Unexplored pharmaceutical potential of phytocompounds extracted from the mushroom, *Geastrum saccatum*

Pramod C. Mane[1], Ashok N. Khadse[2], Deepali D. Kadam[1], Shabnam A. R. Sayyed[1], Vrushali T. Thorat[1], Sunita D. Sarogade[1] and Ravindra D. Chaudhari[1,*]

[1]P.G. Department of Zoology and Research Centre, Shri Shiv Chhatrapati College of Arts, Commerce and Science, Junnar, Savitribai Phule Pune University, Pune 410 502, India
[2]Chandrapur Forest Academy, Mul Road, Chandrapur 442 401, India

The phytochemical content and medicinal properties of the mushroom *Geastrum saccatum*, collected from the northern Western Ghats were evaluated. The mushroom powder was extracted in different solvents separately and assessed for the presence of phytochemicals. Anti-inflammatory, anti-diabetic, antioxidant and iron chelating activities of the mushroom extract were evaluated. The results revealed that chloroform extract of *G. saccatum* (CEGS) exhibited the maximum number of phytochemicals compared to the other extracts and hence was selected for further studies. The mushroom analysed contains different types of phytoconstituents having pharmaceutical activities. Maximum activity for the studied bioassays was found at 50 µg/ml of CEGS concentration. Thus chloroform extract of *G. saccatum* has potential pharmaceutical properties and thus can be used for the treatment of different diseases.

Keywords: Chloroform extract, medicinal properties, mushroom, pharmaceutical potential, phytoconstituents.

AT present the world trade in medicinal products is more than US$ 6 billion and their demand has also increased. Several mushrooms contain β-glucan, lectin, phenolic compounds, alkaloids, flavonoids, xanthones, glycosides, etc. with potential biological activities[1]. Due to special characteristics of medicinal and edible mushrooms, the United States National Cancer Institute, USA has chosen them as an important source of new drugs[2]. Mushrooms have tremendous untapped potential of novel pharmaceutical products and are known as biological response modifiers; hence they have gained importance in modern medicine[3–5].

Around 14,000–22,000 species of mushrooms are known to the world, while the real number may be high. Around 300 species of mushrooms possess medicinal properties and 2000 species are found to be safe for human health[6,7]. The mushroom *Geastrum saccatum*, also called rounded earth star mainly grows in humus-rich soil[8].

Inflammation is a complex protective response of the body against the materials which harms our body, including bacteria, viruses, injured cells and irritants, etc. Anti-inflammatory compounds used in traditional drugs for the treatment of pain and swelling lead to several side effects, and hence bioactive molecules which do not cause any adverse effects can be used[9].

Antioxidants are important for repairing body damage caused due to the reactive oxygen species (ROS). Synthetic antioxidants exhibit harmful effects on both humans and the environment. Hence, at priority basis synthetic antioxidants must be replaced by natural oxidizing agents[10,11].

Nowadays diabetes mellitus has become a serious problem for human beings. Globally more than 190 million people are affected, while this will rise to about 380 millions by 2030 and 629 million by 2045. Hence on a priority basis effective treatment options are necessary. The bioactive compounds like polysaccharides, proteins, lipids, fibres, alkaloids, terpenoids, lactones, lectines and phenolic substances will play an important role in the treatment of diabetes[12–14].

Keeping in mind the medicinal importance of mushrooms, herein we report the presence of bioactive compounds from *G. saccatum* with its pharmacognostic importance.

Materials and methods

The mushrooms were collected from June to November 2014 using standard methods[15]. The samples were dried at 40–50°C and pulverized to powder form, stored in airtight containers and used for further studies.

Extraction of bioactive compounds from mushrooms

Five grams of mushroom powder was soaked in different solvents, viz. methanol, ethyl acetate, chloroform,

*For correspondence. (e-mail: rdchaudhari2004@yahoo.co.in)

acetone, petroleum ether and hexane separately. All the extractions were carried out at 4°C for 24 h and centrifuged at 4°C for 10 min at 12,000 rpm. The collected extract was concentrated and dried in a rotary evaporator (RV10 control, IKA) and then dissolved in Milli Q water for further studies.

Phytochemical analysis

Presence or absence of phytocompounds in extracts of *G. saccatum* was determined using standard methods[16–20].

In vitro antioxidant and iron chelating activity

Spectrophotometric method was used to determine the total antioxidant activity. Iron chelating activity was evaluated by standard colorimetric method. Ascorbic acid was used as the standard[21,22].

Inhibition of albumin denaturation

The process includes fixed aliquot of egg albumin, phosphate buffer solution (PBS; pH 6.4) and varying concentrations of mushroom extract. The formulation was kept in a shaking incubator at 37°C for a fixed amount of time and then heated for 5 min. At room temperature the absorbance of the mixture was noted at 600 nm against vehicle as a blank. The final result was obtained using the following formula[23]:

$$\text{Percentage inhibition} = 100 \times (\text{Abs}_t/\text{Abs}_c - 1),$$

where Abs_t is the absorbance of the sample and Abs_c is the absorbance of the control.

Inhibition of protein denaturation

The chemical process includes fixed aliquot of 5% bovine serum albumin with different concentrations of mushroom extract; the pH was maintained at 6.3. The samples were put in an incubator for 20 min at 37°C, which was then heated at 57°C for 3 min. After cooling, an aliquot of PBS was mixed in each test tube. The absorbance was measured at 600 nm against distilled water as a blank and product control tests did not contain bovine serum albumin[24].

In vitro anti-diabetic activity

The reaction mixture contained starch solution (1 ml), mushroom extract (1 ml) of different concentrations and 1 ml of α-amylase solution. The whole mixture was incubated at 25°C for 3 min. Next, 1 ml of 96 mM DNS reagent was added to the above mixture and heated for 15 min in a boiling water bath. The absorbance was measured at 540 nm using a spectrophotometer and percentage inhibition was calculated.

Results and discussion

Recently, due to worldwide adverse economic conditions, and harmful effects of modern drugs, medicines from natural sources like plants, mushrooms, etc. have gained importance in healthcare[25]. In many Asian countries, wild edible mushrooms were traditionally used in food as well as medicine because they contain several secondary metabolites[26].

The present study revealed that proteins, phytosterols, saponins, glycosides, flavonoids, carbohydrates, terpenoids, phenols and tannins are present while alkaloids, quinones, cumerin, anthocynins and emodins were absent in chloroform extract of *G. saccatum* (CEGS). Preliminary screening tests were useful for the detection of bioactive compounds and their subsequent use in drug discovery and development.

Phytochemical analysis of *G. saccatum* was carried out. Table 1 shows the results.

This study is mainly focused on the search for a new generation of anti-inflammatory agents from natural sources with maximum efficacy and no side effects. The percentage protection of CEGC was found to be 93.20 at 50 µg/ml, while the lowest percentage protection was 26.71 at 10 µg/ml (Figure 1).

In this study, CEGS exhibited the presence of terpenoids which have a wide range of medicinal uses, including anti-inflammatory activity[27]. The phytochemical analysis revealed the presence of terpenoides in CEGS which are responsible for anti-inflammatory activity[28]. Inflammation is related to the action of the cells for maintenance of tissue structure and function. It was observed that there is a link between development of cancer and long-term

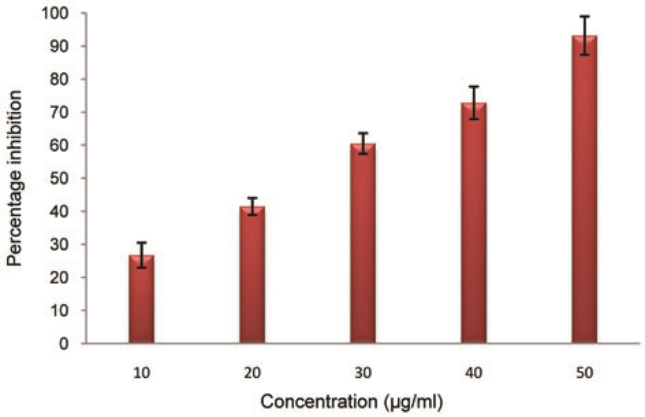

Figure 1. *In vitro* anti-inflammatory activity of chloroform extract of *Geastrum saccatum* (CEGS).

Table 1. Phytochemical analysis of *Geastrum saccatum* in various solvents

Parameters	Acetone	Chloroform	Hexane	Methanol	Petroleum ether	Ethyl acetate	Ethanol
Phytosterols	–	+	–	+	–	+	–
Saponins	–	+	–	–	–	–	–
Alkaloids	–	–	–	–	–	+	–
Phenolic compounds and tannins	–	+	–	–	–	+	–
Proteins	–	+	–	–	–	–	–
Glycosides	–	+	–	–	–	–	–
Flavonoids	–	+	–	+	–	+	–
Fehlings test	–	+	–	–	–	+	–
Benedicts test	–	–	–	–	–	–	–
Quinines	–	–	–	–	–	–	–
Coumerin	–	–	–	–	–	–	–
Terpenoids	+	+	+	+	+	+	+
Anthocyanins	–	–	–	–	–	–	–
Emodins	–	–	–	–	–	–	–

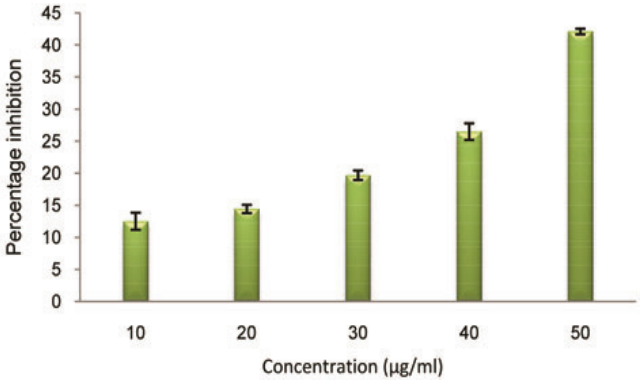

Figure 2. Effect of CEGS on protein denaturation.

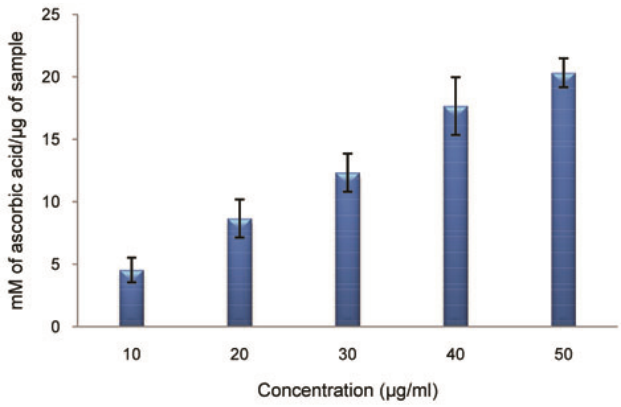

Figure 3. Total antioxidant capacity of CEGS.

inflammation. Due to the effects of inflammation, bioactive compounds having anti-inflammatory potential are gaining importance. Within this framework it was observed that mushrooms are the best source for natural and safe anti-inflammatory compounds[29,30].

Denaturation of proteins is responsible for inflammation. The potential of CEGS with regard to protein denaturation was evaluated using egg albumin. It was found that inhibition of protein denaturation is concentration-dependent. Herein, we observed that CEGS possessed maximum percentage inhibition of 42.11 at 50 µg/ml (Figure 2).

Mushrooms contain various types of bioactive compounds which are responsible for anti-inflammatory activity. These compounds have terpenoids which act as anti-inflammatory agents. They suppress the secretion of inflammatory cytokine tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL6) and also the inflammatory mediators nitric oxide (NO) and prostaglandin E_2 (PGE2)[31].

In this study, phytochemical screening revealed the presence of phenols. Many mushrooms exhibit anti-inflammatory activity as they contain phenolic compounds, including pyrrogallol[32].

The flavonoids, saponines and steroids also possess anti-inflammatory properties[33]. Most researchers have reported that denaturation of proteins is also responsible for inflammation, and the mechanism of denaturation of proteins mainly involves alternation in electrostatic, hydrogen, hydrophobic and disulphide bonding. Some researchers have mentioned that mushrooms having immunomodulatory effects are mainly related with excitation of the immune system by different polysaccharides. Such consequences mainly encompass maturation of dendritic cells, stimulation of natural killer cell activity, and activation of T and B-lymphocytes[34,35].

Antioxidants defend our body against free radicals and various mushrooms are a rich sources of antioxidants. Low levels of antioxidants lead to damage or kill the cells due to oxidative stress[36]. Our results also support the findings of other studies that *G. saccatum* possesses antioxidant activity[37,38].

In the present study, the total antioxidant activity was found to be highest (20.33 mM of ascorbic acid/µg of sample) at a concentration of 50 µg/ml (Figure 3).

CEGS shows good antioxidant activity which is due to the presence of phenolic components and flavonoids. The

chain reaction of lipid oxidation at the initial stage was halted due to phenolic components and flavonoids by donating hydrogen to the free radical[39,40].

As CEGS showed dose dependant antioxidant activity, antioxidant agents may be developed from these mushrooms to treat the disorder associated with free radicals like ageing, cancer and diabetes[41]. Recently, it was reported that the most commonly used synthetic antioxidants show side effects like liver damage and carcinogenesis; hence much attention has been paid to natural antioxidants[42].

A study reported that the extracts of *Agaricus* species contain total phenolic content and some phenolic acids which are responsible for antioxidant activity and hence useful in protection against oxidative damage[43]. The study highlighted the potential application of *Agaricus* species as food compounds or nutraceuticals.

The results of iron-chelating activity of CEGS extract may be due to presence of phenols which acts in a similar way as it reacts with free radicals and aborts free radical chain reaction. The iron chelating effect of CEGS was observed to be maximum, i.e. 3.16 mM of ascorbic acid/µg at a concentration of 50 µg/ml (Figure 4). In biological systems, transition metal ions catalyse the Haber-Weiss- and Fenton-type reactions, which lead to the generation of hydroxyl radicals. The transition metal ions form a chelate with the antioxidant which can prevent hydroxyl radical generation and hence hinder the peroxidation process of biological molecules. The iron chelating activity is directly related to the concentration of phenolic compounds which can chelate the metal ions[44].

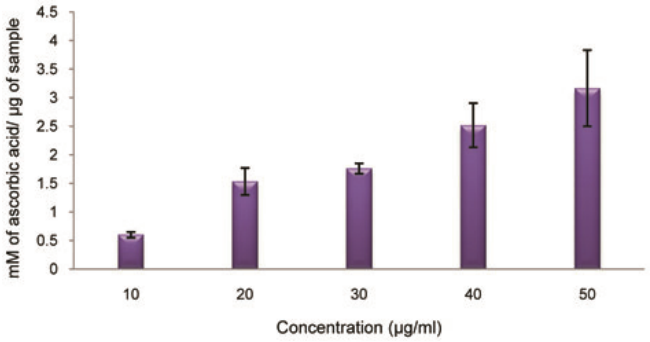

Figure 4. Iron chelating activity of CEGS.

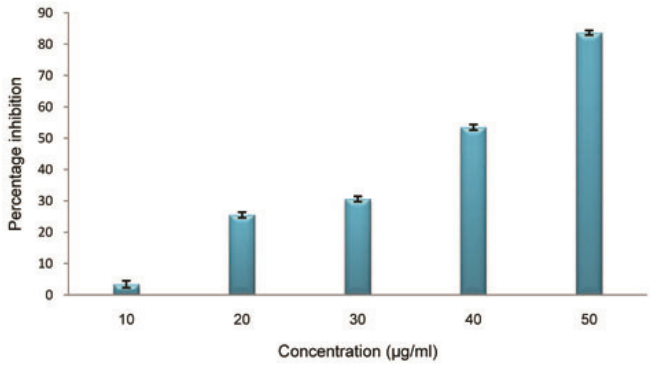

Figure 5. *In vitro* anti-diabetic activity of CEGS.

Iron chelates are small molecules which can bind tightly to metal ions. The important role of chelates is to remove excess iron from the blood and prevent poisoning, thus providing protection to our body[45].

The present study showed that CEGS has a potential for anti-diabetic activity. Recently, much attention has been paid to plants, mushrooms and other natural components for the treatment of diabetes. This disorder can be treated by decreasing post-prandial hyperglycaemia, by slowing down the absorption of glucose through the curbing of carbohydrate hydrolysing enzymes, viz. α-amylase and α-glycosidase in the digestive tract[46].

CEGS revealed a significant inhibitory action on α-amylase enzyme. The percentage inhibition varied from 3.44 ± 1.12 to 83.63 ± 0.71. The maximum percentage inhibition was found at 50 µg/ml of CEGS concentration (Figure 5).

Saponines have broad-spectrum use in pharmacological properties such as anti-inflammatory and anti-diabetic activities. Thus mushrooms can be used for controlling inflammation-related diseases and diabetes[47]. The extracts of medicinal plants contain one or more vital ingredients which lead to the depletion of blood glucose. These components mainly include flavonoids of plant origin and show promising anti-diabetic activity. In this study, CEGS showed the presence of terpenoids and glycosides. It has also been reported that they have hypoglycaemic activity[48–50].

Conclusion

The present study reveals that CEGS possesses most of the phytochemicals compared to methanol, ethyl acetate, acetone, petroleum ether and hexane extracts. The mushroom analysed was found to be a good source of anti-inflammatory, anti-diabetic, antioxidant as well as iron chelating agents and other phytoconstituents. Thus CEGS has some potential pharmaceutical activities and hence can be used for the treatment of various diseases.

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