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Review Article

Antimicrobial activity of Nigerian medicinal plants

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ABSTRACT

Antimicrobial resistance (AMR) is currently one of the major threats facing mankind. The emergence and rapid spread of multi- and pan-drug-resistant organisms (such as vancomycin-, methicillin-, extended-spectrum β-lactam-, carbapenem- and colistin-resistant organisms) has put the world in a dilemma. The health and economic burden associated with AMR on a global scale are dreadful. Available antimicrobials have been misused and are almost ineffective with some of these drugs associated with dangerous side effects in some individuals. Development of new, effective, and safe antimicrobials is one of the ways by which AMR burden can be reduced. The rate at which microorganisms develop AMR mechanisms outpaces the rate at which new antimicrobials are being developed. Medicinal plants are potential sources of new antimicrobial molecules. There is renewed interest in antimicrobial activities of phytochemicals. Nigeria boasts of a huge heritage of medicinal plants and there is avalanche of researches that have been undertaken to screen antimicrobial activities of these plants. Scientific compilation of these studies could provide useful information on the antimicrobial properties of the plants. This information can be useful in the development of new antimicrobial drugs. This paper reviews antimicrobial researches that have been undertaken on Nigerian medicinal plants.

KEY WORDS: Antimicrobial activity, antimicrobial resistance, Nigerian medicinal plants

THE ANTIMICROBIAL RESISTANCE BURDEN

AMR is the ability of microorganisms such as bacteria and fungi to grow despite exposure to antimicrobial (antibacterial or antifungal) agent designed to inhibit their growth [1]. In general, microorganisms exhibit AMR by innate (e.g., absence of drug target site) and/or acquired (e.g., enzymatic degradation of drug) mechanisms conferred by AMR genes (ARGs) acquired via horizontal gene transfer (HGT) (transformation, transduction, and conjugation) from other microorganisms [1,2]. AMR includes two levels of resistance, the cellular level resistance (CELR), and the community level resistance (COLR) [3,4]. CELR develops via endogenous gene mutation or via HGT of resistance determinants from other microorganisms [4] while COLR occurs when a group of organisms become tolerant to environmental stress [5]. COLR is often observed among persisters (organisms that change their physiological state to become tolerant to lethal effect of drugs) in bacterial biofilms [4,5]. It was earlier thought that AMR evolved after the development of penicillin in 1940s, but historical reports, as well as studies on bacterial organisms from permafrost, had revealed that microorganisms exhibited innate AMR long before the development of any antimicrobial agent [6-8]. However, it is inappropriate use (abuse, misuse or overuse) of antimicrobials in human, animal, and plant settings that triggered the emergence of acquired AMR in microorganismcolistin,8].

Currently, AMR is one of the major threats to global health and factors such as global climatic change, globalization (increased international travel and food importation/exportation), and change in demographics are worsening the crisis [4,9,10]. The emergence and rapid spread (due to mobile genetic elements) of multi-drug- and pan-drug-resistant organisms (such as vancomycin-, methicillin-, extended-spectrum β-lactam-, carbapenem-, quinolone- and colistin-resistant bacteria) which exhibit resistance to virtually all antimicrobial agents currently known to man, has put the whole world in a dilemma [11]. These organisms (superbugs) jeopardize antimicrobial therapy resulting in untreatable and fatal infections [7]. There is no need reiterating their involvement in hospital-, nosocomial-, and community-linked infections worldwide [7,8]. The economic and health impact of AMR on a global scale is enormous and dreadful [4,7,10]. A recent review on "global crisis of AMR" chaired by Jim O'Neill, underestimated that about 700,000 lives are lost worldwide annually due to antimicrobial-resistant infections [11]. The report also estimated that by 2050, the societal and financial cost of not tackling the AMR crisis will be US$100 trillion [11,12]. Other recent studies estimated population reduction of between 11 million and 444 million people and a reduction in the size of the global economy by 0.1-3.1% by 2050, if effective antimicrobial agents are not developed [13,14]. The cost of developing a new antimicrobial has been estimated to be US$1 billion [7] and an estimation of US$30 billion is needed to tackle AMR crisis now before it

becomes uncontainable [11,12]. The impact of AMR is worse in developing nations, including Nigeria, where the cost of treatment of resistant infections and associated deaths are unaccounted for [15].

SEARCH FOR NEW PHYTOCHEMICALS WITH ANTIMICROBIAL ACTIVITY

It is largely recognized that most of the currently available antimicrobials which are mainly synthetic are almost inefficient and most of these agents elicit terrible effects to recipients [16-18]. For example, Stevens-Johnson syndrome and toxic epidermal necrolysis, and hypersensitivity reactions are associated with the administration of antimicrobials such as sulfonamide and fluoroquinolones, and penicillin, respectively [19,20]. All the experts that proposed strategies/solutions to tackle the AMR crisis recognized that development of new and safe antimicrobials is more critical than any other proffered solutions/strategies [7,11,12,21]. Many initiatives and programs have been set up by many countries/organizations with the aim of developing new, effective, and safe antimicrobials [21]. For instance, the 10x'20 initiative proposed in 2010 is aimed at developing 10 new, safe, and effective antibiotics by 2020 [22]. Thus, researchers/scientists are now looking at every ecological niche including soil, plant, animal, and marine for potentially new and safe antimicrobial agent [23,24]. Unfortunately, the rate at which microorganisms develop AMR outpaces the rate of discovery/development of new drugs [7,11].

The African traditional medicine is the oldest medicinal system and often culturally referred to as the Cradle of Mankind [16,25]. Traditional herbal medicines have been used to treat infectious diseases for thousands of years in various parts of the world [26,27]. There has been a renewed interest in indigenous medicine worldwide because orthodox medicine is not widespread [17,27]. In poor countries, the health care has been sustained by other practices based on cultural alternatives [27]. In many developing countries, including Nigeria, 80% of patients use indigenous herbal remedies to treat infectious diseases [17,24,28]. Despite the availability of modern medicine in some communities, herbal medicines (medicinal plants) have continued to maintain popularity for historical and cultural reasons, in addition to their efficacy and cheaper cost [17,24,27]. They also represent sources of potentially important new pharmaceutical substances since all parts of a plant, from roots to seed heads and flowers, are employed in traditional remedies and can, therefore, act as sources of lead compounds [17]. Moreover, molecules from natural products have represented about 80% of drugs that have been put into the market [17,29]. The use of plant remedies has steadily increased worldwide in recent years as well as the search for new phytochemicals that potentially could be developed as useful drugs for the treatment of infectious diseases [16,24,28].

Nigeria is located in West Africa on the Gulf of Guinea. It is bordered in the East by Cameroon (1,690 km), Northeast by Chad (87 km), North by Niger (1,497 km), and West by Benin (773 km) and by the Atlantic Ocean in the South [Figure 1] [17]. The country is divided administratively into the Federal Capital Territory (Abuja) and 36 states [17], these states are grouped into 6 geographical regions. Covering an area of 923,768 km^2, Nigeria is a country rich in biodiversity, possessing an array of fauna and flora including about 20,000 species of insects, almost 1,000 species of birds, 247 species of mammals, 123 species of reptiles, about 1,000 species of fish [17,30]. Nigeria boasts a unique and diverse botanical heritage with over 7,895 plant species of which *ca*. 3000 species are used therapeutically [30-32]; although, many of its plant species are at risk of extinction due to inadaptability problems attributed to natural factors such as climate change (e.g., desertification) as well as anthropogenic factors (e.g., deforestation due to timber logging, bush clearing and burning, oil spillage, over-grazing and urbanization), among others [30,33]. The humid tropical climate of Nigeria supports the growth and development of many plant species that have been used in Nigerian traditional medicine even before the advent of Western medicine [17]. Not only is the Nigerian flora rich in diversity but it is also mostly endemic [16,34]. In addition to this unique botanical heritage, Nigeria has a cultural diversity with traditional healing being integral to each ethnic group [16,17].

Despite the well-documented ethnobotanical literature, very little scientific information (e.g., efficacy, phytochemistry) has become available on indigenous medicinally used plants in Nigeria [17,27,35]. From available literature, the earliest documents on antimicrobial activity of Nigerian plants seem to be those of Dalziel [36,37] in 1937 and 1957, respectively. Two decades later, few other publications on chemistry and antimicrobial activities of Nigerian plants appeared in the literature [38,39]. In the 1980s, few studies on antimicrobial activity of Nigerian plants became available in the literature [40-42]. However, from 1990 to date, there has been an avalanche of publications in the literature on the chemistry and antimicrobial properties of Nigerian medicinal plants. This recent emergence in the scientific validation of antimicrobial activities of Nigerian medicinal plants may be a result of increased public awareness, method advancements and a number of citations in local books confirming the need for such studies [16,36,37,43]. Further reasons for advancement of work on Nigerian medicinal plant include searching for new lead compounds to be developed as drugs or as templates for analog synthesis and the evaluation of traditional medicine and herbal medicinal products [17,24,44]. Medicinal effects of Nigerian plants are attributed to interaction of phytochemicals (such as alkaloids, tannins, phenols, saponins, flavonoids, and essential oils) and bioactive compounds contained in their tissues [16,45]. Scientific compilation of studies on antimicrobial activity of Nigerian plants would enhance understanding of the extent of research that has been undertaken to elucidate the antimicrobial potential of these plants. Such study could arouse interest on Nigerian plants with potential antimicrobial activity from which new antimicrobial molecules could possibly be isolated. This review highlights the findings of studies that have been undertaken on antimicrobial activity of Nigerian medicinal plants 1971-2016.

Figure 1: Map showing Nigeria and her neighbors [17,46]

EXPERIMENTAL APPROACH USED IN ANTIMICROBIAL INVESTIGATION OF NIGERIAN PLANTS

Several steps are taken in evaluation of plants for antimicrobial activity. Selection of plant for antimicrobial screening is necessary to avoid unnecessary waste of time and resources [16]. Four standard approaches used for selecting plants include: (1) Random selection followed by chemical screening, (2) random selection followed by antimicrobial assays, (3) follow-up of antimicrobial activity reports, and (4) follow-up of ethnomedical or traditional uses of plants against infectious diseases [27,45-47]. Of these four approaches, the random selection followed by antimicrobial assay of plants against infectious diseases was the most common approach used by studies cited in this review [Table 1]. Selection of plant for antimicrobial investigation based on ethnomedical use is the best approach to avoid waste of resources and time [16,48]. It is important that selected plant is identified by an expert/plant taxonomist and deposited in a reliable herbarium for future identification and reproducibility of study [27]. While most of the studies on antimicrobial activity of Nigerian medicinal plants reported expert identification of selected plant(s), only few papers [49-60] reported deposition of plant in herbarium with the accompanying voucher number. Selection of the plant part to be evaluated may be based on ethnomedical use, randomly or follow-up of antimicrobial activity [61]. Different parts of a plant may contain varying types and amount of phytochemicals [45], thus the extent of extraction of these bioactive substances depends on the type of solvent used for extraction and the degree of binding with other substances in the plant material [24,61]. The majority of the antimicrobial screening studies on Nigerian plants assayed the leaf, while few analyzed the root, stem bark, fruit, and/or seed of selected plant(s) [Table 1].

The process of extraction in antimicrobial studies is critical as it determines to a large extent the result of the study [24,61]. In cases where the study is based on ethnomedical approach, an important factor to consider is the preparation of extract as described by the traditional healers to mimic as much as possible the way the herbal remedy is indigenously used [16,32]. In this way, the use of the plant in the traditional medicine can be correctly validated or invalidated. In cases where the antimicrobial activity of the plant is not based on ethnomedical approach, selection of solvent system largely depends on the specific nature of the bioactive compound being targeted [24]. In general, however, a good solvent used in plant extraction for antimicrobial bioassay should (i) have low toxicity, (ii) have relatively low boiling point so as to be easily removed from the compound after extraction, (iii) promote rapid physiological absorption of the desired compound in the extract in specific body compartments, (iv) have preservative action and inability to cause the quenching or dissociation of active principles, and (v) not interfere with the bioassay as the end product in extraction will contain traces of residual solvent [24,61]. Although aqueous (water) extraction is commonly used by the traditional healers, it has been shown that plant extracts obtained using organic solvents give more potent and consistent antimicrobial activity result than aqueous extract [16,24,62,63]. Studies indicated acetone as the most favorable solvent for plant extraction in antimicrobial studies [16,24,64]. Some of the antimicrobial screening papers in this review instead of using water or ethanol that is used in traditional medicine used organic solvent including ethyl acetate, methanol, butanol, petroleum ether and hexane for extraction [Table 1]. These organic solvents are not acceptable in indigenous preparation of plant extracts, thus the result could have been affected in a way [32].

Consideration should also be given to time and temperature of extraction as these as well as the solvent determines the extraction yield [24]. Some screening papers in this review

Table 1: Antimicrobial screening assays performed on extracts from Nigerian plants

Screening approach	Number of plants studied	Extract tested	Plant part analyzed	Assay	Highest activity	Reference
Antimicrobial activity	6	Ethanol	Leaf	AWD, MIC	*V. amygdalina,* 25 mg/ml against *S. typhi*	[86]
Pharmacological study	5	Methanol	Leaf	AWD	*A. djalonensis,* against *Proteus* spp., *E. coli* and *Shigella* spp.	[163]
Antimicrobial activity	3	Petroleum ether, methanol	Leaf	DD, MIC	*T. roka, T. procumbens* and *M. angolensis* (methanol extracts), 6.25 mg/ml, broad-spectrum	[249]
Antibacterial activity	3	Methanol	Leaf	AWD, MIC	*G. latifolium,* 75 mg/ml against *P. monteilli*	[206]
Pharmacological study	50	Ethanol	Various	MIC	*A. senegalensis,* 0.0625 mg/ml against *Bacillus* (*B.*) *subtilis*	[137]
Pharmacological study	13	Methanol	Various	DD	*A. cissampelli* and *G. arboretum* (root extracts), against *B. subtilis* and *E. coli*	[246]
Antibacterial activity	8	Ethanol	Leaf and bark	AWD	*A. schimperi* (leaf extract), against *P. aeruginosa*	[150]
Spices	5	Aqueous, ethanol, hexane	Various	AWD	*X. aethiopica* (ethanol seed extract), against *B. cereus* and *S. dysenteriae*	[81]
Curcubitaceae	3	Aqueous, ethanol	Leaf	AWD, MIC	*M. charantia, L. cylindrical and T. cucumerina* (ethanol extract), 2-9 mg/ml, broad-spectrum	[250]
Orodental hygiene	18	Aqueous	Various	AWD, MIC	*C. ferruginea* (fruit extract), *B. ferruginea* (stem/twigs extract), *A. leiocarpus* and *T. glaucescens* (root extracts), <2 mg/ml, broad-spectrum	[50]
Pharmacological study	6	Aqueous, ethyl acetate, ethanol, methanol, butanol	Stem bark or root bark	AWD	*M. senegalensis* (ethanol root bark extract), against *S. aureus*	[248]
Antimicrobial activity)	5	Aqueous (hot and cold), ehanol	Leaf	AWD	*A.* africana (ethanol extract), against *K. pneumonia*	[184]
Pharmacological study	11	Methanol, chloroform, hexane, ethanol, aqueous	Various	AWD, MIC	*M. tomentosa* (leaf), broad-spectrum; *T. heudelotti* (leaf), broad-spectrum	[48]
Orodental hygiene	4	Aqueous, ethanol	Root and stem	AWD, MIC	*A. schimperi* (ethanol root and stem extract), 1.56 mg/ml against *S. aureus*	[251]
Orodental hygiene	9	Phosphate buffered saline	Root and stem	MIC	*S. werneckei*, 0.25 μg/ml against *B. gingivalis*	[42]
Pharmacological study	3	Aqueous, ethyl acetate, methanol	Leaf	AD, MIC	*H. opposita* (methanol extract), against *Klebsiella* spp.	[252]
Antimicrobial activity	5	Aqueous, ethanol, methanol, ethanol: Aqueous	Various	AWD	*B. paradoxum* (ethanol extract), against *Klebsiella* spp.	[253]
Antibacterial activity	4	Aqueous (hot and cold), ethanol	Various	AWD	*A. indica* (ethanol leaf extract), against *E. coli*	[180]
Wound healing	9	Aqueous, ethanol	Leaf or stem	MIC	*A. wilkesiana* (ethanol extract) and *P. globosa* (ethanol and aqueous extract), 0.31 mg/ml against *S. aureus;* *A. conyzoides* (ethanol extract), *L. inermis* (ethanol and aqueous extract) and *P. globosa* (aqueous extract), 0.31 mg/ml against *B. subtilis*	[141]
Antimicrobial activity	4	Aqueous, ethanol	Leaf	DD	*C. papaya* (aqueous extract), against *A. butzleri*	[94]
Orodental hygiene	10	Aqueous, methanol	Stem	AWD	*G. kola, A. leiocarpus, T. glaucescens, S. warneckei* and *V. doniana* (aqueous extracts), against methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, multidrug resistant *B. cepacia* and *P. aeruginosa*	[187]
Pharmacological	2	Methanol	Root or stem bark	DD, MIC	*T. avicennioides* and *A. leiocarpus,* 0.3 mg/ml against *S. pyogenes* and *B. subtilis*	[166]
Pharmacological study	4	Aqueous (hot and cold), ethanol, methanol	Leaf	AWD	*P. macrophyla* (methanol extract), against *S. aureus*	[254]
Pharmacological study	5	Ethanol	Leaf	AWD	*B. nitida, C. alata* and *G. arboretum* against *T. rubrum, E. floccosum* and *B. haptosporus*	[255]
Antimicrobial activity	4	Aqueous (hot), methanol	Leaf	AWD	*E. hirta* (methanol extract), against *Pseudomonas* spp.	[161]
Antimicrobial activity	2	Aqueous: methanol	Leaf	MIC	*E. camaldulensis*, <0.0625 μg/ml against *C. albicans*	[112]

(Contd...)

Table 1: *(Continued)*

Screening approach	Number of plants studied	Extract tested	Plant part analyzed	Assay	Highest activity	Reference
Pharmacological study	2	Aqueous, ethanol, acetone, methanol	Leaf	AWD, MIC	*V. doniana* (acetone extract), 0.78 mg/ml against *E. coli*	[256]
Pharmacological study	2	Aqueous, ethanol	Leaf	AWD	*B. alba* (ethanol extract) against *M. luteus*	[158]
Orodental hygeine	2	Methanol	Stem	AWD, MIC	*A. leiocarpus*, 3.125 mg/ml against *C. krusei*	[111]
Spices	4	Aqueous, ethanol	Seed	AWD, MIC	*P. guineense* (aqueous extract), 30 mg/ml against *S. aureus*	[153]
Pharmacological study	4	Ethanol	Leaf	AWD, MIC	*S. mombin*, 4 mg/ml against *S. aureus*	[257]
Anti-infective activity	2	Methanol	Stem and root bark	DD	*A. leiocarpus* against *Lactobacillus* spp.	[92]
Antimicrobial activity	2	Ethanol	Stem	AWD	*O. gratissimum* against *Salmonella* spp.	[168]
Pharmacological study	4	Aqueous	Leaf	DD, MIC	*A. hybridus, C. esculenta,* and *C. bicolor,* 6.33 mg/ml against *C. albicans, E. coli*, and *S. aureus,* respectively	[106]
Orodental hygiene	5	Aqueous, ethanol, ethyl acetate	Root	AWD	*V. doniana* against *S. aureus*	[258]
Pharmacological study	3	Ethanol	Leaf	AWD, MIC	*P. osun*, 0.25 mg/ml against *K. pneumoniae*	[142]
Antifungal activity	8	Methanol	Various	AWD	*C. occidentalis* (root extract) against *A. fumigatus*	[174]
Pharmacological study	4	Aqueous, ethanol	Root and bark, or bark	AWD, MIC	*T. glaucescens* and *A. leiocarpus* (ethanol root extract), 0.625 mg/ml against *E. coli, S. aureus,* and *S. dysenteriae*	[143]
Skin infections	2	Aqueous, ethanol	Leaf and bark	DD	*M. oppositifolius* (aqueous extract), against *Penicillium* spp.	[79]
Pharmacological study	3	Aqueous (hot), ethanol	Seed or leaf	DD	*V. amygdalina* (aqueous and ethanol extracts), against *S. aureus* and *C. albicans*	[181]
Pharmacological	2	Ethanol	Leaf	AW	*S. mahogoni*, against *S. aureus*	[155]
Antimycobacterial	10	Ethanol, hexane, methanol	Various	MIC	*A. leiocarpus* and *T. avicennioides* (hexane extract), 312 μg/ml against *M. tuberculosis* and BCG antigen	[259]
Pharmacological study	3	Ethanol, acetone	Leaf	DD, MIC	*O. basilicum* (ethanol extract), 0.5 mg/ml against *E. coli, V. amygdalina* and *G. latifolium* (ethanol and acetone extract), 0.5-1 mg/ml against *K. pneumoniae, P. aeruginosa,* and *E. coli*	[105]
Orodental hygiene	8	Aqueous	Various	AWD	*T. glaucescens* against *P. gingivalis, P. intermedia, F. nucleatum, E. corrodens* and *C. rectus*	[169]
Antimycobacterial	11	Aqueous: methanol	Various	MIC	*P. corymbosa* (leaf and twist extract), 800 μg/ml against *M. tuberculosis*	[55]
Pharmacological study	4	Aqueous, ethyl acetate, butanol, hexane	Leaf	AWD	*C. acontifolius* and *A. digitata* (butanol extract), against *B. subtilis*	[201]
Antimicrobial activity	3	Aqueous	Leaf and stem bark	AWD	*A. schimperi, C. occidentalis* and *B. thonningi* (leaf extract), against *S. aureus* and *S. typhi*	[176]
Antibacterial activity	3	Aqueous, ethanol	Leaf	AWD	*M. lucida*, against *Flavobacterium* spp.	[262]
Antiviral activity	5	Methanol	Various	Cell culture /RNA probe	*A. muricata* (stem bark methanol extract), 5.8 μg/ml (EC_{50}) against Hepatitis C virus	[261]
Antibacterial	5	Aqueous, ethanol	Leaf or stem bark	MIC	*T. avicennioides* (ethanol stem bark extract), 18.2 μg/ml against methicillin-resistant *S. aureus*	[49]
Orodental hygiene	3	Aqueous, ethanol	Stem	AWD	*A. leiocarpus* against *C. krusei*	[188]
Antimicrobial activity	7	Methanol	Various	MIC	*S. mombin* (stem bark extract), 61.1 μg/ml (91% inhibition) against *M. tuberculosis*	[122]
Orodental hygiene	9	Aqueous	Various	MIC	*S. werneckei* (bark and pulp extract), ≤1% concentration against *B. melaninogenicus, B. oralis, B. ginivalis* and *B. asaccharolyticus*	[133]
Bryophyta	2	Ethanol, methanol, acetone	Whole	MIC	*C. erosum* and *B. coronatum* extracts, <0.0625-5 mg/ml, broad-spectrum	[145]
Antimycobacterial activity	12	Aqueous, ethanol	Various	Proportion	*A. acalonicum* (aqueous and ethanol leaf extract), *T. glaucescens* (aqueous and ethanol stem extract and *A. cepa* (aqueous and ethanol bulb extract), and *S. longepedunculata* (ethanol stem extract), 0.05 g/ml against *M. tuberculosis*	[235]

(Contd...)

Table 1: *(Continued)*

Screening approach	Number of plants studied	Extract tested	Plant part analyzed	Assay	Highest activity	Reference
Pharmacological study	10	Aqueous, ethanol	Stem or root	DD	*D. benthmianus* (ethanol extract), against *T. mentagrophyte*	[80]
Antimicrobial activity	4	Ethanol	Leaf	AWD	*J. nigra* against *C. albicans*	[164]
Antibacterial activity	10	Aqueous, ethanol	Twigs	AWD	*Azadirachta indica* (ethanol extract), against *S. mutans*	[240]
Antibacterial activity	8	Aqueous, methanol	Various	AWD, MIC	*P. africana* (methanol extract), *G. senegalensis* and *D. microcarpum* (aqueous leaf extract), 0.156-0.625 mg/ml against *S. typhi*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *P. vulgaris* and *Citrobacter* spp.	[148]
Antidiarrheic	2	Aqueous, ethanol	Leaf	AWD, MIC	*A. occidentale* (ethanol extract), 0.05-0.10 mg/ml, broad-spectrum	[135]
Pharmacological study	2	Aqueous, ethanol	Leaf	AWD	*S. alata* (aqueous extract), against *A. niger*	[239]
Orodental hygiene	3	Aqueous, ethanol	Stem	AWD	*F. zanthoxyloides* against *S. aureus*	[190]
Antimicrobial activity	6	Ethanol	Various	MIC	*A. indica* and *J. mimosaides* against *S. typhi* and *S. dysenteriae*	[260]
Pharmacological study	3	Ethanol	Various	AWD, MIC	*N. latifolia* (root extract), *C. penduliflorus* and *A. precatorius* (seed extracts), 3.025 mg/ml against *K. pneumoniae*	[247]

AWD: Agar well diffusion; DD: Disc diffusion; MIC: Minimum inhibitory concentration, *E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, C. ulcerans: Candida ulcerans, S. pyogenes: Streptococcus pyogenes, K. pneumoniae: Klebsiella pneumoniae, N. gonorrhoeae: Neisseria gonorrhoeae, M. bovis: Mycobacterium bovis, S. aureus: Staphylococcus aureus, A. niger: Aspergillus niger, C. albicans: Candida albicans, M. tuberculosis: Mycobacterium tuberculosis, S. typhi: Salmonella typhi, P. mirabilis: Proteus mirabilis, S. faecalis: Streptococcus faecalis, A. precatorius: Abrus precatorius, N. latifolia: Nauclea latifolia, C. penduliflorus: Croton penduliflorus, A. indica: Azadirachta indica, S. dysenteriae: Shigella dysenteriae, F. zanthoxyloides: Fagara zanthoxyloides, J. mimosaides: Jacaranda mimosaides, A. occidentale: Anacardium occidentale, S. alata: Senna alata, P. vulgaris: Proteus vulgaris, D. microcarpum: Deterium microcarpum, G. senegalensis: Gueira senegalensis, J. nigra: Juglan nigra, D. benthmianus: Distemonath benthmianus, T. mentagrophyte: Trichophyton mentagrophyte, S. longepedunculata: Securidaca longepedunculata, T. glaucescens: Terminalia glaucescens, A. cepa: Allium cepa, C. erosum: Calymperes erosum, B. coronatum: Bryum coronatum, B. asaccharolyticus: Bacteroides asaccharolyticus, B. ginivalis: Bacteroides ginivalis, B. oralis: Bacteroides oralis, B. melaninogenicus: Bacteriodes melaninogenicus, S. werneckei: Serindeia werneckei, S. mombin: Spondias mombin, A. leiocarpus: Anogeissus leiocarpus, A. digitata: Adansonia digitata, C. acontifolius: Cnidoscolus acontifolius, P. corymbosa: Pavetta corymbosa, C. rectus: Campylobacter rectus, E. corrodens: Eikenella corrodens, F. nucleatum: Fusobacterium nucleatum, P. intermedia: Prevotella intermedia, P. gingivalis: Porphyromonas gingivalis, V. amygdalina: Vernonia amygdalina, O. basilicum: Ocimum basilicum, S. mahogoni: Swietenia mahogoni, M. oppositifolius: Mallotus oppositifolius, P. osun: Pterocarpus osun, V. doniana: Vitex doniana, E. camaldulensis: Eucalyptus camaldulensis, E. hirta: Euphorbia hirta, B. haptosporus: Basidiobolus haptosporus, E. floccosum: Epidermophyton floccosum, C. alata: Cassia alata, T. rubrum: Trichophyton rubrum, G. arboretum: Gossypium arboretum, B. nitida: Baphia nitida, P. macrophyla: Pentaclethra macrophyla, B. cepacia: Bulkhoderia cepacia, S. warneckei: Sorindeia warneckei, G. kola: Garcinia kola, A. butzleri: Arcobacter butzleri, C. papaya: Carica papaya, P. globosa: Parkia globosa, L. inermis: Lawsonia inermis, A. conyzoides: Ageratum conyzoides, A. wilkesiana: Acalypha wilkesiana, B. paradoxum: Butyrospermum paradoxum, B. gingivalis: Bacteroides gingivalis, T. heudelotti: Trichilia heudelotti, M. tomentosa: Markhamia tomentosa, A. africana: Aspilia africana, M. senegalensis: Maytenus senegalensis, B. ferruginea: Bridellia ferruginea, C. ferruginea: Cnestis ferruginea, T. cucumerina: Trichosanthes cucumerina, L. cylindrical: Luffa cylindrical, M. charantia: Momordica charantia, X. aethiopica: Xylopia aethiopica, A. cissampelli: Adenia cissampelli, A. senegalensis: Annona senegalensis, P. monteilli: Pseudomonas monteilli, G. latifolium: Gongronema latifolium, A. fumigatus: Aspergillus fumigatus, C. bicolor: Caladium bicolor, C. esculenta: Colocasia esculenta, A. hybridus: Amaranthus hybridus, O. gratissimum: Ocimum gratissimum, P. guineense: Piper guineense, M. luteus: Micrococcus luteus, B. alba: Basella alba, H. opposita: Hoslundia opposita, M. angolensis: Maerua angolensis, T. procumbens: Tridax procumbens, T. roka: Trichilia roka, A. djalonensis: Anthocleista djalonensis*

reported the use of hot aqueous solvents for extraction may be to increase yield or to mimic the extraction procedure used by the traditional practitioners [Table 1]. Nasir *et al.* [24] suggested that in studies where the aim is initial screening of plants for potential antimicrobial activities, the process may begin by using the crude extracts prepared from different organic and aqueous solvents and then followed by the utilization of various organic solvents for fractionation. Some publications on Nigerian medicinal plants reported fractionation of extracts with various organic solvents [65-72]. The storage conditions of plant material (whole/extract) have been shown to affect the result of antimicrobial studies by impacting microbial efficacy [16,64,73]. Some papers on Nigerian plants reported storage of extract in refrigerator at 4°C [18,59,60,74]. This storage condition is good because the activity/growth of possible extract-contaminating organisms would be inhibited and this in a way enhances the reliability of the result [16,75].

In vitro or *in vivo* method employed in assay of antimicrobial activity of plant extracts is critical. This relates to the fact that microbiological methods incorporate viable test microorganisms; therefore, predictability of the outcome is not always clear and subject to many environmental influences that may impact on a response [16,61]. Hence, there is need for standardization of methods which is often encountered with many problems [16,61]. The Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial

Susceptibility Testing have standardized some of the methods used in antimicrobial assay [76] but it should be noted that these methods are standardized for standard drug preparations and not really for plant extracts [61]. Various *in vitro* methods are employed in assay of plant extracts for antimicrobial activity; these methods have been extensively reviewed [24,61,76,77]. The most critical step in *in vitro* assay of plant extract for antimicrobial activity is the inoculum size quantification of selected organism [24,76]. While bacterial/yeast inoculum size quantification is commonly done using the McFarland's turbidity standard method [76], quantification of mold inoculum size is more difficult and requires biosafety equipment because they produce spores. Hemocytometric method is considered the best for quantifying fungal spores [78]. Few papers on antimicrobial activity of Nigerian plants against mold reported the use of hemocytometeric method in quantifying spores of selected organism [79-81].

Review of over 400 papers on antimicrobial investigation of Nigerian medicinal plants revealed the use of various antimicrobial assay methods. Figure 2 represents proportion of studies and the methods used to assess antimicrobial activity of Nigerian plants from 1971–2016. Minimum inhibitory concentration (MIC) and Agar well diffusion (AWD) assays are the two most common methods used to investigate the antimicrobial activity of Nigerian medicinal plants. The MIC assay is a quantitative method of measuring antimicrobial activity based on the principle of contact of a test organism to a series of dilutions of test substance [16,24,76]. MIC is the lowest concentration of the antimicrobial agent that prevents visible growth of a microorganism under known conditions [24,76,82]. Assays involving MIC methodology (such as macro [test tubes] and micro [microtitre plates] broth dilution and agar dilution) are widely used and an accepted criterion for measuring the susceptibility of organisms to inhibitors [16,83]. This is supported by majority of publications on Nigerian plants representing 58.1% for extract and 1.9% for essential oil. The AWD is a widely used method of assay possibly because it indicates the concentration of the plant extract that exhibit the highest microbiostatic effect on the test organisms [24,76]. The method is qualitative and based on the principle of contact of a test organism to an equal volume of different concentrations of test substance inoculated into wells of equal depth and diffusing into cultured agar [24,76]. Although AWD resembles disc diffusion, it is preferred to disc diffusion because it gives a more consistent result [24,76] and this is supported by high proportion (57.6% for extract and 1% for essential oil) of publications on Nigerian medicinal plants which utilized this method. Some papers presented result obtained using both AWD and MIC assay [84-89]. However, variation in data obtained using MIC assay may be influenced by factors such as the inoculum size, the type of growth medium, the incubation time, and the inoculum preparation method [76,90,91].

The fact that a lower proportion of publications on antimicrobial activity of Nigerian plants (15.7% for extract and 1% for essential oil) used disc diffusion is a further indication that the investigators adopted more quantifiable methods of assaying antimicrobial activity of plants [16]. The use of disc diffusion in these studies may possibly be due to its simplicity and capacity to analyze a large number of test samples [16,24,76]. Some studies on Nigerian plant extract presented only disc diffusion data [80,92-99] while some papers (7.1%) reported results using both disc diffusion and MIC [48,69,100-106]. Although disc diffusion methodology is a quick simple means of screening for antimicrobial activity, it is associated with problems which may arise when investigating oil samples [16]. The associated problems that could yield inconsistent result with disc diffusion assay include variation in diffusion rates due to differences in chemical nature of the particular sample, lipophilic substances like essential oil or water-insoluble samples do not easily diffuse through the agar even with a pre-diffusion time allocation of 1 h [16,76,107]. Thus, false negatives may still be encountered and the possibility of activity could be underestimated [16]. Volatility of oily samples is another prominent factor to be considered [16]. Excessive incubation

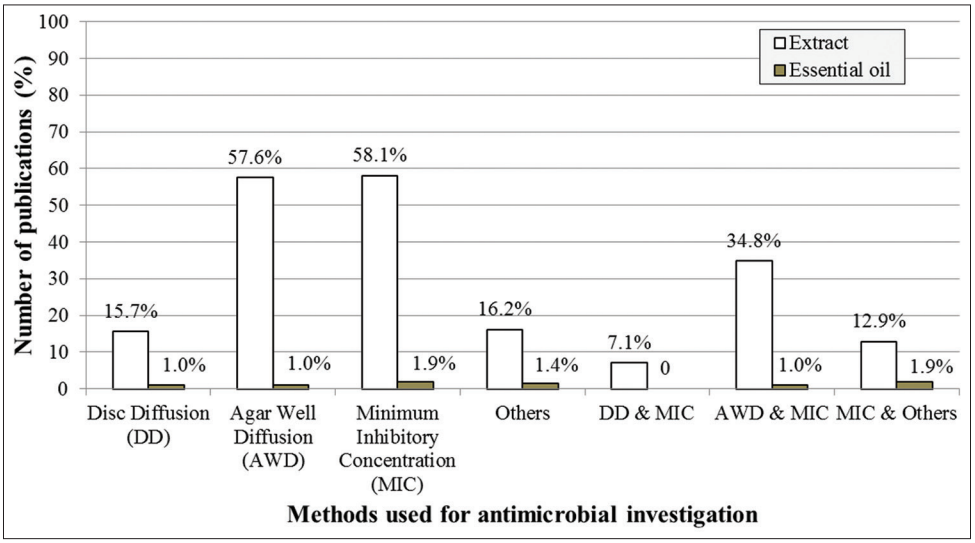

Figure 2: Methods used to assess antimicrobial activity of Nigerian plant extracts

time and temperature (such as during long incubation period 2-7 days of fungal incubation) may result in loss of a proportion of the oil due to evaporation, this too may impact on the false negatives [16,108]. Antimicrobial activity against a proportion of plant sample (especially essential oils) may be assessed following loss of hydrocarbon which is postulated to be very prone to evaporation [16,109].

Other antimicrobial investigation methods used to assess antimicrobial activity of extracts and essential oils of Nigerian plants include killing kinetics and bioautographic assays [16,76]. Although killing kinetics is labour-intensive and requires a number of steps where variables may be introduced, it provides descriptive information on the relationship between bacteriostatic and/or bactericidal activity in relation to the concentration of test substance over time (i.e. the method gives valuable information of the cidal action over time) [16,110]. Some publications on Nigerian plant extract reported data using killing kinetics [42,53,57,111]. Bioautographic assay (involving chromatography and biological systems) is mainly used to evaluate antimicrobial activity of isolated bioactive compounds [16,24,76], but it is also useful for assessing antimicrobial activity of crude plant extracts [24]. Antimicrobial activity of Nigerian plant extracts [65,68,70,71,112-127] and essential oils [32,128] have been assayed using bioautography [Figure 2]. However, no study on Nigerian plants has utilized biointeractive methods [16,129].

ANTIMICROBIAL INVESTIGATION ON NIGERIAN PLANT EXTRACTS

The majority of the papers on Nigerian medicinal plants were dedicated to antimicrobial activity of extracts. Identification of plants with potential antimicrobial activity from screening publications for further investigation is usually the first choice in antimicrobial studies, thus papers on antimicrobial screening are usually sought after [16]. Some screening studies in which several Nigerian medicinal plants were studied are presented in Table 1. It is widely accepted that extracts having activities where MIC values are below 8 mg/ml are considered to possess some antimicrobial activity [16,130] and natural products with MIC values below 1 mg/ml are considered noteworthy [16,131,132]. Some publications reported antimicrobial activities of Nigerian medicinal plant extracts with MIC less than or equal to 1 mg/ml against selected organisms [42,49,51,53,54,58,71,103,104,114,121,133,134-148] [Table 1]. Inhibitory zone diameter (IZD) is measured in millimeter (mm) when DD or AWD assays is used [76,149,150]. Kudi *et al*. [150] reported that plant extracts exhibiting IZD of 6mm and above against a selected pathogen are considered to posses some antimicrobial activity while Udgire and Pathade [151] suggested IZD of 10 mm and above. Because many organisms are now exhibiting high resistance to most antimicrobials, this study proposes that plant extract exhibiting IZD greater than or equal to 10 mm against selected organisms should be considered to possess antimicrobial activity whereas those showing IZD ≥20 mm against selected organisms are considered noteworthy. Some papers [32,101,121,145,152-159] on Nigerian plant extract reported IZD ≥20 mm.

Some publications were dedicated on study of specific genus of Nigerian plant such as *Pterocarpus* spp. [142], *Eucalyptus* spp. [69], and *Amaranthus* spp. [106]. Some Nigerian plant genus whose antimicrobial activity has been investigated by several authors include *Stachytarpheta* spp. [66,69,85,133], *Euphorbia* spp. [96,160-162], *Ocimum* spp. [97,142,105,163-168], *Terminalia* spp. [49,92,111,112,143,165,169-173], *Cassia* spp. [103,114,151,174-177], and *Allium* spp. [57,102,104,178-180]. Some specific species have been investigated by several researchers such as *Vernonia amygdalina* [86,94,97,105,141,179,181,182], *Psidium guajava* [87,121,163,183], *Jatropha curcas* [88,138,184], *Crinum jagus* [59,118,127], *Moringa oleifera* [147,185,186], *Anogeissus leiocarpus* [50,111,171,138,153,167,186-189], *Gongronema latifolium* [128,136], and *Fagara* (*Zanthoxylum*) *zanthoxyloides* [39,190] while some papers were dedicated to the study of specific species such as *Terminalia avicennioides* [58,71,117], *Stachytarpheta angustifolia* [69,70], and *Struchium sparganophora* [191,192].

Geographical and seasonal variations have been reported to affect the phytochemical constituents of plant and this in turn affects the result of antimicrobial activity [16,61]. No study has been conducted to assess the effect of seasonal change on Nigerian medicinal plants. South African studies reported little antimicrobial variability of plant extracts within 2 months interval [16,193]. Some papers on Nigerian plants focused on the antimicrobial screening together with other pharmacological investigations including toxicity [18,42,54,60,85,95,120,191,194,195]. Although medicinal plants are considered generally safe, they are known to contain potentially toxic, mutagenic, and/or carcinogenic substances [16]; therefore, it has been recommended that pharmacological studies should always be accompanied by toxicology screening [16,196,197]. Several papers included additional pharmacological assays to complement the antimicrobial investigations, such as the study on Nigerian *Stachytarpheta* spp. which includes antispasmodic properties [66,85]. Some authors investigated wound healing properties of plants together with antimicrobial property [18,59,60,141,155,198] while some studies reported antioxidant and antimicrobial properties together [48,59,60,99,199-203]. Ajali and Okoye [194] reported the anti-inflammatory and antimicrobial properties of *Olax viridis* and MIC assay showed considerable broad-spectrum activity (MIC 2000-4000 μg/ml) against selected pathogens. Williams *et al*. [204] reported antidiarrheal and antibacterial properties of *Guiera senegalensis* and MIC assay showed broad-spectrum activity (MIC 3.13 mg/ml) against selected pathogens. Kasim *et al*. [191] reported antitumor (using cell lines) and antimicrobial properties of *Struchium sparganophora* and MIC assay showed considerable activity (MIC 6.25 mg/ml) against *P. aeruginosa* and *C. albicans*. Some studies were dedicated to investigation of antimicrobial activity of Nigerian plants against multidrug resistant organisms such as methicillin-resistant *S. aureus* (MRSA) [49,124,147,187,205] and extended-spectrum β-lactamase (ESBLs)-producing organisms [206]. To ensure that resistant isolates were used, some investigators went

further to confirm resistance in isolates using disc diffusion assay [65,119,162,181,182,207].

It was observed that not much have been done on subterranean Nigerian plants. Few studies investigated the antimicrobial activity of subterranean plants such as *Allium* species [57,102,104,178-180] [Table 1] and *Dioscorea bulbifera* (Dioscoreaceae) which neither methanol nor ethanol tuber extracts showed promising antimicrobial activity (MIC 25 mg/ml against *S. aureus* and *E. coli*) [89]. This study recommends that further anti-infective studies be undertaken on medicinal bulbous plants of Nigeria. It was also observed that the majority of researchers on Nigerian plants investigated antimicrobial activity of leaf of selected plants whereas plant roots were barely studied [Tables 1 and 2]. The reason for avoidance of studies using root may be due to the destructive harvesting nature [16]. Elsewhere, it was shown that root and shoot of plants may exhibit similar antimicrobial activities [16,208]. Higher concentrations of secondary metabolites (alkaloids, tannis, flavonoids, saponins, etc.,) that are responsible for the antimicrobial activity of plants occur in bark, heartwood, roots, branch bases, and wound tissues [45,61]. The concentration varies from one plant species to another and from season to season and environment [61,209]. The mechanisms of antimicrobial activities of these metabolites have been extensively reviewed [24,45,210].

Antimicrobial Synergistic Activity of Nigerian Medicinal Plants

Because of the slow pace in development of new antimicrobials, there has been renewed interest in plants that have potential of increasing the effect of available standard (allopathic) antimicrobials. Time-kill kinetics showed that combining plant products together with allopathic antimicrobial drugs is effective in treating multidrug resistant infections [211]. In addition to antimicrobial activity of Nigerian plant extracts, some studies also investigated antimicrobial synergistic effect of these extracts with standard drugs [72,161,212,213] or antimicrobial synergistic effect of extracts from different plants [102]. Some of the studies used the agar diffusion checkerboard (ADCB) method in which the fractional inhibitory concentration (FIC) of the substances is determined by dividing the MIC of each of the substance in combination by the MIC of the substance alone, summation of the FICs gives the FIC index which is then used to classify the effect of the combination ratio as additive, synergistic, antagonistic or indifferent [72,75,76,214]. The overlay inoculum susceptibility disc (OISD) method involves measuring the IZD when antibiotic agar base containing sub-inhibitory concentration of the extract is overlayed with inoculated agar on which standard antibiotic disc is placed, percentage difference of IZD of the test in comparison with the control is then used to classify the effect of the extract as additive, synergistic, indifferent or antagonistic [72,214].

By ADCB method, methanol leaf extract of *Euphorbia hirta* exhibited synergistic effect in combination with nystatin (ratio, 6-9:4-1) against *C. albicans* [160]. Methanol leaf extracts of *Phyllanthus muellerianus* exhibited synergistic effect with ciprofloxacin against *Pseudomonas aeruginosa* and *Proteus mirabilis* using ADCB and OISD methods, respectively [72]. Using OISD method, Nweze and Onyishi [212] demonstrated that ethanol and methanol fruit extracts of *Xylopia aethiopica* exhibited synergistic effect in combination with different antimicrobials (ciprofloxacin, ofloxacin, gentamicin, fluconazole, and ketoconazole) against various organisms including *P. aeruginosa*, *E. coli*, *B. subtilis*, *S. aureus*, *C. albicans*, and *Aspergillus flavus*. The major concern surrounding the combined use of plant extracts and standard drugs is the toxicity effect that could result from interaction of phytochemicals with the active principle(s) in the drug [61,212]. Therefore, there is need for *in vivo* interaction and toxicity studies on Nigerian plants that showed potential antimicrobial synergism with standard drugs.

Antimicrobial Investigation on Essential Oils of Nigerian Medicinal Plants

Essential oils are volatile oils from aromatic plants responsible for the characteristic scent, odor or smell they exude [45]. Essential oil is found in the volatile steam distillation fraction of plants [45]. Several endemic Nigerian plants belonging to the family Lamiaceae, Myrtaceae, Compositae, Asteraceae, Liliaceae, and others are rich in essential oils [16,45]. Phytochemical screening of plants in these families in many publications [Tables 1 and 2], in this review, revealed the presence of terpenoid essential oils both mono and sesquiterpenes. Some studies investigated antimicrobial activity of essential oils of Nigerian plants in different families [32,52,69,128,179,192,215-221]. It was observed that studies on geographical and seasonal variation have not been undertaken on essential oil of selected aromatic Nigerian plants. There is need to conduct these studies because they are important for potential commercialization [16]. Reporting the chemical composition of essential oil (both quantitative and qualitative) together with the antimicrobial activity could give some indication as to how the climatic or geographical factors may influence the phytochemicals and their resultant biological activity [16]. It has been reported that with antimicrobial studies, the chemical composition should ideally be used to correlate any structure activity relationships [16]. Studies on antimicrobial activity of Nigerian plant oils have indicated that the correlation between chemical structure and biological activity are integrated and the essential oil chemistry has provided insight into the antimicrobial activity [16,128,192]. Isolation of the bioactive compound β-caryophyllene from essential oil obtained from *Gongronema latifolium* and *Struchium sparganophora* [128,192] are attributes that enable the plant oils to elicit antimicrobial activity. Nonetheless, it should be noted that bioactive compounds from essential oil cannot independently and in combination be responsible for the overall activity of the plant [16,222].

There has been lack of set criteria in the literature by which essential oil is classified as having good, moderate or poor activity, and many researchers base the assessment on their own

Table 2: Antimicrobial compounds isolated from Nigerian traditional medicinal plants

Plant	Plant part used	Solvent	Compound	Antimicrobial activity			Reference
				Bioactivity	Highest activity	Range	
P. crassipes K. Schum (Rubiaceae)	Leaf	Aqueous (hot)	Quercetin-3-O-rutinoside	Antibacterial	*E. coli P. aeruginosa* and *C. ulcerans* *S. pyogenes, K. pneumoniae* and *N. gonorrhoeae*	6.25 mg/ml 6.25-12.5 mg/ml	[224]
T. avicennioides Guill. & Perr. (Combretaceae)	Root bark	Petroleum ether, ethyl acetate, chloroform, methanol	Friedelin	Antimycobacterial	Bacille Calmette Guerin (*M. bovis*) antigen	4.9 μg/ml	[117]
		Hexane, ethyl acetate, chloroform, methanol	Arjunolic acid			156 μg/ml	[71]
G. latifolium (Benth.) (Asclepiadaceae)	Leaf	Hydroditillation (essential oil)	β-caryophyllene	Antibacterial	*E. coli*	39 μg/ml	[128]
C. alata Linn. (Leguminosae)	Seed	Ethanol	4-butylamine 10-methyl-6-hydroxy cannabinoid dronabinol	Antibacterial and antifungal (yeast and mold)	*P. aeruginosa* *S. aureus, E. coli, K. pneumoniae, A. niger* and *C. albicans*	6.25 mg/ml 12.5-50 mg/ml	[177]
S. mombin Linn. (Anacardaciae)	Stem bark	Methanol	Mombitanes I and II	Antimycobacterial	*M. tuberculosis*	40 μg/ml	[122]
F. zanthoxyloides (Lam.) Zipern and Timler. (Rutaceae)	Root	Petroleum ether, chloroform, ethanol, aqueous	Canthine-6-one, chelerythrine and berberine	Antibacterial			[39]
S. sparganophora Linn. Ktze (Asteraceae)	Aerial part (essential oil from stem and leaf)	Hydrodistillation	β-caryophyllene, germacrene D, a-humulene, caryophyllene oxide and 1,8-cineole	Antibacterial	*S. typhi* *P. aeruginosa, Proteus mirabilis, B. cereus* and *B. subtilis*	0.1 mg/ml 0.1-1 mg/ml	[192]
	Stem and root	Hexane, chloroform, methanol	Vernodalin Luteolin 3 methyl 2,6, hexacosedienol	Antibacterial Antibacterial and antifungal (yeast and mold)	*K. pneumonia* *K. aerogenes* *K. aerogenes, E. coli, C. albicans,* and *A. niger*	25 μg/ml 6.25 μg/ml 50 μg/ml	[191]
R. communis Linn. (Euphorbiaceae)	Seed	Hexane	Cineole Limonene	Antibacterial	*S. aureus* Broad spectrum, mold and yeast	6.25 mg/ml 12.5-25 mg/ml	[207]
C. nigricans Vahl. (Leguminosae)	Leaf	Petroleum ether, methanol	Hydroxyestranic acid ethy ester	Antibacterial	S. pyogenes *P. aeruginosa, C. albicans, N. gonorrhoeae* and *S. typhi*	2000 μg/ml 3000 μg/ml	[114]
P. guineense Schumach. and Thonn. (Piperaceae)	Fruit	Hydrodistillation	Myristicin	Antibacterial	*P. aeruginosa*	5 mg/ml	[215]
J. gossypifolia Linn. (Euphorbiaceae)	Seed	Methanol	9-acetoxynerolidol (from chloroform partition)	Antifungal	*C. albicans*	5 mg/ml	[68]
I. secundiflora Poir. (Fabaceae)	Aerial	Methanol, acetone	Quercetin 3, 3′, 4′-trimethyl ether	Antibacterial	*S. aureus, Bacillus subtilis, E. coli* and *P. aeruginosa*	200 μg/ml	[116]
L. pterodonta (DC.) Sch. Bip. (Asteraceae)	Aerial part (stem and leaf)	Hexane, ethyl acetate	Triacontyl methyl ether	Antibacterial	*K. pneumoniae, K. ozonae* and *B. cereus*	50 μg/ml	[125]
			Di-eicosanyl glycol or ethane-1,2-dieicosanoate		*K. pneumoniae, B. cereus, B. subtilis, S. aureus* and *K. ozonae*	50 μg/ml	

(Contd...)

Table 2: *(Continued)*

Plant	Plant part used	Solvent	Compound	Antimicrobial activity			Reference
				Bioactivity	Highest activity	Range	
			Eicosanoic acid		*K. pneumoniae, K. ozaenae, B. subtilis, B. cereus, S. aureus, E. coli, S. faecalis* and *S. dysentariae*	50-100 μg/ml	
			Ethane-1,2-di-eicosenoate		*K. pneumoniae, S. aureus, B. cereus, K. pnuemoniae* and *K. ozaenae*	50 μg/ml	
			2-triacontoxyethyleicosanoate		*S. aureus, B. subtilis, B. cereus, K. pneumoniae* and *K. ozaenae*	50-100 μg/ml	
		Hexane, ethyl acetate	Taraxasteryl aceteate Ethane-1,2-dieicosanoate	Antimycobacterial	*M. tuberculosis*	691.48 μm 269.23 μm	[126]
		Hexane, ethyl acetate, methanol	Pterodiondol (5βH, 7βH, 10β-epi-cryptomeridiol) Stigmasterol Stigmasteryl-3β-O-D-glucopyranoside	Antibacterial	*S. aureus, K. pneumoniae, K. ozaenae, B. cereus* and *E. coli*	50 μg/ml 50-100 μg/ml 50-100 μg/ml	[123]
B. pinnatum (Lam) Oken. (Crussalaceae)	Aerial part	Aqueous: Ethanol	Gallic acid Luteolin and epigallocatechin 3-O-syringate	Antibacterial	*S. aureus* *S. aureus*	25 μg/ml >400 μg/ml	[263]
C. prostrata (Linn.) Blume (Amaranthaceae)	Aerial part	Ethyl acetate	Ethyl hexadeacanoate and 7, 9-Di-tert-butyl-1-oxaspiro (4, 5) deca-6, 9-diene-2, 8-dione	Antimicrobial	*B. subtilis, S. aureus, E. coli, A. niger, P. aeruginosa, C. albicans*	1-15 mg/ml	[264]

E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, C. ulcerans: Candida ulcerans, S. pyogenes: Streptococcus pyogenes, K. pneumoniae: Klebsiella pneumoniae, N. gonorrhoeae: Neisseria gonorrhoeae, M. bovis: Mycobacterium bovis, S. aureus: Staphylococcus aureus, A. niger: Aspergillus niger, C. albicans: Candida albicans, M. tuberculosis: Mycobacterium tuberculosis, S. typhi: Salmonella typhi, P. mirabilis: Proteus mirabilis, N. gonorrhoeae: Neisseria gonorrhoeae, S. faecalis: Streptococcus faecalis, C. alata: Cassia alata, S. mombin: Spondias mombin, F. zanthoxyloides: Fagara zanthoxyloides, S. sparganophora: Struchium sparganophora, R. communis: Ricinus communis, C. nigricans: Cassia nigricans, J. gossypifolia: Jatropha gossypifolia, I. secundiflora: Indigofera secundiflora, L. pterodonta: Laggera pterodonta, B. pinnatum: Bryophyllum pinnatum, C. prostrata: Cyathula prostrata

particular data attained [16]. Considering that essential oils have lower antimicrobial activities than extracts, they need to be classified differently [16]. It has been proposed that essential oils with MIC value less than or equal to 2mg/ml could be considered noteworthy [16]. Publications on Nigerian medicinal aromatic plant essential oils which reported antimicrobial activities MIC of less than or equal to 2mg/ml against selected pathogens include studies on *Ocimum gratissimum* (Lamiaceae) [223], *Gongronema latifolium* (Asclepiadaceae) [128], *Allium sativum* (Liliaceae), and *Citrus reticulata* (Rutaceae) [179]. There is need for studies on time-kill kinetics of essential oils of Nigerian medicinal plants to be undertaken to elucidate their microbicidal effect.

Bioactive Compounds from Nigerian Medicinal Plants

Different bioactive compounds with antimicrobial properties have been isolated from various Nigerian medicinal plants [Table 2]. This is made possible due to engagement of multidisciplinary approach (by collaboration between chemists, botanists, and microbiologists) which is integral to achieve high-quality research [16]. It has been proposed that isolated compounds with antimicrobial activities of 64–100 μg/ml are accepted as having clinical relevance [16,131]. Some authors specify that compounds with activities less than or equal to 10 μg/ml are noteworthy [16,132]. Reports on isolated bioactive compounds from Nigerian plants exhibiting MIC values less than or equal to 10 μg/ml against selected organisms include Bello *et al*. [224], Mann *et al*. [117], and Kasim *et al*. [191] [Table 2]. Despite the availability of a number of publications on the isolation and identification of bioactive compounds from Nigerian plants, it should be noted that the complexity of plants and a single compound may not be responsible for the observed activity but rather a combination of compounds (either major or minor) interacting in an additive or synergistic manner [16,225].

IN VIVO AND FORMULATION STUDIES ON NIGERIAN MEDICINAL PLANTS

Considering that traditional medicines are esteemed not only for their therapeutic value but also from a holistic administrative approach in which the plant is given to treat the patient on various levels, one must not forget that there may be other physiological effects on the body that act beyond the symptomatic treatment of the disease when studying traditional medicine [16]. Although many studies on Nigerian medicinal plants have identified specific plant species as having antimicrobial activity in an *in vitro* model, it is necessary to subject these plants to animal models and human subjects to determine their efficacy in metabolic environments [16]. Two studies [59,226] on antimicrobial activity of Nigerian plants focused on *in vivo* models. Mice injected intraperitoneally with *S. aureus* and then dosed orally with 25-200 mg/kg body weight of *Alchornea cordifolia* aqueous:ethanol leaf extract improved significantly when compared to the control [226]. Topical treatment of excision wound on rats contaminated with *S. aureus*, *B. subtilis*, *C. albicans* and *P. aeruginosa* with 5% and 10% *Crinum jagus* methanol bulb extract ointment, resulted in significant reduction in isolation rate of these organisms except *P. aeruginosa* [59]. However, these studies used solvents/solvent mixture that are not acceptable in traditional medicine, therefore, there is need to include aqueous extract in *in vivo* screening assays to mimic traditional use of plant material [16]. This will enable adequate assessment of the efficacy of plants as used in traditional medicine. Nevertheless, it has been reported that solvent-derived extracts exhibit more antimicrobial activities than the aqueous extracts [16,227], thus raising concern in terms of antimicrobial efficacy when the traditional method is applied [16]. Therefore, there is great need to translate the applied knowledge gained from intricate assays and make it meaningful to the ethnic people who rely on traditional medicine [16].

Establishing suitable formulations that retain the efficacy demonstrated in the *in vitro* screening procedures has been reported to be the next logical step in the investigation of the antimicrobial efficacy of plant extracts and essential oils [16]. Formulations could be in form of tinctures, concoctions, teas, ointments, capsulation or tablets. Some studies on Nigerian plants focused on ointment formulations of plant extract which is applied topically. Suara *et al*. [228] examined the potential application of methanol extract of *Plukenetia conophora* into cream formulation, the ointment showed highest activity MIC 1mg/ml against *Proteus mirabilis*. Azubuike *et al*. [229] also examined ointment formulation of *Azadirachta indica* and *Aloe barbadensis* which gave highest activity MIC 2.5 mg/ml against *B. subtilis*, *S. aureus* and *C. albicans*, and 2 mg/ml against *C. albicans*, respectively. Muinat *et al*. [230] examined ointment formulation of *Argemone mexicana*, AWD assay showed inhibitory effect of the formulation on *Trichophyton mentagrophyte*. Udegbunam *et al*. [18] examined *Pupalia lappacea* methanol leaf extract ointment formulation on surgical wounds in rats, wound healing parameters assessed as well as microbiological assay (culture) showed inhibitory effect of the ointment on common wound-contaminating pathogens. Soap and ointment formulated using extract from 4 Nigerian medicinal plants proved effective in management of skin infections in selected individuals [165]. Studies are needed on assessment of potential antimicrobial activity of Nigerian plants' essential oil formulations and plant extract formulations in other forms apart from cream ointment. Inclusion of studies assessing the feasibility and bioavailability/stability of the active ingredients of the formulations especially is important [16].

ACTIVITIES ATTRIBUTED TO THE SPECIFIC ETHNOBOTANY OF THE PLANT

It was observed in this review that the majority of plant directed antimicrobial studies focused on screening against a battery of pathogens, while very few ethnobotanical studies have been carried out on pathogen-specific infections where the selection of test organisms relate directly to the traditional use of the plant [16]. For adequate ethnobotanical study, it has been suggested that studies on plants should be done using organisms related to the diseases managed

with the plant in traditional medicine [16,27,61,142]. For instance, plants used for managing diarrhea should be tested against *E. coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae*, *Campylobacter* spp., *Entamoeba histolytica*, etc., which are known to be associated with enteritis [231]. Plants utilized for skin infection should be tested against bacteria such as *Pseudomonas* spp. and *S. aureus* and fungal agents associated with skin diseases including *Epidermophyton* spp., *Trichophyton* spp., and *Microsporum* spp. [16,231]. Plants used for oral complaints should be tested against *Streptococcus mutans*, *Streptococcus sobrinus*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans* [16,232]. Although, studies relating to ethnobotanical use of Nigerian plants have been largely neglected (in terms of using pathogens targeted in ethnomedical setting), few studies focused on ethnobotanical use of studied plant on wound healing [18,60,142] and oral infection [42,133,169,170,233] taking into account the possible impact of microbial infection.

It was observed that few publications [135,168,204] have been dedicated to the antidiarrheal properties of Nigerian plants, and given the severity and mortality rates of diarrheal-related diseases especially in rural areas, not enough has been done on one of the most prevalent diseases affecting rural Nigerians [16]. Omojasola and Awe [135] studied 2 plants (*Anacardium occidentale* and *Gossypium hirsutum*) used traditionally to treat stomach ailments in Southwest Nigeria and reported MIC 0.05-0.10 mg/ml of their ethanol leaf extract against *S. aureus*, *E. coli*, *S. dysenteriae*, *Salmonella* spp., and *P. aeruginosa*.

Candida albicans is an organism (yeast) responsible for infections which are often prolific, requiring long-term antifungal treatment [16,233]. About 120 Nigerian medicinal plants were screened for anticandidal activity [Table 1]. Reference, clinical and nonclinical *C. albicans* strains were used in the studies. The MIC of plant extract against the organism varied depending on the plant, solvent used and the strain of *C. albicans* used [16]. Nigerian plant extracts with the most promising anticandidal activity include *Sesame radiatum* (methanol leaf extract, MIC 28.2 μg/ml) [138], *Amaranthus hybridus* (aqueous leaf extract, MIC 6.33 mg/ml) [106], *Pterocarpus santalinoides* (ethanol fresh leaf extract, MIC 0.75 mg/ml) [142], *Balanites aegyptiaca* (aqueous leaf and root extract, MIC 3.125 mg/ml) [234], *Eucalyptus camaldulensis* (aqueous:methanol leaf extract, MIC 0.0625 μg/ml) [112], *Commiphora africana* (ethanol root extract, MIC 2,000 μg/ml) [65], *Cymbopogon citratus* (chloroform leaf extract, MIC 32 μg/ml) [119], and *Cyathula prostrata* (ethanol leaf and stem extract, MIC 400 μg/ml) [154]. Kubmarawa *et al*. [137] reported anticandidal activity of ethanol extract from various parts of *Acacia tortilis*, *Anogeissus leiocarpus*, *Jatropha curcas*, *Nauclea latifolia*, and *Vitex doniana* with MIC ranging from 0.5 to 2 mg/ml. There is need for further clinical assessment of these Nigerian plant extracts with considerable anticandidal activity.

Some publications have been dedicated to investigation of antimycobacterial activity of Nigerian plant extract [54,55,57,118,122,125,189,235,236] [Tables 1 and 2]. Screening of antimycobacterial activity of 12 medicinal plants used in treating tuberculosis in Southwest Nigerian revealed that both methanol and ethanol extract of *Allium ascalonicum*, *Terminalia glaucescens*, *Allium cepa*, and ethanol extract of *Securidaca longepedunculata* inhibited *M. tuberculosis* at concentration of 0.05 g/ml [235]. On comparison with Bacille Calmette Guerin (BCG) (attenuated *Mycobacterium bovis*) antigen, 10 Nigerian medicinal plants used in herbal antitubercular recipes by traditional healers, showed potential antimycobacterial activity following preliminary MIC assay [189]. Hexane extract of various parts of *T. avicennioides* and *Anogeissus leiocarpus* exhibited activity (MIC 312 μg/ml) against *M. tuberculosis* and BCG antigen [236]. Chloroform root bark extract of *Uvaria afzelii* (Annonaceae) and hexane root bark extract of *Tetracera alnifolia* (Dilleniaceae) exhibited activity MIC 87.5 μg/ml and 93.31 μg/ml against *M. tuberculosis*, respectively [54]. Screening of aqueous:ethanol extract from various parts of 11 plants used for treating tuberculosis in the Northcentral region of Nigeria, showed that leaf/twist extract of *Pavetta corymbosa* exhibited the highest activity (MIC 800 μg/ml) against *M. tuberculosis* [55]. Methanol stem bark extract of *Spondias mombin* exhibited activity (MIC 61.1 μg/ml) against *M. tuberculosis* [122]. Bioactive compounds with potential antimycobacterial activity have been isolated from Nigerian medicinal plants [117,126] [Table 2]. The use of BCG antigen in conducting antimycobacterial test by some investigators may be possibly due to the difficulty (long incubation time, requirement for specialized media, and biosafety cabinet environment to avoid exposure) associated with culturing *M. tuberculosis*. Considering the zoonotic importance and the difficulty (long-term therapy and resistance - multidrug resistant tuberculosis [MDR TB] and extremely-drug-resistant TB [XDR TB]) in treating mycobacterial infections, there is need for more investigations on Nigerian plants for detection of potential antimycobacterial agent. Currently, there is awakened interest in search of new, safe, and effective antitubercular drugs globally [237].

Although Nigerian medicinal plants used in treatment of sexually-transmitted infections (STIs) showed antimicrobial activity [66,69,70,72,79,95,174,238,239], none of the studies used organisms such as *Neisseria gonorrhoeae*, *Treponema pallidum*, *Haemophilus ducreyi*, *Trichomonas vaginalis*, *Ureaplasma urealyticum*, and *Oligella urealytica* which are known to cause STIs [16]. Understandably, these organisms are fastidious and require more intensive culturing techniques, but their use is a necessary progression for further antimicrobial pharmacognosy studies since it can no longer be assumed that broad-spectrum activity is adequate for plants used for specific diseases [16].

STUDIES ON NIGERIAN MEDICINAL PLANTS BASED ON LOCALITY

A few antimicrobial-related papers have focused on the ethnobotany of specific geographical regions within Nigeria [92,117]. These studies investigated the antimicrobial activity of *Terminalia aviccenoides* commonly used in the Nupe

traditional medicine for treating microbial infections, with the root bark extract of *T. avicennioides* yielding Friedelin with strong antimycobacterail activity (MIC 4.9 ug/ml) [Table 2]. Some studies investigated antimicrobial activity of plants used as chewing stick for maintaining oral hygiene within Southwest Nigeria [39,42,124,133,169,170,187,188,190,218,233,240].

ANTIMICROBIAL STUDIES ON NONFLOWERING AND PARASITIC NIGERIAN PLANTS

Two studies reported the antimicrobial activity of the fungus *Ganoderma* (Ganodermataceae) [241,242]. Ofordile *et al*. [241] screened four *Ganoderma* species in which *G. colossum* showed potential antimicrobial activity, further studies on this species resulted in isolation of Colossolactone E and 23-hydroxycolossolactone E which inhibited the growth of *B. subtilis* and *Pseudomonas syringae* [241]. Extracts of bryophytes *Calymperes erosum* (Calymperaceae) and *Bryum coronatum* (Bryaceae) exhibited broad-spectrum activity against selected Gram-positive and Gram-negative organisms with both plants having MIC 0.625 - 5 mg/ml [145] [Table 1]. Screening of the parasitic plant *Viscum album* (Loranthaceae) by diffusion method showed no promising antimicrobial activity [98,243].

FUTURE ANTIMICROBIAL RESEARCH ON NIGERIAN MEDICINAL PLANTS

There are some considerations that investigators of Nigerian plants need to adopt for adequate validation of antimicrobial activity of these plants. Van Vuuren [16] highlighted these considerations: Methods should be standardized and only noteworthy activities considered for publication, disc diffusion assays should be avoided especially when considering essential oil studies, isolation of bioactive compounds should be directed to plants having antimicrobial activity as identified in the screening procedures, attention needs to be directed toward the ethnobotanical use of the plant and the rational antimicrobial screening that follows, the use of nonpathogenic organism such as *Bacillus subtilis* for anti-infectivity studies should be avoided, studies on the efficacy of plants against resident beneficial bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* could yield information that may make plant extracts or oils more appropriate than the presently administered allopathic antimicrobials, which inevitably destroy the beneficial commensal organisms together with the invading pathogens. Moreover, the majority of investigators on antimicrobial activity of Nigerian plants used isolates that were only identified to generic level. This may be due to limited facilities (such as molecular laboratories) for adequate identification of organisms to species level. However, for reproducibility of studies, there is need for the use of adequately characterized organisms in antimicrobial screening studies.

Because the identification of a single active chemical entity responsible for the antimicrobial activity of a plant is less likely, research should be focusing on the investigation of a combination of compounds to achieve a greater efficacy [16]. Incorporation of interactive phytochemical studies with existing practices is crucial in the search for novel chemotherapeutic agents [16]. Regular or consistent administration of antimicrobials to determine if enhanced efficacy would be achieved with regular subtherapeutic administrations in comparison with single acute administrations should be considered in future research designs [16,244,245]. Extended time-kill experiments monitoring the viability of plant extracts overtime with regular dosages is crucial in determining the cidal effect of an antimicrobial [16].

CONCLUSION

The reviewed reports showed that there is a progressive trend in studies on antimicrobial activity of Nigerian medicinal plants. With advancement in laboratory techniques coupled with renewed interest in the field and the scientific validation of the traditional uses of medicinal plants, traditional medicine is increasingly been recognized as an accepted alternate regimen to orthodox health-care systems [16]. It is clear that many endemic Nigerian plants used in treating microbial infections and various ailments in traditional medicine, potentially possess antimicrobial activity. Bioactive compounds with antimicrobial activity have been isolated from Nigerian plants in different families. With further researches, new chemotherapeutic agents could possibly be developed from these plants.

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