IRIDOIDS AND PHENYLETHANOID GLYCOSIDES FROM *PLANTAGO PALMATA* HOOK. F. S.

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Résumé: Quatre iridoïdes [aucubine (1), gardoside (2), acide 8-épi-loganique acid (3) et arborescoside (4)] et deux glycosides de phényléthanoïdes [plantamajoside (5) et actéoside (ou verbascoside, 6)] ont été isolés à partir de feuilles et/ou de racines de *Plantago palmata* par chromatographie liquide à moyenne pression. Les composés obtenus ont été identifiés par comparaison spectroscopique (RMN du proton) avec de standards authentiques et confirmés par chromatographie en phase gazeuse et sur couche mince.
Les propriétés pharmacologiques bien connues de ces composés, notamment l'aucubine et l'actéoside permettent de justifier en partie les usages traditionnels et les activités biologiques de la plante.

Mots clés : iridoïdes, phényléthanoïdes glycosides, Plantaginaceae

Summary: Four iridoids [aucubin (1), gardoside (2), 8-epi-loganic acid (3) and arborescoside (4)] and two phenylethanoid glycosides [plantamajoside (5) and acteoside (verbascoside, 6)] were purified from the leaves and/or roots of *Plantago palmata* by medium-pressure liquid chromatography. The obtained compounds were identified by comparison of ^{1}H-NMR spectra with authentic standards and confirmed by GC and TLC profiles. The well-known pharmacological properties of these compounds, notably aucubin and acteoside, could account for some of the major traditional uses and biological activities of the plant.

Keywords: iridoids, phenylethanoid glycosides, Plantaginaceae

I. INTRODUCTION

Plantago palmata Hook .f. (Plantaginaceae) grows in the humid mountain regions of intertropical Africa at 1800 to 3000 m altitude and is known under several vernacular names : cibarhama or cibarhama-nibinja in mashi (South-Kivu) and mbatama in kinande (North-Kivu)(Congo R. D.); kibatama in kirundi (Burundi); batabata, ikibatama, imbatama, imbatabata in kinyarwanda (Rwanda) [1-3].

The leaves are used in Burundi, Rwanda and in South Kivu (Congo) [3]: *(i)* crushed leaves treat abscesses, wounds, burnings, sting bites; *(ii)* leaves diluted with water enhance milk secretion and treat woman sterility, abortion menace, eye infections, hemorrhoids, dysentery, gonorrhea, ascaridiasis and hepatitis; *(iii)* decoctions are a remedy for ascites, hypertension, malaria and stomach ache; and *(iv)* infusions are used for the treatment of pregnancy troubles, colibacillosis and for the improvement of health after disease [3,4].

Screenings of *P. palmata* showed antibacterial, antifungal and antiviral activities [4] and previously isolated classes of constituents include alkaloids, anthraquinones, flavonoids, leucoanthocyanins, saponosides, steroids and tannins [5]; some polysaccharide

fractions have been shown to be immunomodulatory [6].

Iridoids and caffeoyl phenylethanoid glycosides are widely distributed in the genus *Plantago* and have been proposed as chemotaxonomic markers[7]. Given the pharmacological interest of these classes of secondary metabolites, the present work aims to identify the major compounds from the Congolese *P. palmata*.

II. MATERIAL AND METHODS

2.1 Plant

Plantago palmata Hook. f. (Plantaginaceae) seeds were harvested in the Democratic Republic of Congo and grown in a greenhouse (Experimental Garden Jean Massart, Brussels, Belgium); leaves and roots were collected after three months of culture and stored in acetone. The plant was identified by Professor J. Lejoly in the Laboratory of Systematical Botany and Phytosociology, Free University of Brussels (ULB), Belgium, where a voucher specimen has been deposited.

2.2 Extraction

Freshly collected leaves (205 g) and roots (187 g) of *P. palmata* were crushed and homogeneized twice with EtOH (2 L). The combined filtrates were concentrated under reduced pressure at 30°C and partitioned between Et_2O and H_2O (1 L). The aqueous extract was evaporated to dryness (reduced pressure, 30°C), dissolved in MeOH (100 mL), filtered through activated charcoal and dried to yield 2.81 g and 2.82 g of crude extract for leaves and roots, respectively.

The preparative medium pressure liquid chromatography (MPLC) was performed on a B-size Lobar® RP-18 column (Merck, Darmstadt, Germany), eluting with H_2O: MeOH gradients; in order to improve the retention of acidic glycosides, the crude extracts (leaves, 1.71 g; roots, 2.12 g) were dissolved in 10 % acetic acid (4 mL) [7].

2.3 Spectroscopy

The ^{1}H-NMR spectra, recorded at 300 MHZ in D_2O or in CD_3OD using the residual solvent peak as the reference, were compared with those of authentic standards previously isolated from other *Plantago* species (gardoside from *P. squarrosa*, aucubin from *P. serraria*, 8-epi-loganic acid from *P. spathulata*, plantamajoside from *P. major*, arborescoside from *P. arborescens*) and fully characterized [7].

2.4 Gas chromatography

The presence of aucubin in leaves and roots was confirmed by gas chromatography (GC) profiling according to [8]. Briefly, 100 mg of dried grounded leaves or roots were extracted overnight with 10 mL of MeOH and filtered. The filtrate was evaporated to dryness, partitioned between 10 mL H_2O and 10 mL Et_2O (vortex) and centrifuged (2000 g, 20 min). The aqueous layer was washed twice with 10 mL Et_2O, evaporated to dryness, dissolved in 1 mL MeOH and evaporated to dryness again, added with 0.1 mL of a silylation reagent (TRI-SIL Z, Pierce Chemical Company, USA) and heated at 70-80°C for 20 min. 1µL was injected onto a CP-SIL 5CB WCOT fused silica 25 m x 0.32 mm i.d. column (Varian Inc., USA); injector t°, 250 °C; detector t°, 300 °C; column t° gradient from 80 °C to 250 °C (8 °C/min). The retention times were compared with those of standards.

2.5 Thin-layer chromatography

The major phenylethanoid glycosides acteoside was confirmed by thin-layer chromatography (TLC). 50 mg of leaves or roots powder were extracted with 25 mL methanol (60 min) and centrifuged (2000 g; 15 min); 10 µL of the supernatant were applied on a silica gel 60 F_{254} plate (Merck, Darmstadt, Germany) with a TLC sampler III (Camag, Switzerland).

The mobile phase was acetic acid-formic acid-water-ethyl acetate (7.5 : 7.5 : 18 : 67, v/v) with revelation by spraying methanolic solutions of aminoethanol

diphenylborate (10 g/L) and then macrogol 400 (25 g/L) and observation under 365 nm. Densitometric measurements at 365 nm in fluorescence mode with a TLC scanner III densitometry (Camag, Switzerland) allowed to estimate the content in acteoside in leaves.

III. RESULTS

Tables I and II detail the iridoids and phenylethanoid glycosides that could be isolated from *P. palmata* leaves and roots.

We could not detect catalpol in *P. palmata* by any of the techniques used; this iridoid, structurally near to aucubin, is reported absent in a part of *Plantago* species investigated so far [9].

Figures 2-A and 2-B present GC chromatograms of the crude extracts of *P. palmata* leaves and roots, confirming the presence of aucubin in both organs. The content in aucubin in leaves and roots dry material was estimated at about 0.4 and 0.3 % w/w on dry material, respectively. The TLC profile of the leaves methanolic extract (Figure 3) confirms the presence of acteoside, estimated at ~ 1.5 % w/w on dry material.

Table I : Iridoids and phenylethanoid glycosides isolated from *Plantago palmata* leaves by MPLC (B-size Lobar® RP-18 column, eluted with the indicated H_2O - MeOH mixtures)

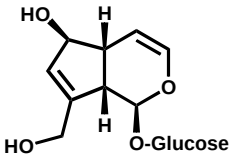
Leaves (1.71 g)			
Eluent (H_2O-MetOH)	Compound	Class	Recovered (mg)
25 : 1	*n.d.* [(a)]		10
15 : 1	Aucubin (1)	Iridoid	63
15 : 1	Gardoside (2)	Iridoid	≤ 1
7 : 1	*n.i.* [(b)]		14
7 : 1	8-epi-loganic acid (3)	Iridoid	26
5 : 1	*n.d.* [(a)]		8
3 : 1	Arborescoside (4)	Iridoid	1
2 : 1	Acteoside (6)	Phenylethanoid glycoside	2

[(a)] n.d. : structure as yet unassigned
[(b)] n.i. : non-iridoid (^{1}H-NMR spectroscopy)

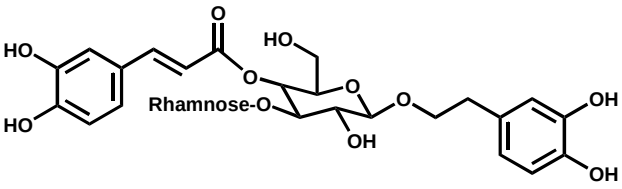
Table II : Iridoids and phenylethanoid glycosides isolated from *Plantago palmata* roots by MPLC (B-size Lobar® RP-18 column, eluted with the indicated H_2O - MeOH mixtures)

Roots (2.12 g)			
Eluent (H_2O-MetOH)	Compound	Class	Yield (mg)
25 : 1	Aucubin (1)	Iridoid	135
3 : 1	*n.d.* [(a)]		≤ 1
2 : 1	*n.d.* [(a)]		≤ 1
2 : 1	Plantamajoside (5)	Phenylethanoid glycoside	1 - 2

[(a)] n.d. : structure as yet unassigned



Aucubin (1)
(iridoid)



Acteoside (6)
(phenylethanoid glycoside)

Figure 1 : Structures of the major iridoid and phenylethanoid glycoside from *Plantago palmata*

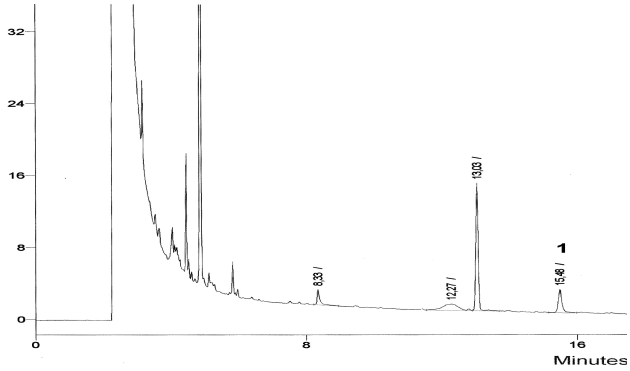


Figure 2-A
GC chromatogram of the *Plantago palmata* roots extract (column CP-SIL 5CB WCOT fused silica 25 m x 0.32 mm i.d.; injector t°, 250°C; detector t°, 300°C; column t° gradient from 80°C to 250°C (8°C/min)
Peak 1 = Aucubin

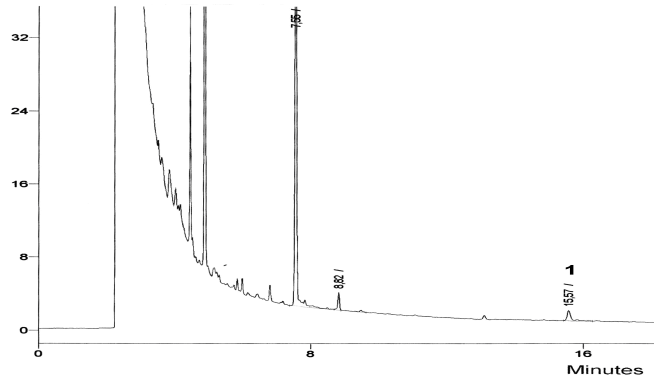


Figure 2-B
GC chromatogram of the *Plantago palmata* leaves extract (column CP-SIL 5CB WCOT fused silica 25 m x 0.32 mm i.d.; injector t°, 250°C; detector t°, 300°C; column t° gradient from 80°C to 250°C (8°C/min)
Peak 1 = Aucubin

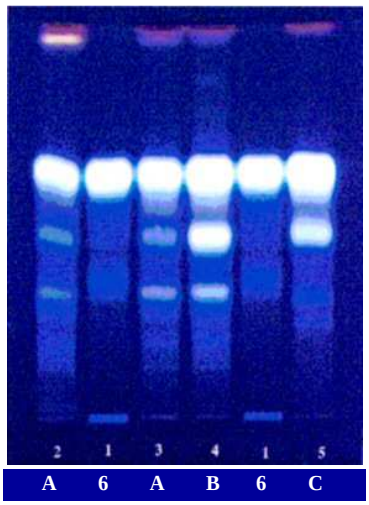


Figure 3
TLC chromatogram of acteoside from *Plantago* leaves [silica gel 60 F_{254} plate; mobile phase, acetic acid-formic acid-water-ethyl acetate (7.5 : 7.5 : 18 : 67, v/v); derivatization by aminoethanol diphenylborate and macrogol 400; observation under 365 nm**].**
Lanes : 6, acteoside; A, *Plantago lanceolata*; B, *Plantago minor*; C, *Plantago palmata*

IV. DISCUSSION

Ethnopharmacological data show clear convergences between the African therapeutical uses of *P. palmata* and those of *Plantago sp*. used in occidental traditional medicine, such as *P. lanceolata* or *P. major* [10-12]. This prompted us to study two groups of metabolites which could be related to the biological activity of *P. palmata* and justify his uses in traditional medicine.

Aucubin is the major iridoid from both leaves and roots of *Plantago palmata*, amounting to 0.2 - 0.4 % for both organs. In a previous study, aucubin could be extracted from the leaves of 34 species of *Plantago* in yields ranging from 0.006 to 0.2 % [7] and it is so far considered a compound typical for the entire genus *Plantago* [9]. It is reported at the levels of 0.3 to 2.5 % in *Plantago lanceolata* [10] and 0.2 to 3.1 % in *Plantago lanceolata*, *Plantago major* and *Plantago media* [13]. Aucubin is active as an antiviral, antiinflammatory, antibacterial, and antihepatotoxic agent [14]; it protects the plant against pathogenic organisms and its activity on insects has been investigated in details [15].

From the phenylethanoid glycosides, acteoside is usually present in *Plantago* species, sometimes accompanied by plantamajoside [9]. In *P. palmata*, acteoside is the major compound from the leaves, amounting to about 1.5 % and plantamajoside could be detected only in the roots; this is also the case for *P. lanceolata* [16]. Acteoside is reported as an antispasmodic, antihepatotoxic, antitumoral, antiviral, antihypertensive agent and plantamajoside has antiinflammatory, antioxidant, antitumoral, and antimicrobial activities [14].

The composition of the Congolese *P. palmata* investigated in this work is practically in line with the composition recently reported from a sample of the Copenhagen Botanical Garden [9], the only compound missing from our leaves extract being plantamajoside, however reported as "trace" only for the Danish sample.

V. CONCLUSION

The composition in secondary metabolites of *P. palmata* shows similarities with the compositions reported for other *Plantago* species. These data support the proposed use of iridoids and phenylethanoid glycosides for the chemotaxonomy of the Plantaginaceae and the *Plantago* genus[9,7-20].

The major leaves and roots iridoids (aucubin) and phenylethanoids glycosides (acteoside) justify some of the principal uses of *P. palmata* in African traditional medicine to treat inflammatory, septic and hepatotoxic disorders.

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