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In vitro and *in silico* cytotoxicity effects of *Zanthoxylum simulans* Hance. fruit bark extract against gastric cancer cell lines

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ABSTRACT: *Zanthoxylum simulans* Hance. is known in folklore as a spicy herb, commonly used to treat cold-induced diseases in the body. In this study, we evaluated the cytotoxic effects of *Zanthoxylum simulans* fruit bark extract. Samples of *Zanthoxylum simulans* were extracted with 70% ethanol and subsequently fractionated with n-Hexane, ethyl acetate (EtOAc), and n-butanol (n-BuOH) solvents. To evaluate the in vitro cytotoxic effect, we performed SRB (Sulforhodamine B) assay on the cell lines of human gastric MKN-7. In this study, we also used molecular docking method to evaluate the inhibition abilities of MAPK1 and AKT1 receptors of 120 compounds in *Zanthoxylum simulans* Hance. The *in vitro* cytotoxic results showed that the n-Hexane total extract had the strongest cytotoxicity effects on gastric cancer cells with an IC_{50} of 23.65±1.75 mg/ml. The results cytotoxic effects on MNK-7 gastric cancer cells of these fractions showed that the EtOAc and EtOH fractions exhibited activity with IC_{50} values of 35.61±2.90 and 50.67±3.82 µg/mL, respectively; while the BuOH and H_2O fractions showed no activity. The molecular docking results showed five compounds that inhibit both MAPK1 and AKT1 targets including Simulanoquinoline, N-acetylanonaine, N-acetyldehydroanonaine, Oxyavicine, and Benzosimuline. Therefore, our results showed that *Zanthoxylum simulans* fruit bark extract has a strong cytotoxicity effect on gastric cancer cells. *In vivo* studies of these potential compounds should be carried out to become anti-cancer drugs in the future.

KEYWORDS: *Zanthoxylum simulans* Hance.; cytotoxicity; molecular docking; gastric cancer cells; MAPK1; AKT1.

1. INTRODUCTION

Cancer is a complex disease caused by genetic alterations such as gene mutations or changes in the expression of cancer-related genes or tumor suppressor genes associated with chromosomes [1]. Gastric cancer, an aggressive form of digestive system tumours, has the third highest lethality and fourth highest morbidity in all cancers worldwide. It is a complex disease with multiple genetic and environmental factors involved in its development and progression.

MAPK1 is a member of the mitogen-activated protein kinase (MAPK) family, which is involved in various cellular processes, including cell proliferation, differentiation, survival, and apoptosis [2]. It is activated by a cascade of phosphorylation events initiated by upstream signaling molecules, such as growth factors, cytokines, and stress stimuli. Several studies have shown that MAPK1 is upregulated in gastric cancer tissues compared to normal gastric tissues. In addition, its expression levels correlate with the stage and grade

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of the disease, suggesting that it may play a role in gastric cancer progression. One of the mechanisms by which MAPK1 promotes gastric cancer is through the regulation of cell proliferation and apoptosis [3].

MAPK1 activation stimulates the expression of genes involved in cell cycle progression, such as cyclin D1 and c-Myc, while inhibiting the expression of pro-apoptotic genes, such as Bax and caspase-3. This leads to increased cell proliferation and decreased apoptosis, which are hallmarks of cancer cells. Targeting MAPK1 signaling pathway has emerged as a promising strategy for the treatment of gastric cancer [4].

AKT1, also known as protein kinase B (PKB), is a serine/threonine protein kinase that plays a crucial role in regulating various cellular processes, including cell proliferation, differentiation, survival, and metabolism [5]. Dysregulation of AKT1 signaling has been implicated in various types of cancer, including gastric cancer. Several studies have reported that AKT1 expression is upregulated in gastric cancer tissues compared to normal gastric tissues. In addition, high AKT1 expression levels have been associated with a poor prognosis in gastric cancer patients, suggesting that AKT1 may be involved in gastric cancer progression. One of the mechanisms by which AKT1 promotes gastric cancer is through its effects on cell proliferation and apoptosis. AKT1 activation leads to the upregulation of cell cycle regulators, such as cyclin D1 and c-Myc, and the downregulation of pro-apoptotic genes, such as Bax and caspase-3. This promotes cell proliferation and inhibits apoptosis, which are hallmarks of cancer cells [6]. AKT1 also plays a role in regulating the invasive and metastatic potential of gastric cancer cells. AKT1 activation stimulates the expression of matrix metalloproteinases (MMPs), which are enzymes that degrade the extracellular matrix and facilitate cancer cell invasion and migration. Targeting AKT1 signaling pathway has emerged as a promising therapeutic approach for the treatment of gastric cancer [7].

Molecular docking is a modeling technique to predict the favorable position and configuration that the substrate molecule can bind to a protein. It can predict the interaction of the compound with acid amine in the protein target. The substrate molecule is displaced through space and encircles the protein molecule to find the position with the most negative binding energy. In most cases of cancer, chemotherapy or radiation therapy is the primary treatment option [8]. However, radiation therapy often causes many side effects, chemotherapy can be costly, and the five-year survival rate for patients remains limited. One approach that is currently receiving special attention is the use of whole-plant extracts or compounds extracted from medicinal plants to treat cancer [9]. Medicinal plants are a readily available source of materials, cost-effective, have good efficacy, and have few undesirable side effects. *Zanthoxylum simulans* Hance. is a wild-growing plant that is found in tropical climates. It is commonly found in eastern China, Taiwan, North Korea, Cambodia, and Laos. In Vietnam, this plant is found in the central region. Chemical analysis of the Xuyen tieu plant has shown that it contains main compounds belonging to the groups of alkaloids, amides, lignans and neolignans, coumarins, peptides, terpenoids, and flavonoids [10-12]. Pharmacological studies have shown that *Zanthoxylum simulans* has many important effects, including anti-inflammatory, antibacterial, antiparasitic, antiviral, antioxidant, anticancer/antitumor, and cytotoxic effects [10]. In this study, we conducted to evaluate the in vitro cytotoxic effects of *Zanthoxylum simulans* fruit bark extract against gastric cancer cell lines. We also performed *in silico* molecular docking, and absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies to demonstrate the probable interactions between promised compounds in *Zanthoxylum simulans* with MAPK1 and AKT1 and their ADMET profile.

2. RESULTS

2.1. *In vitro* cytotoxicity effects on gastric cancer cells of *Zanthoxylum simulans*

In vitro cytotoxicity effects on gastric cancer cells of the total ethanolic extract and fractions of *Zanthoxylum simulans* fruit bark were demonstrated in Table 1.

Table 1. *In vitro* cytotoxicity effects of the total ethanolic extract and fractions of *Zanthoxylum simulans* fruit bark on gastric cancer cells

MNK-7	EtOH	n-Hexan	EtOAc	BuOH	H_2O	Ellipticine
IC_{50}	50.67±3.82	23.65±1.75	35.61±2.90	>100	>100	0.40±0.03

From Table 1, the positive control drug Ellipticine showed significant toxicity against the human gastric cancer cell line with an IC_{50} of 0.40±0.03. The n-Hexane total extract displayed cytotoxicity against the human gastric cancer cell line with an IC_{50} of 23.65±1.75 mg/ml. Results in Table 1 also showed that the EtOAc and EtOH fractions demonstrated activity with IC_{50} values of 35.61±2.90 and 50.67±3.82 µg/mL, respectively. The BuOH and H_2O fractions did not exhibit activity against the tested cell line.

2.2. Evaluation of the docking model

Before screening compounds, the co-crystallized ligands (Ipatasertib and Ravoxertinib) were redocked to the active site of the target by Chimera. We received the root mean square deviation (RMSD) of AKT1 and MAPK1 are 0.432 Å and 0.464 Å, respectively (Figure 1 & Figure 2). All RMSD values are less than 1.5 Å proving that molecular docking results to the target are reliable.

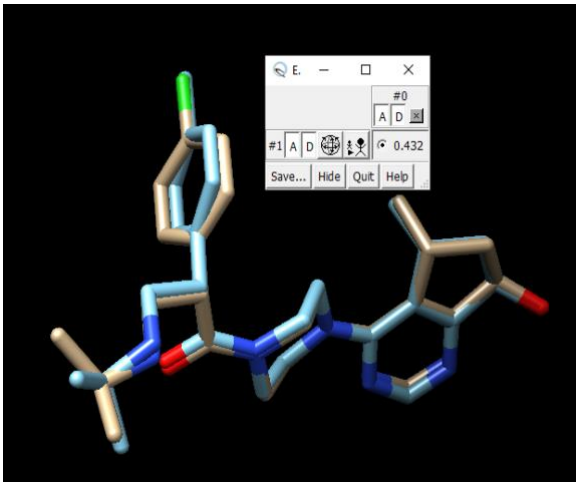


Figure 1. Co-crystallized ligand redock results of AKT1

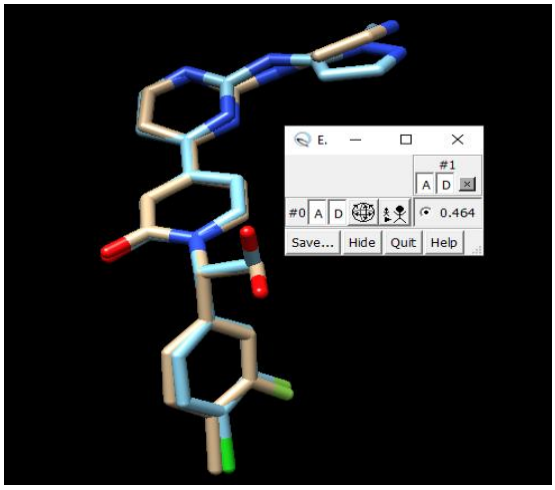


Figure 2. Co-crystallized ligand redock results of MAPK1

2.3. Molecular docking of compounds to the target protein

After preparing the proteins, we docked 120 natural compounds from *Zanthoxylum simulans* Hance. to screen inhibitory activity in AKT1 and MAPK1 targets. The result is shown in Table 2.

Table 2. The docking results of 120 compounds and reference compounds with AKT1 and MAPK1.

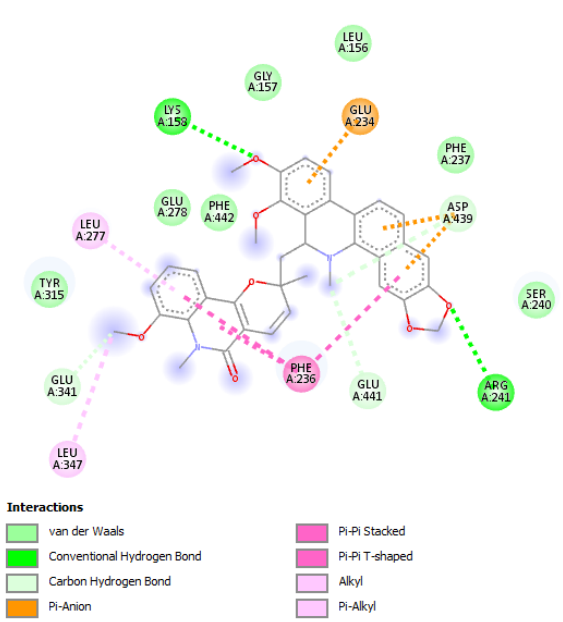
No.	Name	PubChemID	Binding energy (kcal/mol)	
			AKT1	MAPK1
1	Zanthosimuline	5315426	-8.5	-8.2
2	Huajiaosimuline	5318093	-8.9	-8.2
3	Simulanoquinoline	5321314	-9.5	-10.3
4	N-acetylanonaine	6453733	-9.6	-9.6
5	Chelerythrine	2703	-8.6	-8.7
6	Norchelerythrine	443719	-8.4	-8.6
7	Bocconoline	181121	-8.3	-8.6

8	Skimmianine	6760	-7.4	-6.7
9	N-acetylnornuciferine	101630664	-8.1	-8.8
10	Arnottianamide	3085181	-8.5	-8.1
11	Decarine	179640	-9.0	-8.6
12	N-acetyldehydroanonaine	5315739	-9.8	-9.1
13	Simulansine	5321315	-8.7	-8.1
14	Oxychelerythrine	147279	-9.0	-8.8
15	Dihydrochelerythrine	485077	-8.9	-8.5
16	Zanthobisquinolone	54688597	-9.0	-8.4
17	γ-fagarine	107936	-7.2	-6.6
18	Toddaquinoline	11390791	-8.6	-8.4
19	Dictamnine	68085	-7.1	-6.6
20	4-methoxy-2-quinolone	600167	-6.4	-6.3
21	Benzophenanthridine	12407248	-8.7	-8.7
22	Noravicine	14037817	-9.1	-9.6
23	Rhoifoline B	377308819	-9.5	-9.3
24	8-methoxyisodecarine	136854509	-9.1	-8.7
25	Dihydronitidine	99641	-9.4	-8.5
26	5,6-dihydro-6-methoxynitidine	38845	-7.8	-8.8
27	6-acetonyldihydronitidine	101664489	-9.0	-7.9
28	6-acetonyldihydroavicine	101212618	-9.8	-9.5
29	6-acetonyldihydrochelerythrine	185516	-8.9	-7.9
30	8-hydroxydihydrochelerythrine	15940321	-8.9	-8.8
31	Ethoxychelerythrine	160921	-7.8	-7.6
32	Oxynitidine	97597	-9.5	-9.1
33	Oxyavicine	12313849	-9.8	-9.5
34	Isoarnottianamide	14189418	-8.7	-7.2
35	Integriamide	155897	-8.9	-8.2
36	Citronellol	8842	-4.7	-4.9
37	Spathulenol	92231	-7.5	-7.0
38	Robustine	164950	-7.1	-6.9
39	N-methylflindersine	72819	-8.0	-7.9
40	Liriodenine	10144	-9.2	-9.2
41	Hinokinin	442879	-9.0	-8.6
42	Lysicamine	122691	-8.7	-8.4
43	Simulenoline	5321316	-8.7	-8.3
44	Peroxysimulenoline	101936038	-9.1	-8.2
45	Benzosimuline	5321951	-9.4	-9.3
46	Edulitine	826073	-6.6	-6.2
47	Arborinine	5281832	-8.2	-7.6
48	Scoparone	8417	-6.6	-6.4
49	β-amyrone	12306160	-8.3	-8.0
50	β-amyrin	73145	-8.5	-8.1
51	β-sitosterol	222284	-7.3	-7.0
52	β-sitostenone	5484202	-7.9	-7.3
53	Tetracosyl ferulate	14238617	-6.7	-6.6
54	Zanthobungeanine	5315422	-8.0	-7.6
55	Pyrrolezanthine	636825	-6.7	-5.8
56	(-)-Simulanol	636826	-7.9	-7.7

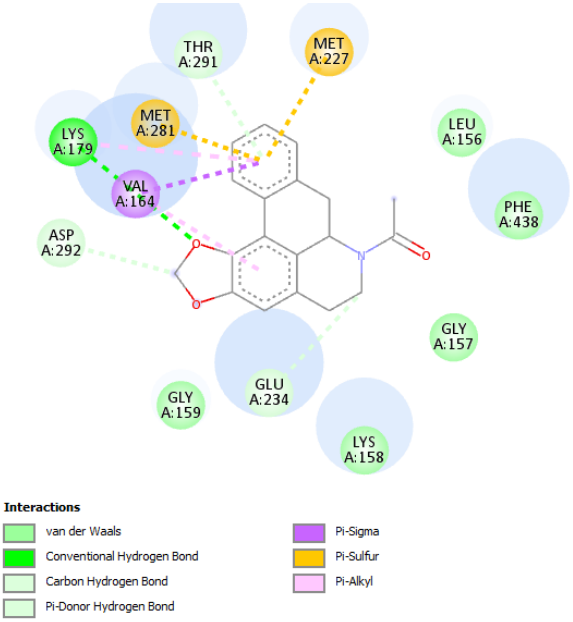
57	Zanthopyranone	10419590	-5.2	-4.7
58	Sinapic aldehyde	5280802	-5.7	-5.5
59	Vanillic acid	8468	-6.0	-5.4
60	Syringic acid	10742	-5.8	-5.5
61	Isofraxidin	5318565	-6.7	-6.4
62	(-)-Balanophonin	23252258	-7.9	-8.2
63	haplopine	5281846	-7.5	-6.7
64	(+)-Platydesmine	6451457	-7.9	-7.4
65	(-)-Syringaresinol	11604108	-7.9	-7.9
66	Flindersine	68230	-8.0	-8.0
67	Zanthodioline	78384601	-8.0	-7.7
68	Scopoletin	5280460	-6.5	-6.4
69	(+)-abscisic acid	5280896	-7.7	-6.9
70	Caryophyllene oxide	1742210	-6.5	-6.3
71	Isobutyl acetate	8038	-4.6	-4.0
72	Kobusin	182278	-8.6	-8.2
73	(+)-Fargesin	5320622	-8.7	-8.3
74	Epieudesmin	7000209	-8.3	-8.0
75	Simulansamide	5321312	-7.8	-7.3
76	α-pinene	6654	-5.2	-5.3
77	Camphene	6616	-5.0	-5.4
78	β-pinene	14896	-5.3	-5.1
79	Sabinene	18818	-4.8	-5.2
80	Isoamyl acetate	31276	-4.5	-4.1
81	β-myrcene	31253	-4.9	-4.8
82	α-terpinene	7462	-5.9	-5.7
83	Limonene	22311	-5.7	-5.6
84	β-phellandrene	11142	-5.8	-5.5
85	1,8-cineole	2758	-4.8	-5.5
86	(Z)-β-ocimene	5320250	-5.0	-4.9
87	γ-terpinene	348276756	-5.9	-5.6
88	(E)-β-ocimene	5281553	-5.3	-5.2
89	p-cymene	7463	-5.9	-5.7
90	Terpinolene	11463	-6.0	-5.8
91	(Z)-3-tridecen-1-yne	5367347	-5.4	-4.5
92	Heptyl acetate	8159	-4.6	-4.5
93	3,4-dimethyl-2,4,6-octatriene	5371124	-5.5	-5.3
94	(E)-sabinene hydrate	6430763	-5.3	-5.7
95	Linalyl acetate	8294	-5.2	-5.1
96	N-octanol	957	-4.1	-4.0
97	Linalyl formate	61040	-5.1	-4.6
98	4-terpineol	11230	-5.8	-5.2
99	β-caryophyllene	5281515	-6.9	-6.5
100	Sabina ketone	92784	-5.1	-4.9
101	α-humulene	5281520	-6.7	-6.2
102	α-terpineo	17100	-6.3	-5.9
103	3-thujen-2-ol	561871	-5.5	-5.5
104	α-terpinyl acetate	111037	-6.3	-5.9

105	Germacrene D	5317570	-6.9	-6.9
106	Neryl acetate	1549025	-5.6	-5.1
107	Zingiberene	92776	-6.2	-6.7
108	Geranyl acetate	1549026	-5.6	-5.5
109	δ-cadinene	8145817	-7.5	-7.0
110	Allethrolone	11083	-5.9	-5.4
111	Geraniol	637566	-4.9	-4.7
112	Elemol	92138	-6.1	-5.8
113	Allo-ocimene	5368821	-5.4	-5.2
114	α-thujene	17868	-5.5	-5.2
115	α-fenchol	439711	-5.5	-5.7
116	Trans-2-Pinanol	1268143	-5.2	-5.5
117	Camphor	2537	-5.0	-5.2
118	Camphene hydrate	101680	-4.8	-5.5
119	Isoborneol	6321405	-5.0	-5.0
120	Carvone	7439	-6.0	-5.7
121	Ipatasertib		-8.6	
122	Ravoxertinib			-8.0

Therefore, in this study, we compared the binding energies of potential compounds with positive controls (Ipatasertib and Ravoxertinib) to evaluate the inhibition of AKT1 and MAPK1 ability. Based on Table 2, there are 29 natural compounds with the most negative binding energy with AKT1 than positive control Ipatasertib, 37 natural compounds with the most negative binding energy with MAPK1 than positive control Ravoxertinib. From these results, the five strongest natural compounds that inhibit all both targets AKT1 and MAPK1 receptors were Simulanoquinoline (3), N- acetylanonaine (4), N-acetyldehydroanonaine (12), Oxyavicine (33), Benzosimuline (45). The ligand-amino acid interactions between them and the AKT1 and MAPK1 receptors (Figure 3 & Figure 4) show mainly interactions with π-bonding and hydrogen bonding. The amino acids of these both targets with 5 potential compounds are shown in Table 3.



Simulanoquinoline



N-acetylanonaine

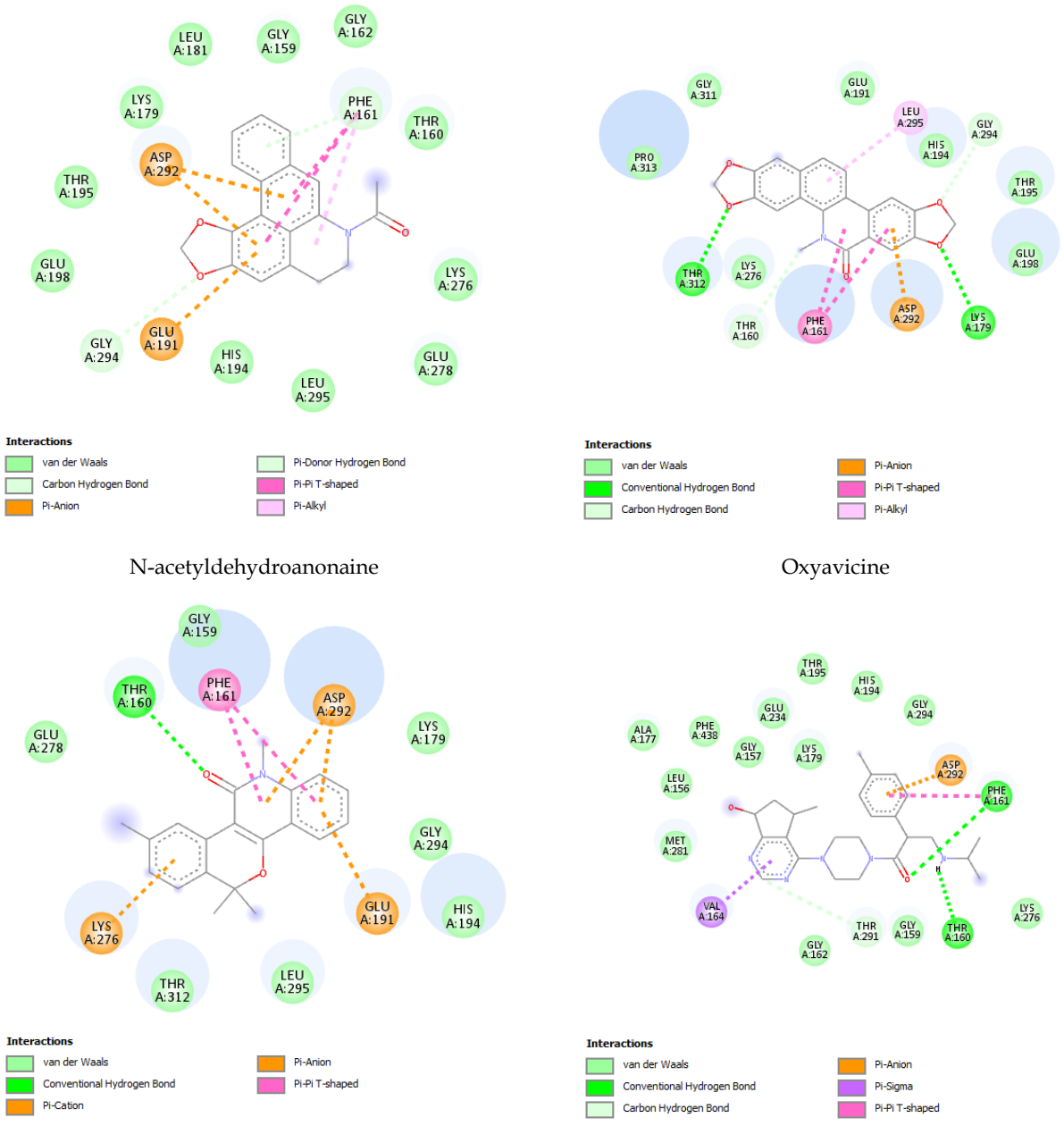
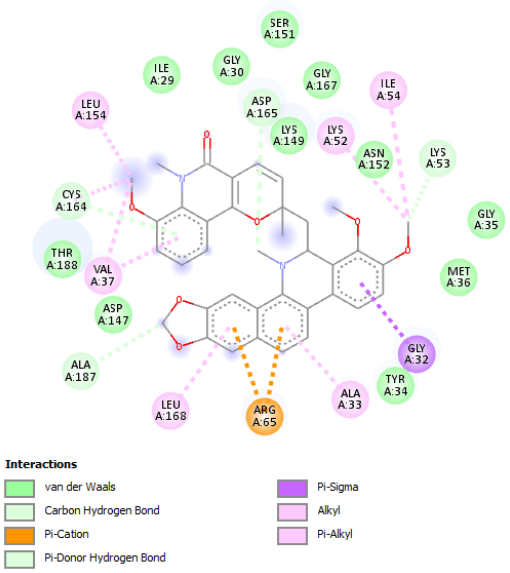
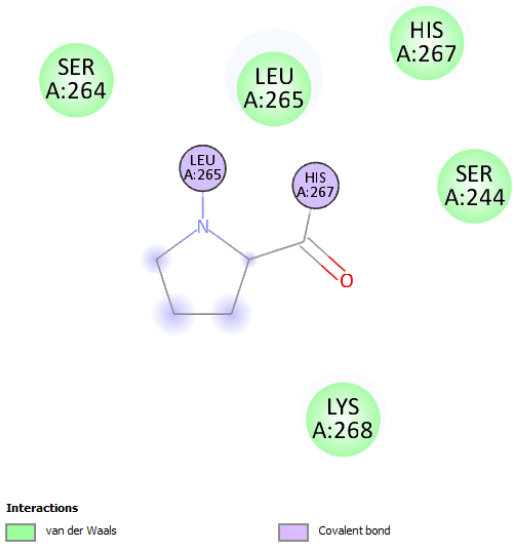


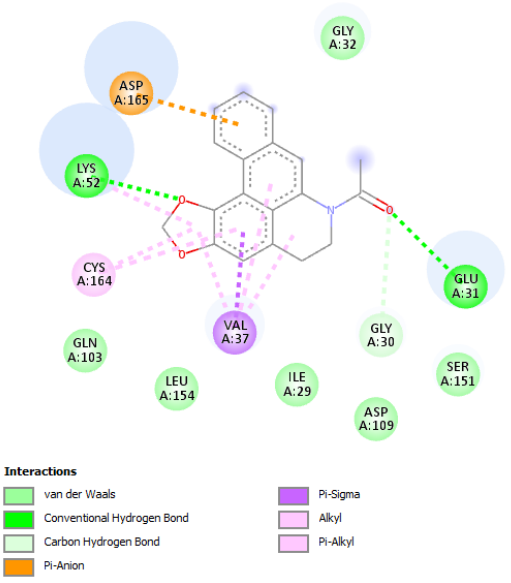
Figure 3. Interactions between 5 compounds and Ipatasertib with AKT1



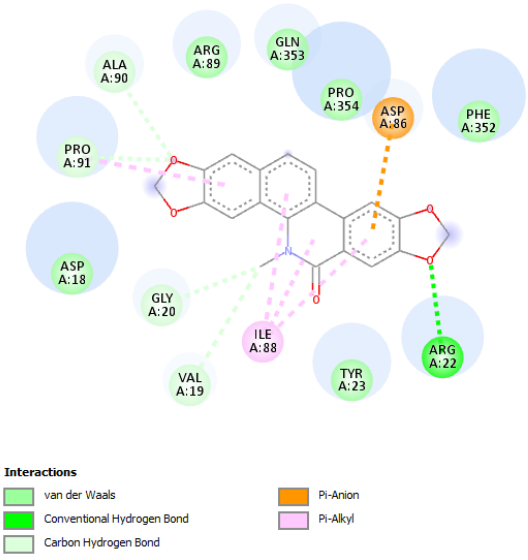
Simulanoquinoline



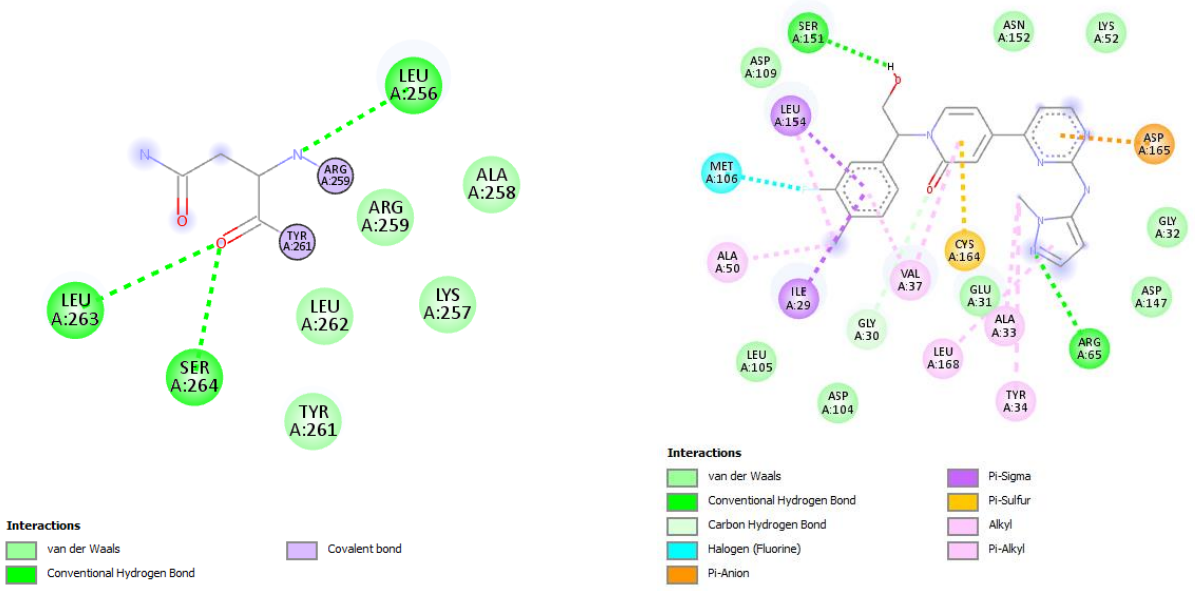
N-acetylanonaine



N-acetyldehydroanonaine



Oxyavicine



Benzosimuline Ravoxertinib

Figure 4. Interactions between 5 compounds and Ravoxertinib with MAPK1

Table 3. Amino acids of the 5 potential compounds that bind to AKT1 and MAPK1 receptors.

Compounds	The important amino acids binding	
	AKT1	**MAPK1**
Simulanoquinoline	LYS 158, LEU 227, GLU 234, PHE 236, ARG 241, GLU 341, LEU 347, ASP 439, GLU 441	GLY 32, ALA 33, VAL 37, LYS 52, LYS 53, ILE 54, ARG 65, LEU 154, CYS 164, ASP 165, LEU 168, ALA 187
N-acetylanonaine	VAL 164, LYS 179, MET 227, GLU 234, MET 281, THR 291, ASP 292	LEU 265, HIS 267
N-acetyldehydroanonaine	PHE 161, GLU 191, ASP 292, GLY 294	GLY 30, GLU 31, VAL 37, LYS 52, CYS 164, ASP 165
Oxyavicine	THR 160, PHE 161, LYS 179, ASP 292, GLY 294, LEU 295, THR 312	VAL 19, GLY 20, ARG 22, ASP 86, ILE 88, ALA 90, PRO 91
Benzosimuline	THR 160, PHE 161, GLU 191, LYS 276, ASP 292	LEU 256, ARG 259, TYR 261, LEU 263, SER 264
Ipatasertib	THR 160, PHE 161, GLY 162, VAL 164, ASP 292	
Ravoxertinib		ILE 29, GLY 30, ALA 33, TYR 34, VAL 37, ALA 50, ARG 65, MET 106, SER 151, LEU 154, CYS 164, ASP 165, LEU 168

Comparing the interactions of these 5 compounds and AKT1-targeted positive controls, it can be seen that the ligand amino acid binding of most compounds are similar. Those are THR 160, PHE 161, ASP 292. For the MAPK1 target, these compounds also have relatively low binding energies to this enzyme through similar amino acids: ASP 165, GLY 30, ALA 33 (Table 3).

2.4. Lipinski's rule of five

Compounds are considered to be "drug-like" when they have at least 2 of the 5 criteria of Lipinski's 5-criteria rule: Molecular mass (MW) below 500 Daltons; high lipophilicity (expressed as LogP less than 5); less than 5 hydrogen bond donors (HBD); less than 10 hydrogen bond acceptors (HBA1) and molar refractivity

(MR) should be between 40-130. Table 4 showed that all 5 compounds satisfy Lipinski's 5-criteria rule. Next, these compounds were further evaluated for their pharmacokinetic-toxicological properties by predicting ADMET.

Table 4. The result of Lipinski's rule five.

Name	MW	HBD	HBA1	LogP	MR	Drug-likeness
Simulanoquinoline	618.0	0	9	6.8751	176.33	Yes
N-acetylanonaine	307.0	0	4	3.0840	85.5	Yes
N-acetyldehydroanonaine	305.0	0	4	3.6307	89.3	Yes
Oxyavicine	347.0	0	6	3.5541	94.36	Yes
Benzosimuline	305.0	0	3	4.1050	91.95	Yes

2.5. Prediction of ADMET profile

To evaluate the effectiveness of the above 5 substances, we continuously evaluate the pharmacokinetic and toxicological parameters (ADMET) through pkCSM. Table 5 is the ADMET prediction result.

In the absorption process, human colon adenocarcinoma-2 cell line (Caco2) permeability and human intestinal absorption (HIA) are crucial benchmarks to determine the entire bioavailability of a drug [13, 14]. Caco2 membrane permeability (log Papp in 10^{-6} cm/s) with a higher value than 0.9 is said to have good permeability. Table 5 indicates that five compounds have good permeability to the CaCo2 cell membrane, with value log Papp in 10^{-6} cm/s in range from 1.022 to 1.937. Five compounds also showed good absorption through the human intestine with absorption percentages from 97.303 to reach 100%. For distribution, a logBBB value greater than 0.3 is said to be well absorbed across the blood-brain barrier and less than -1 is considered not to cross the blood-brain barrier. The results showed that five compounds had little permeability through the blood-brain barrier except Benzosimuline has logBBB is 0.285. In terms of metabolism, the cytochrome P450 system is an important enzyme system in drug metabolism in the liver, with two important CYPs, CYP3A4 and CYP2D6. Here, all of the above compounds are substrates of CYP3A4, indicating that they can be metabolized in the liver. Furthermore, Benzosimuline and Oxyavicine may inhibit CYP3A4. In terms of elimination and toxicity, all compounds are capable of renal elimination. The ADMET prediction results also show that simulanoquinoline gives the best results with predictive properties of no AMES toxicity and no liver toxicity and could not cause skin irritation. Four compounds including N-acetyldehydroanonaine, N-acetylanonaine, Benzosimuline and Oxyavicine may have AMES toxicity and Hepatotoxicity but not cause skin sensitisation.

Table 5. Pharmacokinetic and toxicological prediction results.

Properties	Simulanoquinoline	N-acetyldehydroanonaine	N-acetylanonaine	Oxyavicine	Benzosimuline
Absorption					
Water solubility (log mol/l)	-4.743	-4.831	-3.764	-5.507	-4.647
Caco2 permeability (log Papp in 10^{-6} cm/s)	1.022	1.719	1.937	1.284	1.532
Intestinal absorption (human) (%)	100	99.256	97.303	100	99.096
Distribution					
VDss (human) (log L/kg)	-0.899	0.353	0.436	-0.165	0.469
BBB permeability (log BB)	-1.238	-0.15	-0.149	-0.389	0.285
Metabolism					
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	Yes	Yes
Excretion					
Total clearance (log ml/min/kg)	0.373	0.14	0.136	0.08	0.307
Toxicity					
AMES toxicity	No	Yes	Yes	Yes	Yes
Hepatotoxicity	No	Yes	Yes	Yes	Yes
Skin sensitisation	No	No	No	No	No

3. DISCUSSION

We conducted the SRB method to evaluate the cytotoxicity of different fractions of *Zanthoxylum simulans* fruit bark extract on gastric cancer cells. The total n-hexane extract exhibited inhibitory activity on MNK-7 gastric cancer cells line with an IC_{50} value of 23.65±1.75 µg/mL. The EtOAc and EtOH fractions showed cytotoxicity activities with IC_{50} values of and 35.61±2.90 and 50.67±3.82 µg/mL, respectively. Our results are consistent with previous studies. Chao Wang and colleagues extracted acridone alkaloids from *Zanthoxylum simulans* fruit root bark and evaluated their inhibitory effects on prostate cancer PC-3M and LNCaP cells as well as *Plasmodium falciparum* parasites. These authors showed that five acridone alkaloids, including normelicopidine, normelicopine, melicopine, melicopidine, and melicopicine, exhibited inhibitory effects on cancer cells and anti-malarial activity *in vitro* [15]. In another study, Nguyen Bich Thu and colleagues screened medicinal plants for their anti-cancer effects and found that methanol extracts leaves and seeds of *Zanthoxylum simulans* inhibited HepG2 cancer cells with IC_{50} values of 7.1 ± 0.1 and 28.5 ± 4.3 µg/mL, respectively. Furthermore, they showed that the extracts also showed inhibitory effects on other cancer cell lines, such as lung cancer A549, liver cancer Huh-7, fibrosarcoma HT1080, and breast cancer MCF-7 [16]. Recently, Yong-Qiang Tian showed that chelerythrine, a compound in *Zanthoxylum simulans* exhibited stronger inhibitory effects on gastric cancer cells than Cerdulatinib, a positive control. Chelerythrine inhibited cell adhesion, migration, invasion, and induced programmed cell death in AGS gastric cancer cells, reduced the expression of estrogen receptors (ER-α36, ER-α66, and ER-β1) and Src proto-oncogene [17].

In this study, we evaluated the inhibitory ability of 120 compounds in *Zanthoxylum simulans* Hance. with both MAPK1 and AKT1 targets. After performing molecular docking, we obtained five compounds showing high inhibitory ability on these 2 targets, with lower binding energy than positive controls and drug-like properties, including Simulanoquinoline, N-acetyldehydroanonaine, N-acetylanonaine, Benzosimuline, and Oxyavicine. Simulanoquinoline is a benzophenanthridine alkaloid. The simulanoquinoline dimeric profile was first identified in *Zanthoxylum simulans* in 1993 by Shwu-Jen and Ih-Sheng [18]. This compound is found in many plants of the genus Zanthoxylum such as *Zanthoxylum simulans* Hance., and *Zanthoxylum rhetsa* (Roxb.) DC [19]. Similar to simulanoquinoline, N-acetyldehydroanonaine is also found in many plants such as *Zanthoxylum nitidum, Zanthoxylum simulans* [11, 20]. The genus Zanthoxylum has been studied with many biological effects such as anti-cancer cell proliferation, cytotoxicity, anti-inflammatory, antioxidant, antibiotic, hepatoprotective, and antiviral,... mainly due to the presence of alkaloids and essential oils [21]. In our study, Simulanquinoline showed the ability to inhibit both AKT1 and MAPK1 targets simultaneously with docking scores of -9.5 and -10.3 kcal/mol, respectively. Meanwhile, N-acetyldehydroanonaine has docking scores of -9.6 with both targets. Besides, these two compounds also show the potential to become an anti-cancer drug when satisfying the criteria of Lipinski's rule of five, predicting a positive AMET (well-absorbed, less metabolized by the liver, eliminated by the kidney). Therefore, further studies need to be conducted to more accurately assess the drug potential of this compound.

N-acetylanonaine is an alkaloid, found in many plants such as *Zanthoxylum simulans,* and *Magnolia kachirachirai* [22, 23]. This compound has been reported for its complete inhibitory ability on platelet aggregation induced by arachidonic acid and collagen as well as antimicrobial activity in both bacteria and fungi such as *C. albicans* and *S. aureus* [23, 24]. In our study, N-acetylanonaine exhibited the ability to inhibit both AKT1 and MAPK1 targets with docking scores of -9.8 and -9.1 kcal/mol, respectively. Besides, this compound also shows the potential to become an anti-cancer drug when satisfying the criteria of Lipinski's rule of five, predicting a positive AMET (non-skin sensitisation, well-absorbed, less metabolized by the liver, eliminated by the kidney). Although N-acetylanonaine may have AMES toxicity and hepatotoxicity, further studies need to be evaluated its potential to become an anti-gastric cancer drug in the future.

Oxyavicine is an alkaloid with antinociceptive and anti-inflammatory abilities [25]. In this study, oxyavivine inhibits both AKT1 and MAPK1 targets with docking scores of -9.8 and -9.1 kcal/mol, respectively through binding acid amines similar to positive control. Moreover, this compound also showed the potential

to become a drug through drug-likeness, and positive pharmacology. Therefore, oxyavicine is a potential compound to treat gastric cancer in the future.

Benzosimuline is anti-platelet aggregation activity that has been isolated from the plant *Zanthoxylum simulans* [26]. Our results showed that benzosimuline strongly inhibits both AKT1 and MAPK1 with a free binding energy of -9.4 and -9.3 kcal/mol, which is much more negative than the positive controls, Ipatasertib and Ravoxertinib. The ADMET profile suggested that this compound has renal elimination and is well-absorbed. Therefore, we can see the great potential of benzosimuline in inhibiting gastric cancer cells.

4. CONCLUSION

Our study evaluated the *in vitro* anticancer effects of *Zanthoxylum simulans* fruit bark extract on gastric cancer cells line. The total n-Hexan extract and two fractions, EtOAc and EtOH, showed the strongest activity against gastric cancer cells line with IC_{50} values of 23.65±1.75; 35.61±2.90 and 50.67±3.82 µg/mL, respectively. Through the results of screening 120 compounds from *Zanthoxylum simulans*, we have found the five most potential compounds, including Simulanoquinoline, N-acetyldehydroanonaine, N-acetylanonaine, Benzosimuline and Oxyavicine. These compounds could simultaneously inhibit MAPK1 and AKT1 targets with the most negative binding energy, drug-likeness and have the best ADMET results including good absorption, renal elimination, and low toxicity. Therefore, *in vitro* and *in vivo* studies are needed to develop these potential compounds as anti-cancer drugs.

5. MATERIALS AND METHODS

5.1. Plant material

The fruit bark of *Zanthoxylum simulans* was purchased in December 2022 from Hanoi Vietnam. The plant samples were authenticated, and a voucher specimen (No: DLPC23UMP-VNU) has been deposited at the Department of Pharmacology, University of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam.

5.2. Extraction

The dried fruit bark of *Zanthoxylum simulans* (500 g) was extracted with 70% EtOH at room temperature, 3 times x 3 days with a ratio of medicinal plants/solvent 1:10 (kg/l). The combined extracts were filtered and evaporated under reduced pressure to yield a green residue (23.7 g), which was suspended in water and successively partitioned with organic solvents, then concentrated to yield three extracts of *n*-hexane (1.2 g), ethyl acetate (EtOAc, 7.6 g), *n*-butanol (4.7 g), and a water-soluble layer (8.1 g).

5.3. Cytotoxicity assay

The SRB assay is used for cell density determination, based on the measurement of cellular protein content [27]. The method described here has been optimized for the toxicity screening of compounds to adherent cells in a 96-well format. After an incubation period, cell monolayers were fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min; then, excess dye was removed by washing the cells repeatedly with 1% (vol/vol) acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution for optical density (OD) determination at 540 nm using a microplate reader. Ellipticine was used as control positive. The cytotoxicity activity was expressed as IC_{50} values.

5.4. Molecular docking study

Compounds were docked into protein binding cavities using Autodock Tools software.

5.4.1. Preparation of protein structures

The 3D structures of AKT1 (ID: 4EKL) [28, 29] and MAPK1 (ID: 6OPH) [30-32] were retrieved from the Protein Data Bank RCSB (https://www.rcsb.org/). We removed water molecules and co-crystal ligands from the protein molecule by using Discovery Studio software. Next, we used MGL Autodock Tools 1.5.6 software

to add hydrogen atoms, optimize polar hydrogens, and Kollman charges. The active region of AKT1 and MAPK1 were located in grid boxes of corresponding sizes (40Å×40Å ×40Å) and (60Å×60Å ×60Å), the grid center (x,y,z) corresponding (x = 20.918; y = 2.534 ; z = 16.36) and (x = 5.953, y = 18.56, z = 23.812). This protein was saved in pdbqt format to prepare for the docking program. The selection of grid box indexes is based on previous publications [32-34].

5.4.2. Preparation of ligands

Based on previous publications, we collected 120 natural compounds in the plant *Zanthoxylum simulans* [11, 18, 21, 25, 36-40]. The ligand structures of these molecules were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format. Then we converted them to pdb format using Discovery Studio Visualizer 2021 software. Finally, the ligands were optimized by Avogadro software using Conjugate Gradients method and converted to the pdbqt format using Autodock Tools 1.5.6.

5.4.3. Evaluation of docking results

To evaluate the docking results, the co-crystal ligand after being separated from the protein was redocked to the active site of the target. The process was performed successfully if the root-mean-square deviation (RMSD) value was less than or equal to 1.5 Å. For substances that need docking, their binding ability is assessed through interaction with amino acids, and the interaction energy is calculated by the scoring function of Autodock vina.

5.4.4. Evaluation of Lipinski's rule of five

Lipinski's rule of five was used to study the drug-like and non-drug-like molecules which help to evaluate the potential molecular to become a therapeutic drug. We used the online tool (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) and through criteria: molecular mass (MW), high lipophilicity (LogP), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA1); molar refractivity (MR). After selecting the drug-like compounds, we continued to analyze the pharmacokinetic and toxicological parameters to give the final results.

5.4.5. Prediction of ADMET by computational analysis

We used the online tool pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) to predict the pharmacokinetic and toxicological properties of the compounds as input data SMILES formulas. Predictive results of pharmacokinetic-toxicological (ADMET) parameters including absorption, distribution, metabolism, elimination, and toxicity of potential compounds were analyzed.

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