RESEARCH ARTICLE

Isolation, In Silico Design and Anti-Inflammatory Activity of Spirosolenol from the Roots of Solanum anguvi

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Isolation, *In Silico* Design and Anti-Inflammatory Activity of Spirosolenol from the Roots of *Solanum anguvi*

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Abstract
The aim of our research was the isolation of the steroidal alkaloid glycosides such as anguivine and isoanguivine from the dried roots of *S. anguvi* and evaluation of their anti-inflammatory activity by HRBC and carrageenan induced paw edema method. The PASS programme predicts the anti-inflammatory activity of the aglycon part of spirosolenol, on the basis of a similarity structure based drug design. Molecular docking studies with human phospholipase A_2 enzyme were carried outby utilizing Argus lab 4.0.1.version. The in *silico* study and pharmacological activity were correlated with good agreement.

Keywords: *Solanum anguvi*; PASS; Argus lab; anti-inflammatory activity.

1. Introduction

S. anguvi is a non-tuberous plant belonging to the family *Solanaceae*. The ethnobotanical survey revealed that the crushed root was used for curing diarrhoea and skin ailments [1]. Fruits of the plant were reported to possess antiproliferative activity [2], antioxidant activity [3] and inhibit basal erythropoiesis [4]. The isolation and chemical characterisation of steroidal glycosides from the fruits of *S. anguvi* such as anguiviosides A, B, C, III, IX and XV [5, 6] has been reported earlier.

The isolation of steroidal alkaloid glycosides such as anguivine and isoanguivine from the dried roots of methanolic extract of *S. anguvi* has been reported earlier [7]. The literature also gave an insight into the bioactive nature of spirosolenol, the aglycone part of the above mixture of steroidal alkaloid glycosides. Phospholipase A_2 is an enzyme that plays a major role in the formation of pro-inflammatory mediators such as prostaglandins, leukotrienes, platelet-aggregating factor and lysophospholipids [8]. The PASS programme predicted anti-inflammatory activity which is a novel pharmacological profile of spirosolenol nucleus. So the present work was focused on isolation and evaluation of anti-inflammatory activities of the isolated steroidal alkaloid glycosides from methanolic extract of roots of *S. anguvi* and the docking studies on Phospholipase A_2 focused on its enzyme binding interactions.

2. Methods

The fresh roots of *S. anguvi* were collected from Palakkad, Kerala, India in the month of March and it was authenticated by Dr. P. Sujanapal, Taxonomist, Kerala Forest Research Institute, Thrissur, Kerala. A voucher specimen was deposited in our lab for future reference.

2.1. Isolation of steroidal alkaloid glycosides

Fresh roots of the *S. anguvi* were dried at 60°C and were extracted with methanol at room temperature. The slurry obtained under *vacuo*, gave a residue which was partitioned between water and benzene/diethyl ether (1:1). After the addition of potassium bicarbonate to the aqueous layer, the latter was extracted with chloroform: ethanol (2:1). Evaporation of solvents *in vacuo* gave a mixture of alkaloids. The aglycone part of the glycosides is shown in Figure 1.

2.2. *In silico* design

The computational studies have been made to establish the therapeutic potential of the chemical substances.

2.2.1. Prediction activity spectra of the substances

Estimation of general biological potential for drug-like compounds on the basis of their structural formulae and can be performed with a computer program PASS (Prediction of Activity Spectra for Substances) that predicts more than 780 pharmacological effects. This program is based on a robust analysis of structure-activity

relationships in a heterogeneous training set. A biological spectrum for a substance is a list of biological activity types, for which the probability to be revealed (Pa) and not to be revealed (Pi) values are independent and their values range from 0 to 1. The more is the Pa value, the less is the probability of false positives in the set of compounds selected for study [9]. The predicted pharmacological activities of the spirosolenol aglycone part of the steroidal alkaloidal glycosides are shown in Table 1.

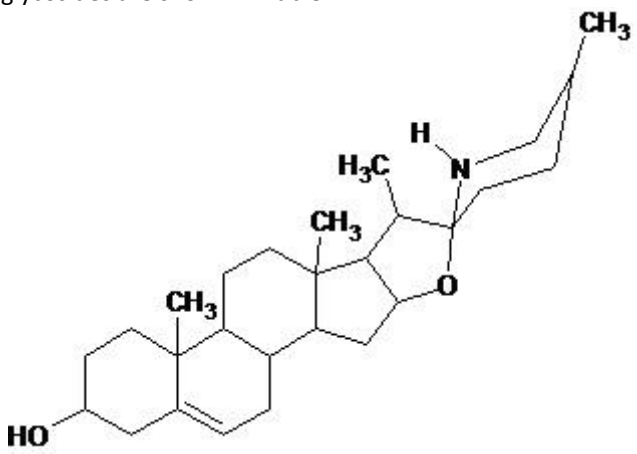

Figure 1: Structure of spirosolenol.

Table 1: PASS prediction.

Biological Activity	Pa	Pi
Embryotoxic	0.966	0.002
Teratogen	0.965	0.002
Hematotoxic	0.937	0.007
Anti-inflammatory	**0.916**	**0.004**
Antineoplastic	0.909	0.005
Hypertensive	0.805	0.016
UGT1A4 substrate	0.784	0.003
CYP17 inhibitor	0.777	0.003

2.2.2. Molecular docking methodology

The crystal structure of Human phospholipase A_2 complexed with a highly potent analogue [10] was retrieved from the Protein Data Bank (PDB entry: 1kvo). The pdb file was refined by removing the water molecules and inhibitors, and then hydrogen atoms were added [11]. The energy minimization of the edited protein was done by energy minimizing tool of Swiss-Model workspace [12] with the GROMOS96 implementation. The active sites of the enzyme were predicted by CASTp database [13]. These predicted amino acid residues were selected and saved as the binding site for the docking study. The active moiety of the aglycon part of the steroidal alkaloidal glycoside was saved as mol format and opened in the Argus lab 4.0.1. version. Geometry, hybridization and valency of the structure were corrected initially. The final energy optimization was done by PM-3 semi empirical QM method. Argus lab was employed in the docking between protein and ligand using Argus dock with a fast and simplified potential of mean force (PMF). Docking of ligand in to binding site was done by 'Argus Dock' as the docking engine. 'Regular precision' was selected in docking precision menu, 'Dock' was selected as calculation type, 'Flexible' for the ligand and 'Ascore' for the scoring function. The process of docking was repeated until a constant value of docking score was obtained. The resulted final docked structures were saved in pdb format and the docking snap shots were imported into Molegro virtual viewer version 2010 2.0.0 for interpreting the different drug-receptor interaction such as hydrogen bonding, hydrophobic interaction and electrostatic force of attraction, etc.

2.3. Anti-inflammatory activity

2.3.1. HRBC stabilization technique (*in vitro*)

This technique [14] was employed to evaluate the anti-inflammatory activity of the isolated steroidal alkaloid glycosides from the roots of the *S. anguvi*. The human blood was collected from a healthy volunteer who has not taken any NSAIDS for two weeks prior to the experiment and was mixed with equal volume of Alsever

solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of the isolated compound were prepared (50, 100 and 150 µ/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension were added. It is incubated at 37^0C for 30 minutes and centrifuged at 3000 rpm for 20 minutes. The percentage inhibition was calculated spectrophotometrically at 560 nm. Indomethacin (50 & 100 µ/ml) was used as the standard. The results are shown in the Table 2.

Table 2: *In vitro* anti-inflammatory activity of steroidal alkaloidal glycosides from the roots of *S. anguvi*.

Treatment	Concentration (µ/ml)	Percentage of Inhibition
Control	-	-
Isolated fraction	50	56.3
	100	87.6
	150	98.3
Indomethacin	50	61.4
	100	98.7

2.3.2. Carrageenan induced paw edema (*in vivo*)

The study was carried out by carrageenan induced paw edema models in rat [15]. The Wistar rats were divided into 4 groups of 6 animals each. Group I served as the control, Group II, III received the isolated fraction of 50 and 100 mg/kg, and Group IV was served with standard, indomethacin 50 and 100 mg/kg. Carrageenan was injected into the sub planter aponeurosis of the right hind of paw rats. An hour before carrageenan injection, animals were given the isolated fraction and standard orally. The paw volumes were measured before and three hours after carrageenan administration by volume displacement method. The results are shown in Table 3.

Table 3: *In vivo* anti-inflammatory activity of steroidal alkaloidal glycosides from the roots of *S. anguvi*.

Treatment	Dose (mg/kg)	Edema volume (ml)	% inhibition
Control	-	0.76±0.012	-
Isolated fraction	50	0.26±0.004	65.7
	100	0.10±0.021	86.8
Indomethacin	50	0.23±0.016*	70
	100	0.11±0.032*	85.5

Values are expressed as mean±SEM; n=6 animals in each group; *p<0.001 compared to control group.

3. Results and Discussion

The current study described the isolation of steroidal alkaloid glycosides such as anguivine and isoanguivine from the dried roots of methanolic extract of *S.anguvi* from a reported method and evaluation of their *in vitro* anti-inflammatory activity.

3.1. *In silico* studies

The PASS programme of the aglycon part, spirosolenol significantly predicted the anti-inflammatory action with Pa score of 0.916. Molecular docking studies with Human phospholipase A_2 enzyme were carried out with Argus lab 4.0.1.version. A binding score of -12.56 Kcal/mol revealed good interaction between the enzyme and the ligand, spirosolenol nucleus. Amino acid residues which are enveloped with each fragment of the nucleus are shown in Figure 2. A hydrogen bonding with a bond length of 2.56Å was obtained between Gly 401 and the OH group of the steroid nucleus. It has been noted that the docking study showed a crucial hydrophobic interaction between the angular methyl group of the ligand and the non-polar alkyl part of LYS 434. The alkaloid ring which is present in the apex of the steroid ring was sandwiched between PHE 311 and PHE 435. So, we can conclude that there is a strong π-π interaction between the aromatic nucleus of PHE and the alkaloidal part, which can contribute a significant role in the pharmacological evaluation. The hydrophobic interaction of the ligand is shown in Figure 3.

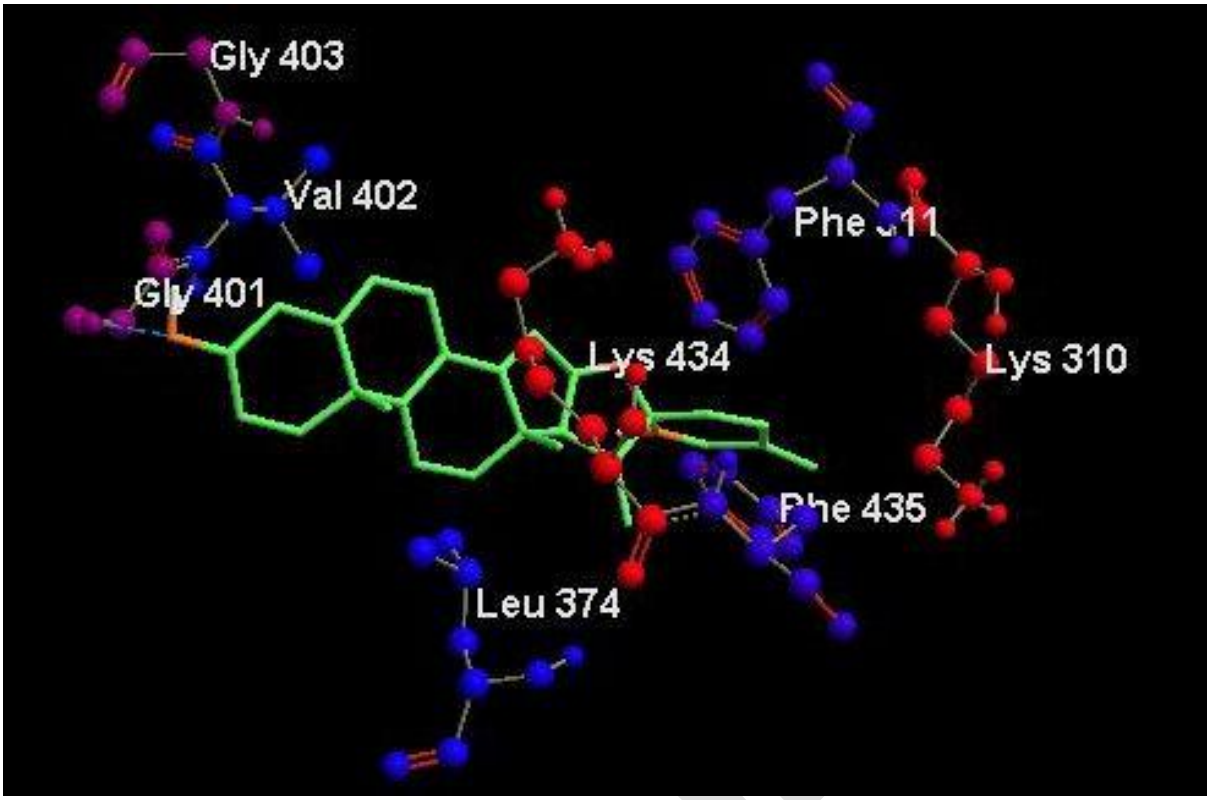

Figure 2: Docking view of spirosolenol with Human phospholipase A_2 enzyme.

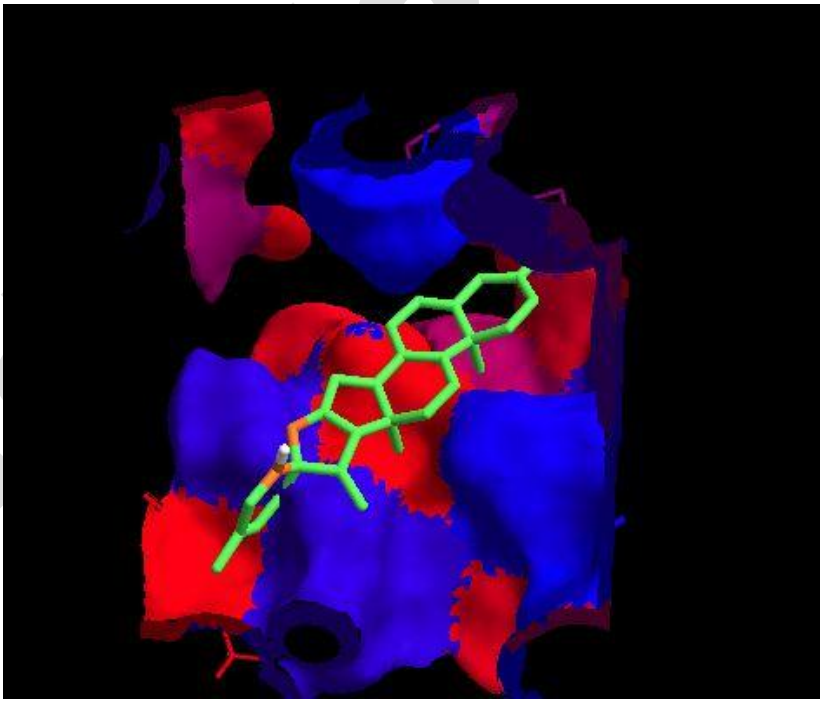

Figure 3: Docking of spirosolenol with its hydrophobic interaction.

3.2. Anti-inflammatory activity

Interestingly, it has been noted that the isolated steroidal alkaloid glycosides exhibit a significant pharmacological action with a percentage inhibition of 56.3, 87.6 & 98.3 at a dose level of 50, 100 and 150 µ/ml respectively in HRBC method. The *in vivo* result suggested that the isolated fraction at a dose level of 100

mg/kg in the experimental animal model can produce a percentage inhibition of 86.8 when compared to the standard.

4. Conclusion

Our study explains the classical effect of spirosolenol on the anti-inflammatory activity. The utilization of computer tools predicted its bioactive nature and the experimental data have supported the prediction. So, it has been concluded that the significant anti-inflammatory activity of spirosolenol may be due to the prominent hydrophobic interaction of its angular methyl group and alkaloid part towards the non-polar chain of enzyme. In the present research work, we did an *in silico* study of spirosolenol inhibiting Phospholipase A_2 enzyme. The *in silico* study and pharmacological activity were correlated with good agreement.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

BM and GEM carried out the project. SG developed the *in silico* studies. VBAR and SM assisted with the preparation of manuscript. GEM carried out the pharmacological screening in the project.

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