Caliphate Journal of Science & Technology (CaJoST)

ISSN: 2705-313X (**PRINT**); 2705-3121 (**ONLINE**) **Research Article**

Open Access Journal available at: https://cajost.com.ng/index.php/files and https://www.ajol.info/index.php/cajost/index

DOI: https://dx.doi.org/10.4314/cajost.v6i1.7

Article Info

Received: 30th September 2023

Revised: 28th January 2024

Accepted: 31st January 2024

Characterization and Evaluation of Antimicrobial Activity of Sorbitan Isolated from the Leaves of *Securidaca longipedunculata* (Fresen)

Lawal G. Hassan[1], Kabiru J. Umar[2], Amina. J. Yusuf[3], Ephraim Okolo[4] and Umar A. Umar[5]*

[1]Department of Pure and Environmental Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Pure and Industrial Chemistry, Federal University Birnin Kebbi, Nigeria.
[3]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.
[4]School of Chemistry Stores, University of Nottingham, United Kingdom.
[5]Department of Chemistry, Sokoto State University, Sokoto, Nigeria.

***Corresponding author's email:**

umarkebbe@gmail.com

Cite this: *CaJoST*, 2024, 1, 54-60

Securidaca longipedunculata is a shrub of the family *Polygalaceae*. It is a potent medicinal plant used in management of microbial infection others. The aim of this study is to characterize, and evaluate the antimicrobial activity of sorbitan isolated from the leaves of *Securidaca Longipedunculata* (Fresen). The methanol extract of *S. longipedunculata* was subjected to chromatographic purification, which led to isolation of compound identified as sorbitan by analysis of the 1D and 2D-NMR spectral data. The antimicrobial screening against bacterial pathogens (*Methicillin-resistant staphylococcus aureus (MRSA), Vancomycin-resistant enterococci (VRE) S. aureus, S. feacalis, E. coli, S. typhimurium, P. fluorescens, K. pneumoniea*) and fungal pathogens (*C. albicans, C. krusei, A. niger, A. fumigates,* and *Microsporumcanis*) was performed using agar well diffusion and broth dilution method. In-vitro inhibition of these pathogenic microorganisms produced inhibition zone ranging from 25 - 32 mm for sorbitan; the standard drugs sparfloxacin and ciprofloxacin (500µg/mL) had zone of inhibition ranging from 28 - 40 mm. While the antifungal drugs fluconazole and Fulcin exhibited zone of inhibition ranging from 27 mm to 34 mm. Minimum inhibition concentration (MIC) values of sorbitan ranged from 25 - 12.5 mg/mL. The minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) of sorbitan was observed to be 50 - 25 mg/mL. Sorbitan was isolated and characterized from *S. longipedunculata* leaves extract and it demonstrated good antimicrobial activity which validates the used of the plant leaves in the treatment of microbial infections in traditional medicine.

Keywords: *Phytochemical, Isolation, Sorbitan, Microbial infection, Securidaca longipedunculata.*

1. Introduction

Medicinal plants have long been used as health remedies for diverse disease condition. These medicinal plants contain bioactive compounds with antimicrobial properties that can be utilized for managing and treating various ailments and aimed at promoting the health of mankind (Kebede *et al.,* 2021). The emergence of multi drug-resistance bacteria/fungi has compromised the accessibility and affordability of many currently prescribed antibiotics have worldwide (Van duin and Doy, 2017; Poulakou *et al.,* 2018). Resistance to antibiotics can be naturally developed through the acquisition as well as mutation of plasmids which code genes responsible for resistance to drugs (Chung *et al.,* 2004).

According to the National Action plan for antimicrobial resistance report (2017-2022) found out that 42% of adults and 46.7% of five-year children of peoples acquire serious infections resistant to one or more of the antibiotics used for the treatment of infections. This situation is further complicated in low-income society as a result of the lack of effective surveillance systems, laboratory diagnostics, and access to appropriate antimicrobials in the face of financial limitations (Bakal *et al.,* 2017). A concerted effort to reverse this trend is a worthy cause of scientific investigations. To this effect, the search for novel antibiotics from natural source is eventually an important section of modern medicine to overcome the socio-economic and health crash caused by multidrug-resistant microbes.

Securidaca longipedunculata is a shrub of the family *Polygalaceae*. It is used ethnomedicinally for the management of microbial infection among others. The plant grows up to 10 cm high, 2 - 9 cm long, with 0.5 - 2.5 cm broad leaves (Anjarwalla *et al.,* 2015). It is locally known as "*Uwar magunguna*" by the hausa speaking people of northern Nigeria. It grows in varying climatic environment, from hot arid to humid climates, and in broad vegetation range, from semi-arid to dense forest. The plant leaves is locally used to treat epilepsy, headaches, tuberculosis (Asres *et al.,* 2001), convulsion in children and the decoction of the plant is used to accelerate labour (Degu and Manash, 2015), snakebite, toothache, cancer, stomach ache, infertility, skin infections, dislocated jaw, contraceptive purposes and to expel the placenta (Abubakar *et al.,* 2019). The aim of this study is to characterize, and evaluate the antimicrobial activity of sorbitan isolated from the leaves of *S. longipedunculata*.

2. Materials and Methods

2.1. Sample Collection, Identification and preparation

The samples of *S. longipedunculata* were collected from the Zuru local government region of Kebbi State in August 2021. Subsequently, the sample was transported to the laboratory and authenticated by a taxonomist at the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. A voucher specimen with the identification number PCG/UDUS/POLY/0001 was meticulously created and stored at the herbarium of the department for future reference. The leaves of *S. longipedunculata* were thoroughly washed to eliminate any soil contaminants. The leaves were air-dried in the shade and then crushed into a fine powder using a wooden pestle and mortar. The pulverized specimen was subsequently placed in a sealed container until it was required for analysis.

2.2. Extraction procedure

Following the maceration and exhaustive percolation technique outlined by Nweze *et al.,* (2004), a weight of 200 g of the finely ground sample, made up of *S. longipedunculata* leaves, was immersed in about 2000 cm^3 of n-hexane (with a ratio of 1:10, w/v of plant material to solvent) within a glass beaker. The beaker was securely closed and stored for 72 hours with periodic stirring to extract the soluble metabolites into the solvent. The solution underwent filtration and was subsequently concentrated using solvent evaporation. The same extraction processes were carried out using ethyl acetate and methanol, respectively. The extracted samples were dried and preserved for subsequent analysis.

2.3. Chromatographic studies

Methanol extract was subjected to purification using low pressure column chromatography. The procedure described by Hassan *et al.* (2018) was adopted for column chromatography.

The methanol extract (10.00 g) was gradiently eluted in a silica gel packed column starting with chloroform (100%), chloroform: methanol (9:1) to chloroform: methanol (1:1). Eluates were collected in 40 cm^3 portion. A total of 323 fractions were collected and combined based on their TLC profile to give (29) major fractions coded M_1 - M_{29}. Fractions M_{27} (0.8 g) was further purified using silica gel column and the same mobile phase as above repeatedly, which led to the isolation of compound Ab. TLC analysis of Ab using two solvent systems, viz; chloroform: methanol (5:1) and (9:1) and sprayed with 10% sulphuric acid revealed single homogenous spot. The compound (Ab) was further subjected to chemical test and spectroscopic analysis (1D and 2D-NMR) to elucidate its structure.

2.4. Characterization of Ab

2.4.1. Spectral Analysis

Foueier-transform Infrared Spectroscopy (FT-IR), 1D and 2D-Nuclear Magnetic Resonance and Mass Spectrometry were used to elucidate the structure of the compound.

2.5. Antimicrobial Studies

2.5.1. Test Organisms

Clinical isolates of bacterial pathogens (*Methicillin-resistant staphylococcus aureus (MRSA), Vancomycin-resistant enterococci (VRE), S. aureus, S. feacalis, E. coli, S. typhimurium, P. fluorescens, K. pneumoniea*) and fungal pathogens (*C. albicans, C. krusei, A. niger, A. fumigates,* and *M. canis*) was obtained from Department of Medical Microbiology of Ahmadu Bello University Teaching Hospital Zaria. All the isolates were checked for purity and maintained in Mueller Hinton agar (for bacteria) and in slants of sabouraud dextrose agar for fungi.

2.5.2. Susceptibility test

The antimicrobial activity of the compound was carried out using stock concentration of 500 µg/mL. Mueller Hinton agar was used as the growth medium for the microbes. The medium was prepared according to the manufacturer's instructions and sterilised at 121°C for 15 min. It was poured into sterile petri dishes and then allowed to cool and solidify. The sterilised medium was seeded with 0.1 mL of standard inoculum of the test microbe; the inoculum was spread evenly over the surface of the medium

using a sterile swab. A standard sterile cork borer of 6 mm diameter was used to bore a well at the centre of each inoculated medium. The wells were filled with 0.1 mL of the solution of the compound and allowed to diffuse for 1 hour. Incubation of the inoculated medium was made overnight at 37°C and 25°C for bacteria and fungi, respectively, after which the medium was observed for the zone of inhibition of growth; the tests were conducted in duplicates and the zone of inhibition was measured with a transparent ruler. The mean of the results was recorded in millimetres (mm) (Yusuf *et al.,* 2015; Muhammad *et al.,* 2019).

2.5.3. Minimum Inhibitory Concentration (MIC)
The minimum inhibitory concentration (MIC) of compound was determined using the broth dilution method (Yusuf *et al.,* 2015; Muhammad *et al.,* 2019). Two-fold serial dilutions of the compound in the sterile broth were made to obtain the concentrations of 500, 250, 125, 62.5 and 31.25 µg/mL. An amount of 0.1 mL suspension of the standard inoculum of the test microbe was then inoculated into the different concentrations of the compound. The tubes were incubated at 37°C for 24 hours and 25°C for 48 hours for bacteria and fungi, respectively, after which the plates were observed for turbidity (growth). The MIC was defined as the lowest concentration of the compound inhibiting the visible growth of each micro-organism.

2.5.4. Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC)
Minimum bactericidal and fungicidal concentration was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar broth was prepared, sterilised at 121°C for 15 min and transferred into sterile Petri dishes to cool and solidify. The contents of the MIC in the serial dilution were subcultured into the prepared medium and incubated at 37°C for 24 hour; the plates were observed for colony growth; the MBC/MFC was the plate with lowest concentration of the compound in serial dilution without colony growth (Yusuf *et al.,* 2015; Muhammad *et al.,* 2019).

3. Results and Discussion

3.1 Results

3.1.1 Isolation and purification of compound Ab

Compound Ab gave a single homogeneous spot on TLC using chloroform: methanol (9:1) and the result are presented in Table 1).

Table 1: R_f value of Ab

Solvent systems	No sport	color of spot after spraying with 10 % H_2SO_4	R_f value
Chloroform: Methanol (9:1)	1	Black	0.56

3.1.2. Spectral Analysis of Ab

3.1.2.1. FTIR analysis of Ab

The Infrared signal observed from the isolate Ab is presented in Table 2.

Table 2: Infrared Peaks of compound Ab with Reported literature data

Bands	#υ (cm^{-1})	*υ(cm^{-1})
O-H stretching	3401	3350-3450
CH and CH_2 stretching aliphatic	2922	2850-2950
C-O-C	1133	1120-1160

* * = (Yunus and Nulamuga, 2020) # = compound Ab

3.1.2.2. ^{1}H-NMR analysis of Ab

The ^{1}H-NMR (400 MHz, CD_3OD) of the compound revealed resonances at δ_H 3.91 (dd, *J* =11.0, 5.4 Hz, 1H), 2.85 (dd, *J* = 11.9, 2.3 Hz, 1H) 3.63 (dd, *J* = 11.8, 5.8 Hz, 1H), 3.47 (ddd, *J* = 10.5, 8.6, 5.4 Hz, 1H), 3.32 – 3.13 (m, 4H) (Table 3).

3.1.2.3. ^{13}C-NMR analysis of Ab

The ^{13}C-NMR (125 MHz CD_3OD) and ATP experiments of the compound indicated the presence of 6 carbon atoms: δ_C 71.84 (C-1), 71.42 (C-2), 79.98 (C-3), 82.49 (C-4), 70.92 (C-5) and 63.09 (C-6) (Table 3).

3.1.3. Antimicrobial activity of Sorbitan from the leaves of *S. longipedunculata* extract

The compound, sorbitan, exhibited varying degrees of antimicrobial activity against the test microbes. Susceptibility test result showed inhibition ranging from 25 mm to 32 mm against all the organisms; the most sensitive organism was *MRSA* while the least sensitive organism was *S. typhimurium*. The results of the antimicrobial activity of *S. longipedunculata* leaves extracts and that of sorbitan are presented in Table 5-8.

Table 3: 1D-2D-NMR data of Compounds Ab

Position	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)	APT	HSQC	^{1}H-^{1}H-COSY	NEOSY
1	3.48, 3.22 (2H)	71.84	CH_2	-	H-2,H-3	H-5
2	3.19 (1H, s)	71.42	CH	-	H1,H2	-
3	3.63 (1H, s)	79.98	CH	C_3-1H	H-5	-
4	2.85 (1H, s)	82.49	CH	-	-	-
5	3.91 (1H, s)	70.92	CH	C_5-1H.	H-3	H-1
6	3.32 - 3.13 (4H, m)	63.09	CH_2	-	-	-

Table 4: ^{1}H and ^{13}C-NMR data of compound Ab compared with reported literature

Position	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)
	Compound U1c		Literature	
1	3.48, 3.22 (2H, d)	71.84	3.39, 3.27 (2H, M)	75.96
2	3.19 (1H, s)	71.42	3.39	73.48
3	3.63 (1H, s)	79.98	3.5	76.55
4	2.85 (1H, s)	82.49	3.43	80.48
5	3.91 (1H, s)	70.92	4.36	69.35
6	3.33-3.13 (4H, m)	63.09	3.8 (2H, M)	64.29

Table 5: Zone of inhibition of sorbitan against the test organisms

Test organisms	Ab	SF	CF	FZ	FC
Bacteria					
MRSA	32	35	0	0	0
VRE	26	0	28	0	0
S. aureus	0	31	0	0	0
S. feacalis	0	30	30	0	0
E. coli	26	34	37	0	0
S. typhimurium	25	0	40	0	0
P. fluorescens	27	31	0	0	0
K. pneumoniea	0	0	31	0	0
Fungi					
C. albicans	27	0	0	32	0
C. krusei	31	0	0	34	32
A. nigre	0	0	0	0	27
A. fumigatus	0	0	0	0	31
M. canis	30	0	0	0	30

Key: SF = Sparfloxacin, CF = Ciprofloxacin, FZ = Fluconazole, FC = Fulcin, Ab = Sorbitan

Table 6: Minimum inhibition concentration of sorbitan against the test organisms

Compound	Test organisms	Concentration (µg/mL)				
		100	**50**	**25**	**12.5**	**6.25**
Sorbitan	*MRSA*	-	-	-	+	++
	VRE	-	-	0*	+	++
	E. coli	-	-	0*	+	++
	S. typhimurium	-	-	0*	+	++
	P. fluorescens	-	-	-	0*	+
	C. albicans	-	-	-	0*	+

	C. krusei	-	-	-	0*	+
	M. canis	-	-	-	0*	+

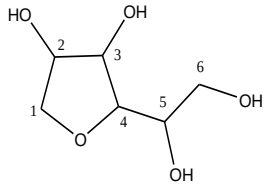
Table 7: Minimum bactericidal and fungicidal concentration of sorbitan against the test organisms

Compound	Test organisms	Concentration (µg/mL)				
		100	**50**	**25**	**12.5**	**6.25**
Sorbitan	*MRSA*	-	-	0*	+	++
	VRE	-	0*	+	++	+++
	E. coli	-	0*	+	++	+++
	S. typhimurium	-	0*	+	++	+++
	P. fluorescens	-	0*	+	++	+++
	C. albicans	-	0*	+	++	+++
	C. krusei	-	-	0*	+	++
	M. canis	-	-	0*	+	++

Key: Ab = Sorbitan - = No turbidity (no growth), 0* = Minimum bactericidal and fungicidal concentration, + = light colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth

3.2. Discussion

The isolated compound Ab appeared as a white crystalline solid at room temperature, it was partially soluble in methanol and ethanol with an uncorrected melting point ranging from 112–113 °C it and gave a positive reaction when tested with Fehling's reagent, an indication of the presence of a carbohydrate (sugar moiety) (Silver *et al.,* 1998; Hassan *et al.,* 2018). The frequencies of the functional groups of Ab compared with literature (Table 2) showed a absorption band at 3401 cm^{-1} which was attributed to O-H group. Absorption at 2922 cm^{-1} and 1133 cm^{-1} were due to stretching CH and CH_2, respectively. The ^{1}H-NMR (400 MHz CD_3OD) of Ab revealed resonances at δ_H 3.91 (dd, *J* =11.0, 5.4 Hz, 1H), 2.85 (dd, *J* = 11.9, 2.3 Hz, 1H) 3.63 (dd, *J* = 11.8, 5.8 Hz, 1H), 3.47 (ddd, *J* = 10.5, 8.6, 5.4 Hz, 1H), 3.32 – 3.13 (m, 4H) (Table 3), characteristic of a Sorbitan (Charlotte *et al.,* 2015). The integration under the spectrum also confirms the ratio of proton approximately to be eleven hydrogen atoms. The ^{13}C-NMR and APT experiments of Ab showed 6 carbon signals constituting six methylene carbons at δ_C 70.92 (C-1, CH_2), 70.01 (C-2, CH), 78.57 (C-3, CH_2), 81.09 (C-4, CH), 69.52 (C-5, CH) and 61.69 (C-6, CH_2) respectively, which is in consistent with the proton NMR, typical of sorbitan (Charlotte *et al.,* 2015). The attachment of each proton to their respective carbon atoms was done via HSQC analysis (Table 3). Major correlations observed include; the proton at δ_H 3.91 correlated with the carbon at δ_C 70.92, δ_H 3.30 correlated with δ_C 79.98 among others. COSY spectrum of compound Ab established the correlations between protons that are situated in the same environment. Cross peak correlations observed between δ_H 3.91 and 3.63 confirmed the assignment of H-5 and H-3 on the side chain of the molecule; Assignment of H-1, H-2, and H-3 was confirmed via cross peak observed at δ_H 3.48 and 3.19 and 3.36 respectively. NEOSY spectrum of compound Ab revealed the correlation between protons in space; major cross peaks/correlations observed include δ_H 3.91 and 3.22 and 3.48 which confirm the placement on H-5 and H-1 respectively. The structure of compound Ab was confirmed via mass spectrometry analysis which revealed the molecular formula and mass of compound Ab to be $C_6H_{12}O_5$ and m/z 187.0575 $[M - H]^+$; The value was in close agreement with the value reported for sorbitan by Charlotte *et al.* (2015). Based on the analysis of IR, ID- and 2D-NMR data of Ab and confirmation via MS, the structure of compound Ab was confirmed to be (3s)-2-(1,2-dihydroxyethyl) oxalane-3,4-diol (Sorbitan).



Proposed srtucture of Ab: Sorbitan

The susceptibility test results, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of Sorbitan was presented in Tables 5, 6, and 7. Agar well diffusion method was used to assess the antimicrobial activity of sorbitan

against the selected pathogens. Sorbitan from the methanol leaves extract of *S. longipedunculata* exhibited higher antimicrobial activity compared to the extracts with mean zone of inhibition ranging from 25 – 32 mm (Hassan *et al.,* 2024); the most sensitive organism was *MRSA* while the least sensitive organism was *S. typhimurium*. No activity was observed against *S. aureus, S. feacalis, K. pneumoniea, A. niger,* and *A. fumigates.* The standard drugs, Sparfloxacin and Ciprofloxacin (at 500µg/cm^3), indicate zone inhibition ranging from 31 – 35 nm and 28 - 40 mm, respectively against the bacterial isolates; while the standard antifungal agents, Fluconazole and Fulcin (at 500µg/cm^3) had 32 - 34 nm and 27 – 32 nm against the test organisms (Table 5). The antimicrobial activity of the sorbitan is evident from the minimum inhibition concentration (MIC) values, which range from 25 - 12.5 mg/cm^3; while the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) ranges from 50 - 25 mg/cm^3. The lower value of the MIC and MBC/MFC point out that the compound has excellent antimicrobial activity against the susceptible organisms since compounds with MICs less than 100 µg/cm^3 are regarded as having strong antimicrobial property (Yusuf *et al.,* 2018). The antimicrobial of *S. longipedunculata* extracts from this work, possess good antimicrobial activity against the tested organisms, with inhibition range of 18–29 mm; the compound sorbitan isolated from methanol extract displayed an improved inhibitory effect and significantly lower MIC value (25-12.5 mg/ml) when compared to the extract. The isolation of sorbitan from the extract of *S. longipedunculata* has therefore allowed for an accurate activity evaluation of the compound, which reveals that the antimicrobial activity observed with the extract might be attributed to its sorbitan content.

Bacteria such as *MRSA* can cause hospital acquired infections like surgical site infections (Yusuf *et al.,* 2015; Brambilla, 2017), which is leading to cause of morbidity and mortality, and which can lead to high cost of seeking health care among individual and families. *E. coli* is a bacterium that is commonly found in the lower intestine and it causes dysentery, diarrhea, and urinary tract infection (Junaid *et al.,* 2008; World Gastoroenterology Organization Global Guidelines, 2012). Finding from this study has shown the potential of sorbitan as an antimicrobial agent against *MRSA* among others. It is worth noting that the development of antimicrobial resistance is a significant concern in the field of antimicrobial activity. Microorganisms can develop mechanism to resists the effects of antimicrobial agents (Kebede *et al.,* 2021), rendering them less effective or ineffective. Thus, the appropriate and judicious use of Plants-based products is crucial to mitigate the emergence of resistance and ensure their long-term effectiveness.

4. Conclusion

Sorbitan was isolated from methanol leaves extract of *S. longipedunculata* and it exhibited good antimicrobial activity. This study validates the use of the leaves of the plant in the treatment of microbial infection and should study further for possible utilization for use as a herbal therapy. To the best of our knowledge, this is the first report of isolation and characterization of sorbitan from natural sources.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge the support and love shown by the laboratory personnel of Pharmaceutical and Medicinal Chemistry, Department at the Faculty of Pharmaceutical Sciences, UDUS. The support of the Department of Medical Microbiology of Ahmadu Bello University Teaching Hospital, Zaria is also appreciated.

References

Abubakar, U.S., Danmalam, U.H., Ibrahim, H. and Maiha, B.B. (2019).A Review on African Violet Tree (*Securidaca longipedunculata*) : A Traditional Drug with Multiple Medicinal Uses. *Specialty Journal of Chemistry.* 4 (3): 7-14. https://www.researchgate.net/publication/336284980

Anjarwalla, P., Belmain, S., Jamnadass, R. and Stevenson, P.C. (2015).Pesticidal Plant Leaflet – *Securidaca longipedunculata.*Technical report at: https://www.researchgate.net/publication/282855101.

Asres, K., Bucar, F., Kartnig, T., Witvrouw, M., Pannecoupue, C., and de Clercq, E. (2001). Antiviral activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) of ethno botanically selected Ethiopian medicinal plants. *Phytother. Research.*15: 62 69. 10.1002/1099-1573(200102)15:1<62::aid-ptr956>3.0.co;2-x.

Bakal, S. N., Bereswill, S. and Heimesaat, M. M. (2017). Finding novel antibiotic substances from medicinal plants—Anti-microbial properties of Nigella sativa directed against multidrug-resistant bacteria. *Eur Journal of*

MicrobiolImmunol. 7(1): 92–98. 10.1556/1886.2017.00001

Brambilla, L. Z. S. (2017) 'Anti-biofilm activity against Staphylococcus aureus MRSA and MSSA of neolignans and extract of Piper regnellii', *Revista Brasileira de Farmacognosia*. Sociedade Brasileira de Farmacognosia, 27(1): 112–117. http://dx.doi.org/10.1016/j.bjp. 2016.08.008 0

Charlotte, G., Elsa, D., Marie-Christine, D., Valérie, M., Jean-Marie, A., Andreas, Redl., Nicolas, D. and Marc, L. (2015). Preparation of Amphiphilic Sorbitan Monoethers through Hydrogenolysis of Sorbitan Acetals and Evaluation as Bio-Based Surfactants. *Royal Society of Chemistry.* Pp. 4-16. **https://doi.org/10.1039/C5GC02131F**

Chung, P.Y., Chung, L.Y., and Ngeow, Y.F (2004). Antimicrobial activity of Malaysian plant specoies. *Pharmaceutical Biology.* 42: 292-300.

Degu L. K. and Manash K. C. (2015).Phytochemical investigation of *Securidaca longipedunculata* (*Polygalaceae*) and structure elucidation of benzyl 2-hydroxy-5-methoxy benzoate. *International Journal of Current Microbiology and Applied Science.* 4(1): 690-695. http://www.ijcmas.com

Hassan, L. G., Msheila, H. E., Umar, K. J., Umar, A. U. and Ogbiko, C. (2018). Isolation and Characterization of α-Amyrin and βSitosterol from Ethyl Acetate Extract of the Leaves of *Monechma ciliatum* (Jacquin). *International Journal of Pharmacy and Pharmaceutical Research.* 12(1): 298-306.

Junaid, S., Ofodile A., Olabode A., Echeonwu G. and Okwori A. (2008). Evaluation of Securidacalongipenduculata leaf and root extracts for antimicrobial activities. African Journal of Microbiology Research. 2 (12) 322-325.

Hassan L. G., Umar K. J., Yusuf A. J., Rabah A. B., Umar A. U. (2024). Comparative Antimicrobial Activity of *Securidaca longipedunculata* (Fresen) Extracts on Selected Human Pathogens. *Saudi J Med Pharm Sci,* 10(1): 26-31. 10.36348/sjmps.2024.v10i01.005

Kebede, T., Gadisa, E. and Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. PLoS ONE 16(3): 1-16. https://doi.org/10.1371/journal.pone.0249253

Lee, R.W., Leach, D.N., Meyer, P., Lin, D.G., Brushett, D.J. and Waterman, P.G. (2004). Anti-inflamatory activity, Cytoxicity and active compounds of *Tinospora Smilacina Benth. Phytother Research.* 18: 78-83. 10.1002/ptr.1373

Muhammad, A., Sani, U.D., Sumaayya, A.W. and Muhammad, S.A. (2019). Phytochemical screening and Antimicrobial Activity of Bitter leaf (Vernoniaamygalina). *Annals Microbiology and Infectious Diseases.* 2(4): 01-07.

Poulakou, G., Lagou, S., Karageorgopoulos, D. E. and Dimopoulos, G. (2018). New treatments of multidrug-resistant Gram-negative ventilator-associated pneumonia. Ann Transl Med. 6(20): 423–423. 10.21037/atm.2018.10.29 Ab

Van Duin, D. and Doi, Y. (2017). The global epidemiology of carbapenemase-producing Enterobacteriaceae. Viru- lence [Internet]. 8(4):460–9. 10.1080/21505594.2016.1222343

World Gastroenterology Organization Global Guidelines . (2012). Acute diarrhea in adults and children: a global perspective. Pp 1-24.

Yunus, M.M. and Nulamuga, B. (2020). Extraction and Characterization of Caffeine: A Biochemical Compound contained in some Locally Consumed Tea Leaves. (*Camellia sinensis*). *International Journal of Research and Scientific Innovation* (IJRSI). **7**(1):136-140.

Yusuf, A. J., Abdullahi, M. I. Nasir, I., Yunusa, A., Alebiosu, C. O. and Muhammad, A. A. (2023). Isolation and Characterization of Prophylactic Antimalarial Agents from Ochna kibbiensis Leaves. Drugs Drug Candidates. 2: 37–51.10.3390/ddc2010003

Yusuf, A. J., Abdullahi, M. I.,Haruna, A. K., Musa, A. M., Abdullahi, M. S., Ibrahim, Z. Y. Y., Halilu, M. E. and Odiba O. J. (2015). Phytochemical and antimicrobial evaluations of the methanol stem bark extract of N*eocaryamacropylla. Journal of Chemical and Pharmaceutical Research.* 7(1): 477-481. 10.7324/JAPS.2015.54.S10

Yusuf, A.J., Abdullahi, M.I., Aleku, G.A., Ibrahim, I.A.A., Alebiosu, I .O. and Yahaya, M. (2018). Antimicrobial activity of stigmasterol from the stem bark of *Neocarya macrophylla*. *Journal of Medicinal Plants For Economic Development.* 2(1): 38. **10.2174/1573407218666220426132448**

