

## Evaluation of *In vitro* Antiulcer Activity of Ethanolic Extract of *Pleurotus florida*

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**ABSTRACT:** Mushrooms have been playing an important role in several aspects of human activity. One of the most common culinary mushrooms is the oyster mushrooms. They are good sources of dietary fiber and other valuable nutrients. *Pleurotus* species is commonly known as giant mushroom. Edible ligninolytic mushrooms with high medicinal properties using the biotechnological and environmental field. It is an abundance of protein, minerals and elements of C and B complex. They are frequently as a therapeutic food having anticarcinogenic, anti cholestrolaemic and antimicrobial properties and also prophylactic properties. The present study investigates the antiulcer properties of edible mushroom *Pleurotus florida*, commercially cultivated in Trichy, Tamilnadu. The acid-neutralizing capacity (ANC) method and the H<sup>+</sup>/K<sup>+</sup> -ATPase inhibitory activity methods were used to test the antiulcer activity of an ethanol-based extract of grown, dried, and powdered mushrooms. Results indicated that the ethanolic extract, with respect to H<sup>+</sup>/K<sup>+</sup> -ATPase inhibitory action, extract considerably reduced ANC to 10.7 at a concentration of 1500 mg compared to standard, and at a concentration of 200 g compared to 60.8% with standard Omeprazole.

**Keywords:** Mushroom, *Pleurotus florida*, antiulcer activity, acid -neutralizing capacity, H<sup>+</sup>/K<sup>+</sup> -ATPase inhibitory action, ethanol extract.

### INTRODUCTION

In this multiple causes, cultivation of mushrooms is a prominent commodity in emerging economies. The fact that they are produced on agricultural wastes is one of their most endearing qualities. Also, can acquire substrate materials for cheap or even free as a result of it, and we can recycle garbage to help the environment. More than any other mould, oyster mushrooms (*Pleurotus* spp.) may use an array of basal substrates (Ashraf *et al.*, 2010; Bonatti *et al.*, 2004).

Since quite a-while, Consumption of the shiitake was advised for long life and good health in traditional Chinese medicine and shiitake have been employed effectively as food or as food additives in many different culinary products. Owing of their antitumor, antifungal, and lowering hypercholesterolemia properties, certain culinary mushrooms have been applied in research (Elmastas *et al.*, 2007). The infinite array of enzymes observed within *Pleurotus* species allow them to utilise the complex organic chemicals found in industrial waste and agricultural waste (Rahman and Choudhury 2012). This renders pre-processing of substrates for *Pleurotus* species cultivation unnecessary (Khan and Chaudhary 1989; Yalinkilic *et al.*, 1994).

*Pleurotus florida* is an edible mushroom which is the third largest cultivated mushroom in the world. However, the limiting factor for their cultivation is presence of weeds and contaminants (Malsawmtluangi *et al.*, 2021). In mushroom cultivation, the biological efficiency of mushrooms is an important one. The challenging issue in mushroom cultivation is the selection of substrate and container, which give a high percentage biological efficiency (Ramesh Kumar *et al.*, 2022)

An oozing sore on the skin or mucous membrane known as an ulcer is characterised by the shedding of inflammatory dead tissue. Despite the fact that they can occur practically everywhere, ulcers are most frequently found on the skin of the lower limbs and in the gastrointestinal tract (Abdulla *et al.*, 2010). Peptic and duodenal ulcers are the two different forms of ulcers. Based on the point of presence, adults occasionally have both abscesses and gastric ulcers, which are both present in the stomach and are painful in older individuals (de Lira Mota *et al.*, 2009). Enjoying spicy foods exacerbates ulcer manifestations, which often include nausea, vomiting, and weight loss. Whereas patients with stomach ulcers produce normal or reduced amounts of gastric acid, ulcers can still form even when there is no acid present at all (Vyawahare *et*

al., 2009). Given people's bad eating habits, the rate of peptic ulcers is rising among the population nowadays (Yang *et al.*, 2017).

The aim of this research was done in order to establish *Pleurotus* species therapeutic value as an ulcer-curing agent in traditional medicine.

## MATERIALS AND METHODS

### *Pleurotus florida* extract preparation:

The *Pleurotus florida* edible mushroom was nurtured in an aseptic manner (Ritota and Manzi 2019), and the fruit sections were air dried, ground up, and extracted with ethanol using a soaking process (Cheng *et al.*, 2015). Further research was conducted using the resultant extract.

### Assessment of antiulcer activity *in vitro*:

**Acid Neutralizing Capacity.** The quantity of aqueous extract that can neutralise acid is 100 mg, 500 mg, 1000 mg, and 1500 mg. Magnesium hydroxide (500 mg) and aluminium hydroxide have been assessed for the standard (Singhal *et al.*, 2022). Following applying 5ml of the mixture and adding the remaining 70ml of water, the total volume was 70ml. This was blended for one minute. That 30ml of 1.0 N HCl was added to the standard and test preparation and swirled for 15 minutes. Then, phenolphthalein was added and combined. The surplus HCl was promptly titrated till the pink colour was achieved using 0.5N sodium hydroxide (Thabrew and Arawwawala 2018 ; Garad *et al.*, 2012).

Moles of HCl neutralised divided by grammes of antacid/extract equals acid neutralising capacity (ANC) per gramme of antacid.

**H<sup>+</sup>/K<sup>+</sup> - ATPase Inhibition Activity:** The gastric mucosa of the fundus was cut off and opened, and the inner layer of the stomach was scraped out for the parietal cell in order to prepare the H<sup>+</sup>/K<sup>+</sup> - ATPase enzyme sample. The fresh goat stomach was acquired at the nearby abattoir. After being extracted from the stomach, the parietal cell was homogenised in 16 mM Tris buffer with a pH of 7.4, 10% Triton X-100, and centrifuged at 6000 rpm for 10 minutes. The supernatant solution was then used to inhibit the H<sup>+</sup>/K<sup>+</sup>

ATPase. Bradford's technique is used to determine protein content, and BSA is used as a reference. Evaluation of the H<sup>+</sup>/K<sup>+</sup> ATPase inhibition. The reaction mixture of the sample comprising 0.1 ml of enzyme extract (300 µg) and ethanolic extract of *Pleurotus florida* with varying concentrations (50 µg, 100 µg, 150 µg, 200 µg) was per-incubated for 60 min at 37°C. 2 mM ATP was added as the substrate, along with 200 µl each of 2 mM MgCl<sub>2</sub> and 10 mM each of KCl, to start the reaction. After 30 minutes at 37°C, the reaction was halted with 4.5% ammonium molybdate. Then, 60% perchloric acid was added, and the mixture was spun at 2000 rpm for 10 minutes to liberate the inorganic phosphate, which was then detected at 640 nm using a spectrophotometer. In a nutshell, 1ml of supernatant, 4ml of Millipore water, 1ml of 2.5% ammonium molybdate, and 0.4ml of ANSA were added after 10 min at room temperature. At different extract concentrations, the absorbance at 640 nm inorganic phosphate has been measured; the enzyme activity has been estimated as micromoles of Pi released per hour. Outcomes were evaluated against Omeprazole and expressed as Mean ± SD (Gupta and Hanumanthappa 2013). Percentage using the formula: one has estimated enzyme inhibition.

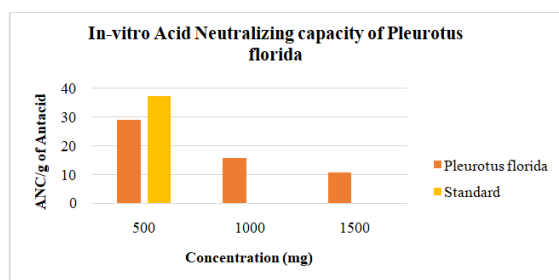
Percentage of inhibition = (Activity (control) - Activity (test)/Activity (control)) × 100.

## RESULTS

**Acid Neutralising Capacity:** Three concentrations of the ethanolic *Pleurotus florida* extract (500 mg, 1000 mg, and 1500 mg) as well as the industry standard of Aluminium Hydroxide + Magnesium Hydroxide (Al(OH)<sub>3</sub>+Mg(OH)<sub>2</sub>) (500 mg) were examined for the neutralising impact of the extract. According to the outcomes, the acid neutralising capacity (ANC) of the extract at concentrations of 500 mg, 1000 mg, and 1500 mg was significantly lower than the standard value of Al(OH)<sub>3</sub>+Mg(OH)<sub>2</sub> (500 mg), which is 37.3, at 29, 15.65 and 10.7, respectively. It has been discovered that the extract neutralises acid more effectively when diluted to a concentration of 1500 mg. The outcomes are displayed in Table 1 and Fig. 1.

**Table 1: *In vitro* Acid Neutralizing capacity of *Pleurotus florida*.**

Sr. No.	Concentration (mg)	Volume of NaOH consumed (ml)	mEq of Acid Consumed	ANC per gram of Antacid
1.	500	31	14.5	29
2.	1000	28.7	15.65	15.65
3.	1500	27.9	16.05	10.7
4.	500(Al(OH) <sub>3</sub> +Mg(OH) <sub>2</sub> )	22.7	18.65	37.3

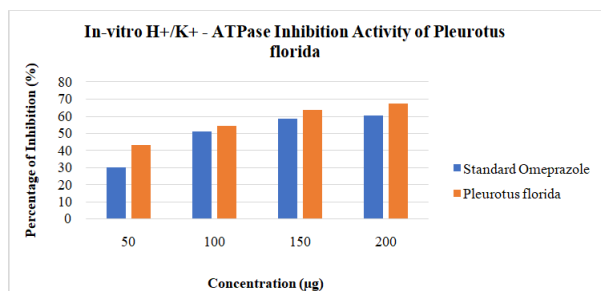


**Fig. 1. *In vitro* Acid Neutralizing capacity of *Pleurotus florida*.**

**H<sup>+</sup>/K<sup>+</sup> - ATPase Inhibition Activity:** The H<sup>+</sup>/K<sup>+</sup> - ATPase inhibitory activity of ethanolic extract of *Pleurotus florida* was compared with Omeprazole as the gold standard at concentrations of 50g, 100g, 150g, and 200g. Significant action in a dose-dependent manner was demonstrated by the extract. At a concentration of 200 g, extract demonstrated the highest percentage of inhibition at 68.02%, whereas normal omeprazole showed 60.81%. The outcomes are displayed in Table 2 and Fig. 2.

**Table 2: In vitro H<sup>+</sup>/K<sup>+</sup> - ATPase Inhibition Activity of *Pleurotus florida*.**

Sr. No.	Concentration (µg)	Percentage of Inhibition (%) (Mean±SD)	
		Standard Omeprazole	Extract of <i>Pleurotus florida</i>
1.	50	30±1.41	43.25±1.26
2.	100	51.25±0.96	54.5±1.29
3.	150	58.6±1.14	64±0.91
4.	200	60.8±1.3	68±0.82



**Fig. 2.** In vitro H<sup>+</sup>/K<sup>+</sup> - ATPase Inhibition Activity of *Pleurotus florida*.

## DISCUSSION

The results of the current investigation demonstrated that the ethanolic extract of *Pleurotus florida* possess gastroprotective action (Gregori *et al.*, 2007). Acidity is a prevalent digestive issue linked to a functional impairment that can happen for a number of different reasons (El-Maraghy *et al.*, 2015; Thabrew and Arawwawala 2018). excessive HCl secretion, which results in ulcers and inflammation of the stomach lining (Houshia *et al.*, 2012). Antacids work by neutralising stomach acid and lowering the stomach's pH. When therapeutic medicines are used instead of inhibiting stomach acid secretion, the restored equilibrium is maintained (Konozy *et al.*, 2022; Rahman and Choudhury 2012; Sharath *et al.*, 2015).

The volume of acid that an antacid can neutralise is known as its acid-neutralizing capacity (ANC), and it has been evaluated using a technique called back titration. At 1500 mg of concentration, the ethanolic extract of *Pleurotus florida* displayed a much lower ANC of 10.7.

Via the proton pump, the parietal cells of the stomach mucosa secrete excessive amounts of hydrochloric acid, which is what is known as hyperchlorhydria. An essential enzyme for producing acidity is H<sup>+</sup>/K<sup>+</sup> - ATPase, which is found on the apical secretory membrane of parietal cells (Yadav *et al.*, 2012). At a concentration of 200 µg, the extract exhibited a maximum percentage inhibition of 68% in H<sup>+</sup>/K<sup>+</sup>-ATPase activity.

Owing to the outcomes, *Pleurotus florida*'s ethanolic extract may have antacid, antisecretory, and antiulcer properties (Dharmani and Palit 2006). This could be because the mushroom contains bioactive chemicals.

## CONCLUSION AND FUTURE SCOPE

In accordance to the outcomes of the current investigation, *P. florida* ethanolic extract was shown to

have the most potential antiulcer agent. Analysing findings, the investigators led to the premise that *Pleurotus florida*'s ethanolic extract may be used as a source of antiulcer medications. This study showed that mushrooms can be used for both food and medicine (Tundis, *et al.*, 2008; Vyawahare *et al.*, 2009; Zayachkivsk *et al.*, 2005) and that they may be a good natural antiulcer alternative to currently available synthetic antiulcer medications in the food and pharmaceutical industries (Yalinkilic *et al.*, 1994). Nevertheless, a thorough investigation of the bioactive components from *Pleurotus florida* and their mode of action, which is accountable for their antiulcer efficacy, needs to be done in the future.

**Conflict of Interest.** None.

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