

Isovitexin from *Kigelia pinnata*, a potential eco-friendly nematocidal agent

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Synthetic pesticides are usually environmentally hazardous. In an attempt to obtain an eco-friendly alternative to synthetic nematocidal agents, isovitexin, a flavonoid glycoside isolated from butanol fraction of the leaf extract of *Kigelia pinnata* was examined for its *in vitro* nematocidal potential. The chemical structure of the isovitexin was confirmed by comparing its UV, IR, MS and NMR spectroscopic data with that reported in literature. The compound induced a hundred percent mortality at 24 h of exposure and inhibited egg hatch of *Meloidogyne incognita* at a concentration of 0.16 mg/mL. There was no significant difference ($p < 0.05$) in the action of isovitexin and the synthetic nematocide, oxamyl, when tested at the same concentration. The bioactivity and toxicity evaluation on 'molinspiration' and 'toxpredict' evaluators indicated that isovitexin is a potentially bioactive compound with low genotoxic carcinogenicity and high enzyme inhibition activity. The biodegradability evaluation indicated that it belongs to a class 2 persistent chemical. The results of this study could be harnessed to make isovitexin commercially available as a natural nematocide. This is the first account of isovitexin being isolated from *Kigelia pinnata* and evaluated for nematocidal potential.

Keywords: *Kigelia pinnata*, *Meloidogyne incognita*, flavonoid glycoside, nematocide, genotoxic carcinogenicity.

Plant parasitic nematodes are responsible for annual crop damage, less productivity and high cost of food worldwide (Sasser and Freckman 1987, 7-14). Root-knot nematode, *Meloidogyne incognita* is one of the most economically important class of nematodes with grave impact on food production. However, the control of root-knot nematodes with various synthetic nematocides has had a positive impact with highly significant reduction of nematode population where applied, and consequently leading to increase in crop yield (Mani et al. 1987, 239-248). The success recorded was however not without attendant environmental implications on non target important organisms in the ecosystem. For instance, oxamyl, a pesticide belonging to the carbamates is a "restricted use" chemical due to its acute toxicity to birds and mammals (EFSA 2005, 1-78; USEPA 2007, 1-138). Hence, the application of environmentally less toxic bio-nematocides has been encouraged (Ploeg 2007, 1028). In an attempt to obtain an eco-friendly and safer alternative to synthetic nematocidal agents, a C-glycoside isolated

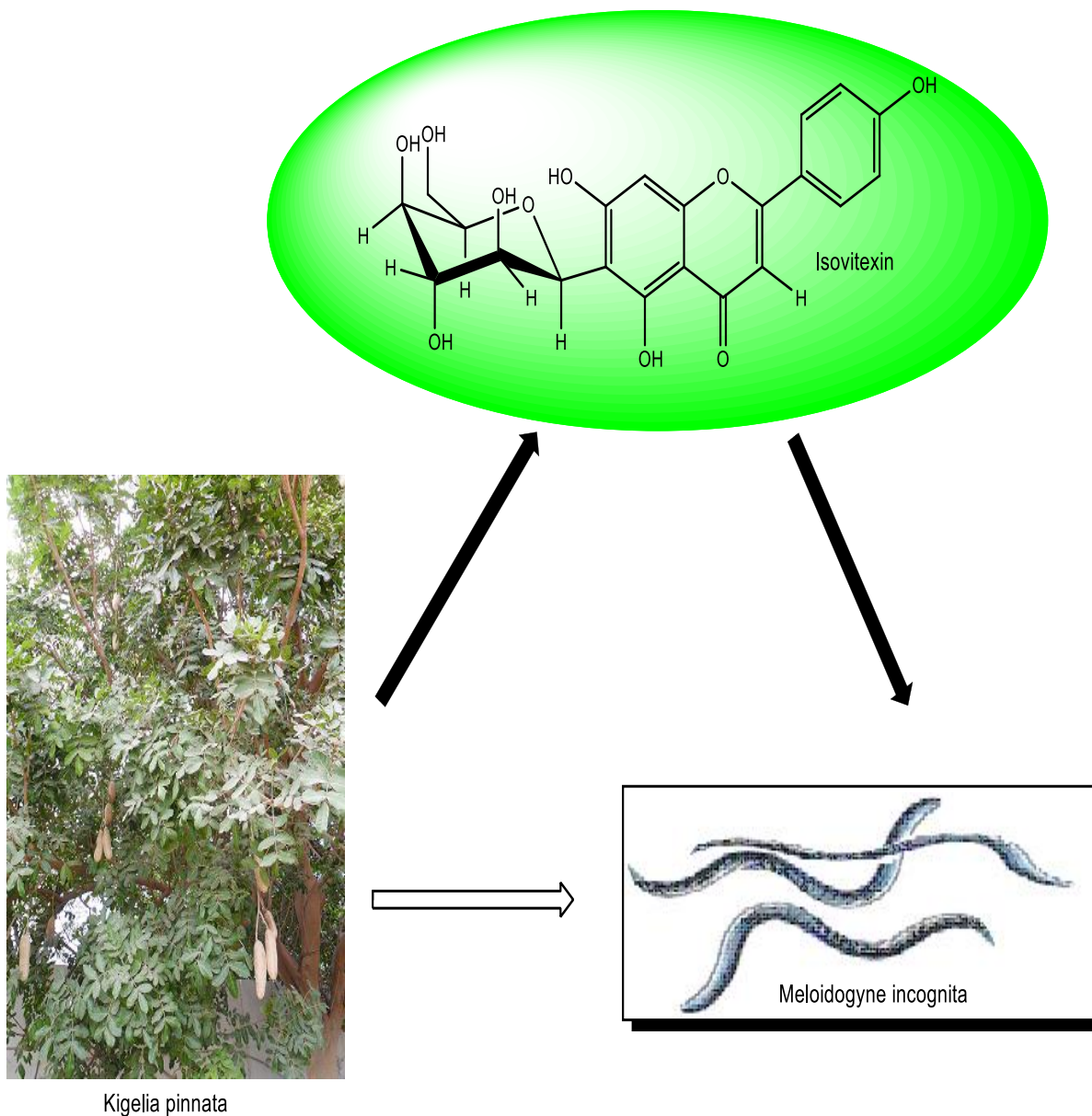
from *Kigelia pinnata* was evaluated for its nematocidal property. *Kigelia pinnata* (Lam.) Benth (Bignoniaceae) has been reported for the presence of various compounds (Atolani et al. 2009, 187-194; 2011, 264-273; 2012, 47-53) with interesting biological activities such as antimicrobial, anti-cancer and anti-inflammatory among others (Olatunji and Atolani 2009, 158-164).

Materials and methods

FTIR spectrum (KBr) was obtained on a JASCO-320-A spectrophotometer while UV Spectrum was recorded on a Hitachi U-3200 spectrophotometer. Bruker AMX 400 MHz instrument was used to acquire ¹H-NMR data while EI-MS spectral data was obtained on a Finnigan MAT 312 double-focusing mass spectrometer. TOF-MS spectrum was obtained on QqTOFMS/MS instrument at room temperature. The sample was dissolved in an appropriate solvent, and working dilution was prepared in acetonitrile-water containing 0.1% trifluoroacetic acid.

Graphical Abstract

Isovitexin from *Kigelia pinnata*, A Potential Eco-friendly Nematicidal Agent



Analysis was carried out by electrospray ionization (ESI) and collision-induced dissociation (CID) mass spectrometry on a positive ion mode of the instrument. TLC analyses were carried out on silica gel 60 F₂₅₄ pre-coated plates (Merck Darmstadt, Germany), while Silica gel 60 (0.063D, 0.200 mm; Merck Darmstadt, Germany) was used for column chromatography (CC) elution.

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Plant material

Kigelia pinnata (Lam.) Benth leaves were collected from Ado Ekiti, Nigeria and identified (voucher specimen: UIH 958) at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Extraction and isolation

The pulverized plant material (0.2 Kg) was extracted successively with hexane, dichloromethane, ethyl acetate, methanol and water at room temperature for five days each. The concentrated aqueous crude extract (11.2 g) was partitioned with butanol and the resulting butanol fraction was concentrated under reduced pressure. The concentrated butanol crude extract was fractionated on a silica gel column chromatography (CC). The column was eluted with increasing polarity of the mixture of hexane, ethyl acetate and methanol. Twenty-three fractions were obtained and grouped into nine, fractions A to I, based on their TLC profile. Fraction I which appeared as a single spot on TLC with R_f 0.8 (EtOAc: EtOH; 1:1) afforded a yellow powder, whose spectroscopic data was concluded to be isovitexin.

Isovitexin: yellowish brown powder (16 mg) MF: $C_{21}H_{20}O_{10}$; MW: 432; UV λ_{max} (MeOH, nm) (log ϵ): 211 (1.64), 277 (0.64), 325 (0.4). IR ν_{max} (KBr, cm^{-1}) 3397, 2922, 1650, 1598, 1520, 1490, 1083, 1020, 823 cm^{-1} . EI-MS m/z 43 (96), 60 (72), 73 (100), 97 (38), 185 (6), 236 (5), 256 (5), 320 (7), 337 (12), 368 (5); TOF-ESI-MS m/z 433 $[M + H]^+$; 1H -NMR ppm; 400 MHz, MeOD, δ 6.7 (s, 2H, H-3, 8), 5.8 (s, 3H, OH, H-5, 7, 4'), 3.5 – 3.8 (m, 9H, glycosyl protons), 7.6 – 7.8 (m, 4H, H-2', 3', 5', 6').

Nematicidal Assay

The eggs and second stage juveniles of *Meloidogyne incognita* were extracted from a pure culture raised on eggplant (*Solanum melongena*) using the sodium hypochlorite method (Hussey and Baker 1973, 1025-1028). 6.3 mg of the crude methanol extract and isovitexin were dissolved in 40ml distilled water each to obtain the concentration, 0.16 mg/mL accordingly. Treatment concentrations were varied by diluting the stock solution to obtain 75%, 50% and 25% concentrations; whilst using distilled water as a negative control. Similar concentrations of oxamyl were prepared and used as positive control. Data obtained were statistically analyzed using SAS software

program (SAS Institute, 1998). Comparisons among mean values were made with the Duncan's multiple range tests at 5% level of probability.

Molecular Properties and Estimated toxicological hazard of Isovitexin and Oxamyl

The chemoinformatics software, molinspirationTM (version 2011.06) was used to establish the molecular properties and bioactivity score of the glycoside and the standard, oxamyl. Bioactivity score is based on Lipinski's "Rule of 5" (Lipinski et al. 1997, 4-25), which considers whether various molecular properties and structural features of a particular molecule are similar to known drugs. The properties, such as hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility influences the absorption, bioavailability, transport properties, reactivity, toxicity, metabolic stability of the molecule and thus potential for use in living systems. Lipinski's rule, formulated by Christopher Lipinski in 1997, describes molecular properties important for a compound's pharmacokinetics such as absorption, distribution, metabolism and excretion (ADME). Isovitexin and oxamyl were checked for the bioactivity by calculating the activity score for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand (Lipinski et al 1997, 4-25). The toxicological hazard of isovitexin and oxamyl were also estimated using the "Toxpredict evaluator" (<http://apps.ideaconsult.net:8080>) as previously reported by other authors (Borani et al 2013, 134-142).

Results and discussion

The glycoside, isovitexin was obtained as a yellowish brown powder (16 mg). The molecular formula was determined as $C_{21}H_{20}O_{10}$ by using the combination of various spectral data and comparing with literature data (Lin et al. 2000, 253-256; Pereira et al. 2012, 652-659).

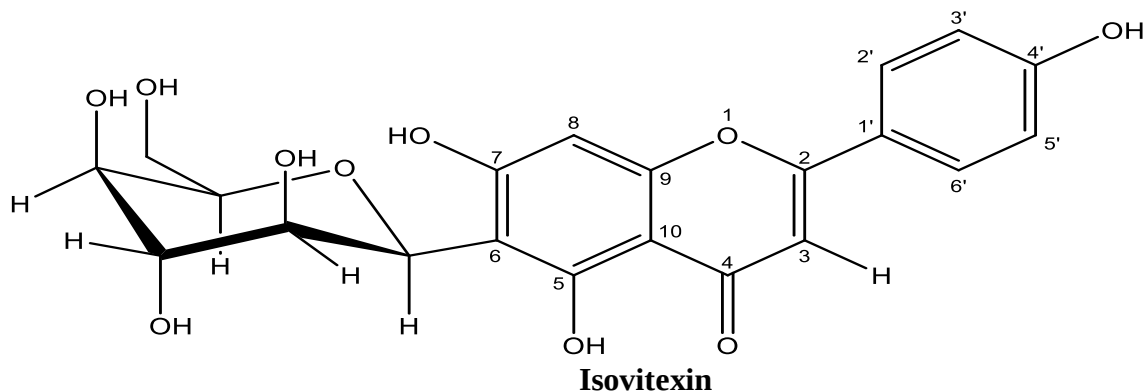


Table 1: The effect of oxamyl, isovitexin and methanolic crude extract of *Kigelia pinnata* on percentage mortality of *Meloidogyne incognita* juveniles

Time of Exposure of Juveniles to Extract						
Treatments	1 h	2 h	3 h	4 h	Day 1	Day 2
Isovitexin	39.76 ^a	52.50 ^a	61.67 ^a	76.18 ^a	100.00 ^a	100.00 ^a
Crude	25.67 ^b	31.15 ^b	37.36 ^b	45.83 ^b	58.36 ^b	64.00 ^b
Oxamyl	40.13 ^a	53.19 ^a	62.08 ^a	75.69 ^a	100.00 ^a	100.00 ^a
S.E.M.	1.09	1.28	1.43	1.71	2.07	2.11
Treatment level (%)						
0	0.00 ^d	0.00 ^d	0.00 ^d	0.00 ^d	1.11 ^d	2.18 ^c
25	20.38 ^c	30.17 ^c	38.14 ^c	44.19 ^c	79.07 ^c	94.26 ^b
50	26.10 ^b	37.61 ^b	46.02 ^b	53.12 ^b	86.00 ^b	100.00 ^a
75	30.13 ^a	43.26 ^a	51.00 ^a	59.44 ^a	91.00 ^a	100.00 ^a
S.E.M.	0.34	0.51	0.69	0.85	1.19	1.36

Values with different letters along the same column are statistically different at $p < 0.05$

Key: Crude = *Kigelia pinnata* methanol extract; S.E.M. = Standard error of mean

Nematicidal Activity

Isovitexin produced a significant ($p < 0.05$) percentage mortality (39.76%) at the first hour of exposure which was not significantly different from oxamyl (40.13%). This trend was observed throughout the period of the experiment. The methanolic crude extract was however not as effective as the isolate and synthetic nematicide (Table 1). The highest concentration (75%) was significantly more effective than all the other concentrations. The control (0%) recorded the least percentage mortality of 2.18% on day two of the experiment while there was 100% mortality in the 75% and 50% concentrations. There was total egg hatch inhibition by oxamyl and isovitexin, with a few hatches using the crude extract (Table 2). Egg hatch inhibition was observed at all concentration levels except the

0% concentration. It is clear from the result that isovitexin is effective in controlling the root-knot nematode, *Meloidogyne incognita* at concentration that is as low as 0.16 mg/mL. Glycosides have been reported to be nematicidal. The glycosides, schaffoside and isoschaffoside were reported to be effective against *Meloidogyne incognita* at 114.66 and 323.09 $\mu\text{g/mL}$ respectively (Du et al. 2011, 5079-5086). Lanatoside exhibited significant toxicity against *M. incognita* and 90% mortality was observed at a concentration of 10 mg/mL (Begum, et al. 2000, 765-767). It is obvious that bio-nematicides could control nematode growth and population effectively and also minimise environmental hazards caused by synthetic nematicides. Isovitexin isolated from *Kigelia pinnata* here has shown potential for development as a natural nematicide.

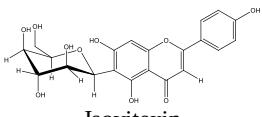
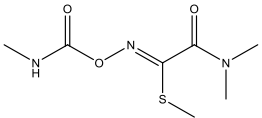
Table 2: The effect of oxamyl, Isovitexin and methanolic crude extract of *Kigelia pinnata* on percentage egg hatch of *Meloidogyne incognita*

Exposure Time of Eggs to Extract				
Treatments	Day 1	Day 2	Day 3	Day 4
Isovitexin	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Crude	0.00 ^a	0.00 ^a	0.08 ^a	0.13 ^b
Oxamyl	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
S.E.M.	0.00	0.00	0.00	0.02
Treatment Level (%)				
0	12.86 ^b	18.67 ^b	25.02 ^b	38.33 ^b
25	0.00 ^a	0.00 ^a	0.00 ^a	0.06 ^a
50	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
S.E.M.	0.02	0.06	0.11	0.17

Values with different letters along the same column are statistically different at $p < 0.05$

Key: Crude = *Kigelia pinnata* methanol extract; S.E.M. = Standard error of mean.

Table 3: Bioactivity score of isovitexin and oxamyl

Compound	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
 Isovitexin	0.12	0.02	0.15	0.23	0.03	0.47
 Oxamyl	-0.37	0.04	-0.72	-0.95	-0.38	0.14

Oxamyl, belongs to a group of pesticides called carbamates and is reported to be rapidly absorbed and excreted in mammals with the highest concentration in blood, heart, liver, kidney, lungs, spleen and the gastro-intestinal tract (EFSA 2005, 1-78; USEPA 2007, 1-138). In experiments involving rat, the oral toxicity was high, with an oral LD₅₀ of 3.1 mg/kg bw and 2.5 mg/kg bw in male and female rats, respectively. The toxicity during inhalation in rats was also high, LC₅₀ 0.056 mg/L air (EFSA 2005, 1-78). Signs of oxamyl intoxication observed in animal studies include soiled fur, lacrimation, salivation, tremors, incoordination or impaired mobility, and change in papillary response. However, the dermal toxicity on the other hand was found to be low. There may be a high risk of contamination by oxamyl and its metabolites in certain

circumstances, particularly if application is made on acidic soils that have not been fully assessed. The long term risk to earthworms is considered high and it is not readily biodegradable (EFSA 2005, 1-78).

Molecular Descriptor and Estimated toxicological hazard of isovitexin and Oxamyl

A vast and structurally diverse class of compounds with varying bioactivity potential exists in natural products. Their physical, chemical and biological properties are usually conducted on established platform in order to display unforeseen properties and thus elicit the scope of the chemistry involved. The bioactivity and molecular properties (Table 3 and 4) of the

isovitexin estimated on molinspiration evaluator indicated that isovitexin is a good enzyme and kinase inhibitor. This implies that the compound's principal mechanism of action might be by inhibiting enzyme and kinase (Table 3) activity in the nematode. However, similar mode of action would be observed if the compound is exposed to humans. The score for nuclear receptor ligand was also high, while its potential as ion channel modulator and protease inhibitor was low. The log P value was below five, suggesting that the molecule should have good permeability across the cell membrane in order to initiate bioactivity. In contrast, the standard nematicide, oxamyl had a negative kinase inhibitor and nuclear receptor ligand potential. Oxamyl did not violate any of the Lipinski's rules, while isovitexin molecular polar

surface area 181Å does not fulfil the optimal requirement for absorption. Compounds violating more than one Lipinski's rule may have limited bioavailability at the site of action (Ertl et al 2000, 3714-3717). Toxicological evaluation (Table 5) revealed that isovitexin has low genotoxic carcinogenicity and acute toxicity (LC₅₀ 1.76 mmol/L) to fish with no potential as carcinogen, based on quantitative structure activity relationship, QSAR. Biodegradability evaluation also indicated that it belongs to a 'class 2 persistent chemical' which implies that it is not highly bio-accumulative (CSCL 2010, 1-35). It also did not have functional groups associated with enhanced toxicity on human. This implies that the use of isovitexin as a nematicidal agent may not pre-dispose to toxicological and environmental hazards.

Table 4: Molecular properties of isovitexin and oxamyl

Compound	MW/ Formula	Log P ^a	Molecular Polar surface area/volume	No. non- H atoms	No. H bond acceptors ^b	No. H bond donors ^c	Rotatable bonds	Lipinski Failures ^d
Isovitexin	432.38 C ₂₁ H ₂₀ O ₁₀	0.518	181.041/ 355.203	31	10	7	3	1
Oxamyl	219.26 C ₇ H ₁₃ N ₃ O ₃ S	- 0.721	71.005/192.406	14.0	6	1	4	0

a Log P is based on octanol-water partition coefficient; b H bond acceptors include O & N atoms; c H bond donors include OH and NH groups; d Rule of 5 violations are based on the molecular descriptors used by (Lipinski et al. 1997, 4-25) for "drug-like" molecules (log p ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5).

Table 5: Estimated toxicological hazard of isovitexin and oxamyl

Toxicity Parameter	Isovitexin	Oxamyl
CAS RN	38953-85-4	23135-22-0
Biodegradability	Class 2 persistent chemical	Class 2 persistent chemical
Ecotoxic effects: Acute toxicity to fish (F96h-LogLC ₅₀ mmol/L)	1.76	1.06
Negative for genotoxic carcinogenicity	Yes	Yes
Negative for nongenotoxic carcinogenicity	Yes	Yes
Potential carcinogen based on QSAR	No	
Unlikely to be a S. typhimurium TA100 mutagen based on QSAR	No	No
Skin irritation / skin corrosion	Not corrosive to skin	Not Irritating
Contains functional groups associated with enhanced toxicity on human	No	No
Aqueous Solubility log(mg/L) (TAZ & BTAZ)	0.09	2.30
Cytochrome P450-Mediated Drug Metabolism (SMARTCyp. Rank1, 2 and 3 Reaction)	Alcohol oxidation, O-dealkylation	S-oxidation, Aliphatic hydroxylation, N-dealkylation

CAS RN: Chemical Abstract Services Registry Number.

Conclusion

Isovitexin was isolated from the butanol fraction of the leaf of *Kigelia pinnata* and the purity was ascertained by TLC. The structure was confirmed by comparing the UV, IR, MS and NMR spectroscopic data with literature. The nematicidal activity of isovitexin compared favourably with oxamyl, a standard synthetic nematicidal agent. The predicted toxicity of isovitexin was very minimal. There would be need to further study the mechanism of action of the compound as a nematicidal agent and also examine the metabolite produced in *in vitro* and *in vivo* systems. This result further strengthen the fact that natural compounds hold promise as potential nematicidal agents.

Conflict of Interest

Authors declare no conflict of interest.

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References

- Olatunji, G.A., and O. Atolani. 2009. "Comprehensive scientific demystification of *Kigelia africana*: A Review." *African Journal of Pure and Applied Chemistry*. **3**: 158-164.
- Atolani, O., O.S. Adeyemi, E. Akpan, and C.B. Adeosun, and G.A. Olatunji. 2011. "Chemical Composition and Antioxidant Potentials of *Kigelia pinnata* Root Oil and Extracts." *EXCLI Journal*. **10**: 264-273.
- Atolani, O., S. Oladoye, A.P. Oluyori, and G.A. Olatunji. 2012. "New Constituents of *Kigelia pinnata* Leaves." *Singapore Journal of Scientific Research*. **2**: 2, 47-53.
- Atolani, O., G.A. Olatunji, O.S. Adeyemi, and O. Fayemi. 2009. "Antioxidant and Antimicrobial Activity of Cuticular Wax from *Kigelia africana*." *FABAD Journal of Pharmaceutical Science*. **34**: 4, 187-194.
- Begum, A., A. Wahab, B.S. Siddiqi, and F. Qamar. 2000. "Nematicidal constituents of the aerial parts of *Lantana camara*." *Journal of Natural Product* **63**, 765-767.
- Boriani, E., E. Benfenati, D. Baderna, and M. Thomsen. 2013. "Application of ERICA index to evaluation of soil ecosystem health according to sustainability threshold for chemical impact." *Science of the Total Environment* **443**: 134-142.
- CSCL. 2010. "Chemical Substances Control Law. Chemical Safety Office, Chemical Management Policy Division, Manufacturing Industries Bureau. Ministry of Economy, Trade and Industry." P. 1-35. http://www.meti.go.jp/policy/chemical_management/chemical_wondertown/index.html
- Du, S.S., Hai Ming Zhang., Chun Qi Bai., Cheng Fang Wang., Qi Zhi Liu., Zhi Long Liu., Yong Yan Wang and Zhi Wei Deng. 2011. "Nematicidal Flavone-C-Glycosides against the root-knot nematode (*Meloidogyne incognita*) from *Arisaema erubescens* Tubers." *Molecules*. **16**: 5079-5086.
- EFSA (European Food Safety Authority). 2005. "Scientific Report. (2005) Conclusion regarding the peer review of the pesticide risk assessment of the active substance, Oxamyl." **26**:1-78. <http://www.efsa.eu.int>
- Hussey, R.S., and K.R. Barker. 1973. "A comparison of methods of collecting inocula on *Meloidogyne* spp., including a new technique." *Plant Disease Reporter* **57**: 1025-1028.
- Lin, Y., Y. Kuo, M. Shiao, C. Chen, and J. Ou. 2000. "Flavonoid Glycosides from *Terminalia catappa* L." *Journal of the Chinese Chemical Society*. **47**: 253-256.

- Lipinski, C.A., F. Lombardo, B.W. Dominy, and P.J. Feeney. 1997. "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings." *Adv. Drug Del. Rev.* **23**: 4–25.
- Mani, A., P.H. Naidu, and S. Madhavachari. 1987. "Occurrence and control of *Meloidogyne incognita* on turmeric in andhra pradesh, India." *Inter. Nematol. Network Newslett.* **2**: 11-12
- Pereira, O.R., A.M.S. Silva, M.R.M. Domingues, and S.M. Cardoso. 2012. "Identification of phenolic constituents of *Cytisus multiflorus*." *Food Chemistry* **131**: 652–659.
- Ploeg, A. 2007. *Bio-fumigation to manage plant-parasitic nematodes. Integrated management and biocontrol of vegetable and grain crops nematodes*. Integrated Management SAS Institute (1998). *SAS/STAT Users Guide*. 6th Ed. SAS Institute Inc. Carry, NC, USA.1028pp.
- Sasser, J. N., and D.W. Freckman. 1987. "A world perspective on nematology: The role of the society." In *Vistas on nematology*, edited by J.A. Veech, and D.W. Dickson, 7-14. Society of Nematologists, Hyattsville.
- USEPA, United State Environmental Protection Agency. 2007. Interim Reregistration Eligibility Decision for Oxamyl. Washinton, 1-138.
- Insecticide/nematicide: Protect your crops against damage from insects, mites and nematodes. Technical Bulletin. 1-21. <http://apps.ideaconsult.net:8080/ToxPredict>