Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 11, Issue 2, 2023

Antimicrobial Assessment of *Commiphora pedunculata* and *Pergularia tomentosa*

[1]Okunola O. J., [1]Ali T., [2]Mann A. and [1]Bello O. M.

[1]Department of Applied Chemistry, Federal University Dutsin-Ma, Katsina State, Nigeria

[2]Department of Chemistry, Federal University of Technology, Minna, Niger State, Nigeria

Corresponding Author: ookunola@fudutsinma.edu.ng

ABSTRACT

In this study, the phytochemical screening and antimicrobial activity of the leaves of *Commiphora pedunculata* and *Pergularia tomentosa*, extracted with solvents of varying polarities, were examined against eight organisms (*Methicillin Resist Staph. Aureus, Streptococcus pyogenes, Aspergillus fumigatus, Aspergillus niger, Candida tropicalis, Microsporum audouinii, Microsporum canis and Tricophyton rubrum).* The antimicrobial activity was determined by disc diffusion method. Minimum inhibitory concentrations (MIC), minimum bactericidal and fungicidal concentration were determined by serial dilution method. The methanol, ethyl acetate and hexane fractions of the stem bark of *C. pedunculata* exhibited the highest inhibition zone ranging from 10-19 mm against the tested microorganism. At dose 60 mg/ml, the MIC against *Microsporum canis, Candida tropicalis, Streptococcus pyogenes were* the most significant with 3, 4 and 4 mm respectively. This work suggests that new antimicrobial leads can be sourced from plants and further phytochemistry on these plants is recommended.

Keywords: Infectious diseases, MIC, Antimicrobial drugs, *Commiphora pedunculata*, *Pergularia tomentosa*

INTRODUCTION

Diseases that are caused by bacteria and microbes are major and growing burden in the world. The increasing incidence of multidrug resistance of bacterial and fungal strains have made this challenge a more complex one especially in the scientific world [1,2]. Greater number of available antibiotics currently in use are suffering from this endemic of resistance [1,3,4]. Children are the most vulnerable among human populace, millions of children die yearly of infectious diseases while antibiotics could have little or no cure to these [5]. It has become so important and necessary, to combat emerging infectious diseases with a view to ascertain and invent new leads of more robust and effective therapeutic precis to challenge this growing threat to global health [6,7]. The use of herbs and plants' concoction against infection and management of microbial-caused

diseases is as old as life itself. Medicinal plants have structurally diverse constituents that have the large range of activity and usefulness. Medicinal and higher plants have been one of the primary sources for novel antimicrobial leads in the past couple of years [8]. The quest for new lead and effective agents against microorganism and therapeutic constituents from natural sources i.e. plants, that are effective against antibiotic resistant bacteria, safe and cost-effective have been of great interest in the last few decades [3,9-11].

Commiphora pedunculata stem bark has been reported to treat infected skin [12]. The root is usually chewed for treating cough and it is also used for treating jaundice, nausea and yellow fever [13]. The decoction of the leaves and stem bark is used for the treatment of dysentery and diarrhea. *Pergularia tomentosa* is a genus of the botanical family *Apocynaceae.* Pergularia is a perennial twinning herb that grows along the roadsides of tropical and sub-tropical regions [14]. *Pergularia tomentosa* is a climbing to semi erect perennial herb. It is used in northern Nigeria for treatment of skin diseases and arrow poisoning [15].

This study is to assess and evaluate the traditional use of these medicinal plants for skin infections. Previous studies on *Commiphora pedunculata* reported phytochemicals of the stem bark and Minimum Bactericidal/Fungicidal Concentration of the plant extracts in the different solvents [16]. The aim of this study, therefore, is to establish the scientific justifications of the whole plant use of *Commiphora pedunculata* and *Pergularia tomentosa* in the treatment of skin-related microbial infection and diseases.

MATERIALS AND METHODS

Collection and Extraction

Fresh plant material was collected in the month of March, 2018 from the bushes around Dutsin-Ma Local Government Area, Katsina State, Nigeria. The leaf and stem bark were identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria. The plants parts were air-dried for 21 days and crushed to coarse powder. The dried powder (766.02 g) was macerated successively in hexane, ethyl acetate and methanol exhaustively until complete extraction.

Phytochemical Screening

The extracts of the leaf and stem bark were subjected to phytochemical screening using standard techniques of plant secondary metabolites [17-19]. The metabolites tested for were alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, anthraquinones, cardiac glycosides and terpenes.

Test Organisms

The antimicrobial activity of methanol, ethyl-acetate and n-hexane of the two medicinal plants' extracts were determined using some pathogenic microbes. The microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Sensitivity Test of the Extract Using Agar Well Diffusion Method

The agar well diffusion method was used. The standardized inoculum of the isolates was uniformly streaked onto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (8 mm in diameter), five appropriately labelled wells were punched into each agar plate. Then, 0.2 ml of the appropriate extract concentration was placed in each well and allowed to diffuse into the agar. The plate was incubated at 37 ºC for 24 hours. While for the fungi, Sabouraud Dextrose Broth was used and the incubation period was 48 hours at 25 ºC. The antimicrobial activity was expressed as diameter of inhibition zones produced by the plant extracts [20, 21].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the methanol and water crude extracts of the stem bark of *Commiphora pedunculata* and *Pergularia tomentosa* were verified using Broth Dilution method [23]. The broth labelled "Mueller Hinton Broth" was made by strictly following the manufacturer's instructions. The MIC of the various solvents fractions of the plants were determined using the modified agar well diffusion method [18, 20, 24-25].

Determination of Minimum Bactericidal Concentration (MBC).

The minimum bactericidal concentrations of the water and methanol extracts were ascertained by selecting and sub-culturing the tubes that did not show growth during MIC determination, into fresh medium which contained no extract. Wire–loop was used to transfer from these tubes, these were sub-cultured over the surface of extract free nutrient agar, in petri dishes. These were incubated for 24 h at 37 ^{0}C. The lowest extract concentration from which the organism did not recover and grow on the nutrient agar was documented as MBC [18, 20, 24-25].

Determination of the Minimum Fungicidal Concentration (MFC).

Sabour and Dextrose Agar (SDA) plates were made following the manufacturers' instructions. The lowest dilution that did not show growth during MIC was inoculated onto the SDA plates. These

plates were incubated at ambient temperature for 24 hours. The plates were then observed for growth. The lowest extract concentration from which the fungi did not recover and grow on the agar was noted as the minimum fungicidal concentration (MFC). The result is recorded in Table 2 [18, 20, 24-25].

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical constituents' extracts of *C. pedunculata* and *P. tomentosa* are shown in Table 1. On the whole, coumarins, alkaloids, saponins, tannins, flavonoids, tannins, anthraquinones, cardiac glycosides and triterpenes were identified in all extracts. The ethyl acetate of both extracts gave poor result for most groups of secondary metabolites investigated. The phytochemical screening reveals that saponins and sugars were present in recognizable amount in the methanol extract of C*ommiphora pedunculata* while tannins and flavonoids in the methanol stem bark extract of *Pergularia tomentosa*. This screening may be useful in the discovery of the bioactive compounds and subsequently may lead to drug discovery and development.

Table 1: The Phytochemical Screening of Combined Plant Parts of with Solvents of Different Polarities

S/N	Constituents	*Pergularia tomentosa,*			*Commiphora pedunculata*		
		MeOH	E.A	Hex	MeOH	E.A	Hex
1	Alkaloids	-	-	-	-	-	-
2	Saponins	+	-	-	+++	-	-
3	Tannins	+++	++	+	+	-	-
4	Flavonoids	+++	++	+	++	-	++
5	Carbohydrates	++	++	+	+++	++	+
6	Steroids	++	-	++	+	++	+++
7	Anthraquinones	++	-	-	-	++	+
8	Cardiac glycosides	+	+	+	+	+	+
9	Terpenes	++	-	-	++	-	++

H_20: Water, MeOH: Methanol, E.A: Ethyl acetate, Hex: Hexane, +++= Very Good, ++=Good, +=Fair, - =Not present

MIC, MBC and MFC's Results

Table 2 shows the sensitivity and resistance test of the employed organisms towards the various solvent extracts of the two medicinal plants. Methicillin Resist Staph. Aureus and *Microsporum audouinii* were sensitive in all the solvent extracts used though *Tricophyton rubrum* was resistance to all the solvent extracts employed. *Streptococcus pyogenes* and *Candida tropicalis* showed that it has a high level of resistance in the three solvents used in *C. pedunculata* but were sensitive in *P. tomentosa. Microsporum canis* displayed sensitivity to the solvent extracts of *P. tomentosa.*

Table 2: The Antimicrobial Activities of Ethyl-Acetate, Methanol and n-Hexane the Plants' Extracts

Test Organism	Ethyl Acetate		Methanol		n-Hexane	
	PT	CP	PT	CP	PT	CP
Methicillin Resist Staph. Aureus	S	S	S	S	S	S
Streptococcus pyogenes	S	R	S	R	S	R
Aspergillus fumigates	S	S	S	R	S	R
Aspergillus niger	S	R	S	S	S	S
Candida tropicalis	R	R	S	R	R	R
Microsporum audouinii	S	S	S	S	S	S
Microsporum canis	R	S	R	S	R	R
Tricophyton rubrum	R	R	R	R	R	R

- *Key: S → Sensitive, R → Resistance*

Table 3: Zone of Inhibition of the Extract against the Test Micro-organism

Test Organism	Methanol		Ethyl acetate		n-Hexane	
	PT	CP	PT	CP	PT	CP
Meth. Resist Staph. Aureus	21	21	25	25	21	18
Streptococcus pyogenes	22	0	0	0	0	0
Aspergillus fumigatus	28	20	23	23	22	16
Aspergillus niger	20	20	21	21	28	17
Candida tropicalis	22	0	0	0	0	0
Microsporum audouinii	21	21	23	23	0	18
Microsporum canis	21	19	21	24	0	0
Tricophyton rubrum	0	0	0	0	0	0

PT: P. tomentosa; CP: C. pedunculata; Values are means of three replicates (N=3 ± SD).

The zone of inhibition of the extracts of *C. pedunculata* and *P. tomentosa* on the eight test organisms are shown in Table 3. The bacteria inhibition produced by the extracts of the medicinal plants varied in relation to the type of extract and to the micro-organism strain used. Though most of the crude extracts exhibited varying degree of inhibition zones, methanol and hexane extracts of the stem bark of *C. pedunculata* had the highest inhibition zone ranging from 10-19 mm against the tested bacteria employed, implying that it is the more likely active fraction. The ethyl acetate extracts of both the stem bark of *C. pedunculata* and the leaves of *P. tomentosa* showed a moderate zone of inhibition ranging 20-28 mm against the tested organisms. The antibacterial activity exhibited by the methanol and n–hexane of the stem bark of *C. pedunculata* is commendable hence the need to further exploit its antibacterial activity. For the leaves of *P. tomentosa,* no need to further assess its activity since from here it has shown a moderate activity. The generally accepted diameter zone of inhibition for plant extract against tested organisms is >21 mm [26]. However, for the plant extracts, the average zone of inhibitions observed against these pathogens ranged from 10-19 mm.

Table 4: Minimum Inhibition Concentration of *C. pedunculata* extracts against the Test Microorganisms

Extract	Conc (mg/ml)	*MRSA*	*SP*	*AF*	*AN*	*CT*	*MA*	*MC*	*MR*
Hexane	60	6	5	6	7	6	7	6	5
	30	7	6	5	5	7	6	6	7
	15	12	12	12	12	12	12	12	12
	7.5	20	21	23	21	20	21	24	25
	3.75	25	26	-	-	-	-	-	-
Ethylacetate	60	5	4	6	7	4	7	3	6
	30	6	5	5	6	5	5	5	7
	15	10	11	10	12	12	11	10	13
	7.5	20	21	23	21	20	21	24	25
	3.75	ND	ND	ND	ND	ND	ND	ND	ND
Methanol	60	9	5	8	7	6	8	7	8
	30	8	6	7	8	7	8	7	8
	15	16	17	15	-	-	14	14	-
	7.5	21	21	24	-	-	21	24	28
	3.75	25	26	>30	>30	>30	>30	ND	ND
Amphotericin B	10 µg/ml	34	33	35	22	29	28	36	25

MRSA=Methicillin Resist Staph. Aureus; SP=Streptococcus pyogenes; AF=Aspergillus fumigatus; AN=Aspergillus niger; CT=Candida tropicalis; MA=Microsporum audouinii; MC=Microsporum canis; TR=Tricophyton rubrum; -=No Inhibition; ND=Not Determined; Values are means of three replicates (N=3 ± SD).

Table 4 showed the MIC values for different solvents of stem bark extract of *C. pedunculata*. The study showed that the values ranges from 3 - >30 mg/ml. Maximum Inhibition Concentration was observed with methanol extract of this medicinal plant against *Methicillin Resist Staph. Aureus, Streptococcus pyogenes, Aspergillus fumigatus, Aspergillus niger, Candida tropicalis, Microsporum audouinii, Microsporum canis, Tricophyton rubrum (*20 - >30 mg/ml) (Table 4). Resistance is a growing menace. MICs are often used by laboratories to check resistance, though it is also employed as a means to evaluate the antimicrobial activity (*in vitro*) of new agents [22]. The most significant result i.e. Minimum Inhibition Concentration was observed with ethyl acetate extract of *C. pedunculata* against the tested organism at dose 60 and 30 mg/ml though hexane extract gave a good MIC result also. At dose 60 mg/ml, the MIC against *Microsporum canis, Candida tropicalis, Streptococcus pyogenes* were the most significant with 3, 4 and 4 mm respectively. These values may fall within the sensitive or resistance range when compared to the positive control. The values gotten may be credited to crude plant extracts which may contain a large amount of active constituents. Many authors have reported the significant activity of many medicinal plants' extracts within such diameter inhibition zone range [27-30].

Table 5: Minimum Bactericidal/Fungicidal Concentration of *C. Pedunculata* Extract against the Test Micro-Organism

Extract	Conc (mg/ml)	*MRSA*	*SP*	*AF*	*AN*	*CT*	*MA*	*MC*	*MR*
Hexane	60	3	3	4	-	-	4	4	2
	30	1	-	4	-	5	6	5	6
	15	-	11	9	8	-	-	10	10
	7.5	ND	ND	ND	ND	ND	ND	ND	ND
	3.75	ND	ND	ND	ND	ND	ND	ND	ND
Ethylacetate	60	2	2	2	-	-	4	-	3
	30	3	-	-	6	4	3	3	2
	15	12	10	11	-	-	9	9	10
	7.5	ND	ND	ND	ND	ND	ND	ND	ND
	3.75	ND	ND	ND	ND	ND	ND	ND	ND
Methanol	60	4	5	4	4	-	-	3	5
	30	7	6	10	6	8	7	-	-
	15	14	15	17	-	-	-	-	-
	7.5	ND	ND	ND	ND	ND	ND	ND	ND
	3.75	ND	ND	ND	ND	ND	ND	ND	ND
Amphotericin B	10 µg/ml	33	32	30	21	21	25	21	22

MRSA=Methicillin Resist Staph. Aureus; SP=Streptococcus pyogenes; AF=Aspergillus fumigatus; AN=Aspergillus niger; CT=Candida tropicalis; MA=Microsporum audouinii; MC=Microsporum canis; TR=Tricophyton rubrum; - =No Inhibition ND=Not Determined; Values are means of three replicates (N=3 ± SD).

It is important to note that MBC/MFC values gotten for the extracts of *C. pedunculata* against the tested organism are higher than MIC. This suggests that the extracts are capable of inhibiting the growth or reproduction of microorganism at lower concentrations and kill them at higher concentrations. This implies that when this medicinal plant is used traditionally against microbes, it inhibits the growth of microbes without necessarily killing them since large quantity of this extract are been taken orally in traditional medicine. Ethyl acetate extract gave the best activity against *Methicillin Resist Staph. Aureus, Streptococcus pyogenes, Aspergillus fumigatus* and *Tricophyton rubrum* at 2, 2, 2 and 3 mm while hexane extract displayed a significant activity too against *Methicillin Resist Staph. Aureus, Streptococcus pyogenes* and *Tricophyton rubrum* at 3, 3 and 2 mm respectively. *Methicillin-resistant S. aureus* isolates have been noticed to be resistant to many classes of antibiotics as the name implies, threatening antibiotic treatments worldwide hence the search for new constituents against these strains, *C. pedunculata* can serve as a source of new leads against this as shown in Table 5.

Streptococcus pyogenes is a positive gram bacteria responsible for many skin infections. It was noticed in this study, that ethyl acetate and hexane extracts gave significant results against this organism hence the need to search further for lead template against the source of skin infections. *Aspergillus fumigatus* and *Tricophyton rubrum* are fungi responsible for many illness i.e. respiratory illnesses bloodstream infections, ringworm, nail fungal infection and athlete's foot. Phytochemicals can be harvested from ethyl acetate and hexane extracts of this medicinal plant to combat these fungi.

Making comparisons with existing literature, this study compliments the thoughts of many authors that *C. pedunculata* and *P. tomentosa* leaf and stem barks are reservoir of antimicrobial agents. Though, most of the authors used different parts of the medicinal plant i.e. leaves, roots, flowers, root-bark, stem-bark and roots [16,31-33]. However, this study considered the use of leaf and stem bark.

CONCLUSION

C. pedunculata and *P. tomentosa* extracts were examined against eight microorganisms, consisting of both + Gram, – Gram bacteria and fungi, (*Methicillin Resist Staph. Aureus, Streptococcus pyogenes, Aspergillus fumigatus, Aspergillus niger, Candida tropicalis, Microsporum audouinii, Microsporum canis, Tricophyton rubrum).* The solvent extracts of *C. pedunculata* exhibited the highest inhibition zone ranging from 10-19 mm against the tested microorganisms. These were

further assessed by determining their MIC, MBC and MFC. The facts in this study confirm the use of the whole plant of *C. pedunculata* and *P. tomentosa* extracts in the treatment of skin-related microbial infection and diseases.

Acknowledgement

The authors appreciate the funding made available by the Institutional Based Research (IBR)-Tetfund for this work.

REFERENCES

1. Chaturvedi, H.K., Mahanta, J. & Pandey, A. (2009). Treatment-seeking for febrile illness in north-east India: an epidemiological study in the malaria endemic zone. *Malar. J.* 8, 301.
2. Girish, K. & Bhavya, B.M. (2018). Antifungal activity of *Lantana camara L., rhizosphere bacteria. Eurasian Journal of Biosciences,* 12(2),245 – 251.
3. Bello, O.M., Ibitoye, T. & Adetunji, C. (2018). Assessing antimicrobial agents of Nigeria flora. *Journal of King Saud University-Science*. https://doi.org/10.1016/j.jksus.2018.04.017.
4. Brent, A.J., Oundo, J.O., Mwangi, I., Ochola, L., Lowe, B. & Berkley, J.A. (2006). Salmonella bacteremia in Kenyan children. *Pediatr. Infect. Dis. J.* 25, 230–236.
5. WHO (2005). Department of Communicable Disease Surveillance and Response. WHO Global Strategy for Containment of Antimicrobial Resistance. [Accessed: December 20, 2013].Available at: http://www.who.int/csr/resources/publications/drugresist/en/EGlobal_Strat.pdf.
6. Mohanta, T.K., Petra, J.K., Rath, S.K., Pal, D.K. & Thatoi, H.N. (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium* L.f. *Sci. Res. Essays* 2(11), 486-490
7. Boyko, N.N., Zhilyakova, E.T., Bondarev, A.V., Kazakova, V.S., Pisarev, D.I., Novikov, O.O., Osolodchenko, T.P., Sahaidak-Nikitiuk, R.V. & Nefedova, L.V. (2019). Study of antimicrobial activity technology optimization of *Calendulae flos* galenicals. *Eurasian Journal of Biosciences* 13(2), 969 – 974.
8. Hasan, S.A., Najati, A.M. & Abass, K.S. (2019). Isolation and identification of mult-drug resistant "pseudomonas aeruginosa" from burn wound infection in Kirkuk City, Iraq. *Eurasian Journal of Biosciences* 13(2), 1045-1050.

9. Bello, O.M., Zaki, A.A., Khan, I.S., Fasinu, P.S., Ali, Z., Khan, I.A., Usman, L.A. & Oguntoye, O.S. (2017). Assessment of selected medicinal plants indigenous to West Africa for antiprotozoal activity. South Africa. *Journal of Bot.* https://doi.org/10.1016/j.sajb.2017.08.002.
10. Keong, B.C.M. & Sulaiman, W. (2006). Typhoid and malaria co-Infection – an interesting finding in the investigation of a tropical fever. *Malays. J. Med. Sci*. 13, 74–75.
11. Maltha, J., Guiraud, I., Kaboré, B., Lompo, P., Ley, B., Bottieau, E., VanGeet, C., Tinto, H. & Jacobs, J. (2014). Frequency of severe malaria and invasive bacterial infections among children admitted to a rural hospital in Burkina Faso. *PLoS One* 9, e89103.
12. Bhatia, S.C. (2008). Handbook of food processing technology Published by Atlantic Publishers, India.
13. Assob, J.C.N. , Kamga, H.L.F., Nsagha, D.S., Njunda, A.L., Nde, P.F., Asongalem, E.A., Njouendou, A.J., Sandjon, B. & Penlap, V.B. (2011). Antimicrobial and toxicological activities of fivemedicinal plant species from Cameroon Traditional Medicine. *Complementary and Alternative Medicine* 11(70), 1-11. doi:10.1186/1472-6882-11-70.
14. Endree, M.E. (2000). A Revised Classification of the Apocynaceae S.I. *The Botanical Review* 66(1), 1-56.
15. Hussein, H.T., Alrajhay, D. & Hashem, S.M. (1999). Molluscidal activity of *Pergularia tomentosa* (L). *Int J. Pest manage*. 45, 211-213.
16. Sallau, M.S., Tajuddeen, N., Ndukwe, G.I., Musa, A.M., Dambatta, B.M. & Sani, Y.M. (2014). Phytochemical and Antimicrobial Properties of *Commiphora pedunculata* (Engl) Stem Bark Extracts. *Bayero Journal of Pure and Applied Sciences* 7(1), 101 – 104.
17. Harborne, J.B. (1998). Phytochemical methods. Chapman and Hall Ltd., London. pp. 100-200.
18. Sofowora, A. (1993). Medicinal Plants and Traditional Medicines in Africa.Chichester John, Willey & Sons New York 256.
19. Trease, G.E. & Evans, W.C. (1989). A Textbook of Pharmacognosy, 13th ed. Bailliere Tindall Ltd, London, 134.
20. Lalnundanga, L. N. & Thanzami, K. (2015). Antimicrobial Activity of Methanol Extract of Root Bark of *Hiptage benghalensis* (L) Kurz. *Journal of Pharmacognosy and Phytochemistry* 3(6), 119-121.
21. Mathur, R. (2013). Phytochemical and Antimicrobial Evaluation of Plant Extracts of *Enicostemma hyssopifolium*. *Journal of Pharmacognosy and Phytochemistry* 2(4), 30-36.

22. Ravathie, A., Sevvel, P., Nirmala, R. & Kularajany, N. (2012). Alternative Culture Growth for Bacteria using different Formulation of Protein Sources. *Journal of Natural Product and Plant Resources*, 2(6), 697-700.
23. Donald, R.S. & Thornsberry, C. (1975). Broth-Dilution Method for Determining the Antibiotic Susceptibility of Anaerobic Bacteria. *Antimicrobial Agents and Chemotherapy* 7(1), 15-21.
24. Umaiyambigai, D. & Saravanakumar, K. (2015). Screening of phytochemical and antimicrobial activity from the leaves of *Pleiospermium alatum* (Wall. & Arn.) Swingle. *Journal of Chemical and Pharmaceutical Research* ,7(12), 498-504.
25. Mathur, R. (2013). Phytochemical and Antimicrobial Evaluation of Plant Extracts of *Enicostemma hyssopifolium*. *Journal of Pharmacognosy and Phytochemistry*, 2(4), 30-36.
26. NCCLS (1993). Performance Standards for Antimicrobial Disc Suspectibility Tests. Approved Standard NCCLS Publication M2-A5, Villanova, PA, USA.
27. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC and Fasure AK (2005). Screening of crude extracts of some medicinal plants used in SouthWest Nigerian Unorthodox medicine for anti-methicilin resistant Staphylococcus aureus. *BMC Compl. Alternative Medicine* 5, 6 doi:10.1186/1472- 6883-5-6.
28. Ilori, M.O, Shiteolu, O.A., Omonigbehin, E.A. & Adeneye, A.A. (1996). Antidiarrhoeal activity of *Ocimum gratissimum* (Lamiaceae). *J. Diarrhoeal Dis. Res*. 14, 54-58.
29. Ogbeche, A.K., Ajayi, G.O. & Onyeneta, P. (1997). Antibacterial activities of the leaf extract of Ageratum conyzoides *Nig. Qt. J. Hosp. Med*., 7,: 397-399.
30. Olukoya, D.K., Idika, N. & Odugbemi, T. (1993). Antimicrobial activity of some medicinal plants from Nigeria. *J. Ethnopharmacol*. 39, 69-72.
31. India Jacqueline (2005). Efficacy of Some Medicinal Plants Used in Various Parts of Kenya in Treating Selected Bacterial and Fungal Pathogens. M. Sc Microbiology. School of Pure and Applied Sciences of Kenyatta University, Kenya.
32. Akor, J.S. & Anjorin, T.S. (2009). Phytochemical and antimicrobial studies of *Commiphora africana* (A. Rich) Engl. root extracts. *Journal of Medicinal Plants Research* 3(5), 334-337
33. Ibrahim, S. & Abubakar, A. (2016). Phytochemical Compositions and Antibacterial Activities of Some Medicinal Plants Found in Aliero Area, Kebbi State, Nigeria. *International Journal of Pharma Sciences and Research* 7(2), 82-87