Albanian j. agric. sci. 2015; 14 (2): 149-156 *Agricultural University of Tirana*

RESEARCH ARTICLE (Open Access)

Toxicity of *Acanthus ilicifolius* (L) Fractions Against *Pratylechus* spp. on Maize (*Zea mays*)

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Abstract

Pollution problems associated with the control of plant parasitic nematodes, necessitated research into alternative nematode management methods. The roots of *Acanthus ilicifolius* were evaluated for its phytochemical constituents and nematicidal activity. Methanol and n-hexane extracts of *A. ilicifolius* roots were chromatographed on silica gel column (100-120µm mesh grades). Six fractions were tested on *Pratylenchus* spp infecting maize in the field. Generally enhanced plant development was observed in the treated plants. The polar fractions were significantly ($P<0.05$) more effective and compared well with a commercial nematicide (Mocap). Maize treated with the polar fractions tasselled earlier, with significantly higher yield. In the laboratory the polar fractions produced significantly higher mortality than non polar fractions with 48.07% mortality at three hours of exposure, while the non polar fractions recorded 15.22% mortality. Results of the phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids and anthraquinone, while friedlan-3-one, oleanolic acid, β-sitosterol, lupeol, quercetin, 3,3-dimethyl hexanone, and octadecanoic acid,2-hydroxyl-1,3-propanediyl ester are the bioactive compounds identified by gas chromatography-mass spectroscopy. The bioactivity of the fractions from *A. ilicifolius* is attributed to the presence of the above named compounds. Bio compounds from plants will go a long way in the management of plant parasitic nematodes.

Keywords: Chromatography; toxicity, nematodes; bio-pesticide.

1. Introduction

Maize (*Zea mays* L.) is the most important cereal crop in Nigeria on the basis of its economic value. It has been in the diet of Nigerians for centuries, and has now risen to a commercial crop on which many agro-based industries depend as raw materials [11]. It is an important source of carbohydrate, proteins and essential minerals. It provides useful quantities of vitamins A, C and E. It is also rich in dietary fibre and calories which are a good source of energy [16]. Approximately 4.5million tons of maize grain are produced in Nigeria annually. Yearly there is an increase in the demand for maize as the population increases, thus acquiring higher yield is a function of proper farm management. Plant parasitic nematodes are one of the major factors militating against higher output of maize grain in Nigeria; they are present in all maize production areas of the country. Several nematode species are known to damage maize but *Pratylenchus* spp may be particularly damaging, they infect about 400 plant species including some important crops [15]. They are one of the most widely distributed and economically important groups of plant parasitic nematodes; which interact with various fungi in disease complexes [15]. They have been recorded around cereals, legumes, temperate fruits, sunflower, natural grassland and even in forest plants [12]. *Pratylenchus* spp may also cause significant damage to tomatoes and other important crops and vegetables [19]. A decrease in maize weight and size, as well as delay in fruit ripening when nematode populations reached 230–590 nematodes per gram of root has been reported [20]. Fungal and bacterial root pathogens in maize can also be enhanced by the presence of *P. penetrans* [20]. *Pratylenchus* spp cause root lesion through feeding on the root cells and thus impairing the uptake of nutrients from the soil. Infected plants are characterized by chlorosis, root necrosis, reduced yield and finally death of the crop.

Several methods are employed in the control of root lesion nematode, one of which is the use of synthetic nematicides. The high cost of synthetic nematicide is a major factor to the economic control of nematodes on maize. However this method has so far been the most effective but with its attendant pollution problems. The secondary metabolites produced by synthetic nematicides pollute the environment. The conventional farming practices

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(Accepted for publication June 05, 2015)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

which uses chemical methods do result in the malfunctioning of the food chain. Bio-control is the best method to cope with the losses done by chemicals. There is thus the need to investigate plants for cheaper nematicides which are also environmentally friendly. The use of natural compounds as nematicides is receiving increased attention due to their non-toxicity to non target organisms in the ecosystem and humans in general. In view of this; investigation was made into the roots of *Acanthus ilicifolius* for its possible nematicidal potential. *A. ilicifolius* belongs to the family Acanthaceae. The roots of *A. ilicifolius* are used in the treatment of rheumatism, kidney stones, asthma, neuralgia, snake bites and arrow poison wounds. The pounded seeds have been indicated in the treatment of boils, while juice from leaves prevent hair loss [13]. Water extracted from the bark is used for colds and skin allergies. Ground fresh root serves as antiseptic, while tea brewed from the leaves relieves pain and purifies the blood [10]. Several studies have documented the use of plant metabolites in the control of plant parasitic nematodes, but extensive literature review revealed that the nematicidal potential of *A. ilicifolius* has not been investigated. This research was carried out to investigate the possible nematotoxic and nematostatic potential of the roots of *A. ilicifolius*.

2. Material and Methods

2.1 Extraction of Plant Materials

A. ilicifolius plants were collected from a private home garden in Ibadan Oyo state Nigeria, and identified at the herbarium unit of the University of Ibadan. The roots were washed free of adhering soil particles and then cut into tiny pieces. Six hundred grams (600g) of the plant material was soaked separately in n-hexane and methanol for five days. Each extract was evaporated under reduced pressure using rotary evaporator. The n-hexane and methanol crude extracts were weighed (121 g and 160 g respectively). Fifty grams each of the crude extracts were subjected to open column chromatography over silica gel (110-120µm mesh grade), which afforded eight fractions each and the individual fractions were purified over preparative thin layer chromatographic plates.

2.2 Spectroscopic measurements:

The purified fractions were subjected to GC/MS analysis on a Gas Chromatography-Mass Spectroscopy system; GCMS-QP 2010 Plus (Shimadzu Japan) interfaced with a finigan MAT ion trap detector ion source Temp., RTX5MS column packed with 100% dimethylpolysiloxane with the following settings; 200^{o}C, interfaced Temp., 250^{o}C, solvent cut time; 2.50 min; relative detector mode, ACQ mode; Scan; start time - end time; 3.00 min - 46.00 min, event time, 0.50 sec; scan speed, 1428. Identification of the components was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC-MS; their spectral were compared with those of NIST library mass spectra.

2.3 Laboratory test:

Matured maize plants were removed from the soil, taking care not to damage fine roots. The root systems were removed from the plants, placed in plastic bags, and stored at 25^{0}C until processed. The roots were washed, cut into 2-3 cm length and macerated with some distilled water in a warring blender for 20 seconds. The water in the blender was decanted onto a 45µm aperture sieve and then washed onto a wet two-ply facial tissue paper in a modified Baermann tray. After 24 hours nematodes were drawn off from the water, this was standardized and 1 mL contains 28 nematode juveniles and 20 mL nematode suspension (560 juveniles) was used for each assessment *in vitro*. Three fractions were taken from each extract plus their crudes and mocap, these gave a total of nine treatments. The experimental design was a 9x4x3 factorial design conducted in randomised complete design (RCD), involving nine treatments, at four levels and each replicated three times. Thirty milligrams each of the fractions was dissolved in 50 mL distilled water, made up to 100mL solution by the addition of 50mL of emulsifier to aid solubility of the fractions. This forms hundred percent concentration from which serial dilutions were made as follows: 2mL concentrated fraction + 6mL distilled water = 25%; 4mL concentrated fraction + 4mL distilled water = 50%; 6mL concentrated fraction + 2mL distilled water = 75%. Distilled water served as 0%. The same dilution was used for the crude extract and mocap. Counting of nematodes was done under the stereomicroscope at x50 magnification, nematodes

which did not respond to the touch of fine needles were considered dead.

2.4 Field sampling:

The experimental field was sampled for Initial nematode population before and after inoculation. Soil samples were taken systematically at a depth of 8 inches each in between the rows and on the ridges to represent the experimental field. Nematodes in the soil samples were extracted using the modified Baerman method of extraction [23].

2.5 Field Experiment:

Experiments were conducted during the 2011 and 2012 growing season at the University of Ilorin teaching and research farm, Ilorin **(**Lat 8^0, 29^1 N of the Equator; Long: 4^0, 40^1 E of the Greenwich Meridian), in the southern guinea savannah ecological zone. Maize plots consisted of four 15 m long rows spaced 1 m apart with 30 cm plant spacing within the rows. Four seeds were sown per hole, and this was thinned down to two plant stand after two weeks of germination. The experimental design was also a 9x4x3 factorial design conducted in randomised complete block design (RCBD), involving nine treatments, at four levels and each replicated three times. This gave a total of one hundred and eight (108) plots for each of the experiments. Fifty milligrams each of the fractions was dissolved in 100 mL distilled water, made up to 200mL solution by the addition of 100mL of emulsifier to aid solubility of the fractions. This forms the stock solution from which serial dilutions were made as follows: 5ml concentrated fraction + 25ml distilled water = 25%; 15ml concentrated fraction + 15ml distilled water = 50%; 25ml concentrated fraction + 5ml distilled water = 75%. Distilled water served as 0%. The same dilution was used for the crude extract and mocap. Data were taken from the field on plant height. Days to 50% tasseling was recorded as tasseling was observed. Fruit weight per plant was taken after harvest while nematode population per 300g of soil sample and nematode population in 20g root samples were taken and recorded after the experiment in the laboratory.

2.6 Statistical Analysis:

All data collected were subjected to analysis of variance (ANOVA) and significant means separated with the Duncan's multiple range tests [7].

2.7 Phytochemical analysis:

Preliminary Phytochemical screening was carried out on the concentrated extracts according to standard methods [21].

3. Results and Discussion

A. ilicifolius was found to contain terpenoids, tannins, saponins, alkaloids and anthraquinone in larger quantity while flavonoids occur in traceable amount in the n-hexane and methanol extract (Table 1). The results of the in-vitro nematicidal assay of chromatographic fractions from *A. ilicifolius* n-hexane and methanol extracts are shown in Tables 2 and 3. From Table 2, the chromatographic fractions were toxic to *Pratylenchus zea* juveniles. The toxicity of the fractions increased with the concentration and time of exposure. The third fraction from *A. ilicifolius* methanol extract (ACNI/$MeOH_3$/CC) was the most effective. The other fractions from methanol extract were significantly more effective than the fractions from n-hexane (ACN1/Hex_1/CC; ACN1/Hex_2/CC; ACN1/Hex_3/CC) while the crude extracts (ACNI/Hex/CRD; ACNI/MeOH/CRD) exhibited weak toxicity on the juveniles. The rate (level) of application of the treatments were also significantly ($p<0.05$) effective with the highest level of concentration being the most effective with 14.43 % mortality at three hours of exposure. The chromatographic fractions also inhibited egg hatching, there was no egg hatch recorded in the fractions throughout the period of observation, although a few egg hatches were recorded in the n-hexane crude extract **(**ACNI/Hex/CRD) (Table 3). Maize plant heights were significantly ($p<0.05$) higher in plants treated with polar fractions and commercial synthetic nematicide (ACNI/MeOH3/CC; ACNI/MeOH2/CC; MOCAP) while the heights of maize plants treated with non fractionated crude extracts were significantly ($p<0.05$) low. Days to 50 percent tasseling was also significantly earlier in plants which received mocap and methanol fractions of *A. ilicifolius* in the first and second trial (Table 5). Yield was lower in maize plants treated with ordinary crude extracts, but the chromatographic fractions produced significantly

higher yield at harvest (Table 7). Nematodes were significantly fewer in 300g soil and 20g root sample of maize plants which received the fractions, more nematodes were recovered from the root and soil of plants treated with crude extracts, while nematode population in plants treated with methanol crude was significantly lower than those in the soil and root of plants treated with n-hexane crude (Table 6); this was however significantly lower than the initial nematode population on the field before and at planting which was 189.54 and 351.22 respectively. The GC/MS results revealed the presence of compounds such as octadecanoic acid ethyl ester (8.10%); 3, 3-dimethyl hexanone (18.22%); hexadecanoic acid ethyl ester (3.07%); Octadecanoic acid, phenyl methyl ester (21.04%); 12-Oleanen-3yl acetate (33.15%) and beta-amyrin acetate (16.42%).

Phytochemicals	**n-Hexane extract**	**Methanol extract**
Anthraquinone	+	+++
Alkaloids	+++	+++
Flavonoids	+	+
Glycosides	+++	+++
Saponins	+++	+++
Tannins	+++	+++
Terpenoids	+++	+++

$^{+}$traceable amount; $^{+++}$appreciable amount

Explanation of abbreviations:

ACNI/Hex_1/CC-*Acanthus ilicifolius* n-hexane fraction one;
ACNI/Hex_2/CC-*Acanthus ilicifolius* n-hexane fraction two;
ACN1/Hex_3/CC-*Acanthus ilicifolius* n-hexane fraction three;
ACNI/$MeOH_1$/CC-*Acanthus ilicifolius* methanol fraction one;
ACNI/$MeOH_2$/CC-*Acanthus ilicifolius* methanol fraction two;
ACNI/$MeOH_3$/CC-*Acanthus ilicifolius* methanol fraction three;
ACNI/Hex/CRD-*Acanthus ilicifolius* n-hexane crude extract;
ACNI/MeOH/CRD-*Acanthus ilicifolius* methanol crude extract;
MOCAP- synthetic nematicide.

Table 1: Phytochemicals in *A. ilicifolius*

Table 2: Effect of treatment and level of application of chromatographic fractions from *A. ilicifolius* on Juvenile mortality of *Pratylenchus zea*

	Exposure Time				
Treatments	**3hrs**	**6hrs**	**9hrs**	**12hrs**	**24hrs**
ACNI/Hex_1/CC	15.22^{f}	24.39^{f}	33.02^{f}	38.00^{f}	55.12^{f}
ACNI/Hex_2/CC	20.10^{e}	29.00^{e}	36.14^{e}	43.10^{e}	59.29^{e}
ACN1/Hex_3/CC	26.33^{d}	35.04^{d}	42.22^{d}	49.15^{d}	66.70^{d}
ACNI/$MeOH_1$/CC	32.68^{c}	40.61^{c}	46.09^{c}	55.23^{c}	74.00^{c}
ACNI/$MeOH_2$/CC	39.12^{b}	46.30^{b}	53.32^{b}	61.48^{b}	79.19^{b}
ACNI/$MeOH_3$/CC	48.07^{a}	55.16^{a}	61.21^{a}	72.74^{a}	86.52^{a}
ACNI/Hex/CRD	8.51^{h}	11.09^{h}	19.07^{h}	26.20^{h}	44.72^{h}
ACNI/MeOH/CRD	13.00^{g}	20.33^{g}	25.02^{g}	36.63^{g}	53.03^{g}
MOCAP	47.59^{a}	55.45^{a}	60.83^{a}	73.30^{a}	87.13^{a}
S.E.M	2.99	3.10	3.25	3.51	3.95
Treatment level (%)					
0	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
25	1.51^{c}	2.78^{c}	4.67^{c}	8.41^{c}	12.89^{c}
50	7.56^{b}	13.98^{b}	18.22^{b}	24.76^{b}	37.90^{b}
75	14.43^{a}	19.26^{a}	27.64^{a}	33.00^{a}	46.18^{a}
S.E.M.	0.32	0.91	0.99	1.13	1.91

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new DMRT.

DMRT=Duncan's multiple range test.

Table 3: Effect of treatment and level of application of chromatographic fractions from *A. ilicifolius* on egg hatch of *Pratylenchus zea*

	Time of Exposure				
Treatments	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**
ACNI/Hex$_1$/CC	0.00	0.14^{b}	0.20^{b}	0.24^{b}	0.31^{b}
ACNI/Hex$_2$/CC	0.00	0.06^{b}	0.10^{c}	0.15^{c}	0.19^{c}
ACN1/Hex$_3$/CC	0.00	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}
ACNI/MeOH$_1$/CC	0.00	0.00$_{b}$	0.00^{c}	0.00^{c}	0.00^{c}
ACNI/MeOH$_2$/CC	0.00	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}
ACNI/MeOH$_3$/CC	0.00	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}
ACNI/Hex/CRD	0.00	1.24^{a}	2.33^{a}	3.42^{a}	5.51^{a}
ACNI/MeOH/CRD	0.00	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}
MOCAP	0.00	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}
	N.S				
S.E.M	0.00	0.00	0.00	0.01	0.02
Treatment Level (%)					
0	12.33^{b}	16.19^{b}	28.64^{b}	37.11b	43.22^{b}
25	0.00^{a}	0.00^{a}	0.00^{a}	0.05^{a}	0.09^{a}
50	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
75	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
S.E.M	0.16	0.19	0.21	0.27	0.32

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the new DMRT.

Table 4: Effect of treatments and level of application of fractions from *A. ilicifolius* and mocap on plant height (cm) of maize infected with *Pratylenchus* spp (1st and 2nd trials) in the field.

Treatments	**5wap**		**7wap**		**9wap**		**13wap**	
	1st	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
ACNI/Hex$_1$/CC	17.12^{b}	14.00^{b}	34.04^{b}	32.30^{b}	55.12^{c}	59.05^{c}	103.17^{d}	100.01^{d}
ACNI/Hex$_2$/CC	17.16^{b}	14.02^{b}	34.20^{b}	32.59^{b}	55.25^{c}	59.00^{c}	103.23^{d}	100.29^{d}
ACN1/Hex$_3$/CC	17.25^{b}	14.11^{b}	34.34^{b}	32.15^{b}	55.42^{c}	59.23^{c}	103.00^{d}	100.18^{d}
ACNI/MeOH$_1$/CC	25.04^{a}	23.01^{a}	41.86^{a}	45.13^{a}	64.22^{b}	68.09^{b}	119.28^{c}	115.60^{c}
ACNI/MeOH$_2$/CC	25.32^{a}	23.09^{a}	41.16^{a}	45.06^{a}	64.38^{b}	68.34^{b}	130.00^{b}	124.12^{b}
ACNI/MeOH$_3$/CC	25.28^{a}	23.24^{a}	41.31^{a}	45.26^{a}	72.31^{a}	77.72^{a}	141.80^{a}	137.72^{a}
ACNI/Hex/CRD	11.06^{c}	10.01^{c}	25.18^{c}	21.22^{c}	46.11^{d}	49.29^{d}	90.14^{f}	88.11^{f}
ACNI/MeOH/CRD	11.16^{c}	10.33^{c}	25.21^{c}	21.39^{c}	46.29^{d}	49.30^{d}	96.26^{e}	92.43^{e}
MOCAP	25.07^{a}	23.31^{a}	41.22^{a}	45.10^{a}	72.56^{a}	77.00^{a}	141.12^{a}	137.29^{a}
S.E.M	0.13	0.17	0.21	0.28	0.32	0.26	0.33	0.20
Level (%)								
0	3.14^{d}	3.08^{d}	10.18^{d}	11.21^{d}	21.21^{d}	24.89^{d}	55.15^{d}	52.28^{d}
25	9.07^{c}	7.19^{c}	16.07^{c}	19.26^{c}	37.31^{c}	40.05^{c}	109.11^{c}	103.79^{c}
50	14.31^{b}	12.22^{b}	23.31^{b}	26.09^{b}	44.08^{b}	47.39^{b}	116.00^{b}	112.33^{b}
75	19.22^{a}	17.57^{a}	30.00^{a}	33.17^{a}	51.29^{a}	54.00^{a}	123.19^{a}	119.94^{a}
S.E.M	0.08	0.11	0.16	0.12	0.09	0.10	0.03	0.15

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the new DMRT.

Table 5: Effect of treatments and level of application of fractions from *A. ilicifolius* and mocap on number of days to 50% tasseling of maize infected with *Pratylenchus* spp (1st and 2nd trials) in the field.

Treatments	Days to 50% tasselling	
	1st trial	2nd trial
ACNI/Hex_1/CC	64.16[d]	64.23[d]
ACNI/Hex_2/CC	64.28[d]	63.89[d]
ACNI/Hex_3/CC	63.52[d]	62.71[d]
ACNI/$MeOH_1$/CC	60.01[c]	60.19[c]
ACNI/$MeOH_2$/CC	57.14[b]	56.88[b]
ACNI/$MeOH_3$/CC	55.02[a]	55.17[a]
ACNI/Hex/CRD	75.28[f]	76.09[f]
ACNI/MeOH/CRD	70.14[e]	70.25[e]
MOCAP	54.71[a]	55.11[a]
S.E.M	0.31	0.29
Level (%)		
0	55.21[d]	56.05[d]
25	49.36[c]	50.16[c]
50	44.18[b]	45.33[b]
75	39.04[a]	38.25[a]
S.E.M	0.22	0.19

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the new DMRT.

Table 6: Effect of treatments and level of application of fractions from *A. ilicifolius* and mocap on nematode populations of maize infected with *Pratylenchus* spp (1st and 2nd trials) in the field at harvest.

Treatments	Nematode population in 300g soil		Nematode population in 20g root sample	
	1st trial	2nd trial	1st trial	2nd trial
ACNI/Hex_1/CC	8.28[c]	11.19[c]	3.15[c]	5.49[c]
ACNI/Hex_2/CC	3.11[b]	5.10[b]	1.27[b]	2.07[b]
ACNI/Hex_3/CC	3.09[b]	4.89[b]	1.31[b]	1.84[b]
ACNI/$MeOH_1$/CC	2.60[b]	4.74[b]	1.21[b]	1.67[b]
ACNI/$MeOH_2$/CC	2.71[b]	5.13[b]	0.89[b]	2.14[b]
ACNI/$MeOH_3$/CC	3.26[b]	4.68[b]	1.10[b]	2.10[b]
ACNI/Hex/CRD	19.18[e]	23.08[e]	11.00[e]	16.12[e]
ACNI/MeOH/CRD	13.34[d]	19.17[d]	8.11[d]	11.06[d]
MOCAP	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M	0.06	0.01	0.22	0.18
Level (%)				
0	345.09[d]	329.18[d]	119.39[d]	131.05[d]
25	78.11[c]	82.43[c]	44.15[c]	47.23[c]
50	14.00[b]	10.00[b]	3.00[b]	2.03[b]
75	3.18[a]	1.02[a]	0.24[a]	0.76[a]
S.E.M	1.14	1.19	0.98	1.04

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the new DMRT.

Table 7: Effect of treatments and level of application of fractions from *A. ilicifolius* and mocap on yield of maize infected with *Pratylenchus* spp (1st and 2nd trials) in the field.

Treatments	Yield (kg/ha^{-1}) 1st Trial	Yield (kg/ha^{-1}) 2nd Trial
ACNI/Hex_1/CC	29.79^{d}	31.29^{d}
ACNI/Hex_2/CC	30.02^{d}	32.09^{d}
ACNI/Hex_3/CC	30.16^{d}	32.18^{d}
ACNI/$MeOH_1$/CC	34.22^{c}	36.27^{c}
ACNI/$MeOH_2$/CC	39.14^{b}	41.10^{b}
ACNI/$MeOH_3$/CC	44.07^{a}	47.06^{a}
ACNI/Hex/CRD	16.28^{f}	18.13^{f}
ACNI/MeOH/CRD	23.12^{e}	25.03^{e}
MOCAP	43.89^{a}	47.29^{a}
S.E.M	1.07	1.12
Level (%)		
0	9.11^{d}	10.21^{d}
25	14.27^{c}	16.33^{c}
50	20.12^{b}	21.04^{b}
75	27.05^{a}	29.18^{a}
S.E.M	0.10	0.13

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05 using the new DMRT.

Chromatographic fractions from *A. ilicifolius* have been found to be effective in inhibiting egg hatch and inducing mortality. The potency of the chromatographic fractions could be attributed to the presence of secondary metabolites in the plant. The nematicidal activities of plants in general have been linked to the presence of secondary metabolites [5]. The result of the phytochemical constituents of *A. ilicifolius* reported in this study is in agreement with the findings of Dharya and Vidhu [4]. Several plants have been established to possess nematicidal activity against a number of nematodes. Strong nematicidal compounds such as alpha-terthienyl and some derivatives have been shown to be present in *Tagetes* species and toxic to nematodes [22, 3, 8, and 2]. Essential oils from *Ocimum* spp was established to be toxic to *P. brachyrus* on tomato at 100 µg mL^{-1}, while the aqueous extracts of *Tagetes* spp was also indicated to be toxic to root knot nematode *Meloidogyne incognita* [18, 19]. Neem triterpenes used as coating materials, root dips and seed treatments have been found to be nematicidal against several species of plant parasitic nematodes of vegetables and legumes [1, 9].The compounds identified by GC/MS in *A. ilicifolius* are mainly fatty acid esters; many of these have been demonstrated to be nematicidal. Fabiyi *et al*., [6] reported the nematicidal activity of fatty acid esters from *Alstonia boonei* and *Bridelia ferruginea*. The anti microbial activity of the n-hexane, chloroform, and methanol extracts of the roots and leaves of *A. ilicifolius* was established by Khajure and Rathod. The extract exhibited strong activity against *Aspergillus niger, Bacillus subtilis, Staphylococcus aureus, Candida albicans, and Aspergillus fumigates [14]*. The nematicidal activity of the fractions from *A. ilicifolius* is being reported in literature for the first time.

4. Conclusions

The results obtained in this study showed that the column chromatographic fractions of n-hexane and methanol extract of *A. ilicifolius* is nematicidal and this supports the hope that bio-nematicides would effectively control nematodes on agricultural fields. Further investigation of the chromatographic fractions on other plant parasitic nematodes is suggested.

5. References

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