GC-MS DETERMINATION OF CHEMICAL COMPONENTS IN THE BIOACTIVE SECRETION OF *ANOPLODESMUS SAUSSURII* (HUMBERT, 1865)

Rukhsana, K., Vijesh Varghese, Akhilesh, V. P., Jisha Krishnan, E. K., Priya Bhaskaran, K. P., Bindu, P. U. and Sebastian, C. D.*

Molecular Biology and Biochemistry Laboratory, Department of Zoology,
University of Calicut, Kerala, 673635 India
drcdsebastian@gmail.com
Cell: 919447648961

ABSTRACT

The secretory bioactive components of the hexane extract of *Anoplodesmus saussurii* was analyzed using GCMS. From the study, presence of ten components in the secretion was confirmed. N-[3-(4-Butoxyphenyl) -10- Oxo-10H-9-oxa-2,4-diaza-phenanthren-1-yl] propionamide and 8,9-Octadecenamide are the major components and was found in 22.646% and 20.023% in peak area. These components showed a variety of functional as well as physiological properties like antioxidants, steroidal antimineralocorticoid agents, plasticizers, antibacterial agents, antimicrobial agents and ectoparasiticides.

Key words: *Anoplodesmus saussurii*, Hexane extract, GC-MS analysis

INTRODUCTION

Millipedes, commonly called as the 'thousand-legged worms' are well known for secreting biochemical compounds against predators and parasites as a mean of defence [1]. These bioactive secretions include different quinones, phenolic compounds, organic acid, quinazolines, monoterpens, or cyanogenic compounds [2]. Various biochemical studies indicate that the quantitative and qualitative differences in millipede allomones show some evolutionary patterns with respect to genus/species. The restricted production of hydrogen cyanide and other cyanogenic compounds by the members of the order Polydesmida is well established [1,3,4,5]. In millipedes usually the predominant secretory components are hydrogen cyanide and benzaldehyde, along with phenol, benzoic acid, benzoylnitrile and mandelonitrile. Studies have revealed that the composition of biosecretions in some species indicates interspecific or intraspecific differences and this information is very useful in their chemotaxonomy [6]. Hydrogen cyanide is a toxic component, generally believed to function as a defensive agent against predators and parasites [7]. In millipedes, defense glands are present in the pleurotergites of somites 5, 7, 9, 10, 12, 13, and 15-18 on its lateral sides of paranotal expansions.

Anoplodesmus saussurii is a millipede found in South India, Ceylon, Singapore and Mauritius [8]. They are found in large aggregations under the cover of decaying litter layers in the agricultural and horticultural land areas and forests on humid soils in India. They tend to keep away from light and release an array of chemical substances to avoid predators and parasites. The species feeds on various kinds of leaf litter, rotten vegetables, tree stems, wood, decaying fish, and cow dung [9,10,11,12,13]. It plays an important role in the litter breakdown of the ecosystems in which it lives. Populations of *A. saussurii*, which occur often in high densities, can consume and breakdown up to 1 kg of dry litter per square meter per year [14]. The present study is the GCMS analytical separation of biochemically active components from the secretion of *A. saussurii*.

MATERIALS AND METHODS

The millipede, *A. saussurii* were collected from Malappuram, Kerala by hand picking. From the whole body, the p^H of body fluid was measured by p^H indicator paper (p^H 1-14; Johnson Test Papers Ltd.). The Hydrogen Cyanide (HCN) secreted from the live millipedes was qualitatively examined by the picric acid test [15]. Filter paper, previously impregnated with a saturated solution of picric acid, was sprayed with 5% sodium bicarbonate. Live millipedes were placed on to the wet filter paper and squeezed forcefully. If the millipede secretions contained HCN, the colour of the portion of the paper stained by the secretions gradually turns orange. For hexane extraction millipede was shaken vigorously in a glass specimen tube for 2 minutes. Hexane was then added (volume of the hexane depends upon the size of the specimen). The tubes were kept for 3 minutes at room temperature. Secretion smeared on the inner wall of the tube was collected by decantation and simultaneously subjected to GC-MS analysis. The GC-MS analysis of secretions were carried out on a GC Clarus 500 Perkin Elmer comprising a AOC-20i Autosampler and Gas Chromatograph interfaced to a Mass Spectrometer (GC-

MS) instrument employing the following conditions: Column Elite-5MS fused silica capillary column (30mm x 0.25mm x 0.25 µm df composed of 5% Diphenyl/95% Dimethyl poly siloxane), operating in electron impact mode at 70eV. Helium (99.999%) was used as carrier gas at a constant flow rate of 1 ml per minute and an injection volume of 3 µl (split ratio 10:1). An injector temperature of 250°C and an ion-source temperature of 280°C were employed. The percentage of each chemical constituent was calculated by comparing the average peak area to the total areas.

RESULTS AND DISCUSSION

The secretions of *A. saussurii* include different biochemical components such as quinines, organic acids and phenolic compounds. GCMS chromatogram of the hexane extract of *A. saussurii* showed ten peaks indicating the presence of ten chemical constituents (Figure 1). On comparison of the mass spectra of the constituents, the various chemical constituents were identified and characterized. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the hexane extract of *A. saussurii* are presented in Table-1. Figures 2 exhibits the mass spectrum and structure of ten chemical constituents, *viz.* Benzene (1-butylpentyl); Phenol, 2,4-bis (1,1-dimethylethyl)-; Tri isobutyl (3-phenylpropoxy) silane; 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione; Benzeneacetonitrile, α-(benzoyloxy)-; N-[3-(4-Butoxyphenyl)-10-oxo-10H-9-oxa-2,4-diaza-phenanthren-1-yl]-propionamide; Dibutyl phthalate; Hexadecanamide; 8,9 Octadecenamide and Phenol, 4,4'-methylenebis [2,6-bis (1,1-dimethylethyl). There was no cyanide presence detected from the secretions of *A. saussurii*.

The physiological and biochemical properties of the major components identified from the secretion of *A. saussurii* through GC-MS analysis ranging between antioxidants, steroidal antimineralocorticoid agent, plasticizer, antibacterial agent, antimicrobial agent, ectoparasiticide etc. Phenol, 2,4-bis (1,1-dimethylethyl)- is used as a chemical intermediate for the synthesis of UV stabilizers or antioxidants which included the alkylated phenol category. 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione is a synthite, it has steroidal antimineralocorticoid property in addition to anti-androgen and weak progesterone properties and some indirect estrogen and glucocorticoid effect. Dibutyl phthalate is esters of phthalic acid and it is used as a plasticizer, antibacterial agent, additive to printing inks and ectoparasiticide. 8 9-Octadecenamide is a amide and has antimicrobial effect. There are studies stipulated that mandelonitrile benzoate is produced in large amounts together with hydrogen cyanide following shake-disturbances administered to several millipede species [16]. On the other hand, the presence of mandelonitrile benzoate in defensive fluids may be the mechanism that stabilizes mandelonitrile if the specimen is consumed by a predator; in such cases this compound will be degraded during passage through the digestive tract [7]. Some investigators showed that between the genera *Polydesmus* and *Brachydesmus* there exist differences in the chemical composition of the defensive secretion, including the presence of mandelonitrile only in the genus Polydesmus, and benzyl alcohol and benzoic acid only in the genus *Brachydesmus* [6]. There are accepted hypotheses that adaptive responses to local conditions are important in the evolution of the diplopod secretions which are employed by millipedes for defense against a variety of predators and natural enemies [1].

CONCLUSION

A. saussurii contains various bioactive compounds of which ten components are the major part. It can be attributed that the species secretes hydrogen cyanide when disturbed, but there was no cyanide related compounds secreted otherwise. All these components are important for the organism for their defence, immunity and communication. An evaluation of the pharmacological and biomedical properties of these compounds is suggested, which may lead to the discovery of drug molecules as chemotherapeutic agents in combating various diseases of mankind.

ACKNOWLEDGEMENTS

The financial assistance from University Grants Commission, New Delhi and Kerala State Council for Science Technology and Environment under Research Projects and Ministry of Minority Affairs, Government of India in the form of MANF are gratefully acknowledged.

REFERENCES

[1] W. A. Shear, T. H. Jones, H. M. Miras et al. A possible phylogenetic signal in millipede chemical defenses: The polydesmidan millipede Leonardesmus injucundus Shelley & Shear secretes p-cresol and lacks a cyanogenic defense (Diplopoda, Polydesmida, Nearctodesmidae). Biochem. Syste. Ecol., 2007, 35: 838-842.

[2] T. Eisner, D. Alsop, K. Hicks, J. Meinwald et al. Defensive secretions of millipedes. In: Bettini S. (Ed.) - Arthropod Venoms. Berlin, Springer, 1978, 41-72.

[3] H. Omura, Y. Kuwahara, T. Tanabe et al. 1-Octen- 3-ol together with geosmin: new secretion compounds from a polydesmid millipede, Niponia nodulosa. J. Chem. Ecol, 2002, 28: 2601-2612.

[4] H. Omura, Y. Kuwahara, T. Tanabe et al. Species-specific chemical composition of defense secretions from Parafontaria tonominea Attems and Riukiaria semicircularis Takakuwa (Polydesmida: Xystodesmidae). Appl. Entomol. Zool., 2002, 37: 73-78.

[5] J. Taira, K. Nakamura, Y. Higa et al. Identification of secretory compounds from the millipede, Oxidus gracilis C. L. Koch (Polydesmida: Paradoxosomatidae) and their variation in different habitats. Appl. Entomol. Zool., 2003, 38: 401-404.

[6] S. E. Makarov, B. P. M. Curcic, V. V. Tesevic, M. B. Jadranin, L. V. Vujisic, S. B. Curcic, B. M. Mandic, T. L. Sekulic, B. M. Mitic et al. Defensive Secretions in Three Species of Polydesmids (Diplopoda, Polydesmida, Polydesmidae). J. Chem. Ecol., 2010, 36: 978–982.
[7] M. Zagrobelny, B. Soren, B. L. Moller et al. Cyanogenesis in plants and arthropods. Phytochemistry, 2008, 69: 1457-1468.
[8] R.M. Shelley, P.T. Lehtinen et al. Introducing millipedes of the family Paradoxosomatidae on the Pacific Islands (Diplopoda: Polydesmida). Arthropoda Selecta, 1998, 7: 81-94.
[9] K. Bano, R. V. Krishnamoorthy et al. Consummatory responses of the millipede Jonespeltis splendidus (Verhoeff) in relation to soil organic matter. Proc. Nat. Ac. Sc. India, 1981, 90: 631–640.
[10] K. Bano, R. V. Krishnamoorthy et al. Relative rates of litter decomposition and humification of various soils by the millipede Jonespeltis splendidus. UAS Technical Series, University of Agr. Sc. Hebbal, 1981, 37: 87–92.
[11] M. P. Prasad, T. N. Khan et al. Bioecology of banana inhabiting millipede Anoplodesmus saussurei (Humb.) (Diplopoda: Paradoxosomatidae). Bull. Zoological Survey of India, 1981, 3: 125–135.
[12] A. K. Dutta, V. C. Joy et al. Food preference and assimilation efficiency of Anoplodesmus saussurei (Humb) on decomposing leaf litter. Proc. Zool. Soc. (Calcutta), Supplement A, 1992, 45: 447–455.
[13] A. K. Dutta, V. C. Joy et al. Distribution of Anoplodesmus saussurii (Humb) (Diplopoda: Polydemida [Polydesmida]) in decomposing leaf litter of forest stands. J. of Soil Biol. and Ecol., 1993, 13: 31–38.
[14] K. Bano. Feeding rates and nutrient assimilation in the millipede Jonespeltis splendidus (Diplopoda, Paradoxosomatidae). Acta Myriapodologica. Memoires du Museum National de Histoire Naturelle. 1996, 169: 561–564.
[15] S. Noguchi, N. Mori, Y. Higa, Y. Kuwahara et al. Identification of mandelonitrile as a major secretory compound from Chamberlinius hualienensis Wang (Polydemida Paradoxosomatidae). Japan J. Environ. Entomol. Zool., 1987, 8: 208-214.
[16] Y. Kuwahara, N. Shimizu, T. Tanabe et al. Release of Hydrogen Cyanide via a Post-secretion Schotten-Baumann Reaction in Defensive Fluids of Polydesmoid Millipedes. J. Chem. Ecol., 2011, 37: 232–238.

Table 1: Chemical constituents in the Hexane extract of *Anoplodesmus saussurii*

Sl. No.	Name of the compound	RT	Peak area %
1.	N-[3-(4-Butoxy-phenyl)-10-oxo-10H-9-oxa-2,4-diaza-phenanthren-1-yl]-propionamide	33.584	22.646%
2.	8 9-Octadecenamide (Z)-	41.176	20.023%
3.	Phenol, 4,4'-methylenebis[2,6-bis(1,1-dimethylethyl)-	44.236	8.965%
4.	Hexadecanamide	38.054	5.508%
5.	Benzeneacetonitrile, α-(benzoyloxy)-	33.378	3.616%
6.	Phenol, 2,4-bis(1,1-dimethylethyl)-	24.117	3.077%
7.	Triisobutyl(3-phenylpropoxy)silane	26.607	2.103%
8.	Benzene(1-butylpentyl)-	12.608	0.889%
9.	Dibutyl phthalate	33.842	0.888%
10.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	32.852	0.766%

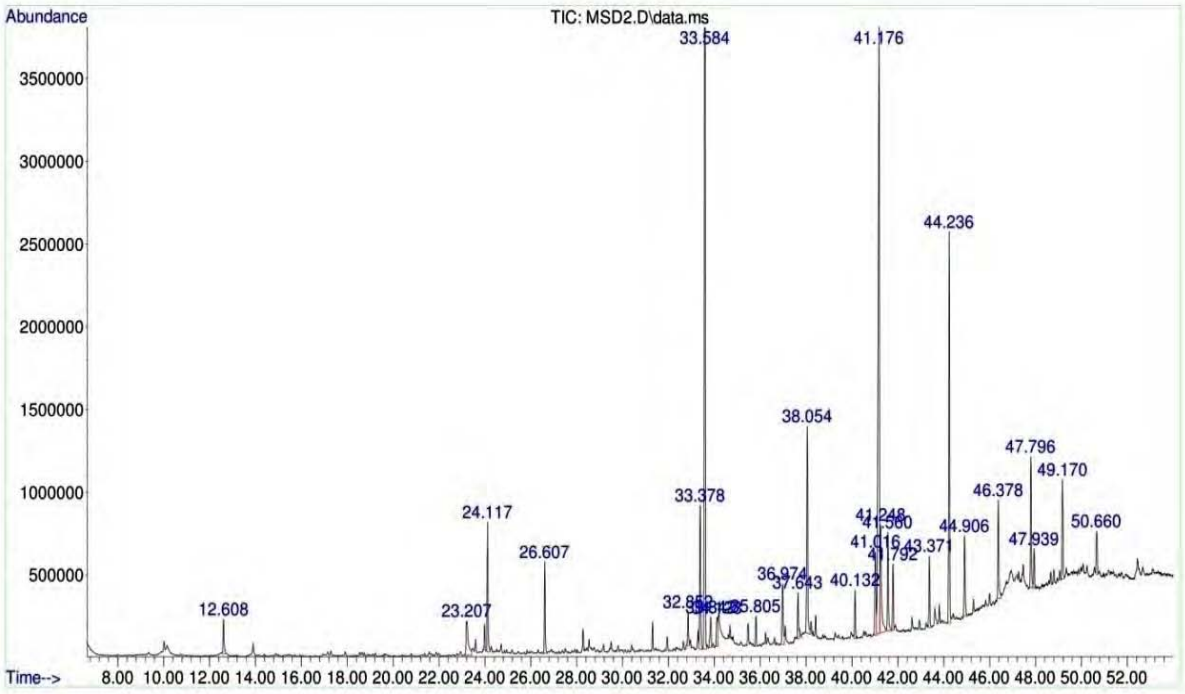

Figure 1: GC-MS Chromatogram of the Hexane extract of *Anoplodesmus saussurii*

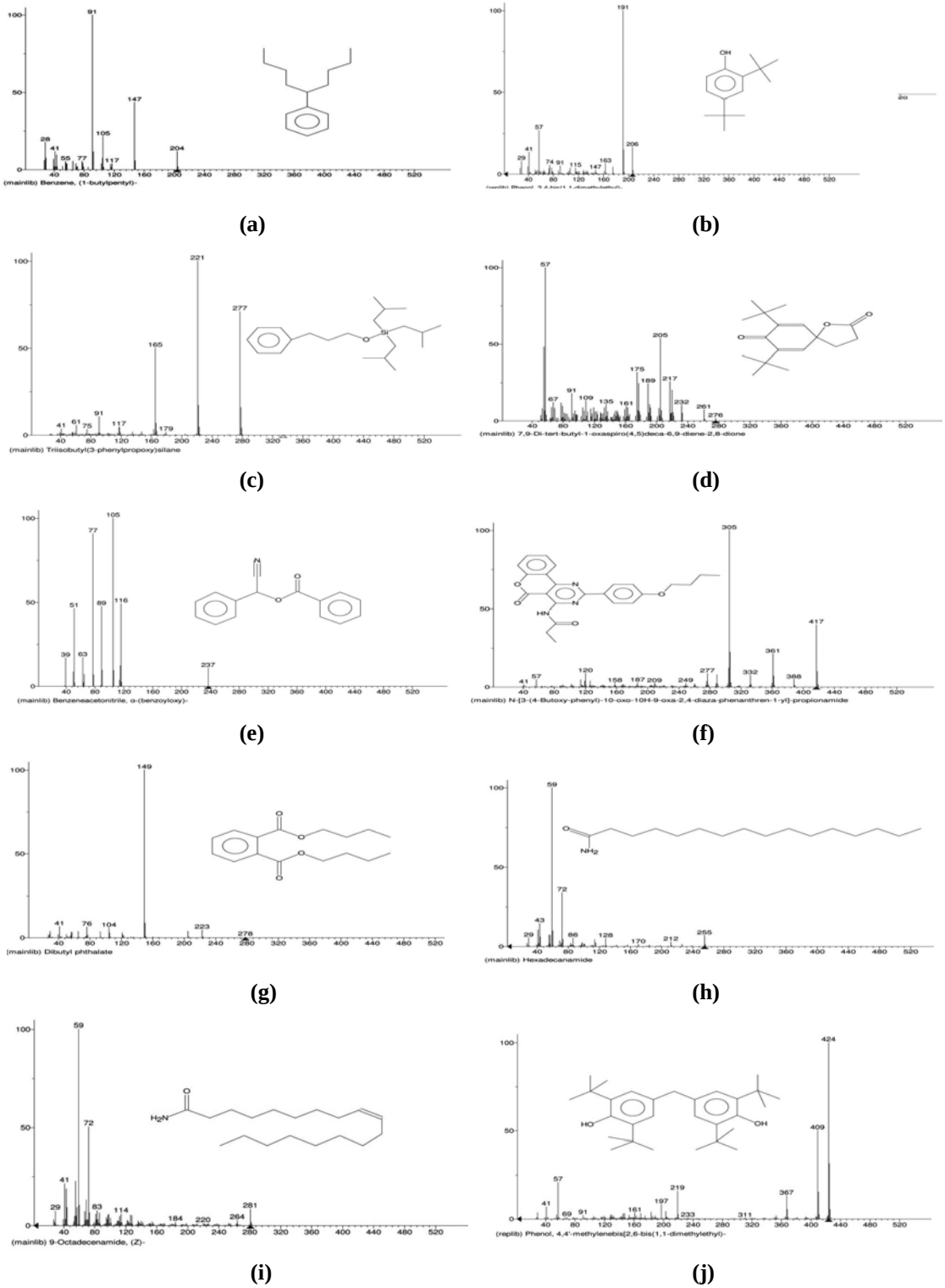

Figure 2: Mass spectrum of: (a) Benzene (1-butylpentyl); (b) Phenol, 2,4-bis (1,1-dimethylethyl); (c) Tri isobutyl (3-phenylpropoxy) silane; (d) 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione; (e) Benzeneacetonitrile, α-(benzoyloxy)-; (f) N-[3-(4-Butoxy-phenyl)-10-oxo-10H-9-oxa-2,4-diaza-phenanthren-1-yl]-propionamide; (g) Dibutyl phthalate; (h) Hexadecanamide; (i) 8,9Octadecenamide-Z; (j) Phenol, 4,4'-methylenebis [2,6-bis (1,1-dimethylethyl)