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Phytochemical Screening and Pharmacological evaluation of anti-ulcer activity of ethanolic extract of *Pistia stratiotes*

G. Sreelekha[1], P. Sailaja*[2], Y. Prapurnachandra[3]
[1]Master of Pharmacy, Ratnam Institute of Pharmacy, Pidathapolur(V), Muthukur(M), SPSR Nellore-524346 A.P. India.
[2]Associate Professor, Department of Pharmacology, Ratnam Institute of pharmacy, Pidathapolur(V), Muthukur(M), SPSR Nellore-524346 A.P. India.
[3]Professor & Principal, Department of pharmacology, Ratnam Institute of pharmacy, Pidathapolur(V), Muthukur(M), SPSR Nellore-524346 A.P. India

A B S T R A C T

Peptic ulcer is the most common, chronic gastrointestinal disorder and has become a common global health problem affecting a large number of people worldwide and still a major cause of morbidity and mortality. The anti-ulcer activity of ethanolic extract of *Pistia stratiotes (*araceae) leaves of **EEPS** was investigated in pylorus ligation and ethanol-induced ulcer models in Wistar rats. In this model, the parameter determined was ulcer index **EEPS** at doses of 200,400 mg/kg p.o produced significant inhibition of the gastric lesions induced by pylorus ligation-induced ulcers. The extract (200, 400 mg/kg) showed a significant ($P < 0.001$) reduction in gastric volume, free acidity and ulcer index as compared to the control. This present study indicates that ***Pistia stratiotes*** leaves extract has potential antiulcer activity. These results may further suggest that ethanolic extract was found to possess antiulcer genic as well as ulcer healing properties.

Keywords: EEPS, *Pistia stratiotes*, araceae, anti-ulcer activity, ethanolic extract

A R T I C L E I N F O

Corresponding Author
P. Sailaja
Associate Professor, Department of Pharmacology
Ratnam Institute of Pharmacy,
Pidathapolur (V), Muthukur (M), Nellore-524346, A.P, India.

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CONTENTS

1. Introduction

Peptic ulcer is the most common, chronic gastrointestinal disorder and has become a common global health problem affecting a large number of people worldwide and still a major cause of morbidity and mortality (Chan F.K.L., 2012). Geographically, the disease is prevalent throughout the work, in USA annually 3.7 million people are affected by this disease. Most patients with peptic ulcer disease present with abdominal discomfort, pain or nausea. The goal of therapy for peptic ulcer disease is to relieve symptoms, heal craters, prevent recurrences, and prevent complications. Medical therapy should include treatment with drugs, and attempt to accomplish the following: 1) reduce gastric acidity by mechanisms that inhibit or neutralize acid secretion, 2) coat ulcer craters to prevent acid and pepsin from penetrating to the ulcer base, 3) provide a prostaglandin analog, 4) remove environmental factors such as NSAIDs and smoking, and 5) reduce emotional stress (in a subset of patients).

It is estimated by World Health Organization that, 80% of the world population must rely on traditional medicines for health care; these traditional medicines are mainly plant based. Most of the studies demonstrate the importance of natural products in drug discovery. The use of phytoconstituents as drug therapy to treat major ailments

has proved to be clinically effective and less relatively toxic than the existing drugs.

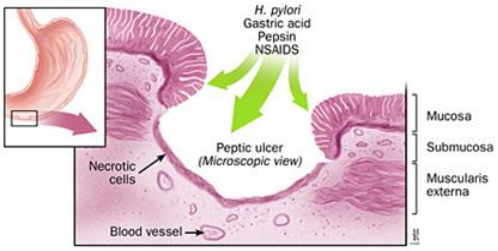

Fig.1: Pathogenesis of peptic ulcer disease.

Fig.2: Plant image of *Pistia stratiotes*

Common Names: Water cabbage, Water lettuce, Nile cabbage, Shellflower

Taxonomical classification

Kingdom : Plantae – Plants
Subkingdom : Angiosperms
Super division : Monocots
Order : Alismatales
Family : Araceae
Genus : Pistia
Species : stratiotes
Binomial name : Pistia stratiotes

Description:

It is a perennial monocotyledon with thick, soft leaves that form a rosette. It floats on the surface of the water, its roots hanging submersed beneath floating leaves. The leaves can be up to 14 cm long and have no stem. They are light green, with parallel veins, wavy margins and are covered in short hairs which form basket-like structures which trap air bubbles, increasing the plant's buoyancy. The flowers are dioecious, and are hidden in the middle of the plant amongst the leaves. Small green berries form after successful fertilization. The plant can also undergo asexual reproduction. Mother and daughter plants are connected by a short stolon, forming dense mats.

2. Materials and Methods

Ethanolic extraction: About 300g of air dried powdered material was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days. At the end of second day the powder was taken out and it was dried. After drying it was again packed and extracted by using ethanol as solvent, till colour disappeared. The temperature was maintained at 55ºC-65ºC. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield of ethanolic extract were noted.

Chemical Tests

A) Test for carbohydrates

1**. Molisch Test:** It consists of treating the compounds of α-naphthol and concentrated sulphuric acid along the sides of the test tube. Purple colour or reddish violet colour was produced at the junction between two liquids. (Kokate, C.K *et al*, 2000)

2**. Fehling's Test:** Equal quantity of Fehling's solution A and B is added. Heat gently, brick red precipitate is obtained.

3**. Benedict's test:** To the 5ml of Benedict's reagent, add 8 drops of solution under examination. Mix well, boiling the mixture vigorously for two minutes and then cool. Red precipitate is obtained.

4. **Barfoed's test:** To the 5ml of the Barfoed's solution add 0.5ml of solution under examination, heat to boiling, formation of red precipitate of copper oxide is obtained.

B) Test for Alkaloids

1. **Dragendroff's Test:** To the extract, add 1ml of Dragendroff's reagent Orange red precipitate is produced.

2. **Wagner's test:** To the extract add Wagner reagent. Reddish brown precipitate is produced.

3. **Mayer's Test:** To the extract add 1ml or 2ml of Mayer's reagent. Dull white precipitate is produced.

4. **Hager's Test:** To the extract add 3ml of Hager's reagent yellow precipitate is produced.

C) Test for Steroids and Sterols

1. **Liebermann Burchard test:**

Dissolve the test sample in 2ml of chloroform in a dry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

2. **Salkowski test:**

Dissolve the sample of test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish red, cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

D) Test for Glycosides

1. **Legal's test:** Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red colour is produced.

2. **Baljet test:** To the drug sample, sodium picrate solution is added. Yellow to orange colour is produced.

3. **Borntrager test:**

Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.

4. **Killer Killani test:**

Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

E) Test for Saponins

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes.1 cm layer of foam indicates the presence of saponins.

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes.1 cm layer of foam indicates the presence of saponins.

F) Test for Flavonoids

Shinoda test: To the sample, magnesium turnings and then concentrated hydrochloric acid is added. Red colour is produced.

G) Test for Tri-terpenoids

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

H) Tests for Tannins and Phenolic Compounds

The Phenol content in the raw material of extract was estimated spectroscopically. To 2-3 ml of extract, add few drops of following reagents:

a). 5% $FeCl_3$ solution: deep blue-black color.

b). Lead acetate solution: white precipitate.

c). Gelatin solution: white precipitate

d). Bromine water: decolouration of bromine water.

e). Acetic acid solution: red color solution

I) Test for Fixed Oils and Fatty acids

1. **Spot test:** Small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

2. **Saponification test:**

Few drops of 0.5N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution. Later the mixture is heated on water bath for 1-2 hours soap formation indicates the presence of fixed oils and fats in the extracts.

J) Test for Gums and Mucilage

Ruthenium red test: Small quantities of extract are diluted with water and added with ruthenium red solution. A pink colour production shows the presence of gums and mucilage.

K) Test for Proteins and Amino acids

1. **Biuret test:** Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.
2. **Ninhydrin test:** Add 2 drops of freshly prepared 0.2% Ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.
3. **Xanthoprotein test:** To the extract, add 20% of sodium hydroxide or ammonia. Orange colour indicates presence of aromatic amino acid.

Acute toxicity study:

This study is needful before pharmacological screening on animals. The acute oral toxicity study was carried out according to OECD 423 guideline (Organization for Economic Cooperation and Development) which is based on a stepwise procedure with the use of a minimum number of animals per step. Absence or presence of compound related mortality of the animal's dose at one step will determine the next step (Ghosh MN, 1984). Healthy, young, male mice were used for this study. Animals were fasted prior to dosing. On next day, the fasted body weight of each animal was determined and the dose was calculated according to the body weight. Animals were divided into 5 groups for giving dose 5, 50, 300, and 2000 mg/kg (Ghosh MN, 1984).Dosing was started with first group and we moved to next group only after confirming that at that dose all animals survived.

Group 1: Three animals were given 5 mg/kg of extract, orally

Group 2: Three animals were given 50 mg/kg of extract, orally

Group 3: Three animals were given 300 mg/kg of extract, orally

Group 4: Three animals were given 2000 mg/kg of extract, orally

Animals were observed individually 30 minutes after dosing, periodically during the first 24 hours and daily thereafter for total of 14 days.

Dose selection:

Dose was selected on the basis of maximum tolerable dose (NOAEL), as there was no lethality observed up to 2000 mg/kg. Thus dose was selected as $1/10^{th}$ and $1/5^{th}$ of 2000 mg/kg, i.e. 200 mg/kg, 400 mg/kg for further investigation.

Evaluation of Anti-Ulcer Activity

Animals: Male Albino rats, weighing 150-200g were used in the present study. All the rats were kept at room temperature ($22^{\circ}C$) in the animal house. All the animals were housed and treated as per the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food and were acclimatized to laboratory conditions. All the experimental procedures were reviewed and approved by Institutional Animal Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals.

Experimental Procedure

Pylorus Ligation Induced Ulcer Formation

Male Albino rats were divided in to five groups of six animals per group and animals were fasted for 24hr prior to the experiment in perforated steel cages to avoid coprophagy. Five groups were made as below.

Group I - received 1% Acacia (1.0ml/kg p.o) as normal control.

Group II - received 1% Acacia (1.0ml/kg p.o) as vehicle control. .

Group III - received (200mg/kg, p.o) ethanol extract of ***Pistia stratiotes***

Group IV - received (400mg/kg, p.o) ethanol extract of ***Pistia stratiotes***

Group V - received (30mg/kg, I.P.) Ranitidine as standard

The pyloric ligation was carried out 1h after the drug administration in each group animals. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. After 4 hrs of pyloric ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out. The gastric juice thus collected was centrifuged and the volume of gastric juice, pH of gastric juice was noted .The stomach was opened along the greater curvature and the severity of hemorrhagic erosions in the acid secreting mucosa was assessed on a scale of 0 to 3 as given below.

Measurement of gastric juice volume and pH

Gastric juice was collected from pylorus lygated rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100g body weight. The pH of the supernatant was measured using digital pH meter. (Canmon DC *et al*., 1969; Kannappan *et al*., 2008; Patil K.S. *et al*., 2008; Paul V. *et al*., 2000).

Ulcer index (UI):

The mucosa was flushed with saline and stomach was pinned on frog board. The lesion in glandular portion was examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined. (Malairajan *et al*., 2007).

0 – Normal coloured stomach
0.5 – Red colouration
1 – Spot ulceration
1.5 – Haemorrhagic streak
2 – Ulcers
3 – Perforations

Calculation of ulcer Index:

U1 = UN + US + UP x 10-1
U1 = Ulcer Index
UN = Average of number of ulcer per animal
US = Average of severity score
UP = Percentage of animal with ulcer

Statistical Analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison test. The values are statistically significant at three levels, ***p<0.001. **p<0.01. *p<0.05. But non-significant if p > 0.05.

3. Results and Discussion

Preliminary Phytochemical Studies

Pistia stratiotes was subjected for hot continuous extraction using ethanol as solvent. The yield for ethanol extract was found to be 10.75% w/w. The extracts obtained were subjected to various phytochemical tests and the results were given in the table.

Table 1: Phytochemical screening of *Pistia stratiotes*

S.No	phytochemical constitunts	ethanolic extract
1	Alkaloids	++
2	Saponins	_ _
3	Tannins	++
4	Terpenoids	++
5	Flavonoids	++
6	Carbohydrates	_ _
7	Cardiac glycosides	_ _
8	Phytosteroids	_ _
9	Amino acids	_ _
10	Gums	_

Pharmacological Studies

Acute oral toxicity studies:

The acute oral toxicity of the ethanolic extract of *Pistia stratiotes* was carried out as per OECD 423 – guidelines (Acute toxic class method). The acute toxicity studies revealed that LD_{50} > 2000mg/kg for the extract. Hence, the biological dose was fixed at 200 and 400mg/kg of body weight for the extract.

Anti-Ulcer Screening

Pylorus lygation induced ulcer in Rats

Effects of ethanol extract of *Pistia stratiotes* on ulcer index by using pylorus lygation induced ulcer method in rats are shown in Table. Pylorus lygation induced gastric damage showed gross mucosal lesion, including long haemorrhage bands and petechial lesion. Animals pretreated with ethanol extract of *Pistia stratiotes* and standard drug Ranitidine showed very mild lesions and sometimes no lesion at all, when compared to ulcer control group. *Pistia stratiotes* showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 26.53 and 53.06 at doses of 200 and 400mg/kg doses respectively.

The ulcer protective action of extracts at different doses was better as that of standard drug, Ranitidine, which exhibited an inhibition percentage of 77.5. Pylorus ligated rats showed severe gastric haemorrhagic lesions. (Mozafar khazaei *et.al*., 2006) The pathogenesis of pylorus ligation induced gastric damage in rats is complicated and involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leucotriene C4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage. (Oates *et.al*., 1988) So the cytoprotective mechanism of the *Pistia stratiotes* extract may therefore include mechanisms other than simple acid neutralization.

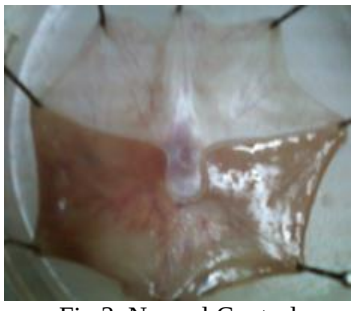

Fig.3. Normal Control

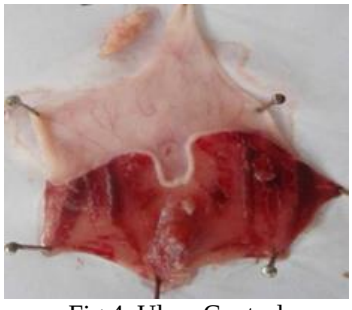

Fig.4. Ulcer Control

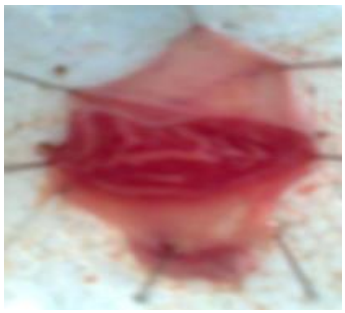

Fig.5. EEPS (200mg/kg)

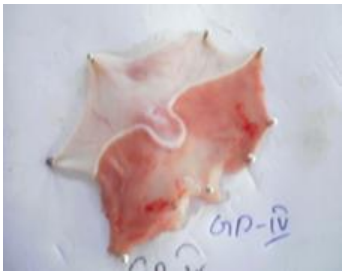

Fig.6. EEPS (400mg/kg)

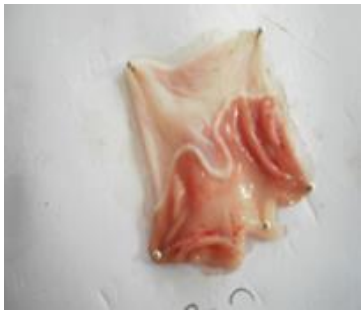

Fig.7. Ranitidine (30mg/kg)

Fig.3-7 Effect of EEPS on pylorus ligation induced ulcer in rats

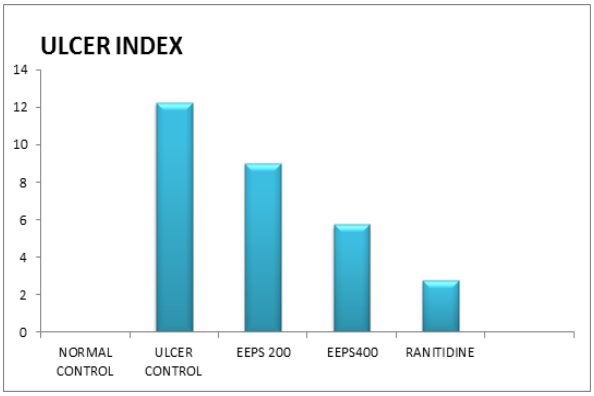

Fig.8: Effect of EEPS on Ulcer Index in pylorus ligated rats

Ulcer index (UI) and acid parameters

The effects of ethanolic extract of *Pistia stratiotes* on acid parameters showed significant effect at 200mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH of the gastric juice was increased compared to ulcer control group. But, in this gastric environment also able to induce ulcer, so it can be thought that the antisecretory activity might not be the main mechanism of action of these extracts.

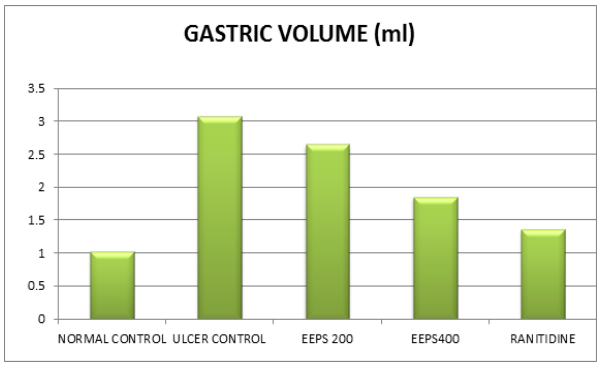

Fig.9: Effect of EEPS on Gastric secretion in pylorus ligated rats

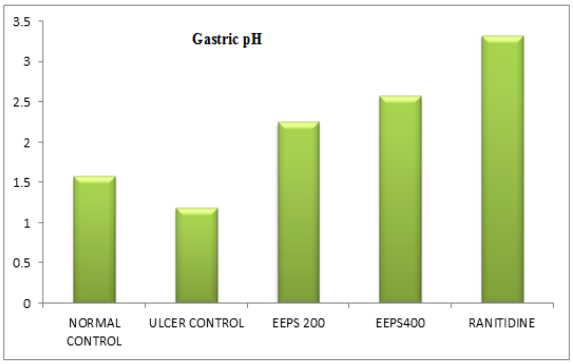

Fig.10: Effect of EEPS on Gastric pH in pylorus ligated rats

Table 1: Design matrix summarizing the levels of 13runs of CCD for optimization of THC loaded LNs.

Group	Ulcer index (UI)	Percentage inhibition (%)
Normal Control	00.00 ± 0.00	-
Ulcer Control	12.25 ± 0.95	-
***EEPS* (200mg/kg)P.O.**	9 ± 0.81**	26.53
***EEPS* (400mg/kg)P.O.**	5.25 ± 0.95***	53.06
Ranitidine (30mg/kg) i.p.	2.75 ± 0.50***	77.5

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. Significant as compared to control P*** < 0.001, P** < 0.05

Table 2: Effect of EEPS on Gastric secretion, pH in pylorus ligated rats

Group	Gastric volume (ml/100g)	pH of gastric juice
Normal Control	1.025±0.29	1.575±0.22
Ulcer control	3.075 ± 0.206	1.175±0.095
***EEPS* (200mg/kg)P.O.**	2.65±0.208***	2.25±0.129**
***EEPS*(400mg/kg)P.O.**	1.85 ± 0.129***	2.575 ± 0.125***
Ranitidine (30mg/kg)I.P.	1.35 ± 0.2***	3.32 ± 0.309***

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. Significant as compared to control P*** < 0.001, P** < 0.05.

4. Conclusion

The present study was undertaken to determine the antiulcer activity of the ethanol extract from the leaves of *Pistia stratiotes*. The preliminary phytochemical investigation showed the presence of alkaloids, flavonoids, terpenoids, tannins, cardiac glycosides, gums and phytosteroids. The pharmacological and acute toxicity studies of ethanol extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed (3 days) up to 2000mg/kg of body weight. The phytoconstituents like flavonoids, tannins and terpenoids, have been reported in several anti-ulcer literatures as possible gastroprotective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which antiulcerogenic efficacy has been extensively confirmed. (Borelli F. *et al.*, 2000). It is suggested that these compounds will be able to stimulate mucus, bicarbonate and prostaglandin secretion, and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. (Suja pandian *et. al*., 2002) Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants. (Berenguer B *et.al*., 2005) Similarly, the ethanol extract of *Pistia stratiotes* showed the presence flavonoids and their glycosides, tannins and triterpenoids. These phytoconstituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by pylorus ligation. *Pistia stratiotes* showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 26.50 and 53.06at doses of 200 and 400mg/kg doses respectively. The ulcer protective action of extracts at 400mg/kg was good to that of standard drug, Ranitidine, which exhibited an inhibition percentage of 77.50.

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