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Antibacterial Activities of *Asmina triloba* against Some Bacterial Pathogens

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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 saponin, 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/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 moderate the effects of the active ingredients.

North American pawpaw (*Asmina trilobota*) 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-

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pended in 750ml of 75% ethanol for 120hours. The extracts were decanted, filtered and evaporated in vacuole at 450C.

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 analysed for were alkaloids, tannins, phenolics, glycosides. saponins, flavonoids, steroids, phlobatanins 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℃ for 15 minutes. It was then poured on to plates and allowed to solidify. Standardized inoculum of each test organisms was spread on to 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℃ 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 pippeted 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 organisms diluted at 10^6cfu/ml was added to the test tubes and then incubated at 37℃ for 24 hours. The least concentration of the extract that did not indicate any visible growth of the incubated organisms in broth culture was taken as the minimum inhibitory concentration (MIC) (Hugo and Russel, 1983).

3. Results

Phytochemical Screening of the Extracts

Table 1 shows the phytochemical screening of the extract of *A. triloba*. The phytochemical components of A. triloba showed the presence of alkaloid and phlobatanin but the absence of tannins, phenolics, glycosides, saponin, flavonoids and triterpenes.

Antimicrobial Activities of the Extracts

Table 2 shows the zones of inhibition (mm) of extract of *A. triloba* at different concentrations (mg/ml). At 500mg/ml, *P. aeruginosa* and *E. coli* had a zone of inhibition of 11 ± 2.08mm and *S. aureus* had the least zone of inhibition of 5 ± 1mm. At 50 mg/ml, *P. aeruginosa* had the highest zone of inhibition of 12 ± 8mm and the least zone of inhibition of 6 ± 1mm was obtained by *S. aureus*. At 5mg/ml, *S. aureus* was not sensitive to extract of *A. triloba* but *P. aeruginosa* had the highest zone of inhibition of 9 ± 1.5mm.

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

S/no	Component	*A. triloba*
1	Alkaloid	+
2	Tannins	-
3	Phenolics	-
4	Glycoside	-
5	Saponin	-
6	Flavonoid	-
7	Phlobtanin	+
8	Triterpenes	-

Key: + = Present - = Absent

Table 2. Antimicrobial activities of the extracts

Organisms	Concentration of extract of *A. triloba*		
	500mg/l	50mg/l	5mg/l
P. aeruginosa	11±2.08mm	12±8mm	9±1.5mm
K. ozanae	7.7±0.6mm	8±2mm	5±1mm
S. aureus	5±1mm	6±1mm	-
E. coli	11±2.08mm	11±2.08mm	6±1mm

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

Table 3 shows the minimum inhibitory concentration and minimum bactericidal concentration of the test organism on the extract of *A. triloba*. The minimum inhibitory concentration for *Pseudomonas aeruginosa, K. ozanae*, *S. aureus* and *E. coli* was 500mg/l. *Pseudomonas aeruginosa* and *K. ozanae* had no MBC but *S. aureus* and *E. coli* had MBC values of 500mg/ml.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Organisms	MIC (mg/l)	MBC (mg/l)
P. aeruginosa	500	-
K.ozanae	500	-
S. aureus	500	500
E. coli	500	500

Susceptibility Testing using Standard Antibiotics (Positive control)

Table 4 shows the susceptibility result of standard antibiotics against the test organisms. *P. aeruginosa* was only sensitive to Amoxacillin (AMX). *K. ozanae* was sensitive to all antibiotics tested except Augmentin (AU) and Ceporex (CEP), *E. coli* was also sensitive to all the antibiotics except Amoxacillin (AMX) and Ceporex (CEP) while *S. aureus* was not sensitive to Ciprofloxacin (CPX), Ceporex (CEP) and Ampicillin (PN).

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	-	-	-	-	-	-	-	-
K.ozanae	10±2	19±2	19±2	-	-	4±2.52	11±4.5	5±3.05	6±1.00
S. aureus	10±2	-	10±2	8±2	-	7±2.52	-	7±2.52	6±1.00
E. coli	-	8±2	8±2	12±8	-	10±2	6±1.00	7±3.00	6±2.0

Key: AMX- Amoxacillin, CPX- Ciptrofloxacin, 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).

Table 5. Susceptibility testing using standard antibiotic

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 use 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.*

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