Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221−1691.303604

Impact Factor: 1.90

Screening of phytocompounds, molecular docking studies, and *in vivo* anti−inflammatory activity of heartwood aqueous extract of *Pterocarpus santalinus* L.f.

Shanti Vasudevan C.N.[1]✉, Bibu John Kariyil[2], Athira Nair D.[1], I'ma Neerakkal[1]

[1]*Department of Botany, Sacred Heart College, Thevara, Kochi 682013, Kerala, India*

[2]*Department of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Pookode 673576, Wayanad, Kerala, India*

ABSTRACT

Objective: To evaluate the anti-inflammatory potential of aqueous extract of *Pterocarpus santalinus* L.f. heartwood using molecular docking and *in vivo* experiment.

Methods: An aqueous extract of *Pterocarpus santalinus* heartwood was prepared using a Soxhlet apparatus. Phytocompounds in the extract were tentatively identified using high-resolution mass spectrometry. Molecular docking experiments were carried out to evaluate the binding affinity of selected compounds, phloridzin to cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), prostaglandin E synthase-1 (PGES-1) and 5-lipoxygenase (5-LOX). Anti-inflammatory potential was evaluated by carageenan induced paw edema model in rats.

Results: The presence of major component phloridzin along with quercetin, parthenin, ginkgolide B, picrotoxinin, usnic acid, octopine, and epigallocatechin was detected in the extract. Molecular docking study showed that phloridzin inhibited COX-1, COX-2, PGES-1 and 5-LOX with more affinity than ibuprofen and paracetamol. *Pterocarpus santalinus* heartwood extract at 200 and 400 mg/kg BW showed significant reduction in carageenan-induced hind paw edema in a dose-dependent manner, but the effect was slow when compared with the standard ibuprofen (30 mg/kg *p.o.*).

Conclusions: The study indicated that after clinical trials, the aqueous extract of *Pterocarpus santalinus* heartwood can be effectively used in phytotherapy to treat inflammation.

KEYWORDS: Phloridzin; COX-1; COX-2; PGES-1; 5-LOX

1. Introduction

The body produces inflammation as a defense mechanism to protect from harmful stimuli. It forms an important part of the body's immune system[1]. Pyrexia is produced as a result of various biochemical reactions in response to infectious or inflammatory stimuli[2]. The most common anti-inflammatory and antipyretic drugs (NSAIDs) inhibit the cyclooxygenase enzymes isoforms cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) to varying degrees[3] and thereby stop or decrease the formation of the principal fever mediator, PEG-2. COX inhibition can cause various health complications like gastrointestinal bleeding, nephrotoxicity, and cardiovascular side effects[4]. Reye's syndrome (liver damage) may be caused due to long term usage of ibuprofen[5]. The strategy of third-generation NSAIDs includes development of dual cycloxygenase–lipoxygenase and prostaglandin synthase inhibitors[6].

Flavonoids show anti-inflammatory effects due to their potential for inhibiting proinflammatory enzymes like COX-1, COX-2, and 5-lipoxygenase (5-LOX)[7]. Several plants are used for treating inflammation[8] and fever since ancient times[9]. Hence investigating the anti-inflammatory and antipyretic potential of such plants can prove to be useful in finding natural substitutes for synthetic drugs with less toxicity and side effects.

Pterocarpus santalinus (*P. santalinus*) L.f. (Fabaceae), commonly known as 'red sanders', was listed as endangered by the IUCN but reclassified as near threatened[10]. It is an endemic species to southeastern mountain range of South India. In the traditional system of Ayurvedic medicine, the decoction prepared from the heartwood of *P. santalinus* has various medicinal properties. Ayurvedic text

✉To whom correspondence may be addressed. E-mail: shantivasudevan@shcollege.ac.in

How to cite this article: Vasudevan CNS, Kariyil BJ, Nair DA, Neerakkal I. Screening of phytocompounds, molecular docking studies, and *in vivo* anti-inflammatory activity of heartwood aqueous extract of *Pterocarpus santalinus* L.f. Asian Pac J Trop Biomed 2021; 11(2): 59-65.

Article history: Received 3 January 2020; Revision 11 February 2020; Accepted 29 June 2020; Available online 29 December 2020

Madanapala Nighantu suggests *jwaraghna* (antipyretic) property for the heartwood of *P. santalinus*. It is reported to have antipyretic, anti-inflammatory, antihelminthic, anti-hemorrhoidal, antidysenteric, aphrodisiac and diaphoretic activities[11].

Phytochemical studies on the bark of *P. santalinus* have identified the presence of isoflavone, isoflavone glucosides, aurone glycosides, lignans, yellow or orange pigments, and terpenoids. The heartwood contains isoflavone glucosides and two antitumour lignans, *viz.*, savinin and calocedrin[12].

Little information is available on the phytochemical and pharmacological studies of aqueous extract of heartwood of *P. santalinus*. In this context, this study was aimed to identify the phytochemical components in the aqueous extract of *P. santalinus* heartwood by high-performance liquid chromatography-mass spectrometry (HPLC-MS) and to investigate the possible mechanism behind traditionally known anti-inflammatory and antipyretic action using molecular docking experiments. The binding affinity of the major component detected in the extract was evaluated with COX-1, COX-2, prostaglandin E synthase-1 (PGES-1) and 5-LOX. To validate the anti-inflammatory potential of the aqueous extract of *P. santalinus* heartwood, an *in vivo* experiment was carried out using carageenan-induced paw edema model in Wistar Albino male rats.

2. Materials and methods

2.1. Plant material collection and extraction

The heartwood samples of *P. santalinus* were collected, identified, authenticated, and submitted at Kerala Forest Research Institute, Peechi, Kerala (A voucher specimen - Accession No:16686). The plant sample was washed, dried under shade and ground to a fine powder in an electric blender. The aqueous extract was prepared in distilled water using a Soxhlet apparatus by continuous heat application. The extract was further concentrated in a rotary evaporator (Hanvapor HS-2005V, Hahnshin Scientific) and lyophilized.

2.2. HPLC–MS analysis of aqueous extract of P. santalinus heartwood

To identify the phytocomponents present in the aqueous extract of *P. santalinus* heartwood, high-resolution LC-Q-ToF-MS analysis was carried out at Sophisticated Analytical Instrument Facility, IIT Bombay, India. The analysis was performed using Agilent Technologies, USA, Model:1290 Infinity nano HPLC (Binary) with Chipcube (Microfluidic column), 6550 iFunnel Q-TOFs. The solvent system was used as follows: solvent A: water + 0.1% formic acid and solvent B: 90% acetonitrile + 10% water + 0.1% acetonitrile; a gradient started with 95% solvent A and ended with 100% solvent B. The flow rate was maintained at 0.3mL/min and injection volume was 5 μL. The column used was hypersil GOLD column (C18 100 mm × 2.1 mm, 3-micron). The sample was run in both positive and negative ionization modes from 140 *m/z* to 1 000 *m/z*. The analysis was performed using the following tuning parameters: gas temperature (250 ℃), gas flow (13 L/min), nebulizer (35 psig), and nozzle voltage (1 000 V). Total LC running time was 30 min. The MS spectra of the analyzed sample was searched against the Metlin database to find the probable compounds present in the sample.

2.3. Molecular docking studies

Ibuprofen and paracetamol, widely used standards for inflammation and pyrexia respectively, were docked into the binding pockets of COX-1, COX-2, PGES-1 and 5-LOX. The most abundant compound with known anti-inflammatory and antipyretic effect in the aqueous extract of *P. santalinus* heartwood was also docked with the same enzymes to compare its binding affinity with the standards used. Molecular docking simulations were carried out using AutodockVina[13]. Crystal structures of COX-1 (PDB ID 2OYE), COX-2 (PDB ID 3LN1), PGES-1 (PDB ID3DWW), and 5-LOX (PDB ID3V99) were obtained from PDB and the structures of ibuprofen, paracetamol and phloridzin were downloaded from PubChem database. The predicted docked poses of ibuprofen, paracetamol and phloridzin against COX-1, COX-2, PGES-1, and 5-LOX were analysed in Discovery studio visualizer.

2.4. In vivo experiment

The anti-inflammatory activity of the extract was tested using carageenan-induced rat paw edema model as described previously with slight modifications[14]. A total of twenty-four Wistar Albino male rats weighing 100-200 g were used in this study. Rats were randomly divided into four groups of six rats each. Group Ⅰ, Group Ⅱ, Group Ⅲ, and Group Ⅳ received distilled water (control), ibuprofen (30 mg/kg BW *p.o.*), 200 and 400 mg/kg BW *p.o.* of the aqueous extract of *P. santalinus* heartwood, respectively. After 1 h, 0.1 mL of 1% (w/v) carageenan was injected in the subplantar tissue of the right hind paw of each rat. The thickness of hind paw was measured at 1-hour interval for 4 h using vernier callipers. The percentage inhibition (PI) was calculated according to the below equation[14].

$$PI = \frac{(T_t - T_o)\text{control} - (T_t - T_o)\text{treated}}{(T_t - T_o)\text{control}} \times 100$$

where T_o = mean paw thickness at 0 h; T_t = mean paw thickness at a particular time interval.

2.5. Statistical analysis

Measurements of paw thickness were expressed as mean ± standard error of the mean (SEM). Statistical analysis of results was performed by one-way analysis of variance (ANOVA) followed by multiple Tukey's comparison tests. $P<0.05$ was considered statistically significant.

2.6. Ethical statement

The experiment was performed in accordance with the CPCSEA

norms and approved by the Institute of Animal Ethical Committee of the College of Veterinary and Animal Sciences, Thrissur, Kerala, India where the study was conducted [Approval Order No. Acad (3)/6554/04 of Kerala Veterinary & Animal Science University, dated 27/09/18].

3. Results

3.1. Secondary metabolites identified from the aqueous extract of P. santalinus heartwood

The compounds identified using HPLC-MS analysis are presented in Table 1. Phloridzin was detected both in positive and negative ionization mode. In negative ionization mode, compound 3, phloridzin, at retention time 4.775, showed the highest intensity (Figure 1). Its MS spectra showed the molecular ion peak –ve ESI– *m/z* 435.128 and the fragment ions *m/z* 435, 190, 221, 315 (Figure 2).

3.2. Molecular docking

Binding energy scores and amino acid interactions for ibuprofen, paracetamol, and phloridzin docked to COX-1, COX-2, PGES-1, and 5-LOX are shown in Table 2. Two-dimensional binding site interaction models are shown in Figure 3. Phloridzin had a higher affinity with COX-1, COX-2, PGES-1, and 5-LOX than the standard drugs ibuprofen and paracetamol. For phloridzin, the highest binding energy score of -8.7 kcal/mol was observed with COX-2. In addition, ibuprofen showed a better affinity with COX-1, COX-2, and PGES-1 than paracetamol.

3.3. Anti–inflammatory activity

Anti-inflammatory effect of the aqueous extract of *P. santalinus* heartwood on carageenan-induced paw edema is presented in Table 3. The PI values obtained for the standard drug ibuprofen at a dose of 30 mg/kg BW from 1 h to 4 h were 45.5%, 68.5%, 82.4% and

Table 1. Secondary metabolites identified from the aqueous extract of *Pterocarpus santalinus* heartwood and their retention time, *m/z* value and elemental composition.

Compounds	Retention time	Mass	Formula	*m/z*	ESI mode
Flavonoids					
Phloridzin	4.775	436.135	$C_{21}H_{24}O_{10}$	435.128	+ & -
Isotectorigenin, 7-methyl ether	7.836	328.092	$C_{18}H_{16}O_6$	329.099	+
Quercetin tetramethyl (5,7,3',4') ether	8.605	358.103	$C_{19}H_{18}O_7$	359.110	+
Terpenes					
Ginkgolide B	4.211	424.135	$C_{20}H_{24}O_{10}$	451.123	-
3Alpha-hydroxy-4,4-bisnor-8,11,13-podocarpatriene	6.697	216.149	$C_{15}H_{20}O$	217.157	+
Picrotoxinin	8.024	292.095	$C_{15}H_{16}O_6$	315.084	+
Parthenin	8.380	262.116	$C_{15}H_{18}O_4$	263.123	+
Punctaporin B	8.686	252.170	$C_{15}H_{24}O_3$	275.160	+
Muurolladie-3-one	9.414	218.166	$C_{15}H_{22}O$	219.173	+
Phenolic compounds					
Epigallocatechin	6.788	306.072	$C_{15}H_{14}O_7$	329.061	+
Baeomycesicacid	8.454	374.097	$C_{19}H_{18}O_8$	375.105	+
Carotenoids					
Bacteriorubixanthinal	5.466	596.423	$C_{41}H_{56}O_3$	619.412	+
Diketospirilloxanthin/2,2'-diketospirilloxanthin	6.017	624.417	$C_{42}H_{56}O_4$	625.425	+
Aldehydes					
2,6-Nonadienal	7.561	138.105	$C_9H_{14}O$	161.094	+
2-Tridecenal	8.292	196.183	$C_{13}H_{24}O$	219.173	+
Fatty acid					
2-Methyldodecanedioic acid	6.629	244.168	$C_{13}H_{24}O_4$	267.157	+
Ester					
Butacaine	18.76	306.229	$C_{18}H_{30}N_2O_2$	365.245	-
Others					
4'-Hydroxyfenoprofen glucuronide	4.266	434.120	$C_{21}H_{22}O_{10}$	433.113	-
1-Naphthoic acid glucuronide	7.259	348.083	$C_{17}H_{16}O_8$	329.065	-
Calcipotriene	20.44	412.292	$C_{27}H_{40}O_3$	393.276	-
3-N-decyl acrylic acid	6.599	212.178	$C_{13}H_{24}O_2$	235.166	+
(-)-Usnic acid	7.159	344.087	$C_{18}H_{16}O_7$	367.076	+
Sotalol	7.624	272.120	$C_{12}H_{20}N_2O_3S$	273.127	+
Hematoporphyrin	7.981	598.274	$C_{34}H_{38}N_4O_6$	621.263	+
Mesoporphyrin Ⅸ	8.251	566.284	$C_{34}H_{38}N_4O_4$	567.292	+
5,4'-Dimethoxy-7-hydroxyflavone	9.773	298.082	$C_{17}H_{14}O_5$	299.090	+
Octopine	4.179	246.135	$C_9H_{18}N_4O_4$	247.142	+
1-(13Z-docosenoyl)-2-(7Z,10Z,13Z,16Z,19zdocosapentaenoyl)-sn-glycerol	19.598	724.600	$C_{47}H_{80}O_5$	747.590	+

Table 2. Binding energy and amino acid interactions for ibuprofen, paracetamol, and phloridzin docked to selected targets.

Compound	Enzymes	Binding Energy (kcal/mol)	Type of bonds	Interacting residues
Phloridzin	COX-1	-7.9	Conventional hydrogen bond	AsP-58, Thr60, Cys 41
			Vander Waals interaction	Tyr-39, Gln-42, Gln-44, Gly-45, Ile- 46, Cys-47, Arg-61, Cys-59, Thr-62, Thr-129, Tyr-130, Ile-137, Ile-151
			Pi-alkyl interaction	Pro-125, Leu-152, Pro-153
			Carbon hydrogen bond	Thr-60
	COX-2	-8.7	Conventional hydrogen bonds	Arg 106, Tyr 341, Tyr 371, Ile 503
			Vander Waals interaction	His-75, Met-99, Gln-178, Tyr-334, Leu-338, Ser-339, Tyr-341, Leu-345, Phe-367, Leu-370, Trp-373, Arg-449, Ala-502, Phe-504, Met-508, Gly-512, Ser-516
			Pi-sigma	Val-509
			Pi-alkyl	Val-335, Ala-502, Ala-513, Leu-517
	PGES-1	-6.1	Conventional hydrogen bond	Leu-17, Cys-83
			Vander Waals interaction	Phe-16, Leu-23, Val-24, Ile-79, Leu-83, Phe-84, Val-88, Phe-91
			Pi-pi stacked	Phe-87
	5-LOX	-8.0	Conventional hydrogen bond	Iso-406, Ala-410, Gln-413, Asn-554, Leu-607
			Vander Waals interaction	Phe-169, Ser-171, Phe-His-372, Phe-555, Phe-610, Val-671
			Carbon hydrogen bond	His-550, Ala-672
			Pi-sigma	Ile-406
			Pi-pi Stacked	Phe-177
			Pi-alkyl	Lys-409
Ibuprofen	COX-1	-6.9	Vander Waals interaction	Val-349, Ser-353, Ser-355, Ile-523, Ser-530, Leu-531, Ser-553
			Pi-sigma	Tyr-385
			Amide-pi-staked	Gy-526
			Alkyl & pi-alkyl	Leu-352, Phe-381, Leu-384, Trp-387, Phe-518, Met-522, Ala-527
	COX-2	-7.7	Conventional hydrogen bond	Arg-499, Tyr-341
			Vander Waals interaction	His-75, Ser-339, Phe-367, Arg-499, Ile-503, Phe-504, Gly-512, Ala-513, Ser-516
			Alkyl & pi-alkyl	Tyr-334, Val-335, Leu-338, Tyr-371, Trp-373, Val-509
	PGES-1	-5.2	Conventional hydrogen bond	Ser-20
			Vander Waals interaction	Cys-19, Ser-20, Leu-23, Val-24, Met-27, Ile-79, Phe-84
			Pi-sigma	Leu-83, Phe-87
			Pi-alkyl	Leu-83, Phe-87
	5-LOX	-6.3	Conventional hydrogen bond	Asn-554, Tyr-558
			Vander Waals interaction	Gly-556, Gln-557, Val-604, Ser-608, Val-671, Ala-672
			Pi-alkyl	His-550, Phe-555, Leu-607, Phe-610
Paracetamol	COX-1	-6.3	Conventional hydrogen bond	Leu-384, Trp-387
			Vander Waals interaction	Tyr-348, Tyr-348, Val-349, Phe-381, Phe-518, Ile-523, Ala-527, Leu-352, Ser-530
			Pi-sulphur	Met-522
			P-pi-T shaped & amide pi-staked	Trp-387, Tyr-385, Gly-526
			Pi-alkyl	Leu-384
	COX-2	-6.3	Conventional hydrogen bond	His-75
			Vander Waals interaction	Val-335, Leu-338, Tyr-341, Phe-504, Gly-512, Ala-513, Ser-516
			Pi-sigma	Ser-339, Val-509
	PGES-1	-4.4	Conventional hydrogen bond	Trp-146, Glu-147
			Vander Waals interaction	Tyr-89, Pro-94, Val-98, Gly-143, Arg-150
			Pi-anion	Glu-147
	5-LOX	-6.4	Conventional hydrogen bond	Arg-370, Thr-545
			Vander Waals interaction	Val-243, Arg-370, Ser-447, Phe-450, Ala-453, Arg-457, trp-478, Phe-544, Gln-549
			Pi-alkyl	Leu-448

Table 3. Effect of aqueous extract of *Pterocarpus santalinus* heartwood on carrageenan-induced paw edema in rats.

Groups	0 h	1 h	2 h	3 h	4 h
Control	4.30 ± 0.07	5.53 ± 0.07	5.89 ± 0.06	5.44 ± 0.04	5.16 ± 0.07
Standard (30 mg/kg BW)	4.43 ± 0.02	5.10 ± 0.06*	4.93 ± 0.06*	4.63 ± 0.07	4.50 ± 0.07*
PSAE (200 mg/kg BW)	4.21 ± 0.07	5.19 ± 0.06*	5.39 ± 0.06*	4.95 ± 0.03*	4.71 ± 0.04*
PSAE (400 mg/kg BW)	4.44 ± 0.05	5.33 ± 0.07*	5.41 ± 0.07*	5.06 ± 0.08*	4.84 ± 0.07*

Values are expressed as mean ± SEM (n = 6). One-way ANOVA analysis was carried out followed by multiple Tukey's comparison test. *$P < 0.05$ compared to the control group. PSAE: aqueous extract of *Pterocarpus santalinus* heartwood.

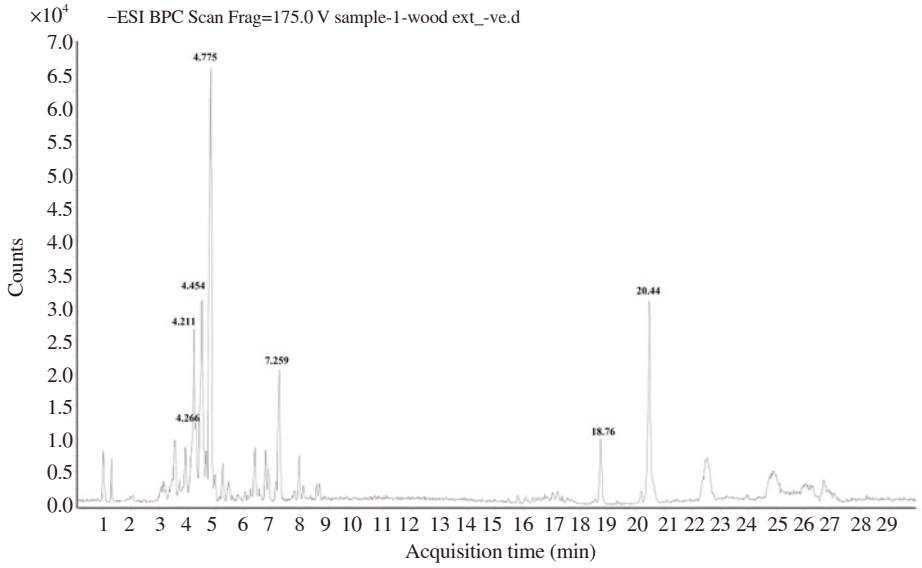

Figure 1. HRLC-ve ESI-MS-MS chromatogram of the aqueous extract of *Pterocarpus santalinus* heartwood.

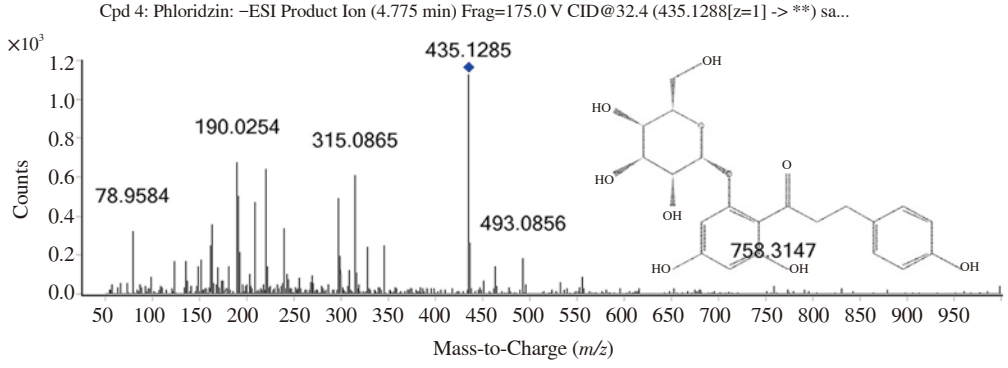

Figure 2. MS/MS spectra and spectrum peak list of phloridzin (-ve ESI) with *m/z* 435.128.

91.8%, respectively. The PI values obtained for the aqueous extract of *P. santalinus* heartwood at the same time interval at doses of 200 and 400 mg/kg BW were 20.3%, 25.8%, 35.1%, 41.8% and 27.6%, 38.9%, 45.6%, 53.5%, respectively. Thus, the aqueous extract of *P. santalinus* heartwood at doses of 200 and 400 mg/kg BW inhibited paw edema in a dose-dependent manner. But the effect was slow when compared to the standard drug ibuprofen.

4. Discussion

In the present study, active constituents of the aqueous extract of *P. santalinus* heartwood were tentatively identified and the metabolite responsible for producing the anti-inflammatory and antipyretic effect was predicted. Flavonoids, terpenoid derivatives, phenolic compounds, and carotenoids prevailed among the extract. Quercetin tetramethyl (5,7,3',4') ether, epigallocatechin and 5,4'-dimethoxy-7-hydroxyflavone identified in the aqueous extract were in conformity with the earlier reports of similar compounds in the genus *Pterocarpus*[15–17]. Among the compounds detected, phloridzin is reported to have anti-inflammatory[18] and antipyretic[19] activity. Phloridzin is a febrifuge like salicin. It was reported by De Koenick and Stass in the root bark of apple, pear, cherry and plum tree[20]. Mode of action of phloridzin as an antimalarial agent is reported *in vitro* cultures of *Plasmodium falciparum*[21]. Anti-inflammatory action of phloridzin by decreasing the synthesis of PGE_2 and IL-8 is also reported[22].

Phloridzin possesses structural features that interact with COX-1, COX-2, PGES-1 and 5-LOX indicating that it can be a potent inhibitor of these enzymes. The presence of phloridzin in the aqueous extract of *P. santalinus* heartwood may be responsible for the antipyretic effect shown by the extract as reported in our previous *in vivo* study[23].

In vivo study indicated that the aqueous extract of *P. santalinus* heartwood showed a dose-dependent anti-inflammatory activity, but less effective than ibuprofen. The anti-inflammatory activity may be due to phloridzin content in the extract. Phloridzin showed more negative binding energy *in silico*, probably indicating the inhibition of COX-1, COX-2, and 5-LOX. Phloridzin showed more affinity with enzymes than the standards. However, the extract showed lower anti-inflammatory activity than the standards, which may be due to the synergistic effect of other compounds in the crude extract or lower concentration of phloridzin in the extract administered. The present molecular docking study supported the earlier reports of

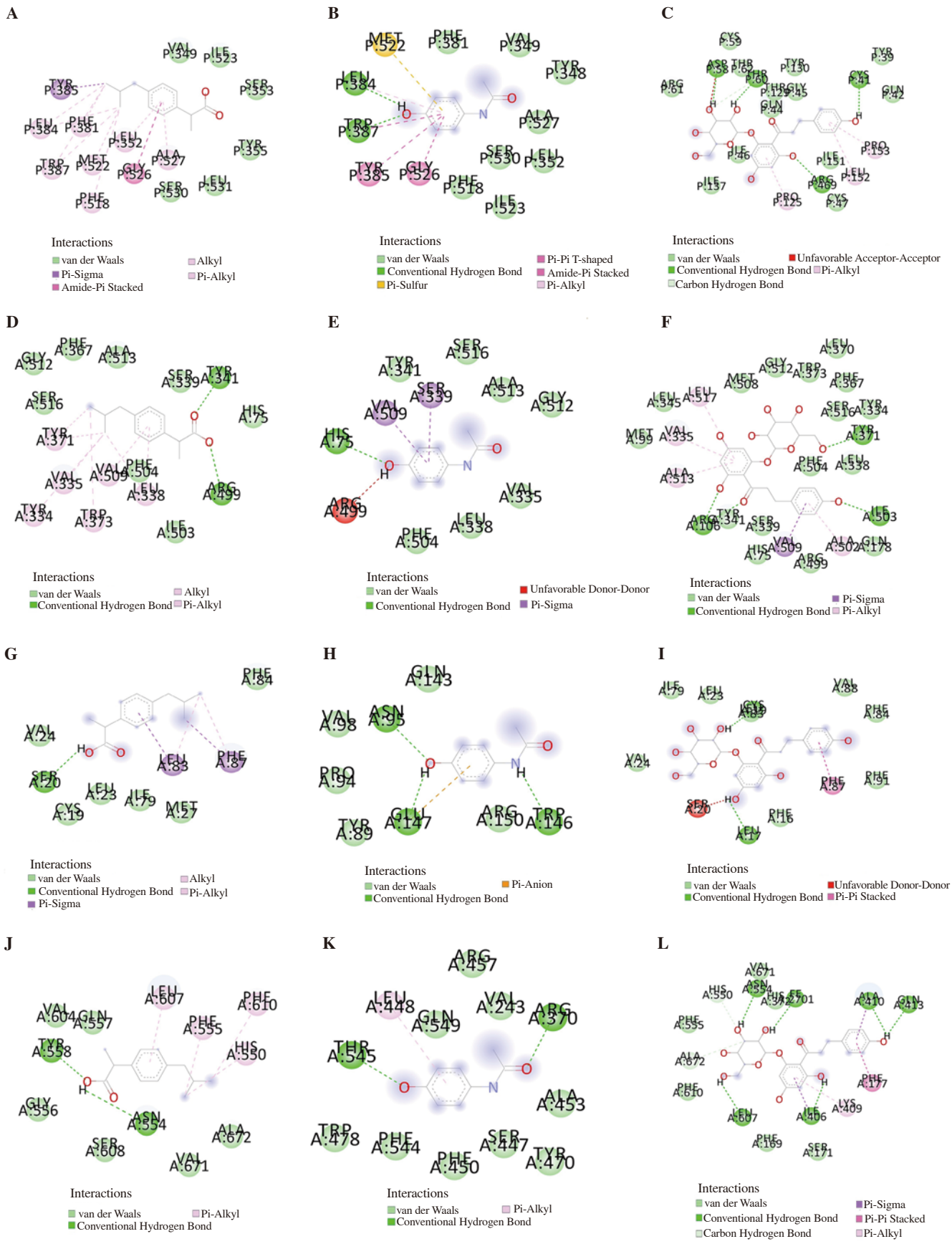

Figure 3. Two-dimensional binding site interaction models. (A) ibuprofen with COX-1; (B) paracetamol with COX-1; (C) phloridzin with COX-1; (D) ibuprofen with COX-2; (E) paracetamol with COX-2; (F) phloridzin with COX-2; (G) ibuprofen with PGES-1; (H) paracetamol with PGES-1; (I) phloridzin with PGES-1; (J) ibuprofen with 5-LOX; (K) paracetamol with 5-LOX; (L) phloridzin with 5-LOX. COX-1 and COX-2: cyclooxygenase-1 and 2, PGES-1: prostaglandin E synthase-1, 5-LOX: 5-lipoxygenase.

phloridzin being used against fever and inflammation. Its role in inhibiting cytokines responsible for inflammation process is also reported[24].

The study revealed the presence of several metabolites in the aqueous extract of *P. santalinus* heartwood. Molecular docking showed that phloridzin inhibits COX-1, COX-2, PGES-1 and 5-LOX with more affinity than ibuprofen and paracetamol. *In vivo* experiment proved anti-inflammatory effect of the extract. More studies, especially clinical trials, are needed to further confirm anti-inflammatory activity of the aqueous extract of *P. santalinus* heartwood.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgments

The corresponding author acknowledges UGC, Bangalore for granting Teacher Fellowship during the research work. Authors thank Sophisticated Analytical Instrument Facility, IIT Bombay for HRLCMS/MS service provided. The lab facility and animal house provided by the Dept. of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India is duly acknowledged.

Authors' contributions

SVCN carried out the experiments and wrote the entire manuscript. BJK supervised the animal study experiment. AND carried out the molecular docking experiment. IN gave overall direction and helped in interpreting the results. All the authors provided critical feedback.

References

[1] Huang MT, Ghai G, Ho CT. Inflammatory process and molecular targets for anti-inflammatory nutraceuticals. *Compr Rev Food Sci Food Saf* 2004; **3**(4): 127-139.

[2] Zampronio AR, Hoadley ME, Luheshi G, Rothwell NJ, de Souza GE, Hopkins SJ. Interleukin (IL)-6 release and fever induced by a pre-formed pyrogenic factor (PFPF) derived from LPS-stimulated macrophages. *Eur Cytokine Netw* 2000; **11**(4): 589-596.

[3] Cryer B, Feldman M. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. *Am J Med* 1998; **104**(5): 413-421.

[4] Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. *Aging Dis* 2018; **9**(1): 143.

[5] Hongo T, Momoki N, Mae S, Nozaki S, Takahashi K, Fujiwara T. A rare case of Reye's syndrome induced by influenza A virus with use of ibuprofen in an adult. *Acute Med Surg* 2020; **7**(1): e457.

[6] Groesch S, Niederberger E, Geisslinger G. Investigational drugs targeting the prostaglandin E2 signaling pathway for the treatment of inflammatory pain. *Expert Opin Invest Drugs* 2017; **26**(1): 51-61.

[7] Hanáková Z, Hošek J, Kutil Z, Temml V, Landa P, Vaněk T, et al. Anti-inflammatory activity of natural geranylated flavonoids: Cyclooxygenase and lipoxygenase inhibitory properties and proteomic analysis. *J Nat Prod* 2017; **80**(4): 999-1006.

[8] Gacche R, Shaikh R, Pund M, Deshmukh R. Cyclooxygenase inhibitory, cytotoxicity and free radical scavenging activities of selected medicinal plants used in Indian traditional medicine. *Pharmacogn J* 2011; **3**(19): 57-64.

[9] Chhetri DR. Medicinal plants used as antipyretic agents by the traditional healers of Darjeeling Himalayas. *Indian J Tradit Know* 2004; **3**(3): 271-275.

[10]Barstow M. *Pterocarpus santalinus*. The IUCN Red List of Threatened Species; 2018, e.T32104A67803072. [Online] Available from: https://www.iucnredlist.org/species/32104/67803072 [Accessed on 1 February 2018].

[11]Pullaiah T, Reddy VD. Pharmacology of red sanders. In: Pullaiah T, Balasubramanya S, Anuradha M (eds.) *Red sanders: Silviculture and conservation.* Springer, Singapore; 2019, p. 57-76.

[12]Arunakumara KK, Walpola BC, Subasinghe S, Yoon MH. *Pterocarpus santalinus* Linn. f.(Rathhandun): A review of its botany, uses, phytochemistry and pharmacology. *J Korean Soc Appl Biol Chem* 2011; **54**(4): 495-500.

[13]Trott O, Olson AJ. AutoDockVina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2010; **31**(2): 455-461.

[14]Arpitha S, Srinivasan K, Sowbhagya HB. Anti-inflammatory effect of resin fraction of cardamom (*Elettaria cardamomum*) in carrageenan-induced rat paw edema. *Pharma Nutrition* 2019; **10**: 100165.

[15]Singh P, Bajpai V, Gupta A, Gaikwad AN, Maurya R, Kumar B. Identification and quantification of secondary metabolites of *Pterocarpus marsupium* by LC–MS techniques and its *in–vitro* lipid lowering activity. *Ind Crops Prod* 2019; **127**: 26-35.

[16]Sheehan EW, Zemaitis MA, Slatkin DJ, Schiff Jr PL. A constituent of *Pterocarpus marsupium*, (-)-epicatechin, as a potential antidiabetic agent. *J Nat Prod* 1983; **46**(2): 232-234.

[17]Seshadri TR. Polyphenols of *Pterocarpus* and *Dalbergia* woods. *Phytochemistry* 1972; **11**(3): 881-898.

[18]Chang WT, Huang WC, Liou CJ. Evaluation of the anti-inflammatory effects of phloretin and phlorizin in lipopolysaccharide-stimulated mouse macrophages. *Food Chem* 2012; **134**(2): 972-979.

[19]Gosch C, Halbwirth H, Stich K. Phloridzin: Biosynthesis, distribution and physiological relevance in plants. *Phytochem* 2010; **71**(8-9): 838-843.

[20]Graham T. Salicin and bodies obtained from its decomposition. In: Henry W (ed.) *Elements of chemistry: Including the applications of the science in the arts.* Philadelphia: Lea & Blanchard; 1843, p. 595.

[21]Kutner S, Breuer WV, Ginsburg H, Cabantchik ZI. On the mode of action of phlorizin as an antimalarial agent in *in vitro* cultures of *Plasmodium falciparum*. *Biochem Pharmacol* 1987; **36**(1): 123-129.

[22]Zielinska D, Laparra-Llopis JM, Zielinski H, Szawara-Nowak D, Giménez-Bastida JA. Role of apple phytochemicals, phloretin and phloridzin, in modulating processes related to intestinal inflammation. *Nutrients* 2019; **11**(5): 1173.

[23]Shanti VCN, Bibu JK, I'ma N. Antipyretic activity of aqueous extract of heart wood of *Pterocarpus santalinus* L. in yeast induced pyrexia. *J Pharmacogn Phytochem* 2019; **8**: 244-246.

[24]Milani R, Marcellini A, Montagner G, Baldisserotto A, Manfredini S, Gambari R, et al. Phloridzin derivatives inhibiting pro-inflammatory cytokine expression in human cystic fibrosis IB3-1 cells. *Eur J Pharm Sci* 2015; **78**: 225-233.