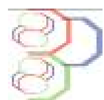

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Antibacterial activities of seed-pod extracts of *Acacia nilotica Willd* to *Artemia salina* larvae

[1]Mbatchou V. Chi and [2]Adoum O. Moubarak

[1]Department of Applied Chemistry and Biochemistry, University for Development Studies, P. O. Box 24, Navrongo, Ghana; [2]Department of Chemistry, Bayero University Kano, P. M. B. 3011 Kano, Nigeria.

*Corresponding author email: mbatcham@yahoo.fr
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ABSTRACT
Objectives: To evaluate the antibacterial activities of solvent partitioned fractions of the seed-pod extracts of *Acacia nilotica Willd.*
Methodology and results: The EtOH (ethanol) soluble extract, the $CHCl_3$ (chloroform) phase, the distilled H_2O-EtOAc (water-ethyl acetate) interphase and the EtOAc (ethyl acetate) soluble fractions of the seed-pod of *Acacia nilotica Willd* obtained by extraction and fractionation processes respectively, were investigated using paper disk-plate technique for growth inhibitory effect on *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In addition, 25g of the EtOH soluble extract of the seed-pod of A. nilotica Willd was column chromatograghed using different solvents and solvent mixtures (petroleum ether, petroleum ether-$CHCl_3$, $CHCl_3$-EtOAc, EtOAc, EtOAc-MeOH and MeOH), and column chromatographed fractions were tested on *Artemia salina Leach (brine shrimp)* larvae using brine shrimp bioassay technique which is indicative of pesticidal, cytotoxic and other pharmacological effects. The EtOH soluble fraction of the extract inhibited the growth of *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This extract inhibited the growth of *Staphylococcus aureus* at 2,000µg/ml concentration, whereas for *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa,* it inhibited growth at 5,000µg/ml concentration. The respective growth inhibitory mean values recorded were 3±0.42mm, 5±0.27mm and 8±0.44mm for *Staphylococcus aureus*, 7±0.60mm and 8±0.18mm for *Escherichia coli*, 7±0.20mm and 8±0.05mm for *Klebsiella pneumonia,* 7±0.40mm and 8±0.60mm for *Proteus vulgaris*, and 7±0.40mm and 8±0.44mm for *Pseudomonas aeruginosa*. The $CHCl_3$ soluble fraction inhibited the growth of *Staphylococcus aureus* at the concentrations of 5,000µg/ml and above with the minimum zone of inhibition being 6±0.5mm. The distilled H_2O-EtOAc interphase soluble fraction demonstrated growth inhibitory activity on *Escherichia coli* at concentrations of 5,000µg /ml and above with the minimum zone of inhibition being 6±0.09mm. The EtOAc soluble fraction demonstrated growth inhibitory activity on *Staphylococcus aureus* only at 10,000µg/ml, both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at 5,000µg /ml and above, and both *Escherichia coli* and *Proteus vulgaris* at all the tested concentrations. The respective growth inhibitory mean values recorded were 7±0.40mm for *Staphylococcus aureus*, 7±0.27mm and 8±0.20mm for *Pseudomonas aeruginosa*, 7±0.17mm and 8±0.44mm for *Klebsiella pneumoniae*, 2.1±0.20mm, 3±0.10mm, 4.5±0.20mm and 7±0.10mm for *Escherichia coli*, and 3±0.05mm, 4±0.10mm, 6±0.20mm and 9±0.10mm for *Proteus vulgaris*. The growth inhibitory effect demonstrated by the EtOH soluble extract and other solvent soluble fractions on the bacterial isolates indicated that the seed-pod of

the plant contained antibacterial agents. The activity of column chromatographed fractions AN-8-7, AN-8-12 and AN-8-14 of the seed-pod on *Artemia salina Leach* larvae was also an indication of pesticidal, cytotoxic and Pharmacological effects, and were correlated with the traditional medicinal uses of the plant, with fraction AN-8-12 being the most active at 95 % confidence interval and LC_{50} value of 126 µg/ml concentration.

Conclusion and application of findings: These results supported the use of the seed-pods of *Acacia nilotica Willd* by cattle rearing Fulani men in Northern Nigeria to treat foot and mouth cow disease. It also revealed that the seed-pods can be used to treat diseases caused by the beta-lactamase producing bacteria used in the research which are known to be resistant to some penicillin antibiotics.

Key words: Seed-pod extracts, *Acacia nilotica Willd,* antibacterial effect, column chromatography, *Artemia salina* and cytotoxicity

INTRODUCTION

African plants constitute rich untapped pools of natural products that are yet to be explored. Some of these plants are already used by traditional medical practitioners for the treatment of different ailments including bacterial diseases (Bringmann and Pokorny, 1995). The plant *Acacia nilotica Willd* is a widely used tree from Africa, and the Indian sub-continent. Extracts of various parts of this plant e.g. fruits, gum, roots and stem-bark are used in the form of infusions, decoctions or concoctions to combat dysentery, leprosy, pneumonia, meningitis and malaria in Guinea Bissau, Sudan and Nigeria (Watt *et al*., 1962; Kokwano, 1977; Etkin, 1997 and Taura *et al*., 2004). Currently, natural product chemistry is undergoing a metamorphosis as it is becoming increasingly multidisciplinary with biochemical, pharmacological, medical and biophysical techniques being used. In medicinal plant chemistry for instance, it is now possible to isolate and structurally elucidate microgram quantities of potent bioactive natural products with medicinal effects (Cragg *et al*., 1997). Moreover, it is often mentioned that herbalists prescribing drugs do not have concrete evidences to rule out potential toxicities such as allergic reactions and hepatoxicity (Shaw *et al*., 1997), unlike orthodox drugs that have optimal doses which reduce the risk of extrapyramidal side effects (Lancet, 2003). However, increased abuse of orthodox drugs in the developing countries has raised serious concern about their safety effects on man.

The objective of this study was to screen soluble solvent extracts, and column chromatograghed fractions of the seed-pod of *A. nilotica Willd* for antibacterial activities and toxicity to *Artemia salina Leach* larvae (Brine shrimp), using paper disk-plate and brine shrimp lethality techniques (Pelczar *et al*., 199; Meyer *et al*., 1982; McLaughlin, 1991).

MATERIALS AND METHODS

General experimental procedures: Analytical grade solvents bought from commercial suppliers were redistilled before use. The seed-pod of *A. nilotica Willd* obtained from Worno Bornu about 6 Km South of Azare town, Bauchi State, Nigeria was pulverized and subjected to extraction process using 95% EtOH. The soluble solvent extract obtained from it was then partitioned in to (1) $CHCl_3$, (2) distilled H_2O, (3) distilled H_2O-EtOAc interphase and (4) EtOAc soluble fractions. Soluble solvent extract and fractions were tested for growth inhibition on five clinical bacterial isolates.

Column chromatography was carried out on silica gel (60-120 Merck, net surface area $500m^2/g$, pore volume $0.75cm^3$). Filter agent (celite) was mixed with the EtOH soluble extract before loading on the column. Thin layer chromatography (T.L.C) was performed on 20 x 5cm glass plates coated with 0.5mm silica gel (Merck, T.L.C grade, with gypsum binder and fluorescent indicator). T.L.C bands were viewed under ultraviolet (U.V) lamp (254 and 365nm), and by exposure to iodine. The silica gel plates were prepared by coating slurry of silica gel with distilled H_2O (1g: 3 ml) on plates and activating them at about 120º C for at least 24 hours before use. Brine shrimp eggs (*Artemia salina*), and instant ocean sea salt purchased from Aquarium Systems, Ohio,

U.S.A were used in the brine shrimp lethality test (B.S.T) against samples derived from the column.

Plant material: The seed-pods of *A. nilotica Willd* were collected at random from Worno Bornu about 6 Km South of Azare town, Bauchi State, Nigeria. The part of plant was identified by Baba Ali and authenticated by B.S. Aliyu both of the Biological Sciences Department of Bayero University Kano, Nigeria. A voucher specimen of the seed-pods with voucher number 3/80 was deposited in the herbarium of the same university.

Extraction procedure: Seed-pods of *A. nilotica Willd* air dried at room temperature were pulverized and 500g of the fine power was extracted with 1,500ml of 95 % EtOH for two weeks. The percolate was concentrated on a "Bucchi rotary evaporator (R 110)" at 40 º C, and the EtOH soluble extract obtained (a brown solid of 57.98g in mass) was stored in a sterilized container. Subsequently, 25g of the EtOH soluble extract was partitioned between $CHCl_3$ and distilled H_2O (300ml, 1: 1) using a separating funnel. The $CHCl_3$ soluble fraction was concentrated as the percolate, while the distilled H_2O soluble fraction was washed with 150ml of EtOAc and later partitioned in to the distilled H_2O, the distilled H_2O-EtOAc interphase and the EtOAc soluble fractions. Residues of these three soluble solvent fractions were then derived by separately concentrating them as the percolate.

Antibacterial bioassay: Five bacterial strains *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used in the screening. All the organisms, β-lactamase producing bacteria were clinical isolates obtained from the Department of Microbiology, Amino Kano Teaching Hospital, Kano, Nigeria. A sterile inoculating loop was used to transfer a portion of the colony of each isolate in to a trypton soya broth (T.S. B) that was incubated at room temperature (25◦ C) for three days. Zero point one milliliter (0.1 ml) of the broth was diluted with 1ml of distilled water in a ratio of 1 : 100 (Adoum *et al*., 1997).

A paper punch was used to prepare disks of about 6mm diameter from Whatman number 1 filter paper. Batches of prepared disks were put in to a screw-capped bottle and sterilized in an oven at 140º C for 60 minutes.

The stock solution for the bioassay was prepared by dissolving 10 mg of each test extract in 1ml of dimethyl sulphoxide (D.M.S.O) (i. e. 10,000µg/ml). Three different concentrations of 5,000, 2,000 and 1,000 µg / ml of the plant extracts were prepared using D.M.S.O and the stock solution already prepared. Zero point one milliliter (0.1ml) of each concentration of test extract was introduced in to labeled screw-cap bottles containing 10 disks. The prepared disks were stored in a refrigerator until required for test.

Paper disk-plate method was used (Adoum *et al*., 1997). Saboraud's Dextrose agar (S.D.A) plates were inoculated with standard test inocula by direct streaking. The prepared disks were then introduced on to the inoculated surfaces, and the plates were incubated at room temperature (25◦C) for 48 hours (Pelczar *et al*., 1993).

Cultures were examined for areas of no growth around the disks (zones of inhibition). Organisms sensitive to the test extracts were inhibited at some distance away from the disks, whereas organisms that were not affected grew up to the edges of the disks. In this method, disks impregnated only with D.M.S.O served as control disks (Cheesbrough, 2,000). Diameter of zones of inhibition was measured in millimeters using a ruler.

Column chromatography of EtOH soluble extract: The column (38'' length, 2'' i.d) was packed with 1kg of silica gel in a slurry of petroleum ether. Its content was washed severally with petroleum ether and then chloroform to remove oily materials. The petroleum ether and chloroform washings were redistilled separately using a water bath. The silica gel was later removed from the column and allowed to dry for 4 days. Twenty five grams (25.0g) of the EtOH soluble extract (crude extract) of the seed-pods was mixed with equal portions of celite and silica gel until a homogenous solid mixture was formed. The sample mixture was then carefully loaded on the column that has been packed with the washed silica gel. An additional portion of silica gel (10.0 g) was packed to form a protective layer on top of the adsorbent. The column was then eluted in this order with petroleum ether (1.5 liters), petroleum ether-chloroform (1: 1, 1.5 liters), chloroform (1.5 liters), chloroform-ethyl acetate (1: 1, 1.5 liters), ethyl acetate (1.5 liters), ethyl acetate-methanol (1: 1, 1.5 liters) and methanol (1.5 liters). Eluents were collected in 250 ml portions. Each pooled fraction was concentrated and analyzed on T.L.C. Similar pooled fractions were combined on the basis of T.L.C patterns and given pool numbers (AN) (Table 3).

Isolation of components of AN-8 from EtOH soluble extract: On the basis of T.L.C pattern of AN-8 in different solvent systems, it was selected for further purification. A mass of 0.47 kg of silica gel was packed on a column (length = 8 cm, diameter = 4 cm) in a slurry of petroleum ether. The silica gel was washed

severally with petroleum ether and later with chloroform to remove oily materials. The petroleum ether and chloroform washings were separately distilled using a water bath. The silica gel was then removed from the column and allowed to dry for 4 days. A mass of 2.4749 g of AN-8 was mixed with equal portions of celite and silica gel until a homogenous solid mixture was formed. The sample mixture was then carefully loaded on the column that had been packed with the washed silica gel. An additional portion of silica gel (0.8 g) was packed to form a protective layer on top of the adsorbent. The column was eluted in the following order with eluents collected in portions of 15 ml, 25 ml, 20 ml, 20ml and 100ml: (1: 1, 150ml), (1: 2, 225ml), (1: 4, 400ml) petroleum ether–chloroform, ethyl acetate (80ml), (4 : 1, 150ml), (3: 2, 125ml), (1: 1,150ml) ethyl acetate-methanol and methanol (100ml) respectively. Each pooled fraction was concentrated and analyzed on T.L.C. Similar pooled fractions were combined on the basis of their T.L.C patterns and given pool numbers (Table 4).

Brine Shrimp Lethality Bioassay: *Artemia salina Leach* eggs (50mg) were placed in a hatching chamber containing a solution of Instant Ocean Sea Salt, prepared by dissolving 2.86 g of the salt in 75 ml of distilled water (Meyer *et al*.,1982). The hatching chamber was kept under a fluorescent bulb for 48 hours for the eggs to hatch in to shrimp larvae. A total of 20 mg of pooled factions with pool numbers AN-8-2, AN-8-7, AN-8-12, AN-8-13 and AN-8-14 were separately dissolved in 2 ml of methanol from which 500, 50 and 5µl of each solution was transferred into vials corresponding to 1,000, 100 and 10 µg /ml respectively. Each concentration was tested in triplicate. The vials (9 per test fraction) and one control containing 500µl of solvent were allowed to evaporate to dryness in about 48 hours at room temperature. A 4.5 ml volume of Instant Ocean Sea Salt solution was added to each vial, and 10 larvae of *A. salina* (taken 48-72 hours after the initiation of hatching) were added to each vial. The content in each vial was made up to 5 ml with Sea salt solution after adding the shrimp. After 24 hours, the number of surviving shrimp at each concentration was recorded and LC_{50} values were determined at 95 % confidence intervals by analyzing the data on a Kingtech AT-Compatible Computer loaded with a "Finney Program'' (Table 5).

RESULTS AND DISCUSSION

Distilled H_2O and the distilled H_2O-EtOAc interphase soluble fractions of the seed-pod of *A. nilotica* exhibited no growth inhibitory effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa* at the tested concentrations of 1,000, 2,000, 5,000 and 10,000µg/ml and were not different from the control (Table 1). There was also no growth inhibitory effect demonstrated by the CHCl3 soluble fraction on *Pseudomonas aeruginosa* at these concentrations. Also, it was noted that at 1,000µg /ml, all the soluble extracts or fractions showed no growth inhibitory effect on the bacterial isolates.

The EtOH soluble extract showed no inhibition of growth on *Staphylococcus aureus* at 1,000 µg /ml concentration, whereas at higher concentrations there were zones of inhibition observed. The soluble extract gave mean zones of inhibition of 3 ± 0.42, 5 ± 0.27 and 8 ±0.44 at the respective concentrations of 2,000, 5,000 and 10,000 µg / ml. The EtOH soluble extract in addition to the $CHCl_3$ and EtOAc soluble fractions showed no growth inhibitory effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 1,000 and 2,000 µg / ml concentrations. Both the EtOH soluble extract and the EtOAc soluble fraction exhibited no growth inhibitory effect on *Pseudomonas aeruginosa* at these concentrations, just as the $CHCl_3$ soluble fraction didn't inhibit growth of *Staphylococcus aureus*. On the contrary, at higher concentrations of 5,000 and 10,000 µg / ml, the EtOH soluble extract and the EtOAc soluble fraction inhibited *Pseudomonas aeruginosa* with mean zones of inhibition of 7 ± 0.4 and 8 ± 0.0 4 for the EtOH soluble extract, and 7 ± 0.27 and 8 ± 0.2 for the EtOAc soluble fraction. The $CHCl_3$ soluble fraction inhibited *Staphylococcus aureus* with mean zones of inhibition of 6 ± 0.5 and 7 ± 0.27 at 5,000 and 10,000 µg /ml respectively. 10,000 µg / ml of the EtOAc soluble fraction inhibited *Staphylococcus aureus* with a mean zone of inhibition of 7 ± 0.40. Results (table 2) clearly showed that the $CHCl_3$ and distilled H_2O soluble fractions of the seed-pod of *Acacia nilotica Willd* did not inhibit the growth of *E. coli, K. pneumoniae* and *P. vulgaris* at the concentrations of 1,000 , 2,000 ,5,000 and 10,000µg /ml.

Table 1: Zones of inhibition (mm) exhibited by seed-pod extracts of *Acacia nilotica* on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Soluble solvent extract/fractions	***Staphylococcus aureus*** **Concentration (µg/ml)**				***Pseudomonas aeruginosa*** **Concentration (µg/ml)**				
	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative control
EtOH	0	3±0.42	5±0.27	8±0.44	0	0	7±0.40	8±0.04	0
$CHCl_3$	0	0	6±0.50	7±0.27	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0
Distilled H_2O-EtOAc	0	0	0	0	0	0	0	0	0
EtOAc	0	0	0	7±0.40	0	0	7±0.27	8±0.20	0

Key: ± = standard error mean; 0 = no growth inhibitory effect

Table 2: Zones of inhibition (mm) exhibited by seed-pod extracts of *Acacia nilotica Willd* on *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*

Soluble solvent extract/fraction	**E. coli Concentration (µg/ml)**				**K. pneumonia Concentration (µg/ml)**				**P. vulgaris Concentration (µg/ml)**				
	1000	2000	5000	10000	1000	2000	5000	10000	1000	2000	5000	10000	-ve control
EtOH	0	0	7±0.60	8±0.180	0	0	7±0.40	8±0.60	0	0	7±0.20	8±0.050	
CHCl3	0	0	0	0	0	0	0	0	0	0	0	0	0
Distilled H2O	0	0	0	0	0	0	0	0	0	0	0	0	0
Distilled H2O-EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0
EtOAc	3±0.050	4±0.10	6±0.20	9±0.10	2.1±0.20	3±0.10	4.5±0.20	7±0.10	0	0	7±0.170	8±0.440	0

Key: ± = standard error mean; 0 = no growth inhibitory effect

No growth inhibitory effect was exhibited by the distilled H_2O-EtOAc interphase fraction on *K.pneumoniae* and *P. vulgaris* at all the tested concentrations. At 1,000 and 2,000 µg /ml, the EtOH soluble extract and the distilled H_2O-EtOAc interphase soluble fraction did not inhibit growth of *E. coli, K.* pneumonia and *P. vulgari.* The trend of no growth inhibitory effect was also exhibited by the EtOAc soluble fraction on *K. pneumoniae* at concentrations of 2,000µg /ml and below. On the contrary, the EtOH soluble extract presented growth inhibitory effects on the three clinical isolates at higher concentrations of 5,000 and 10,000 µg /ml. The recorded mean zones of inhibition were 7±0.40 and 8±0.60 for *E. coli*, 7±0.60 and 8±0.180 for *P. vulgaris,* and 7±0.20 and 8±0.050 for *K. pneumoniae.* The distilled H_2O-EtOAc interphase and the EtOAc soluble fractions separately recorded mean zones of inhibition of 6±0.09 and 7±0.10 for *E. coli,* and 7±0.10 and 8±0.44 for *K. pneumoniae* at the respective concentrations of 5,000 and 10,000µg/ml. The EtOAc soluble fraction inhibited *E. coli* and *P. vulgaris* with increasing inhibition as concentrations increased. For *E. coli,* the mean zones of inhibition were 2.1±0.20, 3±0.10, 4.5±0.20 and 7±0.10 at concentrations of 1,000 , 2,000 , 5,000 and 10,000 µg /ml respectively, whereas for *P. vulgaris* the means were 3±0.05, 4±0.10, 6±0.20 and 9±0.10 at the respective concentrations 1,000, 2,000, 5,000 and 10,000 µg /ml. The EtOH soluble extract and other solvent soluble fractions of the seed-pod of *Acacia nilotica Willd* contained antibacterial agents.

Table 3: Column chromatography results of the EtOH soluble extract

Column eluents	Single or pooled fraction	Pool number	Weight (g)	% Recovery
100 % Petroleum ether	1, 2, 3 & 4	-	-	-
Petroleum ether-$CHCl_3$(1:1)	5	-	-	-
100 % $CHCl_3$	6	-	-	-
$CHCl_3$-EtOAc (1:1)	7	AN-1	0.006	0.0024
$CHCl_3$-EtOAc (1:1)	8	AN-2	0.0077	0.0308
$CHCl_3$-EtOAc (1:1)	9	-	-	-
$CHCl_3$-EtOAc (1:1)	10	AN-3	0.1018	0.4072
$CHCl_3$-EtOAc (1:1)	11	AN-4	2.0793	8.3172
$CHCl_3$-EtOAc (1:1)	12	AN-5	2.2891	9.1584
$CHCl_3$-EtOAc (1:1)	13	AN-6	2.2277	8.9108
$CHCl_3$-EtOAc (1:1)	14	AN-7	2.2896	9.1584
100 % EtOAc	15 & 19	AN-8	2.4749	9.8996
100 % EtOAc	16, 17 & 18	AN-9	2.2325	8.930
EtOAc-MeOH (1 : 1)	20	AN-10	0.1728	0.6912
EtOAc-MeOH (1 : 1)	21 & 22	AN-11	0.0945	0.3780
EtOAc-MeOH (1 : 1)	23	AN-12	0.1568	0.6272
EtOAc-MeOH (1 : 1)	24	AN-13	0.4621	1.8484
EtOAc-MeOH (1 : 1)	25	AN-14	0.9765	3.9060
100 % MeOH	26, 27 & 28	AN-15	2.8798	11.5172
100 % MeOH	29	AN-16	0.1991	0.7964
100 % MeOH	30 & 31	AN-17	4.1325	16.530

Table 4: Column chromatography results of AN-8 fraction

Column eluents	Single or pooled Fraction	Pool number	Weight (g)	% Recovery
Petroleum ether-$CHCl_3$ (1:1)	1, 2, 3, 4, 5, 6, 7, 8, 9 & 10	-	-	-
Petroleum ether-$CHCl_3$ (1 : 2)	11, 12, 13, 14, 15, 16, 17, 18 & 19	-	-	-
Petroleum ether-$CHCl_3$ (1: 4)	20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 & 31	-	-	-
100 % EtOAc	32	AN-8-1	0.0541	2.185
	33	AN-8-2	0.1047	4.230
	34	AN-8-3	0.0630	2.545
	35	AN-8-4	0.1009	4.076
EtOAc-MeOH (4 : 1)	36	AN-8-5	0.0484	1.955
	37	AN-8-6	0.328	1.325
	38	AN-8-7	0.2167	8.755
	39	AN-8-8	0.0720	2.909
	40	AN-8-9	0.0584	2.359
	41	AN-8-10	0.03924	1.585
EtOAc-MeOH (3 : 2)	42	AN-8-11	0.0401	1.620
	43	-	-	-
	44	AN-8-12	0.1009	4.076

	45	AN-8-13	0.1301	5.256
	46	-	0.0476	1.925
	47	-	0.0131	0.529
	48	-	0.0438	1.769
100 % MeOH	49	AN-8-14	0.173	6.994

The EtOH soluble extract was chromatograghed on silica gel and eluents were analyzed by T.L.C. Similar pooled fractions, 15 and 19 eluted with 100 % EtOAc were combined to give fraction, AN-8 (Table 3), which was further chromatographed. Fractions AN-8-2, AN-8-7, AN-8-12, AN-8-13 and AN-8-14 were eluted using 100 % EtOAc, EtOAc-MeOH (4:1), EtOAc-MeOH (3:2) and 100 % MeOH respectively (Table 4).

Table 5: The activity of some column chromatographed fractions of the seed-pods of *Acacia nilotica Willd* on *Artemia salina Leach* larvae

Column chromatographed fraction	**$^{a}LC_{50}$ Value (µg/ml)**
AN-8-2	> 1,000
AN-8-7	291 (125-117)
AN-8-12	126 (231-69)
AN-8-13	> 1,000
AN-8-14	331 (545-204)

$^{a}LC_{50}$ µg /ml (95 % confidence interval)

Of the different available procedures, the brine shrimp lethality bioassay was selected for screening the seed-pods of *Acacia nilotica Willd* because it is a convenient general bioassay and it is indicative of pesticidal, cytotoxic and other pharmacological effects (Mikolojczak *et al*., 1988, 1989; Rupprect *et al*., 1990). Results presented in table 4 show active cytotoxic components which are column chromatographed fractions AN-8-7, AN-8-12 and AN-8-14, and from the fore going, the most potent fraction is identified as AN-8-12 with an LC_{50} value of 126 µg /ml at 95 % confidence interval. Fifty percent and above of the *Artemia salina Leach* larvae were killed by these fractions at concentrations below 1,000µg/ml, whereas column chromatograghed fractions AN-8-2 and AN-8-13 were inactive at these concentrations. Work on the structure of bioactive component AN-8-12 will be reported in future communications.

CONCLUSION

The growth inhibitory effect shown by the EtOH soluble extract and other solvent soluble fractions of the seed-pod of *Acacia nilotica Willd* on *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* indicated the presence of antibacterial agents in the seed-pod. Also, the activity of column chromatograghed fractions, AN-8-7, AN-8-12 and AN-8-14 of the seed-pod at concentrations of 291, 126 and 331µg/ml respectively on *Artemia salina Leach* larvae was an indication of pesticidal, cytotoxic and Pharmacological effects. These results supported the use of the seed-pod by cattle rearing Fulani men in Northern Nigeria to treat mouth cow disease, and also revealed that the seed-pod can be used to treat diseases caused by the beta-lactamase producing bacteria used in the research which are known to be resistant to some penicillin antibiotics
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