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EVALUATE THE ANTI-ULCER ACTIVITY OF AERIAL PART OF *CANSCORA DECUSSATA* EXTRACT IN WISTER ALBINO RAT

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ABSTRACT

Ulcer can be developed inside the inner lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer). Both the ulcers are also cumulatively referred as peptic ulcers. It affects nearly 10% of world population. The present study was to investigate the antiulcer activity of methanolic extract of *Canscora decussata* (MECD) on albino Wistar rats. The present study was carried by pylorus ligation ulcer models in albino Wistar rats. The antiulcer activity of MECD (100 and 200) was compared with standard drugs (Ranitidine). In pyloric ligation induced ulcer model, the studied parameters were gastric volume, pH, total acidity, free acidity, and ulcer index was determined for severity of ulcers. In pyloric ligation model the volume of gastric content, total/free acidity and pepsin activity was significantly decreased at $p<0.05$ and $p<0.01$ and pH of the gastric juice was significantly increased at $p<0.05$ and $p<0.01$ in MECD treated groups as compared to control group. All the doses of MECD showed dose dependent antiulcer effect as well as significant ($p<0.05$ and $p<0.01$) reduction in the ulcer index as compared to control group in all the experimental models. The results of the study indicate that the MECD have better potential against ulcer which supports the traditional claims in folklore medicine.

Keywords: *Canscora Decussata,* Antiulcer Effect, Pylorus Ligation, Ulcer Index.

I. INTRODUCTION

Herbal medicine, also known as botanical medicine or phytomedicine, involves utilizing various parts of plants such as seeds, berries, roots, leaves, bark, or flowers for medicinal uses. Since ancient times, individuals globally have utilized herbal medicine for treating, controlling, and managing various health conditions. Abundant archaeological proof suggests that early humans utilized plants and herbs for medicinal reasons. An example is the examination of pollen from various plants discovered in the burial site of a Neanderthal man in Iraq 60,000 years ago revealed that all the plants placed with the body had medicinal properties. Medicinal herbs discovered in the possessions of the frozen "Ice man" in the Swiss Alps for over 5300 years, are believed to have been utilized for treating the parasites in his intestines [1].

A peptic ulcer is a wound in the digestive system caused by acid, typically found in the stomach or upper part of the duodenum. It features bare mucosa, with the damage reaching into the submucosa or muscularis propria. Recent epidemiological studies have indicated a reduction in the occurrence, hospitalization rates, and death rates linked to peptic ulcer, despite the estimated prevalence of the disease in the general population being 5-10%. This is probably due to the development of new treatments and better hygiene, leading to a decrease in Helicobacter pylori (H. pylori) infections [2, 3].

Historically, it has been believed that the mucosal damage seen in individuals with acid peptic disease is caused by excessive acid production, along with dietary habits or stress. Factors that increase the risk of developing peptic ulcers include H. pylori infection, alcohol and tobacco use, taking NSAIDs, and having Zollinger-Ellison syndrome [4, 5]. The primary factors that pose a risk for both gastric and duodenal ulcers are H. pylori infection and the use of NSAIDs. Yet, only a small percentage of individuals who have H. pylori or take NSAIDs will develop peptic ulcers, highlighting the significance of individual susceptibility in the initiation of mucosal damage. Peptic ulcers are linked to functional variations in various cytokine genes. An instance would be polymorphisms in interleukin 1 beta (IL1B) which impact mucosal production of interleukin 1β, leading to H. pylori-related gastroduodenal diseases [6].

Conversely, the likelihood of peptic ulcer complications is multiplied by four in NSAID users and doubled in aspirin users [7]. Using NSAIDs or aspirin together with anticoagulants, corticosteroids, and selective serotonin reuptake inhibitors can raise the likelihood of upper gastrointestinal bleeding [8]. While there are a lot of individuals who have both H. pylori infection and use NSAIDs or aspirin, the role they play together in causing

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peptic ulcer disease is still a topic of debate. An analysis of multiple studies found that NSAIDs, aspirin use, and H. pylori infection all independently raise the risk of peptic ulcer disease.

Peptic ulcer disease classified as an idiopathic ulcer can be diagnosed in approximately 20% of cases when patients are negative for H. pylori, NSAIDs, and aspirin. The imbalance between factors contributing to mucosal integrity and aggressive insults causes idiopathic peptic ulcers, but the pathogenic mechanisms remain unidentified [9]. A study conducted in Denmark found that emotional stress may raise the likelihood of developing peptic ulcers [10]. Additional causes include blood flow restriction, medications like steroids and chemotherapy, radiation therapy, viral infections, histamine, eosinophilic infiltration, surgical procedures like gastric bypass, and metabolic imbalances [11].

In India, the plant *Canscora decussata Schult*. is commonly referred to as "Shankhpushpi" and can be found all over India, reaching heights of up to 1300 m. It is cultivated in Sri Lanka and Myanmar as well. It is a highly branched, one-year plant that reproduces through seeds. This plant blooms from October to December and is grown in gardens for its decorative flowers. This is a tall yearly plant with a stem that has four wings and is half a meter long, with branches arranged in opposite pairs. It thrives in wet environments. The leaves are without stalks, 2.5-4 cm long, lance-shaped, arranged in pairs with 3 distinct vertical lines; the flowers are found in the leaf axils, single, and either white or yellowish. Both the whole plant and its fresh juice are utilized in traditional medicine to treat insanity, epilepsy, and nervous debility. This plant has bitter compounds and an oleoresin. Additionally, triterpines, alkaloids, and xanthones have been discovered in it. Furthermore, it serves as a natural provider of pentaoxygenated, hexaoxygenated, and dimeric xanthones [12, 13].

The main aim of the preset study was to evaluate the anti-ulcer activity of aerial part of *canscora decussata* extract in wister albino rats.

II. MATERIALS AND METHODS

Collection and authentication of plant material:

The plant *canscora decussata* was collected from Vatemla town, near Vemulawada, Rajanna Sircilla district, Telangana State, India. The plant material was authenticated by……..

Preparation of different *canscora decussata* extracts

The aerial components of *canscora decussata* were dried in shade at room temperature followed by reducing them to a fine powder using a mixer grinder. Next, the powder was taken out using a soxhlet apparatus in the order of petroleum ether, chloroform, ethanol, and water. The sample was put into the beaker that was weighed beforehand and evaporated on a water bath at 50^{o}C until it became a thick paste to produce the extracts. After air drying the extracts completely to eliminate all solvent residue, the percentage yield was determined before moving on to the next solvent extraction. The marc was dried in a hot air oven at temperatures below 50^{o}C, and each extract was concentrated by distilling the solvent and evaporating it on a water bath to obtain the final extracts.

These samples were kept in sealed containers in a refrigerator at a temperature lower than 10 degrees Celsius. The samples were analyzed for their hue and texture. The percentage yield was determined based on the air-dried powder sample utilized in the extraction process. [14].

Percentage yield

The percentage of yield was calculated using the following formula: -

Yield (g/100 g) = $(W_1 \times 100)/W_2$

Where,

W_1 = weight of the crude extract residue obtained after solvent removal

W_2 = weight of plant powder packed in the Soxhlet

Preliminary phytochemical screening:

Preliminary phytochemical tests were performed on *canscora decussata* extracts using petroleum ether, chloroform, ethanolic, and aqueous solvents to identify phytoconstituents qualitatively. Standard methods were followed for conducting the tests. All chemicals and reagents utilized were of analytical quality. [15]

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Experimental animals

Institutional Animal Ethical Committee (IAEC) has given its approval to the experimental protocol with ethical clearance No: …… Adult, healthy, male Albino-Wistar rats with average weight of 150-200gms were acquired. Animals have been provided with 24-hour access with water and standard nutritional pellets, prior to and during the treatment. They were acclimatized under a time period of one week under approved laboratory environment, i.e., $25^0C \pm 1^0C$ temperature, 45-55% RH and also free access to food and water, after which they have been employed in the experiment.

Dose selection

From the literature review 100 and 200mg/kg doses were selected.

Anti-Ulcer activity

Pylorus ligated induced ulcer model in experimental rats

In this approach, albino rats were not fed for 24 hours while receiving ESIL, a reference drug, and a control vehicle 1 hour before pyloric ligation. Next, the animals that had been pre-treated were put under anesthesia using ether; a small incision was made below the xiphoid process to open the abdomen. The pyloric section of the stomach was tied off without harming its blood vessels. The stomach was carefully separated and the abdominal wall was closed with interrupted sutures. The animals did not have access to water after the surgery. Four hours post ligation, the stomach was removed and its contents were gathered in new tubes. The gastric juice's volume, pH, and total acid content were measured. The materials were spun in a centrifuge, strained, and then analyzed through titration to measure the overall acidity. Samples of 1 ml each were extracted from the supernatant for measuring pH, total or free acidity, and pepsin activity [16]. Every stomach was inspected for sores in the front part of the stomach and categorized based on how serious they were [17].

Table 1: Treatment Schedule

S. No.	Name of Group	Treatment	Dose	Duration of th e study	No. of animals i n each group
1	Standard Group	Ranitidine	10 mg/kg b. wt (i. p.)	21 days	6
2	Control	Distilled water	1ml/kg	21 days	6
3	Test Group I	*MECD*	100mg/kg b. wt (p. o)	21 days	6
4	Test Group II	*MECD*	200mg/kg b. wt (p. o)	21 days	6

Biochemical evaluation of serum parameters

pH, gastric volume, Free acidity and total acidity and Macroscopic Evaluation of Stomach was evaluated.

III. RESULTS AND DISCUSSION

Extraction yield

Table 2 displays the percentage of crude successive extracts yield of *canscora decussata* using Methanol, Acetone, Chloroform, Hexane, Water, and Ethyl acetate. Methanolic extract of *canscora decussata* (MECD) had a higher yield at 14.4%w/w compared to chloroform extract at 4.8%w/w. The ethyl acetate extract had a yield of only 0.8%w/w, the lowest among all the extracts. So further studies were carried out in Methanolic extract of *canscora decussata* (MECD)

Table 2: Extraction Physical properties and Percentage yield

Extract	Colour	Consistency	Percentage
Methanol	Thick Green	Liquid	14.4% w/w
Acetone	Thick Green	Liquid	3.2% w/w
Chloroform	Brownish Green	Liquid	4.8% w/w

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Hexane	Green	Liquid	2.4% w/w
Water	Pale Brownish Yellow	Liquid	1.6% w/w
Ethyl acetate	pale Green	Liquid	0.8% w/w

Preliminary Phytochemical Screening

During the initial phytochemical study of *canscora decussata*, various phytochemical compounds were found in different extracts such as Water, Methanol, Hexane, Chloroform, and Acetone. Methanolic extraction revealed the presence of all the phytochemicals.

Table 3: Phytochemical Screening

S. No	Phyto constituents	Tests	Water	Methanol	Hexane	Chloroform	Acetone
1.	Carbohydrates	Molish Test	**+ve**	**+ve**	**+ve**	**-ve**	**+ve**
2.	Proteins	Xnthoprotic Test	**+ve**	**+ve**	**-ve**	**-ve**	**+ve**
		Biuret Test					
3.	Amino Acids	Millons Test	**+ve**	**+ve**	**-ve**	**-ve**	**+ve**
		Ninhydrin Test					
4.	Steroids	Libermann's buchards Test	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
		Salkoski reaction					
5.	Glycosides	Saponin Test	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
6.	Alkaloids	Dragendroff Test	**+ve**	**+ve**	**-ve**	**-ve**	**+ve**
		Wagners Test					
		Mayers Test					
		Hagers Test					
7.	Tanins	Lead acetate test	**+ve**	**+ve**	**-ve**	**-ve**	**+ve**
8.	Phenols	FeCl3 Test	**+ve**	**+ve**	**+ve**	**-ve**	**+ve**
9.	Flavonoids	Schinoda Test	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**

Effect of the MECD on Pylorus Ligation Induced Gastric Ulceration

The volume of stomach contents, pH levels, pepsin enzyme activity, and both total and free acidity levels are displayed in [Table 3, 4] for rats with pylorus ligation. In groups treated with MECD, the amount of acid secretion, overall acidity, and pepsin activity were reduced while the pH of the stomach fluid was higher compared to the control group with ulcers. At doses of 100 and 200 mg/kg, the impact of MECD on acid parameters was found to be statistically significant ($p<0.01$ and $p<0.05$) compared to the ulcer control group. Furthermore, MECD exhibited a curative ratio that was dependent on the dose in comparison to the control groups with ulcers.

Table 4: Effect of MECD on % of ulcer protection

S. No	Treatment	Dose	Ulcer index	% of ulcer protection
1.	Control	Distilled water	4.750±0.5284	0
2.	Standard control	Ranitidine 20mg/kg	0.58±0.3005**	86.73**
3.	*MECD* Low-Dose	100mg/kg	1.50±0.00*	69.14*
4.	*MECD* High-Dose	200mg/kg	0.83±0.33**	82.52**

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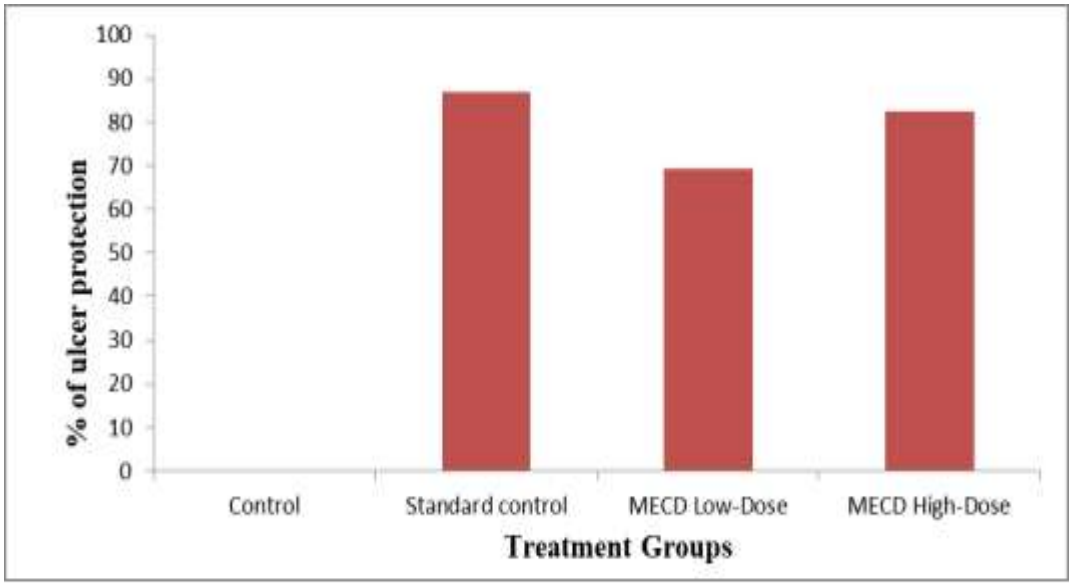

Figure 1: Effect of MECD on % of ulcer protection

Table 5: Effect of MECD on different bio chemical parameters

Groups	Treatment	Gastric Content(ml)	pH	Free Acidity (meq/L)	Total Acidity (meq/L)
Control	Distilled water	10.58±0.81	1.915 ±0.30	45.36±0.56	86.84±1.28
Standard	Omeprazole 20mg/kg	5.83±0.21	2.86 ±0.18**	19.0±0.68	42.46±0.71
Extract Low-dose	100mg/kg	7.06±1.38	2.21 ±0.27*	25.67±0.64	68.2±1.55
Extract High-dose	200mg/kg	7.28±0.86	2.48 ±0.45**	28.63±0.50	52±1.524

Values are the mean ± S.E.M., n=6, significant at *P < 0.05 and **P < 0.01

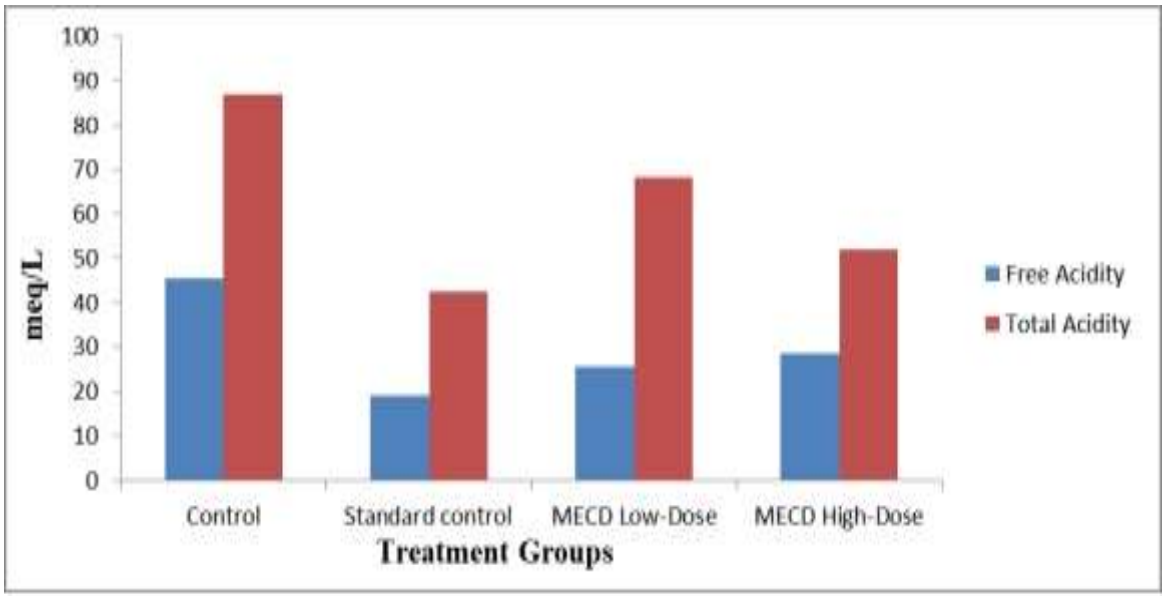

Figure 2: Effect of MECD on Free acidity and total acidity of pylorus ligated rats

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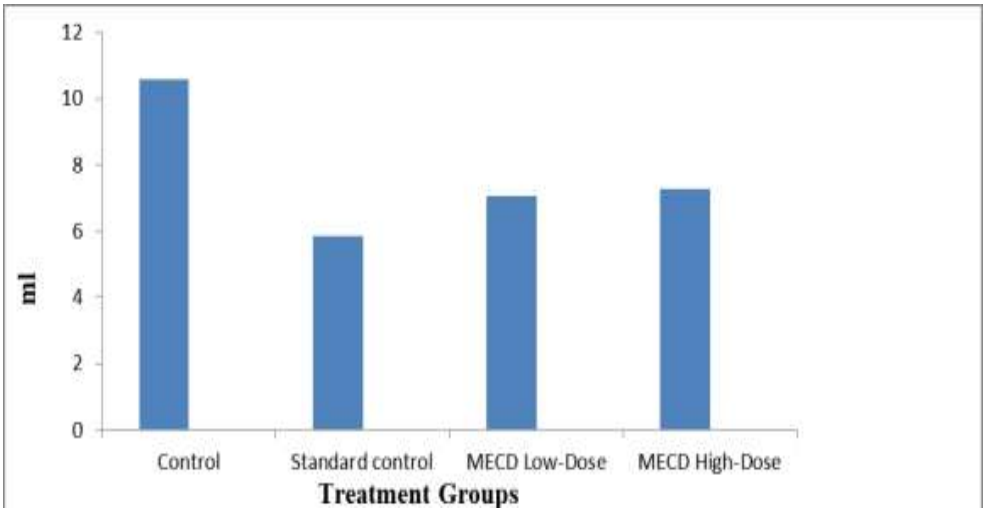

Figure 3: Effect of MECD on Gastric content of pylorus ligated rats

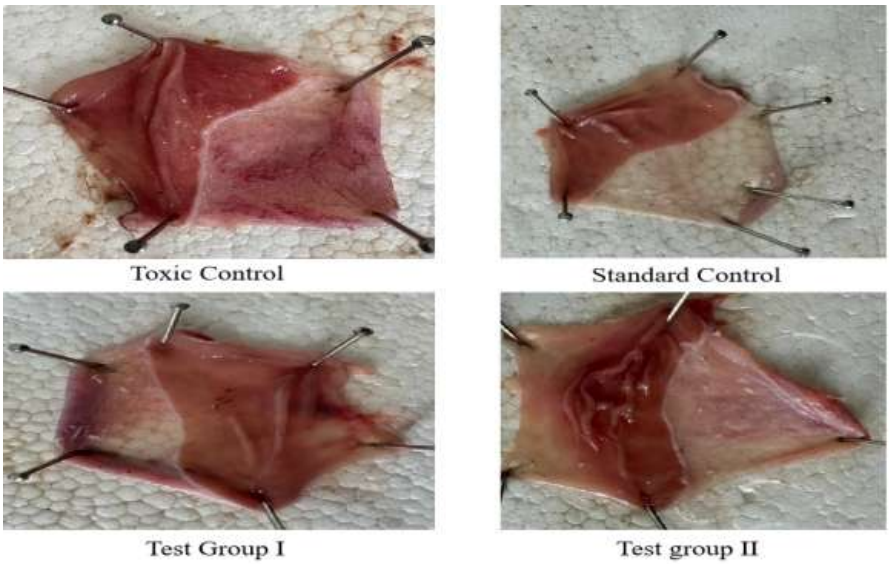

Figure 4: Effect of MECD on Ulcer Index

Discussion

An initial qualitative phytochemical examination conducted on the aerial parts of *canscora decussata* showed the existence of Carbohydrates, Proteins, Amino Acids, Steroids, Glycosides, Alkaloids, Tanins, Phenols, and Flavonoids. These secondary compounds have a variety of biological and therapeutic benefits [18-24], indicating that this species is likely to have numerous medicinal applications. The percentage of yield from the Methanol extract of aerial parts of *canscora decussata* was higher than the yield from extracts of Acetone, Chloroform, Hexane, Water, and Ethyl acetate. The high polarity of methanolic solvent may result in attracting a wider range of plant constituents compared to other solvents [25]. The level of polarity and the type of species are said to have a significant influence on the extraction of secondary metabolites. Despite being considered safe, medicinal plants have the potential to be toxic, highlighting the need to study their safety. Hence, it is crucial to effectively assess the safety and effectiveness of plants used in traditional medicine. An acute toxicity study showed that 2000 mg/kg of both aqueous and methanol extracts did not show any signs of toxicity or mortality in rats within 14 days, indicating a wide safety margin and LD50 value greater than 2000 mg/kg. Pylorus ligation is a key procedure that can indicate changes in parameters such as volume of gastric juice, total acidity, and pH of gastric content [26].

Pyloric ligation-induced ulcers result from a higher buildup of gastric acid and pepsin, which causes the self-digestion of the gastric mucosa [27]. Preventing high levels of stomach acid is crucial for protecting against ulcers, as excessively acidic environments can overwhelm the body's defenses. This model was crucial for

evaluating the key parameters needed to assess the overall anti-ulcer activity of plant extracts, including pH, total acidity, volume, ulcer index, and percentage of protection, all of which were analyzed in the study. In this study, MECD 100mg/kg did not display a noticeable increase in gastric juice pH and decrease in total acidity ($P > 0.05$) compared to the negative control. However, MECD 200mg/kg showed a significant increase in pH and decrease in total acidity, at the highest dose of extracts.

Multiple research studies found that compounds found in plants, such as flavonoids, tannins, terpenoids, and saponin, were accountable for agents that protect the stomach [28, 29]. Tannins act as an antiulcer agent due to their astringency and vasoconstriction properties. Because micro proteins precipitated on the ulcer site, a protective layer was created that blocks gut secretions and shields the mucosa from toxins and irritants. Prior research has suggested that the active compounds mentioned can enhance mucus, bicarbonate, and prostaglandin secretion, as well as protect against the harmful effects of reactive oxidants in the gastrointestinal tract [30, 31]. Hence, MECD exhibits antiulcer properties, possibly because of the existence of tannins, flavonoids, and terpenoids.

IV. CONCLUSION

The current research found that MECD's ability to treat ulcers may be due to its ability to reduce stomach acid, protect the stomach lining, and act as an antioxidant. Bioactivity-directed screening of MECD identified flavonoids, tannins, and triterpenoids that could contribute to its anti-ulcer properties. These compounds could be isolated and studied to better understand their potential in various anti-ulcer mechanisms.

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