

EVALUATION OF THE ANTIMICROBIAL POTENTIALS OF 35 MEDICINAL PLANTS FROM NIGERIA.***Obiukwu, C. E and Nwanekwu, K. E.**

Department of Microbiology, Faculty of Science, Evan Enwerem University, Owerri, Imo State.

E-mail: aerohaccs@yahoo.com

ABSTRACT: Crude ethanol extracts of 35 plant species were screened *in-vitro* for their antimicrobial activities against Gram negative bacterial strains- *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and Gram positive bacterial strains- *Staphylococcus aureus* and *Streptococcus pyogenes* known to be pathogenic. The extracts showed stronger activity against the Gram positive organisms than the Gram negative bacteria which were more resistant. The results obtained showed that, 33(94.3%), of the plants species were active against *Staphylococcus aureus*, 28(80%) were active against *Streptococcus pyogenes* and 29(82.9%) were active against *Escherichia coli*. *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were also susceptible to 27(77.1%), 27(77.1%) and 22(62.9%) respectively of the plant species screened.

KEY WORDS: Medicinal plants, antimicrobial potentials, *In vitro*, pathogenic, Gram positive, Gram negative.

INTRODUCTION

Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species. Millions of rural households use medicinal plants in a self-help mode. Over one and a half million local medicine practitioners use medicinal plants in preventive and curative applications (Akinyemi *et al.*, 2005). In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded within and across the countries (Suresh *et al.*, 2008).

Plants are indeed the first source for preparing remedies in the form of alternative medicine. The search for plants with antibacterial activity has gained increasing importance in recent years due to the development of antimicrobial drug resistance and often the occurrence of undesirable side effects of some antibiotics (Soberon *et al.*, 2007). With the advent of ever increasing resistant bacterial strains, there has been a corresponding rise in the universal demand for natural antimicrobial therapeutics which may constitute a reservoir of new antimicrobial substances to be discovered (Akharaiyi *et al.*, 2010).

In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al.*, 2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections (Balandrin *et al.* 1985).

Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests (Suffredini *et al.*, 2004). The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant pathogenic bacteria and fungi.

In Nigeria as well as in most countries of sub-Saharan Africa, bacterial and fungal infections represent an increasing problem, particularly with patients suffering from severe immune deficiencies, such as AIDS (Hostettmann *et al.*, 2002). Moreover, the validation of

medicinal plant based therapy is imperative for African developing countries as more than 70% of the population use such remedies (Pousset, 1994).

However, studies on the biological, pharmacological, phytochemical analysis and some important information on traditional medicinal plants from Africa in general are still inadequate, under reported or lacking in some cases (Neuwinger, 1996). As traditional medicine is mostly the single therapeutic alternative, increasing knowledge of the plants used is an important issue and constitutes the aim of this study. Therefore, the antibacterial potential of 35 plants used in Nigerian traditional medicine has been evaluated. However, these studies discuss only a few of the available medicinal plants and many investigations still need to be done.

MATERIALS AND METHODS**Plant Materials**

Medicinal plants from Nigeria particularly the Southern part of the country were collected, identified and the voucher specimens deposited at the Imo State University Herbarium Nigeria. Roots, leaves, stem bark, bulbs, fruits; seeds of the plants were air-dried and then ground into fine powders. The plant powders were extracted by a 10-fold excess of 90% ethanol at room temperature with mechanical stirring for 12 hours. The alcohol solutions were evaporated and lyophilized (Hostettmann *et al.*, 2002).

Organisms

The organisms used were clinical strains of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* obtained from the Federal Medical Centre Owerri, Imo state, Nigeria. (They were taken to the lab immediately within 2hours for the evaluation).

Evaluation of Antimicrobial Activity

The preliminary antimicrobial screenings of the plant extracts were carried out using the agar diffusion technique (Singleton, 1999).

Mueller Hinton agar plates were seeded with 0.1ml of 1/100 dilution of an overnight culture of the bacterial isolate respectively and allowed to stand. A standard cork borer of 6mm diameter was used to cut uniform wells on the agar surface into which 0.2ml of the test solution of each extract (100mg/ml conc.) was added. The plates were incubated at 37°C for 24 hours after which diameter of zone of inhibition were measured. Ethanol was included separately in each plate as solvent control.

RESULTS AND DISCUSSION

The antimicrobial effects of 35 locally used medicinal plants in Nigeria were evaluated against 6 bacterial pathogens. The ethanol extracts of the 35 plant species (Table 1) showed varying antimicrobial activities against the test organisms as shown in their varying diameter zones of inhibition (Table 2). Of the 35 tested plant extracts, 33 were active against *S. aureus* (94.3%), 28 were active against *Strep. pyogenes* (80%), 29 were active against *E. coli* (82.9%), 22 were active against *S. typhi* and *P. aeruginosa* (62.9%), 27 were active against *K. pneumonia* (77.1%). From the results, the most active species were *Spondias mombin*, *Xylopi aethiopic a*, *Uvaria chamae*, *Picrilima nitida*, *Cassia agustifolia*, *Carica papaya*, *Alcomea cordifolia*, *Manniophyton fluvum*, *Ocimum gratissimum*, *Ocimum basilicum*, *Allium sativum*, *Azadirachta indica*, *Piper guineense*, *Nauclea latifolia*, *Zingiber officinale*, *Bryophyllum pinnatum*, *Chrysophyllum albidum*, *Acalypha ciliate* and *Cymbopogon citratus* (Table 2). Some of the active plant species have already been reported to have similar antimicrobial properties (Okeke *et al.*, 2001; Onyeagba *et al.*, 2004; Poonam *et al.*, 2010).

The extracts showed stronger activity against the Gram positive organisms (*S. aureus* and *Strep. pyogenes*) than the Gram negative bacteria (*E. coli*, *S. typhi*, *P. aeruginosa* and *K. pneumonia*). This observation could be due to the high lipid content of the walls of the Gram negative bacteria or the formation of protective slimy layer by some of the organisms especially *E. coli* which reduced the penetration or affinity of the active constituents to the organisms (Tamokou *et al.*, 2008). The resistance demonstrated by these organisms particularly Gram negative bacteria to some plant extracts was reported by Adoni (2006), who also observed the inhibitory activity and detrimental effects of some plants to other organisms. This shows that these organisms possess resistance factors which might pose a serious health concern mainly in the rural areas where modern health care practices/facilities are non-existent or inadequate. These test isolates are pathogenic and can induce infections of increasing importance due to the resurgence of immunodeficiency situations. Others such

as *P. aeruginosa* and *K. pneumonia* are implicated in nosocomial and community acquired infections (Akharaiyi *et al.*, 2010).

This research work has succeeded in the identification and confirmation of Nigerian plant species used in traditional medicine practices. The antimicrobial potentials of these plants against bacterial pathogens justifies their use as alternative therapy for infectious diseases. Further work needs to be carried out to identify the active principles in these plants.

Table 1: List of Tested Plant species

Family	Species	Tested part
Anarcardiaceae	<i>Mangifera indica</i>	L
	<i>Spondias mombin</i>	L
Annonaceae	<i>Xylopi aethiopic a</i>	F
	<i>Uvaria chamae</i>	R
Apocynaceae	<i>Alstonia boonei</i>	SB
	<i>Picrilima nitida</i>	S
Asteraceae	<i>Vernonia amygdalina</i>	L
Caesalpiniaceae	<i>Cassia agustifolia</i>	R
Caricaceae	<i>Asmina triloba</i>	L
Culsiaceae	<i>Garcinia kola</i>	S, R, SB, L
Compositae	<i>Chromaleana odorata</i>	L
	<i>Amillia africana</i>	L
Cucurbitaceae	<i>Momordica charantia</i>	L
Euphorbiaceae	<i>Alchomea cordifolia</i>	R
	<i>Manniophyton fluvum</i>	L
	<i>Phyllanthus niruri</i>	L
Fabaceae	<i>Tetrapluera tetraptera</i>	P/S
Gnetaceae	<i>Gnetum africanum</i>	L
Labiatae	<i>Ocimum gratissimum</i>	L
	<i>Ocimum basilicum</i>	L
Liliaceae	<i>Allium cepa</i>	B
	<i>Allium sativum</i>	F
Meliaceae	<i>Azadirachta indica</i>	SB
Piperaceae	<i>Piper guineense</i>	S
Rubiaceae	<i>Nauclea latifolia</i>	L
Rutaceae	<i>Citrus aurantifolia</i>	F
Sapotaceae	<i>Gongronema latifolia</i>	L
Zingiberaceae	<i>Zingiber officinale</i>	F
Others include:	<i>Bryophyllum pinnatum</i>	L
	<i>Chrysophyllum albidum</i>	L
	<i>Acalypha ciliata</i>	L
	<i>Cymbopogon citratus</i>	L
	<i>Asmina triloba</i>	L
	<i>Xanthosoma sagittifolium</i>	R, L
	<i>Persea americana</i>	L

L: leaves; R: root; S: seeds; SB: stem bark; F: fruits; P/S: pod/seed

Table 2: Antibacterial Screening of Ethanol Crude Extracts

Plants	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>S. pyogenes</i>	<i>P. aeruginosa</i>	<i>K. pneumonia</i>
	Diameter Zone of Inhibition (mm)					
<i>Mangifera indica</i>	15	-	-	-	-	-
<i>Spondias mombin</i>	20	20	18	19	20	25
<i>Xylopi aethiopic a</i>	15	18	11	15	14	11
<i>Uvaria chamae</i>	20	21	15	10	19	12

Plants	<i>S. aureus</i>	<i>E. coli</i>	Diameter Zone of Inhibition (mm)		<i>P. aeruginosa</i>	<i>K. pneumonia</i>
			<i>S. typhi</i>	<i>S. pyogenes</i>		
<i>Alstonia boonei</i>	21	9	-	11	21	16
<i>Picrilia nitida</i>	25	20	10	20	19	20
<i>Vernonia amygdalina</i>	16	18	-	13	9	15
<i>Cassia agustifolia</i>	19	12	19	11	12	10
<i>Asmina triloba</i>	16	19	19	18	10	19
<i>Garcinia kola</i>	14	15	-	-	-	18
<i>Chromaleana odorata</i>	15	12	14	-	-	9
<i>Aspillia africana</i>	17	17	-	16	-	15
<i>Momordica charantia</i>	10	11	-	10	-	-
<i>Alcomea cordifolia</i>	22	12	15	20	15	12
<i>Manniophyton fluvum</i>	19	11	13	9	12	10
<i>Phyllanthus niruri</i>	12	-	-	-	-	-
<i>Tetrapluera tetraptera</i>	-	-	-	-	-	-
<i>Gnetum africanum</i>	31	25	-	20	-	-
<i>Ocimum gratissimum</i>	13	15	15	14	10	19
<i>Ocimum basilicum</i>	10	13	11	10	7	8
<i>Allium cepa</i>	20	18	21	17	-	15
<i>Allium sativum</i>	25	25	18	20	10	15
<i>Azadirachta indica</i>	20	20	9	21	19	20
<i>Piper guineense</i>	20	27	18	18	19	20
<i>Nauclea latifolia</i>	20	25	18	21	25	20
<i>Citrus aurantiifolia</i>	-	-	-	-	-	-
<i>Gongronema latifolia</i>	18	20	18	-	19	20
<i>Zingiber officinale</i>	26	21	25	15	13	21
<i>Bryophyllum pinnatum</i>	18	11	11	15	18	10
<i>Chrysophyllum albidum</i>	18	12	12	15	15	10
<i>Acalypha ciliata</i>	18	12	14	18	11	15
<i>Cymbopogon citratus</i>	25	25	20	30	21	20
<i>Asmina triloba</i>	8	-	-	-	-	-
<i>Xanthosoma sagittifolium</i>	11	11	-	12	-	13
<i>Persea americana</i>	15	-	-	19	-	-

- ; No zone of inhibition

REFERENCES

- Adoni, O.A. (2006). Antimicrobial Activity of Aqueous and Ethanol Extracts of the Stem bark of *Alstonia boonei* and *Morinda lucida*. *Scientific Research and Essey*. 1(2): 50-53.
- Akharaiyi, F.C., Makanjuola, O.Y and Dada, O.E. (2010). Antibacterial Potentials of *Parquetina nigrescens* Extracts on Some Selected Pathogenic Bacteria. *Journal of Natural Products*. 3: 124-129.
- Akinpelu, D.A. and Onakoya, T.M. (2006). Antimicrobial Activities of Medicinal Plants Used in Folklore Remedies in South-Western Nigeria. *Afr. J. Biotechnol.* 5(11):1078-1081.
- Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C. and Fasura, K.A.(2005). Screening of Crude Extracts of Six Medicinal Plants used in Southwest Nigerian Unorthodox Medicine for Anti-Methicilin Resistant *Staphylococcus aureus* Activity. *Complement. Alt. Med.* 5:5-8.
- Balandrin, M F., Kjocke, A. J. and Wurtele, (1985). Natural Plant chemicals sources of Industrial and mechanical materials, *Science* 228-1154-160.
- Hostettmann, K., Kamanzi, K.A., Kone, M., Terreaux, C. and Dosso, M. (2002). Evaluation of the Antimicrobial Potential of Medicinal Plants from the Ivory Coast. *Phytother. Res.* 16, 497-502.
- Krisharaju, A, V., Rao T V N, and Sundararaju. (2005) Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Altenaria salania*) lethality assay. *Int. J. Appl. Sci Eng.* 2: 125-134..
- Neuwinger, H.D. (1996). In African Ethnobotany-Poisons and Drugs-Chemistry-Pharmacology-Toxicology. Chapman & Hall: London.
- Okeke, M.I., Iroegbu, C.U., Jidefor, C.O., Okoli, A.S. and Esimone, C.O. (2001). Antimicrobial Activity of Ethanol Extracts of Two Indigenous Nigerian Spices. *Journal of Herbs, Spices and Medicinal Plants.* 8: 39-46.
- Onyeagba, R.A., Ugbogu, O.O., Okeke, C.U and Iroakasi, O. (2004). Studies on the Antimicrobial Effects of Garlic, Ginger and Lime. *Afr. J. Biotechnol.* 3(10): 552-554.

- Poonam, G.D., Brijesh, S., Pundarikaksh, Udu, T., Noshir, H.A. and Tannaz, J.B. (2010). Antidiarrhoeal Activity of *Zingiber officinale* (Rosc.) *Current Science* 98(2): 222-229.
- Pousset, J.L. (1994). In *Plantes Medicinales Africaines. Le Pharmacein d'Afrique: Abidjan*.
- Singleton, P. (1999). *Bacteria in Biology, Biotechnology and Medicine*. 4th ed. John Wiley and sons publishers Ltd pp. 331-337.
- Soberon, J.R., Sgariglia, M.A., Sampietro, D.A., Quiroga, F.N. and Vattuone, M.A. (2007). Antibacterial Activity of Plant Extracts from Northwest Argentina. *J. Appl. Microbiol.* 102: 1450-1416.
- Suffredini, J.B., Sader, H.S., Goncalves, A.G., Reis, A.O., Gales, A.C., Varella, A.D. and Younes, R.N. (2004). Screening of antimicrobial extracts from plants native to the Brazilian Amazon rainforest and Atlantic forest. *Brazil. J. Med. Biol. Res.* 37: 379-384.
- Sures,h K., S. Saravana Babu and Harisaranraj, R. (2008). Studies on *In Vitro* Antimicrobial Activity of Ethanol Extract of *Rauvolfia tetraphylla*. *Ethnobotanical Leaflets* 12: 586-90.
- Tamokou, J.D., Kuiata, J.R., Njateng, G.S.S., Mpetga Simo, D.J., Njouendou, A.J., Jane, P. and Amvam Zollo, P.H. (2008). Antimicrobial Activity of Dichloromethane-Methanol (1:1 v/v) Extract from the Stem bark of *Coula edulis* Bail. (Olacaceae). *Research Jour. Microbiol.* 3(6): 414.