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Original article

Assessing antimicrobial agents of Nigeria flora

Oluwasesan M. Bello [a,b,c,*], Tosin Ibitoye [d], Charles Adetunji [e]

[a] Dept. of Applied Chemistry, Federal University Dutsin-Ma, Katsina State, Nigeria
[b] Dept. of Chemistry, University of Ilorin, Kwara State, Nigeria
[c] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA
[d] Dept. of Zoology, University of Ilorin, Ilorin, Kwara State, Nigeria
[e] Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Landmark University, Omu-Aran, Nigeria

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ABSTRACT

The search for new antimicrobial agents for the management of infections is of increasing interest because of the intense growing resistance of antibiotics to bacteria. Eighteen medicinal plants' extracts, these plants belonging to different families were fractionated using different solvents with varying polarity (hexane, chloroform and methanol), with historical use against infections in a primary and secondary screening for activity against *Candida albican, Aspergillus fumigatus, Crytococcus neoformans, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, Vancomycin resistance *enterococcus* and Methicillin-resistance *staphylococcus aureus* in an *in vitro* assay. The methanol extract of leaves of *Leptadenia hastata* (IC_{50} (μg/mL) = 22.53 (*MRS*), 11.89 (*VRE*)) and chloroform extract of seeds of *Parkia biglobosa* (IC_{50} (μg/mL) = 28.25 (*C. neoform.*), 85.68 (*VRE*)) exhibited significant inhibition against the microbes. Though hexane fractions of *P. biglobosa* and *Crotalaria mucronata* show selective inhibition of IC_{50} (μg/mL) = 28.71 (*C. neoform.*) and 58.13 (*C. alb*) respectively. The antimicrobial nature of extracts from the seeds of *P. biglobosa*, leaves of *B. ferruginea* and *T. preussi* were ascertain in this study for the first time as far as we know. The positive results of these medicinal plants constitute vital reference for ongoing phytoanalysis and biological studies.

1. Introduction

Africa is blessed with enormous biodiversity resources, vegetation and it is estimated to contain between 40,000 - 45,000 species of different plant genus with a need for exploitation, large numbers of these species are medicinally used in managing many ailments. Nigeria, a country in West Africa, represents a hot spot for biodiversity, where unexplored medicinal plants are dominant. Western medicines and its prescriptions are enormous and quite popular but medicinal herbs and its use are gaining rapid attention worldwide (Bello et al., 2017). The use of medicinal plants and herbs in the management of diseases and ailments has been a major source of cheap and affordable means of health care system in developing nations most especially from the sub-Sahara Africa. Global interest in the use of medicinal plants or botanicals has increased so much due to easy accessibility, there is a general believe that they are less toxic, cheaper than synthetic drugs and less side effects when compared to orthodox medicines. Extracts from these plants are mostly considered safe due to their historical use as natural sources of antimicrobial agents, they degrade easily (Croft and Yardley, 2002).

Microorganisms are mostly pathogenic, such as bacteria, fungi, parasites and viruses have great impact on health of the populace because most of the high and growing incidences of infectious diseases (Brent et al., 2006; Chaturvedi et al., 2009). In countries such as Nigeria, there are serious evidences of invasive bacterial infections with Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli*, and other Gram-negative bacteria which are enteric in nature (Bello et al., 2017; Berkley et al., 1999; 2005; Church and Maitland, 2014; Crawley et al., 2010; Evans et al., 2004; Gwer et al., 2007; Uneke, 2008). The discovery of new medicines i.e. antibiotics most times from natural sources, have ameliorated humanity's health status and quality of life. Over the years, the frequent use of antibiotics as resulted in the emergence

* Corresponding author at: Dept. of Applied Chemistry, Federal University Dutsin-Ma, Katsina State, Nigeria.
E-mail address: obello@fudutsinma.edu.ng (O.M. Bello).

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of microbial resistance to most available medicines in the market. Thus, the search for novel antimicrobial and therapeutic agents from natural origins i.e. plants, that are effective against antibiotic resistant bacteria, safe and cost-effective have been of great interest in the last few decades (Keong and Sulaiman, 2006; Maltha et al., 2014).

Medicinal plants do possess interesting and structurally diverse compounds that have the broad spectrum of biosynthetic capability and activity. Thus, it has become imperative to investigate the antimicrobial activity of these medicinal plants (18) which have long history of use as traditional medicines in Nigeria against infectious diseases. Different solvent fractions (chloroform, methanol and hexane) of each plant species was prepared based on ethnopharmacological knowledge, and screened for antimicrobial activity in an *in vitro* assay against *Candida albican, Aspergillus fumigatus, Crytococcus neoformans, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, Vancomycin resistance *enterococcus* and Methicillin-resistance *staphylococcus aureus*. Information about the selectivity of the different solvent extracts were obtained by determining the cytotoxic activity.

2. Materials and methods

2.1. Collection and extraction of plant materials

Parts of different plants (fruits, leaves, root and stem bark, seed with whole plant) were collected from knowledge gained from the locals, constituents of concoctions used against various infections in Nigeria. The plants species employed in this study are eighteen which are from fourteen plants' families, these were obtained between around May–June 2014 in Ilorin Metropolis, Nigeria. The plants obtained were ascertained by a taxonomic botanist in the dept. of plant biology, University of Ilorin, where voucher specimens were left. Plants' extracts were made by maceration method. These plants' parts were air- dried and the fine powder obtained. Each of the powdered plant are weighed and placed into different conical flask containing 250 ml of the different solvent. Each conical flask is then covered with aluminum foil and placed on mechanical shaker for 48 h at 190 rev. per. min. Each of these extracts were poured and passed through unsimiliar clean muslin cloth and filtered with fitter paper (whatman). The filterate gotten was allowed to evaporate at 50 °C and the residues found were saved in an aluminum foil. The methanol extract of each of the plant sample was fractionated with organic solvents with varied polarity (chloroform, hexane and methanol)

2.2. Antimicrobial

The antimicrobial activity was investigated against some fungi and bacterial *in vitro*. Microorganism employed are: fungi; *Aspergillus fumigatus* (ATCC 204305) (*A. fumigatus*) *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258) and *Cryptococcus neoformans* (ATCC 90113) and bacteria: *Staphylococcus aureus* (ATCC 29213) (*S. aureus*), methicillin resistant *S. aureus* (ATCC 33591), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Mycobacterium intracellulare* (ATCC 23068) (*M. intracellulare*), the potency of these extracts against the microorganism used was determined using the improved forms of the CLSI/NCCLS approaches (National Committee for Clinical Laboratory Standards (NCCLS), 2002). *M. intracellulare* and *A. fumigatus* was tested using an Alamar Blue method. The method employed here is an adapted one by Jain et al., (2005) though modified (Jain et al., 2005; CLSI, 1997, 2000; Bello, 2016).

3. Result

The initial screening which is the primary assessment for antimicrobial activities of the extracts at a single concentration of 200 μg/ml on the 8 different microorganisms: *Candida albican, Aspergillus fumigatus, Crytococcus neoformans, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vancomycin resistance enterococcus* and *Methicillin-resistance staphylococcus aureus* in an *in vitro* assay was stated in percentage growth inhibition, the results are shown Table 1. The secondary screening involves the medicinal plants' extracts which displayed a growth inhibition of >50% against the parasite, they were estimated in IC_{50} values as revealed in Table 2.

All the extracts shown in Table 2 showed >50% growth inhibition hence secondary test. The methanol extract of *Laptadenia hastata* (IC_{50} (μg/mL) = 69.59 (*C. neoform*), 22.53 (*MRS*), 11.885 (*VRE*) and chloroform extract of *Parkia biglobosa* (IC_{50} (μg/mL) = 80.28 (*C. albican*), 28.25 (*C neoformans*), 191.89 (*MRS*), 85.68 (*VRE*)) exhibited the most inhibition against the microbes. Though hexane fractions of *Parkia biglobosa* and *Crotalaria mucronata* show selective inhibition of IC_{50} = 28.71 μg/mL (*C. neoformans*) and 58.13 μg/mL (*C. albican*) respectively. The chloroform extracts of *balanite aegyptiaca* (L) and *indigofera astragalina* show inhibition against *Vancomycin resistance enterococcus* with IC_{50} (μg/mL) = 71.6, 107.5 and hexane extracts of *balanite aegyptiaca (S)* and *Tapinanatus preussii* showed inhibition against *Candida albican* with IC_{50} values of 168, 185 (μg/mL) respectively.

4. Discussion

Crotalaria mucronata Desv. is a synonym of *Crotalaria pallida* Aiton (Theplantlist). Methanol extract of *Crotalaria mucronata* Desv. shows selective inhibition of IC_{50} 58.13 μg/mL against *C. albicans* and the hexane extract of this plant displayed a good inhibition with IC_{50} value of 180.15 μg/mL against Methicillin-resistance *staphylococcus aureus* (MRS). Bhacca and Sharma (1968) isolated mucronatinine which is a new alkaloid from *Crotalaria mucronata* and the antibacterial and antifungal activity of mucronatinine was confirmed. The new alkaloid was tested up to 1600 μg/mL concentration against six microorganisms which includes both bacterial and fungal using serial dilution method" (Bhacca and Sharma, 1968). These author reported that the compound may be the main reason for the expected the antimicrobial activity in the plant.

Aliero and Wara (2009) studied the antimicrobial activity of the leaves extracts of *L. hastata* on bacterial and fungal microorganism (Aliero and Wara, 2009). Aqueous extract (the most polar) exerted inhibition on the development of both *E. coli* and *S. paratyphi* at the concentration of 30 mg/ml and *P. aeruginosa* at the concentration of 60 mg/ml. The methanol and acetone extract displayed a low antimicrobial activity against the microorganisms used. These authors investigated the antifungal activity of the leaves extarcts of *L. hastata* with *Aspergillus niger* and *Fusarium oxysporum*. The outcome of their assays revealed that methanol extract inhibited the growth of *F. oxysporum* and *A. niger* at 80 mg/ml with inhibition proportions extending from 58.89 to 73.30%. The polar part i.e. methanol extract of *Laptadenia hastata* was found to exhibit good antimicrobial activity with IC_{50} value of 69.59 μg/mL (*C. neoform.*), 22.53 μg/mL (*MRS*), 11.89 μg/mL (*VRE*). It was suggested that polar extracts of *L. hastate* could be explored in the management of different types of infectious diseases (Aliero and Wara, 2009).

The genus *Indigofera* belonging to the family *Leguminosae,* this family is renowned for its antimicrobial activity. Extract of *Indigofera caerulea* was tested against both Gram negative and Gram positive bacterial strains by performing cup plate method. Natarajan

Table 1
Antimicrobial Activity of Plants' Extracts (Showing % micro-organism growth).

Genus species (Synonym names and parts used)	*Primary Candida albicans*			*Primary Aspergillus fumigatus*			*Primary Cryptococcus neoformans*			*Primary MRS*			*Primary E. coli*			*Primary Pseudomonas aeruginosa*			*Primary Kp*			*Primary_VRE*		
	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M
Balanites aegyptiaca (L.) Delile (Leaves)	3	3	3	3	0	6	0	0	0	86	86	85	16	9	21	0	0	1	0	7	0	14	87	12
Balanites aegyptiaca (L.) Delile (Seeds)	53	0	42	3	0	0	0	0	0	0	0	76	**26**	**36**	14	0	0	0	13	20	0	10	1	0
Bridelia ferruginea Benth (Leaves)	0	0	0	2	13	13	0	0	0	0	0	0	6	4	21	14	0	0	0	0	7	0	5	11
Byrsocarpus coccineus (Schumach. & Thonn.) (leaves)	0	4	0	7	7	13	0	0	0	5	8	0	7	7	21	29	2	0	2	0	7	9	10	11
Cassia obtusifolia L. (Syn. Senna obtusifolia, Chamaecrista obtusifolia) (Leaves)	11	9	11	6	3	2	0	0	11	0	0	0	26	27	4	0	0	0	13	18	0	7	10	3
Corchorus walcotti F. Muel. (Leaves)	0	10	0	8	0	8	0	4	0	21	0	7	18	23	18	16	0	10	2	0	0	26	14	14
Crotalaria mucronata Desv. (Syn. Crotalaria pallida Aiton) (leaves)	75	31	0	6	5	12	0	0	11	70	60	4	32	22	17	0	0	1	15	3	0	4	3	11
Ficus vallis-choudae Delile. (Whole Plant)	40	0	5	6	8	8	6	0	25	5	0	0	21	12	16	0	0	0	0	0	0	11	3	14
Indigofera astragalina D.C (leaves)	0	37	0	10	8	11	4	0	8	0	85	3	18	17	16	4	1	7	0	0	0	13	75	12
Kigelia Africana (Lam.) Benth (Kigellia pinnata) (leaves)	0	1	0	6	4	4	0	0	0	57	0	0	37	7	10	0	0	0	31	0	0	7	12	7
Lannea microcarpa Engl. & K. Krause (bark)	0	9	0	7	5	12	0	0	1	0	0	1	14	39	16	0	0	0	0	32	0	5	9	13
Launaea taraxacifolia (Willd.) Amin ex C. Jeffrey (Leaf)	25	0	0	9	11	12	10	21	0	4	0	0	13	14	25	13	14	0	0	0	14	7	4	9
Leptadenia hastate (Pers.) Decne (leaf)	29	0	24	7	8	0	12	4	89	3	0	96	17	30	23	44	0	0	0	9	0	10	13	99
Luffa aegyptiaca Mill. (Luffa cylindrical, Luffa aegyptiaca (whole plant)	1	4	51	12	4	11	6	17	0	3	0	84	14	9	24	0	0	0	0	0	13	8	12	10
Parkia biglobosa G. Don (seeds)	0	86	0	4	6	5	99	98	1	25	57	0	41	49	11	0	0	0	31	45	0	12	52	1
Pseudocedrela kotschyi (Schweinf.) Harms (Leaves)	42	0	3	10	5	11	0	0	21	64	3	0	34	5	14	0	0	0	29	0	0	11	4	11
Tapinanthus preussii (Engl.) Tiegh. (whole plant)	63	0	4	7	6	10	0	2	22	0	0	0	34	17	21	0	0	0	17	0	0	11	10	9
Vitex grandifolia Gurke (Leaves)	0	0	0	8	13	5	0	3	2	3	0	0	16	16	11	0	0	0	0	0	0	11	12	5
Amphotericin B																								
Pentamidine																								

H = Hexane C = Chloroform M = Methanol, Data shown are mean values of two independent experiments run in triplicate, test concentration is 200 μg/mL.
Bolded Values: Plants' extracts which showed a growth inhibition of ≥ 50% against the parasite employed.

Table 2
Secondary Antimicrobial Activity of Plants' Extracts.

Genus species	*C. albicans IC50*	*C. neoformans* IC50	*MRS IC50*	*VRE IC50*
Crotalaria mucronata Desv. (Hexane fraction)	59.13	>200	190.60	>200
Crotalaria mucronata Desv. (Chloroform fraction)	>200	>200	180.15	>200
Leptadenia hastate (Pers.) Decne (Methanol fraction)	>200	69.59	22.53	11.89
Indigofera astragalina DC. (Chloroform fraction)	>200	>200	104.38	107.47
Balanite aegyptiacae (L.) Delile (Leaves) (Methanol fraction)	>200	>200	>200	162.74
Balanite aegyptiacae (L.) Delile (Leaves) (Hexane fraction)	>200	>200	>200	129.85
Balanite aegyptiacae (L.) Delile (Leaves) (Chloroform fraction)	>200	>200	161.73	71.59
Balanite aegyptiacae (L.) Delile (Seeds) (Methanol fraction)	>200	>200	>200	174.85
Balanite aegyptiacae (L.) Delile (Seeds) (Hexane fraction)	185.86	>200	>200	>200
Parkia biglobosa (Jacq.) G. Don (seeds) (Hexane fraction)	>200	28.71	>200	>200
Parkia biglobosa (Jacq.) G. Don (seeds) (Chloroform fraction)	>200	28.25	191.55	85.68
Kigellia Africana (Lam.) Benth (Leaves) (Hexane fraction)	>200	>200	193.90	>200
Luffa aegyptiacae Mill. (Whole plant) (Methanol fraction)	197.66	>200	116.08	>200
Tapinanthus preusii (Engl.) Tiegh. Whole plant) (Hexane fraction)	167.99	>200	>200	>200
Pseudocedrela kotschyi (Schweinf.) Harms (Leaves) (Hexane fraction)	>200	>200	157.97	>200
Bridellia ferruginea Benth (Leaves) (Hexane fraction)	>100	>100	>100	>100

Data shown are mean values of two independent experiments run in triplicate, test concentration is 200–8 μg/mL.

et al. (2010) discovered that the aqueous and hexane extract of *Indigofera caerulea* has the best inhibition against the bacterial strains employed. Kumar et al. (2013) studied the antimicrobial nature of *Indigofera trita* against more than twenty microorganisms. Most of the extracts showed moderate antifungal activity when compared to antibacterial activity which displayed very low activity. These species of *Indigofera* i.e. *I. glandulosa, I. hirsuta L, I. suffruticosa, I. aspalathoides, I. dendroides, I. Arrecta* have antimicrobial activity as revealed in the literature (Latha and Yasodamma, 2015; Prabakaran et al., 2011; Suvarnalatha et al., 2014; Efuntoye et al., 2014; Vieira et al., 2007).

Doughari et al., 2007 stated the antibacterial activity of both aqueous and ethanol parts of the leaves of *B. aegyptiaca* against *Salmonella typhi* by the disc diffusion method, this microorganism was isolated from blood clot culture (Doughari et al., 2007). The antibacterial activity was noticed highest with the ethanol extracts of *B. aegyptiaca* while the aqueous extract showed low activity at the dose of 100 mg/ml, these were compared with controls drugs popular used against typhoid fever (Doughari et al., 2007). Most

studies revealed the leaves and seeds of *B. aegyptiaca* displayed antibacterial activity against most strains of bacteria used (Daya et al., 2011; Speroni et al., 2005; Doughari et al., 2007; Noor Jahan et al., 2012; Karuppusamy et al., 2002).

Antimicrobial activity has been documented for *Kigelia africana,* many biological studies on its various parts have been established. Many studies have shown that its stembark possess antibacterial and antifungal properties and attributed these activities to dihydro-isocoumarins, naphthoquinones, iridoids and phenylpropanoids (Inoue et al., 1981, Akunyili et al., 1991, Houghton and Akunyili, 1993, Binutu et al., 1997, Moideen et al., 1999, Jeyachandran and Mahesh, 2007, Kela et al., 1989). Grace et al. (2002) have attributed the antimicrobial activity of the fruits of *Kigelia africana* to a blend of three fatty acids while palmitic acid, which is a compound already known to have antibacterial activity, was the prime constituent in the blend. This was the first reported isolation of Palmitic acid from *K. africana* though the compound is known already to have antibacterial activity (Grace et al., 2002). Binutu et al. (1996) reported the isolation of some compounds through antibacterial and antifungal activity guided column of the polar part (methanol) of the roots and fruits of *K. pinnata* D. C. This lead to the isolation of some interesting secondary metabolites which are liable for the antimicrobial effect of the roots amongst are naphthoquinones kigelinone, isopinnatal, dehydro-a-lapachone, lapachol and the phenylpropanoids *p*-coumaric acid and ferulic acid. Kigelinone and caffeic acid are the reason for the antimicrobial activity of fruits of this plant (Binutu et al., 1996).

In Nigeria, chewing sticks are easily accessible hence the most common means of maintaining healthy buccal cavity i.e. oral hygiene. Mostly all parts i.e. flowers, sap, twigs, leaves, roots and stems, of numerous plants are often used. World Health Organization recommend chewing sticks for oral hygiene, and most of these medicinal plants have historical importance for managing oral infections (Ndukwe et al., 2004). Many authors have confirmed the use of some parts of *Pseudocedrela kotschyi* as chewing stick or maintaining oral health (Akande and Hayashi, 1998; Akande and Ajao, 2011; Ndukwe et al., 2004). The leaves, stembark and roots of *P. kotschyi* has been a subject for research in many studies, literatures complement the antibacterial nature of *P. kotschyi* which was discovered in this study too (Akande and Hayashi, 1998; Akande and Ajao, 2011; Asase et al., 2008; Ayo et al., 2008; Adeniyi et al., 2010).

Mimosaceae is one of the plant family that is the subject of much studies because of its seeds' nutritional gains (Krans and Reiboth, 1973; Fetuga et al., 1974; Aiyelaagbe et al., 1996; Bello et al., 2017). In Nigeria, the people in villages use the seeds of *P. biglobosa* for food seasoning and as food condiments, this is obtained after the seeds are boiled and fermented. Extracts from leaves, stembark and roots of *P. biglobosa* inhibits both gram negative and gram positive organisms causing infections, this was reported by many authors confirming them to have large spread of activity (Millogo-Kone et al., 2006; Ajaiyeoba, 2010; Udobi and Onaolapo, 2009; El-Mahmood and Ameh, 2007). Most studies are yet to assess the antibacterial and antifungal nature of the seeds hence the dire need for this work. The hexane fraction of *P. biglobosa*'s seeds displayed a good selective inhibition of IC_{50} value of 28.71 μg/mL against *C. neoform* revealed from the study.

Antimicrobial activity of stembark of *B. ferruginea* had been investigated, many authors confirmed its stembark antibacterial and antifungal nature through various methods i.e. agar diffusion, *in vitro* assay, disc diffusion technique (Jose and Kayode, 2009; Owoseni et al., 2010; Kayodé and José, 2009; Adébayo and Ishola, 2009; Owoseni et al., 2010; Irobia et al., 1994). In this study, the leaves of *B. ferruginea* were investigated contrary to much investigated stembark yet it gave a promising antimicrobial activity. Mankilik et al. (2014) reported the antimicrobial investigations on extracts of *Luffa aegyptiaca* with vary solvents' polarity against *Staphylococcus species, Corynbacterium ulcerans, Bacillus subtilis, Salmonella typhi, E-coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Neisseria gonorrhaeae*, and *Candida albicans.* The chloroform extract of *L. aegyptiacae* showed good activities with exhibiting the most potent antimicrobial activity (Mankilik et al., 2014). *Luffa cylindrical* is the same as *Luffa aegyptiacae* This was the only reference literature to complement the antimicrobial activity of *L. aegyptiacae* showed in this study.

Loranthus pachycaulis Engl. & Krause and *Loranthus preussii* Engl. are synonym to *Tapinanthus preussii* (Engl.) Tiegh (Theplantlist). All these names were queried to check for any reference on the antimicrobial nature of these plants, this study is the first work on the antimicrobial activity of *T. preussi* to the best of our knowledge.

5. Conclusion

In this work, different solvents' fractions of eighteen medicinal plants' extracts, with traditional use against infections were tested against nine (9) different microorganisms, to justify their tradition applications. The various fractions displayed different levels of activities against the strains of bacteria and fungal used. *Leptadenia hastate,* seeds of *Parkia biglobosa* and leaves of *Crotalaria mucronata* showed efficacy activities against these microorganisms, and hold promising features for antimicrobial drug discovery. The results from this study supports the historical use of these medicinal plants in the management of infectious diseases. The findings from this study suggest that these plants species should be investigated to identify the antibacterial and antifungal compounds.

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Conflicts of interest statement

No potential conflict of interest was reported by the authors.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jksus.2018.04.017.

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