JBiopest 6(2):211-218

Preliminary chemical analysis of extracts from *Encosternum delegorguei* using Gas Chromatography Mass Spectroscopy

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ABSTRACT

The methanol extract of *Encosternum delegorguei* was quantitively analyzed through GCMS which had plasma-ionization detector (PID), quartz capillary column ZB-5 (polydimethylsyloxane, 5 % of phenyl groups), identified and produced the following 18 compounds which were C_2-C_{13} aliphatic hydrocarbons, aldehydes, furanones, aromatic, oxo-alkenals, esters, ketones, lactones and ethers. The compounds were identified as 2-hexenal, 1-methylbutyl formate, 3-methyl-3-heptanol, octenal, 2,4-dimethyl-3-heptanol, 1,1-diethoxy butane, tridecane, 6-trideceny-4-ne, 4,6-nonadien-8-yn-3-ol, 4,5-dimethyl-1,3-dioxolane-2-cyclohecyl, 3-heptanol, 2-hexenal diethyl acetal, 3-ethoxy pentane, 1,1-dimethoxy heptanes, 2-heptanol acetate, 2-methyl-4-pentanal, 5-ethyl-2-furanone and 2-butoxy pentane. FTIR spectra of the chitin sample from *E. delegorguei* indicated their good defatting with alcohol and deacetylation process. According to the spectra obtained, yield of chitin is low because of poor acetone treatment. The characteristic absorption bands at 1560, 1655 cm^{-1} and in the vicinity of 3265 and 3100 cm^{-1} corresponding to the stretching vibration C=O and NH (-$NHCOCH_3$) were of low intensity, and C-H stretching vibrations at 2926 and 2864 cm-1 showed a high intensity. The elemental analysis produced the following percentages of chitin elemental constituents of carbon 46.11 $\pm$ 0.055; hydrogen 6.63 $\pm$ 0.002; nitrogen 7.06 $\pm$ 0.077; with a C/N = (46.11/12)/(7.06/14) =7.62. Currently we are determining the chemical compounds using standards to elucidate, evaluate the qualitative constituents and in near future the plant can be utilized for pest management.

MS History:25.8.2013 (Received)-11.10.2013 (Revised)-30.11.2013 (Accepted)

Key words: Phytochemical analysis, extraction, *Encosternum delegorguei*, GCMS, chitin

INTRODUCTION

Encosternum delegorguei is commonly distributed in the sub-tropical open woodlands and bushvelds of Zimbabwe (Kwashirai, 2007). The insect is widely found in the southern part of the country in Bikita area and is known to feed on *Combretum imberbe* plant (Chavhunduka, 1975). *E. delegorguei* is also found in South Africa's Northern provinces (Teffo *et al*., 2007; Dzerefos *et al*., 2009). *E. delegorguei* is known to constitute of essential oils, volatile compounds, fatty acids, many phytochemicals, amino acids like threonine and valine (Teffo *et al*., 2007; Teffo *et al*., 2009; Dzerefos *et al*., 2009; Zvidzai *et al*., 2013 in press). Besides these mentioned compounds, other insects are known to include adipokinetic peptides for the kissing bugs of *Rhodnius prolixus, Triatoma infestans, Dipetalogaster maxima* and *Panastrongylus megistus* which are known to have a hormonal like role (Marco *et al*., 2013).

The Indian stink-bug, *Tessaratoma javanica*, share some common compounds with the *Encostrenum delegorguei*. Studies have shown that *T. javanica* scent glands and nymphs contain the following chemical compounds: 2-hexenal, hex-2-enyl acetate, n-tridecane, and oct-2-enal (Janaiah *et al*, 1979). The scent is reported to have exhibited a lethal effect on small red and black ants. Much of the chemical analytical research work involving *E. delegorguei* has been focussed on its nutritional composition (Teffo *et al*. 2007; Dzerefos *et al*., 2009). However, an emerging interest of late has been on agricultural and the medicinal value of compounds derived from this insect species. Our previous work on qualitative analysis of phytochemicals of *E. delegorguei* realised the presence of alkaloids, flavonoids, cardiac glycosides, steroids, triterpenoids and free reducing sugars were found in high concentrations (Zvidzai *et al*., 2013 in press). *E. delegorguei* employ the use of chemicals that they can synthesis or obtain from plants that they feed on. Such foraging behaviour results in accumulation of phytochemicals and the defense chemicals that may include peptides.

Besides phytochemicals and other nutritional compounds, insects are known to constitute of chitin that has many functions as in aiding digestion, pharmaceutical role and wastewater processing (Zvezdova, 2010; Biniaś *et al*., 2007). The potential to harvest chitin for pharmaceutical and medicinal roles meanwhile co-harvesting phytochemicals for agricultural applications can maximise on insect utilisation. Although *E. delegorguei* is a delicacy for many communities in Southern Africa, there is no detailed profiling of its chemical composition. In addition, there are reports on the possible use of its extracts for medicinal as well as insecticidal applications (Teffo *et al*., 2009; Zvidzai *et al*., 2013 in press). We previously reported that aqueous extracts of *E. delegorguei* are traditionally applied on tomatoes to control aphids. This study, therefore sought to fully profile the chemical composition of the aqueous and methanol extracts of this edible insect using GCMS. Further studies after isolating the compounds was conducted to assess the insecticidal properties of the extracts for tomato aphid control and other medicinal uses.

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MATERIALS AND METHODS

Origin and collection of *E. delegorguei*

The samples of *E. delegorguei* insects were collected from Bikita (approximately 20^o 1' 23.22" S 31^o 41' 17.65" E), southern part of Zimbabwe during winter season and transported the following day when they were still alive in a perforated sack. A traditional process of processing the insects for consumption was prepared with the help of the experienced local consumers and community residents and an aqueous sample was kept in a cooler box for subsequent analysis.

Preparation of insects traditional aqueous extraction

A portion of 500 g of live insects were gradually killed with 5 litres of lukewarm water (37 ^{o}C) and stirred for 5 min until they turned off green to brown in colour. A flame was then used to burn off the volatiles from the insects meanwhile stirring for about 3 min. The aqueous liquid was kept as the traditional extract. The raw insects were kept in perforated plastic bags that were kept at ambient temperatures until use for experimental purposes. Raw and boiled samples were oven-dried at 60 °C overnight followed by grinding separately using the pestle and mortar. The pulverised materials were stored at 4 °C in bottles for use in experiments.

Extraction of phytochemicals

An amount of 10 g averaging to 28 raw *E. delegorguei* insects, were 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).

Gas chromatography mass spectroscopy analysis

The analysis of water, ethanol and methanol extracts were performed by gas chromatography connected to flame-ionization (GCh-FID) and mass spectrometric (GCh-MS) detectors. The identification was performed using GCh-MS and the data base of mass-spectra NIST05 and Wiley. The quantitative analysis was performed by internal normalization using peaks areas in GCh-MS analysis. Gas chromatograph Shimadzu GC-2010, Japan, was connected to plasma-ionization detector (PID), quartz capillary column ZB-5 (polydimethylsyloxane, 5 % of phenyl groups), length 30 m, diameter 0.25 mm, film thickness 0.25 μm. Oven temperature was programmed to be held at 40°C for 3 minutes and then increased to 280°C at a rate of 10 ^{0}C/min. Vaporizer and detector (PID) temperatures were 250°C and 300 ^{0}C, respectively. Nitrogen was used as carrier gas with a split ratio of 1:30 and a gas flow rate of 1.0 ml/min. The GCMS was injected with 1.0 μl of the methanol extract.

Chitin extraction and analysis using elemental analyzer and FTIR

The chitin analysis was done at the Department of Technology for Organic Synthesis at the Ural Federal University, Yekaterinburg, Russia. Extraction of chitin from *E. delegorguei* was extracted as described elsewhere (Teslenko and Voevodina, 1995).). A 150.0 g of ground raw biomass of insects were successively treated twice with 8 (w/v) % solution of NaOH at 90 ºC for 1.5 hours (deproteinization process). Then demineralization was carried out with 7 (v/v) % solution of HCl at room temperature for 3 hours. The extracted product was washed with ethanol to remove fats and lipids (defatting processs). Then followed by filtering and washing twice with distilled water to remove ethanol and then subsequently deacetylating with 29 (w/v) % solution of NaOH. After extraction Fourier transformation infra red functional groups analysis was performed using "Spectrum One" FTIR spectrometer (Perkin Elmer) using Diffuse Reflectance Sampling Accessory (DRA) or by use of Smart Orbit Accessory for Single-Reflection Attenuated Total Reflectance (ATR) respectively. The following instrument was used for elemental analysis, Automatic Perkin-Elmer CHN PE 2400 Elemental Analyzer (USA).

RESULTS AND DISCUSSION

GCMS qualitative analysis

Figure 1 below shows a typical chromatogram after GCMS analysis of the methanol extract from *E. delegorguei*. The identification of the components of the methanol extracts and their quantitative content are given in table 1 below. The methanol extract was analyzed through GCMS which had plasma-ionization detector (PID), quartz capillary column ZB-5 (polydimethylsyloxane, 5% of phenyl groups), and produced the following 18 compounds: 2-hexenal, 1-methylbutyl formate, 3-methyl-3-heptanol, octenal, 2,4-dimethyl-3-heptanol, 1,1-diethoxy butane, tridecane, 6-trideceny-4-ne, 4,6-nonadien-8-yn-3-ol, 4,5-dimethyl-1,3-dioxolane-2-cyclohecyl, 3-heptanol, 2-hexenal diethyl acetal, 3-ethoxy pentane, 1,1-dimethoxy heptanes, 2-heptanol acetate, 2-methyl-4-pentanal, 5-ethyl-2-furanone and 2-butoxy pentane. The compounds that were identified from the chromatography are listed in Table 1 below with their respective retention times, chemical structures, chemical names, chemical formulae, mass and percentage content as obtained from the elution profile.

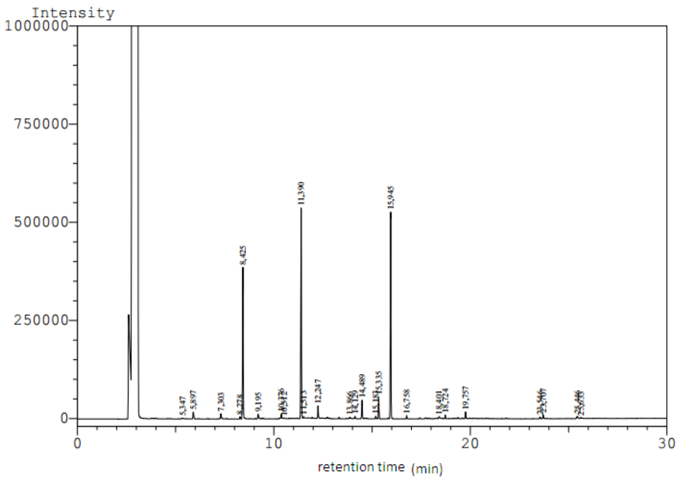
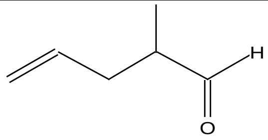
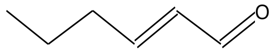
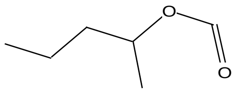
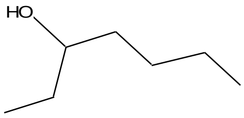
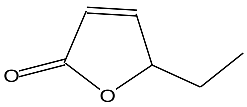
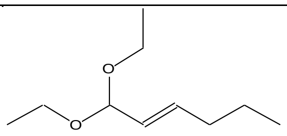
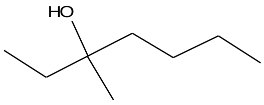
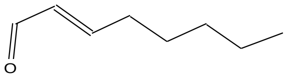
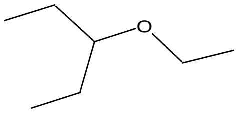
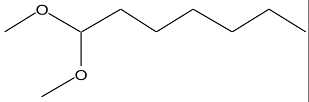
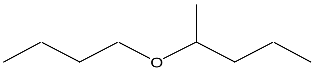
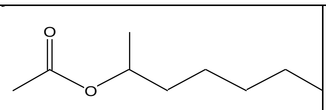
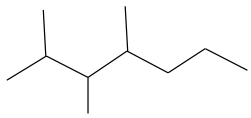
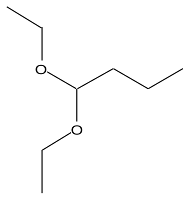
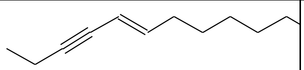
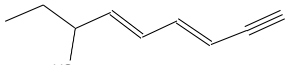
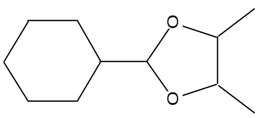


Fig. 1. Gas chromatography mass spectroscopy analysis of methanol extract of *E. delegorguei*

These compounds include are C_2-C_{13} aliphatic hydrocarbons, aldehydes, furanones, aromatic, oxo-alkenals, esters, ketones, lactones and ethers. The compounds were found at different content. *De novo* biosynthesis of chemical defense compounds via shikimic and isoprenoid pathways have arisen through adaptation of the insect's glands and storage mechanisms of the endogenous metabolites for sequestering plant compounds (Williams *et al*., 2005; Teffo *et al*., 2009; Aldrich *et al*., 1979; Aldrich, 1995). Similar range of compounds have been noted by other workers on *Eurygaster integriceps* a heteropera stink bug and these were 2-hexenal, 2(5)-furanone, 5-ethyl, 2-hexe-1-ol, 5-decyne, tridecane and nonadecane (Hassani *et al*., 2010). The occurrence of 2-hexenal is known to play a role for defense purposes and as an alarm pheromone to warn and disperse others in case of danger (Hassani *et al*., 2012; Kye and Hardie, 2002; Durakand Kalender, 2007a). Tridecane is a known insect repellent or an effective deterrent towards predators (Zarbin *et al*., 2000). Similar compounds to 6-trideceny-4-ne which are 1-decyne are known

Table 1. Chemical components identified and their qualitative content of methanol extracts from *E. delegorguei*

Retention time (GCh-PID), min	**Chemical Structure**	**Compound name (IUPAC)**	**Chemical formulae**	**Mass (m/z)**	**% Content, (GCh-PID)**
7.296		4-pentenal, 2-methyl-	$C_6H_{10}O$	98	0.18
8.425		2-hexenal	$C_6H_{10}O$	98	23.10
10.376		1-methyl butyl formate	$C_6H_{12}O_2$	116	0.81
10.459		3-heptanol	$C_7H_{16}O$	116	56.77
10.491		2(5H)-furanone, 5-ethyl-	$C_6H_8O_2$	112	5.95
11.311		2-hexenal diethyl acetal, trans	$C_{10}H_{20}O_2$	172	0.46
11.390		3-heptanol, 3-methyl-	$C_8H_{18}O$	130	30.03
12.247		Octenal	$C_8H_{14}O$	126	1.82
12.370		pentane, 3-ethoxy-	$C_7H_{16}O$	116	2.78
13.098		heptane, 1,1-dimethoxy-	$C_9H_{20}O_2$	160	2.58
13.318		pentane, 2-butoxy-	$C_9H_{20}O$	144	1.22

13.718		2-heptanol, acetate	$C_9H_{18}O_2$	158	3.94
14.489		3-heptanol, 2,4-dimethyl-	$C_9H_{20}O$	144	2.81
15.335		butane, 1,1-diethoxy-	$C_8H_{18}O_2$	146	2.81
15.945		Tridecane	$C_{13}H_{28}$	184	28.42
16.758		6-tridecen-4-yne, (E)-	$C_{13}H_{22}$	178	0.46
18.724		4,6-nonadien-8-yn-3-ol, (E,E)-	$C_9H_{12}O$	136	0.52
19.757		1,3-dioxolane, 2-cyclohexyl-4,5-dimethyl-	$C_{11}H_{20}O_2$	184	0.98

to be involved in maintaining the aggregation cohersion noted in other stink bugs of *Dysdercus cingulatus* as is the case with *E. delegorguei* (Farine *et al*., 1992). However, other insects like the red-shouldered stink bug, *Thyanta pallidovirens* (Stål) (Hemiptera: Pentatomidae) produces a male sex pheromone a blend of methyl (E2,Z4,Z6)-decatrienoate (E2,Z4,Z6–10:COOMe), and the sesquiter-penes (+)-α-curcumene, (-)-zingiberene, and (-)-β-sesquiphellandrene (McBrien *et al*., 2002; Millar *et al*., 2010).

These compounds include are C_2-C_{13} aliphatic hydrocarbons, aldehydes, furanones, aromatic, oxo-alkenals, esters, ketones, lactones and ethers. The compounds were found at different content. *De novo* biosynthesis of chemical defense compounds via shikimic and isoprenoid pathways have arisen through adaptation of the insect's glands and storage mechanisms of the endogenous metabolites for sequestering plant compounds (Williams *et al*., 2005; Teffo *et al*., 2009; Aldrich *et al*., 1979; Aldrich, 1995). Similar range of compounds have been noted by other workers on *Eurygaster integriceps* a heteropera stink bug and these were 2-hexenal, 2(5)-furanone, 5-ethyl, 2-hexe-1-ol, 5-decyne, tridecane and nonadecane (Hassani *et al*., 2010). The occurrence of 2-hexenal is known to play a role for defense purposes and as an alarm pheromone to warn and disperse others in case of danger (Hassani *et al*., 2012; Kye and Hardie, 2002; Durakand Kalender, 2007a). Tridecane is a known insect repellent or an effective deterrent towards predators (Zarbin *et al*., 2000). Similar compounds to 6-trideceny-4-ne which are 1-decyne are known to be involved in maintaining the aggregation cohersion noted in other stink bugs of *Dysdercus cingulatus* as is the case with *E. delegorguei* (Farine *et al*., 1992). However, other insects like the red-shouldered stink bug, *Thyanta pallidovirens* (Stål) (Hemiptera: Pentatomidae) produces a male sex pheromone a blend of methyl (E2,Z4,Z6)-decatrienoate (E2,Z4,Z6–10:COOMe), and the sesquiter-penes (+)-α-curcumene, (-)-zingiberene, and (-)-β-sesquiphellandrene (McBrien *et al*., 2002; Millar *et al*., 2010).

In other paper we reported the high efficacy noted on several microbes by the *E. delegorguei* extracts and this can be attributed to the 5-ethyl-2-furanone which is a lactone which is known to have such antibacterial and antifungal activity on *Trichoderma* sp, *Rhizoctonia* sp, *Thielaviopsis* sp as was observed elsewhere (Paulitz *et al*., 2000; Johne *et al*, 2006).

It can be noted that some of them are precursors for the others e.g. dehydrogenation or hydrogenation of tridecane to 6-tridecen-4-yne is interconvertable. These two fatty acids like compounds can be generated from the normal biosynthetic mechanisms of the insects. Such similar compounds are known to be involved in sex pheromones the likes of methyl-2,6.10-trimethyltridecanoate identified in stink bugs *Euschistus heros* and *Euschistus obscures* (Favaro *et al.*, 2012).

A biochemical interconversion biosynthetic process can occur between 3-heptanol and 2-hexenal. The removal of ethyl groups and aldehyde conversion can result in the generation of one or the other. Cyclization of 2-methyl-4-pentenal and subsequent aldehyde generation can highly likely produce 5-ethyl-2-furanone. Although some of these compounds are for defensive mechanism, they might be produced and not accumulate and will have other pheromone purposes for alarm, mating, sex and aggregation as noted with *Euschistus conspersus*, *Chlorochoa ligata*, *Chlorochroa uhleri* and *Chlorochroa sayi* (Buxton *et al*. 1983; Aldrich *et al*., 1989; Millar *et al*., 2010). Since the insects mate and interact in their aggegarian tendencies, 2-cyclohecyl-4,5-dimethyl-1,3-dioxolane is a complex structure that can possibly be generated via combining two cyclic compounds or most likely through the acquisition of the biochemical compounds from natural plants that are capable of such complex reactions. During feeding on the plants *E. delegorguei* might acquire the plant extracts through feeding and be part of the insect's chemical compounds in its body.

Naturally S-adenosyl methionine, methylene cobalamin and N^5, N^{10}-methylene tetrahydrofolate anyone of them can be utilized for methylation reactions hence *E. delegorguei* might possibly generate the following compounds through methylation as part of their biochemical intermediate reactions: 2-methyl-4-pentenal, 3-methyl-3-heptanol, 2,4-dimethyl-3-heptanol, and 1-methyl butyl formate.

The insects can perform biological and typical esterification as well as etherification reactions making use of its biosynthetic machinery to generate compounds that include: 1-methylbutyl formate, 2-hexenal diethyl acetal, 2-heptanol acetate and 1,1-diethoxy butane. Insects can possibly generate ether compounds as noted from the presence of 2-hexenal diethyl acetal in the *E. delegorguei* extracts.

Chitin Analyses

The source of chitin from the *Econsternum delegorguei* species had an approximate 40 (w/w) % chitin content and FTIR spectra of the samples indicated a good defatting process with alcohol. Apparently, deacetylation of the sample went on well. According to the spectra obtained, yield of chitin is low because of poor acetone treatment. The characteristic absorption bands at 1560, 1655 cm^{-1} and in the vicinity of 3265 and 3100 cm^{-1} corresponding to the stretching vibration C=O and NH(-NHCOCH3) are low intensity, and C-H stretching vibrations at 2926 and 2864 cm-1 have a high intensity. The FTIR spectrum is quite comparable to those of other researchers that show equally good FTIR spectra of chitin extracted from other insects that include krill, crabs and shrimp shells (; Biniaś *et al*., 2007; Skorik, 2010; Zvezdova, 2010).

The elemental analysis produced the following percentages of chitin elemental constituents of carbon 46.11 $\pm$ 0.055; hydrogen 6.63 $\pm$ 0.002; nitrogen 7.06 $\pm$ 0.077; with a C/N = (46.11/12) / (7.06/14) = 7.62. The experimental C/N ratio is within the range of the theoretical value of chitin whose value is known to be C/N = 7-8. The proportions confirm the presence of chitin and this was further noted from the FTIR analysis which produced the spectrum in Figure 2.

Though the investigators were not concerned with the chemical composition of *E. delegorguei*, it will be interesting to study the monthly or seasonal variation in the chemical profile of the insect to determine the optimum period for the extraction of chemicals of interest. Scientists need to study biosynthetic genetic machinery for the chemical defense to manipulate the metabolic pathways of these stink bug insects. Further work can encompass researching on the possible peptides that the insect might produce which might be used as chemicals for various functions (Bártu *et al.*, 2010).

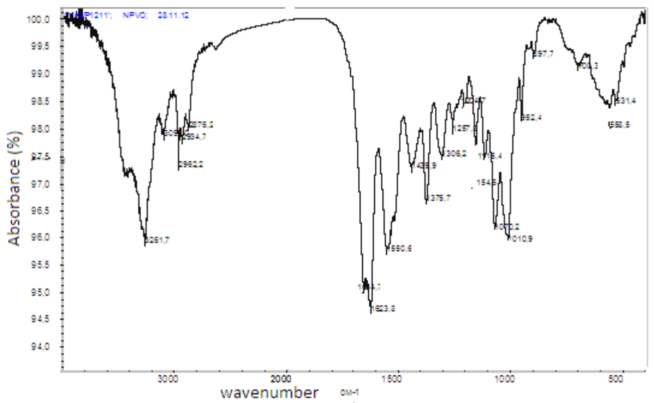


Fig. 2. FTIR analysis of chitin extracted from the *E. delegorguei*

Acknowledgments

The authors would like to express their sincere gratitude towards the provision of funds from the Directorate of Research and Resource Mobilisation office, Chinhoyi University of Technology, Zimbabwe under the project SERC/12/07/12.

REFERENCES

Aldrich J. R., Blum M. S. and Fale H. M. (1979) Species-specific natural products of adult male leaf-footed bugs (Hemiptera: Heteroptera). *Journal of Chemical Ecology*, **5**: 53 – 62.

Aldrich, J. R. (1995) *Chemical communication in the true bugs and parasitoid exploitation*I n R. T. Card´e and W. J. Bell (eds.). Chemical Ecology of Insects II. Chapman & Hall, New York, 318–363 **PP**.

Aldrich J. R., Lusby W. R., Marron B. E., Nicolau K. C., Hoffman M. P. and Wilson L. T. 1989. Pheromone blends of green stink bugs and possible parasitoid selection. *Naturwissen schaften*,**76**:173–175.

Bártu I.,Tomcala A., Socha R., Šimek P. and Kodrík D. 2010. Analysis of the lipids mobilized by adipokinetic hormones in the firebug *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae). *European Journal of Entomology*. **107**: 509 – 520.

Biniaś D., Wyszomirski M, Biniaś W. and Boryniec S. (2007). Supermmolecular structure of chitin and its derivatives in FTIR spectroscopy studies. *Polish Chitin Society, Monograph*, **12**: 95 – 108.

Chavunduka D. M. 1975. Insects as a source of food to the African. *Rhodesian Science News*. **9**: 217–220.

Dzerefos C. M., Witkowski E. T. F. and Toms R. (2009) Life-history traits of the edible stinkbug, *Encosternum delegorguei* (Hem., Tessaratomidae), a traditional food in southern Africa. *Journal of Applied Entomology*, **133**: 749 – 759.

Durak, D. and Kalender, Y. 2007. Fine structure and chemical analysis of the metathoracic scent gland of *Eurygaster maura* (Linnaeus, 1758) (Heteroptera: Scutelleridae). *Folia biologica*, **55**: 133-141.

Farine J. P., Bonnard, O., Brossut, R. and Le Quere, J. L. 1992. Chemistry of pheromonal and defensive secretions in the nymphs and the adults of *Dysdercus cingulatus* Fabr. (Heteroptera, Pyrrhocoridae). *Journal of Chemical Ecology*, **18**: 65-76.

Fávaro C. F., Santos T. B. and Zarbin P. H. 2012. Defensive compounds and male-produced sex pheromone of the stink bug, *Agroecus griseus*. *Journal of Chemical Ecology*, **38**: 1124 – 1132.

Harbone J. B. (1973) *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. (2nd Eds.). New York: Chapman A. & Hall, London, UK, 278 **PP**.

Hassani S., Abad R. F. P., Djozan D. and fazel M. M. (2010) Compounds in metathoracic glands of adults of the sunn pest, *Eurygaster integriceps* (Puton) (Heteroptera: Scutelleridae). *Munis of Entomology and Zoology*, **5**: 232 – 238.

Janaiah C., Rao P.S., Chari N and Reddy P.V. (1979) Chemical composition of the scent glands of adults and nymphs of the bug *Tessaratoma javanica* Thunberg. *Indian Journal of Experimental Biology*. **17**: 1233–1235.

Johne, A. B., Weibecker, B. and Schutz, S. 2006. Microorganisms on *Aesculus hippocastanum*–olfactory perspective of *Cameraria ohridella* (Deschka & Dimic). *Mitt. Dtsch. Ges. Allg. Angew. Entomology*, **15**: 147-151.

Kwashirai C. V. 2007. Indigenous management of teak woodland in Zimbabwe, 1850–1900. *Journal of Historical Geography*, **3:** 816 – 832.

Kye, C. P. and Hardie, J. 2002. Functional specialization and polyphenism in aphid olfactory sensilla. *Journal of Insect Physiology,* **48**: 527-535.

Marco H. G., Simek P., Clark D. K. and Gade G. 2013. Novel adipokinetic hormones in the kissing bugs *Rhodnius prolixus*, *Triatoma infestans*, *Dipetalogaster maxima* and *Panastrongylus megistus*. *Peptides*, (*in press* on-line).

McBrien H. L., Millar J. G., Rice R. E., Mcelfresh J. S., Cullen E. and Zalom F. G. 2002.Sex attractant pheromone of the red-shouldered stink bug *Thyanta pallidovirens*: a pheromone blend with multiple redundant components. *Journal of Chemical Ecology*, **28**: 1797 – 1818.

Millar J. G., McBrien H. M. and McElfresh J. S. 2010. Field trials of aggregation pheromones for the stink bugs *Chlorochroa uhleri* and *Chlorochroa sayi* (Hemiptera: Pentatomidae*). Journal of Entomology*, **103**: 1603 – 1612.

Paulitz T., Nowak-Thompson B and Gamard P. 2000. A novel antifungal furanone from *Pseudomonas aureofaciens*, a biocontrol agent for antifungal plant pathogens. *Journal of Chemical Ecology,* **26**: 1515 – 1524.

Skorik, Yury A.; Pestov, Alexander V andYatluk, Yury G. 2010. Evaluation of various chitin-glucan derivatives from *Aspergillus niger* as transition metal adsorbents. *Bioresource Technology*, **101**: 1769-1775.

Teffo L. S., Toms R. B. and Eloff J. N. 2007. Preliminary data on the nutritional composition of the edible stink-bug, *Encosternum delegorguei* Spinola, consumed in Limpopo province, South Africa. *South African Journal of Science*, **103**: 434–436.

Teffo L. S. Aderogba M. A. and Eloff J. N. 2010. Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonae viscosa* var. *angustifolia* leaf extracts. *South African Journal of Botany*, **76**: 25 – 29.

Teslenko A. Y. and Voevodina I. N. (1995) Method for preparing glucan-chitosan complexes. Patent RU 2043995 C1, 1-6 **PP**.

Williams L., Rodriguez-Saona C., Pare P. W. and Crafts-Brandner S. 2005. The piercing-sucking herbivores *Lygus Hesperus* and *Nezara viridula* induce volatile emissions in plants. *Archives of Insect Biochemistry and Physiology*, **58**: 84 – 96.

Zarbin, P. H. G., Borges, M., Santos, A. A., Oliveira, A. R. M., Simonelli, F. and Marques, F. A. 2000. Alarm Pheromone system of stink bug *Piezodorus guildinii* (Heteroptera: Pentatomidae). *Journal of the Brazilian Chemical Society*, **11**: 424-428.

Zvezdova D. 2010. Investigation of some physicochemical properties of chitin from crab shells. *Scientific works of University of Rousse*, **49**: 36 – 40.

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