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Unveiling the therapeutic profile of *Oxyspora paniculata*: Insights into neuropharmacological, antidiarrheal, and thrombolytic activities *In Silico*

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Abstract: The application of supplementary herbal medicines has lately expanded in an effort to discover viable alternative therapies that lessen the side effects of chemical pharmaceuticals. *Oxyspora paniculata* is a rich source of phytochemicals with pharmacological action; therefore, it may have health advantages. This current research was performed to analyze the *in silico* evaluation of phytoconstituents present in *Oxyspora paniculata* for neuropharmacological, antidiarrheal, and thrombolytic activity. *In silico* activity of the isolated constituents for antioxidant activity was carried out by PyRx AutoDock Vina, and the protein-ligand interactions were examined using BIOVIA Discovery Studio Visualizer. ADME analysis was performed utilizing the SwissADME free online web server, and a toxicology study was done using the admetSAR online server. In the computational approach, among all the proteins, a docking score was found ranging from −4.6 to −10.3 kcal/mol. Besides, all the compounds were found safe in the ADME/T study. The absorption, distribution, metabolism, excretion/toxicity evaluation of phytoconstituents assures that they have obeyed Lipinski's guideline of five, suggesting their safe consumption. The results of our scientific research validate the suitability of this plant as an alternative source of novel therapeutics. It was determined that *Oxyspora paniculata* contained active phytochemicals, which might provide a variety of pharmacological uses as a hidden source of substances with significant medicinal value. This needs more investigation in order to identify secondary metabolites that can be used to treat various illnesses.

Keywords: Antidiarrheal, antidepressant, thrombolytic, SwissADME, molecular docking, PyRx Autodock Vina.

1. Introduction

Medicinal plants offer promising chemicals for medicinal and pharmacological applications [1]. According to estimates from the World Health Organization (WHO), up to 80% of people in underdeveloped

nations still get their primary medical treatment from locally grown medicinal herbs [2]. Plants are the source of around 25% of prescription medications and 11% of drugs classified as essential by the World Health Organization. Additionally, a significant portion of synthetic pharmaceuticals are generated from plant precursor molecules. The primary arguments in favor of traditional medicine over contemporary therapy are its accessibility, effectiveness, and low cost [3].

There are two distinct words for two different mental illnesses: anxiety and depression. That being said, they may both happen simultaneously. There is a complex relationship between these two conditions [4]. People with anxiety disorders often develop depression [5]. The sensation of being threatened never goes away for those who suffer from anxiety [6]. Furthermore, the World Health Organization lists depressive disorders as a major contributor to nonfatal illnesses globally [7]. Regular experience of anxiety may lead to its classification as a psychiatric condition. Anxiety is a natural emotional state. The presence of anxiety in conjunction with depression leads to a variety of symptoms, such as a decrease in response to treatment or medicine, a drop in the actual prognosis, and an elevated risk of suicide [8].

One of the most common infectious disorders among children in third-world nations is diarrhea, which is brought on by abnormalities in the intestine's secretion and absorption, increasing the volume of excrement produced [9]. According to the World Health Organization (WHO), Bangladesh has a high prevalence of diarrhea in children, with 17% of children under the age of five hospitalized in pediatric wards [10]. Diarrhea can be acute or chronic, with the former caused by epidemiological factors, including travel. Chronic diarrhea lasts for more than four weeks [11, 12]. Infections with bacteria, viruses, and parasites are the causes of diarrhea. Noroviruses and rotaviruses are two examples of viruses that can cause viral illnesses. The primary bacterial causes of diarrhea include species like *Salmonella typhi*, *Helicobacter pylori*, *Clostridium difficile*, and *Escherichia coli*; parasitic diseases are caused by *Entamoeba histolytica* and *Giardia intestinalis* [13]. Diarrhea is a significant issue in underdeveloped nations, despite progress in health services and economic prosperity.

Generally speaking, thrombosis is the term for localized blood clotting that can happen in the venous or arterial circulation and has serious medical consequences. Acute arterial clotting is the primary risk factor for myocardial infarction (heart attack), and 80 percent of strokes occur in the majority of affluent nations [14]. If treatment for this condition is delayed, it will finally result in death. Thrombosis is often brought on by a platelet abnormality or blood coagulation protein, which obstructs the circulatory vessel and prevents the body's normal blood flow. Nevertheless, in addition to heart attacks and strokes, thrombosis can result in cardiac impairment, bleeding ulcers, blindness, and a few other symptoms [15]. Antithrombotic medicines are used to treat thrombosis by targeting particular proteins in the human body's coagulation cascade. The administration of these medications causes the drug molecule to connect with a target protein, thus promoting clot breakup. There are several antithrombotic medications on the market that are effective in treating thrombosis in people with cardiovascular problems. However, several of the existing medications have been accused of causing severe bleeding after administration [14, 16, 17].

Molecular docking facilitates the identification, screening, designing, prediction, and synthesis of chemical compounds, among other processes that lead to the discovery of medicinal medications. Drugs with significant therapeutic potential can be designed, synthesized, and discovered more effectively with the help of molecular docking [18]. It is being used in several biological and medical domains, including bioremediation, protein engineering, medicinal chemistry, and cheminformatics. Using the molecular docking approach, potent therapeutic molecules, particularly those derived from naturally occurring chemicals, have been predicted to combat various diseases. Using molecular docking to assess the intricacy of protein-ligand interactions saves money and time. As a result, the neuropharmacological, antidiarrheal, and thrombolytic effectiveness of *Oxyspora paniculata* has been sought through an *in silico* docking model to determine how they are implicated in such biological activity.

2 Materials and Methods

2.1 Molecular docking: protein preparation

Human serotonin transporter (PDB ID: 5I6X) [19], potassium channel enzyme (PDB ID: 4UUJ) [20], μ-opioid receptor (μOR) (PDB ID: 5C1M) [21], and tissue plasminogen activator (PDB ID: 1A5H) [22] have been derived from RCSB Protein Data Bank (https://www.rcsb.org/structure) in PDB format for the antidepressant, anxiolytic, antidiarrheal, and thrombolytic studies, respectively. All of the water and heteroatoms have been removed from proteins using BIOVIA Discovery Studio 2020. Additionally, using SWISS PDB Viewer, all proteins were reduced to the lowest possible energy level in preparation for additional analysis [23].

2.2 Molecular docking: ligand preparation

Eight chemical components of *Oxyspora paniculata* were simultaneously determined in a previous study using the HPLC technique [24]. The structures of eight compounds of *Oxyspora paniculata*, namely gallic acid (PubChem CID: 370), dihydromyricetin (PubChem CID: 161557), protocatechuic acid (PubChem CID: 72), rutin (PubChem CID: 5280805), myricitrin (PubChem CID: 5281673), oleanolic acid (PubChem CID: 10494), quercetin (PubChem CID: 5280343), and kaempferol (PubChem CID: 5280863), were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Besides, diazepam (PubChem CID: 3016), loperamide (PubChem CID: 3955), and streptokinase (PubChem CID: 9815560) have been studied to compare and contrast the docking of the compounds of *Oxyspora paniculata*. The ligands have been downloaded in 3D SDF format and minimized by the PyRx tool to find the best possible hit for these targets. The virtual screening software PyRx from MGLTools (https://sourceforge.net/projects/pyrx/) has been kept in default format [25].

2.3 Molecular docking: docking analysis

PyRx AutoDock Vina was used to dock the chosen protein-ligand complexes [25]. The docking study was carried out using a semi-flexible docking system. PDB files containing phytochemicals and proteins were

reduced and then converted to PDBQT format using the PyRx AutoDock Vina program. This analysis kept the protein stiff and the ligand flexible. The ligand molecules have been granted ten degrees of freedom. AutoDock describes measures for automatically transforming molecules into PDBQT-format molecules, such as box type and grid box construction. The grid box was designed with an active location in its middle. In addition, BIOVIA Discovery Studio Visualizer 2020 [26] was expedited to find the optimal docking places.

2.4 Pharmacokinetics and toxicity measurement

The pharmacokinetic properties of the substances were investigated using the SwissADME online method (ADME). Compounds having desirable drug-like properties are evaluated using Lipinski's five criteria (molecular weight < 500 daltons, H-bond donors ≤ 5, H-bond acceptors ≤ 10, molar refractivity between 40 and 130, and lipophilicity<5) [27]. Furthermore, the online application admetSAR (http://lmmd.ecust.edu.cn/admetsar2) was utilized to compute the toxicological properties of every material.

3. Results

3.1 Molecular docking analysis for antidepressant and anxiolytic study

The docking analysis results for antidepressant and anxiolytic activity are presented in **Table 3** and **Figure 1**. In this investigation, two receptors namely human serotonin transporter (PDB ID: 5I6X) and potassium channel (PDB ID: 4UUJ) have been used to screen antidepressant and anxiolytic docking analysis, respectively. In the case of the human serotonin transporter (PDB ID: 5I6X), the ranking of the docking score is as follows: rutin > diazepam> quercetin > dihydromyricetin > oleanolic acid > myricitrin > kaempferol > gallic acid > protocatechuic acid. On the contrary, the docking of the selected compounds against the potassium channel (PDB ID: 4UUJ) is as follows: oleanolic acid > diazepam >myricitrin > quercetin > rutin > dihydromyricetin > kaempferol > protocatechuic acid > gallic acid. Amino acid residues namely gly338, tyr95, phe341, val501 and ile172 established the interaction between rutin and 5I6X. Furthermore, oleanolic acid binds to the enzymatic pocket of 4UUJ receptor by means of phe103 and val106 residues with a docking score of (-7.2) kcal/mol.

3.2 Molecular docking analysis for the antidiarrheal study

The docking analysis of antidiarrheal activity has been illustrated in **Table 3** and **Figure 1**. In this investigation, μ-opioid receptor (μOR) (PDB ID: 5C1M) was used to quest of binding interaction with the selected compounds of *Oxyspora paniculata*. Oleanolic acid explores best binding affinity with the μ-opioid receptor (μOR) (5C1M) proteins which interact with the amino acid residues namely asn109, leu116, tyr149, ile146, met203, trp152 and leu112. The ranking of the docking score is as follows: loperamide > oleanolic acid > dihydromyricetin > kaempferol > quercetin > rutin > myricitrin > gallic acid > protocatechuic acid.

3.3 Molecular docking analysis for thrombolytic study

The docking analysis results for the thrombolytic activity have been presented in **Table 3** and **Figure 2**. In case of tissue plasminogen activator (PDB ID: 1A5H), the highest score has been obtained −8.9 kcal/mol for myricitrin. The ranking of the docking score is as follows: myricitrin > oleanolic acid > quercetin > rutin > dihydromyricetin > kaempferol > streptokinase > gallic acid > protocatechuic acid. Myricitrin interacts with the protein (1A5H) through a series of amino acid residues (tyr99, tyr151, leu41, gly219, gly216) of conventional hydrogen and van der Waals as well as cys191, trp215, and gln192 of amide-Pi stacked and carbon hydrogen bond.

3.4 Pharmacokinetics and toxicity measurement

The study shows that all the compounds agree with Lipinski's rules and claim that these compounds are orally bioavailable. In order to predict the toxicological properties of the eight compounds, the online admetSAR (http://lmmd.ecust.edu.cn/admetsar1) server is also used. The analysis showed that the selected compounds are non-Ames toxic and noncarcinogenic (**Table 1**). The investigation also revealed the GI absorption (GA) and blood-brain barrier (BBB) of eight phytoconstituents in *Oxyspora paniculata* (**Table 2**).

Table 1: Absorption, digestion, metabolism, excretion, and toxicological analysis of phytoconstituents in *Oxyspora Paniculata*

Ligands	Molecular formula	MW(g/mol)	HBD	HBA	LogP (o/w)	AMT	CAR	Lipinski violation
Gallic acid	$C_7H_6O_5$	170.12 g/mol	4	5	0.21	No	No	0
Dihydromyricetin	$C_{15}H_{12}O_8$	320.25 g/mol	6	8	0.9	No	No	1
Protocatechuic acid	$C_7H_6O_4$	154.12 g/mol	3	4	0.66	No	No	0
Rutin	$C_{27}H_{30}O_{16}$	610.52 g/mol	10	16	1.58	No	No	3
Myricitrin	$C_{21}H_{20}O_{12}$	464.38 g/mol	8	12	0.92	No	No	2
Oleanolic acid	$C_{30}H_{48}O_3$	456.70 g/mol	2	3	3.89	No	No	1
Quercetin	$C_{15}H_{10}O_7$	302.24 g/mol	5	7	1.63	No	No	0
Kaempferol	$C_{15}H_{10}O_6$	286.24 g/mol	4	6	1.7	No	No	0

Note: PID = PubChem ID, MW = molecular weight (acceptance range: <500), HBD = hydrogen- bond donor (acceptance range: ≤5), HBA = hydrogen-bond acceptor: (acceptance range: ≤10), LogP = high lipophilicity (acceptance range: <5), AMT, AMES toxicity; CAR = carcinogens

Table 2: GI absorption (GA) and Blood brain Barrier (BBB) of phytoconstituents in *Oxyspora Paniculata*.

Ligands	PubChem ID	Canonical SMILES	GA	BBB permeant
Gallic acid	370	C1=C(C=C(C(=C1O)O)O)C(=O)O	High	No
Dihydromyricetin	161557	C1=C(C=C(C(=C1O)O)O)C2C(C(=O)C3=C(C=C(C=C3O2)O)O)O	Low	No
Protocatechuic acid	72	C1=CC(=C(C=C1C(=O)O)O)O	High	No
Rutin	5280805	Oc1cc(O)c2c(c1)oc(c(c2=O)OC1OC(COC2OC(C)C(C(C2O)O)O)C(C(C1O)O)O)c1ccc(c(c1)O)O	Low	No
Myricitrin	5281673	Oc1cc(O)c2c(c1)oc(c(c2=O)OC1OC(C)C(C(C1O)O)O)c1cc(O)c(c(c1)O)O	Low	No
Oleanolic acid	10494	OC1CCC2(C(C1(C)C)CCC1(C2CC=C2C1(C)CCC1(C2CC(C)(C)CC1)C(=O)O)C)C	Low	No
Quercetin	5280343	Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O	High	No
Kaempferol	5280863	Oc1ccc(cc1)c1oc2cc(O)cc(c2c(=O)c1O)O	High	No

Table 3: Docking scores of the selected ligands with the human serotonin transporter (PDB ID: 5I6X), potassium channel (PDB ID: 4UUJ), μ-opioid receptor (μOR) (PDB ID: 5C1M), and tissue plasminogen activator (PDB ID: 1A5H).

Ligands	**Antidepressant (5I6X)**	**Anxiolytic (4UUJ)**	**Antidiarrheal (5C1M)**	**Thrombolytic (1A5H)**
Gallic acid	-6	-4.6	-5.3	-6.4
Dihydromyricetin	-8.9	-5.7	-7.5	-8.1
Protocatechuic acid	-6	-4.7	-5.1	-6.2
Rutin	**-10.3**	-5.8	-6.6	-8.2
Myricitrin	-8.5	-6.3	-6.2	**-8.9**
Oleanolic acid	-8.7	**-7.2**	**-7.7**	-8.7
Quercetin	-9	-5.9	-6.9	-8.2
Kaempferol	-8	-5.6	-7	-7.4
Standard (Diazepam/Streptokinase/ Loperamide)	-9.1	-7	-8	-6.5

Note: The most significant values of docking scores have been marked in bold letter.

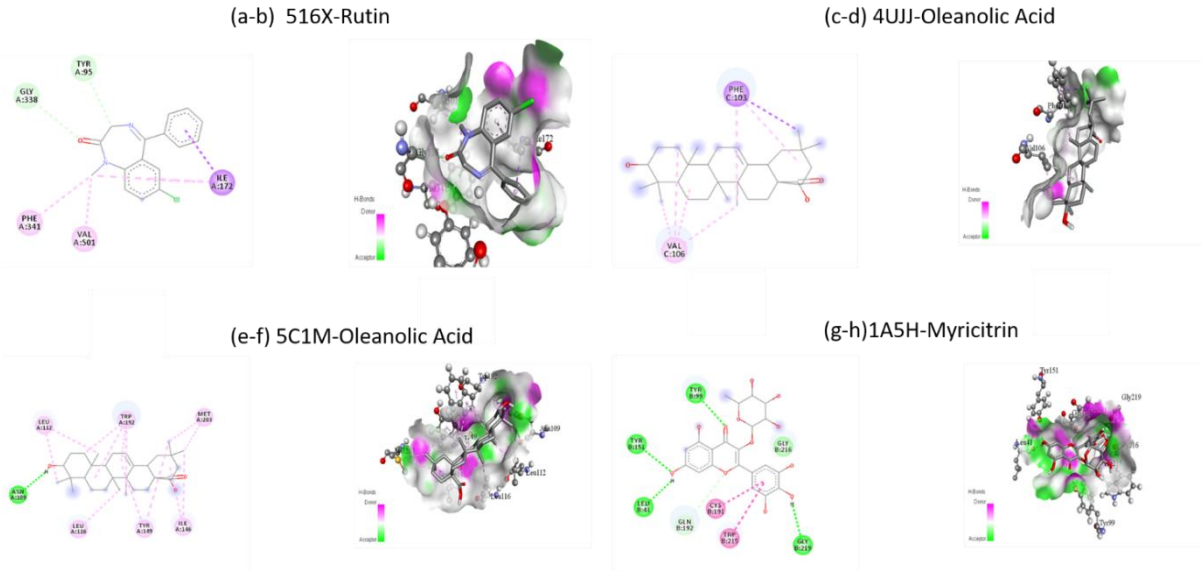

Figure 2: 3D and 2D presentations of the best ligand–receptor interactions (a, b, c, d, e, f, g, and h represent 5I6X-rutin, 4UUJ-oleanolic acid, 5C1M-oleanolic acid, and 1A5H-myricitrin interactions, respectively).

4. Discussion

Even if there are several therapy options for depression, anxiety, diarrhea, and coronary artery disease, it is still unclear how to completely relieve the symptoms of the condition without causing adverse consequences. As a result, these medications' poor pharmacokinetics and adverse effects limit their therapeutic use. Because of this, the need for new medications is growing, and concerns about the safety, effectiveness, duration of action, and side effects of existing medications have become crucial [28]. Herbal therapy has emerged as a potentially effective treatment for many ailments because of the variety of neurological targets it targets [29]. However, *Oxyspora paniculata*, a prominent ethnomedicinal plant with a wide range of therapeutic uses, was used in this investigation. The current research demonstrated that the neuropharmacological, antidiarrheal, and thrombolytic efficiency of *Oxyspora paniculata* was explored by an *in silico* docking model to establish whether they have been connected to such biological activity.

Molecular docking studies are commonly used to forecast ligand-target interactions and deepen our understanding of the bioactivities of natural products. It also provides more information on potential binding processes inside the protein binding pockets [30]. To understand the consequences of this result, biological investigations were described and validated using molecular docking research. Eight common *Oxyspora paniculata* compounds were chosen for the docking research in order to conduct a more in-depth analysis of their biological properties (antidepressant, anxiolytic, anti-diarrheal, and thrombolytic). The compounds were docked against four targets: human serotonin transporter (PDB ID: 5I6X), potassium channel (PDB ID: 4UUJ), μ-opioid receptor (PDB ID: 5C1M), and tissue plasminogen activator (PDB ID: 1A5H) for antidepressant, anxiolytic, antidiarrheal, and thrombolytic studies, respectively. Human serotonin transporters (PDB ID: 5I6X) interact with ligands through several linkages, resulting in docking values ranging from -6 to -10.3 kcal/mol. These findings suggest that these phytoconstituents play a significant role in antidepressant activity via their interactions with target proteins. To analyze the anxiolytic docking investigation, the chosen phytoconstituents were docked with the potassium channel (PDB ID: 4UUJ). The docking score ranged from -4.6 to -7.2 kcal/mol. The docking score for the antidiarrheal trial ranged from -5.1 to -7.7 kcal/mol. In the thrombolytics docking study, myricitrin, oleanolic acid, quercetin, and dihydromyricetin all had a significant docking score against plasminogen tissue activator (PDB ID: 1A5H), with myricitrin having the highest score. This study reveals that *Oxyspora paniculata's* thrombolytic effect may be attributed to its bioactive components (myricitrin, oleanolic acid, quercetin, dihydromyricetin, and rutin).

The pharmacokinetics and toxicological characteristics of the drugs have been verified in relation to the results of molecular docking investigations against the human serotonin transport, potassium channel, μ-opioid receptor, and tissue plasminogen activator. Every compound complied with Lipinski's guidelines for drug-likeliness. As a result, these analyses of the compounds that have been found are very helpful in the development of a novel pharmaceutical agent [27, 28, 29, 30, 31].

The results imply that the compounds are safe for oral use and satisfy Lipinski's criteria, which suggest that they may serve as useful medication candidates (**Table 1**). Additionally, as medication safety is a crucial component of what makes a successful medical product, we evaluated the toxicological parameters of the assigned plant components using the admetSAR online tool [32]. As a result, the thorough investigation produced important information on the chosen chemicals. The combined orientation of several phytochemicals, including established and unreported phytochemicals, may become a contributing factor in the outcomes.

5. Future implication and limitations of study

Our findings have interesting implications for future *tabble* and clinical studies, potentially leading to the development of new treatment drugs and combination therapies for neurological disorders, diarrhea, and thrombosis. However, the study's in-silico nature restricts its immediate usefulness, as the effectiveness and safety of the compounds must be verified in experimental and clinical trials. Further study might include *in vitro* and *in vivo* studies to establish the physiological consequences of these findings.

6. Conclusion

The biological results of this scientific study indicate that *Oxyspora paniculata* can be a significant source of antidepressant, anxiolytic, antidiarrheal, and clot-lytic medicines. Furthermore, the molecular docking analysis of the bioactive phytoconstituents revealed promising binding affinity to certain proteins, and the ADMET research demonstrated their drug-like properties. Thus, the computational investigation validated the experimental results of biological activities and gave a potential insight into contemplating *Oxyspora paniculata* as a significant therapeutic candidate. Additional research is also encouraged, as structural modifications to these compounds may result in a higher docking score and greater medicinal relevance.

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