Evaluation of Antioxidant and Anti-ulcerative Potential of Millingtonia Hortensis Leaf Methanolic Extract: An In Vitro and In Silico Study

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Abstract

The results of this study included an extract from Millingtonia hortensis leaves that was prepared using methanol. Research on the antioxidant, anti-ulcer, and in silico properties of this extract (called MEMH) has shown a significant ability to scavenge free radicals. MEMH counteracts the effects of artificial stomach acid, demonstrating significant anti-ulcer potential. Molecular docking was carried out using Pyrx software, using particular ligands that interact with the H+/K+ ATPase proton pump related protein (PDB ID: 2XZB). The Flavone earned the highest docking score, at -8.7. The study suggests that a methanolic extract of Millingtonia hortensis leaves could be a helpful phytomedicine for treating ulcers. Nevertheless, further research is needed to pinpoint the exact mechanism of this extract's activity in vivo before recommending it as a therapeutic substitute.

Keyword: *Antiulcer, Antioxidant, Pyrx, In silico, Millingtonia hortensis, Molecular docking*

1. Introduction

Worldwide, hospitalisations due to ulcers are growing more and more frequent. They are characterised by recurrent episodes of epigastric discomfort that are frequently alleviated by eating or taking antacids. They are a medical ailment that is not lethal but can cause significant pain, emotional distress, bewilderment, and interruption of everyday activities. Ulcers may be the result of several things, including stress or long-term usage of anti-inflammatory medications. It's widely acknowledged that ulcers arise from an imbalance between the body's mucosal defence mechanisms and aggressive forces, even if the precise cause is still mostly unknown. A treatment for peptic ulcers should either lessen the effects of stimuli on the gastroduodenal mucosa or raise mucosal resistance against them. An injury to the stomach or duodenum's lining is known as a peptic ulcer. Organ bleeding and perforation can result from ulcers, which vary in severity from profound abrasions to mild irritations of the epithelium [1-3].

The well-known ornamental tree *Millingtonia hortensis Linn*, which is a member of the Bignoniaceae family and is native to Southern Asia, is extensively grown in India. The tree's hepatoprotective, larvicidal, antioxidant, and antifungal effects are well documented. The leaves of *Millingtonia hortensis* are used in traditional medicine as a cholagogue, sinusitis therapy, tonic, and antipyretic. Many chemicals, including terpenoids, glycosides, phenols, and flavonoids, have been extracted from the plant. Furthermore, the In silico method of molecular docking using Pyrx provides detailed insights into how specific phytoconstituents bind to the interacting amino acids of a target protein molecule[4-7].

The antioxidant and antiulcer activities of *Millingtonia hortensis* were synthesised and investigated in this study using a methanolic leaf extract. The ability of the methanol extract of *Millingtonia hortensis* to neutralise acid was used to measure its antiulcer efficacy. Thus, certain techniques were applied in order to validate its historical application as an anti-ulcer substance. To evaluate the binding affinities of flavone, N-[4-[4,4-Diethyl-1,4-dihydro-2H-benzo[d][1,3] oxazin-2-yl]- phenyl]-acetamide, 2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene, Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5- dimethylphenyl) methyl], coumarine, and 8-methoxy-3-(4-nitrophenyl) against the gastric H+/K+ ATPase proton pump, however, in silico molecular docking using Pyrx was carried out in order to ascertain their important function in treating ulcer.

2. Materials and Methods

2.1. Collection of plants

Botanist Harikishna from Osmania University confirmed the authenticity of *Millingtonia hortensis* leaves collected in October from the Hyderabad area of Telangana. The leaves were dried for about 20 days at room temperature in the shade and then finely ground using a mixer grinder. The decision was then made to retain or utilize the powdered leaves for the extraction process.

2.2. Preparation of plant extract

Twenty grammes of dry *Millingtonia hortensis* leaf powder and two hundred millilitres of methanol were added to a beaker. Then, after sealing it with aluminium foil, it was allowed to stand for the full day. It was periodically shook throughout this time to help the ethanol-soluble chemicals dissolve. The combination was then filtered. During the following four to five days, the filtered product should be let evaporate at room temperature. After the water has completely evaporated, the extract needs to be scraped off and weighed [8].

2.3. Invitro antioxidant activity

2.3.1. DPPH method

This method has evaluated the sample extracts' ability to decrease DPPH. 4 mg of DPPH were dissolved in 100 millilitres of methanol to create 0.004% of the DPPH solution. Different concentrations of plant extract (20, 40, 60, 80 and 100µg/ml) were used, and methanol was added to make the volume up to 3mL. The standard was ascorbic acid. Fill each test tube with 1 ml of methanolic DPPH. After 30 minutes of room temperature incubation, measure the absorbance at 517 nm to get the IC50 value.

The formula Y = 100*A1/ (X + A1), where A1 = IC 50, Y = response (Y = 100% when X = 0), and X = inhibitory concentration, was used to compute the IC 50 values. The antioxidant activity was reported in terms of IC50 (µg/mL concentration required to prevent the production of radicals by 50%), and the IC50 values were compared using paired t tests [9-10].

2.3.2. Hydrogen peroxide scavenging assay

Using a balance, 10 mg of MEMH were weighed and then diluted in 1000 millilitres of distilled water to determine the hydrogen peroxide scavenging test. Different concentrations of MEMH solutions (20, 40, 60, 80, and 100 µg/mL) were produced. 100 µL of each MEMH concentration and 400 µL of phosphate buffer were added. The standard solution of ascorbic acid was used to duplicate the process. The tubes were then vortexed, filled with 600 µL of a hydrogen peroxide solution containing 40 mM, and left for ten minutes to incubate. Using a UV-visible spectrophotometer, absorbance was measured at 230 nm using 100 µL of each sample and a blank that included only phosphate buffer and no hydrogen peroxide. Positive control, or standard, was ascorbic acid. Hydrogen peroxide-free samples served as the negative control. The following equation was used to determine the hydrogen peroxide scavenging abilities: % scavenged (H_2O_2) = (Ao − A1)/Ao × 100, where Ao denotes the sample's absorbance and A1 the control [11].

2.4. Invitro antiulcer activity

2.4.1. Acid neutralizing capacity

The ANC values of the methanolic extract of *Millingtonia hortensis* were measured at different concentrations (100 mg/ml, 200 mg/ml, 500 mg/ml, and 1000 mg/ml) in order to compare it with the standard antacid MCCC (magnesium carbonate + calcium carbonate at 500 mg/ml). To get a final volume of 70 millilitres, every concentration was diluted with 5 millilitres of water. After that, the test and standard samples each received 30 millilitres of 1N HCl, and they were shaken for 15 minutes. The last step was to add one or two drops of phenolphthalein solution. Immediately after, the surplus HCl was titrated drop by drop with 0.5N sodium hydroxide solution until a pink hue developed. The method used to compute the moles of acid neutralised is the formula for moles of acid neutralised is (vol. of HCl × Normality of HCl) - (vol. Of NaOH × Normality of NaOH). The formula for acid neutralising capacity (ANC) per gramme of antacid is moles of HCl neutralised / grammes of antacid/extract [12].

2.4.2. Neutralizing effects on artificial gastric juice

Preparation of artificial gastric acid

NaCl (two grammes) and pepsin (3.2 milli grammes) were added to a 500millilitre solution of filtered water. Enough water was added to create a 1000 mL solution, and 7.0 mL of hydrochloric acid was added. The pH of the solution was adjusted to 1.20.

pH determination of the GIE

The pH of GIE (400 mg and 800 mg) was measured at temperatures ranging from 25^0C to 37^0C. The pH values of the active control solution, sodium bicarbonate (SB), and water were also measured for comparison

Determination of the neutralizing effects on artificial gastric acid

The freshly prepared test solutions GIE [400 mg and 800 mg (90 mL)], water (90 mL), and the active control SB (90 mL) were mixed separately to 100 mL of artificial gastric juice at pH 1.2. The pH values were measured to investigate the neutralizing effects of artificial gastric juice.

Determination of the neutralization capacity in vitro using the titration method of Fordtran's model

The freshly prepared test solutions GIE [400 mg and 800 mg (90 mL)], water (90 mL), and the active control SB (90 mL) were mixed separately with 100 mL of artificial gastric juice at pH 1.2. The pH levels were monitored to determine the neutralizing effects of artificial gastric juice [13].

2.5. Molecular docking using Pyrx

The RSCB (PDB) website provided the enzyme proton pump model, which was retrieved from the Protein Data Bank (coded 2xzb). The chemical compound models were updated using Millingtonia hortensis phytoconstituents that were downloaded in three dimensions in SDF format from the Pubchem website. Docking was carried out and the outcomes were visualised using the Pyrx software [14].

3. Results and discussion

Invitro antioxidant and antiulcer activity of methanolic extract of *Millingtonia hortensis* leaves was explored for its antiulcer activities. All the results obtained in the studied were included below.

Preliminary Phytochemical analysis

The preliminary phytochemical investigation of MEMH extract of *Millingtonia hortensis* leaves showed the presence of phenolic compounds, glycosides, flavonoids, terpenoids, tannins, steroids etc.

Table 1. Preliminary Phytochemical analysis

Phytochemical Constituents	Results
Tannins	++
Flavonoids	++
Terpenoids	++
Phenols	++
Glycosides	++
Steroids	++

Note: + indicates present; - indicates absent

***In vitro* antioxidant assay**

The MEMH was subjected to invitro antioxidant assay. In vitro antioxidant assay was performed using DPPH and H_2O_2 scavenging assay.

DPPH scavenging assay

Table 2. DPPH Method of Ascorbic acid, MEMH

S.No.	Compounds	Concentration (µg/ml)	%inhibition	IC50 values
1.	Ascorbic acid	20 40 60 80 100	58 63 72 91 98	51
2.	Methanolic extract of *Millingtonia hortensis* (MEMH)	20 40 60 80 100	55 60 67 91 97	87

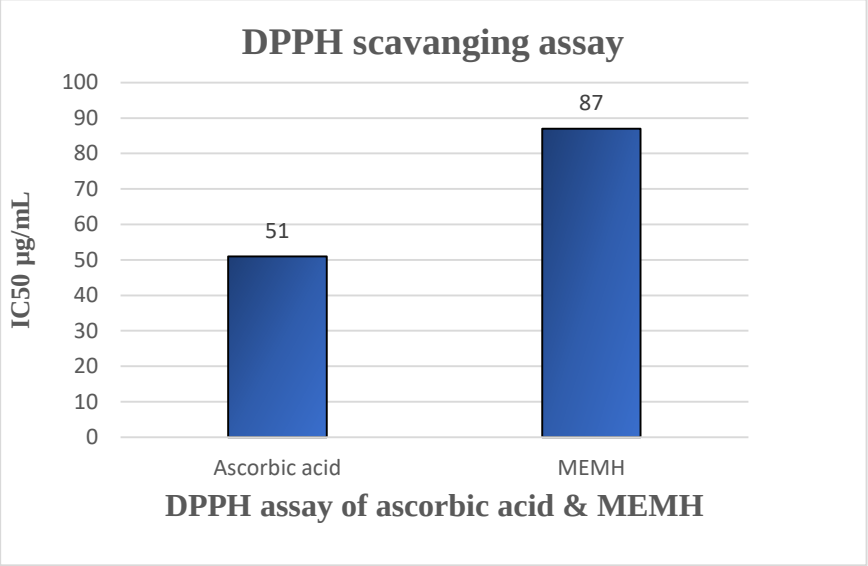


Figure 1. DPPH assay of Ascorbic acid, MEMH

DPPH Method, IC_{50}values of standard drug, MECE and NPCE was found to be 51 and 87 ug/ml respectively. From the above result it is clear that NPCE showed more antioxidant activity compared to MECE. The results were expressed in the table 2 and the profile was expressed in the figure 1.

H_2O_2 Scavenging Assay:

Table 3. H_2O_2 Scavenging Assay of Ascorbic Acid, MEMH

S.No.	Compounds	Concentration (µg/mL)	%inhibition	IC50 values
1.	Ascorbic acid	20 40 60 80 100	17 20 55 65 74	61
2.	Methanolic extract of *Millingtonia hortensis* (MEMH)	20 40 60 80 100	14 27 50 55 60	75

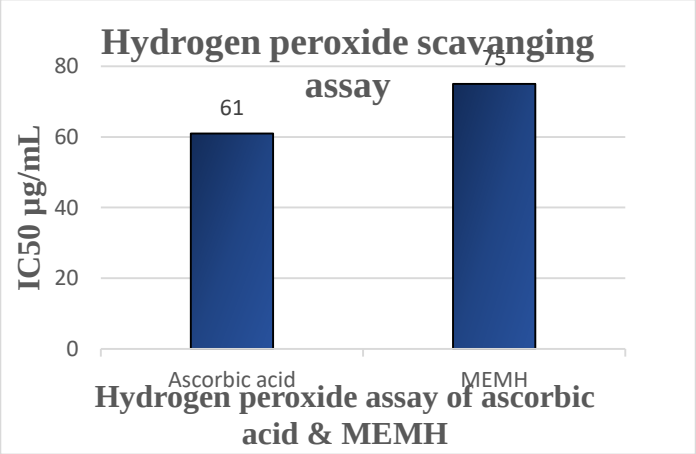


Figure 2. H_2O_2 Scavenging Assay of Ascorbic Acid, MEMH

H_2O_2 Scavenging Assay Method, IC50values of standard drug, MEMH was found to be 61 and 75 ug/ml respectively. From the above result it is clear that MEMH showed more antioxidant activity compared to standard. The results were expressed in the table 3 and the profile was expressed in the figure 2.

***Invitro* antiulcer activity**

The MEMH was subjected to invitro antiulcer activity. In vitro antiulceractivity was performed using Calcium carbonate and Magnesium Carbonate in combination as a standard

Acid Neutralizing Capacity

MECE (100, 200, 500, and 1000 mg) and a normal dose of 500 mg of calcium carbonate + magnesium carbonate [$Caco_3$+ $Mgco_3$], the neutralizing impact of the methanolic extract was investigated. Using standard and four concentrations (100, 200, 500, and 1000 mg), the neutralizing impact of the methanolic extract was investigated.

The results demonstrated thatthe extract's capacity to neutralize acid dropped considerably at doses of 100, 200, 500, and 1000 mg, by 85,65,15 and 7 mg/mg, respectively, when compared to the standard of10 mg/mg. It has been discovered that when diluted to a concentration of 1000 mg, the extractsignificantly increases the ability of acid to be neutralized. The outcomes are listed in Table No 4.

Table 4. Effect of MEMH on acid neutralizing capacity

S. No	Compounds	Concentration mg	Volume of NaOH consumed (mL)	meq of acid consumed	ANC per gram of acid
1.	MEMH	100	43	8.5	85
2.	MEMH	200	34	13	65
3.	MEMH	500	45	7.5	15
4.	MEMH	1000	46	7	7
5.	Standard	500	50	5	10

Neutralizing effects on artificial gastric acid

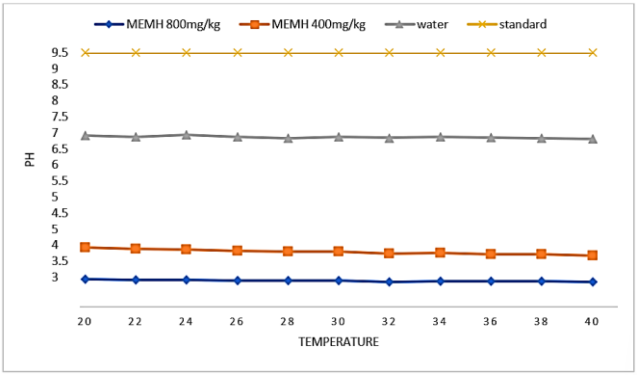


Figure 3. pH values of water, standard and GIE (400mg and 800mg) determined at temperatures ranging from 25^{0}C to 37^{0}C.

The pH values of the GIE 400 mg and GIE 800 mg solutions at temperatures from 25^{0}C to 37^{0}C ranged from 1.59 to 2.02 and 1.36 to 1.75 respectively. The pH values of water and SB solutions at temperatures from 25^{0}C to 37^{0}C ranged from 1.54 to 1.78 and 1.55 to 1.97, respectively (Figure 3). The results indicate that temperature did not affect pH significantly.

Table 5. pH values with 90 ml water, standard and GIE (400mg/kg and 800mg/kg) added to 100 ml of artificial gastric juice

Drug	pH value
Water	1.68 ± 0.02
Standard	1.66 ±0.02
GIE 400mg/kg	1.76 ±0.01
GIE 800mg/kg	1.75 ±0.02

Neutralizing effects on artificial gastric acids

When 90 mL of the test solution was added to 100 mL of the artificial gastric juice (pH 1.2), the pH values of GIE 400 mg and GIE 800 mg solutions were found to be 1.76 ±0.01 and, 1.75 ±0.02 respectively. The pH values of water and SB solutions were 1.68 ± 0.02 and 1.66 ±0.02, respectively. This result shows that the neutralizing effect of 400 mg and GIE 800 mg was significantly better than that of water (Table 5).

Table 6. Consumed volume of artificial gastric juice and H+ (mmol) in the titration of 90 mL water, standard and GIE (400mg and 800mg) with artificial gastric juice (pH 1.2) to the end point of pH 3

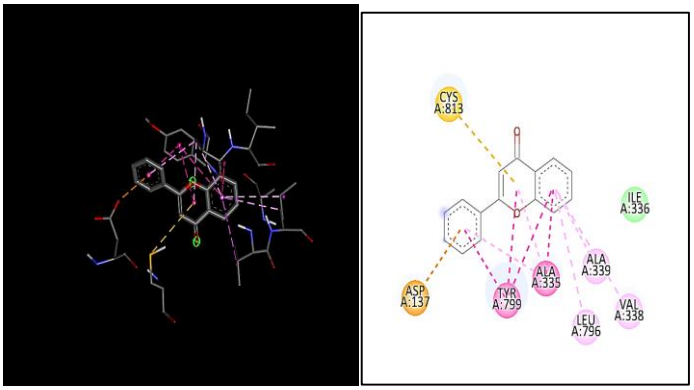
Drug	Consumed volume of artificial gastric juice (mL)	mmol of H
Water	2.32 ±0.23	0.14 ±0.002
Standard	15.22 ±2.03	0.95 ±0.06
GIE 400 mg	9.08 ±1.05	0.57 ±0.02
GIE 800 mg	4.02±0.34	0.27 ±0.06

Physical neutralization capacity in vitro

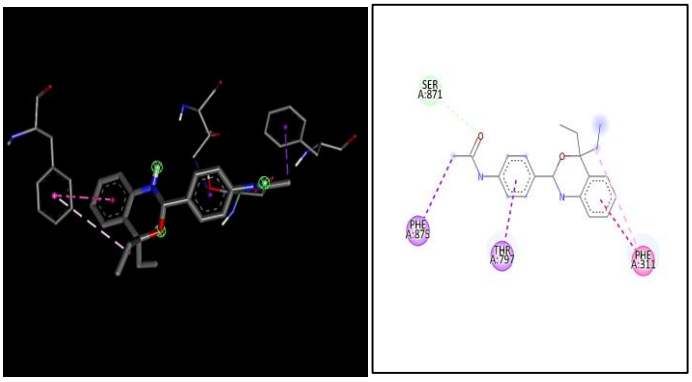
The consumed volumes of artificial gastric juices to titrate to pH 3.0 for water, GIE 400 mg, GIE 800 mg and SB solutions were2.32 ±0.23,9.08 ±1.05,4.02±0.34 and 15.22 ±2.03 respectively. The consumed H+ were 0.14 ±0.002, 0.57 ±0.02, 0.27 ±0.06 and 0.95 ±0.06 mmol, respectively (Table 6). The active control SB and both tests (GIE 400 mg and 800 mg), exhibited significant antacid potency. The neutralization capacities of GIE 400 mg and 800 mg were lesser than that of SB but significantly better than that of water.

Molecular docking studies using Pyrx

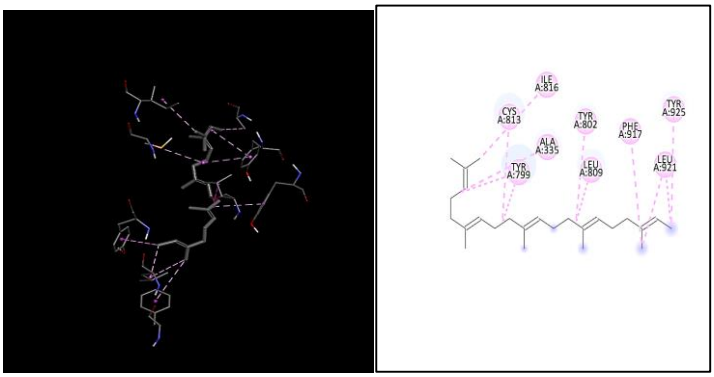
Pyrx conducted the studies on docking. Through docking tests, we were able to determine which of the ligands from our study (flavone, N-[4-[4,4-Diethyl-1,4-dihydro-2H-benzo[d][1,3] oxazin-2-yl]- phenyl]-acetamide, 2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene, Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5- dimethylphenyl) methyl], coumarine, and 8-methoxy-3-(4-nitrophenyl) had a stronger binding affinity with the gastric proton pump protein with the PDB id 2xzb. This was demonstrated by binding scores that were closer to omeprazole, the anti-ulcer drug that is frequently prescribed, and by negative binding scores. A table 7 and a figure 4 showed the outcomes. The highest binding score for flavone was found to be -8.7, higher than the score for omeprazole (-7.2) and N-[4-[4,4-Diethyl-1,4-dihydro-2H-benzo[d][1,3] oxazin-2-yl]- phenyl]-acetamide (-8.4), 2,6,10,14,18-pentamethyl-2,6,10,14,18 eicosapentaene(8.4), Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5- dimethyl phenyl) methyl] (-7.9), coumarine, and 8-methoxy-3-(4-nitrophenyl) (-7.5).



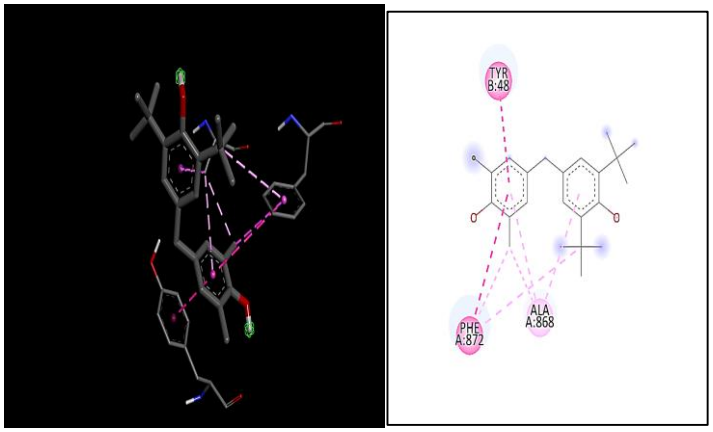
(a) Flavone: -8.7



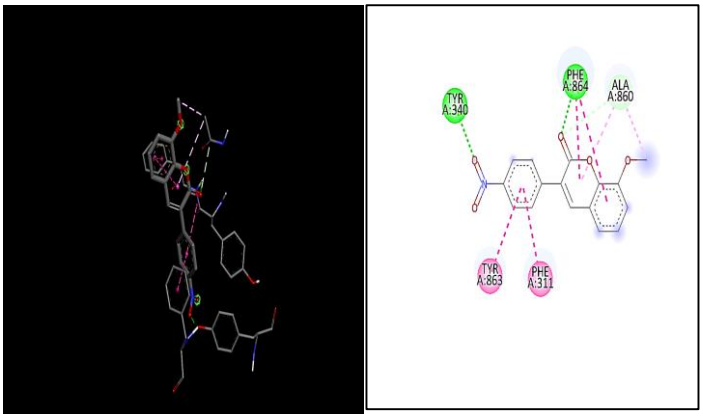
(b) N-[4-[4,4-Diethyl-1,4-dihydro-2H-benzo[d] [1,3] oxazin-2-yl]- phenyl]-acetamide: -8.4



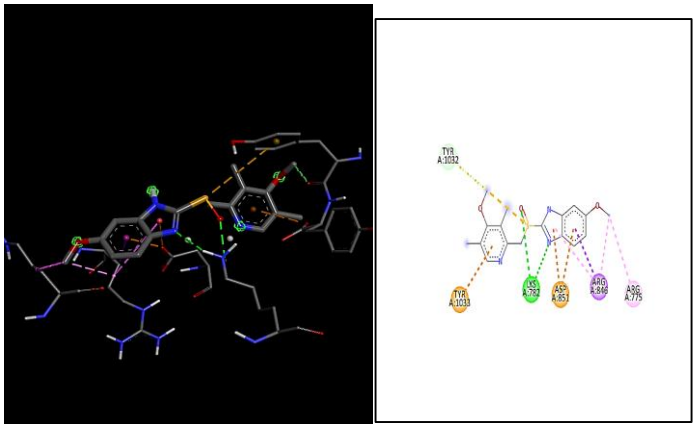
(c) 2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene: -8.4



(d) Phenol,2,6-bis(1,1-dimethylethyl)-4- [(4-hydroxy-3,5- dimethylphenyl) methyl]: -7.9



(e) Coumarine,8-methoxy-3-(4-nitrophenyl): -7.5



(f) Omeprazole: - 7.2

Figure 4. Docking modes of respective ligands with H+/K+ gastric proton pump.

Table 7. Docking result between ligand with the gastric proton pump protein (PDB: 2ZXB).

S.no	Ligand / chemical compound	Gibbs Free Energy (ΔH)
1	Flavone	-8.7
2	N-[4-[4,4-Diethyl-1,4-dihydro-2H-benzo[d][1,3] oxazin-2-yl]- phenyl]-acetamide	-8.4
3	2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene	-8.4
4	Phenol,2,6-bis(1,1-dimethylethyl)-4- [(4-hydroxy-3,5- dimethyl phenyl) methyl]	-7.9
5	Coumarine,8-methoxy-3-(4-nitrophenyl)	-7.5
6	omeprazole	-7.2

4. Conclusion

The plant was collected, and its constituents were extracted using a methanol-based maceration method. Conventional methods were then applied to the resulting extract for further analysis. An initial phytochemical examination of the *Millingtonia Hortensis* methanolic extract was conducted, revealing the presence of phenols, terpenoids, flavonoids, tannins, glycosides, and steroids. The in vitro antioxidant activity was assessed using the hydrogen peroxide scavenging assay and the DPPH technique. An antioxidant investigation revealed strong free radical scavenging properties in a methanolic extract of Millingtonia hortensis leaves. The extract, known as MEMH, has shown significant antiulcer efficacy by neutralizing acid and counteracting the effects of artificial gastric acid. When compared to a standard, the antiulcer properties of MEMH were evident. It can be concluded that MEMH possesses potent antioxidant and antiulcer effects. Additionally, the phytoconstituents in MEMH showed anti-ulcer efficacy by binding to the H+/k+ ATPase gastric proton pump protein and obtaining a higher binding score in Pyrx-based in silico molecular docking studies, which is comparable to omeprazole's. Although the results are promising, further research is necessary to determine the exact mechanism of action using in vivo models before recommending this extract as a therapeutic agent for ulcer treatment.

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