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ORIGINAL PAPER

Virtual screening and in vitro assay of potential drug like inhibitors from spices against glutathione-S-transferase of filarial nematodes

Shamina Azeez · Rosana O. Babu · Riju Aykkal · Reena Narayanan

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Abstract Glutathione-S-transferase(s) (GST) enzyme from *Brugia malayi* has been exploited as a target in lymphatic filariasis therapeutics. An active GST is a homodimer of a 208 residue long monomer consisting of two domains, a smaller α/β domain and a larger α domain. The components of the glutathione (GSH) system, mainly GST enzymes, are critical antioxidant and detoxification system responsible for the long-term existence of filarial worms in mammalian host; hence they are major chemotherapeutic targets in filarial species. In the present study, 58 phytochemicals from 10 plants, predicted and reported to have potential nematicidal activity and ADMET satisfaction, have been docked to GST enzyme of *B. malayi* to assess their binding affinity and consequently their inhibitory activity. A comparative study has been made with commonly employed chemotherapeutic GST inhibitors such as cibacron-blue, butylated hydroxyanisole, hexyl glutathione and ethacrynic acid. In vitro effects of potential drug like compound from *in silico* results have been done for validation of docking studies. In vitro assay revealed efficacy in GST inhibition in the following compounds: linalool (97.50%), *alpha*-pinene (90.00%), strychnine (87.49%), vanillin (84.99%), piperine (79.99%), isoeugenol (62.49%), curcumin (57.49%), *beta*-caryophyllene (39.50%), cinnamic acid (27.49%), capsaicin (19.99%), citronellol (19.99%) and geraniol (17.49%). An online database (www.spicebioinfo.res.in/gstleadbase) has been developed, which will serve as a useful repository of information on GST inhibitors for future development of drugs against filarial nematodes. These findings thus suggest that the above phytochemicals could be potentially developed as lead molecules for targeting GST of lymphatic filarial parasites.

Keywords Brugia malayi · Dirofilaria immitis · Docking · Glutathione S-transferase(s) · Phytochemicals

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Introduction

Lymphatic filariasis (LF) is a mosquito-borne tropical disease caused by the nematode parasites *Wuchereria bancrofti, Brugia malayi* and *B. timori* [1]. It is the major cause of acute and chronic morbidity in 81 countries in Asia-Pacific, Africa and the Americas. Approximately 1.3 billion people living in these regions are at risk of infection [2]. The adult parasites live 5 to 10 years, of which the fecund life span is 4 to 6 years. Several hundreds to thousands of infective mosquito bites are necessary to establish infection. Of these, three parasites *W. bancrofti* accounts for nearly 90% of LF infections worldwide. *B. malayi* is prevalent only in some parts of South and Southeast Asia, and *B. timori* is found only in Indonesia. The drugs used for treating LF include annual doses of diethylcarbamazine (DEC), DEC plus albendazole, or ivermectin plus albendazole; none of these is effective in killing adult worms, and treatments are therefore aimed at reducing transmission and pathology [3, 4]. Since an effective treatment for filarial adult worms is currently unavailable, new chemical classes of compounds with macrofilaricidal activities are now required [5]. Recently Srinivasan et al. [6] reported ethacrynic acid, plumbagin and curcumin as inhibitory compounds against GSTs of bovine filarial worms *Setaria digitata.* Only a few studies have reported the use of phytochemicals as GST inhibitors.

The components of the glutathione (GSH) system GSTs (glutathione-S-transferase(s)) and GSHPx (glutathione peroxidases) are the major defense systems present in filarial nematodes. The role of this secreted enzyme is the inhibition of the oxidative burst of leukocytes and neutralization of secondary products of lipid peroxidation, thus providing an explanation for the resistance of these parasites to immune effector mechanisms and their persistence in the mammalian host [7]. The mechanism of action of GST(s) (E.C.2.5.1.18), a large family of multifunctional dimeric enzymes includes defense against oxidative attack via conjugation of electrophiles to glutathione and reduction of lipid hydroperoxides [8]. Due to their primary role in drug metabolism, GSTs have been the recent focus of research as a potential drug target for anti-schistosomal [9], antimalarial [10, 11], and antifilarial [12–14] drug development. In addition to their isomerization and GSH conjugation activities, in mammals these enzymes contribute to defense against oxidative stress, by virtue of both their selenium-independent GSH peroxidase activities [15]. Inhibition of parasitic GST affects the survival of the parasites or helps in the enhancement of activity of presently available antifilarial drugs [16]. GST from human filarial parasites is significantly different from human GST in sequence and structure [17]. Hence *B. malayi* GST was exploited to design new target based chemotherapeutic agents.

An active GST is a homodimer of a 208 residue long monomer consisting of two domains (smaller α/β domain and larger α domain) (Fig. 1). The N-terminal small domain (residues 1 to 74) is an α/β structure with the folding topology βαβαββα arranged in the order β2, β1, β3 and β4 with β3 anti-parallel to the others, forming a regular β-sheet with a right-handed twist surrounded by three α-helices. The C terminal, large domain 2 (82–208 residues) is α-helical. The residues that interface the two βαβ and ββα motifs are Trp38, Phe8, Val33, Cys47, Leu52 and Leu43 in human π GST. In Bm-GST the residues Val33, Cys47 and Leu43 are replaced by Ile 38, Phe47 and Met43 [17]. The secondary structure of *B. malayi* GST has been generated by GenTHREADER – Protein fold recognition software (http://bioinf.cs.ucl.ac.uk/threader/) [18].

A wide range of chemical compounds including alkaloids, coumarins, flavonoids, benzofurans, terpenoids and steroids have been isolated from various plant extracts and these have been found to possess various pharmacological, nematicidal and insecticidal activities. A comprehensive review of chemical constituents and pharmacological profiles of 10 selected medicinal plants, including spices, have led to the identification of potential nematicides. These new nematicides of natural origin may lead to higher safety and efficiency in nematode control and nematicidal drug development.

Historically, herbs, shrubs and spices have enjoyed a rich tradition of use for their flavor enhancement characteristics and medicinal properties [19, 20]. Spices hold the promise of providing both significant clinical benefits and key insights into the pathophysiology of cancer, arthritis, inflammation, respiratory disorders, gastrointestinal disturbances, allergy and microbial infections. Numerous demonstrations of preclinical efficacy of turmeric and various spices in animal models for preventing cancer and cardiovascular disorders have been reported [21]. This study was conducted with the objective of exploring the nematicidal activity of herbs and spices, with special reference to its potential to inhibit GST activity.

Materials and methods

Database screening and activity prediction

Initially, a review of plants with nematicidal property was made, which helped us identify 10 spices and medicinal plants: coriander, cassia, turmeric, allspice, cinnamon, strychnous, lemongrass, garlic, litsea and vanilla. The chemical compounds from these plants were collected through literature search and from Dr. Duke's phytochemical and ethno-botanical databases (http://ars-grin.gov/duke/). The screening results revealed the presence of 128 nematicidal phytochemicals in these plants. The PASS server [22] was used to predict nematicidal activity and GST substrate activity of the phytochemicals (http://195.178.207.233/PASS/AP.html); PreADMET server (http://preadmet.bmcrd.org/) was used to predict the drug-likeness and ADME-Tox (Absorption, Distribution, Metabolism Excretion and Toxicity) properties [23]. The ADME-Tox properties of a compound together with its pharmacological properties such as drug likeness are conventionally a part of drug development. The compounds obeying the ADMET rules and drug likeness rules were short listed for docking studies.

Ligand structure

The canonical smiles notations of phytochemicals were collected from PubChem (http://pubchem.ncbi.nlm.nih.gov/), ChemSpider (http://chemspider.com) and DrugBank (http://www.drugbank.ca/). The 3D structures of compounds were developed by 3D Structure Generator CORINA [24, 25] using canonical smiles of the compound. Energy minimization and molecular optimization of all compounds were done using Arguslab 4.0.1 [26]. Geometry optimization was carried out using AM1 (Austin Model 1), semi-empirical quantum mechanics force field in Arguslab4.0.1. The best conformer thus obtained was based on energy minimization

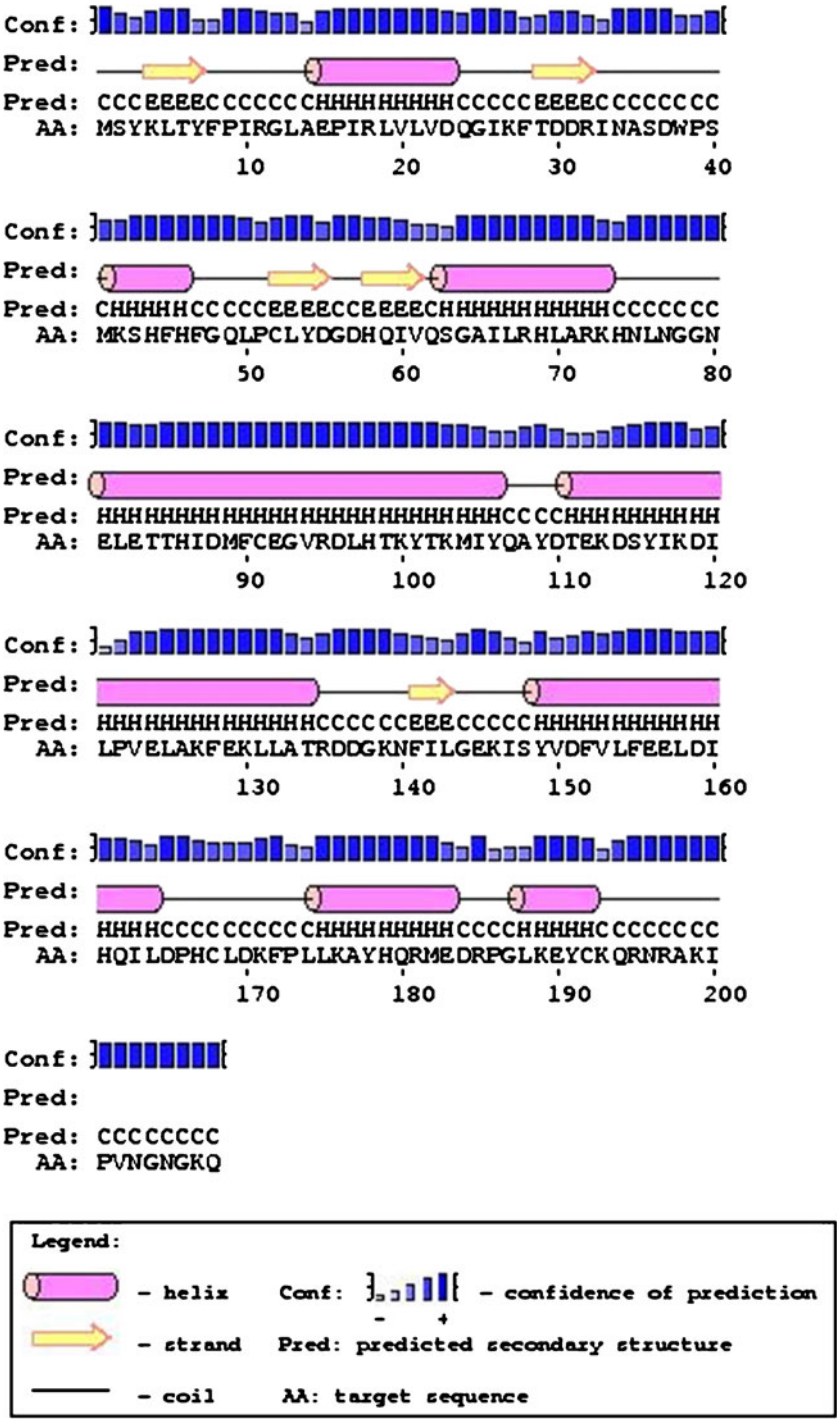


Fig. 1 Secondary structure of *Brugia malayi* GST: Generated by GenTHREADER – Protein fold recognition software (http://bioinf.cs.ucl.ac.uk/threader/) [18]

and geometry optimization. The final structures exhibiting lowest energy were saved in *.pdb format for input in to MVD environment.

Target protein structure

Theoretically solved structure of *Brugia malayi* glutathione-S-transferase was selected as the target for docking study, since to date there are no experimentally solved structures for *Wuchereria bancrofti, Brugia malayi* or *B. timori*. The GST structure was downloaded from Protein Data Bank (PDB id - 1SJO) and the structure optimized using Swiss PDB viewer software. Three active sites were detected in the GST enzyme of *B. malayi* using Molegro Virtual Docker. Active site residues of the GST enzyme were predicted using WHATIF server (http://swift.cmbi.ru.nl/servers/html/index.html) [27]. Active site residues predicted by WHATIF were Tyr7, Tyr101, Tyr106,

Table 1 Characteristics of phytochemicals docked with GST of *B. malayi*

Serial number	Ligand	Moldock score (kJ/mol)	H-bond interaction energy (kJ/mol)	Number of H- bonds
1.	1,8-Cineole	−51.9902	−0.948	1
2.	2-Furfuraldehyde	−64.8983	−4.449	3
3.	2-Methoxycinnamaldehyde	−64.8564	−2.953	3
4.	Acetyl-eugenol	−82.2233	−2.196	4
5.	Alpha-copaene	−53.9326	0	0
6.	Alpha-humulene	−68.188	0	0
7.	Alpha-pinene	−75.5588	−2.772	3
8.	Alpha-terpinene	−58.5699	0	0
9.	Alpha-terpineol	−59.8115	−2.688	2
10.	Benzaldehyde	−58.3635	−2.5	2
11.	Brucine	−91.7289	−4.112	6
12.	Brucine-n-oxide	−95.1105	−4.2539	6
13.	Capsaicin	−58.5662	−2.4545	3
14.	Carvacrol	−63.5588	−2.772	3
15.	Cinnamaldehyde	−66.6676	−2.5	2
16.	Cinnamic acid	−66.1044	−1.657	1
17.	Cinnamyl acetate	−71.9436	−1.588	3
18.	Cinnamyl alcohol	−62.8854	−2.326	2
19.	Cis asarone	−66.448	−1.465	3
20.	Citral	−67.7443	−2.141	2
21.	Citronellal	−70.7249	−2.231	1
22.	Citronellol	−73.133	−2.5	2
23.	Curcumin	−137.66	−8.4343	7
24.	Decanal	−71.6631	−2.5	1
25.	Diaboline	−67.3305	−0.783	2
26.	Diallyl disulfide	−59.5614	0	0
27.	Diallyl trisulfide	−61.3798	0	0
28.	Diallylsulfide	−56.062	0	0
29.	Dodecanal	−81.4478	−2.5	1
30.	Eugenol	−76.089	−5.745	3
31.	Genostrychnine	−86.3784	−2.000	4
32.	Geraniol	−69.8422	−2.5	2
33.	Icajine	−83.174	−3.109	4
34.	Isoeugenol	−75.9879	−5.557	3
35.	Isopulegone	−57.0261	−1.219	1
36.	Limonene	−58.8357	0	0
37.	Linalool	−80.895	−2.240	2
38.	Methyl-eugenol	−70.2178	−1.423	2
39.	Methyl-isoeugenol	−66.8614	−1.522	3
40.	Myristicin	−69.8614	−1.7384	2
41.	Neral	−72.4322	0	1
42.	Nonanal	−65.4356	0	1
43.	NVA	−78.8236	−5.557	3
44.	Octanal	−62.3393	0	0
45.	p-cymene	−58.6187	0	0
46.	Piperine	−79.985	−3.139	4
47.	pseudo strychnine	−78.8246	−1.350	4
48.	Strychnine	−84.0994	−1.436	3
49.	trans- 2-decan-1-ol	−75.983	−2.5	2

Table 1 (continued)

Serial number	Ligand	Moldock score (kJ/mol)	H-bond interaction energy (kJ/mol)	Number of H- bonds
50.	trans-anethole	−64.2715	−1.221	2
51.	Turmerone	−79.985	−1.653	2
52.	Undecanal	−79.5591	−2.5	1
53.	Vanillin	−89.5321	−7.0792	5
54.	Verbenol	−53.0257	−2.357	2
55.	Vomicine	−79.4487	−5.469	6
56.	Zingiberene	−74.8799	0	0
57.	β-caryophyllene	−51.5322	0	0
58.	β-colubrine	−91.7316	−1.247	4

Phe8, Phe45, Phe47, Phe155, Pro9, Pro51, Pro201, Ile10, Ile33, Ile105, Ile200, Arg11, Arg32, Arg95, Gly12, Gly48, Gly64, Leu13, Leu50, Asn34, Asn203, Ala35, Try38, Lys42, Lys103, Gln49, Gln62, Ser63, His98, Thr99, Thr102, Asp159, Val61, and Val202. Docking was carried out using single active-site having large volume (79.608 $Å^3$) among the three cavities. This cavity was chosen since it binds glutathione with the highest specificity compared to the other sites, when docked with the whole protein using MVD. Amino acid residues present in the active site selected for docking and grid generation were Gln49, Gln12, Ile10, Ile105, Gly12, Ser63, His98, Tyr7, Tyr101, Tyr106, Pro9, Pro51, Pro201, Arg11, Arg32, Arg95, Thr99, Thr102, Val61, Val202, Lys42 and Lys103.

Molecular docking

Molecular docking study was carried out by using Molegro Virtual Docker [28]. The entire protein structure was loaded on to MVD platform for docking process. MVD performs flexible ligand docking, so the optimal geometry of the ligand is determined during the docking. MVD includes MolDock Score [28] and PLANTS Score [29] for evaluating docking solutions. MVD returns multiple poses representing different potential binding modes. This can be useful when the best-scoring (i.e., lowest-energy) pose does not represent the native binding mode or when multiple binding modes exist. Here clustering has been used to reduce the number of poses found during the docking run and only the most promising ones are reported. Compounds with the lowest dock score and high interaction with active-site was taken for in vitro studies based on the availability of the compound.

In vitro GST assay

The phytochemicals β-caryophyllene, capsaicin, cinnamic acid, citronellol, curcumin, eugenol, geraniol, isoeugenol, linalool, myristicin, neral, α-pinene, piperine, terpineol, vanillin and strychnine were purchased in the pure form from Sigma Chemicals, USA; glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) were purchased from Sisco Research Laboratories Pvt. Ltd., (Mumbai, India). *Dirofilaria immitis* microfilaria, the canine filarial nematode used for in vitro study, was obtained from the District Veterinary Centre Campus, Calicut, Kerala.

GST crude enzyme was obtained by centrifuging the serum containing ~2000 filarial nematodes at 1000 rpm for 2 min, and washing twice with phosphate buffered saline (PBS) at pH 7.4. The nematodes were ground with micro pestle and glass powder. The solution was centrifuged at 10000 rpm at 4 °C for 30 min. Supernatant was dialyzed against PBS overnight and made up to 2 ml. The following phytochemicals were used to study their GST inhibitory activity, at a concentration of 0.001 mg ml^{-1} in ethanol: β-caryophyllene, capsaicin, cinnamic acid, citronellol, curcumin, eugenol, geraniol,

Table 2 Binding energy scores of GST-inhibitors

Serial number	GST-inhibitor	Dock score (kJ/mol)	H-bond interaction energy (kJ/mol)	Number of H-bonds
1.	Cibacron-blue	−129.656	−1.561	5
2.	Hexyl glutathione	−113.777	−6.320	10
3.	Ethacrynic acid	−87.569	−2.424	5
4.	Butylated hydroxyanisole	−68.431	−3.640	4

isoeugenol, linalool, myristicin, neral, α-pinene, piperine, terpineol, vanillin and strychnine (dissolved in water).

The dialyzed enzyme fraction (0.1 ml) was incubated in the presence of 1 ml of 0.001 mg ml^{-1} concentration of the phytochemicals listed above, in the presence of 1 mM glutathione reduced (GSH), and 0.1 M phosphate buffer, pH 6.5, for 1 hour at room temperature. A control containing ethanol was also maintained. GST activity was measured using the method of Habig et al. [30], by initiating the reaction with the addition of 1 mM 1-chloro-2,4-dinitroben-

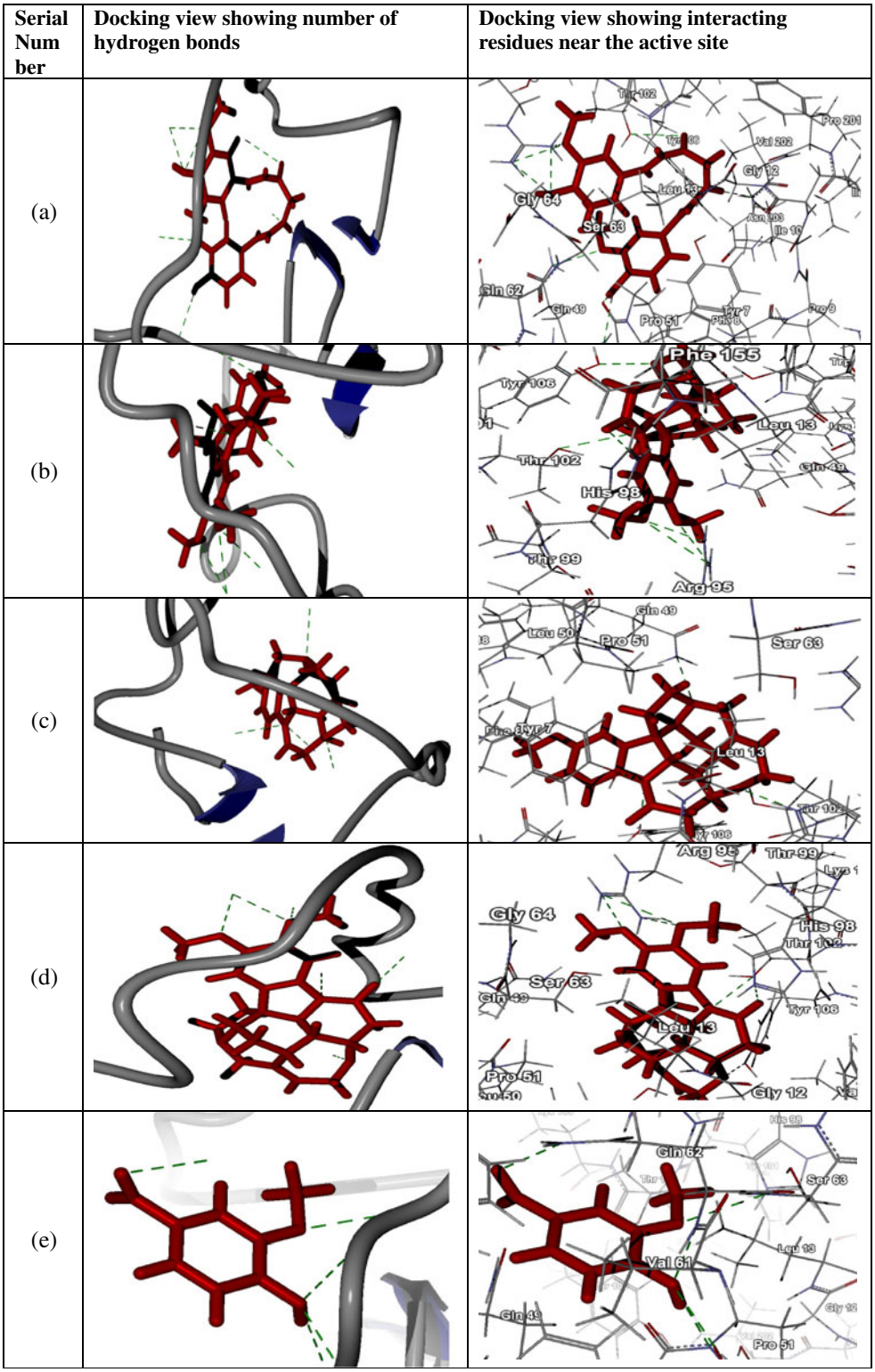
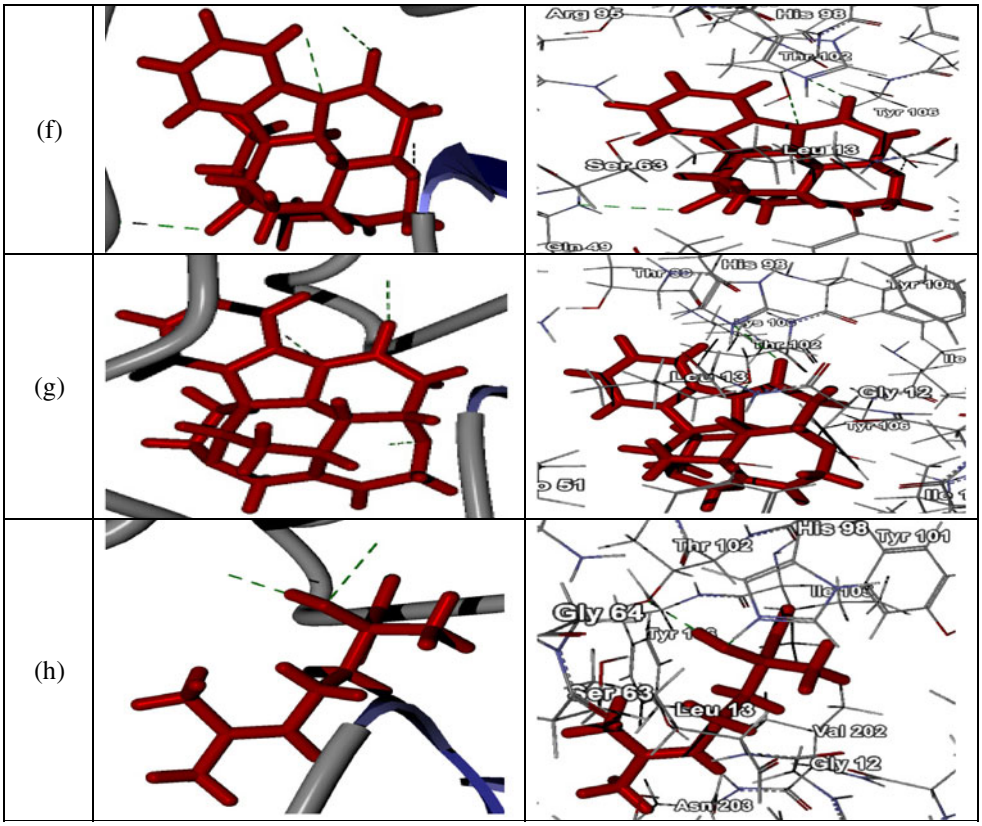


Fig. 2 Docking view showing Hydrogen bond interaction of ligands with residues in active site of GST enzyme. (**a**) curcumin, (**b**) brucine-n-oxide,(**c**) *beta*-colubrine, (**d**) brucine, (**e**) genostrychnine, (**f**) strychnine, (**g**) vanillin and (**h**) linalool

Fig. 2 (continued)



zene (CDNB) and following the change in absorbance at 340 nm, in a Shimadzu 1601 UV-Visible spectrophotometer. The GST activity was expressed as change in absorbance at 340 nm per minute per ml crude enzyme extract. Two replicates of each treatment were maintained.

Results and discussion

Biological activity prediction yielded 58 of the 128 phytochemicals with nematicidal, anti-helmintic and GST substrate activities. These phytochemicals also satisfied both ADME-Tox and drug likeness rules and were selected for docking studies. Docking results showed that all 58 compounds docked satisfactorily to the GST enzyme active site with good docking scores of less than −51.532 kcal mol^{-1}. Hence these phytochemicals of comparatively less docking energy and greater number of hydrogen bond interactions were selected as promising lead compounds after docking studies (Table 1).

An *in silico* study was performed to compare the binding affinity of commonly employed chemotherapeutic GST

Table 3 Binding energy scores of eight phytochemicals and interacting residues

Serial number	Phytochemical	Dock score (kJ/mol)	H bond interaction energy (kJ/mol)	Common interacting residues	Number of H bonds
1.	Curcumin	−137.66	−8.4343	Gln62, Gln64, Pro51, Val202, Tyr106, Pro201, Tyr7	7
2.	Brucine N oxide	−95.1105	−4.2539	Thr102, His98, Arg95 (3 H bonds), Tyr106	6
3.	beta-Colubrine	−91.7316	−1.247	Tyr7, Gln49, Thr102, Tyr106	4
4.	Brucine	−91.7289	−4.112	Arg95 (2 H bonds), Thr102 (2 H bonds), Tyr106, His98	6
5.	Vanillin	−89.5321	−7.0792	Gln62 (2 H bonds), Ser63, Pro51(2 H bonds)	5
6.	Genostrychnine	−86.3784	−2.000	Gln49, His98, Thr102, Tyr106	4
7.	Strychnine	−84.0994	−1.436	Thr102, Tyr106, His98	3
8.	Linalool	−80.895	−2.240	His98, Thr102	2

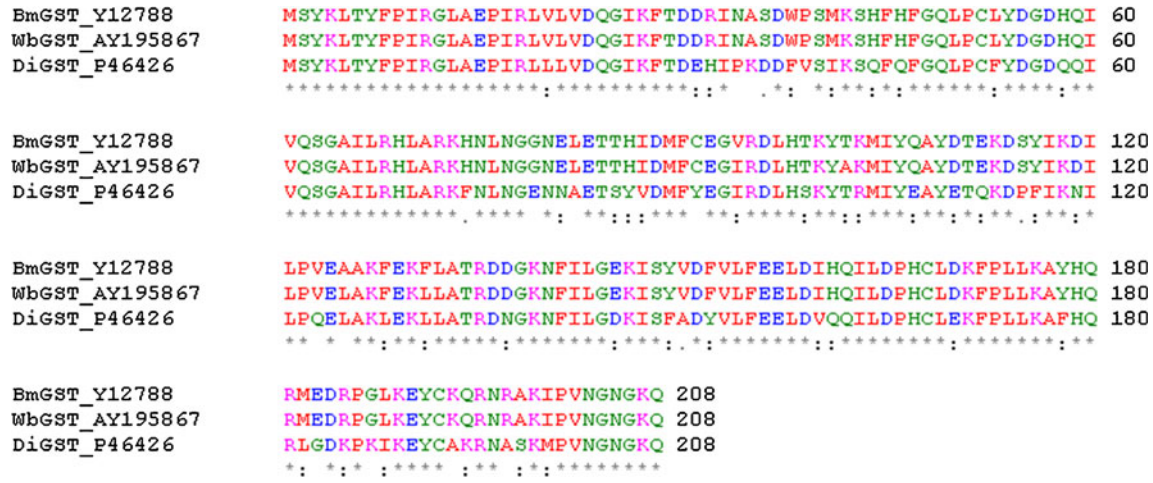


Fig. 3 Multiple sequence alignment (ClustalW) of GST sequences of *B. malayi, D. immitis* and *W. bancrofti*, which show that these sequences are identical. BmGST (Y12788) and WbGST (AY195867) show 98% similarity, BmGST and DiGST (P46426) share 74% similarity and WbGST and DiGST 75% similarity. The (*) denotes identical bases, (:) denotes strongly similar amino acids and (.) denotes weakly similar amino acids

inhibitor substances such as cibacron-blue, butylated hydroxyanisole, hexyl glutathione and ethacrynic acid, with the phytochemicals used for this study. Several potent phytochemicals that possess docking scores very similar to the current GST inhibitor drugs were identified (Table 2). Among the GST inhibitors butylated hydroxyanisole (BHA) markedly reduces worm viability [31]. BHA had a docking score of −68.431 kcal mol^{-1}; phytochemicals with lower docking score than BHA are potential GST inhibitors, and represent promising starting points as lead compounds to treat LF. Thus phytochemicals which exhibit low dock scores and strong hydrogen bond interaction energy and greater number of hydrogen bonds in docking studies such as curcumin (PubChem CID: 969516; MW: 368.380 g mol^{-1}), vanillin (PubChem CID: 1183; MW: 152.147 g mol^{-1}), strychnine (PubChem CID: 5979; MW: 334.412 g mol^{-1}), genostrychnine (PubChem CID: 73393; MW: 350.411 g mol^{-1}), brucine (PubChem CID: 442021; MW: 394.464 g mol^{-1}), brucine-n-oxide (PubChem CID: 161215; MW: 410.463 g mol^{-1}), *beta*-colubrine (PubChem CID: 10512; MW: 364.438 g mol^{-1}) and linalool (PubChem CID: 6549; MW: 154.249 g mol^{-1}) are promising hits as GST inhibitors of natural origin. The hydrogen bond interaction of these lead compounds with the target residues is shown in Fig. 2. The analysis showed that curcumin has greater number of H-bond interactions and strychnine the least. Table 3 shows interacting properties of the eight highly docked phytochemicals to the target protein.

Table 4 GST activity in *Dirofilaria immitis*, treated with phytochemicals

Serial number	Treatments	GST-activity (units[a])	Inhibition (% of control)
1.	Control	13.333	
2.	Linalool	0.333	97.50
3.	Alpha-pinene	1.333	90.00
4.	Strychnine	1.667	87.49
5.	Vanillin	2	84.99
6.	Piperine	2.667	79.99
7.	Isoeugenol	5	62.49
8.	Curcumin	5.667	57.49
9.	Beta-Caryophyllene	8.333	37.50
10.	Cinnamic acid	9.667	27.49
11.	Capsaicin	10.667	19.99
12.	Citronellol	10.667	19.99
13.	Geraniol	11	17.49
14.	Alpha-Terpineol	ND	
15.	Neral	ND	
16.	Myristicin	ND	

[a] Units=x 10^{-3} dA/minute/ml crude enzyme extract

ND=Not Detectable

GST is extensively investigated as a major target against several parasitic infections [31–35]. GST of filarial nematodes has very similar function and multiple sequence analysis revealed its similarity in sequence level (Fig. 3). There is no sequence in public domain of GST of *B. timori* to compare its relatedness to other LF GSTs. GST protein of *B. malayi* (BmGST) and *W. bancrofti* (WbGST) shares 98% similarity and GST of the canine filariasis nematode, *Dirofilaria immitis* (DiGST) shares only 74% similarity to BmGST and 75% to WbGST. We used *D. immitis* GST for in vitro studies, since the other filarial nematodes were not available.

In vitro studies indicated that linalool (97.50%), *alpha*-pinene (90.00%), strychnine (87.49%), vanillin (84.99%), piperine (79.99%), isoeugenol (62.49%), curcumin (57.49%), *beta*-caryophyllene (39.50%), cinnamic acid (27.49%), capsaicin (19.99%), citronellol (19.99%) and geraniol (17.49%) have good potential as nematicidal compounds against filarial GST (Table 4). Molecular structures of these compounds are given in Fig. 4. Terpineol, neral and myristicin had no detectable inhibitory effect. These in vitro studies help validate the results obtained from *in silico* docking studies. The reason why in vitro activities do not correlate closely with *in silico* docking could be because we have used *B. malayi* GST for *in silico* studies, while in vitro studies were carried out with *D. immitis*, due to the difficulty in obtaining samples of *B. malayi*. Multiple sequence alignment of GST

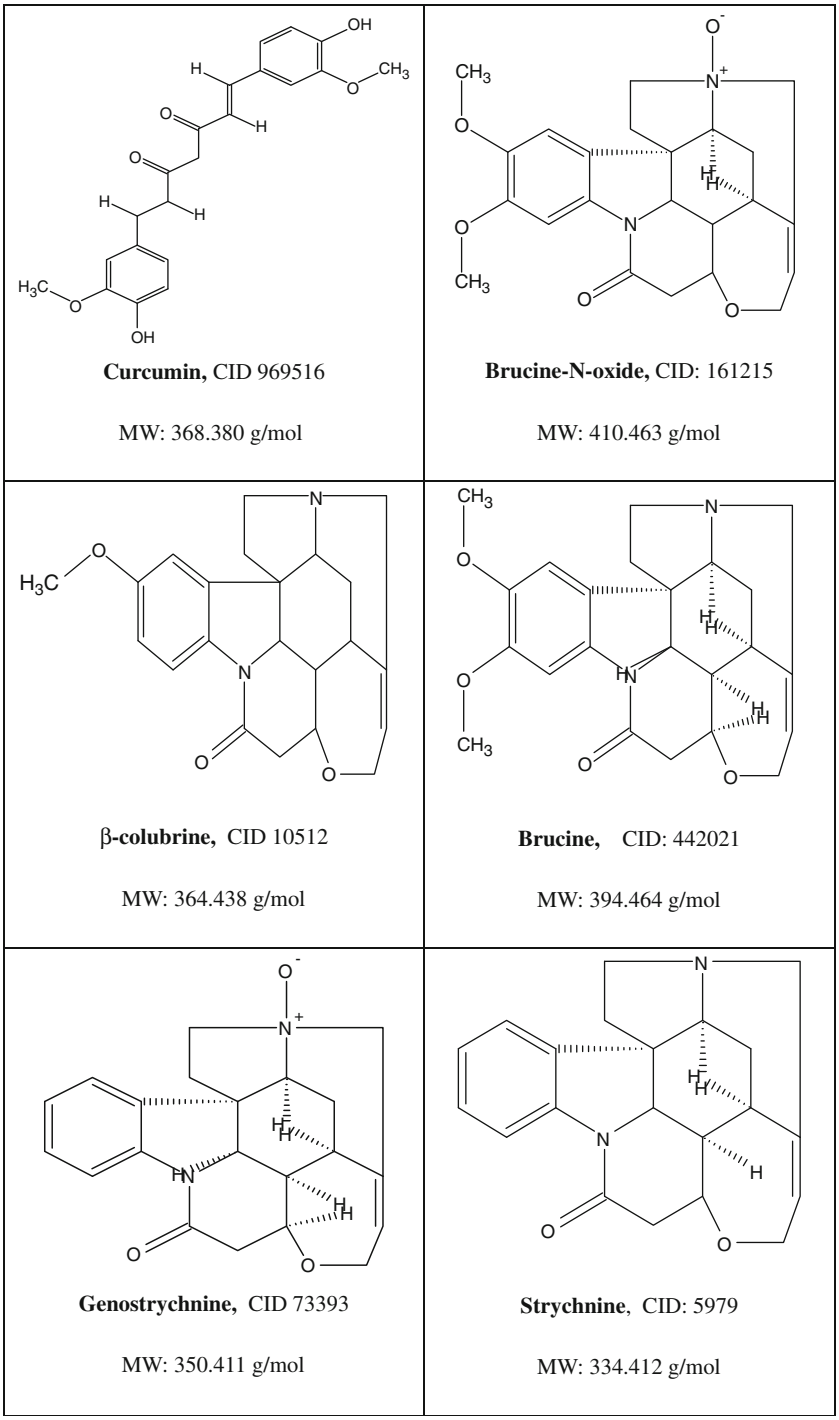
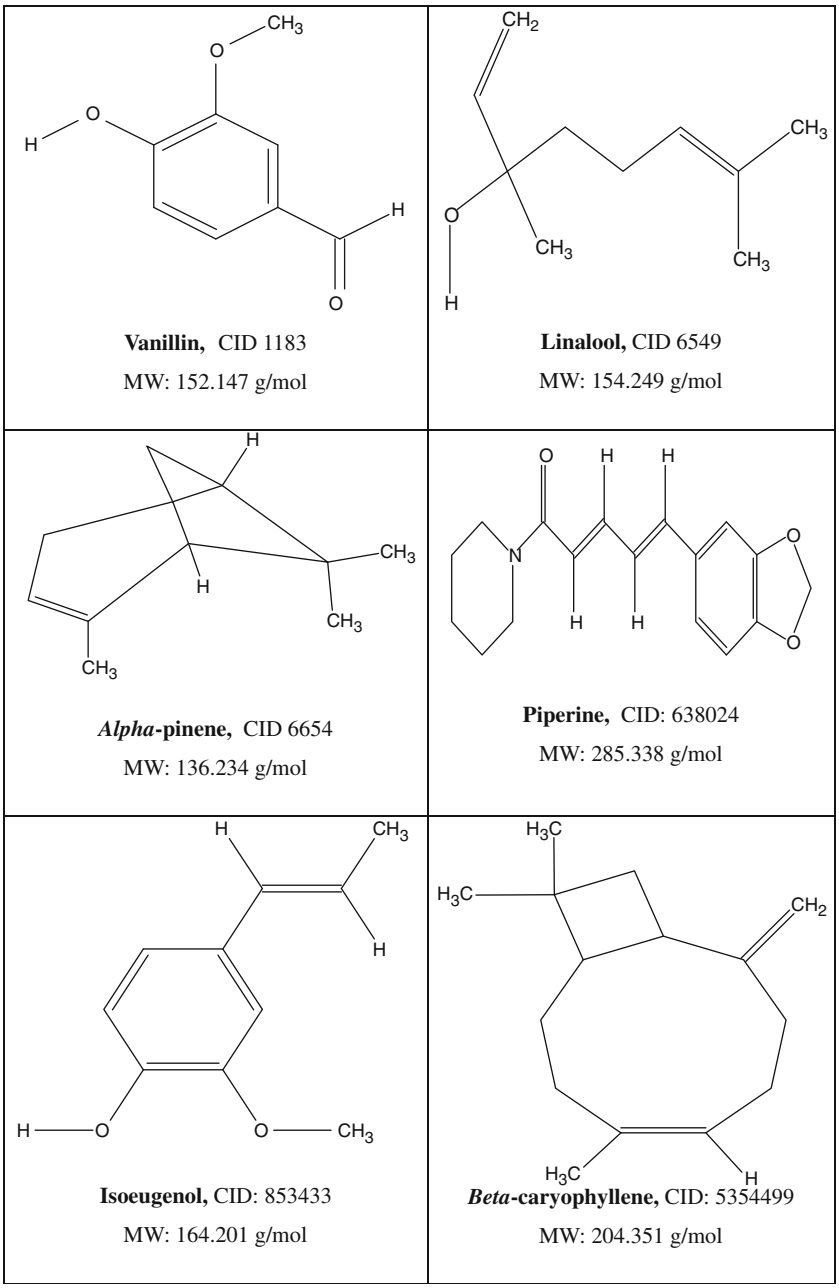


Fig. 4 Molecular structures of assayed compounds - Structures drawn using ChemSketch software

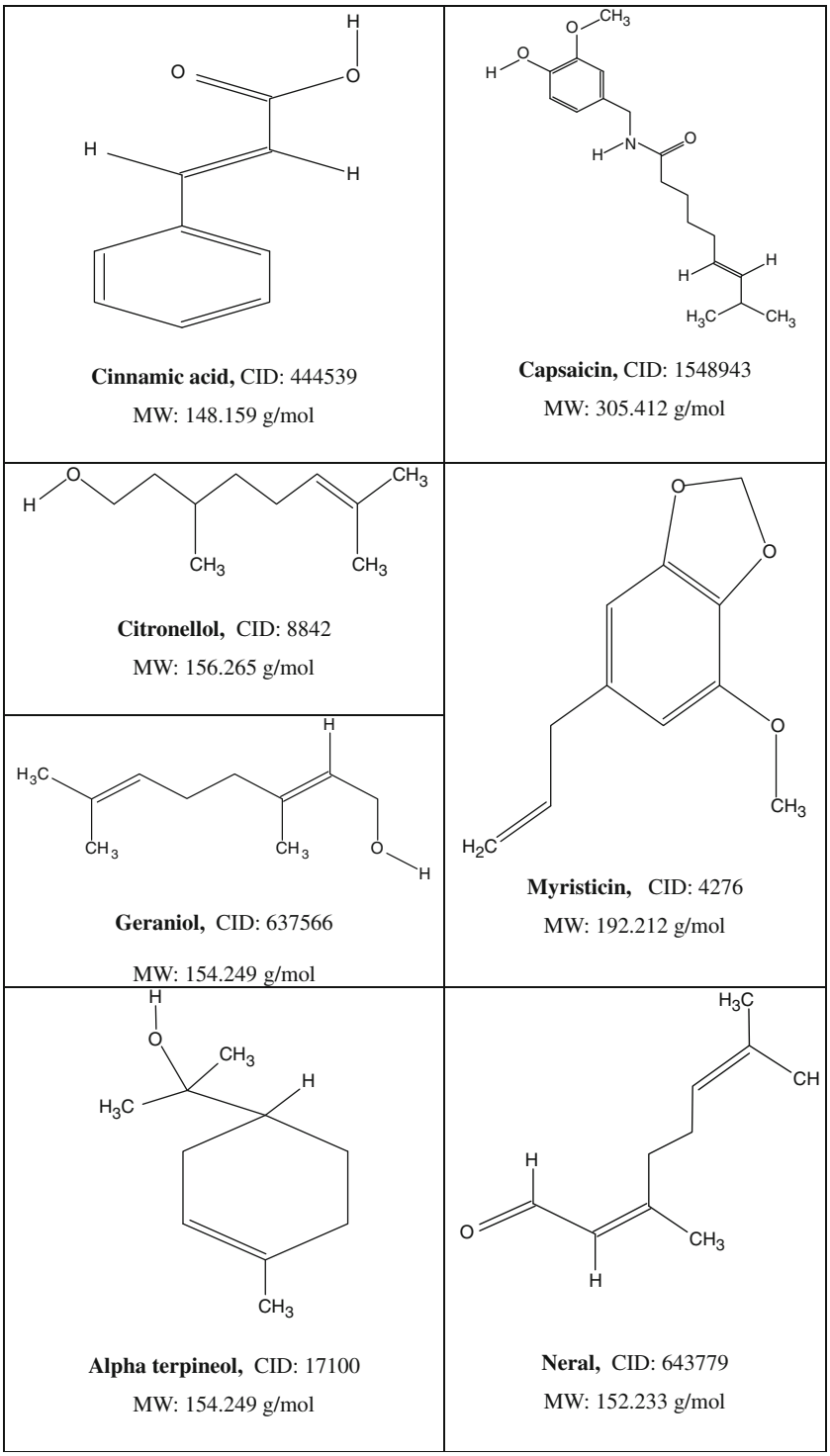
Fig. 4 (continued)



sequences revealed that BmGST and DiGST (P46426) share only 74% similarity (Fig. 3). The difference in the correlation between the *in silico* and in vitro results could be attributed to the structural differences among the BmGST and DiGST.

Curcumin is being used for treatment of cancer, wounds and as a cosmetic [36] among other medicinal uses. GST inhibiting activity of curcumin has been identified in various organisms [37, 38] and its worm motility inhibition was found to be effective at 54.29 μM [6]; the dried seed of *Strychnos nux-vomica* L., has been effectively used in Chinese folk medicine for the treatment of liver cancer and associated pathological abnormalities for ages [39]. Vanilla is a valued spice for its aroma and flavor. The anti-inflammatory activity of linalool has been reported earlier [40]. Piperine, a bioavailability enhancer from black pepper (Piper spp.), has already been reported to inhibit glucuronidation activity in rats and guinea pigs [41]. Singh et al. [42] reported that piperine inhibited rat hepatocyte-mediated glucuronidation of 3-hydroxybenzo[a]pyrene with an IC_{50} of 50 μmol L^{-1}. Co-administration of piperine and curcumin to humans and rats enhanced the bioavailability of curcumin by 2000% and 154%, respectively [43]. Isoeugenol is a well-known antioxidant and its other biological activities include anti-inflammatory, antibiotic, antioxidant, anti-carcinogenic and local anaesthetic activities.

Fig. 4 (continued)



Since the above studied compounds are of natural origin, which satisfies both ADMET and drug likeness properties, these compounds can be used as potent lead compounds against filarial parasites. An online database (www.spicebioinfo.res.in/gstleadbase) has been developed, which it is hoped will serve as a useful repository of information on GST inhibitors for future development of drugs against filarial nematodes.

Conclusions

To summarize, we have employed virtual screening protocol, molecular docking to identify potential drug-like inhibitors of the detoxifying enzyme - GST - of *Brugia malayi*. Several potential drug-like inhibitors have been screened and found to interact with GST satisfactorily. Phytochemicals like curcumin, brucine-n-oxide, *beta*-colu-

brine, brucine, genostrychnine, strychnine, vanillin and linalool revealed strong binding with less docking scores and more number of hydrogen bond interactions to GST of *B. malayi*. This in vitro and *in silico* docking study validates GST inhibitory activity of compounds such as linalool, *alpha*-pinene, strychnine, vanillin, piperine, isoeugenol, curcumin, *beta*-caryophyllene, cinnamic acid, capsaicin, citronellol and geraniol, hence these compounds are novel, alternative drug therapy, of natural origin, for treatment of filariasis through inhibition of GST. Further studies are required to mark them as lead compounds for the development of novel drugs against lymphatic filariasis.

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