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## Study on preliminary phytochemical screening and anti ulcer activity of *Agaricus bisporus*

**Jaffar Shaik****Abstract**

Peptic ulcer is the most prevalent gastrointestinal disease. Even though a wide range of drugs are available for the treatment of peptic ulcer, but many of these do not fulfill all the requirements and have side effects. These factors have attracted researchers to investigate the natural products which have more efficacy, less side effects and less expensive for the treatment of peptic ulcer disease. In the present study the anti-ulcer activity of *Agaricus bisporus* investigated in the aspirin pylorus ligated induced ulceration in rats. The administration of *A. bisporus* 250 mg/kg and 500 mg/kg and observed the anti ulcer activity. *A. bisporus* 500 mg/kg showed significant decreased the offensive factors like ulcer index and acid secretion and also reduced gastric Ph. The efficacy of *Agaricus bisporus* was comparable with the reference drug Ranitidine. The results of the present study reveal that the *A. bisporus* having efficiency in the gastro protective activity. It is recommended that the above mushroom can be further studied for their anti ulcer efficacy in human subjects.

**Keywords:** *Agaricus bisporus*, Gastric PH, ulcer index, Aspirin, peptic ulcer

**Introduction**

Ulcer is defined as erosion in the lining of the stomach or duodenum and is caused by the disruption of the gastric mucosal defense systems. Ulcer in the stomach is called gastric ulcer and in the duodenum is called duodenal ulcer and together it is named as peptic ulcer. Ulcer incidence varies with the type of ulcer, gender and age. Peptic ulcer has initiated as open craters or sores in the inner lining (mucosa) of the stomach or the duodenum. A coating of mucus and other biochemicals normally shield the stomach and duodenum from digesting themselves. When these protective mechanisms are disturbed, powerful digestive acids can erode into the lining of these organs and cause ulcers.

Ulceration is an imbalance between the rate of secretion of gastric juice and the degree of protection afforded by the gastro duodenal mucosal barrier as well as the neutralization of the gastric acid by duodenal juice. Infection by the bacterial pathogen *Helicobacter pylori*, frequent usage of Non-Steroidal Anti-inflammatory Drugs (NSAIDs) and high acid secretion are main reasons for induction of ulcer. Other causes of peptic ulcer are smoking, alcohol consumption, psychological stress and irregularity in diet. Reduction of gastric acid production as well as re-enforcement of gastric mucosal production has been the major approaches to cure peptic ulcer. As a result, more and more synthetic drugs are introduced and offering newer options for treatment of peptic ulcer. Because of several side effects of synthetic medicines, there is new thought of better natural alternative for the treatment of peptic ulcer.

Medical mushrooms are mushrooms or extracts from mushrooms that are used or studied as possible treatments for disease. Some mushroom materials include polysaccharides, glycoprotein and proteoglycans modulated immune system. Some medicinal mushrooms isolates that have been identified also show cardiovascular, antiviral, antibacterial, antiparantic, antiinflammatory and antidiabetic properties.

The arising awareness of the relationship between diet and diseases has evolved the concept of functional foods and the development of a new scientific discipline, Functional Food Science [1]. A food may be considered to be functional if it contains a food component (whether a nutrient or not) which affects one or more identified functions in the body in a positive manner, which are in different name forms, e.g. dietary supplements, nutraceuticals, medicinal foods, vita foods, pharma foods, phytochemicals, mycochemicals and foods for specific health uses [2]. Mushroom is a macro fungus with a distinctive fruiting body that is large enough to be seen by the naked eyes. It includes both edible and non edible species. Some mushrooms serve as food because of their nutrient contents while some have been used extensively in traditional medicine [3]. Of the hundreds of known mushroom varieties, several have been studied for their ability to enhance the human immune system and fight infections.

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There are a number of studies on the biological activities and chemical constituents of *A. bisporus*. White button mushroom lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemia rats [4]. *A. bisporus* (brown) polysaccharide possessed strong immunostimulatory and anti-tumor bioactivity *in vivo* and *in vitro* [5]. The white button mushroom is protective against hepatic steatosis and nonalcoholic fatty liver disease in ovariectomized mice as a model for postmenopausal women [6]. It also showed antioxidant properties and reported to contain phenolics, ergothioneine and minerals [7]. The exopolysaccharides from *A. bisporus* exhibited antioxidants and anti-diabetic properties [8]. Another study reported that extracts of *A. bisporus* stipe showed antibacterial property [9]. The volatile components of *A. bisporus* were reported as 18- or 16-carbon compounds, such as octadecanoic acid, hexadecanoic acid derivatives, and other volatiles such as dl-limonene, n-nonane, benzenedicarboxylic acid, and cis-linoleic acid esters [10]. *A. bisporus* also afforded  $\beta$ -glucosidase [11]. Furthermore, ergosterol was isolated in both white and brown *A. bisporus* mushrooms. The ergosterol concentration was higher in early growth stages and accumulated more in the caps after maturation [12]. The fatty acid contents of these mushrooms are mainly linoleic, palmitic, and stearic acids [12]. Recently, it was reported that the most abundant vitamin in *A. agaricus* is niacin, followed by riboflavin. Other vitamins include vitamin B1, vitamin B3, L-ascorbic acid and  $\alpha$ -tocopherol [13].

*Agaricus bisporus*, known as table mushroom, cultivated mushroom or button mushroom, is an edible basidiomycete fungus which naturally occurs in grasslands, fields and meadows. It has spread much more widely and is one of the most widely cultivated mushrooms in the world. The original wild form bears a brownish cap and dark brown gills but more familiar is the current variant with a white form, having white cap, stalk and flesh and brown gills. *Agaricus* is the most cultivated mushroom and accounts for the 38% of worlds cultivated mushrooms. From this literature review, it has been observed that some interesting compounds were isolated from this genus.

The main purpose of the selection of this *Agaricus bisporus*, there was no systematic and scientific investigation has been carried out so far. Thus, the present study focused on evaluation of phytochemical and antiulcer activity of the methanolic extract of *Agaricus bisporus*.

## Materials and Methods

### Collection of Fungi

The fungi were collected in Vijayawada, Krishna district, Andhra Pradesh., India in November 2016.

### Phytochemistry

#### Extraction Process

The freshly collected fungi were shade dried and powdered in a grinder. The powdered material was then subjected to hot extraction.

#### Hot extraction

The dried powdered materials was extracted with methanol in soxhlet apparatus for 24 hrs. The extract thus obtained was concentrated under vacuum, dried completely and weighed.

### Qualitative chemical tests for phytoconstituents

#### Test for steroids

- Salkowski test:** Few drops of concentrated  $H_2SO_4$  are added to the chloroform extract, shaken and on standing, lower layer turns red in colour.

- Liebermann buchard's test:** To the chloroform solution of the extract, few drops of acetic anhydride are added mixed well. 1 ml of con  $H_2SO_4$  is added from the sides of test tube a reddish brown ring is formed at the junction of two layers.

#### Test for triterpens

- Salkowsti test:** Few drops of con  $H_2SO_4$  are added to the chloroform extract, shaken and on standing, lower turns golden yellow color.
- Brickorn and brinar test:** To the chloroform solution few drops of chlorosulfonic acid in glacial acetic acid (7:3) are added, red color is produced.

#### Test for Alkaloids

- Mayer's test:** The acid layer when mixed with mayer's reagent gives creamy white precipitate.
- Dragendroff's test:** The acid layer with few drops of dragendroff's reagent gives reddish brown precipitate.
- Wagner's test:** The acid layer when mixed with few drop's of wagner's reagent gives yellow precipitate.

#### Test's for carbohydrates

- Molisch's test:** The extract is treated with molisch's reagent and con  $H_2SO_4$  along the sides of the test tube, a reddish violet ring shows the presence of carbohydrate.
- Fehling's test:** The extract when heated with Fehling's A and B solution's gives an orange red precipitate showing the presence of reducing sugar.
- Benedicts test:** The extract on heating with benedict's reagent, brown precipitate indicate the presence of sugar.

#### Test for flavonoids

- Shinoda test:** The alcoholic solutions with few fragments of magnesium ribbon and con HCL produced magenta colour after few minutes.
- Ferric chloride test:** Few drops of neutral ferric chloride solutions are added to little quantity of alcoholic extract. A balackish green colour producted indicates the phenolic nucleus.ss

#### Test for tannins

**Ferric chloride test:** With 1% ferric chloride solution the extract gives blue, green or brownish color indicating the presence of tannins.

#### Test for glycosides

- Baljet test:** To the extract sodium picrate solution is added. It shows yellow to orange colour.
- Legal test:** The extract is dissolved in pyridine, sodium Nitroprusside solution is added to it and made alkaline, pink or red color is produced.

### Anti-ulcer activity

#### Study design

The rats were divided in 5 groups (n=5) and were fasted for 24 hrs prior to the experiment.

Group 1 animals served as normal controls.

Group 2 received 1% SCMC (1ml/kg p.o) as vehicle control,

Group 3 received 20mg/kg ranitidine as standard,

Group 4 received 250mg/kg p.o methanolic extract of *A. Bisporus*,

Group 5 received 500mg/kg p.o methanolic extract of *A. Bisporus*.

### Induction of Ulcers

Aspirin plus pylorus ligation-induced gastric ulcer in rats  
Wistar albino rats weighing 100–200g of either sex were divided into five groups, each group consists of six animals. All groups of animals received treatments as shown below along with 200mg/kg of aspirin once daily for 3 days. Group 1 animals served as normal control, Group 2 received 1.0mL/kg p.o. 1% SCMC as vehicle control; group 3 received 20mg/kg p.o. ranitidine as standard; group 4 and group 5 received 250mg/kg, and 500 mg/kg p.o. methanol extracts of *A. bisporus* respectively. Ulceration in rats was induced as described by Goel *et al.* [14]. On the fourth day, pylorus part was ligated after 36h fasting Shay *et al.* [15]. Four hours after the pyloric ligation, the animals were sacrificed by decapitation. The stomach was opened, and the ulcer index was determined. The gastric content was titrated against 0.01 N NaOH to find out the free acidity and total acidity by Ganguly & Bhatnagar [16].

### Biochemical parameters

#### Measurement of gastric juice volume and pH

Gastric juice was collected from aspirin plus pylorus ligation induced ulcer rats. The gastric juices thus collected were centrifuged at 3000 rpm for 10min. the volume of supernatant was measured and expressed as ml/100g body weight. The pH of the supernatant was measured using digital pH meter.

#### Free and total acidity

An aliquot of 0.1 ml of gastric juice was pipetted out into a 50ml conical and 2/3 drops of Topfers reagent was added and titrated with 0.01N NaOH until all red color disappeared and solution turns to yellowish orange in color.

The volume of 0.01N NaOH was noted which corresponds to free acidity. Then 2/3 drops of phenolphthalein was added and titration was continued until a permanent pink colour was developed. The volume of total alkali consumed was noted which corresponds to total acidity. The free acidity and total acidity was determined using the formula and values are expressed as meq/1/100g

Acidity = Volume of NaOH × Normality of NaOH

#### Ulcer index

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 gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined

0 – Normal coloured stomach

0.5 – Red colouration

1 – Spot ulceration

1.5 – Hemorrhagic streak

2 – Ulcers

### 3 – Perforations

Percentage inhibition: percentage inhibition was calculated using the following formula

#### Determination of ulcer index in the stomach

The stomach was cut opened and the mucosa was washed slowly with saline. The stomach was pinned on frog board and observed under microscope (10×) for ulcer mean score for each animal. The result is expressed as ulcer index by using following formula.

$$\% \text{ Ulcer index} = \frac{(\text{USc} - \text{Ust})}{\text{USc}} \times 100$$

USc = Ulcer surface area in control and USt = Ulcer surface area in treated animals.

### Results and Discussion

**Table 1:** Qualitative phytochemical screening of *Agaricus bisporus*

Name of the Test	Methanolic Extract
<b>Steroids</b>	
a) Salkowski test	+
b) Liebermann Buchard's test	+
<b>Triterpenes</b>	
a) Salkowski test	+
b) Brickorn and Brinar test	+
<b>Alkaloids</b>	
a) Mayer's test	-
b) Dragnendrott's test	-
c) Wagner's test	-
<b>Carbohydrates</b>	
a) Molisch's test	+
b) Fehling's test	+
c) Benedict's test	+
<b>Flavonoids</b>	
a) Shinoda test	+
b) Ferric chloride test	+
<b>Tannins</b>	
Ferric chloride test	+
<b>Glycosides</b>	
a) Baljet test	+
b) Legan test	+

**Table 2:** Effect of *A. bisporus* on ulcer index and % of inhibition using aspirin and pylorus ligation induced ulcer.

Group	Ulcer index	% of inhibition
Normal control	00.00±0.00	-
Ulcer control	47±1.9	-
AB (250 mg/kg)	19±1.6***	59.5****
AB (500 mg/kg)	15±1.6***	68****
Ranitidine (100 mg/kg)	11±1.7***	76.5****

**Table 3:** Effect of *A. bisporus* on gastric secretion, total and free acidity using aspirin and pylorus ligation induced ulcer.

Group	Gastric volume (ml/100 g)	PH of gastric juice	Total acidity	Free acidity
Normal Control	1.2±0.12	2.0±0.03	56±2.9***	26±1.7***
Ulcer control	3.1±0.16***	0.98±0.06***	70±2.1***	48±3.3***
<i>A. Bisporus</i> (250 mg/kg)	2.2±0.08**	1.8±0.03**	58±1.7**	35±1.8**
<i>A. Bisporus</i> (500 mg/kg)	1.6±0.27***	2.3±0.10***	54±1.7***	27±1.8***
Ranitidine	0.97±0.13***	3.0±0.14***	45±2.8***	20±1.4***

In the present study, 500mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH of gastric juice was increased

compared to ulcer control group. Although extracts could significantly reduce the gastric acidity, the volume of gastric juice and increase the gastric juice pH, the stomach pH was

sufficiently high to be able to induce ulcers. So it can be thought that the anti-secretory activity might not be the main mechanism of action of these extracts.

The effects of methanolic extract of *A. bisporus* on acid parameters were less significant at 250mg/kg dose. But methanolic extract of *A. bisporus* showed significant ( $p < 0.001$ ) effect at 500mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH of gastric juice was increased compared to ulcer control group. Although extracts could significantly reduce the gastric acidity, the volume of gastric juice and increase the gastric juice pH, the stomach pH was sufficiently high to be able to induce ulcers. So it can be thought that the anti-secretory activity might not be the main mechanism of action of these extracts.

Rivera-Pastrana *et al.* [17] reported that flavonoids like  $\beta$ -carotene, rutin (quercetin 3-o-rutinoside), lutein, zeaxanthin and cryptoxanthin are reported in the papaya fruit. Flavonoids have been reported as highly useful in the therapy of acute and chronic gastric ulceration [18-20]. The antiulcer and gastro protective effects of quercetin and its glucosides are reported by many researchers. Martin *et al.*, [21] and Kahraman *et al.*, [22] reported the antioxidant mechanisms involved in gastroprotective effects of quercetin in ethanol induced ulcerative rats.

Antiulcer activity of flavonoids by stimulating Platelet Activator Factor (PAF) in acid-ethanol induced ulcerative animal model was reported by Izzo *et al.*, [23]. Effects of quercetin and other flavonoids on the reserpine induced ulcerogenic mice was recorded by Barnaulov *et al.*, [24]. Motilva *et al.*, [25] recorded the effects of naringenin and quercetin on the acetic acid induced ulcerogenic rats.

Further, *Agaricus bisporus* is also an excellent source of Vitamin-A and vitamin C. Vitamin A is required for maintaining healthy mucus membrane. Vitamin C has many important functions like scavenger of free radicals, immune booster, and anti-inflammatory agent. The wound healing efficacy may be further attributed to the availability of micro and macro nutrients and other nutraceutical constituents from the *Agaricus bisporus*.

Preliminary phytochemical investigations showed the presence of steroids, hence, the antiulcer activity of *A. bisporus* in this experimental model may be due to the steroids. The results demonstrated that *A. bisporus* extract produced antiulcerogenic effects possessing antisecretory, cytoprotective, and proton pump inhibition mechanism. This interesting observation indicates that *A. bisporus* methanolic extract can be a potential source for the treatment of ulcer. However, a detailed study such as isolation of active molecules and characterization is required to confirm the phytochemicals responsible for the activity.

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#### Conflict of Interest

The author declare that no conflict of interest.

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