International Journal of Physical Sciences Research, 7 (1), 1-10, 2023
Print ISSN 2515-0391 (Print)
Online ISSN 2515-0405 (Online)
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development-UK

Antimalarial, Antibacterial, and Phytochemical Contribution of *Prosopis Africana* Stem Bark Methanolic Extract

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DOI: https://doi.org/10.37745/10.37745/ijpsr.17/vol7n1110 Published August 10 2023

Citation: Bunu M.I., Suleiman E., Garba M.H., Garba Y., Gana J., Charlotte M.T., Ndinteh D.T. (2023) Antimalarial, Antibacterial, and Phytochemical Contribution of Prosopis Africana Stem Bark Methanolic Extract, *International Journal of Physical Sciences Research*, 7 (1), 1-13

ABSTRACT: The *Prosopis africana (Fabaceae*) plant is traditionally used in the treatment of Hepatic disease, malaria, new wounds and fever [1][2][3][4][5]. Phytochemical screening, antibacterial and *In vitro* antiplasmodial activities of *Prosopis africana stem bark* methanolic extract was investigated against *S. epidermidis, M. smegmatis, E. faecalis, S. aureus, B. subtilis)* and Gram-negative strains *K. aerugninosa, P. vulgaris, K. pneumonia, K. oxytoca, E. cloacae, P. asaccharolyticus, E. coli, P. mirabilis*. Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the extract showed activities against *S. epidermidis, M. smegmatis, S. aureus, B. aureus,)* and Gram-negative strains *P. mirabilis, K. oxytoca, E. cloacae, P. asaccharolyticus, E. coli).* The extract has broad spectrum against bacteria. The LD_{50} value was greater than 5000mg/kg body weight. It also has a high potent for antiplasmodial activities with *P. bargie* inhibition of 76.52%. The phytochemical screening and GC-MS profiling indicated the present of secondary metabolites that may be responsible for the antimalarial and antibacterial activities of the extract. The results explicitly indicated that *Prosopis africana* stem bark methanolic extract can be used as a source of cheaper, less toxic antimalarial and antibiotic agent for drug development.

KEYWORDS*:* Antibacterial, Antimalarial, phytochemical, *Prosopis africana*, GC-MS RT, Medicinal plants

INTRODUCTION

Traditional medicine began when man started searching for food in the bush by plugging and eating all types of leaves and fruits [6]. Traditional medicine is a precursor and major raw material that gives breakthrough to modern medicine [7]. Natural products are vital tools in the production of pharmaceuticals Plant-derived constituents have traditionally been the primary source of pharmaceuticals. About (30–40%) proportion in the pharmaceuticals in present-day medicine are derived from natural sources [7][8]. Due to their large diversity in nature, they serve as source for the identification of lead molecules of interest for the development of new

therapeutic agents. Orthodox healers use them to treat malaria fever and other ailments. The stem bark on the other hand has a variety of applications and has not been validated scientifically. Therefore, it was critical to conduct this research in order to determine the biological or pharmacological foundations for these medicinal plants' use in the treatment of malaria and bacterial infection. *Prosopis africana* is a flowering plant species in the genus Prosopis and family Fabacae found in the African region. It has a common names African mesquite, iron tree and it is called "kirya" in Hausa language. Extract of aqueous stem / leaves of *Prosopis africana* are used for wound healing by traditional healers [9].

Materials and procedures

Plant content collection and preparation

The stem bark of *Prosopis africana* collected from Kontagora LGA, Niger state, Nigeria in March 2017. Identification was done by Mr Idris M. Sabi, Department of Forest Resources Management, Forestry Research Institute of Nigeria and Mr Mukaila Yusuf, Department of Forestry , Federal College of Wildlife Management, New Bussa, Niger State, Nigeria where voucher specimens [*Prosopis africana (Musa /KNT/ FHI:1469)]*

Extraction and Isolation of the plant's extract

The stem bark (1kg) was air-dried at 37^{o}C and ground to powder. Extraction was carried out using the method described by [10] with slight modification.

Qualitative examination of phytochemicals

The method of [11][12][13][14] was adopted for the test of flavonoids, terpenoids, tannins, saponnins, Steroids, Alkaloid, Phenolic;Anthraquinone

GC- High Resolution TOF-MS Profile screening of the extracts

The method of [15] was adopted and the NIST (National Institute of Standards and Technology) mass spectral library (2014) was used to make the identification, with a cutoff of 700.

Acute toxicity tests of the crude extracts

The extract's toxic effect was assessed using OECD procedures, which included oral administration of the extract at a single high dose of 5,000 mg/kg body weight [17].

Anti-Plasmodial Screening of the extracts

Parasite Inoculation

Highly parasitized (20-30% parasitemia) blood was obtained by cardiac puncture from *Plasmodium berghei* infected mice. The blood was diluted with phosphate buffer saline and 0.2ml of the diluted blood was intra-partitionally inoculated into mice [16].

Treating of inoculated mice with plant extracts

Four days (4) suppressive test were carried out to evaluate the antimalarial properties of the extracts according to the method described by [16][17].

Antibacterial Assay

Antibacterial activity of the crude extracts was evaluated by the serial micro-dilution method [18][19][20][21].

RESULTS AND DISCUSSION

Phytochemical constituents

Prosopis africana are used widely in traditional medicine. Phytochemicals found in this plant includes alkaloids, flavonoids, tannins, saponins, Phenolic; Anthraquinone and steroids & triterpenes [2][3][4][5][7][8][9][20][21][22][23][24].

Table1 Phytochemical screening of PaM04

Plant extract PaM04	Alkaloid	Saponin	Phenolic;Anthraquinone	Flavonoids	Polyphenone	Tannins	Steroids & Triterpenes
	++	+++	+	++	++	++	+++

+ = mild, ++ = medium, +++ = high intensity, ND= Not Detected, PaM04= methanolic extract of *Prosopis africana.*

GC-MS Profiling Result

The GC-MS results of the *Prosopis Africana stem bark* crude methanolic extract (PaM04). The main secondary metabolites detected in this crude extract was biologically active compounds which have 4-Chloro-l-proline, Glufosinate, Resorcineol and1,3-dioxalane they are used in antibacterial, antineoplastic and antiviral drugs [25].8-Trifluoromethylchinchoninic acid is a quinoline-4-carboxylic acid derivative. The antimalarial assay of 8-Trifluoromethylchinchoninic which is primary used to test for delayed death inhibitors of the malaria parasite plastid, was carried out using 96 hours incubation (Activity is inconclusive) [28]. Pyranone derivatives, furan and its derivative are reported to possess various biological activities such as antibacterial [27]

2-coumaranone (used as antioxidant, anti-inflammatory, anticancer, anti-HIV, and antibacterial [30]), Piperidine, Pyrrolidine derivatives are used for treating anti-inflammatory and hepatoprotective properties [31]. 1H-Indole, 4-methyl- is used for antibacterial, antitumor, antioxidant and anti-inflammatory [32]. Benzofuran derivatives are used as anticancer, antiviral, anti-inflammatory, anti-ulcer, anti-alzheimer, anti-tubecular, antioxidant and antimicrobial [33]. 4-[p-fluorophenyl]-2H-1,3[3H]-Oxazine-2,6-dione (oxazines derivatives have documentary as worthy synthetic intermediate with notable sedative analgesis, anticulvulsant, antipyretic, antimicrobial, antioxidant, anticancer and antimalarial activities [34].

Pam04	Name	R.T.s	Base mass	Conc	Sample conc	Match	Quant mass	Area	Baseline m.	Quantification
	1,3-Dioxolane, 4-methyl-/Malam frf v3	150.326				700	BPI(88.052047±3 ppm)		1,3-Dioxolane, 4-methyl-	
	2(5H)-Furanone, 5-methylene-/Malam frf v3	160.886				700	BPI(96.020655±3 ppm)		2(5H)-Furanone, 5-methylene-	
	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one/Malam frf v3	239.378				700	BPI(144.041857± 3ppm)		2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	
	Benzofuran/Malam frf v3	314.969				700	BPI(118.041217± 3ppm)		Benzofuran	
	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-:2/Malam frf v3	407.188				700	BPI(142.026123± 3ppm)		4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-:2	
	Resorcinol/Malam frf v3	420.096				700	BPI(110.036043± 3ppm)		Resorcinol	
	Trimethyl(3,3-difluoro-2-propenyl)silane/Malam frf v3	508.632				700	BPI(150.067297± 3ppm)		Trimethyl(3,3-difluoro-2-propenyl)silane	
	Norfuraneol/Malam frf v3	671.449				700	BPI(114.031076± 3ppm)		Norfuraneol	
	1-(2-furyl)-1,2-propanedione/Malam frf v3	737.784				700	BPI(138.031072± 3ppm)		1-(2-furyl)-1,2-propanedione	
	2-Coumaranone/Malam frf v3	743.485				700	BPI(134.036374± 3ppm)		2-Coumaranone	
	5-Acetoxymethyl-2-furaldehyde/Malam frf v3	773.868				700	BPI(168.041543± 3ppm)		5-Acetoxymethyl-2-furaldehyde	
	Glufosinate/Malam frf v3	973.078				700	BPI(181.049739± 3ppm)		Glufosinate	
	2H-1-Benzopyran-7-ol, 3,4-dihydro-3-(2-hydroxy-4-methoxyphenyl)-/Malam frf v3	1431.94				700	BPI(272.104512± 3ppm)		2H-1-Benzopyran-7-ol, 3,4-dihydro-3-(2-hydroxy-4-methoxyphenyl)-	
	4-Chloro-l-proline/Malam frf v3	1509.1				700	BPI(149.023603± 3ppm)		4-Chloro-l-proline	

PaM04= Methanolic extract of Prosopis africana

Parasitaemia

Table 5 presents the parasitaemia counts of *Plasmodium berghei* infected mice treated with extracts from *Prosopis Africana* plants. Infected untreated mice showed progressive increase in Parasitaemia count from 6.00 ±1.00 to 57.50±0.50%. Treatment of the infected mice with 5mg/kg bw chloroquine (standard drug) produced significant antiplasmodial activities with 97.39% inhibition of the parasite. The methanolic extract of *Prosopis africana (*PaM04) showed a high potent of antiplasmodial activities with *Plasmodium bergei* inhibition of 76.52%. [6] Found that the stem bark methanolic extract of Prosopis africana had excellent antiplasmodial efficacy in vitro, with an IC50 of 0.70 g/ml against Plasmodium falciparum strains. [5][35][36] Reported that the aqueous and methanolic extracts Prosopis africana also show an excellent inhibition of malaria parasite. Extracts with IC50 values less than 10g/ml are classified as active, whereas those with IC50 values less than 25g/ml are classified as partly active [37]. According to [38], a compound is active when parasitemia is reduced by 30% or more [39]. The extract contained-saponins, sesquiterpenes, alkaloids, and tannins, all of which have been linked to antiplasmodial action. A plant extract's chemo suppression is linked to its innate capacity to remove parasites from contaminated cells. The failure of a plant extract to fully clear parasites from contaminated cells may be attributed to the rudimentary plant extract's increased biotransformation [39].

Table 5.5: Effect of plant extracts on parasitaemia count in *P. berghei* infected mice

	Parasitaemia			
Samples	ONE	THREE	FIVE	% Parasite Inhibition
PaM04	7.50±0.50	29.50±1.50	13.50±2.50	76.52 a
standard control	5.00±3.00	11.50±0.50	1.50±0.03	97.39 b
Negative	6.00±1.00	25.50±2.50	57.50±0.50	-

Data are Mean±SEM of triplicate determination. The mean parasite inhibition with different superscript alphabet are significantly ($p<0.05$) difference

Table 5.10 shows the antibacterial activities Minimum Inhibitory Concentration of *Prosopis africana stem bark methanolic extract (*PaM04). The plants crude extracts were evaluated for antibacterial activities against 13 pathogens: *Enterococcus faecalis, Styplococcus aureus, Escherichia coli, Proteus mirabilis, Salmonella typhi, Klebsiella pneumonia, Proteus vulgaris, Bacillus subtilis, Entrobacter cloacae, Mycobacterium smegmatis, Klebsiella oxytoca, Peptostreptococcus asaccharolyticus* and *Styplococcus epidermidis.* The MIC and MBC values of the crude extracts against bacterial strains are 125–500 g/mL (Table 5a and 5b) respectively. The *methanolic extract (*PaM04) had very good activity against *Styplococcus epidermidis, Bacillus subtilis, Mycobacterium smegmatis, Styplococcus aureus and Salmonella typhi.* Literature has shown that the plant is used in treating wounds and wound contaminant bacteria are *Escherichia coli, Styplococcus aureus* [40]. Since phytochemical studies revealed that the plant possessed alkaloids, saponins, tannins, flavonoids, steroids, and carbohydrates [40], the results of this analysis attested to the usage of the Prosopis Africana by traditional healers to treat wounds. The GC-MS table revealed that most of the phytochemicals have bacterial infections potent, these phytochemicals with antibacterial activities are;-pyrimidine derivatives [41], Nicotinic acid [42], 2-Aziridineethanol [43], Tryptamine [44], pyrimidinone [45], pyrrolo[1,2-a] pyrazine which control the activity of drug resistance staphylococcus aureus [46], benzofuran [47], 1,3-dioxane [48], pyranone derivatives [24], Adenine [49], lupeol [50], oxazines [34], These phytochemicals may work independently or together with other compound

Table5.1 MIC values of *Prosopis africana stem bark methanolic extract (*PaM04)

			MIC (µg/ml)		
Bacterial strain	ATCC	Gram	PaM04	STM	NLD
E. c	25922	-	125	64	>512
K. p	13048	-	500	64	64
P. v	33420	-	500	30	8
E. f	14506	+	500	128	>512
S.e	12228	+	250	8	64
M. s	14468	+	250	<4	>512
E. cl	13047	-	250	>512	16
S. a	25923	+	250	128	32
K.o	8724	-	250	16	8
P. m	7002	-	250	16	256
B. s	19659	+	250	16	16
P. a	1496	-	500	8	64
S. typhi	39183	-	500	75	73

E. f = Enterococcus faecalis, S.a = Styplococcus aureus, E. c= Escherichia coli, P.m=Proteus mirabilis, S.t = Salmonella typhi, K.P=Klebsiella pneumonia, P.v= Proteus vulgaris, B. s= Bacillus subtilis, E.cl= Entrobacter cloacae, M.s= Mycobacterium smegmatis, K.O= Klebsiella oxytoca, P. a= Peptostreptococcus asaccharolyticus, S.e= Styplococcus epidermidis, STM= Streptomycin, NLD= Nalidixic acid. -= Gram-negative, + = Gram-positive

			MBC g/Ml
Bacterial strain	ATCC	Gram	PaM04
E. c	25922	-	125
K. p	13048	-	250
P. v	33420	-	250
E. f	14506	+	250
S.e	12228	+	125
M. s	14468	+	125
E. cl	13047	-	125
S. a	25923	+	250
K.o	8724	-	250
P. m	7002	-	250
B. s	19659	+	250
P. a	1496	-	500
S. typhi	39183	-	500

E. f = Enterococcus faecalis, S.a = Styplococcus aureus, E. c= Escherichia coli, P.m=Proteus mirabilis, S.t = Salmonella typhi, K.P=Klebsiella pneumonia, P.v= Proteus vulgaris, B. s= Bacillus subtilis, E.cl= Entrobacter cloacae, M.s= Mycobacterium smegmatis, K.O= Klebsiella oxytoca, P. a= Peptostreptococcus asaccharolyticus, S.e= Styplococcus epidermidis, STM= Streptomycin, NLD= Nalidixic acid. -= Gram-negative, + = Gram-positive

Acute Toxicity

In general, for in vitro and in vivo experiments, the smaller the LC_{50} and LD_{50} value, the more dangerous the sample is, the worse it is. The reverse is also true: the lower the toxicity, the higher the LC50 and LD50 value. Plant extracts with LC50 values of over 1000 and >5000 mg/mL are considered non-toxic, while those with LC50 values of between 500 and 1000 mg/mL, LD50 values of between 2,500 and >5000 mg/mL are considered weakly toxic, those with LC50 values of between 100 and>500 mg/mL, LD50 values of between 1000 and >5000 mg/mL are considered moderately toxic, and those with LC50 values of less than 100 mg/m [17].

Therefore, the methanolic stem bark crude extract of *Prosopis africana is safe and not toxic*

Table 5.8: Acute toxicity profile of some plant extracts

Sample	**0bservation (>5000) mg/kg bw**	**Mortality**	**LD_{50} (**mg/kg)
PaMO4	Weakness	Nil	>5,000

PaM04= Methanolic extract of *Prosopis africana*

CONCLUSION

It suffice to state that from the result in this context that anti plasmodial potentials of most of the tested extract is quite promising with PaM04 displaying efficacies of 76.52 with a negligible acute toxicity except. However, in the context of bacterial activity, PM04 has been found to have significant broad spectral activities against tested bacterial organisms. Therefore, summing up the observations together, it could be asserted that when properly harnessed and effectively purified, such studied plants samples could be a potent reliable candidates for both antiplasmodial and antibacterial agents especially in the era of climate change/ environmentally-induced drug resistance exhibited by pathogenic organisms.

REFERENCE

1. A.M. Balde, M.S. Traore, M.S.T. Diallo, E.S. Balde, S. Diallo, L. Pieters, (2013). Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of Malaria. *Journal of ethnopharmacology. Vol. 150 (3).Pp 11455-115*
2. J.O. Adebayo, A.U. Krettli (2011). Potential antimalarials from Nigerian plants: A review. *Journal of Ethnopharmacology 133 (2011) 289–302*
3. Kari Inngjerdingen ,Cecilie Sogn Nergård, Drissa Diallo, Pakuy Pierre Mounkoro, Berit Smestad Paulsen (2004). An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. *Journal of Ethnopharmacology 92, 233–244*
4. T.A. Tor-Anyiin, R. Shaato, H.O.A. Oluma (2003). Ethnobotanical survey of antimalarial medicinal plants amongst the Tiv people of Nigeria. *Journal of Herbs, Spices and Medicinal Plants 10, 61–74.*
5. E.O. Uzodinma, E.I. Mbaeyi-Nwaoha, E.U. Onwurafor(2020). Suitability ofbacterial fermentation and foil packaging of condiment from African mesquite *(Prosopis africana)* seeds for nutritional retention and commercialization. *African Journal of Microbiology Research. Vol. 14(7), pp.348*

6. A-J. Abogo Mebale, A.S. Ondo AZI, T. Ndong, NLO, H. Massimba, Dibama, J.A. Ondo, and R. MenyeBiyogo, (2013). Phytochemical constituents of Uapaca le-testuana (A. Chev.) extracts from Gabon. *Journal of Research in Biochemistry; 1(2), 106-109.*
7. H.O. Edeoja, D.E. Okwu, and B.O. Mbalebie, (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology, 4(7),Pp 685-688.*
8. A.C. Ezike, P.A. Akah, C.O. Okoli, S. Udegbunam., N. Okwume, C. Okeke and O. Iloani (2010). Medicinal Plants Used in Wound Care: A Study of *Prosopis africana (Fabaceae)* Stem Bark. *Indian J Pharm Sci. 72(3): 334–339.*
9. A.A. Ajiboye, D.A Agboola, O.Y. Fadimu and A.O. Afolabi, (2013). Antibacterial, Phytochemical and Proximate Analysis of *Prosopis Africana* (Linn) Seed and Pod Extract *Futa J. Res. Sci., Vol 9, No. 1, pp 101-109*
10. P. J. Wright, D. T. Plummer, Biochem Pharmacy, **1972**, 23, 65-73
11. J. B. Harborne, (2005). Phytochemical methods– A guide to modern techniques of plant analysis. 3rd edition New Delhi: Springer Pvt.Ltd.Pp280
12. F.O. Jimoh, A.A. Adedapo, A.A. Aliero, and A.J. Afolayan,. (2008). Polyphenolic contents and biological activities of *Rumex ecklonianus*. Pharmamcutical Biolology 46(5): 333-340.
13. I. Kostova, and D. Dinchev, (2005). Saponins in *Tribulus terrestris*—chemistry and bioactivity. Phytochemistry Review 4(2-3): 111–137.
14. A. Kumar, R. Ilavarasn, T. Jayachandran, M. Decaraman, P. Aravndhan, N. Padmanaban, and M.R.V. Krishnan, (2009). Phytochemical investigation on trophical plant. Pakistan Journal of Nutrition 8(1): 1-23.
15. O.A. Adebo, E. Kayitesi, F. Tugizimanab, P. B. Njobeh, (2019). Differential metabolic signatures in naturally and lactic acid bacteria (LAB) fermented ting (a Southern African food) with different tannin content, as revealed by gas chromatography mass spectrometry (GC–MS)-based metabolomics. *Food Research International 121, 326–335*
16. A. Hilou, O.G. Nacoulma, T.R. Guiguemde. (2006). In vivo antimalarial activities of extracts from Amaranthus spinosus L. and Boerhaaviaerecta L. in mice. *Journal of Ethnopharmacology,*;103:236-40.
17. A.A. Jigam, T.A. Usman, N.E. Martins In-vivo antimalarial and toxicological evaluation of Chrozophora senegalensis A. Juss (euphorbiaceae) extracts. J Appl Pharm Sci 2011;01:90-4
18. M.A. Ekpo, PC. Etim (2009). Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections. J Med Plant Res 3(9):621-624. Encyclopedia Britannica, 2010 Anthelmintic. Encyclopedia Britannica Ultimate Reference Suite, Chicago.
19. J.V. Dacie, & S.M. Lewis, (2000). Practical hematology. 9th edition Churchill, Livingstone
20. Andrews, J.M., (2001). Determination of minimal inhibitory concentration. *Journal Antimicrob Chemother., 48, Pp. 5-6*
21. D. Kubmarawa, G. A. Ajoku, N. M. Enwerem and D. A. Okorie (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology Vol. 6 (14), Pp. 1690-1696*
22. K.I. Eghianruwa Veterinarskiarhiv, **2012**, 82, 5, 519-529.
23. M.A., Akanji, O.S., Adeyemi, S.O. Oguntoye, F., Sulyman, EXCLI J, **2009,** 8, 148-154.
24. J.N. Eloff, Planta Medica, **1998**, 64, 711–713.
25. Hadissa Tapsoba, Jean-Pierre Deschamps (2006). Use of medicinal plants for the treatment of oral diseases in Burkina Faso. *Journal of Ethnopharmacology 104, 68–78.*

26. Kuntal Manna, YadvenK Angrawal (2009).Microwave assisted synthesis of new indoophenazine 1,35-trisubstituted pyazoline derivatives of benzofuran and their antimicrobial derivatives Bioorganic and medicinal chemistry ;19:2688-2692
27. 2-methylquinoline-4-carboxilic acid. Publichem. https://pubchem.ncbi.nlm.nih.gov (Retrieved on 3/10/2020)
28. L. Xu, X. Zhao, W. Zhang, (2015). The study on Biological and pharmacological activity of Coumarins in: Proceedings of Asia-pacific Energy equipment Engineering Research conference, Zuhani, China, carl J.,M Ed. Atlantis press, pp135-138.
29. S. Haider, ZS. Saify, N. Begun, , S. Ashraf, T. Zarreen, , SMG, Saeed (2014). Emerging pharmaceutical application of piperidine, pyrrolidine and its derivatives. World journal of pharmaceutical research vol 3, issue 7;pp 987-1024
30. K. Lalit, B. Shashi, J. Kamal, (2012). The Diverse pharmacological importance of indole derivatives. A Review. *International Journal of Research in pharmacy and science; Vol. 2 (2), 23-33*
31. J. Reshma, Neragi., Santosh N. Dighe (2015). Biological and medicinal significance of Benzofuran. European Journal of medic inal chemistry vol.97;561-581.
32. Dhafer S. Zinad, Ahmad Mahal, Ranjan K. Mohapata, Ashish K. Sarangi, Mohammad Rizki Fadhil Pratama (2019). Medicinal chemistry of Oxazines as promising agents in drug discovery. Chemical Biology & Drug Design Vol. 95, issue 1
33. C. Vonthron-Senecheau, B. Weniger, M. Ouattara, F.T. Bi, A. Kamenan, A. Lobstein, R. Brun, R. Anton (2003). In vitro antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. *Journal of Ethnopharmacology 87, 221–225.*
34. G.G. Mwangi, A.M. Wagacha, J.M. Nguta, J.M. Mbaria,. (2015). Brine shrimp cytotoxicity and antimalarial activity of plants traditionally used in treatment of malaria in Msambweni district. *Pharm Biol,; 53(4): 588–593. DOI: 10.3109/13880209.2014.935861*
35. G. Garavito, J. Rinco´n, L. Arteaga, et al. (2006). Antimalarial activity of some Colombian medicinal plants. *J Ethnopharmacol 107:460–2.*
36. A.U. Krettli, J.O. Adebayo, L.G. Krettli,. (2009). Testing of natural products and synthetic molecules aiming at new antimalarials. Curr Drug Targets; 10(3):261-270
37. J.M. Nguta, J.M. Mbaria, D.W. Gakuya, et al. (2011). Biological screening of Kenya medicial plants using Artemia salina L. (Artemiidae). Pharmacology online 2:458–78.
38. F.E. Emele, M.I. Izomoh, E. Alufolai, (1999). Microorganism associated with wound infec*tion in Ekpoma, Nigeria. West Afr. I. Med. 18:97-100.*
39. C. Mallikarjunaswamy, L. Mallesha, D.G. Bhadregowda, Othbert Pinto (2012).Studies on synthesis of pyrimidine derivatives and their antimicrobial activity. Arabian Journal of Chemistry, http://dx.doi.org/10.1016/j.arabjc.2012.10.008.
40. M. Ahmed, M. Naglah, Hassan. A. Awad, Mashooq, A. Bhat, Mohamed Al-Omar, and E.. Abd El-Galil Amr (2015). Microwave-Assisted Synthesis and Antimicrobial Activity of Some Novel Isatin Schiff Bases Linked to Nicotinic Acid via Certain Amino Acid Bridge. *Journal of Chemistry Volume 2015, Article ID 364841, 8 pages http://dx.doi.org/10.1155/2015/364841*
41. Sun-HeangHeo .Joung-PyoNam . Dong-Gon Kim' Young-II Jeong Yang-Bae Kim' Young-HoonPark , Mi-Kyeong Jang . Jae-Woon Nah (2008). Chemical Modification of Chitosan with 2-Aziridineethanol for the Increase of Amine Values *Applied Chemistry, Vol. 12, N0. 2, 241-244*

42. Shazia Kousara, Sadia Noreen Anjuma, Farrukh Jaleela, JallatKhana and Sidra Naseema (2017). Biomedical Significance of Tryptamine: A Review *J Pharmacovigil, 5:5* DOI: 10.4172/2329-6887.1000239
43. Kyungmin Kim, Daseul Kim, Hyunjin Lee, Tae Hoon Lee, Ki-Young Kim, and Hakwon Kim (2020). New Pyrimidinone-Fused 1,4-Naphthoquinone Derivatives Inhibit the Growth of Drug Resistant Oral Bacteria. *Biomedicines 8,160*
44. George Seghal Kiran, aSethu Priyadharsini, Arya Sajayan,a Amrudha Ravin dranaand Joseph Selvin (2018) An antibiotic agent pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro isolated from a marine bacteria Bacillus tequilensis MSI45 effectively controls multi-drug resistant Staphylococcus aureus. RSC Adv., 8, 17837
45. Pratibha Sanjenbam, Krishnan Kannabiran (2016). Bioactivity of Pyrrolo[1,2- a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)- Extracted from *Streptomy ces*sp. VITPK9 Isolated from the Salt Spring Habitat of Manipur, India. *Asian Journal of Pharmaceutics 10 (4) | 265*
46. J. Reshma. Neragi., N Santosh. Dighe (2015). Biological and medicinal significance of Benzofuran. European Journal of medic inal chemistry vol.97;561-581.
47. Hatice Baspınar Küçük, Ayse Yusufoglu, Emel Matarac and Sibel Dösler (2011). Synthesis and Biological Activity of New 1,3-Dioxolanes as Potential Antibacterial and Antifungal Compounds. *Molecules*, *16*, 6806-6815; doi:10.3390/molecules16086806. www.mdpi.com/journal/molecules
48. G. Chioma. Anusionwu, A. Blessing, Aderibigbe and Xavier Y. Mbianda (2019). Hybrid Molecules Development: A Versatile Landscape for the Control of Antifungal Drug Resistance: A Review. *Mini-Reviews in Medicinal Chemistry,19,* 450-464
49. N. Sowrirajan, T. Chinnamani, G. Vijayakumar, T. Kolochi, (2018). Antibacterial and antifungal activities of Derivatives of Adenine. *International Journal Advance multidiscipline Re*search 5 (5):30-37.
50. A. Anas, A. Ahmed, S. Umar, U.M. Jajere, E.H. Mshelia, and O. Natasha,.(2017). Inhibitory Effect of Isolated Lupeol from Stem Bark of *Diospyros mespiliformis* Horsch (Ebenaceae) Against Some Microbial Pathogens. *Bayero Journal of Pure and Applied Sciences, 10(1): 293 – 299*