EVALUATION OF *IN-SILICO* ANTICANCER POTENTIAL OF PYRETHROIDS: A COMPARATIVE MOLECULAR DOCKING STUDY

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ABSTRACT

Pyrethroids have shown promising potential to induce apoptogenic signaling pathways in various cells. Therefore, present study on pyrethroids was designed to unlock better alternative agents against cancer disease. Different targets such as estrogen (PDB: 3ERT), androgens (PDB: 2PIT) & cervix (PDB: 3F81) cancer receptors were used in the study. Type 1 & type 2 pyrethroids were subjected to docking simulations using Maestro 9.2 version (Schrodinger's LLC). Pyrethroids (Type 1 & type 2) docking studies have revealed varying glide score to cancer receptors. Resmethrin exhibited better binding interaction to estrogen (Glide Score: -7.32) & androgens (Glide Score: -7.47) while fluvalinate against cervix (Glide Score: -4.54) protein receptors. Decrease in glide score be evidence for greater bond stability with protein. Based on the current finding from docking studies, these preliminary results may act as effective precursor tool for development of pyrethroids as promising anticancer agents. However, furthermore experimental validation using in-vitro & in-vivo studies is needed to explore their therapeutic & toxic effects.

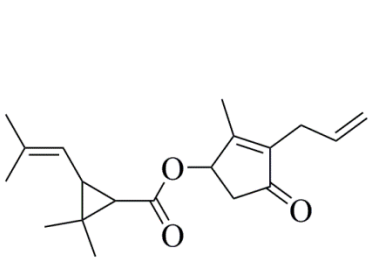
Keywords: Pyrethroids, anticancer agents, docking, cancer receptors

INTRODUCTION

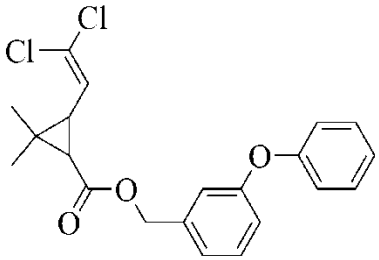
In the last few years, Cancer has become an important leading precursor towards mortality rate (8.2 millions, 2012) & estimated to increase via almost 70% over the next two decades.[1] The exact cause is mysterious but mutation in the cell proteins which encodes specific gene plays a crucial role towards its pathogenesis.[2-3] The serious problem arises due to action of anticancer

drugs on the proliferating cells of body like alimentary tract, bone marrow cells, and epidermal cells which lead to adverse effects such as hair loss, anemia & other infectious diseases.[4] Although, the various synthetic drugs have shown promising potential against this disease but poor selectivity issue remains a major concern. To date, there is no safe and effective cancer therapy available, thus, there is an urgent need to explore the anticancer properties of the existing molecules on which safety data is already available.

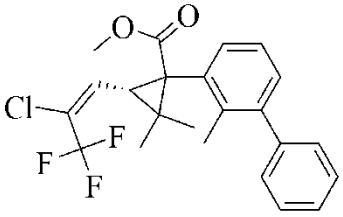
Pyrethroids (type 1 & type 2) are sound synthetic insecticide compounds derived from the chief phytoconstituent (pyrethrins) of *Chrysanthemum cineraraefolum*. Chemically, the type 2 pyrethr oids such as deltamethrin and fenvalerate show an α-cyano phenoxy benzyl moiety while the type 1 pyrethroids such as permethrin lack this moiety. All synthetic pyrethroids usually deal with chiral nature and exist in different forms of enantiomers.[5] The main reward with pyrethroid insecticides are their photo stability, high efficacy at low concentrations, easy disintegration and low toxicity to birds and mammals.[6-7]



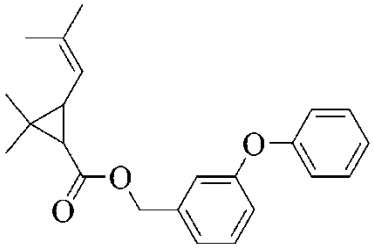
Allethrin



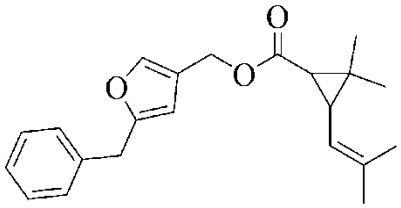
Bifenthrin



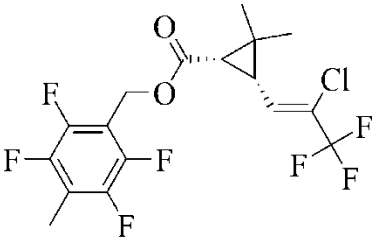
Permethrin



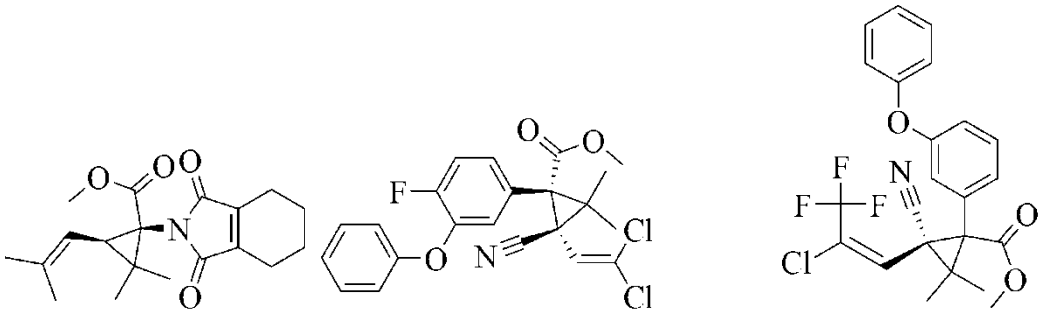
Phenothrin



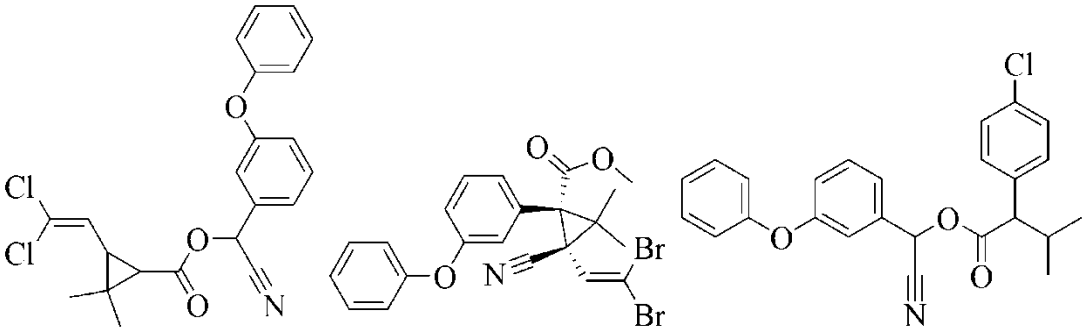
Resmethrin



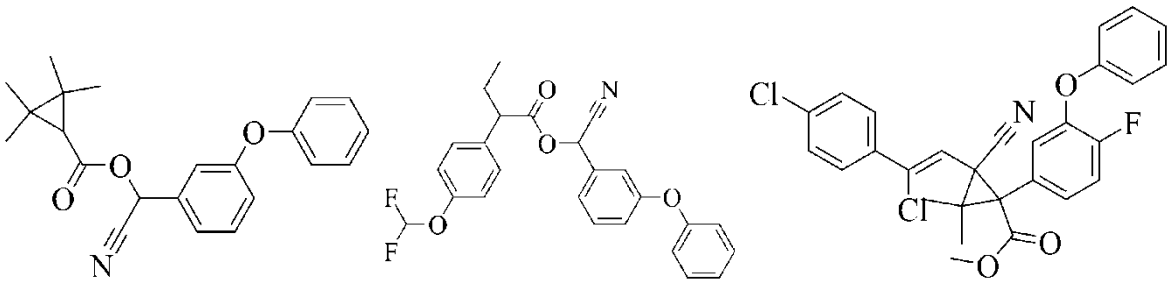
Tefluthrin



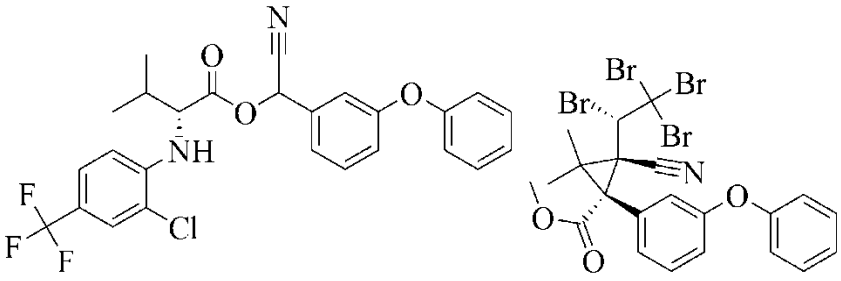
Tetramethrin Cyfluthrin Cyhalothrin



Cypermethrin Deltamethrin Fenvalerate



Fenpropathrin Flucythrinate Flumethrin



Fluvalinate Tralomethrin

Figure 1: Chemical structures of type 1 & 2 Pyrethroids

Pyrethroid insecticides are widely used in home and agricultural pest control. Humans are exposed to pyrethroid insecticides by various modes like air, water, vegetables, etc. at low concentrations. Pyrethroid insecticides have been considered as safe for humans at low concentrations.[8] At the higher concentration, in the literature, various reports have shown that its toxicity is mediated by oxidative stress caspase dependent apoptotic signaling pathways.[9,10] Recently, Kumar et al., (2015) have proposed a hypothesis in which it has been shown that at lower concentration, deltamethrin can induce apoptogenic signaling pathways in various cancer cells.[11] Chi et al., (2014) demonstrated that pyrethroid insecticide at low concentration (5–10 μM) induced calcium dependent apoptogenic signaling pathways in OC2 human oral cancer cells.[12] Another study conducted by Hsu et al. (2012) also demonstrated the anticancer activity of pyrethroid insecticide in Human Glioblastoma Cells via inducing intrinsic pathways of apoptosis .[13] These results indicate that pyrethroid insecticide at lower concentration have potential to act as anticancer agent.

Docking is now commonly used in virtual screening or lead optimization for drug screening and design. It is used to predict the preferred orientation of ligand (Protein-ligand docking) or protein (protein–protein docking) towards a relevant target to form a stable complex.[14] In most cases, one can choose the best 'binding affinity' to be the potent ligand for further development.[15] Therefore in the present investigation, we have tried to assess the *in-silico* interactions of pyrethroid derivatives against different cancer proteins (estrogen, androgen & cervix).

MATERIALS & METHODS

Maestro 9.2 version software (Schrodinger LLC suite) was used for docking simulation. Molecular docking software was installed in single machine running on core ^{TM} processor with 2 GB RAM and 180 GB with centrp linux as the operating system. The pyrethroid chemical entities (type 1 & type 2) data were collected from the literature.

Protein preparation

The crystal structures of cancer receptors (PDB: 3ERT-1.9A^{o}; 2PIT-1.76A^{o}; 3F81-1.9A^{o}) were retrieved from RCSB protein bank.[16] The crystal structures of estrogen, androgen & cervix receptors were reported to complex with 4-HydroxyTamoxifene, 5-Alpha-Dihydrotestosterone &

2-(5-methyl-4-oxo-2-thioxo-2,4-dihydro-3H-1lambda~4~,3-thiazol-3-yl)ethanesulfonic acid, respectively. Protein pre-process was completed by addition of polar hydrogen and removal of metal ions, cofactor and water molecule outside $5A^0$. The ionization (pH: 6.7-7.3), optimization of hydrogen bond and restorative energy minimization steps were applied to correct the geometry of receptors.

Ligand library

The structure of all the tested compounds were drawn in chem draw ultra 8.0 (Cambridge soft), saved in three dimensional structures (.mol file) and finally imported into maestro project table. Ligands preparations plus energy minimization were completed by using least square OPLS_2005 force field. The conformers (max 32/ligand) were generated and filtered to their energy minima with possible state generation (pH 7±2.0).

Grid generation & docking calculation

The electrostatic and vander wall's potential of binding pocket was assigned through grid box with maximum 14E edge length around the active site of internal ligand. Extra precision (XP) glide docking was applied. The docking pose analysis was done through XP visualizer. The *in-silico* docking results have analyzed not only glide score basis but in addition different possible interactions of the pyrethroids with the different residues of cancer receptors were too seen.

RESULT & DISCUSSION

The reference ligands along with type 1 & type 2 pyrethroids were docked against cancer proteins (PDB: 3ERT, 2PIT & 3F81). The ranking were evaluated by top HITs glide score of the test ligands. Table 1 reports have revealed that resmethrin possess higher binding affinity with estrogen (Glide Score: -7.32) & androgens (Glide Score: -7.47) proteins while fluvalinate (Glide Score: -4.54) against cervix receptor.

Table1: Glide score of type1 & type2 pyrethroids with cancer receptors (PDB: 3ERT, 2PIT & 3F81).

Sr No.	Name of the compounds	Glide Score (3ERT)	Glide Score (2PIT)	Glide Score (3F81)
1	Resmethrin	-7.32	-7.47	-2.78
2	Cyhalothrin	-7.16	-7.17	-1.12
3	Permethrin	-6.27	-5.13	-2.45
4	Cyfluthrin	-6.02	-	-2.52
5	Fluvalinate	-5.81	-	-4.54
6	Flucythrinate	-5.70	-	-2.27
7	Tetramethrin	-5.07	-2.25	-2.15
8	Bifenithrin	-4.12	-	-2.62
9	Fenproparthrin	-3.99	-5.61	-2.65
10	Tefluthrin	-2.87	-	-1.73
11	Phenothrin	-	-6.04	-2.57
12	Deltamethrin	-	-6.17	-2.50
13	Flumethrin	-	-	-2.46
14	Tralomethrin	-	-	-2.14
15	Cypermethrin	-	-2.19	-1.96
16	Fenvalerate	-	-	-1.80
17	Allethrin	-	-6.43	-
18	*Reference	-9.19	-8.25	-3.18

* Reference (**3ERT**: 4-HydroxyTamoxifene, **2PIT**: 5-Alpha-Dihydrotestosterone & **3F81**: 2-(5-methyl-4-oxo-2-thioxo-2,4-dihydro-3H-1lambda~4~,3-thiazol-3-yl)ethanesulfonic acid)

Docking analysis of the compounds is not an easy task but rather quite tricky process because inaccuracies of glide score may result in false results. One should always evaluating the docking results via glide score (energy), hydrogen & hydrophobic bonding interactions. In addition, non polar, polar atom interactions & binding pocket analysis are also of major concern.[17]

Top ranked pyrethroid HITs

Estrogen (PDB: 3ERT) cancer receptor

Resmethrin

This compound was placed the most potent hit against estrogen cancer receptor (PDB: 3ERT) with glide score as mentioned in table1. The hydrophobic interactions such as Met388, Leu391, Leu387, Leu349, Met343, Leu346, Met528, Leu525, Met522, Leu536, Trp83, Leu354, Ala350, Leu384 & Ile424 were observed.

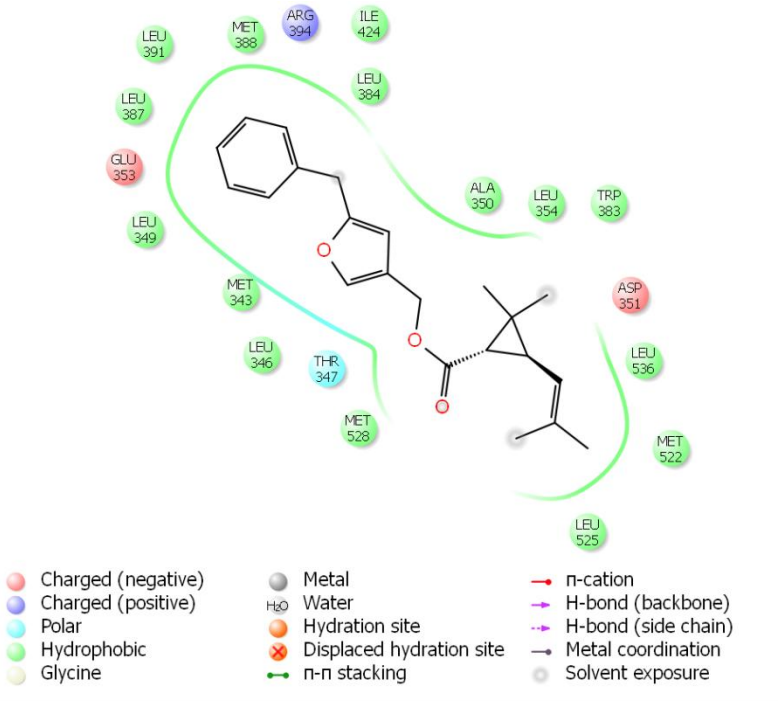


Figure 2: Binding interactions of pyrethroid (Resmethrin) with estrogen cancer cell receptor (PDB: 3ERT)

Cyhalothrin

It was also placed among top ranked hits with glide score as indicated in table 1. Interestingly, hydrophobic interactions with different amino acid residues like Met388, Leu387, Leu384, Ala350, Met528, Leu525, Val534, Leu539, Val533, Leu536, Trp383, Leu354, Met343, Leu346, Leu349, Phe404 & Leu391, correspondingly were reported.

Permethrin

Permethrin was also capable of showing hydrophobic interactions (Leu346, Leu391, Leu349, Leu539, Leu536, Leu534, Trp383, Ala350, Met528, Leu525, Met343, Met421, Phe404, Ile424, Met388, Leu384, Leu428 & Leu387) with above discussed cancer proteins.

Androgen (PDB: 2PIT) cancer receptor

Resmethrin

Numerous types of hydrophobic interactions (Phe764, Met749, Val746, Met745, Met742, Phe876, Leu873, Met780, Leu701, Leu704, Met787, Pro49, Ile48, Ile83, Leu880, Met895, Phe891, Ile899 & Leu707) were observed with this most potent Hit against androgen receptors proteins (Figure3). In addition, π-π interaction of furan ring (Trp741) was also observed.

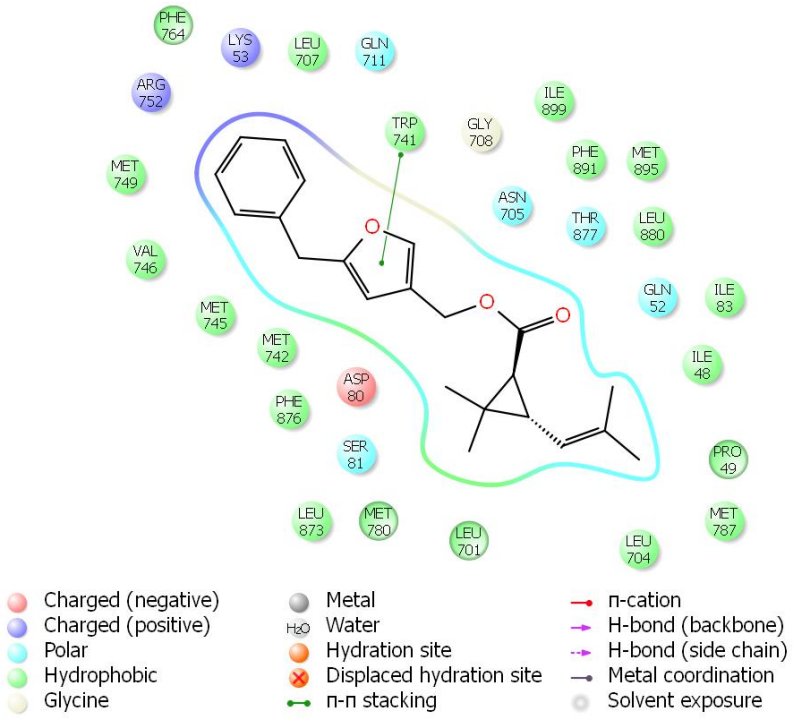


Figure 3: Binding interactions of pyrethroid (Resmethrin) with androgen cancer cell receptor (PDB: 2PIT)

Permethrin

The compound showed a very extensive hydrophobic bonding with Met745, Ala748, Met749, Phe764, Met749, Met895, Leu701, Leu880, Leu873, Met780, Phe876, Leu704, Val746, Met787, Phe891, Ile899 & Leu707, respectively. Notably, π-π interaction was also confirmed.

Allethrin

Allethrin has resulted in hydrophobic bonding with Met749, Val746, Met787, Met742, Trp741, Met745, Leu704, Ile899, Phe899, Leu880, Leu701, Met780, Phe876, Leu873, Leu707 & Phe764 against androgen protein.

Cervix (PDB: 3F81) cancer receptor

Fluvalinate

This compound was examined as apex strong hit and involved in hydrophobic interactions such as Tyr23, Cys124, Phe68, Met69, Leu25 & Cys22, correspondingly. Additionally, π-π interaction (Tyr128 with phenyl ring) was also present.

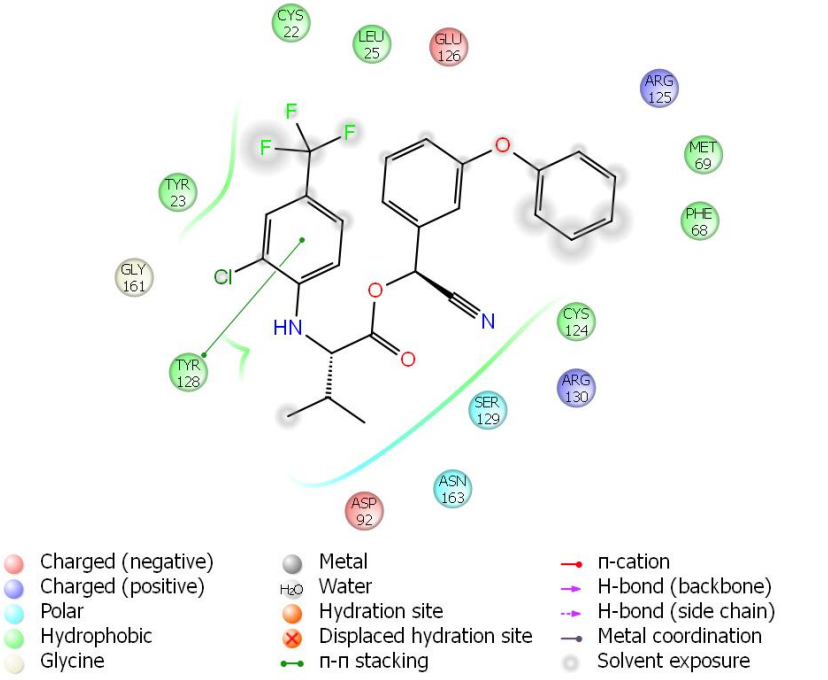


Figure 4: Binding interactions of pyrethroid (Fluvalinate) with cervix cancer cell receptor (PDB: 3F81)

Resmethrin

The hydrophobic interactions (Pro26, Leu25, Leu16, Pro162, Tyr23 & Cys22) and π-π interaction (Tyr128) were examined.

Fenpropathrin

This ranked compound was simply capable of hydrophobic interactions (Tyr128, Leu25, Pro26, Met69, Tyr23, Leu16, Leu167 & Pro167) with cervix cancer protein.

CONCLUSIONS

The glide score of tested pyrethroids were obtained from -1.12 to -7.47 which indicate the strong affinity towards cancer cell receptors. Top screened pyrethroids like resmethrin (3ERT & 2PIT) & fluvalinate (3F81) have resulted in most hopeful hits to anticancer assessment. The widespread applications of pyrethroids among population have turned researchers focus to unlock its novel potential and thus this recent preliminary molecular docking study can serve as an important breakthrough to further understand its anticancer nature with its clear mechanism. However, the complete efficacy and safety studies should be assessed to start a clinical trial for these compounds.

References

1. Siegel, R.; DeSantis, C.; Virgo, K.; Stein, K.; Mariotto, A.; Smith, T.; Cooper, D.; Gansler, T.; Lerro, C.; Fedewa, S.; Lin, C.; Leach, C.; Cannady, R. S.; Cho, H.; Scoppa, S.; Hachey, M.; Kirch, R.; Jemal, A.; Ward, E. Cancer treatment and survivorship statistics. *CA. Cancer J. Clin.*, **2012**, 62, 220–41.
2. Danaei, G.; Vander Hoorn, S.; Lopez, A. D.; Murray, C. J.; Ezzati, M. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. *Lancet*, **2005**, 366, 1784–93.
3. Boffetta, P.; Nyberg, F. Contribution of environmental factors to cancer risk. *Br. Med. Bull.*, **2003**, 68, 71–94.
4. Donaldson, K. L.; Goolsby, G. L.; Wahl, A. F. Cytotoxicity of the anticancer agents cisplatin and taxol during cell proliferation and the cell cycle. *Int. J. Cancer*, **1994**, 57, 847–55.
5. Saillenfait, A. M.; Nadiaye, D.; Sabate J. P. Pyrethroids : Exposure and health effect-an update. *Int. J. Hyg. Environ. Health*, **2015**, 218, 281-292.
6. Bradburry, S. P.; Coast, J. R. Comparative toxicology of pyrethroid insecticides. *Rev. Environ. Contam. T.*, **1989**, 108, 133-177.

7. Maud, S. J.; Hamer M. J.; Wariton J. S. Aquatic ecotoxicology of the pyrethroid insecticide lamdaccyhalothrin: consideration for higher-tier aquatic risk assessment. *J. Pest. Sci.,* **1998**, 54, 408-417.
8. Kumar, A.; Sasmal, D.; Sharma, N. An insight into deltamethrin induced apoptotic calcium, p53 and oxidative stress signalling pathways. *Toxicol. Environ. Health Sci.*, 2015, 7, 25-34.
9. Kumar, A.; Sasmal, D.; Sharma, N. Deltamethrin induced an apoptogenic signalling pathway in murine thymocytes: exploring the molecular mechanism. *J. Appl. Toxicol.*, **2014**, 34, 1303-1310.
10. Kumar, A.; Sasmal, D.; Bhaskar, A.; Mukhopadhyay, K.; Thakur, A.; Sharma, N. Deltamethrin-induced oxidative stress and mitochondrial caspase-dependent signaling pathways in murine splenocytes. *Environ. toxicol*., **2014**, DOI: 10.1002/tox.2209.
11. Kumar, A.; Sasmal, D.; Sharma, N.; Bhaskar, A.; Chandra, S.; Mukhopadhyay, K.; Kumar, M. Deltamethrin, a pyrethroid insecticide, could be a promising candidate as an anticancer agent. *Med. hypotheses*, **2015**, 85, 145-147.
12. Chi, C. C.; Chou, C. T.; Liang, W. Z.; Jan, C. R. Effect of the pesticide, deltamethrin, on Ca^{2+}signaling and apoptosis in OC2 human oral cancer cells. *Drug Chem. Toxicol*., **2014**, 37, 25–31.
13. Hsu, S. S.; Chou, C. T. Deltamethrin induced $[Ca^{2+]}$ rise and death in HGB human glioblastoma cells. *Chin. J. Physiol.*, **2012**, 55, 294–304.
14. Ganesh, S. M.; Awasthi, P.; Timiri, A. K.; Ghosh, M. A novel approach for rationale selection of medicinal plants against viruses via molecular docking studies. *The Pharmstudent* **2015**, 18-30.
15. Sliwoski, G.; Kothiwale, S.; Meiler, J.; Lowe, E. W. Computational methods in drug discovery. *Pharmacol. Rev.*, **2014**, 66, 334-395.
16. RCSB Protein Data Bank - RCSB PDB. http://www.rcsb.org/pdb/. (Last assessed on **05/10/2015**)
17. Kumar, M.; Dagar, V.; Gupta, VK.; Sharma, A. *In silico* docking studies of bioactive natural plant products as putative DHFR antagonists. *Med. Chem. Res.*, **2014**, 23, 810-817.