

Antibacterial Activity of the Ethanolic and Methanolic Leaf Extracts of Some Tropical Plants on Some Human Pathogenic Microbes

¹Odunbaku, O.A and ²Ilusanya O.A

¹Department of Plant Science and Applied Zoology,

²Department of Microbiology, Olabisi Onabanjo University, Ago-iwoye, ogu-state, Nigeria.

Abstract: Ethanol and Methanol Extracts of *Chromolaena odorata*, *Azadirachta indica*, *Cymbopogon citratus*, *Piper guineense*, *Psidium guajava*, *Morinda lucida* and *Gynandropsis gynandra* were screened for antibacterial activity against six human pathogenic microbes. The highest inhibition was obtained with the ethanol extracts(300-500 mg/ml) The study also revealed that all the six plants used had similar inhibitory activities with Tetracycline, Chloramphenicol, Erythromycin, Gentamycin and Cotrimoxazole against *E.coli*, *S. aureus*, *P.mirabilis* and *S.albus*

Key words: Antibacteria activity, Tropical plants, Pathogenic microbes

INTRODUCTION

In a constant attempt to improve the quality of life, men have used plants as source of food, shelter, clothing, medicine, cosmetic and for seeking relief from hardship of life. Some plants are known as medicinal because they contain active substance that causes certain reaction from relenting to the cure of diseases of man^[1]. Knowledge of medicinal plants sometimes means the only therapeutic resource of some communities and ethnic groups^[2].

Therefore, searches for substance with antimicrobial activity are frequently considered interesting by some researchers since they are frequently used in medicine as remedies for many infections diseases.

Studies of plants used in traditional medicine have been carried out in the field of microbiology, especially on pathogenic bacterial growth and some of these studies were about the antimicrobial activities of some plants e.g. *Psidium guajava* L.^[3,4,5], *Gynandropsis gynandra*.^[6], *Cymbopogon*^[2,7,8,9,11]. Thus, this research work seeks to establish the microbial activities of some plants and to justify the ethnobotanical uses of some of these plants.

MATERIALS AND METHODS

Plant Samples: *Chromolaena odorata*, *Azadirachta indica*, *Cymbopogon citratus*, *Piper guineense*, *Psidium guajava*, *Morinda lucida* and *Gynandropsis gynandra* were collected in 2007 from Ago-Iwoye and voucher specimens were deposited at the Herbarium of the Department of Plant Science and Applied Zoology,

Olabisi Onabanjo University, Ago-Iwoye. The leaves of the plants were air dried and triturated in a mechanical mill.

Preparation of Plant Extracts: Triturated plant materials were extracted using Soxhlet extraction apparatus 70% of methanol was use for the extraction. The filtrates were concentrated on a rotary evaporator at 45°C and the extracts were then kept in sterile bottles under refrigerated conditions until use.

Bacterial Used: Pure culture of *EschericiaS coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus albus* were obtained from the Department of Medical Microbiology, Ogun State University Teaching Hospital, Sagamu. They were kept in McCartney bottles with slant preparation of nutrients agar to maintain their growth.

Bacterial Inoculation and Incubation with Extracts: Nutrient agar and nutrient broth (oxid) were prepared according to the manufacturers' recommendations. The agar-well diffusion method was used for the inoculation of the bacteria. Plates containing 0.5 ml of sterile nutrient agar each were inoculated with standardized inocula (1.5 x 10⁸ cells/ml)^[12] using sterile Pasteur pipette. Wells of 5 mm diameter were made at the centre of each plate and 0.15 ml of the various concentrations of the plant extracts were dispensed into each well.

The extracts were allowed to diffuse into the medium for 1hr at room temperature. This was then

Table 1: Antibacterial Activity of some plant extracts on some pathogenic bacterial

Ethanollic Plant Extracts	Conc. mg/ml	<i>E. coli</i>	<i>S. aureus</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>K. pneumonia</i>	<i>S. albus</i>
<i>Psidium guajava</i>	300	++	++	-	-	-	-
	400	++	++	++	-	-	-
	500	++	++	++	+	+	+
<i>Morinda lucida</i>	500	-	++	-	-	-	-
	750	-	++	-	-	-	-
	1000	+	+++	-	-	-	-
<i>Chromolaena odorata</i>	650	-	-	-	-	+	-
	800	-	++	+	++	+	+
<i>Piper guineense</i>	600	+	+	+	+	+	+
	700	++	+++	++	++	+++	+++
<i>Gynandropsis gynandra</i>	300	+	+	+	+	-	-
	400	+	++	+	+	+	+
	500	+	++	+	+	++	++
Methanolic Plant Extracts							
<i>Chromolaena odorata</i>	500	-	+++	++	-	++	++
	650	-	+++	++	+	+++	++
	800	+++	+++	++	++	+++	+++
<i>Azadirachta indica</i>	100	+	-	-	+	+	+
	500	+++	-	-	+++	+++	++
<i>Cytopogon citrates</i>	500	+	-	-	+	++	-
	650	++	++	-	++	++	+
	800	++	++	+	++	++	++
<i>Psidium guajava</i>	500	++	++	++	++	++	++
	700	++	++	++	+++	+++	+++
<i>Morinda lucida</i>	500	+	-	-	+	-	-
	750	++	+	-	+	-	-
	1000	+++	+	-	+	-	-
<i>Gynandropsis gynandra</i>	300	-	-	-	-	-	-
	400	-	-	-	-	+	+
	500	-	-	-	-	+	+
	700	+	++	+	+	+	+
Tetracycline		++	+	+	-	++	++
Chloramphenicol		++	++	++	-	-	++

Table 1: Continued

Erythromycin	++	+	++	-	++	++
Gentamycin	+++	+++	+	+++	+++	+++
Penicillin	-	+	-	-	++	-
Ampicillin	-	+	+	-	++	+
Cotrimoxazole	++	++	-	-	-	++

Diameter of cork borer = 6mm

10 – 23mm = + (low inhibition)

24 – 35mm = ++ (moderate inhibition)

36mm = +++ (high inhibition)

(-) = No activity

incubated at for 24 h at 37°C after which the zones of growth inhibition were measured and recorded in millimeter. The control was set up in a similar manner except that the extract was replaced with sterile distilled water.

Antibiotic Assay: The selected antibiotics are obtained from a chemist. These drugs, in their high concentration, were diluted with sterile water reducing them into a lower concentration. Wells were bored on the prepared agar with a cork borer and with the use of sterile needle and syringe; the antibiotics were poured into the well. The zone of inhibition was observed after 24 hours and recorded.

RESULTS AND DISCUSSION

Results: The antibacterial activities of the Ethanolic leaf extracts at different concentration are presented in Table 1. The bacteria used were clinical stains of *Escherichia coli*, *Staphylococcus aureus*, *proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus albus*. The table revealed that the plant extracts had activities against *E. coli* except *Chromolaena odorata*. However the activity of *Morinda lucida* against this organism (*E. Coli*) was at a high concentration (1000mg/ml). It was only *Morinda lucida* that did not have activity against *P. mirabilis*, *P. aeruginosa*, *K. pneumonia* and *S. albus*.

The table shows the antibacterial activities of the methanolic leaf extracts at different concentrations. The table revealed that all the six plants used had inhibitory activity against *E. coli*, *A. indica* had no activity against *S. aureus*, and *P. mirabilis*, *M. lucida* had no inhibitory activity against *P. mirabilis*, *K. pneumonia* and *S. albus*.

The action of the antibacterial drugs on the growths of the microorganisms revealed that only gentamycin had activity against *P. geruginosa* while Chloramphenicol did not have activity against *K. pneumonia*. The two cell walls synthesis inhibitors did not have activity against *E. coli* and *P. aeruginosa*

while penicillin alone did not have activity against *P. mirabilis*, *P. aeruginosa* and *S. albus* cotrimoxazole (folic acid and nucleic acid inhibitor had activity against *E. coli*, *S. aureus* and *S. albus*

Discussion: From the results of the antibacterial studies as shown in table 1 the ethanolic extracts had more activity on the organisms than the methanolic extracts. Methanolic leaf extract of *Morinda lucida* had activity against *Pseudomonas aeruginosa* while the ethanolic leaf extract did not show any activity on these organisms.

The result also revealed that the methanolic extract of the plants were active at very high concentrations (500 – 1000mg/ml) unlike the Ethanolic extracts (300 – 500mg/ml) which means that ethanol extracted the active components of the plants responsible for their antimicrobial activities.

The study also revealed that all the six plants used had similar inhibitory activities with Tetracycline, Chloramphenicol, Erythromycin, Gentamycin and Cotrimoxazole against *E.coli*, *S. aureus*, *P.mirabilis* and *S.albus*. The above known antibiotics are reported to have inhibitory effects on cell wall synthesis and the nucleic acid production^[14] hence these plant extracts might be exhibiting similar effect. Furthermore, this result is in consonance with the reports^[7,8,13,14,15,16,17] on the activities of the plant extracts on *E.coli*.

Gynandropsis gynandra methanolic leaf extracts was active against *S. aureus* and *P. aeruginosa* at high concentration (700mg/ml), which is in variance with^[6] 200mg/ml. This could be to time of collection of plant material.

The antibiotic activity of the drugs on the bacteria revealed that Gentamycin is the best choice of drug among the protein synthesis inhibitor drugs to be used in treating diseases caused by *P. aeruginosa*. The study also revealed that *S. aureus* and *P. aeruginosa* have become resistant to Ampicillin and Penicillin drugs^[6]. Cotrimoxazole is best used to treat diseases caused by *E. coli*, *S. aureus* and *S. albus*.

REFERENCES

1. Sliva Junior, AA, VJ Vizotto, E Giorgi, SG Macedo and LF Marques 1994. plantas medicinals, caracterizacao e cultivo EPAGRI. Bol Tecnico Florianopolis, 68: 1-71. 79: 213-220.
2. Di Stasi, LC, 1996. Arte, ciencia e magia. In LC Di Stasi, CA Hiruma-Lima (eds), plantas Mediciniais: Arte e Ciencia, Unesp, Sao Paulo, pp: 15-21.
3. Gnan and Demello, 1999. inhibition of Staphylococcus aureus by aqueous Goiaba extracts. Journal of Ethnopharmacology., 68,73: 103-108
4. Lin, J., 2002. Anti-diarrhea evaluation of some medicinal plants used by Zulu traditional healers. Journal Of Ethnopharmacology, 79,1: 53-56.
5. Vieira, R.H.S., Rodrigues, J.S. Aragno and O.U. Sousa, 2001. Microbial effect of medicinal plant extracts (*Psidium guajava* and *Carica papaya*) upon bacteria isolated from fish muscle and known to induce diarrhea in Children. Revista do Institution de Medicina. Tropical de sao Paulo. 43,3: 145-148.
6. Ajaiyeoba, E.O., 2000. Phytochemical and anti microbial studies of Gynandroosis gynandra and Buchholzia coriaceae extracts. African journal of biomedical research., 3(3): 161-165.
7. Hammer, K., 1999. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbial, 86: 985-990.
8. Wannissorn, B. and T. Artikou, 1996. Anti fungal activity of essential oils obtained from officinal plants against dermatophytes. Mycoses 36: 333-336.
9. Kishore, N. and X. Charle, 1993. Fungi toxicity of essential oil against dermatophytes. Mycoses, 36: 211-215.
10. Cimanga, K, K Kambu, L Tona, S Aper, T de Bruyne, N Hermans, J Totte, Pieters L, Vlietinck AJ 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 79: 213-220.
11. Hiruma-Lima, CA, 2002. plantas Mediciniais na Amazonia e na Mata Atlantica, 2nd ed., Unesp, Sao Paulo, pp: 604.
12. Olafimihan, CA, Fawole MO (2003). Antibacterial properties of the stem bark of *Azadirachta indica* (The Neem Tree). Niger. J. Pure Appl. Sci., 18: 1407-1412
13. Joyce Elaine, C.B., P.M. Rebecca, N.B Lidiane, Luize, C.D. Claudi and F.J. Ary, 2000. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Mem inst Oswaldo cruz. Rio de Janeiro. 10(4): 387-390.
14. Burkill, H.M. 1997. The useful plants of west tropical Africa, edition 2 vol. 4 families M-K Royal botanical gardens kew.
15. Ilori, M. AO Sheteolu., EA Omonibgehin and AA. Adeneye, 1996. Anti diarrhea activities of ocimum gratissimum (Lamiaceae) J. Diarrhoea dis res. 14: 283-285.
16. Ramanoelina, AR, GP Terrom, JP Biancuini and P Coulanges, 1987. Antibacterial action of some essential oils extracted from Madagascar plants. Arch. Inst Pasteur Madagascar, 53: 217-226
17. Janssen, A.M, JJ Scheffer, LI Ntezurubanza and A Baeheim suendsen, 1989. Anti microbial activies of some ocimum species grown in Rwanda. J. Ethnopharmacol, 26: 57-63.