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Isolation of Rutin from *Neocarya macrophylla* leaves (Sabine) Prance (Chrysobalanaceae)

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Abstract

Neocarya macrophylla is a medicinal plant which belong to the Chrysobalanaceae family. It has many ethnomedicinal uses such as treatment of snake bite, pain and inflammation. In this work, a flavonoid diglycoside was isolated from the ethylacetate fraction of the methanol leaf extract of the plant. The compound was isolated using a combination of silica gel and sephadex LH-20 column chromatography. Using 1H and 13C-NMR spectroscopy followed by comparison with previously established data in the literature, the structure of the compound elucidated was, 3′, 4′, 5, 7-tetrahydroxyflavone-3-(α-L-rhamnopyronosyl-(1→6)-β-D-glucopyranose) (quercetin-3-O-rutinoside) known as Rutin. It is a flavonoid diglycoside that is used to treat and manage a variety of conditions such as pain and inflammation. To the best of our knowledge, this is the first report of isolation and characterization of rutin from the leaves of N. macrophylla.

Keywords: Neocarya macrophylla, Isolation, Chracterization, Rutin, NMR Spectroscopy

Introduction

Neocarya is a category in the Chrysobalanaceae family, which was described by Prance ex White in 1976. The genus consists of just one species, known as *Neocarya macrophylla*, which was identified by Dressler *et al*. [1]. It is a small tree that usually grows up to 10 meters in height. Its crown is rounded and bushy, and the branches are covered in fine, matted hairs, according to Bayero *et al*. [2]. The Chrysobalanaceae family is made up of about 525 species and 17 genera, mostly consisting of woody plants, shrubs, and trees found in tropical and subtropical regions, as reported by Yakandalawa *et al*. [3]. The characteristics of the plant include a glabrous skin with gray warts and a yellowish-brown ellipsoid drupe fruit. The leaves grow up 10 to 25 cm in length and 5 to 15 cm in width which are coriaceous, ovate, or elliptic and have a downy underside Arbonnier [4]. The leaf blade typically has a rounded, acuminate apex, and a cordate base, as described by Burkill [5]. These characteristics collectively provide a comprehensive description of *N. macrophylla*. The plant is traditionally used as food, for medicinal, spiritual and industrial purposes. It is notable for its use as good tannin agent in soap making, dye, glue, firewood, fodder, insecticide and for structural materials. Yusuf *et al*. [6] reported recent studies

on the physicochemical, nutritional contents, phytochemical and pharmacological activities which have validated the benefits of *N. macrophylla* to humanity in food, cosmetics and pharmaceutical products. Steroids and flavonoids (including stigmasterol, catechin, quercetin and its glycosides) are the primary bioactive components found in the plant thus far. The plant extracts exhibit positive effects in combating venom, microbes, pain, inflammation, mycobacteria, parasitic worms, and oxidative stress. However, studies have proven that the plant is toxic in cases of acute exposure. [6]

In this study, we detail the process of isolating and analysing Rutin from the ethylacetate fraction of the methanol leaf extract of N. macrophylla through the application of chromatographic methods and ^{1}H and ^{13}C-NMR spectroscopy. The isolation and characterization of Rutin can provide valuable information for determining its suitability as a potential medication for various therapeutic or pharmacological purposes within the human body.

Materials and Methods

General procedures

Sigma Aldrich in Germany pre-coated aluminium sheets with silica gel 60 GF_{254} was used for the purpose of conducting Thin Layer Chromatography (TLC). To visualize the TLC plates, a general spraying reagent of 10% H_2SO_4 was sprayed followed by heating at 102 ˚C for 10 min. Sephadex LH-20 was used for Gel Filtration Chromatography. ^{1}H and ^{13}C- NMR data was recorded on a Bruker AVANCE spectrometer (500 MHz) with residual solvent being used as the internal standard. The Melting point of the isolated compound was determined using an Electro thermal melting point apparatus.

Plant sample

In June 2019, plant samples of *N. macrophylla* were obtained from Jega Local Government area located in Kebbi State, Nigeria. The identification of the plant was carried out by Musa Magaji who works at the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. A voucher specimen number (PCG/UDUS/CHRY/001) was obtained. Following the gathering of the plant, the leaves were washed thoroughly with water to get rid of any impurities on the surface and were dried in the shade for three weeks. After drying, they were ground into a fine powder and stored in a plastic container before use.

Extraction and isolation

The maceration method was used to extract 1961 grams of pulverized leaves with 90% methanol over 7 days, with occasional stirring. The resulting extract was filtered using Whatman No.1 filter paper and then evaporated using a rotary evaporator at 40 ˚C until a residue was obtained, which was called the methanol leaf extract (MEL). Following the extraction method of Yusuf et al. [7], a portion of the MEL (60 g) was suspended in

distilled water, filtered, and the resulting filtrate was partitioned into chloroform, ethyl acetate, n-butanol, and aqueous fractions.

The ethylacetate fraction (EF, 4.50 g) was gradiently eluted in a silica gel packed column using different solvent combinations starting with hexane: ethylacetate (9:1), chloroform (100 %) to chloroform: methanol (9.5: 0.5). Thirty (30) cm^3 each of a total of 277 fractions were collected and merged based on their TLC profile to give 18 major fractions coded E1-E18. Fraction E8 was subjected to further purification with sephadex LH-20 using methanol as solvent, 2 cm^3 each of a total of 33 collections were made and combined based on their TLC profile to afford 5 major fractions coded F1-F5. Fraction F4 was purified using gel filtration repeatedly to give fractions F4A-F4E and further purification of F4C using sephadex LH-20 led to the isolation of a yellow amorphous substance (5 mg) coded compound C_2. TLC analysis of the compound using ethylacetate: chloroform: methanol: water (15: 4: 4: 1) and chloroform: methanol (1: 1) as mobile phase gave single homogenous spot and the compound was subjected to characterization using ^{1}H and ^{13}C-NMR spectroscopic analysis and the melting point was determined.

Results and Discussion

Spectral data

Proton NMR analysis of C_2

M.p. 240 – 242 ^{o}C. ^{1}H-NMR (500MHz CD_3OD) revealed resonanses at δ_H 7.78(1H, *d*, *J*=9.0Hz,H-2″), 7.53(1H, *dd*, *J*=2.0, 8.5Hz, H-6′), 6.95(1H, *d*, *J*=8.5Hz, H-5′), 6.39(1H, *d*, *J*=1.5Hz, H-8), 6.21(1H, *d*, *J*=2.0Hz, H-6), 5.47 (1H, *s*, H-1″), 3.60 (1H, *m*, H-2″), 3.58 (1H, *m*, H-3″), 4.33 (1H, *br s*, H-1‴), 3.57 (1H, *m*, H-3‴), 3.50 (1H, *m*, H-4‴), 3.51 1H, *m*, H-5‴), 1.22 (3H, *br s*, H-6‴).

^{13}C and APT NMR spectral analysis of C_2

The ^{13}C-NMR (500 MHz, CD_3OD) and APT experiments of C_2 indicated the presence 27 carbon atoms; δ_C 177.8 (C-4), 165.1 (C-5), 160.8 (C-7), 157.0 (C-2), 146.5 (C-4′), 145.5 (C-3′), 130.5 (C-3), 121.5 (C-1′),115.1 (C-2′), 115.4 (C-5′), 102.1 (C-10), 108.1 (C-1″), 98.6 (C-1‴),93.4 (C-8), 71.8 (C-4″), 77.3 (C-5″), 63.0 (C-6″), 70.7 (C-2‴), 70.5 (C-3‴), 70.6 (C-4‴), 16.3 (C-6‴).

The isolated compound (C_2) was obtained as a yellow amorphous substance, which gave a single homogenous spot with two different solvent systems Gibbons and Gray [8] and the uncorrected melting point of the compound was between 240 – 242 ˚C indicating it to be pure. The ^{1}H-NMR spectrum of compound C_2 indicated the presence of 1,2,3,5-tetrasubstituted benzene ring A via the meta-coupled protons at δ_H 6.39 (1H, *d*, *J*=1.5 Hz) and δ_H 6.21 (1H, *d*, J=2.0 Hz) ,which were assigned to H-8 and H-6 respectively and 1,3,4-trisubstituted benzene ring B was observed via protons at δ_H 7.78(1H, *d*, *J*=9.0 Hz, H-2″), δ_H 6.95(1H, *d*, *J*=8.5 Hz, H-5′) and δ_H 7.53(1H, *dd*, *J*=2.0, 8.5 Hz, H-6′). These values were in total agreement with those reported for quercetin nucleus [9]. It also revealed two signals at δ_H 5.47

(1H, *s*, H-1″) and δ_H 4.33 (1H, *br s*, H-1‴) and typical of β- and α- anomeric protons of sugar respectively. Overlaping signals due to carbinol protons were observed between δ_H 3.51 – 3.57 confirmimg the presence of sugar moiety; the resonances at δ_H 1.22 (3H, *m*, H-6‴) was ascribable to rhamnose [10] (see Table 1).

The ^{13}C-NMR of compound C_2 revealed the presence 27 carbon atoms and APT experiments revealed their multiplicities as one methyl, one methylene, fifteen methine, and ten quaternary carbons. The down field signal due to carbonyl carbon resonated at 177.8 (C-4), other signals observed were 165.1 (C-5), 160.8 (C-7), 157.0 (C-2), 130.5 (C-3), 102.1 (C-10), 98.6 (C-6), 93.4 (C-8), 121.5 (C-1′), 115.1 (C-2′), 145.5 (C-3′), 146.5 (C-4′), 115.4 (C-5′), typical of a flavonoidal-quercetin nuclues [11]. This was in aggreement with those reported for quercetin by Abdullahi *et al*. [10] and Abdullahi *et al*. [12]. The chemical shift values at 108.1 (C-1″), 72.5 (C-3″), 71.8 (C-4″), 77.3 (C-5″), 63.0 (C-6″), 98.6 (C-1‴), 70.7 (C-2‴), 70.5 (C-3‴), 70.6 (C-4‴), and 16.3 (C-6‴) were characteristic of sugar absorptions and the -CH_2 (63.0) and CH_3 (16.3) suggests the sugars to be glucose and rhamnose, in aggrement with previously reported work by Abdullahi *et al*. [10] and Abdullahi *et al*. [12] (Table 2). Based on the ^{1}H and ^{13}C-NMR data of compound C_2, and comparison with establised data in the literature by Abdullahi *et al*. [10] and Abdullahi *et al*. [12] (Table 2), a tentative structure of compound C_2 was proposed to be quercetin-3-*O*--(α-L-rhamnopyronosyl-(1→6)-β-D-glucopyranose) (Rutin) (Figure 1).

Rutin, a recognized member of the flavonoid family, possesses a quercetin nucleus and is known for its anti-oxidant activity that safeguards the body from cellular damage due to free radicals [15]. It also has pain-relieving [13] inflammation-reducing [14]and cancer-fighting properties [15]. Rutin is helpful in treating hypertension [13], infections [14], atherosclerosis [15], osteoarthritis, and haemorrhoids [15]. It can also prevent stroke and high cholesterol [16].

Conclusion

Chromatograhic separation of the ethylacetate fraction of the methanol leaf extract of *N. macrophylla* led to the isolation and characterization of quercetin-3-*O*-(α-L-rhamnopyronosyl-(1→6)-β-D-glucopyranose) (Rutin). To the best of our knowledge, this compound is isolated for the first time from ethyl acetate fraction of the plant extract. However, further studies on isolation and characterization should be conducted on other fractions of the plant extracts such as chloroform and n-butanol. Overall, *N. macrophylla* is a valuable plant species with a range of traditional, industrial, and medicinal uses. Its physical characteristics make it easily identifiable, and its bioactive constituents provide a basis for the plant's potential medicinal benefits. While it is noted as toxic, further studies on its toxicity and potential therapeutic effects may pave

the way for its use in future pharmaceutical research.

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Conflict of Interest

The authors declared that they have no conflict of interests.

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Table 1: Summary of ^{1}H and ^{13}C-NMR data of compound C_2

Position	^{1}H-NMR (*J* in Hz)	^{13}C-NMR	APT
2	-	157.0	C
3	-	130.5	C
4	-	177.8	C
5	-	165.1	C
6	6.21(1H, *d*, *J*=2.0)	98.6	CH
7	-	160.8	C
8	6.39(1H, *d*, *J*=1.5)	93.4	CH
9	-	-	-
10	-	102.1	C
1′	-	121.5	C
2′	7.78(1H, *d*, *J*=9.0)	115.1	CH
3′	-	145.5	C
4′	-	146.5	C
5′	6.95(1H, *d*, *J*=8.5)	115.4	CH
6′	7.53(1H, *dd*, *J*=2.0, 8.5)	-	-
1″	5.47(1H, *s*)	108.1	CH
2″	3.60(1H, *m*)	-	-
3″	3.58(1H, *m*)	72.5	CH
4″	-	71.8	CH
5″	-	77.3	CH
6″	-	63.0	CH_2
1‴	4.33(1H, *br s*)	98.6	CH
2‴	-	70.7	CH
3‴	3.57(1H, *m*)	70.5	CH
4‴	3.50(1H, *m*)	70.6	CH
5‴	3.51(1H, *m*)	-	CH
6‴	1.22(3H, *m*)	16.3	CH_3

Table 2: ^{1}H and ^{13} C-NMR data of compound C_2 compared with reported literature

Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
	Compound C_2		**Literature 1**		**Literature 2**	
2	-	157.0	-	156.4	-	157.1
3	-	130.5	-	133.2	-	134.5
4	-	177.8	-	177.3	-	178.1
5	-	165.1	-	161.2	-	164.8
6	6.21(1H, *d*, *J*=2.0)	98.6	6.19	98.8	6.23	98.6
7	-	160.8	-	164.2	-	161.6
8	6.39(1H, *d*, *J*=1.5)	93.4	6.38	94.1	6.43	93.4
9	-	_	-	156.6	-	157.6
10	-	102.1	-	103.9	-	104.1
1′	-	121.5	-	121.1	-	121.4
2′	7.78(1H, *d*, *J*=9.0)	115.1	7.55	116.2	7.90	116.6
3′	-	145.5	-	144.7	-	144.4
4′	-	146.5	-	148.4	-	148.6
5′	6.95(1H, *d*, *J*=8.5)	115.4	6.84	115.2	6.89	114.7
6′	7.53(1H, *dd*, *J*=2.0, 8.5)	-	7.55	121.5	7.62	121.6
1″	5.47(1H, *s*)	108.1	5.34	101.2	5.10	104.6
2″	3.60(1H, *m*)	-	3.22	74.0	3.60	73.7
3″	3.58(1H, *m*)	72.5	3.23	76.4	3.58	72.5
4″	-	71.8	3.32	70.5	3.61	71.7
5″	-	77.3	2.96	75.9	3.67	73.9
6″	-	63.0	3.70	66.9	3.85	62.9
1‴	4.33(1H, *br s*)	98.6	4.36	100.7	4.55	100.5
2‴	-	70.7	2.96	70.2	3.77	70.9
3‴	3.57(1H, *m*)	70.5	3.23	70.6	3.55	68.8
4‴	3.50(1H, *m*)	70.6	3.07	71.8	3.53	70.7
5‴	3.51(1H, *m*)	-	3.23	68.2	3.51	68.3
6‴	1.22(3H, *m*)	16.3	0.99	17.7	1.21	16.6

Compound C_2: CD_3OD, 500 MHz

Literature 1: DMSO-*d6*, 400 MHz reported by Abdullahi *et al*. [10]

Literature 2: CD_3OD, 400 MHz reported by Abdullahi *et al*. [12]

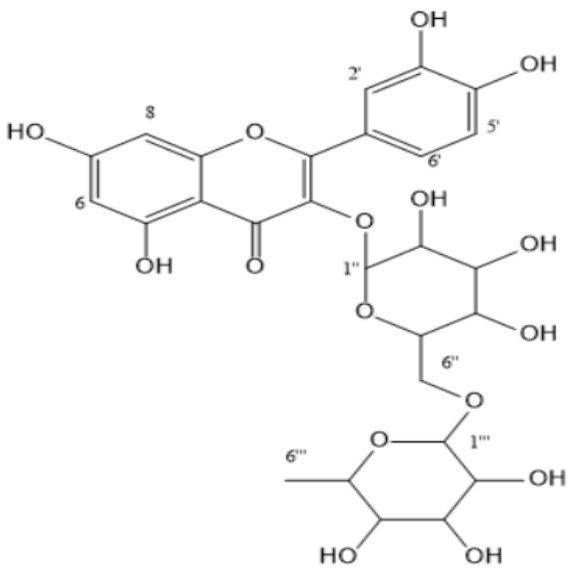

Figure 1: 3′, 4′-5, 7-tetrahydroxyflavone-3-*O*-(α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranose) (quercetin-3-*O*-rutinoside)

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