Indian Journal of Natural Products and Resources
Vol. 5(3), September 2014, pp. 258-261

In silico and *in vitro* anthelmintic activity of β-sitosterol isolated from rhizomes of *Hedychium spicatum* Buch.-Ham.

T Sravani[1], Padmaa M Paarakh[1,*] and S D Shruthi[2]

[1]Department of Pharmacognosy, The Oxford College of Pharmacy, Hongasandra, Bangalore-560 068, Karnataka, India
[2]Microbiology and Cell Biology Department, Indian Institute of Science, Bangalore

Received 1 November 2013; Accepted 18 February 2014

Hedychium spicatum Buch.-Ham. (Family-Zingiberaceae), commonly known as spiked ginger lily, is found in the entire Himalayan region. The rhizomes are reported to be used as tranquilizer, hypotensive, antispasmodic, CNS depressant, analgesic, anti-inflammatory, antimicrobial, antioxidant, antifungal, pediculicidal, cytotoxic and anthelmintics. The present study is an attempt to explore the anthelmintic activity of β-sitosterol isolated from the rhizomes of *H. spicatum* by molecular docking with tubulin and *in vitro* activity against adult Indian earthworms, *Pheretima posthuma*. β-sitosterol binds very efficiently within the active pocket of tubulin which is better when compared to orientation of standard drug, Piperazine citrate. These results were also confirmed with *in vitro* study. The time taken for each worm for paralysis and death were determined. The time taken for paralysis and death with β-sitosterol at 40 mg/mL was comparable to standard Piperazine citrate.

Keywords: Anthelmintic activity, β-Sitosterol, Docking, *Hedychium spicatum*, Zingiberaceae, Tubulin.
IPC code; Int. cl. (2014.01)–A61K 36/00, A61P 33/10

Introduction

Hedychium spicatum Buch.-Ham. (Family-Zingiberaceae) also known as spiked ginger lily, has a rich history of use in India. The plant is a perennial rhizomatous herb, up to 1 m tall with elongate stem. The rhizomes are 15-20 cm long and 2-2.5 cm in diam. and externally yellowish brown. The edge of each piece is covered by rough reddish brown layer marked with numerous scars and circular rings, rudiments of rootlets are visible[1]. The essential oil from rhizomes are reported to be used as tranquilizer[2], hypotensive, anti-spasmodic, CNS depressant, analgesic[3], anti-inflammatory[4], antimicrobial[5], antioxidant[6,7], antifungal[8], pediculicidal[9] and cytotoxic activities[10].

The anthelmintic activity of methanol extract and isolation of phytoconstituents from rhizomes of *H. spicatum* and has already been reported[11,12]. Tubulin is a known anticancer and anthelmintic drug target, the investigation of tubulin inhibitors could lead to the development of new anthelminthic drugs. Inhibitors bind selectively to β-tubulin of nematodes, cestodes and fluke, a protein subunit of microtubule and thereby disrupting microtubule structure and function[13-15]. Microtubules are highly dynamic, ubiquitous cellular organelles serving a variety of vital functions including mitosis, motility and transport, in all eukaryotes. Many of these structures exist in a dynamic equilibrium in which assembly and disassembly of the soluble subunits are balanced. In such systems, the drug–tubulin interaction results in a shift of this equilibrium with a net loss of microtubules and accumulation of free tubulin. In view of the crucial roles, that microtubules play in many cellular processes, their drug-induced destruction eventually leads to the death of the organism[15]. Some anthelmintic drugs act rapidly and selectively on neuromuscular transmission of nematodes. Levamisole, pyrantel and morantel are agonists at nicotinic acetylcholine receptors of nematode muscle and cause spastic paralysis. Dichlorvos and haloxon are organophosphorus cholinesterase antagonists[16]. Diethylcarbamazine blocks host and possibly parasite, enzymes involved in arachidonic acid metabolism and enhances the innate, non-specific immune system. Some drugs are known to affect the fatty acid oxidation pathway in mammals, caused a reduction in oxygen consumption rates in *Caenorhabditis elegans* and genome-wide gene expression profiles provided an additional confirmation of its mode of action[17].

*Correspondent author:
E-mail: padmaparas@hotmail.com
Phone: 09880681532

In silico molecular docking technique play an important role in the drug design and discovery to predict the conformations of each ligand molecule at the active site, hence, the molecular docking study was carried out to predict the β-tubulin inhibitory activity and results are reported. Even though Benzyl derived compounds are known to have antiparasitic effect, it is now banned in many countries. Piperzine has broad spectrum activities like anthelmintic, antiallergenic, antibacterial, antihistamic, antiemetic and antimigraine agents. It is used as an anthelmintic for humans and farm animals against intestinal roundworms and pinworms infection and it is administered orally. Because of its broad spectrum usage it is used as a standard drug in our study and there are various research articles available which support our study. Since in our previous study[11], we used piperazine citrate as reference standard, present study was also studied with same standard as this compound was isolated from the methanol extract only.

The present study is an attempt to explore the anthelmintic activity of β-sitosterol isolated from the rhizomes of *H. spicatum* by *in silico* anthelmintic activity with Tubulin and also against adult Indian earthworms, *Pheretima posthuma*.

Material and Methods

Collection of plant material

The rhizomes of *H. spicatum* Buch.-Ham. were collected, identified and authenticated by Dr Shiddamallayya N (SMPU/NADRI/BNG/2010-11/307) at National Ayurveda Dietetics Research Institute, Bengaluru, Karnataka. A voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bengaluru. The rhizomes were dried under normal environmental conditions and powdered to store in a closed container for further use.

Extraction and isolation procedure

The dried rhizomes of *H. spicatum* were coarsely powdered and subjected to successive extraction by soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, benzene, chloroform, methanol and distilled water. Each time the marc was dried and later extracted with other solvents. All the extracts were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 2.176, 0.831, 0.861, 6.06 and 5.41 % w/w, respectively with reference to the air dried plant material.

Crude methanol extract (10 g) was subjected to column chromatography over silica gel (60-120 mesh) using petroleum ether, petroleum ether: benzene (different ratio), benzene (100 %) and chloroform: methanol (different ratio), taking 500 mL fraction each time. From petroleum ether: benzene: 8:2, fractions 63-73 (HS-1; 1 g) was isolated and characterized as β-sitosterol[12] (10 % yield).

Earthworm collection and authentication

Healthy adult Indian earthworm (*P. posthuma*; Annelida; Megascolecidae) were collected from Microbial Resources Division, Gandhi Krushi Vijnana Kendra (GKVK), Government of Karnataka, Bengaluru. Earthworms in moist soil were washed with normal saline and used for the study. The earthworms 3-5 cm in length and 0.1-0.2 cm width were used due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[18, 19].

In vitro anthelmintic activity

The anthelmintic activity of β-sitosterol isolated from *H. spicatum* was evaluated as per the method reported[11]. The compound was suspended in Tween 80 (0.1 %) in normal saline. Both the isolated β-sitosterol and Piperazine citrate were freshly prepared before starting the experiment. Four groups of six earthworms each were released into 20 mL of desired formulation as follows; vehicle (0.1 % Tween 80 in normal saline), piperazine citrate (40, 60 mg/mL) and β-sitosterol (40 mg/mL). Only one concentration of β-sitosterol was chosen as the crude methanol extract was found to be very effective at 40 mg/mL[11]. Observations were made for the time (in minutes) taken to paralysis and death of individual worms up to 4 h of the test period. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body color[19].

In silico docking studies

Molecular docking study was done to determine the orientation of β-sitosterol bound in the active site of tubulin as target for antihelminthic activity. A Lamarckian genetic algorithm method, implemented in the program AutoDock 3.0, was employed. The ligand molecules β-sitosterol and piperazine citrate were designed and the structure was analyzed using ChemDraw Ultra 6.0. 3D co-ordinates were prepared using PRODRG server[20]. The protein structure file (1SA0 for tubulin) was taken from PDB (www.rcsb.org/pdb) and was edited by removing the heteroatoms and adding C terminal oxygen[21]. For docking calculations, Gasteigere–Marsili partial charges were assigned to the ligands and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The grid map

was centered at particular residues of the protein which was predicted from the ligplot and was generated with AutoGrid. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters[22,23].

Results and Discussion

The results of the anthelmintic activity are given in the Table 1. The perusal of the data revealed that β-sitosterol at a dose of 40 mg/mL produced paralysis within 6.8 ± 0.37 min and the corresponding death time was 29.2 ± 0.39 min. The standard drug Piperazine citrate of dose 40 and 60 mg/mL showed paralysis at 9.2 ± 0.58 and 3.0 ± 0.96 min and death occurred at 33.4 ± 0.24 and 26.8 ± 0.48 min, respectively. As the yield obtained was only 1 g, higher concentration of 60 mg/mL (20 mL) could not be tried.

The *in silico* analysis showed that β-sitosterol, even after showing good effects in *in vitro* studies it does not inhibit tubulin efficiently. The binding energy obtained is comparable with the standard, where as it has not formed hydrogen bonds with the protein target (Fig. 1-3). The

Table 1—*In vitro* anthelmintic activity of β-sitosterol and piperzine citrate

S. No.	Treatment	Time taken for paralysis [mean ± SD]	Time taken for death [mean ± SD]
1.	Vehicle	—	—
2.	Piperazine citrate 40 mg/mL 60 mg/mL	9.20 ± 0.58 3.00 ± 0.00	33.4 ± 0.24 26.8 ± 0.48
3.	β-sitosterol 40 mg/mL	6.80 ± 0.37	29.2 ± 0.37

Results are expressed as mean ± SD of six determinations; vehicle worms were alive up to 24 h of observation.

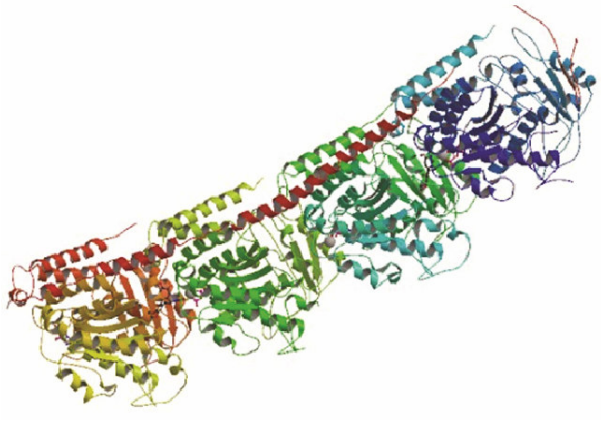

Fig 1—Structure of tubulin taken for *in silico* study

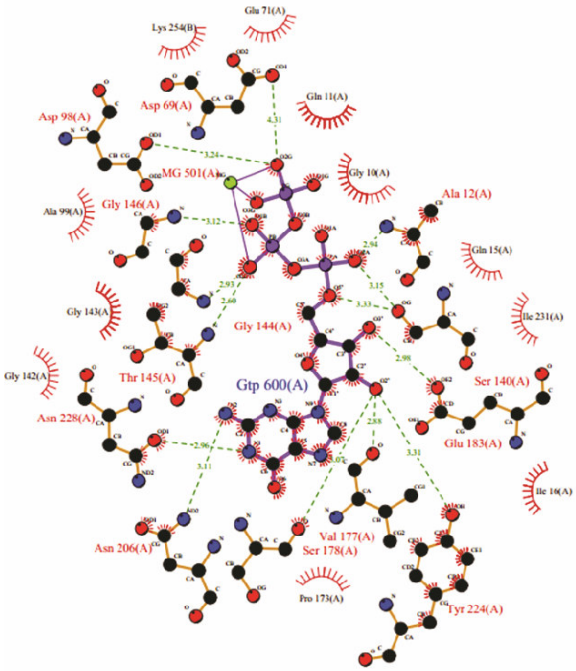

Fig 2—Predicted LigPlot of tubulin from PDBSum

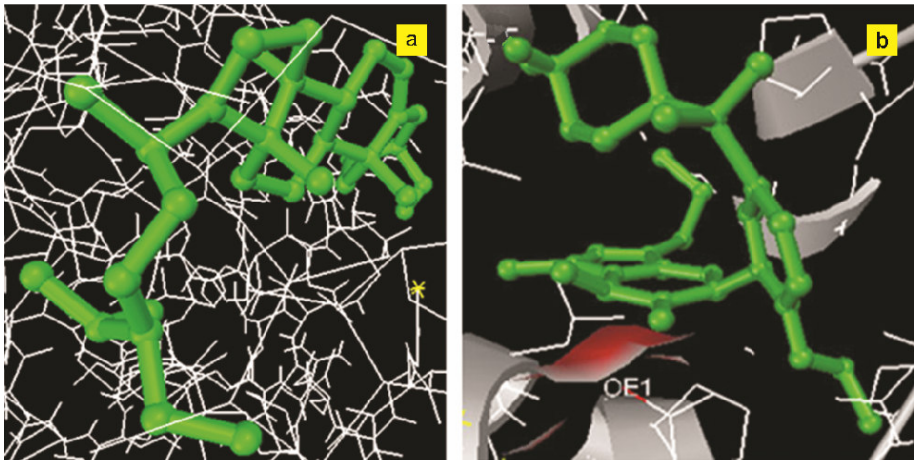

Fig 3—(a) Orientation of β-sitosterol in the active pocket of tubulin; (b) Piperzine citrate with tubulin as obtained in AutoDock

Table 2—Docking parameters of β-sitosterol and piperzine citrate as obtained by AutoDock

S No.	Compounds	Binding energy	Docking energy	Inhibitory constant	Intermol energy	Hydrogen bonds
1	β-sitosterol	-10.35	-11.79	2.58e-008	-12.22	—
2	Piperzine citrate	-8.23	-10.94	9.32e-007	-10.41	TB:A:GLU107:OE1::PC:DRG1:HAK

values obtained in AutoDock are shown in Table 2. Probably β-sitosterol is showing activity by inhibiting other proteins of the cascade and exhibiting activity. Further studies towards finding those targets and improving the structure of β-sitosterol to minimize its energy requirement are in progress.

Conclusion

β-sitosterol isolated from the methanol extract of rhizomes of *H. spicatum* showed remarkable anthelmintic activity. The *in vitro* activity was found to be better than piperazine citrate. From the *in silico* analysis, it showed that β-sitosterol, even after showing good effects in *in vitro* studies does not inhibit tubulin efficiently. As β-sitosterol is very common in plants, the anthelmintic activity of the compound will definitely become a very useful lead for eradicating the helminthiasis from the world.

References

1 Anonymous, The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, National Institute of Science Communication and Information Resources, CSIR, New Delhi, 2004 (Reprint), Vol. V, 13-14.

2 Dixit VK and Varma KC, Effect of essential oils of rhizomes of *Hedychium spicatum* on central nervous system, *Indian J Pharmacol*, 1979, **11**(2), 147-149.

3 Rastogi RP and Mehrotra BN, Compendium of Indian Medicinal Plants, Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi, 1980, 369-370.

4 Tandon SK, Chandra S, Gupta S and Lal J, Analgesic and anti-inflammatory effects of *Hedychium spicatum*, *Indian J Pharma Sci*, 1997, **59**(3), 148-150.

5 Bishit GS, Awasthi AK and Dhole TN, Antimicrobial activity of *Hedychium spicatum*, *Fitoterpia,* 2006, **77**(33), 240-242.

6 Joshi S, Chanotiva CS, Agarwal G and Prakash O, Terpenoid compositions and antioxidant and antimicrobial properties of the rhizome essential oils of *Hedychium spicatum*, *Chem Biodiv*, 2008, **5**(2), 299-309.

7 Sravani T and Padmaa MP, Antioxiant activity of *Hedychium spicatum* Buch.-Ham. rhizome, *Indian J Nat Prod Resour*, 2012, **3**(3), 354-358.

8 Aqil F and Ahmad I, Broad spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants, *World I Micro and Biotech*, 2003, **19**(6), 653-657.

9 Jadhav V, Kore A and Kadam VJ, *In vitro* pediculicidal activity of *Hedychium spicatum* essential oil, *Fitoterpia*, 2007, **78**(7-8), 470-3.

10 Reddy PP, Rao RR, Shashidhar J and Sastry BS, Phytochemical investigation of labdane diterpenes from the rhizomes of *Hedychium spicatum* and their cytotoxic activity, *Bio Org and Med Chem Let*, 2009, **19**(21),6078-81.

11 Sravani T and Paarakh PM, Evaluation of anthelmintic activity of *Hedychium spicatum* Buch., *Int J Res Pharm Sci*, 2011, **2**(1), 66-68.

12 Sravani T, Padmaa MP and Vedamurthy AB, Isoaltion of phytoconstituents from rhizomes of *Hedychium spicatum* Buch.-Ham., *J Pharm Res*, 2012, **5**(1), 526-527.

13 Friedman PA and Platzer EG, Interaction of anthelmintic benzimidazoles and benzimidazole derivatives with bovine brain tubulin, *Bioch Biophy Acta*, 1978, **544**, 605-614.

14 Kohler P and Bachmann R, Intestinal tubulin as possible target for the chemotherapeutic action of mebendazole in parasitic nematodes, *Mol Biochem Parasitol*, 1981, **4**, 325-336.

15 Lacey E, The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of the drug resistance to benzimidazoles, *Int J Parasitol*, 1988, **18**, 885-936.

16 Martin RJ, Modes of action of anthelmintic drugs, *Vet J,* 1997, **154**(1), 11-34.

17 Taylor CM, Wang Q, Rosa BA, Huang SC, Powell K Schedl T, Pearce EJ, Abubucker S and Mitreva M, Discovery of anthelmintic drug targets and drugs using chokepoints in nematode metabolic pathways, *PLoS Pathog*, 2013, **9**(8): e1003505. doi: 10.1371/journal.ppat.1003505.

18 Thorn GW, Adams RD, Brundwal E, Isselbacher KJ and Petersdort RG, Harrison, Principles of internal medicine, New York, McGraw Hill Co, 1977.

19 Tambe VD, Nirmal SA, Jadhav RS, Ghogare PB and Bhalke RD, Anthelmintic activity of *Wedelia trilobata* leaves, *Indian J Nat Prod*, 2006, **22**, 27-29.

20 Ghose AK and Crippen GM, Atomic physicochemical parameters for three dimensional structure directed quantitative structure activity relationships. 2. Modelling dispersive and hydrophobic interactions, *J Chem Inf Comput Sci*, 1987, **27**, 21-35.

21 Binkowski TA, Naghibzadeg S and Liang J, CASTp computed atlas of surface topography of proteins, *Nucleic Acid Res*, 2003, **31**, 3352-3355.

22 Vidya SM, Krishna V, Manjunatha BK, Rajesh KP, Bharath B R and Manjunatha H, Antibacterial and molecular docking studies of entagenic acid, a bioactive principle from seed kernel of *Entada pursaetha* DC, *Med Chem Res*, 2012, **21**(7), 1016-1022.

23 Shruthi SD, Padmalatha RS and Ramachandra YL, Isolation, characterization, antibacterial, antihelminthic and *in silico* studies of polyprenol from *Kirganelia reticulata* Baill., *Med Chem Res*, 2013, **22**(6), 2938-2945.