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Research Article

# Enzyme Urease Activity and Phytochemical Investigation of the Leaves of Baissea Mortehanii (Apocynaceae)

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## Abstract

The phytochemical investigation of a previously unstudied species of the genus Apocynaceae, *Baissea mortehanii* de Wild was undertaken and eight known secondary metabolites were isolated from leaves of this plant including one alkaloid, N-Feruloyltryptamine (1); one aromatic ester, Dibutyl phthalate (2); two flavonoids, Genistein (3) and Gerontoisoflavone A (4), four sterols, *β*-Sitosterol (5), Sitosterol-3-O-*β*-D-glucopyranoside (6), Stigmasterol (7) and Stigmasterol-3-O-*β*-D glucopyranoside (8). The structures of compounds were determined by means of spectroscopic methods :NMR analysis ($^{1}$H and$^{13}$C NMR, 1H-1H-COSY, HSQC, HMBC), spectrometric methods such as UV, IR, ESI-MS, EI, and by comparing their data with those reported in the literature. All the isolated compounds were tested for their potential to inhibit the enzyme urease. Urease activity was determined by measuring ammonia production using the indophenol method and thiourea was used as standard inhibitor of urease. Compounds 5 and 7 showed the best urease inhibition with an $IC_{50}$ value 17. 2 and 18.5 µM respectively, which is higher than that of the potent inhibitor, thiourea ($IC_{50}$ = 21.5 µM); Compounds 3, 4, 6 and 8 showed a good urease inhibition with an $IC_{50}$ value 26.9, 29.7, 32.8 and 34.3 µM respectively; Compounds 1 and 2 showed a moderate urease inhibition with an $IC_{50}$ value 49.1 and 46.8 µM respectively.

## Keywords

Apocynaceae, Baissea Mortehanii, Enzyme Urease

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# 1. Introduction

The family Apocynaceae comprises about 3700 species in 424 genera [1], distributed throughout the tropics and some subtropical regions, mainly in America, Africa, and Asia. As in all apocynaceous plants, this plant exudes a milky juice from all parts when broken. Alkaloids have been reported to be present in a number of species of this genus [2]. Alkaloids have been reported to be present in a number of species of this genus [2]. The genus Baissea (Apocynaceae) is found in continental Africa and eastern Asia, particularly in humid forests and shady places. It includes 62 species including Baissea axillaris Hua, Baissea baillonii Hua, Baissea klaineana Pierre, Baissea floribunda Hua, Baissea leonensis Bent, Baissea zygodioides Stapf without forgetting Baissea mortehanii de Wild which is the species which was the subject of our study [3]. A phytochemical study on Baissea mortehanii de Wild was carried out. As results eight known compounds 1-8. In this article we have described the isolation, structural elucidation and biological activity.

# 2. Experimental

## 2.1. General Methods

UV spectra were measured on a Hitachi U-3200 spectrophotometer. IR spectra were recorded ona Shimadzu 8900 FT-IR spectrophotometer in KBr disks. Melting points were performed on a Buchi-M560 melting point apparatus. The NMR spectra in MeOD, DMSO-d6 and pyridine-d5 were obtained using Bruker Av-500, Avance-500 Cryo-Probe and AV-III-HD 800 Cryo-Probe instruments, operating at 400, 500, 600 and 800 MHz for 1H NMR and 75, 125 and 200 MHz for $^{13}$CNMR. Chemical shifts are given in (ppm) using tetramethylsilane (TMS) as internal standard. EI-MS, HR-EI-MS, HR-ESI-MS were obtained with a JEOL JMS-600H mass spectrometer. Column chromatography was carried out using silica gel (70–230 mesh; Merck). Chromatograms were visualized by spraying with a solution of 1% vanillin-$H_2SO_4$ or under ultraviolet light of wavelength 254 and 366 nm.

## 2.2. Plant Material

The leaves of *Baissea mortehanii* de Wild was collected from Kala Mountain (Nkolbison, at 8 km. W. of Yaounde), Centre region, Cameroon in August 2017. The plant was authenticated by M. Victor Nana (Botanist at National Herbarium, Yaounde, Cameroon), plant taxonomist at the National Herbarium of Cameroon, where a voucher specimen was deposited under the No. 20037 SRF/CAM.

## 2.3. Extraction and Isolation

Dried and powdered leaves (1.50 kg) of *Baissea mortehanii* de Wild were extracted exhaustively with MeOH at room temperature during 48 h. The solvent was removed under vacuum giving a residue (110 g). The residue was partitioned with hexane. The methanolic residue was partitioned with ethyl acetate to yield an EtOAc soluble residue (30.4 g). The EtOAc residue was analyzed by TLC and fractionated on a silica gel column using the mixture $CH_2Cl_2$/MeOH solvent system with increasing polarity (1% to 100%) to provide five main fractions I (1.7 g), II (3.6g), III (2.8 g), IV (4.4 g) and V (6.3 g).

Fraction II (3.6 g) was submitted to silica gel using the solvent system $CH_2Cl_2$/MeOH

(30/1 to 15/1) to give three sub-fractions (IIa, IIb and IIc). Sub-fraction IIc (1.1 g) was further

chromatographed on a silica gel column by using hexane: ethyl acetate as mobile phase with gradient elution (35% to 5%) to furnish 4 compounds were identified as 1 (7 mg), 2 (5 mg), 3 (8 mg) and 4 (10 mg).

Using the same process, fraction III (3.8 g) gave three sub-fractions (IIIa, IIIb and IIIc). Fraction IIIb (1.20 g) was further chromatographed on Sephadex LH-20 to yield two sub-fractions (IIIb1and IIIb2). Sub-fraction IIIb1 (0.4 g) was subjected to a silica gel column and eluted with $CH_2Cl_2$/MeOH and gradient elution (50 % to 30 %) to afford 4 compounds, 5 (11 mg); 6 (15 mg); 7 (7 mg) and 8 (12 mg).

## 2.4. Urease Inhibitor Assay

The urease activity was determined by measuring ammonia production using the indophenol method, as previously described [4, 5]. Thiourea was used as the standard inhibitor of urease. For kinetic studies, the concentration of compounds that inhibited the hydrolysis of substrates (jack bean urease) by 50% ($IC_{50}$) was determined by monitoring the inhibition effect of various concentrations of both compounds in the assay. The $IC_{50}$ (required inhibitor concentration that inhibits 50% activity of enzyme) values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, MA, USA).

# 3. Results and Discussion

The phytochemical investigation of the leaves of *Baissea mortehanii* was carried out and we identified 8 knowns compounds as N-Feruloyltryptamine 1 [6], Dibutyl phthalate 2 [7], Genistein 3 [8] and Gerontoisoflavone A 4 [9], $\beta$- Sitosterol 5 [10], Sitosterol-3-O-$\beta$-D-glucopyranoside 6 [11], Stigmasterol 7 [12] and Stigmasterol-3-O-$\beta$-D glucopyranoside 8 [13].

The eight isolated compounds were evaluated in vitro for their inhibitory property against urease (Table 1).

It appears from this table that all the compounds tested for their inhibitory activity against urease showed better, good and moderate activities with $IC_{50}$ values between 49.1 and

17.2 µg/ml, compared to the activity of thiourea whose $IC_{50}$ value is 21.5 µg/ml, it is more precisely

*β*-sitosterol and Stigmasterol which exhibit better inhibitory activity than thiourea with $IC_{50}$ values of 17.2 and 18.5 µg/ml, respectively;

Genistein and Gerontoisoflavone A which have good inhibitory activity with $1C_{50}$ values of 26.9 and 29.7 µg/ml, respectively,

However, N-Feruloyltryptamine, Dibutyl phthalate, *β* sitosterol 3-O-*β*-D-glucopyranoside and stigmasterol 3-O-*β* -D glucopyranoside showed moderate inhibitory activity with $IC_{50}$ values of 49.1; 46.8, 32.8 and 34.3 µg ml, respectively.

The various data obtained during this analysis show that the hydroxylated compounds are mainly responsible for the urease inhibition activity. This could be due to monodentate bonding via the oxygen atom of the molecule to the nickel atoms of urease [14, 15].

The same work was carried out on the species indigofera atriceps Hook. f and indigofera spicata Forsk [16].

## 4. Conclusion

Phytochemical investigation of the leaves of Baissea mortehanii de Wild led to the isolation and identification of eight known compounds, N-Feruloyltryptamine (1), Dibutyl phthalate (2), Genistein (3) and Gerontoisoflavone A (4), *β*-Sitosterol (5), Sitosterol-3-O-*β*-D-glucopyranoside (6), Stigmasterol (7) and Stigmasterol-3-O-*β*-D glucopyranoside (8). Further biological study revealed significant enzyme urease activity of these compounds, contributing further insight towards the pharmacological properties of secondary metabolites from this plant and we should note that this is the first time that this species has undergone a phytochemical study.

***Table 1.*** *Urease inhibitory activity of the isolated compounds.*

| Compounds | Urease inhibitory activity values IC50 ± SEM (µg/mL) |
|---|---|
| 1 | 49.1 ±0.76 |
| 2 | 46.8 ±0.04 |
| 3 | 26. 9 ±0.21 |
| 4 | 29.7 ±0.54 |
| 5 | 17. 2 ±0.71 |
| 6 | 32.8 ±0.78 |
| 7 | 18.5±0.28 |
| 8 | 34. 3 ±0.18 |
| Thiourea | 21.5 ±0.47 |

*$IC_{50}$ = concentration of the compound exerting 50% inhibition, SEM = Standard Error of the Mean; Red = Excellent Activity; Green = good activity; Black = moderate activity; Blue = reference molecule.

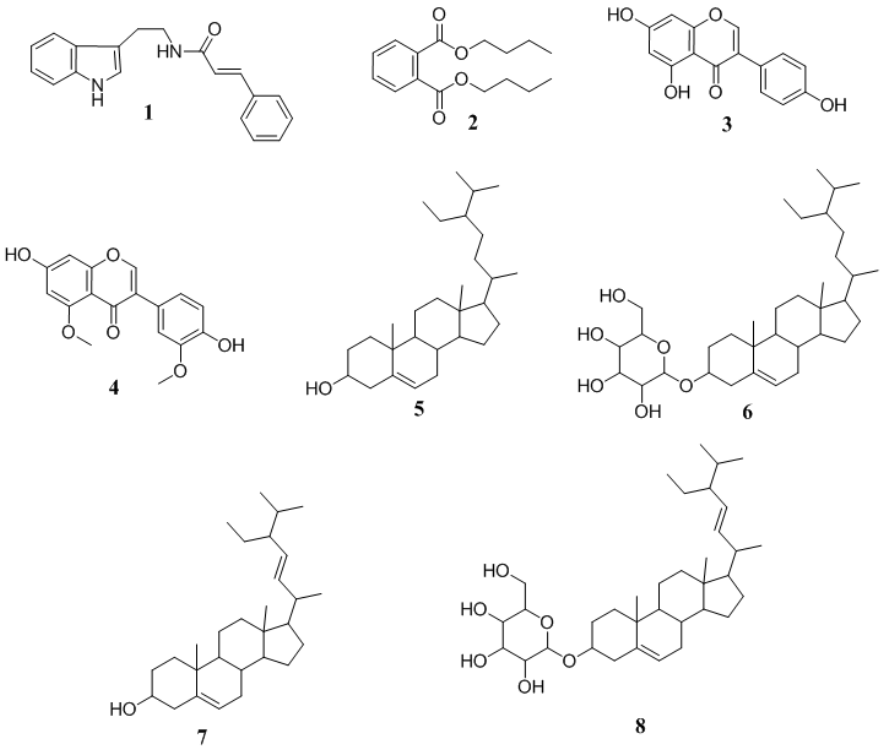


***Figure 1.*** *Compounds isolated from the leaves of Baissea mortehanii (1–8).*

## Abbreviations

NMR: Nuclear magnetic resonance
Mp: Melting points
UV: Ultraviolet
IR: Infra-red
EI-MS: Electron Ionization Mass Spectrometer
HR-EI-MS: High resolution Electron Ionization Mass Spectrometer
HR-ESI-MS: High resolution Electrospray ionization Mass Spectrometer

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## Conflicts of Interest

The authors declare no conflicts of interest.

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