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Research Article

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Thalictramine, A new alkaloid from *Thalictrum rhyncocarpum* (Dill & Rich) and its anti-bacterial activity

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ABSTRACT

*Thalictrum rhyncocarpum, a creeping plant with fern-like leaves, is used in herbal medicine to treat various infections in parts of Africa. Previously, we reported In-vitro anti-bacterial activity of different parts (root, stem bark and leaves) of the plant. In this study, bioassay-guided chromatography of an active fraction of ethanol extract of the plant against Staphylococcus aures-SG 511, Pseudomonus aeruginosa-K799/61 and Mycobacterium vaccae-10670 (using tube dilution method) was undertaken. Two bioactive constituents were isolated from an active fraction of the extract and characterized using spectrometric methods as thalictramine (**1**) and berberine. In addition, 6-O-β-D-glucopyranosyl-D-glucose (gentiobiose) was also isolated from the same fraction. The antibacterial activities of the active fraction, thalictramine and berberine against P. aeruginosa and M. vaccae were higher than that of ciprofloxacin.*

Key words: *Thalictrum rhyncocarpum*, thalictramine anti-bacteria activities

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INTRODUCTION

The genus *Thalictrum* comprises 120 species of perennial herbaceous plants. Over 200 alkaloids, almost all belonging to the isoquinoline group, have been reported from this genus and a large number have biological activities, mainly hypotensive, antimicrobial and antitumor properties [1] Bis-benzylisoquinoline and aporphine-benzylisoquinoline alkaloids associated with the genus are structurally rare type of alkaloids with interesting pharmacological activities [2]. A more recent study revealed that in addition to the alkaloids, the genus is also rich in triterpenoids, flavanoids, cyanogenic glycosides and saponins [2].

Different species of the genus are used to treat different diseases in the tropics. In India, some of them are used as sources of tonics, diuretics and antiseptics, as well as for the treatment of stomach discomforts, snake bites, jaundice, rheumatism and microbial infections [3]. In China, as many as 43 species of this genus are used to treat lung cancer, cough and dysentry [4]. In Africa, aqueous extracts of different parts of some species are used to accelerate wound healing and treat stomach ulcers, snake bites, dysentery, skin rashes and different bacterial infections [5, 6]. Previous *in vitro* anti-bacterial screening of crude extract of *T. rhyncocarpum* revealed impressive activities against a range of bacteria [7]. This study reports on bioassay-guided isolation and characterization of

some constituents of the ethanolic extract of the plant and their anti-bacterial activities in comparison with that of ciproflaxin, a synthetic fluoroquinolone antibiotic.

EXPERIMENTAL SECTION

2.1 Plant collection

T. rhyncocarpum was collected in November 2010 in Ngong forest in Nairobi County, Kenya (37 ° 08 E and 0 ° 13 S). The identity of the plant was authenticated at University of Nairobi Herbarium, where a specimen is preserved (Voucher number TR-NG-203).

2.1 Preparation of plant material

The plant was dried under shade for a period of 14 days and ground into powder using an electric grinder.

2.3 Extraction and partitioning

The powdered plant material was repeatedly extracted with analytical grade absolute ethanol and the combined extract concentrated under vacuum. The dry crude ethanol extract of *T. rhyncocarpum* was partitioned between methanol and hexane to afford a polar and non-polar fraction. Of the two, the polar fraction was found to have potent antibacterial activities.

2.4 Isolation of pure compounds

The polar fraction was sequentially chromatographed on silica gel (mesh 60-230;Merck), sephadex LH-20 using methanol/dichloromethane (1:1) as the eluent, followed by High Pressure Liquid Chromatography (HPLC) on C_{18} coated column using methanol in water (7:3) as the mobile phase at a flow rate of 10 ml/ min.

2.5 Tube dilution assay

The Minimum Inhibitory Concentrations (MIC) of crude extract, fractions and pure compounds were determined using a serial micro-plate dilution assay [8] against each test bacterial species. This was determined by 2-fold serial dilution of the compounds beyond the level where no inhibition of growth of the bacterial strains was observed. Each test sample was reconstituted to 100 µg/ml in DMSO and 100 µl aliquot serially diluted with equal amounts of water in 96-well microplates. The test bacteria (*Staphylococcus aures*-SG 511, *Pseudomonus aeruginosa*-K799/61 and *Mycobacterium vaccae*-10670) were inoculated into Muller-Hinton (MH) broth culture (1%), incubated at 37^{o}C overnight and 100 µl aliquots of the resulting culture added to each well. Ciprofloxacin at a concentration of 100 µg/ml was used as a positive control and untreated wells used as negative controls. The microplates were sealed and incubated at 37 ^{o}C and 100% relative humidity for 18 h. As an indicator of bacterial growth, 40 µL aliquots of a 0.2 mg/mL solution of *p*-iodonitrotetrazolium violet (INT) dissolved in water was added to the microplate wells and incubated at 37 ^{o}C for 30 min. The MIC value was recorded as the lowest concentration of the extract at which bacterial growth was inhibited.

Thalictramine (1)

MP: 278-280^{o}C

Rf: 0.7 ($CHCl_3$-MeOH, 8:2).

IR (film): 3378 cm^{-1}, 2253 cm^{-1}, 1650 cm^{-1}, 1597 cm^{-1}, 1496 cm^{-1}, 1358 cm^{-1}, 1269 cm^{-1}, 1211cm^{-1}, 1171 cm^{-1}, 1118 cm^{-1}, 1016 cm^{-1}, 814 cm^{-1}, 668 cm^{-1}

UV/Vis λ_{max} (MeOH) nm(log ε): 227 (3.1), 276 (3.824), 313 (2.79).

^{1}H NMR (500 MHz, CD_3OD): 3.80 (3H, s, OMe), 6.20 (1H, s, H-2$^{/}$), 6.40 (1H, s, H-5$^{/}$), 6.90 (1H, d, *J* = 8.4 Hz, H-8), 7.53 (1H, d, *J* = 8.4, H-7), 7.62 (1H, s, H-5).

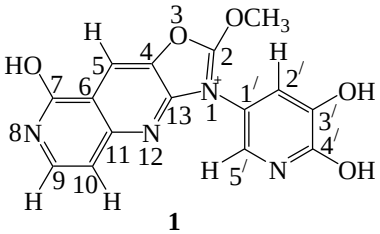
^{13}C NMR (75 MHz, CD_3OD): 60.5 (OCH_3), 94.7 (CH), 99.8 (CH), 105.8 (C), 116.4 (C), 116.5 (CH), 122.3 (CH), 122.9 (C), 139.5 (C), 146.5 (C), 149.9 (C), 158.0 (CH), 158.4 (C), 163.1 (C) 166.0 (C).

HRMS-ESI: *m/z* [M + H^{+}] calcd for $C_{15}H_{11}O_5N_4$: 328.316; found: 328.000.

RESULTS AND DISCUSSION

Repeated column chromatography on silica gel followed by purification using sephadex-LH 20 and High Performance Liquid Chromatography (HPLC) of a polar fraction of ethanol extract of *T. rhyncocarpum* gave a new alkaloid (**1**), a known one berberine (**2**) and a known disaccharide, gentiobise (**3**).

The new alkaloid (**1**) was obtained as a yellow crystalline solid. HR-MS of the compounds revealed a molecular ion peak $(M + H)^+$ calcd for $C_{15}H_{11}O_5N_4$: 328.316; found: 328.000. The ^1H NMR spectrum of **1** (Table 1) revealed signals due to five aromatic methines (δ_H 7.62, 7.53, 6.90, 6.20, 6.40, H-5, H-9, H-10, H-2′ and H-5′ respectively). The signal (δ_H 3.80 H_3-methoxyl) was associated with one methoxyl group [9]. The ^{13}C NMR spectrum of **1** revealed 15 carbons consisting of 14 aromatic carbons (δ_C 139.5, C-2; δ_C 158.4, C-4; δ_C 116.4, C-5; δ_C 158.0, C-6; δ_C 146.5, C-7; δ_C 122.3, C-9; δ_C 116.5, C-10; δ_C 149.9, C-11; δ_C 122.9, C-13; δ_C 105.8, C-1′; δ_C 99.8, C-2′; δ_C 163.1, C-3′; δ_C 94.7, C-5′) and 1 methoxy carbon (δ_C 60.5). COSY and HMBC (**1A**) correlations revealed atomic connectivity and substitution pattern of **1**. The COSY spectrum indicated correlation between C(9)H and C(10)H doublets with the same $J_{1\text{-}2}$ value of 8.4 suggesting an *ortho* arrangement of the δ_H 7.53, H-9 and δ_H 6.90, H-10 hydrogens [10]. In the HMBC spectrum, the hydrogen H-9 showed correlation with quaternary carbons at δ_C 146.5 (C-7) and δ_C 149.9 (C-11), while H-10 only showed correlation with the carbon at δ_C 158.0 (C-6). This fact enabled placement of the nitrogen in ring A (position 8) and the one in ring B (position 12). HMBC correlation between H-5 hydrogen with two carbons at δ_C 149.9 (C-11) and δ_C 122.9 (C-13) suggested its position on ring B. Lack of an expected third three bond correlation of the H-5 in the HMBC spectrum enabled placement of oxygen in ring C (position 3). The placement of a methoxyl group on ring C was revealed by HMBC correlation between the quartenary carbon at δ_C 139.5, (C-2) and the methoxy hydrogens (δ_H 3.80). This was further strengthened by existence of correlation between methoxy hydrogens (δ_H 3.80) and δ_H 6.20 (2′-H) in NOESY spectrum of **1.** The singlets at δ_H 6.20 (2′-H) and δ_H 6.40 (5′-H) only showed two 3-bond correlations in the HMBC spectrum **1**. Correlation was observed between the singlet at δ_H 6.20 (2′-H) and carbons at δ_C 166.0, (C-4/) and δ_C 94.7, (C-5′), while the one at δ_H 6.40 (5′-H) showed correlation with carbons at δ_C 99.8 (C-2′) and δ_C 166.0, (C-4′). The absence of expected third 3-bond C/H correlation of these two protons in the HMBC spectrum of **1** justified the placement of the nitrogen in ring C. In the IR spectrum of **1**, bands at 1496.49cm^{-1}, 1597.73cm^{-1}, and 1650.77cm^{-1} with aromatic system [11]. C-N stretch was associated with the absorption bands at 3378.67cm^{-1}, 1016.30cm^{-1}, 1118.51cm^{-1}, 1171.54cm^{-1}, 1211.08cm^{-1},1269.90cm^{-1}, and 1358.60cm^{-1} [12]. Bands at 668.21cm^{-1} and 814.78cm^{-1} revealed out of plane aromatic bends [13]. The strong peak at2253.48cm^{-1} was associated with carbon dioxide absorption, a common background band in JASCO FT/IR-4100 acquired IR spectra [13]. From this data, the structure of thalictramine was determined as **1**.



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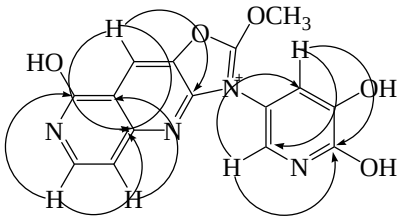
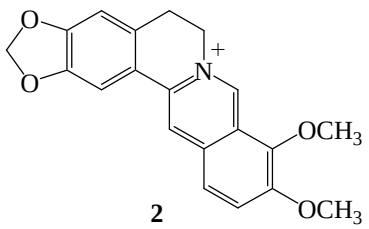


1A



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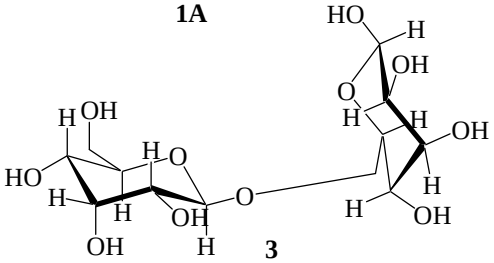

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Table 1: ^{1}H (300MHz) and ^{13}C (75MHz) NMR data for thalictramine in CD_3OD

Carbon No	δ_H	δ_C
1	-	
2	-	139.5
3	-	
4	-	158.4
5	7.62 s (1H)	116.4
6	-	158
7	-	146.5
8	-	
9	7.53 d *J* = 8.4 (1H)	122.3
10	6.90 d *J* = 8.4 (1H)	116.5
11	-	149.9
12	-	
13	-	122.9
1′	-	105.8
2′	6.20 s (1H)	99.8
3′	-	163.1
4′	-	166
5′	6.40 s (1H)	94.7
2-OCH_3	3.80 s (3H)	60.5

The anti-bacterial activities of the crude extract, a polar fraction, less polar fraction and constituents in comparison with that of ciprofloxacin, a synthetic fluoroquinolone antibiotic are summarized in Table 2.

Table 1: *In-vitro* anti-bacterial activity of crude extract, fractions and pure compounds from *T. rhyncocarpum*

Sample	*S. aures* (µg/ml)	*P. aeruginosa* (µg/ml)	*M. vaccae* (µg/ml)
A	$13.4^{b} \pm 0.6$	$12.8^{c} \pm 1.6$	$11.3^{c} \pm 1.7$
B	$17.4^{c} \pm 1.6$	$5.2^{a} \pm 0.4$	$4.2^{a} \pm 1.3$
C	$>100^{d}$	$>100^{d}$	>100
D	$>100^{d}$	$8.2^{b} \pm 2.3$	$7.4^{b} \pm 1.2$
E	$>100^{d}$	$6.2^{a} \pm 1.4$	$12.8^{c} \pm 0.5$
F	$>100^{d}$	$>100^{d}$	>100
G	$11.3^{a} \pm 1.4$	$12.4^{c} \pm 3.2$	$10.3^{c} \pm 1.3$

A: crude extract; B: polar fraction; C: less polar fraction; D: thalictriamine; E: berberine; F: gentiobiose; G: ciprofloxacin

The results showed that the more polar fraction was significantly more active than the less polar counterpart and more active against *P. aerruginosa* and *M. vaccae* than ciprofloxacin. However, the activity of the fraction against *S. aures* was lower than that of the crude extract, indicating that some constituents of the less polar fraction may be responsible for the overall activity of the crude extract. The more polar ethanolic fraction, thalictrimine and berberine showed higher activities against *P. aeruginosa* and *M. vaccae* compared to ciprofloxacin. Interestingly, the activity of the polar fraction against *P. aeruginosa* and *M. vaccae* was significantly higher than each of the three compounds isolated from the fraction, which suggests additive or synergistic effects of these constituents against the two bacteria species.

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