

Isolation and Characterization of Some Methoxylated Flavones from *Centaurea perrottetii* D. C. (Asteraceae)

Alebiosu C. Oluranti^{1*}, Sule M. Ibrahim², Pateh U. Umar², Abdullahi M. Ismail¹, Idowu Thomas³ and Yusuf A. Jega¹

¹Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

²Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria

³Department of Pharmaceutical and Medicinal Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

*Corresponding Author's Email & Phone No.: celestina.alebiosu@udusok.edu.ng; Tel:+2348036058858

Received on: January, 2024

Revised and Accepted on: March 2024

Published on: March 2024

ABSTRACT

Centaurea perrottetii, which is commonly known as star thistle, belongs to the compositae family and it is used in traditional medicine to treat malaria, microbial infections among others. This study aimed to isolate and characterize compounds from the whole plant of *C. perrottetii*. The whole plant extract of *Centaurea perrottetii* was collected, dried, pulverized and extracted with methanol using maceration method. The extract was concentrated in *vacuo* with the aid of rotary evaporator to afford a greenish crude methanol extract (CME). The extract was successively partitioned into hexane, chloroform, ethylacetate and n-butanol fractions respectively. These fractions were subjected to preliminary phytochemical investigation using standard procedures. A combination of silica gel and sephadex LH-20 column chromatography was employed which led to the isolation of two methoxylated flavones, 8,4'-dihydroxy-6,7,3'-trimethoxyflavone and 3',4'-dihydroxy-6,7,8-trimethoxyflavone, from the ethylacetate soluble fraction of the methanol extract of the whole plant of *Centaurea perrottetii*. The identity of the compounds was determined via chemical tests, spectroscopic techniques and by comparison with existing data in the literature. To the best of our knowledge, this is the first report of isolation of these compounds from the whole plant of *C. perrottetii*.

Keywords: *Centaurea perrottetii*; whole plant; flavones; NMR

1.0 INTRODUCTION

The Asteraceae family, formerly known as the Compositae, is the family of flowering plants with more than 1600 genera and 23,000 species (Melaku *et al.*, 2022). *Centaurea perrottetii* D. C. is a member of this family. The plant, which is extensively dispersed from Mauritania to Niger, Northern Nigeria, Western Cameroons, and throughout the Sahara, is also known as star-thistle in English and Danyii or Danar maguzawa in Hausa (Alebiosu *et al.*, 2019). The plant furnishes a common cattle fodder in Northern Nigeria and it has been reported that the whole plant is bitter (Burkil, 2000). The plant is traditionally valued by the Wolof of Senegal for use in the treatment of gonorrhoea, syphilis, and fevers when taken orally with other plants (Alebiosu *et al.*, 2019); the whole plant can be ground into a paste and applied externally to treat ear problems; and the leaves can be used to treat wounds, parasitic infections, and dermal infections (Burkil, 2000; Yosif, 2022). The herb is also used to treat fever, ulcers, malaria, and skin infections in Northern Nigeria (Alebiosu *et al.*, 2019).

Extensive literature search on the plant revealed little information on pharmacological studies, isolation and characterization of compounds from the plant. Pharmacologically, antimalarial activity of the crude methanol extract of *C. perrottetii* was reported (Alebiosu *et al.*, 2019).

A Quercetin-Derivative (amino-flavone) was reported to have been isolated from the n-hexane soluble fraction of the whole plant of *Centaurea perrottetii* (Celestina *et al.*, 2022). Also, isolation of Taraxasterol and Stigmasterol were reported from the chloroform fraction of the aerial part of *Centaurea perrottetii* (Aduku *et al.*, 2020). The isolation and structural elucidation of two methoxylated flavones from *Centaurea perrottetii* whole plant are described in this research.

2.0 METHODOLOGY

2.1 Plant material

In November 2014, a fresh whole plant of *C. perrottetii* was collected from Illela Local Government Area in Sokoto State, Nigeria. The whole plant was identified and verified by a Botanist at the Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher samples (UDUH/ANS/0034) were created and deposited. The whole plant was shade dried for two weeks, pulverized labeled, and kept at room temperature for future use.

2.2 Extraction and Isolation

Using the maceration process, 2.1 kg of the powdered whole plant was successively extracted for 7 days with 70 % methanol with occasional stirring. The crude methanol extract, also known as CME, was obtained after the solvent was filtered and concentrated in-vacuo using

a rotary evaporator at 40 °C. About 120 g of CME were suspended in distilled water, filtered, and then partitioned into n-hexane, chloroform, ethylacetate, n-butanol, and residual aqueous fractions.

The ethylacetate fraction (4 g) was gradiently eluted on a silica gel packed column using different solvent combinations starting with hexane: ethyl-acetate (9:1): ethyl-acetate (100 %) to ethyl-acetate: methanol (8:2) and methanol (100%); 20 mL each of a total of 68 fractions were collected and combined based on their TLC profile to afford ten (10) major fractions coded E1 to E10. Compounds 1 and 2 were obtained by repeatedly subjecting fractions E3 and E5 to gel filtration using sephadex LH-20. To identify their structures, the compounds underwent physicochemical tests (such as appearance, Shinoda and alkaline test, melting point determination) and spectroscopic analysis (NMR) as previously described by Yusuf et al. (2021).

2.3 General procedures

Thin Layer Chromatography (TLC) was performed using silica gel 60 GF254 pre-coated aluminum sheets from Sigma Aldrich, Germany. While sephadex LH - 20 (Sigma, Space Street, St. Louis, MO, USA) was used for gel filtration chromatography, LOBA cheme silica gel (60 - 200 mesh) was used for column chromatography. Spraying 10% H₂SO₄ on TLC plates and then heating them for ten minutes at 105 °C allowed for the visualization of the spots. With residual solvent serving as the internal standard, NMR data were collected on a Bruker AVANCE spectrometer (III) (600 MHz).

2.4 Spectral data

8,4-dihydroxy-6,7,3'-trimethoxyflavone (1). Yellow solid substance, m.p 319 – 320 °C; ¹H-NMR spectrum (in CD₃OD); δ_H 6.67 (1H, s, H-3), 6.84 (1H, s, H-5), δ_H 7.45 (1H, s, H-2'), 7.12 (1H, d, J = 8.4 Hz, H-5'), δ_H 7.56 (1H, d, J = 8.10 Hz, H-6'), δ_H 4.01 (3H, s, 7-OCH₃), δ_H 3.91 (3H, s, 6-OCH₃), δ_H 3.98 (3H, s, 3'-OCH₃). ¹³C-NMR (150 MHz, CD₃OD); δ_C 182.8 (C-4), 164.9 (C-2), 159.3 (C-7), 153.5 (C-9), 152.3 (C-8), 151.4 (C-3'), 146.9 (C-4'), 132.4 (C-6), 123.5 (C-1'), 118.7 (C-6'), 112.6 (C-2'), 111.3 (C-5'), 105.3 (C-10), 103.0 (C-3), 90.9 (C-5), 59.7 (6-OCH₃), 55.6 (7-OCH₃), 55.1 (3'-OCH₃).

3',4'-dihydroxy-6,7,8-trimethoxyflavone (2). Yellow solid substance, m.p 317 – 318 °C; ¹H-NMR spectrum (in CD₃OD); δ_H 6.93 (1H, s, H-5), 6.82 (1H, s, H-3), 7.60 (1H, dd, J = 8.4, 2.2 Hz, H-6'), 7.49 (1H, d, J = 2.28 Hz, H-2'), 7.12 (1H, d, J = 8.52 Hz, H-5'), 3.95 (3H, d, J = 3.06 Hz, 7-OCH₃), 3.89 (3H, s, 8-OCH₃), 3.75 (3H, s, 6-OCH₃). ¹³C-NMR (150 MHz, CD₃OD); δ_C 182.7 (C-4), 164.4 (C-2), 159.2 (C-7), 153.2 (C-9), 152.5 (C-8), 151.8 (C-3'), 147.4 (C-4'), 132.4 (C-6), 123.4 (C-1'), 119.3 (C-

6'), 113.6 (C-2'), 112.6 (C-5'), 105.6 (C-10), 103.8 (C-3), 92.1 (C-5), 60.5 (6-OCH₃), 56.9 (7-OCH₃), 56.3 (8-OCH₃)

3.0 RESULTS AND DISCUSSION

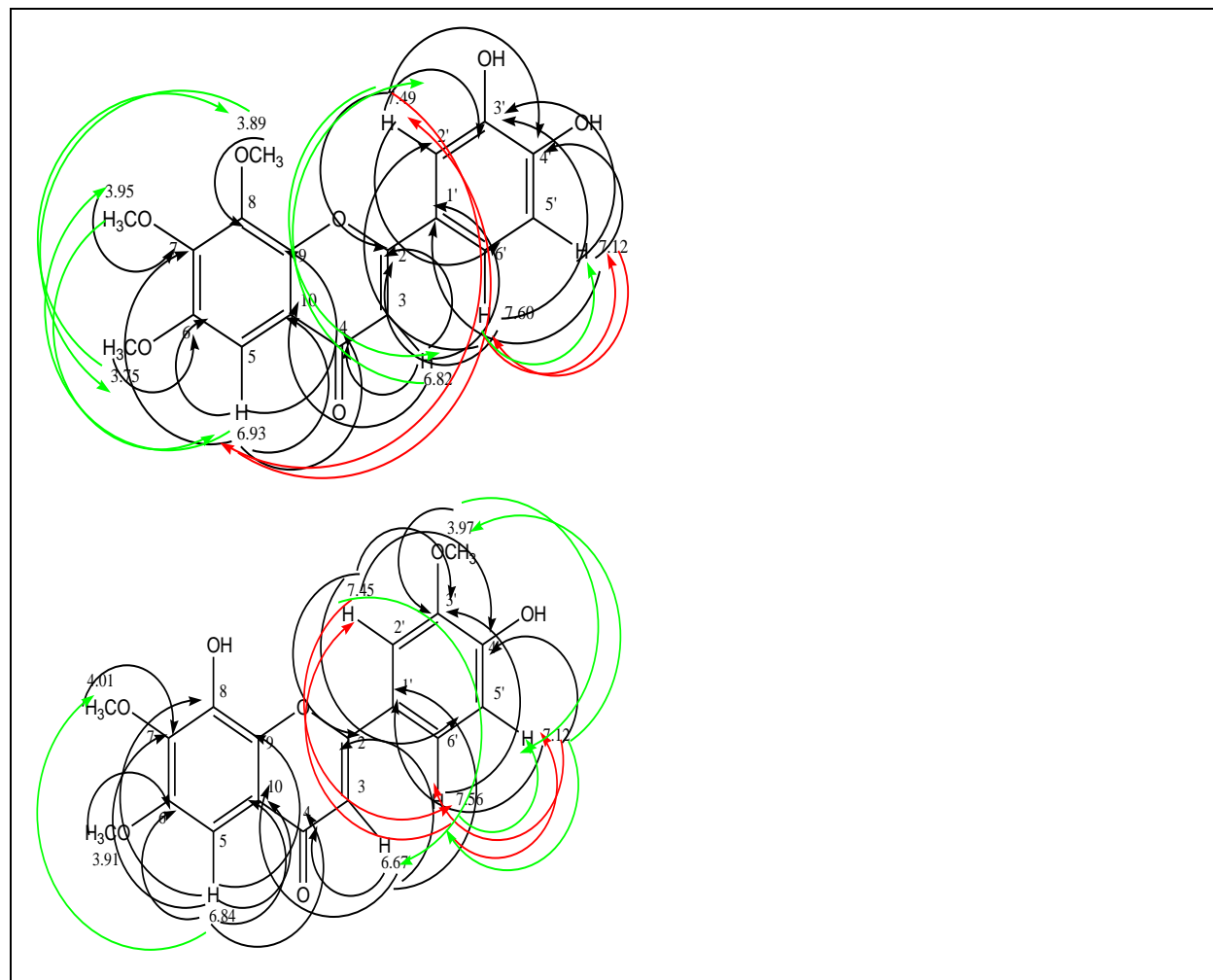
A yellow solid material coded as compound 1 was obtained, and it tested positive for flavonoids using the Shinoda and alkaline reagents (Silva *et al.*, 1998). Its purity can be determined by the uncorrected melting point, which ranges from 319 to 320 °C. The proton NMR of compound 1 displayed chemical shift values typical of flavonoids (Dias *et al.*, 2021; Mohammed *et al.*, 2022; Bationo *et al.*, 2021). The presence of two singlets was observed at δ_H 6.67 (1H, s) and 6.84 (1H, s) which were assigned to H-3 (of ring C) and H-5 (of ring A), respectively (Bationo *et al.*, 2021). An aromatic ring B was clearly depicted via the proton signals at δ_H 7.45 (1H, s, H-2'), 7.12 (1H, d, J = 8.4 Hz, H-5') and 7.56 (1H, d, J = 2.1 Hz, H-6'). The proton NMR depicted chemical shift values of three methoxyl groups at δ_H 4.01 (3H, s, 7-OCH₃), 3.91 (3H, s, 6-OCH₃) and 3.98 (3H, s, 3'-OCH₃). These values were in close agreement to the values reported for methoxylated flavones and derivatives (Dias *et al.*, 2021).

The ¹³C-NMR (150 MHz, CD₃OD) and DEPT experiments revealed the presence of 18 carbon atoms including ten quaternary, five methine and three methoxy carbons (Table 1). Thus, these chemical shift values were in consistent with those of the ¹H-NMR and were similar to those reported for eupatorin (Dias *et al.*, 2021). HSQC experiment of compound 1 established the attachment of various protons to their respective carbon atoms (Table 1). Major correlations observed include δ_H 7.56 and δ_C 118.7, δ_H 7.45 and δ_C 112.6, δ_H 7.12 and δ_C 111.3, δ_H 6.84 and δ_C 90.9, δ_H 6.67 and δ_C 103.0, δ_H 3.91 and δ_C 59.7, δ_H 4.01 and δ_C 55.6, δ_H 3.98 and δ_C 55.1.

The correct assignment of protons on ring B was confirmed via ¹H-¹H-COSY experiment which indicated cross peak correlations between the protons at δ_H 7.56 (H-6') and δ_H 7.12 (H-5') and 7.45 (H-2') at an ortho-coupled and meta-coupled position, respectively. HMBC spectrum of compound 1 established the long-range connectivity between the various units of the molecule; the attachment of ring B to C-2 of ring C was confirmed via long range correlations observed between δ_H 7.56 (H-6') and δ_C 164.9 (C-2), 118.7 (C-6') and 151.4 (C-3'); the correct assignment of ring B was confirmed via correlations observed between δ_H 7.45 (H-2') and δ_C 112.6 (C-2'), 146.9 (C-4'), 118.7 (C-6'); the correlations observed between δ_H 7.12 (H-5') and δ_C 123.5 (C-1') and 146.9 (C-4') further substantiated the assignment of protons and carbons on ring B.

Long range correlations observed between the proton at δ_H 6.84 (H-5) and δ_C 105.3 (C-10), 132.4 (C-6), 153.5 (C-9), 159.3 (C-7) and 182.8 (C-4) confirmed the attachment of ring C to ring A as well as the assignment of a singlet (δ_H 6.84) at position 5 of ring A. The correct assignment of δ_H 6.67 to position 3 (H-3) of ring C was confirmed via the long-range correlations observed with the carbons at 105.3 (C-10), 164.9 (C-2) and 182.8 (C-4). There was a long-range correlation between δ_H 4.01 and δ_C 159.3 (C-7), δ_H 3.98 and δ_C 151.4 (C-3'), and δ_H 3.91 and δ_C 132.4 (C-6) which confirmed the attachment of

methoxy groups at positions 7, 3' and 6, respectively (Figure 1). The nuclear Overhauser effect spectroscopy (NOESY) of compound **1** revealed the correlations between protons that are in close proximity through space interactions. Major cross peaks observed include δ_H 7.56 (H-6') and 7.12 (H-5'), δ_H 7.45 (H-2') and 6.67 (H-3), and δ_H 7.12 (H-5') and 3.98 (3'-OCH₃) (Figure 1). Based on the 1D and 2D-NMR data (Table 1), the structure of compound **1** was confirmed to be 8,4'-dihydroxy-6,7,3'-trimethoxyflavone (**1**) (Figure 2).



Compound **2** exhibited similar chemical shift values with that of Compound **1** (Table 2). The major difference observed was on the ring A and B of compound **2** which indicated the presence of three methoxy groups at δ_H 3.89 (3H, s, 8-OCH₃), 3.95 (3H, d, J = 3.06 Hz, 7-OCH₃) and 3.75 (3H, s, 6-OCH₃) assignable to positions 8, 7 and 6 of ring A, respectively. The long range HMBC correlations observed by compound **2** was used to confirm the assignments of the methoxy groups on ring A; the methoxy protons at δ_H 3.89 (H-8-OCH₃), 3.95 (H-7-OCH₃) and 3.75 (H-6-OCH₃) correlated with the carbons at 152.5 (C-8), 159.2 (C-7) and 132.4 (C-6), respectively (Figure 1). NOESY experiment of compound **2** was used to confirm the various attachments of protons (in space) within the molecule. Cross peaks observed between δ_H 7.60 (H-6') and 7.12 (H-5') confirmed their assignment on ring B; other major correlations observed include δ_H 6.93 # 3.95 and δ_H 3.89 # 3.75 (Table 2). Based on the NMR data as indicated in Table 2, the structure of compound **2** was confirmed to be 3',4'-dihydroxy-6,7,8-trimethoxyflavone (**2**) (Figure 1).

Table 1: 1D and 2D-NMR spectral data summary for compound 1 (CD₃OD)

Position	δH (J in Hz)	δC	DEPT	¹ H- ¹ H-COSY	HMBC	NEOSY
2	-	164.9	C			
3	6.67 (s)	103.0	CH		C-10, C-4, C-2, C-1'	
4	-	182.8	C			
5	6.84 (s)	90.9	CH		C-10, C-9, C-8, C-7, C-6, C-4	H-7''
6	-	132.4	C			
7	-	159.3	C			
8	-	152.3	C			
9	-	153.5	C			
10	-	105.4	C			
1'	-	123.5	C			
2'	7.45 (s)	112.6	CH	H-6'	C-6', C-4', C-3', C-2	H-3
3'	-	151.4	C			
4'	-	146.9	C			
5'	7.12 (d, 8.40)	111.3	CH	H-6'	C-1', C-4'	H-6', H-3''
6'	7.56 (d, 8.10)	118.7	CH	H-2', H-5'	C-3'	H-5'
6''	3.91 (s)	59.7	OCH ₃	-	C-6	
7''	4.01 (s)	55.6	OCH ₃	-	C-7	
3''	3.98 (s)	55.1	OCH ₃	-	C-3'	H-5'

Table 2: 1D and 2D-NMR spectral data summary for compound 2 (CD₃OD)

Position	δH (J in Hz)	δC	DEPT	¹ H- ¹ H-COSY	HMBC	NEOSY
2	-	164.4	C			
3	6.82 (s)	103.8	CH		C-10, C-4, C-2, C-1'	H-2'
4	-	182.7	C			
5	6.93 (s)	92.1	CH	H-2'	C-10, C-9, C-7, C-6, C-4	H-7''
6	-	132.4	C			
7	-	159.2	C			
8	-	152.5	C			
9	-	153.2	C			
10	-	105.6	C			
1'	-	123.4	C			
2'	7.49 (d, 2.28)	113.6	CH	H-5	C-6', C-4', C-3', C-2	H-3
3'	-	151.8	C			
4'	-	147.3	C			
5'	7.12 (d, 8.52)	112.6	CH	H-6'	C-1', C-4', C-3'	H-6
6'	7.60 (dd, 8.4, 2.2)	119.3	CH	H-5'	C-2', C-3', C-2	H-5'
6''	3.75 (s)	60.5	OCH ₃	-	C-6	H-8''
7''	3.95 (d, 3.06)	56.9	OCH ₃	-	C-7	H-5'
8''	3.89 (s)	56.3	OCH ₃	-	C-8	H-6''

Flavones have been reported to possess a wide range of pharmacological actions such as antioxidant, anticancer, antimicrobial and anti-inflammatory activities and thus, their presence may contribute to the ethnomedicinal uses of the plant (Shamsudin *et al.*, 2022; Achika *et al.*, 2023).

4.0 CONCLUSION

Two flavones, 8,4'-dihydroxy-6,7,3'-trimethoxyflavone and 3',4'-dihydroxy-6,7,8-trimethoxyflavone were isolated from the ethylacetate soluble fraction of the methanol extract of the whole plant of *Centaurea perrottetii* for the first time.

5.0 ACKNOWLEDGEMENTS

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

6.0 DECLARATION OF COMPETING INTEREST

None declared

References

- Achika, J.I., Yusuf, A.J., Ayo, R.G., Liman, D.U. (2023). Flavonoids from Nigerian indigenous medicinal plants and their bioactivities: A review, *Phytomedicine Plus*, Volume 3, Issue 2, 100405, <https://doi.org/10.1016/j.phyplu.2023.100405>.
- Aduku, O.G., Abdullahi, M.I., Sule, M.I., Atiku I. and Anyam, J.V. (2020). Isolation of taraxasterol and stigmasterol from the aerial part of *Centaurea perrottetii* DC. (Asteraceae). *Bima Journal of Science and Technology*, 4:2; 86 – 93. ISSN: 2536-6041
- Alebiosu, C.O., Sule, M.I., Pateh, U.U., Abdullah, M.I., Yusuf, A.J. and Umaru, M.L. (2019). In vivo antimalarial studies of the methanol extract of *Centaurea perrottetii* DC (Asteraceae). *Nig J Pharm Biomed Research* 4(1) 102 – 107. DOI: 10.4314/cajost.v4i2.14
- Bationo RK, Dabiré CM, Hema A, Ch Nébié RH, Palé E, Nacro M. (2022). HPTLC/HPLC-mass spectrometry identification and NMR characterization of major flavonoids of wild lemongrass (*Cymbopogon giganteus*) collected in Burkina Faso. *Heliyon*. 25;8(8):e10103. doi: 10.1016/j.heliyon.2022.e10103. PMID: 35991980; PMCID: PMC9382276.
- Burkil, H.M. (2000) *The useful plants of West Tropical Africa*. 2nd Edition. Volume 5, Families S–Z, Addenda, Royal Botanic Gardens, Kew, Richmond, United Kingdom. 686. <https://doi.org/10.1038/141054a0>
- Celestina O. A., Mohammed I. S., Umar U. P., Musa I. A., Aliyu M. M. and Amina J. Y. (2022). Isolation and structure elucidation of quercetin-derivative (amino-flavone) from *Centaurea perrottetii* DC (Asteraceae). *Caliphate Journal of Science and Technology*. 2; 238-243. DOI:<https://dx.doi.org/10.4314/cajost.v4i2.14>
- Dias M. C, Pinto D.C.G.A. and Silva A.M.S. (2021). Plant Flavonoids: Chemical Characteristics and Biological Activity. *Molecules*. 26(17):5377. doi: 10.3390/molecules26175377. PMID: 34500810; PMCID: PMC8434187.
- Melaku M. W., Mohammed K. G. , Shibani M., Tapan K. B., Prasanta R. and Alok K. P. (2022). Ethnobotanical study of endemic and non-endemic medicinal plants used by indigenous people in environs of Gullele botanical garden Addis Ababa, central Ethiopia: A major focus on Asteraceae family. PMID: 36506590 - PMCID: PMC9727095; DOI: 10.3389/fphar.2022.1020097
- Mohammed H. A., Almahmoud S. A., El-Ghaly E. M., Khan F. A., Emwas A. H., Jaremko M., Almulhim F., Khan R. A. and Ragab E. A. (2022). Comparative Anticancer Potentials of Taxifolin and Quercetin Methylated Derivatives against HCT-116 Cell Lines: Effects of O-Methylation on Taxifolin and Quercetin as Preliminary Natural Leads. *ACS Omega*. 7;7(50):46629-46639. doi: 10.1021/acsomega.2c05565. PMID: 36570308; PMCID: PMC9774329.
- Shamsudin, N. F., Ahmed, Q.U., Mahmood, S., Shah, S.A.A., Sarian, M.N. Khattak, M.M.A.K., Khatib, A., Sabere, A.S.M., Yusoff, Y.M. and Latip, J. (2022) Flavonoids as Antidiabetic and Anti-Inflammatory Agents: A Review on Structural Activity Relationship-Based Studies and Meta-Analysis. *Int. J. Mol. Sci.*; 23, 12605. <https://doi.org/10.3390/ijms232012605>
- Silva, G.L. Lee, J. and Douglas, K.A. (1998). Special problems with extraction of plants. In: Cannell J.P.R (eds). *Natural Products Isolation*, Human Publishers, New Jersey USA. (1998) 251 - 293. DOI:10.1007/978-1-59259-256-2_12
- Yosif A. (2022). Medicinal plants used for dermatological disorders among the people of the kingdom of Saudi Arabia: A narrative review. *Saudi J Biol Sci* 29 (6); PMID: 35592741. doi: 10.1016/j.sjbs.2022.103303
- Yusuf A.J., Abdullahi M.I., Musa A.M., Haruna A.K. (2021). Biochemical evaluation and molecular docking assessment of glucosamines from *Neocarya macrophylla* fruits against *Naja nigricollis* venom. *Carbohydrate Research*, 509, <https://doi.org/10.1016/j.carres.2021.108436>.