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IN SILICO STUDIES OF IDENTIFIED COMPOUNDS FROM ETHANOLIC EXTRACT OF CELOSIA TRIGYNA AGAINST NEMATODE (*Meloidogyne incognita*)

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ABSTRACT

Plant-parasitic nematodes (PPNs) have been found to be a major cause of considerable economic burden on the horticultural crops industry. *Celosia* genus have been renowned to possess antimicrobial activity. This study tends to swing the antimicrobial activity of one species of this genus i.e. *Celosia trigyna* against root-knot nematodes employing molecular docking methods. The identification of compounds in the leaf extract with the potential to inhibit *Meloidogyne incognita* was carried out *in silico*. Liquid Chromatography Mass Spectrometry (LCMS) was employed for analysis of the ethanolic leaf extract of this plant, it revealed the presence of cis-9-tetradecanoic acid, vitexin-glucoside, 6^o-O-deoxyhexoside, gallic acid, malic acid, sinapic acid, 3-feruloylquinic acid in the extract. Site-directed multiligand docking of the identified compounds was performed on protein 2MIF of *Meloidogyne incognita* and employing Bifenazate and Boscalid as positive controls. The binding affinity of 3-feruloylquinic (-4.2 kcal/mol) was significantly higher than the positive control compounds i.e. Bifenazate and Boscalid (-4.0 kcal/mol and -3.9 kcal/mol). The interactions of this molecule with the amino acids of the protein showed that the mechanism of its inhibitory action is similar to that of the cocrystallized ligand. This result validates that the antimicrobial activity of the ethanolic extract of the leaves of *C. trigyna* could be employed against root-knot nematodes for sustainable agriculture.

Keywords: *Meloidogyne incognita*; *Celosia trigyna*; *in silico*; LCMS

INTRODUCTION

A diverse array of crops with significant economic value are facing a high risk of infestation by nematodes, which are parasitic to plants and have a detrimental impact on agricultural yields worldwide. Numerous species of plant-parasitic nematodes are known to cause serious reductions in the yields of over 2000 types of vegetables, field crops, grasses, and trees (Moens et al., 2009). In particular, root-knot nematodes, known as *Meloidogyne* spp., pose a significant problem in agricultural crop production (Nicol et al., 2011). *Meloidogyne* spp. are endoparasites with a worldwide geographical distribution and a broad host range (Aeron et al., 2011). They are notorious for causing substantial yield damage in vegetable crops (Sahebani and Hadavi, 2008), including tomatoes, which are the most widely grown commercial crop, accounting for 14% of the world's vegetable production (Sikora and Fernandez, 2005). Root-knot nematode infections weaken plants by disrupting their water and nutrient supply, rendering them more susceptible to other opportunistic pathogens (Walters et al., 1993).

Root-knot nematodes can be effectively managed through soil and chemical management practices, as well as the selection of resistant varieties (Khan et al., 2008). Conventional farming has preferred chemical management for root-knot nematodes as it can increase earnings (Droby et al., 2009). However, due to the nematodes' broad host range, short generation times, high reproductive rates, and endoparasitic

habits, chemical nematicides' control efficiency is not fully effective. Furthermore, these chemicals can lead to severe human health effects and environmental contamination. Therefore, it is necessary to always have environmentally safe and economically feasible alternative control measures. Antagonistic microorganisms have been identified as one of the potential alternatives to control root-knot nematodes, and extensive research has been conducted in this regard (Siddiqui and Mahmood, 1999).

The flora belonging to the *Amaranthus* family can be discerned across both tropical and cool-temperate regions. The *Amaranthaceae*'s nativity is rooted in the tropical and subtropical regions of Central America, Africa, and Australia. The main species, *Amaranthus tricolor*, thrives predominantly in Asia, whilst *Amaranthus dubius* is cultivated in the Caribbean territories (Souza et al., 2007; Simpson, 2010). *Amaranthus cruentus*, on the other hand, flourishes predominantly in Africa. Some *Amaranthus* species, serving as grain crops, are present in South America. The plant genus *Celosia*, belonging to the *Amaranthaceae* family, comprises about 60 species (*Caryophyllales*) worldwide; a genus of annual or perennial herbs, edible and ornamental plants (Koh et al., 2009; Wee, 1992). The genus is indigenous to subtropical and temperate zones of Africa, South America, and South East Asia. The flowers of the *Celosia* genus species are both edible and ornamental (Simpson, 2010; Uusiku et al., 2010). The generic name is derived from the Greek word *kelos*, meaning

"burned," which refers to the flame-like flower heads. Commonly known as wool-flowers, brain celosia, or cockscombs, the flower heads are crested by fasciation. If the flower heads bear velvet-like texture, it is referred to as Velvet flower (Souza et al., 2007). The antimicrobial activities of the species in the genus *Celosia* have been established hence in this study, swing this activity against nematodes i.e. *Meloidogyne incognita*. Therefore, the identified secondary metabolites by LCMS from the ethanolic extracts of *Celosia trigyna* will be assessed against *Meloidogyne incognita* employing computational techniques

MATERIAL AND METHODS

Collection, Identification and Drying of the Plant

The leaves of *C. trigyna* were collected in November 2021, at Ilorin, Kwara State, Nigeria. It was identified at the Department of Life Sciences at the Federal University of Dutsin-Ma, Katsina. In the laboratory, the leaves were cleansed with water and air-dried for two weeks. They were ground with a pestle and mortar. The powdered samples were maintained at room temperature in clean, airtight containers until they were needed.

Extraction and Concentration

Ethanol was used to extract the powdered leaf sample. Ethanol was utilized as the extraction solvent for 7 days on 1 kg of powdered leaf sample packed in Bama

bottles. The solvent was collected by a rotary evaporator at the end of the period. The extract was fractionated using a separation funnel and Hexane as the solvent, the polar and the non-polar fraction were collected. After that, the extracts were placed in a desiccator and allowed to dry fully before being tested.

Liquid Chromatography-Mass Spectrometry (LCMS)

Protocol for LCMS Analysis (Generic Method) using LC Waters's e2695 separation module with W2998 PDA and couple to ACQ-QDA MS

The ethanol extract of the leaves of *C. trigyna* was analyzed using liquid chromatography (LC) tandem mass spectrophotometer (MS) as described by (Piovesana et al., 2018) with some modifications. The extracted samples were reconstituted in methanol and filtered through a polytetrafluoroethylene (PTFE) membrane filter with 0.45 µm size. After filtration, the filtrate (10.0 µl) was injected into the LC system and allowed to separate on Sunfire C18 5.0 µm 4.6 mm x 150 mm column. The run was carried out at a flow rate of 1.0 mL/min, with Sample and Column temperature at 25°C. The mobile phase consists of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) with a gradient as below

Time	% A	% B
0	95	5
1	95	5
13	5	95
15	5	95
17	95	5
19	95	5
20	95	5

The ratio of A/B 95:5 this ratio was maintained for a further 1 min, then A/B 5:95 for 13 min, to 15 min. then A/B 95:5 to 17 min, 19 min and finally 20 min. the PDA detector was set at 210-400 nm with a resolution of 1.2 nm and a sampling rate of 10 points/sec. The mass spectra were acquired with a scan range from m/z 100 – 1250 after ensuring the following settings: ESI source in positive and negative ion modes; capillary voltage 0.8 kv (positive) and 0.8 kv (negative); probe temperature 600oC; flow rate 10 mL/min; nebulizer gas, 45 psi. MS set in automatic mode applying a fragmentation voltage of 125 V (Piovesana et al., 2018). The data was processed with Empower 3. The compounds were identified based on the following information, elution order, and retention time (Rt), fragmentation pattern, and Base m/z.

Computational methods

Identification and preparation of molecular target

The molecular target protein human pancreatic alpha-amylase (PDB ID: 1Z32) (Fig. 2) was identified from

literature ((Nahoum et al., 2000) and downloaded from the Protein Data Bank (PDB) (<http://www.rcsb.org/>). The crystallographic water molecules and co-crystallized ligands that were interfering were eliminated, and the protein's energy was reduced through the utilization of UCSF Chimera 1.14. The minimization process involved 300 steepest descent steps at 0.02 Å. Ten conjugate gradient steps were conducted at 0.02 Å and ten update intervals. To ensure a suitable structure conformation, Gasteiger charges were integrated using Dock Prep, as suggested by Duru et al. (2021a).

Ligand Identification and preparation

The 3D structure-data files (SDF) of the compounds identified in the ethanol extract of the leaves of *C. trigyna* from the LC-MS analysis were selected and downloaded from the PubChem database. They were minimized in PyRx virtual screening tool, using Universal Force Field at 200 steps, then converted to

AutoDock ligands (pdbqt) and used for the docking analysis.

Docking procedure and analysis of results

The compounds identified from the ethanol extract of the leaves of *C. trigyna* underwent screening on the enzyme pocket that bears the cocrystallized ligand EEK. The amino acids located at this binding site were identified through the utilization of UCSF Chimera 1.14 software, and the site underwent validation using PyMOL software. The multiple docking of the ligands on the enzyme was carried out with Autodock Vina in PyRx software (Tsao et al., 2020; Duru et al., 2020), with center grid box sizes of x center: 9.7977, y center: 42.5668, and z center: 19.5188 and the dimension x centre: 31:0360, y centre: 28.5115, and z centre: 23.7148 The binding affinities of the compounds on the protein target were acquired subsequent to docking, and the outcomes were systematically organized on an Excel spreadsheet.

Analysis of protein-ligand interactions

The examination of protein-ligand interactions in the optimal compounds of the extract was carried out by analyzing the distinct amino acid residues of the protein using Biovia Discovery studio client 20.1 (BIOVIA, 2020).

RESULT AND DISCUSSION

Chromatography functions as a pivotal analytical instrument utilized for the purpose of separating and quantifying mixture components. The resultant product of chromatography, which is represented by a chromatogram, illustrates the segregated components of the mixture. The conspicuous peaks discerned on the chromatogram are suggestive of the compounds which are present within the scrutinized crude extract. The numerical values that are attributed to the peaks concur with the elution times of the compounds throughout the course of the chromatographic process. The Liquid chromatogram for the phytochemicals present in the ethanolic leaf extract of *C. trigyna* exhibited 7 peaks, which are exhibited in **Figure 1**.

From the Table 1, seven (7) compounds were tentatively identified by comparing the mass to charge m/z of the compounds as perceived by the LC-MS spectrometer and the structures elucidated above. The classes in which these compounds fall include: Hydroxybenzoic acid: compounds found under this class include Gallic acid (7), Hydroxycinnamic acid: Sinapic acid (8), Fatty acids: Cis-9-tetradecanoic acid (4), Flavonoid: vitexin glucoside (5)

Table 1: Compound identified from ethanolic leaf extract of *C. trigyna*

	Identified compounds	Molecular formula	Calculated mass	Precursor ion, m/z [M-H]⁻[M+H]⁺	Fragmentation
4	[cis-9-tetradecanoic acid]	C ₁₄ H ₂₆ O ₂	227.434	227	209; 181; 155; 199; 181; 127
5	Vitexin glucoside	C ₂₇ H ₃₀ O ₁₅	593.352	594.5181	415; 353; 283; 265; 176
6	6''-O-deoxyhexoside [apigenin 6-C-glucoside 6''-O-deoxyhexoside]	C ₂₇ H ₃₀ O ₁₄	579.252	579	415; 297; 177; 397; 344; 362
7	Gallic acid	C ₇ H ₆ O ₅	172.156	171	126
1	Malic acid	C ₄ H ₆ O ₅	134.102	133	115
8	Sinapic acid [trans-sinapic acid]	C ₁₁ H ₁₂ O ₅	225.357	225	179; 153; 115; 133; 115
3	3-feruloylquinic acid	C ₁₄ H ₂₅ NO ₁₀	367.511	366	186; 142

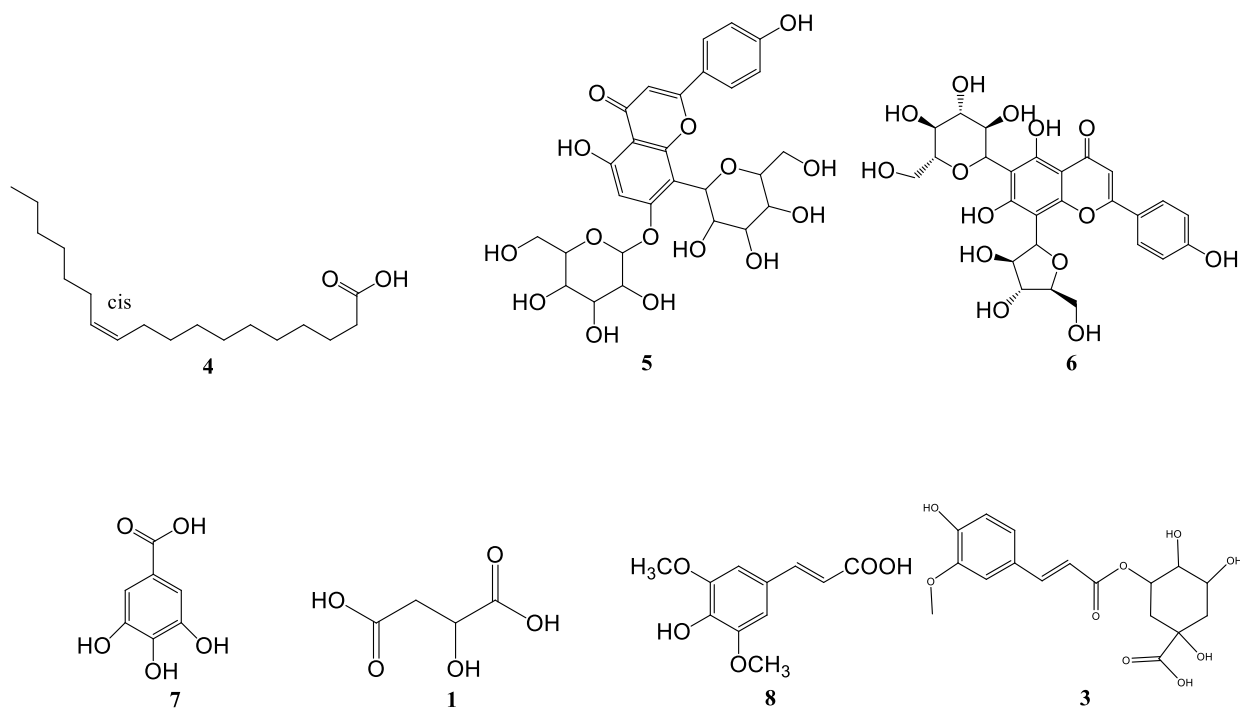


Fig 1: Structures of compounds from the ethanolic extract of *Celosia trigyna* leaves.

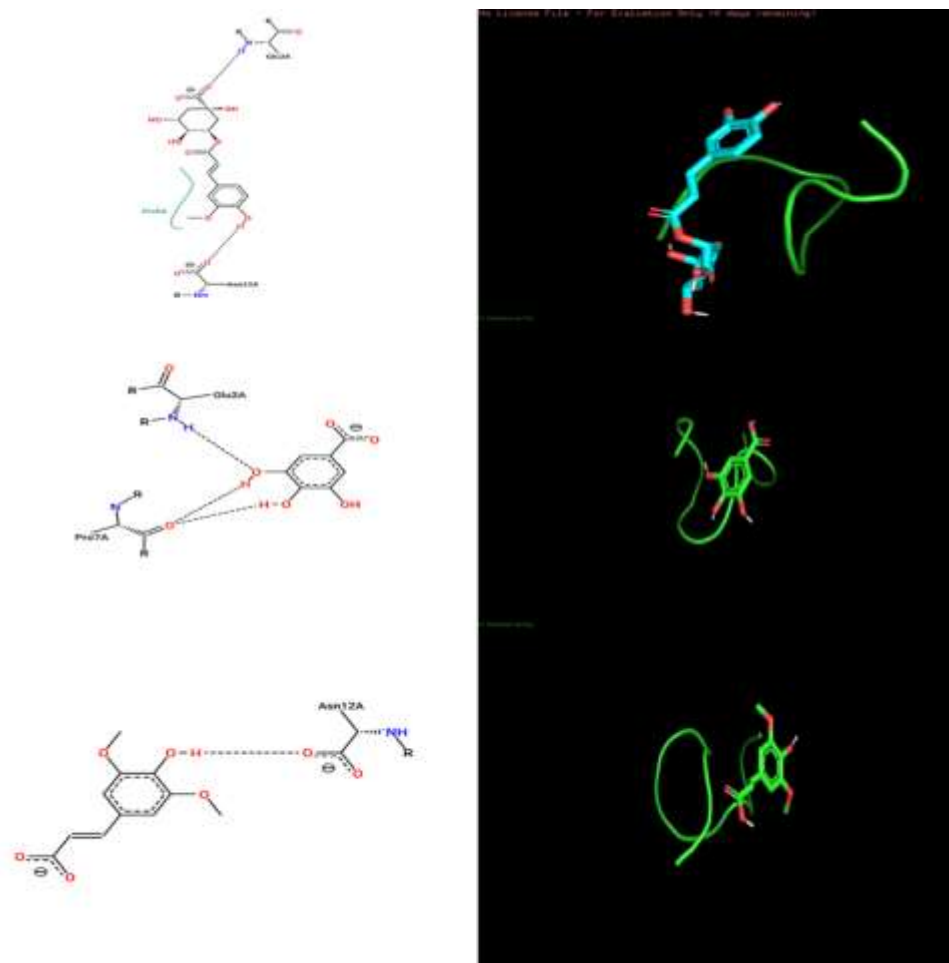


Figure 2: 2D (left) and 3D (right) views of molecular interactions of (a) 2MIF (b) 3-Feruloylquinic Acid (c) Sinapic acid (d) Gallic acid

Table 2: Details of the best three protein-ligand interactions with protein 2MIF of *M. incognita* and their Binding energies

Ligand	Binding Affinity ΔG (kcal/mol)	Interacting residues	Category	Type of Interaction
1 3-Feruloylquinic Acid	-4.2	Glu2A, Pro9A, Asn12A,	Hydrophobic Electrostatic Electrostatic	Pi-sigma Van der waal Van der waal
2 Bifenazate	-4.0	N.A	N.A	N.A
3 Boscalid	-3.9	N.A	N.A	N.A
4 Sinapic acid	-3.8	Asn12A, Glu2A,	Electrostatic, Hydrophobic	Van der waal, Pi-sigma
5 Gallic acid	-3.4	Pro7A, Glu2A	Electrostatic, Hydrophobic	Van der waal, Pi-sigma

Table 2 provides details of the best three protein-ligand interactions with protein 2MIF of *Meidogyne*

incognita. The table presents information about different ligands, their corresponding binding

affinities (ΔG , measured in kcal/mol), the interacting residues of the protein, and the categories and types of interactions involved.

3-Feruloylquinic Acid: Binding Affinity ΔG : -4.2 kcal/mol with Interacting residues: Glu2A, Pro9A, Asn12A, Category: Hydrophobic, Electrostatic, Electrostatic; Type of Interaction: Pi-sigma, Van der Waals, Van der Waals. The ligand 3-Feruloylquinic Acid exhibits the highest binding affinity with protein 2MIF, having a ΔG of -4.2 kcal/mol. It interacts with specific amino acid residues of the protein, including Glu2A, Pro9A, and Asn12A. The interactions involve hydrophobic, electrostatic, and Pi-sigma interactions. The hydrophobic interaction likely occurs between the ligand and Pro9A, while the electrostatic interactions involve charged residues like Glu2A and Asn12A. The Pi-sigma interaction may involve a pi electron system interacting with a sigma bond. These interactions contribute to the stabilization of the ligand-protein complex.

Sinapic Acid: Binding Affinity ΔG : -3.8 kcal/mol with Interacting residues: Asn12A, Glu2A; Category: Electrostatic, Hydrophobic; Type of Interaction: Van der Waals, Pi-sigma. Sinapic Acid shows a binding affinity of -3.8 kcal/mol with protein 2MIF. It interacts with the residues Asn12A and Glu2A through Van der Waals forces and likely forms a Pi-sigma interaction as well. These interactions contribute to the binding and stabilization of Sinapic Acid within the active site or binding pocket of the protein.

Gallic Acid: Binding Affinity ΔG : -3.4 kcal/mol with Interacting residues: Pro7A, Glu2A; Category: Electrostatic, Hydrophobic; Type of Interaction: Van der Waals, Pi-sigma. Gallic Acid exhibits a binding affinity of -3.4 kcal/mol with protein 2MIF. It interacts with the residues Pro7A and Glu2A through Van der Waals forces and may also form a Pi-sigma interaction. These interactions contribute to the binding of Gallic Acid with the protein. Overall, the table provides valuable information about the best three protein-ligand interactions with protein 2MIF of *Meidogyne incognita*, highlighting the binding affinities, interacting residues, and the categories and types of interactions involved. Overall, the **Table 2** provides valuable information about the best three protein-ligand interactions with protein 2MIF of *Meidogyne incognita*, highlighting the binding affinities, interacting residues, and the categories and types of interactions involved.

CONCLUSION

The identification of nematicidal compounds in ethanolic leaf extract of *C. trigyna* was carried out employing in silico methods. The molecule 3-Feruloylquinic Acid gave the highest binding affinity on the 2MIF protein of *Meidogyne incognita* to the other components in the extract and the

synthetic compounds used as control. This compound is substantially present in the leaf of this plant and its nematicidal action is similar in mechanism to the synthetic cocrystallized ligand on the enzyme. This result validates that the antimicrobial activity of the ethanolic extract of the leaves of *C. trigyna* could be employed against root-knot nematodes for sustainable agriculture.

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