In vivo and in silico Evaluation of Analgesic and Hypoglycemic Activities of Roots of Acacia nilotica, Azadirachta indica and Justica adhatoda

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ABSTRACT: The present study focuses on the investigation of methanol extracts of roots of three indigenous plants of Bangladesh namely *Acacia nilotica, Azadirachta indica* and *Justicia adhatoda* to evaluate their analgesic and hypoglycemic activities in experimental animal model along with *in silico* modelling of several compounds present in the root extracts of these plants. Analgesic and hypoglycemic activities were evaluated in Swiss albino mice using acetic acid-induced writhing inhibition method and glucose tolerance test, respectively. *In silico* molecular docking and ADME study was conducted to assess the binding affinity with the target receptors and oral bioavailability of the compounds. The methanol extracts of *A. nilotica, J. adhatoda* and *A. indica* roots at a dose of 400 mg/kg body weight reduced the number of writhes by 61.53%, 54.61% and 47.69%, respectively compared to standard diclofenac sodium (70.77% at a dose of 50 mg/kg bw) ($p<0.05$). *A. nilotica* and *A. indica* root extracts showed significant hypoglycemic activity at a dose of 400 mg/kg bw (% reduction of blood glucose 43.66 and 37.55% respectively, $p<0.001$) and *J. adhatoda* root extract reduced the blood glucose level by 33.71% ($p<0.001$) compared to that of standard drug, glibenclamide (57.46% reduction of blood glucose) after 120 minutes of administration. Among the computationally tested compounds, flavan-3-ol showed the lowest binding energy (-8.7 kcal/mol) with both COX-1 and COX-2 compared to standard diclofenac sodium (-7.8 kcal/mol). On the other hand, quercetin demonstrated the lowest binding energy (-8.8 kcal/mol) with ATP-sensitive potassium channel with sulfonylurea receptor 1 subunit among the tested compounds compared to standard glibenclamide (-9.3 kcal/mol). All the compounds showed high oral bioavailability in ADME analysis. Among all the root extracts, *A. nilotica* exhibited the most promising analgesic and hypoglycemic activities and should be employed to future investigation for isolating specific chemical constituents.

Key words: *Acacia nilotica, Azadirachta indica, Justicia adhatoda*, methanol root extracts, analgesic, hypoglycemic

INTRODUCTION

The use of medicinal plants for the prevention and cure of different diseases has been in practice from the time immemorial. There is an uprising demand for the herbal drug treatment of different diseases and a variety of medicinal plants are being explored in Ayurvedic system. Numerous biologically active secondary metabolites have been discovered and identified from hundreds of medicinal plants which are paving the ways to invent safe and efficacious remedies for different ailments.[1] The present estimation of herbal medicines usage for health care is about 4 billion.[2] Medicinal plants are the prominent reservoir of natural molecules with multifarious pharmacological activities, which may appear as potential leads for new drugs discovery and development. Therefore, medicinal plants have attained the center of attention in research and development of new drug to reveal the facts behind their traditional uses[3] and the aims of researchers are to develop of new prototypic compounds with low cost and less side effects.[4] Based on the pharmacological properties of indigenous medicinal plants new herbal, Ayurvedic and Chinese medicines

are incorporated in the market as alternative treatments for many disorders.

To explore the therapeutic potentials of indigenous plants we have been continuously investigating several prominent medicinal plants. Earlier, we have identified the medicinal properties of a number of plant extracts.[5-8] Herein, we have selected three plants namely *Acacia nilotica* (Family: Fabaceae), *Azadirachta indica* (Family: Meliaceae) and *Justicia adhatoda* (Family: Acanthaceae) based on their traditional uses and phytoconstituents. *A. nilotica, A. indica* and *J. adhatoda* plants under investigation are enriched in several secondary metabolites including flavonoids and phenolic compounds. The root of *A. nilotica* contains catechin, ethyl gallate, flavan-3-ol, betulin, octaconsanol, β-sitosterol and β-amyrin which is traditionally used in cancer, tuberculosis and induration of liver and spleen.[9] For more than 4000 years, *A. indica* is considered as natural drug reservoir in Ayurvedic medicine due to broad spectrum of medicinal properties of all parts of this plant. Some compounds isolated from *A. indica* are quercetin, nimbin, azadirachtin, meliantriol and salannin which possess insecticidal and pesticidal activities.[10,11] *J. adhatoda* is a rich source of pyrroquinazoline alkaloids like vasicinol, vasicine, vasicinone, peganine and other constituents in minor amount.[12,13] The traditional uses of this plant in the treatment of asthma, cold, cough, rheumatism, swelling and venereal diseases, joint and lumber pain, eczema, malaria etc. are found in Indian medicine.[14]

In Bangladesh, different parts of the selected plants are used traditionally for the treatment of a number of health ailments. For example, leaves of *J. adhatoda* are used as antitussive medicine[15], leaves and barks of *A. indica* are used against different skin infections, cancer and pests[10,16], and seeds and leaves of *A. nilotica* possess antimalarial, antimicrobial, antidiarrheal, astringent, antihypertensive and antispasmodic properties.[17] Although leaves, barks, stems, flowers, fruits and roots of the three plants have diversified ethno-pharmacological uses, there is lack of sufficient reports on the traditional uses of the

root parts as they have not been investigated adequately for the biological activities. Therefore, we decided to test the analgesic and hypoglycemic properties of the roots of these plants via *in vivo* and *in silico* approaches. We selected these three plants because the root parts of these plants have not been investigated widely. In this study we aimed to investigate and compare the biological activity of the root extracts of these plants in order to determine which root had the highest potential of possessing natural lead compounds.

In silico molecular docking and ADME study are considered as reliable tools to identify active sites of the target receptors and to analyze binding interactions of compounds with the proteins along with prediction of drug like characteristics of the molecules.[18] In this study, several components reported to be present in the roots of the selected three plants were analyzed for their binding affinity with the target receptors for analgesic and hypoglycemic activities. The present study concentrates on the exploration of analgesic and hypoglycemic activities of the methanolic crude extracts of the roots of three conspicuous medicinal plants namely *A. nilotica, A. indica* and *J. adhatoda* including *in silico* analysis of the compounds present in the roots of the plants.

MATERIALS AND METHODS

Drugs and chemicals. The solvents and chemicals were obtained from University laboratory stock. Glibenclamide was collected as Dibenol Tablet and diclofenac sodium as clofenac Injection, from Square Pharmaceuticals Ltd. Bangladesh. Normal saline solution was acquired from Beximco Pharmaceuticals Ltd., Bangladesh. Tween-80 was obtained from BDH Chemicals, UK. All analytical reagent grade chemicals were used in the experiment.

Collection of plant materials. Fresh roots of *A. nilotica* and *A. indica* were collected from Noakhali and fresh roots of *J. adhatoda* were collected from Gazipur, Dhaka. The plants were identified in Bangladesh National Herbarium, Dhaka where voucher specimens have been deposited with the

accession numbers as DACB-38214, DACB-38215 and DACB-38213 for *A. nilotica, A. indica* and *J. adhatoda,* respectively.

Preparation of extract. The collected roots of the three plants were washed, cut into small pieces and air dried and then oven dried at temperature below 30° C for 24 hours to facilitate grinding into coarse powder and was stored in air-tight container in cool, dark and dry place for future use. About 700gm of the powdered root of each plant was taken in three different clean desiccators (5 liters) and soaked in 2.5 liter of methanol each. The containers were covered by aluminum foil and kept for 15 days with random stirring and shaking. The extractable components of the roots in the solvent was filtered using cloth and finally with Whitman filter papers. The weights of the crude extracts of *A. nilotica, A. indica* and *J. adhatoda* were found to be 60 gm, 35 gm and 16 gm, respectively. After drying, the extracts were preserved in the refrigerator to prevent microbial contamination. The test doses were prepared adding the respective extract with normal saline solution and 1% Tween 80 as required.

Experimental animal. Swiss-albino mice (*Mus musculus*) of either sex, 4-5 weeks of age, obtained from Jahangir Nagar University Animal House, Savar, Bangladesh were used in the experiment. The animals were kept in polypropylene cages (30cm x 20cm x 13cm) in standard conditions for (21 ± 1° C with a 12 h light and dark cycle) seven days prior to the experiment. The animals were supplied with standard diet and water ad libitum. The experiment was performed in the Physiology Laboratory of the Faculty of Pharmacy at University of Dhaka. After completion of the experiment the mice were returned to the officer of the animal husbandry for necessary action regarding the animals.

Analgesic activity. Acetic acid induced writhing method was used to evaluate the analgesic activity[19,20]. Forty mice were divided into eight groups randomly by generating random numbers using randomizing function in Microsoft Excel. Each group consisted of five mice receiving a particular treatment. Before each experiment, each mouse was

weighed accurately and the doses of the test samples and control materials were adjusted accordingly. The crude methanolic extracts of *A. nilotica, A. indica* and *J. adhatoda* were administered at both doses of 400 mg/kg and 200 mg/kg bw of mice. Diclofenac sodium was used as standard at the dose of 50 mg/kg bw and 1% Tween 80 in normal saline at the dose of 0.2 ml/10 gm of body weight was used as control. After 30 minutes of administration of the materials to allow proper absorption, acetic acid (0.7 %) was administered intraperitoneally at a dose of 0.1 ml/10 gm to each animal. After 5 minutes, the number of writhing was counted for each mouse for 15 minutes. The writhing counts of the test samples and diclofenac sodium treated groups were compared to those of the animals in the control group. Percent inhibition of writhing in comparison to control group was considered as an index of analgesia and was calculated using the following formula:

$$\% \text{ inhibition} = (Wc-Wt) \times 100)/Wc,$$

Here, Wc denotes the average number of writhing in the control group and Wt denotes the average number of writhing in the test group.

Hypoglycemic activity. Hypoglycemic activity was studied in glucose-loaded hyperglycemic mice.[21,22] Forty mice were fasted for 18 hours with free access to water and divided into eight groups randomly by generating random numbers using randomizing function in Microsoft Excel. Each group consisted of five mice receiving a particular treatment. Fasting blood glucose level was checked by collecting blood samples from tail vein. Before any treatment, each mouse was properly weighed and the doses of the test samples and control materials were adjusted accordingly. The crude methanolic extracts of *A. nilotica, A. indica* and *J. adhatoda* roots were administered at both doses of 400 mg/kg and 200 mg/kg bw of mice. The standard glibenclamide was administered at the dose of 5 mg/kg body weight and 1% Tween 80 in normal saline at the dose of 0.2 ml/10 gm of body weight was used as control. After 30 min of administration, the mice of all groups were orally treated with 10% glucose solution (zero minute) at 2gm/kg bw. Blood

samples were collected from tail vein just before glucose administration and at 30 minutes, 60 minutes, 90 minutes and 120 minutes after glucose loading. Blood glucose level was measured by using glucometer and glucose oxidase-peroxidase reactive strips[23] and compared with the control group to calculate the hypoglycemic activity of the standard and test samples using the following formula:

$$\% \text{ reduction of blood glucose level} = ((Gc-Gt) \times 100)/Gc$$

Here, Gc is the average blood glucose level in the control group and Gt is the average blood glucose level in the test group.

Statistical evaluation. All values from the experiments were demonstrated as the mean ± standard error of the mean (SEM) and the results were analyzed statistically by One Way Analysis of Variance (ANOVA) followed by Dunnett's test by using SPSS (version 19, IBM Incorporation, USA). The $p < 0.05$ was considered to be statistically significant. The outcome assessment and the data analysis were performed blind as the analyst was not aware of the allocation of the groups.

Selection of compounds and target proteins for *in silico* molecular docking. Eight different compounds reported to be present in the selected plants were chosen for computational analysis of analgesic and hypoglycemic activities compared to standard glibenclamide (PubChem CID 3488) and diclofenac (PubChem CID 3033). The compounds were catechin (PubChem CID 73160), ethyl gallate (PubChem CID 13250), flavan-3-ol (PubChem CID 3707243) nimbin (PubChem CID 108058), quercetin (PubChem CID 5280343), vasicine (PubChem CID 442929), vasicinol (PubChem CID 442934) and vasicinone (PubChem CID 10242). The structures of the selected compounds were obtained from PubChem and optimized using the software package Avogadro (Version 1.2), an open-source molecular builder and visualization tool. The MMFF94 force field model was used to optimize the molecules until further optimization runs resulted in no further changes in geometry. The three-dimensional structures of the protein targets selected for the

molecular docking study were obtained from the Protein Data Bank RCSBPDB[24] which included cyclooxygenase-1 (PDB: 1EQG)[25], cyclooxygenase-2 (PDB: 5IKT)[26] for analgesic activity and ATP-sensitive potassium channel with sulfonylurea receptor-1 (SUR1) subunit (PDB: 5YW7)[27] for the hypoglycemic activity. The enzymes/receptor were prepared for docking by first removing all the heteroatoms and water molecules from the crystal structure using PyMol (version 2.4.1) software packages.[28] Swiss-PdbViewer software packages (version 4.1.0) were used to minimize energy where computations were carried out *in vacuo* with the GROMOS 96 43B1 parameters set, without reaction field.[29] Autodock Vina docking protocol was used using the PyRx (Version 0.8) virtual screening tool and grid box dimensions were selected based on the ligand binding site coordinates of the three-dimensional structures of the protein targets obtained through PyMol.[30] BIOVIA Discovery Studio Visualizer 2016 was employed for assessing the docking positions for best ligand macromolecule interactions.

RESULTS AND DISCUSSION

Analgesic activity. The analgesic activity of the methanolic extracts of the roots of three plants are summarized in table 1. All the plant extracts showed statistically significant analgesic activity in a dose dependent manner. *A. nilotica* revealed potent analgesic activity with writhing inhibition of 61.53% at the dose of 400 mg/kg bw compared to standard diclofenac sodium (70.77% writhing inhibition at 50 mg/kg bw). *A. indica* and *J. adhatoda* demonstrated mild to moderate analgesic activity at 400 mg/kg bw with writhing inhibition of 47.69% and 54.61%, respectively. Acetic acid-induced writhing method is mainly used for the evaluation of peripheral analgesic activity of the test samples considering reduction of writhing count as an index of peripheral analgesia.[31] Acetic acid produces pain sensation by activating localized inflammatory response to secret free arachidonic acid which in turn increases PGE_2 and $PGF_{2\alpha}$ in peritoneal fluid.[32] It may also involve the mast cells and macrophages which secret cytokines,

e.g. interleukin 1β (IL-1β), interleukin 8 (IL-8) and tumor necrosis factor α (TNF-α) which is also regulated by prostaglandin pathway.[33] The standard diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID) which acts on cyclooxygenase 2 (COX 2) enzyme to block prostaglandin synthesis causing anti-nociceptive action. The percent writhing inhibitions of the methanol extracts of the plant roots are comparable to that of the standard diclofenac sodium. So, the pain reducing activity might be because of its ability to interfere in the biosynthesis of some chemical agents such as prostaglandins and leukotrienes from cyclooxygenase and lipooxygenase pathway which are accountable for blocking pain sensation and hence exhibited promising analgesic activity.

Table 1. Screening of analgesic activity of plant extracts by acetic acid (0.7%) induced writhing method.

Animal group	Test sample dose (mg/kg bw)	Mean ± SEM	% of writhing	% of inhibition
Control (1% Tween-80 in normal saline)	0.2 ml/10 g of body wt	26 ± 0.7	100	-
Standard (Diclofenac sodium)	50	7.6 ± 0.67*	29.23	70.77
A. nilotica	200	11.4 ± 1.02*	43.85	56.15
	400	10 ± 0.7*	38.46	61.53
A. indica	200	16.6 ± 1.02*	63.84	36.16
	400	13.6 ± 0.92*	52.30	47.69
J. adhatoda	200	15 ± 0.70*	57.69	42.30
	400	11.8 ± 0.86*	45.38	54.61

SEM = Standard error of mean. *$p<0.001$; Probability values calculated as compared to control using one way-ANOVA followed by Dunnett's test.

Hypoglycemic activity. The hypoglycemic activity of the crude methanol extracts of the roots of *A. nilotica*, *A. indica* and *J. adhatoda* are summarized in table 2 and figure 1. All the root extracts reduced blood glucose level in a dose dependent manner. At the dose of 200 mg/kg bw, all the extracts exhibited mild to moderate reduction of blood glucose level. The crude methanol extracts of *A. nilotica* and *A. indica* roots at the dose of 400 mg/kg bw showed significant ($p<0.001$) hypoglycemic activity with 43.66% and 37.55% reduction of blood glucose level compared to standard glibenclamide (57.46% reduction of blood glucose level at 5 mg/kg bw). The extract of *J. adhatoda* displayed moderate hypoglycemic activity with the reduction of blood glucose level of 33.71%. The main mechanism of causing hyperglycemia is the reduction of glucose utilization by the tissues and overproduction of glucose by hepatic glycogenolysis and gluconeogenesis.[34] The plant samples exhibited promising hypoglycemic activity with statistically significant lowering of blood glucose level. The root extracts of *A. nilotica* and *A. inidca* reduced the blood

Table 2. Screening of hypoglycemic activity of methanol extracts of the plant roots using glucose tolerance test (GTT).

Group	Dose (mg/kg bw)	Blood glucose level (mmol/l) Mean ± SEM				
		0 minute	30 minute	60 mins	90 mins	120 mins
Control (1% Tween-80 in normal saline)	0.2 ml/10 g of body wt	6.72± 0.29	17.04 ± 0.5	15.42 ± 0.5	12.44± 0.38	8.84 ± 0.40
Standard (Glibenclamide)	5	6.52± 0.28	10.94±0.38**	7.64 ± 0.31**	5.68 ± 0.16**	3.76 ± 0.33**
A. nilotica	200	6.28 ± 0.19	14.5 ± 0.34*	12.5 ± 0.34**	8.84 ± 0.25**	6.08 ± 0.35**
	400	7.00 ± 0.2	13.4 ± 0.39**	10.9 ± 0.46**	7.8 ± 0.39**	4.98 ± 0.46**
A. indica	200	7.0 ± 0.33	15.5± 0.45	13.4 ± 0.36*	10.42 ± 0.38**	6.8 ± 0.39*
	400	6.46 ± 0.24	12.42 ± 0.32**	10.08 ± 0.38**	7.58 ± 0.29**	5.52 ± 0.46**
J. adhatoda	200	6.18 ± 0.35	13.06 ± 0.45**	11.32 ± 0.35**	9.52 ± 0.37**	7.68 ± 0.26
	400	6.76 ± 0.17	11.52 ± 0.33**	9.96 ± 0.20**	7.98 ± 0.23**	5.86 ± 0.19**

SEM = Standard Error of Mean; One-way ANOVA; ** = statistically highly significant where $P<0.001$, * = statistically significant where $P<0.05$; Probability values calculated as compared to control using one way ANOVA followed by Dunnett's test using SPSS 19 program
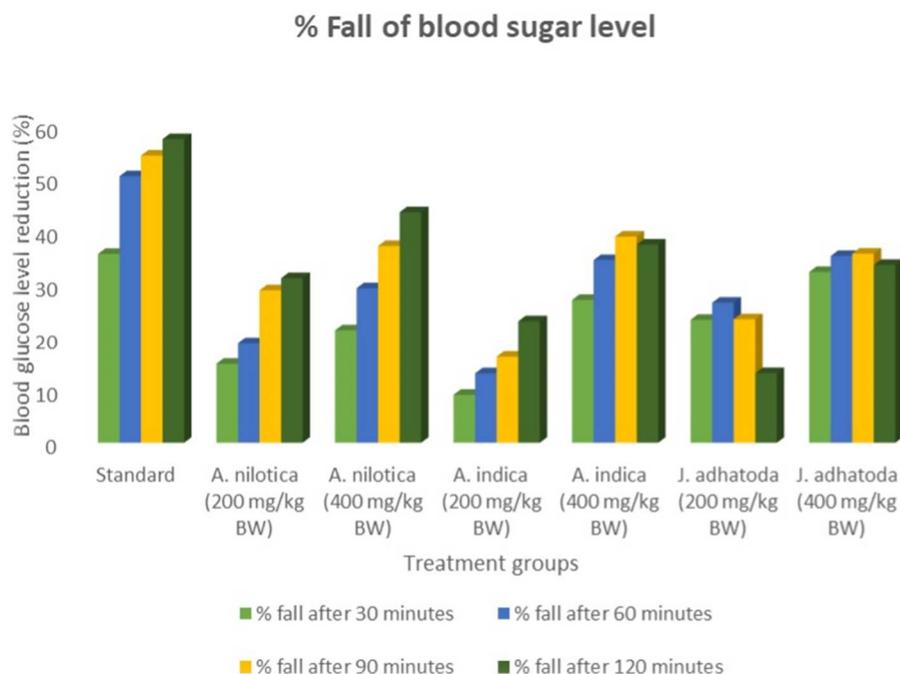
% Fall of blood sugar level

Figure 1. Comparative study of fall of percentage of blood glucose level by standard (Glibenclamide) and crude methanol extracts of *A. nilotica, A. indica* and *J. adthatoda* roots compared to control.

glucose level to normal at the dose of 200 mg/kg bw after 120 minutes of administration in the glucose loaded mice. *J. adhatoda* root extract showed reduction of blood glucose to normal level at the dose of 400 mg/kg bw after 120 minutes of administration. All the plant extracts reduced blood sugar level in a dose dependent manner. This indicates the improvement of external glucose utilizing capacity of the animals under the influence of the plant extracts which is comparable to the glucose lowering activity of the standard glibenclamide. So, it suggests that the plant root extracts may have a similar mechanism of action as the sulphonylureas to exert hypoglycemic activity.[35] Phytoconstituents present in the plant roots are responsible for these activities which should be identified for developing an effective therapeutic agent.

Molecular docking analysis. COX-1 and COX-2 enzymes catalyzes the rate limiting step in the formation of prostaglandins from arachidonic acid and so, these enzymes were selected for predicting the anti-nociceptive activity of the selected phyto-constituents by analyzing their binding affinity in the active sites of the enzymes compared to standard diclofenac. All of the tested compounds except nimbin revealed significant binding affinity towards the target enzymes (-6.6 to -8.7 kcal/mol) (Table 3). Among all the compounds, flavan-3-ol showed the highest binding affinity for both enzymes (-8.6 and -8.7 kcal/mol) compared to standard diclofenac (-8.3 and -7.8 kcal/mol) (Table 4, Table 5). Flavan-3-ol exhibited strong affinity towards the active site of COX-1 by forming stable hydrogen bonds with ALA527 residue and strong amide pi-stacked bonds with GLY526 and ALA527 (Figure 2). The compounds also shared similar hydrophobic interaction sites with diclofenac at amino acids positions VAL349 and ALA527 of the COX-1 receptor which indicate that flavan-3-ol is binding to the same pocket of COX-1 enzyme target site but with a stronger affinity than diclofenac. Even better results were observed in case of COX-2 receptor where flavan-3-ol had much higher docking scores compared to diclofenac mainly because of two strong hydrogen bonds formed with VAL349 and ALA527 residues, respectively (Figure 3). Both diclofenac and

flavan-3-ol showed binding to the same pocket of COX-2 receptor which is evident in the similar hydrophobic interactions seen with both these ligands at VAL349, ALA527, LEU531, LEU352 and VAL523 residues of the receptor.

ATP-sensitive potassium channel with SUR1 subunit was utilized for predicting hypoglycemic activity of the selected compounds compared to standard glibenclamide. Among all the tested compounds, quercetin showed the highest binding affinity with the receptor (-8.8 kcal/mol) (Table 6). All other compounds showed less binding affinity (ranging from -5.7 to -8.3 kcal/mol) (Table 3) than

glibenclamide (-9.3 kcal/mol). Quercetin demonstrated docking with the target receptor by forming strong conventional hydrogen bonds with ARG306, ARG1246 and ARG1246 residues whereas glibenclamide showed docking with active site of the receptor forming conventional hydrogen bonds with ARG306, ARG370 and ASN1273 residues which is comparable to the binding interactions of quercetin (Figure 4). Both glibenclamide and quercetin have similar interaction with ARG306, ALA380 and PRO436 residues of the target receptor which indicates that they are binding to the SUR1 subunit target site.

Table 3. Binding affinities of standard ligands and selected compounds against target proteins.

Compounds	Binding affinity (Kcal/mol)		
	Analgesic activity		Hypoglycemic activity
	COX1 (1EQG)	COX-2 (5IKT)	SUR1 (5YW7)
Standard	-8.3	-7.8	-9.3
Catechin	-6.7	-7.8	-8.3
Ethyl gallate	-6.5	-6.3	-5.7
Flavan-3-ol	**-8.6**	**-8.7**	-8.3
Nimbin	4.2	7	-8.3
Quercetin	-6.6	-8.2	**-8.8**
Vasicine	-7.4	-7.5	-6.8
Vasicinol	-6.8	-6.9	-6.8
Vasicinone	-7.7	-8.1	-7.3

Standard ligands for cyclooxygenase-1 (PDB: 1EQG) and cyclooxygenase-2 (PDB: 5IKT) is diclofenac (PubChem CID 3033). For pancreatic ATP-sensitive potassium channel with SUR1 subunit (PDB: 5YW7) the standard ligand is glibenclamide (PubChem CID 3488).

Table 4. Comparison of binding interactions of standard and the Compound with best binding affinity against cyclooxygenase-1 (PDB: 1EQG).

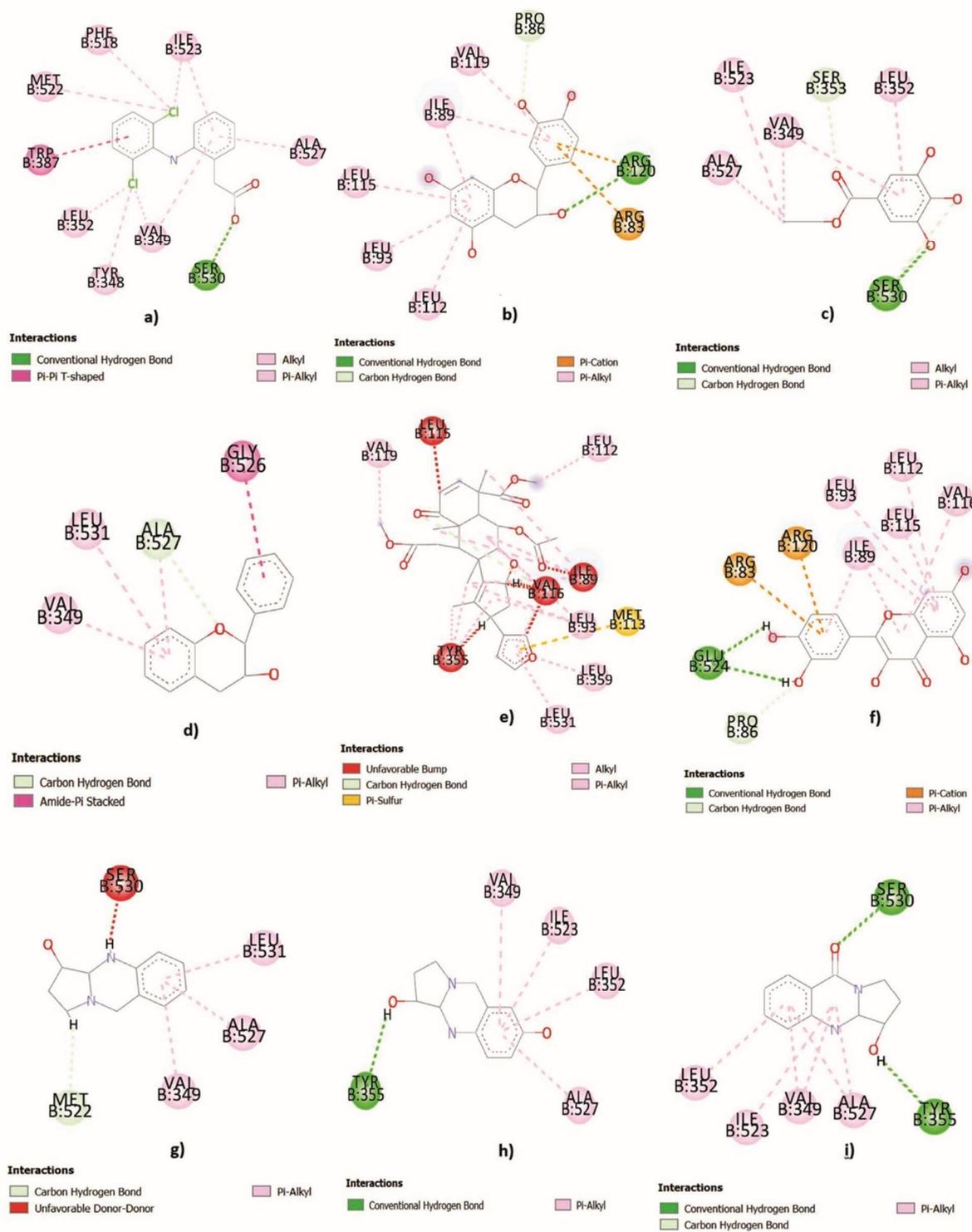| Compounds	Binding energy (Kcal/mol)	Hydrophobic interactions				Hydrogen bond			
		Bonding type	Interacting amino acids	Interacting atoms or rings	Distance (Å)	Bonding type	Interacting amino acids	Interacting atoms or rings	Distance (Å)
Diclofenac	-8.3	Pi-Pi T-shaped	TRP387	Benzene	4.957	Conventional	SER530	H…O	2.261
		Alkyl	VAL349	Cl	4.278				
			LEU352	Cl	5.167				
			MET522	Cl	4.944				
			ILE523	Cl	4.296				
		Pi-Alkyl	TYR348	Cl	4.857				
			PHE518	Cl	4.033				
			VAL349	Benzene	5.157				
			ILE523	Benzene	4.146				
			ALA527	Benzene	4.825				
Flavan-3-ol	-8.6	Amide-Pi Stacked	GLY526; ALA527	Benzene	3.949	Carbon	ALA527	H…O	2.572
		Pi-Alkyl	VAL349	Benzene	4.487				
			ALA527	Benzene	4.027				
			LEU531	Benzene	5.228				

Figure 2. Binding interactions of a) diclofenac, b) catechin, c) ethyl gallate, d) flavan-3-ol, e) nimbin, f) quercetin, g) vasicine h) vasicinol and i) vasicinone with 1EQG.
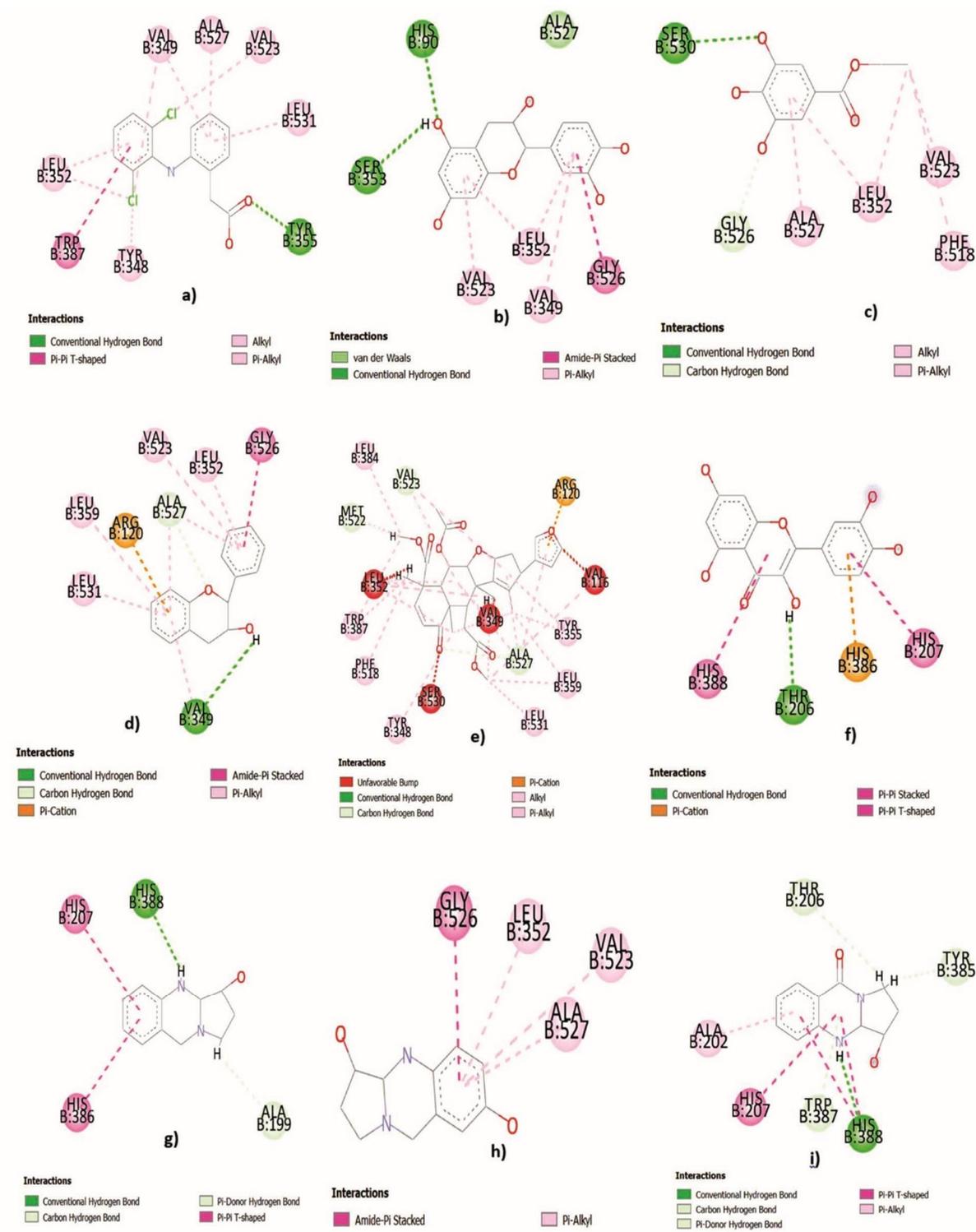
Figure 3. Binding interactions of a) diclofenac, b) catechin, c) ethyl gallate, d) flavan-3-ol, e) nimbin, f) quercetin, g) vasicine h) vasicinol and i) vasicinone with 5IKT.
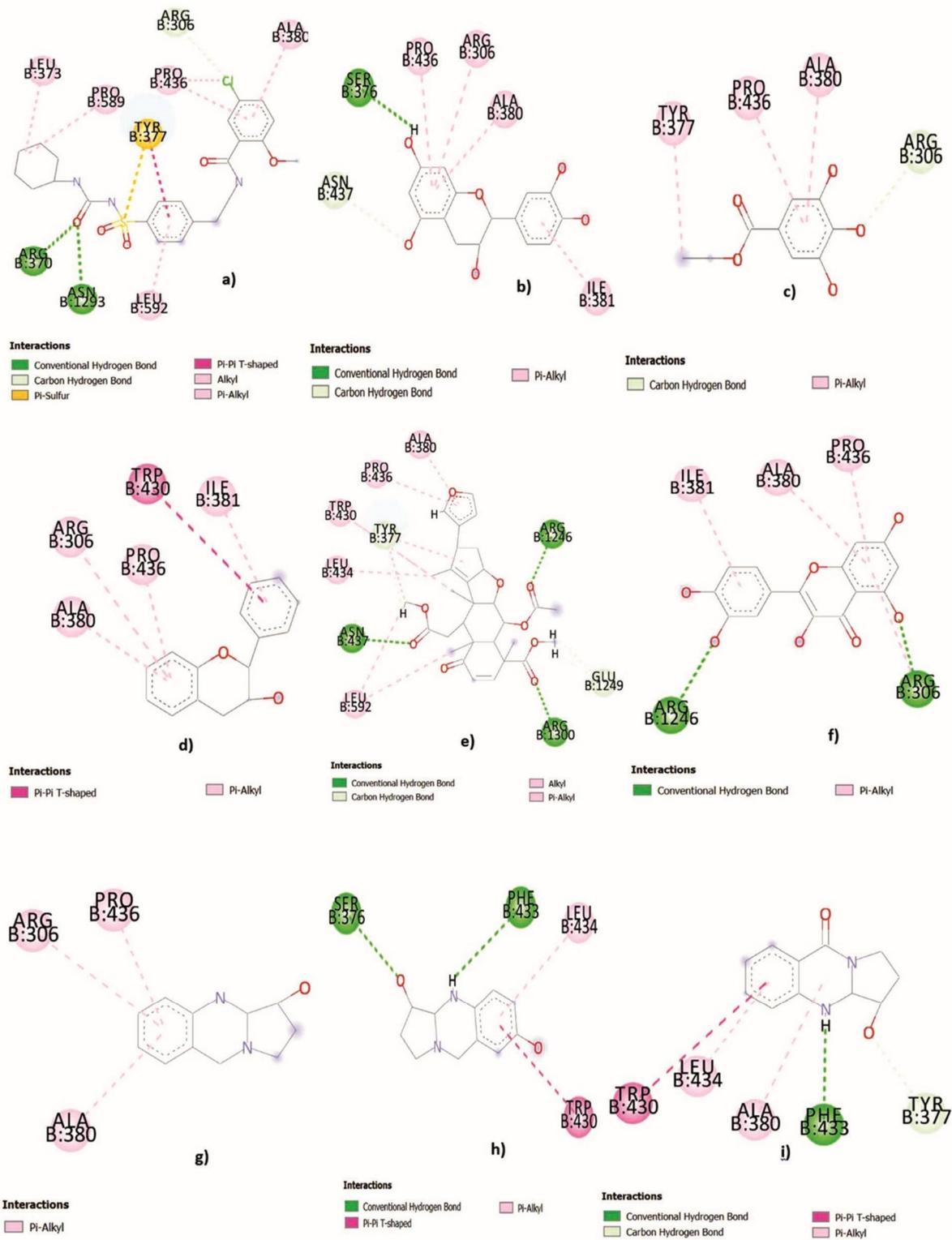
Figure 4. Binding interactions of a) glibenclamide, b) catechin, c) ethyl gallate, d) flavan-3-ol, e) nimbin, f) quercetin, g) vasicine h) vasicinol and i) vasicinone with 5YW7.

Table 5. **Comparison of binding interactions of standard and the compound with best binding affinity against cyclooxygenase-2 (PDB: 5IKT).**

Compounds	Binding anergy (Kcal/mol)	Hydrophobic interactions				Hydrogen bond			
		Bonding type	Interacting amino acids	Interacting atoms or rings	Distance (Å)	Bonding type	Interacting amino acids	Interacting atoms or rings	Distance (Å)
Diclofenac	-7.8	Pi-Pi T-shaped	TRP387	Benzene	5.44	Conventional	TYR355	H…O	2.417
		Pi-Alkyl	TYR348	Benzene	5.182				
			VAL349	Benzene	3.944				
			ALA527	Benzene	3.519				
			LEU531	Benzene	4.976				
			LEU352	Benzene	5.277				
		Alkyl	VAL349	Cl	4.575				
			LEU352	Cl	5.121				
			VAL523	Cl	3.463				
Flavan-3-ol	-8.7	Pi-Cation	ARG120	Benzene	4.94	Conventional	VAL349	H…O	3.085
		Amide-Pi Stacked	GLY526; ALA527	Benzene	4.33				
		Pi-Alkyl	VAL349	Benzene	4.616				
			LEU359	Benzene	5.469				
			ALA527	Benzene	4.354				
			LEU531	Benzene	5.181	Carbon	ALA527	H…O	2.907
			LEU352	Benzene	4.890				
			VAL523	Benzene	5.131				
			ALA527	Benzene	4.875				

Table 6. Comparison of binding interactions of standard and the compound with best binding affinity against the pancreatic ATP-sensitive potassium channel with SUR1 subunit (PDB: 5YW7) receptor.

Compounds	Binding energy (Kcal/mol)	Hydrophobic interactions				Hydrogen bond			
		Bonding type	Interacting amino acids	Interacting atoms or rings	Distance (Å)	Bonding type	Interacting amino acids	Interacting atoms or rings	Distance (Å)
Glibenclamide	-9.3	Pi-Sulfur	TYR377	S	5.87	Conventional	ARG370	H…O	2.063
		Pi-Pi T-shaped	TYR377	Benzene	5.274				
		Alkyl	PRO589	Cyclohexane	5.363		ASN1293	H…O	2.927
			LEU373	Cyclohexane	5.256				
			ARG306	Cl	3.69	Carbon	ARG306	H…Cl	3.07
			PRO436	Cl	3.66				
		Pi-Alkyl	LEU592	Benzene	5.451				
			ALA380	Benzene	4.979				
			PRO436	Benzene	5.318				
Quercetin	-8.8	Pi-Alkyl	ILE381	Benzene	5.314	Conventional	ARG306	H…O	2.188
			ARG306	Benzene	4.733		ARG1246	H…O	2.704
			ALA380	Benzene	4.551		ARG1246	H…O	2.777
			PRO436	Benzene	5.176				

Table 7. *In silico* **prediction of physicochemical properties of the test compounds for good oral bioavailability.**

Compounds	Molecular weight (M.W) (g/mol)	H-bond acceptors	H-bond donors	Molar refractivity	Lipophilicity logP (o/w)	No. of violations	GI absorption
Diclofenac	296.15	2	2	77.55	3.66	0	High
Glibenclamide	494.0	5	3	126.25	3.58	0	Low
Catechin	290.27	6	5	74.33	0.85	0	High
Ethyl gallate	198.17	5	3	48.6	0.92	0	High
Flavan-3-ol	226..27	2	1	66.24	2.66	0	High
Nimbin	540.6	9	0	138.81	3.24	1	High
Quercetin	302.24	7	5	78.03	1.23	0	High
Vasicine	188.23	2	1	62.11	1.17	0	High
Vasicinol	204.23	3	2	64.13	0.77	0	High
Vasicinone	202.21	3	1	56.09	0.94	0	High

***In silico* ADME analysis.** *In silico* physico-chemical properties like molecular weight, H-bond donors and acceptors, molar refractivity, lipophilicity logP, GI absorption etc. were predicted by Swiss ADME analysis. All the tested compounds showed good GI absorption and acceptable lipophilicity logP values ranging from 0.77 to 3.24 for drug like activities (Table 7). Apart from nimbin, the other seven tested molecules did not violate Lipinski's rule of 5 which makes them potential candidates for new drug development by further SAR analysis.

CONCLUSION

From this preliminary study it is evident that the crude methanolic root extracts of the three plants have significant analgesic and hypoglycemic properties. *In* silico analysis of some phytoconstituents of these plants showed promising results in prediction of their binding affinities with the target receptors/enzymes and drug like physicochemical properties. So, further extensive studies are encouraged on isolating chemical constituents from these plants to identify the chemical components accountable for these significant pharmacological actions as well as exploration of these results on higher animal models.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interests.

CONTRIBUTION OF AUTHORS

The whole experiment was designed and supervised by SMAR. FH performed the experiments and MSH analyzed the experimental data. PS performed the literature review and FH and PS wrote down the manuscript. FIR performed the *in silico* studies and molecular docking. All authors have read and approved the manuscript.

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