



## Article Info

Received: 10<sup>th</sup> December, 2021

Revised: 5<sup>th</sup> June 2022

Accepted: 10<sup>th</sup> June 2022

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Cite this: *CaJoST*, 2022, 2, 238-243

## Isolation and Structure Elucidation of Quercetin-Derivative (amino-flavone) from *Centaurea perrottetii* DC (Asteraceae)

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The plant, *Centaurea perrottetii* DC (Asteraceae) is a herbaceous thistle-like shrub used in traditional medicine for the treatment of malaria, fever, syphilis, ulcer and skin infections. The aim of the study is to isolate and elucidate the structure of compound(s) from *C. perrottetii*. Quercetin-derivative (an amino-flavone) was isolated from the *n*-hexane fraction of the methanol extract of the whole plant of *Centaurea perrottetii* using a combination of silica gel column and gel filtration chromatography. The structure of the compound as 2-(3,4-dihydroxyphenyl)-3-((1-ethylcyclopropyl)amino)-5,7-dihydroxy-4Hchromen-4-one was established *via* chemical tests, Ultraviolet (UV), Infrared (IR), 1D- and 2D-dimensional NMR spectroscopy. This is the first report of isolation of this compound from *Centaurea perrottetii*.

**Keywords:** *Centaurea perrottetii*; Quercetin; Amino Flavone.

## 1. Introduction

*Centaurea perrottetii* DC (Figure 1) is a species of the genus *Centaurea* of the family Asteraceae (Burkil,2000). It is a herbaceous thistle-like shrub which is usually prostrate or erect growing up to 50 cm high; it is covered with rough hairs, undulate, spiny margins (and flowers heads) with deeply lobed leaves (Burkil,2000). The plant is widely distributed from Mauritania to Niger, northern Nigeria, Western Cameroon and through the Sahara (Burkil, 2000; Alebiosu *et al.*, 2019). It is commonly known as star thistle and *Danyii* or *Danar Maguzawo* in Hausa (Alebiosu *et al.*, 2019).

The whole plant is used traditionally in Nigeria for the treatment of various ailments such as malaria, ear aches, syphilis, fevers, skin infections and ulcer without scientific validation (Burkil,2000). Thus, the antimalarial activity of the methanol extract of the plant and its fractions have been reported (Alebiosu *et al.*, 2019). We report herein the isolation of a quercetin-derivative (amino flavone) from the *n*-hexane soluble fraction of the methanol extract of the whole plant of *Centaurea perrottetii* for the first time. The study was limited to isolation and characterization of compound(s) from the plant.



Figure 1: Picture of *Centaurea perrottetii*

## 2. Materials and Methods

### 2.1 General Procedures

Nuclear Magnetic Radiation (NMR) data were recorded on a Bruker AVANCE III spectrophotometer (600 MHz) with residual solvent as internal standard. Thin Layer Chromatography (TLC) was carried out using silica gel 60 GF<sub>254</sub> pre-coated Aluminium sheets by Sigma Aldrich, Germany. Column chromatography was conducted using LONA cheme silical gel (60 – 200 mesh), while gel filtration chromatography was performed using sephadex LH – 20 (Sigma, Space street, St. Louis, MO, USA). Spots on TLC plates were visualized using 10 % H<sub>2</sub>SO<sub>4</sub> followed by heating at 105 °C for ten minutes.

### 2.2 Collection and Identification of Plant Material

Whole plant of *C. perrottetii* was collected in November, 2014 from Ilella, Gwadabawa Local Government Area, Sokoto State, Nigeria and was identified and authenticated by a taxonomist, Mal. M. A. Salihu. Voucher specimens were prepared and deposited at the Herbarium of the Taxonomy Unit, Department of Botany, Usmanu Danfodiyo University, Sokoto, Nigeria, and voucher specimen number (UDUH/ANS/0034) was given.

### 2.3 Preparation of the Extract Partitioning

The plant material was shade dried, pulverize to powder (2.1 kg) and it was extracted with 70 %

methanol (25 L) using maceration method for 7 days with constant agitation. The extract obtained was filtered and evaporated *in-vacuo* using a rotary evaporator at 40 °C to yield a dark green residue (147.84 g) subsequently referred to as the crude methanol extract (CME). Some part of the CME (120 g) was suspended in distilled water (500 mL) and partitioned successively with n-hexane (1.5 L), chloroform (500 mL), ethylacetate (1.0 L) and n-butanol (1.5 L) to obtain hexane fraction (HF), chloroform fraction (CF), ethyl-acetate fraction (EF), n-butanol fraction (BF) and the residual aqueous fraction (AQF), respectively.

### 2.4 Isolation and Characterization of Compound SHA

The n-hexane fraction (5.0 g) was subjected to column chromatography on a silica gel (60-120 mesh Merck) column and it was gradually eluted with n-hexane (100 %), n-hexane : ethyl acetate (10:90), ethyl acetate (100 %), ethyl acetate : methanol (5: 95) and methanol (100 %). A total of 125 fractions of 20 mL each, were collected and combined based on their TLC profiles to obtain 17 major fractions coded H1 – H17. Fraction H-4 was subjected to further purification using Sephadex LH-20 and methanol (100 %) as mobile phase; 5 mL each of a total of 100 collections were made and combined based on the TLC profile to afford 7 major fractions coded HA – HG. Repeated gel filtration of fraction HC and HF led to the isolation of a compound labelled as SHA which was characterized using chemical tests, melting point and spectroscopic analysis.

## 3. Results and Discussion

### 3.1 Results

#### Spectral Data

#### **2-(3,4-dihydroxyphenyl)-3-((1-ethylcyclopropyl)amino)-5,7-dihydroxy-4Hchromen-4-one (SHA).**

Yellow solid substance, m.p. 258 - 260 °C. TLC (chloroform-ethyl acetate, 1:6) R<sub>f</sub> = 0.76, UV spectrum (in MeOH) - 307 nm; IR spectrum (in KBr) - 3420 cm<sup>-1</sup>, 2923 cm<sup>-1</sup>, 1650, 1034 cm<sup>-1</sup>. <sup>1</sup>H-NMR spectrum (600 MHz, CD<sub>3</sub>OD); δ<sub>H</sub> 7.76 (1H, d, J=2.4 Hz, H-2'), 7.67 (1H, dd, J=2.1, 8.4 Hz, H-6'), 6.92 (1H, d, J=8.34 Hz, H-3'), 6.42 (1H, d, J=1.68 Hz, H-8), 6.21 (1H, d, J=1.98 Hz, H-6). <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD); δ<sub>C</sub> 175.95 (q, C-4), 164.20 (q, C-7), 161.12 (C-5), 156.85 (C-9), 147.39 (C-2), 146.63 (C-4'), 144.83 (C-5'), 135.84 (C-3), 122.76 (C-1'), 120.23 (C-6'), 114.84 (C-2'), 114.61 (C-3'), 103.13 (C-10), 97.55 (C-6), 93.03 (C-8), 31.65 (C-1''), 29.33 (C-2''), 22.31 (C-3''), 29.04 (C-1'''), 13.01 (C-2''').

**Table 1:** 1D and 2D NMR spectral summary data for compound SHA (CD<sub>3</sub>OD)

Position	δ <sub>H</sub> (J in Hz)	δ <sub>C</sub>	DEPT	HMBC
1	-	-	-	-
2	-	147.39	C	-
3	-	135.84	C	-
4	-	175.96	C	-
5	-	161.12	C	-
6	6.21(d, J=1.98)	97.55	CH	C7, C5, C10
7	-	164.20	C	-
8	6.42(d, J=1.68)	93.03	CH	C7, C9, C10

9	-	156.85	C	-
10	-	103.13	C	-
1'	-	122.76	C	-
2'	7.76(d, $J=2.4$ )	114.84	CH	C2
3'	6.92(d, $J=8.34$ )	114.61	CH	C2
4'	-	146.63	C	-
5'	-	144.83	C	-
6'	7.67(dd, $J=2.1, 8.4$ )	120.23	CH	C2
1''	-	31.65	C	-
2''	1.26	29.33	CH <sub>2</sub>	C1'''
3''	1.64	22.31	CH <sub>2</sub>	-
1'''	1.36	29.04	CH <sub>2</sub>	C2'', C2'''
2'''	0.97	13.01	CH <sub>3</sub>	C1'''

Multiplicity and coupling constants ( $J$ ) in Hz are given in parentheses

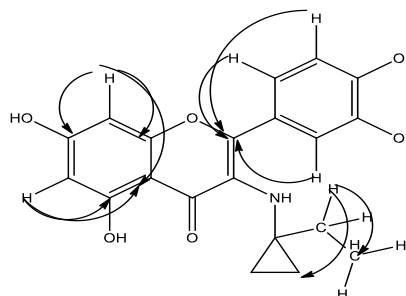
### 3.2 Discussion

Compound SHA was isolated from the *n*-hexane soluble fraction of *C. perrottetii* as a yellow solid substance (5 mg); it reacted positively to Shinoda and Wagner's tests, suggesting the presence of phenolic and alkaloidal nucleus (Silva *et al.*, 1998). The uncorrected melting point of compound SHA was 258 - 260 °C. TLC analysis of SHA using chloroform and ethylacetate (1:6) gave a single homogenous spot with  $R_f$  value of 0.76.

The UV spectrum (in MeOH) of SHA indicates 307 nm as the wavelength of maximum absorption, other absorptions observed were 209 and 256 nm. The IR spectrum (in KBr) values at 3420  $\text{cm}^{-1}$  which is due to OH stretching, 2923  $\text{cm}^{-1}$  (CH bending), 1650 and 1034  $\text{cm}^{-1}$  due to N-H bending and C-OH vibration.

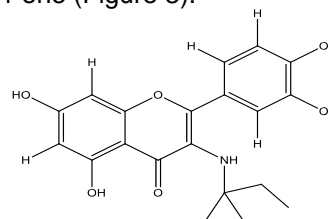
The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectrum of SHA exhibited chemical shift values typical of flavonoids (Tekeli *et al.*, 2011; Sayed and Emadeldin, 2014). The  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta_{\text{H}}$  6.21(1H, d, 1.98 Hz), 6.42 (1H, d, 1.68 Hz) were due to meta-coupled protons of ring-A (H-6 and H-8) of a flavonoid nucleus. Proton signals at  $\delta_{\text{H}}$  6.92 (1H, d, 8.34 Hz), 7.67 (1H, dd, 2.1, 8.4 Hz) and 7.76 (1H, d, 2.4 Hz) were assigned to H-3', H-6' and H-2' of ring B. A prominent proton signal at  $\delta_{\text{H}}$  4.57 which did not appear in the HSQC spectrum suggest the presence of an amino group in the compound (Emani *et al.*, 2007). The  $^{13}\text{C-NMR/DEPT}$  experiments revealed presence of 20 carbon signals. HSQC spectrum of SHA was used to attach each proton to their respective carbons. The downfield signal at 175.96 ppm was due to the carbonyl group at C-4. Lack of carbon signal at  $\delta_{\text{C}}$  101.8, a proton at  $\delta_{\text{H}}$  6.76 and the presence of a signal at  $\delta_{\text{C}}$  135.83 ppm as observed in flavonol and other heteroatoms ( $\delta_{\text{C}}$  136 - 139 ppm) (Eunjung *et al.*, 2008) suggest the presence of a nitrogen at C-3 with the cyclopropyl chain. The  $^1\text{H-}^1\text{H}$  COSY established the correlations between the protons at  $\delta_{\text{H}}$  7.76 (H-2') and 7.67 (H-6'). Other cross peaks correlations were observed between  $\delta_{\text{H}}$  7.67 (H-

6') and 6.92 (H-3');  $\delta_{\text{H}}$  6.42 (H-8) and 6.21 (H-6). Another major correlation observed was between the methyl group at  $\delta_{\text{H}}$  0.97 (H-2''') and a methylene group at  $\delta_{\text{H}}$  1.36 (H-1'''). The HMBC spectrum established the connectivity between the various units of the molecule. Major correlations observed between  $\delta_{\text{H}}$  7.76 (H-2') and 147.38 (C-2),  $\delta_{\text{H}}$  6.92 (H-3') and 147.38 (C-2),  $\delta_{\text{H}}$  7.67 (H-6') and 147.38 (C-2) confirmed the assignment of protons on ring B while the long range correlation observed between proton at  $\delta_{\text{H}}$  6.42 (H-8) and the carbons at 103.13 (C-10), 156.85 (C-9) and 164.19 (C-7) and  $\delta_{\text{H}}$  6.21 (H-6) with 164.19 (C-7), 161.12 (C-5) and 103.13 (C-10) confirmed the attachment of ring C to ring A. The correct assignment of the amino substituent was confirmed via the correlations at  $\delta_{\text{H}}$  1.36 (proton H-1''') and 13.01 (C-2'''), 29.33 (C-2'') (Figure 2).



**Figure 2:** HMBC- correlation of SHA in  $\text{CD}_3\text{OD}$

Based on the above data (Table 1), the structure of SHA was proposed to be an amino flavone (quercetin-derivative), 2-(3,4-dihydroxyphenyl)-3-((1-ethylcyclopropyl)amino)-5,7-dihydroxy-4H-chromen-4-one (Figure 3).



**Figure 3:** 2-(3,4-dihydroxyphenyl)-3-((1-ethylcyclopropyl)amino)-5,7-dihydroxy-4H-chromen-4-one

## 4. Conclusion

An amino flavone derivative, 2-(3,4-dihydroxyphenyl)-3-((1-ethylcyclopropyl)amino)-5,7-dihydroxy-4H-chromen-4-one was isolated from the *n*-hexane soluble fraction of the whole plant of *Centaurea perrottetii* for the first time. This constituent might be linked to the pharmacological effects of the plant.

## Acknowledgements

We acknowledge the efforts of Mr. Dilip Jagjivan and Mr. Viyusa Mzozoyan, School of Chemistry and Physics, University of Kwa-Zulu Natal-Durban, South Africa for running the 1D and 2D-NMR spectroscopy.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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## Supplementary Information

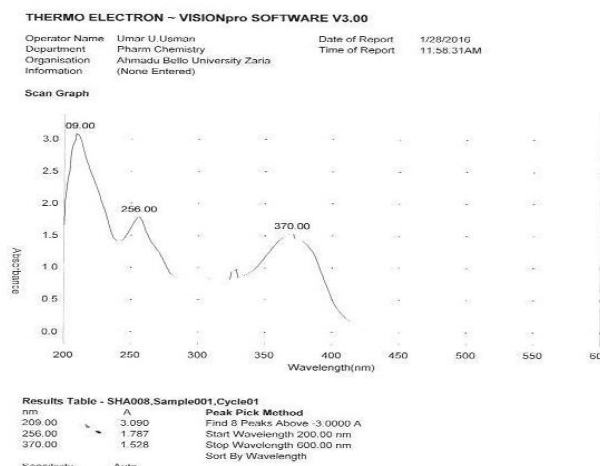


Figure S1: UV spectrum of SHA

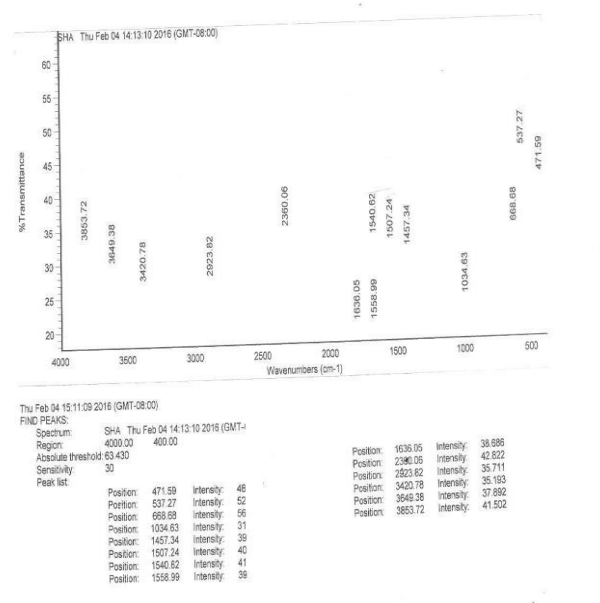
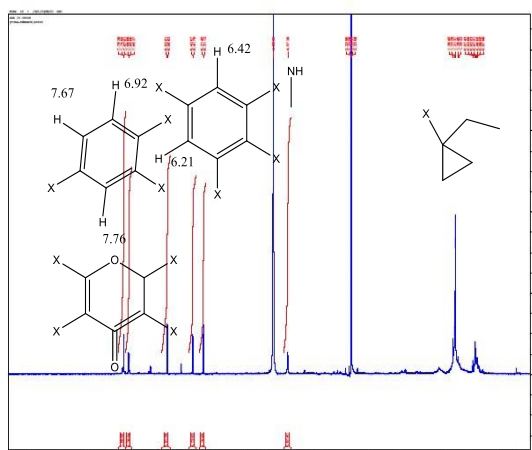
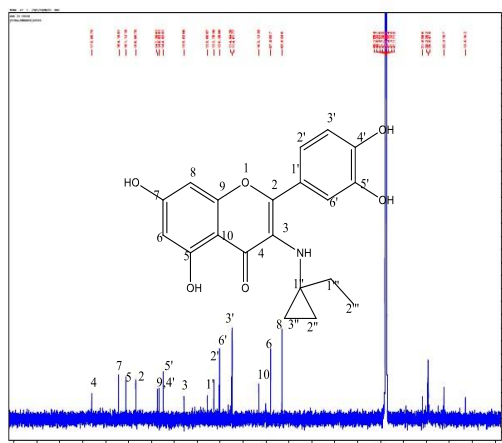


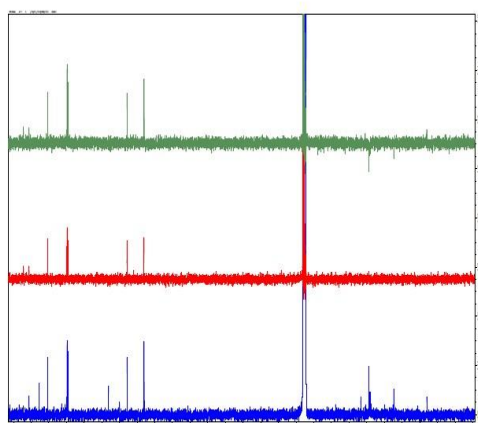
Figure S2: IR spectrum of SHA (in KBr)



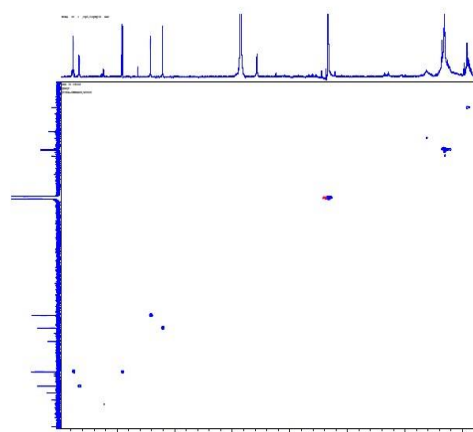
**Figure S3:**  $^1\text{H}$ -NMR spectrum of SHA in  $\text{CD}_3\text{OD}$



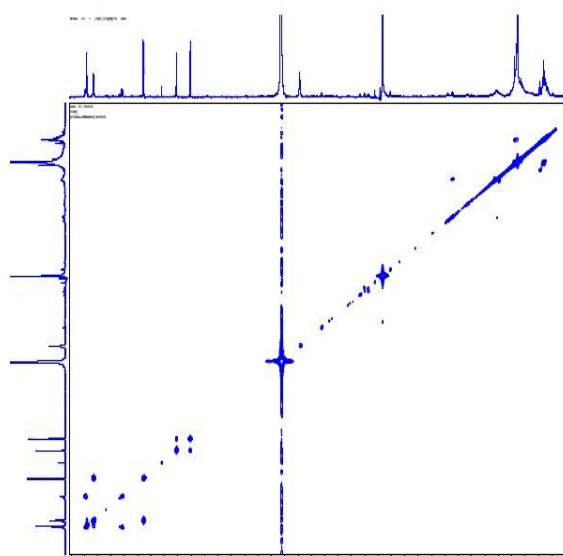
**Figure S4:**  $^{13}\text{C}$ -NMR spectrum of SHA in  $\text{CD}_3\text{OD}$



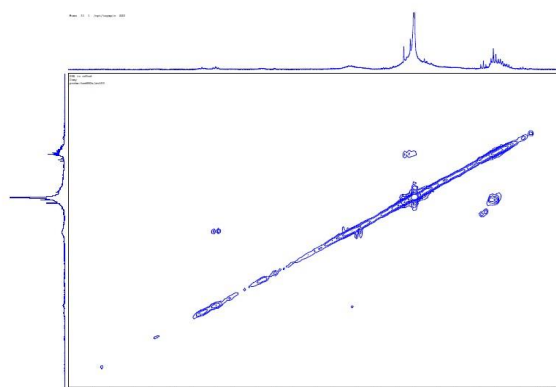
**Figure S5:**  $^{13}\text{C}$ -DEPT spectrum of SHA in  $\text{CD}_3\text{OD}$



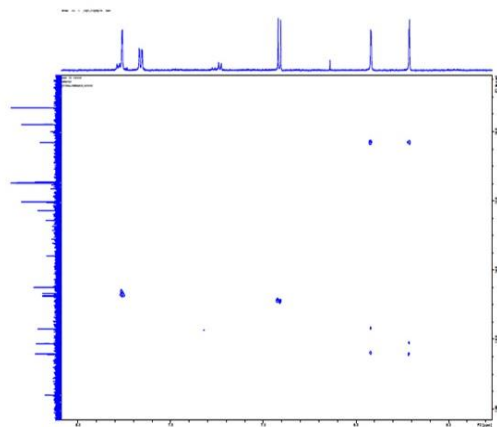
**Figure S6:** HSQC spectrum of SHA in  $\text{CD}_3\text{OD}$



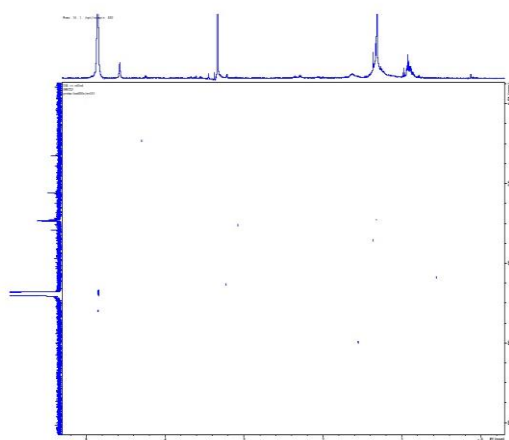
**Figure S7:**  $^1\text{H}$ - $^1\text{H}$ -Cosy of SHA in  $\text{CD}_3\text{OD}$



**Figure S8:**  $^1\text{H}$ - $^1\text{H}$ -Cosy of SHA in  $\text{CD}_3\text{OD}$ -expansion



**Figure S9:** HMBC of SHA in CD<sub>3</sub>OD



**Figure S10:** HMBC of SHA in CD<sub>3</sub>OD-expansion