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In silico molecular docking study and Protective effect of *Piper attenuatum* on aspirin induced gastric ulcer in rats

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A B S T R A C T

Steroid and painkiller abuse induced peptic ulcer disease, which causes abdominal pain, fullness, heartburn, and nausea. Medicinal plants have treated ulcers for centuries. We used *Piper attenuatum* ethanolic plant extracts for aspirin to induce ulcers in Wistar rats to test the leaf ethanolic extract's antiulcer properties. The control group is normal saline, the standard group is ranitidine (20 mg/kg), and the extract-treated groups are 100 mg/kg and 200 mg/kg ethanolic plant extracts. Ulcer Score, gastric juice volume, free and total acidity, ulcer index, ulcer protection, and pH were measured. The ulcer score was determined via rat stomach biopsies. Plant ethanolic extracts are gastroprotective. Only pH increased compared to the control group. *Piper attenuatum* ethanolic extract is the most gastroprotective at 200 mg/kg. Extracts were phytochemically and analytically assessed. Phytochemical screening demonstrates that plant extracts contain alkaloids, amides, glucose, proteins, glycosides, steroids, flavonoids, etc. This research suggests that phytoconstituents may have anti-ulcer potential, although structural elucidation of bioactive chemicals is needed. Molecular docking showed better binding affinity versus the 3D structure of pig gastric H+/K+ ATPase isoforms phytoconstituents Cepharadione A, Cepharadione B, Guineensine, Norcepharadione B, and Piperlonguminine. With these significant results, it may be a drug in the future.

1. Introduction

Peptic ulcer is globally accepted chronic disease which affects millions of peoples worldwide having higher rate of morbidity and mortality.[1] Peptic ulcer is composed of both gastric and duodenal ulcer [2] which is the wound in mucosa of gastrointestinal tract that typically spread throughout the muscularis mucosa of stomach. [3] According to the past study on peptic ulcer it was found that this disease is found in almost 10% of total population.[2] Peptic ulcer disease is commonly found in those peoples whose hydrochloric acid, and bicarbonate, prostaglandin, nitric oxide and growth factor become imbalanced respectively [4]. Despite these many other factors are also causes ulcer like long term use of steroidal and non-steroidal anti-inflammatory drugs, intake of excessive alcohol, bacterial infection.[5] Increase gastric and pepsin secretion, decrease prostaglandin synthesis, gastric cell proliferation, gastric blood flow and mortality impart in ulcer pathogenesis. Aspirin and other non-steroidal anti-inflammatory drugs are used to treat inflammatory and associated disorders like arthritis and gout.[6] Proton pump (H^+/K^+ATPase) located in the stomach is the key factor which makes acidify the stomach content and are responsible for acid production. Inhibition of proton pump plays a major role in management of gastrointestinal disorders like ulcer, dyspepsia and gastroesophageal reflux disease.[7] In market there are many synthetic compounds which are used to treat gastrointestinal disorder including ulcer; they commonly include proton pump inhibitors, histamine (H_2) blockers, anticholinergics, prostaglandin analogue and antacids.[8] Medicinal plants and herbal products are used by peoples from all over the world from ancient time to alleviate many diseases including ulcer. These herbals are easily available, affordable, and have minimum adverse effects so these products are used by nearly 80% of peoples in South Africa.[9] Piper

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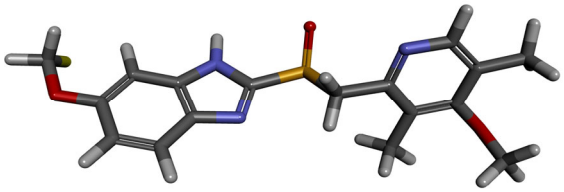
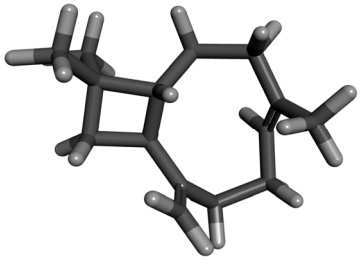
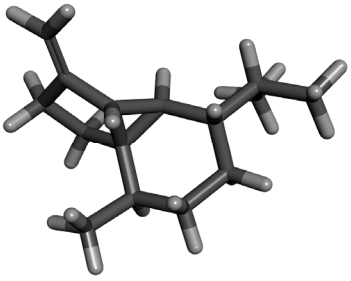
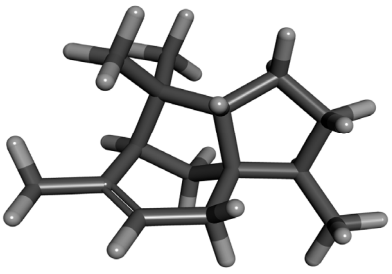
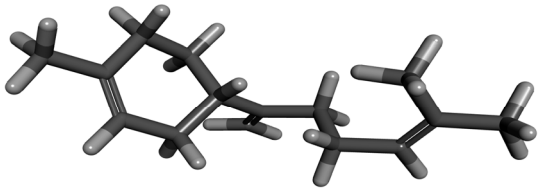
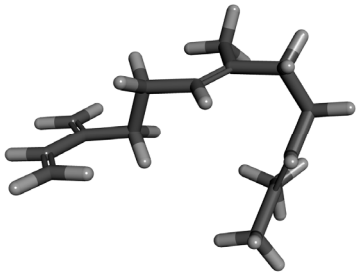
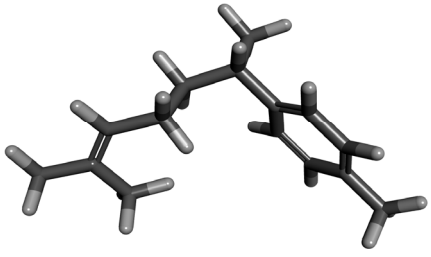
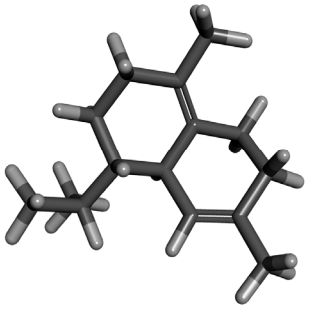
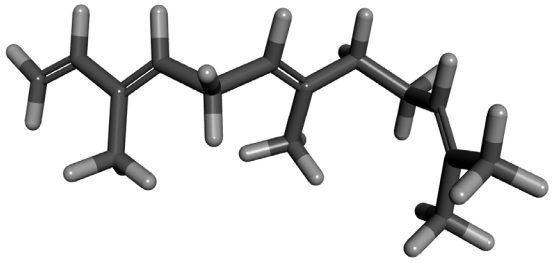
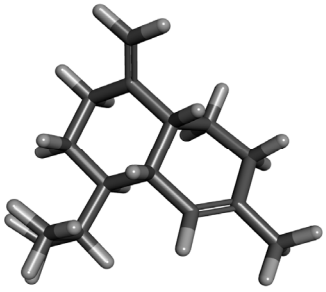
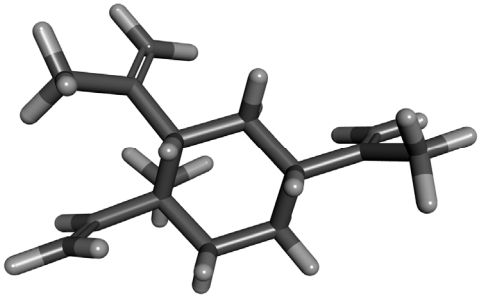
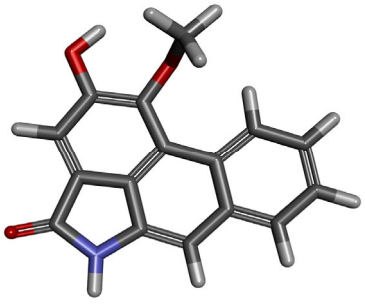
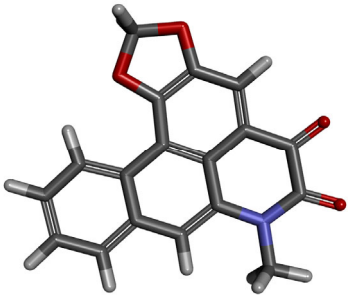
species are one of the common species basically used as condiments globally and also have medicinal value. Most commonly used Piper species are *Piper attenuatum*, *Piper nigrum*, *Piper longum*, *Piper caninum*, *Piper lolot*, *Piper chaba* and many more.[10] *Piper attenuatum* is a rare piper species, globally approx. 500 piper species are there. Plant has curved and hairless branches having leaves size approx 6.5 to 14 cm. *Piper attenuatum* have been reported for various phytochemicals viz alkamides, Piperine, steroids like sitosterol and many other.[11] *Piper attenuatum* have been reported for various pharmacological activities like anti-inflammatory,[12] anti-oxidant, muscle relaxant[13] anti-hyperlipidemic,[14] anti-cancer,[15] and hepatoprotective activities. [11] *Piper attenuatum* is also used as rubefacient, and as diuretic agent while it is reported that whole plant is used to treat headache and muscle pain,[16] Throat pain, heal wound, antibacterial and antihyperglycemic activity.[10] As we know that plants products are the rich source of therapeutic agents which plays an important role in human healthcare system to cure many of diseases with minimum adverse effects and we also know that herbal drugs are safer as compare to allopathic drugs.

Nowadays many synthetic anti-ulcer drugs are popular in the market; but most of them have adverse effects like anaphylaxis, gynecomastia, thrombocytopenia and nephrotoxicity. These antiulcer drugs also required multiple dosages. Due to these factors, we need new antiulcer drugs with minimum adverse effects and at low dosage for treatment of ulcer and this could only be possible with plant resources.[18] *Piper attenuatum*. Standard drug Omeprazole (A) and Phytoconstituents (B-X) extracted from plant extract with 3-D structure and PubChem ID (https://pubchem.ncbi.nlm.nih.gov/) mentioned in **Table 1**.[17]

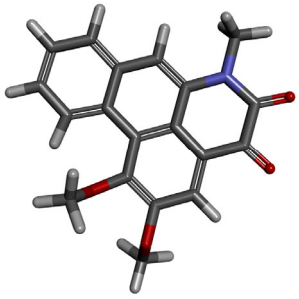
Table 1. Different phytoconstituents extracted from *Piper attenuatum* with 3-D structure and PubChem ID.

S. No.	Compounds Name	PubChem ID	3D Structure
a.	Omeprazole	4594	
b.	β -Caryophyllene	5281515	
c.	b-cubebene	93081	

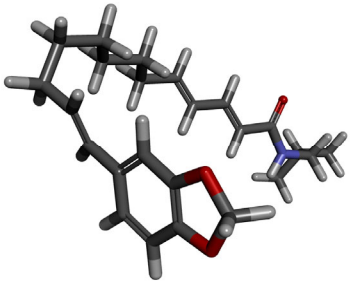
d.	a - cedrene	6431015	
e.	b-bisabolene	403919	
f.	β-farnesene	5281517	
g.	ar - curcumene	92139	
h.	delta-cadinene	441005	

i.	α - farnesene	5281516	
j.	gamma – muurolene	6432308	
k.	β -elemene	6918391	
l.	Aristolactam A II	148657	
m.	Cepharadione A	94577	

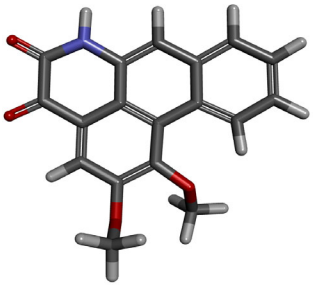
n. Cepharadione B 189151



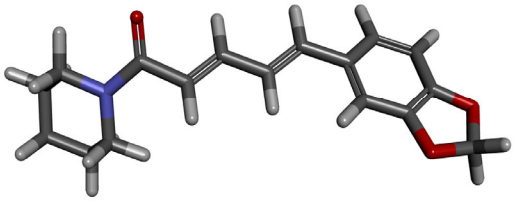
o. Guineensine 6442405



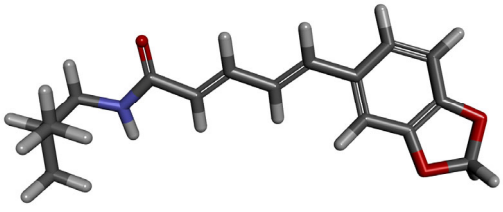
p. Norcepharadione B 189168

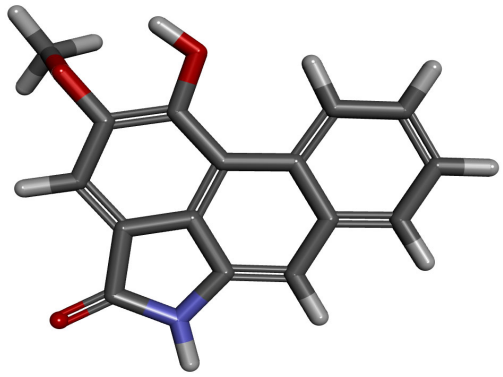
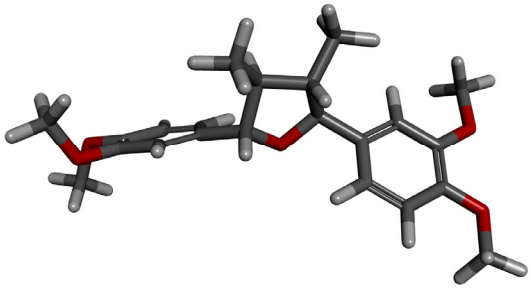
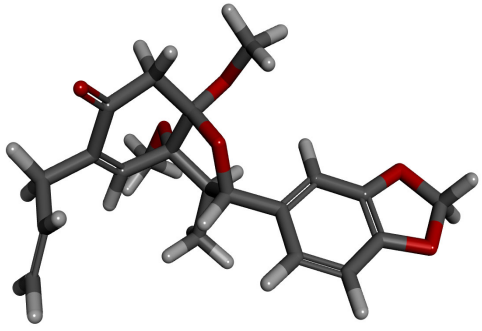
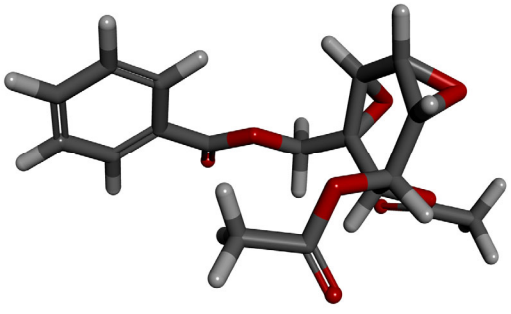


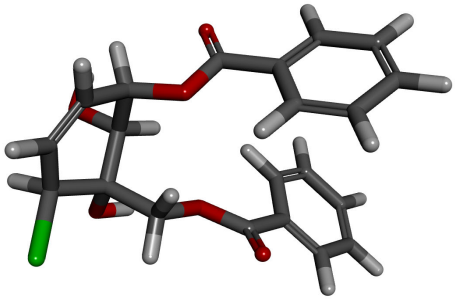
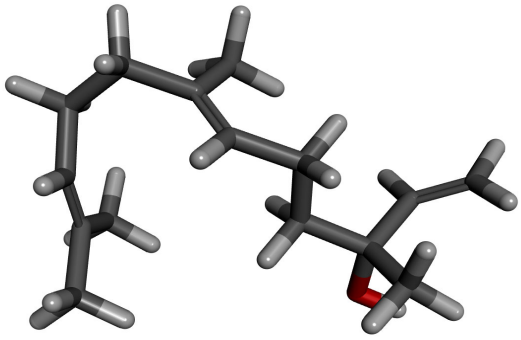
q. Piperine 638024



r. Piperlonguminine 5320621



s.	Piperolactam A	3081016	
t.	Galbelgin	11975378	
u.	Kadsurin A	442885	
v.	Crotepoxide	161314	

w.	Pipoxide chlorohydrin	100854335	
x.	Nerolidol	5284507	

2. Results and Discussion

The % yield for the ethanolic extract of *Piper attenuatum* was found to be 28.33%. Various chemical tests were performed on ethanolic leaf extract of plant *Piper attenuatum* to determine their phytochemical characteristic. Ethanolic extract contains alkaloids, amides, flavonoids, tannins, glycosides and many more. A detailed summary of phytochemicals found in ethanolic extract was given in **Table 2**. Active phytochemicals from extract were isolated by using various analytical methods like thin layer chromatography and column chromatography. In thin layer chromatography nine compounds were spotted with decreasing Rf values and are shown in **Table 3**. Under visible light five bands become visualized (A). Six bands were seen under short ultraviolet radiation at 254nm (B), eight band were seen under long ultraviolet radiation at 366 nm (C) When vanillin-sulfuric acid reagent were sprayed on plate we got seven bands (D) which are presented in **Figure 1**. Compounds with Rf values of 0.92, 0.86, 0.53 and 0.13 were visualized in all thin layer chromatograms. From column chromatography we found different fractions according to their colour and they are subjected to dry under reduced pressure. We select three crystals named S1 (Ethanolic extract of *Piper attenuatum* a), S2 (Ethanolic extract of *Piper attenuatum* b) and S3 (Ethanolic extract of *Piper attenuatum* c) for further study which are clear in color and good in shape and were shown in **Table 4**. These three crystals were again subjected to phytochemical analysis for confirmation of phytochemicals and directed again for thin layer chromatography to separate single compounds. Ethyl Acetate & methanol were used as a solvent system. Their Rf value at 366nm, 254nm and on visible light were shown in **Table 5**. These fractions have an Rf value between 0.05 to 0.89 for S1 at 366nm, at 254nm the Rf range was 0.06 to 0.65 and at visible light it was 0.04 to 0.95. For fraction S2 the Rf range at 366nm was 0.03 to 0.89 at 254nm it was 0.04 to 0.66 and at visible light it was 0.03 to 0.93 and for fraction S3 the Rf range at 366nm was 0.03 to 0.91 at 254nm it was 0.05 to 0.67 and at visible light it was 0.04 to 0.94. When these isolated fractions were subjected to TLC with standard Piperine we found that the Rf value of standard Piperine was near the Rf value of all isolated fractions when seen under visible light which was shown in **Figure 2.** Pharmacological screening of extract was done on aspirin induced ulcer models in rats using aspirin as an inducing agent. All the parameters like Ulcer Score, volume of gastric juice, free and total acidity, ulcer index, ulcer protection and pH were measured and when compared with the control group we found that all parameters were decreased while pH was increased with dose depended on manner and presented in **Table 6**. **Table 7.** Shows Effect of *Piper attenuatum* ethanolic extract on various parameters to evaluate its antiulcer activity.

All non-steroidal anti-inflammatory drugs including aspirin cause gastric mucosal damage that leads to ulcer. Prostaglandin which coats the entire gastric mucosa that prevents the gastric mucosa from gastric hydrochloric acid and prevents ulcer formation. Aspirin is a non-steroidal anti-inflammatory drug which inhibits synthesis of prostaglandin thus causing injury to gastric mucosa and leads to formation of ulcer [33]. Data from research work of Sabina *et al* revealed that ethanolic extract of plants have Piperine alkaloid, and [34] reported that Piperine has antiulcer activity in non-steroidal anti-inflammatory drug induced ulcer model. Biopsy was also done for rats of all groups. Histopathology showed that there was no gastric mucosal epithelial injury for group 1 shown in **Fig. 3** (A) which was termed as a normal control group. Group 2

treated with standard drug Ranitidine (20mg/kg) showed mild gastric lesion directed in **Fig. 3** (B). While group 3 and group 4 both are test groups treated with ethanolic extract at 100mg/kg and 200mg/kg showed moderate gastric ulceration which are directed in **Fig. 3** (C) and **Fig. 3** (D). On the basis of research work data it was found that ethanolic extract of *Piper attenuatum* has many phytochemicals among these Piperine was one of them. It may be concluded that ethanolic leaf extract has gastroprotective effect and it may be possible that Piperine stimulates prostaglandin synthesis to prevent ulcer formation. But further work may be required to predict the exact mechanism of Piperine as an antiulcer agent and also for structure elucidation of Piperine responsible for antiulcer activity.

Table 2. Phytochemicals present in the ethanolic extract of *Piper attenuatum.*

Sr. No	Test Performed	Observation	Confirmed compound
1	Molish Test	+	Carbohydrate
2	Fehling Test	+	Carbohydrate
3	Barfoed's Test	+	Carbohydrate
4	Biuret Test	+	Protein
5	Millons Test	+	Protein
6	Ninhydrin Test	+	Amino acid
7	Salkowski Test	+	Steroids
8	Lead Acetate Test	+	Flavonoids
9	Mayer Test	+	Alkaloids
10	Hager Test	+	Alkaloids
11	Killer Killani Test	+	Glycoside
12	Borntrager's Test	+	Glycoside
13	Foam Test	-	Saponin absent
14	Ferric Chloride Test	+	Tannins
15	Solubility Test 1. In Water 2. In Organic Solvent		Soluble in warm water and alcohol

Table 3. TLC of ethanolic leaves extract of *Piper attenuatum.*

Sr. No.	Rf value	Visual Light	UV-254 nm	UV-366 nm	Vanillin-sulfuric acid spray
1	0.92	Light yellow	Faint dark	Faint red	Violet spot
2	0.86	Dark Grey	Dark gray	Pinkish red	Light brown
3	0.76	----------	Faint dark	Red	Brown
4	0.7	Green	Dark gray	----------	Green
5	0.53	Yellow	Light dark	Green	Light green
6	0.4	----------	---------	Light blue	----------
7	0.25	----------	----------	Blue	----------
8	0.13	Dark Green	Dark gray	Pink	Green
9	0.013	----------	----------	Red	Light green

Table 4. Different main fraction obtained from the ethanolic extract of *Piper attenuatum* by Column Chromatography (CC).

Sr. No	Solvent Used	Ratio	Fraction Colour	Fraction Name	Selected Crystal for Phytochemical study
1	n – H : EA	50:00:00	Colourless	F_1	
2	n – H : EA	40:10:00	Light Orange	F_2	
3	n – H : EA	30:20:00	Light Green	F_3	
4	n – H : EA	20:30	Dark Green	F_4	
5	n – H : EA	10:40	Dark Blue	F_5	
6	n – H : EA	0:50	Yellow	F_6	**S1**
7	EA : Met	40:10:00	Green	F_7	
8	EA : Met	30:20:00	Light Red	F_8	
9	EA : Met	20:30	Red	F_9	
10	EA : Met	10:40	Yellow	F_{10}	**S2**
11	EA : Met	0:50	Green	F_{11}	
12	Met : nH	40:10:00	Dark Green	F_{12}	
13	Met : nH	30:20:00	Orange	F_{13}	**S3**
14	Met : nH	20:30	Yellow	F_{14}	
15	Met : nH	10:40	Colourless	F_{15}	

N - Hexane = nH
Ethylacetate = EA, Methanol = Met
S1 - Ethanolic extract *Piper attenuatum* a
S2 - Ethanolic extract *Piper attenuatum* b
S3 - Ethanolic extract *Piper attenuatum* c

Table 5. TLC of fractions of *Piper attenuatum.*

Sr. No	Fraction Used	Solvent Used	Ratio	Rf value range		
				366nm	254nm	Visible light
1	S1	Ethylacetate & methanol	(10:1)	0.05 – 0.89	0.06 – 0.65	0.04 – 0.95
2	S2	Ethylacetate & methanol	(10:1)	0.03 – 0.89	0.04 – 0.66	0.03 – 0.93
3	S3	Ethylacetate & methanol	(10:1)	0.03 – 0.91	0.05 – 0.67	0.04 - 0.94

Table 6. *Piper attenuatum* ethanolic extract effect on various parameters.

Groups	Ulcer Score	Volume of gastric juice	Free acidity	Total acidity	Ulcer Index	Ulcer Protection	pH
Control	3.83 ±0.023	3.36 ± 0.45	42.8 ± 0.154	54.8 ± 0.148	10.69 ± 0.11		1.34±0.11
Treated	0.17 ± 0.002***	1.54 ± 0.017**	4 ± 0.71***	6.2 ± 1.28**	1.48 ± 0.05***	84.50***	4.3±0.34**
EEPA (100mg/kg)	1.43 ± 0.121**	1.80 ± 0.34**	11.8 ± 2.48**	24.8 ± 0.43**	5.414 ± 0.08***	52.79***	3.30±0.11**
EEPA (200mg/kg)	0.83± 0.001***	1.34± 0.12**	3.2 ± 0.58**	10.6 ± 0.71***	3.451 ± 0.05***	68.38***	4.1±0.22**

Data are represented as mean ± Standard Deviation. One way ANOVA followed by Dunnett's multiple comparison tests and Graph pad prism was used for statistical analysis. ***P <0.001 was considered significant.

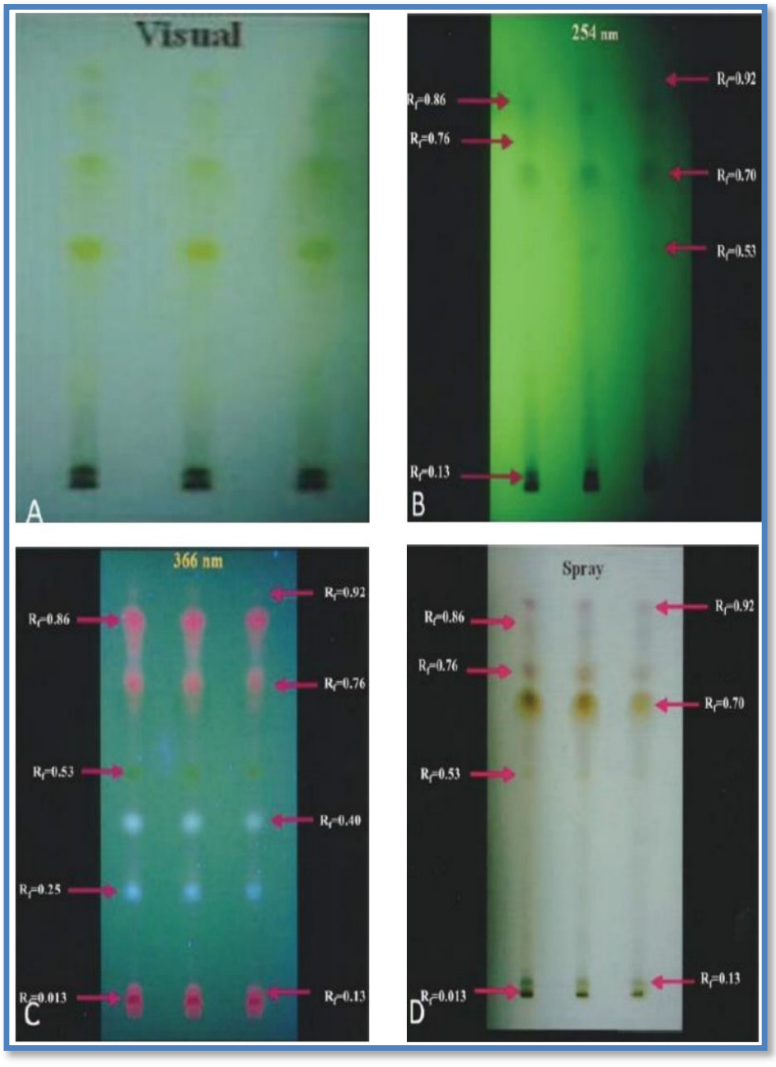


Fig. 1. TLC of Ethanolic leaf extract of *Piper attenuatum*, visualized under the influence of (A) Visible light (B) UV - 254nm (C) UV - 366nm (D) Vanillin - Sulfuric acid spray.

Fig. 2. TLC of Standard Piperine Compound & Isolated fraction (S1), (S2) & (S3) from ethanolic leaf extract of *Piper attenuatum.*

Std – Standard Piperine
S1 = Ethanolic Extract of *Piper attenuatum* a
S2 = Ethanolic Extract of *Piper attenuatum* b
S3= Ethanolic extract of *Piper attenuatum* c

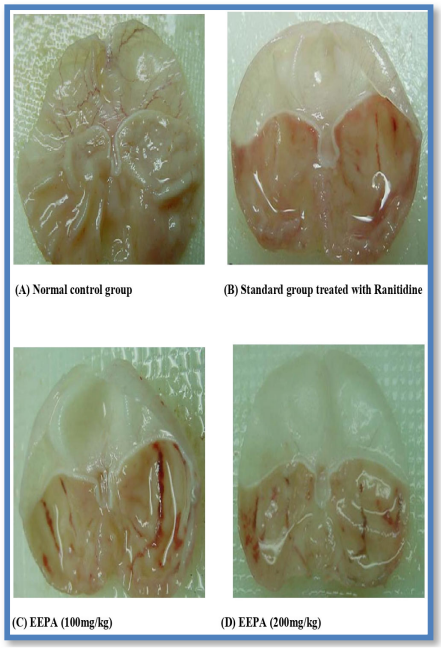
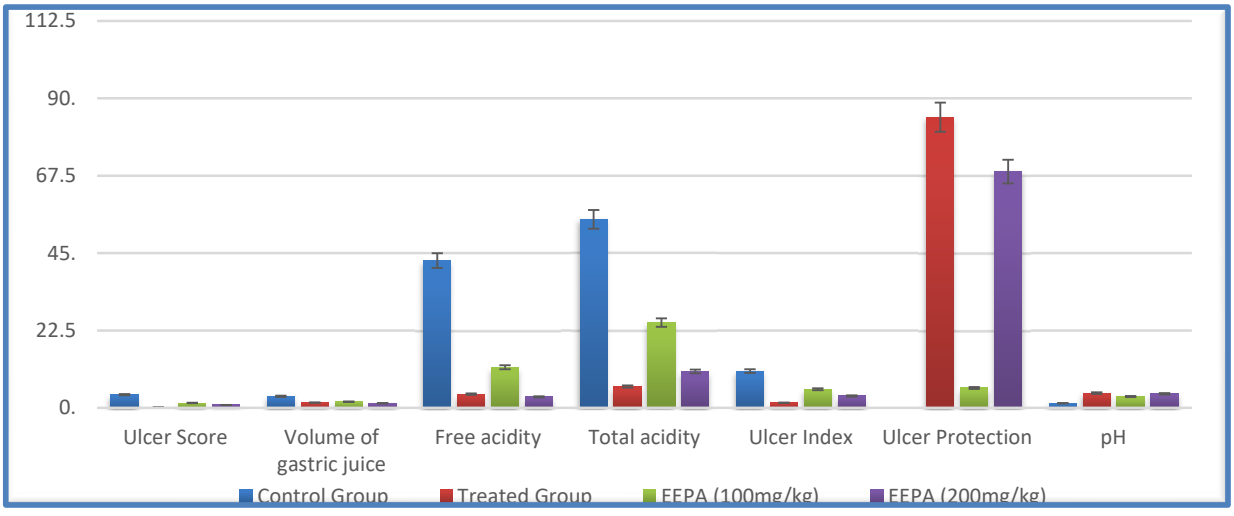


Fig. 3. Effect of ethanolic leaf extract of *Piper attenuatum* on microscopic appearance of gastric mucosa in aspirin induced gastric ulcer in rats

(A) Normal control group, (B) Ranitidine treated group, (C) Ethanolic extract of *Piper attenuatum* (100mg/kg) treated group - EEPA (100mg/kg), (D) Ethanolic extract of *Piper attenuatum* (200mg/kg) treated group – EEPA (200mg/kg)

Table 7. Effect of *Piper attenuatum* ethanolic extract on various parameters to evaluate its antiulcer activity.



2.1. Virtual screening result analysis

After doing a computer-based analysis of the 23 phytochemicals from *Piper attenuatum* to determine their ability to limit cell growth, we found that several of the compounds were almost ready to exhibit drug-like characteristics. Most of the drug-likeness guidelines and ADMET characteristics are adhered to by these substances. Molar refractivity for all these compounds varies from 40 to 120 and molecular weight from 200 to 400 (**Table 2**)[35]. All the selected phytoconstituents follow Lipinski rule which exhibits fine binding of drug-like molecules with protein in blood[36].

2.2. Molecular docking result analysis

Docking has been done numerous times for each ligand to obtain the variance in binding affinity score corresponding to each ligand. Based on the minimum binding affinity score and the maximum dipole moment, the inhibitor-receptor (protein-ligand) structure with the highest degree of stability is chosen. **Table 8** displays interactions between these substances and the target proteins by way of conventional, pi-donor, and carbon H-bonds. with Leucine (LEU), Glutamine (GLN), Threonine (THR), Lysine (LYS), Asparagine (ASN), Histidine (HIS), Tyrosine (TYR), Methionine (MET), and Aspartic acid as the shared active site amino acid residues (ASP). Additionally, Dreiding energy is supplied for various postures that take into account the complex's additive energy under energy components like bond length, angles, etc. Based on the minimum binding affinity score, the optimum poses for each of the phytochemicals under consideration are chosen. Consequently, molecular docking was completed using Pyrx, another docking programme. In among all phyto-constituents Aristolactam A II, Cepharadione A, Cepharadione B, Cepharadione B, Guineensine, Norcepharadione B, Piperlonguminine, Kadsurin A, Crotepoxide, Pipoxide chlorohydrin all these shows minimum binding affinity score. Cepharadione A has binding energy -8.7 kcal/mol two hydrogen bonds formed between Lysine to Arginine. Cepharadione B has binding energy -7.9 kcal/mol three hydrogen bonds formed between aspartate and glutamine. Guineensine having binding energy -6.9 kcal/mol three hydrogen bonds formed phenylalanine and isoleucine. Norcepharadione B having binding energy -8.3 kcal/mol three hydrogen bonds formed, Piperlonguminine having binding energy -7.4 kcal/mol and one hydrogen bond formed.

Table 8. Molecular configuration and Drug-likeness properties of proposed ligand drug molecules.

Physicochemical properties									Drug likeness								
Compound name	Molecular weight /mol)	Hydrogen-Bond Donor count	Hydrogen-Bond Acceptor count	Rotatable bond count	Heavy atom count	Molar refractivity	GI absorption	Permean BBB	Lipinski Rule	Ghose filter	Veber Rule	Egan Rule	Muegge filter	Bioavailability	Pains	Lead-likeness	Synthetic accessibility
Omeprazole	345.42	1	5	5	24	93.7	High	No	0	0	0	0	0	0.55	0	0	3.58
β -Caryophyllene	204.35	0	0	0	15	68.78	Low	No	1	0	0	0	1	0.55	0	2	4.51
b-cubebene	204.35	0	0	1	15	67.14	Low	Yes	1	0	0	0	1	0.55	0	2	4.24
a - cedrene	204.35	0	0	0	15	66.88	Low	No	1	0	0	0	1	0.55	0	2	5.53
b-bisabolene	204.35	0	0	4	15	70.68	Low	No	1	0	0	0	2	0.55	0	2	3.9
β-farnesene	204.35	0	0	7	15	72.32	Low	No	1	0	0	0	2	0.55	0	2	3.42
ar - curcumene	202.34	0	0	4	15	69.55	Low	No	1	0	0	0	2	0.55	0	2	2.31
delta-cadinene	204.35	0	0	1	15	69.04	Low	No	1	0	0	0	1	0.55	0	2	4.14
α - farnesene	204.35	0	0	6	15	72.32	Low	No	1	0	0	0	2	0.55	0	2	3.72
gamma – muurolene	204.35	0	0	1	15	69.04	Low	No	1	0	0	0	1	0.55	0	2	4.35
β -elemene	204.35	0	0	3	15	70.42	Low	No	1	0	0	0	2	0.55	0	2	3.63
Aristolactam A II	265.26	2	3	1	20	79.28	High	Yes	0	0	0	0	0	0.55	0	0	1.96
Cepharadione A	305.28	0	4	0	23	88	High	Yes	0	0	0	0	0	0.55	0	0	2.54
Cepharadione B	321.33	0	4	2	24	94.92	High	Yes	0	0	0	0	0	0.55	0	0	2.36
Guineensine	383.52	1	3	13	28	116.75	High	Yes	0	0	1	0	1	0.55	0	3	3.8
Norcepharadione B	307.3	1	4	2	23	90.02	High	Yes	0	0	0	0	0	0.55	0	0	2.35
Piperine	285.34	0	3	4	21	85.47	High	Yes	0	0	0	0	0	0.55	0	0	2.92
Piperlonguminine	273.33	1	3	6	20	78.77	High	Yes	0	0	0	0	0	0.55	0	1	2.98
Piperolactam A	265.26	2	3	1	20	79.28	High	Yes	0	0	0	0	0	0.55	0	0	2.02
Galbelgin	372.45	0	5	6	27	104.87	High	Yes	0	0	0	0	0	0.55	0	2	4.12
Kadsurin A	372.41	0	6	5	27	98.32	High	Yes	0	0	0	0	0	0.55	0	1	4.86
Crotepoxide	362.33	0	8	8	26	84.23	High	No	0	0	0	0	0	0.55	0	2	4.16
Pipoxide chlorohydrin	402.82	2	6	7	28	101.93	High	No	0	0	0	0	0	0.55	0	1	4.7
Nerolidol	222.37	1	1	7	16	74	High	Yes	0	0	0	0	1	0.55	0	2	3.53

Table 9. Binding mode of each inhibitor-receptor.

S. No.	Compounds	Binding Affinity (kcal/mol) with protein 2XZB
a.	Omeprazole	-7.3
b.	β -Caryophyllene	-7.6
c.	b-cubebene	-7.7
d.	a - cedrene	-7.7
e.	b-bisabolene	-7.7
f.	β-farnesene	-6.8
g.	ar - curcumene	-8.5
h.	delta-cadinene	-8.4
i.	α - farnesene	-7.3
j.	gamma – muurolene	-7.7
k.	β -elemene	-6.7
l.	Aristolactam A II	-8.3
m.	Cepharadione A	-8.7
n.	Cepharadione B	-7.9
o.	Guineensine	-6.9
p.	Norcepharadione B	-8.3
q.	Piperine	-8.4
r.	Piperlonguminine	-7.4
s.	Piperolactam A	-8.3
t.	Galbelgin	-8.1
u.	Kadsurin A	-7.9
v.	Crotepoxide	-6.8
w.	Pipoxide chlorohydrin	-8.4
x.	Nerolidol	-6.7

Table 10. Interaction details of the best pose for different ligands.

S. No.	S. No. in Table 3	Compounds	Hydrogen Bond interactions with Protein 2XZB (Distance in A) [Type of bond]	Total number of Hydrogen-bonds
1	a.	Omeprazole	GLY A:156 (3.78) [C-H-Bond], LEU A:370 (2.11)	**2**
2	l.	Aristolactam A II	GLN A:498 (2.39) [Conventional H-Bond]	1
3	m.	Cepharadione A	LYS A:782 (2.78), ARG A:852 (2.50) [Conventional	**2**
4	n.	Cepharadione B	ASP A:851 (3.31), GLU A:856 (3.44) [C-H-Bond],	3
5	o.	Guineensine	PHE A:864 (3.36), ILE A:869 (3.13) [C-H-Bond], TYR	3
6	p.	Norcepharadione B	GLY A:153 (3.70, 3.47) [C-H-Bond], GLN A:110	3
7	r.	Piperlonguminine	GLU A:856 (3.75) [C-H-Bond], TYR A:340 (2.07)	2
8	u.	Kadsurin A	GLN A:104 (3.36) [C-H-Bond]	1
9	v.	Crotepoxide	GLN A:104 (3.17) [C-H-Bond], GLN A:110 (2.50)	2
10	w.	Pipoxide chlorohydrin	ALA A:868 (3.57) [C-H-Bond]	1

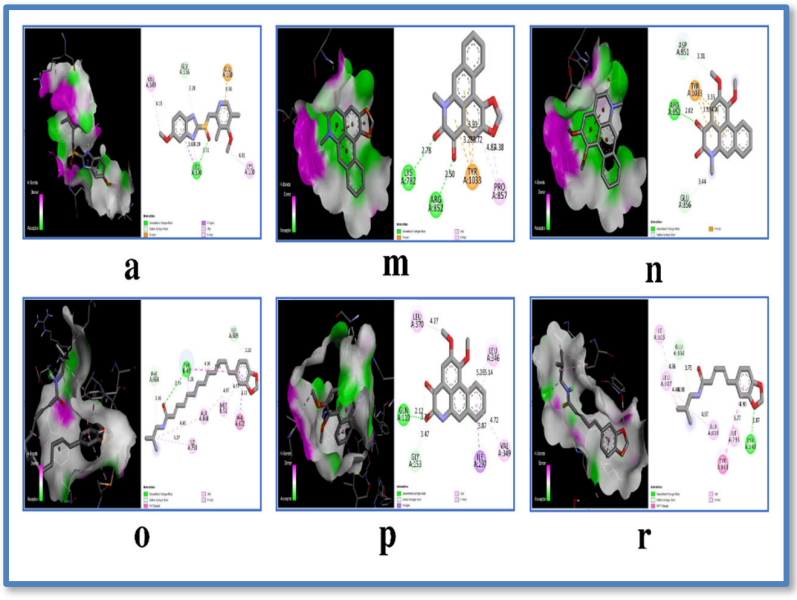


Fig. 4. Donor-acceptor interactions obtained by docking between phytoconstituents and Protein 2XZB.

3. Conclusion

Traditional herbs have long been thought of as a rich source of remedies for a variety of illnesses. For this study, we took into account a few phytochemicals that were taken from a particular plant, *Piper attenuatum*, which has a long history of use as a medicine. On the other hand, a crucial antiulcer medicine is a requirement. With the aid of this work, we have attempted to compile various computational methods used to target the phytochemicals of the plant *Piper attenuatum*. With the best binding affinity score of -8.4 kcal/mol and following the majority of the ADMET features, Cepharadione A, a component of *Piper attenuatum*, has pushed us to assume that it is a better component to be employed as a medication against antiulcer.

4. Materials and Methods

4.1. Plant Sample: Fresh leaves of *Piper attenuatum* were collected by Jawahar Lal Nehru Tropical Botanic Garden and Research Institute, Palode, India. Dr. Mathew Dan authenticated the plant sample. A voucher specimen was also submitted and preserved in the institute as herbarium Nos: LICP/2022/PCOG/22-23.

4.2. Chemicals: All the chemicals required to carry out research work were of standard quality and purchased from local market of Jaipur, Rajasthan, India. All the instruments used in research work were provided by Department of Pharmacy, Lords University, Chikani, Alwar, Rajasthan, India

4.3. Animals: For the Research work wistar rats in range of 170-200gm were used. All the rats were provided by Department of Pharmacy, Lords University, Chikani, Alwar, Rajasthan. They were housed under controlled condition according to OECD 423 guideline. The protocol was approved by IAEC of Faculty of Pharmacy, Lords University, Alwar having approval no 1386/PO/Re/S/10/CPCSEA.

4.4. Ethanolic extract preparation and % Yield determination: Authenticated leaves of *Piper attenuatum* were thoroughly washed with clean water and drying was done in oven at 40^0C so that the moisture content of leaves was reduced to 14% to minimize fungal infection in plant sample then the dried leaves was subject to grind and stored. Ethanolic extract was prepared by using Soxhlet extraction method. Total quantity of powdered drug was 270gm. First the powdered drug defatted with ether for 3 days by soxhlation then extraction was carried out with 99% ethanol for 2 days. Extract become dry when we use flash evaporator under (40^0C-50^0C). % Yield determination was carried out by using following formula.

$$\text{Yield (\%)} = \frac{W_2 - W_1}{W_0} \times 100$$

where

W_2 – Extract and container weight

W_1 – Weight of container

W_0 – Dried sample weight (Initial) [13]

4.5. Phytochemical Characterization: Ethanolic extract of leaves of plant *Piper attenuatum* was subjected to its phytochemical analysis for the presence of various phytochemicals like carbohydrate, alkaloids, amides, saponins, steroids, tannins, phenolic compounds, flavonoids and glycosides which may be responsible its pharmacological activity [19-21].

4.6. Isolation of bioactive compounds by Thin Layer Chromatography (TLC): Silica gel G plates 5cm × 10cm were used for sample application. Ethyl acetate and n-hexane (7:3v/v) were used as mobile phase. Chromatographic plates developed in glass chamber, and separate from chamber when the solvent moved to 15cm from actual exact position and let to dry. Then the plates were sprayed with vanillin Sulfuric acid spraying reagent for color reaction and allow drying. After drying, spots were visualized under visible (white), short UV (254 nm), and long UV (366 nm) light. The movement of each separating spot of the extract was denoted by its retention factor (Rf) value. Values were calculated for each spot using the following formula [22].

$$\text{RF} = \frac{\text{Distance traveled by solute from the point of application to the center of spot}}{\text{Distance traveled by solvent front}}$$

4.7. Isolation of bioactive compounds by Column Chromatography: Silica gel column was used to isolate bioactive compounds from ethanolic extract. Ethanolic extract poured in the silica gel column and eluted with increasing polarity using n-Hexane, Ethylacetate and Methanol as solvent system. Again, we perform TLC on the collected fractions to isolate single compound. Ethylacetate and methanol was used as solvent system in (10:1) ratio. Spots were visualized under visible light, UV light at a wavelength of 254nm and 366 nm depending on the nature of compounds separated [23].

4.8. Pharmacological screening of ethanolic extract

4.8.1. Aspirin induced gastric ulcer model in rats

Aspirin at a dose 200mg/kg by oral route causes ulcer in rats. Albino wistar rats weighing 170 gm -200 gm were used for the research work. All animals were grouped into 4 parts each having six rats. First group received vehicles only and were termed as control groups. Second group of animals was treated as a standard group as it received Ranitidine (20mg/kg), while third and fourth groups were treated as test groups as they received ethanolic extract at 100 mg/kg and 200mg/kg respectively. Treatment was given for 7 days. Aspirin at a dose 200mg/kg was given to treated groups after 7 days before 30 minutes of the last dose. Rats were sacrificed by anesthesia after 6 hr of aspirin dose. Rat stomachs were removed for determination of gastric lesion after washing with water. Other parameters like gastric juice volume, free acidity, total acidity and ulcer index were determined. pH of gastric juice was also measured.

Statistical analysis: ANOVA and multiple comparison tests were employed. Graph pad prism 9 was used to calculate probability (P) value to compare between groups. Probability value <0.05 was considered significant[24].

4.9. Drug-likeness properties and ADMET properties A set of rules and guidelines for determining the structural properties is preferred for initial screening of drug-likeness of compound

Some such Drug-likeness rules are available, for example, Lipinski's rule, MDDR-like rule, Veber's rule, Ghose filter, Egan rule, Muegge rule, Lipophilicity (iLOGP, WLOGP, XLOGP3, MLOGP, Log Po/w), water solubility etc [25]. Any chemical molecule may be utilized as an orally active drug if, and only if, it will not contravene Lipinski's rule (also known as Pfizer's rule or simply the rule of five (RO5))[26]. The chemicals that only follow RO5 will have a greater probability of being consumed orally by humans and making it to the marketplace. The aforementioned regulations initially justify whether or not the molecule is suitable for drug production. Some rules, such as the following ones, molecular weight < 500, hydrogen-bond donors < 5, hydrogen-bond acceptors < 10, MLOGP (n-octanol–water partition coefficient) < 4.15, molar refractivity should be between 40 and 130, log P ranging between − 0.4 to + 5.6, solubility (log S) > − 5.7, also assist us in determining the suitability of drug molecules[27]. These preclinical assays all aid in identifying drug-like and non-drug-like structural differences. With the help of the online tool SWISS-ADME (http://www.swissadme.ch/), all of these qualities are investigated. Our ability to analyze all physiochemical parameters, medicinal chemistry, drug-like qualities, pharmacokinetics, lipophilicity, etc. is made possible by this software. It is frequently seen in virtual drug-likeness screening that a number of approved medications do not totally adhere to all screening requirements, breaking any of the RO5 and other drug-likeness rules[28].

4.9.1. Potential target protein structure

Three-dimensional (3D) structure of Pig Gastric H+/K+ ATPase (PDB code 2XZB) was retrieved from protein data bank[29]. The docking results because the PDB structure (2XZB) used in the docking analysis was obtained from enzyme crystallized inhibitor of gastric H+/K+ ATPase having comparable activity as of omeprazole[30]. **Fig. 5.** Shows the 3D structure of protein obtained from BIOVIA discovery studio visualiser.

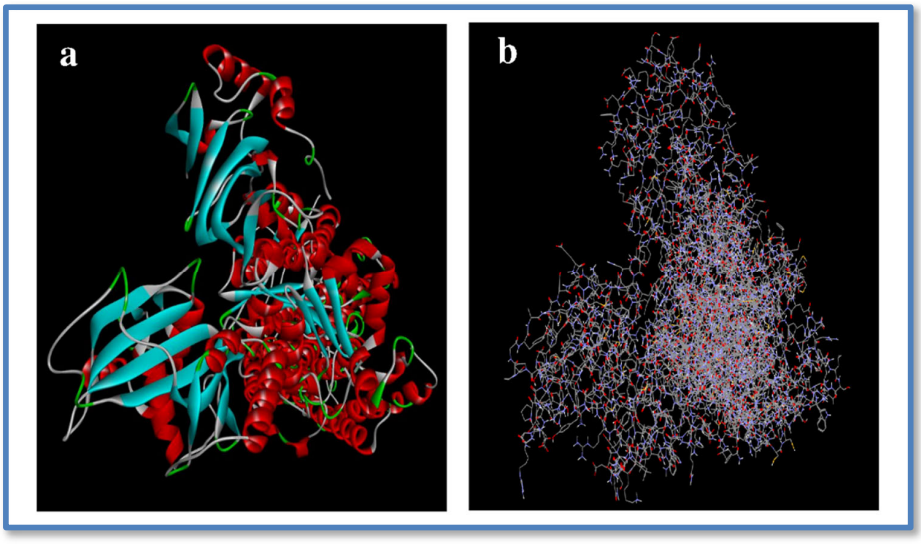


Fig. 5. (a) Protein 2XZB (b) protein after removing water, adding polar Hydrogen and kollman charge

4.9.2. Potential inhibitor

Piper attenuatum All of the chosen phytoconstituents' 3-D structures were obtained from PubChem and displayed in **Table 1** along with their PubChem ID.

4.10. Molecular docking and visualization

Molecular docking is regarded as the molecular modelling approach that is favoured in computer-assisted drug design for the prediction of the ligand-receptor interaction when both molecules are bonded to form a stable complex[31]. In this study, we carried out molecular docking using Pyrx software. The algorithm of Auto Dock Vina for docking of deals with configuration parameters like nine binding modes, exhaustiveness = 8, energy difference = 4 kcal/mol, grid box with center coordinates x = X 27.4518, y = 32.4287, z = 39.7209 of the position of the target protein is used to do the docking-based studies on the suggested. The created protein is stored in the pdbqt file format. Similar to how ligands are created, ligands are also created using Auto Dock Vina and saved in pdbqt format. These variables include dreiding energy, number of H-bonds, ligand dipole moment (in Debye), and binding affinity (G) (Kcal/mol). We used Pyrx, a different docking programme, to perform docking for the same protein-ligand combinations in order to validate our docking results[32]. Pyrx is available for download at https://sourceforge.net/projects/pyrx/files/latest/download.

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