ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

A TRITERPENOID SAPONIN FROM THE ROOT BARK EXTRACT OF *Massularia acuminata spp.*

Harami M. Adamu[1], *Uduak S. Ukekpe[1,2] and Eno O. Ekanem[1].

1. Chemistry Programme, Abubakar Tafawa Balewa University, Bauchi, Nigeria.
2. Department of Science Laboratory Technology, Federal Polytechnic, Damaturu, Nigeria.

Manuscript Info

Manuscript History:

Received: 14 January 2016
Final Accepted: 19 February 2016
Published Online: March 2016

Key words:
Massularia acuminata, Saponins, Aglycone, Oleanolic acid, Antimicrobial, Extract, Root bark.

Corresponding Author

Uduak S. Ukekpe.

Abstract

Compound CFME2 was isolated as the second column fraction of chloroform soluble fraction of methanol extract of the root bark of Massularia acuminata and was identified to be a bioactive triterpenoid saponin. On the basis of chemical and spectroscopic analyses, the structure of the compound was established and the compound identified as 3-O – β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – 28- O – β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl- oleanolic acid. 1D- and 2D- NMR experiments which include ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HSQC and HMBC were used to achieve the structural elucidation. The compound showed good inhibitory ability against the microorganisms, *Salmonella spp, Staphylococcus aureus, Escherichia coli, Aspergillus niger and Candida albicans.*

Introduction:-

Massularia acuminata (D. Don) Bullock ex. Hoyle is a medium size shrub or small tree growing up to 5 m high (Yakubu and Omoniwa, 2012, Iwu, 2014). It is a tropical plant native to the tropical region of West Africa (Wong, 2014) and it is found usually in the undergrowth of close moist forest (Yakubu et al., 2008). The plant's stem has been used as chewing stick to achieve oral health over ages in the Southern part of Nigeria (Aderinokun et al., 1999, Bankole et al., 2012, Ogunshe and Ademiluka, 2013). It is a well known medicinal plant in Akwa Ibom State, Nigeria, as the root bark has been used to treat skin infections. The medicinal value of the root bark of Massularia acuminata is evidenced in the phytochemical constituents and the antimicrobial activities of the extracts as reported by Ukekpe et al. (2015). The work indicated a strong presence of saponins in the extracts as one of the phytochemicals.

Saponins are non-volatile surface active compounds that are widely distributed in nature (Vincken et al., 2007). They are naturally occurring glycosides characterized by their strong foam forming property in aqueous solution (Gl-stndag and Mazza, 2007, Man et al., 2010 and Negi et al., 2013). Many saponins are known to have antimicrobial properties. They inhibit moulds and protect plants from insects and therefore are among the protective molecules found in plants (Negi et al., 2013).

In this work, isolation and characterization of a triterpenoid saponin from the methanol extract of the root bark of *Massularia acuminata* has been described. The antimicrobial activities of the compound against some clinical isolates have also been shown.

Methods:-

Plant Material:-

Fresh roots of *Masssularia acuminata* were collected within Udi and Nto Etuk Udo villages in Ika L.G.A., Akwa Ibom State, Nigeria. The roots were washed with water, the bark peeled-off the roots, air dried and ground to fine powder.

Extraction:-

Soxhlet extraction was carried out exhaustively on the plant part using the solvents, hexane, chloroform, ethyl acetate, acetone, ethanol and methanol sequentially to yield the solvents' crude extracts of the plant part.

Purification and Isolation:-

The methanol crude extract was subjected to solvent fractionation with hexane, chloroform, ethyl acetate, acetone, ethanol and methanol sequentially to yield solvent fractions of the extract.

The chloroform fraction (CFME) was subjected to column chromatography by the method described by Teke et al. (2010) and Ode et al. (2011) using glass column (60 x 8 cm) packed with silica gel (type 60 – 120 mesh, Qualikens) with hexane - methanol (1:4) solvent system as the eluent. This afforded compound CFME2, a white amorphous powder as the second fraction.

Melting Point Determination:-

The melting point of the compound was determined in melting point capillary tube using Graffin – Gallenkamp 350BM2.5 melting point apparatus.

Spectroscopic Analyses:-

The infrared spectroscopic analysis of the compound was carried out using FTIR – ATR Bruker Alpha – p spectrometer.

The ^{1}H, ^{13}C, DEPT and 2D (^{1}H - ^{1}H COSY, HSQC, HMBC) NMR spectroscopic analyses of the compound in DMSO–d6 with TMS internal standard were performed on Bruker 600 MHz spectrometer.

Acid Hydrolysis/Sugar Identification:-

The method of Viana et al. (2004), Li et al. (2010) and Xu et al. (2010) was employed for the acid hydrolysis of the isolated compounds to identify the sugar moieties.

The isolated compound (10 mg) was refluxed in 10 cm^3 2M methanolic HCl for 3hours. The reaction mixture was diluted with distilled water and extracted with chloroform. The chloroform extract was evaporated to yield the aglycone component of the compound, and the aqueous layer neutralized with saturated Na_2CO_3 solution and subjected to TLC analysis with CH_2Cl_2 - MeOH - H_2O (15:6:1) as the developing reagent. The spots were visualized by spraying with 50% ethanolic H_2SO_4, allowed to dry and the plate heated at 100 ^0C for spots to appear. The R_f value was compared with that of standard sugars to identify the sugar moieties.

Antimicrobial Activity:-

The antimicrobial activity of the isolated compound was assayed against Salmonella spp, Staphylococcus aureus and Escherichia coli for the antibacterial activity, and Aspergillus niger and Candida albicans for the antifungal activity.

The modified plate-well diffusion assay described by Uduak and Kola (2010) and Babu et al. (2007) was used for the tests. Wells were made in nutrient agar and sabourand dextrose agar (SDA) plates which had been previously incubated with bacteria and fungi respectively. The sample solutions of different concentrations (0.2cm^3 each) were introduced into the wells and left to pre-diffuse for 30 minutes. Distilled water was used as negative control and standard drugs, streptomycin for bacteria and fulcin for fungi, were used as positive controls.

The bacterial plates were incubated at 37^{o}c for 24h, while the fungi plates were incubated at 25^{o}c for 72 h.

The degree of inhibition was determined by the size of the zone of inhibition measured in mm and was taken as evidence of antimicrobial activity of the compound.

Results and Discussion:-

Table 1: Compound CFME2 ^{1}H and ^{13}C Spectra Data

Carbon (Aglycone)	^{13}C (δ)	DEPT	^{1}H(δ)	Carbon (Sugar moieties)	^{13}C (δ)	DEPT	^{1}H(δ)
1	38.7	$>CH_2$	1.16, 1.37 m	C_1'	104.0	>CH-	5.45 d
2	27.0	$>CH_2$	1.28, 1.50 m	C_2'	83.0	>CH-	4.14
3	89.0	>CH-	2.68 t	C_3'	78.0	>CH-	3.43
4	39.0	>C<	-	C_4'	71.0	>CH-	3.84
5	55.0	>CH-	0.83 t	C_5'	76.0	>CH-	3.63
6	19.0	$>CH_2$	1.22, 1.44 m	C_6'	64.0	$>CH_2$	3.54, 3.60
7	33.0	$>CH_2$	1.16, 1.38 m	C_1''	103.0	>CH-	5.25 d
8	40.0	>C<	-	C_2''	77.0	>CH-	3.34
9	48.0	>CH-	0.94 s	C_3''	77.9	>CH-	2.77
10	37.0	>C<	-	C_4''	72.0	>CH-	3.10
11	24.0	$>CH_2$	1.71, 1.93 d	C_5''	78.0	>CH-	2.93
12	123.0	>CH-	4.48 t	C_6''	62.0	$>CH_2$	2.86, 2.91
13	144.0	>C<	-	C_1'''	102.1	>CH-	4.38 d
14	42.0	>C<	-	C_2'''	72.0	>CH-	3.16
15	28.0	$>CH_2$	0.94, 1.16 m	C_3'''	74.0	>CH-	3.00
16	23.0	$>CH_2$	1.28, 1.47 m	C_4'''	78.8	>CH-	2.92
17	47.0	>CH<	-	C_5'''	76.0	>CH-	2.92
18	42.0	>CH-	2.34 d	C_6'''	64.0	$>CH_2$	2.85, 3.90
19	46.0	$>CH_2$	1.06, 1.28 m	C_1''''	95.0	>CH-	5.40 d
20	30.5	>C<	-	C_2''''	72.0	>CH-	3.65
21	34.0	$>CH_2$	1.23, 1.32 m	C_3''''	75.0	>CH-	2.76
22	32.5	$>CH_2$	1.55, 1.77 m	C_4''''	74.8	>CH-	3.09
23	28.5	$-CH_3$	0.79 s	C_5''''	76.0	>CH-	2.72
24	17.0	$-CH_3$	0.72 s	C_6''''	62.0	$>CH_2$	2.85, 2.90
25	15.6	$-CH_3$	0.74 s	C_1'''''	97.0	>CH-	4.55 d
26	17.5	$-CH_3$	1.02 s	C_2'''''	73.0	>CH-	3.99
27	26.0	$-CH_3$	0.89 s	C_3'''''	74.0	>CH-	3.31
28	176.0	>C<	-	C_4'''''	77.0	>CH-	3.69
29	33.0	$-CH_3$	0.77 s	C_5'''''	78.0	>CH-	3.50
30	24.0	$-CH_3$	0.76 s	C_6'''''	62.0	$>CH_2$	3.41, 3.47
				C_1''''''	99.0	>CH-	4.85 d
				C_2''''''	73.0	>CH-	3.91
				C_3''''''	74.0	>CH-	3.73
				C_4''''''	78.0	>CH-	3.64
				C_5''''''	76.5	>CH-	3.02
				C_6''''''	64.0	$>CH_2$	3.51, 3.57

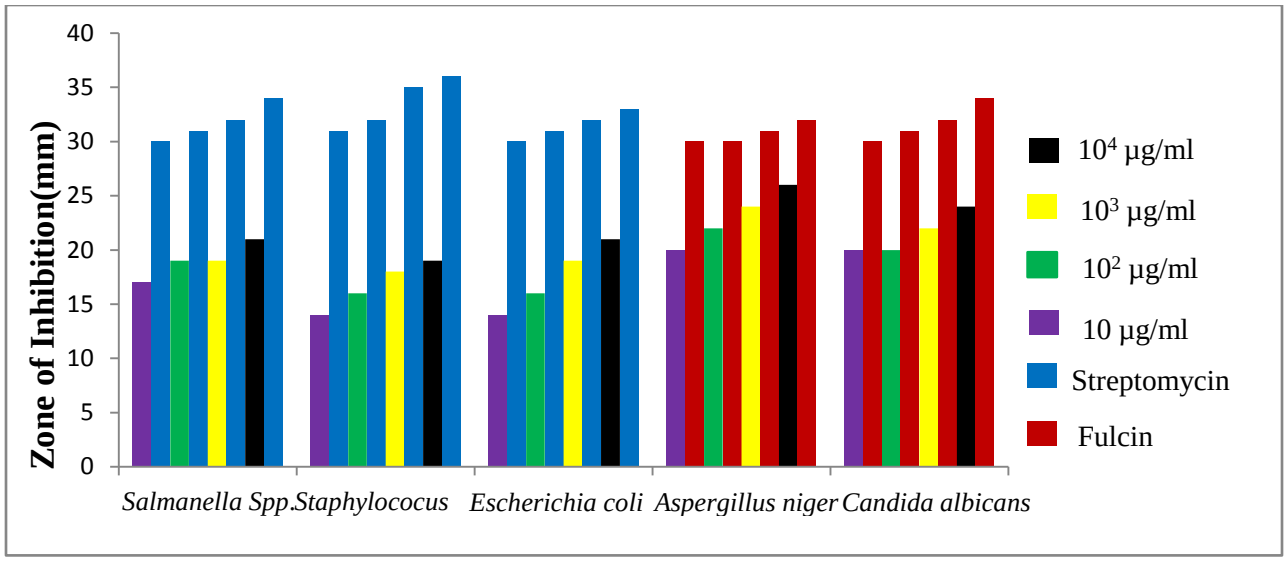

Figure1: Antimicrobial Activity of Compound CFME2.

Discussion:-

The fractionation by column chromatography of chloroform fraction of methanol crude extract of Massularia acuminata afforded compound CFME2 as the second fraction (R_f – 0.674). Compound CFME2 was isolated as white amorphous powder with a melting point of 220 – 222 ^{0}C. The IR spectrum of the compound exhibited absorption bands at 3382 cm^{-1} (OH), 2933 cm^{-1} (C-H stretching), 1704 cm^{-1} (C=O stretching), 1630 cm^{-1} (C=C stretching), 1229 cm^{-1} (C-O stretching) and 766 cm^{-1} (C-H bending). The broad band at 3382 cm^{-1} indicates the glycosidic nature of the compound (Khan, 2011), and the peak at 766 cm^{-1} due to C-H bending is characteristic of olefinic bond at C-12 of a pentacyclic triterpenoid saponin.

The structural elucidation of compound CFME2 was carried out using 1D- and 2D- (^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H-COSY, HSQC and HMBC) NMR experiments. The aglycone portion of the compound was established as olean-12-en-28-oic acid by comparing ^{13}C -NMR spectra data of the compound with reported similar saponins with the same aglycone (Teng et al., 2003, Seebacher et al., 2003, Shah et al., 2009). The sugar moieties were established as glucose as the acid hydrolysis sugar products of the compound indicated only glucose on TLC analysis.

The ^{1}H NMR showed seven methyl singlets at δ 1.02, 0.89, 0.79, 0.77, 0.76, 0.74, 0.72 and a vinyl proton signal at δ 5.5 suggesting that the aglycone is an olean -12- ene skeleton (Debella et al., 2000). It also displayed six anomeric proton signals at δ 5.45 (J= 8.76), 5.25 (J= 7.85), 4.38 (J= 7.14), 5.40 (J= 7.8), 4.55 (J= 6.91) and 4.85 (J= 7.56) signifying the presence of six sugar units (Mehta et al., 2004, Zhoa, 2010).

The ^{13}C –NMR spectrum showed the C-3 signal to be shifted downfield at δ 89 compared with δ 78.5 (Güvenalp et al., 2006) of oleanolic acid. This is as a result of glycosylation shift indicating that saccharide moiety is bonded to oxygen of C-3 of the aglycone (Debella et al., 2000, Alvarez et al., 2003, Mehta et al., 2004). Glycosylation shift was also observed for the C-28 at δ 176.0 as compared with δ 180 of oleanolic acid (Debella et al., 2000, Mehta et al., 2004, Li et al., 2010, Uddin et al., 2011), also indicating the bonding of saccharide at that point. The glycosylations at these carbons have been corroborated by the HMBC experiment which showed correlation between the anomeric proton of C′ and C-3 and the anomeric proton C′′′′ and C-28(Figure 3). The confirmation by HMBC experiment of the glycosylation of the aglycone at two points suggests the compound to be bisdesmosidic glycoside (Debella et al., 2000, Sun et al. 2006).

The signals of the aglycone's C-12 and C-13 at δ 123.0 and δ 144.0 respectively show the presence of two olefinic carbons (Mehta et al., 2004, Xu et al., 2010), indicating that the aglycone is of olean-12-ene skeleton and therefore olean-12-en-28-oic acid aglycone.

The presence of six sugar moieties is evidenced in the signals at δ 95.0, 96.0, 97.0, 102.0, 103.0 and 104.0, confirming the presence of six anomeric carbons (Mehta et al., 2004, Zhoa, 2010). The linkages of the sugars with the aglycone and the sequencing have been established by the HMBC experiment (Shah et al., 2009, Li et al. 2010). Thus, on the basis of the above evidence and analysis, compound CFME2 has been identified as a bisdesmosidic hexasaccharide triterpenoid saponin of olean-12-en-28-oic acid aglycone with the molecular formula, $C_{66}H_{108}O_{33}$, and has been established to be 3-O – β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – 28- O – β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl- oleanolic acid.

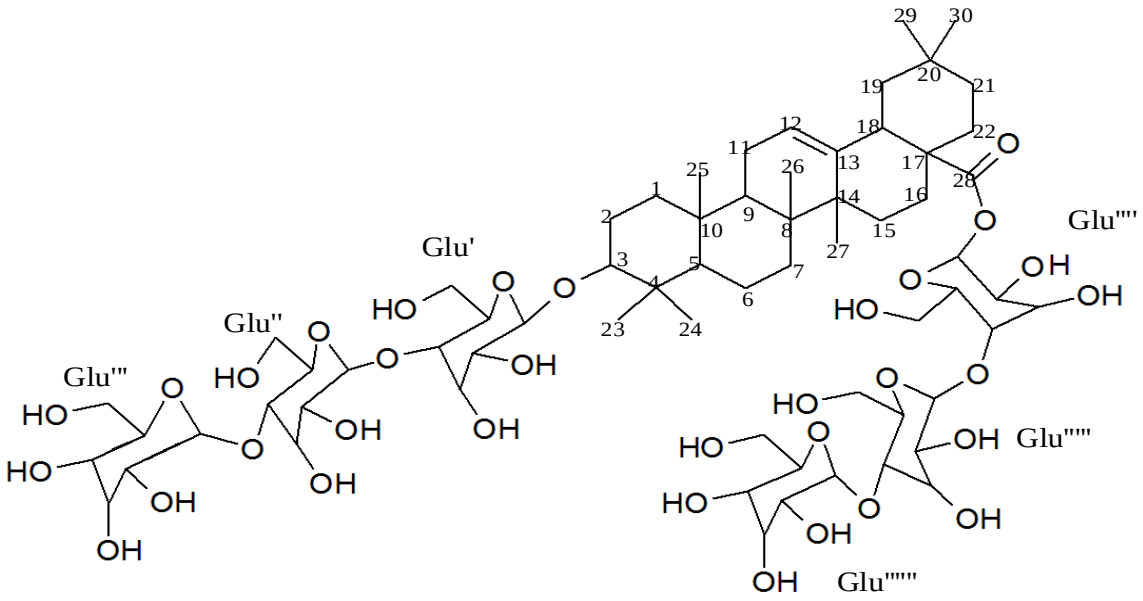

Figure2: Compound CFME2 **(**3-O – β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – 28- O – β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl- oleanolic acid) Structure.

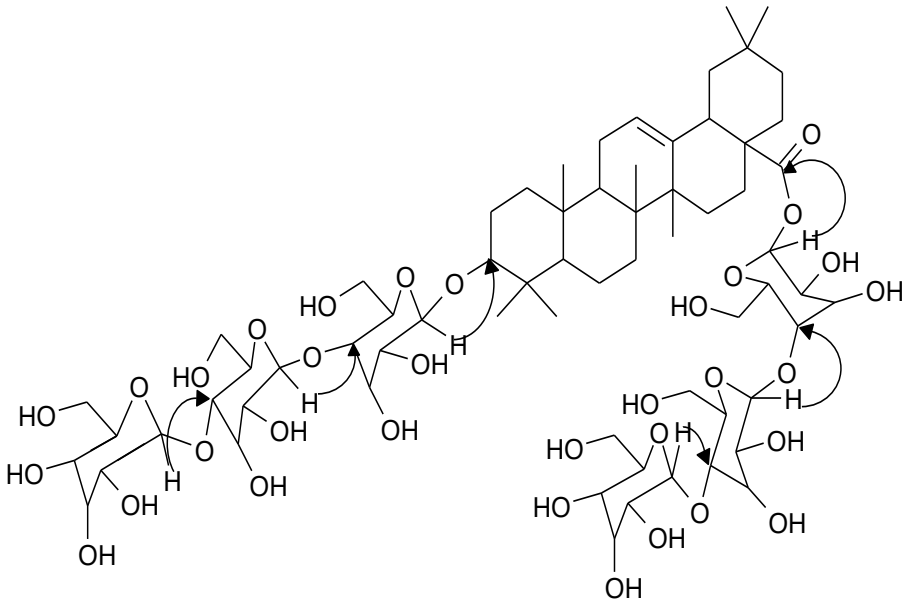

Figure 3: Key HMBC Correlation of Compound CFME2.

Antimicrobial Activities of the Compound:-

The result of antimicrobial assay of the isolated compound (Figure 1) showed the compound to have inhibitory ability against the microorganisms, Salmonella spp, Staphylococcus aureus, Escherichia coli, Aspergillus niger and Candida albicans tested upon. The compound was found to exhibit far more potency against the microorganisms than the methanol crude extracts it was isolated from (Ukekpe et al., 2015). The reason for this could be that as antimicrobial agent, its activity might have been inhibited by low concentration due to the presence of other (non-antimicrobial) substances that might have been present in the crude extract. Similar observation has been made by Manguro et al. (2011) with an isolated triterpene saponin from Maesa lanceolata leaves as the compound, having glucose moieties, was found to be more potent against the test microorganisms than the methanol extract it was isolated from.

Compound CFME2 was found to exhibit higher antifungal activity than antibacterial. This is contrary to the activity of the methanol extract it was isolated from as the extract showed higher antibacterial activity than antifungal (Ukekpe et al., 2015). This suggests that there are other components of the extract that might probably be more of antibacterial than antifungal. Barile et al. (2007) also observed higher antifungal activity for saponins isolated from Allum minatiflorum and Garai (2014) has pointed that saponins exhibit high toxicity against fungi because of their ability to complex with sterols and cause membrane permeabilisation in fungi.

The antimicrobial activity of the compound shows the compound to be a bioactive compound and its presence in the plant part has lent credence to Massularia acuminata as a medicinal plant.

Conclusion:-

Compound CFME2 has been isolated from the methanol extract of the root bark of Massularia acuminata and identified to be a bioactive triterpenoid saponin. This compound, through IR and NMR spectroscopic analyses has been established to be 3-O – β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – 28-O – β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl – (1-4)- β- D- glucopyranosyl- oleanolic acid.

References:-

1. Aderinokun, G. A., Lawoyin, J. O. and Onyeaso, C.O. (1999). Effect of two NigeriaChewing Sticks on Gingival Health and Oral Hygiene. Odontostomatology Tropicals. **22**: 13-18.
2. Alvarez, L., Zamilpa, A., Marquina, S. and González, M. (22003). Two New Oleanolic Acid Saponins from the Root of Viguiera hypargyrea. Revista de la Sociadad Quimica de Mexico. **47**(2): 173 – 177.
3. Babu, S., Satish, S., Mohana, D.C., Raghavendra, M.P., and Raveesha, K.A. (2007) Anti-bacterial Evaluation and Phytochemical Analysis of Some Iranian Medicinal Plants Against Plant Pathogenic Xanthomanas pathovars. Journal of Agricultural Technology. **3**(2): 307 – 316.
4. Bankole, P. O., Adekunle, A. A., Oyede, R.T., Faparais, F. and Adewole, A. (2012). Antimicrobial Activities and Phytochemical Screening of Two Tropical Nigerian Chewing Sticks. International Journal of Applied Sciences and Technology. **2** (6):131- 138.
5. Barile, E., Bonanomi, G., Antignani, V., Zolfaghari, B., Sajjadi, S. E., Scala, F. and Lanzotti, V. (2007). Saponins from Allium minutiflorum with Atifungal Activity. Phytochemistry. **68**:596 – 603.
6. Debella, A., Haslinger, E., Schmid, M. G., Bucar, F., Michl, G., Abebe, D. and Kunert, O. (2000). Triterpenoid Saponins and Sapogenin Lactones from Albizia gummifera. Phytochemistry. **53**: 885 – 892.
7. Garai, S. (2014). Triterpenoid Saponins. Natural Products Chemistry & Research. **2**(6): 1-13.
8. Gl-stndag, Z. and Mazza, G. (2007). Saponins Properties, Application and Processing. Food Science Nutrition. **47**: 231-258.
9. Güvenalp, Z., Kiliç, N. Kazaz, C., Kaya, Y. and Demirezer, Ö. (2006). Chemical Constituents of Galium tortumense. Turk Journaal of Chemistry. **30**: 515 – 523.
10. Iwu, M. M. (2014) Handbook of African Medicinal Plants. 2nd Edition. Boca Raton. CRC Press, Taylor & Francis group. P253-254.
11. Khan, N. A. (2011). In vitro Antimicrobial Activity of Triterpenoid Saponin from Tephrosia purpurea Seed Extract. European Journal of Chemistry. **2**(2): 189 – 192.
12. Li, W., Zhang, W., Xiang, L., Wang, Z., Zheng, Y., Wang, Y., Zhang, J. and Chen, L. (2010). Platycoside N: A New Oleanane – Type Triterpenoid Saponin from the Roots of Platycodon grandiflorum. Molecules. **15**: 8702 – 8708.
13. Man, S., Gao, W., Zhang, Y., Hang, L. and Liu, C. (2010) Chemical Study and Medical Application of Saponins as Anti-cancer Agents. Fitoterapia. **81**: 703-714.

14. Manguro, L. O. A., Midiwo, J. O., Tietze, L. F. and Hao, P. (2011). Triterpene Saponins of Maesa lanceolata leaves. ARKIVOC. **2011(ii)**: 172 – 198.
15. Mehta, B. K., Mehta, D. and Itoriya, A. (2004). Structure Elucidation by NMR Spectroscopy of a New Acetylated Saponin from Centratherum anthelminticum. Carbohydrate Research. **339**: 2871 – 2874.
16. Negi, J. S., Negi, P. S., Pant, G. J., Rawat, M. S. M and Negi, S. K (2013) Natural Occurring Saponins: Chemistry and Biology. Journal of Poisonous and Medicinal Plant Research. **1**(1): 001-006.
17. Ogunshe, A. A. O. and Ademiluka, O. (2013). Evaluation of In vitro Adjunct Antimycotic Effects of most Preferred Chewing Sticks on Human Oral Candida species. Journal of Natural Product Plant Resource. **3**(3): 48-54.
18. Seebacher, W., Simic, N., Weis, R, Saf, R. and Kunert, O. (2003). Spectra Assignments and Reference Data: A Complete Assignments of ^{1}H and ^{13}C NMR Resonances of Oleanolic Acid, 18α- Oleanolic Acid, Ursolic Acid and their 11- Oxo Derivatives. Magnetic Resonance in Chemistry. **41**: 636 – 638
19. Shah, B. A., Chip, R., Gupta, P., Sethi, V. K., Koul, S., Andotra, S. S., Nargotra, A., Sharma, S., Pandey, A., Bani, S., Purnima, B. and Taneja, S. C. (2009). Saponin as Novel TNF-α Inhibitors: Isolation of Saponins and Nor-Pseudoguaianolide from Parthenium hysterophorus. Organic and Biomolecular Chemistry. **7**: 3230 – 3235.
20. Sun, H., Fang, W., Wang, W. and Ha, C. (2006). Structure - Activity Relationships of Oleanane - and ursane - type Triterpenoids. Botanical Studies. **47**: 339 - 368.
21. Teng, R., Xie, H., Liu, X., Wang, D. and Yang, C. (2003). Four New Oleanane Type Saponins from Morina nepalensis Var Alba. Journal of Asian Natural Products Research. **5**(2): 75 – 82.
22. Uddin, G., Waliullah, Siddiqui, B. S., Alam, M., Sadat, A., Ahmad, A. and Uddin, A. (2011). Chemical Constituents and Phytotoxicity of Solvent Extracted Fractions of Stem Bark of Grewia optiva Drummond ex Burret. Middle – East Journal of Scientific Research. **8**(1): 85 – 91.
23. Uduak, A. E. and Kola, K. A. (2010) Antimicrobial Activities of Some Enphobiaceae Plants Used in the Traditional Medicine of Akwa Ibom State of Nigeria. Ethnobotanical Leaflets. **14**: 654 – 664.
24. Ukekpe, U. S., Adamu, H. M., Ekanem, E. O. and Saleh, A. A. (2015). Phytochemical Constituents and Antimicrobial Activities of the Root Bark Extracts of Massularia acuminata spp. International Journal of Advance Research. **3**(8): 557 – 565.
25. Viana, F. A., Braz-Filho, R., Pouliquen, Y. B. M., Neto, M. A., Satiago, G. M. P. and Rodrigues-Filho, E. (2004). Triterpenoid Saponins from Stem Bark of Pentaclethra macroloba. Journal of the Brazilian Chemical Society. **15**(4): 1 – 8.
26. Vincken, J., Heng, L., de Groot, A. and Gruppen, H. (2007). Saponins, Classification and Occurrence in the Plant Kingdom. Phytochemistry. **68**: 275-297.
27. Wong, C. (2014). The Benefits of Massularia Acuminata. Retrieved December 12, 2014 from http.// altmedicine. About.com/od/iterbal Remedies /H/ The-Benefits-of-Massularia-Acuminata, htm.
28. Xu, Y., Bai, L., Liu, Y., Liu, Y. Xu, T., Xie, S., Si, Y., Zhou, H., Liu, T. and Xu, D. (2010). A New Triterpenoid Saponin from Pulsatilla cernua. Molecules. **15**: 1891 – 1897.
29. Yakubu, M. T., Akanji, M, A., Oladiji, A. T. Adesokan, A. A. (2008). Androgenic Potentials of Aqueous Extracts of Massularia acuminata (G. Don) Bullock ex. Hoyle. Stem in Male Wistar Rats. Journals of Ethnopharmacology.**118**: 508-513.
30. Yakubu, M.T. and Omoniwa, B.P. (2012) Effects of Aqueous Stem Extract of Massularia Acuminata on Some Liver Function Indices of Male Rats. Iranian Journal of Toxicology. **6**: 716-722.
31. Zhoa, W. (2010). Saponins. In: Introduction to Natural Product Chemistry. Xu, R, Ye, Yan and Zhoa, V. (Eds). Beijing. Science Press. P 125 – 124.