Special Conference Edition, November, 2019

http://dx.doi.org/10.4314/bajopas.v12i1.86S

Bayero Journal of Pure and Applied Sciences, 12(1): 573 - 576
ISSN 2006 – 6996

ISOLATION OF RUTIN FROM THE LEAF OF *Ziziphus mucronata* WILLD. (Rhamnaceae)

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ABSTRACT

Rutin, a flavonol diglycoside, was isolated from the n-butanol fraction of a methanol extract of the leaf of Ziziphus mucronata, an important medicinal plant of the Rhamnaceae family widely used in ethnomedicine to treat inflammation, diarrhoea, tumour, cough, sores, asthma, measles, fever and urinary problems. Its isolation was carried out by a combination of column chromatography and gel filtration. The structure of the isolated compound was determined by analysis of its UV, IR, 1D and 2D Proton and carbon-13 NMR spectral data, as well as comparison with reported data. This is the first report of isolation of Rutin from the leaf of this species.

Keywords: Rutin, NMR spectral analysis, Ziziphus mucronata

INTRODUCTION

Ziziphus mucronata is one of the 40 species of spiny shrubs and small trees in the buckthorn family, rhamnaceae, distributed in the warm-temperate and subtropical regions throughout the world. The plant, which is 10-20 m high with a spreading canopy, is commonly known as Buffalo thorn in English and Magaryar kura in Hausa. The leaf of the plant is simple, alternate; ovate or broadly ovate; vary enormously in size from tree to tree, 30-90 x 20-50 mm, tapering or having mucronate apex and often asymmetrical at the base. It is chordate to rounded on one side; margin finely serrated, often badly eaten by insects (Plate 1). It is glossy green above but slightly hairy and paler below and 3- to 5-veined from the base; veins covered with fine hairs when young; petiole up to 20 mm long; stipules, when present, take the form of small thorns at the nodes, one straight and one hooked. Leaf turns golden yellow in autumn (Burkill, 1995).

The main use of the leaf in African traditional medicine includes (but not limited to) treatment of diarrhoea, tumour, cough, sores, ear inflammation, asthma, syphilis, gonorrhoea, measles, fever, psychiatric disorder and prevention of abortion (Burkill, 1995). Preliminary phytochemical screening of the methanol extract of the leaf revealed the presence of saponins, flavonoids, tannins, triterpenes and steroids while that of the n-butanol fraction revealed the presence of mainly phenolic compounds and glycosides (Abdullahi *et al*., 2017).

The ethnomedicinal use of the leaf in the treatment of pain and inflammation was validated scientifically (Abdullahi *et al*., 2017). The n-butanol extract has also been found to possess some anti-inflammatory activity (Authors, unpublished). Apart from cyclopeptides identified as chemo markers of the rhamnaceae family, there is dearth of information regarding isolation of potentially bioactive principles from the plant. To the best of our literature search, only the isolation of catechins (Abdullahi *et al*., 2017) and biflavonoid (Abdullahi *et al*., 2018) were previously reported from the leaves of the plant. In continuation of our study aimed at investigating the bioactive principles present in the leaf of *Z. mucronata*, we report herein, the isolation of Rutin from the n-butanol soluble fraction of the methanol extract of the leaf.

MATERIALS AND METHODS

General Experimental Procedures and Spectroscopic Characterization

The solvents used were of high quality (analytical grade) and include: methanol, n - hexane, chloroform, ethyl acetate and n-butanol purchased from Sigma Co. USA; silica gel 60-120 µm (Qualikems, India) was used for column chromatography, sephadex LH-20 (GE Healthcare) was used for purification of isolated compound. Thin layer chromatography (TLC) was carried out on aluminum-backed Kieselgel 60 F_{254} TLC plate (Merck no. 5554, Darmstadt, Germany) and a Gallenkhamp electro thermal melting point apparatus was used to determine the melting point of isolated compound.

UV spectrum was recorded on a Thermo electron-vision Helios zeta scientific ultraviolet (UV) spectrophotometer available at Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria. IR spectrum was recorded on Agilent technologies cary 630 FTIR spectrometer available at Multi-user research science laboratory, Ahmadu Bello University, Zaria. ^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (100 MHz, CD_3OD), HSQC, COSY and HMBC spectra were recorded on a Bruker AVANCE-600 Japan NMR spectrometer available at Universiti Teknologi Malaysia using TMS as reference peak.

Collection and Identification of the Plant Material

The leaf of *Z. mucronata* was collected from Kudingi village of Giwa local government area of Kaduna State, after identification of the tree on the field using descriptions in the monograph (Burkill, 1995). The identity of the leaf was confirmed and authenticated by Messer Musa Muhammad of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, by comparison with the existing herbarium specimen of voucher number 900328.

Preparation of the extract

The leaf was dried at room temperature for several weeks and size reduced manually using mortar and pestle.

The size reduced leaf (1 kg) was extracted with methanol by maceration for 72 hours and concentrated *in vacuo*. This yielded 134 g of a gummy extract. The extract was suspended in distilled water and filtered. The water soluble portion was treated successively with ethyl acetate and n–butanol to yield ethyl acetate fraction (EAF) and n-butanol fraction (nBF) respectively.

Isolation and Purification of Compound S_3

Based on the promising anti-inflammatory activity of nBF, a further study was carried out to investigate the phytochemical constituents in an attempt to isolate the bioactive compound(s) responsible for the anti-inflammatory activity.

7 g of nBF was subjected to column chromatography on silica gel with gradient elution using ethyl acetate and methanol (Salituro and Dufresne, 1998). Eluents were collected in 50 mL aliquot and TLC was used to monitor the progress of elution.

RESULTS

A total of 68 fractions were collected and pooled together into 13 major fractions based on their TLC profiles (Cannell, 1998). Fraction K (9.4 mg), which showed two major spots, was subjected to repeated gel filtration using sephadex and eluted with absolute methanol to give 6.2 mg of yellow needles, coded S_3. S_3 gave a single homogenous spot with solvent system containing ethylacetate: chloroform: methanol: water in the ratio 15:8:4:1 (Figure 1) and 15:4:4:1. The appearance of a single spot on the TLC plate indicates the purity of the sample (Gibbons and Gray, 1998). S_3 was subjected to chemical test and spectral analysis to elucidate its chemical structure.

Chemical Test on S_3

S_3 gave a Prussian blue colour with freshly prepared ferric chloride solution indicating the presence of a phenolic nucleus. It also produced red colour with concentrated Hydrochloric acid in the presence of Magnesium chips (Shinoda test). This is indicative of a flavonoid nucleus (Silva *et al.*,1998).

Plate I: *Ziziphusmucronata* leaf

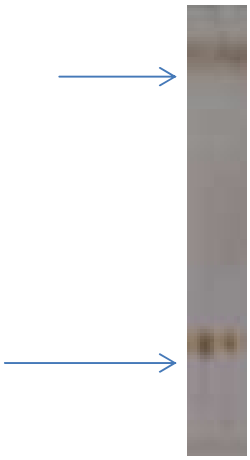

Figure 2: Proposed structure of S_3

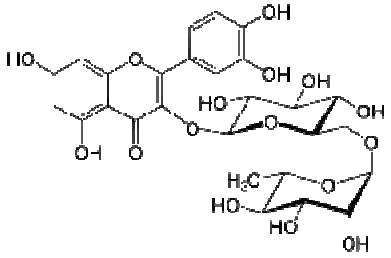

Figure 1: Chromatogram of S_3

Table 1: HMQC and DEPT spectral data of S_3

Position	δ_C	δ_H	DEPT
1			
2	157.08		C
3	134.46		C
4	178.08		C
5	164.81		C
6	98.59	6.23	CH
7	161.55		C
8	93.44	6.43	CH
9	157.57		C
10	104.14		C
1'	121.41		C
2'	116.55	7.90	CH
3'	144.35		C
4'	148.60		C
5'	114.72	6.89	CH
6'	121.63	7.62	CH
1''	104.56	5.10	CH
2''	73.69	3.60	CH
3''	72.47	3.58	CH
4''	71.73	3.61	CH
5''	73.92	3.67	CH
6''	62.91	3.85	CH_2
1'''	100.53	4.55	CH
2'''	70.89	3.77	CH
3'''	68.79	3.55	CH
4'''	70.65	3.53	CH
5'''	68.31	3.51	CH
6'''	16.56	1.21	CH_3

DISCUSSION

UV and IR

The UV spectrum of S_3 shows absorption bands at 257 nm and 359 nm consistent with bands due to benzoyl and cinnamoyl moieties respectively. These values are typical for Flavonol nucleus.

The IR spectrum showed two sharp peaks at 1602 cm^{-1} and 1654 cm^{-1} characteristic of C=C in aromatic ring and alkenes respectively. An intense peak observed at 1712 cm^{-1} is typical of carbonyl frequency. It also displayed coupling bands at 2855 cm^{-1} and 2922 cm^{-1} due to saturated symmetric and asymmetric C-H stretching in glucose; and a broad band at 3272 cm^{-1} due to hydrogen bonded hydoxyl group. These values are in agreement with those reported by Abdullahi *et al.*, 2011.

^{1}H-NMR

The ^{1}H-NMR spectrum of S_3 displayed signals for five aromatic proton and were assigned to an AX type ring A [δ_H 6.23 (1H, d, J = 2.0 Hz, H-6), 6.43 (1H, d, J = 2.0 Hz, H-8)] and an ABX type ring B [δ_H 6.89 (1H, d, J = 8.4 Hz, H-2'), δ_H 7.90 (1H, d, J = 2 Hz, 5'), δ_H 7.62 (1H, dd, J = 8.4 Hz and 2.0 Hz, H-6')]. It also showed two signals at δ_H 4.55 (1H, d, J = 1.2 Hz, H-1''') and 5.10 (1H, d, J = 8 Hz, H-1'') typical of α and β anomeric protons of sugar respectively. Overlapping signals due to carbinol proton were observed at δ_H 3.43-3.85 confirming the presence of sugar moiety.

Other resonances were at δ_H 1.21 (3H, d, J = 6.4 Hz, H-6''') ascribable to Rhamnose and δ_H 1.30 (2H, s, H-6'') ascribable to Glucose.

^{13}C-NMR

The proton-decoupled ^{13}C-NMR spectrum disclosed 27 well resolved peaks separated by the DEPT experiment into one methyl carbon at δ_C 16.56(C-6'''), one methylene carbon at δ_C 62.91(C-6''), 15 methine carbons at δ_C [98.59(C-6), 93.44(C-8), 116.55(C-2'), 114.72(C-5'), 121.63(C-6'), 104.56(C-1''), 72.49(C-2''), 73.69(C-3''), 71.73(C-4''), 73.92(C-5''), 100.53(C-1'''), 70.89(C-2'''), 68.79(C-3'''), 70.65(C-4''') and 68.31(C-5''')] and 10 quaternary carbons at δ_C [157.08(C-2), 134.46(C-3), 178.08(C-4), 161.55(C-5), 164.81(C-7), 157.57(C-9), 104.14(C-10), 121.63(C-1'), 144.35(C-3') and 148.60(C-4')].

The assignment of the carbons and the placement of the sugar moiety were achieved using 2D-NMR experiments (Table 1).

In the COSY spectrum of S_3, the presence of cross peaks between δ_H [6.89 and 7.62]; δ_H [3.60 and 4.55]; δ_H [3.85 and 3.60] and δ_H [3.85 and 5.10] confirm their assignments to adjacent carbon atoms.

In the HMBC spectrum, there was a common J_3 correlation between protons at δ_H 6.23 and 6.43 to carbons at δ_C 98.59 and 93.44 respectively and this confirms their assignment to ring A. Also, a J_2 correlation between protons at δ_H 6.89 to carbon at δ_C 121.63 and a J_3 correlation to carbon at δ_C 144.35 confirm the assignment of the carbons to ring B. A J_3 correlation between anomeric proton at δ_H 5.10 to a quartenary carbon at δ_C 134.46 confirms that the sugar moiety is linked to the flavonoid aglycone through C-3 carbon. Other correlations confirmed by the HMBC are those of the sugar residue.

The foregoing spectral analysis, further substantiated with the result of chemical test led us to propose the structure of S_3 as Rutin (Figure 2).

Rutin is a well-known member of the flavonoid family possessing a quercetin nucleus. Rutin harbours anti-oxidant properties which help to protect the body from cellular damage caused by free radicals. Rutin has anti-inflammatory and anti-carcinogenic properties. It is beneficial for chronic venous insufficiency, hypertension, infections, atherosclerosis, osteoarthritis, haemorrhoids, stroke prevention and high cholesterol (Sattanathan *et al*., 2011). The isolation of this compound from *Z. mucronata* seems to support some folkloric use of the plant in African traditional medicine, e.g. in the management of inflammatory conditions.

CONCLUSION

To the best of our search, this is the first report of the isolation of Rutin from this species. Anti-inflammatory studies and the possible mechanism of action of compound S_3 are in progress in our laboratory.

ACKNOWLEDGMENT

The authors acknowledge the assistance of Dr Kamal Ja'afar Muhammad of Sheda Science and Technology Complex, Abuja for running the NMR spectroscopy of compound S_3.

REFERENCES

Abdullahi MI, Musa AM, Haruna AK, Sule IM, Abdullahi MS, Abdulmalik M, Akinwande Y, Abimiku AG, Iliya I. (2011). Anti-microbial Flavonoid Diglycoside from the Leaves of *Ochna schweinfurthiana* Hoffm. (Ochnaceae). *Nig J Pharm Sci*, *10*(2): 1-7.

Abdullahi SM, Musa AM, Abdullahi MI, Sani YM, Atiku I. (2017a). Catechin from the Leaf Extract of *Ziziphus mucronata* willd.(Rhamnaceae).*Nig J Pharm Sci*.; 16(2):1-5.

Abdullahi SM, Musa AM, Ya'u J, Magaji MG, Hassan HS, Idris AY. (2017b) Phytochemical, Analgesic and Anti-inflammatory Studies on the Methanol Leaf Extract of *Ziziphus mucronata* Willd. (Rhamnaceae). *Jopatrot*.; 6(2):120-128.

Abdullahi SM, Musa AM, Abdullahi MI, Sani YM, Hassan HS, Ya'u J. (2018). A New Biflavonoid Glycoside from the Leaves of *Ziziphus mucronata* Willd. (Rhamnaceae). *Trop J Nat Prod Res*., 2(4):167-170. doi.org/10.26538/tjnpr/v2i4.3

Burkill HM. (1995). *The Useful Plant of West Tropical Africa*, Royal Botanical Gardens Kew, vol. 5. Pp. 94-98.

Cannell RJP. (1998). How to Approach the Isolation of Natural Product.In Cannell R.J.P.(Ed.) *Natural Product Isolation*. Humana Press Totowa, New Jersey (USA). Pp. 1-52.

Gibbons S. and Gray AI. (1998). Isolation by Planar Chromatography. In: Cannell R.J.P.(Ed.) *Natural Products Isolation*; Humana Press, Totowa New Jersey (USA), Pp.209-246.

Salituro GM and Dufresne C. (1998). Isolation by Low-pressure Column Chromatography. In: Cannell, R.J.P. (Ed.). *Natural Products Isolation*.Humana Press Totowa, New Jersey (USA). Pp. 356-358.

Sattanathan K., Dhanapal CK., Umarani R. and Manavalan R, (2011). Beneficial health effects of rutin supplementation in patients with diabetes mellitus. *Journal of Applied Pharmaceutical Science*, 1(8): 227-231. ISSN: 2231-3354.

Silva GL., Lee I. and Douglas KA.(1998). Special Problems with Extraction of Plants. In: Cannell, R.J.P. (Ed.). *Natural Products Isolation.* Humana Press Totowa, New Jersey (USA). Pp. 356-358.