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Araştırma Makalesi

Hypericum perforatum'un *in vitro* sitotoksik değerlendirmesi ve model organizma *Tribolium castaneum* ve *Homo sapiens* üzerindeki PINK-1 inhibitörlerinin moleküler kenetleme ve dinamik analizi

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ÖZ

Hypericum türleri özellikle farmakolojik özellikleriyle bilinmektedir. Bitkinin ana bileşenlerinden biri, güçlü sitotoksik etkileriyle tümör inhibisyonunda kullanılabilen hiperisindir. Bu çalışmada, bu önemli bitki uçucu yağının MCF-7 meme kanseri hücre hattı üzerindeki antikanser etkisi incelenmiş ve *H. perforatum* L'un bileşikleri olan hiperisin, hiperosid ve hiperforinin hem insan hem de model organizma, *Tribolium castaneum*'un (Herbst) (Coleoptera: Tenebrionidae) PINK1 proteinine bağlanma potansiyeli *in silico* olarak araştırılmıştır. Son zamanlarda, tam genom dizilimi yapılan ilk tür olan *T. castaneum* da dahil olmak üzere pek çok böcek türü model organizma olarak önerilmiştir. Böceklerin dünya çapındaki dağılımları, çevresel önemleri ve üretimlerinin nispeten ucuz olması onlara olan ilgiyi arttırmıştır. Bu nedenle, *in silico* çalışmalarda, insan ve model organizma ile bağlanma benzerliklerini karşılaştırmak amacıyla *T. castaneum* model olarak kullanılmıştır. Çalışmada *H. perforatum*'un MCF-7 hücrelerindeki IC_{50} konsantrasyonu 98.765 µg/ml olarak belirlenmiştir. *In silico* bulgularına göre, -12,5 kcal/mol ile en uygun bağlanma afinitesi Hiperisin molekülü ile böcek PINK1 proteini arasında gözlenmiştir. Söz konusu bitki bileşenlerinin hücreleri stres kaynaklı mitokondriyal fonksiyon bozukluğundan koruduğu düşünülen PINK1 proteinine yüksek enerji ile bağlanıyor olması bitkisel orijinli tıbbi ilaçların ve biyopestisitlerin geliştirlmesi için umut vericidir.

Anahtar kelimeler: *Hypericum perforatum, Tribolium castaneum*, antikanser etki, *in silico,* PINK1

In vitro cytotoxic evaluation of *Hypericum perforatum* and molecular docking and dynamic analysis of PINK-1 inhibitors on model organism *Tribolium castaneum* and *Homo sapiens*

ABSTRACT

Hypericum species are especially known for their pharmacological characteristic. One of the major component of the plant is hypericin that can be used in tumor inhibition with its potent cytotoxic effects. In this study, the anticancer effect of *H. perforatum* essential oil on the MCF-7 breast cancer cell line was examined, and the binding potential of the compounds of *Hypericum perforatum* L., hypericin, hyperoside and hyperforin, to the PINK1 protein of both human and model organism, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) was investigated *in silico*. Recently, many insect species have been proposed as model organisms, including *T. castaneum*, that the first species with whole genome sequenced. Worlwide distribution of insects, their environmental importance and realtively inexpensive cultivation have increased the interest in them Therefore, in *in silico* studies, *T. castaneum* was used as a model to compare binding similarities with humans and model organisms. In the study, the IC_{50} concentration of *H. perforatum* L. on MCF-7 cells was determined to be 98.765 µg/ml. Based on *in silico* findings, the most favorable binding affinity of -12.5 kcal/mol was observed between the Hypericin molecule and the insect PINK1 protein. The fact that these plant components bind with

high energy to the PINK1 protein, which is believed to guard cells from mitochondrial dysfunction triggered by stress, is promising for the development of plant-based medical drugs and biopesticides.

Key words: *Hypericum perforatum, Tribolium castaneum,* anticancer effect*, in silico*, PINK1.

INTRODUCTION

Hypericum spp.*,* belonging to the Hypericaceae family, comprises approximately 484 species (Guedes et al., 2012). *Hypericum* (Hypericaceae), are flowering plants known to be invasive and harmful weeds, spreading prolifically in various environments apart from polar regions, deserts, and tropical lowlands. These plants exhibit a diverse range of structures, such as trees, shrubs, annuals, and perennials. Many woody species of *Hypericum* possess multiple stems originating from a common base, while shrub varieties have upright or spreading stems. Most Hypericum species reproduce through apomixis. Some species have simple single rows of petals, while others feature elongated and slender arrangements of petals. The variable nature of *Hypericum* species contributes to their adaptability and spread in different regions (Crockett and Robson, 2011).

Among these species lots of them are used as therapeutic aromatic plants customarily (Ferreira et al., 2006) and one of these plants is *H. perforatum* L., (St John's wort) that has been incorporated into the pharmacopeia of numerous countries. (Jaric et al., 2007). These plants are known for their abilities to aid in wound healing, act as bactericidal agents, possess diuretic properties, exhibit anti-inflammatory effects, and even offer sedative qualities. The diverse range of medicinal benefits attributed to Hypericum species has made them significant in traditional medicine practices worldwide (Çırak and Kurt 2014).The main bioactive compounds of *H. perforatum* are hypericin, hyperforin, and flavonoids (Kimira et al., 1998, Barnes et al., 2001). Hypericin exhibits potent cytotoxic and pro-apoptotic properties against cancerous cells (Agostinis et al., 2002). It is known that various medicinal and aromatic plants are rich sources of phytochemicals with different biological effects (Yuca et al., 2022). In literature have been implicated apoptotic roles of *H. perforatum* on *in vitro* and *in vivo* several cancer cells (Jang et al., 2002, Borawska et al., 2016, Mirmalek et al., 2016).

In previous researches have explored the effectiveness of insecticides of essential oils extracted from various *Hypericum* species and they have been suggested as alternative method for future works in pest management (Rouis et al., 2013, Parchin and Ebadollahi 2016). *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), significantly impacts the quality and quantity of products derived from beans, grains, dried fruits, nuts, flour, peas, and spices, making it a significant pest in stored goods (Caballero-Gallardo et al., 2011, Khan et al., 2014).

In addition, the importance of *T. castaneum* in this study is its potential as a model organism. Many species of Coleopteran have been used as a model organism especially in biomedical and environmental researches. Pharmacological studies were conducted to assess the impact of novel active substances on beetles. It should be emphasized that the insects themselves may be the origin of substances that exhibit antimicrobial and anticancer effects. Up to the present, the sequence of 11 insect species is available and seven have been released (McKenna 2018). The genome of *T. castaneum*, a significant pest in stored products, was the first beetle genome to be sequenced (Tribolium Genome Sequencing Consortium, 2008). Some developmental characteristics of *T. castaneum* were found to be more comparable to mammals than *Drosophila* (Schroder et al., 2008). Genetic characteristic of *T. castaneum* made this beetle an important model in many research areas.

The molecular docking method is a method used to predict the best-matching binding mode of a ligand to macromolecules. Nowadays, 3D structures of various compounds can be designed *in silico* and their physicochemical structures can be determined. In addition to studies on the use of various substances as active pharmaceutical ingredients, *in silico* tools are also used in the characterization of plant viral disease agents (Güller et al., 2024).

In the research, the impact of *H. perforatum* was investigated about its cytotoxic activity against breast cancer cell line. In addition, we used molecular docking analyses to determine the interaction between PINK1 protein of both cancer and *T. castaneum* and hypericin, hyperoside and hyperforin compounds of *H. perforatum*.

MATERIALS AND METHODS

Plant material

H. perforatum were collected in June 2017 in Uşak region of Turkey by Hatice Baş. The species identification was conducted by Prof. Dr. Ümit BUDAK (Yozgat Bozok University, Faculty of Science and Arts, Department of Biology). Dried aerial parts of plants were exposed for 4 hours to water distillation using with a Clevenger type apparatus. The derived essential oil was stored at 4 °C until beginning of the study.

Cytotoxic effect of *Hypericum perforatum* on cancer cells

MCF-7 cancer cells were cultured in 75T cell culture flasks using RPMI/1640 culture medium, which was enriched with 10% fetal bovine serum (FBS) and 1% solution of gentamicin. The cells were maintained at 37°C in an environment with 5% CO2. The impact of *H. perforatum* L. on the viability of MCF-7 cells was assessed using the Cell Proliferation Kit (XTT), following the guidelines provided by the manufacturer. In brief, cells were seeded in 96-well plates at 5 x 104 cells/cm2 and the final exposure concentrations of H. perforatum ranged from 0 to 500 µg/mL. On each plate, an assay was conducted featuring a blank medium control column and a cell control column. Subsequently, XTT reagent was added and the soluble product was quantified at 482 nm using the BIOTEK 96-well plate reader.

In our previous study, the *H. perforatum*'s essential oil was tested for its fumigant efficacy against adult stage of the *T. castaneum*. Different concentrations of essential oil (0-30 µL/L air) were exposed to insect adults for 24 hours. It was observed that the mortality percentage of adults increased with increasing dose of essential oil. Based on the findings from probit analysis, LC_{50} and LC_{99} values of essential oil were calculated as 16,512-32,732, µL/L air against *T. castaneum* adult stage, respectively. Determination of binding energy by molecular docking of a known essential oil with insecticidal effect proves the accuracy of this effect.

Molecular Docking Studies

Molecular docking computations were executed using the Lamarckian Generic Algorithm (Morris et al., 1998) implemented in Autodock Vina (Trott and Olson, 2010). Water molecules and cofactors were excluded from the protein structure to enhance the visibility of the interactions between the ligand and the receptor. Binding affinities were determined using Autodock Vina. Additionally, the PINK1 protein's 3D theoretical model can be obtained freely from the RCSB Protein Data Bank (PDB ID: 5yj9 for T. castaneum) (PDB ID: 6glc for Homo sapiens) (https://www.rcsb.org).

The 3D molecular structure of hypericin, hyperforin and hyperoside metabolites were retrieved from chemical databases namely PubChem. And *in silico* docking was conducted utilizing Autodock Vina in combination with protein cavity modeling software. The present work describes the molecular interaction of hypericin, hyperforin and hyperoside metabolites of *H. perforatum* L. with PINK1 protein using an molecular docking analysis softwares, Autodock Vina, Molegro Molecular Viewer 2.5 (Molegro Molecular viewer free software) (http://www.molegro.com) Thomsen and Christensen 2006). In this study, the grid size of PINK-1 was set to 124x124x80 points with 1.000 Å spacing centered on hyperforin, 126x126x94 points with 1.000 Å spacing centered on hypericin, 126x116x82 points with 1.000 Å spacing centered on hyperoside for T. castaneum, 126x102x96 points with 1.000 Å spacing centered on hyperforin, 126x104x94 points with 1.000 Å spacing centered on hypericin and 126x86x114 points with 1.000 Å spacing centered on hyperoside for Homo sapiens.

Molecular Dynamic Studies

Molecular docking computations were executed using Lamarck's generic algorithm in our previous study (Ercan et al. 2019). The structure of the PINK1 protein is freely available in the RCSB Protein Data Bank as a theoretical 3D model (PDB ID: 5yj9 for *T. castaneum*) (PDB ID: 6glc for Homo sapiens) (https://www.rcsb.org). The 3D molecular structure of hypericin, hyperforin, and hyperoside metabolites was retrieved from chemical databases, namely PubChem, and used for dynamic in silico studies. The simulation of the complex formed by the ligand and protein was carried out using webgro (Bekker et al. 1993; Abraham et al. 2015; Lindorff-Larsen et al. 2010; Bjelkmar et al. 2010; Oostenbrink et al. 2004). The MD simulation lasting 20 ns was conducted to assess the stability of the complexes formed by the ligand and protein.

RESULTS AND DISCUSSION

The antiproliferative effects of the *Hypericum perforatum* extracts on MCF-7 cell line

In this research, we explored the impact of *H. perforatum* L. extracts on the proliferation of the MCF-7 cell line, assessed through the XTT Cell Proliferation Kit following the guidelines provided by the manufacturer. HeLa cells were seeded into 96-well microtiter plates at a concentration of 5.0X104. The impact of *H. perforatum* L. on the viability of MCF-7 cells was explored through the XTT cell proliferation assay, and the IC_{50} values were determined. In this study, the IC_{50} value of *H. perforatum* L. was found as 98.765 µg/ml on MCF-7 cells (Figure 1). This study provides new evidence for the antiproliferative effect of *H. perforatum* on MCF-7 cell line and could serve as a promising naturally derived antitumor agent against cancer.

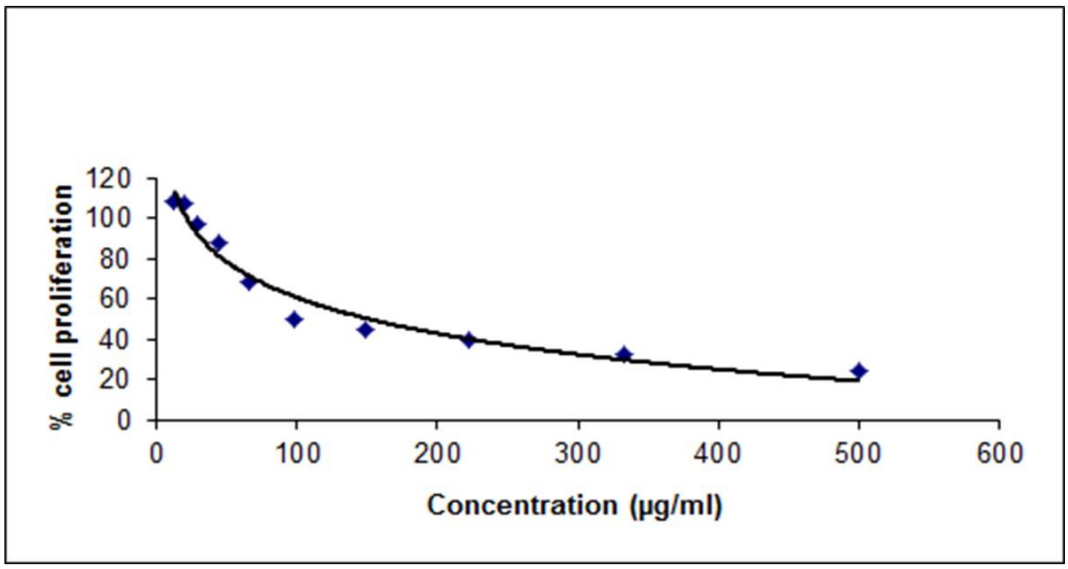

Figure 1. Cytotoxicity analyses of *H. perforatum* on MCF-7 cells

Molecular Docking Studies

Docking outcomes were acquired through Autodock Vina, Molegro, and VMD. The binding strength was determined using a scoring function derived from the Lamarckian Generic Algorithm. The binding free energy might encompass electrostatic, hydrogen bonding, and Van der Waals interactions. The highest scoring indicates a strong binding affinity between the protein and the ligand. In our investigation, the most significant binding score was observed between *T. castaneum* PINK1 protein with the hypericin molecule. The docking results computed by the Vina for PINK1 proteins of *T. castaneum* and human with hypericin, hyperforin and hyperoside metabolites of *H. perforatum* (Table 1.)

Table 1. Docking binding energy results of hypericin, hyperforin and hyperoside metabolites as inhibitor with PINK1 proteins of both human and *T. castaneum*.

PINK1	Binding Energy (K.Cal/mol) Human	Binding Energy (K.Cal/mol) Tribolium castaneum
Hypericin	-10.8	-12.5
Hyperforin	-9.1	-8.5
Hyperoside	-10.4	-9.9

Based on these findings, the most significant binding score was achieved in the interaction between the Hypericin molecule and the insect PINK1 protein, with an affinity energy of -12.5 kcal/mol. Hyperforin molecule showed

lower binding free energy for insect PINK1 protein. All tested molecules were found to be docked at both human and insect PINK1 protein with good information. Figure 2 and Figure 3 illustrate the interactions between Hypericin and the PINK1 protein. In the hypericin and human PINK1 compound, hydrogen bonds with oxygen atom of the ester and Gln 327 residue was identifed. Electrostatic interaction with amino acid residue Lys 219 was also determined. For *T. castaneum*, both hydrogen and electrostatic interactions can be seen with hypericin and amino acid residue Tyr 297 (Figure 3).

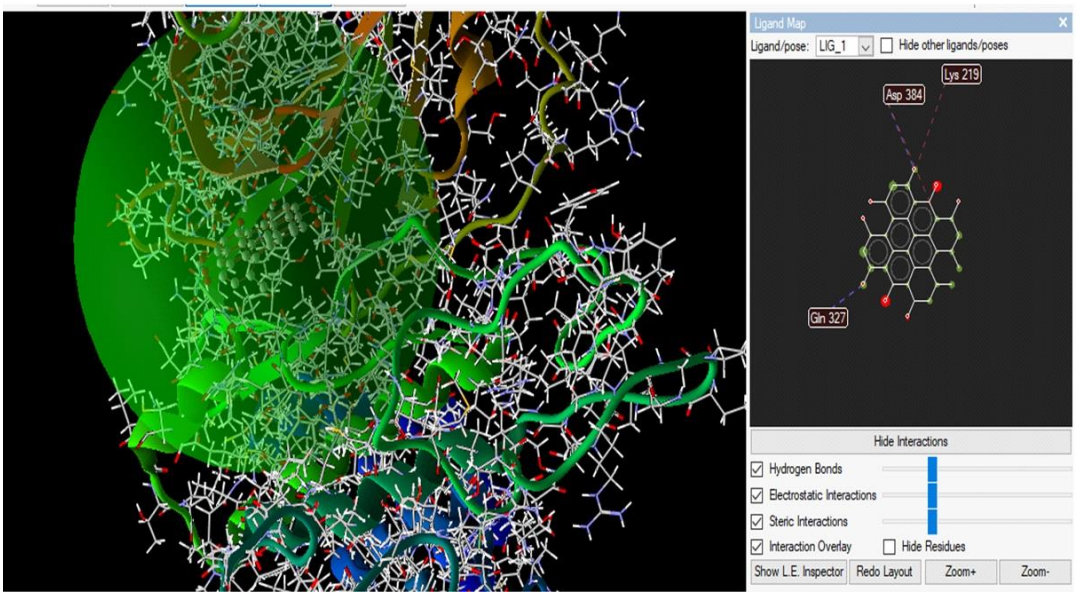

Figure 2. The 3D representation of Hypericin at the active site of human PINK1 protein in molecular docking.

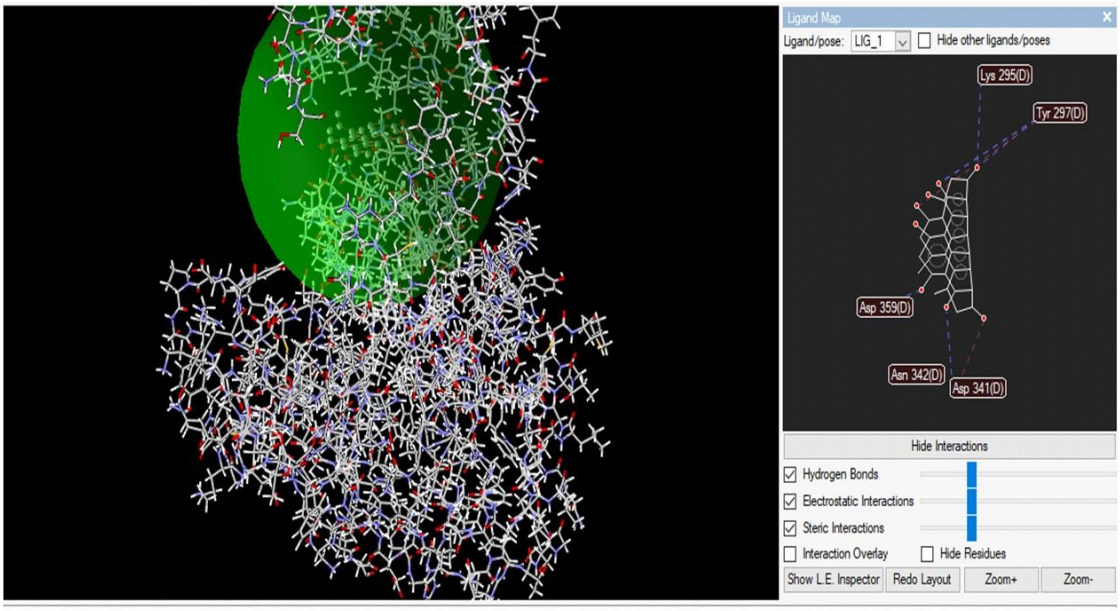

Figure 3. The 3D representation of Hypericin at the active site of insect PINK1 protein in molecular docking.

Hyperforin and PINK 1 protein docking results were summarized in Figure 4 and Figure 5. For human PINK1 protein, only an electrostatic interaction was identified with amino acid residue Val 247. In human, the highest count of hydrogen bond interactions was observed in the interaction between hyperoside and the PINK1 protein. Hydrogen bonds with hyperoside and Asp 362, Asp 366, Asp 384, Lys 219 and Ala 384 were shown in Figure 6. In *T. castaneum* PINK1 protein and hyperoside interaction, hydrogen bonds can be observed with residue Glu 502, Glu 530 and Ser 509; electrostatic interactions were seen between ligand and residue Pro 487 and Lys 528 (Figure 7).

Molecular docking is a structure based method that makes possible to determine the molecules will fit with an enough binding energy. In the present study, the cytotoxicity and docking results disclosed the significant anti-neoplasic activity of the H. perforatum. Our findings indicate that hypericin exhibits a strong affinity for the PINK1 protein, suggesting its potential utility as an inhibitor of PINK1 and a novel, natural chemotherapy agent. Hypericin is naturally occured in H. perforatum and is known that can be used as a photosensitizer in the photodynamic treatment of tumors, inflammatory diseases and infections (Miskovsky 2002).

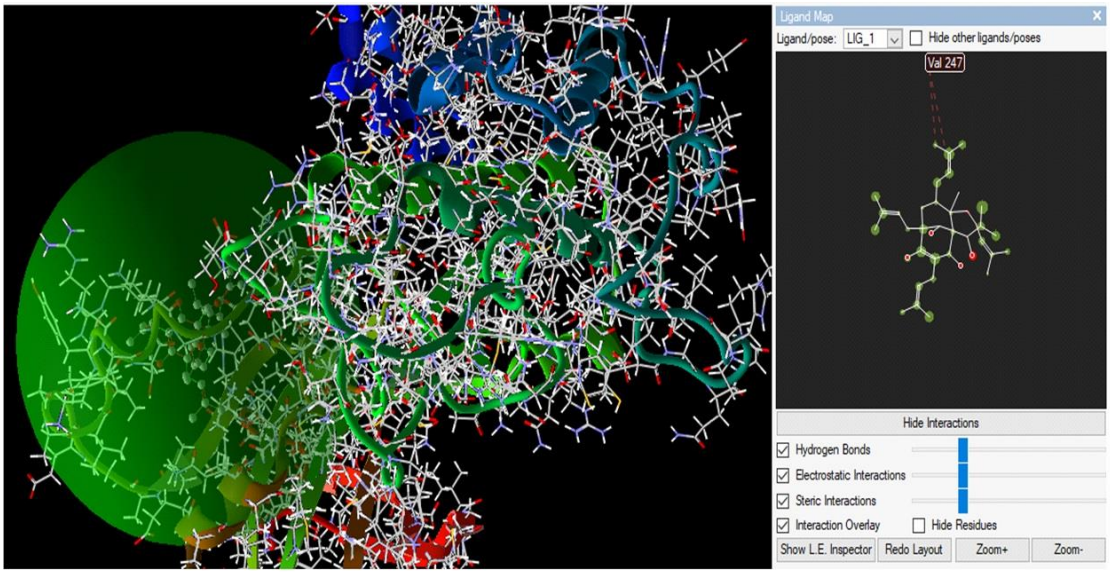

Figure 4. The 3D representation of Hyperforin at the active site of human PINK1 protein in molecular docking

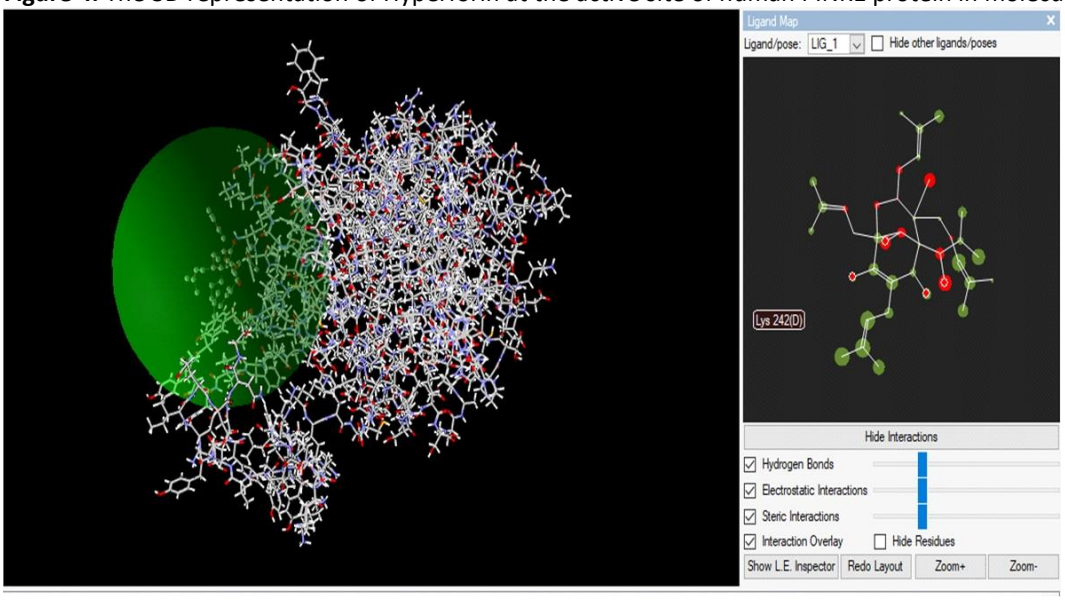

Figure 5. The 3D representation of Hyperforin at the active site of insect PINK1 protein in molecular docking

Previous studies have shown that Hypericin also exhibits strong anti-proliferative and anti-metastatic effects without light activation (Blank et al., 2004). Like hypericin, hyperforin is one of the most important active components of *H. perforatum*. This compound is known to have several important medicinal properties such as antidepressant, anticarcinogenic and proapoptotic (Medina et al., 2006). In the study, Hyperforin, a phloroglucinol compounds of H. perforatum, shown the lowest binding energy against PINK1 protein of both human and insect.

Hyperoside, is a flavonol glycoside that has been isolated from many medicinal plants such as H. perforatum (Zhou et al., 2006). In previous studies, anti-inflammatory, anti-depressant, cardio-protective, neuroprotective,

anti-diabetic, anti-fungal, anti-cancer and antioxidant properties of this compound are known (Huang et al., 2008). Both cytotoxicity and docking results of our study support the potential of this plant, in particular the hypericin, to be used in anticancer drug studies.

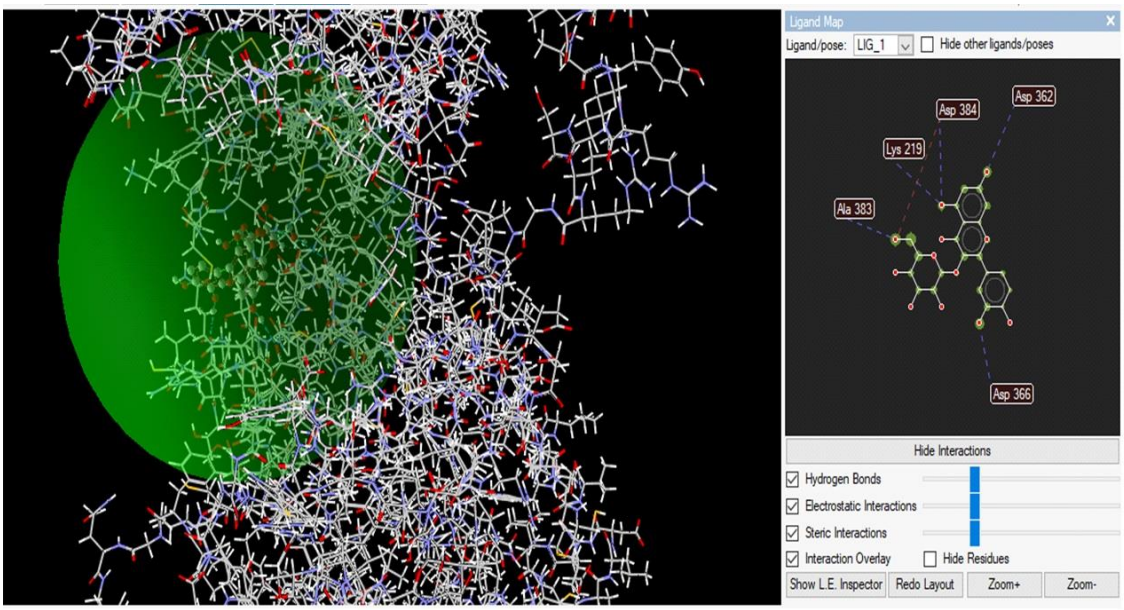

Figure 6. The 3D representation of Hyperoside at the active site of human PINK1 protein in molecular docking

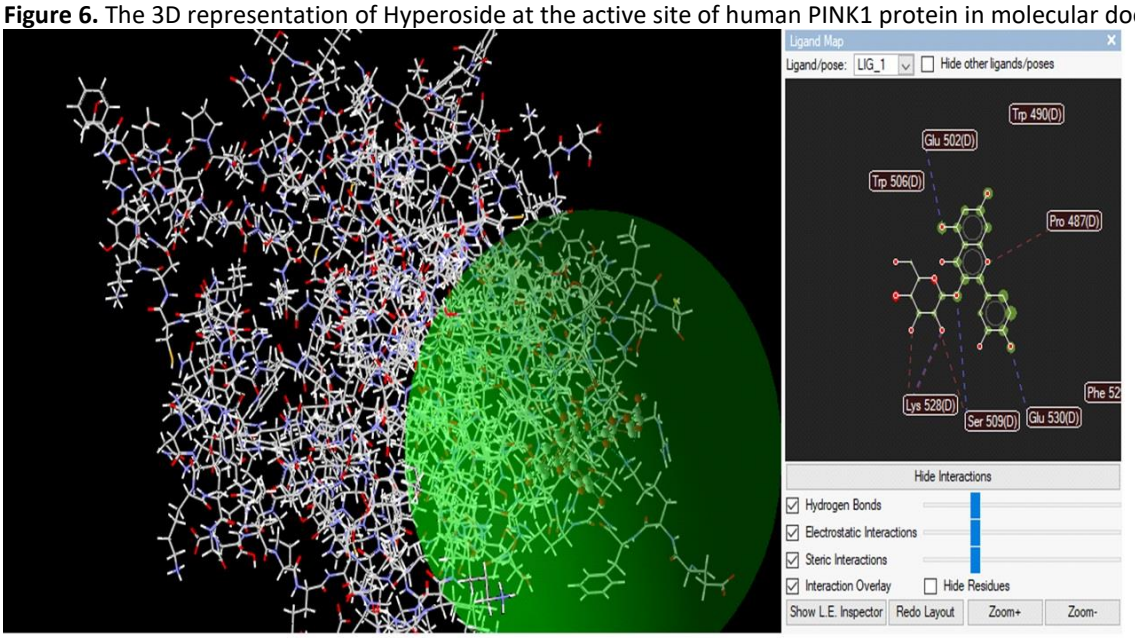

Figure 7. The 3D representation of Hyperoside at the active site of insect PINK1 protein in molecular docking

Molecular Dynamic Studies

Molecular dynamics simulations were executed for protein-ligand complex using WebGro, incorporating GROMOS9643a1 force field for 20 ns at physiological pH of 7.4. For dynamic analysis, root mean square deviation (RMSD) and the root means square fluctuations (RMSF) values were obtained. RSMD is a vital parameter for analyzing the stability of MD orbitals and is predicted for backbone atoms of protein and ligand-protein complexes. The comparisons of the RMSD and RMSF value of ligands-protein were shown in Figures 8 and 9 in both human and model organism. To determine the flexibility of the protein backbone structure, the RMSF of the backbone atoms of each residue within the Ligand-protein complex was examined. A high RMSF value suggests increased flexibility, whereas a low RMSF value indicates restricted movements. RMSD of backbone atoms of Hyperforin was observed between 0,25 and 0.6 Å, whereas for Hypericin 0,25–0.6 Å and Hyperocide 0,25-0,6 Å values were observed in human. RMSD of backbone atoms of Hyperforin, Hypericin, and Hyperocide

was observed between 0,2 and 0.4 Å in T. castaneum. In homo sapiens, RMSF of Hypericin was within the limit of 2.5 Å, but RMSF for other residues exceeds 3.0 Å (Figure 8-9).

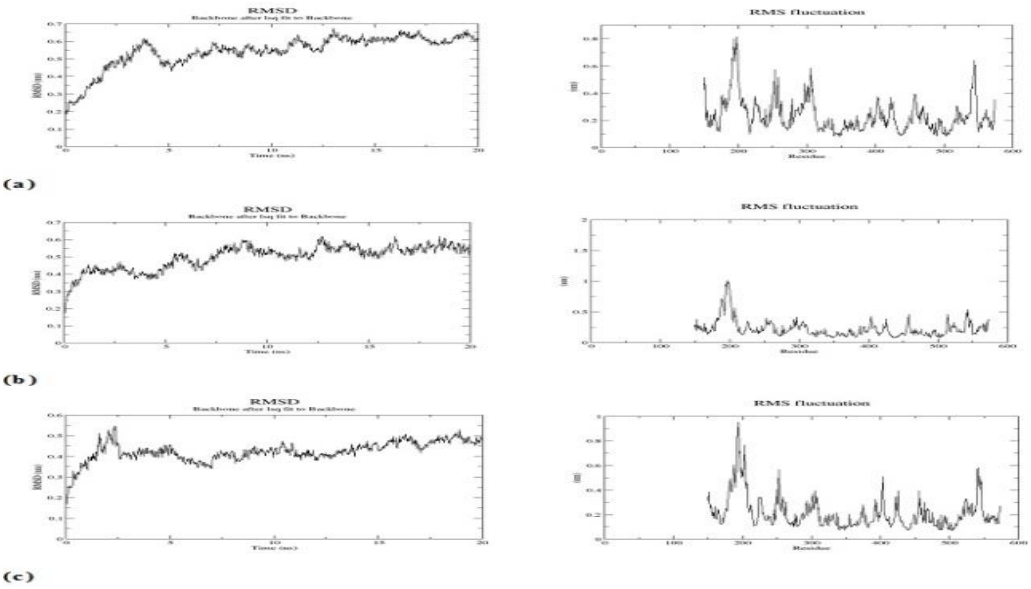

Figure 8. (a) Hyperforin and Human PINK 1 protein Molecular dynamic results. (b) Hypericin and Human PINK 1 protein Molecular dynamic results. (c) Hyperocide and Human PINK 1 protein Molecular dynamic results.

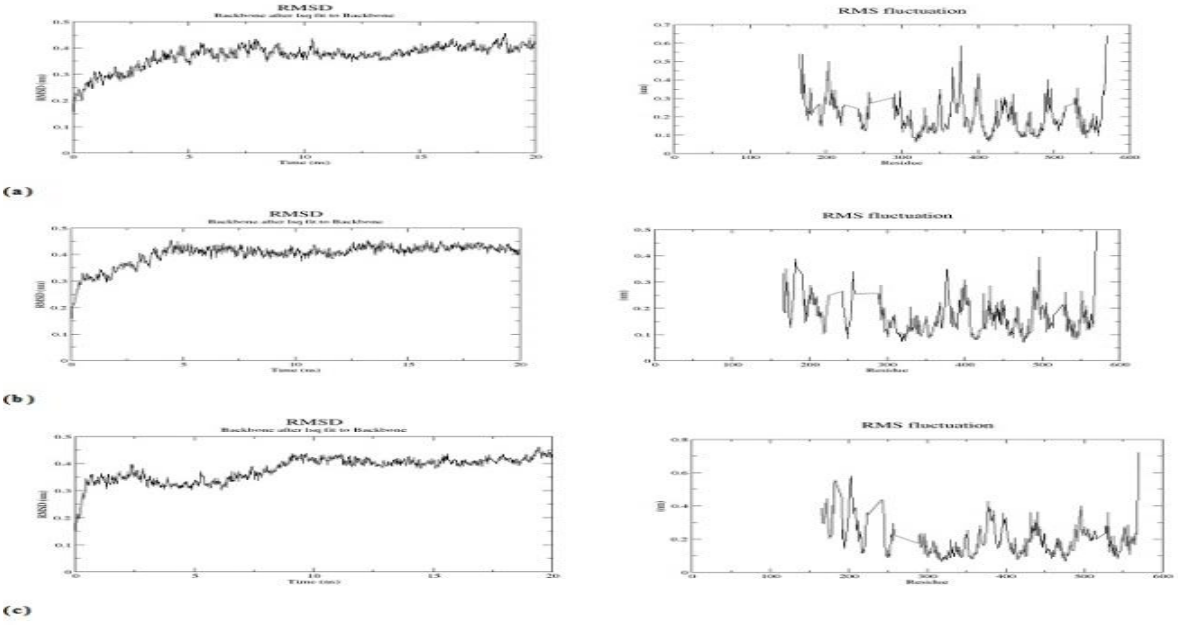

Figure 9. (a) Hyperforin and *T. castaneum* PINK 1 protein Molecular dynamic results. (b) Hypericin and Human *T. castaneum* PINK 1 protein Molecular dynamic results. (c) Hyperocide and Human *T. castaneum* PINK 1 protein Molecular dynamic results.

CONCLUSION

In recent years, biologic potential of active components of *H. perforatum* has major effect. Within the framework of this information, we analysed on the biological potential of *H. perforatum* both cancer cells and *T. casteneum*. Our findings suggest that *H. perforatum* could be valued as excellent source as therapeutic agent and fumigant. However, these findings should be supported by animal experiments in future studies..

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Conflict of Interest Statement: The authors declare that they have no conflict of interest.

Contribution Rate Statement Summary of Researchers: FSE was responsible for molecular docking and prepared the manuscript. SYA prepared the in vitro analyses. HB prepared in silico analyses. SY was responsible for in vitro analyses. All authors contributed to writing and editing the manuscript. All authors read and approved the final manuscript.

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