RESEARCH PAPER

Phytochemical Investigation and In-vitro Antiulcer Activity Evaluation of Flavonoids Rich Fraction of Alternanthera sessilis Leaf Ethanolic Extract

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ABSTRACT
Objectives: Examining the acid-neutralizing ability, H^+/K^+ ATPase and Na^+/K^+ ATPase modulation of the flavonoid-rich fraction of *Alternanthera sessilis* leaf ethanolic extract, as well as its phytochemical profile and in-vitro antiulcer efficacy, were the primary goals of the research.
Methods: Leaves of *A. sessilis* were collected, shade-dried, and extracted using Soxhlet extraction with ethanol. Preliminary phytochemical screening and TLC profiling were performed, followed by column chromatography to obtain two major fractions. GC-MS analysis identified key bioactive compounds in both fractions. Among them, Fraction 2, enriched in flavonoids, was selected for *In-vitro* evaluation. Antiulcer assays included Acid Neutralizing Capacity (ANC), H^+/K^+ ATPase and Na^+/K^+ ATPase inhibition models using standard protocols with omeprazole and ouabain as reference drugs.
Results: Fraction 2 demonstrated the highest ANC of 120 mEq/g at 100 mg concentration compared to calcium carbonate (37.8 mEq/g). In H^+/K^+ ATPase inhibition assay, Fraction 2 showed 58.90% inhibition at 100 µg/mL with an IC_{50} of 91.1 µg/mL, whereas omeprazole had an IC_{50} of 55.9 µg/mL. Similarly, in the Na^+/K^+ ATPase assay, Fraction 2 exhibited 43.50% inhibition at 100 µg/mL (IC_{50}: 91.1 µg/mL), compared to 80.51% inhibition by ouabain (IC_{50}: 55.9 µg/mL). The optimized fraction was selected based on GC-MS profiling and comparative bioactivity across all *In-vitro* assays.
Conclusion: The optimized flavonoid-rich fraction of *A. sessilis*shows significant antiulcer potential via multi-targeted mechanisms and offers a promising, natural alternative for acid-related disorders. These findings support further in-vivo validation and highlight the potential for future clinical translation as a standardized phytopharmaceutical.
Keywords: *Alternanthera sessilis; Flavonoids; Antiulcer; H^+/K^+ ATPase; Na^+/K^+ ATPase; GC-MS; Phytotherapy.*
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INTRODUCTION
The disease of peptic ulcer is one offurthermostextensive diseases of GIT around the world, as a result, almost every tenth person of the global population experienced the manifestation of the disease during his/her life[1]. The major reason it happens is because the mucosal barrier can't withstand the harsh stomach elements, such pepsin and hydrochloric acid[2]. Common triggers include *Helicobacter pylori* infection, chronic NSAID usage, emotional stress, and alcohol use. Proton pump inhibitors as well as H2 receptor antagonists are extensively used, however they come with a risk of adverse effects, medication resistance, and relapse when stopped[3]. Besides, most patients in LMICs cannot afford long term treatment giving rise to high morbidity. Peptic ulcer disease has a heavy economic burden on health systems globally because billions are wasted each year on diagnosis and treatment of the disease and lost productivity[4]. Recent tendencies in gastrointestinal pharmacotherapy focus on plant-based interventions specifically because of their safety and side-effects. Nevertheless, there is an urgent necessity to study and confirm new phytomedicinal possibilities and explore new solutions with a strong gastroprotective potential to address current limitations in treatment[5].
*Alternanthera sessilis*is a well-knowntherapeutic plant species that has received notable evaluation due to its phytochemical contents and the abundance of flavonoids in it. Flavonoids are representatives of polyphenolic compounds, which are characterized by antioxidative, anti-inflammatory and cytoprotective effects critical to

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overcoming oxidative damage and both mucosal injury and oxidative stress in the gastrointestinal tract[6]. These compounds have structural characteristics including hydroxyl and conjugated double whose structure allows them to react as radical scavengers and metal chelators[7]. Research has shown that flavonoids have the potential to stimulate the secretion of mucus, inhibit gastric proton pumps, as well as interfere with major inflammatory processes and hence, promote the healing of the mucosa, and alleviate ulcer index[8]. *A. sessilis*leaves ethanolic extracts contain such bioactive flavonoids in abundance and this fact justifies the development of the ethanolic extract in the treatment of gastrointestinal disorders. Its chemical composition comprising of bio-components such as quercetin, kaempferol and luteolin derivatives indicate multiple action effect on the body that has potential of relieving gastric lesions and healing mucosal integrity. Thus, the flavonoid-containing fraction of *A. sessilis* can be considered a potential, natural approach to treating peptic ulcers[9].

In-vitro assessment models play a precisesignificant role in explaining mechanistic joining of antiulcer activity particularly when the investigation focus is on the extract of plants like the flavonoid-rich frack of *Alternanthera sessilis*[10]. Among the most popular methods, ANC assay can be mentioned, which allows defining the extract potential to buffer extremestomach acid one of main factors of ulcer pathogenesis[11]. The evaluation of ANC offers preliminary information on the properties of the formulation in terms of lowering it as well as preventing gastric injury due to low gastric-acidity[12]. Moreover, the H ATPase inhibition model is also a valid parameter of verifying the possibility of natural compounds to disrupt the proton pump-promoted acid production[13]. Given the fact that flavonoids have been postulated to modulate enzyme activities, then the inhibitory effect of flavonoids on this gastric proton pump may also lead to the suppression of acids[14]. More to the point, the Na+/K+ ATPase activity test provides an opportunity to examine effect of extract on cellular ionic homeostasis and coating of the mucosa that is critical in the gastric integrity. These models together constitute a scientifically sound platform on which the gastroprotective effect of natural flavonoid rich preparations can be evaluated[15].

The given research study explores the phytochemical background and will merge to review the *In-vitro* antiulcer activity of the flavonoids-enriched fraction of the ethanolic leave of *Alternanthera sessilis*. Specific goals will be qualitative and quantitative identification of flavonoids, investigating the cytoprotective effect on gastric models, and determining comparative effectiveness with standard antiulcer agents to determine the relevance of therapeutic effects and the degree of innovation.

MATERIALS AND METHODS

MATERIALS

Ethanol (analytical grade, 99.9% purity), silica gel (60–120 mesh, analytical grade), quercetin (analytical grade, MW 302.24 g/mol), aluminum chloride (analytical grade, ≥99% purity), methanol (HPLC grade, ≥99.8% purity), vanillin, benzene, acetic acid, formic acid, ethyl acetate, chloroform, and n-butanol (all analytical grade), omeprazole (USP grade, MW 345.42 g/mol), calcium carbonate (analytical grade, 99% purity), and ouabain (analytical grade, ≥98% purity) were purchased from Research Lab, Mumbai, India.

METHODS

Collection and Authentication

The aerial parts of a recently harvested *Alternanthera sessilis* were collected in September at Deolali Camp in Nashik, Maharashtra, India. Ethical and environmental collecting criteria were followed when plant was harvested. The Department of Botany at the PadmashriVikhe Patil College of Arts, Science and Commerce in Pravaranagar, Maharashtra, was consulted for botanical verification after a specimen voucher had been made and sent there. Dr. A. S. Wabale verified the plant's authenticity and taxonomic status, and it was assigned the reference number PVP/BOT./2024-25/310.The verified specimen was maintained in a herbarium of the department as reference material. After authentication, the leaves were completely washed using running tap water to clear attached debris. The washed leaves were further finely chopped into small bits equal size and dried under shade conditions at a room temperature of (25 ± 2 °C) at room temperature over a period of 15 days in an attempt to inhibit degradation of the phytochemicals. The material was allowed to completely dry then mechanically grinded to coarse-powder form by using a mechanical grinder (RemiElektrotechnik Ltd., Mumbai, India) and reserved in sealed, light-resistant containers at room temperature until further use in the experiments[16,17].

Extraction of plant material using Soxhlet

100 g of *Alternanthera sessilis* leaves that had been shade-dried and roughly crushed were extracted using 500 mL of analytical grade ethanol in a Soxhlet apparatus. Extraction was carried using a thermsteadily regulated heating pad at a constant temperature of 8 hours and a standard temperature of 78 ± 1 °C. A thick crude extract was obtained by pipetting a further concentrated sample under low pressure using a rotary vacuum evaporator at 40 °C after ethanolic extract had been fully extracted. Filter used was Whatman No. 1. After that, semi-solid extract was transferred to a glass vial with an amber hue and stored at 4 °C in a regular refrigerator until pharmacological and phytochemical testing could be conducted. To validate reproducibility and consistency of the extract, every extraction step was done in triplicate (n = 3)[18,19,20].

Phytochemical screening of *Alternanthera sessilis*leaves extract

By following the established qualitative protocols outlined in the phytochemical literature and pharmacopoeia guidelines, we performed an initial phytochemical examination of ethanolic extract of *Alternanthera sessilis* leaves to determine presence of major classes of bioactive compounds. Using the conventional reagent-based methods, analysis was carried out to detect

metabolites.Analyses were done in triplicate (n = 3) in order to improve reproducibility and reliability of results[21,22].

Quantitative estimation of flavonoid in *Alternanthera sessilis* leaves ethanolic extract

A colorimetric approach based on aluminum chloride and quercetin was used to quantify flavonoid contented of ethanolic extract from *Alternanthera sessilis* plant's leaves. The mixture was kept in the dark at 25 ± 2 °C for 30 minutes after an aliquot of 1mL of the extract (1 mg/mL) was added to 1mL of a 2% solution of aluminum chloride in methanol. A UV-Visible spectrophotometer (Systronics UV-1900, Ahmedabad, India) that was blanked against a reagent blank was used to measure absorbance at 415 nm. Used quercetin concentrations ranging from 10-100 µg/mL to create a standard calibration curve, and we displayed the total flavonoid content as mg quercetin equivalent/g extract. The data that were obtained as mean±SD were based on three measurements each (n = 3)[23,24].

Thin-Layer Chromatography (TLC) for Selection of Solvent System

TLC analysis was carried out to determine the optimal solvent system for fractionating the ethanolic *Alternanthera sessilis* leaf extract. 5 µL of the extract solution was added to silica gel 60 F254 TLC plates made by Merck Millipore in Mumbai, India, after its dissolution in ethanol at a concentration of 10 mg/mL. Benzene:aceticacid:water (125:72:3), methanol:chloroform: hexane (7: 2: 1), butanol:ethyl acetate: water (1: 2: 3), and ethyl acetate:aceticacid:formicacid:water (100:11:11:27) were the solvent systems used to perform development on pre-saturated chambers. After development and drying, the plates were exposed to UV light at 254 and 366 nm.The plates were subjected to heat at 110°C for 5 minutes after being coated with the vanillin-sulfuric acid reagent.R_f values were noted and the system which gives the best resolution and distinct band separation was selected on which column chromatography was subsequently performed. To get consistency, all the procedures were done in triplicate (n=3)[25,26,27,28,29].

Thin-Layer Chromatography (TLC) for Selection of Solvent System

Column chromatography of ethanolic extract of *Alternanthera sessilis* leaf on increasing solvent polarity was performed. Silica gel (60-120 mesh, Qualikems Fine Chem Pvt. Ltd., Vadodara, India) was wet packed (with benzene) into a glass column (60 cm x 2.5 cm). About 5g of crude extract was adsorbed, in silica gel and loaded to the column. The solvent systems were applied in the following order of polarity: benzene:aceticacid:water (125:72:3), methanol: chloroform: hexane (7:2:1), n-butanol:ethylacetate:water (1:2:3), and ethyl acetate: acetic acid: formic acid: water (100:11:11:27). They were eluted sequentially. Using the appropriate solvent systems, TLC observed the phytochemical separation of the 10 mL extraction fractions. Medica Instrument Manufacturing Co., Mumbai, India's EquitronRoteva rotary vacuum evaporator was used to mix and concentrate fractions with identical Rf profiles at 40 °C. Reproducibility was checked by repeating each method three times (n=3)[30,31].

Gas Chromatography–Mass Spectrometry

GC-MS was used to recognize phytochemical constituents in ethanolic extract fractions of *Alternanthera sessilis* leaves. The work was accompaniedby a GC–MS apparatus. The device included an HP-5MS fused silica capillary column with an i.d. of 30 m x 0.25 mm and a film thickness of 0.25 um. Helium was as carrier gas, and the flow rate was maintained at 1.0 mL/min. The injector's temperature was 250 °C, and the injections had a volume of 1 µL. The oven was set to 60 °C for two minutes, 280 °C for ten minutes, and then maintained at that temperature. The whole process took thirty-two minutes. Mass spectra obtained using the electron impact mode at 70 eV encompassed a range of m/z 50 to 600. By comparing the components' mass spectra to those in the NIST database, able to identify each one.To make sure the findings could be repeated, we ran the analysis three times (n = 3)[32].

***In-vitro* antiulcer action**

Acid neutralizing capacity

To evaluate *Alternanthera sessilis*'s capacity to neutralize stomach acid, the value needed was the buffering power of flavonoid-rich fractions of ethanolic leaf extract. To conduct the test, the extract was diluted with standard 0.1 N NaOH after reacting with excess 0.1 N HCl. A 100 mL solution of distilled water and 1 gram of extract were combined with 100 mL of 0.1N hydrochloric acid, to sum it up. The mixture was mixed in a water bath maintained at 37 ± 1 °C and agitated every 5 minutes for 1 hour. After incubation, the residual acid was adjusted by titrating it with 0.1 N NaOH using phenolphthalein as an indicator.The formula used to calculate the ANC was:

$$\text{ANC (mEq/g)} = \frac{V1 - V2}{N}$$

whereV_1 is the volume of NaOH required for blank, V_2 for the test sample, and N is the normality of NaOH. All tests were done in triplicate (n = 3), and values were stated as mean ± SD[33,34].

***In-vitro* H^+/K^+ ATPase Inhibitory Action**

Action of the flavonoid-rich fraction of the *Alternanthera sessilis* against H + / K + ATPase was tested with goat gastric mucosal homogenate. Centrifuged in ice-cold Tris-HCl buffer (20 mM, pH7.4) at 18,000 rpm speed for 30 minutes at 4 °C, the cleansed and homogenized mucosa was acquired by scraping it out of fresh goat stomach that was procured locally at a slaughterhouse. The enzyme source was the liquid portion above the solid. Protein (40 ug), Tris-HCl buffer (40 mM, pH 7.4), Magnesium chloride (2 mM), potassium chloride (10 mM), and ATP (20 mM) made up the test mixture. The extract was added in various amounts and left to incubate at 37 ± 1 °C for 30 minutes in order to carry out the test.The reaction was terminated with 10% TCA and liberated inorganic phosphate was analyzed at 400nm employing ammonium molybdate reagent. The reference standard was omeprazole. Each test was carried out three times (n = 3) and percent inhibition was carried out against control[35,36].

***In-vitro* Na^+/K^+ ATPase Action**

The flavonoid-rich fraction of *Alternanthera sessilis* was tested as a source of Na + /K + ATPase by making use of the goat gastric mucosal homogenate as the enzyme source. The mucosal scrapings were then homogenized using ice-cold Tris-HCL buffer (20 mM pH 7.4) After 30 minutes at 4 °C, the mixture was centrifuged at 18,000 rpm. In the supernatant, the experiment was carried out. Here is what the reaction mixture was made of: 40 units of protein were added to the following reaction components: 30 millimolarTris-HCl buffer (pH 7.4), 5 millimolar MgCl2, 10 millimolarNaCl, 10 millimolarKCl, and 20 millimolar ATP. After adding the test extract, it was incubated at 37± 1 °C for 30 minutes. A spectrophotometer reading taken at 400 nm in ammonium molybdate reagent was used to assess the amount of inorganic phosphate freed after the reaction was halted with 10% TCA. Ouabain was said to be the reference standard. The enzyme inhibition was calculated as a percentage relative to the control after each experiment was repeated three times (n=3)[37].

RESULTS AND DISCUSSION

RESULTS

Phytochemical investigation

Phytochemical analysis of ethanolic leaf extract of *Alternanthera sessilis*showed the following components: positive alkaloid, carbohydrates, glycosides, steroids, flavonoids, saponins, tannins, phenols, terpenoids and negative proteins. Presence of flavonoids confirms its possible antiulcer action as flavonoids are antioxidant and cytoprotective. These results prove the highly phytochemically diverse nature of the extract (Table 1) and justify further testing of the gastroprotective activity.

Table 1:Preliminary phytochemical investigation of *Alternanthera sessilis* leaves ethanolic extract

Phytochemicals	Test/reagent	Ethanolic extract of *Alternanthera sessilis*
Alkaloids	Dragendorff Mayer Hager Wagner	+ + + +
Steroids	Libermann-Burchard Salkowski	+ +
Glycosides	Keller-Killiani Borntrager	+ +
Carbohydrates	Molisch Fehling	+ +
Saponins	Foam	+
Flavonoids	Shinoda Lead acetate	+ +
Phenols	Ferric chloride	+
Tannins	Lead acetate	+
Terpenoid	Copper Acetate	+
Protein	Biuret Xanthoprotein Ninhydrin	- - -

Quantitative estimation of flavonoid

The whole extract of ethanolic extract of *Alternanthera sessilis* contained 0.702 mg/100 mg of dry extract as measured by the colorimetric technique through gauging aluminum chloride with quercetin as the standard. The moderate level of flavonoids sustains the doubt that the plant may have gastroprotective potential given the antioxidant and antiulcer qualities of flavonoids. Table 2 shows the results.

Table 2: Total Flavonoid Content of *Alternanthera sessilis* Ethanolic Extract

Sr.no	Extract	Total Flavonoid Content (mg/100 mg of dried extract)
1	*Alternanthera sessilis*leaves ethanolic extract	0.702

TLC Profiling of *Alternanthera sessilis* Ethanolic Extract

TLC profiling of ethanolic extract of *Alternanthera sessilis* revealed single distinct spots in all tested solvent systems, indicating the presence of specific phytochemicals. With a ratio of 125:72:3, the Rf value was 0.42 in the benzene:aceticacid:water solution. In the methanol:chloroform:hexane solution, the Rf value was 0.59. In the n-butanol:ethylacetate:water solution, the Rf value was 0.57. The optimal resolution was achieved with ethyl acetate:aceticacid:formicacid:water (100:11:11:27), with an Rf value of 0.76. Table 3 summarizes these results.

Table 3: TLC Profiling of *Alternanthera sessilis* Ethanolic Leaf Extract Using Different Solvent Systems

Solvent System Used	Number of Spots Observed	R_f Value
Benzene : Acetic acid : Water (125:72:3)	1	0.42
Methanol : Chloroform : Hexane (7:2:1)	1	0.59
n-Butanol : Ethyl acetate : Water (1:2:3)	1	0.57
Ethyl acetate : Acetic acid : Formic acid : Water (100:11:11:27)	1	0.76

TLC profiling after collection of *Alternanthera sessilis* Ethanolic Extract fractions

TLC profiling of column fractions of *Alternanthera sessilis* ethanolic extract showed consistent single spots for each solvent system, allowing for pooling based on similar Rf values. Fractions eluted with benzene:aceticacid:water (Rf = 0.42) and n-butanol:ethylacetate:water (Rf = 0.57) were grouped as Fraction 1 (F1), while those with methanol:chloroform:hexane (Rf = 0.63) and ethyl acetate:aceticacid:formicacid:water (Rf = 0.76) were pooled as Fraction 2 (F2). Detailed data are presented in Table 4.

Table 4: TLC Profiling and Pooling of Column Fractions from *Alternanthera sessilis* Ethanolic Extract

Solvent System Used	Number of Spots Observed	Rf Value	Assigned Fraction
Benzene : Acetic acid : Water (125:72:3)	1	0.42	F1
n-Butanol : Ethyl acetate : Water (1:2:3)	1	0.57	F1
Methanol : Chloroform : Hexane (7:2:1)	1	0.63	F2
Ethyl acetate : Acetic acid : Formic acid : Water (100:11:11:27)	1	0.76	F2

GC-MS analysis

GC-MS examination of column fractions of ethanolic extract of *Alternanthera sessilis* was done to determine the main phytoconstituents that existed in Fraction 1 (F1) and Fraction 2 (F2). Compounds found in F1 included 2,2-dimethoxybutane, (+)-2-bornanone, caryophyllene oxide, whereas F2 contained large bioactive molecules that included eucalyptol, neophytadiene and tau-cadinol amongst others and significant quantity of 3,3-4-5-7-pentahydroxyflavone. Since the flavonoid concentration and larger concentration of known gastroprotective and anti-inflammatory agents were found in F2, Fraction 2 was chosen to investigate *In-vitro* antiulcer activity. Their tables with more detailed profiles ofcomposites are presented in Table 5 and Table 6 and their spectra are presented as Figure 1 and Figure 2.

Figure1: GC-MS spectra of F1 fraction

Table 5: GC-MS profile of *Alternanthera sessilis* ethanolic extract (Fraction 1) showing identified compounds with retention time, molecular details, and peak scores.

Peak No.	Compound Name	Retention Time	Molecular formula	Molecular Weight	Score
1	2,2-Dimethoxybutane	3.19	$C_6H_{14}O_2$	118.17	Area%
2	(+)-2-Bornanone	13.48	$C_{10}H_{16}O$	152.23	0.52
3	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-me	20.19	$C_{15}H_{24}$	204.35	2.30
4	3,3′,4′,5,7-pentahydroxyflavone	20.99	$C_{15}H_{10}O_{17}$	286.23	30.39
5	Naphthalene,decahydro-4a-methyl-1-methyle	22.78	$C_{15}H_{24}$	204.35	28.91
6	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimet	22.94	$C_{15}H_{24}$	204.35	0.69
7	Bicyclo[5.3.0]decane,2-methylene-5-(1-meth	23.21	$C_{15}H_{24}$	204	0.78
8	Caryophylleneoxide	25.09	$C_{15}H_{24}O$	220.35	0.46
9	11,14,17-Eicosatrienoicacid,methylester	37.23	$C_{21}H_{36}O_2$	320.50	30.62

Figure 2: GC-MS spectra of F2 fraction

Table 6: GC-MS profile of *Alternanthera sessilis*ethanolic extract (Fraction 2) showing identified compounds with retention time, molecular details, and peak scores.

Peak No.	Compound Name	Retention Time	Molecular Weight	Molecular formula	Score
1	2,2-Dimethoxybutane	3.20	118.17	$C_6H_{14}O_2$	4.09
2	Eucalyptol	9.98	154	$C_{10}H_{18}O$	7.65
3	Bicyclo[2.2.1]heptan-2-ol,1,3,3-trimethyl-,(1	12.74	220.35	$C_{15}H_{24}O$	1.23
4	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-m	23.35	204.35	$C_{15}H_{24}$	3.44
5	tau.-Cadinol	26.50	222.37	$C_{15}H_{26}O$	9.78
6	Neophytadiene	31.00	278.51	$C_{20}H_{38}$	1.22
7	3,3′,4′,5,7-pentahydroxyflavone	41.35	286.23	$C_{15}H_{10}O_{17}$	72.59

***In-vitro* antiulcer activity**

Acid Neutralizing Capacity

ANC of *Alternanthera sessilis* ethanolic extract (Fraction 2) was considered at different concentrations and comparisons were made with that of standard calcium carbonate (500mg/100mL). Fraction 2 was utilized with a dose effect of acid neutralization and it had the highest ANC of 120 mEq/g at 100 mg concentration. Comparatively, ANC of the standard was 37.8mEq/g. The findings seem to indicate potent acid buffering capacity of the extract at low doses. Values are shown in Table 7 and Table 3 respectively.

Table 7: ANC of *Alternanthera sessilis* Ethanolic Extract (Fraction 2)

Sample	Volume of NaOH (mL)	Neutralizing Capacity (mEq)	ANC (mEq/g)
Standard (Calcium carbonate 500 mg)	43.7	18.9	37.8
100 mg	30.0	12.0	120.0
500 mg	34.8	14.4	28.8
1000 mg	32.8	13.4	13.4
1500 mg	41.4	17.7	11.8

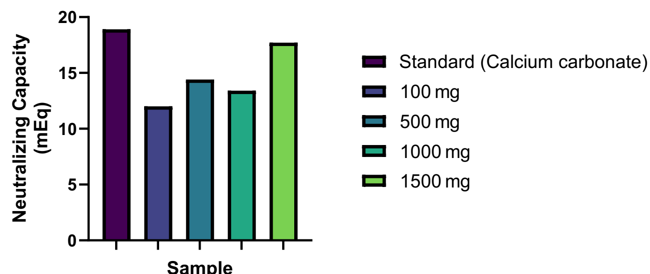

Figure 3: Comparison of Neutralizing Capacity (mEq) of *Alternanthera sessilis* Ethanolic Extract (Fraction 2) at Various Concentrations with Standard Calcium Carbonate.

H+/K+ATPase Action

Alternanthera sessilis in Fraction 2 ethanolic extract inhibitory activity against the H+ /K+ ATPase enzyme was evaluated and compared with controls, omeprazole. The proton pump activity was fractions of inhibition in a concentration-dependent methodby a maximum of 58.90 % inhibition achieved at 100 µg/mLand an IC_{50} value of 91.1 µg/mL. Comparatively, inhibition of omeprazole at the identical concentration was 80.82%by an IC_{50} of 55.9 µg/mL. These results show that extract has a moderate inhibitory outcome on proton pumps. Table 8 and Figure 4 summarize the results.

Table 8: H+/K+ ATPase Inhibitory Activity of *Alternanthera sessilis* Ethanolic Extract (Fraction 2) Compared with Standard Omeprazole

Sample	Concentration (µg/mL)	Mean Absorbance at 660nm	%Inhibition	IC_{50}(µg/mL)
Control		1.46	------	-
Standard (omeprazole)	20	1.23	15.75%	55.9
	40	0.82	43.83%	
	60	0.67	54.10%	
	80	0.46	68.49%	
	100	0.28	80.82%	
Fraction 2	20	1.39	4.79%	91.1
	40	1.27	13.01%	
	60	1.07	26.71%	
	80	0.83	43.15%	
	100	0.60	58.90%	

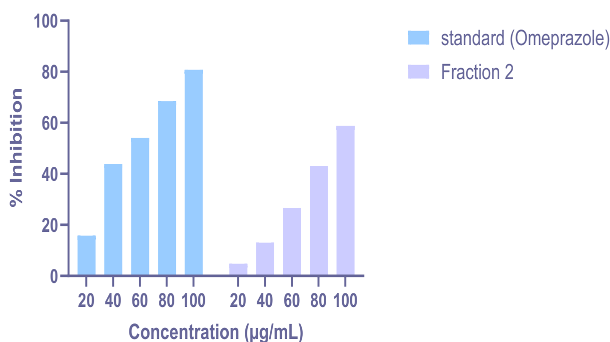

Figure 4: Comparative H^+/K^+ ATPase inhibitory activity of *Alternanthera sessilis* ethanolic extract (Fraction 2) and standard Omeprazole at varying concentrations (20–100 µg/mL).

Na+/K+ ATPase Activity

Inhibitory activity of Na+/K+ ATPase of ethanolic extract of *Alternanthera sessilis*(Fraction 2) was tested and compared with the known standard ouabain. Fraction 2 exhibited dose-dependent inhibition and reached up to 43.50 % at 100 µg/mL and IC_{50} value of 91.1 µg/mL. Comparatively, ouabain showed 80.51 % inhibition at an equivalent concentration with an IC_{50} of 55.9µg/mL. These findings indicate that Fraction 2 has moderate inhibition of Na + /K + ATPase, which adds value to its possible anti-protective activity in the body. Table 9 shows data and Figure 5 illustrates them.

Table 9: Na^+/K^+ ATPase Inhibitory Activity of *Alternanthera sessilis* Ethanolic Extract (Fraction 2) Compared with Standard Ouabain

Sample	Concentration (µg/mL)	Mean Absorbance at 660 nm	% Inhibition	IC_{50} (µg/mL)
Control		1.54	------	-
Standard (Ouabain)	20	1.26	18.18%	55.9
	40	0.91	40.90%	
	60	0.65	57.79%	
	80	0.44	71.42%	
	100	0.30	80.51%	
Fraction 2	20	1.47	4.54%	91.1
	40	1.33	13.63%	
	60	1.21	21.42%	
	80	0.97	37.01%	
	100	0.87	43.50%	

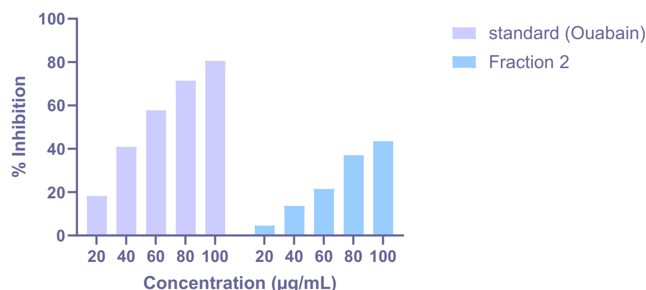

Figure 5: Comparative Na^+/K^+ ATPase inhibitory activity of *Alternanthera sessilis*ethanolic extract (Fraction 2) and standard ouabain at different concentrations (20–100 µg/mL).

DISCUSSION

Aim of current research paper was to explore phytochemical profile and antiulcer activity of flavonoid rich-fraction of ethanolic extract of *Alternanthera sessilis*leaves *In-vitro* against a standard panel of experimental model. These components were then validated using preliminary phytochemical screening (Table 1) that showed presence of different bioactive constituents that synergistically play a gastroprotective and cytoprotective activity[38]. It is remarkable that proteins were not present in the extract and there must have been little interferences by nitrogenous macromolecules in biological tests. The flavonoid content as identified with the help of aluminum chloride colorimetric assay was 0.702 mg/100 g of dried extract (Table 2). This significant content of moderate flavonoid levels is a sign of the possible mechanism of the noted pharmacological effects of these polyphenolic compounds. Flavonoids like quercetin, kaempferol derivatives have been found to possess antiulcer effects by acting at various mechanisms viz. antioxidative effect, prostaglandins stimulation and inhibition of acid release[39]. The choice of appropriate solvent systems was directed with the help of TLC profiling (Table 3), and after fractionation by column chromatography, two major pools were obtained because of pooling by similar Rf values (Table 4). When Fraction 1 and Fraction 2 were subjected to GC-MS analysis (Tables 5 and 6; Figures 1 and 2), it was was found that though both fractions had various phytoconstituents, Fraction 2 however had very high amounts of biologically active compounds like 3, 3, 4, 5, 7-pentahydroxyflavone (a well-known flavonol), tau- cadinol, eucalyptol, and neophytadiene, in it[40]. They are reported to have anti-inflammatory, antioxidant and mucosa-protective effects and it is Fraction 2 that is, therefore, most logical to proceed with testing and verification of this biological activity[41]. As shown in Table 7 and Figure 3 (ANC test), Fraction 2 was clearly a potent buffer to extra gastric acid and strongly Buffered the extra acid in a concentration-

dependent manner. Surprisingly, ANC of extract even stood at 120mEq/g (100mg), higher than the standards calcium carbonate (37.8mEq/g). It shows good acid neutralizing property, especially, at the lower concentrations, which would give instant relief in those ulcerative conditions to increase acidity of gastric secretions and to lower the irritation of mucosal membranes[42].

The extract also exhibited notable activity in the H^+/K^+ ATPase inhibition assay, which models the suppression of acid secretion via the proton pump pathway. At 100 µg/mL, Fraction 2 inhibited the enzyme by 58.90% with an IC_{50} of 91.1 µg/mL, while omeprazole showed 80.82% inhibition and an IC_{50} of 55.9 µg/mL (Table 8, Figure 4)[43]. Although omeprazole remains more potent, the extract displayed substantial enzyme inhibition, likely due to the flavonoids and terpenoids interfering with the enzyme's functional groups, indicating a plausible mechanism of anti-secretory activity.Further, the Na^+/K^+ ATPase inhibition assay (Table 9, Figure 5) demonstrated Fraction 2's ability to modulate ion exchange and maintain mucosal homeostasis, with a maximum inhibition of 43.50% at 100 µg/mL and an IC_{50} of 91.1 µg/mL. In comparison, the standard ouabain achieved 80.51% inhibition (IC_{50} = 55.9 µg/mL). While the extract exhibited moderate activity, its ability to inhibit this membrane-bound enzyme suggests its role in reducing mucosal permeability and preventing proton back-diffusion, thus aiding ulcer healing[44].

CONCLUSION

The current investigation proves that the ethanolic fraction of *Alternanthera sessilis* leaves is rich in flavonoids; therefore, it exhibits great *In-vitro* antiulcer activity by modulating H +/K + ATPase, acid neutralization, and Na+/K+ ATPase. It was demonstrated that these potent bioactives like 3,3, 4, 5, 7 -pentahydroxyflavone and tau-cadinol make it pharmacologically effective as tested through GC -MS profiling. The refined fraction provides a natural, low cost option to chemical ant-acids and user goals include less side effects, greater mucosal protection and multi-target action. Analyzing its positive results may indicate that it can be useful in treating acid-related GI diseases. Nonetheless, additional preclinical studies in-vivo such as reduction of ulcer index, histopathological considerations, and toxicity are required to confirm its relevance to translations and therapeutic safety. These studies will open up the procedure of developing a formula of phytopharmaceutical that is applicable in clinical setting and standardized.

Abbreviations

ANC: Acid Neutralizing Capacity; TLC: Thin Layer Chromatography; GC-MS: Gas Chromatography–Mass Spectrometry; Rf: Retardation Factor; H^+/K^+ ATPase: Hydrogen-Potassium Adenosine Triphosphatase; Na^+/K^+ ATPase: Sodium-Potassium Adenosine Triphosphatase; ATPase: Adenosine Triphosphatase; IC_{50}: Half maximal inhibitory concentration; UV: Ultraviolet; nm: Nanometer; µg/mL: Micrograms per milliliter; mEq: Milliequivalent; mg: Milligram.

Author's Contributions

All author contributed equally

Conflict of interest

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