International Journal of Innovation Engineering and Science Research
(ISSN: 2581-4591)
www.ijiesr.com

Chemical characterization of bioactive compounds from *Distephanus garnierianus*, an endemic Malagasy antidiarrheal plant

Haingo Lovatiana RASOANARIVO[1], David RAMANITRAHASIMBOLA[2], Maonja Finaritra RAKOTONDRAMANGA[3], Riana ANDRIANJATO[3], Mahefarivo ANDRIANJAKANIAINA[4], Harald GROSS[5], Herisetra Lalaina TSIRINIRINDRAVO[1]

[1]Laboratoire de Biotechnologie Microbiologie, Département de Biochimie Fondamentale et Appliquée, Faculté des Sciences, Université d'Antananarivo, Madagascar
[2]Laboratoire de l'Institut Malgache de Recherche Appliquée (IMRA), Avarabohitra, Itaosy, BP 3833, Antananarivo 102, Madagascar
[3]Laboratoire de Chimie Organique Substances Marines, Mention Chimie, Université d'Antananarivo, BP 906, Madagascar
[4]Laboratoire du Centre National de Recherche Pharmaceutique (CNARP), Analamahitsy, BP 702, Antananarivo 101, Madagascar
[5]Pharmaceutical Institute, Department of Pharmaceutical Biology, University of Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany

ABSTRACT

Distephanus garnierianus is an endemic species of Madagascar belonging to the Asteraceae family. Traditionally valued in ethnopharmacology, it is primarily used for its potent antidiarrheal properties. The main objective of this study is to isolate and identify the chemical nature of its active constituents. This research aims to scientifically validate its traditional medicinal use while evaluating its broader pharmacological potential. Ultimately, characterizing these secondary metabolites could pave the way for the discovery of novel therapeutic agents. The crude extract was obtained by maceration of powdered plant material in methanol at room temperature, with an extraction yield of 13%. Phytochemical screening revealed the presence of flavonoids, steroids including sterols, terpenoids, unsaturated sterols, and saponins. The isolation and purification of the active principles were bio-guided by microbiological assays (antibiograms against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (CA-SFM). Separation of compounds by silica gel column chromatography yielded six isolated constituents, four of which were pure. Structural elucidation by mass spectrometry and nuclear magnetic resonance (1D and 2D NMR) allowed the structural identification of these four pure compounds: two baicalein-derived flavonoids $C_{16}H_{14}O_5$ and $C_{17}H_{14}O_5$ and two triterpenes $C_{30}H_{48}O_3$ and $C_{30}H_{50}O$. These compounds were isolated for the first time from this species.

KEYWORDS: *Distephanus garnierianus*, Asteraceae, diarrhea, terpenes, flavonoids, Madagascar.

I. INTRODUCTION

Distephanus garnierianus is an endemic plant species of Madagascar belonging to the Asteraceae family. This family has been extensively studied worldwide, both from a botanical perspective (Bremer, 1994) and from biological and chemical viewpoints (Harborne and Mabry, 1982; Vestri Alvarenga and *al.,* 1995). It represents one of the largest and most important plant families. Several of its species possess medicinal properties and are used in the treatment of various ailments. Members of the Asteraceae family are rich in terpenoids, steroids, flavonoids, glycosides, simple phenols, and hydrocarbons (Tsirinirindravo and *al, 2009)*. Due to their structural diversity in triterpenes and flavonoids, and because they have been isolated in large quantities from species of this family, flavonoids can serve as taxonomic markers at lower hierarchical levels (Crawford, 1978). Consequently, our attention focused on *Distephanus garnierianus*, an endemic species of Madagascar traditionally used in ethnopharmacology for its antidiarrheal properties, according to ethnobotanical surveys conducted among traditional healers. In this context, we aimed to investigate its secondary metabolites in order to contribute to the enrichment of scientific data regarding antidiarrheal plants in the Malagasy traditional pharmacopeia.

II. MATERIALS AND METHODS

The study material consisted of *Distephanus garnierianus* (formerly named *Vernonia garnieriana*), locally known by the Malagasy vernacular name "rambiazina". It is an endemic shrub of Madagascar belonging to the Asteraceae family and the genus *Distephanus*, which comprises 35 species: 2 in Southeastern Africa, 1 in Mauritius, and 32 endemic to Madagascar (Schatz, 2001). Plant material was collected during the vegetative growth period in the Central Highlands of Madagascar, within the Alaotra-Mangoro region, Moramanga district, Ambohibary commune, inside the Ambatomainty forest (Alt: 999 m; Lat: 18°52’29.8’’S; Long : 048°17’37.7’’E). In accordance with indications provided by traditional healers during the ethnobotanical survey, leaves were selected for this study regarding the traditional treatment of diarrhea.

II.1. Crude extract preparation

The crude extract was obtained by macerating 500 g of powdered plant material in methanol (4×1000 ml), at room temperature, with the solvent renewed every 24 hours. This was followed by filtration and vacuum evaporation at 40°C to prevent thermal degradation of the metabolites.

II.2. Phytochemical screening

Phytochemical screening was conducted to identify the major chemical families present in the crude extract.
The presence of these classes was revealed by color changes or precipitation resulting from reactions between bioactive compounds in the extract and specific reagents.

II.3. Polarity-based solvent partitioning

The crude extract was re-solubilized in distilled water and subjected to sequential liquid–liquid extraction using solvents of increasing polarity. Solvents were selected based on their capacity to preferentially extract specific compounds according to their polarity: hexane (non-polar), dichloromethane (DCM, intermediate polarity), and ethyl acetate (EtOAc, higher polarity). The flow diagram in Figure 1 illustrates the extraction process.

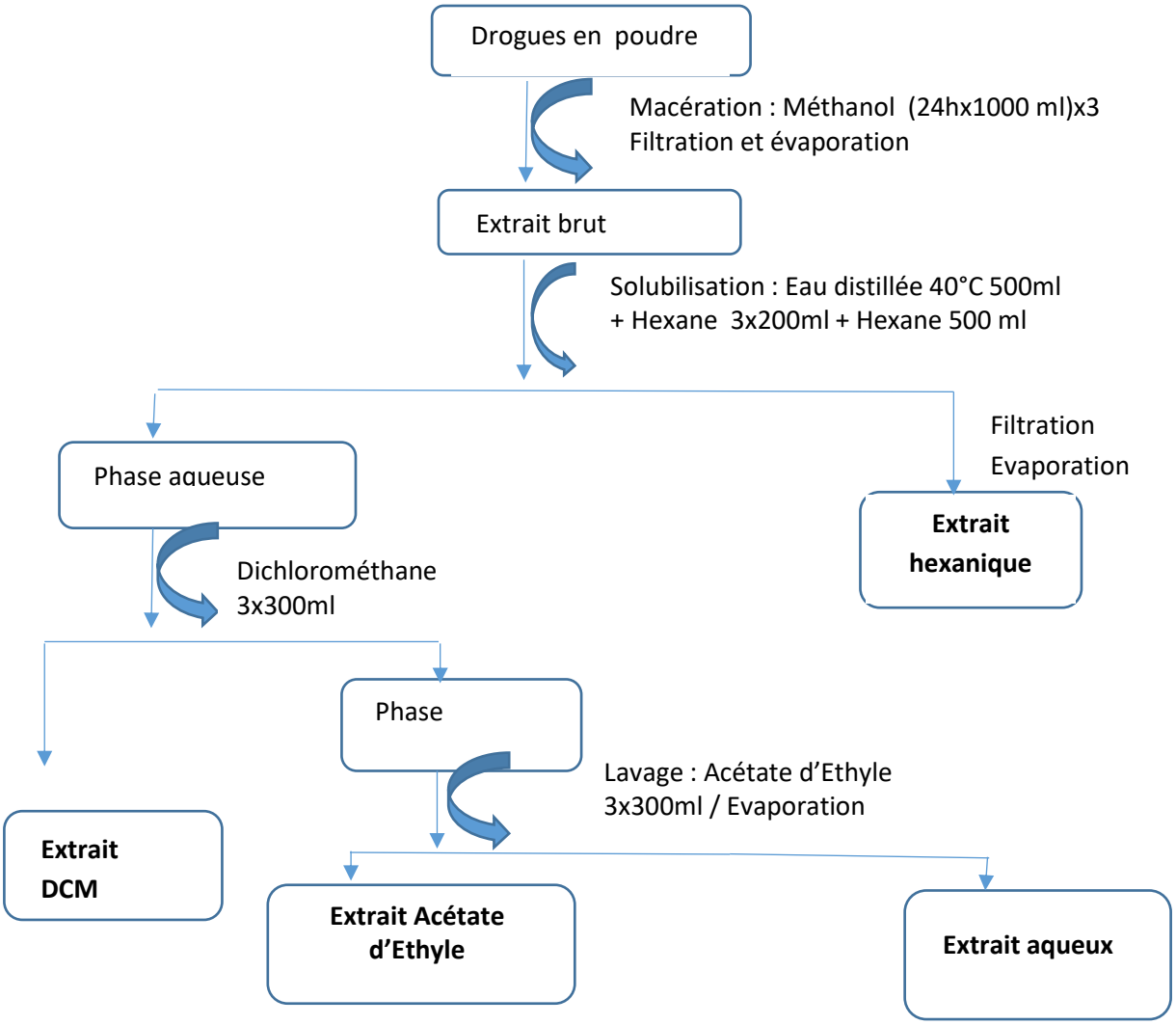


Figure 1: *Flowchart of liquid–liquid extraction partitioning.*

II.4. Isolation and Purification of Compounds

The isolation and purification of active principles were bio-guided by microbiological assays (antibiograms against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM). Low-pressure silica gel column chromatography was used to separate organic molecules. The general principle relies on adsorptive separation based on compound polarity (Fried and Sherma, 1999; Sarker et al., 2006; Cseke et al., 2006).

II.4.1. Column Preparation

- **a) Mobile Phase Optimization:** Thin-Layer Chromatography (TLC) was used to achieve optimal separation. Various solvent mixtures were evaluated: DCM/Acétate d'éthyle (1/9 ; V/V ; DCM/Acétate d'éthyle (0.5/9,5 ; V/V) ; DCM/Acétate d'éthyle (9.5/0.5 ; V/V), DCM/méthanol (95/5 ; V/V) ; Acétate 100%. Aluminum plates pre-coated with silica gel 10-40 µm, fluorescent at 254nm were used as the stationary phase.

Extracts were dissolved in methanol (10mg/mL) and spotted 1cm from the bottom edge (spot diameter 5mm). Visualization was performed using vanillin-sulfuric acid reagent and Neu's reagent (2-aminoethyl diphenylborinate / polyethylene glycol) specific for flavonoids. Plates were inspected under UV light at 254nm and 365nm before and after spraying.

- **b) Column Packing:** A clean, dry glass column was packed with silica gel using a silica-to-sample ratio of 30:1 to 40:1 (w/w). Silica powder was slurried in DCM (initial eluant) to form a gel, which was loaded into the column while avoiding air bubbles. The initial eluant possessed a lower polarity than the optimal mobile phase determined via TLC; the optimized mixture was introduced starting from the second loading step.
- **c) Sample Application:** A 5 g portion of extract was moistened with a minimal volume of DCM and mixed with 15 g of silica gel powder. The mixture was homogenized using a mortar and pestle to obtain a free-flowing dry powder, which was dry-loaded onto the column bed.
- **d) Compound characterization:** Fractions were collected and combined based on matching TLC profiles. Melting points were measured using a Büchi 510 melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 341 spectrometer. RMN^{1}H, RMN^{13}C and RMN 2D spectra were recorded on a Bruker AM 400 spectrometer at 400MHz and 100.5MHz, using tetramethylsilane (TMS) as an internal standard. Electron Impact Mass Spectrometry (EI-MS) data were obtained using Finnigan MAT 312 and Autospec (VG) mass spectrometers. Analytical TLC was carried out on silica gel 60 F254 pre-coated plates (0.2mm, Merck). Column chromatography (CC) was performed using Merck silica gel 60 (0.063-0.040 mm or 0.04-0.02mm).

III. RESULTS

III.1. Phytochemical Screening

Preliminary phytochemical testing revealed several major chemical classes, as summarized in Table 1.

Table 1: *Phytochemical screening of Distephanus garnierianus.*

Natural Product Class	Test / Reagent	Expected Observation	Interpretation	Result
Alkaloids	Mayer, Wagner & Dragendorff reagents	Precipitate	Presence of alkaloids	-
Flavonoids & Leucoanthocyanins	Wilstater Test HCl + tournures de Mg	Red coloration	Presence of flavones	++
		Purple-red coloration	Presence of flavonols	-
		Violet-red coloration	Presence of flavanones & flavanols	-
	Modified Wilstater test HCl + tournures de Mg+H2O+ alcool isoamylique	Red coloration	Presence of flavones	++
		Purple-red coloration	Presence of flavonols	-
	Bate-Smith test	Violet-red coloration	Presence of leucoanthocyanins	-
		Red coloration	Presence of anthocyanins	-
Tannins &	Gelatin 1%	Precipitate	Presence of polyphenols	-

Polyphenols	Salted gelatin	Precipitate	Presence of tannins	+++
	$FeCl_3$ in MeOH	Blue-green coloration	Presence of condensed tannins (catechic)	+++
		Black-bluish coloration	Presence of hydrolyzable tannins	-
Quinones	NH_4OH	Violet-red coloration	Presence of quinones	-
Steroids & Terpenoids	Liebermann–Burchard test	Purple coloration	Presence of triterpenoids	-
		Violet / Blue-green	Presence of steroids	++
	Salkowski test	Red ring interface	Presence of unsaturated sterols	+++
	Kedde Baljet / Picric acid test	Red coloration	Presence of lactone steroids	-
	Keller–Kiliani test	Purple-red ring interface	Presence of 2-deoxy sugars	-
	HCl	Blue coloration	Presence of iridoids	-
Saponins	Indice de mousse	Persistent after 30 min	Presence of saponins	+
Polysaccharides	Water / Ethanol precipitation	Precipitate	Presence of polysaccharides	-

Key: Absent; (+) Low presence; (++)$ Moderate presence; $(+++)$ High abundance.

Four main classes of organic secondary metabolites were detected:

1. Flavonoids (specifically flavones) in moderate amounts;
2. Tannins and polyphenols (specifically condensed/catechic tannins) in high abundance;
3. Steroids and terpenoids in moderate amounts, with unsaturated sterols in high abundance;
4. Saponins in minor amounts.

III.2. Liquid–Liquid Partitioning Yields

Fractionation using solvents of increasing polarity yielded the mass distributions shown in Table 2.

Table 2: *Extraction yields by solvent fraction.*

Extract Fraction	Crude Extract	Hexane	DCM	EtOAc	Intermediate	Aqueous
Yield (%)	13.00	26.44	12.90	11.65	6.83	31.23

Note: The presence of an intermediate phase indicates compounds insoluble in the target solvent pairs. The majority of the crude extract constituents (31.23%) remained in the aqueous phase.

III.3. Isolation and Structural Elucidation

Bio-guided assays showed significant antibacterial activity in the DCM fraction against the tested bacterial strains (*E. coli*, *P. aeruginosa*, *S. aureus*). Thus, purification focused on this fraction.

Silica gel column chromatography of the DCM fraction yielded 336 sub-fractions. TLC profiling allowed grouping into six major fractions: **H67**, **H129**, **H137A**, **H138A**, **H151**, and **H172**.

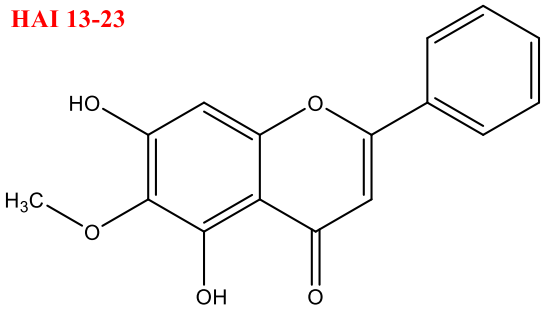
Preparative HPLC purification conducted at the Department of Pharmaceutical Biology, University of Tübingen (Germany), yielded four pure compounds. Their structures were elucidated via UV, IR, NMR, and Mass Spectrometry.

a) Compound 1 ("HAI 13-23")

Flavone derivative with the molecular formula $C_{16}H_{12}O_5$

- **ESI-MS (+)**, m/z : 282 $[M - 2H]^+$, 591 $[2M + Na]^+$;
- **Spectre RMN ^{1}H** (500 MHz, $CDCl_3$, 298 K, δ en ppm, J en Hz) : 4.01 (3H, s, 6-OCH_3), 6.56 (1H, s, H-8), 6.68 (1H, s, H-3), 7,55 (1H, m, H-4′), 7.90 (1H, dd, J=2, 8.1, H-2′, H-6′), 7.54 (1H, dd, J=2, 8.1, H-3′, H-5′), 13,09 (1H, br s, 5-OH).
- **Spectre RMN ^{13}C** (125 MHz, $CDCl_3$, 298 K, δ en ppm) : 164.1 (C-2), 105,0 (C-3), 183,0 (C-4), 152.9 (C-5), 130.69 (C-6), 155.4 (C-7), 93.44 (C-8), 153.1 (C-9), 105.9 (C-10), 131.3 (C-1′), 126.3 (C-2′ et C-6′), 148.7 (C-3′ et C-5′), 132.5 (C-4′).

The molecular structure is shown in Figure 2.



5,7-dihydroxy-6-methoxy-2-phenyl-4*H*-chromen-4-one
Chemical Formula: $C_{16}H_{12}O_5$
Exact Mass: 284,07

Figure 2 : Molecular structure of the Compound 1 ("HAI 13-23")

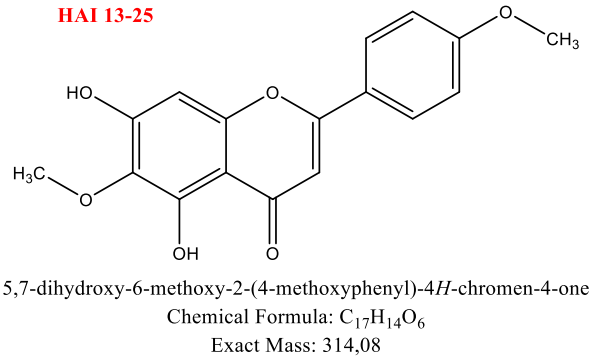
b) Compound 2 ("HAI 13-25")

Flavone derivative with the molecular formula $C_{17}H_{14}O_6$:

- **ESI-MS (+)**, m/z : 312 $[M - 2H]^+$, 651 $[2M + Na]^+$;
- **Spectre RMN ^{1}H** (500 MHz, $CDCl_3$, 298 K, δ en ppm, J en Hz) : 4.07 (3H, s, 6-OCH_3), 6.59 (1H, s, H-8), 7.54 (1H, s, H-3), 8.08 (1H, dd, J=2, 8.1, H-2′, H-6′), 7.54 (1H, dd, J=2, 8.1, H-3′, H-5′), 12,88 (1H, br s, 5-OH), 3.88 (1H, m, 4′-OCH_3).
- **Spectre RMN ^{13}C** (125 MHz, $CDCl_3$, 298 K, δ en ppm) : 156.09 (C-2), 131.00 (C-3), 179.01 (C-4), 151.79 (C-5), 130.04 (C-6), 155.4 (C-7), 93.17 (C-

8), 152.1 (C-9), 106.39 (C-10), 130.46 (C-1′), 128.34 (C-2′ et C-6′), 128.68 (C-3′ et C-5′), 139.24 (C-4′), 60.99, (6-OCH_3), 60.41 (4′-OCH_3).

The molecular structure is shown in Figure 3.



5,7-dihydroxy-6-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one
Chemical Formula: $C_{17}H_{14}O_6$
Exact Mass: 314,08

Figure 3 : Molecular structure of the Compound 2 ("HAI 13-25").

c) Compound 3 ("H1E33")

With the molecular formula $C_{30}H_{48}O_3$, 3-hydroxyolean-12-en-28-oic acid (oleic acid / oleanolic acid), whose characteristic MS values and RMN ^1H et ^{13}C are :

- **EI-MS**: m/z: 414 [M^+] ($C_{30}H_{48}O_3$),
- **^1H NMR** (500 MHz, $CDCl_3$) δ 5.0 (t, J = 3.3 Hz, 1H), 3.42 (dd, J = 10.6, 5.5 Hz, 1H), 3.29 (dd, J = 13.8, 4.0 Hz, 1H), 2.07-2.20 (m, 2H), 2.00-2.06 (m, 1H), 1.93-1.96 (m, 3H), 1.78-1.83 (m, 4H), 1.68 (t, J = 8.9 Hz, 1H), 0.85-1.58 (m, 11H), 1.27 (s, 3H), 1.22 (s, 3H), 1.02 (s, 3H), 1.01 (s, 3H), 1.00 (s, 3H), 0.94 (s, 3H), 0.89 (s, 3H);
- **^{13}C NMR (125 MHz, $CDCl_3$) :** 171.1 (C-28): 144.56 (C-13),123.98 (C-12), 80.9 (C-3), 55.39 (C-5), 50.36 (C-9,), 48.3 (C-17), 48.2 (C-19), 42.8 (C-14), 40.86 (C-18), 40.01 (C-8), 38.47 (C-1), 38.05 (C-4) , 37.83 (C-10), 37.10 (C-22), 35.5 (C-21), 34.22 (C-29), 31.9 (C-22), 31.4 (C-7), 29.8 (C-20), 29.7 (C-23), 27.9 (C-15), 27.4 (C-2), 26.1 (C-27), 23.7 (C-30), 21.3(C-16), 20.9 (C-11), 18.5 (C-6), 17.3 (C-26), 16.5 (C-26), 16.1 (C-24), 14.5 (C-25),

The molecular structure is shown in Figure 4.

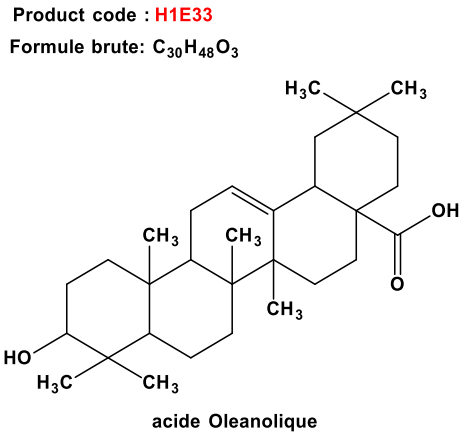


Figure 4 : Molecular structure of the Compound 3 ("H1E33")

d) Compound 4 ("H4399")

This compound corresponds to alpha-amyrin with the molecular formula $C_{30}H_{50}O$. According to the following data :

- **EI-MS**: m/z: 426 [M $^+$] ($C_{30}H_{50}O$),
- **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.0 (t, J = 3.13 Hz, 1H), 3.42 (dd, J = 10.6, 5.5 Hz, 1H), 3.19 (dd, J = 13.8, 4.0 Hz, 1H), 2.06-2.17 (m, 2H), 1.90-2.00 (m, 1H), 1.83-1.86 (m, 3H), 1.73-1.75 (m, 4H), 1.60 (t, J = 8.9 Hz, 1H), 0.85-1.58 (m, 11H), 1.25 (s, 3H), 1.18 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.80 (s, 3H), 0.73 (s, 3H);
- **^{13}C NMR (125 MHz, $CDCl_3$) :** 139.60 (C-13),124.43 (C-12), 79.07 (C-3), 59.08 (C-18), 55.19 (C-5), 47.7 (C-9), 42.09 (C-14), 41.54 (C-22), 40.03 (C-8), 39.68 (C-19), 39.62 (C-20), 38.08 (C-1,4) , 36.91 (C-10), 33.77 (C-17), 32.95 (C-7), 31.2 (C-21), 28.7 (C-15), 28.14 (C-23), 28.1 (C-2), 23.38 (C-16), 23.7 (C-30), 21.3(C-16), 20.9 (C-11), 18.5 (C-6), 17.3 (C-26), 16.5 (C-26), 15.7 (C-24), 15.6 (C-25)

The molecular structure is shown in Figure 5.

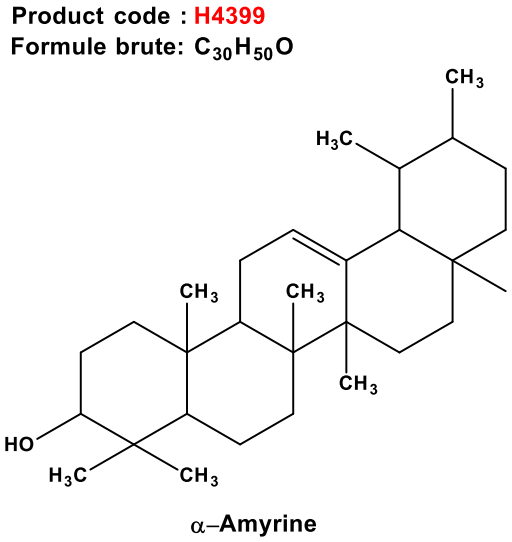


Figure 5 : Molecular structure of the Compound 4 ("H4399")

IV. DISCUSSION

The crude extract yield of 13% for *Distephanus garnierianus* is comparable to values reported for other species in the same genus, such as *Distephanus polygalifolius* (14.57%; Rafalimanana, 2020) and *Distephanus trinervis* (13.8%; Ramanantsoa, 2015).

Distephanus garnierianus contains secondary metabolites including phenols, tannins, and flavonoids, which are well-documented for their antidiarrheal efficacy (Biaye, 2002; Dougnon, 2012). Tannins act as astringents, precipitating mucosal proteins in the intestinal tract and reducing secretions. Flavonoids modulate membrane permeability in intestinal mucosal cells, thereby decreasing hydro-electrolytic hypersecretion (Maha and *al.,* 2013). Additionally, saponins contribute to antisecretory action by inhibiting active transport across the intestinal mucosa, enhancing absorption mechanisms (Georges and *al.,* 2002).

These findings support the traditional ethnopharmacological use of *D. garnierianus* as an antidiarrheal remedy. Its chemical profile aligns with previously studied *Distephanus* species, including *Distephanus glutinosus* (Rajosefa, 2014) and *Distephanus polygalifolius* (Rakotondramasy, 2015; Rafalimanana, 2020), which share major bioactive constituents such as flavonoids, steroids, unsaturated sterols, and tannins.

Furthermore, literature reports indicate that the co-occurrence of phenols, tannins, flavonoids, steroids, and terpenes imparts anti-inflammatory, antioxidant, and antiseptic activities to plant extracts (Biaye, 2002; Dougnon, 2012; Hennebelle, 2006). Saponins have also been associated with antiviral, anti-inflammatory, hepatoprotective, anti-ulcer, antibacterial, hypoglycemic, anti-fertility, and anti-cariogenic properties (Segal and Schlösser, 1975).

V. CONCLUSION

Distephanus garnierianus is an endemic plant of Madagascar belonging to the Asteraceae family, traditionally used as an antidiarrheal remedy. Phytochemical analysis confirmed the presence of secondary metabolites including terpenes, phenols, tannins, and flavonoids. Bio-guided isolation and purification of the dichloromethane (DCM) extract led to the structural identification—for the first time in this species—of four pure compounds: two baicalein-derived flavones (5,7-dihydroxy-6-methoxy-2-phenyl-4*H*-chromen-4-one and 5,7-dihydroxy-6-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one) and two pentacyclic triterpenoids (oleanolic acid and alpha-amyrin).

Elucidating the chemical structure of active metabolites from this endemic plant contributes to expanding the scientific knowledge base of the Malagasy traditional pharmacopeia. Given their biological potential, these secondary metabolites could serve as active precursors or lead compounds for phytotherapeutic formulations.

REFERENCES

[1.] **Biaye, M.** (2002), Actions Pharmacologiques des Tanins. Ph.D. Thesis, Faculté de Médecine, de Pharmacie et d'Odontostomatologique, Universite Cheikh Anta Diop (UCAD), Dakar, Sénégal ; p. 44.
[2.] **Bremer, K**. (1994) Asteraceae: Cladistics and Classification. Timber Press, Portland.
[3.] **Crawford, D. J.** (1978). Flavonoid chemistry and angiosperm evolution. *The Botanical Review*, *44*(4), 431-456.
[4.] **Cseke L.J., Kirakosyan, A., Kaufman, P.B**. (2006). *Natural Products from Plants* (2nd ed.). CRC Press, Boca Raton.
[5.] **Fried B., sherma J.** (1999). Thin-Layer Chromatography (4th ed.). Marcel Dekker, New York.
[6.] **Harborne H., Mabry.** (1982), Vestri Alvarenga, S. A., Rodrigues, G. D. V., Gastmans, J. P., & Emerenciano, V. D. P. (1995). Natural Product Substitution Pattern and Skeletal Recognition Method. *Natural Product Letters*, *7*(2), 133-140.
[7.] **Hennebelle T.** (2006) Investigation Chimique, Chimiotaxonomique et Pharmacologique de Lamiales Productrices d'antioxydants: *Marrubium Peregrinum, Ballota Larendana, Ballota Pseudodictamnus* (Lamiacées) et *Lippia alba* (Verbénacées). Ph.D. Thesis, Chimie Organique et Macromoléculaire, Université de Lille, Sciences et Technologies, Villeneuve-d'Ascq, France, p. 280.
[8.] **Kebieche M**. (2009). Activité Biochimique des Extraits Flavonoïdiques de la Plante *Ranunculus repens* L.: Effet sur le Diabète Expérimental et l'hépatotoxicité Induite par l'Epirubicine. Ph.D. Thesis, Faculté des Sciences de la Nature et de la Vie, University of Constantine, Cité Frére Arrafa, Algeria ; p. 123.
[9.] **Leboeuf M.; Cave A.; Forgacs P.; Tiberghien, R.; Provost, J.; Touche, A.E.; Jacquemin, H.** (1982) Alcaloides des Annonacees. XL. Etude chimique et pahrmacologique des alcaloides de l'Annona montana Macf. *Plantes Med. Phytother.* , *16*, 169–174.
[10.] **Maha Y. A., Babyker M. E., Dalia J. K., Damia E. M. B. (2013).** Activité antidiarrhéique de l'extrait éthanolique d' *Adansonia digitata* pâtes de fruits chez les rats. J. Pharm. Phy. Adv., 3 (6) : 172-178.

[11.] **Piotr Migas, Wojciech Cisowski, Wanda Dembińska-Migas, 2005**. Isoprene derivatives from the leaves and callus cultures of *Vaccinium corymbosum var. Bluecrop* in *Acta Poloniae Pharmaceutica - Drug Research, 62(1):45-51*

[12.] **Rafalimanana M. N**.(2020), Etude de la plante *Distephanus polygalifolius* (Less.) Recherche de lactones sesquiterpéniques et évaluation de l'activité antimicrobienne , ESPÄ, Université d'Antananarivo

[13.] **Rajosefa N. A.** (2014). Contribution à l'étude chimique et biologique d'une plante endémique de Madagascar : *Vernonia glutinosa* Université d'Antananrivo, ESPA

[14.] **Rakotondramasy H. R**., 2015, Contribution à l'étude d'une plante endémique antidiabétique : *Vernonia polygalifolium* Less (Compositeae), Mémoire de Master en chimie, Faculté des sciences, Université d'Antananarivo

[15.] **Ramanantsoa N. J.P**. (2015). 2-(3,4-dihydroxyphényl)-5,7-dihydroxy-6-methoxychromen-4-one, un flavonO-methylé isolé à partir de *Vernonia trinervis*, ESPÄ, Université d'Antananarivo.

[16.] **Sarker S.D., Latif, Z., Gray, A.I**. (2006). Natural products isolation. Methods in Biotechnology, Vol. 20, Humana Press, Totowa, NJ.

[17.] **Schatz** 2001.Flore générique des arbres de Madagascar, Royal Botanic gardens, Kew, 503p.

[18.] **Segal, R.; Schlösser, E**.(1975) Role of glycosidases in the membranlytic, antifungal action of saponins. *Arch. Microbiol.* , *104*, 147–150.

[19.] **Tsirinirindravo H.L**., **Andrianarisoa B.** : Activités antibactériennes de l'extrait de feuilles de *Dalechampia clematidifolia* (EUPHORBIACEAE). Int. J. Biol. Chem. Sci, 2009.

[20.] **Vestri Alvarenga, S. A., Rodrigues, G. D. V., Gastmans, J. P., Emerenciano**. (1995). Natural Product Substitution Pattern and Skeletal Recognition Method. *Natural Product Letters*, *7*(2), 133–140.