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Original Research Article

GC-MS Analysis and Molecular Docking Studies to Identify Potential SARS-CoV-2 Nonstructural Protein Inhibitors from *Icacina trichantha* Oliv Tubers

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ABSTRACT

The COVID-19 pandemic, caused by the SARS-CoV-2, has prompted international concern. This research aims to find bioactive phytocompounds from the traditional herb *Icacina trichantha* (Oliv) that could be used as a possible SARS-CoV-2 nonstructural protein inhibitor. GC-MS analysis identified fifteen (15) phytocompounds. *In silico* molecular docking, drug-likeness, toxicity and prediction of these compounds' substance activity spectra (PASS) were evaluated. The phytocompounds all have good binding energies, according to molecular docking. The phytocompound, 9,12-octadecanoic acid gave the best binding affinity of -24.98 kcal/mole. All of the identified compounds conformed to Lipinski's Rule of Five (RO5). This showed that the identified *I. trichantha* (Oliv) compounds would have lower attrition rates during clinical trials and thus have a better chance of being marketed. The current findings suggest that the discovered phytocompounds of *I. trichantha* (Oliv) could be developed as a novel COVID-19 medication.

Keywords: Docking,GC-MS,*Icacinatrichantha* Oliv, SARS-CoV-2, Tubers.

Introduction

The coronavirus disease of 2019 (COVID-19) is a new global public health hazard. The SARS-CoV-2 is responsible for this pandemic called Covid-19. SARS-CoV-2 has currently resulted in around 16.6 million deaths worldwide, with over 760.2 million confirmed cases, posing a major concern to public health.[1] Till date, there are no clinically authorized vaccinations or medical treatments for COVID-19. Natural sources are increasingly being considered as a potential source of new lead compounds for the treatment of COVID-19. *I. trichantha* is a species of *Icacinaceae* found in Central and West Africa. It is a medicinal shrub used by people in Nigeria. *I. trichantha* can grow up to two meters in height.[2] Carbohydrates (primarily starch), proteins, lipids, and mineral components like sodium, potassium, and calcium were found in *I. trichantha*.[3] In recent years, research on this plant has shown several remarkable pharmacological and chemical capabilities, suggesting some practical use for the plant material and compounds.[2] This plant's leaves and tubers are said to be aphrodisiacs.[4]The leaves and seeds are used for the management of asthma and hypertension when grounded and macerated in local alcoholic beverages.[5] Traditional herbal medicine vendors utilize tubers to treat rheumatism, malaria, constipation, poisoning, and toothache.[6] Mumps can be cured by drinking the tuber juice.[7]The first pharmacological report on *I.trichantha* was reported in 1990 (Asuzu and Ugwueze, 1990).[8] An aqueous extract of *I.trichantha* tubers influenced loss of the righting reflex caused by pentobarbital in mice, according to Asuzu and colleagues.[8]

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The activity of the extracts on the CNS was demonstrated by extending pentobarbitone sleeping time in rats, causing local anaesthetic outcomes in guinea pigs, and protecting rats and mice from pentylenetetrazole poisoning.[9]When mice were given a 51% methanol extract of the plant's leaves after being pretreated with pentylenetetrazole, the extract reduced convulsions and increased pentobarbitone-induced sleep time.[2] The emetic effect of the methanol extract was ascribed to the increased number of retches in guinea pigs, and this extract also ledtothe histological growth of the liver and kidney impacted by tetrachloromethane.[2] An ethyl ethanoate extract of the leaf component was found to protect rats' livers from paracetamol-induced liver damage.[10]Similarly, a methanol extract *of I. trichantha*leaf was discovered to have hepatoprotective effects against arsenic poisoning in rats, as the extract reduced the enzyme activities of aspartate aminotransferase (AST),-glutamyltransferase serum alanine, and aminotransferase.[11] Furthermore, it was discovered that the amount of micronucleatedpolychromatophilic red blood cells obtained from the bone marrow smear of *Icacina*-treated rats was lower than that of untreated arsenic-poisoned controls.[11] The 2,2-diphenyl-picryl-hydroxyl radical analysis revealed that the vegetative parts of *I. trichantha* had average levels of antioxidant activity.[10,12] The antioxidant activity of the leaf was proportional to total phenol content[13]and n-hexane extract was found to be effective in three nearly identical studies.[14] The first microorganisms employed to demonstrate the *in vitro* antibacterial activity of *I. trichantha* leaf were *Pseudomonas aeruginosa* and *Escherichia coli*.[15]

GC-MS is a technique that has been utilized by several researchers to identify phytocompounds in plants.[16-26] However, *I. trichantha* tubers have not been fully explored. There is very little research on the structures of the bioactive chemicals found in the tubers of *I. trichantha*. Additionally, GC-MS data on *I.trichantha* tubers and the bioactive phytocompounds' molecular docking investigations are unknown till date. To the best of our knowledge, this is the first evaluation of *I. trichantha* tuber using gas chromatography-mass spectrometry analysis, *in silico* molecular docking, drug-likeness, toxicity and prediction of substance activity spectra (PASS). As a

result, the goal of this research is to use GC-MS and molecular docking to find potential SARS-CoV-2 nonstructural protein inhibitors from *I.trichantha* tubers

Materials and Methods

Sample collection and extraction

Fresh tubers of *I. trichantha* were harvested at Umunakwukwu Chokoneze Mbaise, Imo state on February, 2017. The plant was identified and assigned a herbarium number ICA DALZ 1094 by the Taxonomy section of the Michael Okpara University of Agriculture, Umudike (MOUAU) Forestry Department. After washing the tubers to remove grit, they were peeled and grated. The grated tuber after four weeks of air drying was weighed (1.2 kg). It was macerated in chloroform for 2 days, then decanted, filtered using Whatman No.1 filter paper, and concentrated with a rotary evaporator under lower pressure to get 9 g of extract.

GC-MS analysis

The test was carried out on a 7890A GC-MS Triple Quad instrument (Agilent Technologies, Santa Clara, USA). Chemically coupled with a 5% diphenyl, 95% dimethylpolysiloxane cross-linked stationary phase (0.25 mm film thickness), an HP-5MS 30 m–250 mm (i.d.) fused-silica capillary column (Agilent J&W Scientific, Folsom, CA, USA) was employed. Exactly 1.5 µL of the sample was manually inserted in the split less mode, Helium was used as a carrier gas at 1.0 mL/min in split mode. The injector and supply were both at 250°C. The oven's temperature was initially set at 40°C, and then gradually raised to 300°C at a rate of 10°C/min per minute, for a total of 60 minutes. The temperature was set to 305°C after the run and stayed for 1 minute. The mass spectrometer was operated in EI mode (70 eV). Data was collected in full scan mode with a scan time of 0.5 seconds from m/z 50 to 650. Agilent Mass Hunter Qualitative Analysis was used to evaluate the data (Version B.04.00). By comparing the average peak area of each component to the total areas, the relative percentage amounts of each component were computed.

Identification of phytochemical components of the GC-MS

The compounds from the GC-MS spectra were identified by comparing mass spectral data and retention indices with the Wiley Registry of Mass Spectral Data 8th edition and the NIST Mass Spectral Library, and compounds were identified. Calculation of retention indices (RI) relative to a homologous sequence of n-alkanes under identical experimental conditions, as well as comparison with the literature, further verified the identification.

Preparation of SARS-CoV-2 viral protein and identified compounds

SARS-CoV-2 Nonstructural protein 1 (NSP1) (PDB ID: 7K3N) was obtained from the RCSB Protein Databank. Water molecules and ions were removed, and polar hydrogens were added using ArgusLab 4.0.1 software.[27]ACD lab ChemSketch software was used to draw the structures of the identified compounds. Energy minimization was done using ArgusLab 4.0.1 software.[27] ArgusLab 4.0.1 software [27]was used to convert the structures of the identified compounds to PDB format.

Molecular docking study

The identified compounds were docked to the SARS-CoV-2 Nonstructural protein 1 using the PatchDock server, a molecular docking tool based on shape complementarily principles. [28] The compounds were free to explore the whole surface area of the target protein on the PatchDock server (blind docking). The compound and protein's PDB files were uploaded to the PatchDock site for docking analysis, with a cluster RMSD value of 1.5 and a protein-small ligand complex type as the analysis settings. Patchdock server findings were fine-tuned with the Firedock server.[29]The bond lengths, interactions and 3D interaction of all docked complexes were visualized using the protein-ligand interaction profiler (PLIP) Server.[30] We also docked oleic acid with its original target protein, bovine beta-lactoglobulin (4DQ3), as a control, to validate the docking protocol in this work.

Drug-likeness prediction study

Using Lipinski's RO5,[31] the drug-likeness parameters of the phytocompounds were assessed using the web server of Swiss ADME.[32]

In Silico toxicity prediction study

ProTox-II was used to predict the toxicity and lethal dose (LD_{50}) for the identified chemicals.[33]

In Silico prediction of substance activity spectra (PASS) study

The potential bioactivities of docked compounds were assessed using the internet program Prediction of Substance Activity Spectra (PASS).[34]

Results and Discussion

GC-MS analysis

The GC–MS chromatogram of chloroform extract from *I. trichantha* tubers revealed a total of 15 peaks corresponding to bioactive compounds. Figure 1 depicts the GC chromatogram. The compounds have been listed in Table 1. Structures of compounds isolated from GC-MS of *I. trichantha* tubers are presented in Figure 2.

Molecular docking studies

SARS-nonstructural CoV-2's protein 1 (NSP1) was docked with all the phytocompounds. The global energies of the docked compounds from *I. trichantha* tubers with SARS-CoV-2 are listed in Table 2. The 3D interactions of the five best-docked compounds are shown in Figure 3.

Protein-ligand interaction of oleic acid with NSP1 of SARS-CoV-2 (Figure 3a) showed the involvement of hydrophobic interactions, water bridges and salt bridges. Hydrophobic interactions were observed with protein residues GLN 6A (3.41 Å), TYR 109A (2.92 Å) and TYR 109A (3.71 Å). Water Bridge was observed with protein residue GLY 103A (2.73 Å). Salt Bridge was observed with protein residue HIS 101A (5.30 Å).The global energy value was -17.28 Kcal/mol. The interaction of 9,12-Octadecanoic acid with NSP1 of SARS-CoV-2 (Figure 3b) showed the involvement of hydrophobic interactions and hydrogen bonds. Hydrophobic interactions were observed with protein residues LEU 7A (2.94 Å), PRO 10A (3.41 Å) and TYR 109A (2.81 Å). Hydrogen bond occurred with protein residue VAL 5A with a bond distance of 2.48 Å. The global energy value was -24.98 Kcal/mol. The interaction of methyl stearate with NSP1 of SARS-CoV-2 (Figure 3c)showed the involvement of hydrophobic interactions.

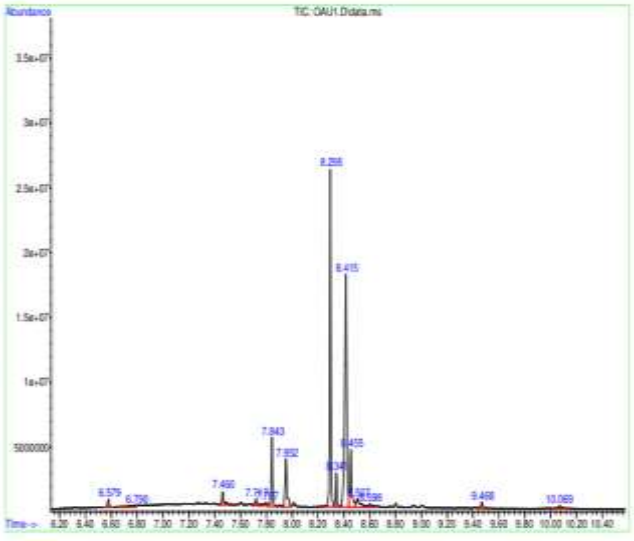

Figure 1: GC chromatogram of chloroform extract of *I. trichantha* tubers

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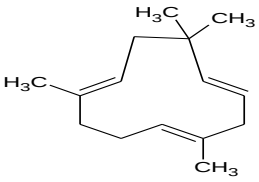
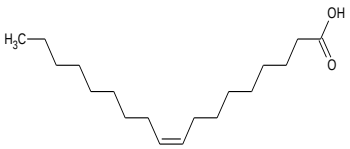
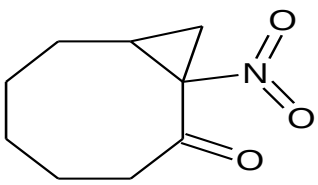
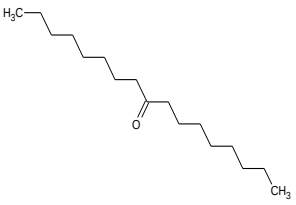
Table 1: Identified compounds in the GC-MS of *I. trichantha* Tubers

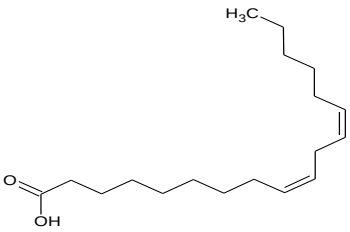
S/No	Compound	Mol. Weight (g/mol)	Retention Time	Composition (%)
1	Humulene	204.35	6.579	0.98
2	Oleic acid	282.46	6.790	0.88
3	1-Nitro-bicyclo[6.1.0]nonan-2-one	183.20	7.460	2.27
4	9-Heptadecanone	254.45	7.717	1.30
5	9,12-Octadecanoic acid	280.45	7.797	0.93
6	Hexadecanoic acid, methyl ester	270.45	7.843	6.11
7	Hexadecanoic acid, methyl ester	270.45	7.952	6.85
8	Methyl (E)-octadec-9-enoate	296.49	8.295	27.92
9	Methyl stearate	298.50	8.341	3.26
10	cis-Vaccenic acid	282.46	8.415	34.75
11	Octadecenoic acid	282.46	8.455	8.13
12	Oleic acid	282.46	8.507	2.34
13	Acetic acid,(2,4-dichlorophenoxy), isooctyl ester	333.25	8.598	0.83
14	Bis(2-ethylhexyl)phthalate	390.56	9.468	0.82
15	9-Octadecenoic acid(Z), 2-hydroxyethyl ester	326.51	10.069	0.81

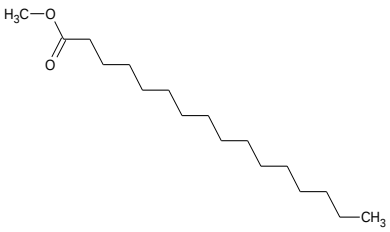

Humulene

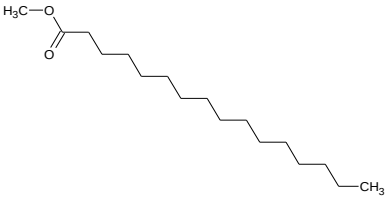

Octadecenoic acid

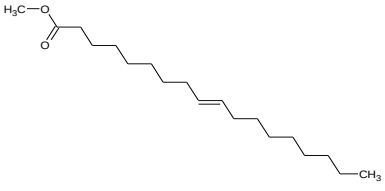

1-Nitro-bicyclo[6.1.0]nonan-2-one

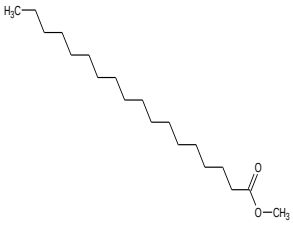

9-Heptadecanone

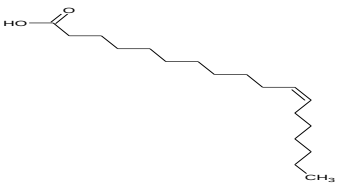

9,12-Octadecanoic acid

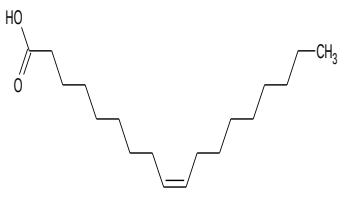

Hexadecanoic acid, methyl ester

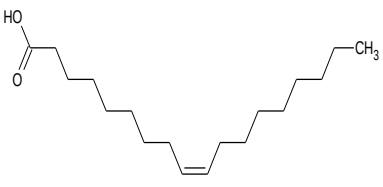

Hexadecanoic acid, methyl ester


Methy (E)-octadec-9-enoate


Methyl stearate


cis-Vaccenic acid


Octadecenoic acid


Octadecenoic acid

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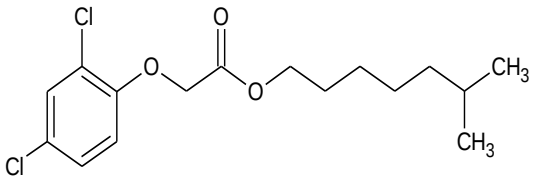
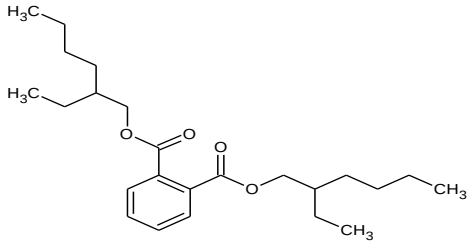
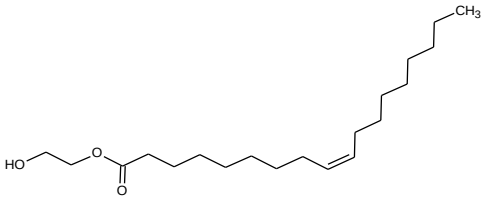


Acetic acid,(2,4-dichlorophenoxy), isooctyl ester



Bis(2-ethylhexyl)phthalate



9-Octadecenoic acid(Z), 2-hydroxyethyl ester

Figure 2: Structures of compounds isolated from GC-MS of *I. trichantha* tubers

Hydrophobic interactions were observed with protein residues TYR 88A (3.24 Å), TYR 88A (3.74 Å), and ARG 115A (3.13 Å). The global energy value was -16.37 Kcal/mol. Protein-ligand interaction of Bis(2-ethylhexyl)phthalate with NSP1 of SARS-CoV-2 (Figure3d) showed the involvement of hydrophobic interaction and hydrogen bond. Hydrophobic interactions were observed with protein residues ARG 64A (3.17 Å), ARG 90A (2.96 Å), ARG 90A (3.63 Å), and GLU 93A (3.46 Å). Salt bridge was observed with protein residue ARG 90A (4.63 Å). The global energy value was -18.99 Kcal/mol. Protein-ligand interaction of 9-Octadecenoic acid(Z), 2-hydroxyethyl ester with NSP1 of SARS-CoV-2 (Figure3e) showed the involvement of hydrophobic interaction and hydrogen bond. Hydrophobic interaction was observed with protein residues GLU 93A (2.98 Å). A hydrogen bond was observed with protein residue GLU 93A (3.53 Å). The global energy value was -16.91 Kcal/mol. Results of the docking score suggested that *I. trichantha* contained lead compounds that can be a potential drug candidate against SARS-CoV-2.

Table 3 shows the drug-likeness property predictions for the phytocompounds. The RO5 is a thumb's rule developed by Lipinski for determining whether a compound with a particular bioactivity has physical and chemical characteristics that are expected to be an orally active medication. Table 3 showed that the five best-docked compounds meet the requirements of RO5. This showed that the identified *I. trichantha* compounds will have a low attrition rate for further studies in the drug development process.

Toxicity prediction

Toxicity prediction of phytocompounds by ProTox-II is shown in Table 4.The compound, 9,12-Octadecanoic acid was anticipated to be non-lethal (LD_{50}> 5000 mg/kg) in this investigation. Toxicity predictions revealed that methyl stearate and 9-octadecenoic acid (Z), 2-hydroxyethyl ester could be harmful if taken (2000 < LD_{50}≤ 5000). The predicted toxicity result suggested that 9,12-Octadecanoic acid is safe for consumption.

Biological activity prediction

Predictions of bioactivity of the five best-docked compounds are shown in Table 5. Prediction of PASS, a structure-based bioactivity prediction online tool, was used to evaluate the five best-docked compounds' potential biological activity. The PASS analysis identified each compound's potential targets and biological activity. Based on (Pa) Possibility of activity > (Pi) Possibility of inactivity and Pa > 0.7 values. We studied the biological activity for each molecule. With Pa >0.951, the results showed various major actions, implying that the identified compounds of *I. trichantha* tubers had a broader potential (Table 5).

Table 2: Global energies of phytochemical from *I. trichantha* tubers with SARS-CoV-2

S/No	Compound	Global energies (kcal/mol)
1	Humulene	-11.32
2	Octadecenoic acid	-17.28
3	1-Nitro-bicyclo[6.1.0]nonan-2-one	-10.24
4	9-Heptadecanone	-11.20
5	9,12-Octadecanoic acid	-24.98
6	Hexadecanoic acid, methyl ester	-13.82
7	Hexadecanoic acid, methyl ester	-13.82
8	Methy (E)-octadec-9-enoate	-10.50
9	Methyl stearate	-16.37
10	cis-Vaccenic acid	-11.70
11	Octadecenoic acid	-12.28
12	Octadecenoic acid	-12.28
13	Acetic acid,(2,4-dichlorophenoxy), isooctyl ester	-10.19
14	Bis(2-ethylhexyl)phthalate	-18.99
15	9-Octadecenoic acid(Z), 2-hydroxyethyl ester	-16.91

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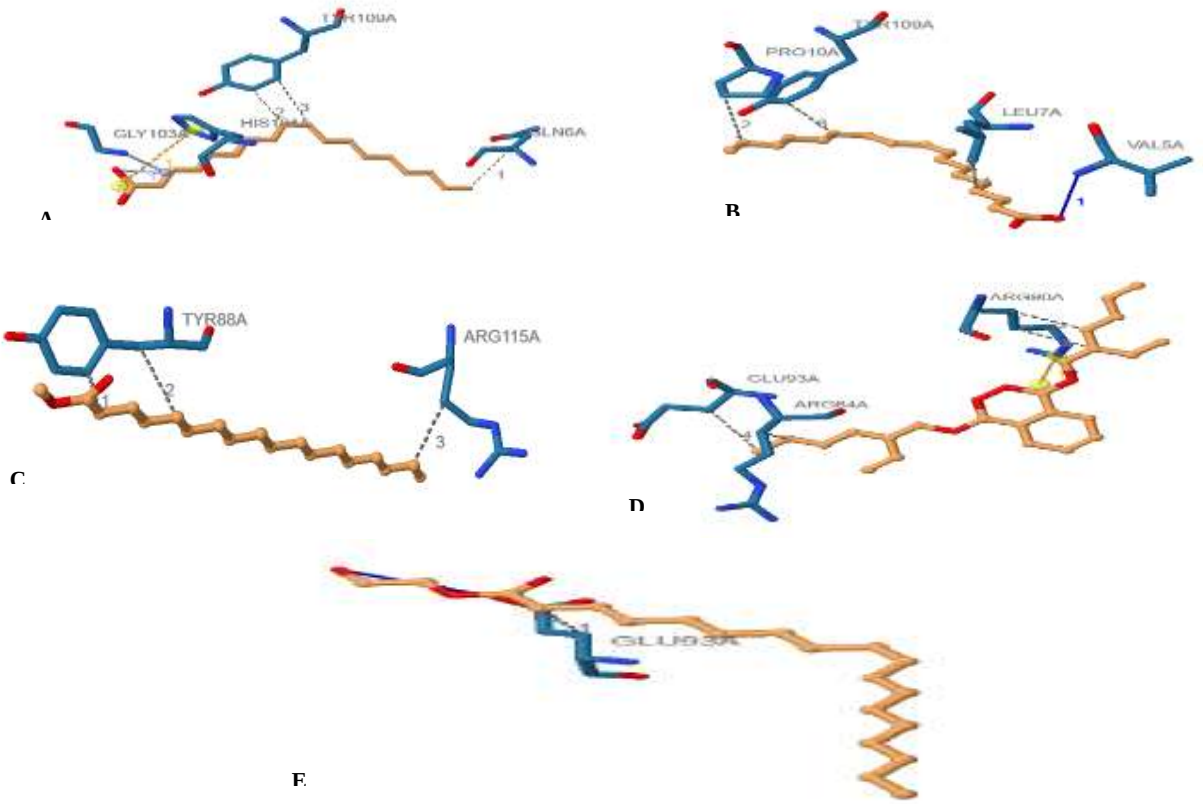

Figure 3: The bioactive compounds from *I. trichantha* (oliv) tubers docked with SARS-CoV-2. a) Octadecenoic acid b) 9,12-Octadecanoic acid c) Methyl stearate d) Bis(2-ethylhexyl)phthalate d) 9-Octadecenoic acid(Z), 2-hydroxyethyl ester

Table 3: Drug-likeness property prediction for the five best-docked compounds.

Compound	Mol. Weight [1](g/mol)	HB Acceptor [2]	HB Donor [3]	Lipophilicity[4]	Molecular Refractivity [5]	Rule of Five [6]
Octadecenoic acid	282.46	2	1	6.11	89.94	1
9,12-Octadecanoic acid	280.45	2	1	5.88	89.46	1
Methyl stearate	298.50	2	0	6.42	94.73	1
Bis(2-ethylhexyl)phthalate	390.56	4	0	6.43	116.3	1
9-Octadecenoic acid(Z), 2-hydroxyethyl ester	326.51	3	1	5.56	100.23	1

[1]Molecular weight (acceptable range: <500). [2] HB, Hydrogen bond acceptor (acceptable range: ≤10). [3] HB, Hydrogen bond donor (acceptable range: ≤5). [4]Lipophilicity (Log Po/w, acceptable bounds <5). [5] Molar refractivity, acceptable bounds 40 - 130.[6] RO5: Number of RO5 violations ideal range: 0–4.

Table 4: Toxicity prediction of the five best-docked compoundsProTox-II.

Compound	Predicted LD_{50}, mg/kg [a]	Predicted Toxicity Class [a]
Octadecenoic acid	48	2
9,12-Octadecanoic acid	10000	6
Methyl stearate	5000	5
Bis(2-ethylhexyl)phthalate	1340	4
9-Octadecenoic acid(Z), 2-hydroxyethyl ester	5000	5

[a]ProTox (http://tox.charite.de/protox_II, accessed on 7 March, 2022) Class 1: deadly if consumed (LD_{50}≤5); Class 2: deadly if consumed (5 < LD_{50}≤ 50); Class 3: lethal if consumed (50 < LD_{50}≤ 300); Class 4: harmful if consumed (300 < LD_{50}≤2000); Class 5: maybe harmful if consumed (2000 < LD_{50}≤ 5000); Class 6: non-lethal (LD_{50}> 5000)

Table 5: Prediction of bioactivity of the five best-docked compounds

Compound	Pa [a]	Pi [b]	Biological activity
Oleic acid	0.974	0.001	CYP2J substrate
9,12-Octadecanoic acid	0.951	0.001	Phosphatidylglycerophosphatase inhibitor
Methyl stearate	0.962	0.002	Saccharopepsin inhibitor
Bis(2-ethylhexyl)phthalate	0.966	0.002	Eye irritation, inactive
9-Octadecenoic acid(Z), 2-hydroxyethyl ester	0.971	0.002	Eye irritation, inactive

Conclusion

GC-MS study of the tubers of *I. trichantha* demonstrated that this plant is a rich source of bioactive phytocompounds. Docking tests revealed excellent binding affinity to the NSP1 SARS-CoV-2. Drug-likeness conformed to RO5. The results of the molecular docking revealed that *I. trichantha* would be a promising natural antiviral candidate against SARS-CoV-2. However, more research is needed to isolate the pure chemical responsible for the identified bioactivity, as well as to determine its toxicity profile and long-term safety.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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