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Research Article

In-Vivo Antinociceptive Activity and *In-Silico* Molecular Docking of Selected Phytoconstituents of Methanolic Extract of *Hypericum Japonicum*

Shaik Aminabee*, Raveesha Peeriga, V. Adithya, M. Mohansai, Shaherbanu, K. Harshitha, K. Himaja Kasthuri, M. Lakshmi Priya, G. Chandini Naga Mallika and Lakshmana Rao Atmakuri

Department of Pharmacology, V. V. Institute of Pharmaceutical Sciences, India

**Address Correspondence to Shaik Aminabee, aminaammi786@gmail.com*

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Abstract

This research work was carried out to analyse and evaluate the antinociceptive activity of methanolic extract of *Hypericum Japonicum* (MEHJ) and *in-silico* molecular docking of selected phytoconstitutents with cyclooxygenase-2 (COX-2) enzyme along with absorption (A), distribution (D), metabolism (M), excretion (E) and toxicity (T) studies. *In-vivo* antinociceptive activity was performed by hot plate method, tail immersion method and acetic acid induced writhing response method in rat. In-silico molecular docking was done by using Autodock Vina and Discovery Studio Visualizer. Absorption, distribution, metabolism, excretion and toxicity (ADMET) studies were examined by Swiss ADME software. The results proved that methanolic extract of *Hypericum Japonicum* has dose dependent antinoceptive activity at all doses. Among all the phytoconstitutents saroaspidin B has very best docking rate of -7.1 kcal/mol which was better virtually than standard celecoxib which has docking rate of -7.4 kcal/mol. This shows that there is good binding affinity between ligand and receptor than the standard i.e celecoxib. ADMET evaluation using swissADME and admeSAR software assures that saroaspidin B has followed all the 5 Lipinski's guidelines suggesting that it is safety for consumption. Hence by this research, we conclude that *Hypericum Japonicum* can be a potent agent as antinociceptive activity and further studies are required to for the development of performance of saroaspidin B.

Keywords: *Hypericum Japonicum*; Kielcorin; Mesuaxanthone B; Analgesic; Celecoxib

Abbreviations: (TAE) Tannic Acid Equivalent; (GAE) Gallic Acid Equivalents; (CE) Catechin Equivalents; (AE) Atropine Equivalent; (RT) Retention Time; (PA) Peak Area

Introduction

To recognize the location that is damaged and harmed by numerous stimulations, pain is a tool that is beneficial in body's immune system. For the treatment of pain, many drugs like non-steroidal anti-inflammatory drugs (NSAIDs), analgesics opioid in nature, opioid anaesthetics and steroidal medicines are used [1]. They are having many harmful effects like kidney failure, liver damage, cardiac problems, high blood pressure, erectile dysfunction, skin degeneration, manic depression, reduced bone density, constipation, abscess and respiratory problems. So it gained importance for herbal based antinoceptive drug which can be available at low cost, more potent and has less negative effects [2]. In order to generate highly active compound with minimum adverse effects, drug design has become a vital tool in medicinal chemistry field where novel compounds are synthesized by chemical or molecular modification of lead moiety. *In-silico* docking method was a huge breakthrough in drug design and development to predict therapeutic efficacy of the novel molecules [3]. To design new drugs, molecular docking has become an essential element where selected protein will show binding affinity for ligand. To understand chemical properties and drug receptor interactions, *In-silico* docking methods are largely useful [4]. *Hypericum Japonicum* is an annual herb flowering plant belonging to the family hypericaceae. It is only 2-5 cm long. Its stems are green, 4 angled and 2-52 mm long internodes that exceed the leaves. The leaves are persistent, spreading and sessile. This species is 30 flowered and flowers are branched upto 3 nodes. The flowers are 4-8 mm in diameter and petals are bright orange or yellow. The stamens are 5-30 in number arranged in irregular groups. Seeds are 50 mm long approximately. It is distributed in India, China, Laos, Japan, Vietnam, Myanmar, Thailand, Malaysia, and Indonesia, Philippines to New Guinea, New Zealand, and Australia. It is well grown in good drained and soil that retains moisture. Flourish and semi shade under sun. Sunny region is better foe flowers. Mostly this species was distributed in wet soils. The plant is harvested from the wild for local use as a medicine. The plant is antiphlogistic, alternative, astringent, febrifuge, depurative, vulnerary and stomachic. For use it can be boiled with water. Also used in the therapy of dysentery, appendicitis, acute hepa-

titis, asthma, pain in the liver region, abscesses and boils. Also used as a styptic. In the treatment of malaria it was combined with Zingiber officinale (ginger) and ash salt. It is also having antitumour activity. Applied externally, it is used to treat leech, wounds, swellings, snake bites, scrofula abscesses and fungal skin diseases.

Materials and Methods

Collection and identification of plant material

Whole plant of *Hypericum Japonicum* was collected from Tirumala hills, Tirupathi, Andhra Pradesh. The plant was confirmed and acknowledged by Prof. K. Madhava Chetty, Plant Taxonomist, Professor, Department of Botany, SV University, Tirupathi, and Andhra Pradesh, India [5]. *Hypericum Japonicum* was thoroughly washed and cleaned with water, air dry under room temperature and grounded into powdered using home appliance. It was preserved for pharmacognostical and pharmacological study.

Preparation of extracts

By using home appliances the shade dried *Hypericum Japonicum* was powdered. Soxhlet apparatus was used for extraction of coarse powder (1000 gm) with methanol. The final extract was filtered. In rotating evaporator the extract was concentrated by vaporization of solvent and maintained in refrigerator [6].

Phytochemical analysis

For the existence of saponins, alkaloids, terpenoids, glycosides, flavonoids, tannins, carbohydrates, proteins, amino acids and fixed oils phytochemical analysis of extracts were carried out by different methods [7].

Experimental animals

From livestock building of Vallabhaneni Venkatadri Institute of Pharmaceutical Sciences, Gudlavalleru albino wistar rats (180-200 gm) are obtained. Animals are fasted for a period of 12 hrs before and after treatment water was ad libitum.

Acute toxicity studies

As per OECD guidelines 423, acute toxic studies are performed on MEHJ taking albino mice of body weight 20-25 gm as experimental animals. Adult mice which are healthy are divided into groups and each group contained 5 mice. Each group received MEHJ at different doses of 5, 50, 200 and 300 mg/kg body weight orally. For mortality and physical/behavioural changes animals were observed for over 14 days [8].

Evaluation of antinociceptive activity

Acetic acid induced writhing response: The method described by Dharmasiri et al., was followed for the evaluation of analgesic activity of MEHJ with slight modifications. Number of rats in each group are 5. Normal saline (2 ml/kg) was given to control group orally. Standard group received diclofenac 5 mg/kg and test groups received MEHJ at different doses of 50, 100, 200, 400 mg/kg. After 30 min, all the animals in different groups received 0.7% 10 ml/kg acetic acid solution intraperitoneally [9]. The writhing's induced by acetic acid include back limb stretching's and stomach restrictions which are considered for about 30 min with 5 min latency period. A notable deduction of writhes in test group animals analogized to normal saline group (control) was appraised as a significant antinociceptic reaction.

Eddy's hot plate test

Six groups of animals are taken, each group contain 5 animals. Control is group I. Standard is group II and received tramadol 20 mg/kg intraperitoneally. Test groups are III, IV, V and VI and they received MEHJ orally of doses 50, 100, 200 and 400 mg/kg body weight respectively. All the animals 1 hour after their respective treatments are positioned separately on the hot plate kept at 55°C. The response of the animal to pain stimulus either by jumping or paw licking (whichever appeared first) was noted as the time at which the animal reacted. 15 seconds was the cut off time for the reaction [10]. The response was noted before (0 min) and after drug administration for 0.5, 1, 1.5, 2 and 3 hr.

Tail immersion test

Six groups of animals are taken, each group contain 5 animals. Control is group I. Standard is group II and received tramadol 20 mg/kg intraperitoneally. Test groups are III, IV, V and VI and they received MEHJ orally of doses 50, 100, 200 and 400 mg/kg body weight respectively. After 1 hour of therapy, the tip of animal tail approximately up to 5 cm was dipped in hot water which was sustained at 58°C. Unexpected removal of tail from 58°C hot water was noted as the response time. In order to circumvent injury to the tail cut off time was 10 seconds for all groups of animals [11]. To assess response to noxious stimulus, the time taken for flicking the tail was noted.

In-silico molecular docking

Computational study: The molecular docking studies are carried on cyclooxygenase-2 (COX-2) enzyme. The structure of COX-2 was acquired via protein data bank (ID: 4PH9). Three dimensional structure of COX-2 (4PH9) was given in Figure 1. The plant constituents from *Hypericum japonicum* was collected from literature wok testimonial [12].

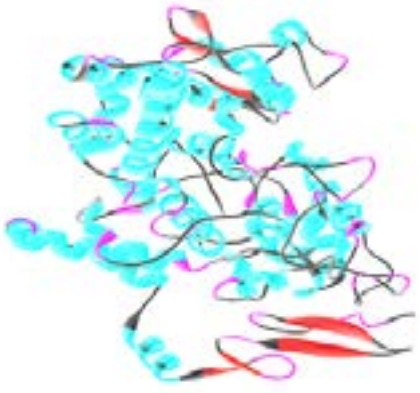

Figure 1: Three dimensional structure of COX-2 (4PH9)

Preparation of ligand structures

Selected ligand structures are downloaded from PubChem database in 3D SDF format. 3D SDF chemical structures of ligands are transformed to .pdb format by using BIOVIA Discovery Studio Visualizer. After that ligand in .pdb format is opened in AutoDock Tools (ADT) for amplification Gasteiger moderation and rotatable bonds [13]. Ligand structure in .pdb format is converted to .pdbqt format by using AutoDock vina. Three dimensional structures of selected ligands are given in Figure 2.

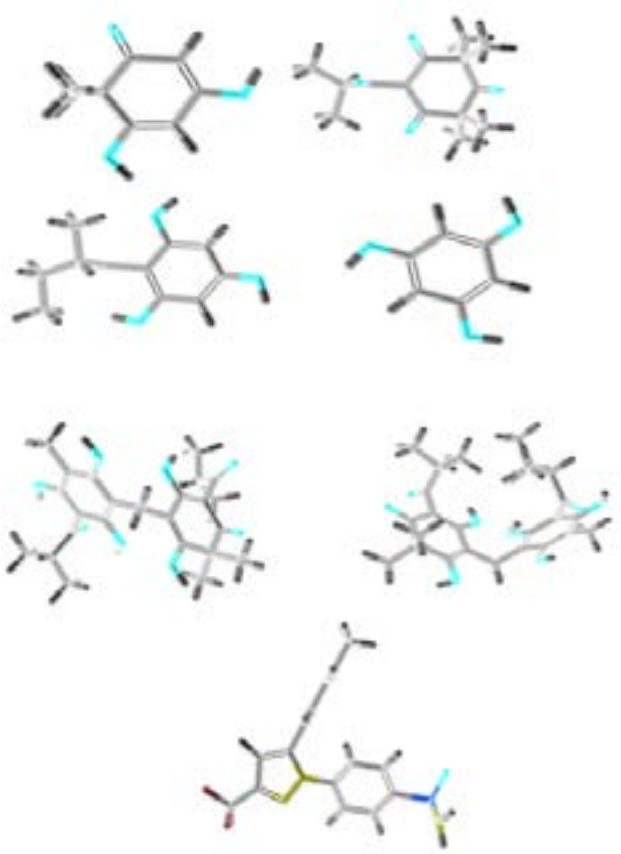

Figure 2: Three dimensional structure of ligands A. Filicinic acid, B. Flavesone, C. Multifidol, D. Phloroglucinol, E. Saroaspidin A, F. Saroaspidin B, G. Celecoxib

Preparation of COX-2 protein

The 3D structure of protein COX-2 (Id: 4PH9) was downloaded from RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Base). The COX-2 was opened in BIOVIA Discovery Studio Visualizer. Then remove water molecules, next find the list of ligands inside by define and edit the binding sites. Binding sphere is generated; make sure that all the ligands are enclosed in the binding sphere. Click on the sphere, it turns yellow in colour and then right click it to get the attributes x, y and z. Note x, y and z parameters in the notepad [14]. Then remove the ligand and add polar hydrogen molecules. Now this is the prepared protein so save this as protein. pdb format. Open protein.pdb in Auto Dock vina, go to grid macromolecule choose. Select protein molecule. Initializing pop up will appear on screen. Now save this as protein.pdbqt.

Docking methodology

Ligand.pdbqt and protein.pdbqt are ready now. In command prompt, by using various command instructions complex formed is split into poses. Protein.pdbqt and poses are opened in BIOVIA Discovery Studio Visualizer. By using receptor-ligand interaction we can find out which pose is having best binding affinity with receptor [15]. The rest of the poses are deleted. 2D and 3D structures of the selected pose are taken and results are interpreted.

ADMET analysis

Pharmacokinetic properties of the ligands in order to understand the function of ligands inside the body ADMET analysis was done by using admetSAR. Lipinski's rule of five (RO5), is a rule that a chemical compound with certain physical properties, chemical properties and pharmacological activity that can be orally active drug in humans to evaluate drug likeness [16].

The rule outlines the pharmacokinetic of drugs such as absorption (A), distribution (D), metabolism (M) and excretion (E) in the human body but pharmacodynamics properties of a drug cannot be confirmed. In our study, we used swissADME and admeSAR software to assess the ADME and noxious effects of the isolated compounds from *Hypericum japonicum*. In this swissADME and admeSAR software, we used the canonical smiles to for ADME/T analysis.

Statistical analysis

By using one way ANOVA and multiple comparisons by Dunnetts test data comparison was done as mean ± SEM. At $P<0.05$, <0.01 and <0.001 values are considered to be significant statistically.

Results

Phytochemical analysis

The phytochemical screening of methanolic extract of *Hypericum Japonicum* revealed the appearance of saponins, alkaloids, terpenoids, glycosides, flavonoids, tannins, carbohydrates, proteins, amino acids and fixed oils (Table 1). Specific phytoconstituents present in *Hypericum Japonicum* are obtained from literature survey [17].

Table 1: Phytochemical screening of successive solvent extraction of MEHJ

Phytoconstituents	Method	Methanolic extract
Flavonoids	Shinoda Test	+
	Zn. Hydrocholride Test	+
	Lead acetate Test	+
Alkaloids	Wagner Test	+
	Hager's Test	+
Steroids	Salkowski Test	+
Saponins	Foaming Test	+
Tannins & Phenols	Fecl3 Test	+
	Pot.Dichromate Test	+
Volatile Oil	Stain Test	+
Glycoside	Keller-Killani Test	+
Carbohydrates	Molish Test	+

Acid Compounds	Litmus Test	-
Proteins	Biuret	+
Amino Acids	Ninhydrin Test	+

In-vivo analgesic activity

In acetic acid induced writhing response, all rats in different groups displayed writhings in significant number compared to that of control. Standard group treated with diclofenac showed low number of writhings collated to other treated groups. Between the treated groups, group treated with MEHJ 400 mg/kg showed significant inhibition of writhings. The results are presented in Table 2.

Table 2: Effect of methanolic extract of *Hypericum Japonicum* by acetic acid induced writhing response

Experimental Groups	Dose (mg/kg)	Number of Writhes	% inhibition
Control (acetic acid)	0	25.2 ± 2.11	0
Diclofenac	5	7.63 ± 1.56	69.72
MEHJ	50	23.12 ± 1.36	8.2
MEHJ	100	19.25 ± 0 .99	23.61
MEHJ	200	18.36 ± 1.11	27.14
MEHJ	400	10.11 ± 1.45	59.88

Table 3: Effect of methanolic extract of *Hypericum Japonicum* by hot plate method

Treatment	Reaction time					
	0 min	0.5 hr	1 hr	1.5 hr	2 hr	3 hr
Normal Saline (2 ml/kg)	3.17 ± 0.01	3.12 ± 0.05	3.18 ± 0.08	3.20 ± 0.04	3.21 ± 0.08	3.23 ± 0.05
Tramodol (20 mg/kg)	3.16 ± 0.05	5.12 ± 0.12 (62.02)	5.53 ± 0.05 (75.00)	5.82 ± 0.09 (84.17)	4.98 ± 0.11 (57.59)	4.85 ± 0.08 (53.48)
MEHJ (50 mg/kg)	3.34 ± 0.06	3.41 ± 0.08 (2.09)	3.62 ± 0.07 (8.38)	3.99 ± 0.08 (19.46)	3.68 ± 0.07 (10.17)	3.55 ± 0.12 (6.28)
MEHJ (100 mg/kg)	3.21 ± 0.11	3.32 ± 0.09 (3.42)	4.23 ± 0.05 (31.77)	4.45 ± 0.11 (38.62)	4.26 ± 0.12 (32.71)	3.91 ± 0.11 (21.8)
MEHJ (200 mg/kg	3.43 ± 0.06	3.81 ± 0.08 (11.07)	4.13 ± 0.07 (20.4)	4.82 ± 0.09 (40.52)	4.29 ± 0.14 (25.07)	4.09 ± 0.12 (19.24)
MEHJ (400 mg/kg	3.53 ± 0.07	4.23 ± 0.09 (19.83)	4.84 ± 0.08 (37.11)	5.75 ± 0.06 (62.88)	5.23 ± 0.15 (48.15)	4.96 ± 0.11 (40.5)

Results of antinociceptive activity by Eddys hot plate method are presented in Table 3. Different doses of MEHJ extract and standard group showed notable elevation in latency time collated to control group at 1.5 hr. between the extracts MEHJ (400 mg/kg) manifested the best elevation in latency time.

Table 4: Effect of methanolic extract of *Hypericum Japonicum* by tail immersion test

Treatment	Reaction time					
	0 min	0.5 hr	1 hr	1.5 hr	2 hr	3 hr
Normal Saline (2 ml/kg)	2.51 ± 0.08	2.62 ± 0.07	2.70 ± 0.07	2.53 ± 0.04	2.49 ± 0.11	2.56 ± 0.12
Tramodol (20 mg/kg)	2.39 ± 0.11	3.85 ± 0.12 (61.08)	4.36 ± 0.09 (82.42	4.21 ± 0.12 (76.15)	3.99 ± 0.04 (66.94)	3.76 ± 0.14 (57.32)
MEHJ (50 mg/kg)	2.36 ± 0.09	2.45 ± 0.07 (3.81)	2.54 ± 0.09 (7.62)	2.61 ± 0.14 (10.59)	2.51 ± 0.09 (6.35)	2.45 ± 0.15 (3.81)
MEHJ (100 mg/kg)	2.33 ± 0.07	2.49 ± 0.09 (6.86)	2.65 ± 0.08 (13.73)	2.71 ± 0.09 (16.3)	2.51 ± 0.09 (6.35)	2.45 ± 0.09 (5.15)
MEHJ (200 mg/kg)	2.35 ± 0.06	2.85 ± 0.11 (21.27)	3.11 ± 0.07 (32.34)	3.48 ± 0.11 (48.08)	3.23 ± 0.07 (37.44)	2.89 ± 0.08 (22.97)
MEHJ (400 mg/kg)	2.29 ± 0.13	3.32 ± 0.13 (44.97)	3.62 ± 0.06 (58.07)	3.74 ± 0.03 (63.31)	3.59 ± 0.09 (56.76)	3.21 ± 0.06 (40.17)

In Table 4, the results of analgesic activity by tail immersion test are represented. Different doses of MEHJ extract and standard group showed notable elevation in latency time collated to control group at 1.5 hr. Between the extracts MEHJ (400 mg/kg) manifested the best elevation in latency time.

In-silico study for the evaluation of analgesic activity

Tables 5 and 6 represents the physicochemical properties of phytoconstitutents of *Hypericum Japonicum* required for drug likeliness and ADME/T analysis. By analyzing the results given in these tables we can predict the eligibility of a plant constituent to pass as a safe compound.

Table 5: Drug likeliness analysis of separated phytoconstituents from *Hypericum Japonicum*

Compound	Total Mol. Weight	H-Acceptors	H-Donors	cLogP	Rotatable Bonds	Molar Refractivity	No. of Deviations
1,2,5-Trihydroxyxanthone	244.2	5	3	1.99	0	66.06	0
Euxanthone	228.2	4	2	2	0	64.04	0
Filicinic acid	154.16	3	2	1.02	0	40.59	0
Flavesone	252.31	4	0	2.3	2	67.58	0
Isoquercitin	464.38	12	8	2.11	4	110.16	2
Kielcorin	436.41	8	2	3.45	4	116.32	0
Mesuaxanthone B	244.2	5	3	1.43	0	66.06	0
Multifidol	210.23	4	3	1.55	3	57.13	0
Phloroglucinol	126.11	3	3	0.66	0	32.51	0
Saroaspidin A	446.49	8	5	2.52	6	120.16	0
Saroaspidin B	460.52	8	5	2.65	7	124.96	0
Sarothralin G	602.71	8	5	4.89	11	172.54	1
2.29 ± 0.13	2.29 ± 0.13	2.29 ± 0.13	2.29 ± 0.13	2.29 ± 0.13	2.29 ± 0.13	2.29 ± 0.13	2.29 ± 0.13

Table 6: ADMET properties of separated phytoconstituents from *Hypericum Japonicum*

Compound	HIA	BBB	AMES Toxicity	Carcinogenicity	LD50 in rats
Filicinic acid	0.9831	0.918	Non-toxic	Non-carcinogenicity	2.538
Flavesone	0.9825	0.984	Non-toxic	Non-carcinogenicity	1.455
Multifidol	0.996	0.3391	Non-toxic	Non-carcinogenicity	5.282
Phloroglucinol	0.9853	0.7298	Non-toxic	Non-carcinogenicity	4.374
Saroaspidin A	0.9916	0.2444	Non-toxic	Non-carcinogenicity	5.281
Saroaspidin B	0.9916	0.8103	Non-toxic	Non-carcinogenicity	5.846

Table 7: Binding affinities of separated phytoconstituents from *Hypericum Japonicum*

Compound	1	2	3	4	5	6	7	8	9
1,2,5-Trihydroxyxanthone	-8.2	-7.8	-7.3	-7.1	-7	-7	-6.6	-6.5	-6.4
Euxanthone	-7.7	-7.7	-7.1	-6.6	-6.5	-6.4	-6.4	-6.4	-6.4
Filicinic acid	-6.3	-6.3	-6.1	-6.1	-5.7	-5.7	-5.4	-5.3	-5.3
Flavesone	-6.3	-5.5	-5.4	-5.4	-5	-4.9	-4.9	-4.9	-4.5
Isoquercitin	-7.8	-7.8	-7.7	-7.7	-7.7	-7.6	-7.5	-7.4	-7.2
Kielcorin	-8.2	-8	-7.9	-7.8	-7.6	-7.1	-6.9	-6.7	-6.6
Mesuaxanthone B	-8.2	-7.4	-6.7	-6.7	-6.4	-6.3	-6.3	-6.3	-6.2
Multifidol	-6.2	-6.2	-6.2	-6	-6	-5.7	-5.7	-5.7	-5.7
Phloroglucinol	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5
Saroaspidin A	-6.8	-6.5	-6.4	-6.4	-6.4	-6.3	-6.3	-6.3	-5.9
Saroaspidin B	-7.1	-7	-6.9	-6.7	-6.7	-6.6	-6.6	-6.6	-6.6
Sarothralin G	-8.9	-8.4	-8.4	-8.3	-8.3	-8.1	-8	-7.9	-7.9
Celecoxib	-7.4	-7.0	-6.9	-6.6	-6.5	-6.3	-6.2	-6.1	-6.1

Docking rates are given in Table 7. Saroaspidin B had the most excellent docking score of -7.1 kcal/mol, which showed both hydrogen bond and hydrophobic interactions with COX-2 enzyme. The standard celecoxib revealed the

Table 8: Interactions of COX-2 with ligands at receptor sites

Compound	Hydrogen Binding Interaction	Hydrophobic Interaction
Filicinic acid	TYR (A:386), MET (A:523)	---
Flavesone	THR (A:213), HIS (A:387)	---
Multifidol	TRP (A:388)	LEU (A:392), ALA (A:200)
Phloroglucinol	TRP (A:388), ALA (A:203), HIS (A:389), HIS (A:208)	---
Saroaspidin A	HIS (A:387)	HIS (A:215), VAL (A:292), ARG (A:223)
Saroaspidin B	HIS (A:208), ASN (A:383), GLN (A:455)	THR (A:213), HIS (A:387)
Celecoxib	GLN (A:290), ASN (A:383), THR (A:213)	VAL (A:448)

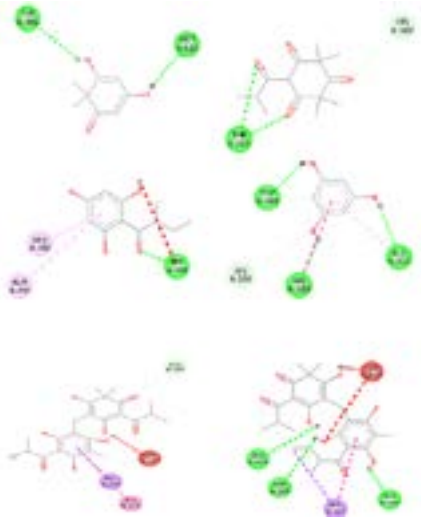

Figure 3: Two dimensional interactions of ligands with COX-2 (4PH9) A. Filicinic acid, B. Flavesone, C. Multifidol, D. Phloroglucinol, E. Saroaspidin A, F. Saroaspidin B G. Celecoxib

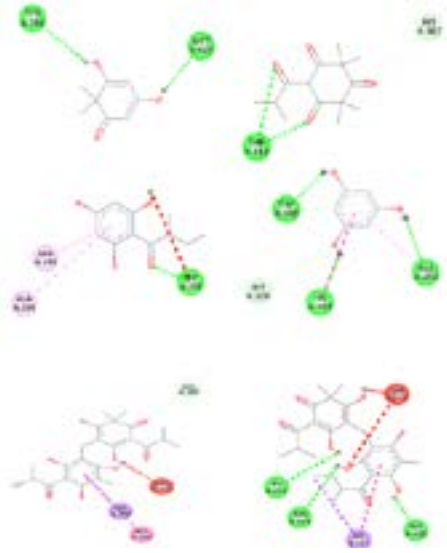

Figure 4: Three dimensional interactions of ligands with COX-2 (4PH9) A. Filicinic acid, B. Flavesone, C. Multifidol, D. Phloroglucinol, E. Saroaspidin A, F. Saroaspidin B, G. Celecoxib

docking score of -7.4 kcal/mol which is comparable to saroaspidin B.

Protein ligand interactions revealing hydrogen binding and hydrophobic interactions are given in Table 8 and published in Figures 3 and 4.

Discussion

By extraction of different plant parts various phytoconstituents are obtained that are responsible for therapeutic activity when used in medical purpose. For pharmacological action secondary metabolites are vitally responsible. Practically all the plants primary metabolites are established in every plant not all the secondary metabolites were found, they differ from plant to plant. So this is the reason that all plants do not show every kind of therapeutic or pharmacological reaction. Hence identification of phytoconstituent is the major step in the area of drug discovery. In the evaluation of *in-vivo* analgesic activity by acetic acid induced writhing response, from tissue phospholipids there is liberation of arachidonic acid (AA) which is considered a pain stimuli when 0.7% acetic acid is injected intra peritoneally. It is established that prostaglandins (PG) pathway or acid sensing ion channels or peritoneal most cells are responsible for induction of peripheral pain [18]. Peripheral analgesic effect of MEHF is given in Table 2. Opioid receptor agonist and centrally acting analgesics are effectively evaluated by Hot plate method and Tail immersion test. It is know that spinal and supra spinal receptors are the targets for opioid receptor agonist. For thermal induced nociception μ receptor agonist are more effective among these opioid receptor agonists. Hence this is the reason why hot plate method and tail immersion test are appraised to be very effective in the evaluation of peripheral analgesic activity [19]. Antinociceptive activity of MEHJ by hot plate method and tail immersion test are represented in Tables 3 and 4 respectively.

To identify the potential phytoconstituents from MEHJ for antinociceptive activity, first we investigated the compatibility of a phytoconstituent to be used as drug molecule. For this objective, some parameters are taken into consideration like molecular weight (<500 gm/ml), hydrogen bond acceptor (≤10), hydrogen bond donor (≤5), clogP (<5) and molar refractivity (40-130) [20]. These values are given in Tables 5 and 6. 6 out of 12 compounds have the capability to be used as a drug molecule. Next, we illustrated *in-silico* molecular docking rate, glide energy rate and glide e-model score on phytoconstituents of MEHJ with COX-2 enzyme in order to evaluate antinociceptive activity of *Hypericum Japonicum* in molecular level [21]. Selective COX-2 inhibitors are preferable option to stop pain stimulus as COX-2 enzyme is the main culprit for acute pain [22]. Since COX-2 inhibitors are having extensive scope of adverse effects, finding replacement for selective COX-2 inhibitors is obligatory [23]. In our research saroaspidin B manifested very appreciable docking scale of -7.1 kcal/mol against COX-2 enzyme where as our standard drug celecoxib manifested -7.4 kcal/mol docking score which can be compared to our phytoconstituents. So, it may be possible that these phytoconstitutents saroaspidin B behave as favourable hit molecules for selective COX-2 inhibitors [24].

Conclusion

Medical plant had extensive scope of activity so they are considered as best source of medications for humans. It is our interest to select the plant, isolate the suitable phytoconstituents for the desirable activity, enhance them and formulate that phytoconstituent better with good potency and lesser side effects. In this study, we found that methanolic extract of *Hypericum Japonicum* has significant *in-vivo* antinociceptive activity. In addition to that, *in-silico* molecular docking and ADMET analysis were performed on various phytoconstituents present in MEHJ to find out the suitable phytoconstituent responsible for this antinociceptic activity. Our investigations revealed that saroaspidin B had good possibility to act as selective COX-2 inhibitor. In order to establish these phytoconstitutents as potent antinoceptive drug molecules vigorous study is suggested as this is a crude research.

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Conflict of Interest

None

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