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IN-VIVO ANTI-ULCER SCREENING OF METHANOLIC EXTRACT OF *AMARANTHUS SPINOSUS*

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Keywords:

Amaranthus spinosus, Amaranthaceae, Antioxidant, Antiulcer, Extraction

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ABSTRACT: Background and Aim: The Amaranthaceae family have gained attention for their potential antiulcer properties. Peptic ulcers, a prevalent gastrointestinal disorder, necessitate the exploration of alternative therapies, and Amaranthaceae species may offer promising leads in this regard. Therefore, we have selected *Amaranthus spinosus* from the Amaranthaceae family for the evaluation of antioxidant and antiulcer properties. **Method:** The plant material was successively extracted by the Soxhlet extraction technique with Petroleum ether, chloroform, methanol, and water. The antioxidant activity of the plant extracts and the standard drugs were based on consideration of the radical scavenging influence of the stable "1,1-diphenyl-2-picrylhydrazyl" (DPPH) free radical action and hydrogen peroxide process. Two models, and non-steroidal anti-inflammatory induced ulcer and pyloric ligation, were analysed for methanolic extract of *Amaranthus spinosus* for its antiulcer activity. **Result:** The phytochemical examination of methanolic extracts reveals the presence of triterpenoids, flavonoids, alkaloids, tannins, glycosides, and proteins. The antioxidant study results revealed that among all the four extracts, the MEAS possesses significant antioxidant potential. Compared with control, there were a significant ($p<0.05$) decrease in gastric volume, total and free acidity in the extract-treated classes and standard class. The MEAS revealed significant ($p<0.05$) percent inhibition, i.e. 59.42 and 69.59 percent with 500 mg mL-1 PL and NSAID, respectively. **Conclusion:** The present research findings demonstrated that the methanolic extract of *Amaranthus spinosus* possesses significant antioxidant and antiulcer activity.

INTRODUCTION: Traditional knowledge about herbal remedies in India has contributed enormously towards the worldwide development of a very reliable complementary and alternative system of medicine.

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A peptic ulcer is a remitting, relapsing wound that is most often recognized in center-aged to older adults which impairs the high-quality of lifestyles; it's miles one of the leading reasons for gastrointestinal surgical operation with excessive morbidity and mortality fees [1].

The pathogenesis of ulcers consists of mainly competitive factors (acid, pepsin, bile, and Helicobacter pylori infection) which can be complemented through factors which include a demanding lifestyle, alcohol intake, smoking, use of steroidal and nonsteroidal anti-inflammatory

drugs (NSAIDs) and lower socioeconomic fame [2, 3, 4, 5]. Further to allopathy capsules, herbal flowers are also well-liked within the anti-ulcer drug market due to their safer ability, effectiveness, and comfort. Although ulcer is not a deadly disease, it can lead to more serious complications like gastrointestinal bleeding, perforations, penetration of ulcer into adjacent organs and gastric outlet obstruction [6]. Medications are used to relieve the pain, heal ulcerations and delay recurrence of ulcerations. These include antibiotics [1], antacids and proton pump inhibitors [7]. Several drugs are available in the market for gastric ulcer therapy; however, most of these drugs are associated with unwanted side effects [8].

Amaranthaceae is a family of flowering plants that includes numerous species with diverse traditional uses in folk medicine. Over the years, various members of the Amaranthaceae family have gained attention for their potential antiulcer properties. Peptic ulcers, a prevalent gastrointestinal disorder, necessitate the exploration of alternative therapies, and Amaranthaceae species may offer promising leads in this regard [9]. Therefore, we have selected *Amaranthus spinosus* from the Amaranthaceae family for the evaluation of antioxidant and antiulcer properties.

MATERIALS AND METHODS:

Chemicals: DPPH (1,1-diphenyl-2-picrylhydrazyl) obtained from Sigma Chemical Co. was the drug and chemical used. All the reagents used were of analytical grade obtained from S.D Fine Chemicals, Ltd, and Hi Media, Mumbai.

Plant Materials: The plant materials were collected from roadside areas of Madhya Pradesh, India. They were positively identified by Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra". The voucher specimen of the plant has been deposited for future identification.

Preparation of Plant Extracts: Immediately after collection, to eliminate the exterior soil and undesirable things, the plant material was washed and cleaned twice in tap water and once in distilled water. The whole plant was dried in the shade for 72 h. The plant content was successively extracted with petroleum ether, chloroform, methanol, and water using Soxhlet's device. The extract of Soxhlet was evaporated over a rotary evaporator at 60°C to dryness and the yield was recorded. The extract collected were labelled as: PEAS: Petroleum ether extract of *Amaranthus spinosus*; CEAS: chloroform extract of *Amaranthus spinosus*; MEAS: Methanol extract of *Amaranthus spinosus*; WEAS: Water extract of *Amaranthus spinosus.*

Antioxidant Study:

DPPH Solution (0.1 mM): By dissolving DPPH (33 mg) in one litre of methanol of analytical grade, the DPPH solution was prepared and placed in an amber-coloured bottle to shield it from sunlight.

Ascorbic Acid: A 100 µg/ml stock solution was prepared by dissolving 10 mg of ascorbic acid in 100 ml of distilled water. This solution prepared 10, 20, 40, 80, 100 and 200 µg/ml of ascorbic acid.

Sample Preparation: A 1 mg/ml stock solution was prepared by adding 10 mg of different *Amaranthus spinosus* extracts to 10 ml of methanol. Solutions of different extract concentrations, such as 10, 20, 40, 80, 100, 200 µg/ml were then prepared from the stock solution.

DPPH Radical Scavenging Assay Method: An antioxidant activity of plant extracts was evaluated on the basis of a radical scavenging effect *via* the modified process of stable DPPH-free radical activity [10]. The optical density was reported and the formula depicted below calculated the percent inhibition [11].

$$\text{Percentage inhibition of DPPH activity} = (A-B) / A \times 100$$

Where A = the blank absorbance and B = the sample absorbance. Compared to positive controls, the real reduction in absorption caused by the test was compared. The IC50 (50 percent inhibition concentration) values were determined using the linear dose inhibition curve by plotting the concentration of the extract versus the corresponding scavenging effect.

Hydroxyl Peroxide Method: Various concentrations of methanolic extract (10-200 µg/mL) were applied to of 0.1 M phosphate buffer of pH 7.4 (2.4 mL) and combined with 43 mM hydrogen peroxide solution (0.6 mL). At 230 nm after 10 min, the optical density was measured. A

blank sample was carried out. The standard drug used was ascorbic acid.

Antiulcer Property:

Animals: Wistar albino male rats (160 to 200 g), collected from the institutional facility of the animal house, were randomly allocated into 5 classes of 6 animals. They were housed in "polypropylene cages" over husk bedding and unless otherwise specified, provided with regular pellet feed and water "*ad libitum"*. With a 12-hour dark and light period, the animals were held at 25 ± 2 °C. Animal experiments were conducted after approval by the "Institutional Animal Ethics Committee" (IAEC) and in full agreement with its guidelines.

Antiulcer Activity Evaluation: Experimental setup for "pyloric ligation induced gastric ulcer". The animals were allocated into 5 classes, each consisting of 6 rats.

Class I: Vehicle (0.9% w/v, p.o normal saline.) administered 1 h prior to pyloric ligation on the day of the procedure.

Class II: For ulcer induction, rats are subjected to pyloric ligation.

Class III: MEAS (250 mg/kg, p.o.) administered 1 h before pyloric ligation on the day of the procedure.

Class IV: MEAS (500 mg/kg, p.o.) administered 1 h before pyloric ligation on the day of the procedure.

Class V: Standard administration (ranitidine 50 mg/kg, p.o.) 1 h before pyloric ligation on the day of experimentation.

Ulcers were caused after 18 hours of fasting and the experimentation was performed as per given in the study of Baliyan *et al.*, 2022 [10] and Devhare and Gokhale 2022 [11].

Similarly, for "NSAID-induced ulcer model" experimental design, the animals were allocated into five classes, with 6 rats each as follows-

Class I: Vehicle administered (0.9 percent w/v, p.o normal saline) 30 min prior ulcers caused by Indomethacin.

Class II: Indomethacin-administered disease control group (25 mg/kg, p.o.) for gastric ulcers induction.

Class III: 250 mg/kg, p.o. MEAS was administered 30 min before ulcers caused by Indomethacin.

Class IV: 500 mg/kg, p.o MEAS administered 30 minutes before ulcers caused by Indomethacin.

Class V: Standard administration (50 mg/kg, p.o. ranitidine) 30 min prior ulcers caused by Indomethacin

According to Tripathi *et al* 2021 [12], the experimental design was carried out for the NSAID-induced ulcer model.

Estimation of Gastric Volume: Stomachs were dissected four hours after ligation and material was obtained to determine the amount of gastric content in the measuring cylinder.

Measurement of Total Acidity and Free Acidity: The gastric content was centrifuged and underwent to titration for calculation of total and free acidity. The 1 ml of supernatant liquid was dissolved in distilled water and pipetted out (10 mL). Using "Topfer's reagent" as an indicator, the liquid was then titrated against 0.01N NaOH to the equivalence point where the solution converted orange in colour. With the addition of 1 percent phenolphthalein solution till the liquid obtained pink colour, titration was further carried out. The appropriate volume of NaOH was recorded and taken as referring to the overall acidity. Absolute acidity was referred by the addition of two titrations [13].

Ulcer Index: As per the stated technique given by Al-Thobaiti *et al.,* 2022 [14], the number of ulcers was counted and scoring was carried out. The percentage of ulcer defence was measured using the formula given by Joshi *et al.,* 2022 [15].

Statistical Analysis: The data was interpreted as "mean ± SEM" and the "p value<0.05" was considered to be significant. The data was subjected to a "one-way analysis of variance" (ANOVA). "Tukey's multiple range tests" evaluated data to measure the significance level of

mean differences between different treated classes using a statistical package of SPSS statistics (Version 7.5).

RESULTS:

Preliminary Phytochemical Analysis: The phytochemical examination of methanolic extracts reveals the presence of triterpenoids, flavonoids, alkaloids, tannins, glycosides, and proteins. The Chloroform extracts contain flavonoids, triterpenoids, tannins and carbohydrates. The water extracts contain alkaloids, glycosides, flavonoids and triterpenoids. It was therefore further examined for different activities like antioxidants and antiulcer.

Antioxidant Activity: The antioxidant activity of different solvent extracts of *Amaranthus spinosus* was carried out by in vitro models of Hydrogen peroxide and DPPH radical methods at different concentrations ranging from 10 to 200 µg/ml. The results of the DPPH method revealed the percent inhibition at 200 µg/ml for MEAS extracts was 58.67±1.95 **Table 1**, and shows a significant decrease ($p<0.05$) in the DPPH radical concentration because of the scavenging capacity of MEAS compared to the standard. The optimal scavenging capability was 60.42±0.12 for the H2O2 method at 200 µg/mL, as depicted in **Fig. 1**. A comparison between these two methods demonstrated a significant ($p<0.05$) higher antioxidant capacity of the MEAS.

The antioxidant study results revealed that among all the four extracts, the methanolic extract (MEAS) possesses significant antioxidant potential. Hence for further study, the same extract is undertaken for antiulcer potential.

TABLE 1: PERCENT INHIBITION OF EXTRACTS BY USING DPPH RADICAL SCAVENGING ASSAY

S. no.	Samples	Percent Inhibition					
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
1	PEAS	1.53±0.44	4.57±0.69	9.65±1.83	13.36±1.32	17.8±2.29	21.13±1.37
2	CEAS	1.85±0.85	2.55±0.85	3.56±0.45	7.56±0.25	9.25±0.56	13.25±1.25
3	MEAS	3.006±0.83	3.74±0.18	9.85±1.94	17.80±1.44	29.45±1.67	60.21±3.005
4	WEAS	4.33±0.37	7.25±1.23	11.23±1.23	18.16±1.46	29.98±1.26	45.36±0.13
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
5	Ascorbic Acid	27.96±0.21	37.14±0.38	50.51±3.21	57.29±0.25	63.42±1.27	72.13±1.5

The values are expressed as Mean±SEM.

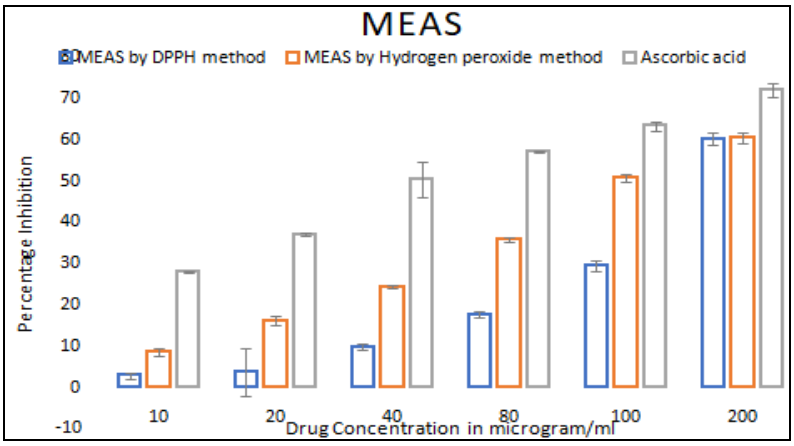

FIG. 1: PERCENT INHIBITION OF MECT BY HYDROGEN PEROXIDE AND DPPH METHOD

Antiulcer Activity: Two models of non-steroidal anti-inflammatory and pyloric ligation mediated ulcer were used for the ulcerogenic effect of MEAS. In the case of pylorus ligation, gastric volume, free and total acidity were observed in the control class. The extracts provided a significant reduction ($p<0.05$) in the ulcer score relative to the control score **Table 2**. Ranitidine followed by MEAS lowered the ulcer index (250 and 500 mg mL^{-1}, respectively). Compared with control, there were a significant ($p<0.05$) decrease in gastric volume, total and free acidity in the extract-treated

classes and standard class. The MEAS revealed significant ($p<0.05$) percent inhibition, i.e. 59.42 and 69.59 percent with 500 mg mL-1 PL and NSAID, respectively, as shown in **Table 3.**

TABLE 2: INFLUENCE ON GASTRIC VOLUME, TOTAL ACIDITY AND FREE ACIDITY IN PL INDUCED ULCERS

Group	Dose (mg/kg)	Gastric Volume (ml/100 gm)	Total Acidity (mEq/L/100gm)	Free Acidity (mEq/L/100gm)
Normal	-	4.29±0.68	74.68±0.93	42.36±0.56
Control	-	8.01±0.98[a]	98.56±1.25[a]	55.02±0.91[a]
MEAS	250	3.02±0.25[b]	70.89±1.02[b]	39.23±0.37[b]
MEAS	500	1.97±0.56[c]	62.85±1.49[c]	35.26±0.45[c]
Ranitidine	50	1.49±0.85[b]	57.29±1.78[b]	29.49±0.39[b]

"Data presented as Mean±SEM, n = 6; [a]$p<0.05$ compared to control group, [b]$p<0.05$ compared to treated group, [c]$p<0.05$ compared to ranitidine group".

TABLE 3: INFLUENCE OF MEAS ON PERCENTAGE INHIBITION AND ULCERATIVE INDEX IN PL AND NSAID

Group	Dose (mg/kg)	Ulcer index		Percentage inhibition	
		PL	NSAID	PL	NSAID
Normal	-	0.78 ± 0.05	0.78 ± 0.05	-	-
Control	-	8.28 ± 0.42[a]	15.23 ± 1.29[a]	-	-
MECT	250	5.27 ± 0.64[b]	7.56 ± 0.87[b]	36.23	50.36
MECT	500	3.36 ± 0.52[c]	4.63 ± 0.61[c]	59.42	69.59
Ranitidine	50	3.03 ± 0.49[b]	4.29 ± 1.19[b]	63.40	71.83

"Data presented as Mean±SEM, n = 6; [a]$p<0.05$ compared to control group, [b]$p<0.05$ compared to treated group, [c]$p<0.05$ compared to ranitidine group".

DISCUSSION: Peptic ulcers, including gastric ulcers and duodenal ulcers, result from an imbalance between aggressive factors, such as gastric acid secretion, and defensive mechanisms, including mucosal integrity and blood flow [1]. The primary causative factor historically identified was excessive acid secretion leading to mucosal damage. However, the discovery of Helicobacter pylori infection and the widespread use of nonsteroidal anti-inflammatory drugs (NSAIDs) expanded our understanding of ulcer pathogenesis. *H. pylori* colonization and NSAID-induced mucosal injury disrupt the mucosal barrier, allowing acid and other aggressive factors to damage the underlying tissue, leading to ulcer formation [16].

The management of peptic ulcers often involves the use of conventional medications such as proton pump inhibitors (PPIs), antibiotics, and cytoprotective agents. However, herbal drugs have long been employed in traditional medicine systems worldwide for the treatment of ulcers. In the current investigation, pre-treatment of pylorus-ligated rats with ranitidine (group V) and 500 mg per kg MEAS (group IV) showed equivalent antiulcer action, as indicated by a decrease in the rise in stomach volume, pH, and free and total acidity. "The methanol bark extract of *Mimus opselengi L. (Sapotaceae)* [17] and flower extract of *Hemidesmus indicus R. Br. (Asclepia daceae)* [18] were reported to reduce total acidity and volume of gastric acid secretion in gastric ulcer-induced rats, and this antiulcer activity was attributed to the strengthening of the mucosal defence mechanism by these plant extracts". According to Suzuki and Ishii (1996) [19], "an increase in bicarbonate ion concentration plays an important role in protecting the gastric and duodenal mucosa from hydrochloric acid, and the mucosal defence mechanism may be due to the epithelial cells of the gastric mucosa, which are impermeable to hydrogen ions, forming a physical barrier [20].

Furthermore, protection against experimental ulcers may be related to the activity of prostaglandins in parietal cells, since they promote mucosal resistance, perhaps by increasing mucus and bicarbonate production and reinforcing the mucosal barrier" [21, 22]. Based on these findings, it is hypothesized that the anti-ulcer action of MEAS reported in the present investigation is attributable to the strengthening of the mucosal defence system. Prior studies have shown that the whole plant has different pharmacological activities such as anti-inflammatory [23], Antibacterial activity [24],

Antifertility activity [25], Phytochemical investigation showed that amaranthine is included as a marker constituent, while Quercetin, Kaempferol and Isoquercitrin are also included [26]. The separation of flavonoids from *Amaranthus spinosus* by several investigators established the framework for the validation of the antioxidant potential of the whole plant [27, 28]. The modes of action included in the defence of cellular processes by the MEAS from oxidative stress is not fully understood. The models such as DPPH and hydrogen peroxide were therefore used to evaluate antioxidant function of *Amaranthus spinosus*. At 517 nm, the free radical DPPH gives high maximum absorption and is purple in colour. DPPH is reduced to DPPH-H when the radical electron is combined with hydrogen which leads to a purple to yellow colour transition [29]. In the present investigation, the highest activity was measured at 200 µg/mL in the case of the hydrogen peroxide process. The findings revealed optimum antioxidant capacity, i.e. 60.21±3.005 by MEAS by DPPH method. While 58.69±0.23 percent inhibition by MEAS by hydrogen peroxide method, concluding that extract demonstrated an outstanding activity of free radical scavenging.

CONCLUSION: The present research findings demonstrated that the methanolic extract of *Amaranthus spinosus* possesses significant antioxidant and antiulcer activity. The antiulcer activity of MECT results in a reduction in gastric acid secretion; inhibition of free radical generation or lipid peroxidation prevention; defence of the mucosal barrier and mucosal secretions restoration; and free radical scavenging or antioxidant properties. Furthermore, "non-steroid anti-inflammatory induced ulcer and pylorus ligation induced gastric ulcer" were examined for antiulcer activity, which showed a potent anti-ulcer effect compared to normal. These findings include pharmacological evidence and support the potent antioxidant and antiulcer agent *Amaranthus spinosus*. In addition, further experiments can be carried out to determine the drug's toxic and non-toxic nature.

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CONFLICT OF INTEREST: Declared None

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