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NEMATICIDAL ISOCHROMANE GLYCOSIDE FROM *Kigelia pinnata* LEAVES

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Nematicidal isochromane glycoside from Kigelia pinnata leaves

Synthetic nematicides such as oxamyl and carbofuran play significant roles in the management of plant-parasitic nematodes. However, their negative environmental impacts have it imperative to search for safer alternatives. As part of our contribution in the search for bio-nematicides, compounds from plant extract were screened for possible potent nematicidal agent. A new isochromane carboxylic acid glycoside, isolated from the leaves of *Kigelia pinnata* (Lam.) Benth (Bignoniaceae) was evaluated for its nematicidal activity. The structure of the proposed compound was characterized by various spectroscopic methods, which included UV, FTIR, 1D-, and 2D-NMR, FAB-MS, TOF-ESI-MS and TOF-ESI-MS/MS (TANDEM). The *in vitro* experiment conducted on the glycoside against *Meloidogyne incognita* juveniles and eggs indicated an induced mortality. Its activity can be compared favourably with oxamyl, when tested at 0.1 mg/mL concentration. At four hours of observation, no significant difference ($P < 0.05$) between oxamyl and the glycoside was observed. The present data sustains that natural glycoside is a promising oxamyl alternate for controlling nematode-induced plant root knots and may contribute to integrated pest management.

Key words: plant protection / nematodes / *Kigelia pinnata* / isochromane glycoside / nematicides

Nematocidni i-kromanski glikozid iz listov rasline Kigelia pinnata

Sintetični nematocidi, kot na primer oxamyl in karbofuran igrajo pomembno vlogo pri zatiranju nematod, ki parazitirajo na rastlinah, zaradi njihovega negativnega vpliva na okolje pa je nujno iskanje varnejših alternativ. Naš prispevek na področju raziskav bioloških nematocidov je poskus osamitve potencialnih nematocidnih substanc rastlinskega izvora. Nov glikozid, izoliran iz listov rastline *Kigelia pinnata* (Lam.) Benth (Bignoniaceae) smo proeizkusili na nematocidno aktivnost. Strukturo predlagane spojine smo opredelili z različnimi metodami, vključno z UV, FTIR, 1D in 2D-NMR, FAB-MS, TOF--ESI-MS in TOF_ESI_MS/MS (TANDEM). *In vitro* poskus z glikozidom na jajčecih in juvenilnih osebkih *Meloidogyne incognita* je nakazal povečano smrtnost. Njegova aktivnost je primerljiva z oxamylom v koncentraciji 0,1 mg/mL. Po štirih urah opazovanja nismo opazili nobenih značilnih razlik med glikozidom in oxamylom. Dosedanji podatki kažejo, da je naravni glikozid obetavna alternativa za oxamyl za kontrolo z nematodami povzročenih koreninskih vozlov in da lahko prispeva k naravnemu načinu zatiranja škodljivcev.

Ključne besede: varstvo rastlin / nematode / *Kigelia pinnata* / i-kromanski glikozid / nematocidi

1 INTRODUCTION

Plant parasitic nematodes are important contributors to crop loss globally. Over 90 species of the genus *Meloidogyne* have been described worldwide (Sikora and Fernadez, 2005). Precisely, the root knot nematode *Meloidogyne incognita*, has been reported to infect about 232 genera of plant species (Swarup *et al.,* 1989). The problem of root-knot nematode in Nigeria is well established. Severe growth reduction, wilting, unfilled spikelets and poor yield of crop are attributed to root-knot nematode (Babatola, 1984). Several other crops such as yam, maize, cassava and vegetables have also been affected by *Meloidogyne* spp. The use of synthetic nematicides in the

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control of soil nematodes is a major concern because of the health and environmental hazards. The use of synthetic nematicides, such as oxamyl and carbofuran, could produces toxic metabolites in ground water and aquatic bodies. Oxamyl, which belongs to a group of pesticides called carbamates is reported to be rapidly absorbed and excreted in mammals where the highest concentration has been reported to be in blood, heart, liver, kidney, lungs, spleen and the gastro-intestinal tract (EFSA, 2005; USEPA, 2007). Several nematicides are phytotoxic and require a long time for degassing and decomposition. The increasing incidence of pesticide poisoning and mortality (Kottegoda, 1985) highlights the risks associated with their use. Natural products have been investigated as alternatives to synthetic nematicides (Abdel-Rahman *et al.*, 2012). *Kigelia pinnata* (Lam.) Benth (Bignoniaceae) is a rich source of diverse phytochemicals including naphthoquinones and essential fatty acids (Atolani *et al*., 2011, 2012) with antioxidant and anticancer properties (Atolani *et al*, 2009, 2013; Olatunji and Atolani, 2009). We here describe the isolation of a new glycoside from *Kigelia pinnata* and its nematicidal activity.

2 MATERIAL AND METHODS

2.1 GENERAL EXPERIMENTAL PROCEDURES

UV Spectra were recorded on a Hitachi U-3200 *spectrophotometer*. FTIR Spectra (KBr) were measured on a JASCO-320-A spectrophotometer. Bruker AMX 500 instrument (300 MHz for ^{1}H and 75 MHz for ^{13}C) was used to acquire (1D and 2D) NMR data. FAB-MS was recorded on MAT 312 mass spectrometer using glycerol-water (1:1) in the presence of KI as matrix, while EI-MS spectral data was obtained on a Finnigan MAT 312 double-focusing mass spectrometer. ESI-MS and ESI-MS/MS data were obtained on QqTOFMS/MS instrument (QSTAR XL mass spectrometer Applied Biosystem/MDS Sciex, Darmstadt, Germany) at room temperature. Samples were dissolved in appropriate polar solvent, and working dilutions were prepared in acetonitrile-water containing 0.1 % trifluoroacetic acid. Analysis was carried out by electrospray ionization (ESI) and collision-induced dissociation (CID) MS, positive ion mode on the instrument at room temperature. MS/MS Experiment was conducted by selecting the product ion. TLC Analyses were carried out on silica gel 60 F_{254} precoated plates (Merck, Darmstadt); detection by ceric sulphate/H_2SO_4. Silica gel 60 (0.063D 0.200 mm; Merck, Darmstadt, Germany) was used for open column chromatography (CC).

2.2 PLANT MATERIAL

Kigelia pinnata (Lam) Benth. leaves were collected from Ado Ekiti, in Ekiti State, Nigeria, and identified by Mr Ajayi, a plant taxonomist at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen (UIH 958) was previously deposited at the Herbarium.

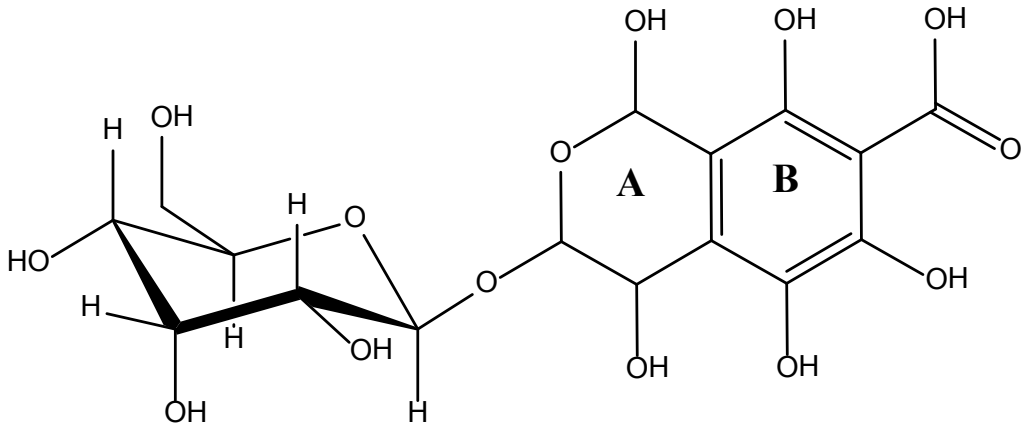
2.3 EXTRACTION AND ISOLATION

The air-dried and powdered leaves (205 g) were sequentially extracted with adequate volume of hexane, dichloromethane, ethyl acetate, methanol and water at room temperature for five days each. The aqueous extract was concentrated and partitioned with butanol. The resulting aqueous fraction was filtered and concentrated under reduced pressure to obtain a crude extract, which was further subjected to a silica gel column chromatography (CC). The column was eluted with the increasing polarity of the mixture of DCM, ethyl acetate, methanol and water. Forty-seven fractions were obtained and pooled to a group of eight (fractions A to H), based on their TLC profile. Further purification of fraction C (CC, MeOH) afforded a yellow syrup, a glycoside tagged "tolaside" with R_f 0.5 (DCM : MeOH, 1:1)

Tolaside (**1**): yellow syrup (95 mg) MF: $C_{16}H_{20}O_{14}$; MW: 436.09; FAB-MS *m/z* 459 $[M+Na]^+$; EI-MS *m/z* 256 $[M^+ - 180]$; TOF-ESI-MS *m/z* 437 $[M + H]^+$; 1D and 2D NMR ppm (Results and discussion); UV λ_{max} (MeOH, nm): 208, 275. IR ν_{max} (KBr, cm^{-1}) 3383, 2927, 1598, 1403, 1051, 718, 669. ^{1}H-NMR: 500 MHz, $CDCl_3$, δ 2.90 (*s*, 1H, OH, H-2), 2.91 (*s*, 1H, OH, H-5), 3.0 – 3.18 (*m*, 2H, CH, H-3', H-4'), 3.31 – 3.43 (m, 3H, CH, H-2', H-6'), 3.55 – 3.79 (m, 4H, OH, H-2, 5, 2', 3' 4' 6'), 3.98 (d, 2H, CH, H-1, H-1'), 4.26 (d, 1H, CH, H-2), 4.43 (*sss*, 3H, ArOH, H-6, 8, 9), 4.88 (*s*, 1H, CH, H-5), 8.39 (s, 1H, OH, H-10). ^{13}C-NMR: (125 MHz, $CDCl_3$): δ 173.1 (C-10), 104.0 (C-6), 97.0 (C-8), 92.4 (C-3), 89.3 (C-9), 76.9 (C-4), 76.7 (C-1), 75.3 (C-1'), 74.0 (C-7), 73.5 (C-5), 72.9 (C-5'), 72.8 (C-3'), 72.4 (C-2'), 71.9 (C-2), 56.52 (C-4'), 52.6 (C-6').

2.4 NEMATICIDAL ASSAY

Meloidogyne incognita 'race one' eggs were extracted from *Solanum melongena* L. roots by agitating in 0.06 % sodium hypochlorite solution for three minutes. The eggs were collected and rinsed with tap water on nested mesh 75 µm and 25 µm. Root particles were retained in 75 µm sieve, while the eggs were collected in the 25 µm sieve. The eggs were later standardized to 250 eggs per mL. The



(1)

extracted eggs were incubated at room temperature for twenty-four hours (24 hrs) to hatch out the second stage juveniles. The juveniles were poured into a 500 mL beaker for standardization and 1 mL was standardized to contain 250 juveniles. The experimental design was a 3 by 4 by 3 factorial experiment in a completely randomised design (CRD) involving three treatments at four levels and each replicated three times. 4 mg of the isolate was dissolved in 40 mL distilled water which is equivalent to 0.1 mg per mL concentration. 250 eggs and juveniles were used for each of the assay. Different doses of treatments were prepared by diluting the stock solution as follows: 6 mL fraction with 2 mL water to afford 75 %; 4 mL fraction with 4 mL water to afford 50 % and 2 mL fraction with 6 mL water affording 25 %, while distilled water served as control (Fabiyi *et al.*, 2012). Data obtained were subjected to analysis of variance. Significant means were separated using Duncan's multiple range test at 5 % level of probability ($P < 0.05$).

3 RESULTS AND DISCUSSION

The glycoside, tolaside (**1**) was obtained as yellow syrup (95 mg). The molecular formula was determined as $C_{16}H_{20}O_{14}$ by using the combination of various mass spectral data. FAB-MS showed *m/z* 459 $[M + 23]^+$, calculated for $C_{16}H_{20}O_{14}Na$, *m/z* 258 calculated for $C_{10}H_{10}O_8$ (aglycone) and *m/z* 180 for the sugar unit; EI-MS showed *m/z* 256 $[M^{+\cdot} - 180$ (one glucose unit)$]^+$, 236 (1), 185 (4), 157 (4), 103 (8), 97 (8), 85 (7), 73 (100), 60 (49), 57 (20), 44 (45); TOF-ESI-MS showed *m/z* 437 $[M + H]^+$ calculated for $C_{16}H_{21}O_{14}$. In order to obtain structural feature of the compound, TOF-ESI-MS/MS (TANDEM) for *m/z* 437 $[M+H]^+$ was carried out. MS/MS indicated major peaks at *m/z* 359, calculated for $C_{16}H_{23}O_9$ (indicating loss of five hydroxyl groups on the aglycone). Based on the spectroscopic data obtained, the structure **1** was proposed for the new isochromane glycoside, tolaside.

The UV spectrum exhibited maxima at 207 and 275 nm. The FTIR spectrum showed absorption bands

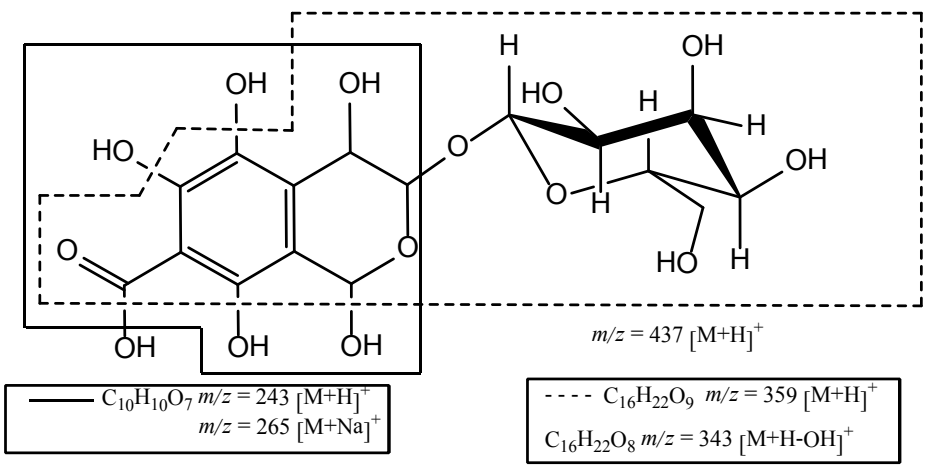


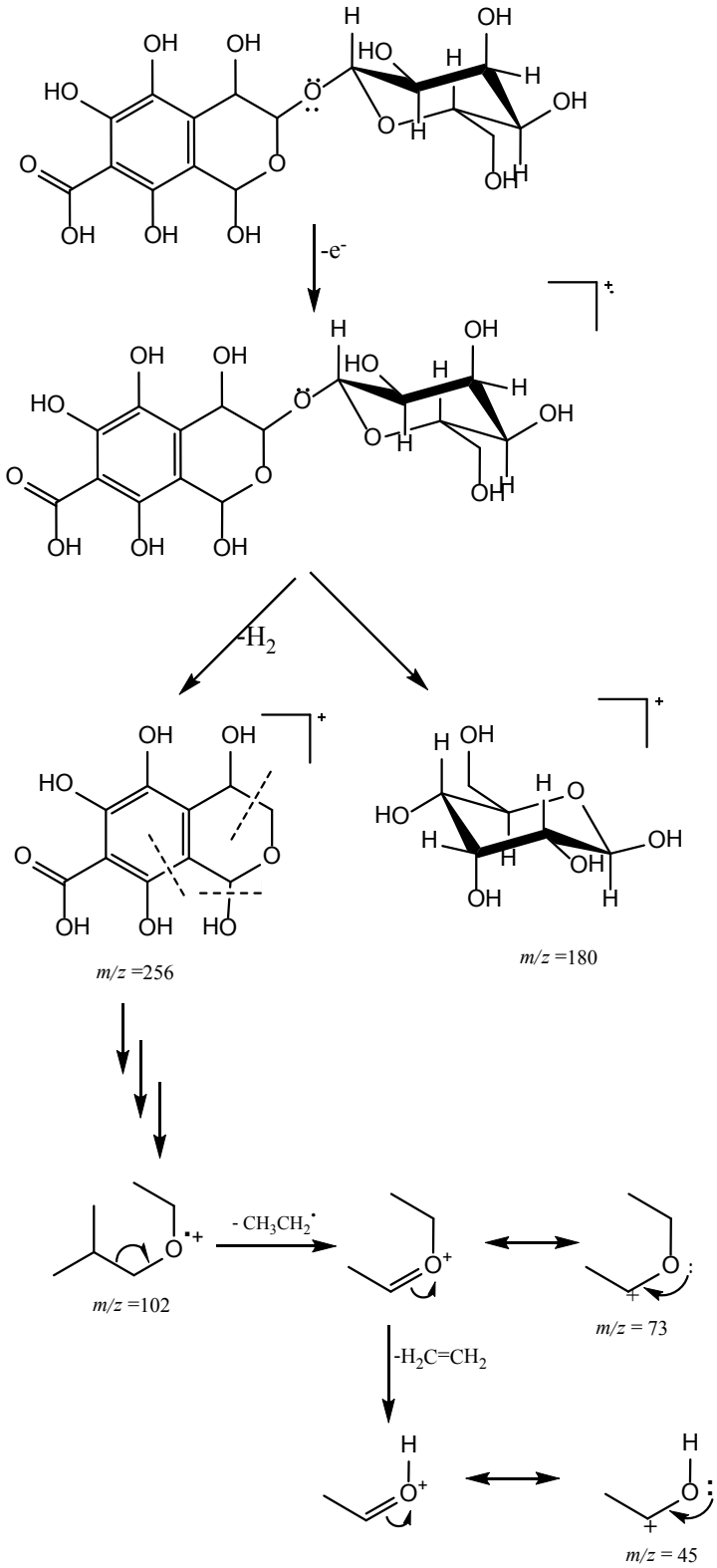
Figure 1: *Proposed ESI-MS-MS fragmentation pattern for tolaside (1)*
Slika 1: *Predlagani ESI-MS-MS fragmentacijski vzorec za tolazid (1)*

for hydroxyl at 3383 cm^{-1}, C-H stretching of aliphatic at 2927 cm^{-1}, aromatic C=C stretching at 1599 cm^{-1}, characteristic O-H bonding of the carboxylic at 1402 cm^{-1}, C-O stretching of alcohol at 1051 cm^{-1} (broad) and aromatic CH bending at 718 cm^{-1}. ^{1}H-NMR spectrum displayed three hydroxyl protons on the aromatic ring at δ_H 4.43, and the carboxylic proton at δ 8.39. The methine protons germinal to –OH and ether in ring A appeared at δ_H 3.55–3.79. Methine protons of the glycone appeared between δ_H 3.0–3.18 and 3.31–3.43, while hydroxyl protons of the glycone moiety resonated between δ_H 3.55–3.79. Three methine proton signals of the aglycone appeared at δ_H 3.98, 4.26, and 4.88. The ^{13}C NMR spectrum displayed 16 signals of which 10 were ascribed to the aglycone. The carbonyl appeared at δ_C 173.07. DEPT 90° and 135° indicated corresponding eight signals representing eight methine carbons, while one signal at δ_C 61.4 for one methylene of the sugar. HMBC experiment also indicated the long range heteronuclear coupling.

3.1 EI-MS AND TANDEM-MS FRAGMENTATIONS

The ejection of a single electron from the O-linkage oxygen of the glycoside induced the production of the molecular ion [M^+]. The M^+ was unstable due to its high polarity as a result of the multiple hydroxyl ends. Cleavage of the C-O bond of the ether-linkage followed by double deprotonation, induced the formation of the cation, *m/z* 256. This fragment further undergoes multiple cleavages to produce the ethyl ethyl sec-buthyl ether ion at *m/z* 102. The α C-C bond of the fragment cleaved to eliminate (-$CH_3CH_2^{\cdot}$) ethyl radical yielding an intermediate cation, which rearranges to the ether cation ($CH_3CHOCH_2CH_3^+$) at m/z 73 as the base peak. This confirms the presence of oxygen-containing fragment cation. The previous intermediate oxygen containing cation at m/z 102 decomposes with hydrogen migration and elimination of ethene to form an enolate, which rearranges to produce an alcohol cation with m/z 45 (Scheme 1).

The ESI-MS spectrum of the compound **1** showed the [M+H]$^+$ at *m/z* 437, with other major ions at *m/z* 386, 341, 308, 292, 252, 199,



Scheme 1: *Proposed EI-MS fragmentation pattern for tolaside 1*
Shema 1: *Predlagani EI-MS fragmentacijski vzorec za tolazid*

Table 1: *Effect of tolaside (1), oxamyl and Kigelia pinnata crude extract on Meloidogyne incognita juveniles*
Preglednica 1: *Učinek tolazida (1), oxamyla in ekstrakta Kigelie pinnate na juvenilne osebke Meloidogyne incognita*

	Exposure time of juveniles to various agents						
	30 min	1 hr	4 hrs	8 hrs	Day 1	Day 2	Day 3
Treatments							
Tolaside (1)	29.43[a]	38.08[a]	52.71[a]	74.18[a]	100.00[a]	100.0[a]	100.00[a]
Crude	10.00[c]	19.19[b]	32.74[b]	40.29[b]	51.10[b]	60.23[b]	72.00[b]
OXML	20.22[b]	37.62[a]	53.24[a]	73.62[a]	100.00[a]	100.0[a]	100.00[a]
S.E.M.	0.91	1.12	1.23	1.42	1.59	1.76	2.06
Treatment level, %							
0	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.97[d]	1.26[d]	2.63[b]
25	7.37[c]	12.61[c]	22.11[c]	41.39[c]	61.22[c]	83.29[c]	100.00[a]
50	11.09[b]	18.11[b]	37.33[b]	54.08[b]	70.01[b]	90.07[b]	100.00[a]
75	16.33[a]	23.00[a]	42.00[a]	60.23[a]	78.26[a]	96.18[a]	100.00[a]
S.E.M.	0.24	0.41	0.56	0.70	0.81	1.12	1.36

Crude = *Kigelia pinnata* crude methanolic extract / surovi metanolni ekstrakt *Kigelie pinnate*; Oxml = Oxamyl; S.E.M. = Standard error of mean / standardna napaka sredine;
Values with different alphabets along the same column are statistically different at P < 0.05 / Vrednosti, označene z različnimi črkami v isti koloni so statistično različne (p < 0,05)

157, 129, 118 and 101. The full scan of the MS-MS spectrum at *m/z* 437 $[M+H]^+$ produced daughter ions at *m/z* 377, 359, 343, 283, 265, 189 and 171. The loss of five -OH on the aglycone produced the ion *m/z* 359, while further loss of another -OH produced ion at the *m/z* 343. The dehydroxylation of carboxylic hydroxyl on the aglycone fragment yielded the ion *m/z* 243 $[M+H]^+$ and the corresponding fragment at *m/z* 265 $[M+Na]^+$ (Fig. 1).

A comparison of the EI and ESI mass spectra indicated that ESI-MS^2 produced $[M+H]^+$ as the main peak, while the M^+ produced in the EI quickly decomposed losing $H^{\cdot}$, $CH_3^{\cdot}$, R'-O-$R^{\cdot}$ and $OH^{\cdot}$. The FAB-MS was also indicative of the presence of the glycone and aglycone, as

Table 2: *Effect of tolaside, oxamyl and Kigelia pinnata crude extract on Meloidogyne incognita eggs*
Preglednica 2: *Učinek tolazida (1), oxamyla in ekstrakta Kigelie pinnate na jajčeca Meloidogyne incognita*

	Exposure time of eggs to treatment					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Treatments						
Tolaside (1)	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
Crude	0.00[a]	0.00[a]	0.03[a]	0.07[a]	0.12[b]	0.19[b]
OXML	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
S.E.M.	0.00	0.00	0.00	0.03	0.05	0.09
Treatment level, %						
0	13.33[b]	19.30[b]	26.15[b]	39.02[b]	47.28[b]	56.22[c]
25	0.00[a]	0.00[a]	0.00[a]	0.03[a]	0.09[a]	0.11[b]
50	0.00[a]	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]	0.00[a]
S.E.M.	0.00	0.00	0.10	0.15	0.18	0.26

Crude = *Kigelia pinnata* crude methanolic extract / surovi metanolni ekstrakt *Kigelie pinnate*; Oxml = Oxamyl; S.E.M. = Standard error of mean / standardna napaka sredine;
Values with different alphabets along the same column are statistically different at P < 0.05 / Vrednosti, označene z različnimi črkami v isti koloni so statistično različne (p < 0,05)

well as the identification of pseudo molecular ion $[M^+]$ at *m/z* 459 $[M+Na]^+$ of the glycoside.

3.2 NEMATICIDAL ACTIVITY OF TOLASIDE (1)

The effect of oxamyl, tolaside and *Kigelia pinnata* crude extract on *Meloidogyne incognita* juveniles is presented in Table 1. Tolaside (**1**) was significantly ($P < 0.05$) more active than the oxamyl at 30 minutes of exposure with a percentage mortality of 29.43 %, while oxamyl showed 20.22 % mortality. From one hour to the end of the observation on day three, there was however no significant difference in the action of the glycoside and oxamyl on *Meloidogyne incognita* juveniles. All the levels of concentration induced mortality, but the 75 % concentration was significantly ($P < 0.05$) more effective with a 60.23 % mortality at 8 hours of observation. The glycoside and oxamyl are both effective inhibitors of egg hatch as there was no record of hatching in the two treatments throughout the period of study (Table 2). The crude extract of *Kigelia pinnata* showed only a few hatches. The lowest concentration (25 %) allowed some hatching, while the higher doses (50 and 75 %) completely inhibited the hatching of eggs.

Previous studies revealed that natural glycosides have nematicidal activity. Green manures of Sudangrass (*Sorghum sudanense*, Poaceae) are being examined as nematode suppressants. It contains the active glycoside, dhurrin (Widmer and Abawi, 2000). Natural glycosides; asparanin I and asparanin B, from *Asparagus adescendens* seed, as well as two triterpenoid glycosides albichinin II from *Albizia chinensis* and sonunin III from *Acacia concinna* have been found to be active at doses as low as 200 µg/mL against *M. incognita* nematodes (Meher *et al.*, 1988). A mixture of two furastanol glycosides, protodioscin and deltoside from *Dioscorea deltoidea*, (Dioscoreaceae) inhibited *M. incognita* motility at 5000 µg/mL but decreased nematode infection of tomato roots at much lower doses through a host-mediated effect (Zinovieva *et al.*, 1997). Other natural glycoside with nematicidal properties included 3-*β*-[*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[α-L-arabinofuranosyl-(1→2)]-*O*-*β*-D-arabinopyranosyl) oxy] jujubogenin active against *C. elegans* (Renukappa *et al.*, 1999) and flavone glycosides linaroside and lantanoside from *L. camara*, which were lethal to *M. incognita* juveniles at 1.0 % (Begum *et al.*, 2000).

4 CONCLUSION

The mass spectrometry analyses with the 1D, 2D NMR and FTIR spectroscopic analyses has unequivocally enabled, the establishment of the proposed structure of newly isolated isochromane carboxylic glycoside. The nematicidal activity of the glycoside **1** can be compared favourably with that of oxamyl, a standard nematicidal agent. This result therefore indicates that natural glycosides holds promise as potential nematicides and can successfully replace the toxic synthetic counterpart.

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