wjpmr, 2024, 10(6), 01-04

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

# A NOVEL PYRAZINE ISOLATED FROM THE ROOTS OF *ASTEROPEIA DENSIFLORA* BAKER (ASTEROPEIACEAE) AND ANTIBACTERIAL ACTIVITIES OF THE ETHYL ACETATE EXTRACT

**Ralambomanana Dimby Andrianina[1]*, Razaka Harisoa Koloina[1], Rakotondramanana Andriamahavola Dina[2], Randrianasolo Rivoarison[2]**

[1]Laboratory of Chemistry of Natural Products and Biotechnology, Faculty of Sciences, University of Antananarivo.
[2]Analytical Chemistry and Formulation Laboratory, Faculty of Sciences, University of Antananarivo.

***Corresponding Author: Ralambomanana Dimby Andrianina**

Laboratory of Chemistry of Natural Products and Biotechnology, Faculty of Sciences, University of Antananarivo.

**Article Received on 24/03/2024** **Article Revised on 14/04/2024** **Article Accepted on 04/05/2024**

## ABSTRACT

A new alkaloid, 2-butoxy-3-chloropyrazine (P1) together with Supinine (P2) and Indicine (P3) were isolated from the ethyl acetate extract of the roots of *Asteropeia densiflora* BAKER (ASTEROPEIACEAE) an endemic plant of Madagascar. This plant is applied by villagers as timber and by woodworkers as a fortifier. The use of chromatographic technique allowed the isolation of the three alkaloids. The structures of these secondary metabolites were established using detailed spectroscopic analysis and by comparison with published data. These antibacterial activities of the ethyl acetate extract was evaluated by Agar diffusion tests. Antibacterial tests have shown that the extract inhibit the strains of *Bacillus subtilis*, *Staphylococcus*, *Streptomyces viridochromogenes*, and *Escherichia coli*. The chemical and biological results indicate that *Asteropeia densiflora* contains bioactive molecules like other studied species of this genus.

**KEYWORDS:** *Asteropeia densiflora*, *2, 3-disubstituted pyrazine*, antimicrobial.

## 1. INTRODUCTION

Madagascar has one of the richest ecosystems in the world with more than 12,000 plant species listed. The degree of endemicity has been estimated between 80 and 90%.[1] Researchers found 120 species of plants at Ibity Antsirabe, represented by 41 families and 83 genera. Seven species belong to endemic families including six SARCOLAENACEAE and one ASTEROPEIACEAE.[2] ASTEROPEIACEAE family are classified as one of the new ectomycorrhizal endemic plants[3] that contains only one genus *Asteropeia*.[4,5] The *Asteropeia* genus has been the victim of an overexploitation due to a strong demand of its precious woods in the local and international market.[6] *Asteropeia densiflora* is used by villagers as timber and by woodworkers as a fortifier.[7] Ethnobotanical surveys have revealed that this species is used to poison rats and to intoxicate freshwater fish. The widespread use of *Asteropeia densiflora* justified the aims of this work to isolate and identify compounds. The present paper deals with the isolation and structural elucidation of one new alkaloid and the antibacterial activities of the ethyl acetate extract. To the best of our knowledge, no previous phytochemical study has been reported on *Asteropeia densiflora*.

## 2. MATERIALS AND METHODS

### 2.1. General

TLC were performed on aluminium silica gel 60 $F_{254}$ (Merck) plates (0.2 mm layer thickness). Spots were visualized using UV lamp (254 and 366 nm) and spraying with Dragendorff's reagent. Column chromatography was performed on silica gel 60 (6.3-20µm) (Merck, Darmstadt, Germany). NMR spectra were recorded with a Brüker AV-400 with a cryoprobe for $^{1}H$, $^{13}C$, HMBC. Chemical shift values are in δ (ppm) using the peak signals of the solvent DMSO (δ-H=2.50 ppm ; 3.47 ppm and δ-C=39.79 ppm) as reference, and coupling constants are reported in Hz. EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosene as a reference substance for HREIMS.

### 2.2. Plant material

The roots of *Asteropeia densiflora* were collected in September 2021 in Ibity, Region of Vakinankaratra, Madagascar. An herbarium of the plantes was deposited at the Laboratory of Natural Product and Biotechnology for a reference.

### 2.3. Extraction and Isolation

300 g of dried powder of the roots of *Asteropeia densiflora* were macerated in 3L of 90% ethanol under the pale agitator, at room temperature for three days. The filtrate was recovered by Buchner filtration and evaporated using the rotary evaporator. The hydroalcoholic extract collected was weighed and preserved. A second maceration was carried out with the residue of the previous extraction in 2L of 90% ethanol for 72 h.

A phytochemical screening was performed to determine the main chemical families in the plant.[8,9,10]

The liquid-liquid extraction was carried out using increasing polarity solvent: hexane, dichloromethane and ethyl acetate.

3.5g of ethyl acetate extract (EtOAc) was applied to a silica gel column with Hexan/EtOAc/MeOH as an eluent of increasing polarity afforded 39 fractions. Fractions were monitored by TLC and similar fractions were combined. Fractions (12 – 18) (235 mg) were chromatographed on silica gel and eluted with a mixture of $CH_2Cl_2$/MeOH in increasing polarity, yielding, compounds P1 (16mg), P2 (12mg) and P3 (13mg).

### 2.4. Antibacterial assay[11]

Agar diffusion tests were performed in the usual manner with *Bacillus subtilis*, *Escherichia coli* (on peptone agar), *Staphylococcus aureus* (Bacto nutrient broth), *Streptomyces viridochromogenes* (M test agar). The ethyl acetate extract were dissolved in an azeotrope chloroform : methanol (87 : 13) and 40 mg aseptic paper disks (8 mm in diameter) were impregnated with 25 µL of substance using a 100 µL syringe, dried for 1 h under sterile conditions and placed on the pre-made agar test plates. Plates with bacteria were kept in an incubator at 37 °C for 12 h. The diameter of inhibition zones was measured.

## 3. RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of alkaloids, flavonoids, desoxy-2-sugars, tannins, polyphenols, terpenoids, polysaccharides and saponins.

Compound P1 was obtained as a white powder. Its HREIMS showed the quasi-molecular ion peak at m/z: 187,6312 [M＋H]+ consistent with the molecular formula $C_8H_{11}N_2OCl$. The $^1H$ (ppm) NMR spectrum shift values for P1 are 7.67 (d, H-5), 7.72 (d, H-6), 4.22 (t, H-1'), 1.66 (q, H-2'), 1.37 (m, H-3'), 0.91 (t, H-4'), indicate the presence of two aromatic protons and aliphatic protons. The proton at 4.22 (t, H-1') is attributed to a $CH_20$- group. The allure of the aliphatic protons allow to propose the presence a group -$0CH_2$-$CH_2$-$CH_2$-$CH_3$ as a substituent of the aromatic squelet.

The $^{13}C$ broadband decoupling spectrum reports to carbon number and the $^{13}C$ DEPT 135 spectrum informs about the nature of carbon.[12] The chemical shift values of the $^{13}C$ NMR spectrum of P1 and the corresponding interpretations allow to attribute four aromatic carbons respectively at 167.45 ppm, 132.13 ppm, 132.00 ppm, and 129.11ppm. The signal at 167.45 ppm was attributed as an aromatic carbon linked with a heteroatom. Two more signal were attributed as aliphatic carbon respectively at 30.45 ppm and 19.11 ppm. Two others were aliphatic carbon linked to a heteroatom at 70.21 ppm and 65.11 ppm.

Based on this information collected from the $^1H$ and $^{13}C$ monodimensional spectra, P1 is suggested to be the molecule presented in Figure 1.

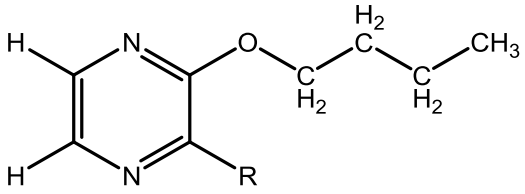
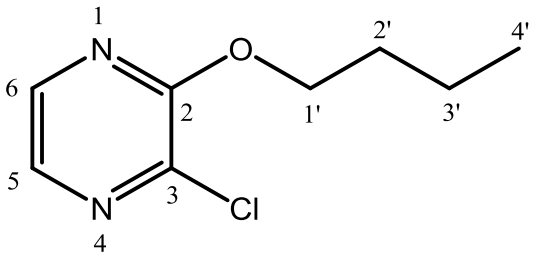



**Fig. 1: Structure hypothesis of P1.**

The positions of each substituent and the assignment of chemical shift values of protons and carbons were possible by exploiting the correlation $^1H$-$^{13}C$ in HMBC spectrum. The 0-$CH_2CH_2CH_2CH_3$ group is connected at the Carbon number 2 according to HMBC Correlation.

Thus, the combination of information obtained from NMR spectra and the determination of molecular weight by high-resolution mass spectrometry allowed us to identify the structure of the P1 product (Figure 2).

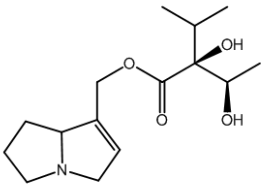



P1: 2-Butoxy-3-chloropyrazine

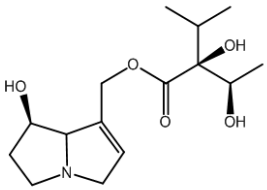
**Fig. 2: Structure of the compound P1.**

**P1:** HREIMS m/z 187.6312 $[M+H]^+$, $^1H$ NMR (DMSO) $\delta$ (ppm) 7.67 (d), 7.72 (d), 4.22 (t), 1.66 (q), 1.37 (m), 0.91 (t), $^{13}C$ NMR (DMSO) $\delta$ (ppm) 167.45, 132.13, 132.00, 129.11, 70.21, 65.11, 30.45, 19.11.

Comparison of chemical shift values with literature data identified P2 and P3 as pyrrolizidine alkaloids: Supinine[13] and Indicine[14] (Figure 3).




P2 : Supinine
Formula : $C_{15}H_{25}NO_4$




P3 :Indicine
Formula: $C_{15}H_{26}NO_5$

**Fig. 3: P2 and P3 structure.**

Ethyl acetate extract showed activities against *Bacillus subtilis* (15 mm inhibition diameter), *Staphylococcus aureus* (14 mm), *Streptomyces viridochromogenes* (Tü 57) (14mm), and *Escherichia coli* (15 mm).

## 4. CONCLUSION

The Results of this study contribute to validate the traditional use of *Asteropeia densiflora* The Aklaloids P2 and P3 isolated during this study are known by their biological activity. In perspective, this plant has an particular interest for the research of anti-cancer drug.

## REFERENCES


1. Ministère de l'Environnement et des Forêts. Cinquième rapport national de la Convention sur la Diversité Biologique - MADAGASCAR. Convention sur la Diversité Biologique. Published March 31, 2014. Accessed April 10, 2024. https://mg.chm-cbd.net/implementation/Documents_nationaux/Rapports_nationaux/mg-nr-05-fr.pdf
2. Alvarado ST, Buisson E, Carrière SM, et al. Achieving Sustainable Conservation in Madagascar: The Case of the Newly Established Ibity Mountain Protected Area. *Tropical Conservation Science*., 2015; 8(2): 367-395. doi:10.1177/194008291500800207
3. Bâ A, Duponnois R, Diabaté M, Dreyfus B. *Les champignons ectomycorhiziens des arbres forestiers en Afrique de l'Ouest: Méthodes d'étude, diversité, écologie, utilisation en foresterie et comestibilité*. IRD Éditions; 2011. doi:10.4000/books.irdeditions.10404
4. Schatz GE, Lowry II PP, Wolf AE. Endemie families of Madagascar. IV. A synoptic revision of Asteropeia (Asteropeiaceae). *Adansonia, sér 3*, 1999; 21(2): 255-268.
5. Ducousso M, Ramanankierana H, Duponnois R, et al. Mycorrhizal status of native trees and shrubs from eastern Madagascar littoral forests with special emphasis on one new ectomycorrhizal endemic family, the Asteropeiaceae. *New Phytologist*, 2008; 178(2): 233-238. doi:10.1111/j.1469-8137.2008.02389.x
6. Lemmens RHMJ, Louppe D, Oteng-Amoako AA. Ressources végétales de l'Afrique tropicale. Prota bois d'oeuvre 2. Published online 2012. Accessed April 11, 2024; 7(2). https://agris.fao.org/search/ru/records/6473b07213d110e4e7a986af
7. Ministère de l'Environnement, des Eaux et Forêts. Kit pédagogique : Région Vakinankaratra. Madagascar. Published online, 2006; 34-37.
8. Guignard JL. *Abrégé de phytochimie*. Masson, 1985.
9. Fong HHS, Tin-Wa M, Farnsworth NR. *Practical Manual for Phytochemical Screening*. College of Pharmacy, University of Illinois, 1974.
10. Harborne JB. *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis*. 2nd Ed. London New York Chapman And Hall, 1984.
11. Maskey RP, Asolkar RN, Kapaun E, Wagner-Döbler I, Laatsch H. Phytotoxic Arylethylamides from Limnic Bacteria Using a Screening with Microalgae. *J Antibiot*. 2002; 55(7): 643-649. doi:10.7164/antibiotics.55.643
12. Chachaty C. Spectrométrie par résonance

magnétique nucléaire, Analyses chimiques et caractérisations. *Techniques de l'Ingénieur, P4, Paris*., 1985; 2880: 1-22.
13. Liu K, Roeder E, Chen THL, Xiu XJ. Pyrrolizidine alkaloids from eupatorium fortunei. *Phytochemistry*., 1992; 31(7): 2573-2574. doi:10.1016/0031-9422(92)83336-W
14. Kazuhito O, Miyagi Y, Higa M, Yogi S. Pyrrolizidine alkaloids from *Messerschmidia argentea*. *Phytochemistry*., 1997; 44(3): 545-547. doi:10.1016/S0031-9422(96)00493-1