

Isolation and Characterization of Secondary Metabolites from *Cola minor* Stem ExtractsTeresa K. Mokaya^{1*}, Leonidah K. Omosa², Joanne Ogunah¹, George S. Nyamato¹¹Department of Physical Sciences, University of Embu, P. O. Box 6-60100, Embu, Kenya²Department of Chemistry, School of Physical Sciences, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya

ARTICLE INFO

Article history:

Received 17 February 2021

Revised 15 March 2021

Accepted 07 April 2021

Published online 03 May 2021

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ABSTRACT

Although several medicinal and pharmacological values have been observed in the *Cola* genus, there is no literature report on the phytochemical screening of *Cola minor*. It is against this background that this study was conducted to determine the possible bioactive compounds in the methanol/dichloromethane extract of the stem of *Cola minor*. The stem of *Cola minor* was air-dried and then ground into a fine powder using an electrical grinder. The secondary metabolites were isolated by subjecting the plant extract separately to column chromatography, preparative chromatography, Sephadex LH-20, and chromatotron. Upon isolation, four compounds were identified as friedelin (1), stigmaterol (2), friedelanol (3), and β -armyryn (4), using spectroscopic techniques, which validates the use of the members of the *Cola* genus in traditional herbal medicine to manage various ailments for centuries. This is the first report on the phytochemical investigations of *Cola minor*.

Keywords: *Cola minor*, Isolation, Secondary metabolites, Plant extracts

Introduction

In Africa and other developing countries, it is estimated that 70 to 80% of people use plants as sources of remedies for their healthcare.¹ Plants continue to provide lead molecules with multiple properties for adoption in the pharmaceutical industry^{2, 3} because they are associated with secondary metabolites that are effective, chemically unique, and with minimal side effects as compared to synthetic drugs.⁴ These compounds, often referred to as phytochemicals, have been associated with the antioxidant, anticancer, antimicrobial, anti-inflammatory, antiulcer, antidiabetic, antispasmodic, and antihypertensive properties of plants.⁵ Medicinal plants contain most of these phytochemicals which make them a vital antimicrobial agent in traditional medicine. *Cola* is one of the largest genera in the family *Sterculiaceae* and is related to the South American genus *Theobroma*.⁶ Members of the *Cola* genus have played a significant role in traditional herbal medicine to manage various ailments for centuries.^{7, 8} For example, the leaves, trigs, and the bark of both *C. nitida* and *C. acuminata* were used as a remedy for dysentery, coughs, diarrhea, and vomiting.⁹ The genus is made up of moderately sized trees that are evergreen and often growing to a height of 20 m with glossy ovoid leaves up to 30 cm long.⁶ The majority of *Cola* species are found in West and Central Africa.¹⁰ The genus is almost entirely restricted to moist evergreen and semi-deciduous forest formations.¹¹ The leaves of *Cola* species are very simple, entire, and narrowed or rounded towards the base.⁹ Despite the wide pharmacological activities of the plants from the *Cola* genus reported in the literature, and due to the lack of any phytochemical investigations of *Cola minor*, this present study was carried out to establish the secondary metabolites in the stem of *Cola minor*.

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Citation: Mokaya TK, Omosa LK, Ogunah J, Nyamato GS. Isolation and Characterization of Secondary Metabolites from *Cola minor* Stem Extracts. Trop J Nat Prod Res. 2021; 5(4):621-625. doi.org/10.26538/tjnpr/v5i4.5

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Plant collection and identification

The stem of *Cola minor* was collected in May 2017 from Nzombo forest in Kwale County along the coastal region in Kenya approximately 35 km from Mombasa city. The plant was then identified by Mr. Patrick Mutiso, the chief taxonomist of the University of Nairobi Herbarium where a voucher specimen, TKM 2017/001, was deposited. The plant material (8 kg) was air-dried to obtain 3.5 kg under a shade in the Department of Chemistry, University of Nairobi, Kenya. Thereafter, the dry material was ground into a fine powder using an electrical grinder.

General

NMR experiments were performed in either CDCl₃ or CD₂Cl₂ at room temperature using a Bruker 500 MHz spectrometer (Avance I) using TMS as the internal standard. TopSpin was used to acquire and process the NMR spectra data. Spectra referencing were done using the residual solvent peaks. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Silica gel 60 F₂₅₄ and Silica gel 60 were used for analytical TLC and column chromatography, respectively, while Sephadex LH-20 was used for gel chromatography.

Extraction and isolation

The fine powder that was weighing 3.5 kg was extracted by cold percolation using a mixture of 1:1 CH₂Cl₂/MeOH at room temperature for 72 hours. The extract was then filtered and concentrated in a vacuum on a rotary evaporator at 30 °C and combined to give a total weight of 99 g of extract, which was 2.83 percent of the total plant material. The extract obtained using 50% MeOH in CH₂Cl₂ was adsorbed into an equivalent amount of silica gel and loaded onto ten times the quantity of silica gel packed column using 100% *n*-hexane. The column was then eluted in a gradient of increasing polarities from 5% then 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, up to 100% ethyl acetate (EtOAc),¹² with different fractions collected of 500 mL each, using conical flasks.

The obtained fractions were concentrated using the rotary evaporator in a vacuum under reduced pressure at 50 °C and spotted on TLC plates. The fractions which had the same retention factor (RF) values

were combined. Further purification was carried out using further column chromatography, preparative chromatography, and chromatotron under different chromatographic conditions and Sephadex LH-20. In a typical experiment, the fractions that were not fully separated with 20-35 g were again subjected to column chromatography. Once again, fractions that showed identical TLC data were pulled together and then purified using preparative chromatography and Sephadex LH-20. This involves Sephadex LH 20 as the stationary phase and the mobile phase consisted of EtOAc in *n*-hexane solvent system in increasing polarity. The separation is based on the different molecular weights of the constituent compounds. Much smaller samples of 1 g and below were purified using chromatotron. In this case, the sample was applied as a solution using *n*-hexane: EtOAc in the ratio of 2:3 on silica gel and centrifugally accelerated. The separated components formed circular bands which were spun off from the edge of the rotor together with the solvent. The structures of the isolated compounds (Figure 1) were determined by analyses of their spectroscopic data and comparison with those reported in the literature.

Results and Discussion

Silica gel chromatography of the CH₂Cl₂/MeOH extracts of the stem of *Cola minor* afforded four compounds which were identified, using spectroscopic techniques, as friedelin (1), stigmaterol (2), friedelanol (3), and β -armyirin (4) (Figure 1).

Compound 1 was isolated as a white powder that crystallized out from ethyl acetate. The ¹H NMR spectrum of this compound showed seven singlets for the methyl groups at δ_H 0.87(s, H-23), 0.74 (s, H-24), 0.89 (s, H-25), 1.01 (s, H-26), 1.02 (s, H-27), 1.17 (s, H-28) and 0.98 (s, H-29); one methyl appeared as a doublet at 0.87 (d, *J* = 5.0 Hz, H-23). The proton and carbon peaks were allocated based on the ¹H-¹H COSY, DEPT, HSQC, and HMBC (Figures 4-6) analyses as shown in Table 1. From the ¹³C NMR and HSQC spectra (Figures 3 and 5), thirty carbons were identified, among them a ketone carbonyl group at δ_C 213.4. DEPT analysis indicated the presence of seven quaternary carbons, four methine carbons, eleven methylene carbons, and eight methyl carbons. Besides, ¹H-¹H COSY-NMR spectrum showed correlations of the following protons; H3-24/H3-25, H3-26/H-18, H3-25/H3-26, and H-18/H3-30 in association with coupling constants of H-10 (δ_H 1.42, *dd*, *J*=3.3, 12.8 Hz) for axial-axial and axial-equatorial coupling with H2-1 determined that A, B, C, and D ring fusion to be trans, and shown that H3-24, H3-26, H3-25, and H-18 were in the same side whereas H-10, H-8, and C-27 at C-13 were in the opposite side. HMBC indicated correlations between C-24, C-23, and C-5. Based on the above spectral data and comparison with literature values,^{13, 14} compound 1 was elucidated as a triterpene friedelin.

Compound 2 was obtained as white crystals in 15% EtOAc in *n*-hexane. The compound was not sensitive to UV₂₅₄ light hence was visualized using iodine vapor. The ¹H NMR spectrum (Figure 7) exhibited two olefinic protons at δ_H 5.31 for H-6 and 5.14 for H-21, a hydroxymethine proton at δ_H 3.49 (H-3) and a vinylic proton at δ_H 5.35 (*t*, ¹H, *J* = 3.0 Hz), 5.21 (*dd*, ¹H, *J* = 24 Hz) assigned to protons at H-5 and 20 positions, respectively (Table 1). Moreover, the ¹H-NMR spectrum of compound 2 showed a signal at δ 3.49 corresponding to the proton attached to the C-3 hydroxy group. The presence of these groups was confirmed by the ¹³C-NMR spectrum which showed peaks corresponding to δ_C 141.0 and 138.5, respectively. The characteristic

methyl protons were observed at δ_H 0.75(s, 3H), 0.83(s, 3H), 0.85(s, 3H), 0.87 (d, 3H) and 0.88 (d, 3H) and 1.04 (d, 3H, *J* = 5 Hz). Analysis of the ¹³C NMR data further indicated the presence of a quaternary carbon at δ_C 141.0 (C-5). ¹H-¹H COSY-NMR spectrum showed correlations of five spins spin systems: H₂-1/ H-3/ H₂-2/H₂-4, H-6/H₂-7/H-8/H-9/H₂-2/ H₂-11/H₂-12/H-16/H₂-15/H₂-14, H-20/H₃-21, H-22/H-23/H-24/H-25/H₃-27 and H₂-28/H₂-29. Verifications of ¹H and ¹³C assignments were done by 2D HSQC while connectivity was verified by HMBC. These spectral data confirmed that the hydroxyl was bonded to C-3 due to the long-range correspondence between C-3 and the protons at H-1 and H-2. H-4 and H-19 showed long-range correlations with C-5 thus placed the double bond at C-5. H-20 and H-21 had long-range correlations with C-22 thus placed the second double bond at this carbon atom. H-28 and H-29 correlations with C-24 determined the position of the isopropyl. Based on this spectral 1D and 2D data (Figures 7-11), as well as corroboration with literature reports,¹⁵⁻¹⁸ compound 2 was identified as stigmaterol with a molecular formula C₂₉H₄₈O.

Compound 3 was isolated as a white powder. From the ¹³C-NMR spectrum, 30 carbon signals were identified which included six quaternary carbons, five CH carbons, eleven CH₂ carbons, and eight CH₃ carbons. Noteworthy was the identification of an oxygenated carbon at δ_C 74.9. The presence of this hydroxyl group was further established by the characteristic hydroxyl group proton at δ_H 3.63 that corresponds to C-3. The position of this hydroxyl group was confirmed from the strong HMBC correlation of the hydroxyl proton at 3.63 with the carbon at 74.9 ppm. Furthermore, the HMBC spectrum of this compound showed that the proton at 1.22 ppm had a strong correlation with the carbons at 42.8 ppm, 35.6 ppm, 29.7 ppm, and 32.8 ppm. HMBC correlations were also observed between the proton at 1.22 ppm and the carbons at 28.0 ppm, 31.6 ppm, and 35.2 ppm; between the proton at 1.05 ppm and the carbons at 31.8 ppm and 36.7 ppm. From the ¹H-¹H COSY spectrum, the proton at δ_H 1.53 had a cross peak with δ_H 0.98 while that at δ_H 2.35 had a cross peak with the one at δ_H 0.94. Another cross peak was observed between the proton at δ_H 1.52 and that at δ_H 1.32. The NMR spectral data (Figures 12-16) for this compound suggested that is friedelanol, based on the good agreement of its ¹H and ¹³C NMR data with literature reports.¹⁹⁻²¹ The ¹H-NMR spectra of compound 4 indicated that the compound contained eight methyl singlets at δ 0.88, 0.99, 1.03, 1.05, 1.06, 1.14, 1.15, and 1.21. A protonated oxygen residue exhibited a signal at δ 3.46 (s, 1H) and is placed at C-3. An unsaturated proton at δ 5.35 (*t*, 1H, *J*=3.5 Hz) suggesting the presence of a double bond. The analysis of the ¹³C-NMR spectrum of compound 4 (Table 1) revealed the presence of 30 carbon signals including two olefinic carbons, ten methylene carbons, and four methine carbons, all suggestive of oleanane type triterpenoid. From the ¹H-¹H COSY spectrum the proton at δ_H 5.67 had a cross peak with δ_H 1.97 while the proton at δ_H 1.21 had a cross peak with the one at δ_H 1.05. Besides, the proton at δ_H 3.44 showed a cross peak with that at δ_H 1.68. HMBC correlations were also observed between the proton at δ_H 1.21 and the carbons at δ_C 35.0, δ_C 40.6 ppm, δ_C 41.8 and δ_C 38.8; between the proton at δ_H 1.05 with carbon at δ_C 76.2, δ_C 40.6, and δ_C 25.2. Moreover, HSQC correlations were also observed between the proton at δ_H 5.65 and the carbons at δ_C 121.6, δ_C 2.1 and δ_C 49.7; between the proton at δ_H 3.46 and the carbon at δ_C 76.2. Thus, the structure of compound 2 was identified as β -armyirin after the spectral data (Figures 16-20) compared favorably with those previously reported.²²⁻²⁶

Table 1: ^1H and ^{13}C -NMR chemical shift values for compounds 1-4.^{a,b}

Position	1		2		3		4	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	1.97, 1.68	22.3		37.3	0.86	17.5		38.8
2	2.37	41.6		32.0		35.2		27.9
3	-	213.4	3.49(<i>td</i> , 1H, $J = 6.5$ Hz)	71.6	3.63 (<i>dd</i> , $J = 11.5$ Hz)	74.9	3.46 (<i>s</i> , 1H)	76.2
4	2.24	58.2		42.2	3.84	49.4		39.3
5	-	42.2	5.35(<i>t</i> , 1H, $J = 1.5$ Hz)	141.0		37.3	1.15 (<i>s</i> , 3H)	56.2
6	1.76, 1.28	41.3		121.4	2.35	41.1	1.03 (<i>s</i> , 3H)	18.5
7	1.38, 1.47	18.3		31.7	0.75	14.3		32.8
8	1.38	53.1		31.9	5.33 (<i>t</i> , 1H, $J = 2$ Hz)	55.6		40.6
9	-	37.5		50.2		37.3		47.5
10	1.52	59.5		36.6	6.00	61.0		37.1
11	-	35.6		21.1	1.41(<i>d</i> , 3H, $J = 5$ Hz)	35.6		23.6
12	1.33	30.5		39.8		29.7	5.65 (<i>t</i> , 1H, $J = 6$ Hz)	121.6
13	1.49, 0.94	39.7		42.3		39.7		146.9
14	-	38.3		56.8		38.3		41.8
15	-	32.4		24.3	2.50, 2.41	32.0		26.9
16	1.55, 1.34	36.0		29.1	2.51(<i>d</i> , 3H, $J = 10$ Hz)	36.0		27.9
17	-	30.0		56.0	1.30	29.7		32.5
18	1.56	42.8		40.5		42.8		49.7
19	0.95	35.1	1.04 (<i>d</i> , 3H, $J = 5$ Hz)	21.7		35.0		47.5
20	-	28.2	5.21 (<i>dd</i> , 1H, $J = 9$ Hz)	138.7		28.0	1.21(<i>s</i> , 3H)	31.8
21	-	32.8	5.07 (<i>dd</i> , 1H, $J = 9$ Hz)	129.6	2.48	32.8		37.9
22	1.47, 0.92	39.3		46.1	0.96	39.1		35.0
23	0.87	6.8		25.4	1.05	10.4		15.9
24	0.74	14.7	0.75 (<i>s</i> , 3H)	12.1	0.87 (<i>s</i> , 3H)	16.4	1.05 (<i>s</i> , 3H)	28.5
25	0.89	17.9		29.6	1.10 (<i>s</i> , 3H)	18.1		15.9
26	1.01	20.3	0.88 (<i>d</i> , 3H)	20.2	0.98 (<i>s</i> , 3H)	20.0	0.88 (<i>s</i> , 3H)	17.2
27	1.02	18.7	0.85 (<i>d</i> , 3H)	19.8	1.05	18.7	1.14 (<i>s</i> , 3H)	25.2
28	1.17	32.1	0.83 (<i>s</i> , 3H)	18.9	1.32, 2.53 (<i>d</i> , $J = 5$ Hz)	32.3		28.8
29	0.98	31.8	0.87 (<i>s</i> , 3H)	12.2		35.2	0.99 (<i>s</i> , 3H)	33.9
30	1.20	35.4	-	-	1.21	31.6		23.6

^aAssignments made based on ^1H - ^1H COSY, DEPT, HSQC, and HMBC correlations; ^bChemical shift values are in δ (ppm).

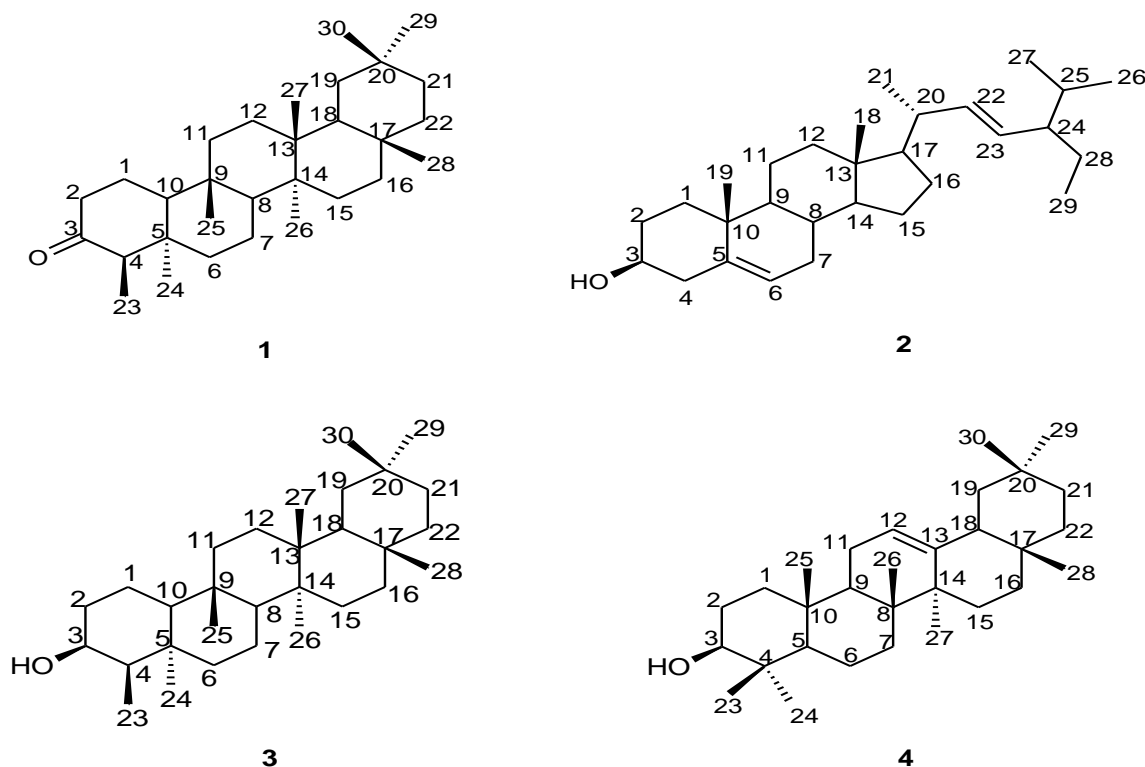


Figure 1: Chemical structures of isolates from the stem of *Cola minor*

Conclusion

Four compounds were isolated from the stem of *Cola minor* obtained from the Coastal region of Kenya at Nzombo forest. The structures of the isolated compounds were identified as friedelin (1), stigmasterol (2), friedelanol (3), and β -armylin (4) based on their spectroscopic data. The complete ^1H and ^{13}C NMR spectral assignments of the four isolated compounds were made based on ^1H - ^{13}C HCOSY, DEPT, HSQC, and HMBC analyses. Although these phytochemicals are widely distributed in the plant kingdom, this is the first report on their isolation from *Cola minor*.

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.

Acknowledgements

The authors would like to thank Potsdam University, Germany, for NMR spectroscopy.

References

- Mbuni YM, Wang S, Mwangi BN, Mbari NJ, Musili PM, Nyamolo OW, Hu G, Zhou Y and Wang Q. Medicinal Plants and their traditional uses in local communities around Cherangani Hills, Western Kenya. *Plants*. 2020; 9:331-347.
- Akthar MS, Degaga B, Azam T. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review. *Issues Biol Sci Pharm Res*. 2014; 2:1-7.
- Al-Snafi AE. Medicinal plants with antimicrobial activities (part 2): Plant-based review. *Sch Acad J Pharm*. 2016; 5:208-239.
- Hamayun M, Khan SA, Sohn EY, Lee I-J. Folk medicinal knowledge and conservation status of some economically valued medicinal plants of District Swat, Pakistan. *Lyonia*. 2006; 11:101-113.
- Banik B, Das S Das MK. Medicinal Plants with Potent Anti-inflammatory and Anti-arthritis Properties found in Eastern Parts of the Himalaya: An Ethnomedicinal Review. *Pharmacogn Rev*. 2021; 14:121-137.
- Sonibare M, Soladoye M, Esan O, Sonibare O. Phytochemical and antimicrobial studies of four species of *Cola* Schott & Endl. (Sterculiaceae). *Afr J Trad CAM*. 2009; 6:518-525.
- Agyare C, Koffuor GA, Boamah VE, Adu F, Mensah KB, Adu-Amoah L. Antimicrobial and anti-inflammatory activities of *Pterygota macrocarpa* and *Cola gigantea* (Sterculiaceae). *Evid-Based Compl Altern Med*. 2012; 2012:1-9.
- Adebola PO. *Cola*. In *Wild Crop Relatives: Genomic and Breeding Resources*. Springer, Berlin, Heidelberg. 2011; 63-71p.
- Victoria AO, Rufina A-SY, Oluwaseun OR, Olusola OB, Anderson EL, Sina, OJ and Fakunie JB. Phytochemical screening and proximate analysis of young *Cola acuminata* leaves. *Unique Res J Med Med Sci*. 2016; 4:029-034.
- Pan AD and Jacobs BF. The earliest record of the genus *Cola* (Malvaceae sensu lato: Sterculioideae) from the Late Oligocene (28–27 Ma) of Ethiopia and leaf characteristics within the genus. *Plant Syst Evol*. 2009; 283:247-262.
- Kenfack D, Sainge MN, Chuyong GB, Thomas DW. The genus *Cola* (Malvaceae) in Cameroon's Korup National Park, with two novelties. *Plant Ecol Evol*. 2018; 151:241-251.
- Azimi M, Ahmadi GM, Varasteh MA, Ebadi M, Zafar MR. Separation and Purification of Effective Ingredient of *Galegine*

- from *Galega officinalis* L. by Column Chromatography Tandemed with Molecularly Imprinted Polymer Enforced by Graphene Oxide (GO-MIP) Technique. *J Med Plants By-Prod.* 2020; 10:77-91.
13. Ambarwati N, Elya B, Malik A, Hanafi M and Omar H. Isolation, characterization, and antibacterial assay of friedelin from *Garcinia latissima* Miq. leaves. *J Phys Conf Ser. IOP Publishing*, 2019; 1402:055078-055091.
 14. Atwolara-Odule O, Aiyelaagbe O, Olubomehin O, Ogunmoye A, Feyisola R and Sanusi A. Antioxidant Activity of some Secondary metabolites from *Tapinanthus bangwensis* (Engl., and K. Krause)[Loranthaceae] Grown in Nigeria. *Sci Afr.* 2020; 8:00348-00354.
 15. Akwada UC, Igwe OU, Chisom F, Ibe-Diala JC. Isolation and Characterization of Stigmasterol and B-Sitosterol from the leaves of *Emilia coccinea* (Sims) G. Don. *Comm Phys Sci.* 2020; 6:863-868.
 16. Khan ME, Bala LM, Maliki M. Phytochemical analyses of *Terminalia schimperiana* (Combretaceae) root bark extract to isolate stigmasterol. *Adv J Chem-A.* 2019; 2:327-334.
 17. Okoro IS, Tor-Anyiin TA, Igoli JO, Noundou XS, Krause RWM. Isolation and Characterization of Stigmasterol and β -Sitosterol from *Anthocleista djalensis* A. Chev. *Asian J Chem Sci.* 2017; 3:1-5.
 18. Kaur G, Gupta V, Singhal R, Bansal P. Isolation and Characterization of Stigmasterol from *Fritillaria roylei*. *Biol Med Nat Prod Chem.* 2020; 9:77-80.
 19. Vasu K, Govardhan P, Reddy CS, Nath AR, Reddy R. *In-vitro* and *in-vivo* anti-inflammatory activity of *Syzygium alternifolium* (wt) Walp. *J Med Plants Res.* 2012; 6:4995-5001.
 20. Van Kiem P, Van Minh C, Huong HT, Nam NH, Lee JJ, Kim YH. Pentacyclic triterpenoids from *Mallotus apelta*. *Arch Pharm Res.* 2004; 27:1109-1113.
 21. Ngah L, Songue JL, Ekon JPL, Tsopgni WDT, Langat MK, Mpondo EM, Wansi JD, Ndom JC and Waffo AFK. The chemistry and biology activities of *Vitex phaseolifolius* Hildbr. *J Pharmacogn Phytochem.* 2020; 9:745-749.
 22. Okoye NN, Ajaghaku DL, Okeke HN, Ilodigwe EE, Nworu CS, Okoye FBC. Beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of *Alstonia boonei* display profound anti-inflammatory activity. *Pharm Biol.* 2014; 52:1478-1486.
 23. Dias M, Hamerski L, Pinto A. Separacao semipreparativa de α e β -amyrina por cromatografia líquida de alta eficiencia. *Quim Nova.* 2011; 34:704-706.
 24. Henneh IT, Huang B, Musayev FN, Hashimi RA, Safo MK, Armah FA, Ameyaw EO, Adokoh, CK, Ekor M, Zhang Y. Structural Elucidation and *In Vivo* Anti-arthritis Activity of β -Amyrin and Polpunonic Acid Isolated from the Root Bark of *Ziziphys abyssinica* HochstEx. A Rich (Rhamnaceae). *Bioorg Chem.* 2020; 103744:1-15.
 25. Hossain MA and Ismail Z. Isolation and characterization of triterpenes from the leaves of *Orthosiphon stamineus*. *Arab J Chem.* 2013; 6:295-298.
 26. Ebajo Jr VD, Shen C-C, Ragasa CY. Terpenoids and sterols from *Hoya multiflora* Blume. *J Appl Pharm Sci.* 2015; 5:33-39.