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Isolation and Characterization of Some Flavonoids from the Leaves of *Globimetula braunii* (Loranthaceae) Growing on *Terminalia catappa* L. (Combretaceae)

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ABSTRACT

Isolation and purification of natural products from medicinal plants are important components of drug development. Many important phytochemical compounds have been isolated from medicinal plants and are used as active therapeutic substances. This study aimed to isolate and characterize some of the phytochemical compounds present in the n-butanol soluble fraction of the ethanol extract of the leaf of *Globimetula braunii* (Loranthaceae) growing on *Terminalia catappa*. The dried pulverized leaf of *Globimetula braunii* was extracted with 90% ethanol using cold maceration. The ethanol leaf extract was partitioned using n-hexane, chloroform, ethyl acetate and n-butanol. The n-butanol fraction was subjected to silica gel column chromatography. The major fractions were further purified using Sephadex gel filteration. Three flavonols; rhamnetin, quercetin and rhamnetin-3-*O*-α-L-rhamnopyranoside were isolated. The structures of these compounds were established by spectroscopic analysis including 1D and 2D NMR. This is the first report of the isolation of these compounds from *Globimetula braunii* (Loranthaceae) growing on *Terminalia catappa*.

***Keywords*:** *Globimetula braunii*, Loranthaceae, Flavonols, Nuclear Magnetic Resonance.

Introduction

Natural products are chemical substances derived from plants or animals or micro-organisms having pharmacological or biological activity.[1] In most cases the term natural products refer to secondary metabolites (such as flavonoids, glycosides, steroids, triterpenoids, alkaloids e.t.c) isolated from plants, animals, or microorganisms. The use of natural products, especially plants, for healing is as ancient and universal as medicine itself.[2] Natural products have provided the primary sources for new drug development.[3] Drug discovery involves the isolation of pure compounds and testing their pharmacological and biological activity. Isolation of a single chemical compound from a plant is very difficult and time-consuming because of the complex mixtures of secondary metabolite it contains. Several fractionation and isolation methods are developed after which isolation of the active moiety and their chemical examination is performed.[1] Following many outstanding developments within the areas of separation and spectroscopic techniques, natural product research has gained new attention for providing novel chemical entities.[4] These chemical entities or secondary metabolites are reported to possessed several biological and pharmacological properties.[5-7] *Globimetula braunii* is a parasitic plant growing on many dicotyledones trees.[8] It is widely distributed in tropical countries and traditionally is used in the treatment of various illnesses such as hypertension ulcer and cancer.[9] Scientific studies have shown that *Globimetula braunii* growing on *Terminalia catappa* possessed antiepileptic activity.[10]

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The leaf extract and fractions of *Globimetula* braunii leaf growing on *Azadirachta indica* was reported to have a significant blood glucose reduction effect in adult Wistar rats.[11] Furthermore, the ethanol leaf extract *Globimetula braunii* growing on *Leucena leucocephala* was found to have an antihyperglycemic effect comparable to glibenclamide.[12] Moreover, the ethyl acetate and methanol leaf extracts of *Globimetula braunii* growing on *Piliostigma thonningii* were found to have strong antioxidant activity.[13] Phytochemical studies of *Globimetula braunii* on *Terminalia catappa* revealed that the plant contained steroids, triterpenes, tannins, saponins, alkaloids and flavonoids.[10] From the limited literature search, there is no compound isolated from *Globimetula braunii* growing on *Terminalia catappa*. However, two lactones; (R)-6-[(S)-2-hydroxy-4-(4-hydroxyphenyl)butyl]-5,6-dihydropyran-2-one and (1R,5S,7S)-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]-nonan-3-one, and five flavonoids; quercetin, (+)-catechin, quercitrin, rutin and avicularin were isolated from the leaf extract of *Globimetula braunii* growing on *Piliostigma thonningii*.[14] Additionally, two compounds (13, 27-cycloursan-3-one and methyl 2, 6-dihydroxy-4-methoxybenzoate) were isolated from ethanol leaf extract of *Globimetula braunii* growing on *Leucena leucocephala*.[12]
This study aimed to isolate some phytochemical compounds present in *Globimetula braunii* (Loranthaceae) growing on *Terminalia catappa* using chromatographic techniques and to characterize them using chemical and spectroscopic techniques.

Materials and Methods

Collection and identification of plant material
Globimetula braunii growing on *Terminalia catappa* was collected in December 2017, from its natural habitat around Aminu Kano Teaching Hospital, Zaria Road, Tarauni Local Government Area, Kano State. The plant was identified and authenticated by Mallam Namadi Sunusi of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, by comparing with voucher specimen number (2839) already deposited in the herbarium. The

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leaves of *Globimetula braunii* were washed with water, air-dried and pulverized.

Extraction and fractionation

The pulverized sample (2500 g) was extracted by maceration with 20 L 90% v/v ethanol. The filtrate collected was concentrated in a rotatory evaporator at 40°C and reduced pressure. The ethanol crude extract was successively partitioned with n-hexane, chloroform, ethyl acetate and n-butanol. The fractions (n-hexane, chloroform, ethyl acetate and n-butanol) were concentrated and dried.

Isolation of flavonols from butanol fraction

The n-butanol fraction (3.0 g) was subjected to silica gel (60-120 mesh size) column chromatography (2.9 cm by 70 cm). The column was eluted first with 50 ml of chloroform 100%, then chloroform-methanol mixture (95:5, 90:10, 85:15, 80:20, 75:25 and 70:30) and finally methanol 100% as solvent systems to give a total of 128 fractions. The collections were pooled together based on their TLC profiles to give 21 major fractions coded A-U. Repeated gel filtration of three major-fraction (J, K and L) with Sephadex LH-20 using methanol as eluting solvent led to the isolation of three compounds; GB_1, GB_2 and GB_3 (5 mg, 7 mg and 10 mg respectively).

Spectroscopic characterization

The isolated compounds (GB_1, GB_2 and GB_3) were characterized using chemical and spectroscopic techniques (UV, IR and NMR). The NMR was recorded on a Bruker Avance spectrometer (400 MHz) for ^{1}H-and (100 MHz) for ^{13}C-NMR using the residual solvent peak as reference. UV spectra were recorded on Jenway 7315 Spectrophotometer. The IR spectra were measured on a Cary630 Agilent technologies Fourier transform infrared spectrophotometer.

Results and Discussion

Compound GB_1 was isolated as a yellow crystalline powder. It was found to melt between 294-296 °C. GB_1 gave a positive $FeCl_3$ test for phenols. The UV spectrum shows absorption at λ max 255 nm and 365 nm. The absorption at 255 nm is due to the ring A (benzoyl system) whereas absorption at 365 nm is considered to be due to the ring B (cinnamoyl) system, this suggested that GB_1 has a flavonoid nucleus.[15] The IR showed weak broad absorption at 3210 cm^{-1} which is O-H vibration, moderate narrow absorption at 2914 cm^{-1} and 2847 cm^{-1} was due to the C-H stretching and aromatic overtone was observed at 1987 cm^{-1} and absorption at 1663 cm^{-1} was due to C=O.[16]

The ^{1}H-NMR (DMSO, 400 MHz) spectrum showed five protons at the aromatic region. δ 6.34 (1H) and δ 6.70 (1H) were assigned to H-6 and H-8 protons respectively. The doublet signals at δ 6.89 (1H, d, J = 8.28 Hz) and δ 7.57 (1H, d, J = 8.04 Hz) were attributed to H-5' and H-6' protons of ring B. And $J \approx 8$ Hz indicated that the protons are ortho-coupled. The signal at δ 7.7I (1H) was assigned to H-2'. The presence of broad singlet at δ 12.46 (1H brs) indicated the presence of phenolic OH.

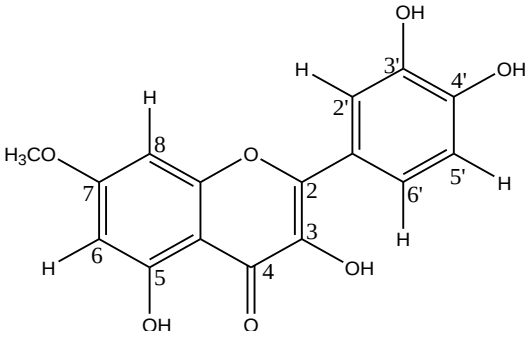
The ^{13}C-NMR spectrum showed signals at δc 176.41 (C=O), δc 56.47 (OCH3). The signals at δc 165.36, δc 160.80, δc 156.53, δc 148.37, δc 147.78, δc 145.56, δc 138.48 δc 122.28, δc and 104.45 were due to quartenery carbon atoms. The signals at δc 120.56, δc 116.03, δc 115.64, δc 97.90 and δc 92.35 were due to methine carbon atom. The analysis of ^{1}H and ^{13}C NMR spectra and comparison with literature,[17] led to the conclusion that GB_1 is Rhamnetin (Figure 1).

Compound GB_2 was isolated as a yellowish powder substance weighed 10 mg. It was found to be sparingly soluble in chloroform and completely soluble in methanol. The compound was found to melt between 315-318. The UV spectrum of GB_2 showed two major absorption maxima typical of flavonoids. The absorption at λ max 255 nm is due to A-ring (benzoyl system) and at 370 nm due to ring B (cinnamoyl system) system.[15] The IR demonstrated weak broad absorption at 3280 cm^{-1} which is due to O-H vibration, weak absorption at 2962 cm^{-1} and 2921 cm^{-1} was due to the C-H stretching respectively. Aromatic overtone was observed at 1772-1994 cm^{-1} and absorption at 1514 cm^{-1} was due to C-H bending in the aromatic ring.[161]H NMR (MeOD, 400 MHz) and ^{13}C-NMR spectra of GB_2 showed signals similar to those of GB_1. However, the methoxy group is absent on both ^{1}H and ^{13}C-NMR spectra of GB_2. This showed that GB_2 signals are similar to quercetin. δ 6.08 (1H, H-6), δ 6.29 (1H, H-8), δ 7.64 (IH, H-2'), δ 6.78 (1H, d, J = 8.44 Hz, H-5') and δ 7.53, (IH, d, J = 8.50 Hz, H-6').

The ^{13}C-NMR: The presence of a signal at 175.91 indicated that the compound has a carbonyl group. The presence of signals at the downfield (δ 161.09 and δ 164.32) suggested that the carbon in the aromatic ring is attached to the OH group.

The COSY spectrum showed correlation between δ 6.78 (1H, d, J = 8.44 Hz, H-5') and δ 7.53 (IH, d, J = 8.50 Hz, H-6'). Both H-5' and H-6' protons have $J \approx 8$ Hz indicating that they are ortho coupled.

HSQC spectrum established protons that are attached to their respective carbon. The following signals were found to have cross peak correlation δH 6.08 and δc 97.91, δH 6.29 and and δc 93.07, δH 7.64 and δc 114.61, δH 6.78 and δc114.84, δH 7.53 and δc 120.29.



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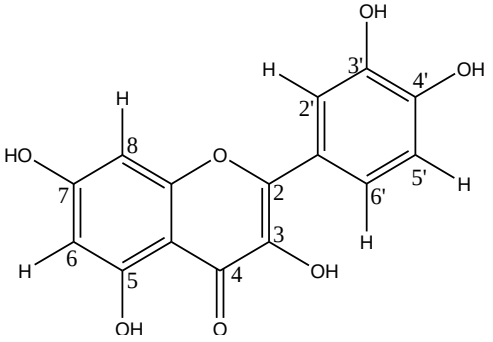

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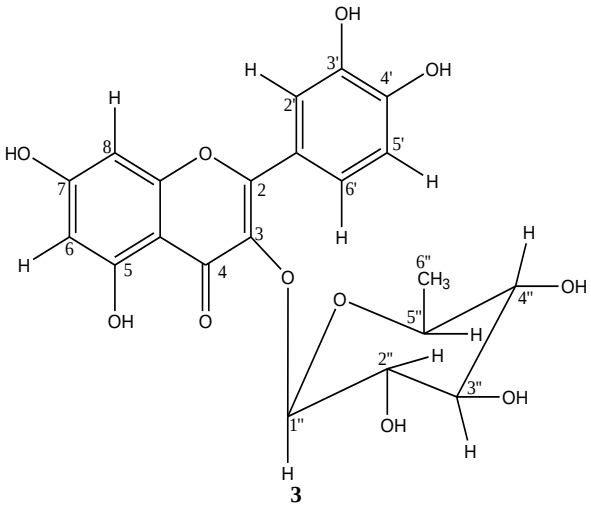

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Figure 1: Chemical structures of flavonoids isolated from the leaves of *Globimetula braunii* (**1**) Rhamnetin (**2**) Quercetin (**3**) Rhamnetin-3-*O*-α-L-rhamnopyranoside

HMBC correlation showed that H-5′ (δ 6.78, 1H, d, *J* = 8.44 Hz) correlated with C-1′ (δ 122.77), C-3′ (δ 144.83) and C-2 (δ 147.37). The long-range correlation between H-5′ and C-2 showed that ring B is connected to ring C. H-6′ (7.53, IH, d, 8.50 Hz) correlated with C-2′ (δ 114.61), C-4′ (δ 146.61). Additionally, H-2′ (7.64, IH) correlated with C-6′ (δ 120.29). The H-8 (6.29, 1H) also correlated with C-2 (δ 147.37), whereas H-6 (6.08, 1H) showed correlation with C-5 (δ 161.09), C7 (164.32), C-8 (δ 93.07) C-10 (δ 103.09). The analysis of ^{1}H, ^{13}C-NMR, COSY and HMBC spectra and comparison with literature,[17] led to the conclusion that GB_2 is Quercetin (Figure 1).

Compound GB_3 was found to be a yellowish amorphous substance weighing 8 mg. It was found to be sparingly soluble in chloroform and completely soluble in methanol. The compound was found to melt between 202-207°C. GB_3 gave a positive $FeCl_3$ test for phenol. The UV spectrum of GB_3 is similar to that of GB_1 and GB_2; it showed two major absorption maxima at λ max 230 nm and 350 nm for A-ring (benzoyl system) and B-ring (cinnamoyl system) respectively.[15] The IR demonstrated weak broad absorption at 3294 cm^{-1} which is O-H vibration, very weak absorption at around 2900 cm^{-1} due to due to C-H stretching. Aromatic overtone was observed at a range of 1830 to 2113 and absorption at 1595 was due to C-H bending in the aromatic ring.[16]

^{1}H-NMR (MeOD, 400 MHz) showed similar signals with GB_1 in the aromatic region 6-7 ppm; H-6 (δ 6.22 1H), H-8 (δ 6.45, 1H), H-2′ (δ 7.33, 1H), H-5′ (δ 6.82, 1H, d, *J* = 8.16 Hz), H-6′ (δ 7.23, 1H, d, *J* = 8.16 Hz) and OCH_3 at δ 3.77 (3H, s). This showed that GB_3 has a rhamnetin aglycon portion. The signal for anomeric proton appears at δ 5.27 (1H, s, H-1′′) indicating α-configuration and presence of doublet at δ 0.85 (3H, d, *J* = 6.04 Hz) suggested that, the α-L-rhamnose sugar is present.

The ^{13}C-NMR spectrum showed signals at δ 102.15, δ 70.52, δ 70.66, δ 70.73, δ 70.87 and δ 16.26 suggested that the GB_3 has a sugar portion possibly rhamnose. Other signals seem to be quite similar to that of GB_1. This suggested that GB_1 and GB_3 have a similar flavonoid (Rhamnetin).

HMBC spectrum showed that there is a correlation between δ 3.77 (3H, s) and C-7 (δ 165.84), indicating that, OCH_3 group is attached to C-7 (Ring A). Anomeric proton H-1′′ (δ 5.27, 1H, s) shows correlation with C-3 (**δ** 135.05) suggesting that the glycon moiety (rhamnose sugar) is attached to C-3 of aglycone moiety (ring C) by a C-O-C linkage. H-6 (δ 6.22, 1H) shows correlation with C-5 (δ 161.53), C-7 (δ 165.84), C-8 (δ 91.72) and C-10 (δ 105.35). Additionally, H-8 (δ 6.45, 1H) shows correlation with C-6 (δ 97.60), C-7 (δ 165.84), C-9 (δ 157.00) and C-10 (δ 105.35). In ring B correlations were observed, H-2′ (δ 7.33, 1H) correlated with C-4′ (δ 148.50) and C-6′ (δ 121.54). Moreover, H-5′ (6.82, 1H, d, *J* = 8.16 Hz) showed long range correlation with C-3′ (δ 145.04) and C-6′ (δ 121.54). H-6′ (δ 7.23, 1H, d, *J* = 8.16 Hz) shows correlation with C-2 (δ 158.23) and C-4′ (δ 148.50) indicating the link between ring B and C. The analysis of ^{1}H, ^{13}C NMR, COSY and HMBC spectra and comparison with literature, [17,18,19,20] led to the conclusion that GB_3 is rhamnetin-3-*O*-rhamnoside (Figure 1).

Flavonoids are important secondary metabolites with various biological and pharmacological activities such as antioxidant, anticancer and sedative activity.[21] In the CNS several flavones bind to the benzodiazepine site on the γ-aminobutyric acid type A (GABAA) receptor resulting in sedation, anxiolytic or anti-convulsive effects.[22] The CNS activity of flavonoids is due to their ability to traverse the BBB and enter the CNS.[22] The flavonoids isolated in this study might possess anxiolytic as well as sedative properties. As reported from the literature quercetin-3-O-(6′′-feruloyl)-*β*-D-galactopyranoside possessed CNS depressant activity.[23] Moreover, some glycosides such as 2S-neohesperidin, 2S-naringin, diosmin, gossipyn and rutin were found to have CNS depressant activity in mice.[24] Rhamnetin was also found to suppress the growth of human breast cancer.[25] Therefore, *Globimetula braunii* growing on *Terminalia catappa* can be considered as an important herbal medicinal plant for the treatment of various diseases due to the phytochemical constituents it contained.

Conclusion

In this study, three flavonols (rhamnetin, quercetin and rhamnetin-3-*O*-α-L-rhamnoside) were isolated from ethanol leaf extract of *Globimetula braunii* (Loranthaceae) growing on *Terminalia catappa* (Combretaceae). The compounds were characterized using chemical and spectroscopic techniques.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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