Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 4 Issue 11 November 2021

Research Article

Anogeissus leiocarpus: A Potential Inhibitor of Some Extended Spectrum β-Lactamase Bacteria

Dahiru M[1]*, MT Adamu[2], Kolawole OS[1] and Isyaka MS[1,3]

[1]*Department of Biological Sciences, Federal University of Kashere, Gombe State, Nigeria*

[2]*Department of Microbiology, Faculty of Science, Gombe State University, Nigeria*

[3]*Department of Chemical Sciences, Federal University of Kashere, Gombe State, Nigeria.*

***Corresponding Author:** Dahiru M, Department of Biological Sciences, Federal University of Kashere, Gombe State, Nigeria.

Received: September 06, 2021

Published: October 26, 2021

Abstract

The plant of *Anogeissus leiocarpus*is widely used for medicinal purposes by Kashere people in Gombe State, Nigeria. To provide scientific barking for its utilization, ten different concentrations of ethanolic stem bark extract were prepared and tested on *E. coli, K. pneumoniae, P. aeruginosa* and *S. typhi* ESBLs bacteria, using standard procedure and phytochemical components of the plant extract was also determined using GC-MS. The results obtained indicate an antibacterial activity at varying concentrations w/v and zones of inhibition diameter (ZID) ranging from 15.67 ± 0.57 mm to 17.00 ± 1.00 mm with a significance difference ($p < 0.05$) across bacteria tested. Minimum inhibitory concentration (MIC) were between 66.67 ± 23.1 mg/L to 16.67 ± 5.77 mg/L. Minimum Bactericidal Concentration and Minimum Bacteriostatic Concentration were between 266.67 ± 2.38 mg/L to 106.67 ± 46.19 mg/L and 266.67 ± 92.37 mg/L to 80.00 ± 0.00 mg/L respectively. The MBC/MIC ratio was bacteriostatic for *E. coli* (0:53) and *S. typhi* (7:1) while bactericidal for *K. pneumonia* (4:1) *P. aeruginosa* (3:1). Twenty five compounds were identified with Myristoleic acid; Z-7-Tetradecenal; 4,5-Dimethyl-4-Hexen-3-one; 1,15-Hexadecadiene and Linoleoyl chloride as most abundant. A more in depth assessment of *A. leiocarpus* should be carried out to isolate bioactive individual compounds responsible for specific antibacterial activity.

Keywords: Bioactive; ESBLs; Bactericidal; Fatty Acid; Herbal

Introduction

In Nigeria, as in many other countries, the importance of natural products as an alternative source of medicines cannot be overestimated due to the large number of people living in rural areas. This is believed to be due to the plant`s ability to prevent and treat some common diseases [1], particularly before and during Nigerian colonial times. This can be seen in the current orientation of traditional medicine worldwide, in addition to the persistent antibiotic resistance to synthetic drugs [2]. The antimicrobial resistance (AMR) phenotype has recently emerged as one of the most important factors in the fight against infectious diseases, with some of the newer drugs of some known and easy-to-treat bacterial infectious agents, such as *E. coli, Klebsiella* spp. and some other species of enterobacteriaceae [3-5]. This bring about the need to offer alternative or solution to AMR crisis [3,6]. The plant (*A. leiocarpus*)

parts are used to cure coughs, and the powdered bark is sometimes rubbed to reduce toothache that is normally applied to the gums, while the crushed root is used to treat wounds and ulcers, in some communities it is used as an anti-helmintic [7]. The disease treatment sources currently available in Kashere still have strong support from traditional medicine, regardless of the presence of federal university and two community health centers, whose major depriving factor to primary health care remains affordability. Consequently, the root, stem bark, and leaves are widely used to treat skin infections such as wounds, boils, and sores, as well as diarrhea, worm infection, and toothache.

In light of this claim by traditional healers, an ethanol extract from the stem bark of *A. leiocarpus* was tested on some of the bacterial isolates normally associated with such diseases. The antibacterial activity on the producers of β-lactamase (ESBL) with an extended spectrum, was determined and possible antimicrobial compounds identified.

Material and Methods

Preparation of plant material

The stem bark of *A. leiocarpus* of the family Combretaceae was collected in the Kashere area and identified in the Department of Biological Sciences of the Faculty of Sciences of the Federal University of Kashere, Gombe. About 50 g of the dried and powdered material was subjected to ethanolic extraction in Soxhlet extractor for 74 hours at 60°C. Extracts were filtered, concentrated and dried using desiccator [8].

Test organisms

Bacterial isolates were collected from Bayero University Microbiology Laboratory in Kano, Kano State, Nigeria, and were confirmed using cultural and biochemical profiles and tested for Extended Spectrum β-Lactamase Production (ESBL) using the Double Disc Synergy Test [9,10].

Standardization of inocula

The concentration for the inoculation was adjusted using a standard equivalent 5% McFarland cell concentration [11]. For each sample, 3 pure colonies were taken from Mueller Hinton (MH) agar and suspended in 4 ml of NaCl in a tube and mixed to homogenize until properly adjusted to the McFarland 0.5 standard.

Antibacterial assay

The antibacterial assay was performed by the Kirby Bauer method (agar disk diffusion) (using two-fold serial dilution techniques). Number 1 Whatman filter paper discs of 6 mm diameter were prepared, sterilized and filled with 0.2 ml with 10 different concentrations of 320, 160, 80, 40, 20, 10, 5, 2.5, 1.3 and 0.63 63 (w/v) respectively [12]. Plates were each inoculated with bacterial isolates and there after inoculated with extract impregnated paper discs and allowed to stand for 30 minutes before incubating for 18 h at 37**°C,** zones of inhibition were observed and recorded according to the CLSI guideline [13]. The minimum inhibitory concentration was measured by preparing 10 different concentrations of 0.63-320 mg/ml of the extract in Müller-Hinton broth (MHB) by two-fold serial dilution. One ml of standardized inocula of each bacterium was added and incubated at 37**°C** for 18-24 hours. Visible increases in the turbidity of the samples were observed compared to un-inoculated MHB [14,15].

GC-MS preparation and identification of compounds

Extract was analyzed by GC-MS machine (GCMS-QP2010 Plus Shimadzu, Japan). The data were obtained on an Elite-1(100% Dimethyl poly siloxane) column (30 0.25 mm 1µmdf), a total running time of 36 minutes was observed [16]. Compounds were identified and GC-MS interpreted in accordance with National Institute Standard and Technology (NIST05s.LIB) database. The molecular weight, name and chemical formulae of compounds from the extract were determined. The spectra of compounds (unknown) were compared with spectra in the database of NIST library Version (2005), Software.

Results

Test organisms

All bacterial isolate were confirmed to be Extended Spectrum β-Lactmase.

Disc diffusion zones of inhibition

The active concentrations vary for *K. pneumonia* (46.67 ± 30.55 mg/Disc), *P. aeruginosa* (2.93 ± 1.89 mg/Disc) and *S. typhi* (5.83 ± 3.82 mg/Disc) but not significance at ($p < 0.05$), while that of *E. coli* (267.67 ± 92.38 mg/Disc) was statistically significant at $p < 0.05$. The zones of inhibition ranged from 15.67 ± 0.57 mm to 17.00 ± 1.00 mm and were statistically significant as contained in the table 1 below.

Table 1: Concentrations and Zones of inhibition diameter for *Anogeissus leiocarpus* Stem bark extract.

Mean standard deviation				
Bacteria	**Concentrations (mg/Disc)**	**Sig (P < 0.05)**	**ZID (mm)**	**Sig (P < 0.05)**
E. coli	267.67± 92.38	.038	16.67 ± 1.15	.002
K. pnuemoniae	46.67 ± 30.55	.118	16.33 ± 0.57	.000
P. aeruginosa	2.93 ± 1.89	.115	15.67 ± 0.57	.000
S. typhi	5.83 ± 3.82	.118	17.00 ± 1.00	.001

The MIC, MBC and MBCs

Minimum Inhibitory Concentrations (MIC) observed ranged from 16.67 ± 5.77 mg/L to 66.67 ± 23.1 mg/L, and were statistically significance only for *E. coli* and *S. typhi* ($p < 0.05$). MBC and MBCs were not observed across concentrations used for *E. coli.* The MBC and MBCs for *K. pneumonia* was 266.67 ± 2.38 each and statistically significant at $P < 0.05$, the result of *P. aeruginosa* (106.77 ± 46.19 mg/L and 80.00 ± 00 mg/L) and *S. typhi* (106.67 ± 46.19 mg/L and 106.67 ± 46.19mg/L) for MBC and MBCs respectively and were not statistically significant as shown in table 2.

Table 2: Antibacterial results of *Anogeissus leiocarpus* Stem bark extract on ESBLs Bacteria.

Mean standard deviation						
Bacteria	**MIC (mg/L)**	**Sig (P < 0.05)**	**MBC (mg/L)**	**Sig (P < 0.05)**	**MBCs (mg/L)**	**Sig (P < 0.05)**
E. coli	53.33 ± 23.1	.057	0	0	0	0
K. pnuemoniae	66.67 ± 23.1	.038	266.67 ± 2.38	.038	266.67 ± 92.37	.038
P. aeruginosa	40.00 ± 0.00	0	106.77 ± 46.19	.057	80.00 ± 00	0
S. typhi	16.67 ± 5.77	.038	106.67 ± 46.19	.057	106.67 ± 46.19	.057

GC-MS results

The results indicated the presence of twenty five compounds (phytochemical constituents) most of which are fatty acids and their derivatives. The retention time, molecular weight and chemical formula of the identified compounds were identified as indicated below. Myristoleic acid was the most abundant compound present in the stem bark extract, followed by Z-7-Tetradecenal; 4,5-Dimethyl-4-Hexen-3-one and 1, 15-Hexadecadiene in order of decreasing quantity. Other bioactive compounds identified were Lineoleoyl chloride, alpha.-D-Galactopyranoside, Aliphatic aldehydes, stearic acid and palmitic acid derivatives.

Table 3: Phytocompounds identified from the ethanol's extract of *A. leiocarpus* stem bark.

S/N	**R/T**	**Area %**	**M/W**	**Compound Name**	**Nature of compound**	**Chemical formula**
1	3.625	0.18	114	Caprolactone	Fatty aldehyde	$C_6H_{10}O_2$
2	3.920	0.12	126	Butylcyclopentane	Alkane	C_9H_{18}
3	5.433	0.26	124	2-Propionyl-furan	Ketone	$C_7H_8O_2$
4	5.654	0.63	126	1-(6-Oxabicyclo[3.1.0]hex-1-yl) ethanone	Ketone	$C_7H_{10}O_2$
5	6.337	0.82	144	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one		$C_6H_8O_4$
6	7.693	9.43	126	4,5-Dimethyl-4-Hexen-3-one	Ketone compound	$C_8H_{14}O$
7	8.258	0.14	150	Cumic alcohol	Benzyl Alcohol	$C_{10}H_{14}O$
8	9.793	2.10	164	beta.-D-Ribopyranoside, methyl	Suger	$C_6H_{12}O_5$
9	10.187	0.70	140	Cyclopentane, 1-methyl-3-(2-methylpropyl)	Alkane	$C_{10}H_{20}$

10	11.276	1.99	342	alpha.-D-Glucopyranoside, .beta.-D-fructofuranosyl	Glycoside	$C_{12}H_{22}O_{11}$
11	13.183	3.59	194	alpha.-D-Galactopyranoside, methyl	Glycoside	$C_7H_{14}O_6$
12	15.715	0.49	270	Pentadecanoic acid, 14-methyl-, methyl ester	Fatty acid ester	$C_{17}H_{34}O_2$
13	17.098	4.73	256	n-Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$
14	18.927	0.89	294	Linolelaidic acid, methyl ester	Fatty acid ester	$C_{19}H_{34}O_2$
15	18.986	0.75	296	11-Octadecenoic acid, methyl ester	Fatty acid ester	$C_{19}H_{36}O_2$
16	19.352	0.20	298	Stearic acid, methyl ester	Fatty acid ester	$C_{19}H_{38}O_2$
17	20.222	32.90	338	Myristoleic acid	Fatty acid	$C_{14}H_{426}O_2$
18	21.058	6.31	222	1,15-Hexadecadiene	Fatty acid	$C_{16}H_{30}$
19	21.708	4.32	624	Glycerin 1,3-distearate	Stearic acid	$C_{39}H_{76}O_5$
20	22.716	3.28	337	*cis* 13-Docosenamide	Fatty acid amide	$C_{22}H_{43}NO$
21	23.121	2.09	158	1-Decanol	Fatty alcohol	$C_{10}H_{22}O$
22	23.577	6.12	298	Linoleoyl chloride	Fatty acid	$C_{18}H_{31}ClO$
23	24.112	4.31	330	Glycerol 1-palmitate	Fatty acid	$C_{19}H_{38}O_4$
24	25.379	2.60	236	cis,cis-7,10,-Hexadecadienal	Aliphatic aldehyde	$C_{16}H_{28}O$
25	25.73	11.04	210	Z-7-Tetradecenal	Fatty aldehyde	$C_{14}H_{26}O$

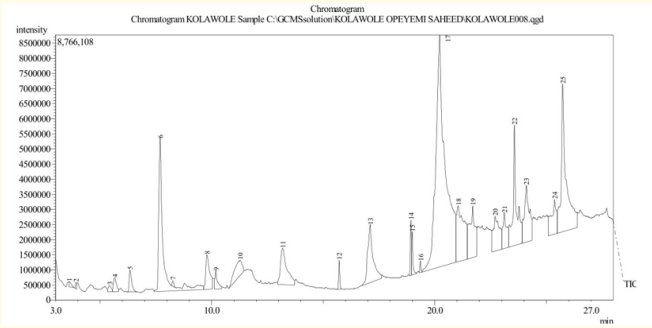

Figure 1: Mass Spectra Profile of methanolic extract of A. leiocarpus stem bark.

Discussion

The plant (*A. leiocarpus*) is said to contain many biologically active compounds including alkaloids, anthraquinones, phenolics and glycosides and is said to have antibacterial, hepatoprotective, anthelmintic, antifungal, leishmanicidal and antiplasmodial antioxidant potential [17-21]. The average inhibition of 16.42 mm observed *A. leiocarpus* across isolates agrees with the findings of other studies. For example Sani and Aliyu, [22] had reported *A. leiocarpus* activity against *E. coli* (12 mm to 16 mm) and (9 mm to 14 mm) for *S. aureus* respectively. Similarly, leaf extract have been reported to be active against *P. aeruginosa* and *K. pneumonia* [23], and Mann and colleagues [18] reported high antimicrobial activity of *A. leiocarpus* leaves, root and stem bark extract against *S. aureus* versus several other were tested together, though this study did not confirm the MRSA or ESBLs status of the isolates used. The observed mean active concentration variation between isolates tested may be influenced by the differences in the cell membrane structure of each bacterial species and the phytochemical constituents of the extract [24,25]. Part of the concentration-dependent gradient inhibitory activity of aldehydes, ketones and fatty glycosides, particularly in gram-negative bacteria (*E. coli, K. pneumonia and S. typhi*), may have been influenced by their potency and the presence of double bonds, the chain length of the group enal [26-32]. Long chain fatty acids such as n-Hexadecanoic acid, linoleoyl chloride, glycerol 1 palmitic and *cis* 13-Docosenamide acid have been reported to inhibit the growth of gram-positive bacteria at

lower concentrations, whereas methyl esters generally decrease activity and sucrose esters increase activity [33]. For example, *E. coli* and other gram-negative bacteria were observed to be sensitive from 5 mg/L and above [33-37] effects that can be attributed to the fatty acid components of the plant. In particular, stearic acid was reported to inhibit the growth of *E. coli and P. aeruginosa* [34].

Linoleic and oleic acids have been reported to exhibit selective antibacterial activity by being active against *B. subtilis* and *S. aureus* and no activity against *E. coli*, *P. aeruginosa*, *K. pneumoniae* [33,36]. Other researchers have reported inhibition of *S. aureus* (MRSA) of linolenic acid, linolenic acid and fatty acid ester forms [38,39] [40]. Similarly, Giamarellos and colleagues [41] reported the susceptibility of *E. coli* into gamma-linolenic acid at a concentration of 1 mgL 5 mgL. Lineoleoyl chloride has great potential as potent antimicrobial agent due to its cleansing properties, amphipathic properties, creation of temporary or permanent pores, disruption of the electron transport chain, disruption of oxidative phosphorylation pathways and inhibition of enzymatic activity and cell lysis [42-46]. *A. leiocarpus* as a natural bioactive compound, easily available and containing beneficial properties [1] can be recommended for the treatment of systemic as well as localized infections [7,22] as is currently the practice in most of northern Nigeria.

Conclusion

Potential *A. leiocarpus* as a candidate drug should be studied further, occurs naturally, is available in most rural communities in northern Nigeria [1,7,22], can be used affordably by our rural people to address availability accessibility and affordability of primary health care.

Acknowledgement

This research was supported by Tertiary Education Trust Fund (TET Fund) grant in collaboration with Federal University of Kashere, Gombe State Nigeria, We also appreciate the support given by staff of Biological Sciences Department, herbarium and laboratory sections, during the research.

Bibliography

1. Chaman Lal and Verma LR. "Use of certain bio-products for insect-pest control". *Indian Journal of Traditional Knowledge* 5.1 (2006): 79-82.
2. Sermakkani M and Thangapandian V. "GC-MS Analysis of *Cassia italica* Leaf Methanol Extract". *Asian Journal of Pharmaceutical and Clinical Research* 5.2 (2012): 0974-2441.
3. Huttner A., *et al.* "Antimicrobial resistance: A global view from the 2013 World Healthcare-Associated Infections Forum". *Antimicrobial Resistance and Infection Control* 2.31 (2013): 1-13.
4. World Health Organization. "Antimicrobial resistance: global report on surveillance". WHO Press, World Health Organization, 20 Avenue Appia (2014): 3-5.
5. Roca I., *et al.* "The global threat of antimicrobial resistance: Science for intervention". *New Microbes and New Infections* 6 (2015): 22-29.
6. Piddock L J. "Reflecting on the final report of the O'Neill Review on Antimicrobial Resistance". *Lancet Infectious Disease* 16 (2016): 767-768.
7. Ibrahim MB., *et al.* "Antibacterial effect of extract of leaf, stem and roof bark of *Anogeissus leiocarpus* on some bacterial organisms". *Journal of Pharmaceutical Research and Development* 2.1 (1997): 20-23.
8. Selvakumar S and Sindhuja J. "Determination of Bio Pharmaceutical Components of A Novel Poly Herbal Formulation by GC-MS Analysis". *IAJPR* 7.11 (2017): 830-835.
9. Drieux L., *et al.* "Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench guide". *Clinical Microbiology and Infection* 14 (2008): 90-103.
10. Adhikari R., *et al.* "Detection of Methicillin Resistant *Staphylococcus aureus* and Determination of Minimum Inhibitory Concentration of Vancomycin for *Staphylococcus aureus* Isolated from Pus/Wound Swab Samples of the Patients Attending a Tertiary Care Hospital in Kathmandu, Nepal". *Canadian Journal of Infectious Diseases and Medical Microbiology* (2017): 1-6.
11. Ramalivhana J N., *et al.* "Antibacterial activity of honey and medicinal plant extracts against Gram negative bacteria". 3.4 (2014): 616-625.
12. Delnavazi M R., *et al.* "Antioxidant and antibacterial activities of the essential oils and extracts of *Dorema ammoniacum* roots and aerial parts". *Research Journal of Pharmacognosy (RJP)* 1.4 (2014): 11-18.
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement: M100-S21 31.1 (2011).

14. Yusha'u M., *et al.* "Biological activity and phytochemical constituents of *Tamarindus indica* stem bark extracts". *Sky Journal of Microbiology Research* 2.9 (2014): 067-071.

15. Saleem U., *et al.* "In-Vitro Antimicrobial Susceptibility Testing of Leaves Methanol Extract and Latex of *Euphorbia Helioscopia* Using Agar Well Diffusion and Broth Dilution Methods". *The Journal of Animal and Plant Sciences* 25.1 (2015): 261-267.

16. Devender R and Ramakrishna H. "GC-MS analysis of bioactive compounds in methanolic extract of *Leucaslavandula efolia* Rees.-A potential folklore medicinal plant". *Journal of Pharmacognosy and Phytochemistry* 6.4 (2017): 405-406.

17. Okpekon T. "Antiparasitic activities of medicinal plants used in Ivory Coast". *Journal of Ethnopharmacology* 90.1 (2004): 91-97.

18. Mann A., *et al.* "Phytochemical and antibacterial screening of *Anogeissus leiocarpus* against some microorganisms associated with infectious wounds". *African Journal of Microbiology Research* 2 (2008): 60-062.

19. Shuaibu M N. "Castalagin from *Anogeissus leiocarpus* mediates the killing of Leishmania in vitro". *Parasitology Research* 103.6 (2008): 1333-1338.

20. Ademola I O and Eloff J N. "In Vitro Anthelmintic Effect of *Anogeissus leiocarpus* (DC.) Guill. and Perr. Leaf Extracts and Fractions on Developmental Stages of *Haemonchus Contortus"*. *African Journal of Traditional, Complementary and Alternative Medicines* 8.2 (2011): 34-43.

21. Arbab AH. "Review on *Anogeissus leiocarpus* A Potent African Traditional Drug". *International Journal of Research in Pharmacy and Chemistry* 3 (2014): 496-500.

22. Sani H D and Aliyu BS. "A Survey of Major Ethno Medicinal Plants of Kano North, Nigeria, Their Knowledge and Uses by Traditional Healers. *Bayero Journal of Pure and Applied Sciences* 4.2 (2011): 28-34.

23. Edewor T I., *et al.* "Determination of antibacterial activity, total phenolic, flavonoid and saponin contents in leaves of *Anogeissus leiocarpus* (DC.) Guill and Perr". *Journal of Coastal Life Medicine* 4.4 (2016): 310-314.

24. Novo D J., *et al.* "Multiparameter flow cytometric analysis of antibiotic effects on membrane potential, membrane permeability, and bacterial counts of *Staphylococcus aureus* and *Micrococcus luteus"*. *Antimicrobial Agents and Chemotherapy* 44 (2000): 827-834.

25. Parsons J B., *et al.* "Membrane Disruption by Antimicrobial Fatty Acids Releases Low-Molecular-Weight Proteins from *Staphylococcus aureus"*. *Journal of Bacteriology* 94.19 (2012): 5294-5304.

26. Bisignano G., *et al.* "In vitro antibacterial activity of some aliphatic aldehydes from Oleaeuropaea L". *FEMS Microbiology Letters* 198.1 (2001): 9-13.

27. Lóránd T., *et al.* "Synthesis and antibacterial study of unsaturated Mannich ketones. *European Journal of Medicinal Chemistry* 36.9 (2001): 705-717.

28. Trombetta D., *et al.* "Study on the mechanisms of the antibacterial action of some plant α,β-unsaturated aldehydes". *Letters in Applied Microbiology* 35 (2002): 285-290.

29. Szakiel A., *et al.* "Antibacterial and Antiparasitic Activity of Oleanolic Acid and its Glycosides isolated from Marigold (*Calendula officinalis*)". *Planta Medica* 74 (2008): 1709-1715.

30. Baden C U and Dobler S. "Potential benefits of iridoid glycoside sequestration in Longitarsus melanocephalus (*Coleoptera*, *Chrysomelidae*)". *Basic and Applied Ecology* 1 (2009): 27-33.

31. Wang Q., *et al.* "Antibacterial effects of two monoterpene glycosides from *Hostap lantaginea* (lam.) Aschers". *Journal of Food Biochemistry* 41.2 (2016).

32. Tagousop C N., *et al.* "Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action". *BMC Complementary and Alternative Medicine* 18 (2018): 252.

33. McGaw LJ., *et al.* "Antibacterial effects of fatty acids and related compounds from plants". *South African Journal of Botany* 68 (2002): 417-423.

34. Kabara JJ., *et al.* "Fatty acids and derivatives as antimicrobial agents". *Antimicrobial Agents and Chemotherapy* 2 (1972): 23-28.

35. Cerdeiras MP., *et al.* "A new antibacterial compound from *Ibicella lutea"*. *Journal of Ethnopharmacology* 73 (2000): 521-525.

36. Dilika F., *et al.* "Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites". *Fitoterapia* 71 (2000): 450-452.

37. Yff B T S., *et al.* "The pharmacological screening of Pentanisiaprunelloides and the isolation of the antibacterial

compound palmitic acid". *Journal of Ethnopharmacology* 79 (2002): 101-107.

38. Lacey RM and Lord V L. "Sensitivity of staphylococci to fatty acids: novel inactivation of linolenic acid by serum". *Journal of Medical Microbiology* 14 (1981): 41-49.

39. McDonald M I., *et al.* "Antibacterial activity of hydrolysed linseed oil and linolenic acid against methicillin-resistant *Staphylococcus aureus"*. *The Lancet* 2 (1981): 1056.

40. Ohta S., *et al.* "Antibiotic effect of linolenic acid from *Chlorococcum* strain HS-101 and *Dunaliella* primolecta on methicillin-resistant *Staphylococcus aureus"*. *Journal of Applied Phycology* 7 (1995): 121-127.

41. Giamarellos-Bourboulis EJ., *et al.* "In-vitro inhibitory activity of gamma-linolenic acid on *Escherichia coli* strains and its influence on their susceptibilities to various antimicrobial agents". *Journal of Antimicrobial Chemotherapy* 36 (1995): 327-334.

42. Peters J S., *et al.* "Inhibition of photosynthetic electron transport by palmitoleic acid is partially correlated to loss of thylakoid membrane proteins". *Plant Physiology and Biochemistry* 41 (2003): 117-124.

43. Zheng C J., *et al.* "Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids". *FEBS Letter* 579 (2005): 5157-5162.

44. Schönfeld P and Wojtczak L. "Fatty acids as modulators of the cellular production of reactive oxygen species". *Free Radical Biology and Medicine* 45 (2008): 231-241.

45. Kenny J G., *et al.* "The *Staphylococcus aureus* response to unsaturated long chain free fatty acids: survival mechanisms and virulence implications". *PLoS One* 4 (2009): e4344.

46. Jenecius A and Mohan VR. "GC-MS Analysis of Bioactive Componentson the Stem Extract of *Bacolepis Nervosa* (Wight and Arn.) Decne.Ex Moq. (Periplocaceae)". *Word Journal of Pharmacy and Pharmaceutical Sciences* 3.4 (2014).

Volume 4 Issue 11 November 2021