IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 2 Ver. IV (Mar -Apr. 2015), PP 59-64
www.iosrjournals.org

GC-MS Determination of Bioactive Constituents of Giant African Snail (*Archachatina maginata)* Haemolymph

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Abstract: *Giant African snail (Archachatina maginata) is of high medicinal value, it haemolymph has been used in folk medicine for the treatment of liver disorders, whooping cough, anaemia, constipation, restore vitality and stop bleeding. In tune with this effect, the objective set for the present study is to identify the bioactive constituents of A. marginata haemolymph in order to understand the nature of the principle component responsible for its medicinal property. The haemolymph was extracted from the snail (A. maginata) and subjected to Gas Chromatography- Mass Spectrometry (GC-MS) analysis using a GC-MS (Model: QP2010 PLUS SHIMADZU, JAPAN) comprising a AOC-20i auto-sampler and gas-chromatograph interfaced to a mass spectrometer. GC–MS analysis provided of seven peaks. On comparison of the mass spectra of the constituents with the NIST library twenty six (26) constituents including 7 ester, 7 fatty acid, 5 alcohol, 6 alkane and 1 phthalate were characterized and identified. The presence of various bioactive compounds justifies it uses for various ailments by traditional practitioners. However isolation of individual constituents and subjecting it to biological activity will definitely give fruitful results and helpful to find anew drugs.*

Keywords: *Snail, Haemolymph, GC-MS analysis, bioactive constituents.*

I. Introduction

Natural products, owing to their medicinal value have continued to play a dominant role in the maintenance of human health since ancient times. The search on natural product have led to the discovery of novel drug candidates used against diverse diseases, as over 50% of all modern clinical drugs are of natural product origin [1]**.**

A knowledge of the chemical constituents of natural products is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of bioactive principle for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [2]. Hence a thorough validation of the natural products with medicinal reputation has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the bioactive agents have complementary and overlapping mechanism of action [3]. Several wild animals and their secreation have been used in folklore medicine. The African giant snail (*Archachatina marginata*) is one of the most important minor forest products in West Africa and Nigeria in particular [4].

The haemolymph of A. marginata contains a myriad of metabolites and other constituents which provide a valuable medium for clinical investigation and nutritional status of human beings and animals [5].In addition to the nutritional value, haemolymph of *A. marginata* cause agglutination of certain bacteria which are responsible for various ailments, including whooping cough. The high iron content is considered important in the treatment of anaemia [6]. It has also been reported to arrest constipation, restore vitality and stop bleeding when applied to a fresh cut. Previous biological studies reported that the acharan sulphate and mucin motifs isolated from *A. marginata* exhibited anti-tumor properties [7], and consistent blood glucose lowering effect [8] respectively. Recently the haemolymph of *A. marginata* has also been reported to have hepatoprotective effect and produce a dose dependent effect on haematological and biochemical parameters when administered to albino rats [3;9]. However, a literature survey has shown that there is no report on the bioactive constituents of the haemolymph thus the present investigation was carried out to identify active ingredients present in the *Archachatina maginata* haemolymph by (GC-MS) analysis

II. Materials And Methods

2.1 Snail Collection

African Giant Snails (*Achachatina maginata*) weighing 110-200g were bought from Kure market, Minna Niger state in September, 2014. They were housed in a ventilated container and fed with cucumbers and melon.

2.2 Haemolymph Collection

The haemolymph of *Archachatina maginata* was obtained as described by Bashir et al., [3]. The apex shell of the snails was opened; the haemolymph was drained into a clean conical flask and stored in the refrigerator.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried out on a GC-MS (Model: QP2010 PLUS Shimadzu, Japan) comprising a AOC-20i auto-sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) The instrument is equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The temperatures employed were; column oven temperature 80ºC, Injection Temp 250ºC at a pressure of 108.0 kPa, with total flow and column flow of 6.20 ml/min and 1.58 ml/min respectively. The linear velocity was 46.3 cm/sec and a purge flow of 3.0 ml/min. The GC program ion source and interface temperature were 200.00ºC and 250.00ºC respectively with solvent cut time of 2.50 min. The MS program starting time was 3.00min which ended at 30.00 min. with event time of 0.50 sec, scan speed of 1666 µl/sec, scan range 40-800u and an injection volume of 1 µl of the plant extract (split ratio 10:1). The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

2.4 Identification of the components

Interpretation on the mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST library. The relative percentage amount of each bio-component was calculated by comparing its average peak area to the total area. The name, molecular weight and structure of the components of the test materials were ascertained.

III. Results And Discussion

GC–MS chromatogram of the *Archachatina maginata* haemolymph (Fig. 1) showed seven peaks. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the haemolymph. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library [10] On comparison of the mass spectra of the constituents with the NIST library twenty six (26) constituents including 7 ester, 7 fatty acid, 5 alcohol, 6 alkane and 1 phthalate were characterized and identified. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the haemolymph are presented in Table 1.The mass spectrum and structure of the individual components are shown in Fig. 2. The first compound identified with less retention time (16.876s) was Methyl 14-methylpentadecanoate whereas 2-Methyloctadecane was the last compound which took longest retention time (25.254s) to identify. Literatures are scarce regarding the identified chemical constituents of *Archachatina maginata* haemolymph, however, The activities of some of the compound are given in table 2.The compound bioactivity prediction is based on Dr. Duke"s Phytochemical and Ethnobotanical Databases [11]

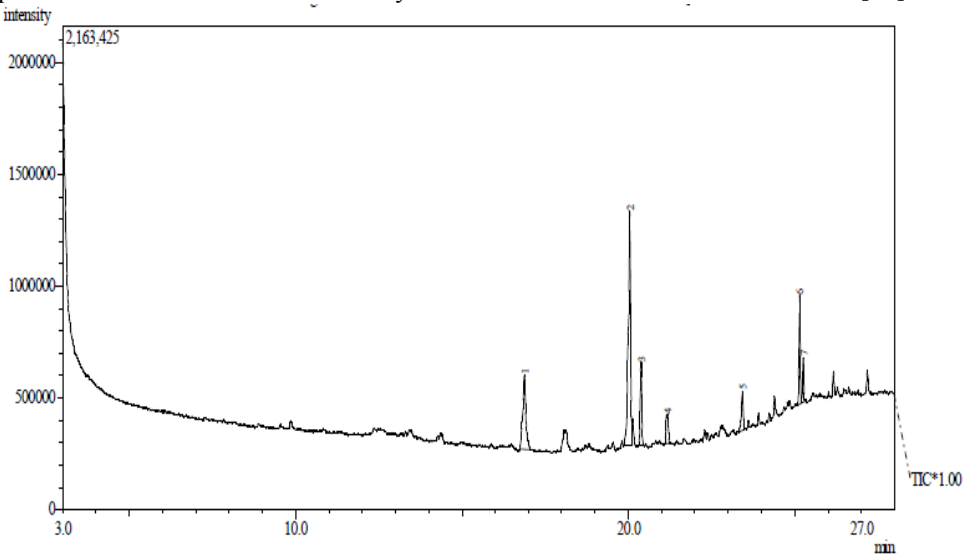


Figure 1.GC-MS Chromatogram of Archachatina maginata haemolymph

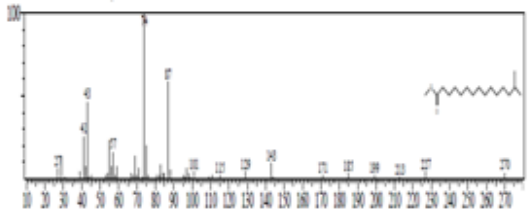
Table 1. Bio-active components identified in the Archachatina maginata haemolymph by GC-MS analysis

	Peak no	**Retention (RT)(s)**	**Compound name**	**Nature of compound**	**Molecular formula (MF)**	**Molecular weight (MW-g/mol)**	**Peak area**
1	1	16.876	Methyl 14-methylpentadecanoate	Ester	C17H34O2	270	19.21
2		16.876	n-Hexadecanoic acid methyl ester	Ester	C17H34O2	270	19.21
3		16.876	Capric acid methyl ester	Ester	C11H22O2	186	19.21
4		16.876	Methyl isoheptadecanoate	Ester	C18H36O2	284	19.21
5			Methyl tridecanoate	Ester	C14H28O2	228	19.21
6	2	20.041	Methyl 11-octadecenoate	Ester	C19H36O2	296	44.10
7		20.041	9-Octadecenoic acid	Fatty acid	C19H36O2	296	44.10
8		20.041	10-Octadecenoic acid	Fatty acid	C19H36O2	296	44.10
9		20.041	Methyl (7E)-7-hexadecenoate	Ester	C17H32O2	268	44.10
10	3	20.378	Octadecanoic acid	Fatty acid	C19H38O2	298	10.13
11		20.378	Hexadecanoic acid	Fatty acid	C18H36O2	284	10.13
12		20.378	Pentadecanoic acid	Fatty acid	C17H34O2	270	10.13
13		20.378	Heneicosanoic acid	Fatty acid	C22H44O2	340	10.13
14		20.378	Docosanoic acid/Behenic acid	Fatty acid	C23H46O2	354	10.13
15	4	21.149	1-Chloroeicosane	alkane	C20H41Cl	316	5.24
16		21.149	2-Ethyl-1-decanol	alcohol	C12H26O	186	5.24
17		21.149	1-Iodo-2-methylundecane	alkane	C12H25I	296	5.24
18		21.149	2-Butyl-1-octanol	alcohol	C12H26O	186	5.24
19	5	23.425	2-Methyloctadecane	alkane	C19H40	268	5.66
20		23.425	11,20-Di-n-decyltriacontane	alkane	C50H102	702	5.66
21		23.425	n-Nonadecane	alkane	C19H40	268	5.66
22	6	25.143	Bis(2-ethylhexyl) 3-nitrophthalate	Phthalate	C24H37NO6	435	11.21
23	7	25.254	2-Butyl-1-octanol	Alcohol	C12H26O	186	4.45
24		25.254	2-Hexyl-1-octanol	Alcohol	C14H30O	214	4.45
25		25.254	2-Hexyl-1-decanol	Alcohol	C16H34O	242	4.45
26		25.254	2-Methyloctadecane	Alkane	C19H40	268	4.45

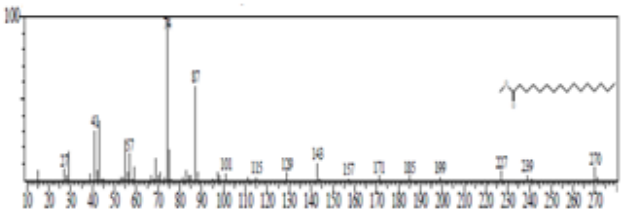
Table 2. Activities of some identified compound in Archachatina maginata haemolymph

compound name	Activities
n-Hexadecanoic acid methyl ester	Antioxidant, hypocholesterolemic, nematicide, hemolytic, 5-alpha reductase inhibitor
Capric acid methyl ester	Calcium antagonist
Methyl 11-octadecenoate	Allelopathic, pesticide
9-Octadecenoic acid	Anti-inflammatory, Anti-alopecic, Anemiagenic, 5 reductase inhibitor, α-reductase inhibitor lubricant, Antitumour, Choleretic, Dermatitigenic, Immunostimulant, Anti-leucotriene-D4, Antiandrogenic, Lipoxygenase inhibitor, Allergenic, Flavour, Hypocholesterolemic, Insectifuge, Irritant, Percutaneo-stimulant, Perfumery and Propecic
Hexadecanoic acid	Lubricant, antiandrogenic, antioxidant, 5- alpha-reductase inhibitor.12
Pentadecanoic acid	Antioxidant
Docosanoic acid/Behenic acid	Hair moisturizer
1-Iodo-2-methylundecane	Sex hormones
n-Nonadecane	Antimutagenic

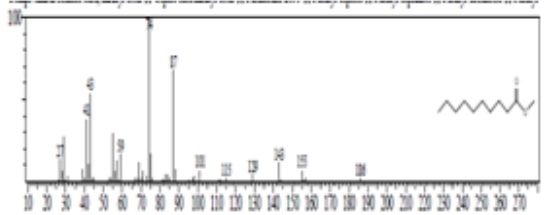
Sources: Dr. Duke‟s Phytochemical and Ethnobotanical Databases [11].



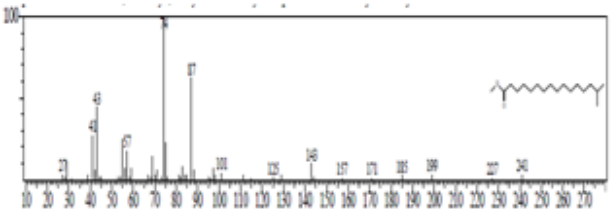
Methyl 14-methylpentadecanoate



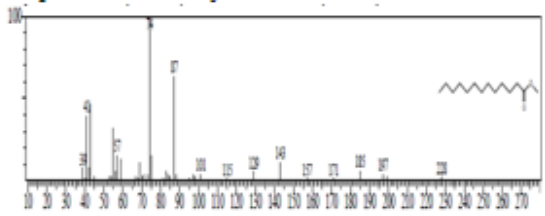
n-Hexadecanoic acid methyl ester



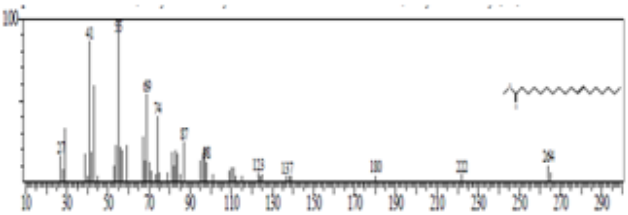
Capric acid methyl ester



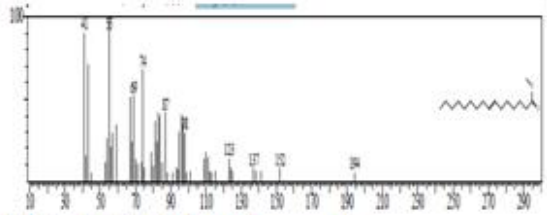
Methyl isoheptadecanoate



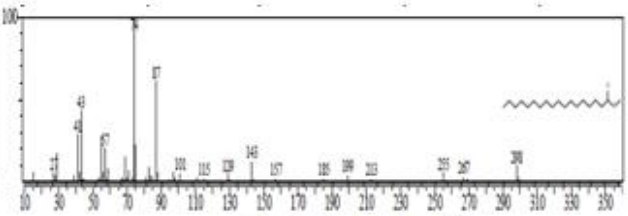
Methyl tridecanoate



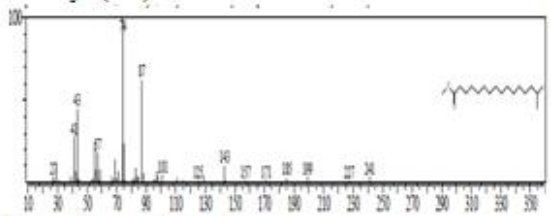
Methyl 11-octadecenoate



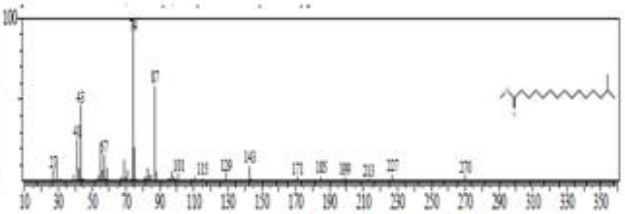
Methyl (7E)-7-hexadecenoate



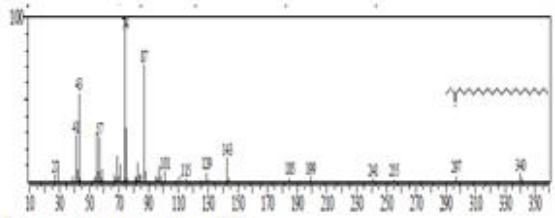
Octadecanoic acid



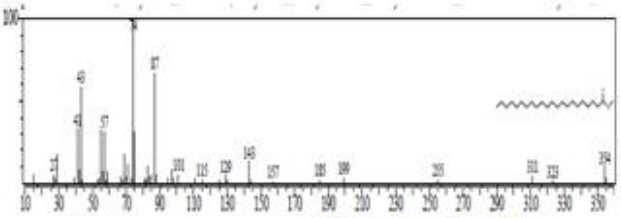
Hexadecanoic acid



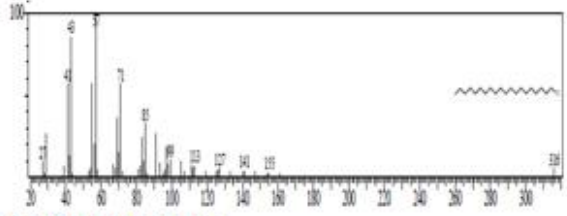
Pentadecanoic acid



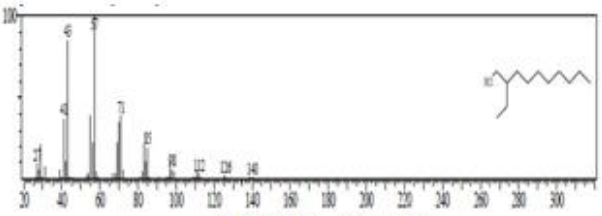
Heneicosanoic acid



Docosanoic acid



1-Chloroeicosane



2-Ethyl-1-decanol

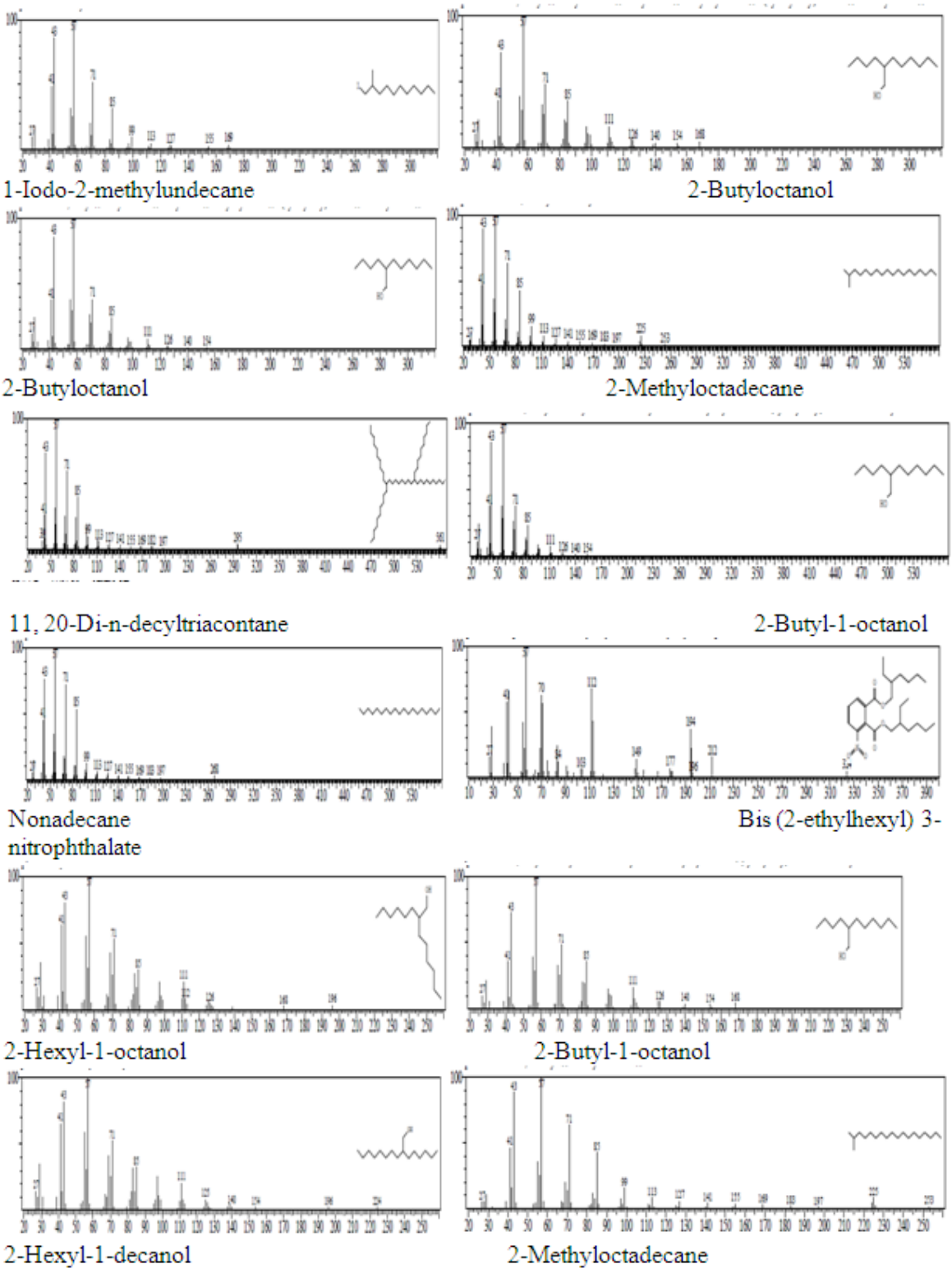


Figure 2; Mass fragmentation of the active components identified in the Archachatina maginata haemolymph

IV. Conclusion

In the present study twenty six chemical constituents have been identified from *Archachatina maginata* haemolymph by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies it uses for various ailments by traditional practitioners. However isolation of individual constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that

Achachatina maginata haemolymph contains various bioactive compounds. So it is recommended as a wild animal of pharmaceutical importance.

Competing interest
The authors declare that they have no competing interest

ACKNOWLEDGEMENTS
Thanks are due to Adamu Mohammed of National Cereals Research Institute Zaria, Nigeria for help rendered during the GCMS analysis.

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