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ORIGINAL RESEARCH ARTICLE

Targeting H3N2 influenza virus RNA dependent RNA polymerase dependent inhibitory activity by principal components from latex of *Calotropis gigantean*

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ABSTRACT

The H3N2 influenza virus is spiking dramatically, which is a major concern worldwide and in India. The multifunctional hetero-trimer influenza virus RNA-dependent RNA polymerase (RdRP) is involved in the generation of viral mRNA and is crucial for viral infectivity, which is directly related to the virus's ability to survive. The goal of the current work was to use molecular docking to determine how the RdRP protein might be affected by powerful bioactive chemicals found in *Calotropis gigantia* latex. By applying CB-dock 2 analysis and 2D interactions, an in-silico docking study was conducted using a GC-FID (gas chromatography with flame-ionization detection) based composition profile. Tocospiro A (15%), Amyrin (7%), and Gombasterol A were found by GC-FID to be the main phytocompounds in the latex of *Calotropis gigantia*. The docking result showed that ligands were effectively bound to RdRP. According to interaction studies, RdRP/ligand complexes create hydrogen bonds, van der Waals forces, pi-alkyl bonds, alkyl bonds, and pi-Sigma bonds. Therefore, it was suggested that *Calotropis gigantia* latex may represent a possible herbal remedy to attenuate H3N2 infections based on the above findings of the fragrance profile and docking.

Keywords: docking; calotropis; latex; herbal drug

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1. Introduction

Acute respiratory infections caused by influenza viruses are of great concern worldwide[1,2]. Recently, the H3N2 influenza virus, belonging to the orthomyxoviridae family, created much havoc, particularly in India, hence being deemed a serious public health concern. According to the ministry of health and sciences, 28,955 viral infection cases are reported[3].The associated symptoms of H3N2 infection include cough, runny or congested nose, sore throat, headache, body aches and pains, fever, chills, fatigue, diarrhea, and vomiting[4]. Symptoms that can signal an emergency and warrant prompt medical attention include: feeling short of breath or having trouble breathing; pain or pressure in your chest or abdomen; dizziness that comes on suddenly; persistent, severe vomiting; feelings of confusion; and symptoms that begin to improve but then return with a worsened cough and fever. At the same time, this virus is easily transmissible from person to person and can infect people of any age[1]. Earlier research reported that the H3N2 influenza virus is prone to periodic outbreaks due to frequent mutations that escape the host immune system[5].

Current treatments to mitigate H3N2 infections are limited, and drug resistance is a major problem worldwide. Recently developed anti-

influenza drugs are mainly RdRp drugs that target the virus[6]. It was reported that all circulating influenza viruses, including H3N2, are resistant to most of the drugs, such as Favipiravir and Baloxavir. However, all synthetic drugs, including Favipiravir, are ineffective owing to their disadvantages, such as high toxicity, teratogenicity, abnormal behavior, etc.[7]. As per reports of the Chinese National Influenza Center's resistance surveillance, H3N2 viruses are resistant to amantadine analogues[8], highlighting the inevitability of novel natural drug therapies. Consequently, the development of novel anti-influenza drugs is a vital task. The multifunctional heterotrimer protein RNA-dependent RNA polymerase (RdRP), which the H3N2 influenza virus possesses and uses to generate viral mRNA via a cap-snatching mechanism, is essential for viral replication in the host cells[9]. The three different subunits that make up H3N2 RdRP are basic polymerase 1 (BP1), basic polymerase 2 (BP2), and acidic polymerase (PA). These domains must interact for full RdRP activity. The successful disruption of protein-protein interactions (PPIs) during protein synthesis was therefore suggested to have antiviral effects. Using deletion analyses, Poole et al.[10] showed lower PB2 binding, which limited the activity of influenza RdRp and prevented virus replication. Many of the novel medicine formulations, including those for antibacterial, antifungal, antiviral, anticancer, and antihypertensive purposes, were previously made from natural materials[11]. Nonetheless, natural bioactives have been newly more methodically explored as hopeful agents than synthetic drugs[12]. Additionally, as microbial resistance to synthetic medications has increased, the scientific community has been increasingly interested in the search for novel, economically viable drugs with natural origins[13]. Natural latex is produced by over 2500 plants from the Apocynaceae, Euphorbiaceae, and Asclepidiaceae families. In addition to numerous microbial diseases, the biological potential of latex-producing plants is discussed in the scientific literature[14]. However, most of these latexes endure to be examined for their biological practicality.

Calotropis gigantia (L.) R. Br. (*Calotropis*), a large shrub up to 4 m and a member of the Apocynaceae family, is a plant native to Malaysia, Indonesia, China, India, Thailand, Nepal, Sri Lanka, Pakistan, and tropical Africa[15]. White latex from stems and leaves is rich in various bioactive molecules. Hence, for the past 2000 years, latex from calotropis has encompassed a number of bioactives with abundant pharmacological potential implicated in various systems of medicine. Due to the richness of a complex mixture of various bioactive components, the latex of Calotropis is used as a pesticide, anti-inflammatory, anti-fertility activity, antiulcer activity, fungicide, antiseptic, insecticide, anti-diabetic, and antihelminthic activity[16,17]. Various parts of C. *gigantea* (L.) Dryand are documented for the treatment of sprain, agitation, fatigue, epilepsy, mental conditions, diarrhea, analgesic intervention, and the interceptive properties of pregnancy, toothache, and earache[18]. In the literature, it was also reported that the alcoholic root extract of *C. gigantea* showed analgesic, anticonvulsant, anxiolytic, and sedative effects in albino rats[19]. The anti-viral (H3N2) mechanism of action of latex is still not fully understood because of its complexity. We hypothesize that bioactives from *Calotropis gigantia* (L.) R. Br. have the capability to prevent infection with H3N2. However, in the present study, we investigated α-Tocospiro A, α-Amyrin, and Ergost-5-en-3-ol as potential inhibitor candidates for H3N2 RdRP proteins.

2. Materials and methods

2.1. Extraction of latex

Latex was aseptically collected from *C. gigantia* (L.) R. Br. plants growing wild in the vicinity of the college area. The wild plants were identified by the botany department of the college, where a voucher was preserved (BT 107). The latex was collected from the aerial parts of wild plants by deliberatingly breaking the aerial shoots, and milky sap oozed out from the stem; this was repeated continuously until the required volume of white latex was collected. One mL of methanol was used to extract about 200 L of fresh latex. The final mixture was vortexed, kept at 4 °C for 12 h, then centrifuged for 15 min at 5000 rpm. For additional analysis, the supernatant was taken out and utilised.

2.2. GC-FID analysis

GC-FID was used to identify bioactive components in the methanol-latex extract (Chemtron 2045). Column requirements were as follows: A 2 m long, 10% OV-17 on 80–100% mesh Chromosorb W (HP) piece of stainless steel 35 mL/min of nitrogen was employed as the carrier gas. The latex samples utilised were 0.2 µL. Detector and injector temperatures were 220 ℃ and 270 ℃, respectively. Oven ramping conditions were as follows: ramping from 100 ℃ (kept initially) to 210 ℃ at 3 ℃/min. By comparing the relative retention times (RT) of latex GC-FID spectra with real standards and published data, bioactive components in latex were found.

2.3. Ligand preparation

Bioactive substances such as α-Tocospiro A, α-Amyrin, Ergost-5-en-3-ol, which are mostly present in latex, were employed as ligands for structures for the viral receptor (RdRP, pdb id: 2ztt). SMILES of the ligands were collected from the NCBI-Pubchem database in order to construct the 3D structure of the ligand.

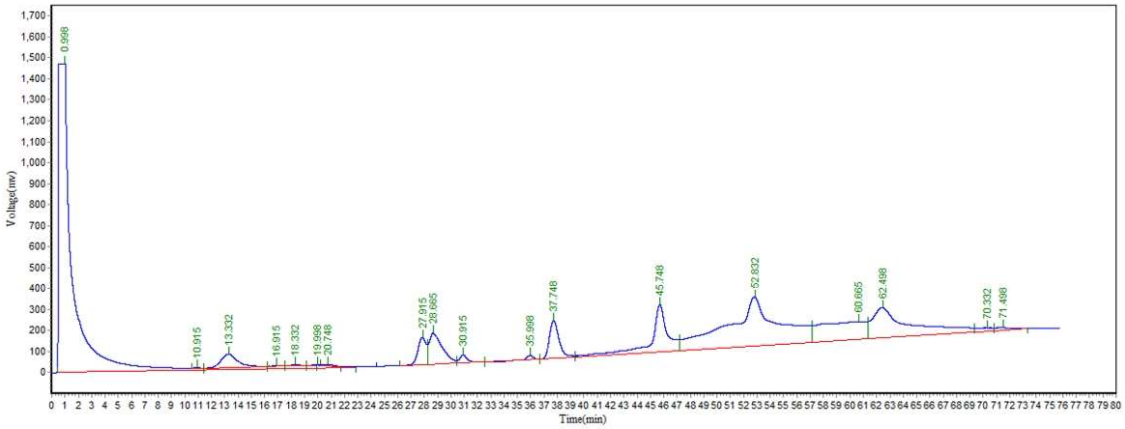
2.4. Molecular docking

RdRP protein crystal structures were found in the PDB. Prior to docking analysis, RdRP was freed of cofactors, co-crystallized ligands, and selected H_2O molecules. The dock prep setup in UCSF-chimera was then used to create all protein target structures. The method being optimised corrects atomic structure, abnormal charges, and bond length. For docking, the CB-DOCK 2 tool (available at https://cadd.labshare.cn/cb-dock2/php/index.php) was used to dock ligands over RdRP. Utilising the technologies from Biovia 2020, UCSF Chimaera, and Plip, 2D and 3D interactions in docked complexes were studied.

3. Results and discussion

3.1. GC-FID analysis of latex

Figure 1 shows the calotropis latex's aroma profile. Calotropis latex (CL) underwent GC-FID analysis, which identified 18 chemicals, including major and minor peaks. The dominant identified compounds in CL were α-Tocospiro A (15%), and α-Amyrin (7%), and Gombasterol A (5%), along with many minor components. As described in the literature, CL is rich in these types of bioactives[16]. Due to the richness of bioactives molecules, CL has immense applications in the pharmaceutical industry[20,21]. Kareem et al.[14] studied the encouraging antibacterial activity of latex against tested pathogenic fungi and bacteria. Because all of the major and minor components are present, biological activity can also be attributed to them. Based on GC-FID analysis, in this study, two major compounds (α-Tocospiro A and α-Amyrin) and one very minor component (Ergost-5-en-3-ol) were selected as ligands for docking studies against the H3N2 RdRP enzyme receptor.



S. No	R.T	Bioactive Compound	Concentration (%)
1	0.998	methanol	57.8405
2	10.915	2-METHOXY-4-VINYLPHENOL	0.0251
3	13.332	3',5'-DIMETHYLACETOPHENONE	1.4000
4	16.915	L-Borneol	0.0233
5	18.332	Cyclohexanol, 3-methyl	0.0565
6	19.998	Ergost-5-en-3-ol	0.0073
7	20.748	Cedr-8(15)-ene	0.0554
8	27.915	Neophytadiene	1.4209
9	28.665	Sulfurous acid, nonylpentyl ester	2.6054
10	30.915	Heptadecyl acetate	0.4014
11	35.998	Tritriacontane	0.1676
12	37.748	Octadecanoic acid, 2,3-dihydroxypropyl ester	2.3328
13	45.748	Guanidine nitrate	4.7754
14	52.832	α-Tocospiro A (alpha-tocopheroids)	15.6999
15	60.665	Gombasterol A	5.3150
16	62.498	α-Amyrin	7.1846
17	70.332	Unknown	0.3879
18	71.498	Unknown	0.3011

Figure 1. GC-FID profile of latex from *Calotropis gigantean*. Letter in bold red denotes the principal components used for docking.

3.2. Molecular docking

The most popular method for creating medications based on 3D structures is called structure-based drug design (SBDD), which is an in-silico methodology[22]. The molecular docking was carried out to examine the potential interaction between RdRP and the bioactive components of calotropis latex. The current investigation sought to dock important viral inhibitor candidates against H3N2 RdRP—Tocospiro A, Amyrin, and Ergost-5-en-3-olbioactive compounds from CL. **Table 1** provides an overview of the binding energies for the bioactive compounds based on docking studies. Less binding energy indicates more efficient ligand-receptor binding. It was clear from the docking analysis that bioactive compounds effectively docked with H3N2 RdRP. According to the 3D docking results and docking scores, viral enzymes exhibited significant binding with ligands. The three phytochemicals discovered in the CL were found to have binding scores ranging from –8.4 to –9.07, with –9.0 being the lowest binding energy for α-Tocospiro A. Therefore, out of the three phytochemicals, α-Tocospiro A showed significant binding to H3N2 RdRP. Docking scores for Ergost-5-en-3-olwas with α-Amyrin were –8.2 and –8.4, respectively. **Figures 2** and **3** show a 3D model showing the ideal docking stance and 2D interactions of α-Tocospiro A, α-Amyrin, and Ergost-5-en-3-ol with RdRP. The docking view showed that the ligands were tightly coupled to the receptor's binding pocket. Tocospiro is docked with the RdRP PA and PB2 domains. The other two ligands, α-Amyrin and Ergost-5-en-3-oldocked at the BP2 domain, however, were not. Chains A, B, and C make up the PA, BP1, and BP2 domains of RdRP[23]. While PA has an endonuclease domain and BP2 has a cap-binding domain, BP1 has polymerase activity.

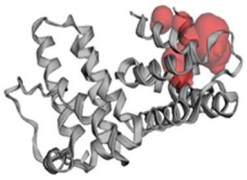
According to Venkataraman et al.[24], the N- and C-terminal domains of BP1 are where the PA and BP2 domains are located. These findings were consistent with earlier research that reported quercetin and chlorogenic acid from the plants Forsythia suspense, *Mangifera indica*, *Hypericum perforatum*, and *Chaenomele speciosa* have docking interactions with viral enzymes[25]. Calotropis latex can be employed as an effective source of anti-H3N2 chemicals, according to the analysis noted. Through 3D docking, ligands can create H-bonds or Van der Waals forces with receptor site residues, designating their affinity for the receptor[26]. Therefore, the docking interactions of Ergost-5-en-3-ol, Tocospiro A, and Amyrin with RdRP were further investigated. **Figure 3** shows the chemical bonding manner of the complexes created between the investigated chemicals and the binding pocket residues of RdRP. Van der Waals' interaction (VDW) and Pi-Alkyl, Alkyl played a significant role in the interaction between RdRP and bioactive compounds. It was found that the A-chain and B-chain residues in ligands α-Tocospiro A form VDW, Pi-Alkyl, Alkyl, and Pi-Sigma interactions with RdRP. Chains B and C of α-Amyrin demonstrated VDW, Pi-Alkyl, and Alkyl interactions with RdRP. In addition to VDW, Pi-Alkyl, Alkyl, and Pi-Sigma interactions with RdRP, Ergost-5-en-3-ol also demonstrated hydrogen bond (HB) interactions. All of the abundant phytoconstituents were mostly bound to the active site of RdRP by interactions between VDW, Pi-Alkyl, and Alkyl. Additionally, it was shown that these compounds interacted with comparable residues in very modest ways. The amino acids Tyr689, Cys692, Cys693, Phe696, Glu697, Phe700, Pro701, Ser702, Ser703, Ser704, Arg707, Pro708, Ser712, Ser713, Val715, Ile19, Leu20, Thr23, Thr24, Val25, Ala29, and Lys32 stabilised the most stable complex (9.0) of α-Tocospiro A—RdRP. In the RdRP receptors' active site cavities, interaction residues were measured by CASTp active site prediction (**Table 2**). A major pocket in the RdRP enzyme was measured to have a volume of 342 and an area of 427. It was hypothesised that because ligands including α-Tocospiro A, α-Amyrin, and Ergost-5-en-3-ol have excellent affinity for the RdRP enzyme upon binding, RdRP will become closed, which would then cause a change in the conformation of the H3N2 enzyme. All of these things stop H3N2 from being viable, which lessens the virus's capacity to infect the host cell.

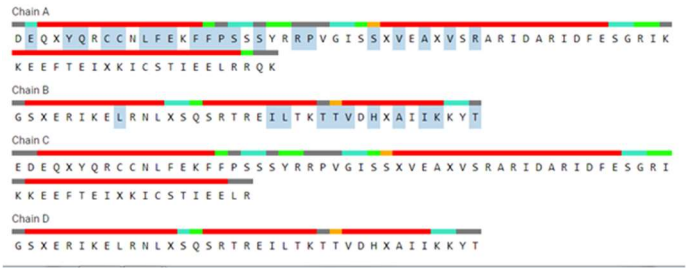
Table 1. Molecular docking of RdRp with ligands.

Ligand	Binding energy (Vina score)	Cavity volume (Å^3)	Center (x, y, z)	Dockingsize (x, y, z)	Involved receptor residues	Type of interactions
α-Tocospiro A	–9.0	714	–2, –3, 12	24, 24, 24	Chain A: TYR689, CYS692, CYS693, PHE696, GLU697, PHE700, PRO701, SER702, SER703, SER704, ARG707, PRO708, SER712, SER713, and VAL715 Chain B: ILE19, LEU20, THR23, THR24, VAL25, ALA29, and LYS32	VDW, Pi-Alkyl, Alkyl, Pi-Sigma
α-Amyrin	–8.2	685	14, 31, 7	22, 22, 22	Chain C: TYR689, CYS692, CYS693, PHE696, GLU697, PHE700, PRO701, SER702, SER704, ARG706, ARG707, and PRO708 Chain D: ILE19, LEU20, THR23, THR24, VAL25, HIS27, ALA29, ILE31, and LYS32	VDW, Pi-Alkyl, Alkyl
Ergost-5-en-3-ol	–8.4	685	14, 31, 7	23, 23, 23	Chain C: CYS692, CYS693, LEU695, PHE696, GLU697, PHE699, PHE700, PRO701, SER702, ARG707, PRO708, SER713, VAL715, ALA717, and VAL719 Chain D: LEU7, ILE19, LEU20, THR23, THR24, VAL25, ALA29, and LYS32	VDW, Pi-Alkyl, Alkyl, HB, Pi-Sigma

Abbreviation: HB, hydrogen bond; VDW, Van der Waals forces.

Table 2. Active site analysis of protein target structure. Letters in red font indicates residues involved in 2D interactions.

H3N2 Receptor	3D model	Interacting active site residues	Cavity	
			Area Å²	Volume Å²
RdRP		686GLU, 689TYR, 690GLN, 692CYS, 695LEU, 696PHE, 697GLU, 699PHE, 701PRO, 702SER, 707ARG, 708PRO, 713SER, 715VAL, 717ALA, 719VAL, 21ARG, 7LEU, 19ILE, 20LEU, 23THR, 24 THR, 25VAL, 27HIS, 29ALA, 31ILE, 32LYS, and 35THR	427.24	342.12



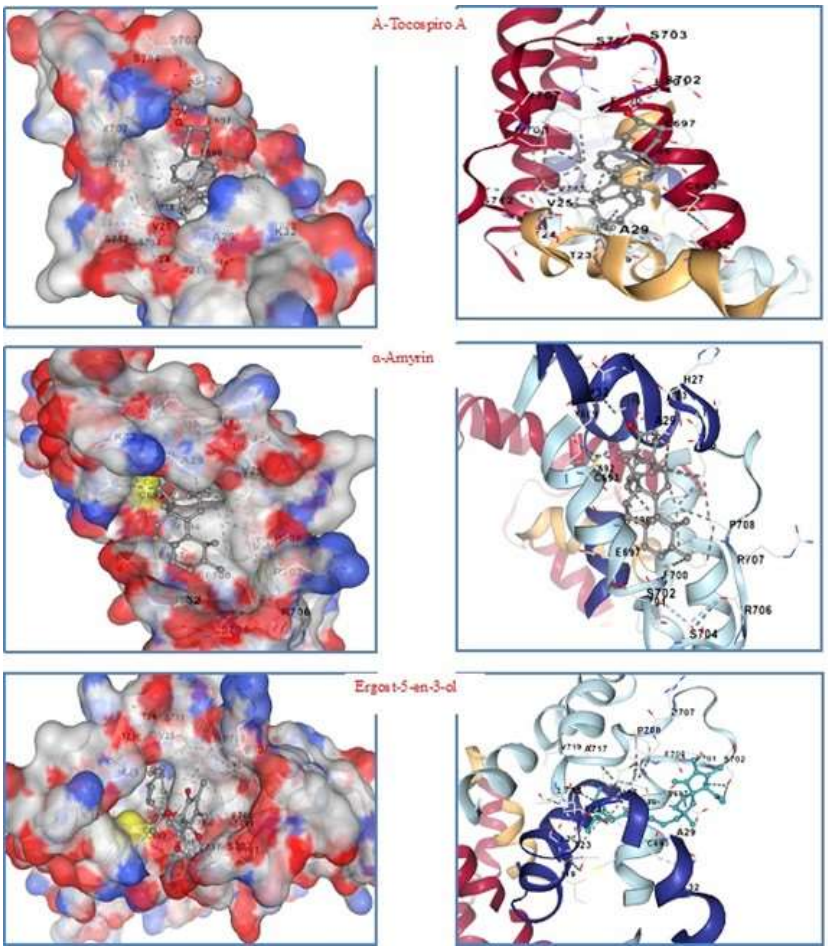


Figure 2. Best fit 2D model depicting interaction of RdRP with ligands.

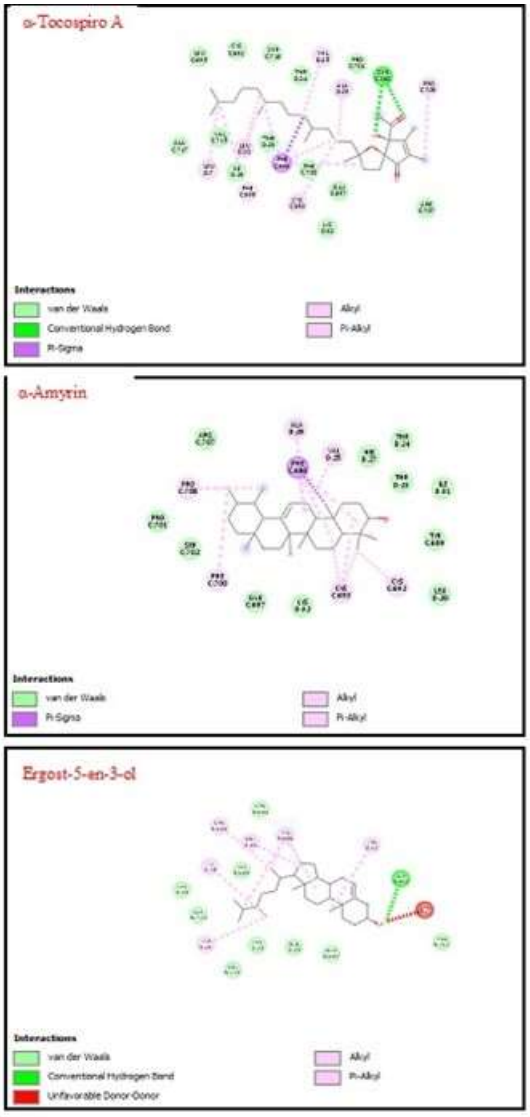


Figure 3. 2D interactions of ligands with RDRP.

4. Conclusions

H3N2 has currently entered the human population and poses a possible global health concern. This study looked at bioactive compounds from calotropis latex (CL) that might be exploited to block the RdRP-targeted H3N2 infection pathway. Bioactive substances such α-Tocospiro A, α-Amyrin, and Ergost-5-en-3-olin aromatic plants were discovered using GC-FID analysis. All bioactive chemicals were shown to effectively bind with RdRP in in-silico docking simulations. As a result, we proposed that bioactive substances from CL might represent prospective therapeutic choices and be present in medicinal plants where they might potentially operate as H3N2 RdRP enzyme inhibitors.

Author contributions

Conceptualization, ADS; designed study, IK; interpreted study, AC; All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

Authors declare no conflict of interest.

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