Chemistry Research Journal, 2017, 2(5):270-274

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Quercetin from *n*-butanol Soluble Fraction of Crude Ethanol Leaf Extract of *Cadaba Farinosa* Forssk

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Abstract The plant *Cadaba farinosa* Forssk (Capparaceae) is distributed throughout the world in mostly tropical and sub-tropical regions. It is used in treatment of pains, dysentery, rheumatism, cough, fever, as antidote and neurological disorders. Phytochemical screening of the leaf revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins and tannins. Silica gel column chromatography of the *n*-butanol soluble fraction (NBU) followed by gel filtration using sephadex LH-20 and PTLC led to isolation of a Flavonol aglycone characterised as Quercetin by spectroscopic techniques. The complete ^{1}H and ^{13}C NMR spectral assignments of the isolated compound is reported herewith on the basis of 1D (^{1}H and ^{13}C) and 2D (COSY, NOESY, HSQC, HMBC) NMR.

Keywords *Cadaba farinosa*, ^{1}H NMR, ^{13}C NMR, TLC plates, sephadex LH-20, silica gel

Introduction

Cadaba farinosa Forssk belong to the family Capparidaceae (Capparaceae). It is distributed throughout the world, mostly in tropical and subtropical region. The plants are usually herbs, erect or scan dent, shrubs and rarely trees in dry short grass savannas. The Leaves are entire, simple, silvery gray and with simple scales [1]. It is locally called *bagayi* or *hanza* in HAUSA while KANURIS call it *bultu* in Nigeria [2].

Materials and Methods

Collection, Identification and Preparation of Plant Material

Fresh leaf samples of *Cadaba farinosa* were collected from Maiduguri Metropolitan Council Area of Borno State, Nigeria. The plant specimen was identified and authenticated at the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria which corresponded with that of voucher specimen number V/No: 2744. The leaf was air-dried under shade for several days and pulverised into fine powder for extraction.

Extraction of Plant Material

The air-dried ground powdered leaf material (1,500 g) was extracted exhaustively with 70 % ethanol using cold maceration method for several days with occasional shaking. The crude ethanol leaf extract was concentrated to

dryness on water bath at 50 ^{o}C and coded CEE – crude ethanol leaf extract of *Cadaba farinosa*. The coded extract (CEE) served as the working sample for the chemical investigations of the plant. This crude extract (CEE) was further subjected to solvents partitioning. After concentration on water bath at 50 ^{o}C to a dark gummy mass, it was dissolved in 500 ml distilled water and partitioned with ethyl acetate (EAE) to give 2.97 g dark green mass. The aqueous portion was further partitioned with *n*-butanol (NBU) to give 34.33 g dark brown mass and an aqueous (residual) portion.

Reagents

Unless otherwise stated in the text, all chemicals and reagents used were of Analar grade (Riedel-de-Häen, Sigma-Aldrich, Fluka, Germany). The Silica gel for column chromatography was BDH laboratory reagents (silica gel of mesh size 60-120 µm) by BDH Chemicals Ltd Poole England (Product No: 15049) and that of thin layer chromatography (TLC) was Kieselgel 60 G (Merck®) made to a thickness of 0.25 mm, pre-coated aluminium TLC plate {20 x 20 cm} (Merck® Germany); TLC Silica gel 60 F_{254} on 20 X 20 cm 25 Aluminium sheets (1.05554.0007) Merck KGaA 64271 Darmstadt, Germany; Sephadex LH-20 for column chromatography (Pharmacia, Biotech, Sweden), Maceration apparatus, Whatman's qualitative filter paper (24.0 cm; Cat No 1001 240) Whatman International Ltd Maidstone England, Burette diameter used for Sephadex LH-20 (Fluka) = 1 cm (50 mls Borosilicate glass); Column (4.5 cm = 1.85 inches diameter with capacity of 500 mls - West Germany).

Equipments

Electro thermal melting point machine, Bruker (AVANCE II/III) NMR spectrophotometer at School of Chemistry and Physics, University of Kwa-Zulu Natal-Durban, South Africa was used for 1D- and 2D-NMR spectroscopy, Oven, ChemDraw Ultra 7.0.

Phytochemical Screening

A little quantity each of the CEE (crude ethanol leaf extract) was subjected to qualitative phytochemical screening to test for the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins and tannins as described [3-7].

Column Chromatographic Separation of *n*-butanol (NBU)

The NBU was used in the isolation procedure. This fraction was obtained as outlined previously. The conventional separation techniques (column and thin layer chromatography) were employed using gradient flow procedure utilising silica gel (60-120 µm mesh size) and sephadex LH-20 as adsorbent where possible

NBU was subjected to column chromatographic separation using Column of dimension 500 mls X 4.5 cm *id*., was packed manually with 500 g silica gel with mesh size of 60-120 µm, mounted unto a stand and allowed to settle and pack for 24 hrs. A 15 g of NBU was weighed, mixed with 5 g silica gel and grounded using pestle and mortar then carefully applied unto the packed column. It was gradiently and successively eluted with chloroform (100 %), chloroform: methanol mixtures (by increasing the polarity by 5 %) until methanol (100 %) was attained at a flow rate of 1 ml/min.

Each eluent's fraction (50 ml aliquots) was collected and the separation monitored by analytical TLC plates which were pre-coated aluminium TLC plates. Eluents of similar R_f values from same TLC solvents system were pooled together. The complete elution profile yielded a total of 235 fractions (50 mls/fraction) which were pooled based on the R_f values on analytical TLC plates to give 35 sub-fractions. Among the 35 sub-fractions, the pooled fraction coded 86-103 was large and gave variety of spots on TLC plates using a number of solvent systems e.g. $CHCl_3$: CH_3OH: H_2O (4: 2: 1 v/v/v); BAW {n-but: CH_3COOH: H_2O (4: 1: 5 v/v/v) upper layer} and EtOAc: CHCl3: MeOH (4: 8: 1 v/v/v) solvent systems and 10 % conc. H_2SO_4 as developmental solvent. This pooled fraction was subjected to further purification over sephadex LH-20 using methanol as eluting solvent.

Isolation of compound D67M

Pooled fraction coded 86-103 was dissolved in MeOH. A 5 mls each was separately loaded unto sephadex LH-20 eluted with methanol. Eluents were collected and monitored on TLC plates using BAW as the solvent system and PTLC which subsequently led to the isolation of D67M (kaempferol).

RESULTS

Phytochemical Screening

The extractive value for the CEE of *Cadaba farinosa* from 1,500 g plant material was found to be 12.63 % w/w (189.37 g; dark gummy mass). The preliminary phytochemical examinations of the crude and the solvents partitioned portions revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins and tannins.

Table 1: Phytochemical constituents of CEE of *Cadaba farinosa* Forssk

S. No.	Group Constituents	Inference CEE
1	ALKALOIDS	+
2	ANTHRAQUINONES	+
3	CARBOHYDRATES	+
4	CARDIAC GLYCOSIDES	+
5	FLAVONOIDS	+
6	GLYCOSIDES	+
7	SAPONINS	+
8	TANNINS	+

Discussion

^{1}H NMR spectral analysis of D67M

The ^{1}H NMR spectrum of D67M in $CDCl_3$ revealed the presence of resonances at δ 6.20 (H-6) (1H, *d*, *J* = 1.3 Hz); δ 6.41 (H-8) (1H, *d*, *J* = 1.4 Hz); δ 6.91 (H-5') (1H, *d*, *J* = 8.5 Hz); δ 7.66 (δ 7.65, δ 7.67) (H-6') (1H, *dd*, *J* = 1.8, 1.4 Hz) and δ 7.76 (2'-H) (1H, *d*, *J* = 1.3 Hz) in the aromatic region of δ 6-8 ppm.

^{13}C NMR spectral analysis of D67M

The ^{13}C NMR (δ ppm, 600 MHz, CD_3OD) experiment showed the presence of 15 carbon atoms; δ_C 93.20 (CH), δ_C 98.09 (CH), δ_C 102.99 (C), δ_C 114.61 (CH), δ_C 114.87 (CH), δ_C 120.30 (C), δ_C 122.78 (CH), δ_C 135.77 (C), δ_C 144.85 (C), δ_C 146.60 (C), δ_C 147.41 (C), δ_C 156.91 (C), δ_C 161.06 (C), δ_C 164.77 (C), δ_C 175.90 (C).

^{1}H ^{1}H COSY spectral analysis of D67M

The nature of aromatic substitution and protons situated in the same chemical environment are indicated in a ^{1}H ^{1}H Correlation Spectroscopy spectrum. Major correlations include the following: 6.20 (H-6) and 6.41 (H-8); 6.91 (H-5') and 7.66 (H-6'); 7.66 (H-6') and 7.76 (H-2') among others established the presence of 2 fragments (A and B).

^{1}H ^{1}H NOESY spectral analysis of D67M in CD_3OD

The Nuclear Over-Hauser Effect Spectroscopy result of D67M revealed correlations between protons at δ_H 6.20 (H-6) and δ_H 6.41 (H-8); δ_H 6.41 (H-8) and 6.91 (H-5'); δ_H 6.91 (H-5') and δ_H 7.66 (H-6'); δ_H 7.66 (H-6') and δ_H 7.76 (H-2') among several other correlations.

^{1}H ^{13}C HSQC analysis of D67M

The attachment of protons to their respective carbons was established from the Heteronuclear Spin Quantum Correlation spectrum of **D67M** in CD_3OD. Established attachments among others include δ_H 6.20 and δ_C 98.09 (H-6 and C-6), δ_H 6.41 and δ_C 93.19 (H-8 and C-8), δ_H 6.91 and δ_C 114.61 (H-5' and C-5'), δ_H 7.66 and δ_C 120.30 (H-6' and C-6') and δ_H 7.76 and δ_C 114.87 (H-2' and C-2').

^{1}H ^{13}C HMBC spectral analysis of D67M

The Heteronuclear Multi Bond Correlation spectral data allowed the establishment of long range connectivity between various units of the molecule (correlation in space). Hence, δ_H 6.20 (H-6) correlated with δ_C 93.19 (C-8), δ_C

102.99 (C-10), δ_C 161.06 (C-5) and δ_C 164.77 (C-7); δ_H 6.41 (H-8) with δ_C 98.09 (C-6), δ_C 102.99 (C-10), δ_C 156.91 (C-9) and δ_C 164.77 (C-7); δ_H 6.91 (H-5') with δ_C 122.78 (C-1'), δ_C 144.85 (C-3'), δ_C 146.60 (C-4') and δ_C 147.41 (C-2); δ_H 7.66 (H-6') with δ_C 114.61 (C-5'), δ_C 114.87 (C-2'), δ_C 146.60 (C-4') and δ_C 147.41 (C-2); δ_H 7.68 (H-2') with δ_C 120.30 (C-6'), δ_C 144.85 (C-3'), δ_C 146.60 (C-4') and δ_C 147.41 (C-2).

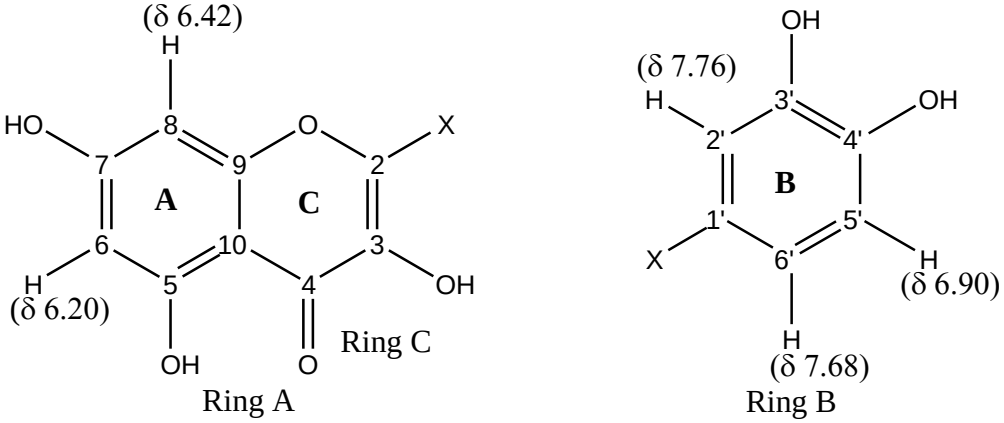
Discussion

Compound D67M

The ^{1}H NMR spectrum exhibited a *meta*-coupled system at δ 6.20 (1H, *d, J* = 1.3 Hz) and δ 6.41 (1H, *d, J* = 1.3 Hz) assignable to H-6 and H-8 respectively in A-Ring. A *tri*-substituted coupling system was observed in B-Ring at δ 7.76 (1H, *d, J* = 1.3 Hz), δ 6.90 (1H, *d, J* = 8.5 Hz) and δ 7.68 (1H, *dd, J* = 1.4, 1.8 Hz) representing H-2', H-5' and H-6' respectively. These coupling systems indicated a 3, 5, 7, 3', 4' penta-oxygenated flavonol (typical of quercetin nucleus) [8-10].

The ^{13}C NMR analysis revealed the presence of 15 carbon atoms in the aromatic region with five CH absorptions having chemical shifts of δ_C 93.20, δ_C 98.09, δ_C 114.61, δ_C 114.87 and δ_C 122.78 ppm. While, all the remaining ten with chemical shift values of δ_C 102.99, δ_C 120.30, δ_C 135.77, δ_C 144.85, δ_C 146.60, δ_C 147.41, δ_C 156.91, δ_C 161.06, δ_C 164.77 and δ_C 175.90 ppm are quartenary (C) carbons.

^{1}H-^{1}H COSY established single bond correlations between one *ortho*-coupled protons 6.91 (H-5') and 7.66 (H-6') of Ring B. Then, two *meta*-coupled protons 6.41 (H-8) and 6.20 (H-6) of Ring A; 7.76 (H-2') and 7.66 (H-6') also of Ring A, established the presence of two fragments viz rings A and B.



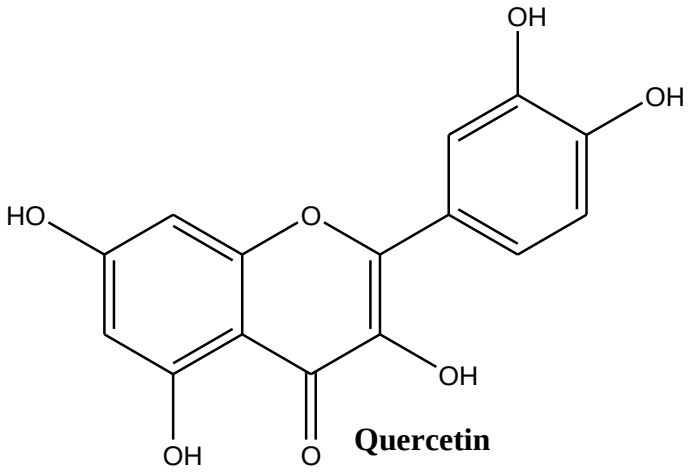
Compound D67M thus can be deduced to be quercetin. However, other available 2D NMR spectral data provided below attested to same.

Using HSQC the assignment of various protons to their respective carbons was achieved. Among these attachments are δ_H 6.20 to δ_C 98.09 (H-6 to C-6); δ_H 6.41 to δ_C 93.19 (H-8 to C-8); δH 6.91 to δ_C 114.61 (H-5' to C-5'); δ_H 7.66 to δ_C 120.30 (H-6' to C-6') and δ_H 7.76 to δ_C 114.87 (H-2' to C-2') typical of Quercetin nucleus.

NOESY: δ_H 6.20 (H-6) and δ_H 6.41 (H-8); δ_H 6.41 (H-8) and 6.91 (H-5'); δ_H 6.91 (H-5') and δ_H 7.66 (H-6'); δ_H 7.66 (H-6') and δ_H 7.76 (H-2').

In the HMBC linkages between the various fragments was established. Result of the HMBC showed a long range correlation between δ_H 6.20 (H-6) correlating with δ_C 93.19 (C-8), δ_C 102.99 (C-10), δ_C 161.06 (C-5) and δ_C 164.77 (C-7); δ_H 6.41 (H-8) with δ_C 98.09 (C-6), δ_C 102.99 (C-10), δ_C 156.91 (C-9) and δ_C 164.77 (C-7); δ_H 6.91 (H-5') with δ_C 122.78 (C-1'), δ_C 144.85 (C-3'), δ_C 146.60 (C-4') and δ_C 147.41 (C-2); δ_H 7.66 (H-6') with δ_C 114.61 (C-5'), δ_C 114.87 (C-2'), δ_C 146.60 (C-4') and δ_C 147.41 (C-2); δ_H 7.68 (H-2') with δ_C 120.30 (C-6'), δ_C 144.85 (C-3'), δ_C 146.60 (C-4') and δ_C 147.41 (C-2).

Utilisation of the 1D and 2D NMR spectra allowed the structure of D67M to be established as 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one (Quercetin):



Quercetin

2-(3,4-Dihydroxy-phenyl)-3,5,7-trihydroxy-chromen-4-one

Conclusion

Purification of NBU pooled fraction coded 86-103 through repeated gel filtration over Sephadex LH-20 eluted with methanol and PTLC led to the isolation of D67M (quercetin).

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