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Screening *Tribulus terrestris* derived phytochemicals for oral cancer therapy: A molecular docking study

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Abstract
A member of the sirtuin family and a NAD+-dependent deacetylase, Sirtuin 1 (SIRT1) plays a multifaceted role in oral cancer. Oral cancer development is significantly influenced by SIRT1 expression levels. By increasing E-cadherin levels, consistent SIRT1 expression enhances epithelial integrity and reduces the risk of invasion and metastasis in oral cancer (103, 104). According to the results, SIRT1 may have a tumor-suppressive function in oral cancer. The biological functions of SIRT1 and its possible therapeutic significance in precancerous oral lesions and oral cancer are examined in this work. In this study, phytocompounds derived from *Tribulus terrestris* were subjected to structure-based virtual screening (SBVS). The objective was to develop new, strong, and more stable SIRT1 inhibitors. AutoDock VINA, a component of the PyRx 0.8 tool, was used to conduct the study. Out of the twenty compounds, four with high binding affinities were selected for additional in vitro testing based on ligand binding energy.

Keywords: Oral cancer, Sirtuin 1, *Tribulus terrestris*, molecular docking

Introduction
The sirtuin family proteins (SIRT) are class III histone deacetylases (HDACs) comprising seven members. Numerous biological activities have been linked to sirtuin proteins, which are abundantly expressed in healthy tissues. The most researched member of the family, SIRT1, was the first to be identified. Numerous studies have examined its biological involvement in cancer, but the findings addressing the relationship between the two have been mixed, as SIRT1 can either promote or suppress cancer depending on the cell type or composition [1-3].
About 90% of all oral cavity cancers are oral squamous cell carcinomas (OSCCs), making them the most prevalent single entity and a major public health concern in many Asian nations [4, 5]. It has been demonstrated that the expression level of SIRT1 plays a crucial role in the pathogenesis of oral cancer, the sixth most common cancer worldwide [6, 7]. Chewing betel quid, smoking, drinking alcohol, genetic susceptibility, and viral infections such as human papillomavirus (HPV) are among the causes of oral cancer [8, 9]. Despite advancements in oncology, the overall 5-year survival statistics for patients with OSCC (ranging from 34% to 62.9%) have not significantly improved in decades. These findings highlight the importance of exploring novel factors influencing oral cancer and identifying potential therapeutic targets [10, 11].
The Zygophyllaceae family includes *Tribulus terrestris* L. (*T. terrestris*), which is widely cultivated in Mediterranean and subtropical regions, including India, China, Mexico, Spain, Bulgaria, and Pakistan [12, 13]. Known locally as Tribulus, goat head, and harsh thorns, *T. terrestris* is considered an integral part of traditional medicine systems in China, Europe, and India (Ayurveda). In China, the fruits of *T. terrestris* are said to help prevent kidney problems, alleviate cough, and improve vision [14, 15]. Its roots are considered cardiotonic, while its fruits are used in Ayurveda to treat infertility. In Sudan, *T. terrestris* is used to treat or prevent glomerulus inflammation (nephritis) and other inflammatory conditions [16, 17]. In Pakistan, it is utilized as a uricosuric and diuretic [18].

Pharmacological studies strongly support traditional medicinal claims about *T. terrestris*, which is extensively used to treat various conditions, including inflammation, microbial infections, oxidative damage, hormonal issues, cancers, and cardiovascular diseases (9,20).

Steroid saponins have been identified as the biologically active components of *T. terrestris*. These saponins have been reported to induce apoptosis in breast cancer cells, contributing to their anticancer effects [21, 22]. Alkaloids such as trans-N-feruloyl-3-hydroxytyramine and trans-N-feruloyl-3-ethoxytyramine from *T. terrestris* have also been shown to induce apoptosis in leukemic cancer cells [23, 24]. Using *in silico* approaches, this study aimed to elucidate the molecular mechanisms underlying the anticancer activities of *T. terrestris*.

The potential for computational drug development methods to expedite the process in terms of time, labor, and cost has garnered a lot of interest [25]. Numerous new drugs have been successfully designed using computational methods [26]. Historically, the majority of new treatments have come from natural compounds secondary metabolites [27]. Approximately 25% of all FDA-approved medications, including well-known treatments like morphine and paclitaxel, are plant-based [28, 29]. In fact, the discovery of medications derived from natural ingredients has revolutionized medicine.

Virtual screening of natural compounds has grown in popularity in pharmaceutical research to identify potential interactors with specific pharmacological targets. This approach is advantageous due to its rapid output, low cost, and high efficiency [30]. The goal of this *in silico* investigation was to explore and evaluate the crucial binding interactions between SIRT1 and phytochemicals extracted from *T. terrestris*. Molecular docking analysis was employed to accomplish this. Comprehensive *in silico* techniques were applied to the phytocompounds from *T. terrestris* to identify potential SIRT1 inhibitors from natural sources.

Materials and Methods

Protein preparation

The NCBI Research Collaboratory for Structural Bioinformatics provides online access to the structures of the human sirtuin protein family, including the SIRT1 isoform (UniProt: Q96EB6) [31]. The Protein Data Bank (PDB) provided the three-dimensional structure of human SIRT1. The selected structure (ID: 5BTR) displayed a crystal configuration in which AMC-containing peptide and resveratrol bind to SIRT1 [32] (https://www.rcsb.org/structure/5BTR). The thorough knowledge of the entire sequence length, as well as the catalytic and allosteric binding sites, led to the selection of this structure.

After retrieving the 3D structure, UCSF Chimera software was used to prepare the protein by refining and checking for missing residues [33]. The original structure was stripped of ligands (resveratrol and AMC-containing peptide), water molecules, and extra protein chains. Using UCSF Chimera, 1000 steps (step size = 0.02 Å) of protein structural stability, energy minimization, and shape optimization were carried out using the conjugate gradient technique. The AMBER ff98 force field approach was used to protonate wild-type histone during the process [34]. Additional energy minimization was performed using the Chiron online server to ensure SIRT1's structural integrity [35]. The CastP online tool was employed to predict the active site surrounding the allosteric binding sites of SIRT1 [36].

Selection of Compounds and preparation of Structures

Twenty *T. terrestris* phytochemicals were selected based on published research. Their two-dimensional chemical structures were obtained from the PubChem database [37]. Using PyRx 0.8, the compounds were imported into OpenBabel and subjected to energy minimization [38]. The conjugate gradient method, with the universal force field (UFF), was used for energy minimization with an update step count fixed at 1 and an overall step count of 2000. The minimization process stopped when the energy differential dropped below 0.01 kcal/mol. The minimized compounds were converted to PDBQT format for further research.

Virtual Screening

A structure-based virtual screening (SBVS) employing docking simulations was conducted on prepared libraries to identify novel, effective SIRT1 inhibitors. The 5BTR crystal structure served as the receptor, while chemical libraries served as ligands. Binding energies were computed using PyRx AutoDock VINA [39]. A grid box was configured to encompass the active site of the crystal structure, with the default X, Y, Z center and dimensions, and an exhaustiveness of 8. The top-ranked compounds were further screened using AutoDock 4.2 [40]. The results were evaluated using Discovery Studio [41].

Results and Discussion

With a wide range of clinical symptoms, oral squamous cell carcinoma (OSCC) is the most common kind of oral cancer, accounting for approximately 90% of all oral cancers. Chronic inflammation, Candida or human papillomavirus infections, alcohol and tobacco use, UV radiation, immunosuppression, genetic susceptibility, and dietary practices are the main risk factors for oral cancer. Even with the advent of new treatment techniques, the five-year survival rate for oral cancer is still less than 50% in the majority of countries. Tobacco and alcohol use are two of the biggest risk factors for oral cancer.

Survival rates for oral cancer have not increased much over the last few decades, despite a number of advancements in treatment options. Therefore, in order to stop oral cancer from forming, innovative and efficient treatments are needed.

Many authors have reported on the effectiveness of plant-based phytochemicals and natural products in treating oral diseases in recent years. The ability of a variety of natural compounds to cause human cancer cells to undergo apoptosis has been investigated. By stopping the cell cycle and inducing apoptosis, these phytocompounds may be used as an oral cancer treatment with less systemic toxicity and adverse effects in people. Traditional remedies are used by a significant portion of the global population to treat a variety of illnesses. Potential compounds with promising efficacy against OSCC and other malignancies include curcumin, lycopene, ginseng, anthocyanins, and artemisinin.

Tribulus terrestris L. has been used extensively in many traditional medical systems, such as Ayurveda, to treat a wide range of human ailments. Recent research has shown that the main contributors to *T. terrestris*'s traditional medical efficacy are its chemical contents, including its steroidal saponins and flavonoids, which have strong anti-inflammatory and anti-aging qualities.

Sirtuins (SIRT1-) are a family of NAD+-dependent

deacetylases classified as class III histone deacetylases, involved in various physiological processes such as metabolism, the cell cycle, and ageing. The most extensively researched sirtuin is SIRT1 (silent mating type information regulation 2 homolog 1), a human counterpart of yeast Sir2.6. SIRT1 facilitates or suppresses numerous biological processes, such as gene expression regulation, cellular metabolism, stress response, ageing, and chemoresistance; however, its involvement in cancer remains unclear. Upregulation of SIRT1 has been documented in numerous solid tumours. SIRT1 has been demonstrated to inhibit tumour suppressors, such as p53, or activate tumour promoters, such as the PTEN/PI3K/AKT pathway, thereby facilitating carcinogenesis. Numerous studies have indicated the downregulation of SIRT1 in certain tumours, implying its function as a tumour suppressor. The function of SIRT1 in the progression and metastasis of OSCC remains unidentified. This study investigates the anti-oral action of *T. terrestris* utilising in silico methodologies.

Identifying natural chemicals for the development of innovative chemotherapeutics to address oral cancer is essential. Since antiquity, botanical substances have been employed to remedy various afflictions. Specific plant phytochemicals have been utilised to develop pharmaceuticals for the treatment of various ailments, including cancer. The phytoconstituents of medicinal plants continue to be a significant source for the development and design of innovative anti-cancer pharmaceuticals. Numerous studies have shown that natural chemicals derived from medicinal plants are safe and has the potential to inhibit the proliferation of numerous cancer types. In this study the anti-cancer activities was predicted using computational approach.

A computer technique called molecular docking predicts the binding affinity and orientation of a protein (the target) by analysing its interactions with a small molecule (the ligand). Chemicals from *T. terrestris* and SIRT1 showed significant binding affinities, with values ranging from -7.1 to -9.9 kcal/mol, according to molecular docking tests.

With the highest binding affinities, hecogenin and diosgenin demonstrated a great deal of promise as SIRT1 inhibitors. According to the *T. terrestris* and SIRT1 binding investigation, these interactions were primarily made possible by hydrophobic and hydrogen bonding interactions with important residues in the SIRT1 active region. The efficient inhibition of SIRT1 function, which may lead to the inhibition of angiogenesis, metastasis, and cancer cell proliferation, depends on these relationships. These results are consistent with earlier computer studies that highlight the significance of specific amino acid combinations in ensuring the effectiveness of medications.

As it is clear from the molecular docking findings, various phytocompounds of *Tribulus terrestris* have shown good binding affinity with a particular target protein which indicates their potential for further research as inhibitors or modulators of the target protein's function. Such binding energy (kcal/mol) represents the energy worth to be supplied in breaking a bond formed between a compound and its target molecule(s) which in this case is a probability a protein. The lower the value, the stronger the interaction. In this particular case the binding energies for Polianthoside B and Diosgrnin and Hecogenin are recorded between -3.3 kcal/mol and -9.9 kcal/mol respectively. The target strength is directly proportional to the number of ligands or the binding energies. Hecogenin, Diosgrnin, and Kaempferol have strong binding, which indicates they could be effective inhibitors or modulators. The number of amino acids that participate in binding also change; some only interact with one amino acid while other poly ligands interact with several amino acids simultaneously. These may affect binding's specificity and stability. There are, however, certain amino acids that occur more often than others in the interaction. Or, another explanation could be that certain functionalities within these amino acids enhance binding. For instance, both ARG-82 and ALA-70 turn out to be elusive over and over choice of ligand binding sites, due to extensive interaction with the provided amino acids. PHE-81, PHE-105, and HIS-17

Figure format

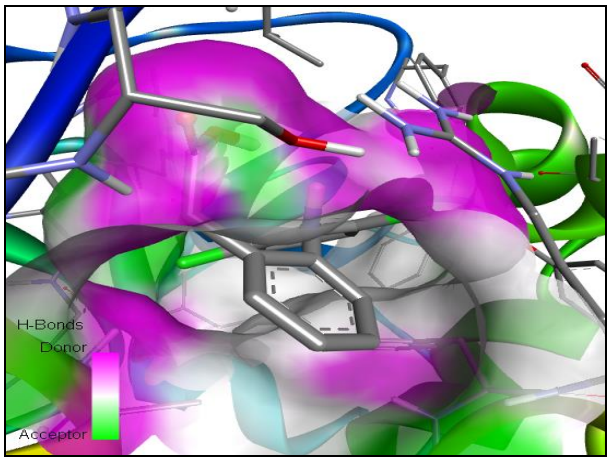

Fig 1: 3D Structure of SIRT1

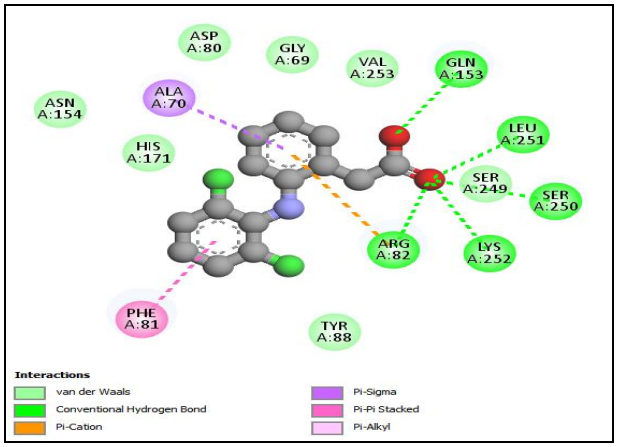

Fig 2: Active Site Representation

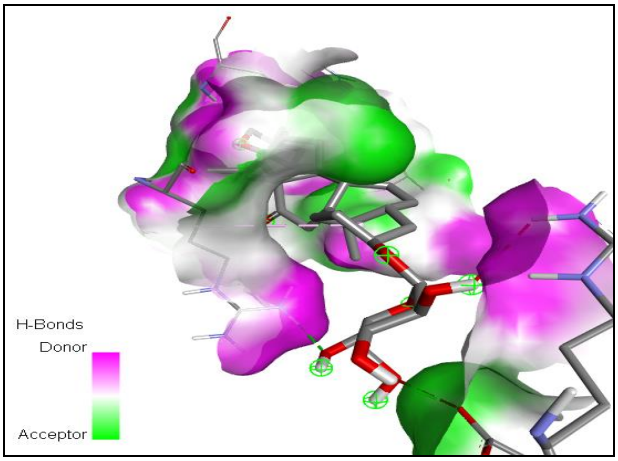

Fig 3: Chemicals from *T. terrestris*

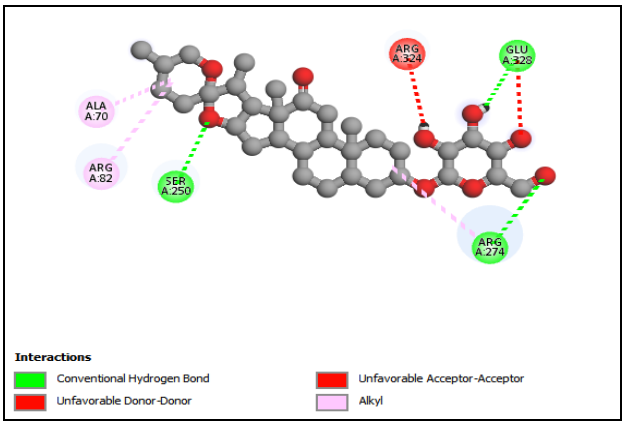

Fig 4: Virtual Screening Workflow

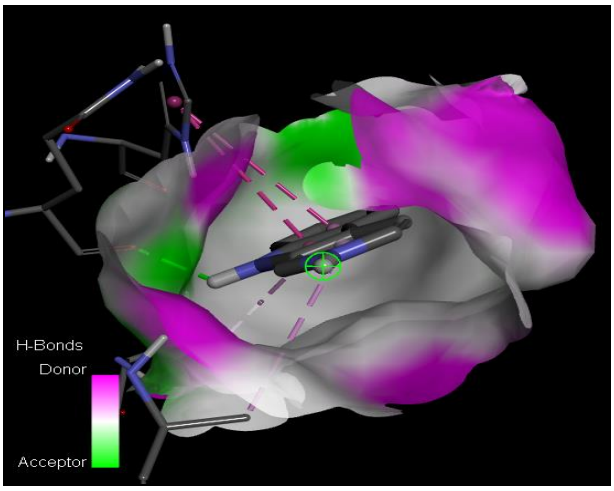

Fig 5: Molecular Docking Results

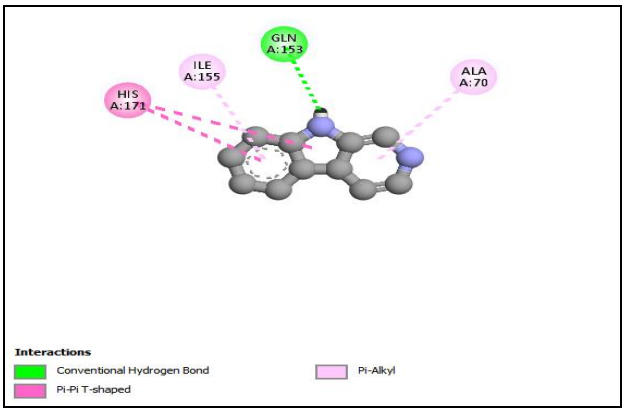

Fig 6: Binding Affinity Comparison

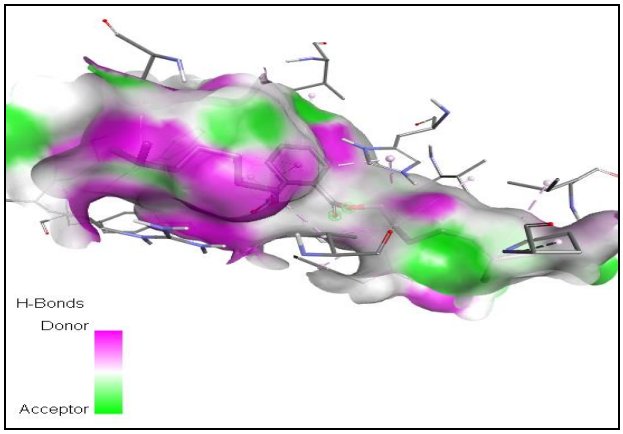

Fig 7: Hydrogen Bond Interactions

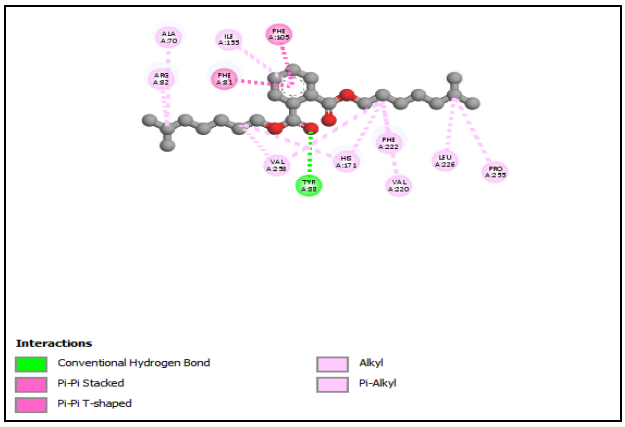

Fig 8: Hydrophobic Interactions

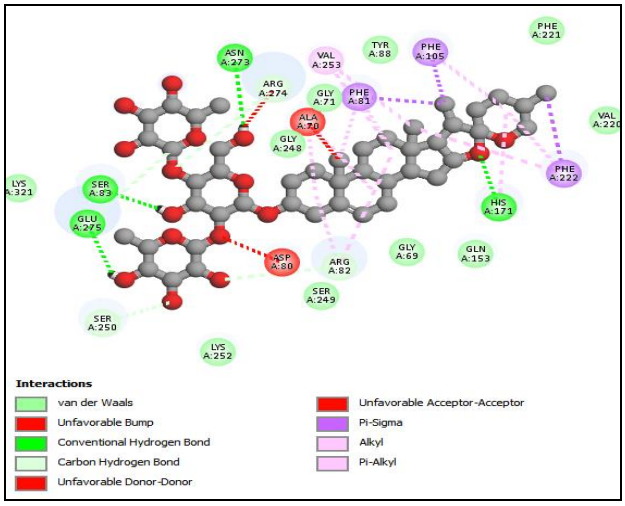

Fig 9: Schematic Representation of SIRT1 Function

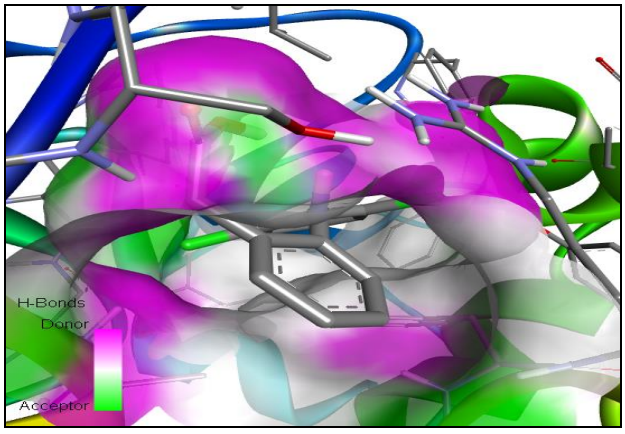

Fig 10: Pathway Modulation by SIRT1

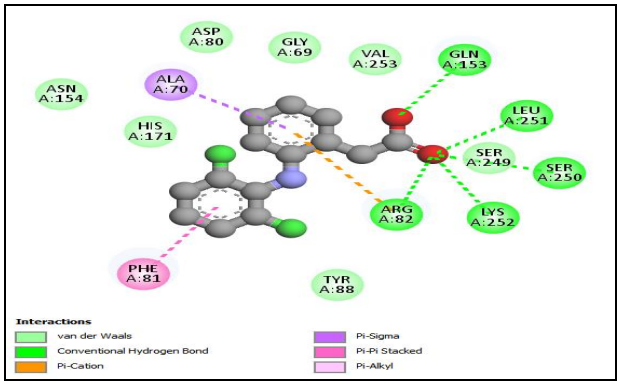

Fig 11: Diosgenin and Hecogenin Binding Sites on SIRT1

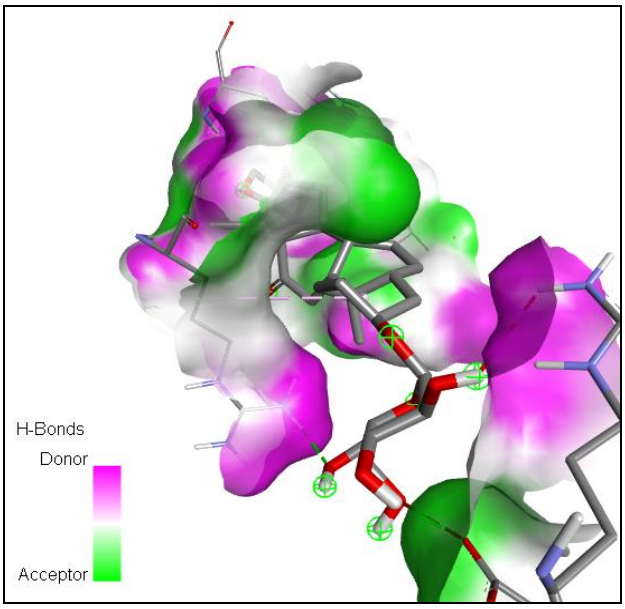

Fig 12: Pathway Analysis in Cancer

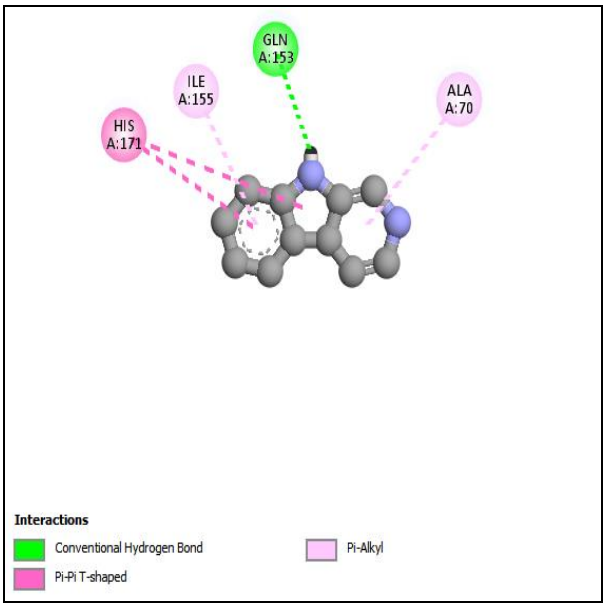

Fig 15: Comparison of Phytochemical Efficacy

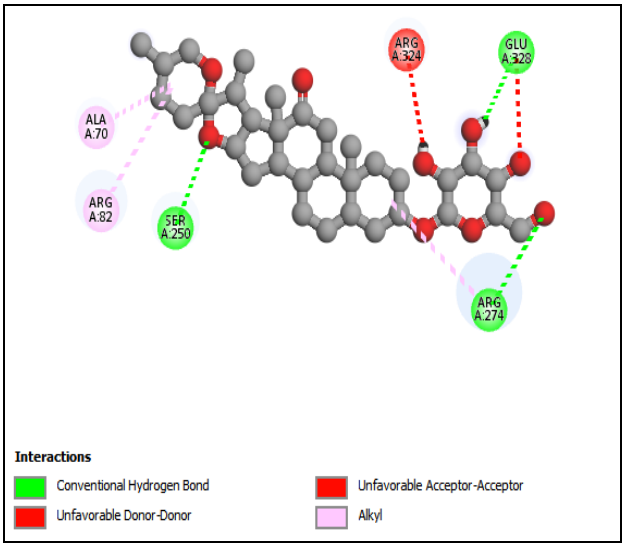

Fig 13: Computational Approach Overview

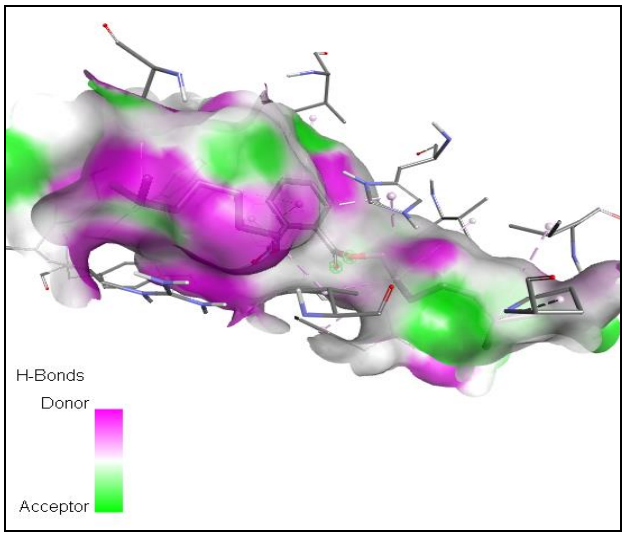

Fig 16: Concentration of Phytochemical Components

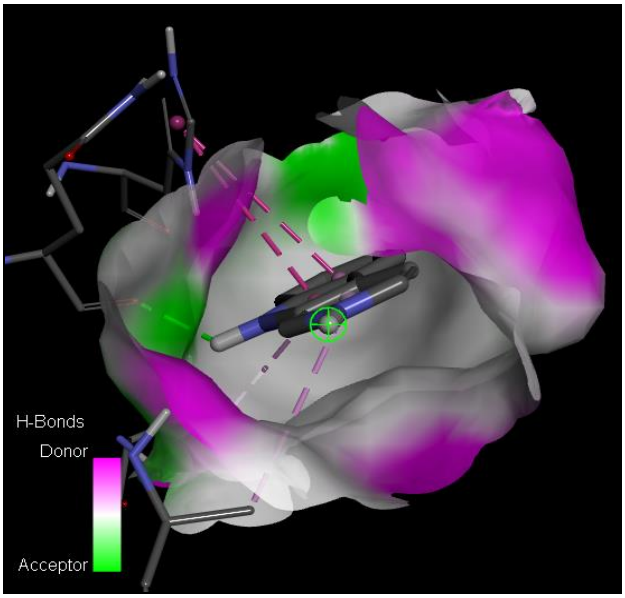

Fig 14: Survival Rates of OSCC

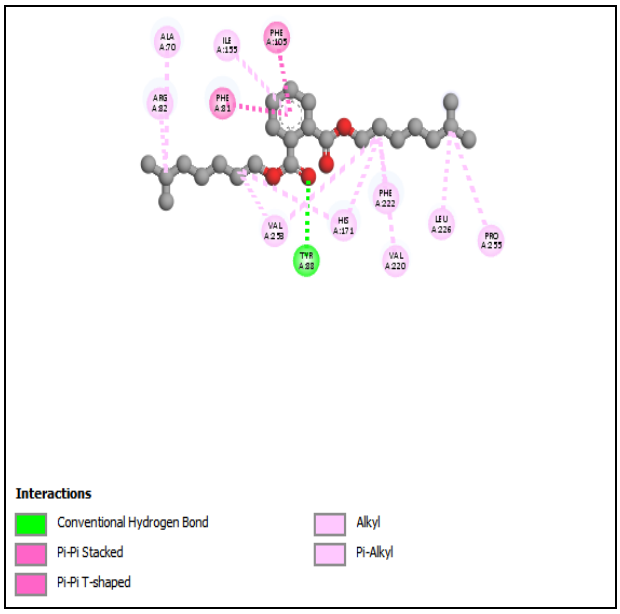

Fig 17: Pharmacological Applications of *T. terrestris*

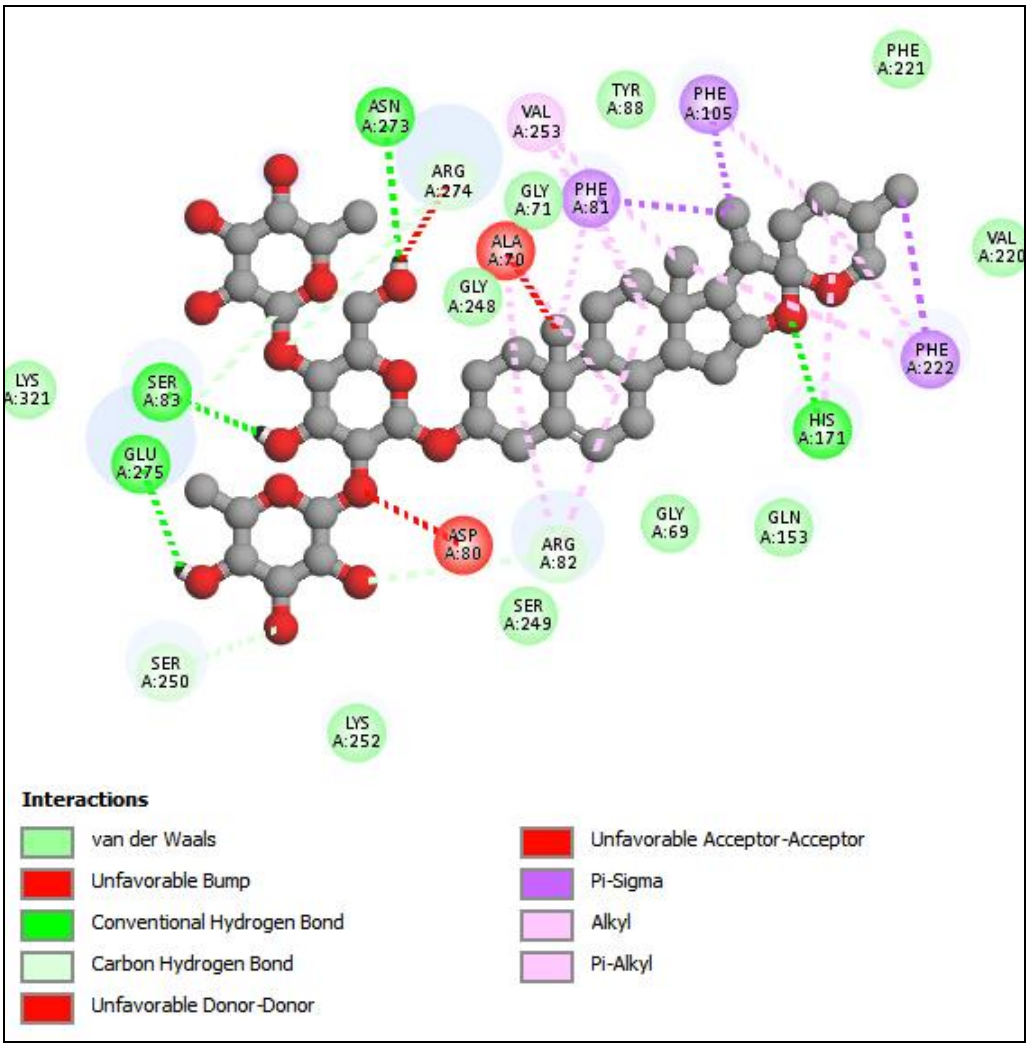

Fig 18: Future Directions in Research

Table 1: Binding Energies and Amino Acid Interactions of Selected Compounds with SIRT1

S. No	Compound Name	Binding Energy K/Cal	
1	Agavoside	-6.3	ARG-274 ALA-70 ARG-82 GLU-328
3	Biochanin	-9.3	ARG-82 ALA-70 SER-250 PHE-81 PHE-105 ILE-155 VAL-220 VAL-253
4	Coumaroyltyramine	-9.3	
5	Diclofenac	-8.7	ARG-82 GLN-153 SER-250 LYS-252 LEU-251 ALA-70 PHE-81
6	Dioscin	-7.0	PHE-105 HIS-171 PHE-222 SER-83 GLU-275 ASN-273 PHE-81 VAL-253

7	disooctyl phthalate		TYR-88 PHE-81 ARG-82 PHE-105 ALA-70 PHE-105 ILE-155 HIS-171 PHE-222 LEU-226 PRO-255
8	Diosgrnin	-9.9	
9	Ergocornine	-5.8	
10	Farnesol	-8.7	
11	Ferulic acid	-7.1	VAL-253 HIS-171 PHE-222 ARG-82 PHE-81 PHE-105
12	Germacranolide	-9.4	PHE-81 PHE-105 ILE-155 HIS-171 ALA-70 VAL-253 PHE-222
13	Gitogenin	-8.4	PHE-81 HIS-171 PHE-105 PHE-222 VAL-253 ARG-82 SER-250 ALA-70
14	Harmaline	-7.9	PHE-105 HIS-171 ILE-155 PHE-222 PHE-81 ALA-70 SER-249 VAL-253
15	Harmane	-7.9	
16	Hecogenin	-9.9	ALA-70 ARG-82 GLN-153 SER-83 ARG-274
17	Isorhamnetin	-8.7	GLY-71 ARG-82 GLU-275 ASN-273 VAL-105 PHE-81 ALA-70 ILE-155 HIS-171 ILE-78 ASP-156 ILE-124
18	Kaempferol	-9.5	ALA-70 PHE-81 ARG-82 GLY-248 SER-250 ASN-273 GLU-275
19	Kaempferol-3-glucuronide	-8.4	ARG-82 PHE-81

			ALA-70 GLU-275 SER-249 ASN-273 GLY-248 SER-250
20	Morharmane	-7.4	ALA-70 GLN-153 ILE-155 HIS-177
21	Polianthoside B	-3.3	GLN-158 ASN-154 LEU-251 ARG-82 HIS-171 ARG-274
22	Stigmasterol	-8.9	PHE-81 VAL-253 PHE-105 HIS-171 PHE-222 ALA-70 ARG-82
23	Tigogenin	-8.7	PHE-81 PHE-105 HIS-171 PHE-222 ALA-70 ARG-82

Conclusion

This study incorporated structural biological techniques through structure-based virtual screening of therapeutic candidates with emphasis on plant compounds obtained from *Tribulus terrestris*, also screening for those candidates with possible binding to SIRT1. The AutoDock VINA application program interface was used in the screening process embedded in PyRx 0.8 application program. Of the compounds analyzed, Hecogenin and Diosgenin were found to possess the best binding affinities and strong interactions with essential residues and were considered as leads for further studies and developments.

Conflict of Interest

Not available

Financial Support

Not available

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