



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2026): 4.82
 Indexing: Embase
 IJBS 2026; 8(9): 118-130
www.biologyjournal.net
 Received: 11-06-2026
 Accepted: 03-08-2026
 Published: 28-09-2026

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Antiviral compounds of *Wrightia tinctoria*(Roxb.) R.Br. against SARS CoV-2: An *in vitro* and *in silico* studies

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DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i9b.997>

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, continues to pose a global health challenge, prompting the search for novel antiviral agents. The present study investigated the phytochemical composition and antiviral potential of *Wrightia tinctoria* against SARS-CoV-2. Extracts of leaf, bark, and root obtained using methanol, aqueous, and petroleum ether solvents were subjected to proximate, phytochemical, and quantitative analyses. Methanol extracts showed the highest yields, while aqueous extracts were rich in flavonoids, phenols, and tannins. GC-MS/MS profiling identified key bioactive compounds including γ -sitosterol, stigmasterol, campesterol, lupeol, and phytol, along with triterpenoids and fatty acid esters, known for their immunomodulatory and antiviral properties. Molecular docking revealed strong binding affinities of these compounds with SARS-CoV-2 target proteins ACE2, TMPRSS2, 3CLpro, PLpro, and RdRp, indicating their potential to inhibit viral entry and replication. Stigmasterol exhibited the most potent multi-target interactions, highlighting its role as a promising lead compound. Overall, *W. tinctoria* demonstrates significant potential as a natural source of antiviral agents against COVID-19.

Keywords: SARS-CoV-2, GC-MS/MS, Molecular docking, Antiviral activity

1. Introduction

The exploration of natural sources for new safe and effective pharmaceutical broad spectrum antiviral candidates against widespread Severe Acute Respiratory Syndrome Coronavirus - SARS-CoV-2 is desirable. Currently, viral infection is the most serious health issue especially SARS-CoV-2 a β -Coronavirus, caused severe and life-threatening respiratory infections in humans which caused unexpected higher rate of death across the globe [1]. SARS-CoV-2, a zoonotic positive-sense single-stranded RNA (+ssRNA) enveloped virus invade the pulmonary epithelial cells of the Upper respiratory system (Shah *et al.*, 2020) [2].

Its spike (S) protein is cleaved into S1 and S2 subunits, which attach to the receptors and facilitate membrane fusion. Viral genetic materials are then delivered to the host's cytoplasm through the membrane fusion pore so that they can multiply (Jackson *et al.*, 2022) [3]. These viral proteins can be the primary targets of effective drugs to suppress viral entry and replication. Only a few antiviral therapeutic drugs may be helpful in controlling the spread of SARS-CoV-2 infection. Unfortunately, the toxicities, countless adverse effects, expenses and ineffective responses of emerging viral resistant strains to synthetic antiviral drugs have reinforced the search of effective and affordable alternative treatment options, such as plant-derived antiviral drug molecules (Kurokawa *et al.*, 2010) [4].

The quest for novel antiviral phyto-compounds depends on the comprehensive screening of plant derived metabolites that comprises several functional bioactive compounds that can impede viral reproduction through modulating the immune responses of host against viral infections without affecting the host physiology with minimal side effect (Kurokawa *et al.*, 2010; Hussain *et al.*, 2017; Newton *et al.*, 2016) [4, 5, 6].

Various literatures stated that *W. tinctoria*, an important medicinal plant with rich source of phytochemical constituents used for the treatment of variety of diseases and found effective against severe viral diseases (Devi *et al.*, 2014; Vignesh *et al.*, 2022) [7, 8]. Considering the broad biological activity of *W. tinctoria*, the present study intended to explore the bioactive compounds of the *W. tinctoria* leaf, bark, and root extracts against SARS CoV-2, causative for pandemic COVID-19 through molecular docking via protein-ligand interactions analysis.

SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV) are zoonotic β -corona viruses that continue to have a significant impact on public health. Timely development and introduction of vaccines and antivirals against SARS-CoV-2 into the clinic have substantially mitigated the burden of COVID-19. However, rapidly arising SARS-CoV-2 variants calls for an expanded and diversified portfolio of antivirals, such as direct-acting antivirals (DAAs) against SARS-CoV-2 variants, the unprecedented pub

lic health and economic impact of the COVID-19 pandemic caused by infection of SARS-CoV-2 has been met with an equally unprecedented scientific response.

2. Materials and Method

2.1. Collection and preparation of plant materials

The leaf, stem bark and root of *W. tinctoria* were collected from Periya Thadagam, Coimbatore district, Western Zone of Tamil Nadu situated between 11°03'32.2" N latitude and 76°50'59.8" E longitude. The collected plant samples were shade dried at room temperature, ground into fine powder and stored for further experimental analysis.



Figure 1. Collection of *W. tinctoria* leaves, stem and root

2.2. Preparation of the extracts

The powdered plant samples of *W. tinctoria* leaf, stem and root (20 g each) were subjected to hot extraction with 350 ml of solvents i.e. aqueous, methanol and petroleum ether using Soxhlet apparatus at 65–80 °C for 8–10 h. The solvents were evaporated in rotary vacuum evaporator until complete removal of solvents and stored at 4 °C for further use. The extracts were dissolved in respective solvents while experimentation to get the solution with a concentration of 10 mg/10 ml.

2.3. Proximate analysis

The analysis of the proximate composition of *W. tinctoria* leaf, stem bark and root was carried out using the standard method.

2.4. GC/MS/MS analysis

The GC-MS/MS analysis was performed using a Varian 3800 coupled with a Mass spectrum 4000 GC-MS/MS system equipped with a fused silica capillary column (15m x 0.2 mm ID x 1µm) linked to an EI detector. Helium gas (99.99% purity) was used as a carrier gas at a constant flow rate of 1 ml/min and the sample injected was 1µl; the instrument was set to an initial temperature of 110 °C, and maintained at this temperature for 2 min. Then the oven temperature was rose up to 280 °C, at the rate of an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured as 250 °C. The ionization

voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45–450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS/MS, the bioactive compounds present in the plants sample were identified. The name, molecular weight and structure of the components were ascertained from these comparisons.

2.5. In silico docking for binding efficacy of SARS CoV-2 proteins

The three-dimensional (3D) structures of receptor proteins were retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org/>) in pdb format. The molecular docking analysis was performed as previously described by Ganesan *et al.*, 2020. The Schrodinger virtual screening tool software was used to predict the conformation of the target ligands within the appropriate target binding site of SARS-CoV-2 protein (3CLpro, ACE2, RdRp, TMPRSS2 and PLpro). The structures retrieved from the RCSB protein data bank was a monomer with co-crystallized ligand. The proteins were prepared by using the protein preparation wizard by refining bond orders, addition of hydrogens, and deleting water molecules beyond 5Å, and missing chains are included by using the prime module of Schrödinger suite 2019-4 (Kumar *et al.*, 2022) [9]. Protein minimization was performed using optimized potentials for liquid simulations (OPLS3) molecular force field with RMSD of crystallographic heavy atoms kept at 0.30 Å. A grid box was generated to define the centroid of the active site. All the compounds were docked into the catalytic pocket of 3CLpro, ACE2, RdRp and PLpro by using the Glide module of Schrödinger suite 2019 in extra precision (XP) mode. The ligands with significant Glide scores (< -4.0) were considered to have more binding affinity towards SARS-CoV-2 protein involved in the coronavirus disease - COVID 19 pathway (Vellanki *et al.*, 2018) [10].

3. Results and Discussion

3.1. Extract yield

The extraction yield of *W. tinctoria* was influenced by both the type of solvent and the plant part used. Methanol extracts showed the highest yields in all parts tested, followed by aqueous, while petroleum ether consistently produced the lowest values. In leaves, the yield was 0.76% in methanol, 0.32% in aqueous, and 0.11% in petroleum ether. Bark extracts recorded the maximum yield in methanol (0.82%), with comparatively lower values in aqueous (0.21%) and petroleum ether (0.10%). Similarly, root extracts gave 0.68% in methanol, 0.31% in aqueous, and 0.08% in petroleum ether. The consistently higher yield obtained with methanol can be attributed to its ability to solubilize a wide range of phytochemicals due to its polarity, whereas aqueous extraction recovered mainly hydrophilic constituents and petroleum ether extracted fewer non-polar compounds (Nagarajan *et al.*, 2016) [11]. Among the plant parts, bark gave a slightly higher methanol yield compared to leaves and roots, which may reflect its richer composition of bioactive metabolites such as alkaloids and tannins. These findings suggest that methanol is the most effective solvent for recovering phytoconstituents from *W. tinctoria* and can be considered the preferred choice for further phytochemical and biological evaluations.

Table 1. Plant extract preparation and determination of yield

Parts used	Solvent System (mL)	Yield (%)*
Leaf	Aqueous	0.32 ± 0.01
	Methanol	0.76 ± 0.02
	Petroleum ether	0.11 ± 0.02
Bark	Aqueous	0.21 ± 0.01
	Methanol	0.82 ± 0.01
	Petroleum ether	0.10 ± 0.02
Root	Aqueous	0.31 ± 0.02
	Methanol	0.68 ± 0.01
	Petroleum ether	0.08 ± 0.02

3.2. Proximate analysis

The phytochemical profiling of *W. tinctoria* extracts revealed the presence of key secondary metabolites such as flavonoids, phenols, sterols, saponins, tannins, and terpenoids, which are known for their broad-spectrum bioactivities and potential antiviral effects. Among the plant parts tested, root aqueous extract exhibited the highest diversity of compounds (seven classes), while bark methanolic extract was also rich in metabolites, highlighting the influence of solvent polarity on compound recovery. Flavonoids and phenolic compounds, consistently detected across different extracts, are of particular interest due to their reported ability to interfere with viral entry, replication, and

protease activity in SARS-CoV-2. Terpenoids and sterols, detected mainly in methanolic and petroleum ether extracts, have also been linked to membrane stabilization and inhibition of viral enzymes. The presence of alkaloids and saponins further strengthens the antiviral potential, as these compounds are known to modulate immune responses and disrupt viral envelopes. The phytochemical diversity observed in *W. tinctoria* suggests its potential as a source of natural compounds that could be further explored for inhibitory effects against SARS-CoV-2 through *in vitro* and *in silico* approaches, emphasizing its relevance in the search for plant-based therapeutics during the COVID-19 pandemic.

Table 2. Phytochemical screening of the *W. tinctoria* extracts

S. No.	Plant parts used	Solvent system	Phytochemicals										Total
			Alkaloids	Flavonoids	Tannins	Saponins	Sterols	Phenols	Glycosides	Proteins	Carbohydrates	Terpenoids	
1	Leaf	Methanol	-	+	-	-	+	+	-	+	-	+	5
		Pet. ether	-	+	-	-	+	+	-	-	-	+	4
		Aqueous	-	+	-	-	+	+	-	+	-	+	5
2	Bark	Methanol	+	+	-	+	+	-	-	+	-	+	6
		Pet. ether	-	+	-	-	-	+	-	-	+	+	4
		Aqueous	-	+	-	+	-	-	-	+	+	+	5
3	Root	Methanol	-	+	+	+	+	+	-	-	-	+	4
		Pet. ether	-	+	+	-	-	+	-	-	-	+	4
		Aqueous	+	+	+	+	+	+	-	-	-	+	7

The quantitative phytochemical evaluation of *W. tinctoria* revealed solvent- and tissue-specific variations that are relevant to antiviral applications, particularly against SARS-CoV-2. Leaf aqueous extracts showed the highest flavonoid (0.680 ± 0.002 mg/g) and phenolic (4.274 ± 0.002 mg/g) contents, while petroleum ether extracts contained notable levels of steroids (1.008 ± 0.01 mg/g), compounds reported to modulate viral replication. Bark extracts were especially rich in phenols when extracted with petroleum ether (6.952 ± 0.004 mg/g), whereas methanol favored flavonoid recovery (0.820 ± 0.002 mg/g). Root extracts exhibited remarkable levels of tannins (2.284 ± 0.002 mg/g) and phenols (5.129 ± 0.03 mg/g) under aqueous solvents, alongside moderate flavonoid recovery. The high content of

flavonoids and phenols across leaf, bark, and root is of particular interest, as these metabolites have been widely associated with inhibition of SARS-CoV-2 main protease (Mpro) and spike-ACE2 interactions, thereby suppressing viral entry and replication. Similarly, tannins and terpenoids, especially abundant in roots and leaves, are known to disrupt viral proteins and enhance host immune defense. These findings suggest that *W. tinctoria* is a promising source of diverse phytoconstituents with potential inhibitory roles against SARS-CoV-2, and the observed solvent-dependent variation provides a scientific basis for selecting appropriate extraction systems for antiviral drug development.

Table 3. Quantitative analysis of extracts of *W. tinctoria* plant samples

S. No	Plant parts	solvents	Phytochemicals				
			Flavonoids (mg/g)	Tannins (mg/g)	Steroids (mg/g)	Phenols (mg/g)	Terpenoids (mg/g)
1	Leaf	ME	0.400 ± 0.02	0.000 ± 0.000	0.564 ± 0.001	2.685 ± 0.003	0.225 ± 0.001
		PE	0.500 ± 0.01	0.000 ± 0.000	1.008 ± 0.01	0.310 ± 0.01	0.007 ± 0.001
		AE	0.680 ± 0.002	0.000 ± 0.000	0.001 ± 0.001	4.274 ± 0.002	0.036 ± 0.002
2	Bark	ME	0.820 ± 0.002	0.000 ± 0.000	0.345 ± 0.001	0.000 ± 0.000	0.013 ± 0.002
		PE	0.740 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	6.952 ± 0.004	0.013 ± 0.02
		AE	0.260 ± 0.002	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.028 ± 0.03
3	Root	ME	0.070 ± 0.02	1.572 ± 0.04	0.574 ± 0.001	3.744 ± 0.02	0.000 ± 0.000
		PE	0.930 ± 0.001	0.541 ± 0.002	0.000 ± 0.000	0.375 ± 0.001	0.006 ± 0.002
		AE	0.870 ± 0.002	2.284 ± 0.002	0.001 ± 0.001	5.129 ± 0.03	0.104 ± 0.02

3.3. GC-MS/MS analysis

The GC–MS/MS analysis of *W. tinctoria* methanolic extracts of leaf, bark, and root revealed a wide spectrum of bioactive compounds with potential antiviral implications against SARS-CoV-2 (Figure 2 & Table 4-6). Leaf extracts were dominated by phytosterols such as γ -sitosterol (46.05%), campesterol (10.49%), and stigmasterol (7.49%), along with triterpenoid 12-oleanen-3-yl acetate (17.38%), all of which have been reported to exhibit immunomodulatory and viral enzyme inhibitory activities, particularly against SARS-CoV-2 main protease (Mpro) and RNA-dependent RNA polymerase. Bark extracts showed an even higher abundance of oleanane-type triterpenoids including 12-oleanen-3-yl acetate (32.23% and 13.93%) and lupeol acetate (28.24%), compounds known to disrupt viral protein assembly and

modulate host inflammatory pathways, which are critical in COVID-19 pathology. Additionally, bark extracts contained germanicol and cycloartane derivatives, triterpenes previously linked with antiviral and anti-inflammatory properties. Root extracts were enriched in fatty acid esters such as ethyl oleate (11.63%) and hexadecanoic acid ethyl ester (8.56%), alongside sterols like cholest-5-en-3-ol (7.10%) and stigmasterol, suggesting roles in maintaining membrane integrity and interfering with viral entry. The consistent detection of phytosterols, triterpenoids, and fatty acid derivatives across different plant parts highlights *W. tinctoria* as a promising reservoir of phytoconstituents capable of modulating viral replication, protease activity, and host immune response. These findings provide a biochemical rationale for further *in vitro* and *in silico* validation of *W. tinctoria* compounds as potential therapeutic candidates against SARS-CoV-2.

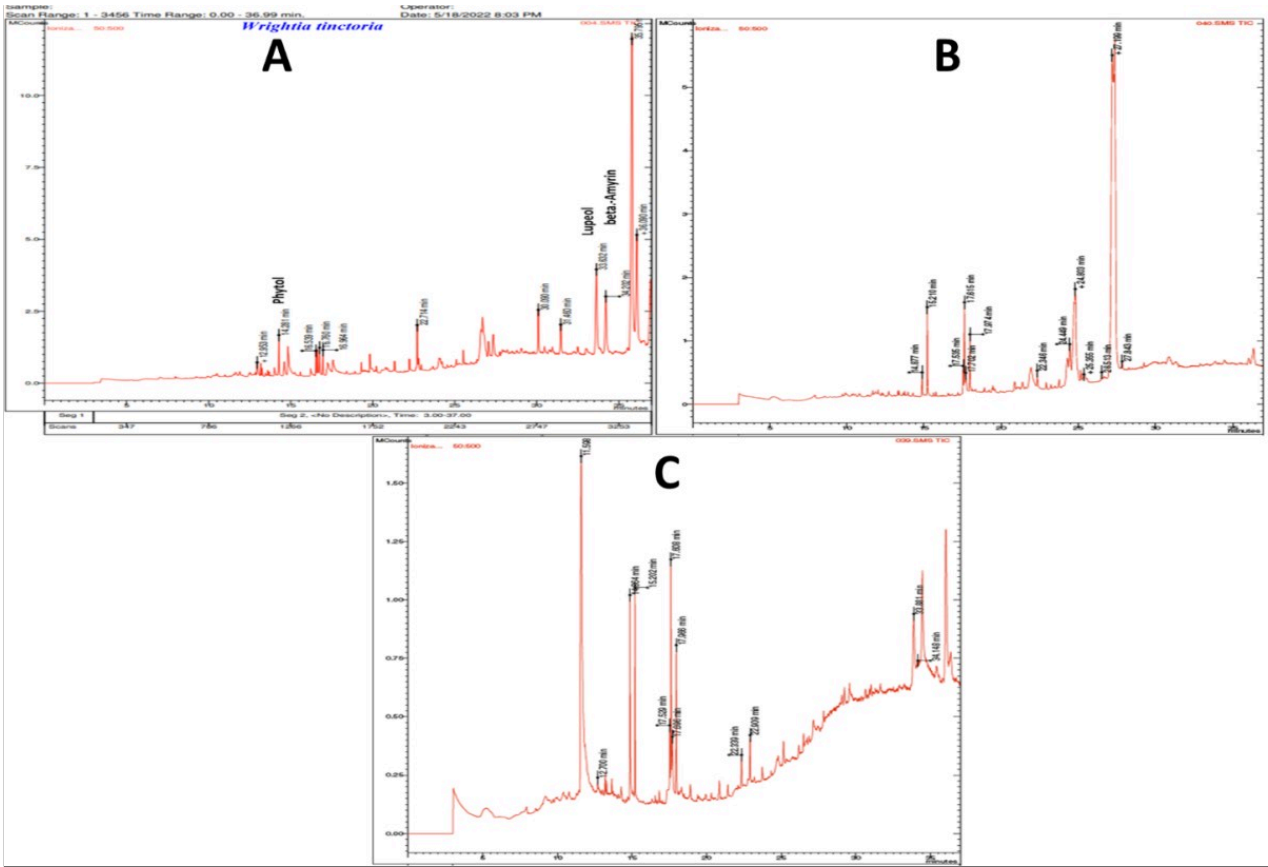


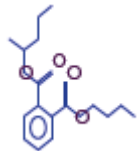
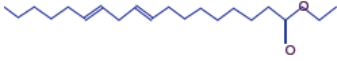
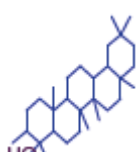
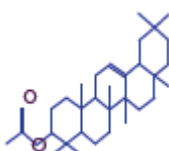
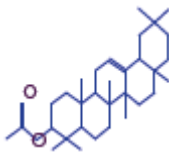
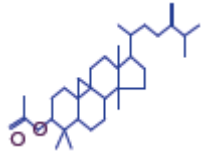
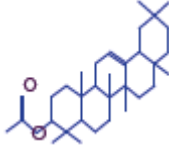
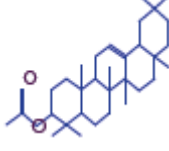
Figure 2. GC-MS Chromatogram of *W. tinctoria*; A- Leaf, B-Bark, C-Root

Table 4. GC/MS/MS analysis of methanol extract of leaf sample of *W. tinctoria*

S. No	Retention time (min)	Name of compound	% of total	Molecular Weight	Molecular Formula	Structure
1	12.949	Aspidospermidine-3-carboxylic acid, 2,3-didehydro-1-methyl-, methyl ester, (5.alpha.,12.beta.,19.alpha.)-	1.082	352	C ₂₂ H ₂₈ N ₂ O ₂	
2	14.283	Hexadecanoic acid, methyl ester	1.723	270	C ₁₇ H ₃₄ O ₂	
3	16.540	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	1.050	294	C ₁₉ H ₃₄ O ₂	
4	16.761	Phytol	1.286	296	C ₂₀ H ₄₀ O	
5	16.966	Octadecanoic acid, methyl ester	1.191	298	C ₁₉ H ₃₈ O ₂	
	22.713	Docosanoic acid, methyl ester	2.126	354	C ₂₃ H ₄₆ O ₂	
7	30.091	Beryllium, .mu.4-oxohexakis[.mu.-(propanoato-O:O')]tetra-	3.039	490	C ₁₈ H ₃₀ Be ₄ O ₁₃	
8	31.415	Silanamine, N-[(17.beta.)-3,17-bis[(trimethylsilyl)oxy]estra-1,3,5(10)-	2.661	503	C ₂₇ H ₄₉ NO ₂ Si ₃	
9	33.630	Campesterol	10.490	400	C ₂₈ H ₄₈ O	
10	34.200	Stigmasterol	7.490	412	C ₂₉ H ₄₈ O	
11	35.798	γ -Sitosterol	46.056	414	C ₂₉ H ₅₀ O	
12	36.088	12-Oleanen-3-yl acetate, (3.alpha.)-	17.389	468	C ₃₂ H ₅₂ O ₂	
13	36.930	Stigmasterol	3.915	412	C ₂₉ H ₄₈ O	

(continued on next page)

Table 5.GC/MS/MS analysis of methanol extract of bark sample of *W. tinctoria*

S.No	Retention time (min)	Name of compound	% of total	Molecular Weight	Molecular Formula	Structure
1	14.881	Phthalic acid, butyl 2-pentyl ester	0.767	292	C ₁₇ H ₂₄ O ₄	
2	15.212	Hexadecanoic acid, ethyl ester	2.625	284	C ₁₈ H ₃₆ O ₂	
3	17.533	9,12-Octadecadienoic acid, ethyl ester	0.862	308	C ₂₀ H ₃₆ O ₂	
4	17.614	Ethyl Oleate	3.068	310	C ₂₀ H ₃₈ O ₂	
5	17.704	Ethyl Oleate	0.909	310	C ₂₀ H ₃₈ O ₂	
6	17.978	Heptadecanoic acid, 15-methyl-, ethyl ester	1.842	312	C ₂₀ H ₄₀ O ₂	
7	24.446	Germanicol	1.748	410	C ₃₀ H ₅₀ O	
8	24.724	12-Oleanen-3-yl acetate, (3.alpha.)-	3.959	468	C ₃₂ H ₅₂ O ₂	
9	24.802	12-Oleanen-3-yl acetate, (3.alpha.)-	7.034	468	C ₃₂ H ₅₂ O ₂	
10	25.360	9,19-Cyclolanostan-3-ol, 24-methylene-, acetate, (3.beta.)-	1.265	482	C ₃₃ H ₅₄ O ₂	
11	27.203	12-Oleanen-3-yl acetate, (3.alpha.)-	32.231	468	C ₃₂ H ₅₂ O ₂	
12	27.289	12-Oleanen-3-yl acetate, (3.alpha.)-	13.933	468	C ₃₂ H ₅₂ O ₂	

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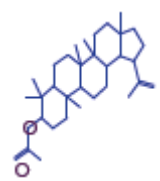
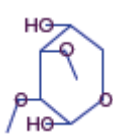
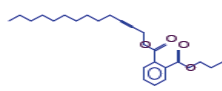
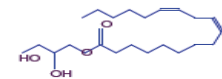
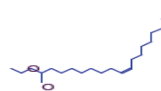
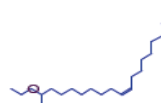
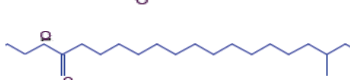
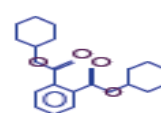
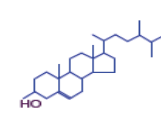
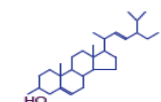
13	27.366	Lup-20(29)-en-3-ol, acetate, (3.β.)-/ Lupeol	28.247	468	C ₃₂ H ₅₂ O ₂	
14	27.845	1-Bromodocosane	0.318	388	C ₂₂ H ₄₅ Br	

Table 6. GC/MS/MS analysis of methanol extract of Root sample of *W. tinctoria*

S. No	Retention time (min)	Name of compound	% of total	Molecular Weight	Molecular Formula	Structure
1	11.602	2,3-Di-O-methyl-D-xylopyranose	44.688	178	C ₇ H ₁₄ O ₅	
2	12.702	Oleic Acid	0.633	282	C ₁₈ H ₃₄ O ₂	
3	14.860	Phthalic acid, butyl tridec-2-yn-1-yl ester	9.690	400	C ₂₅ H ₃₆ O ₄	
4	15.204	Hexadecanoic acid, ethyl ester	8.564	284	C ₁₈ H ₃₆ O ₂	
5	17.532	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	2.838	354	C ₂₁ H ₃₈ O ₄	
6	17.604	Ethyl Oleate	11.630	310	C ₂₀ H ₃₈ O ₂	
7	17.695	Ethyl Oleate	3.862	310	C ₂₀ H ₃₈ O ₂	
8	17.964	Heptadecanoic acid, 15-methyl-, ethyl ester	6.693	312	C ₂₀ H ₄₀ O ₂	
9	22.342	Tritetracontane	1.654	604	C ₄₃ H ₈₈	
10	22.909	1,2-Benzenedicarboxylic acid, dicyclohexyl ester	2.534	330	C ₂₀ H ₂₆ O ₄	
11	33.876	5-Cholestene-3-ol, 24-methyl-	7.109	400	C ₂₈ H ₄₈ O	
12	34.446	Stigmasterol	0.105	412	C ₂₉ H ₄₈ O	

3.4. Molecular docking of *W. tinctoria* plant samples

GC-MS/MS analysis of various parts of the medicinal plant *W. tinctoria* revealed the presence of various compounds having biological activities such as antimicrobial, anti-inflammatory, anticancer, antibacterial, anthelmintic, hepatoprotective, immune potential, antipyretic, antioxidant, antifungal, anticancer, anti-HIV activity etc. Phytocompounds viz., Phytol, Gamma-sitosterol, Lupeol, Campesterol and Stigmasterol were reported to have antiviral activity. To confirm the viral inhibitory activity of these compounds, molecular docking study was conducted to evaluate their Binding affinity against the targeted proteins which somehow or other play's crucial role in reducing or blocking the activity of SARS-CoV-2. Molecular

docking of the four targeted proteins viz., Angiotensin-converting enzyme 2 (ACE2), 3-chymotrypsin like protease (3CLpro), papain-like protease (PLpro) and RNA-dependent RNA polymerase (RdRp) which play crucial role in viral entry into the host cell and indispensable for the replication revealed that Stigmasterol exhibited a remarkable binding affinity towards all the four targeted protein selected for the study, followed by Lupeol. From the study it was found that the candidate drug compounds campesterol, γ -sitosterol, lupeol, phytol and stigmasterol were exhibited significant binding affinity and secured great gliding score energy against the protein target such as ACE2, 3CLpro, RdRp, TMPRSS2 and PLpro as antiviral agents and suppressed by inhibiting the viral functions such as facilitates the virus spike protein especially receptor binding unit of Spike protein to

bind on the nasal epithelial cell wall which may be a foundation for discovering natural products-based drugs against COVID-19. They also significantly bounded with active sites of viral proteins and in turn which shall suppresses the viral metabolic pathways. All the an-

tiviral compounds identified have rmsd/ub and rmsd/pb value $< 2 \text{ \AA}$. *W. tinctoria* with five promising antiviral compounds can be considered as an effective source for healthcare products.

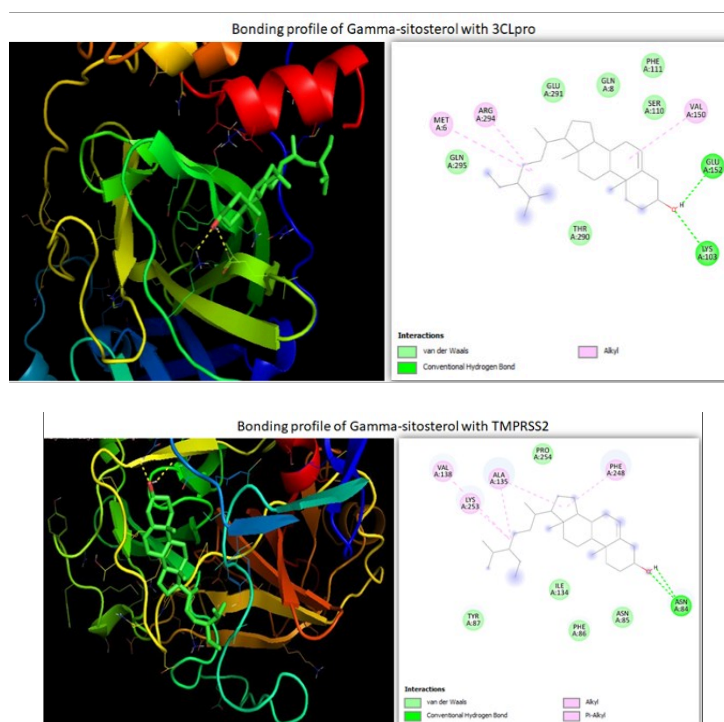


Figure 3. 3D and 2D interaction of Gamma-sitosterol with 3CLpro and TMPRSS2 protein

The ligand γ -sitosterol was reported in methanol extract of *W. tinctoria* leaf (46.056 %). γ -sitosterol was reported to be effective against 3CLpro Protein. The ligand gamma-sitosterol showed binding affinity of -7.4 kcal/mol with 3CLpro protein, -8.3 kcal/mol with ACE2 receptors and -6.5 kcal/mol with TMPRSS2 receptors. γ -sitosterol formed two conventional hydrogen bond with the amino acid residues Glu 152 and Lys 103 with the single hydroxyl group attached with the cyclo hexane ring of the ligand. Three amino acid residues Val 150, Arg 294 and Met 6 formed alkyl bonds and Gln 295, Glu 291, Gln 8,

Thr 290 and Ser 110 formed van der Waals bond with the receptor 3CLpro. γ -sitosterol formed one hydrogen bond with Arg 253, three alkyl/Pi-alkyl bond with His 325, Phe 484 and Val 323 and eight van der Waals force with Leu 483, His 485, Trp 251, Tyr 108, Tyr 31, Ser 109, Ser 105 and Asn 488 amino acid residues of ACE2 receptor. γ -sitosterol formed two conventional hydrogen bond with Asn 84, four alkyl and Pi-alkyl bond with Val 138, Ala 135, Lys 253 and Phe 248 and five van der Waals force with Tyr 87, Ile 134, Phe 86, Asn 85 and pro 254 amino acid residues of TMPRSS2 receptor (Table 7).

Table 7. Binding profile of γ -sitosterol with the receptors

S. No.	Receptor	Binding affinity Kcal/mol	Hydrogen bond	Alkyl / Pi-alkyl bond	Van der Waals force/bond
1.	3CLpro	-7.14	2 Glu 152, Lys 103	3 Val 150, Arg 294 and Met 6	5 Gln 295, Glu 291, Gln 8, Thr 290, Ser110
2.	ACE2	-8.3	1 Arg 253	3 His 325, Phe 484, Val 323	8 Leu 483, His 485, Trp 251, Tyr 108, Tyr 31, Ser 109, Ser 105, Asn 488
3.	TMPRSS2	-6.5	2 Asn 84	4 Val 138, Ala 135, Lys 253, Phe 248	5 Tyr 87, Ile 134, Phe 86, Asn 85, pro 254

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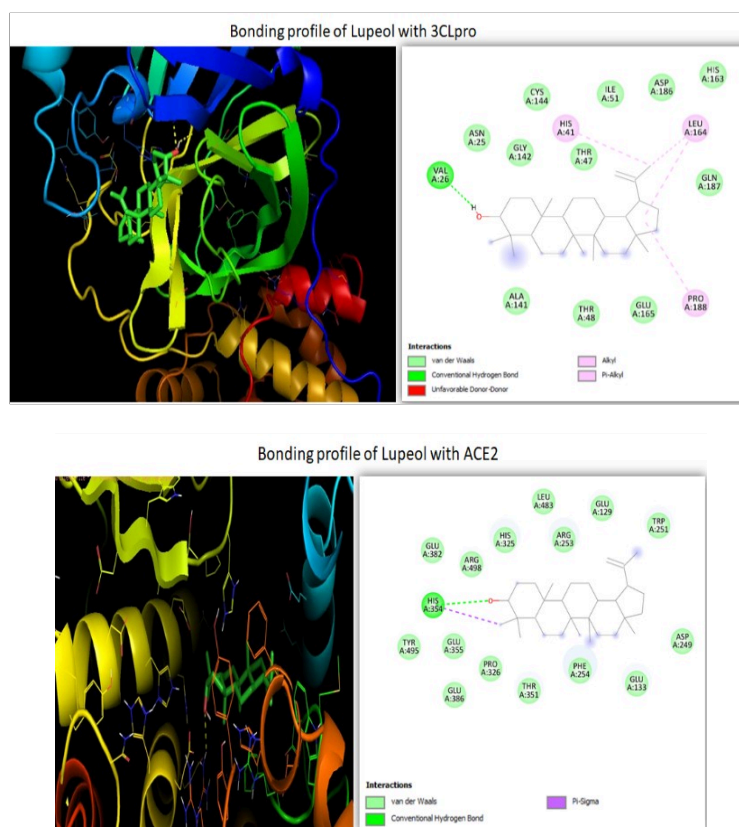


Figure 4. 3D and 2D interaction of Lupeol with 3CLpro and ACE2 receptors

Lupeola naturally occurring pentacyclic triterpenoid and potential entry inhibitor against spike protein of SARS-CoV-2. It is also considered as potential ligands for SARS-CoV-2 spike antagonists and reduces pro-inflammatory cytokines. Lupeol was identified and quantified in *W. tinctoria* leaf (17.389 %). Lupeol showed Binding affinity of -8.8 Kcal/mol with 3CLpro, -8.0 Kcal/mol with ACE2 and -7.2 Kcal/mol with TMPRSS2 receptors (Table 40). Lupeol formed hydrogen bond between the hydroxyl group attached with the cyclo pentane ring of the ligand and Val 26, three alkyl/pi-alkyl bond with His 41, Leu 164 and Pro 188 and van der Waals bond with 11 amino acid residues Asn 25, Gly 142, Cys 144, Thr 47, Ile 51, Asp 186, His 163, Gln 187, Ala 141, Thr 48 and Glu 165 of 3CLpro receptor. Lupeol formed one conventional hydrogen bond and one pi-sigma bond with His 354 and 15 van der Waals bond with Glu 382, Arg 498, His 325, Leu 483, Arg 253, Glu 129, Trp 251, Tyr 495, Glu 355, Glu 386, Pro 326, Thr 351, Phe 254, Glu 133 and Asp 249 of ACE2. Lupeol formed conventional hydrogen bond with Asn 85, alkyl bond with Ala 135, nine van der Waals bonds Tyr 87, Asn 84, Phe 86, Pro 179, Ile

134, Pro 254, Phe 248, Lys 253 and Asn 249 of TMPRSS2 (Table 8). The study was compared with the studies conducted by several research about the antiviral and biological properties of lupeol provides significant activities against some harmful virus was reported to decrease the ROS level and restore the antioxidant enzyme activities in mouse liver against chemical-induced oxidative stress [12]. Also, lupeol treatment is shown to induce growth inhibition and apoptosis in HCC cell line, SMMC7721 (Maurya *et al.*, 2020) [13]. Though lupeol has shown weak antiviral activities in several studies, it has served as a lead drug for the generation of more effective compounds against Influenza A and HSV [14]. On the contrary, lupeol isolated from *Strobilanthes cusia* root has demonstrated a robust anti-HSV activity, *in vitro* (Flekhter *et al.*, 2004) [15]. Notably, to the best of our knowledge, the antiviral activity of lupeol against hepatitis viruses has not been reported, so far. Nevertheless, lupeol (6.72 µg/mg) identified in the antioxidant GSLE, could have a direct or indirect role in the plant's *anti*-HBV activity.

Table 8. Binding profile of Lupeol with the receptors

S. No.	Receptor	Binding affinity Kcal/mol	Hydrogen bond	Alkyl / Pi-alkyl bond	Van der Waals force/bond
1.	3CLpro	-8.8	1 Val 26	3 His 41, Leu 164, Pro 188	11 Asn 25, Gly 142, Cys 144, Thr 47, Ile 51, Asp 186, His 163, Gln 187, Ala 141, Thr 48, Glu 165
2.	ACE2	-8.0	1 His 354	1 His 354	15 Glu 382, Arg 498, His 325, Leu 483, Arg 253, Glu 129, Trp 251, Tyr 495, Glu 355, Glu 386, Pro 326, Thr 351, Phe 254, Glu 133, Asp 249
3.	TMPRSS2	-7.2	1 Asn 85	1 Ala 135	9 Tyr 87, Asn 84, Phe 86, Pro 179, Ile 134, Pro 254, Phe 248, Lys 253 and Asn 249

The viral inhibitor phytol was reported in methanol extract *W. tinctoria* leaf (1.286 %) which work for corona virus and in the manage-

ment of painful inflammatory diseases. Phytol formed conventional hydrogen bond with three amino acid residues between hydroxyl

group and Phe 111, Lys 5 and val 127, two carbon hydrogen bond with Ser 110 and Met 6, alkyl bond with Met 6 and ten van der Waals bond with Ala 7, Val 150, Asn 112, Gly 126, Gln 8, Glu 291, Arg 4, Gln 295, Arg 294, Thr 290 of 3CLpro. Phytol formed conventional covalent bond with Pro 326, two pi-alkyl bonds with Trp 329 and His 381, 8 van der Waals bonds with Asp 330, His 358, Ala 328, Thr 327,

Asp 362, Tyr 365, Glu 355 and His 358 of ACE2. Phytol formed conventional hydrogen bond with Phe 86 and seven van der Waals bonds with pro179, Cys 136, Asn 249, Asn 85, Asn 84, Pro 254 and Asp 250 of TMPRSS2. The amino acid residue Ala 135 formed 4 alkyl/pi-alkyl bonds, Lys 253 two bonds, Van 138, Phe 248 and Ile 134 each one alkyl/pi-alkyl bond with TMPRSS2 (Table 9).

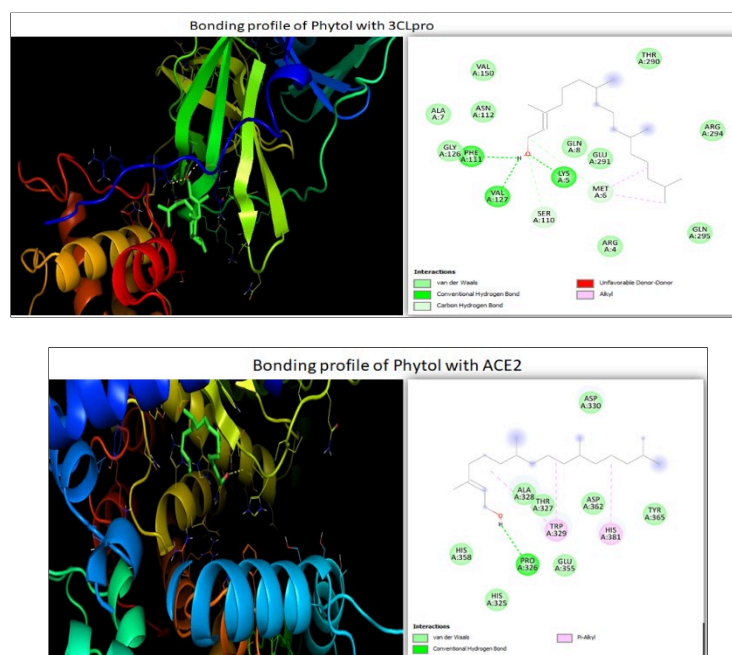


Figure 5. 3D and 2D interaction of Phytol with 3CLPro and ACE2 receptors

Table 9. Binding profile of Phytol with the receptors

S. No.	Receptor	Binding affinity (Kcal/mol)	Hydrogen bond	Carbon hydrogen bond	Alkyl / Pi-alkyl bond	Van der Waals force/bond
1	3CLpro	-5.2	3 Phe 111, Lys 5 and val 127	2 Ser 110 Met 6	1 Met 6	10 Ala 7, Val 150, Asn 112, Gly 126, Gln 8, Glu 291, Arg 4, Gln 295, Arg 294, Thr 290
2	ACE2	-4.8	1 Pro 326	-	2 Trp 329, His 381-	8 Asp 330, His 358, Ala 328, Thr 327, Asp 362, Tyr 365, Glu 355, His 358
3	TMPRSS2	-5.3	1 Phe 86	-	5 Ala 135, Lys 253, Van 138, Phe 248, Ile 134	7 Pro 179, Cys 136, Asn 249, Asn 85, Asn 84, Pro 254 Asp 250

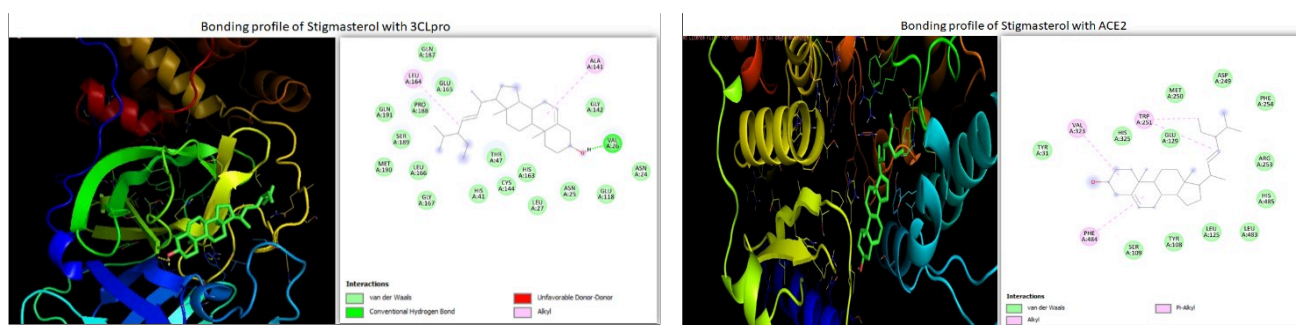


Figure 7. 3D and 2D interaction of Stigmasterol with 3CLpro and ACE2 receptors

The ligand Stigmasterol, a phytosterol has been shown to exhibit *in vitro* and *in vitro* anti-HIV activity by immunomodulatory mechanism through stabilization of CD4⁺ T-lymphocyte counts, and a significant decrease in interleukin-6 level (Tanaka *et al.*, 2004) [16]. Very recently, antiviral effect of β -sitosterol isolated from cottonseed oil against tobacco mosaic virus (TMV) is reported (Donald *et al.*, 1997)

[17]. Interestingly, β -sitosterol has been also shown to attenuate *in vitro* chemical-induced hepatotoxicity [18] and cardiotoxicity by enhancing mitochondrial glutathione redox mechanism (Zhao *et al.*, 2018) [19]. In line with this, our identification of stigmasterol (16.72 μ g/mg) as the abundant compound strongly supports its *in vitro* antioxidative, hepatoprotective and anti-HBV activities, indepen

dently or interdependently. It formed conventional hydrogen bond with Val 26, two alkyl bond with Leu 164 and Ala 141, 17 van der Waals bonds with Gln 187, Leu 164, Glu 165, Gln 191, Pro 188, Ser 189, Met 190, Leu 166, Gly 167, Thr 47, His 163, Cys 144, His 41, Leu 27, Asn 25, Glu 118, Asn 24 of 3CLpro (Table 45). Stigmasterol formed three alkyl/pi-alkyl bonds with val 323, Trp 251 and Phe 484 and 12 van der Waals bonds with Tyr 31, His 325, Glu 129, Met 250, Asp 249, Phe 254, Arg 253, His 485, Ser 109, Tyr 108, Leu 125 and Leu 483 of ACE2. Stigmasterol formed 11 van der Waals bonds with

the amino acid residues Leu 263, Phe 262, lys 310, Tyr 217, Lys 221, Asp 218, Lys 283, Ala 282, Gly 360, Thr 261 and Asp 218 of Plpro. Stigmasterol one conventional hydrogen bond with Arg 184, alkyl/pi-alkyl Phe 153, Ile 172 and pi-sigma bond Phe 153 of RdRp receptor. Stigmasterol formed two conventional hydrogen bonds with Asn 84, two each alkyl bonds with Ala 135 and Lys 253 and one alkyl bond each with Val 138 and Pro 254 and eight van der Waals bonds with the amino acid residues of Gly 137, Cys 136, Tyr 87, Phe 86, Phe 248, Asn 85, Ile 134 and Pro 179 of TMPRSS2 (Table 10).

Table 10. Binding profile of Stigmasterol with the protein receptors

S. No.	Receptor	Binding affinity (Kcal/mol)	Hydrogen bond	Alkyl / Pi-alkyl bond	Van der Waals force/bond
1.	3CLpro	-7.4	1 Val 26,	2 Leu 164 Ala 141	17 Gln 187, Leu 164, Glu 165, Gln 191, Pro 188, Ser 189, Met 190, Leu 166, Gly 167, Thr 47, His 163, Cys 144, His 41, Leu 27, Asn 25, Glu 118, Asn 24
2.	ACE2	-9.1	-	3 Val 323, Trp 251 Phe 484	12 Tyr 31, His 325, Glu 129, Met 250, Asp 249, Phe 254, Arg 253, His 485, Ser 109, Tyr 108, Leu 125, Leu 483
3.	TMPRSS2	-7.4	Asn 84	Ala 135 Lys 253 Val 138 Pro 254	8 Gly 137, Cys 136, Tyr 87, Phe 86, Phe 248, Asn 85, Ile 134 and Pro 179
4	PLpro	-7.5	-	-	11 Leu 263, Phe 262, lys 310, Tyr 217, Lys 221, Asp 218, Lys 283, Ala 282, Gly 360, Thr 261 and Asp 218
5	RdRp	-8.0	Arg 184	Phe 153, Ile 172	-

Campesterol is a plant sterol separated from the methanol extract of *W. tinctoria* leaf (10.49 percent) at 33.630 min. Molecular docking of Campesterol against targeted proteins revealed that it showed a Binding affinity of -7.6 Kcal/mol against 3CLpro and -8.6 Kcal/mol against ACE2 and -6.9 Kcal/mol against TMPRSS2 receptors (Table 11). Campesterol formed conventional hydrogen bond between the hydroxyl group and the amino acid residue Val 26, alkyl bond with three ACE2 amino acid residues Leu 164, Pro 188 and Ala 141 and van der Waals bond with 13 amino acid residues Glu 165, Gln 187,

Ser 189, Met 190, Leu 166, Thr 47, His 41, Cys 144, His 163. Leu 27, Asn 25, Glu 118 and Gly 142 of the 3CLpro receptor. The ligand Campesterol formed alkyl and pi-alkyl bonds with Ala 215, Arg 207, Leu 206 and Phe 210 and van der Waals force with Phe 212, Ile 205 and Gyl 204 of the TMPRSS2 receptor. The ligand Campesterol formed conventional hydrogen bond with the amino acid residue Arg 253 and alkyl and pi-alkyl bond with Trp 251, His 325, Phe 484 and Val 323 of the ACE2 receptor (Table 11).

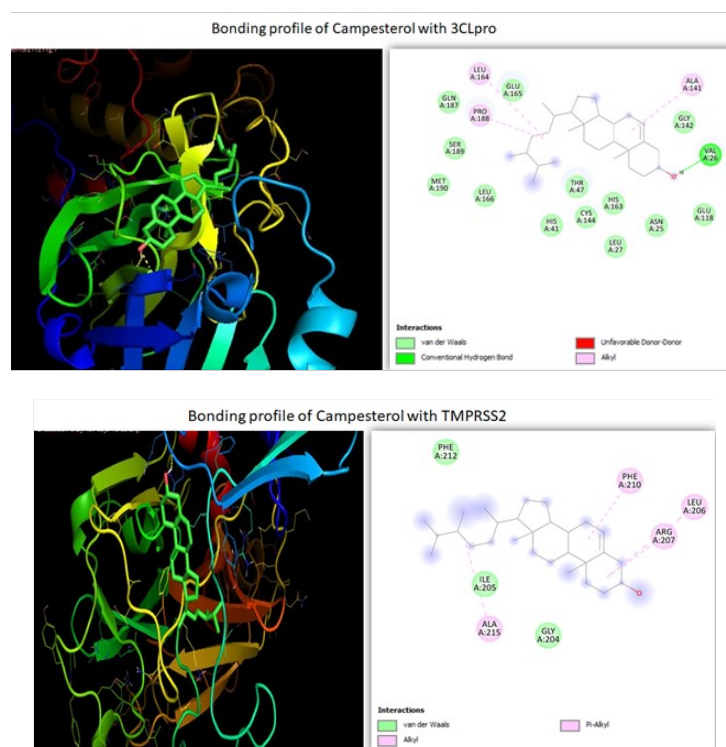


Figure 6. 3D and 2D interaction Campesterol with 3CLpro and TMPRSS2 receptors

Table 11. Binding profile of Campesterol with the receptors

S. No.	Receptor	Binding affinity Kcal/mol	Hydrogen bond	Alkyl/Pi-alkyl bond	Van der Waals force/bond
1.	3CLpro	-7.6	1 Val 26	3 Leu 164, Pro 188 Ala 141 13	13 Glu 165, Gln 187, Ser 189, Met 190, Leu 166, Thr 47, His 41, Cys 144, His 163, Leu 27, Asn 25, Glu 118 and Gly 142
2.	ACE2	-8.6	1 Arg 253	Trp251, His 325, Phe 484, Val 323	Nil
3.	TMPRSS2	-6.9	Nil	4 Ala 215, Arg 207, Leu 206, Phe 210	3 Phe 212, Ile 205 and Gly 204

Molecular docking studies of the identified antiviral compounds Phytol, Gamma-sitosterol, Lupeol, Campesterol and Stigmasterol against the target protein viz., ACE2, TMPRSS2, 3CLpro, PLpro, RdRp revealed that among the 5 antiviral compounds, stigmasterol has significant docking score against 4 target proteins viz., ACE2, 3CLpro, PLpro, RdRp and TMPRSS2 (Table 12). All the 5 antiviral compounds have rmsd/ub and rmsd/pb value < 2 Å. The antiviral compounds selected for molecular docking study showed significant

binding affinity with ACE2 and TMPRSS2 proved that the ligands strongly inhibit the entry of SARS-CoV 2 entry into the host and strong binding affinity with 3CLpro proved the inhibition of further replication the virus in the host cell. The amino acid residues Cys 145, His 41 and Glu 166 are essential for the enzymatic activity of 3CLpro. Stigmasterol with significant Binding affinity towards 4 target proteins may be considered as a promising lead compound with antiviral activity.

Table 12. Antiviral compounds and its binding affinity with target protein in the CoV-19 host

Anti-viral compounds	Target Protein in CoV-19 viral host				Binding distance/ rmsd/ub and rmsd/pb (Å°)			
	Gliding energy/Binding affinity (Kcal/mol)							
	3CLpro	ACE2	Plpro	RdRp	3CLpro	ACE2	Plpro	RdRp
Campesterol	-7.6	-8.6	0	0	< 2	< 2	< 2	< 2
γ-sitosterol	-7.4	-8.3	0	0	< 2	< 2	< 2	< 2
Lupeol	-8.8	-8.0	0	0	< 2	< 2	< 2	< 2
Phytol	-5.2	-4.8	0	0	< 2	< 2	< 2	< 2
Stigmasterol	-7.4	-9.1	-7.5	-8.0	< 2	< 2	< 2	< 2

4. Conclusion

The findings provide biochemical evidence of the presence of five major antiviral compounds such as γ -sitosterol, stigmasterol, campesterol, lupeol, and phytol in *W. tinctoria*. Molecular docking analysis as computational evidence, confirmed the strong binding affinities of these antiviral compounds with key SARS-CoV-2 targets including ACE2, TMPRSS2, 3CLpro, PLpro, and RdRp receptors, thereby indicating their potential to inhibit viral entry, replication, and pathogenesis. Notably, stigmasterol displayed the most promising multi-target activity, suggesting its role as a lead molecule for further development. These data recognizes the potential of *W. tinctoria* and its antiviral compounds against SARS-CoV-2. Future validation through *in vivo* efficacy studies, and formulation development will be essential to translate these insights into therapeutic applications.

5. Acknowledgement

The authors gratefully acknowledge the Director General, Indian Council of Forestry Research and Education (ICFRE), Dehradun, for providing support to carry out this research. The authors also thank the Director, ICFRE–Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu, for institutional support and necessary facilities.

6. Author contributions

R. Sumathi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft preparation; S.P. Subramani: Formal analysis, Investigation, Methodology, Supervision; D. Thangamani: Methodology; Srijita Ganguly: Investigation; C. Bagathsingh: Investigation; N. Senthilkumar: Conceptualization, Supervision, Writing-review and editing. All authors read and approved the manuscript.

7. Data availability

Data produced or examined in the course of this study may be requested from the corresponding author.

References

1. Docea AO, Tsatsakis A, Albulescu D, Cristea O, Zlatian O, Vinceti M, Calina D. A new threat from an old enemy: Re-emergence of coronavirus. *International Journal of Molecular Medicine*. 2020;45:1631–1643.
2. Shah A, Kashyap R, Tosh P, Sampathkumar P, O'Horo JC. Guide to understanding the 2019 novel coronavirus. *Mayo Clinic Proceedings*. 2020;95:646–652.
3. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology*. 2022;23:3–20.
4. Kurokawa M, Shimizu T, Watanabe W, Shiraki K. Development of new antiviral agents from natural products. *Open Antimicrobial Agents Journal*. 2010;2:49–57.
5. Hussain M, Farooq M, Nawaz A, Al-Sadi AM, Solaiman ZM, Alghamdi SS, Siddique KH. Biochar for crop production: potential benefits and risks. *Journal of Soils and Sediments*. 2017;17:685–716.
6. Newton AH, Cardani A, Braciale TJ. The host immune response in respiratory virus infection: balancing virus clearance and immunopathology. *Seminars in Immunopathology*. 2016;38:471–482.
7. Devi SL, Divakar MC. *Wrightia tinctoria* (Roxb.) R.Br.—An updated review. *Hygeia: Journal for Drugs and Medicines*. 2014;6:95–105.
8. Vignesh A, Sivalingam R, Selvakumar S, Vasanth K. A review on ethnomedicinal and phytopharmacological potential of *Berberis tinctoria* Lesch. *Thai Journal of Pharmaceutical Sciences*. 2022;46:137–148.
9. Kumar BK, Faheem N, Sekhar KVG, Ojha R, Prajapati VK, Pai A, Murugesan S. Pharmacophore-based virtual screening, molecular docking, molecular dynamics and MM-GBSA approach for identification of prospective SARS-CoV-2 inhibitor from natural product databases. *Journal of Biomolecular Structure and Dynamics*. 2022;40:1363–1386.
10. Vellanki K, Hou S. Menopause in CKD. *American Journal of Kidney Diseases*. 2018;71:710–719.
11. Nagarajan J, Wah Heng W, Galanakis CM, Nagasundara Raman R, Raghunandan ME, Sun J, Prasad KN. Extraction of phytochemicals using hydrotropic solvents. *Separation Science and Technology*. 2016;51:1151–1165.
12. Mohammad T, Siddiqui S, Shamsi A, Alajmi MF, Hussain A, Islam A, Hassan MI. Virtual screening approach to identify high-affinity inhibitors of serum- and glucocorticoid-regulated kinase 1 among bioactive natural products: combined molecular docking and simulation studies. *Molecules*. 2020;25:823.
13. Maurya SK, Shadab GGHA, Siddique HR. Chemosensitization of therapy-resistant tumors: targeting multiple cell signaling pathways by lupeol, a pentacyclic triterpene. *Current Pharmaceutical Design*. 2020;26:455–465.
14. Akram M, Tahir IM, Shah SMA, Mahmood Z, Altaf A, Ahmad K, Mehboob H. Antiviral potential of medicinal plants against HIV, HSV, influenza, hepatitis and coxsackievirus: a systematic review. *Phytotherapy Research*. 2018;32:811–822.
15. Flekhter OB, Boreko EI, Nigmatullina LR, Pavlova NI, Medvedeva NI, Nikolaeva SN, Tolstikov GA. Synthesis and antiviral activity of lupane triterpenoids and their derivatives. *Pharmaceutical Chemistry Journal*. 2004;38:355–358.
16. Tanaka T, Kanda T, Takahashi T, Saegusa S, Moriya J, Kurabayashi M. Interleukin-6-induced reciprocal expression of SERCA and natriuretic peptides mRNA in cultured rat ventricular myocytes. *Journal of International Medical Research*. 2004;32:57–61.
17. Donald PR, Lamprecht JH, Freestone M, Albrecht CF, Bouic PJD, Kotze D, Van Jaarsveld PP. A randomised placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis. *International Journal of Tuberculosis and Lung Disease*. 1997;1:518–522.
18. Parvez MK, Alam P, Arbab AH, Al-Dosari MS, Alhowiriny TA, Alqasoumi SI. Analysis of antioxidative and antiviral biomarkers β -amylin, β -sitosterol, lupeol, ursolic acid in *Guiera senegalensis* leaves extract by validated HPTLC methods. *Saudi Pharmaceutical Journal*. 2018;26:685–693.
19. Zhao L, Qi Y, Xu L, Tao X, Han X, Yin L, Peng J. MicroRNA-140-5p aggravates doxorubicin-induced cardiotoxicity by promoting myocardial oxidative stress via targeting Nrf2 and Sirt2. *Redox Biology*. 2018;15:284–296.

How to cite this article: Sumathi R, Subramani S, Thangamani D, Ganguly S, Bagathsingh C, Senthilkumar N. Antiviral compounds of *Wrightia tinctoria* (Roxb.) R.Br. against SARS CoV-2: An *in vitro* and *in silico* studies. *International Journal of Biology Sciences*. 2026;8(9):118-130. doi: <https://www.doi.org/10.33545/26649926.2026.v8.i9b.997>