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Rapid isolation of vitexin from leaves of kinkeliba, *Combretum micranthum* G. Don

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Abstract
Vitexin, the main flavonoid was extracted from the leaves of *Combretum micranthum* in soxhlet with ethanol. The extract evaporated to dryness and retrieved thanks to hot water. That liquid extract went through a degreasing and extracted again with butanol. The butanolic extract evaporated to dryness and dissolved in methanol, in which the vitexin precipitated after the washing and the recrystallisation of the first obtained precipitate. From 500g of dry kinkeliba leaves, a series of manipulations led to the isolation of 0.0576 g of vitexin, yielding 0.013% per ounce of the kinkeliba leaves dry powder. The degree of purity of vitexin was 94.79% on the basis of HPLC/UV-Vis/MS of the chemical formula $C_{21}H_{20}O_{10}$. According to the spectral data NMR ^{1}H and ^{13}C the molecular structure of the vitexin is identified.

Keywords: *Combretum micranthum, kinkeliba,* Vitexin, flavonoids

1. Introduction
Combretum micranthum is one of the most widely used medicinal plants in Senegal and in the Sahel and desert region of West Africa. Many herb preparations used as stimulants of bile secretion contain extracts of *Combretum.* Vitexin, one of the main flavonoids in the leaves (Bassene, 1985; Bassene *et al*., 1987) [1, 2] has hypotensive properties (Prabhakar *et al*., 1981) [3]. More recent work shows the plant extract exhibits glucose-lowering activitiy (Welch *et al*., 2017) [4]. In this article, we describe a method for isolation and structure determination of this flavonoid from the leaves.

2. Materials and Methods
2.1 Extraction and isolation by precipitation
First, 500 g of ground *Combretum micranthum* leaves are degreased in a soxhlet extractor with petroleum ether and then extracted with ethanol until exhausted completely. The ethanolic extract was evaporated to dryness (or concentrated to the maximum) then dissolved in 500 ml of hot water. The aqueous extract obtained was degreased with 4 liters of petroleum ether and then extracted with 2 liters of butanol. The butanolic extract was evaporated to dryness, then taken up in 400 ml of methanol and allowed to precipitate after one week at room temperature. This precipitate was collected and washed three times with 2 ml of methanol then recrystallized in the same amount of methanol. After 24 hours, the vitexin precipitates.

2.2 Identification
2.2.1 Identification by HPLC
The chemical formula and the degree of purity were determined on the basis of the comination of HPLC/UV/MS.

2.2.2 Material and Reagent
- Instrument: Agilent 1100 series HPLC/UV-Vis/ MSD (Waldbronn, Germany).
- Column: Prodigy 5u OSD (3) 100 A, 150 * 3.20 mm 5 microns.
- Solvent: distilled water, formic acid, acetonitrile.

2.2.3 Methodology
This test was conducted:
- Mobile phases:

A: water modified with 0.1% Formic acid;
B: acetonitrile with 0.1% Formic acid.

The gradient was 10% for 2 min, 10 to 20% from 2 to 12 min, and 20 to 40% from 12 to 22 min, then 40 to 60% from 22 to 25 min, and keep 60% from 25 to 28min, and 60 to 10% from 28 to 30min.

- Column temperature: 25 ^{0}C
- Injection concentration: 1.0 mg/ml in methanol
- Flow rate: 0.6 ml/mn
- Injection volume: 10 µl
- UV detection: 350 nm

2.2.4 Identification by RMN

The molecular structure was established on the basis of ^{1}H/^{13}C NMR data.

2.3 Material and reagent

- Instrument: Bruker Ultrashield 400 MHz multinuclear NMR
- Precipitation obtained: (10 mg 0.023 mmol) dissolved in 0.8 ml DMSO

2.4 Methodology

We prepare à solution of the substance to identify taking 10 mg representing 0,023 mmol dissolved in 0.8ml of DMSO. That solution will be used for the needs of the NMR.

3. Results and Discussion

0.0576g of recrystallized precipitate was obtained, the yield which is 0.013% per gram of kinkeliba powder material shows us the quantitative value of the vitexin in the leaves of *Combretum micranthum*. Nevertheless, one can have a more interesting yield with HPLC (Bassène, 1985) [4] which found 0.56 µg of vitexin in 45 µg of aqueous crude extract, ie a content of 1.24% per gram of extract. Vitexin was identified with a purity degree of 94.790%, on the spectral basis HPLC/UV-Vis/MSD shown by Figures 1. Based on ^{1}H and ^{13}C NMR data shown by Figures 2 and 3, the structure is identified to vitexin (figure 4), whose chemical formula is: $C_{21}H_{20}O_{10}$.

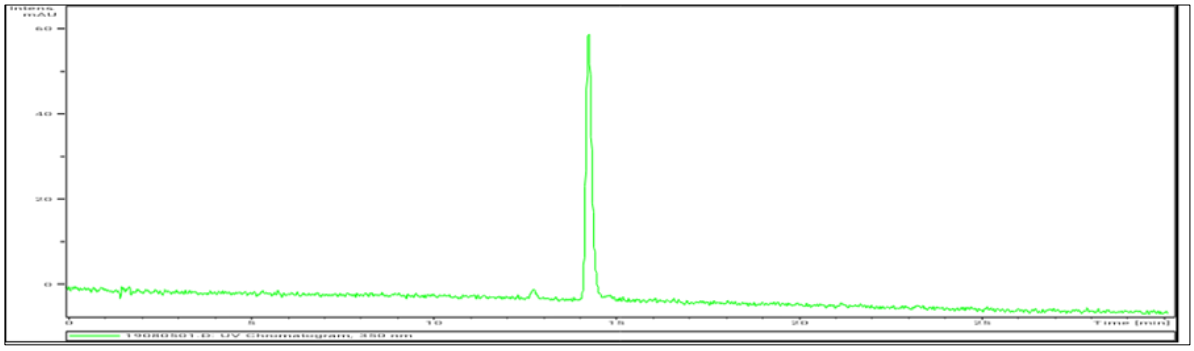

Fig 1: HPLC/UV chromatogram of vitexin at 350 nm

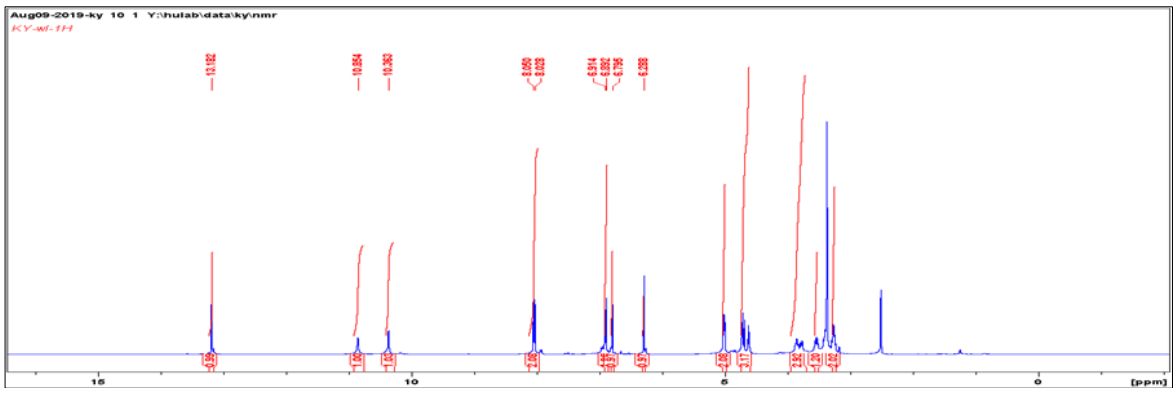

Fig 2: ^{1}H-NMR spectrum of the vitexin protons

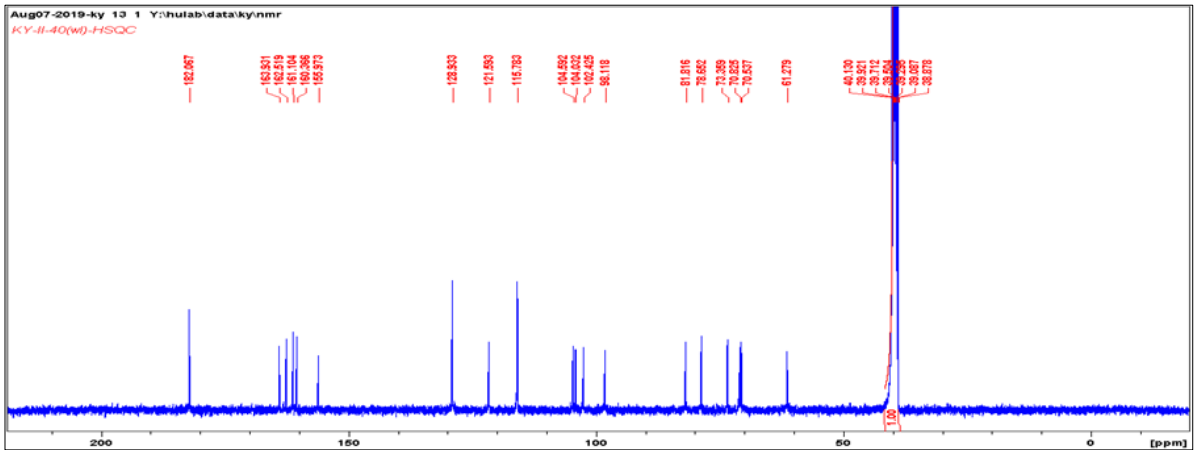

Fig 3: ^{13}C-NMR spectrum of *vitexin*

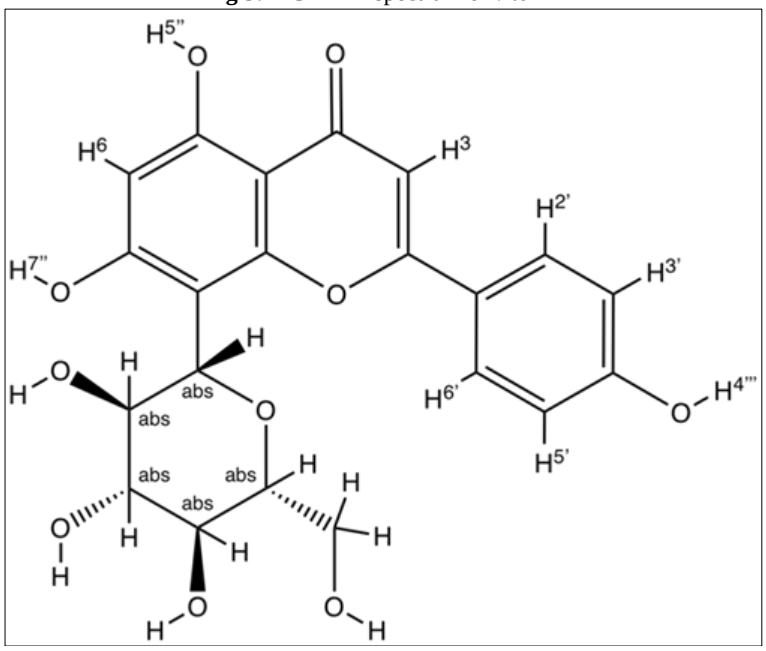

Fig 4: Chemical structure of vitexin (glucosyl-8-apigenin)

Table 1: Assignement of proton and carbon signals

Proton (δ H in ppm)	Fixing carbon (δ C in ppm)	Carbon vicinals (δ C in ppm)
13.182 (S, 1H, -OH^5)	161.1039 (C^5)	104.5919 (C^{10}) - (98,1179) C^6
10.854 (S, 1H, -OH^7)	162.5187 (C^7)	(98.1179) C^6- (102.4253) C^8
10.363 (S, 1H, -OH$^{4'}$)	155,9732 (C$^{4'}$)	(115,7833) C$^{3'}$- (115,7833) C$^{5'}$
8.050 - 8,028 (2H, d, J =8.8 Hz, H$^{2'}$)	128,9325 (C$^{2'}$ et C$^{6'}$)	(121,5925) C$^{1'}$- (115,7833) C$^{3'}$
8,050 - 8,028 (2H, d, J = 8,8 Hz, H$^{6'}$)		(121,5925) C$^{1'}$- (115,7833) C$^{5'}$
6,915 - 6,892 (2H, d, J = 8,8 Hz, H$^{3'}$)	115,7833 (C$^{3'}$ et C$^{5'}$)	(128,9325) C$^{2'}$- 155,9732 C$^{4'}$
6,915 - 6,892 (2H, d, J = 8,8 Hz, H$^{5'}$)		(128,9325) C$^{6'}$- 155,9732 C$^{4'}$
6,795 (S, 1H, H^3)	104,0316 (C^3)	(163,9312)C^2- (182,0666) C^4
6,288 (S, 1H, H^6)	98,1179 (C^6)	(161,1039) C^5- (162,5187) C^7
	121,5925 (C$^{1'}$)	(128.9325) C$^{2'}$-(128.9325) C$^{6'}$- (128.9325) C^2

S: singlet; d: doublet; J: frequency in Hertz; H: proton; C: carbon
On the basis of 1 H and 13 C NMR data, the structure is identified to vitexin

4. Competing interests
The authors declare that they have no competing interests.

5. Authors' contributions
Tine Daba, Fall Alioune Dior, Dieng Serigne Mbacké, Sarr Abdou: Extraction, isolation and preliminar identification of vitexine.
Lyu Weiting, Wu Qingl-Li for spectral identifications in Rutgers University
Simon James E., Bassene Emmanuel: supervision of teams in Rutgers and Cheikh Anta Diop Universities.

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