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Antimicrobial Screening and Therapeutic Potentials of Crude Extracts of Plants Used as Anti-Malarial Remedies in Ibo-Nigeria Folkloric Medicine

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Abstract
An antimicrobial agent is a substance that kills microorganisms or inhibits their growth. The use of substances with antimicrobial properties is known to have been a common practice for at least 2000 years. Ancient Egyptians and Greeks used specific moulds and plant extracts to treat infections. More recently, microbiologists such as Alexander Fleming, Louis Pasture and Jules Francois Joubert have discovered and developed various antimicrobial agents which have been used to treat so many infections that were hitherto untreatable and fatal to mankind. From these initial antimicrobial agents numerous other antimicrobial agents have been derived and used to treat a wide range of infections.Antimalarial agents are amongst the numerous therapeutic agents that abound in plants and herbs. Plants and herbs with antimalarial properties have been successfully employed in the treatment of malaria and other related ailments. It has also been observed that many antimalarial agents possess significant antimicrobial activities and an effort to corroborate this observation is attempted here. The study was designed to evaluate the antimicrobial properties of plants used as anti - malarial remedies in Ibo-Nigerian folkloric medicine. Twenty (20) plants used as anti-malarial remedies in Ibo traditional medicine including Monodora myristica were sun-dried, milled and extracted by cold maceration with 95% methanol. Penicillin G, chloramphenicol and nystatin solution were used as controls for the screening. These solutions together with the controls were standardized to 10mg/ml solution in dimethy sulphoxide solutions. The standardized solutions of the extracts including the controls were then evaluated for anti-microbial activity against some gram-positive bacteria (B. subtilis, Staph aureus), gram-negative bacteria (Klebsiella, Ps. Aeruguosa, S. typhi, E. coli), the yeast (C.albicans) and the mould (Aspergillus niger) using the agar ditch method. The results show that P. guajava (leaves) and M. myristica (seeds) were the most active against B. subtitis. M. myristica (seed) was more active against K. pneumoniae than the control; no other plant extract had significant activity against this organism. None of the extracts was active against S. aureus. Extracts of C. ferruginea (leaves and stem bark), N. latifolia (leaves), N. laeris (leaves) and E. globululus (leaves) were active against S. typhi. C. ferruginea (leaves and stem bark), N. latifolia (leaves), K. senegalesis (leaves) and E. globules (stem bark), P. guajava (leave) and M. myristica (seeds) were active against E. coli with M. myrstica (the most active) being more active than the control. None of the plants extracts was active against C. albicans. Only C. ferrugnea (stem bark) and M. myristica (seeds) were active against A.niger. Monodora myristica (seed extract) was generally the most active and most interesting of all the twenty (20) plant extracts screened. The study concluded that many plants used as anti - malarial remedies in Ibo - Nigeria folkloric medicine possess significant anti - microbial properties against various micro – organisms, the most active being Monodora myristca (seed), followed by Psidium guajava (leaves), and Pterocarpus santalanoides (stem bark).
Keywords: Antimicrobial properties, Anti - malarial remedies, Preliminary screening, Folkloric medicine, Ibo - Nigeria.

1. INTRODUCTION

From time immemorial mankind has benefited immensely from the healing potentials that abound in the different parts of many plants existing in nature. This is not surprising as man and plant are closely biological akin, the life-blood of the plant, its green chlorophyll, has a chemical structure almost identical with the hemoglobin which is the central constituent of the human blood; where chlorophyll has a molecule of magnesium in its central pattern, hemoglobin carries a molecule of iron (Griggs, 1981). Folkloric or traditional medicine is recognized by the WHO as a legitimate primary healthcare delivery system and along with other international bodies has consistently supported its practice in many ways (Nwaogu, 1997). However its practice has been widely criticized due to its inadequate use of modern scientific methods and the involvement of untrained persons in the practice as well as the involvement of rustic medicine men that sometimes associated the practice with various facets of idol worship (Nwaogu, 1997). The benefits of folkloric medicine nevertheless cannot be overemphasized and includes its affordability, effectiveness, efficacy, rare incidence of side / adverse effects and potential for economic improvement through healthcare budget reduction, just to mention but a few.

Little wonder then many industrialized countries of the world like Germany, Russia, China, India, France, America, Japan, etc have not only recovered the considerable healing powers of natural herbs but have also integrated herbal medicine into their medical practice (Nwaogu, 1997).

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials. These consisted of the various parts of twenty (20) plants claimed to possess anti - malarial properties in Ibo – Nigeria folkloric medicine. These plant parts were collected in September at Nsukka in Enugu state of Nigeria with the help of Botanists at the botany department of University of Nigeria, Nsukka. They were then prepared by cutting, sun – drying and milling. The powdered forms were then used in the experiments.

2.1.2. Reagents. Sulphoric acid, Chloroform, Ammonia solution, Ferric chloride, Fehling's solution 1 and 2, Ethylacetate, Hydrochloric acid, Glacial acetic acid, Aluminium chloride, Ethanol, Bromine water, Mayer's reagent, Distilled water, Sodium hydroxide, Tollen's reagent, 2,4 – dinitrophenylhydrazine, Acetic anhydride, acetic acid, silica gel GF_{254}.

2.1.3. Solvents. Methanol, Ethylacetate, Methylethylketone (MEK), MEK / Hexane, Dimethyl sulphoxide (DMSO), Chloroform and Ethanol.

2.1.4. Instrumentation. Separating funnel, Evaporating dish, Rotary evaporator, Water bath, Capillary tubes, Test tubes, Conical flasks, Measuring cylinders, Beakers, Pipettes, Funnels, Filter papers, Weighing balances, Bunsen burner and Spatula.

2.1.5. Microbiological materials. Broth cultures of test organisms [gram-positive bacteria (B. subtilis, Staph aureus), gram-negative bacteria (Klebsiella, Ps. Aeruguosa, S. typhi, E. coli), the yeast (C.albicans) and the mould (Aspergillus niger)], Sterile Petri dishes, Sterile Cork borer, Sterile Forceps, Inoculation loop, Incubator, Autoclave, Indelible Marker, Lighter, Nutrient agar, Paper strip, Penicillin G, Chloramphenicol and Nystatin.

2.2. Method. Twenty (20) plants used as anti-malarial remedies in Ibo traditional medicine including Monodora myristica seeds were sun-dried, milled and extracted by cold maceration with 95% methanol. Penicillin G, chloramphenicol and nystatin were used as controls for the screening. These solutions together with the controls were standardized to 10mg/ml solution in dimethy sulphoxide solutions. The standardized solutions of the extracts including the controls were then evaluated for anti-microbial activity against some gram-positive bacteria (B. subtilis, Staph aureus), gram-negative bacteria (Klebsiella, Ps. Aeruguosa, S. typhi, E. coli), the yeast (C.albicans) and the mould (Aspergillus niger) using the agar ditch method.

3. RESULTS. These are given in table 1 and figure 1 below.

Table 1. Results of anti-microbial screening of crude extracts of Ibo-Nigerian anti-malarial plants

S.N	Plant	Part Use	Conc. (mg/ml)	B.sub	Kleb.a	Staph.a	S.typhi	Pseudo.a	E.coli	C.alb	A.niger
1	Crestis ferruginea	L	10	15	12	13	16	13	14	-	10
2	" "	S.T	10	13	11	11	12	13	17	-	25
3	Nuclear latifolia	L	10	14	13	14	12	12	13	-	8
4	" "	S.B	10	17	11	12	-	-	18	-	8
5	" "	R.B	10	18	11	11	-	12	14	-	12
6	Alstonia boone	S.B	10	13	11	12	-	10	12	-	11
7	Psidium guajava	L	10	18	11	16	-	17	21	-	15
8	Citrus agnensis	L	10	14	11	11	11	13	12	-	10
9	Newbouldia laeris	S.B	10	12	10	12	10	12	17	-	16
10	"	L	10	15	10	-	13	14	12	-	8
11	Carica papaya	L	10	13	11	-	-	12	7	-	12
12	"	FT unriped	10	15	12	16	-	11	11	13	15
13	Khaya senegalensis	L	10	15	8	-	-	12	14	12	15
14	"	S.B	10	12	9	8	-	11	15	14	12
15	Mangifera indica	R.B	10	14	-	7	9	8	17	13	-
16	Eucalyptus globulus	L	10	12	12	14	13	13	11	15	-
17	Quinine plant	S.B	10	18	-	12	-	12	13	-	14
18	Newbouldia laeris	RT	10	8	-	-	-	6	15	-	-
19	Pterocarpus santalonoides	S.B	10	20	15	17	15	12	18	18	22
20	Monodora myristica	S.D	10	28	30	-	-	-	32	17	21
21	PenicillineG	P.D	10	30	25	35	15	-	30	-	-
22	Chloramphenicol	P.D	10	30	21	31	12	12	25	-	-
23	Nystatin	P.D	10	-	7	-	-	13	12	43	35

Key: L = leaves, St = Stem, RB = Root bark, Ft = Fruit, SD = Seed, SB = Stem bark, PD = Pure drug, B. Sub = Bacillus subtillis, Kleb = Kelbsiella pneumoniae Staph. a = Staphylococcus aureus, S. typhi = Salmonella typhimurium, Pseudo. A = Pseudomonas

aeruginosa, E. coil = Eschericia coli, C. alb = Candida albicans, A. niger = Aspergilus niger.
The activities of the most active plants (Monodora myristica seed and Psidium guajava leaves) relative to those of the control drugs are shown in figure 1.

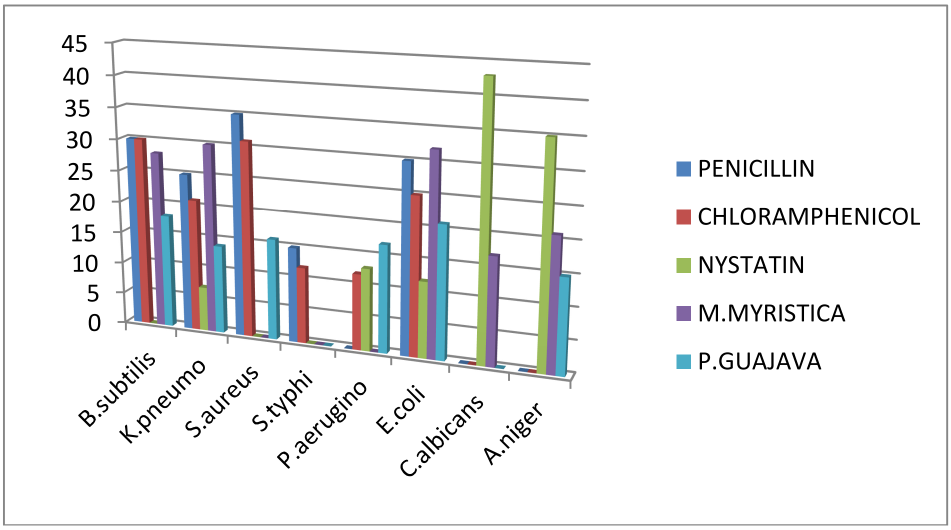

Figure 1. Bar chart showing the activities of the most active plants (Monodora myristica seed and Psidium guajava leaves) relative to those of the control drugs.

4. DISCUSSION

The foregoing study showed that Monodora myristica seeds and Psidium guajava leaves were generally the most active against the various organisms used for the study. Monodora showed activities that were comparable and sometimes greater than those of the controls. This result corroborates the rationale behind its variety of uses in Ibo-Nigerian folkloric medicine.

Against Bacillus subtilis, M. myristica (seed), P. guajava (leaves), N. latifolia (root bark and stem bark), Quinine plant (stem bark) and Pterocarpus santalanoides (stem bark) all showed significant antimicrobial activity. The activity of M. myristica was comparable to that of the control/pure drug. Bacillus organisms are free-living, gram-positive rods that are aerobic and spore-forming. Several of the species produce potent toxins that can be lethal in experimental animals. They are ubiquitous, being found in soil, water, dust and air. They are largely seen as non-pathogenic and as contaminants when isolated in the bacteriology laboratory but occasionally they can be responsible for significant disease. Infections with bacillus organisms have also been associated with intravenous drug abuse, operative procedures, traumatic wounds, burns, hemodialysis and prosthetic heart valves and are characteristically resistant to the penicillins and cephalosporins (Poretz, 1988). These situations may however benefit therapeutically from M. myristica seed and the other plants mentioned above that showed significant activity against B. subtilis.

Against Klebsiella pneumonia, Monodora gave an activity greater than that of the control. This is of very clinical importance considering the problem associated with the treatment of Klebsiella infections. Klebsiella pneumonia is a gram-negative bacilli, one of the few that causes primary lobar pneumonia, a non-motile and encapsulated organism and an important nosocomial pathogen accounting for up to 10% of hospital acquired infections (Silverblast & Weinstein, 1988). Multi-drug resistant strains have become endemic in many hospitals with the persistence of the organism associated with the continued use of large quantities of antibiotics and with the establishment of intestinal carriage among asymptomatic patients (Silverblast & Weinstein, 1988). Klebsiella is resistant to ampicillin, carbenicillin with the strain's resistance to cephalothin, chloramphenicol, tetracycline and gentamicin increasing in frequency probably due to the acquisition of multidrug-resistant R factors. However almost all strains of the organism remain sensitive to Amikacin which has been reserved for only gentamicin-resistant organisms (Silverblast & Weinstein, 1988). The high activity of Monodora against the Klebsiella organism could make it a potential source of alternative effective antibiotics for it.

None of the extracts showed significant activity against Staphyllocus aureus. S. aureus is a highly resistant gram-positive, non-spore forming bacteria with a high prevalence in both communities and hospitals. The high morbidity and mortality associated with it as well as the economic consequences and the virtual absence of a non-human reservoir makes the organism a major subject of epidemiologic studies (Waldvogel, 1988).

Against Salmonella typhi, C. ferruginea (leaves and stem bark), N.latifolia (leaves), N. laeris (leaves), E. globulus (leaves) all showed very significant activity. The activity of C. ferruginea was greater than that of the control. Salmonella typhi is the causative agent of typhoid fever (enteric fever). It is a non-spore forming gram-negative enterobacteria rod. Selection of antimicrobials for the treatment of Salmonella infections has been complicated by the emergence of Salmonella strains that are resistant to multiple antimicrobials (Hook, 1988). This resistance is transferred from organism to organism on plasmids that carry genetic determinants of resistance (R factors). These plants particularly C. ferruginea could be useful in these cases of multidrug resistant Salmonella infections.

Against Pseudomonas aeruginosa, P. guajava (leaves), C. ferruginea (leaves and stem bark), N. latifolia (leaves and root bark), Citrus guinensis (leaves), Newbouldia laeris (stem bark and root bark), Carica papaya (leaves), Khaya senegalensis (leaves), Eucalyptus globules (leaves), Quinine plant (stem bark), Pterocarpus santalanoides (stem bark) and Monodora myristica (seeds) all showed significant activity. P. guajava (leaves) however showed greater activity than the control. P. aeruginosa is a gram-negative aerobic and flagellated rod belonging to the family of pseudomonadeceae. It is cosmopolitan in distribution and is sometimes present as part of the normal microbial flora of man. It rarely causes disease in normal healthy persons despite being a common human saprophyte. However, disease process as a result of infection by it begins with some alteration or circumvention of normal host defenses which may involve a disruption in the integrity of physical barriers to bacteria invasion such as the skin or mucous membranes or their circumvention as in the case of intravenous lines, urinary catheters or endotrachael tubes (Pollack, 1988). The pathogenesis of the infection from this organism is multifactorial as suggested by the large number of potential virulence factors it produces and the broad spectrum of diseases it causes. The incidence and relative frequency of hospital acquired infections from it has also been reported to be on the rise (Pollack, 1988). These hospital acquired infections as well as the other infections caused by P. aeruginosa could therefore benefit from the high antimicrobial activity of P. guajava leaves in particular as well as those of the other plants above that showed significant activity against it.

Monodora myristica (seeds), P. guajava (leaves) and N. latifolia (stem back) all showed significant activity against Eschericia coli. The activity of M. myristica was greater than that of the control indicating its potential therapeutic usefulness in infections caused by E. coli like urinary tract infections (UTIs), bacteremia, neonatal meningitis, traveler’s diarrhea etc. E. coli belongs to the family of enterobacteriaceae which is a diverse group of gram-negative non-spore-forming bacilli, many of which are pathogenic to man, other animals and plants. They are aerobic but can grow under anaerobic conditions (facultative anaerobes). Many members of this group including E. coli possess plasmids which are extrachromosomal genetic elements on which genes expressing virulence properties are carried. Some of these plasmids called R factors encode for resistance to multiple antibiotics. Heavy use of antibiotics in hospitals favors the selection of R factor containing strains which might contribute to the increased antibiotic resistance of resident flora. Other R factor genes encode for the conjugal transfer of plasmids from organism to organisms even members of different species causing widespread outbreaks of nosocomial infections that have involved hundreds of patients, many institutions and several bacteria species. E. coli is the most common cause of urinary tract infections (UTIs), comprising more than 90% of infections arising outside the hospital (Sylverblatt & Weinstein, 1988). It is also the leading cause of gram-negative bacteremia in adults and treatment of these infections could benefit from the high antimicrobial activity of M. myristica seed as well as those of the other plants that showed significant antimicrobial activity against it.

Only Monodora myristica showed activity against Candida albicans. C. albicans is a fungi confined to human and animal sources. They are normal commensals of man and are found on diseased skin, enteric GIT, expectorated sputum, the female genital tract and urine of patients with indwelling foley catheters. Interestingly it rarely colonizes normal skin but damaged skin becomes rapidly colonized with C. albicans (Edwards, 1988). Incidence of diseases due to candida has increased in frequency over the last 50 years with a relatively large number of manifestations. These may therapeutically benefit from the significant antimicrobial activity of M. myristica seed. This strong antifungal activity of M. myristica shown in this study corroborates the results of other works that also demonstrated its antifungal and antibacterial properties (Chalchat et al, 1997) and its use in the the preservation of Okro and other crops (Ejechi et al, 1997).

Against Aspergillus niger, only Monodora myristica (seed) and C. ferruginea (stem bark) showed significant activity. A. niger is a mould that is ubiquitous in nature. It causes aspergillosis, a disease that describes an illness attributed to the antigenic stimulation, colonization or tissue invasion by aspergillus. The disease is acquired by inhalation of airborne spores (conidia) which are small enough (2.5 – 3.0 micrometer) to reach the alveoli or to gain entrance to paranasal sinuses with diverse clinical manifestations. Exposure to aspergillosis nearly universal but the disease is uncommon. When it occurs it could be invasive resulting in serious infections that may require surgical excision of infected body parts to contain the disease (e.g invasive aspergillosis of the brain and paranasal sinuses, non-invasive sinus colonization and possibly aspergillosis of prosthetic cardiac valve), which can aid response to chemotherapy, though prognosis in aspergillus endocarditis remains dreadful (Bennett, 1988). M. myristica seed and C. ferruginea stem bark could be therapeutically relevant in these conditions.

The study in summary has shown that many of the plants parts used as antimalarial remedies in Ibo-Nigeria folkloric medicine do possess significant antimicrobial properties. However further in-vivo and in-vitro tests may be needed to confirm these results.

Of utmost importance in this regard is M. myristica (seed), P. guajava (leaves) and Pterocarpus santalanoides (stem bark). M. myristica seed showed significant activities against Klebsiella pneumonia, P. aeruginosa, E. coli, C. albicans, B. subtilis and A. niger. Its activity was greater than that of the control against K. pneumonia and E. coli. P. guajava on its own was significantly active against B. subtilis, P. aeruginosa and E. coli. Its activity against P. aeruginosa was greater than that of the control. P. santalanoides was also significantly active against P. aeruginosa and B. subtilis. The researchers have also studied the phytochemical and antimicrobial properties of M. myristica fractions separated by preparative thin layer chromatography. These studies are also about to be published.

5. CONCLUSION

Many plants used as anti - malarial remedies in Ibo - Nigeria folkloric medicine possess significant anti - microbial properties against various micro – organisms, the most active being Monodora myristca (seed), followed by Psidium guajava (leaves), and Pterocarpus santalanoides (stem bark). Since most antimalarial agents exhibit antimicrobial activity, it is possible that many of these antimalarial herbs/plants could really posses antimalarial activity. However further in-vitro and in-vivo tests may be needed to confirm this assertion.

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REFERENCES

Bennett, J.E. (1988), "Aspergillus species", in Mandell, G.L., Douglas, R.G and Benneth, J.E (Eds), *Principles and practice of infectious diseases,* 2nd Edition, Churchill and Livingstone, New York, Edinburgh, London, Melbourn, pp. 1447 – 1451.

Edwards, J.E. (1988), "Candida species", in Mandell, G.L., Douglas, R.G and Benneth, J.E (Eds), *Principles and practice of infectious diseases,* 2nd Edition, Churchill and Livingstone, New York, Edinburgh, London, Melbourn, pp. 1435 – 1447.

Griggs, B. (1981), "The green pharmacy", in Jill Norman (Ed), *A history of herbal medicine*", Hobhouse, London, p. 293.

Hook, E.W. (1988), "Salmonella species (including typhoid fever)", in Mandell, G.L., Douglas, R.G and Benneth, J.E (Eds), *Principles and practice of infectious diseases,* 2nd Edition, Churchill and Livingstone, New York, Edinburgh, London, Melbourn, pp. 1256 – 1268.

Nwaogu, M.A. (1997), "Insight into herbal medicine", Whytem prints, Owerri, pp. i – iv, 1 -5.

Pollack, M. (1988), "Pseudomonas aeruginosa", in Mandell, G.L., Douglas, R.G and Benneth, J.E (Eds), *Principles and practice of infectious diseases,* 2nd Edition, Churchill and Livingstone, New York, Edinburgh, London, Melbourn, pp. 1236 – 1250.

Poretz, D.M. (1988), "Other bacillus species", in Mandell, G.L., Douglas, R.G and Benneth, J.E (Eds), *Principles and practice of infectious diseases,* 2nd Edition, Churchill and Livingstone, New York, Edinburgh, London, Melbourn, pp. 1184.

Silverblatt, F.J. and Weinstein, R. (1988), " Enterobacteriaceae", in Mandell, G.L., Douglas, R.G and Benneth, J.E (Eds), *Principles and practice of infectious diseases,* 2nd Edition, Churchill and Livingstone, New York, Edinburgh, London, Melbourn, pp. 1226 – 1236.

Waldvogel, F.A. (1988), "Staphyllococcus aureus (including toxic shock syndrome)", in Mandell, G.L., Douglas, R.G and Benneth, J.E (Eds), *Principles and practice of infectious diseases,* 2nd Edition, Churchill and Livingstone, New York, Edinburgh, London, Melbourn, pp. 1097 – 1116.

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