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Characterization of bioactive compounds in the ant *Leptogenys diminuta* (Smith, 1857) using Gas Chromatography - Mass Spectrophotometry

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Abstract

Ants are one of the diverse groups of insects and are less explored reservoirs of many bioactive natural products, including proteins and peptides. This study was conducted to characterize the compounds of the worker ant, *Leptogenys diminuta* (Smith, 1857). Hence, this study will demonstrate the importance of ants as a potential source of bioactive compounds found in nature. Thirty samples of *L. diminuta* were extracted using hexane solution and the compounds were detected using Gas Chromatography-Mass Spectrophotometry (GC-MS), and the mass spectra of compounds were identified using the NIST08 Standard Reference Database. The analysis identified seven bioactive compounds from *L. diminuta* with pharmacological and industrial applications, including the first reports of the presence of 2,4-di*tert*-butylphenol in Ponerine worker ant's hexane extract. Thus, more studies are needed to explore other essential natural products with various applications found in ants.

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Introduction

Ants are one of the ecologically and taxonomically diverse groups of insects. They dominate much in terrestrial ecosystems, ranging from deserts to subarctic tundra (Shields, 2018). Currently, there are more than 15,752 valid species/subspecies of ants. Of these, the subfamily Myrmicinae and Ponerinae constitute 40% and 17%, respectively (Antweb, 2021). Species from these subfamilies possess sting apparatus. For instance, the sting apparatus of the fire ant *Solenopsis invicta* is used to inject venom against their prey. Venomous sting has been reported to cause inflammation, localized sterile blisters, and sterile pustules that sometimes cause whole-body allergic reactions and even death in humans (Junior and Larsson, 2005; Drees, 2014).

On the other hand, the stinging ant venoms are also a rich source of promising bioactive compounds with therapeutic and industrial applications. In subfamily Myrmicinae, the piperidine alkaloid Isosolenopsin A isolated from the fire ant, *Solenopsis invicta* has cardiovascular depressant and neurologic actions (Yi *et al.*, 2003; Postman, 2009). The specific isomers of synthetic fire ant (*S. invicta*) venom alkaloids demonstrate antibacterial activity against human pathogens (Sullivan *et al.*, 2009). Such as the Solenopsin-induced autophagic and programmed cell death in *Trypanosoma cruzi*, the causative agent of Chagas disease (Silva *et al.*, 2020); and a naturally occurring inhibitor of phosphatidylinositol-3-kinase signaling and angiogenesis (Arbiser *et al.*, 2007). The peptide Bicarinalin was isolated from the ant *Tetramorium bicarinatum* and had antimicrobial activity against Staphylococcus and Enterobacteriacea (Tene *et al.*, 2016). In subfamily Ponerinae, the neuropeptide Poneratoxin isolated from bullet ants, *Paraponera clavate* has potential use for the construction of biological insecticide (Szolajska *et al.*, 2001; Postman, 2009); and affects the excitability of nerve and muscle fibers by its action on sodium channels (Duval *et al.*, 1992). The Poneritoxins from *Anochetus emarginatus* demonstrate a rich source of novel ion channel modulating peptides that are useful in biopesticides development (Touchard *et al.*, 2016). The Ponericins isolated from the venom of the predatory ant *Pachycondyla goeldii* has been found to have antimicrobial, insecticidal, and hemolytic properties (Orivel *et al.*, 2001). The Dinoponeratoxin peptides isolated from the ant *Dinoponera quadriceps* display *in vitro* anti-trypanosomal activity and a broad spectrum of candicidal (Lima *et al.*, 2018; Doduo Lima *et al.*, 2020).

The Philippines currently has 582 species/subspecies, and more than half are stinging ants, with subfamily Myrmicinae constituting 40% and Ponerinae 17% are the most abundant (Antweb, 2021). Despite the promising biologically active natural products, studies on ant venoms in the Philippines remain an underrepresented resource for therapeutics and industry. Thus, this study aimed to characterize the bioactive compounds present in the head, thorax, and sting apparatus of Ponerine ant *Leptogenys diminuta* using Gas Chromatography-Mass Spectrophotometry (GC-MS).

Materials and methods

Collection of samples

Samples of the ant *Leptogenys diminuta* (Smith, 1857) were collected from a decaying wood near a stream of Bolyok falls, Naawan, Misamis Oriental, in October 2019, and placed in 20L plastic box painted with Fluon® to prevent from escaping. Extractions were done 1week after collection from the field.

Ant venom extraction

Thirty workers of *Leptogenys diminuta* were transferred into a rectangular container with a flat lid (500ml) and placed in a freezer for 5-10 minutes to immobilized samples. Crude venom was extracted separately from the head, thorax, and secretory apparatus, which consists of the sting, Dufour's gland, and the venom gland.

The secretory apparatus from 30 worker ants were carefully pulled out of the abdomen by grasping the sting with a pair of forceps. The secretory apparatus was pooled and soaked in 2ml hexane solution; on the other hand, the head and thorax from the same worker ants were soaked separately in 2ml hexane solution and kept in the freezer for 48 hours. The three samples were submitted for Gas Chromatography-Mass Spectrophotometry (GC-MS Ultra QP 2010™) at the Chemistry Analytical and Research Laboratory of Ateneo de Davao University, Davao City. The compounds were identified by interpretation of their mass spectra and using the NIST08 Standard Reference Database.

Results and discussion

The GC–MS profile of the three-sample solvent-extracts of *L. diminuta* workers revealed seven bioactive compounds (Table 1). The comparison of the mass spectra of bioactive constituents was performed using the internal standards of the National Institute of Standard and Technology database (NIST08.library). The spectra of compounds with biological activities that were identified from *L. diminuta* were presented in Fig. 1.

All samples contain 1,4:3,6-Dianhydro-D-glucitol, referred to as Isosorbide. This compound is non-toxic and has pharmaceutical applications, including treatment of hydrocephalus, glaucoma, and as preoperative medication for various surgical procedures, including cataract extraction, retinal detachment, corneal transplant, and glaucoma operations (Becker *et al.*, 1967; Lorber, 1973; Cecutti *et al.*, 1998).

Table 1. Components of Hexane extracts derived from the worker ant, *Leptogenys diminuta*.

	Peak No.	RT(min.)	Compounds	Formula	Retention Index
Head	1	5.61	3, 5-di methyl- 2-propylpyrazine	$C_9H_{14}N_2$	1206
	2	6.08	1,4:3,6-Dianhydro-D-glucitol	$C_6H_{10}O_4$	1216
	3	6.23	2 -butyl-3,5-dimethylpyrazine	$C_{10}H_{16}N_2$	1306
	4	8.065	2,4-d*itert* -butylphenol	$C_{14}H_{22}O$	0
Thorax	1	5.94	1 ,4:3,6-Dianhydro- .alpha, -d-glucopyranose	$C_6H_8O_4$	916
	2	6.045	1,4:3,6-Dianhydro-D-glucitol	$C_6H_{10}O_4$	1216
	3	8.05	2,4-d *itert* -butylphenol	$C_{14}H_{22}O$	0
Sting apparatus	1	5.935	1,4:3,6-Dianhydro-,alpha,-d-glucopyranose	$C_6H_8O_4$	916
	2	6.035	1,4:3,6-Dianhydro-D-glucitol	$C_6H_{10}O_4$	1216
	3	6.615	2,3-diacetyloxypropyl acetate	$C_9H_{14}O_6$	1354
	4	19.995	24-Methyl-9,19-cyclolanost-25-en-3-ol	$C_{31}H_{52}O$	2834
	5	20.405	24-Methyl-9,19-cyclolanost-25-en-3-ol	$C_{31}H_{52}O$	2834
	6	21.435	24-Methyl-9,19-cyclolanost-25-en-3-ol	$C_{31}H_{52}O$	2834
	7	21.55	24-Methyl-9,19-cyclolanost-25-en-3-ol	$C_{31}H_{52}O$	2834

On the other hand, the compound 3,5-dimethyl-2-propylpyrazine and 2-butyl-3,5-dimethylpyrazine [Pyrazine] were found only in the head. The compound Pyrazine has been found in the head of the Australian bull ant *Myrmecia gulosa* (Brophy and Nelson, 1985) and common mandibular components of Ponerine ants (Morgan and Mandava, 1988). Pyrazine has been identified in the mandibular glands of *Odontomachus chelifer*, *O. erythrocephalus*, *O. ruginodis*, *O. bauri*, and *Streblognathus aethiopicus* (Jones *et al.*, 1998). Pyrazine and its derivatives were produced in industries for fragrance, flavor, ingredients in pesticides, insecticides, dyes, and pharmaceutical applications (Ong *et al.*, 2017; Mortzfeld *et al.*, 2020). Also, pyrazine derivatives have been found to possess antibacterial, antifungal, antimycobacterial, anti-inflammatory, analgesic, anticancer for different types, antidiabetic, treatment for arteriosclerosis, antiviral effects (Ferreira and Kaiser, 2012). Furthermore, 2,3-diacetyloxypropyl acetate and 24-Methyl-9,19-cyclolanost-25-en-3-ol were found only in the sting apparatus. These compounds are commonly called Triacetin, which has been used most often as a fungicide, common component of cosmetic formulations, food additive, antifungal and antimicrobial agent (Quinn and Ziolkowski, 2015; National Center for Biotechnology Information, 2021).

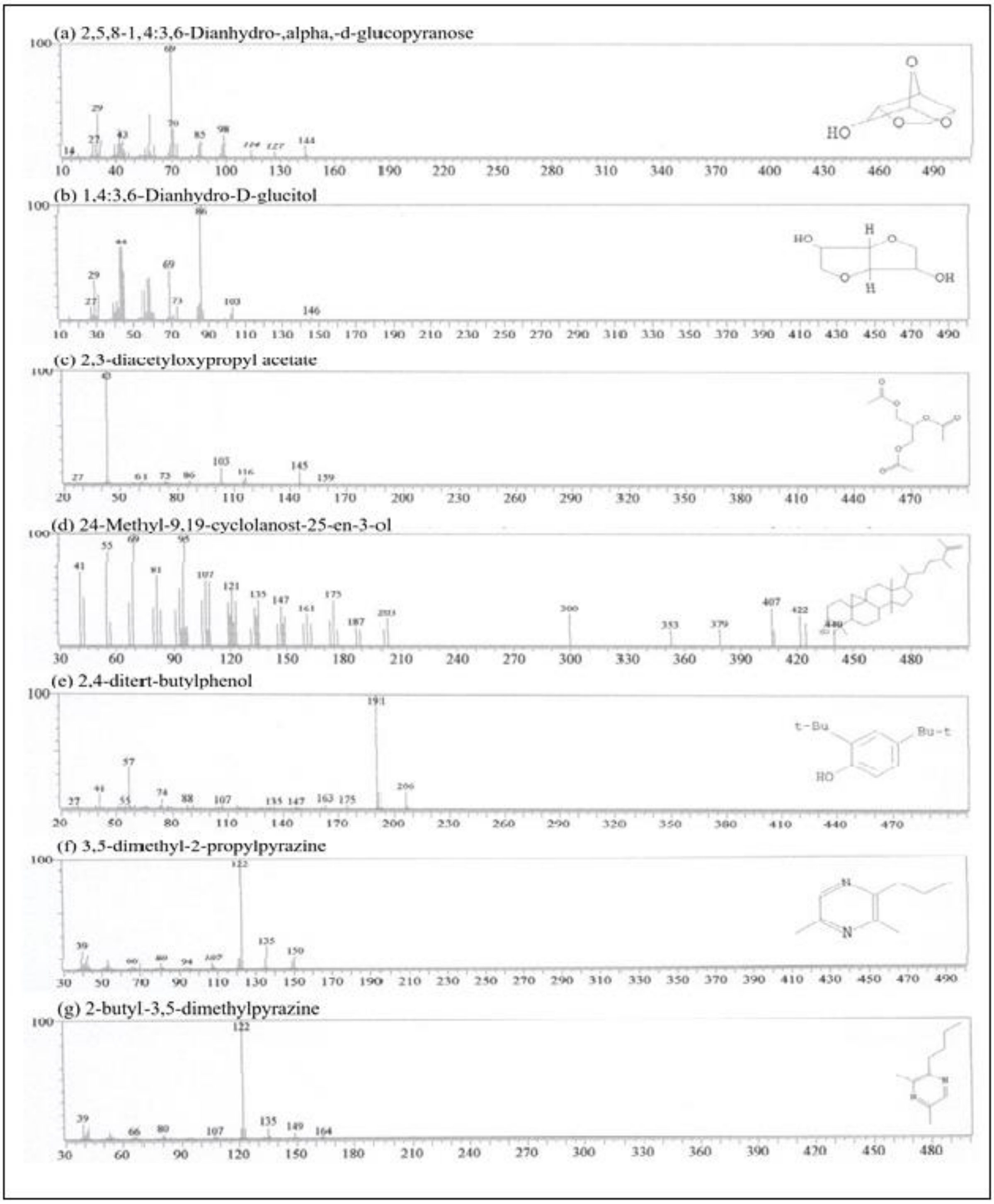

Fig. 1. Mass spectrum of the bioactive compounds identified in worker ants of *Leptogenys diminuta*.

Moreover, this study first reports the presence of 2,4-di*tert*-butylphenol in Ponerine worker ant's hexane extract. This compound has been reported as antimicrobial, antiviral, antibacterial, antifungal, antioxidant, anti-inflammatory, cancer drugs, and a potential application in agriculture as herbicide, pesticide, insecticide and nematocide (Zhao *et al.*, 2020).

Conclusion

The *Leptogenys diminuta* is a generalist predatory ant species found nesting in decaying wood. The GC-MS analysis revealed the presence of seven bioactive compounds in *L. diminuta,* including Isosorbide, Pyrazine, Triacetin, and 2,4-di*tert*-butylphenol. These compounds are essential in the formulation of various industrial and pharmaceutical applications. Thus, exploring rich biological resources is a promising prospect for drug discovery.

References

Arbiser JL, Kau T, Konar M, Narra K, Ramchandran R, Summers SA, Vlahos CJ, Ye K, Perry BN, Matter W, Fischl A, Cook J, Silver PA, Bain J, Cohen P, Whitmire D, Furness S, Govindarajan B, Bowen JP. 2007. Solenopsin, the alkaloidal component of the fire ant (*Solenopsis invicta*), is a naturally occurring inhibitor of phosphatidylinositol-3-kinase signaling and angiogenesis. Blood **109(2),** 560–565. https://doi.org/10.1182/blood-2006-06-029934

AntWeb. Version 8.66. California Academy of Science, online at Accessed 8 November 2021. https://www.antweb.org.

Becker B, Kolker AE, Krupin T. 1967. Isosorbide: An Oral Hyperosmotic Agent. **78(2),** 147–150. http://dx.doi.org/10.1001/archopht.1967.00980030149007

Brophy JJ, Nelson D. 1985. 2,5-Dimethyl-3-n-propylpyrazine from the head of the bull ant *Myrmecia gulosa* (Fabr.). Insect Biochemistry **15(3),** 363-365.

Cecutti MC, Mouloungui Z, Gaset A. 1998. Synthesis of new diesters of 1, 4:3, 6-dianhydro-d-glucitol by esterification with fatty acid chlorides. Bioresource Technology, Elsevier, p 63-67. Ffhal-02089357f

Dodou Lima HV, de Paula Cavalcante CS, Rádis-Baptista G. 2020. Antifungal In Vitro Activity of Pilosulin- and Ponericin-Like Peptides from the Giant Ant *Dinoponera quadriceps* and Synergistic Effects with Antimycotic Drugs. Antibiotics (Basel, Switzerland) **9(6),** 354. https://doi.org/10.3390/antibiotics9060354

Drees BM. 2014. Medical Problems and Treatment Considerations for the Red Imported Fire Ant. Texas A&M AgriLife Extension Service. https://fireant.tamu.edu/files/2014/03/ENTO_005.pdf26.

Duval A, Malécot CO, Pelhate M, Piek T. 1992. Poneratoxin, a new toxin from an ant venom, reveals an interconversion between two gating modes of the Na channels in frog skeletal muscle fibres. Pflügers Arch **420,** 239–247. https://doi.org/10.1007/BF00374453

Ferreira SB, Kaiser CR. 2012. Pyrazine derivatives: a patent review (2008 - present). Expert opinion on therapeutic patents **22(9),** 1033–1051. https://doi.org/10.1517/13543776.2012.714370 https://doi.org/10.1016/0020-1790(85)90027-7.

Jones TH, Garraffo HM, Blum MS., Everett DM, Hasting H, Ware AB. 1998. Elucidation of Dimethylalkylpyrazines from the Ant *Streblognathus aethiopicus* by GC-FTIR. Journal of Chemical Ecology **24,** 125–134. https://doi.org/10.1023/A:1022345114057

Junior V, Larsson C. 2015. Anaphylaxis caused by stings from the *Solenopsis invicta*, lava-pés ant or red imported fire ant. Anais Brasileiros de Dermatologia **90,** 22-5. http://dx.doi.org/10.1590/abd1806-4841.20153420.

Lima DB, Mello CP, Bandeira I, Pessoa Bezerra de Menezes R, Sampaio TL, Falcão CB, Morlighem J, Rádis-Baptista G, Martins A. 2018. The dinoponeratoxin peptides from the giant ant *Dinoponera quadriceps* display in vitro antitrypanosomal activity. Biological Chemistry **399(2),** 187–196. https://doi.org/10.1515/hsz-2017-0198

Lorber J. 1973. Isosorbide in the medical treatment of infantile hydrocephalus, Journal of Neuroscience **39(6),** 702-711. Retrieved Nov 3, 2021, from https://thejns.org/view/journals/j-neurosurg/39/6/article-p702.xml

Morgan D, Mandava H. 1988. CRC Handbook of Natural Pesticides: Pheromone, Part A, Volume IV. CRC Press, Inc., Florida, USA.

Mortzfeld F, Hashem C, Vrankova K, Winkler M, Rudroff F. 2020. Pyrazines – valuable flavour & fragrance compounds: Biocatalytic synthesis and industrial applications. Biotechnology Journal **15(11)**. https://doi.org/10.1002/biot.202000064

National Center for Biotechnology Information. 2021. PubChem Compound Summary for CID 5541, Triacetin. Retrieved July 21, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Triacetin.

Ong KT, Liu ZQ, Tay MG. 2017. Review on the Synthesis of Pyrazine and Its Derivatives. Borneo Journal of Resource Science and Technology **7(2),** 60-75. https://doi.org/10.33736/bjrst.591.2017

Orivel J, Redeker V, Le Caer J-P, Krier F, Revol-Junelles AM, Longeon A, Chaffotte A, Deiean A, Rossier J. 2001. Ponericins, New Antibacterial and Insecticidal Peptides from the Venom of the Ant *Pachycondyla goeldii*. Journal of Biological Chemistry **276(1),** 17823–17829.

Postman TL. 2009. Neurotoxic Animal Poisons and Venoms (Ed. Dobbs, M.R.) Clinical Neurotoxicology, W.B. Saunders, p.463-489, https://doi.org/10.1016/B978-032305260-3.50049-6.

Quinn MJ, Ziolkowski D. 2015. Wildlife Toxicity Assessment for Triacetin. In Williams, M.A., Reddy, G., Quinn, M.J., Johnson, M.S. (Eds.), Wildlife Toxicity Assessment for Chemicals of Military Concerns (pp.291-301). Elsevier, https://doi.org/10.1016/B978-0-12-8000205.00017-X

Shields V. 2018. Introductory Chapter: The Complex World of Ants. 0.5772/intechopen.80387.

Silva R, Fox E, Gomes FM, Feijó DF, Ramos I, Koeller CM, Costa T, Rodrigues NS, Lima AP, Atella GC, Miranda K, Schoijet AC, Alonso GD, de Alcântara Machado E, Heise N. 2020. Venom alkaloids against Chagas disease parasite: search for effective therapies. Scientific Reports **10(1),** 10642. https://doi.org/10.1038/s41598-020-67324-8

Sullivan DC, Flowers H, Rockhold R, Herath HM, Nanayakkara NP. 2009. Antibacterial activity of synthetic fire ant venom: the solenopsins and isosolenopsins. The American Journal of Medical Sciences **338(4),** 287–291. https://doi.org/10.1097/MAJ.0b013e3181af8270.

Szolajska E, Poznanski J, Ferber ML, Michalik J, Gout E, Fender P, Bailly I, Dublet B, Chroboczek J. 2001. Poneratoxin, a neurotoxin from ant venom Structure and expression in insect cells and construction of a bio-insecticide. European Journal Biochemistry **271,** 2127–2136. http://dx.doi.org/10.1111/j.1432-1033.2004.04128.x

Téné N, Bonnafé E, Berger F, Rifflet A, Guilhaudis L, Ségalas-Milazzo I, Pipy B, Coste A, Leprince J, Treilhou M. 2016. Biochemical and biophysical combined study of bicarinalin, an ant venom antimicrobial peptide. Peptides **79,** 103–113. https://doi.org/10.1016/j.peptides.2016.04.001

Touchard A, Aili SR, Fox EGP, Escoubas P, Orivel J, Nicholson GM, Dejean A. 2016. The Biochemical Toxin Arsenal from Ant Venoms. Toxins **8(1),** 30. https://doi.org/10.3390/toxins8010030

Xu S, Errabeli R, Feener DH, Noble K, Attygale AB. 2018. Alkyl-Dimethylpyrazines in Mandibular Gland Secretions of Four *Odontomachus* Ant Species (Formicidae: Ponerinae). Journal of Chemical Ecology **44,** 444–451. https://doi.org/10.1007/s10886-018-0948-y

Yi GB, McClendon D, Desaiah D, Goddard J. Lister A, Moffitt J, Vander Meer RK, deShazo R, Lee KS, Rockhold RW. 2003. Fire Ant Venom Alkaloid, Isosolenopsin A, a Potent and Selective Inhibitor of Neuronal Nitric Oxide Synthase. International Journal of Toxicology **22,** 81–86. http://dx.doi.org/10.1080/10915810390198375.

Zhao F, Wang P, Lucardi RD, Su Z, Li S. 2020. Natural Sources and Bioactivities of 2, 4-Di-Tert-Butylphenol and Its Analogs. Toxins **12(1),** 35. https://doi.org/10.3390/toxins12010035