Natural & Applied Sciences Journal, Vol. II, No. 1,33-39, 2019
Research Article DOI: 10.38061/idunas.570049

A Bioactive (2R, 3R)-dihydroflavonol-3-O-α-L-rhamnoside from Bracystelma togoense Schtlr

Abiche Ekalu[*1], Rachael Gbekele-Oluwa Ayo[2], James D. Habila[2] and Ibrahim Hamisu[2]

Abstract
The Nigerian medicinal plant, Brachystelma togoense has being used to treat various ailments such as typhoid fever, cold and cough, gonorrhoea, skin infections, dysentery and pneumonia. In order to determine the ethnomedicinal potential of Brachystelma togoense. A dihydroflavonol-3-O-α-L-rhamnoside was isolated from the MeOH extract of the plant. The compound was identified using 1D and 2D NMR spectroscopic methods, mass spectrometry and by comparison with literature data. The compound exhibited antimicrobial activity against Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Salmonella typhi, and Candida albicans < 3 mg/ml. The compound had minimum inhibitory concentration ranging from 0.18 to 0.75 mg/ml and minimum bactericidal concentration ranging from 0.75 to 1.50 mg/ml. The compound is reported for the first time from Brachystelma togoense.

Keywords
flavonoid; dihydroflavonol-3-O-α-L-rhamnoside; Brachystelma togoense schtlr

[1] *Nigerian Army School of Education, Ilorin, Kwara, Nigeria*
[2] *Ahmadu Bello University Zaria, Kaduna, Nigeria*
*__Corresponding Author__: ekalumiracle@gmail.com
Manuscript received date: May 25, 2019
Accept Date: June 25, 2019
Published Date: June 30, 2019.

1. INTRODUCTION

Brachystelma was first described by Robert Brown in 1822. The genus Brachystelma R. Br. (Apocynaceae: Asclepiadoideae) is represented by about 100-120 species (Bruyns, 2009). The genus Brachystelma is chiefly distributed in South Africa, South-East Asia and Australasia (Ollerton et al., 2009). A total of 18 species are known in India (Britto and Bruyns, 2016) and out of them, 3 species in Maharashtra. It is an erect perennial herb, growing up to 30 cm, recorded from Ghana to Nigeria, in lowlands to montane situations. The tuber is said to be edible raw (Kew Royal Botanical Gardens, 2019). Many of the tuberous Brachystelma are known to be used medicinally for the treatment of headache, stomach ache and colds in children.

The World Health Organization (WHO) has reported that traditional medicinal plants are those natural plant materials which are used without processing for the treatment of various ailments in our locality. Traditional herbal medicine has being in use for the past years because it is natural and have fewer side effects (Jamshidi-kia et al., 2018). It in view of the uses of these plants for the treatment of various ailments that we decided to isolate the bioactive flavonoid in B.togense. Flavonoids are referred to as the primary class of polyphenolic compounds (Xie et al., 2015). Most flavonoids have been reported to exhibit various microbial activities which includes but not limited to anti-oxidation, anticancer, anti-inflammatory activies (Xiao and Kai, 2012). Over the years, flavonoids have become interesting subject of medical research all over the world. This is because, they have been reported to posses vast antimicrobial activies (Cushnie and Lamb, 2005). Flavonoids have been reported also to posses many biochemical properties. They are known for their antioxidant activity, hepatoprotective activity, antibacterial activity, anti-inflammatory activity, anticancer activity and antiviral activity (Kumar and Pandey, 2013). A similar flavonoid, 2-3-dihydromyricetin-3-O-α-L-rhamnoside isolated from Pradosia huberi (Ducke) Ducke (Sapotaceae) was reported

for rat isolated mesenteric arteries though it was ineffective in the eliciting vasorelaxation (Medeiros et al., 2010). But flavoids antimicrobial activities have been well documented. Therefore, the bilological investigation of the plant which led to the isolation and characterization of a bioactive flavonoid has justified the ethnomedicinal use of B.togense in Nigeria.

2. MATERIALS and METHODS

2.1 Instrumentation

The NMR spectra were recorded in CD3OD on a 400 MHz Bruker AVANCE III NMR instrument at room temperature. HREIMS was recorded on an Agilent Technologies 6550 iFunnel Q-TOF LC/MS with the sample dissolved in CH_2Cl_2. The optical rotation was determined in CH_2Cl_2 on a JASCO P-1020 polarimeter and the infrared spectra was recorded using a Perkin-Elmer (2000 FTIR) spectrometer on NaCl plates. The ECD spectra was measured on a Chirascan CD spectrometer using a 1 mm cell with acetonitrile as solvent at the Department of Chemistry, FEPS, University of Surrey, United Kingdom.

2.2 Plant material

The aerial parts of Brachystelma togoense was collected in the month of April 2018 from Benue State, Nigeria. The plant was identified by the plant taxonomist Mallam Sanusi Namadi and a voucher specimen (25856) is retained in Biological Science Depatment, Ahmadu Bello University, Zaria-Nigeria (Ekalu et al., 2019).

2.3 Extraction and isolation

The air dried Brachystelma togoense (1.0 kg) was extracted on a shaker at room temperature successively with 100 % CH_3OH for 72 hours. The extracts was concentrated using a rotary evaporator at 40 °C to a yield brown gum (9.0 g, 0.9 % MeOH extract). The extract was separated by flash chromatography (Biotage SP1) over silica gel using three solvent mixtures; first with a $CH_2Cl_2/EtOAc$ step gradient starting with 100 % CH_2Cl_2 and gradually increasing the polarity to 100 % CH_2Cl_2 then EtOAc was added gradually until 100 % EtOAc was reached to yield 8 fractions ((Fr.1-Fr.8). Fr.5 was separated successively over Sephadex LH-20 ($CH_3OHEtOAc$ 2:8) to give 1 (50 mg, 0.6 %).

2.4 Antimicrobial screening

The antimicrobial activities of the isolates were determined using some microorganisms. The microorganisms were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. The antimicrobial activity of the isolates were evaluated using the broth dilution assay as described previously for the bacteria and Mueller Hinton broth assay for the fungus (Niaz et al., 2018). The microorganisms tested were: Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Salmonella typhi, and Candida albicans. Different solutions of 6.0 mg of the compound were made using DMSO (10 ml) and the experiment was also set up using DMSO as the control (Bello et al., 2011a).

2.5 Determination of zone of inhibition

The standardized inocula of the isolate were uniformly placed on freshly prepared Mueller Hinton agar plates using a sterile swab stick. Exactly 5 appropriately labelled wells were punched into each agar plate using a sterile cork borer (6 mm in diameter). Aliquot of 0.3 ml of the appropriate isolate concentration was placed in each well and then allowed to diffuse into the agar. An extra plate was streaked with the isolate and ciprofloxacin (10 μ/disc) was placed on it. The plates were incubated at 37 °C for 24 h. While for the fungus, Sabouraud dextrose broth was used and the incubation period was 30 °C and 48 h. The antimicrobial activities were expressed as diameter (mm) of inhibition zones produced by the isolate (Bello et al., 2011a).

2.6 Minimum inhibition concentration (MIC)

The minimum inhibition concentrations of the isolate was carried out using the broth dilution method as outlined by the Clinical and Laboratory Standards Institute (CLSI. Performing Standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute, n.d.). Mueller Hinton broth was prepared; 10 ml was dispensed into tubes and was sterilized at 121 °C for 15 minutes and allowed to cool. The McFarland turbidity standard scale 0.5 was prepared to give turbidity solution. Normal saline was prepared, 10 ml was dispensed into sterile test tube and the test microbes was inoculated and incubated at 37 °C for 24 hours. Dilution of the test microbes was done in the normal saline until the turbidity marched that of the Mc-Farland turbidity scale by visual comparison at this point the test microbe has a concentration of about 1.5x108 cfu/mL. Two-fold serial dilution of the extracts in the sterilized broth was made to obtain the concentrations of 3.00 mg/ml, 1.50 mg/ml, 0.75 mg/ml, 0.37 mg/ml, 0.18 mg/ml and 0.09 mg/ml. The initial concentration was obtained by dissolving 6 mg of the isolate in 10 ml of the sterile broth. Having obtained the different concentrations of the extracts in the sterile broth, 0.3 ml of the standard inoculum of the test microbe in the normal saline was then inoculated into the different concentrations. Incubation was made at 37 °C for 24 h, after which each test tube of the broth was observed for turbidity (growth). The lowest concentration of the isolate in which the broth shows no turbidity was recorded as the Minimum Inhibition Concentration (MIC) (Bello et al., 2011a).

2.7 Determination of minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC)

This was carried out to determine whether the microorganisms could be completely killed or their growth could only be inhibited. The minimum bactericidal concentration of the isolates was determined as outlined by the CLSI on the nutrient agar plates. Minimum bactericidal concentrations were determined by assaying the test tube contents of the MIC determinations. A loopful of the content of each tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 °C for 24 h for bacterial and 30 °C for 48 h for fungus. Then it was observed for microbial growth. The lowest concentration of the subculture with no growth was the minimum bactericidal concentration/ minimum fungicidal concentration (Bello et al., 2011a).

3. RESULTS and DISCUSSION

3.1 Data for compound 1

Dihydroflavonol-3-O-α-L-rhamnoside (1): Yellow solid, $[\alpha]_D$ ^{20} = - 83.6 (c =1.00, MeOH); HRESIMS: m/z 474.1071 [$C_21H_24O_7 + Na$] (Calculated for $C_21H_24O_7Na$ 473.1060 for $C_21H_22O_11Na$); IR (neat): 3382, 2919, 1638 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data are given in Table 1.

Compound 1 was isolated as a yellow solid from the MeOH extract of the aerial parts of Brachystelma togoense and was identified as the known dihydroflavonol-3-O-α-L-rhamnoside, which has been isolated from the methanol extract of Xinjiang wine grapes (Vitis vinifera) (De Britto et al., 1995; Xueyan et al., 2018).

The HRESIMS (spectrum 1.1) showed a molecular ion peak at m/z [M+H+Na]$^+$= 474.1071 (calcd 473.1060 for $C_21H_22O_11Na$) indicating a molecular formula of $C_21H_22O_11$ for the compound. The IR spectrum (spectrum 1.2) showed absorbance bands for hydroxyl (3382 cm^{-1}), conjugated ketone (1638 cm^{-1}), sp^3 CH (2919 and 2851 cm^{-1}) and olefinic carbon (1463 cm^{-1}) groups.

Ring A showed meta-coupled H-6 (δ_H 5.93 d, J = 1.2 Hz) and H-8 (δ_H 5.91 d, J = 1.2 Hz) proton resonances and ring B showed an ABX system with coupled H-2' (δ_H 6.97 d, J = 1.9 Hz), H-5' (δ_H 6.83 d, J = 8.2 Hz) and H-6' (δ_H 6.84 dd, J = 2.1, 8.1 Hz) resonances. As no methoxy groups were present, hydroxyl groups were placed at C-5, C-7, C-3' and C-4'. Hydroxy group proton resonances were not seen in the ^{1}H NMR spectrum (spectrum 1.3) as the solvent used was deuterated methanol and the hydroxyl groups of the molecule undergo proton exchange.

A keto group (δ_C 196.1) was present in the ^{13}C NMR spectrum (spectrum 1.4) that could be assigned to C-4 of the flavonoid skeleton. A pair of doublets (δ_H 5.09 d, J = 10.8 Hz, δ_H 4.59 d, J = 10.7 Hz) could be assigned to H-2 and H-3 of ring B. These large coupling constants showed that H-2 and H-3 were trans to each other.

The NMR spectra showed the presence of one sugar group. The presence of a three proton doublet (δ_H 1.19 d, J = 6.3 Hz) indicated that this was α-L-rhamnose (Xueyan et al., 2018). This was supported by correlations seen in the NOESY spectrum (spectrum 1.9) between the 1" (δ_H 4.06 d, J = 1.6 Hz), 3" (δ_H 3.67 dd, J = 3.4, 9.5 Hz), 4" (δ_H 3.31 dd, J = 6.6, 9.5 Hz) and 5" (δ_H 4.26 dd, J = 6.5, 9.6 Hz) proton resonances. The rhamnose was attached at C-3 β. This was shown by a correlation seen between the H-3 and C-1" ((δ_C 102.3) resonances.

The ^{1}H and ^{13}C NMR resonances were assigned using HSQC and HMBC spectra and are given in Table 1, the structure of compound compound 1 is shown in Figure 1.

The specific rotation was found to be $[\alpha]_D$ ^{20} = - 83.6 (c = 1.00 g/ml, MeOH) and $[\alpha]_D$ ^{20} = - 13.5 was reported (Xueyan et al., 2018) which confirmed the stereochemistry of compound 1. The configurations at the chiral centres were confirmed using the NOESY spectrum.

The ECD spectrum (spectrum 1.10) agreed with the stereochemistry reported in literature (De Britto et al., 1995).

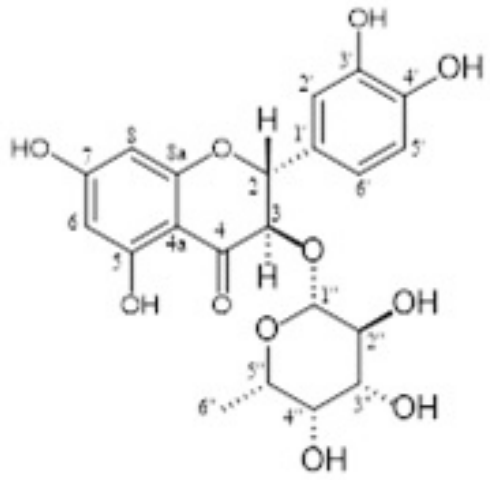

Figure 1. Molecular structure of (2R, 3R)-dihydroflavonol-3-O-α-L-rhamnoside isolated from bracystelma togoense.

Table 1. NMR Data for (2R, 3R)-dihydroflavonol-3-O-α-L-rhamnoside (CD_3OD, 400 MHz J in Hz)

C	^{13}C NMR (100MHz) in CD_3OD	^{13}C NMR (100MHz) in DMSO-6 (Xueyan et al., 2018)	^{1}H NMR (400MHz) CD_3OD (J in Hz)	HMBC (H→C)	COSY	NOESY
1	-					
2	84.1 CH	82.4	5.09 d, J= 10.8	1', 2', 3, 4, 6'	3α	3
3	78.7 CH	76.5	4.59 d, J= 10.7	1', 1", 2, 4	2β	2
4	196.1 C	195.1	-			
5	164.3 C	162.8	-			
6	97.5 CH	96.2	5.93 d, J= 2.1	5, 7, 8		
7	165.7 C	167.0	-			
8	96.4 CH	95.1	5.91 d J= 2.1	6,7,9		
4a	162.5 C	162.8	-			
8a	102.6 C	101.5	-			
1'	129.3 C	128.1	-			
2'	115.6 CH	114.5	6.97 d, J= 1.9	1', 2, 3',6'		
3'	146.7 C	145.9	-			
4'	147.5 C	145.1	-			
5'	116.5 CH	115.2	6.83 d J= 8.2	1', 4', 6'	6'	
6'	120.6 CH	119.5	6.84 dd, J = 2.1, 8.1	1', 2, 4', 5'	5'	
1"	102.3 CH	100.5	4.06 d, J= 1.6			3α, 3", 4", 5"
2"	71.9 CH	70.4	3.54 dd, J= 1.7, 3.3			3α, 3", 5"
3"	72.3 CH	71.3	3.67 dd, J = 3.4, 9.5	4", 5"	4", 5"	3α, 4", 5"
4"	73.9 CH	72.6	3.31 dd, J = 6.6, 9.5	3", 5", 6"	3", 5", 6"	2
5"	70.7 CH	68.9	4.26 dd, J = 6.5, 9.6	3", 4", 6"	3", 4", 6"	3α, 3", 4"
6"	17.9 CH3	17.1	1.19 d, J= 6.3	4",5"	4",5"	3α, 3", 4", 5"

Table 2. Diameter of Zone of Inhibition (mm) of the compound

Concentration (mg/ml)						
Micro organisms	3.0	1.5	0.75	0.375	Ciprofloxacin 10x10-6	Terbinafine 30x 10-6
S. aureus	16	14	13	12	25	
E. coli	18	16	14	13	35	
S. pneumoniae	16	14	12	11	20	
S. typhi	23	20	16	13	24	
C. albicans	20	18	16	14		33

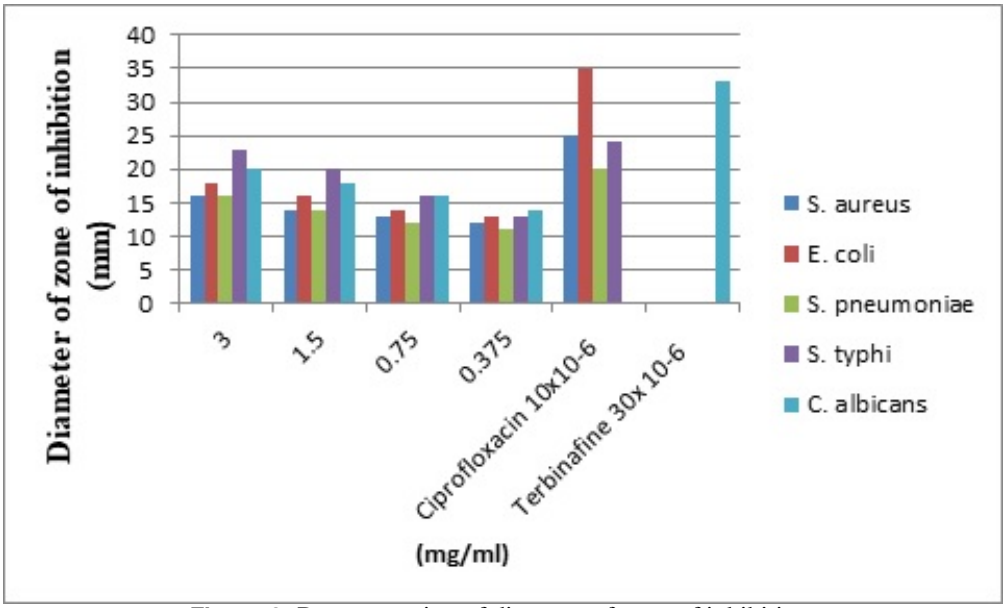

Figure 2. Representation of diameter of zone of inhibition.

Table 3. Summary of MIC, MBC and MFC of the compound (mg/ml)

Micro organisms	MIC	MBC	MFC
S. aureus	0.75	1.50	
E. coli	0.37	0.75	
S. pneumoniae	0.75	1.50	
S. typhi	0.37	0.75	
C. albicans	0.18		0.75

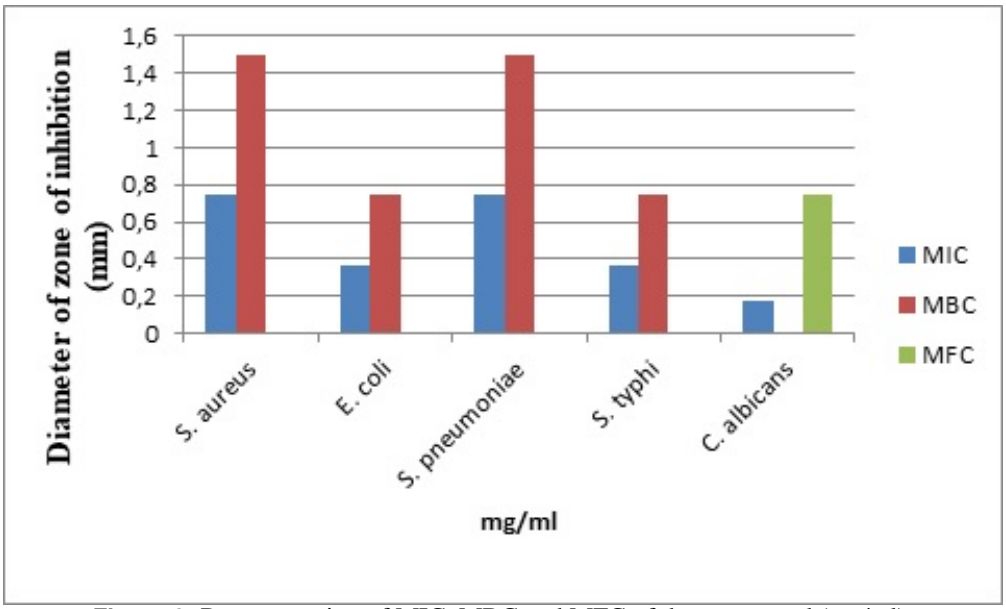

Figure 3. Representation of MIC, MBC and MFC of the compound (mg/ml).

Flavonoids are phenolics widely distributed in plants with reported antimicrobial activity (Cushnie and Lamb, 2005). The results showed that the compound had a remarkable activity against all the five microorganisms tested. Four bacterial strains, Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Salmonella typhi, were used. Ciprofloxacin was used as the positive control against the four bacteria with MIC values of 0.75, 0.37, 0.75, 0.37 mg/ml (Table 3 and figure 3) respectively.

Candida albicans was used as the fungus. Terbinafine was used as the positive control against the fungus with MIC values of 0.18 mg/ml (Table 3 and figure 3). The compound had zones of inhibition ranging from 11 to 23 mm (Table 2 and figure 2). Flavonoids have been reported to exhibite anti-inflammatory, anti-microbial, anti-cancer and anti-allergic activities (Bello et al., 2011b). The antimicrobial activity of flavonoid has been reported (Taleb-Contini et al., 2003) for different flavonoids on microorganisms.There, we can confirm that the isolated compound, a flavonoid has vast medicinal potential which justifies the ethnomedicinal uses of the plant.

4. CONCLUSION

A flavonoid was isolated and identified as a (2R, 3R)-dihydroflavonol-3-O-α-L-rhamnoside from B.togoense. To the best of our knowledge, the compound is reported for the first time in this source. The flavonoid showed significant activities against E. coli, S. typhi and C. albicans. The results from this research have supported the ethnomedicinal uses of this plant in the treatment of, typhoid fever skin infections, abdominal disorders, dysentery, pneumonia, gonorrhea, and as a cough and cold remedy. This justifies the ethnomedicinal uses of the plant in Nigeria.

Conflict of interest

The authors declear no conflict of interest

Acknowledgement

This work was supported by Tertiary Education Trust Fund (TETFUND). The author whishes to thank the Natural product Researcg Group of the Department of Chemistry, University of Surrey, United Kingdom for the laboratory and NMR analyses.

REFERENCES

Bello, I.A., Ndukwe, G.I., Audu, O.T., Habila, J.D., 2011a. A bioactive flavonoid from Pavetta crassipes K. Schum. Organic and Medicinal Chemistry Letters 1, 14. https://doi.org/10.1186/2191-2858-1-14

Bello, I.A., Ndukwe, G.I., Audu, O.T., Habila, J.D., 2011b. A bioactive flavonoid from Pavetta crassipes K . Schum. Organic and Medicinal Chemistry Letters 1:14, 10–15. https://doi.org/10.1186/2191-2858-1-14

Britto, S.J., Bruyns, P. V, 2016. Three new species of Brachystelma from Tamil Nadu, India. Haseltonia 48–54.

Bruyns, P. V, 2009. Three New Species of Brachystelma (Apocynaceae, Asclepiadoideae, Ceropegieae) from South Tropical and Southern Africa. SPIE.

CLSI. Performing Standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute, 2017:32, n.d. PL, 27 th. ed.

Cushnie, T.P.T., Lamb, A.J., 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 26, 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

De Britto, J., Soosai Manickam, V., Gopalakrishnan, S., Ushioda, T., Tanaka, N., 1995. Chemical and chemotaxonomical studies of ferns. Determination of aglycone chirality in dihydroflavonol 3-O-.ALPHA.-L-rhamnosides by 1H-NMR spectroscopy. https://doi.org/10.1248/cpb.43.338

Ekalu, A., Ayo, R.G., Habila, J.D., Hamisu, I., 2019. Phaeophytin and Triterpenoids from Brachystelma togoense Schltr , a Nigerian Medicinal Herb. Asian Journal of Chemical Sciences 6, 1–5. https://doi.org/10.9734/AJOCS/2019/v6i118990

Jamshidi-kia, F., Lorigooini, Z., Amini-khoei, H., 2018. Medicinal plants: Past history and future perspective. Journal of Hermbed Pharmacology 7, 1-7. https://doi.org/10.15171/jhb.2018.01

Kew Royal Botanical Gardens, 2019. Electronic Plant Information Centre (ePIC) [WWW Document]. URL http://epic.kew.org/index.htm (accessed 2.7.19).

Kumar, S., Pandey, A.K., 2013. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal

2013, 1–3. https://doi.org/10.1016/S1572-5995(05)80065-1

Medeiros, A.A.N., Medeiros, F.A., Queiroz, T.M., Tavares, J.F., Silva, M.S., Medeiros, I.A., 2010. Artigo rhamnoside from Pradosia huberi (Ducke) Ducke on rat isolated mesenteric arteries. Animals 20, 542–548.

Niaz, S.I., Zhang, P., Shen, H., Li, J., Chen, B., Chen, S., Liu, L., He, J., 2018. Two new isochromane derivatives penisochromanes A and B from ascidian-derived fungus Penicillium sp. 4829. Natural Product Research 6419, 1–7. https://doi.org/10.1080/14786419.2018.1470173

Ollerton, J., Masinde, S., Meve, U., Picker, M., Whittington, A., 2009. Fly pollination in Ceropegia (Apocynaceae: Asclepiadoideae): biogeographic and phylogenetic perspectives. Annals of Botany 103, 1501–1514. https://doi.org/10.1093/aob/mcp072

Taleb-Contini, S.H., Salvador, M.J., Watanabe, E., Ito, I.Y., Oliveira, D.C.R. de, 2003. Antimicrobial activity of flavonoids and steroids isolated from two Chromolaena species. Revista Brasileira de Ciências Farmacêuticas 39, 403–408. https://doi.org/10.1590/S1516-93322003000400007

Xiao, J., Kai, G., 2012. A review of dietary polyphenol-plasma protein interactions: Characterization, influence on the bioactivity, and structure-affinity relationship. Critical Reviews in Food Science and Nutrition 52, 85–101. https://doi.org/10.1080/10408398.2010.4990

Xie, Y., Yang, W., Tang, F., Chen, X., Ren, L., 2015. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Current medicinal chemistry 22, 132–49. https://doi.org/10.2174/0929867321666140916113443

Xueyan, R., Jia, Y., Xuefeng, Y., Lidan, T., Qingjun, K., 2018. Isolation and purification of five phenolic compounds from the Xinjiang wine grape (Vitis Vinifera) and determination of their antioxidant mechanism at cellular level. European Food Research and Technology 244, 1569–1579. https://doi.org/10.1007/s00217-018-3070-z