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## REVIEW ARTICLE

# IN SILICO STRATEGY AGAINST SEED GERMINATION TARGETING α-AMYLASE AND ASPARAGINYL ENDOPEPTIDASE ENZYMES USING THE NEGATIVE ALLELOPATHIC GC-MS PHYTOCONSTITUENTS OF *Chromolaena odorata* LEAVES

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## ABSTRACT

Studies were conducted to determine and identify the molecular principles or compounds responsible for the notoriously reported negative (inhibitory) allelopathic effect of *Chromolaena odorata* on the germination of both monocotyledonous and dicotyledonous seeds. The aqueous extract of *Chromolaena odorata* leaves were subjected to GC-MS analysis, and 46 compounds were profiled from the extract. Using ArgusLab, these compounds profiled by means of GC-MS were subjected to *in silico* molecular docking targeting an amylase (α-amylase) and a protease (asparaginyl endopeptidase), the key enzymes necessary for starch and protein hydrolyses during germination. The compounds Retinal (Vitamin A aldehyde) showed the least binding energy for α-amylase (-13.5992 kcal/mol) compared to Maltose which showed no acceptable ligand pose; while the compound Tetradecane showed the least binding energy for asparaginyl endopeptidase (-12.6293 kcal/mol) compared to (1R,8S)-bicyclo[6.1.0]nonane (a docked residue in the PDB crystal structure) with binding energy of -9.60911 kcal/mol. This study suggests, therefore, that the inhibitory allelopathic effect of *Chromolaena odorata* on seed germination may be due to the presence of retinaldehyde and tetradecane in the aqueous extract that non/competitively inhibit the functions of α-amylase and cysteine proteinases respectively. Aside proffering a molecular explanation to the inhibitory allelopathy of *Chromolaena odorata* on germinating seeds, this finding has great commercial prospects in the formulation of eco-friendly pre-emergence herbicides and/or the design of preservatives for seed storage.

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# INTRODUCTION

There are a plenitude of reports about the chemical influence some plants have on other plants, of same or different species. This influence, capture under the concept and appellation of "Allelopathy", could either be positive (stimulatory) or negative (inhibitory). Allelopathy has been widely reported to occur in a plethora of weedy species; and one of the reported allelopathic effects of such weeds is their ability to use their chemical influence to subdue the germination of several cultivated agricultural crops in the farms (Ilori *et al.,* 2010; Otusanya, 2014).The germination of seeds starts, from the physiological point of view, from the imbibition of moisture and ends with the protrusion of the radicle. During the formation and maturation of seeds, a lot of deposition takes place as the food (in the form of starch, proteins and lipids) is reserved in the storage tissues, especially the endosperm tissue, the embryonic axis and the aleurone layer (Shewry and Halford, 2002). During germination, when imbibition initiates, the hormone gibberellic acid is synthesized, and, on reaching the aleurone layer, induces *de novo* synthesis of amylases and proteases. These *de novo* enzymes, together with some other stored proteases, begin to hydrolyze the food reserves, splitting them into resultant sub units. The resultant amino acids and energy is dedicated to the growth of the seedling (Bewley, 1997). The desirable nutritional changes that occur during sprouting are mainly due to breakdown of complex compounds into simple forms, transformation into essential constituents, and breakdown of undesirable constituents. As an enzyme, α–amylase catalyzes the hydrolysis of polysaccharides, converting them mainly to maltose and larger oligosaccharides. α–amylase is usually synthesized during germination; and, acts by randomly hydrolyzing the a-1,4-glucan linkages in the starch polymers: amylose and amylopetin; finally converting all the amylose to maltose and the amylopectin to maltose, glucose and alpha-limit dextrins (Helland *et al*., 2002). Germination has a positive impact on the amino acid composition and protein availability in the seeds. In mature dry seeds, proteins are stored in the embryo axis as well as in the endosperm as albumin and globulins, which serve as amino acid reserves, which, if mobilized, can be used to nourish the seedling. The mobilization of seeds storage proteins is a very important germination and post-germination event. Proteolytic enzymes responsible for protein degradation serve an

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essential function in the germination of seeds (Schaller, 2004). The proteolysis of seed storage proteins during germination is very essential in germination as it helps supply amino acids to the embryo in the process of seedling development. This process is overseen by the action of peptidases (Barduche *et al*., 2018). Gimenez *et al*. (2019) have explained that proteases, amylases and other hydrolases are crucial for the survival of the seedling until photosynthesis is fully established. It is therefore very practical to assert that the potential inhibitory effect of certain weedy species on the germination of crop seeds can be traceable to the effect some of the phytoconstituents have on the two key enzymes necessary for germination – amylases (α-amylase, in this case) and proteases (asparaginyl endopeptidase). One of such menacing weeds that has been notoriously reported as an allelopathic weed to the germination of certain important agricultural crops is the *Chromolaena odorata* (Devi and Dutta, 2012; Otusanya *et al*., 2015). The aqueous foliar extract of *C. odorata* has been severally implicated by many reports as the most active and potent source of allelochemicals in the plant. Siam weed (*Chromolaena odorata*) ((L.) R.M. King & H. Robinson) is a herbaceous, perennial, semi woody shrub belonging to the family Asteraceae and forms dense tangled bushes about 1.5 to 2.0 m in height (Phan, 2001). It bears three-veined, ovate-triangular leaves placed oppositely, and with a shallow, fibrous root system (Henderson, 2001). It is a weedy pioneering shrub native to the Americas (Gautier, 1992) which was introduced into diverse ecological areas of tropical lands (Owolabi *et al*., 2010) where it has become one of the worst terrestrial invasive plants (Gautier, 1992). Siam weed is currently recognized as one of the world's worst tropical weeds due to its extremely fast growth rate (up to 20 mm per day) and prolific seed production (Owolabi *et al*., 2010). In the tropics of Africa and Asia it has become agricultural weeds. Considering the possible application of the allelopathy and allelochemicals of *C. odorata* towards the design of an eco-friendly herbicide or a seed storage preservative, this study aims to use GC-MS and *in silico* molecular docking approaches to profile the water-soluble phyto-constituents of *C. odorata* targeting the α-amylase and asparaginyl endopeptidase towards arresting the germination of seeds.

# MATERIALS AND METHODS

***Experimental Site:*** The study was conducted in Petri plates in the Centre for Ecological Studies, Department of Plant Science and Biotechnology, University of Port-Harcourt, Choba, Rivers State, Nigeria. The GC-MS analysis was done at the Chemistry Analytical Laboratory of the Department of Chemistry, Faculty of Science, Yobe State University, Damaturu, Nigeria.

***Plant Material used for the Study:*** The foliage of *Chromolaena odorata* was obtained from the ecological forest of the UniPark Campus, University of Port-Harcourt, Choba, Rivers State, Nigeria.

**Preparation of aqueous foliar extract of *C. odorata:*** With slight modifications, but in accordance to the method as described by Uzoma *et al*. (2018), whole leaves of *C. odorata* were harvested from the ecological forest of the University of Port-Harcourt (4.9069°N, 6.9170°E), Choba, Port-Harcourt, Rivers State. The collected leaves were rinsed in water to remove adhering dust and soil particles, and then air-dried for 30 minutes to remove surface water. The plants were pulverized using an electronic grinder; and 2000g of the pulverized plant samples were macerated in 8000ml (8L) of distilled water for 72 hours (3 days) with intermittent agitation. The setup was filtered using Whatman paper, and the filtrate was steam-dried over a water bath at 40°C to obtain the dry plant extract which served as sample for GC-MS analysis.

**GC-MS analysis:** Aqueous extracts of *C. odorata* was probed for volatile and semi-volatile compounds by GC-MS approach. Using Agilent 7890B gas chromatography connected to a mass spectrometer, 1 µl of the sample was injected in the pulsed spitless mode onto a 30 m × 0.25 mm id DB 5MS coated fused silica column with a film thickness of 0.15 micrometer. Helium gas was used as carrier gas and the column head pressure was maintained at 15 psi to give a constant rate of 1 ml/min. Other operating conditions were preset. The column temperature was initially held at 85 °C for 0 minutes, increased to 110°C at a rate of 25 °C/min, then to 230 °C at a rate of 8 °C/min and 10 min hold, then to 250°C at 5 °C/min rate and 7 min hold and to final temperature of 280 °C at a rate of 25 °C/min. Mass spectra data were acquired in the scan mode in m/z range 50-550. The compounds assayed in the sample were identified by comparing their retention times with those of reference compounds in the library and by comparison of their mass spectra with those of reference substances from the National Institute for Standard and Technology (NIST) library with a quality factor > 80 used as criterion for acceptance (Meyer *et al*., 2010).

**Obtaining the compounds**

**PDBe:** The crystal structures of the enzymes α-amylase and asparaginyl endopeptidase(with PDB IDs 1smd and 6xt5 respectively) were obtained in the ".pdb" format from the European Protein Data Bank (PDBe; https://www.ebi.ac.uk/pdbe/entry/search/index/), a member organization of the Worldwide Protein Data Bank.

**PubChem and NIST Webbook:** The molecular structures of the individual profiled compounds were obtained from PubChem [https://pubchem.ncbi.nlm.nih.gov/] (in the ".sdf" format) or NIST Webbook [https://webbook.nist.gov/] (in the ".mol" format), with their respective IDs shown in the results table.

**Open Babel:** All the molecular structures of compounds in the ".sdf" format were converted to the ".mol" format for easy readability in ArgusLab, using the OpenBabel.

**Molecular Docking:** *In silico m*olecular docking of the profiled compounds into the binding sites of the respective enzymes was carried out using ArgusLab. The receptor (enzyme) molecule was called into the ArgusLab platform: FILE>OPEN>select the saved receptor "1smd.pdb". Water molecules were deleted. The docked "misc" residue of the crystal structure was deleted; leaving only the amino acids. All the amino acids were selected and made into a binding site: "make a group from this residue". The residue was renamed, and the group type set as "binding site". The new binding site group was selected and hydrogens added. The ligand (profiled compound) molecule was called into the ArgusLab platform: FILE>OPEN>select the saved ligand "CID-96170.mol". The geometry of the compound was optimized using the "clean geometry" function. Hydrogens were added to the structure. The compound was made into a ligand: "make a ligand group from this residue". A docking calculation was initiated: "set up a docking calculation" to dock the ligand into the binding site. The scoring function was "Ascore". Bindng site bounding box: "Display box" was checked, and box was set to "calculate size". The following presets were allowed:- Docking engine: ArgusDock; Calculation Type: Dock; Ligand: Flexible. The docking calculation was then started; at the end of which the energy for the "best ligand pose" was recorded and the result of the docking calculation saved. The docked enzyme-ligand complex is saved in the ".pdb" format for further visualization in Pymol.

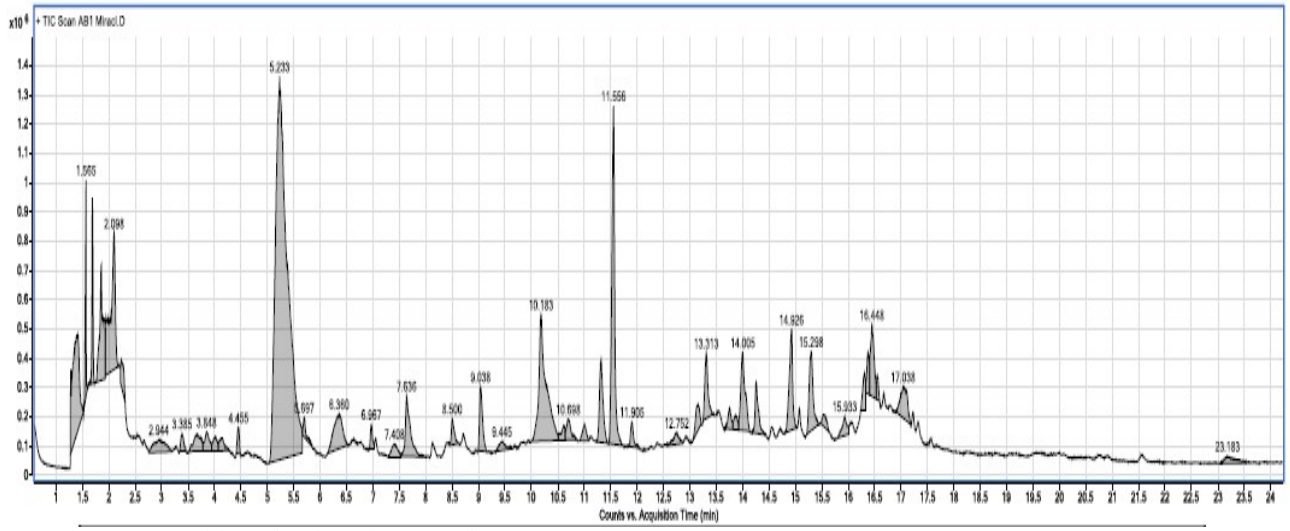
**Molecular Preparation and Visualization**

**Pymol:** The enzyme-ligand complex was called into Pymol. The enzyme structure was shown as cartoon sheets, while the organic ligand was shown as sticks. Using the presets function, a close-up of the hydrogen bonds between the ligand and the amino acid residues were visualized and the bonded amino acids labeled. A view of the ligand pose in the enzyme binding pocket was visualized using the presets>ligand site>solid surface feature; and, images were captured using the Ray feature.

**Chem Sketch:** The 2D diagrammatic representation of the molecular structure of the best three scoring ligands for each enzyme was made using the ACD/ChemSketch.

# RESULTS

## Results for Gc-Ms Analyses




GC chromatogram of the analyzed *C. odorata* sample

**Table 1. Gc-Ms profile of the compounds from the foliage of *C. odorata***

| S/N | NAME | FORMULA | MASS | Retention Time | AREA |
|---|---|---|---|---|---|
| 1 | Silanol, dimethyl- | $C_2H_8OSi$ | 76.0344415 | 1.417 | 3184100.9 |
| 2 | p-Dioxane-2,3-diol | $C_4H_8O_4$ | 120.0422587 | 1.565 | 727784.52 |
| 3 | Oxirane, 2-(1,1-dimethylethyl)-3-ethyl-, cis- | $C_8H_{16}O$ | 128.120115 | 1.686 | 846675.68 |
| 4 | Ethylamine, N,N-dimethyl-2-[4-(chloromethyl)pheno | $C_{10}H_{14}CIN$ | 213 (CID WT 183.68) | 1.857 | 1811792.83 |
| 5 | Methamphetamine | $C_{10}H_{15}N$ | 149.120449 | 2.098 | 2961276.73 |
| 6 | Pentanal, 2-methyl- | $C_6H_{12}O$ | 100.088815 | 2.235 | 402620.56 |
| 7 | Formaldehyde, (2-butenyl)methylhydrazone | $C_6H_{12}N2$ | 112.1000485 | 2.944 | 666642.98 |
| 8 | Tetradecane | $C_{14}H_{30}$ | 198.234751 | 3.385 | 223056.05 |
| 9 | 4-Cyclopentene-1,3-diol, cis- | $C_5H_8O_2$ | 100.0524297 | 3.66 | 569212.28 |
| 10 | trans-1-Butyl-2-methylcyclopropane | $C_8H_{16}$ | 112.1252007 | 3.848 | 414116.23 |
| 11 | 1,6:3,4-Dianhydro-2-deoxy-β-d-lyxo-hexopyranose | $C_6H_8O_3$ | 128.047344 | 4.009 | 271587.09 |
| 12 | Bicyclo[4.1.0]heptan-2-ol, (1α,2β,6α)- | $C_7H_{12}O$ | 112.088815 | 4.152 | 295938.52 |
| 13 | Decane | $C_{10}H_{22}$ | 142.28 | 4.455 | 239895.5 |
| 14 | Catechol | $C_6H_6O_2$ | 110.0367794 | 5.233 | 20446197.65 |
| 15 | 1,2-Benzenediol, mono(methylcarbamate) | $C_8H_9NO_3$ | 167.058243 | 5.697 | 242791.7 |
| 16 | 7-Oxabicyclo[4.1.0]heptan-2-ol | $C_6H_{10}O_2$ | 114.0680795 | 6.36 | 1386361.04 |
| 17c | 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- | $C_{15}H_{24}$ | 204.1878 | 6.967 | 238990.38 |
| 18 | 2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)]]- | $C_{15}H_{26}O_2$ | 238.19328 | 7.408 | 329312.62 |
| 19 | (Z,Z)-α-Farnesene | $C_{15}H_{24}$ | 204.1878 | 7.636 | 1348072.49 |
| 20c | 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- | $C_{15}H_{24}$ | 204.1878 | 8.5 | 337988.7 |
| 21 | Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- | $C_{15}H_{24}$ | 204.1878 | 9.038 | 875699.67 |
| 22 | 8-Azabicyclo[3.2.1]octan-3-ol, 8-methyl-, 8-oxide, (endo,anti)- | $C_8H_{15}NO_2$ | 157.110279 | 9.445 | 261559.49 |
| 23 | 16-Heptadecenal | $C_{17}H_{32}O$ | 252.245316 | 10.183 | 4167714.65 |
| 24± | (-) | (-) | (-) | 10.612 | 251394.51 |
| 25e | 3-Methyl-4-(methoxycarbonyl)hexa-2,4-dienoic acid | $C_9H_{12}O_4$ | 184.073559 | 10.698 | 423873.99 |
| 26 | (Z)-8-Hydroxy-4,7-dimethyl-oct-6-enoic acid lactone | $C_{10}H_{16}O_2$ | 168.115029 | 11.007 | 276152.95 |
| 27 | Bicyclo[5.1.0]octane, 8-methylene- | $C_9H_{14}$ | 122.1095505 | 11.321 | 1314517.85 |
| 28 | Bicyclo[6.1.0]non-1-ene | $C_9H_{14}$ | 122.1095505 | 11.556 | 4264653.36 |
| 29 | 3-Methyl-4-(methoxycarbonyl)hexa-2,4-dienoic acid | $C_9H_{12}O_4$ | 184.073559 | 11.905 | 328159.19 |
| 30 | 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane | $C_{15}H_{26}O$ | 222.198365 | 12.752 | 305944.14 |
| 31f | Cyclopropa[d]naphthalen-2(4aH)-one, 1,1a,5,6,7,8-hexahydro-4a,8,8-trimethyl-, [1aR-(1aα,4aβ,8aS*)]- | $C_{14}H_{20}O$ | 204.151415 | 13.158 | 480188.83 |
| 32 | Epoxy-α-terpenyl acetate | $C_{12}H_{20}O_3$ | 212.141245 | 13.313 | 917947.55 |
| 33a | (2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide | $C_{10}H_{16}O_2$ | 168.115029 | 13.753 | 301392.94 |
| 34 | 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)- | $C_{21}H_{30}O_3S$ | 362.191566 | 13.873 | 239742.45 |

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| | | | | | |
|---|---|---|---|---|---|
| 35 | (2R,4R)-p-Mentha-[1(7),8]-diene, 2-hydroperoxide | $C_{10}H_{16}O_2$ | 168.115029 [168.2328] | 14.005 | 1520240.56 |
| 36f | Cyclopropa[d]naphthalen-2(4aH)-one, 1,1a,5,6,7,8-hexahydro-4a,8,8-trimethyl-, [1aR-(1aα,4aβ,8aS*)]- | $C_{14}H_{20}O$ | 204.151415 | 14.257 | 734819.42 |
| 37 | 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione | $C_{11}H_{16}O_4$ | 212.24 | 14.926 | 1503746.56 |
| 38a | (2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide | $C_{10}H_{16}O_2$ | 168.115029 | 15.298 | 1402499.37 |
| 39 | 1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane | $C_{15}H_{24}O$ | 220.182715 | 15.544 | 236966.15 |
| 40 | Retinal | $C_{20}H_{28}O$ | 284.214016 | 15.933 | 397590.76 |
| 41 | 5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol | $C_{15}H_{28}O$ | 224.214016 | 16.311 | 464255.56 |
| 42 | (1S,15S)-Bicyclo[13.1.0]hexadecan-2-one | $C_{16}H_{28}O$ | 236.214016 | 16.374 | 417306.63 |
| 43d | 2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate | $C_{30}H_{33}ClO_6$ | 524.196568 | 16.448 | 1175121.74 |
| 44b | 1-Hexyl-2-nitrocyclohexane | $C_{12}H_{23}NO_2$ | 213.32 | 16.551 | 216331.4 |
| 45b | 1-Hexyl-2-nitrocyclohexane | $C_{12}H_{23}NO_2$ | 213.32 | 17.038 | 1003336.77 |
| 46d | 2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate | $C_{30}H_{33}ClO_6$ | 524.196568 | 23.183 | 352952.52 |

NB:
1. "*" signifies that the compounds is a control (natural) ligand of the enzyme
2. "±" signifies that the compound was not identified
3. Compounds having the same alphabets attached to their serial number(s) are the same.
4. "NA" signifies that there was no acceptable ligand pose.

**Table 2. Docking scores of the compounds profiled from *c. odorata* leaves**

| S/N | NAME | PubChem ID | DOCKING SCORE (kcal/mol) | |
|---|---|---|---|---|
| | | | α-amylase (1smd) | Asparaginyl endopeptidase (6xt5) |
| * | D-Glucose | CID5793 | NA | (-) |
| * | Maltose | CID6255 | NA | (-) |
| * | (1R,8S)-bicyclo[6.1.0]nonane | CID6432405 | (-) | -9.60911 |
| 1 | Silanol, dimethyl- | CID9989211 | -5.92314 | -6.11863 |
| 2 | p-Dioxane-2,3-diol | CID96170 | NA | NA |
| 3 | Oxirane, 2-(1,1-dimethylethyl)-3-ethyl-, cis- | CID12587200 | -9.16349 | -8.18187 |
| 4 | Ethylamine, N,N-dimethyl-2-[4-(chloromethyl)pheno | CID12467520 | -9.16142 | -10.6479 |
| 5 | Methamphetamine | CID10836 | -8.05919 | -8.59998 |
| 6 | Pentanal, 2-methyl- | CID31245 | -5.4474 | -6.15993 |
| 7 | Formaldehyde, (2-butenyl)methylhydrazone | CID5364995 | -6.6707 | -7.74806 |
| 8 | Tetradecane | CID12389 | -11.8344 | -12.6293 |
| 9 | 4-Cyclopentene-1,3-diol, cis- | CID10148988 | -8.88101 | -10.4902 |
| 10 | trans-1-Butyl-2-methylcyclopropane | CID6432402 | -5.32454 | -5.28046 |
| 11 | 1,6:3,4-Dianhydro-2-deoxy-β-d-lyxo-hexopyranose | CID545707 | -8.83347 | -9.51846 |
| 12 | Bicyclo[4.1.0]heptan-2-ol, (1α,2β,6α)- | CID533432 | -7.7575 | NA |
| 13 | Decane | CID15600 | -9.94522 | -12.0735 |
| 14 | Catechol | CID289 | -8.19956 | -8.25086 |
| 15 | 1,2-Benzenediol, mono(methylcarbamate) | CID82537 | -7.12642 | -7.08981 |
| 16 | 7-Oxabicyclo[4.1.0]heptan-2-ol | CID317053 | NA | NA |
| 17c | 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- | CID5373727 | NA | NA |
| 18 | 2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)]]- | NIST:26184 | -9.61003 | -8.54737 |
| 19 | (Z,Z)-α-Farnesene | CID5317320 | -7.21989 | -6.71972 |
| 20c | 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- | CID5373727 | NA | NA |
| 21 | Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- | CID10223 | -11.5815 | -9.97811 |
| 22 | 8-Azabicyclo[3.2.1]octan-3-ol, 8-methyl-, 8-oxide, (endo,anti)- | CID98058 | NA | NA |
| 23 | 16-Heptadecenal | CID557527 | -7.41445 | -8.22334 |
| 24± | (-) | (-) | (-) | (-) |
| 25e | 3-Methyl-4-(methoxycarbonyl)hexa-2,4-dienoic acid | CID5365038 | -9.00452 | -8.77928 |
| 26 | (Z)-8-Hydroxy-4,7-dimethyl-oct-6-enoic acid lactone | CID5370138 | -9.12603 | -9.67326 |
| 27 | Bicyclo[5.1.0]octane, 8-methylene- | CID556475 | -9.83034 | -12.2951 |
| 28 | Bicyclo[6.1.0]non-1-ene | CID5367451 | NA | NA |
| 29e | 3-Methyl-4-(methoxycarbonyl)hexa-2,4-dienoic acid | CID5365038 | -9.00452 | -8.77928 |
| 30 | 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane | CID550196 | NA | NA |
| 31f | Cyclopropa[d]naphthalen-2(4aH)-one, 1,1a,5,6,7,8-hexahydro-4a,8,8-trimethyl-, [1aR-(1aα,4aβ,8aS*)]- | NIST:4677 | -10.1105 | -10.084 |
| 32 | Epoxy-α-terpenyl acetate | CID538935 | NA | NA |
| 33a | (2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide | NIST:U292744 | -9.8629 | -11.0846 |
| 34 | 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)- | CID5368759 | -8.15538 | -6.94693 |
| 35 | (2R,4R)-p-Mentha-[1(7),8]-diene, 2-hydroperoxide | NIST:U292741 | -9.87781 | -11.0586 |
| 36f | Cyclopropa[d]naphthalen-2(4aH)-one, 1,1a,5,6,7,8-hexahydro-4a,8,8-trimethyl-, [1aR-(1aα,4aβ,8aS*)]- | NIST:4677 | -10.1105 | -10.084 |
| 37 | 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione | CID537288 | NA | NA |
| 38a | (2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide | NIST:U292744 | -9.8629 | -11.0846 |

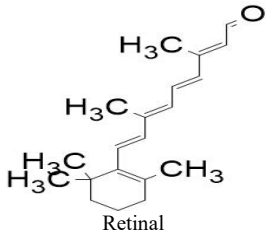
| | | | | |
|---|---|---|---|---|
| 39 | 1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane | CID21681001 | -10.7913 | -10.5223 |
| 40 | Retinal | CID638015 | -13.5992 | -9.48166 |
| 41 | 5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol | CID536434 | -10.7674 | -9.60078 |
| 42 | (1S,15S)-Bicyclo[13.1.0]hexadecan-2-one | CID13760785 | NA | NA |
| 43d | 2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate | CID537118 | -10.2604 | -9.63014 |
| 44b | 1-Hexyl-2-nitrocyclohexane | CID544017 | -7.4204 | -7.68087 |
| 45b | 1-Hexyl-2-nitrocyclohexane | CID544017 | -7.4204 | -7.68087 |
| 46d | 2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate | CID537118 | -10.2604 | -9.63014 |

NB:
1. "*" signifies that the compounds is a control (natural) ligand of the enzyme
2. "±" signifies that the compound was not identified
3. Compounds having the same alphabets attached to their serial number(s) are the same.
4. "NA" signifies that there was no acceptable ligand pose.

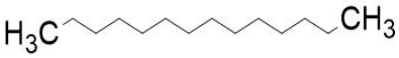
**Table 3. Docking scores of the best three ligands compared to the natural ligand**

| BEST DOCKED LIGANDS (kcal/mol) | | | | | | |
|---|---|---|---|---|---|---|
| α-amylase (1smd) | | | | Asparaginyl endopeptidase (6xt5) | | |
| COMPOUND | SCORE | RANK | | COMPOUND | SCORE | RANK |
| Retinal | -13.5992 | 1 | | Tetradecane | -12.6293 | 1 |
| Tetradecane | -11.8344 | 2 | | Bicyclo[5.1.0]octane, 8-methylene- | -12.2951 | 2 |
| Cadina-1(10),4-diene | -11.5815 | 3 | | Decane | -12.0735 | 3 |
| D-Glucose//Maltose | NA | (-) | | (1R,8S)-bicyclo[6.1.0]nonane | -9.60911 | 16 |

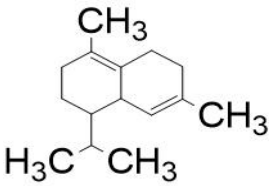
**α-amylase (1smd)**



Retinal

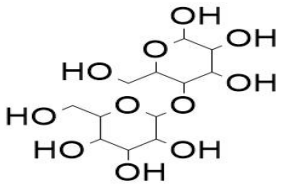


Tetradecane



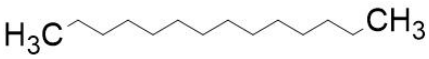
4,7-dimethyl-1-(propan-2-yl)-1,2,3,5,6,8a-hexahydronaphthalene

*Syn: Cadina-1(10),4-diene*

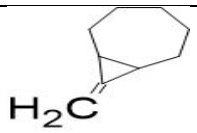


6-(hydroxymethyl)-5-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}oxane-2,3,4-triol

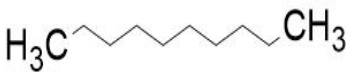
**Asparaginyl endopeptidase (6xt5)**



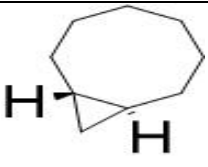
Tetradecane



8-methylidenebicyclo[5.1.0]octane



Decane



(1*S*,8*S*)-bicyclo[6.1.0] nonane
*Syn: trans-Bicyclo[6.1.0]nonane*

**Figure 1. 2D diagrammatic representations of the best docked ligands for α-amylase and asparaginyl endopeptidase (diagrams made with ACD/Chemsketch)**

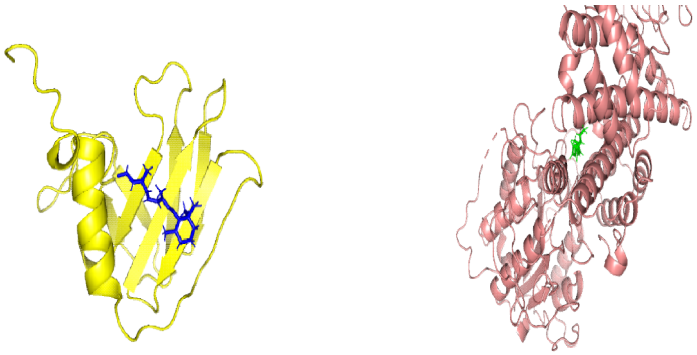



**Figure 2. Poses of best docked ligands in their respective enzymes (A: α-amylase; B: asparaginyl endopeptidase) [Images generated with Pymol]**

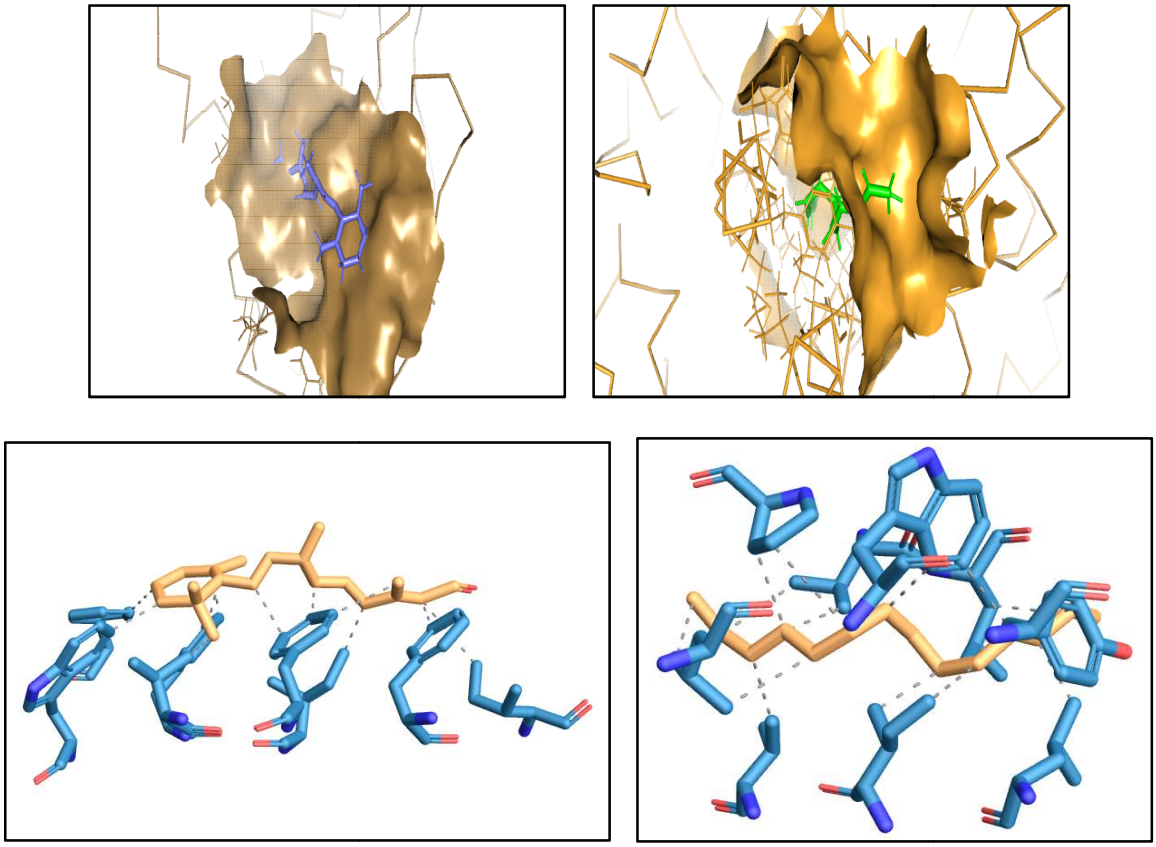


**Figure 4. Ligand interactions with amino acids in their respective enzymatic binding sites (E: α-amylase; F: asparaginyl endopeptidase) [Images generated with PLIP]**

**PLIP:** The molecular interaction between the best scoring ligand and the amino acid residues of the enzymatic binding site was generated using the Protein-Ligand Interaction Profiler (PLIP; https://projects.biotec.tu-dresden.de/plip-web/plip/)

# DISCUSSION

The GC-MS analyses of the aqueous extract of *C. odorata* showed a metabolomic profile of 46 peaks, with the delineation that some peaks were duplicates of the same compound, and one peak was not matched with a spectra in the NIST database; thus, culminating into 39 unique compounds from diverse chemical groups and functionalities that were established from the analyses. Some of these compounds have important biological functions. A compound like retinal is an optical vitamin (vitamin A aldehyde) which serves a plethora of functions in plants and animals; catechol, which has been described as a "benzenediol comprising of a benzene core carrying two hydroxy substituensts ortho to each other", is a plant metabolite that is functionally established as a genotoxin and allelochemical.

Other workers have also used GC-MS metabolomics techniques to profile certain other plants and fungi. Afieroho and Ugoeze (2014) have profiled the n-Hexane extract of the sclerotia of the fungus *Lentinus tuber-regium.* In their study, they characterized seven fatty acids derivatives: heptadecenal, n-hexadecanoic acid, 1-eicosene, linoleic acid, oleic acid, linoleic acid ethyl ester, and octadecanoic acid, and five steroidal triterpenoids: cholesterol, α-ergostenol, anthraergostatetraenol, stigmasterol, and alpha-ergosta-4,6,8(14),22-tetraen-3-one. Considering the importance of exploiting wild mushrooms as potential source of bioactive compounds, another group of workers (Oni *et al*, 2020) employed GC-MS approach to profile the metabolome of the dried sporocarp of six wild edible mushooms: *Lentinus squarrosulus* Mont., *Auricularia auricular-judae* (Bull.) Wettst., *Mycetinis copelandii* (Desjardin) A.W. Wilson & Desjardin, *Baeospora myosura* (Fr.) Singer, *Pleurotus ostreatus* (Jacq. ex. fr) Kummer and *Volvariella volvacea* (Bull. ex. Fr.) Singer. The workers were able to identify 14, 26, 33, 5, 49 and 32 different compounds. The workers explained that the identified compounds showed several bio-activities, some of which include: "antiviral, anticancer, antimicrobial, antioxidant, hypocholesterolemic,

anaphylactic, narcotic, neurostimulant, emollient, expectorant, laxative, pesticidal, insecticidal and insectifugal activities." Olusegun and Musa (2014) also profiled the metabolites in the oil extracted from *C. odorata* using GC-MS approach. They identified 20 compounds in the oils, with the main components being α-pinene (13.60 %,), caryophyllene (9.20%), Bicyclo [7.2.0] undec-4-ene (6.76%), β- pinene (4.83%) and Germacrene D (4.70%). A similar study was done by Joshi (2013).Some of the compounds profiled in the oil content of *C. odorata* were also identified in this study. Many reports have established that the aqueous extract of *C. odorata* has an inhibitory allelopathic effect on the germination of many seeds of important agricultural crops (Devi and Dutta, 2012; Usuah *et al*., 2013; Devi *et al*., 2014). In a bid to determine their role in the inhibitory allelopathic effect on the germination of seeds, the compounds identified by the GC-MS approach were docked into two key enzymes responsible for seed germination – α-amylase and asparaginyl endopeptidase. The protein-ligand docking analyses showed the best three ligands docked to the respective enzymes. The best ligands docked to the enzyme α-amylase, in order of lowest energy levels, are Retinal (-13.5992 kcal/mol), Tetradecane (-11.8344 kcal/mol) and Cadina-1(10),4-diene (-11.5815kcal/mol) which were compared to the natural ligand Maltose, which showed no acceptable ligand pose; while the best ligands docked to the enzyme asparaginyl endopeptidase, in order of lowest energy levels, are Tetradecane (-12.6293 kcal/mol), 8-methylene-Bicyclo[5.1.0]octane (-12.2951 kcal/mol) and Decane (-12.0735 kcal/mol) which compared to the natural ligand trans-bicyclo[6.1.0]nonane (-9.60911 kcal/mol) which ranked the 16th best docked ligand of all the ligands docked to the asparaginyl endopeptidase.

The ligands with the most negative binding energies were considered to have the strongest affinity towards their respective enzymes. This finding points out that the most likely ligands from the leaf of *C. odorata* responsible for the delay or lack thereof of germination in most seed are the Retinal and Tetradecane. Retinal, also called "Vitamin A aldehyde", is a form of vitamin A. Vitamin A has been described as "a group of unsaturated organic compounds which is mostly associated with retinol" (Sajovic *et al*., 2022). Carazo *et al*. (2021) have explained that the Vitamin A (retinol), its natural (including retinal) and synthetic derivatives are termed "retinoids", and that these different forms of Vitamin A can convert from one form to another with the help of specific enzymes. Sajovic *et al*. (2022) has explained that non-retinol retinoids and precursors, such as retinal, usually convert to vitamin A (retinol); but, if they remain unchanged and not converted, then they begin to function as antioxidants. As antioxidants, the retinal will inhibit oxidation, helping to counteract the deterioration of stored food products. This is one of the functions of retinal as reported in this study, where it is serving as an α-amylase inhibitor, helping to stop the enzymatic breakdown and mobilization of glycogen; and, thus inhibiting the germination of the seed by stopping the synthesis of ATP.

Pavirhra and Lalitha (2020) have implicated Tetradecane as a biocontrol agent produced by some *Trichoderma* spp. to tackle the fungal pathogen, *Fusarium oxysporum*, in tomato. The workers reported that tomatoes inoculated with *F. oxysporum*, a fungus responsible for wilt disease in tomato, and treated with *Trichoderma* extract showed strong inhibitory activities against *F. oxysporum*. GC-MS studies of the extract of the *Trichoderma* spp showed presence of Dodecane, Tetradecane and some other metabolites. These findings led the workers to the implication that tetradecane can serve as a bio-control agent against *F. oxysporum* in tomato. Tetradecane has also been reported in the GC-MS profile of a plenitude of plants (Prayitno *et al*., 2021). Kaiira *et al*. (2021) have also reported Tetradecane as one of the allelochemical entities produced by certain crops that inhibit the germination of certain agriculturally important weeds and crops. Evidence from this study showed that, when compared to the natural ligand, Maltose, which showed no acceptable pose, the ligand, Retinal, having the lowest docking score for α-amylase is likely to serve as a non/competitive inhibitor of the enzyme α-amylase and thus stop the mobilization of starch and glycogen which are necessary first steps in the provision of ATP necessary for the germination of most seeds. Also, comparing Tetradecane to the natural ligand of asparaginyl endopeptidase, trans-bicyclo[6.1.0]nonane, which showed a docking energy of -9.60911 kcal/mol and ranked 16th in chronology, Tetradecane, having the lowest docking score for asparaginyl endopeptidase, is likely to serve as a non/competitive inhibitor of the enzyme (asparaginyl endopeptidase), and thus stop the enzyme from mobilizing proteins which is also a necessary first step to the cell division that is concomitant with germination and growth of radicles. Considering that there are a plenitude of literature reporting that *C. odorata* has a negative (inhibitory) allelopathic effect on the seed germination of certain agricultural crops including *Zea mays* (Devi and Dutta, 2012), *Abelmoschus esculentus* and *Citrullus vulgaris* (Usuah *et al*., 2013), and *Solanum lycopersicum* (Devi *et al*., 2014),these two ligands, Retinal and Tetradecane, are therefore the primary chemical constituents of the aqueous foliar extract of *C. odorata* that are responsible for the perceived allelopathic inhibition of seed germination in most plants as reported in several literature. As inhibitors, retinal and tetradecane can be realistically employed as pre-emergence herbicides to arrest the hydrolyses of seed storage starch and proteins, and concomitantly stop the germination of certain agriculturally important weeds.

The desirable nutritional changes that occur during sprouting are mainly due to breakdown of complex compounds into simple forms, transformation into essential constituents, and breakdown of undesirable constituents. As an enzyme, α–amylase catalyzes the hydrolysis of polysaccharides, converting them mainly to maltose and larger oligosaccharides. α–amylase is usually synthesized during germination; and, acts by randomly hydrolyzing the a-1,4-glucan linkages in the starch polymers: amylose and amylopetin; finally converting all the amylose to maltose and the amylopectin to maltose, glucose and alpha-limit dextrins (Helland *et al*., 2002). Germination has a positive impact on the amino acid composition and protein availability in the seeds. In mature dry seeds, proteins are stored in the embryo axis as well as in the endosperm as albumin and globulins, which serve as amino acid reserves, which, if mobilized, can be used to nourish the seedling. The mobilization of seeds storage proteins is a very important germination and post-germination event. Proteolytic enzymes responsible for protein degradation serve an essential function in the germination of seeds (Schaller, 2004). The proteolysis of seed storage proteins during germination is very essential in germination as it helps supply amino acids to the embryo in the process of seedling development. This process is overseen by the action of peptidases (Barduche *et al*., 2018).Gimenez *et al*. (2019) have explained that proteases, amylases and other hydrolases are crucial for the survival of the seedling until photosynthesis is fully established. It is therefore very practical to assert that the potential inhibitory effect of retinal and tetradecane on the α–amylase and asparaginyl endopeptidase respectively can be gainfully employed as potent herbicides or allelochemicals.

The PLIP schematics of enzyme-ligand interactions showed that both ligands, Retinal and Tetradecane, had good hydrogen bonding with 8 amino acid residues from the respective enzyme binding sites which they bound. Retinal was bound in a stable complex by hydrogen bonds to the amino acids Trp-409, Val-417, Phe-429, Phe-487, Ile-453, Ile-427, Phe-419, and Phe-406, thus describing the binding site; while Tetradecane was bound in a stable complex by hydrogen bonds to the amino acids Pro-819, Leu-340, Trp-344, Tyr-347, Leu-361, Ile-364, Val-368 and Leu-815, also describing its binding site. The high hydrogen affinity for these ligands is likely responsible for the very low docking energy and their ability to form stable complexes. Several workers have used molecular docking approaches to probe for phytoconstituents that could serve as possible remedies to diseases or as agents of degradation of environmental pollutants. Kurjogi *et al*. (2018) have use it to search for natural drugs for *Staphylococcus aureus* enterotoxins, and found that 28-Norolean-12-en-3-one (from a flower) and Betulin (from a tree bark) were good inhibitors and potent drug leads. Other workers such as Das *et al*. (2020) and Satapute *et al*. (2019) have employed molecular docking approaches in their

respective probes for the natural anti-sickling agent and a degradation agent of an agrochemical contaminant. The seeming dearth of literature showing the use of molecular docking approach to identify a possible allelochemical lends some novelty to the finding of this study that retinal and tetradecane obtained from the aqueous extract of *C. odorata* are natural inhibitors ofα–amylase and asparaginyl endopeptidase respctively, and which could be gainfully employed as pre-emergence herbicides of weeds or used as preservatives to arrest the germination of seeds of agronomic importance during their period of storage.

# CONCLUSION

This study has laid bare the possible biochemical mechanism and agents behind the negative allelopathic effect of the aqueous extract of *C. odorata* against the germination of the seeds of several crops of agricultural importance. Of the myriad of chemical compounds profiled by GC-MS, retinaldehyde and tetradecane showed the best docking score(s) against α-amylase and asparaginyl endopeptidase, thus hinting on their prospect as inhibitors of starch and protein mobilization in the mature dry seeds of crops; and, therefore, lending credence to the ability of the aqueous extract of *C. odorata* inhibiting the germination of seeds – a negative allelopathy. As inhibitors with the prospect to arrest the hydrolyses of seed storage starch and proteins, and concomitantly stop the germination of seeds, retinal and tetradecane can be realistically employed as pre-emergence herbicides targeting certain agriculturally important weeds and/or they can be used as preservatives to arrest the germination of seeds of agronomic importance during their period of storage. More studies are advised on the *in vitro* and *in vivo* analyses of the potencies of these leads (in their pure form) against the enzyme extracts and seed germination respectively cum subsequent practical field studies to determine if they can serve commercial utility.

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