms of fractions collected from silica gel column chromatography separated by CEF eluent system. Fractions were collected from test tubes 1-34 (a) DCM crude extract; b) collected fractions of test tubes 2-34 (c) further purification of fractions from tubes 23-25 using Sephadex LH20; (d) pure compound Cpd2. 45
- 6.8:** TLC profiles of acetone crude run on Sephadex LH20 column chromatography, fractions from test tubes 1-12 was obtained and pure Compound AB was obtained from test tube 7-10. 46

6.9: Structure of 5-hydroxy -3, 7, 4'-trimethoxyflavone.	46
6.10: Mass Spectrum Fragmentation of 5-hydroxyl-3, 7, 4'-trimethoxyflavone	47
6.11: Structure of 5, 7-dihydroxy-3, 6, 4'-trimethoxyflavone	49
6.12: Mass Spectrum fragmentation of santin.	50
6.13: Structure of 5, 7, 4'-trihydroxy-3, 6-dimethoxyflavone.	52
6.14: Mass Spectrum Fragmentation of 5, 7, 4'-trihydroxy-3, 6-dimethoxyflavone.	53
6.15: Structure of 4'-O-methylkaempferol	55
6.16: Mass Spectrum Fragmentation of 4'-O-methylkaempferol.	56
6.17: Structure of Kaempferol.	58
6.18: Mass Spectrum Fragmentation of Kaempferol.	58
6.19: Five isolated compounds from <i>D. viscosa</i> leaf extracts.	60
7.1 TLC chromatograms showing bioautograms of Compound C against four test microorganisms, (an arrow points the area of growth inhibition).	61
7.2: TLC chromatograms showing bioautograms of Compound E against four test microorganisms.	62
7.3: TLC chromatograms showing bioautograms of Cpd 2.	62

- 7.4: TLC chromatograms showing bioautograms of Compound AB against three test microorganisms. 63
- 7.5: TLC chromatogram separated by CE showing the level of antioxidant activity of Compounds C, E, Cpd2 and AB. 64

LIST OF TABLES

Table:	Page
3.1: Analyzed nutritional components of <i>E. delegorguei</i>	22
3.2: Mineral and Vitamin contents in mg/100g of <i>E. delegorguei</i>	22
3.3: Essential amino acid content of 1009 of <i>E. delegorguei</i>	22
3.4: Nutritional components of some of the commonly consumed insects and other mammals based on 100g serving	23
3.5: Percentage of protein of different essential amino acid of <i>E. delegorguei</i> compared to that of beef and chicken	23
3.6: Nutritional components of some of the commonly consumed insects and other mammals based on 100 g serving	24
5.1: MIC values of <i>D. viscosa</i> leaf extracts	34
5.2 : Total activity of leaf extracts of <i>D. viscosa</i>	35
5.3 : EC ₅₀ of <i>D. viscosa</i> leaf extracts	36
6.1 ¹³ C NMR data of Compound T1055	48
6.2: ¹³ C NMR data of Compound C	51
6.3: ¹³ C NMR data of Compound E	53
6.4: ¹³ C NMR data of Compound Cpd2	57
6.5: ¹³ C NMR data of Compound AB	59
7.1: MIC values of the isolated compounds	64
7.2: Percentage antioxidant activity of Compounds 2-5	64
7.3: EC ₅₀ of compound 4(Cpd2) and 5(AB) from <i>D. viscosa</i> leaf extracts	65

GLOSSARY

List of Abbreviations

- BEA**- benzene/ethanol/ammonia (18:2:0.2)
CE- chloroform/ethyl acetate (8:2)
CEF- chloroform/ethyl acetate/formic acid (10:8:2)
¹³C 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
LD₅₀- the concentration that would kill 50% of the organism
M⁺- Molecular ion
MEDUNSA- Medical University of South Africa
MIC- Minimum Inhibitory Concentration
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 Equivalent 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*