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In silico analysis of Potential Phytochemical Constituents of *Orthosiphon stamineus* as Significant Inhibitors Against Urolithiasis

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ABSTRACT

Naturally occurring bioactives from plant sources have played a significant role in developing multi-targeted drugs towards treatment of various human ailments. Urolithiasis is the process of stone formation in the kidney, urinary tract or in bladder. It occurs to 1 in 20 people at different phases of their life causing pain in the abdomen, flank or groin and bloody urine at times. The current study focuses on pythochemical activity of *Orthosiphon stamineus*(Java tea) that can act against kidney stone. The target proteins responsible for stone formation was identified through literature search and the three dimensional structures available was retrieved from Protein Data Bank and structures unavailable was modelled through computational methods. These proteins were docked against the phytochemicals and certain synthetic compounds which are commercially used for Urolithiasis. The best docked compounds and their interaction have been studied and it was identified that when compared to the synthetic compounds, there is high

competence for phytochemicals in binding to the target proteins which suggests that these compounds can be used further for the process of designing a drug effectively against urolithiasis.
Keywords: Urolithiasis, *Orthosiphon stamineus,* Java tea, Phytochemical and Synthetic compounds

INTRODUCTION

Urolithiasis being the most common disease occurring in the urinary tract is known to affect about 12% of the world population. Less water intake and imbalanced nutrient diet are the major risk factors paving way to urolithiasis(López, M & Hoppe, B, 2010).The symptoms of kidney stone disorder depends on the location where it is formed, whether it is in the kidney, ureter or urinary bladder. Though the initial stage shows no sign of occurrence, the lateral stage causes intensive cramping pain, hematuria, urinary tract related disorders and in severe cases all ending up with kidney dilation(hydronephrosis). The kidney stone formation possess high risk of association with other chronic diseases viz., diabetes, hypertension and renal failure. kidney stone disorder can be cured effectively if the mechanisms involved in stone formation is understood clearly. (Alelign, T, & Petros, B. 2018).

The surgical methods that are used to remove the kidney stones being painful, it is always wiser to prefer the preventive measures that cause no pain. Nowadays the usage of products derived from plants, animals and marine resources are high in use. In traditional Indian medicine, plants play a vital role as the curative agents.**Herbal plants** are an important source of medicines and major health care providers across the globe since ancient times. (Kumar, M, Patel, M et al., 2021). One such plant is Orthosiphon stamineus of the family *lamiaceae*. This plant is known for its diverse bioactive characteristic such as antihypertensive, antioxidant, anti-inflammatory, hepatoprotective and hypoglycaemic. It is reported that *Orthosiphon stamineus* having many compounds that can act as the best inhibitors against the proteins responsible for Urolithiasis.(Hsu, C. L *et al*., 2010)

MATERIAL AND METHODS

Selection of targets

S.No	**Pdb id**	**Swiss-prot id**	**Protein targets**	**Role of target proteins in Urolithiasis**
1.	1KB2	P11473	Vitamin D3 Receptor(Chains A,B)	Increases digestive Calcium absorption and promotes urinary stone formation (Letavernier, E., & Daudon, M. (2018).)
2.	1UUH	P16070	CD44 ANTIGEN(Chains A,B)	A cell surface receptor, play a notable role in Calcium Oxalate binding (Asselman, M *et al*., 2003)
3	-	Q8N6F1	Claudin 19 (Modelled protein)	Renal calcium and magnesium wasting(Hypercalciuria&hypomagnesemia)
4.	5FUO	P02768	SERUM ALBUMIN(Chain A)	Promotes the growth of Calcium Oxalate Monohydrate crystals on hydroxyapatite with Citrate & magnesium inhibitors (Ebrahimpour, A *et al*., 1991)

5.	1BT6	P60903	Annexin II	Binds Calcium Oxalate Monohydrate crystals on renal tubular cells (Kumar *et al*., 2003)
6.	1POZ	P16070	Hyaluronan synthase	Retention of Calcuim Oxalate crystals in tubular epithelial cells (Asselman, M*et al.,* 2003)
7.	1IQT	Q14103	Heterogeneous nuclear ribonucleoprotein DO	It's acidic peptide sequence will mediate the attachment of Calcium Oxalate crystals to the renal tubular cells through selective absorption. (Sorokina, *et al*., 2004)

Target protein structure retrieval:

Three dimensional X-ray crystal structures for the target proteins were obtained from the Protein Data Bank. The PDB ID of corresponding proteins was tabulated above. The protein Claudin 19 does not have experimentally determined 3D structure and hence it was computationally modeled through Swiss model.

Active site prediction

The Computed Atlas of Surface Topography of proteins (CASTp) 3.0 (Binkowski TA, *et al*., 2003&Tian *et al.,* 2018)) was used to predict the active sites that were present in the protein structure. This online server was used in identification and measurement of voids on 3D protein structures. The 3D protein structure was submitted to the CASTp server and binding site residues was predicted.

Selection of ligands

Compounds from *Orthosiphon stamineus was* identified through various literature sources and of about 136 phytochemicals have been retrieved from PubChem and was used for virtual screening against the potential targets for Urolithiasis. (Table 1)

ADME property calculation

ADMET properties help to have insight into the pharmacokinetic nature of the ligand compounds. The phytochemical compounds saved in Canonical SMILES format was uploaded to Swiss ADME available in SIB (Swiss Institute of Bioinformatics) webserver (https://www.sib.swiss) and submitted for ADME estimation. The details of aqueous solubility, blood brain barrier level, gastrointestinal absorption, CYP 2D6, Hepatoxicity, plasma protein binding levels and the drug- likeliness parameters were noted and analyzed. The library was taken for Docking studies along with seven targets using PyRx.

Virtual Screening

Virtual screening was performed using Python Prescription Virtual Screening tool (PyRx 0.8) containing AutoDock Vina module (Dallakyan and Olson, 2015). Protein structure was prepared by adding hydrogen atoms and energy minimization was done. The docking analysis was performed for the chosen compounds with the target proteins using AutoDock Vina module in PyRx 0.8. For docking, the grid map was fixed on the ligand binding site of the receptor. Binding sites were predicted using CASTP server and the same were used for setting grid (XYZ dimensions: 25*25*25) in the Auto Dock Vina for virtual screening experiment with the exhaustiveness value of 8.

RESULTS AND DISCUSSION

Virtual screening of potential herbal ligands against major protein targets for Urolithiasis

Virtual screening of 136 ligands from Java tea 7 major protein targets of Urolithiasis identified potential inhibitors. Information regarding the binding site residues predicted using CASTp server. Top 10 hits reported with higher binding affinity for each target protein is considered for further interaction analysis.

Annexin II

For the target 1BT6 phytochemical compounds such as Beta amyrone, Orthosiphol X, masilinic acid, Catechin gallate have better binding affinity with binding scores of -7.8, -7.7, -7.7, -7.5 Kcal/mol respectively with hydrogen bond formation. (Fig 1)

Heterogeneous nuclear ribonucleoprotein

For the target protein 1IQT phytochemical compounds such as (-)-Catechin gallate, Beta amyrone, Orthosiphol X, Rutin, Sitosterol, cianidanol and Quercetin were predicted as best compounds with

binding affinity values -7.5, -7.4, -7.3, -7.1, -7.1, -7.2 and -7.3 Kcal/mol respectively by forming stable hydrogen bonds.(Fig 1)

Vitamin D3 Receptor

Vitamin D3 receptor chains A and B acts as a main target for urolithiasis and the 3D structure of 1KB2 target was retrieved from PDB. Compounds such as catechin gallate, Quercetin, Orthosiphon_A, neoorthosiphol with least binding affinity were predicted such as -8.1, -7.5, -7.4 and -7.2Kcal/mol respectively. (Fig 1)

Hyaluronan synthase

For the given target 1POZ phytochemical compounds such as Orthosiphol A, Rutin, Orthosiphol B, Masilinic acid, hyperin, eupatorin were found to interact with good binding scores of -6.6,-6.8,-6.4,-6.5,-6.9 and –6.8 Kcal/mol respectively.(Fig 1)

CD44 ANTIGEN

The crystal structure of CD 44 Antigen was retrieved from PDBand phytochemical compounds such as Orthosiphol X, Rutin, Oleanolic acid, Beta amyrone, hyperin, rosmarinic acid, betulinic acid and Orthosiphol Mwere found to interact well with the target protein with binding scores of -8.5,-7.9,-7.8,-7.7,-7.7, -7.8,-7.9 and -7.6Kcal/mol respectively.(Fig 1)

SERUM ALBUMIN

For the target 5FUO phytochemical compounds such as (-)-Catechin gallate, betulinic acid, Orthosiphol A, neoorthosiphol A interacted well with binding scores of -10.5,-10.4,-10.1 and - 8.9 Kcal/mol respectively.(Fig 1)

Claudin 19

The structure of the proteinClaudin 19 was modeled using swissmodel. Thephytochemicals such as Orthosiphol A, Betulinic acid, Stigmasterol, , Orthosiphol N and staminol A shows interaction with binding affinity of -9.8,-9.3,-9.2, -9.1 and -8.9 Kcal/mol respectively.(Fig 1)

CONCLUSION

As a result, phytochemical compounds of *Orthosiphon stamineus* have better binding affinity towards the targets like 1BT6, 1IQT,1KB2, 1POZ, 1UUH, 5FUO, and Claudin 19.In most of the target proteins the compounds such as OrthosipholX, Rutin, (-)-Catechin gallateand Masilinic acid were found to interact with good binding affinity and forming more number of hydrogen bonds. While considering

the side effects of the synthetic drugs, it was obvious that there is still a greater demand for the safer and effective therapy for treating Urolithiasis. In the current study, the ADME profiling of the phytochemical compounds from *Orthosiphon stamineus*ensured its drug- likeliness behavior besides its interaction with the target proteins of Urolithiasis.The results suggested the possibility of employing these compounds as the alternative source of Urolithiasis. Thus, the present preliminary study may direct the research attention on the phytochemical compounds for treating Urolithiasis.

Table1: List of compounds identified from *Orthosiphon stamineus*

S.No	Compound ID	Compound name	Molecular formula	Molecular weight(g/mol)
1.	240	Benzaldehyde	C_7H_6O	106.12
2.	370	Galic acid	$C_7H_6O_5$	170.12
3.	892	Inositol	$C_6H_{12}O_6$	180.16
4.	931	Naphthalene	$C_{10}H_8$	128.169
5.	998	Phenylacetaldehyde	C_8H_8O	120.15
6.	1175	Uric acid	$C_5H_4N_4O_3$	168.11

7.	2153	Theophylline(xanthine)	$C_7H_8N_4O_2$	180.16
8.	2519	Caffeine	$C_8H_{10}N_4O_2$	194.19
9.	2537	Camphor	$C_{10}H_{16}O$	152.23
10.	2758	Cineole	$C_{10}H_{18}O$	154.25
11.	3314	Eugenol(caryophyllic acid)	$C_{10}H_{12}O_2$	164.2
12.	5429	Dimethylxanthine	$C_7H_8N_4O_2$	180.16
13.	6184	Hexanal	$C_6H_{12}O$	100.16
14.	6448	Isobornyl acetate	$C_{12}H_{20}O_2$	196.29
15.	6616	Cophene	$C_{10}H_{16}$	136.23
16.	6654	Alpha pinene	$C_{10}H_{16}$	136.23
17.	6949	Beta guaiene	$C_{15}H_{24}$	204.35
18.	6986	Isomethone	$C_{10}H_{18}O$	154.25
19.	7055	2- methylnaphthalene	$C_{11}H_{10}$	142.2
20.	7127	Methyleugenol	$C_{11}H_{14}O_2$	178.23
21.	7439	Carvone	$C_{10}H_{14}O$	150.22
22.	7463	Para -cymene	$C_{10}H_{14}$	134.22
23.	8175	Decanal	$C_{10}H_{20}O$	156.26
24.	8182	Dodecane	$C_{12}H_{26}$	170.33
25.	8842	Citronellol	$C_{10}H_{20}O$	156.26
26.	8815	Estragole	$C_{10}H_{12}O$	148.2
27.	9064	Cianidanol	$C_{15}H_{14}O_6$	290.27
28.	9895	Beta-cyclocitral	$C_{10}H_{16}O$	152.23
29.	10123	Alpha-selinene	$C_{15}H_{24}$	204.35

30.	10494	Oleanolic acid	$C_{30}H_{48}O_3$	456.7
31.	11527	Octanal-3-ol	$C_8H_{18}O$	130.229
32.	12388	Tridecane	$C_{13}H_{28}$	184.36
33.	14257	Hendecane	$C_{11}H_{24}$	156.31
34.	14896	Beta-pinene	$C_{10}H_{16}$	136.23
35.	14985	5,7,8-trimethyltocol	$C_{29}H_{50}O_2$	430.7
36.	19602	2-pentylfuran	$C_9H_{14}O$	138.21
37.	19725	Alpha-copaene	$C_{15}H_{24}$	204.35
38.	22311	Limonene	$C_{10}H_{16}$	136.23
39.	26447	Menthone	$C_{10}H_{18}O$	154.25
40.	61041	Safranal	$C_{10}H_{14}O$	150.22
41.	62127	Caryophylle acetate	$C_{17}H_{28}O_2$	264.4
42.	64685	Borneol	$C_{10}H_{18}O$	154.25
43.	64971	Betulinic acid	$C_{30}H_{48}O_3$	456.7
44.	68316	Perillene	$C_{10}H_{14}O$	150.22
45.	73170	Alpha amyrin	$C_{30}H_{50}O$	426.7
46.	73659	Masilinic acid	$C_{30}H_{48}O_4$	472.7
47.	75324	Tetrametyluric acid	$C_9H_{12}N_4O_3$	224.22
48.	79730	4,5,7-trimethoxyflavone	$C_{18}H_{16}O_5$	312.3
49.	81722	Alpha,alpha,-dimethyl-4-methyl enecyclohexanemethanol	$C_{10}H_{18}O$	154.25
50.	86609	Alpha cubenene	$C_{15}H_{24}$	204.35
51.	87529	Beta cubenene	$C_{15}H_{24}$	204.35

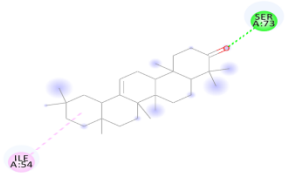
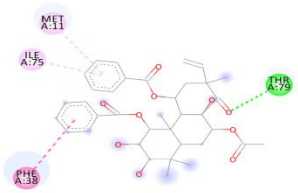
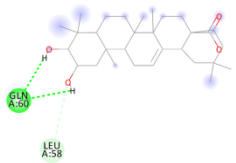
52.	96118	Tetramethyl-o-scutellarin	$C_{19}H_{18}O_6$	342.3
53.	97214	Eupatorin	$C_{18}H_{16}O_7$	344.3
54.	145659	Sinensetin	$C_{20}H_{20}O_7$	372.4
55.	157983	2-pent-1-enylfuran	$C_9H_{12}O$	136.19
56.	161271	Salvigenin	$C_{18}H_{16}O_6$	328.3
57.	177148	1-(7,8-dimethoxy-2,2-dimethyl-chromen-6-yl)ethanone	$C_{15}H_{18}O_4$	262.3
58.	188323	Crisimaritin	$C_{17}H_{14}O_6$	314.29
59.	222284	Beta- sitosterol	$C_{29}H_{50}O$	414.7
60.	292285	Octadecane	$C_{26}H_{54}$	366.7
61.	324224	Beta-bourbonene	$C_{15}H_{24}$	204.35
62.	439378	L-theanine	$C_7H_{14}N_2O_3$	174.2
63.	441005	(+)-delta-cadinene	$C_{15}H_{24}$	204.35
64.	445858	Ferulic acid	$C_{10}H_{10}O_4$	194.18
65.	519382	Dihydro-beta-inone	$C_{13}H_{22}O$	194.31
66.	543423	1,2-15,16-diepoxyhexadecane	$C_{16}H_{30}O_2$	254.41
67.	584957	1,3,3-trimethyl-2-vinyl-1-cyclohexane	$C_{11}H_{18}$	150.26
68.	608753	Beta-vatrienene	$C_{15}H_{22}$	202.33
69.	631170	Luteolin tetramethyl ether	$C_{19}H_{18}O_6$	342.3
70.	637182	Orthosiphol v	$C_{33}H_{42}O_{11}$	614.7
71.	637563	Anethole	$C_{10}H_{12}O$	148.2
72.	638014	Beta-ionone	$C_{13}H_{20}O$	192.3
73.	638072	Squalene	$C_{30}H_{50}$	410.7

74.	689043	Caffeic acid	$C_9H_8O_4$	180.16
75.	1163236	2-(3,4-dimethoxyphenyl)-5,7-di(propan-2-yl)-1,3-diazatricyclo[3.3.1.13,7]decan-6-one	$C_{22}H_{32}N_2O_3$	372.5
76.	1549778	Geranylacetone	$C_{13}H_{22}O$	194.31
77.	1742210	Caryophyllene oxide	$C_{15}H_{24}O$	220.35
78.	2724856	Acetophenone	C_8H_8O	128.199
79.	3084066	Ladanein	$C_{17}H_{14}O_6$	314.29
80.	5280343	Quercetin	$C_{15}H_{10}O_7$	302.23
81.	5280794	Stigmasterol	$C_{29}H_{48}O$	412.7
82.	5280934	Linolenic acid	$C_{18}H_{30}O_2$	278.4
83.	5281167	Cis-3-hexen-1-ol	$C_6H_{12}O$	100.16
84.	5281515	Beta-caryophyllene	$C_{15}H_{24}$	204.35
85.	5281520	Humulene	$C_{15}H_{24}$	204.35
86.	5281764	Chicoric acid	$C_{22}H_{18}O_{12}$	474.4
87.	5281792	Rosmarinic acid	$C_{18}H_{16}O_8$	360.3
88.	5283316	2-heptenal	$C_7H_{12}O$	112.17
89.	5283318	4-heptenal	$C_7H_{12}O$	112.17
90.	5317570	Germacrene D	$C_{15}H_{24}$	204.35
91.	5320945	Rhamnazin	$C_{17}H_{14}O_7$	330.29
92.	5322111	Cis-caryophyllene	$C_{15}H_{24}$	204.35
93.	5364462	Z,E-213-octadecadien-1-ol	$C_{18}H_{34}O$	266.5

94.	5364768	E,E,Z-1,3,12-nondecatriene-5,14-diol	$C_{19}H_{34}O_2$	294.5
95.	5366074	Damascenone	$C_{13}H_{18}O$	190.28
96.	5367460	Ethyl linolenate	$C_{20}H_{34}O_2$	306.5
97.	5378597	Hyperin	$C_{21}H_{20}O_{12}$	464.4
98.	6419835	(-)-catechin gallate	$C_{22}H_{18}O_{10}$	442.4
99.	6428573	2-[(2R,5S)-5-Ethenyl-5-Methyloxolan-2-Yl]Propan-2-Ol;	$C_{10}H_{18}O_2$	170.25
100.	6429376	4,5-di-epi-aristolochene	$C_{15}H_{24}$	204.35
101.	6432254	Trans-linalool oxide	$C_{10}H_{18}O_2$	170.25
102.	6432312	Gamma-elemene	$C_{15}H_{24}$	204.35
103.	6452096	Ethyl cholate	$C_{26}H_{44}O_5$	436.6
104.	6918391	Beta elemene	$C_{15}H_{24}$	204.35
105.	9548705	Geracrene A	$C_{15}H_{24}$	204.35
106.	10030927	Orthosiphol M	$C_{31}H_{38}O_{10}$	570.6
107.	10054823	Orthosiphol X	$C_{36}H_{42}O_{10}$	634.7
108.	10054824	Orthosiphol K	$C_{36}H_{42}O_{10}$	634.7
109.	10055285	Orthosiphonone B	$C_{38}H_{42}O_{11}$	674.7
110.	10055454	Neoorthosiphol A	$C_{38}H_{44}O_{12}$	692.7
111.	10101176	Orthosiphol l	$C_{38}H_{44}O_{12}$	692.7
112.	10258499	Orthosiphol N	$C_{36}H_{40}O_{10}$	632.7
113.	10439492	Orthosiphonone A	$C_{38}H_{42}O_{11}$	674.7
114.	10951947	Orthosiphol V	$C_{31}H_{40}O_{10}$	572.6
115.	11017910	Siphonol B	$C_{38}H_{44}O_{12}$	692.7

116.	11071897	Orthosiphol W	$C_{31}H_{40}O_{10}$	572.6
117.	11090752	Orthosiphol Z	$C_{22}H_{28}O_{7}$	404.5
118.	11115145	Siphonol C	$C_{38}H_{44}O_{12}$	692.7
119.	11144807	Orthosiphol Y	$C_{24}H_{32}O_{8}$	448.5
120.	11802781	Staminol B	$C_{38}H_{44}O_{12}$	692.7
121.	12309449	Delta-elemene	$C_{15}H_{24}$	204.35
122.	15385858	Orthosiphol A	$C_{38}H_{44}O_{11}$	676.7
123.	15385859	Orthosiphol B	$C_{38}H_{44}O_{11}$	676.7
124.	44246135	Isonicotinoylhydrazone of trans-2-hexanal	$C_{12}H_{15}N_{3O}$	217.27
125.	100974770	Neoorthosiphol B	$C_{38}H_{44}O_{12}$	692.7
126.	19725	Alpha-copaene	$C_{15}H_{24}$	204.35
127.	101616676	Beta-amyrone	$C_{29}H_{46}O$	410.7
128.	247573	Isobornyl acetate	$C_{12}H_{20}O_{2}$	196.29
129.	100965638	Orthosiphol H	$C_{45}H_{48}O_{12}$	780.9
130.	10952715	Siphonol A	$C_{40}H_{46}O_{13}$	734.8
131.	10963687	Siphonol E	$C_{39}H_{44}O_{14}$	736.8
132.	10628761	Staminol A	$C_{40}H_{46}O_{13}$	734.8
133.	5320496	Pilloin	$C_{17}H_{14}O_{6}$	314.29
134.	585744	Tetracyclo[6.3.2.0(2,5).0(1,8)]Tridecan-9-Ol, 4,4-Dimethyl-; CTK7J9655	$C_{15}H_{24}O$	220.35
135.	5280805	Rutin	$C_{27}H_{30}O_{16}$	610.5
136.	6427080	2-octenal	$C_{8}H_{14}O$	126.2

Figure 1: Best docked compounds of *Orthosiphon stamineus*with different target proteins targeting Urolithiasis

1bt6_beta-amyrone	1bt6_orthosiphol X	1bt6_masilinic acid
		
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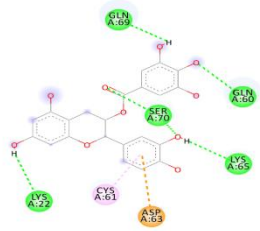	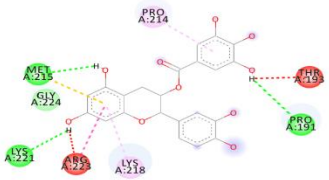	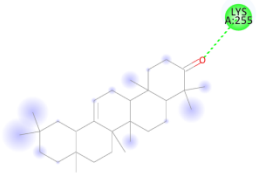
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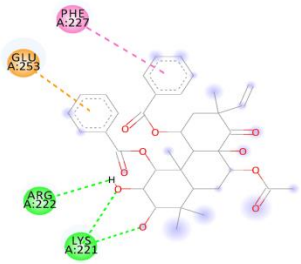	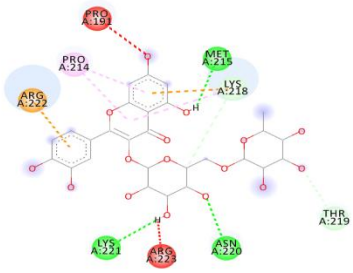	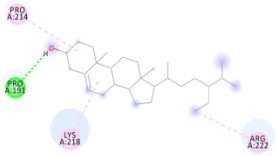
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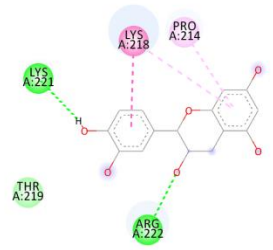	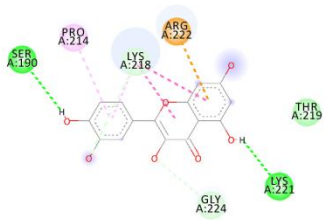	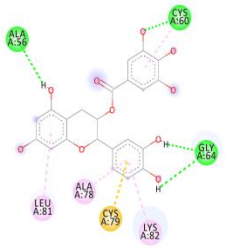
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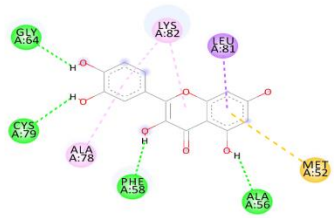	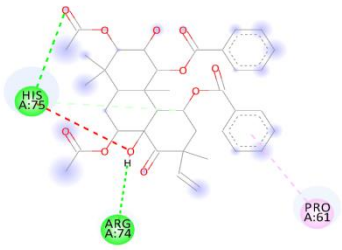	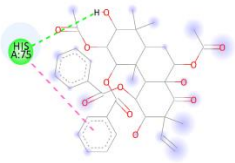
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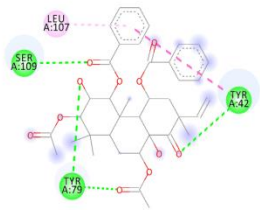	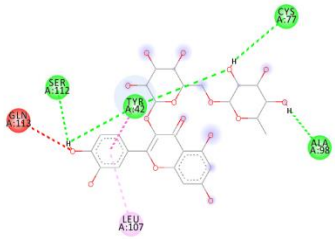	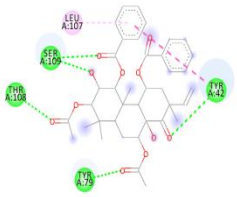
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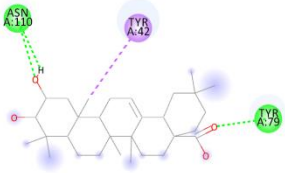	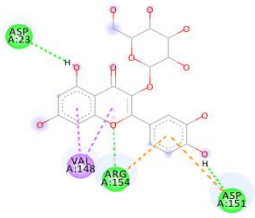	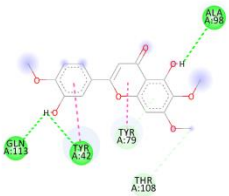

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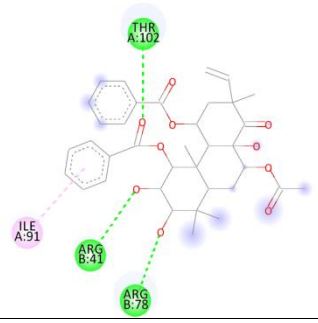	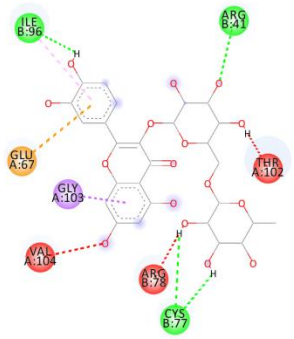	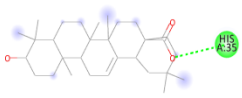
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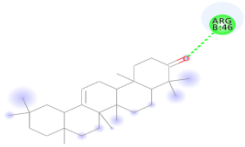	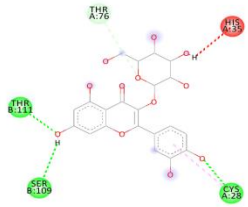	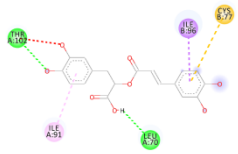
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	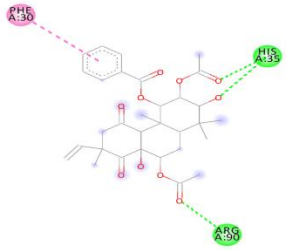	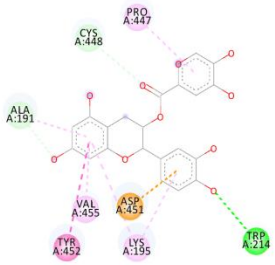
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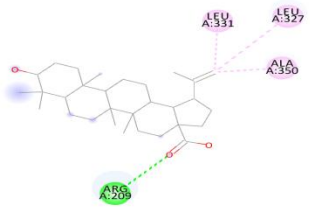	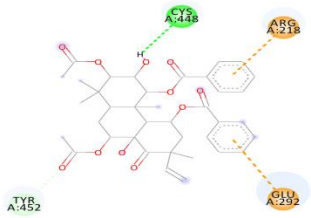	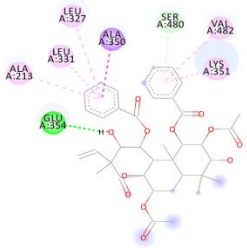
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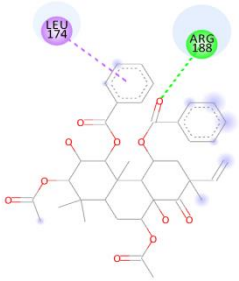	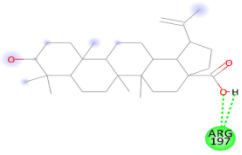	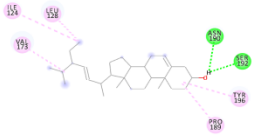
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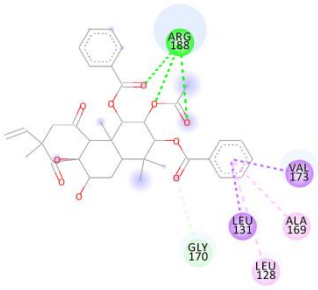	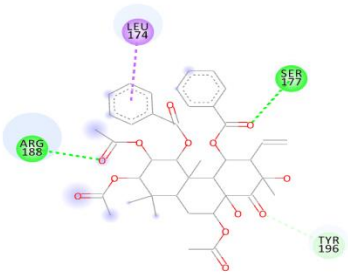	

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflicts of interest in the publication of this content

Author contributions

Designed the project – Bharathi N,Performed the experiments – Bharathi.N &Caroline Nirmala.R , Drafted the manuscript – Bharathi.N &Caroline Nirmala.R. Both the authors discussed the result and reviewed the manuscript.

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