Original Research Article

Anti-ulcer Activity of Ethanolic Extract of *Alstonia scholaris* Bark: An *In vivo* and *In silico* Approach

ABSTRACT

Aims: The present research is focused on screening *in vivo* anti-ulcer activity using pylorus ligation and ethanol induced ulcer model.

Study Design: Fourty eight rats, randomly divided into eight groups, were used in this study. Bark of *Alstonia scholaris* were air-dried, ground into fine powder and used in the preparation of an ethanolic extract.

Place and Duration of Study: Department of Pharmacology, Osmania University, Hyderabad between December 2020 and September 2021.

Methodology: Ethanol related ulcer was induced using 1 mL/kg b.w, *p.o.* Treated rats received ethanolic extract of *Alstonia scholaris* at various doses of 200 and 400 mg/kg b.w. Ulcer index, % ulcer protection were calculated and histological studies were conducted at 6 hr after pylorous ligation respectively.

Results: Pharmacological evaluations were done using 200 mg/kg, b.w. and 400 mg/kg, b.w. The total acidity and free acidity were decreased, pH was increased and ulcer index was decreased by Ethanolic extract of Alstonia scholaris (EEAS 200 and 400 mg/kg b.w., *p.o.*) in pylorus ligation model. Treatment with EEAS (200 and 400 mg/kg b.w. *p.o.*) hassignificantly decreased the ulcer index by ethanol induced ulcer model. To understand the ligand-binding affinity of the active constituents of the extract, docking studies were performed for natural compounds against protein data bank (PDB) ID: 5A5N, PDB ID: 6Q2T, PDB ID: 7MBX, PDB ID: 2FV5. The results revealed that vanillic acid, venoterpine, loganetin, dibutyl phthalate, guaia-3,9- diene, 3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c] pyrrole-1,4- dione, 2H-1-Benzopyran- 2-one,7-acetyl-8-[acetyloxy]-4, n-hexadecanoic acid, stigmasterol, diospyrolide, D- Friedoolean-14-en-3-one, betulin, lupeol acetate, pentanoic acid and standard drug omeprazole had shown highest glide scores with all the selected proteins which indicate a stronger receptor-ligand binding affinity.

Conclusion: From *in vivo* and *in silico* results it is evident that ethanolic bark extract of *Alstonia scholaris* possessed significant anti-ulcer activity.

KEYWORDS: *Alstonia scholaris*, Pylorus ligation, Ethanol, Ulcer index, Docking studies, ADME analysis, PASS (Prediction of Activity Spectra for Substances).

1. INTRODUCTION

The term peptic ulcer refers to acid peptic injury of the digestive tract, resulting in mucosal break reaching the sub mucosa. Traditionally, a hyper secretory acidic environment together with dietary factors or stress were thought to cause most peptic ulcer diseases, but the discovery of *Helicobacter pylori* infection and the widespread use of non- steroidal anti-inflammatory drugs (NSAIDs) in the second half of the 20th century have changed this perception. Lifetime prevalence of peptic ulcer disease in the general population has been estimated to be about 5−10%, and incidence 0·1–0·3% per year. *H. pylori* and consumption of non-steroidal anti-inflammatory drugs (NSAIDs) were common etiologic factors for Peptic ulcer disease (PUD). Stress, tobacco smoking, alcohol intake, Zollinger Ellison syndrome, and age-related decline in prostaglandin levels are also mentioned as risk factors for PUD [1]. Various classes synthetic antiulcer drugs like antacids, proton pump inhibitors, anti-cholinergic, H_2-receptor antagonists and cytoprotective agents are being used in clinical practices, but these entire drugs have been associated with undesirable side effects and drug-drug interaction. Therefore, search for an ideal antiulcer drug continues and has also been extended to herbal drugs for their easy availability, better protection, low cost and lesser toxicity [2].

Alstonia scholaris, commonly called blackboard tree or devil's tree in English, is an evergreen tropical tree in the family *Apocynaceae*. It is native to southern China, tropical Asia and Australia; it is a commonly planted ornamental plant in these areas. It is a toxic plant, but traditionally it is used medicinally for myriad diseases and complaints [3]. The plant is traditionally found to be useful for many ailments like anti-tuberculosis, antibacterial, anticancer, anti-tussive, anti-asthmatic, expectorant, bronchovasodilatory, anti- inflammatory, analgesic, wound healing, anti-diabetic, anti-hyperlipidemic, anti-hypertension, anti-anxiety, antimalarial, hepatoprotective, antidiarrheal and spasmolytic activities etc., [4]. The present study aimed to evaluate the anti-ulcer activity of the ethanolic bark extract of *Alstonia Scholaris* on pylorus ligation and ethanol induced ulcer models and an attempt is made to establish the *in silico* studies of the active constituents of the extract using schrodinger, molinspiration and PASS software.

2. MATERIALS AND METHODS:

2.1 Plant collection and drying

Bark of *Alstonia scholaris* were identified, collected, authenticated by botanist P. Suresh babu, Government degree college, Kukatpally, Medchal district. *Alstonia scholaris* bark were cleaned and dried under shade for about six days and powdered. The powdered material was stored.

2.2 Preparation of ethanolic bark extract of *Alstonia scholaris* (Soxhlet)

The powdered material of *Alstonia scholaris* bark were dried and extracted with ethanol by soxhlation technique [5].

2.3 Preliminary phytochemical analysis of the extract

The extract was subjected to preliminary phytochemical investigations to identify various phytoconstituents present in the ethanolic extract of *Alstonia scholaris* bark.

2.4 Acute toxicity testing

The acute toxicity studies were carried out using OECD 425 guidelines. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India. (Reg.No. 1175/PO/ERe/S/08/CPCSEA).

2.5 Animal housing

The animals (rats and mice) were housed in poly acrylic cages with not more than six animals per cage, with 12 h light/12 h dark cycle. Animals have free access to standard diet and drinking water ad libitum. The animals were allowed to acclimatize the laboratory environment for a week before the start of the experiment. The care and maintenance of the animals were carried out as per the approved guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

2.6 *In vivo* methods for evaluation of anti-ulcer activity

In vivo evaluation of anti-ulcer activity of the ethanolic bark extract of *Alstonia scholaris* was carried out using the following models.

A. Pylorus ligation induced ulcer model.

B. Ethanol induced ulcer model.

2.6.1 Pylorus ligation induced ulcer model:

In the experimental models, male *albino* rats were selected and divided into four groups of six animals each. Animals were fasted for 24 hour before the study, but had free access to water.

Group I received (control) normal saline.

Group II received test drug EEAS at dose of 200 mg/kg b.w., *p.o.*

Group III received test drug EEAS at dose of 400 mg/kg b.w., *p.o.*

Group IV received Omeprazole 20 mg/kg b.w., *p.o.*

Rats were fasted for 18h and the pylorus was ligated under light thiopental sodium anesthesia with care taken not to cause bleeding or to occlude blood vessels. Six hours after ligation, the animals were sacrificed by an overdose of thiopental sodium, and the stomach part was isolated, contents were collected, measured for volume, and subjected to analyze the acidity against 0.1 N NaOH to pH 8 using a pH meter. The total acid output was calculated. Each stomach was examined for lesions. The animals after the treatment were subjected to histopathological studies.

The numbers of ulcers were counted and scoring of ulcer was made as follows: Normal colored stomach (0), Red coloration (0.5), Spot ulcer (1), Hemorrhagic streak (1.5), Deep ulcers (2) and Perforation (3) [6]

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ inhibition of ulceration} = \frac{(UI_{control}) - (UI_{test})}{UI_{control}} \times 100$$

2.6.1.1 Determination of pH

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water, and pH of the solution was measured using pH meter.

2.6.1.2 Determination of total acidity

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water and was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was observed. The volume of 0.01N NaOH consumed was noted.

The total acidity was expressed as mEq/L and calculated by the following formula:

$$\text{Acidity} = \frac{V_{NaOH} \times N \times 100\text{mEq/L}}{0.1}$$

Where V is volume and N is normality

2.6.1.3 Determination of free acidity instead of phenopthalein indicator, the topffer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity. [5]

2.6.2 Ethanol induced ulcer model:

In the experimental models, male *albino* rats were selected and divided into four groups of six animals each. Animals were fasted for 24 hour before the study, but had free access to water.

Group I received (control) + absolute ethanol (1 mL/kg b.w., *p.o*)
Group II received EEAS of 200 mg/kg, *p.o.* for 7 days+ absolute ethanol (1mL/kg b.w., *p.o*)
Group III received EEAS of 400 mg/kg, *p.o.* for 7 days + absolute ethanol (1mL/kg b.w., *p.o*)
Group IV received Omeprazole (20 mg/kg, *p.o.)* + absolute ethanol (1 mL/kg b.w., *p.o*)

On 7th day after 1 hour of administration of EEAS, Omeprazole and control treatment, 1ml of absolute ethanol (1 ml/200g) was administered *orally* to all the animals. After 1 hour, the animals were sacrificed with excess of anesthetics isoflurane and stomach was opened along the greater curvature, cleared of residual matter with saline and the inner surface was examined for severity of ulceration. Ulcer index and % ulcer protection were calculated by using the above formula [5].

2.7 Histopathology studies:

The histopathology studies are carried out in pylorus ligation induced ulcer model. The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the anti-ulcer activity of *Alstonia scholaris*. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-µm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for morphological changes such as inflammation, haemorrhage, and erythema using an arbitrary scale for the assessment of severity of these changes [6].

2.8 *INSILICO ANALYSIS*

2.8.1 Structure based drug design

Initially the protein downloaded from PDB was prepared by removing chain B. Water molecules present in both the chains are removed. Energy minimization was done. Later molecules drawn using chemdraw were converted to mol format and ligprep was created. Grid generation was done by removing crystal ligand and the structures were docked against protein 5A5N, 6Q2T, 7MBX and 2FV5.

2.8.2 Schrodinger XP-docking results

XP docking indicates that some of our compounds have good binding ability with Proton pump inhibitor (PDB ID: 5A5N), Cyt C inhibitor (PDB ID: 6Q2T), Gastrin promoters (PDB ID: 7MBX) and TNF alpha inhibitors (PDB ID: 2FV5).

2.8.3 Ramachandran plot Ramachandran plot has been generated from PROCHECK validation server which was used to access the quality of the model by looking into the allowed and disallowed regions of the plot [7].

2.9 *In silico* ADME study using molinspiration

The ADME properties of selected active constituents of *Alstonia scholaris* were evaluated using the tool Molinspiration Cheminformatics server (http://www.molinspiration.com). There are several pharmacokinetic parameters and physicochemical descriptors which were evaluated for herbal extracts through application of the tool Molinspiration. These properties are mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and of course presence of various pharmacophoric features that influence the behaviour of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others. The Lipinski rule of five deals four simple physicochemical parameter ranges (MWT $\leq$ 500, log P $\leq$ 5, H-bond donor's $\leq$ 5, H bond acceptors $\leq$ 10) [7].

3.0 Bioactivity score using molinspiration

The bioactivity score of selected active constituents were also evaluated using the tool Molinspiration Cheminformatics server (http://www.molinspiration.com). In this computational chemistry technique large chemical databases are analyzed in order to identify possible new drug candidates. Only SMILES or SD file structures of active molecules are sufficient for the training, no information about the active site or binding mode is necessary. This is particularly useful in projects where structure-based approach cannot be applied because information about 3D

receptor structure is not available [7].

3.1 Pass software:

3.1.1 Input and Output of PASS.

PASS uses as input data a MOL- or SD-file23 representing the structural information about the molecules under study. On the basis of these data, MNA descriptors (Multilevel Neighborhoods of Atoms) are generated automatically. Based on the statistics of MNA descriptors for active and inactive compounds from the training set, two probabilities are calculated for each activity: Pa - the probability of the compound being active and Pi - the probability of being inactive. Being probabilities, the Pa and Pi values vary from 0.000 to 1.000 (with three relevant decimals being calculated), and in general 24 Pa + Pi < 1, since these probabilities are calculated independently. Pa and Pi can be considered to be measures of the compound under study belonging to the classes of active and inactive compounds, respectively, or can be seen as estimates for the first and second kinds of errors in the prediction. All MNA descriptors influence the estimates in the activity prediction. Their influence can be either positive (if the descriptors are found in compounds with the particular activity), or negative (if the descriptors are found in compounds without the particular activity), or even neutral (if the descriptors are found in both active and inactive compounds). In the last case, they decrease the relative impact of the “positive” and “negative” descriptors.

3.1.2 Interpretation of Predictions.

The PASS predictions can be interpreted, and used, in a flexible manner. The most probable activities, for a given compound, are characterized by Pa values close to 1, and Pi values close to 0. Let us first consider cases where the Pa value is high and is much larger than Pi. If a statistically significant set of samples with predictions obtained with the threshold Pa > 0.9 is selected from a much larger database and assayed, one has to expect to lose 90% of the active compounds, but the fraction of false-positives will be very small. For a cutoff of Pa > 0.8, only 80% of the actives will be lost, but the fraction of false positives will be a little bit higher. Finally, if one goes down to the criteria Pa > Pi, the probability of the first kind of error equals the probability of the second kind of error, i.e., one is just as likely to miss true actives as to find false positives.

However, maximizing Pa values for the desired activity is not the only criteria for selection of the most promising compounds. Another aspect might be the novelty of a compound. If Pa is very high, the compound might be a close analogue of known pharmaceutical agents. Thus, if one is interested in finding new leads, especially New Chemical Entities (NCE), one may want to choose compounds

for which the specified activity is predicted with lower probability, say, 0.5 < Pa < 0.7. In this case, the probability of false positives is likely to be higher, but if the activity will be confirmed in the experiment, one has a higher chance of having obtained an NCE [8,9,10].

3.2 Statistical analysis

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control and standard by using Dunnett's test. Significant values are expressed as control group (*=p<0.001, a=p<0.0001 b = p<0.001), and standard (A=p< 0.0001, ns- nonsignificant.

4. RESULTS:

Ethanolic bark extract of *Alstonia scholaris* was explored for its *in vivo* anti-ulcer activity using Pylorus ligation model and Ethanol induced model. All the results obtained in the study were included below.

4.1 Preparation of ethanolic bark extract of *Alstonia scholaris*

The ethanolic bark extract of *Alstonia scholaris* was prepared by soxhlation technique. The percentage yield of ethanolic extract was calculated by using the following formula.

% of yield obtained=Amount of extract obtained (gm)/ Total amount powder used × 100

= 60/310 X 100 = **19.35% w/w.**

4.2 Preliminary phytochemical analysis:

The preliminary phytochemical investigation for ethanolic extract of *Alstonia scholaris* bark showed the presence of flavonoids, tannins, steroids, glycosides, alkaloids, saponins, and terpenoids.

4.3 Acute toxicity studies

Ethanolic bark extract of *Alstonia scholaris* was tested on Swiss albino mice up to a dose of 2000 mg/kg bd. wt. The animals did not exhibit any signs of toxicity or mortality up to 2000 mg/kg bd. wt. various morphological and behavioral characters were observed during the study. Hence the extract was found to be safe up to 2000 mg/kg bd. wt.

4.4 Dose selection

From toxicity studies, a dose of 2000 mg/kg bd. wt. was identified to be safe, and the working dose was considered as $1/10^{th}$ i.e., 200 mg/kg, bd. wt. In the present study pharmacological evaluations were done using 200 mg/kg. bd. wt. and 400 mg/kg, bd. wt.

4.5 *In vivo* anti-ulcer activity

The ethanolic bark extract of *Alstonia scholaris* was screened for its anti-ulcer activity using the following models.

4.5.1 Pylorus ligation induced Ulcer model:

Table 1: Effect of EEAS on pylorus ligation induced ulcer model:

Groups	Treatment	Ulcer index	% ulcer protection
I	Control	14.24±0.08	-
II	EEAS 200 mg/kg	11.36±0.04^{*A}	20.22%
III	EEAS 400 mg/kg	9.50±0.14^{*A}	33.28%
IV	Omeprazole 20 mg/kg	5.81±0.01*	59.26%

Values are expressed as Mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as when compared to control group (* = $p<0.001$), and when compared to standard group (A= $p<0.001$).

4.5.1.2 Histopathology studies of pylorus ligation induced gastric ulcerated rat stomach:

The stomach samples were preserved in formaldehyde solution. Later they were washed and thin longitudinal sections were cut and observed under microscope.

Pylorus-ligation causes gastric hyper secretion due to poorly understood mechanisms. The activation of the vagal reflex by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric tonus and secretion Ethanol induced ulcer: Administration of ethanol causes gastric necrotic damage and subsequent inflammatory cell infiltration and reduces the secretion of bicarbonate, gastric mucus, and nitric oxide. In addition, ethanol reduces the gastric blood flow and induces the oxidative stress by increasing the production of malondialdehyde and reducing glutathione production [11]. The various phytochemical active constituents identified in the ethanolic bark extract of *Alstonia scholaris* were saponins, steroids, alkaloids, flavonoids, phenols, fatty acids, carbohydrates, triterpenoids and tannins. Lupeol acetate (Triterpenoid) with its free radical- scavenging property may have prevented in part the GSH depletion thereby affording gastro protection from ethanol damage [12]. Betulinic acid (Triterpenoid) has been reported to have antiulcer activity in several experimental models by the formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting prostaglandin [13]. Vanillic acid (phenol) produced a reduction in the histamine secreting mast cells in ethanol-induced peptic ulcer by

reduction of mast cell degranulation [11]. Vanillic acid (phenol) shows inhibitory action on histamine release which is substantiated by our results of a reduction in the histamine secreting mast cells [14]. Palmitic acid (fatty acids) involved in anti- inflammatory cellular processes by inhibiting proinflammatory cytokines (TNF alpha and IL-6) as well as COX-1 and COX-2. This is able to enhance mucus secretion and reduce Hcl production by the cells of the gastric mucosa [15]. From the above it is clear that terpenoids, phenols, fatty acids present in the extract might be responsible for anti-ulcer activity

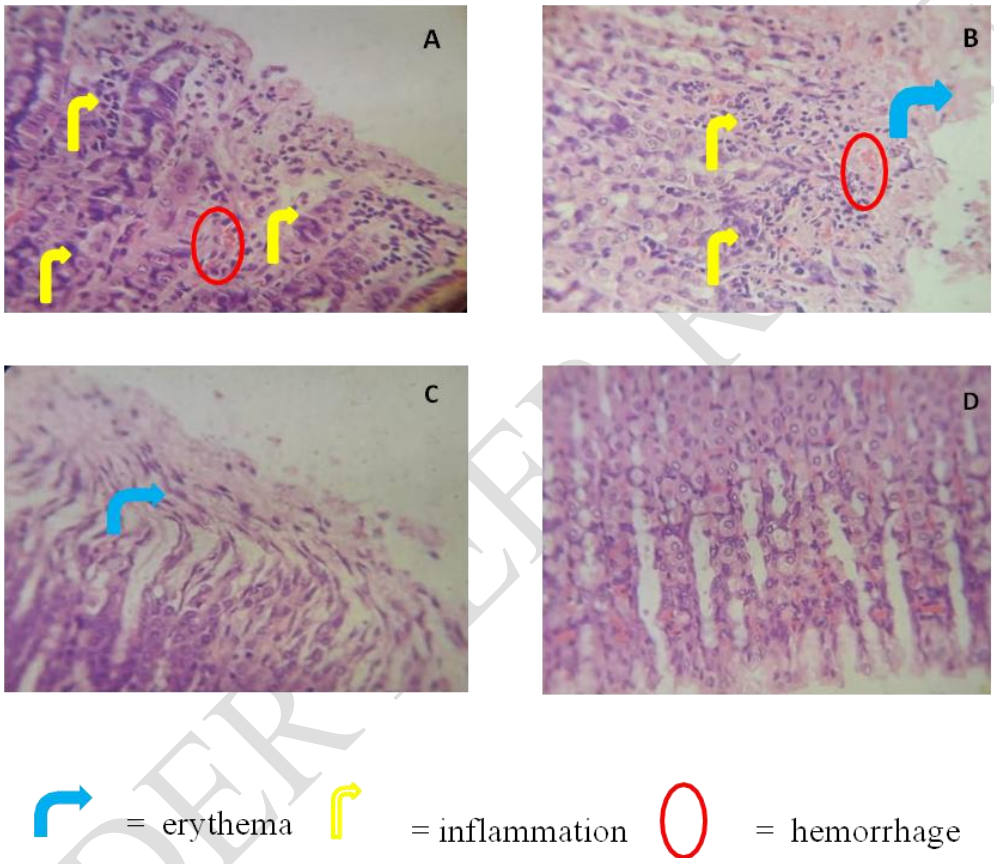


Figure 1: Histopathology of rat's stomach in pylorus induced ulcer model

Group I: Section of gastric mucosa layer shows mucosal ulceration and inflammation was observed. Group II: Inflammation and moderate erythema was observed. Group III: Mild Erythema with no inflammation was observed. Group IV: No significant changes were observed in the section.

4.5.2 Ulcer healing study

Table: 2 Effect of EEAS on gastric contents

Groups	Treatment	pH	Total acidity	Free acidity

I	Control	2.06 ±0.03	67 ±0.5	94.66±0.55
II	EEAS (200 mg/kg)	2.33± 0.03bA	57 ±0.32^{aA}	63.66±0.49^{aA}
III	EEAS (400 mg/kg)	4.83±0.07aA	55.22± 0.16^{aA}	53±0.36^{aA}
IV	Omeprazole 20 mg/kg	5.76±0.02a	50.33± 0.42^{a}	40.16±0.47^{a}

Values are expressed as Mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as when compared to control group (* = p<0.0001), when compared to disease group (a = p<0.0001, b = p<0.001) and when compared to standard group (A = p< 0.0001).

Table: 3 Effects of EEAS on ethanol induced gastric ulcer model

Groups	**Treatment**	**Ulcer index**	**% ulcer protection**
I	Disease control	10.70±0.02	-
II	EEAS (200mg/kg, bd.wt, *p.o.*)	8.96±009*^{A}	16.26%
III	EEAS (400 mg/kg bd.wt., *p.o.*)	5.84±0.02*^{A}	45.42%
IV	Omeprazole (20 mg/kg)	4.81±0.02*	55.04%

Values are expressed as Mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as when compared to control group (* = p< 0.0001), and when compared to standard group (A= p< 0.0001).

4.3 *In silico* analysis

Table 4: Schrodinger XP Docking Scores

Compounds	**5A5N**	**6Q2T**	**7MBX**	**2FV5**
Vanillic acid	-7.42	-6.15	-4.81	-5.46
Venoterpine	-6.58	-6.69	-4.49	-8.32
Loganetin	-6.22	-7.00	-4.13	-7.44
Dibutyl phthalate	-6.00	-	-4.63	-4.29
Guaia-3,9-diene	-5.40	-7.37	-4.82	-7.54

3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	-5.09	-7.80	-5.53	-5.83
2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-methyl-	-4.84	-	-5.20	-5.99
Stigmasterol	-4.46	-5.44	-4.80	-4.80
Diospyrolide	-4.08	-5.10	-4.70	-4.71
D-Friedoolean-14-en-3-one	-3.86	-4.55	-4.58	-4.63
Lupeol acetate	-3.82	-3.93	-3.01	-4.35
Pentanoic acid	-3.72	-4.86	-4.54	-4.14
n-Hexadecanoic acid	-	-	-2.35	-5.28
Betulin	-3.37	-4.87	-4.43	-3.54
Omeprazole	-6.79	-7.76	-5.14	-7.96

G score = glide score, the more negative the Glide score, the more favorable the binding.

Molecular docking continues to holds great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. The docking analysis of isolated compounds from ethanolic bark extract of *Alstonia scholaris* and standard drug Omeprazole was carried out using Schrödinger software. The various constituents identified in the plant extract are Vanillic acid, Venoterpine, Loganetin, Dibutyl phthalate,Guaia-3,9-diene, 3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4- dione, 2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-methyl-, Stigmasterol, Diospyrolide, D-Friedoolean-14-en-3-one, Lupeol acetate, Pentanoic acid, n-Hexadecanoic acid, betulin, and standard drug

Omeprazole was subjected to docking against PDB ID: 5A5N, 6Q2T, 7MBX and 2FV5. The highest glide scores were observed with Vanillic acid, Venoterpine, Loganetin, Dibutyl phthalate, Guaia-3,9-diene, 3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione, 2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-methyl and Omeprazole against all the selected PDB ID: 5A5N, 6Q2T, 7MBX and 2FV5. The glide scores of the venoterpine, vanillic acid, 3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4- dione were found to be more than the glide score of standard drug Omeprazole stating that the compounds might have same affinity to bind to the proteins. These results clearly indicate that the chemical constituents mentioned above might have shown similar mechanism to that of the standard drug Omeprazole as anti-ulcer activity.

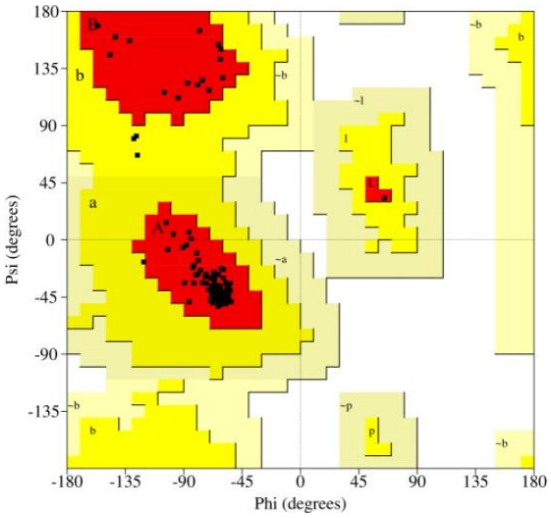
The proteins identified namely PDB ID: 5A5N, 6Q2T, 7MBX and 2FV5 are modeled and the qualities of the 3D model were evaluated using the PROCHECK program and assessed using the Ramachandran plot. It is evident from the Ramachandran plot that predicted models have most favorable regions, additionally allowed regions, generally allowed regions and disallowed regions. Such a percentage distribution of the protein residues determined by Ramachandran plot shows that the predicted models are of good quality. According to Ramachandran plot a good quality model would be expected to have over greater than 90% in the most favored region. Proteins like PDB ID: 5A5N, 6Q2T, 7MBX and 2FV5 showed greater than 90% favored a region which clearly indicates that the selected models in the present study are of good quality.

4.4 Ramachandran plot Analysis

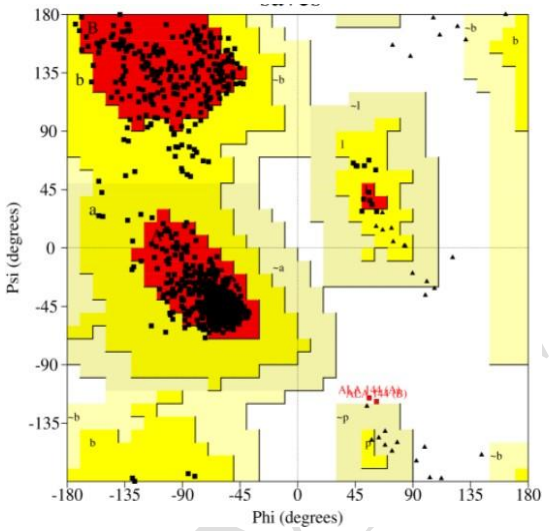
Protein **5A5N, 6Q2T, 7MBX** and **2FV5** were analyzed for Ramachandran plot to know aminoacid presence in different regions of respective protein tabulated in table 5 and pictorial representation in figure 2.

Table 5: Ramachandran plot status with protein with 5A5N, 6Q2T, 7MBX and 2FV5

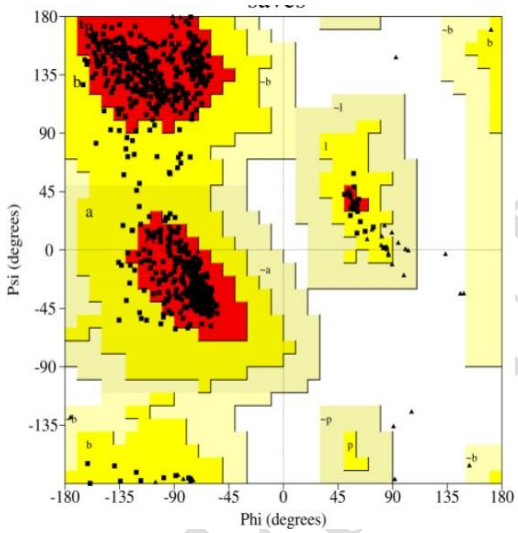
Residues	5A5N	6Q2T	7MBX	2FV5
Most favorable region (%)	96.7	90.9	92.7	87.1
Additional allowed regions (%)	3.3	8.9	7.3	11.8
Generously allowed regions (%)	0.0	0.0	0.0	0.7
Disallowed regions (%)	0.0	0.3	0.0	0.4



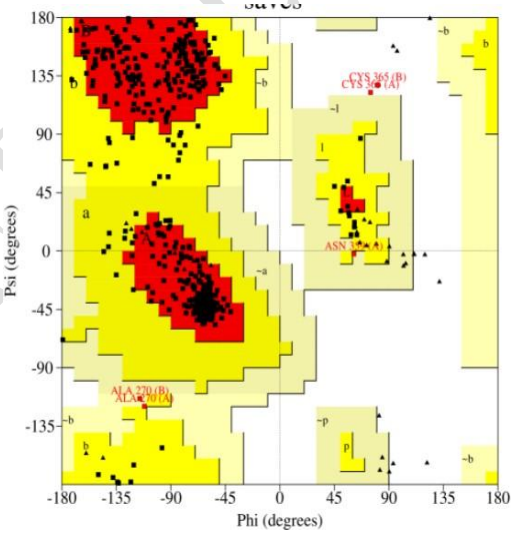
a) PDB 5A5N



b) PDB 6Q2T



C) PDB 7MBX



d) PDB 2FV5

Figure 2: Ramachandran plot of protein 5A5N, 6Q2T, 7MBX and 2FV5

Table 6: ADME properties of compounds from *Alstonia Scholaris* by molinspiration

S.No	Compound	MW	nON	nOH	nV	nrotb	TPSA	miLogP
1	Vanillic acid	168.15	4	2	0	2	66.76	1.19
2	Venoterpine	149.19	2	1	0	0	33.12	0.53
3	Loganetin	228.24	5	2	0	2	76.00	0.45

4	Dibutyl phthalate	278.35	4	0	0	10	52.61	4.43
5	Guaia-3,9-diene	204.36	0	0	1	1	0.00	5.64
6	3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	316.36	4	2	0	2	65.98	4.25
7	2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-	260.25	5	0	0	3	73.59	1.75

	methyl-							
8	Stigmasterol	412.70	1	1	1	5	20.23	7.87
9	Diospyrolide	414.63	3	1	0	0	46.53	4.54
10	D-Friedoolean-14-en-3-one	424.71	1	0	1	0	17.07	7.84
11	Lupeol acetate	468.77	2	0	1	3	26.30	8.71
12	Pentanoic acid	102.13	2	1	0	3	37.30	1.50
13	n-Hexadecanoic acid	256.43	2	1	1	14	37.30	7.06
14	Betulin	442.73	2	2	1	2	40.46	7.16
15	Omeprazole	345.42	6	1	0	5	77.11	2.41

MW = Molecular weight, nON = number of hydrogen bond acceptors, nOH = number of hydrogen bond donors, nV = number of violations of Lipinski's rule of five, nrotb = number of rotatable bonds, TPSA = Total Polar Surface Area and miLogP = Octanol-water partition coefficient logP.

Molinspiration molecular properties were calculated on the bases of Lipinski's rule and its components. Lipinski's rule of five is to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it an orally active drug in humans. In the present study, all the compounds that are docked have lower molecular weight so that they are easily absorbed, diffused and transported. The selected active constituents like Vanillic acid, Venoterpine, Loganetin, Dibutyl phthalate, 3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione, 2H-1- Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-methyl-, Diospyrolide, Pentanoic acid, and standard drug omeprazole has zero violations except Guaia-3,9-diene, Lupeol acetate, n-Hexadecanoic acid, betulin, D-Friedoolean- 14-en-3-one, and Stigmasterol which have one

violation out of five. Any compound with zero violation clearly indicates the probability of its higher oral bioavailability. Topological polar surface area (TPSA) allows the prediction of transport properties of drug candidates in the intestines and blood-brain barrier. The TPSA score in all the selected active constituents of the extract and standard drug omeprazole was found to be less than 140 which clearly indicated better permeability into the tissues. Molinspiration ADME enables the computation of key physicochemical, pharmacokinetic, drug-like and related parameters for one or multiple molecules. Number of H-bond acceptors should be in a range of 0-10 and number of H-bond donors should be 0-5. All the selected active constituents in the present study were found to be within the range. A negative value for ilogP means the compound has a higher affinity for the aqueous phase (it is more hydrophilic); when ilogP equals 0 the compound is equally partitioned between the lipid and aqueous phases; a positive value for ilogP denotes a higher concentration in the lipid phase (i.e., the compound is more lipophilic). In the present study almost all the active constituents and standard omeprazole have shown a positive ilogP value clearly indicating a higher concentration in the lipid phase. [16]

Table 7: Bioactive score of compounds from *Alstonia scholaris* by molinspiration

S.NO	Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	Vanillic acid	-0.85	-0.42	-0.99	-0.61	-1.12	-0.35
2	Venoterpine	-0.43	0.14	-0.57	-0.63	-0.28	0.18

3	Loganetin	0.09	0.03	-0.86	0.03	-0.15	0.28
4	Dibutyl phthalate	-0.16	-0.09	-0.27	-0.12	-0.25	-0.07
5	Guaia-3,9-diene	-0.65	-0.27	-0.97	-0.24	-0.57	-0.17
6	3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	-0.04	-0.07	0.33	-0.04	-0.21	0.10
7	2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-methyl-	-0.79	-0.69	-0.85	-0.33	-0.73	-0.16
8	Stigmasterol	0.12	-0.08	-0.48	0.74	-0.02	0.53
9	Diospyrolide	0.22	0.10	-0.33	0.63	0.15	0.51
10	D-Friedoolean-14-en-3-one	0.07	-0.10	-0.40	0.43	-0.14	0.37
11	Lupeol acetate	0.16	0.08	-0.51	0.72	0.10	0.44
12	Pentanoic acid	-3.06	-3.15	-3.68	-2.91	-3.05	-2.84
13	n-Hexadecanoic acid	0.02	0.06	-0.33	0.08	-0.04	0.18
14	Betulin	0.21	-0.04	-0.41	0.85	0.09	0.51
15	Omeprazole	0.24	-0.23	0.08	-0.21	-0.23	0.43

A few compounds of *Alstonia scholaris* were subjected to bioactivity score using mol inspiration. The scores for the selected compounds can be interpreted as Active (bioactivity score > 0), moderately active (bioactivity score: -5.0-0.0) and inactive (bioactivity score < -5.0). Out of all, the compounds like Loganetin, Diospyrolide, Lupeol acetate, n-Hexadecanoic acid, Betulin was active against all the receptors and enzyme inhibitors. The result of bioactivity score of GPCR ligand, ion channel modulator, and nuclear receptor ligand, inhibitor activities towards kinase, protease and enzymes indicated that the compounds exhibit active to moderate score towards all the receptors.

Table 8: Anti-ulcer activity predicted for the active constituents of *Alstonia scholaris* using PASS

Sl.No	Compound	Probable Activity (Pa)	Probable Activity (Pi)	Biological Activity
1	Vanillic acid	0.937	0.003	Aldehyde oxidase inhibitor
		0.720	0.002	Anti inflammatory, intestinal
		0,709	0,005	Cytoprotectant
		0,715	0,004	H+-exporting ATPase inhibitor
		0,078	0,025	Mucomembranous protector
2	Venoterpine	0,451	0,091	Oxidoreductase inhibitor
		0,397	0,097	Anti inflammatory
		0,435	0,157	Mucomembranous protector
3	Loganetin	0,822	0,005	Anti inflammatory
4	Dibutyl phthalate	0,711	0,052	Mucomembranous protector
5	Guaia-3,9-diene	-	-	-
6	3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	0,379	0,184	Mucomembranous protector
7	2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-methyl	0,489	0,078	Oxidoreductase inhibitor
		0,460	0,069	Anti inflammatory
		0,311	0,075	H+-transporting two-sector ATPase inhibitor
		0,388	0,179	Mucomembranous protector
8	Stigmasterol	0,933	0,001	Oxidoreductase inhibitor
		0,763	0,030	Mucomembranous protector
9	Diospyrolide	0,781	0,008	Oxidoreductase inhibitor
		0,640	0,024	Anti inflammatory
10	D-Friedoolean-14-en-3-one	0,849	0,009	Mucomembranous protector

		0,842	0,005	Anti inflammatory
		0,089	0,006	Oxidoreductase inhibitor
11	Lupeol acetate	0,737	0,012	Antiinflammatory
		0,895	0,005	Mucomembranous protector
12	Pentanoic acid	0,727	0,002	Anti inflammatory, intestinal
		0,933	0,004	Mucomembranous protector
13	n-Hexadecanoic acid	0,789	0,007	Oxidoreductase inhibitor
		0,727	0,002	Anti inflammatory, intestinal
14	Betulin	0,745	0,010	Oxidoreductase inhibitor
		0,700	0,057	Mucomembranous protector
		0,629	0,026	Anti inflammatory
15	Omeprazole	0,849	0.009	Mucomembranous protector
		0,969	0,001	Gastric antisecretory
		0,922	0,000	H+/K+-transporting ATPase inhibitor

Table 9: Adverse effects predicted for the active constituents of *Alstonia scholaris* using PASS (Prediction of Activity Spectra for Substances)

S. No	Compound	Pa	Pi	Adverse effect
1	Vanillic acid	0.424	0.241	Hepatotoxicity
		0.329	0.129	Nephrotoxicity
		0.303	0.300	Arrhythmia
		0.272	0.301	Cardiac failure
2	Venoterpine	0.338	0.138	Cardiac failure
		0.315	0.281	Arrhythmia
3	Loganetin	0.379	0.097	Nephrotoxicity
4	Dibutyl phthalate	0.418	0.064	Myocardial infarction
		0.363	0.219	Arrhythmia

		0.329	0.129	Nephrotoxicity
5	Guaia-3,9-diene	0.430	0.237	Hepatotoxicity
6	3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	0.482	0.205	Hepatotoxicity
7	2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4- methyl	0.558	0.032	Cardiac failure
		0.494	0.198	Hepatotoxicity
		0.384	0.198	Arrhythmia
8	Stigmasterol	-	-	No Adverse effects predicted.
9	Diospyrolide	-	-	No Adverse effects predicted
10	D-Friedoolean-14-en-3-one	-	-	No Adverse effects predicted
11	Lupeol acetate	-	-	No Adverse effects predicted
12	Pentanoic acid	0.500	0.050	Nephrotoxicity
		0.474	0.029	Hepatotoxicity
13	n-Hexadecanoic acid	0.500	0.050	Nephrotoxicity
		0.474	0.209	Hepatotoxicity
14	Betulin	-	-	No Adverse effects predicted
15	Omeprazole	-	-	No Adverse effects predicted

Table 10: Direct and possible target Prediction for the active constituents of *Alstonia scholaris* using PASS

S. No	Compound	Direct Target	Confidence	Possible Target	Confidence
1	Vanillic acid	Prostanoid EP4 receptor	0.1110	Cyclooxygenase-1	0.2499
		Prostanoid EP2 receptor	0.633	Phospholipase C-gamma-2	0.1814
		Cyclooxygenase-2	0.628	Histamine H2 receptor	0.1568
		Prostanoid EP1 receptor	0.0413	Prostanoid EP2 receptor	0.1018
		Cyclooxygenase-	0.284	Prostaglandin E	0.0241

		1		synthase	
2	Venoterpine	Prostanoid IP receptor	0.3210	Prostanoid EP2 receptor	0.0902
		Prostanoid DP receptor	0.0111	Prostanoid FP receptor	0.0645
		Prostanoid EP4 receptor	0.0102	Prostanoid IP receptor	0.0138
3	Loganetin	Prostanoid IP receptor	0.0296	Prostanoid EP2 receptor	0.0556
				Prostanoid FP receptor	0.0507
4	Dibutyl phthalate	Prostanoid EP4 receptor	0.1012	Histamine H2 receptor	0.1547
		Prostanoid IP receptor	0.0733	Prostaglandin E synthase	0.0499
		Prostaglandin E synthase	0.0024		
5	Guaia-3,9-diene	-	-	-	-
6	3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4- c]pyrrole-1,4-dione	-	-	Prostaglandin E synthase	0.0348
7	2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-methyl	-	-	Cyclooxygenase-1	0.0111
				Cyclooxygenase-2	0.0015
8	Stigmasterol	Prostanoid IP receptor	0.1407	-	-
9	Diospyrolide	Prostanoid IP receptor	0.0888	-	-
10	D-Friedoolean-14-en-3-one	Prostanoid IP receptor	0.0517	Cyclooxygenase-2	0.0032
11	Lupeol acetate	Prostanoid IP receptor	0.1050	-	-
12	Pentanoic acid	Prostanoid IP receptor	0.3372	Prostanoid EP2 receptor	0.2239
		Prostanoid EP2 receptor	0.1355	Cyclooxygenase-1	0.1303
		Prostanoid EP4 receptor	0.1349	Histamine H2 receptor	0.1167
		Prostaglandin E synthase	0.1249	Prostanoid FP receptor	0.0672

		Prostanoid DP receptor	0.1185	Prostanoid DP receptor	0.0377
		Cyclooxygenase-1	0.0779	Prostanoid EP4 receptor	0.0331
		Carboxylesterase 2	0.0654	Prostanoid IP receptor	0.0262
		Prostanoid EP3 receptor	0.0038	Prostanoid EP3 receptor	0.0200
		Prostanoid EP1 receptor	0.0026		
13	n-Hexadecanoic acid	Prostanoid EP2 receptor	0.1355	Prostanoid EP2 receptor	0.2239
				Cyclooxygenase-1	0.1303
		Prostanoid EP4 receptor	0.1349	Histamine H2 receptor	0.1161
		Prostaglandin E synthase	0.1249	Prostaglandin E synthase	0.0706
		Prostanoid DP receptor	0.1185	Prostanoid FP receptor	0.0672
		Cyclooxygenase-1	0.0779	Prostanoid DP receptor	0.0377
14	Betulin	Prostanoid IP receptor	0.1391	-	-
15	Omeprazole	-	-	Potassium-transporting ATPase	0.7336
				ATP-binding cassette sub-family G member 2	0.1774

Prediction of activity spectra of substances (PASS) is a valuable interface that should be adopted as an archetypal tool for predicting the potential molecules and to predict the biological activity of certain phytoconstituents for their anti-ulcer effects. The anti- ulcer activity of selected phytoconstituents were predicted by engaging the canonical simplified molecular-input line-entry system obtained from PubChem.com followed by using PASSonline. Several phytoconstituents were predicted to have effects better than marketed drugs under some or the other out of the chosen areas of pharmacological mediation. On the other hand, several new paths were predicted in which the *in vitro* and *in vivo* evaluation of the phytoconstituents can be made on the basis of PASS predicted activities. It would allow the researchers to streamline the lookup more efficiently. Prediction of activity spectra of substances (PASS) is such a device which can predict the pharmacological homes beforehand and would help in screening pharmacological manageable leads for a particular condition [17,18]. It predicts the spectra of organic things to do for a molecule in terms of probable activity (Pa) and probable inactiveness (Pi) [19]. Selected active phytochemical constituents of *Alstonia scholaris* were

subjected to pass software for anti- ulcer activity. The results of these active constituents like probable activity (Pa) and probable inactiveness (Pi) and biological activity were given in table 8. The possible interventions of selected active constituents of *Alstonia scholaris* were found to be Aldehyde oxidase inhibitor, Anti-inflammatory, Intestinal, Cytoprotectant, Mucomembranous protector, H+/K+-transporting ATPase inhibitor.

Selected active phytochemical constituents of *Alstonia scholaris* were subjected to pass software for adverse effects and the results were tabulated in table 9. From the results, the constituent's Stigmasterol, Diospyrolide, D-Friedoolean-14-en-3-one, Lupeol acetate, Betulin, and Omeprazole were found to be free from any adverse effects whereas the remaining Constituents were predicted with hepatotoxicity, nephrotoxicity, Cardiac failure and arrhythmia. Selected active phytochemical constituents of *Alstonia scholaris* were subjected to pass software for direct and possible targets and results were given in table 10. All the constituents were found to have interventions with Prostanoid EP4 receptor, Prostanoid EP2 receptor, Prostanoid EP1 receptor, Cyclooxygenase-1, Cyclooxygenase-2, Histamine H2 receptor, Prostaglandin E synthase, Potassium-transporting ATPase, and ATP-binding cassette sub-family G member 2.

From the above PASS is an important tool for effectively showing the compounds of interest for the biological actions of interest. This helps the researchers to rationalize the research.

5. Conclusion:

From *in vivo* and *in silico* studies it is clear that the ethanolic bark extract of *Alstonia scholaris* possesses anti-ulcer activity in rodent models. Further studies are needed to be carried out to isolate individual phytochemical constituents of the extract and to establish the exact mechanism for its anti-ulcer activity.

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CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

NOTE:

The study highlights the efficacy of " herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

REFERENCES:

1. Mekonnen.N., Atnafie.M., Wahab Atta.S. Evaluation of Antiulcer Activity of 80% Methanol Extract and Solvent Fractions of the Root of Croton *macrostachyus Hocsht*: Ex Del. (Euphorbiaceae) in Rodents Evidence-Based Complementary and Alternative Medicine V 2020:1-11.
2. Mohod M, Bodhankar S. Antiulcer activity of aqueous extract of leaves of *Madhuca indica* J. F.Gmel against naproxen induced gastric mucosal injury in rats Journal of Acute Disease. 2013;127-133.
3. Pankti K, Payal G, Manodeep C, Jagadish K. A Phytopharmacological Review of *Alstonia Scholaris:* A Panoramic Herbal Medicine. 2012;3(3):367-371.
4. Kaushik P, Kaushik D, Sharma N, Rana A. *Alstonia scholaris:* It's hytochemistry and pharmacology. 2011;2(2):361-379
5. Raju MG, Anusha K, Suvarchala Reddy VNVL, Gayathri S and Nikitha K**.** GC-MS Analysis, Gastroprotective and *In Silico* Docking Studies of Phytoconstituents from *Ixora Javanica Flowers*. Int J Life Sci Pharma Res., 2021;11(2):P98-106.
6. Anunya Ch, Tiwari V, Babu PV, Raju MG. Antiulcerogenic activity of ethanolic extract of *moringa oleifera* bark in wistar albino rats. World Journal of Pharmaceutical Research. 2018; 7(1):1368-1378.
7. Raju MG, Yadav V, Suvarchala Reddy VNVL. Natural Compounds AS D_2 Receptor Agonist, M_4 Receptor Antagonist and ACHE Modulator: Mechanistic and *in Silico* Modelling Studies. Journal of Research in Medical and Dental Science 2021;9(6): 26-35.
8. Filimonov DA, Poroikov VV. PASS: Computerized Prediction of Biological Activity Spectra for Chemical Substances. In Bioactive Compound Design: Possibilities for Industrial Use; BIOS Scientific Publishers: Oxford, 1996; 47-56.

9. Gloriozova TA, Filimonov DA, Lagunin AA, Poroikov VV. Evaluation of computer system for prediction of biological activity PASS on the set of new chemical compounds. Chim.-Pharm. J. (Rus); 1998;32(12): 32-39.
10. Poroikov V, Filimonov D. Computer-aided prediction of biological activity spectra. Application for finding and optimization of new leads. Rational Approaches to Drug Design; Prous Science: Barcelona. 2001; 403-407.
11. Bafna PA, Balaraman R. Effect of activity, a herbomineral formulation, on experimentally- induced gastric lesions in rats. Journal of Applied Pharmaceutical Science. 2011;01 (10):134-139.
12. Karampour NS, Arzi A, Rezaie A, Pashmforoosh A, Kordi F. Gastroprotective Effect of Zingerone on Ethanol-Induced Gastric Ulcers in Rats. Medicina. 2019;55(3):64
13. Lira's S, Satyanarayana V, Carvalho S, Guedes M, Morais C, de Souza L, Trevisan S, Lima F, Chaves H. Gastroprotective effect of lupeol on ethanol-induced gastric damage and the underlying mechanism. Inflammopharmacol. 2009; (17) :221–228.
14. Sofidiya.M.O., Orisarem.O., Sansaliyu.I., Adetunde.T. Gastroprotective and antioxidant potentials of ethanolic stem bark extract of *Margaritaria discoidea* (Euphorbiaceae)in rats. Journal of Ethnopharmacology. 2012;1-8.
15. Katary MA, Salahuddin A. Gastroprotective Effect of Vanillin on Indomethacin-Induced Gastric Ulcer in Rats: Protective Pathways and Anti-Secretory Mechanism. Journal of Clinical & Experimental Pharmacology. 2017;7 (2): 1-8.
16. Raju MG, Goud PP, Suvarchala Reddy VNVL. Antihypertensive effect of Rutin: Pharmacological and Computational Approach. Asian Journal of Pharmaceutical and Clinical Research. 2019;12(8): 87-92.
17. Goel RK, Singh D, Lagunin A, Poroikov V. PASS-assisted exploration of new therapeutic potential of natural products, Medicinal Chemistry Research. 2011;20(9): 1509-14.
18. Parasuraman S. Prediction of activity spectra for substances. Journal of Pharmacology & Pharmacotherapeutics. 2011;2(1):52.
19. O'mahony C, Jichi F, Pavlou M, Monserrat L, Anastasakis A, Rapezzi C, Biagini E, Gimeno JR, Limongelli G, McKenna WJ, Omar RZ. A novel clinical risk prediction model for sudden cardiac death in hypertrophic cardiomyopathy (HCM risk-SCD). European Heart Journal 2013;35(30): 2010-20.