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Bio-Active Compounds Composition in Edible Stinkbugs Consumed in South-Eastern Districts of Zimbabwe

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Abstract

Encosternum delegorguei Spinola (Hemiptera: Tessaratomidae) are consumed as relish and with traditional claims of having medicinal roles in the South-Eastern districts of Zimbabwe. However, very little has been explored scientifically to validate these claims. The current study was conducted to investigate bio-active compound composition and diversity of stable antibacterial activity from *E. delegorguei* extracts. Methanol, ethanol and aqueous extractions of *E. delegorguei* were performed followed by qualitative, quantitative analyses of phytochemical/bioactive compounds and determination of antibacterial activities using disc diffusion method on ten clinically important microbes. Alkaloids, flavonoids, anthraquinones, tannins, phlobatannins, steroids, triterpenoids and cyanogen glycosides were detected in the insect extracts. Flavonoids were detected in significantly higher concentrations in unprocessed compared to processed insects. Mean DPPH free radical scavenging activities were 78% and 88% for traditionally processed and raw insect extracts respectively. Traditional processing resulted in reduction of bioactive compounds (22.2% total phenolics; 68.4% flavonoids) and free radical scavenging activities by 10%. However, it resulted in an increase of cyanogen glycosides by 65.7%. Methanol extracts produced highest mean inhibition zones of 20 mm while aqueous and ethanol extracts had mean inhibition of 0 to 15 mm as compared to control with 20-40 mm. High flavonoids levels could be beneficial to consumers. However, a potential trade-off from elevated levels of cyanogen glycosides after processing needs further investigation. The free radical scavenging activity displayed by *E. delegorguei* extracts indicate a potential source of natural anti-oxidants that can be formulated into commercial products.

Keywords: antibacterial, *Encosternum delegorguei*, free radical scavenging, phytochemical

1. Introduction

The Edible Stinkbug, *Encosternum delegorguei* Spinola (Hemiptera: Tessaratomidae) is distributed widely in subtropical woodland and bush veldt with occurrences in Zimbabwe and northern provinces of South Africa (Dzerefos, Wtkowski, & Toms, 2009; Picker, Griffiths, & Weaving, 2004). In Zimbabwe, the Edible Stinkbug is restricted to the southern most parts which include the Nerumedzo region in Bikita (approximately 20°1'23.22"S, 31°41'17.65"E) and Zaka (approximately 20°4'28"S, 30°49'58"E) districts in Masvingo province (Chavhunduka, 1975; Mawere, 2012).

Encosternum delegorguei is an important source of income in parts of Zimbabwe and South Africa. It has also immensely contributed towards attainment of food security to rural communities in these two countries during winter season (Mawere, 2012; Kwashirai, 2007; Defoliart, 1995; Chavhunduka, 1975). Teffo, Toms, and Eloff (2007) reported the nutritional importance of the Edible Stinkbug consumed in Limpopo province, South Africa. The insects were reported to have high protein, fat, amino acid, minerals and vitamin contents.

Although much of the chemical analytical research work involving *E. delegorguei* has been focussed on its nutritional composition (Teffo et al., 2007), an emerging dimension in the research on *E. delegorguei* is on medicinal value of compounds derived from this insect species. A number of insect species are known to sequester compounds from host plants and store them as defence mechanisms. This ability of insects to sequester

and produce allelochemicals and phytochemicals has raised a lot of interest in the research for new drugs and search for alternatives to synthetic pesticides (Elemo, 2011; Silberbush, Markman, Lewinsohn, Bar, & Cohen, 2010; Teffo, Aderogba, & Eloff, 2010; Zaku, Abdulrahaman, Onyeyili, Aguzue, & Thomas, 2009; Moraes et al., 2008).

In the Zimbabwean communities that consume *E. delegorguei*, traditional claims asserts medicinal roles of these insects that include cure for asthmatic and heart diseases, aiding digestive systems, acting as appetizers and enhancing sexual desires. Traditional preparation methods of consumption for these insects are therefore aimed to achieve a quality that will deliver medicinal properties. However, very little has been explored scientifically to validate these practices.

In addition, the Edible Stinkbug is well known for releasing a very offensive smell which is associated with unknown volatile defensive compounds. However, some of these compounds are removed from the insects using a traditional aqueous processing method. Depending on the degree to which the traditional processing steps are followed, insects can either be well-prepared, where the requisite taste and flavour is acquired or can be spoiled. In the latter case, volatile compounds accumulate on the thoracic segments and impart a very bitter taste to the insects. Traditional beliefs also assert stomach currant properties from these spoiled and bitter insects.

This study was born out of the need to investigate the bioactive compound composition and diversity of stable antibacterial activities from *E. delegorguei* extracts as a first step in the validation of traditional claims on medicinal properties associated with this insect species. In addition, the study was also aimed at determining the effect of traditional processing on the quality and quantity of bio-active compounds in order to predict potential implications on health benefits and risks to consumers.

2. Materials & Methods

2.1 Origin and Collection of E. delegorguei

Samples of *E. delegorguei* were collected from Bikita (approximately 20°1'23.22"S, 31°41'17.65"E), during winter season (month of June). Live insects were collected using the jarring and knock down approach from tree branches and transported for laboratory analyses in perforated polypropylene sacks kept in cool boxes.

2.2 Preparation of Insects for Extraction of Phytochemicals

A portion of 500 g of live insects were gradually killed with 5 litres of lukewarm water (~37 °C) and stirred for 5 minutes until the insects were dead. Water was drained from the dead insects giving rise to an aqueous liquid (supernatant) which was kept as the traditional extract while the insects were dried by heating and stirring in traditionally prepared clay pots for 3 minutes. Meanwhile during the heating and drying process, a flame was used to burn off volatile compounds from the dead insects. Heating was stopped when insects changed colour from green to golden brown. The insects killed using this method were kept in perforated plastic bags that were kept at ambient temperatures until use for experimental purposes.

Raw and traditionally prepared insect samples were oven-dried at 60 °C overnight followed by grinding separately using the pestle and motor.

2.3 Extraction of Phytochemicals From Raw E. delegorguei

An amount of 10 g (28 insects), was weighed and homogenised using a pestle and motor in 60 ml of methanol, and this extraction product was centrifuged using bench centrifuge at 3 000 rpm for 10 min (Harbone, 1973). A second extraction was performed using ethanol as the solvent. A third and final extraction was performed on whole organisms with water as a solvent. The extracts were all kept under refrigeration at 4 °C.

2.4 Microbial Strains Tested

Test microorganisms used in this study were *Bacillus subtilis*, *Escherichia coli*, *Faecal streptococcus*, *Lactobacillus* species, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Shigella* species and *Staphylococcus aureus* all obtained from Medical Microbiology Department, University of Zimbabwe. The bacterial isolates were incubated at 37 °C for 18 h during testing.

2.5 Determination of Phytochemical Composition From the Raw and Traditionally Processed E. delegorguei

2.5.1 Assaying for Oxalates

Oxalates were extracted and determined by titration (Amoo & Agunbiade, 2010). Raw and traditionally processed samples (2 g) were digested with 50 ml of 0.75 M H_2SO_4 for 2 hours, stirred and filtered using Whatman No. 1 filter paper. An aliquot of 125 ml of the filtrate was heated until it was close to boiling point (80-90 °C) and titrated against standardised 0.5 M $KMnO_4$ solution to a faint pink colour.

2.5.2 Assaying of Phytates

Phytic acid content was determined by a colorimetric method (Vaintraub & Laptewa, 1988). Raw and traditionally processed samples (1 g) were extracted with 10 ml of 0.5 M HCl for 1 hour at room temperature, diluted, centrifuged and analysed for phytic acid by addition of 1 ml of Wade reagent (0.3 g of ferric chloride ($FeCl_3.6H_2O$) and 3 g of sulphosalycylic acid dissolved in 1L of distilled water) to 3 ml of sample extract. Absorbance was read at 500 nm on a Jenway 6405 UV/VIS spectrophotometer (Jenway Ltd., Essex, UK). The concentration of phytic acid was calculated by comparison with standard solutions of calcium phytate (Sigma –Aldrich Chemie, Steinheim, Germany, www.sigmaaldrich.com).

2.5.3 Assaying for Cyanogen Glycosides

Cyanogen glycosides were quantified as total cyanide (Makkah, 2003). Raw and traditionally processed samples (4 g) were added to 125 ml water followed by 2.5 ml chloroform in a Kjeldahl flask and then distilled. HCN released was absorbed in 2% (w/v) potassium hydroxide (total volume after extraction was 20 ml). An aliquot (5 ml) of the solution was mixed with 5 ml of alkaline picrate and heated in a boiling water bath for 5 minutes. After cooling the absorbance was read at 520 nm. Potassium cyanide (240 mg KCN/ L, Sigma-Aldrich Chemie, Steinheim, Germany, www.sigmaaldrich.com) was used as a standard.

2.5.4 Assaying for Alkaloids

Alkaloids were determined by a gravimetric method (Harbone, 1973). Raw and traditionally processed samples (5 g) were weighed and dispersed into 50 ml of 10% (v/v) acetic acid solution in ethanol. The mixture was vortexed and allowed to stand for 4 hours before it was filtered. The filtrate was evaporated to one quarter the original volume on a hot plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and was then washed with 1% (v/v) ammonium hydroxide solution. The filter paper containing the precipitate was dried in an oven at 60 °C for 30 minutes, followed by transferring to a dessicator to cool and re-weighing until a constant weight. The weight of alkaloid was determined by weight difference of filter paper and expressed as g /100 g fresh weight of sample.

2.5.5 Assaying for Total Phenolics and Flavonoids

For the determination of total phenolics and flavonoids, raw and traditionally processed samples (1 g) were dissolved in 20 ml 50% methanol. The mixture was vortexed for 1 minute and then sonicated for 20 minutes. The mixture was centrifuged at 3000 g for 10 minutes and the supernatant was used for the analysis of total phenolics and flavonoids.

Total phenolics were determined by the Folin-Ciocalteu method using gallic acid (0.5 mg/ml, Sigma-Aldrich Chemie, Steinheim, Germany, www.sigmaaldrich.com) as the standard (Penarrieta, Alvaradoa, Bergenstahlc, & Akkesonb, 2007). The Folin-Ciocalteu reagent, diluted 10 times (2.5 ml), 2 ml of saturated sodium carbonate (75 g/L) and 50 µl of sample (diluted ten times) were mixed and homogenized for 10 seconds and heated for 30 minutes at 45 °C. The absorbance at 765 nm was read after cooling to room temperature.

Total flavonoids were determined according to Jimo, Adedapo, Aliero, Koduru, and Afolayani (2010). To 0.5 ml sample, 0.5 ml of 2% $AlCl_3$ in ethanol solution was added. After 1 hour at room temperature, the absorbance was measured at 420 nm. Total flavonoids content was evaluated as catechin equivalence CE g/100g dry weight of extract.

2.5.6 Assaying for Tannins

Tannins were extracted using methanol and determined by spectrophotometric method (Price, Scoyoc, & Butler, 1978). Raw and traditionally processed samples (1 g) were defatted using diethyl ether and transferred to a 100 ml glass beaker. To the defatted material, 10 ml methanol was added and the beaker placed in an ultrasonic ice-water bath for 30 minutes at room temperature. The contents of the beaker were transferred to centrifuge tubes and centrifuged for 10 minutes at 3000 g. Tannins were determined by the modified Vanillin-HCl method using catechin (5 mg/ml, Sigma-Aldrich Chemie, Steinheim, Germany, www.sigmaaldrich.com) as the standard stock solution (Price et al., 1978).

2.6 Bio-Assaying for DPPH Radical Scavenging Activity

The free radical scavenging activities of methanolic extracts were measured by decrease in the absorbance of methanol solution of DPPH (P. V. Sharma, Paliwal, & S. Sharma, 2011). A stock solution of DPPH (33 mg in 1 L, Sigma-Aldrich Chemie, Steinheim, Germany, www.sigmaaldrich.com) was prepared in methanol, which gave initial absorbance of 0.430, and 5 ml of this stock solution was added to 1 ml of *E. delegorguei* extract solution at different concentrations (100 – 1000 µg/ml). After 30 minutes, absorbance was measured at 517 nm and

compared with standards (100 – 1000 µg/ml). The radical scavenging activity (%) was obtained by expressing the difference between the absorbance of control mixture and that of the test compounds over the absorbance of the control mixture (Anwar, Qayyum, Hussain, & Iqbal, 2010) from the equation:

$$\% \text{ activity} = [(OD_{control} - OD_{sample}) \times 100] / OD_{control} \quad (1)$$

2.7 Disc Diffusion Assaying for Antibacterial Activity

Each organism was maintained on nutrient agar plates and recovered for testing by growth in nutrient broth for 18 hours (Taylor, Manandhar, Hudson, & Towers, 1995). Before use each bacterial culture was diluted 1:100 with fresh sterile nutrient broth and incubated for 3-5 hours to standardise the culture using 0.5 McFarland standard solutions. Test organisms were streaked in a radial pattern on sterile nutrient agar plates. Sterile 6 mm diameter filter paper discs were impregnated with the sterile test materials and placed onto nutrient agar. Negative controls were prepared using the same solvents used to dissolve the plant extracts. Gentamycin (10 µg/ml, Sigma-Aldrich Chemie, Steinheim, Germany, www.sigmaaldrich.com) was used as positive reference standards. The innoculated plates were incubated at 37 °C for 24 hours. The antibacterial activity was measured as the diameter in (mm) of clear zone of growth inhibition. Each test was run in triplicates.

2.8 Statistical Analysis

Statistical analyses of the data were done using GraphPad Prism 5.03 software. The biochemical assays were done in triplicate using three samples. Data obtained were expressed as mean $\pm$ standard deviation, and were subjected to one way analysis of variance (ANOVA) and means were separated by Turkey's pair wise and multiple comparison tests at $P < 0.05$ where appropriate.

3. Results

3.1 Qualitative Analysis of Phytochemicals/Bio-Active Compounds From Raw E. delegorguei Extracts

The initial qualitative analysis of *E. delegorguei* extracts are shown in Table 1. Prior to quantification, alkaloids, flavonoids, cardiac glycosides, steroids, triterpenoids and free reducing sugars were found in varying concentrations as observed from the colour intensities produced. Compounds that were detected in the screening stage were confirmed by the quantitative tests shown in Table 2. Low levels of anthraquinones and phlobatannins were observed in this study. Amino acids were not detected in aqueous and organic solvent extracts.

Table 1. Qualitative amounts of phytochemical compounds from methanol and aqueous extracts of *E. delegorguei*

Phytochemical	Test Done	Methanol extract from raw insects	Aqueous extracts (supernatant after traditional processing)
Alkaloids	Dragendorffs test	+++++	++
	Mayers test	+++	-ve
	Tannic acid test	++++	+++
Flavonoids	NH_4OH test	+	-ve
	NaOH test	+++++	+++
Reducing sugars		+++++	+++++
Tannins	Ferric chloride test	-ve	-ve
	Tannin alkaline reagent test	+	-ve
	Vanillin-Hydrochloride test	+	-ve
Phlobatannins		+	-ve
Steroids		++++	++++
Steroids & Triterpenoids		+++++	+++++
Amino acid using ninhydrin		-ve	-ve
Cardiac glycosides	Keddes test	++++	++++
	Keller- Killan test	+++++	+++++
Anthraquinones		+	+

* + trace amount, ++/+++ moderate amount, ++++/ +++++ appreciable amount, - completely absent.

3.2 Composition of Phytochemical/Bioactive Compounds From E. delegorguei

There were higher quantities of phytochemicals/bio-active compounds in raw *E. delegorguei* for all tests done compared to the traditionally processed samples except for cyanogens which got elevated by approximately three times in processed insects (Table 2). Traditional processing of *E. delegorguei* was noted to reduce significantly the levels of total phenolics, tannins and oxalates through the aid of aqueous system. The extents of reductions in the respective compounds were 22.2% for total phenolics, 29.7% for alkaloids, 30.2% oxalates and 67.7% for tannins (Table 2). However, there was a significant increase (65.7%) in the quantities of cyanogen glycosides after raw insects were processed according to traditional practices.

Phytates were not recorded from both raw and traditionally processed insect extracts while flavonoids occurred in relatively high levels. The traditional processing procedure also significantly reduced the quantity of flavonoids in the processed insects by 68.4% (Table 2).

Table 2. Phytochemical composition of raw and traditionally processed *E. delegorguei*

Phytochemical	Raw insects	Traditionally processed	Percent (%) increase (+)/decrease (-) between raw & traditionally processed insects
Total Phenolics[d] (g GAE/ 100 g)	3.6 ± 0.4^{a}	2.8 ± 0.5^{a}	(-) 22.2
Tannins[e] (g CE/ 100 g)	0.31 ± 0.01^{a}	0.10 ± 0.04^{b}	(-) 67.7
Flavonoids[e] (g CE/ 100 g)	15.20 ± 1.00^{a}	4.80 ± 0.40^{b}	(-) 68.4
Alkaloids (g/ 100g)	7.4 ± 0.6^{a}	5.2 ± 0.2^{b}	(-) 29.7
Oxalates (g/ 100g)	1.26 ± 0.07^{a}	0.88 ± 0.15^{b}	(-) 30.2
Cyanogen glycosides[g] (μg/ 100g)	23 ± 3.1^{a}	67.0 ± 3.4^{b}	(+) 65.7
Phytates (g/ 100g)	Not detected	Not detected	No change

*1 Values are means ± standard deviation. In the same row means with different superscripts are significantly different ($P < 0.05$).

*2 CE- Catechin equivalents, GAE – Gallic acid equivalents.

3.3 DPPH Free Radical Scavenging Assaying From E. delegorguei Extracts

The values of DPPH free radical scavenging activity for *E. delegorguei* extracts were not significantly different for raw and traditionally processed *E. delegorguei* extracts. The free radical scavenging activities were comparable to values of known compounds used as controls in this study (ascorbic acid, catechin and butylated hydroxyanisole) whose values ranged between 87 to 98% (Table 3).

Table 3. DPPH radical scavenging activity (%) produced from methanolic extracts of raw and traditionally processed *E. delegorguei* in comparison to activities of known compounds

Sample (1 mg/ml of each test compound was used)	% DPPH radical scavenging activity
Raw *E. delegorguei* methanolic extract	88 ± 3^{a}
Traditionally processed *E. delegorguei* extract	78 ± 7^{ab}
Ascorbic acid	92 ± 1^{c}
BHA	97 ± 1^{d}
Catechin	92 ± 3^{a}

* 1 Values are means ± standard deviation.

* 2 Means with different superscripts are significantly different ($P < 0.05$).

3.4 Antibacterial Activity Determination of Ethanol, Methanol and Water Extracts

Antibacterial bioactivity of extracts from *E. delegorguei* using disc diffusion assay realised either bactericidal or bacteriostatic action to the following microbes that are Gram positive: *B. subtilis*, *Lactobacillus* sp.,

Staphylococcus aureus, *Salmonella enteritidis*, *Salmonella typhi*, and Gram negative species which included *E. coli* and *P. aeruginosa*, as shown in Table 4.

Table 4. Antimicrobial activity (inhibition diameter (mm)) of *E. delegorguei* extracts using disc diffusion assay on several microbial isolates

Microorganism	Type of extract				Gentamycin
	Methanol extract	Traditional extract	Ethanol extract	Aqueous extract from thoracic glands	
Escherichia coli	20.0 ± 0	9.0 ± 0	10.3 ± 0.6	9.0 ± 0	30.0 ± 0
Salmonella typhi	17.3 ± 0.6	NI	6.3 ± 0.6	NI	30.0 ± 0
Salmonella enteritidis	20.3 ± 0.6	NI	NI	10.0 ± 0	20.0 ± 0
Proteus vulgaris	20.0 ± 0	15.3 ± 0.6	10.0 ± 0	10.0 ± 0	35.0 ± 0
Shigella sp.	20.3± 0.6	10.3 ± 0.6	6.0 ± 0	15.3 ± 0.6	35.3 ± 0.6
Pseudomonas aeruginosa	14.0 ± 0	12.3 ± 0.6	10.3 ± 0.6	14.0 ± 0	35.0 ± 0
Bacillus subtilis	10.7 ± 0.6	NI	NI	NI	35.3 ± 0.6
Staphylococcus aureus	12.0 ± 0	10.3 ± 0.6	15.0 ± 0	6.0 ± 0	40.0 ± 0
Faecal streptococcus	25.0 ± 0	12.3 ± 0.	NI	10.0 ± 0	35.0 ± 0
Lactobacillus sp.	20.3 ± 0.6	10.0 ± 0	7.0 ± 0	9.0 ± 0	25.0 ± 0

* [1] NI = no inhibition.

* [2] Gentamycin antibacterial activity was referenced as 100% inhibition and its inhibition zone was highest at 40 mm diameter.

The methanol extract produced a relatively high comparable zone of inhibition of about 20 mm on *E. coli*, *S. enteritidis*, *P. vulgaris*, *Lactobacillus* sp., *Shigella* sp., and *F. streptococcus* strains to that of the positive control. In general, the aqueous and ethanol extracts had subdued inhibition effect on all the microbial strains and in some instances not having an effect at all. On average, the overall inhibition from the insect extracts was about 10 mm, which was 72% less antibacterial compared to the positive control of 10 μg/ml gentamycin (Table 4).

4. Discussion

Methanol and aqueous extracts of *E. delegorguei* were noted to comprise of alkaloids, flavonoids, cyanogen glycosides, steroids, triterpenoids and free reducing sugars. The defence mechanism employed *E. delegorguei* could possibly make use of some of these chemicals that they can synthesise from plants that they feed on in a manner as suggested by Teffo et al. (2007). In addition, compounds acquired from plants during foraging can function as precursor molecules for the biosynthesis of other compounds that are found in the insects.

Quite a considerable amount of attention has been given on medically applicable phytochemicals from plants (De Britto, Gracelin, & Sebadastian, 2011; Olusesan, Ebele, Onwuegbuchulan, & Olorunmola, 2010; Yusha'u, Hamza, & Abdullahi, 2010; O. O. Igbinosa, E. O Igbinosa, & Aiyegoro, 2009; Teffo et al., 2007; Prabuseenivasan, Jayakumar, & Ignacimuthu, 2006; Akinyemi, Oladapo, Okwara, Ibe, & Fasure, 2005; Zaidan, et al., 2005). However, few studies have focussed on phytochemicals from insects. Much of the studies done so far were centred on the nutritional compositions of edible insects that included the African palm weevil, *Rhychophorus phoenicis* (Elemo, 2011), Edible Stinkbug, *E. delegorguei* (Teffo et al., 2007) and a wide range of other insects (Defoliart, 1995; Banjo, Lawal, & Songonuga, 2006). Our study makes an effort to profile phytochemicals/bio-active compounds from this edible insect species and this could be the foundation of more refined experimental protocols for profiling these compounds in all edible insect species.

The traditional processing procedure which involved the use of warm water and subsequent drying by heating could realise a reduction in the quantities of phytochemicals/bio-active compounds due to chemical degradation. Similar findings on reduction of phytochemicals after aqueous extraction have been noted with plant extracts (Soetan & Oyewole, 2009).

Boiling and sun drying of insects have been shown to eliminate potentially harmful compounds such as

neurotoxins and in some cases improve nutritional quality through partial inactivation of protease inhibitors (Akinnawo, Abatan, & Ketiku, 2002; Marickar & Paltabiraman, 1988). Our study showed similar results for most phytochemicals except for cyanogen gylcosides which have been shown to cause cyanogenesis (liberation of hydrogen cyanide leading to poisoning) if consumed by humans (Lechtenberg, 2011). The accentuation in quantities of cyanogen glycosides due to the traditional processing procedure could indicate a complex degradation and unbinding process of the major chemical constituents in *E. delegorguei* during heating. This observation from our study is quite interesting and contrary to traditional beliefs by consumers that assume that traditional processing assists in removing all harmful compounds from the insects.

Despite the potential threat from increased levels of cyanogen glycosides due to traditional processing, based on analyses of bio-active compound composition, *E. delegorguei* could still be a vital source of flavonoids. These compounds have been shown to have anti-allergic, anti-diarrheal, antiulcer, and anti-inflammatory agents and are considered essential nutrients (Bravo, 1998; Middleton, 2000). From a nutritional perspective, dietary intake of flavonoids has been estimated to range from 23 mg/day in the Netherlands, 28 mg/day in Denmark and 170 mg/day in the US (Cook & Samman, 1996; Leth & Justesen, 1998). Our study shows that the quantities detected even after traditional processing could be enough to meet the minimum acceptable daily intake by consumers. However, bio-availabilities of these flavonoids from *E. delegorguei* need to be determined.

A number of studies have revealed the physiological effects associated with alkaloids, flavonoids, cardiac glycosides, steroids, and triterpenoids. Saponins, oxalates and tannins for example at high levels are known to interfere with digestive processes and thus their presence in food is undesirable (Ijeh, Ejike, Nkwonta, & Njoku, 2010). Reduction in potentially harmful compounds of these groups due to the traditional processing procedure could therefore be beneficial to *E. delegorguei* consumers.

DPPH free radical scavenging activity displayed by *E. delegorguei* methanolic extracts is high, that is, within a range of 13% of activities of currently known scavenging bioorganic molecules that include ascorbic acid (vitamin C), butylated hydroxyanisole (BHA) and catechin (Sharma et al., 2011; Umaru, Adamu, Dahiru, & Nadro, 2007). However, it was noted from our study that subjecting the insects to heat processing reduces the DPPH free radical scavenging activity by 10%. It was also observed that the raw insect extracts expressed the same level of antioxidant activity compared to the known biological antioxidants (that include ascorbic acid) as well as the *Dodonae viscosa* var. *angustifolia* leaf extracts which is one of the insect host plant in South Africa as was recorded by Teffo et al. (2010). Naturally antioxidants have a fundamental physiological role in the human body by reducing tissue damaging free radicals (Tapiero, Tew, Nguyen, & Mathe, 2002). The provision of these possibly free radical quenching agents from the diet can be envisaged to greatly contribute towards the antioxidant preventive measures. In addition, they can also improve the digestion system; can function on reduction of coronary heart diseases, and some types of cancer and inflammation (Jayasri, Mathew, & Radha, 2009; Mattson & Cheng, 2006; Uddin, Akond, Mubassara, & Yesmin, 2008).

Aqueous extracts with antibacterial activity comprising of reducing sugars have been recorded in *A. digitata* (Yusha'u et al., 2010). Ethanol extracts had the least antibacterial inhibition effect followed by aqueous extracts. The poor antibacterial activities expression from ethanol solvents have been observed in several studies (Zaidan et al., 2005; Bhakuni, Dhar, Dhawan, & Mehrotra, 1969; Ahmad, Mehmood, & Mohammad, 1998). Ethanol can therefore be considered to be inappropriate chemical as a solvent for the efficient extraction of a number of phytochemicals from either plants or insects.

Methanol based extracts have been shown to have a broad spectrum of antibacterial activities although these were of plant origin: *Dodonae viscosa* (Teffo et al., 2010). Teffo and co-workers (2010) studied the antimicrobial activities of *E. delegorguei* and its plant food source *D. viscosa* var. *angustifolia*. Based on the bioautography technique, their results indicated antimicrobial activities of methanol extracts on *Staphylococcus aureus* and *Escherichia coli*. Our results using the disc diffusion method show that methanol extracts have some negative effects on some bacteria of clinical interest. These findings lay a foundation for subsequent work towards elucidating the individual components from *E. delegorguei* insects. Further studies are underway to determine minimum inhibitory concentrations and bactericidal concentrations using methanol extracts of whole insects.

5. Conclusions

Encosternum delegorguei has beneficial and harmful bio-active components which are rapidly degraded or extracted (except for cyanogen glycosides) from the insects through a traditional warm water and heating procedure in preparation for consumption. High levels of flavonoids could be beneficial to consumers. However, a potential trade-off resulting from elevated levels of cyanogen glycosides due to processing needs further investigation. The DPPH free radical scavenging activity displayed by *E. delegorguei* methanol extracts is very

comparable to the currently known scavenging bioorganic molecules and could indicate a potential source of natural anti-oxidants that can be formulated into commercial products. However, it was noted that subjecting the insects to heat processing reduces the DPPH free radical scavenging activity by 10%. This could be less beneficial to consumers of this edible insect species.

Currently, further work is in progress to perform quantification and elucidation of chemical structures of phytochemicals from *E. delegorguei*. Research work orientated towards such depth will enable us to determine the discrete compounds that are in *E. delegorguei* using methanol as solvents and come up with a standardized chemical process to harness and concentrate the relevant phytochemicals for specific nutritional and medicinal applications.

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