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Phytochemical Investigation and Invitro Evaluation of Anti Ulcer Activity from The Ethanolic Extract of *Chloris Barbata* (SW)

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ABSTRACT

Medicinal herbs contain a wide variety of bioactive chemical compounds useful for pharmacological studies, *Chloris barbata* belongs to the family of poaceae.

Aim& Objective: This study was aimed to investigate the qualitative phytochemical analysis, and to evaluate the anti-ulcer activity from the *chloris barbata (sw)*

Material and methods: The extracts of *chloris barbata* (sw) were prepared by maceration method using ethanol as solvent; GCMS analysis and Uv spectroscopy were performed. For evaluating the anti-ulcer activity, H+K+ ATPase inhibition assay method were studied in vitro.

Results: Plant extract contains phytochemical constituents includes flavonoids, tannins and saponins, reducing sugar etc., Antiulcer activity, the percentage Inhibition of H+/K+ ATPase activity decreases with increase in concentration of plant extract samples. This proved that the extract has higher ability to inhibit the H+/K+ ATPase enzyme with 85% activity even at concentrations lower than 1 mg/mL.

Conclusion: From this study, the Ethanolic extracts of *chloris barbata* possessing the phytochemicals such as flavonoids, tannins and extracts having the promising potential for antiulcer activity.

KEYWORDS: *Chloris barbata* (sw), phytochemical analysis, GCMS, UV Spectroscopy, antiulcer activity; H+K+ATPase inhibition assay.

I. INTRODUCTION

Plants have always been used as medicine by mankind to treat health-threatening diseases and are still popular to obtain new drug candidates as it is the oldest medical practice for humans. It is known that almost 80% of the populations in developing countries rely on traditional medicine, mainly composing herbal prescriptions[1]

Ulcers are open sores on the skin or mucous membrane. They occur when inflamed dead tissue sloughs off. Ulcers can appear on the surface of the skin or a mucous membrane, leading to a superficial loss of tissue [2].They are most common on the skin of the lower legs and in the gastrointestinal tract, but they can be found almost anywhere on the body.

There are more various types of ulcers, which include mouth ulcer, oesophageal ulcer and peptic ulcer etc. Among these peptic ulcers are common[3]. Peptic ulcers are characterized by inflamed lesions or erosions in the mucosal lining and tissues that protect the gastrointestinal tract. Damage to the protective mucus membrane, which normally shields the oesophagus, stomach, and duodenum from gastric acid and pepsin, results in the formation of peptic ulcer [4].

Chloris barbata is part of the Poaceae family. It is commonly found as a weed in tropical and subtropical regions, including the Indian continent, China, and Southeast Asia[5]. *Chloris barbata* belongs to the Chloridoideae subfamily. The genus Chloris includes 55 species, mainly found in Africa and Australia, thriving in tropical to mild temperate areas, often in dry environments. It is recognized as an invasive weed species and serves as a host for several serious agricultural pests[6]

Chloris barbata, often called swollen finger grass, is a tufted annual grass that can grow about 70 cm tall. Its internodes are longer at the top and shorter at the bottom. The leaves are lanceolate, narrowly linear, and acuminate. The spikes measure 6 cm long and the floral glumes are densely hairy and awned, with oblong grains. This grass is frequently found along cultivated in fields and hilly forest areas[7] In traditional medicine, the whole plant of *Chloris barbata* is used to treat rheumatism[8]. A paste made from the leaves is applied externally for skin disorders[9], while the leaf juice is used for fever, diarrhoea, and diabetes[10]. Traditionally, it has also been used to treat various disease ailments and there is no evidence for treating ulcers. In this study, we have been used to evaluate the anti-ulcer potential of *chloris barbata* (sw)

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II. MATERIALS AND METHODS

Collection and authentication of the plant material: The plant *chloris barbata (*sw) was collected, in and around of Perambalur. The plant was first authenticated by Dr.S. Soosairaj, Department of Botany, St. Joseph's college, Tiruchirappalli. Leaves and roots of *chloris barbata* were cut into pieces, which was shade dried at room temperature for two weeks. The plant was then grinded, to form coarse powder.

Preparation of the plant extract: A 20g of dried powdered sample was subjected to maceration using 200ml of ethanol for extraction. It was macerated for 2 days with occasional stirring. The mixture was filtered through clean muslin cloth and whatmann no: 1 filter paper. Then the filtrate was evaporated to dryness at 40°C.

PHYTOCHEMICAL STUDIES:

The plant extract of chloris barbata, was tested for the preliminary qualitative phytochemical analysis by following standard procedure.

Test for carbohydrates:

Benedict's test: 1ml of extract was added with 1ml of Benedict's reagent and the solution heated for few mins. The colour change was observed. Based on the colour change, presence of carbohydrate as determined.

Test for flavonoids:

1 mL of sample extract was added with 2% sodium hydroxide, Then diluted hydrochloric acid was mixed with the reaction mixture. The disappearance of the yellow colour upon the addition of dilute hydrochloric acid confirms the presence of flavonoids.

Test for alkaloids:

To the 3ml of extract was added with few drops of Mayer's reagent and mixed with dilute.Hcl, and kept aside for 5 mins. The formation of precipitate confirms the presence of alkaloid.

Test for tannins:

To the 2ml of extract, add few drops of 10% ferric chloride solution. The appearance dark bluish black indicates the presence of tannins.

Test for saponins:

2ml of extract was added with equal amount of distilled water, and vigorously mixed to obtain foam. The stable and persistent foam for 5 to 10 mins confirms the presence of saponins.

GC-MS ANALYSIS:

The extract was carried for GC-MS analysis by using the GC-MS instrument. The instrument was operated at ionisation voltage (70eV); an injection volume of1µl was employed at an injector temperature of 280°C. The carrier gas helium at a flow rate of 1ml/min. the oven temperature was initially programmed at 60°C for 3mins and increased at a rate of 6°C/min to 300°C for a holding time of 10 mins. The identification of compounds from the spectral data was based on available mass spectra records (NIST-2008 library).

Anti-ulcer activity

H+K+ATPase inhibition assay

The mucosal layer consisting of parietal cells present in the inner layer of goat stomach was scrapped and it was homogenized with ice – cold phosphate buffer of pH 7.4 with the help of mortar and pestle. After homogenization, the homogenate was centrifuged at 5000 rpm for 20 minutes. The supernatant was collected, and it was subjected to centrifugation at 5000 rpm for 15 to 30 minutes. The pellet (consists of membrane protein) obtained from the centrifugation was resuspended in phosphate buffer and the protein content was estimated by Bradford or Lowry's method using Bovine serum albumin as a standard.

Before centrifugation *After centrifugation*

- Sample Preparation: 20 µL of the pellet was suspended in a solution containing 50 µL of 2mM of magnesium chloride (MgCl2) and 40 mM of Tris – HCl of pH 7.4.
- To the above-mentioned sample, the known concentration of plant extract was added in the range of 1000 –5000 µg/mL and the total volume was made up to 1 mL with Tris – HCl buffer. 100 µL of 2 mM of ATP – disodium salt was added to initiate the process and it was incubated at 37°C for 20 minutes. After incubation, the reaction was terminated by the addition 50 µL of 10 % (w/v) Ice – cold trichloro acetic acid. The same procedure was repeated for different concentrations (1000 - 5000 µg/mL) of Omeprazole (control). The amount of inorganic phosphate from ATP was estimated by measuring the absorbance at 400 nm using ELISA micro plate reader.

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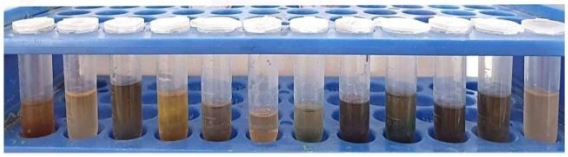
III. Results and discussion:

Phytochemical analysis:

The Ethanolic extract of *chloris barbata* (sw) having the presence of bioactive compounds such as flavonoids, carbohydrates, tannins and saponins as shown in the table.no.1

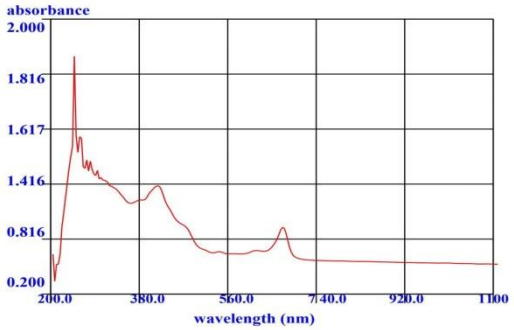
Phytochemicals	observations	Results
Flavonoids	+	Present
Alkaloids	-	Absent
Carbohydrates	+	Present
Tannins	+	Present
Saponins	+	Present
Terpenoids	-	Absent
Phytosterols	-	Absent
Proteins	+	Present
Cardiac glycosides	+	Present

Figure no.1 shows the phytochemical analysis of *chloris barbata* (sw)



There are 15 compounds were identified by GCMS analysis, compounds are phytol, Neophytadiene, Diosgenin, Neodiosgenin acetate, hexadecanoic acid, ethylester,ethyloleate,9,12 octadecadienoicacid (z, z), glycidyl palmitate, beta- Sitosterol, Ergost-5-en-3-ol, (3.beta), Eicosanoic acid ethyl ester,Endo-2-bornyl carbanilate, Cholestan-3-one methoxime,[1,4] Diazepane,1,4-bis (2-methylpyridin-4-yl), 9-octadecenoic acid (z) derivatives.

UV spectroscopy of *chloris barbata* was absorbed at 200-800nm



WAVELENGTH	ABSORBANCE
207.2	0.336
296.5	0.273
376.4	1.573
412	1.373
400	0.482
610.4	0.578
664.4	0.986
663	0.116

Gas chromatography- mass spectroscopy analysis of Ethanolic extract of *chloris barbata*

There are 15 compounds were identified by GCMS analysis, compounds are phytol, Neophytadiene, Diosgenin, Neodiosgenin acetate, hexadecanoic acid, ethyl ester,ethyloleate,9,12octadecadienoicacid(z, z), glycidyl palmitate, beta- Sitosterol, Ergost-5-en-3-ol, (3.beta), Eicosanoic acid ethyl ester,Endo-2-bornyl carbanilate, Cholestan-3-one methoxime,[1,4] Diazepane,1,4-bis (2-methylpyridin-4-yl), 9-octadecenoic acid (z) derivatives.

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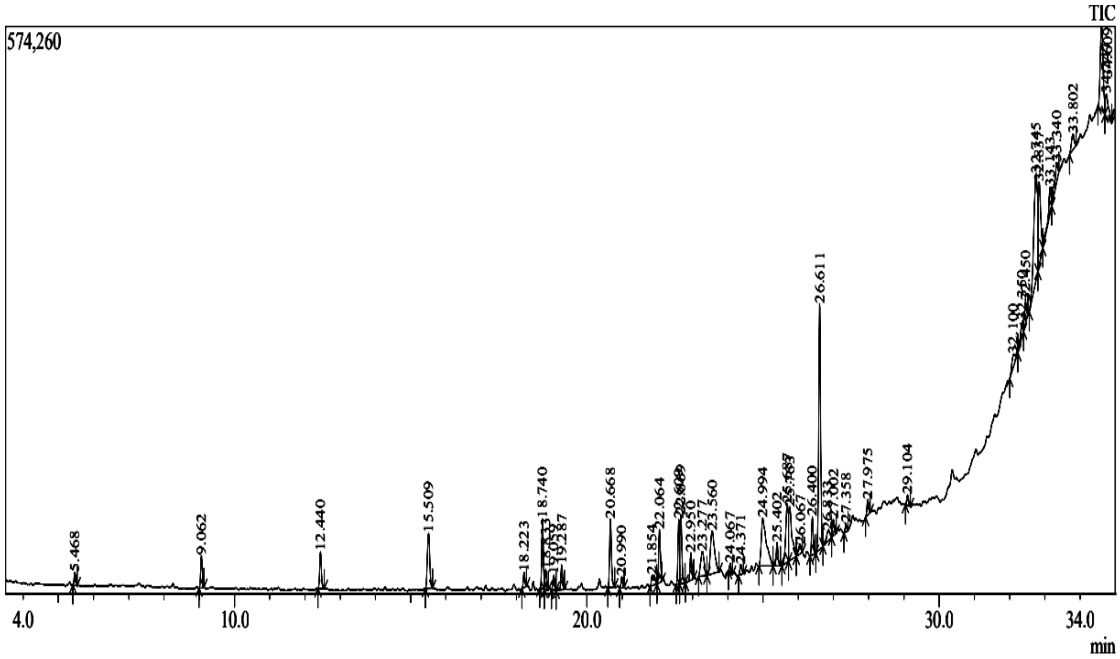
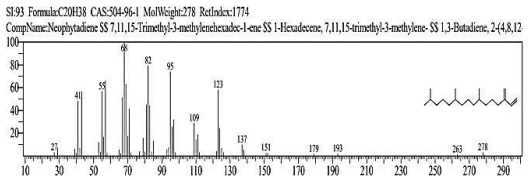
This graph.2 chromatogram shows the Ethanolic extract of *chloris barbata*

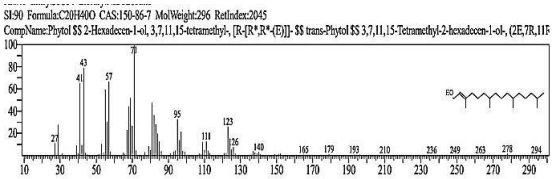


Here the below mentioned table.no.2 shows the presence of bioactive compounds, identified by GC-MS study of Ethanolic extract of *chloris barbata*

S.NO	RT time (min)	Area	Height	Molecular formula	Name of the compound
1.	18.740	227982	70787	$C_{20}H_{38}$	Neophytadiene
2.	20.668	262757	69408	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
3.	22.064	174816	53730	$C_{20}H_{44}O$	Phytol
4.	22.609	207444	64930	$C_{18}H_{32}O_2$	9,12 octadecadienoic acid (z,z)
5.	22.669	258955	68560	$C_{20}H_{38}O_2$	Ethyl oleate
6.	22.950	98204	278051	$C_{22}H_{44}O_2$	Eicosanoic acid, ethyl ester
7.	23.277	164688	24806	$C_{29}H_{44}O_4$	Neodiosgenin(3.beta.,25S)acetate
8.	23.560	355700	43256	$C_{27}H_{42}O_3$	Diosgenin
9.	24.067	24.067	9195	$C_{19}H_{36}O_3$	Glycidyl palmitate
10.	25.687	315008	58925	$C_{29}H_{50}O$	Beta-Sitosterol
11.	26.833	75510	10008	$C_{17}H_{23}NO_2$	Endo-2-bornyl carbanilate
12.	32.450	95049	22210	$C_{17}H_{22}N_4$	[1,4] Diazepene,1,4-bis-(2-methylpyridin-4-yl
13.	33.143	145104	108305	$C_{29}H_{44}NO$	Cholestan-3-one, methoxime
14.	34.609	454119	84675	$C_{28}H_{48}O$	Ergost-5-en-3-ol,(3.beta)-
15.	34.747	140131	22318	$C_{39}H_{74}O_5$	9-Octadecenoic acid(Z), derivative

The below mentioned figures shows the identified bioactive compounds of *chloris barbata* (sw)





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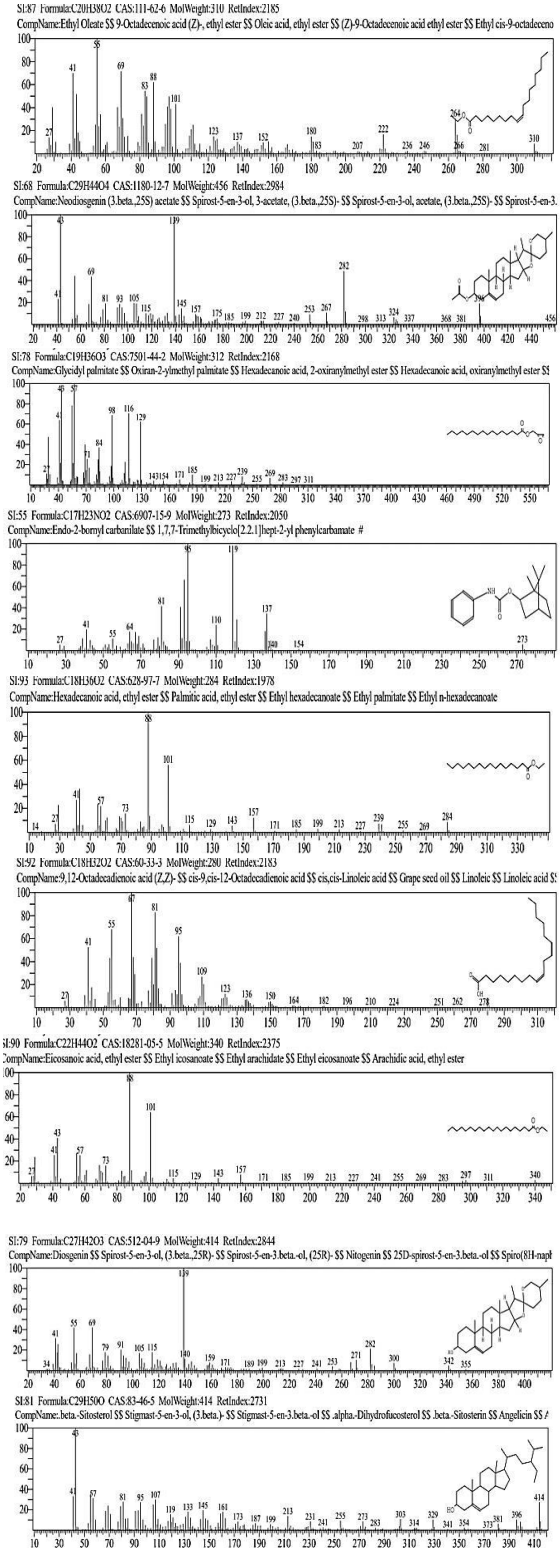
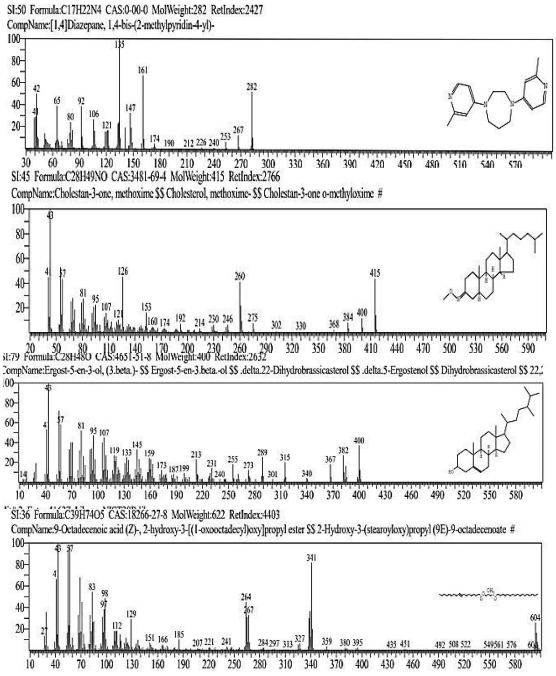
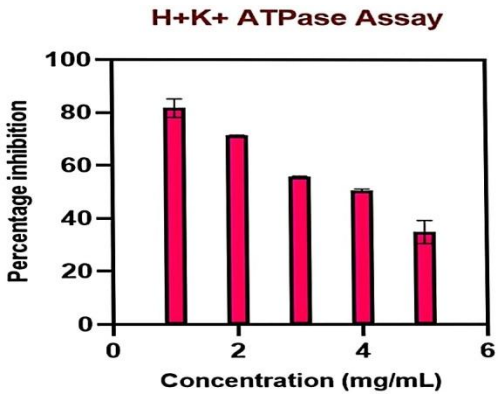




The results of H+K+ATPase inhibition assay for antiulcer activity from the Ethanolic extract of *chloris barbata (sw)*



From the experimental results, it was stated that the percentage Inhibition of H+/K+ ATPase activity decreases with increase in concentration of plant extract samples.

This proved that the extract has higher ability to inhibit the H+/K+ ATPase enzyme with 85% activity even at concentrations lower than 1 mg/mL whereas it highly favours the formation of inorganic phosphate from the ATP than inhibition at concentrations greater than 5 mg/mL.

IV. CONCLUSION

The results of this study, demonstrates that the phytochemicals investigation of *chloris barbata* (sw), may have the presence of

phytochemicals such as flavonoid, tannins, saponins, phenol, and carbohydrates. GCMS analysis of the Ethanolic extract shows the presence of fatty acids and phytol. From these findings, we proved that the Ethanolic extract of *chloris barbata* (sw) having the potential for anti-ulcer activity. On the basis of the results, we conclude that the Ethanolic extract of *chloris barbata* (sw) may be considered as sole source of novel anti-ulcer drugs, detailed study on the isolation of active compounds and their mechanism of action which are responsible for antiulcer activity is to be studied in future.

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