

Full Length Research Paper

Antibacterial screening of the root, seed and stem bark extracts of *Picralima nitida*

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Ethanol, benzene, chloroform and aqueous (cold and hot) extracts of *Picralima nitida* (seed, stem bark and root) were tested against five bacterial strains using the agar-well diffusion method. The ethanol extracts of the root and stem bark (Er and Esb) were active against 100% of the test organisms, respectively. The benzene and chloroform extracts exhibited no activity. Of the fifteen extracts tested, 40.0% were active against *Staphylococcus aureus* ATCC 12600, 20.0% each against *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 11775, 33.3% against *Bacillus subtilis* ATCC 6051 and 13.3% against *Salmonella kintambo* Human 1,13,23:mt: -. The MIC values for the ethanol extracts range from 6.25 to 50 mg/ml, while the MIC values for the cold water seed extract (CWs) was 50 mg/ml. The results provide a rationalization for the traditional use of *P. nitida* for the treatment of various diseases.

Key words: *Picralima nitida*, ethnomedicine, phytochemical analysis, Nigeria.

INTRODUCTION

Picralima nitida, family Apocynaceae, (Common name: *Akuamma* plant, Igbo: *Osi-Igwe*) has widely varied applications in Nigeria folk medicine. Many herbalists have claimed to use the leaves, seed or stem bark as treatment for various fevers, hypertension, jaundice, gastro-intestinal disorders and for malaria (Dalziel, 1961; Iwu, 1993). The seed, stem and roots have been reported to be effective as a cough suppressant anodyne, as well as an aphrodisiac and hypoglycaemic agent in treatment of diabetes (Ayensu, 1978; Oliver, 1960). However, there is apparently no scientific report on the antibacterial properties of the plant. Such lack of scientific knowledge has often constituted a major constraint to consideration of the use of traditional herbal remedies in conjunction with or as an affordable alternative to orthodox medical treatment. Thus, the extracts of the seed, stem bark and root of *P. nitida* were quantitatively screened for activity

against five bacterial strains, including four from the American Type Culture Collection (ATCC), and one serotyped local *Salmonella* strain.

MATERIALS AND METHODS

Plant material

The root, stem bark and seed of *P. nitida* used in this study were obtained from a local herbalist at Egbelubi Eziana in Imo State, in Eastern Nigeria. The plant was identified taxonomically by A. O. Ozioko of the Department of Botany, University of Nigeria, Nsukka, and a voucher specimen was deposited in the Department herbarium.

Extraction of plant materials

The whole root, stem bark and seed of *P. nitida* were prepared by maceration in ethanol, benzene, chloroform, and hot or cold water. The plant parts were first dried in the dark at room temperature (27±2°C) and pulverized to powder using a mechanical grinder. A 15.0 g amount of each of the pulverized plant parts was separately soaked in 80 ml of ethanol, benzene or chloroform, or macerated in equivalent volume of cold water for 18 h. Also, the same amount (15.0 g) of each pulverized plant material was immersed in 80 ml of

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hot water (100°C) and allowed to stand for 2 h with occasional stirring. Each preparation was filtered through a Whatman No 1 filter paper and the filtrate evaporated to dryness in a steady air current for about 24 h in a previously weighed crucible (Okoli et al., 2002). The dried extract was exposed to uv rays for 24 h and checked for sterility by streaking on nutrient agar plate.

Phytochemical screening

The ethanol, cold and hot water stem bark extracts were used for the screening. The dried extracts were first reconstituted in the respective solvents used for their extraction and then tested by standard phytochemical method for the presence of alkaloid, flavonoid, tannin, saponin, glycosides and protein (Bobbit 1964; Harborne, 1984; Touchstone, 1992).

Test bacterial strains

Standard typed cultures of *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145), *Bacillus subtilis* (ATCC 6051), and *Staphylococcus aureus* (ATCC 12600) were obtained from Bioresources Development and Conservation Project (BDPC), Nsukka. *Salmonella kintambo* (human 1,13,23: mt:-) was supplied by the Veterinary Microbiology and Pathology Laboratory of the University of Nigeria, Nsukka. All test strains were re-isolated three successive times on Mueller Hinton agar, MHA (oxid), and identity was confirmed by standard bacteriological methods (Collins and Lyne, 1970).

Screening of extracts for antibacterial activity

The extracts were spot checked for antibacterial activity using the agar well diffusion technique (Okeke et al., 2001). Standardized inoculum (5×10^5 cfu/ml) of each test bacterium was spread on to sterile Muller Hinton agar plates so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0 mm was used to bore wells in the agar plates. The extracts were reconstituted in 20% dimethylsulphoxide (DMSO) and diluted with sterile water to a concentration of 50 mg/ml. Subsequently, a 100 µl volume of the extracts were introduced in triplicate wells into the MHA cultures. The plates were allowed to stand for 1 h or more for diffusion to take place and then incubated at 37°C for 24 h. The zone of inhibition was recorded to the nearest mm. Only extracts exhibiting apparent zone of inhibition were chosen for further evaluation.

Determination of minimum inhibitory concentration (MIC)

The MIC was determined only for the ethanol extracts of the root and stem bark, and the cold water extract of the seed by a modified agar well diffusion technique (Okeke et al., 2001). A two-fold serial dilution of the extracts were prepared by first reconstituting in 20% DMSO then diluting in sterile distilled water to achieve a decreasing concentration range of 50 to 0.781 mg/ml. A 100 µl volume of each dilution was introduced in triplicate wells into MHA plates already seeded with the standardized inoculum (5×10^5) of the test bacterial cells. All test plates were incubated at 37°C for 24 h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

RESULTS

The yield of the extracts is shown in Table 1. The cold water seed extract gave the highest yield (6.8%) while

the least yield (0.2%) was recorded with the stem bark extract of benzene. There was no significant difference ($p > 0.05$) in yield of the aqueous and ethanol extracts as compared to the benzene or chloroform extracts. That is, ethanol and water are better extraction solvents in terms of yield than benzene or chloroform. The phytochemical analysis showed that the ethanol and aqueous (cold and hot) stem bark extracts of *P. nitida* each contained alkaloid, tannin, steroids, flavonoids, protein, carbohydrate and ascorbic acid (Table 2). Cardiac and cyanogenetic glycosides were not detected.

Of the fifteen extracts screened, 6 (40.0%) showed activity against *Staph. aureus*, 3 (20.0%) each against *P. aeruginosa* and *E. coli*, 5 (33.3%) against *B. subtilis* and 2 (13.3%) against *Sal. kintambo* (Table 3). The proportion of test bacterial strains showing susceptibility to DMSO (control) is also shown in Table 3. The benzene and chloroform extracts showed no activity against the test bacterial strains. The ethanol extracts of the root (Er) and stem bark (Esb) had activity to all the standard cultures with highest mean zone of inhibition (25.0 ± 2.65 mm) against *Sal. kintambo*. The cold water extract of the seed (CWs) had activity against the bacterial strains except *Sal. kintambo*, with highest mean zone inhibition (14.0 ± 1.0 mm) against *B. subtilis*. The hot water seed extract (HWs) had activity only against *Staph. aureus* and *B. subtilis*, with mean inhibition of 8.0 ± 0.0 mm and 7.0 ± 0.0 mm, respectively (Table 3).

The MIC was determined for the ethanol extracts of the root and stem bark (Er and Esb), and the cold water extract of the seed (CWs) because these extracts showed a wider spectrum of antibacterial activity compared to others. The MIC values of Er ranges from 25 mg/ml for *B. subtilis* and *Sal. kintambo* to 50 mg/ml for *Staph. aureus*, *P. aeruginosa* and *E. coli*. The Er extract MIC ranges from 6.25 mg/ml for *Staph. aureus* to 50 mg/ml for *P. aeruginosa* while the MIC values for CWs was 50 mg/ml for all the bacterial strains (Table 4).

DISCUSSION

The yield and antibacterial activity of *P. nitida* extracts varied with the plant parts and the solvents used for extraction. The yields of the extracts obtained were relatively low. Maceration has generally been reported to give lower yield of plant extracts compared to Soxhlet extraction (Ibrahim et al., 1997). The higher yield of the aqueous extracts compared with the ethanol, benzene and chloroform extracts, suggest higher proportion of water-soluble plant components. The higher yield achieved with cold water extraction over and above the hot extraction may be explained by the short maceration time (2 h) as compared to 18-24 h for ethanol or cold water. Generally, hot water extracts have been reported to contain higher amounts of plant constituents (Okeke et al., 2001; Okoli et al., 2002).

Table 1. Yield of extracts from ethanol, benzene, chloroform and hot or cold water.

| Extraction solvent | Plant part | code | Yield (g) \pm SD | Yield (%) |
|--------------------|------------|------|--------------------|-----------|
| Ethanol | Root | Er | 0.08 \pm 0.004 | 0.53 |
| | Seed | Es | 0.25 \pm 0.007 | 1.67 |
| | Stem bark | Esb | 0.28 \pm 0.007 | 1.87 |
| Benzene | Root | Br | 0.05 \pm 0.004 | 0.33 |
| | Seed | Bs | 0.09 \pm 0.001 | 0.60 |
| | Stem bark | Bsb | 0.03 \pm 0.007 | 0.20 |
| Chloroform | Root | Cr | 0.15 \pm 0.007 | 1.0 |
| | Seed | Cs | 0.20 \pm 0.007 | 1.33 |
| | Stem bark | Csb | 0.09 \pm 0.007 | 0.60 |
| Hot water | Root | HWr | 0.12 \pm 0.007 | 0.80 |
| | Seed | HWs | 0.40 \pm 0.014 | 2.67 |
| | Stem bark | HWsb | 0.27 \pm 0.007 | 1.80 |
| Cold water | Root | CWr | 0.15 \pm 0.007 | 1.0 |
| | Seed | CWs | 1.02 \pm 0.007 | 6.80 |
| | Stem bark | CWsb | 0.50 \pm 0.035 | 3.33 |

Table 2. Phytochemical screening of ethanol and aqueous stembark extract of *P. nitida*.

| Plant constituent | Esb | CWsb | HWsb |
|----------------------|-----|------|------|
| Alkaloids | ++ | + | + |
| Cyanogenic glycoside | - | - | - |
| Cardiac glycosides | - | - | - |
| Ascorbic acid | + | + | + |
| Saponin | + | ++ | ++ |
| Steroids | + | + | + |
| Carbohydrate | ++ | + | + |
| Tannins | + | + | + |
| Terpenoids | + | + | + |
| Protein | + | + | + |
| Flavonoids | + | + | + |

-: not determined.

+: low concentration.

++: medium concentration

Table 3. Inhibition zone diameter, IZD (mm) a of extracts against the bacterial strains .

| Extract code | <i>Staph. aureus</i> ATCC 12600 | <i>P. aeruginosa</i> ATCC 10145 | <i>B. subtilis</i> ATCC 6051 | <i>E. coli</i> ATCC 11775 | <i>Sal. kintambo</i> Human 1,13,23:mt:- | Proportion susceptible (%) |
|----------------------------------|------------------------------------|------------------------------------|---------------------------------|------------------------------|---|-------------------------------|
| Er | 20 ± 2.0 | 14 ± 1.73 | 16 ± 1.73 | 8 ± 1.73 | 16 ± 2.0 | 5/5 (100) |
| Es | 16 ± 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1/5 (20) |
| Esb | 20 ± 1.73 | 20 ± 1.0 | 16 ± 2.0 | 14 ± 1.73 | 25 ± 2.65 | 5/5 (100) |
| Br | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| Bs | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| Bsb | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| Cr | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| Cs | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| Csb | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| HWr | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| HWs | 8.0 ± 0.0 | 0.0 | 7.0 ± 0.0 | 0.0 | 0.0 | 2/5 (0) |
| HWsb | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| CWr | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |
| CWs | 10 ± 1.0 | 10 ± 1.0 | 14 ± 1.0 | 8 ± 1.73 | 0.0 | 4/5 (80) |
| CWsb | 8 ± 0.0 | 0.0 | 8 ± 0.0 | 0.0 | 0.0 | 2/5 (40) |
| % showing activity per strain | 6/15 (40) | 5/15 (33.3) | 3/15 (20) | 3/15 (20) | 2/15 (13.3) | |
| Control DMSO (20%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0/5 (0) |

^aEach value is a mean of triplicates; test concentration of extract = 50-mg/ml (100µl/well).

Table 4. Minimum inhibitory concentration (MIC) of the ethanol and aqueous extracts against the test bacterial strains.

| Test bacterial strains | MIC (mg/ml) | | |
|------------------------|-------------|------|-----|
| | Er | Esb | CWs |
| <i>Staph. aureus</i> | 50 | 6.25 | 50 |
| <i>P. aeruginosa</i> | 25 | 25 | 50 |
| <i>B. subtilis</i> | 50 | 25 | 50 |
| <i>E. coli</i> | 50 | 50 | 50 |
| <i>Sal. kintambo</i> | 25 | 12.5 | ND |

ND: not determined.

Extracts obtained with analytical grade ethanol gave a relatively wide spectrum of activity (20-100%) against the test strains compared with cold water extract (40-80%) or hot water (40%). The relatively wider spectrum of activity of the ethanol extracts over the aqueous extracts is significant because traditional administration of herbal medicines is in the form of the former (in kai-kai, a locally distilled gin).

Some of the phytochemical compounds detected e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity (Leven et al., 1979). Therefore, the limited spectrum of activity of the aqueous extracts (both cold and hot) compared with the ethanol extracts is difficult to explain since all the extracts contained the metabolites, though not in the same proportions.

Perhaps, the active principles were more soluble in analytical ethanol than the aqueous solvents. This paradox may be resolved when the active constituents have been isolated and the molar activity of the purified form determined.

The antibacterial activity exhibited, particularly by the ethanol extracts of *P. nitida*, is significant for two reasons. First, the more active preparations are the ethanol extracts, the form in which medicinal plant preparations are popularly administered in ethnomedical practice in Eastern Nigeria. Secondly, the bacterial strains used have been implicated in some of the diseases against which *P. nitida* is a preferred herbal remedy. Thus, these preliminary results support the folkloric claims of the use of *P. nitida* as herbal remedy. Further research, probably involving extraction

with the traditional solvent (kai-kai, locally distilled gin) popularly used and the isolation of the active principle in pure form would be needed to establish the antibacterial activity.

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