Antibacterial Activities of *Asmina triloba* against Some Bacterial Pathogens

Abalaka M. E, Oyewole O. A.*

Department of Microbiology, Federal University of Technology, PMB 65, Minna, Niger State, Nigeria

**Abstract** The antibacterial effect of *Asmina triloba* against *Pseudomonas aeruginosa*, *Klebsiella ozanae*, *Staphylococcus aureus* and *Escherichia coli* was determined using the agar cup plate technique. The phytochemical components of *Asmina triloba* showed the presence of alkaloids and phlobatanin and the absence of saponins, tannins, phenolics, glycosides, flavonoids and triterpenes. The results showed that the test organisms were susceptible to 500mg/ml, 50mg/ml and 5mg/ml of the plant extract. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The result showed that the MIC for *Pseudomonas aeruginosa* and *Klebsiella ozanae* was 500mg/l and the MIC of 50mg/l was recorded for *S. aureus* and *E. coli*. No MBC was recorded for both *P. aeruginosa* and *K. ozanae* but MBC for *S. aureus* and *E. coli* was 500mg/l. The results of the study suggest that extracts of *Asmina triloba* could be suitable for the treatment of various infections caused by *P. aeruginosa*, *K. ozanae*, *S. aureus* and *E. coli*.

**Keywords** Antibacterial Effect, *Asmina triloba*, Phytochemical Components, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

1. **Introduction**

   The term herbal drug determines the part or parts of a plant used for preparing herbal and traditional medicines (for examples: leaves, flowers, seeds, roots, barks, stems, etc.) (Kayode and Kayode, 2011) Furthermore, World Health Organization, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds (Sofowora, 1996) which have curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants. Tyler (1999) has reported that plants also contain certain other compounds that modify the effects of the active ingredients.

   North American pawpaw (*Asmina triloba*) is the largest tree fruit native to temperate North America. It is an underutilized plant that has potential as landscape tree, fruit crop and as a source of pharmaceutical products (Finneseth et al., 2000). In addition, *A. triloba* has some identified secondary products (acetoginins) in the bark and leaves that have a wide range of biological activities including anticancer, antimicrobial, immune suppressant and pesticidal properties (Finneseth et al., 2000).

   This study was undertaken therefore, to determine the phytochemical components of the leaf extracts of *A. triloba*, the minimum inhibitory concentration (MIC) of the extract on *Pseudomonas aeruginosa*, *Klebsiella ozanae*, *Staphylococcus aureus* and *Escherichia coli*, the minimum bactericidal concentration (MBC) of the extract on *Pseudomonas aeruginosa*, *Klebsiella ozanae*, *Staphylococcus aureus* and *Escherichia coli*.

2. **Materials and Methods**

   Collection and Preparation of Samples

   The bark of *A. triloba* was collected from different locations in Minna metropolis. It was air-dried for six weeks in microbiology laboratory of Federal University of Technology, Minna. The dried materials were pulverised in mortar and packaged in bottles for analysis.

   Collection of Specimen

   Pure cultures of *Pseudomonas aeruginosa*, *Klebsiella ozanae*, *Staphylococcus aureus* and *Escherichia coli* were obtained from General Hospital Minna, Niger State and were subcultured in agar slants.

   Extraction of Materials

   Ethanol and water were used as solvents for the extraction of the plant materials. 150g of pulverised sample was sus-
pended in 750ml of 75% ethanol for 120hours. The extracts were then filtered, decanted, and evaporated in vacuo at 450°C.

**Phytochemical Screening of Extracts of Asmina triloba**

The phytochemical components of extracts of *A. triloba* was determined using methods described by Odebiyi and Sofowora (1978) and Trease and Evans (1989). The phytochemical components analyzed were alkaloids, tannins, phenolics, glycosides, saponins, flavonoids, steroids, phlobatins, and triterpenes.

**Antimicrobial Susceptibility Test**

Susceptibility test of the test organisms to extracts of *A. triloba* at concentrations of 500mg/ml, 50mg/ml and 5mg/ml was carried out using agar cup plate technique as described by Silver *et al.* (1997). Nutrient agar was prepared using autoclave at 121°C for 15 minutes. It was then poured on to plates and allowed to solidify. Standard inoculum of each test organism was spread on the agar plates so as to achieve a confluent growth. The impregnated discs with different concentration of the extract were placed on the surface of the medium at three points equidistant from one another. The plates were then incubated at 37°C for 24 hours.

**Determination of Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration (MIC) of the test organisms was determined using the tube dilution technique. 9ml of the nutrient broth was pipetted into various test tubes containing concentrations of 500 mg/ml, 50 mg/ml and 5 mg/ml of the extract. The overnight culture of the test organism was diluted at 10^6 cfu/ml was added to the test tubes and then incubated at 37°C for 24 hours. The least concentration of the extract that did not indicate any visible growth of the organism was determined using the tube dilution technique.

**Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Extract of A. triloba on the test organisms**

| Organisms  | Concentration of extract of A. triloba | MIC (mg/l) | MBC (mg/l) |
|------------|--------------------------------------|------------|------------|
| P. aeruginosa | 11±2.08mm | 500 | 500 |
| K. ozanae | 7.5±0.5mm | 50 | - |
| S. aureus | 5±1mm | 5 | - |
| E. coli | 11±2.08mm | 5 | - |

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Extract of *A. triloba* on the test organisms

**Table 1. Phytochemical screening of the extracts of A. triloba**

| Component | A. triloba |
|-----------|------------|
| Alkaloid  | +          |
| Tannins   | -          |
| Phenolics | -          |
| Glycoside | -          |
| Saponin   | -          |
| Flavonoid | +          |
| Phlobatanin | -  |
| Triterpenes | -    |

**Table 2. Antimicrobial activities of the extracts**

| Organisms | Concentration of extract of A. triloba | MIC (mg/l) | MBC (mg/l) |
|-----------|--------------------------------------|------------|------------|
| P. aeruginosa | 11±2.08mm | 500 | 500 |
| K. ozanae | 7.5±0.5mm | 50 | - |
| S. aureus | 5±1mm | 5 | - |
| E. coli | 11±2.08mm | 5 | - |

**Table 4. Susceptibility testing using standard antibiotics**

| Organisms | AMX(mm) | CPX(mm) | RD(mm) | AU(mm) | CEP(mm) | GEN(mm) | PN (mm) | AU (mm) | G (mm) |
|-----------|---------|---------|--------|--------|---------|---------|---------|---------|-------|
| P. aeruginosa | 11±2 | - | - | - | - | 4±2.52 | 11±4.5 | 5±3.05 | 6±1.00 |
| K. ozanae | 10±2 | 19±2 | 19±2 | - | - | 7±2.52 | 7±2.52 | 6±1.00 | - |
| S. aureus | 10±2 | - | 10±2 | 8±2 | - | 10±2 | 6±1.00 | 7±3.00 | 6±2.00 |
| E. coli | 10±2 | 8±2 | 8±2 | 12±8 | - | - | - | - | - |

Key: AMX- Amoxacillin, CPX- Ciprofloxacin, RF- Rifampin, AU- Augmentin, GEN- Gentamycin, CEP-Ceporex, PN- Ampicillin, CH- Chloramphenicol, S- Streptomycin
Susceptibility Testing using Standard Antibiotics (Negative control)

Table 5 shows the susceptibility of the tested organisms on some standard antibiotics. P. aeruginosa, K. ozanae, S. aureus and Escherichia coli were sensitive to Gentamycin (GEN), Ciprofloxacin (CPX) and Streptomycin (S). Only S. aureus and E. coli were sensitive to Ampicillin (PN) and only E. coli was resistant to Augmentin (AU).

| Organisms         | GEN (mm)     | CPX (mm)     | PN (mm)     | AU (mm)     | G (mm)  |
|-------------------|-------------|--------------|-------------|-------------|---------|
| P. aeruginosa     | 14±2.08     | 11±4.5       | -           | 10±2.00     | 5±3.05  |
| K. ozanae         | 14±3.06     | 7±2.52       | -           | 8±2.00      | 6±1.00  |
| S. aureus         | 11±1.00     | 3±2.82       | 9±3.05      | 10±2.00     | 10±1.53 |
| E. coli           | 8±2.00      | 4±1.53       | 10±4.00     | -           | 10±2.00 |

KEY: CPX- Ciprofloxacin, AU- Augmentin, GEN- Gentamycin, CEP-Ceporex, PN- Ampicillin, S- Streptomycin

4. Discussion

The phytochemical components of A. triloba (Table 1) showed the presence of alkaloid and phlobatanin. The presence of these components may be responsible for the antibacterial effects of the bark extract of A. triloba. Avalos et al. (1993) reported that alkaloid have a drastic lethal effect on the central nervous system while phlobatanin have protective ability against bacterial and fungal infections. Sofowora (1996) reported that phytochemical components usually interfere with growth and metabolisms of microorganisms. Oderinde et al. (2002) reported that A. triloba is used tropically in the treatment of cuts, rashes, stings and burns.

A. triloba shows minimum inhibitory concentration (MIC) value of 500mg/ml for P. aeruginosa, K. ozanae, S. aureus and E. coli. MBC values for P. aeruginosa and K. ozanae was 500mg/ml whereas Staphylococcus aureus and E. coli had no MBC value (Table 3). This suggests that the bark extracts of A. triloba is bacteriostatic on the tested organisms. According to Prescott et al. (2005), a bacteriostatic agent kills at a much higher concentration whereas drug kills pathogen at levels only two or four times the MIC.

At 500mg/ml and 50mg/ml and 5mg/ml, the extracts of A. triloba showed a higher susceptibility on P. aeruginosa and E. coli. The least zone of inhibition was recorded in S. aureus while at 5 mg/ml, S. aureus was resistant (Table 2). When compared with standard antibiotics (Table 4), extracts of A. triloba had a higher zone of inhibition on E. coli. This may indicate that extracts of A. triloba has a higher antibacterial effects on gram negative E. coli than the tested antibiotics.

The result of this study shows that at high concentration, bark extract of A. triloba has antibacterial effects against P. aeruginosa, K. ozanae, S. aureus and E. coli.

REFERENCES

[1] Avalos, J., Rupprecht, J.K., Mclaughlin, J.I., Rodrigue, F. (1993). Guinea pig maximazatio test of the bark extract from Pawpaw, Annoceae 29 (1):33-35
[2] Finneseth, C.L.H., Geneve, R.L. and Layne, D.R. C. (2000). Establishment of North American pawpaw (Asimina triloba (L.) Dunal shoots in vitro from mature explants Pp. 1-6
[3] Hugo, S.B. and Russel, A.D. (1983). Pharmaceutical Microbiology 3rd Edition. Blackwell Scientific Publication, London pp. 105-125
[4] Kayode A.A.A. and Kayode, O.T. (2011). Some Medicinal Values of Telfairia occidentalis: A Review. American Journal of Biochemistry and Molecular Biology, 1: 30-38
[5] Kayode, A. and Sofowora, A.E. (1978). Phytochemical screening of Nigeria medicinal plant part III Lioydia 4: 234-246
[6] Oderinde, O., Noronha, C., Oremosu, A., Kusemiju, T. and Okanlawon, A. (2002). Abortifacient properties of aqueos extract of Carica papaya (Linn) seed on female Sprague-Dawley rat. Nig. Postgrad. Med. J., 9: 95-98
[7] Prescott, L. M., J. P. Harley and D. A. Klein. 2005. Microbiology. Sixth Edition. New York: McGraw-Hill
[8] Silver, O.A., Cabrita, T., Pimentel, M., Diniz, A. and Gomes, E. (1997). Antimicrobial activity of Guinea Bissau traditional remedies. Journal of Ethnopharmacology, 50: 55-59
[9] Sofowora, A., 1996. Medicinal Plant and Traditional Medicine in Africa. 2nd Edn., Spectrum Books, Ibadan, Nigeria, pp: 112
[10] Tease, G.E. and Evans, W.C. (1989). A textbook of pharmacognosy 13th edition. Baluiere, Tindali, London pp. 100-101
[11] Tyler, V.E., 1999. Phytomedicines: Back to the future. J. Nat. Prod., 62: 1589-1592
[12] World Health Organization (WHO). 2001. Geneva Legal Status of Traditional Medicine and Complementary /Alternative Medicine: A Worldwide Review. World Health Organisation, Geneva, pp: 129-143