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Review Article

A Multifaceted Fungus Pig's Ears: *Gomphus clavatus* Gray

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Abstract

Gomphus clavatus, commonly known as pig's ears, is a species of fungi in the genus *Gomphus*, family Gomphaceae native to Eurasia and North America. Pig's ear derives its name from the funnel-shaped and folded fruiting body, which resembles a pig's ear in shape and texture. *Gomphus clavatus* mushrooms are edible; these are used as soup, sauce and baking dishes. *Gomphus clavatus* Gray is not only used as a nutritious food but also might be an important source of biologically active compounds with potential additional medical value. In recent research, the fruit bodies of *Gomphus clavatus* Gray were collected from the wild. A novel heteropolysaccharide, namely 'GCG-1', from the fruit bodies of *Gomphus clavatus* Gray was isolated through Sephadex G-200 and DEAE-cellulose columns. Fungal polysaccharide is a type of active organic compound that is composed of long chains of monosaccharide units bound together by glycosidic linkages. Recently, an increasing number of fungal polysaccharides have been identified to show a variety of biological activities, including antitumor and antioxidant properties. Antioxidation test in GCG-1 *in vitro* showed that it has strong free radical scavenging activity. It suggests that *Gomphus clavatus* can be considered as a medicinal food with antioxidant activities.

Keywords: Mushrooms; Fungus; Antioxidant

Introduction

Wild mushrooms have been part of the human diet for centuries because of their taste, texture, nutrient and medicinal values of fruiting body. The annual consumption of wild mushrooms exceeds 10kg per individual in some countries [1]. Successfully controlled condition in mushroom houses have made mushrooms as an economically important food. World production of mushrooms has increased dramatically from 6.1 million tons in 1997 to 12.2 million tons in 2002 [2]. Nutritionally, mushrooms are low in energy and fat but high protein, carbohydrate, and dietary fiber. Carbohydrate is composed of chitin, glycogen, mannitol, and trehalose. In addition, mushrooms contain a variety of minerals and trace elements such as potassium and copper and vitamins such as riboflavin, niacin, and folates [3]. Apart from being as a food, some mushrooms have also been studied to have 'antioxidant activities'. Recent studies showed that extracts prepared from *Gomphus clavatus* fruiting bodies have a high antioxidant activity, and a high concentration of phenolic and flavonoid compounds [4]. Fungal polysaccharide is a type of active organic compound that is found in medicinal fungi, fruiting bodies. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Recently, a novel polysaccharide from *Gomphus clavatus* Gray was isolated through Sephadex G-200 and DEAE-cellulose columns. The *Gomphus clavatus* Gray saccharide (GCG-1) showed antioxidant activities. *Gomphus clavatus* Gray may be an ideal source for antioxidant and anticancer agents [4-7]. The aim of the review is to collect data on fungal biology of *Gomphus clavatus*, belong to basidiomycetes, Agaricomycetes, Gomphales and to study the beneficial effects of *G. clavatus* Gray mushrooms with both edible and medicinal properties.

Taxonomy of the *Gomphus clavatus*, Belong to Gomphus Based on Morphology

The *Gomphus clavatus*, commonly known as pig's ears, is an edible species of fungus in the genus *Gomphus*, the family Gomphaceae (Figure 1 and Table 1). The *Gomphus*, belong to Basidiomycota is characterized by fleshy basidiomata that can have funnel-shaped pilei with wrinkled hymenia. The reduced number of distinctive morphological features and the lack of molecular data (DNA sequences) to clarify the taxonomy of this group have contributed to its confused classification. Nevertheless, the significant contributions by many taxonomists have led to a better understanding of the overall placement of gomphoid fungi. Before Giachini [8] analyzed the molecular phylogeny of *Gomphus* sensu lato (Gomphaceae, Gomphales, Basidiomycota) and related genera in the Gomphales, species of Gomphales were assigned to three different genera: Cantharellus [9], Craterellus [10], and Neurophyllum [11]. Recently Giachini & Castellano [12]. Presented a new classification for *Gomphus* S. l. Giachini [8] and Giachini & Castellano [12]. emphasized that *Gomphus* sensu stricto is the only genus in the Gomphaceae with strictly violet, lavender-brown, or milky-coffee colored hymenia, distinguishing it from other representatives of *Gomphus* s. l. (Gloeocantharellus, Phaeoclavulina, Turbinellus) characterized by orange, brown or greenish olive hymenia [12]. Furthermore, all *Gomphus* species produce clamp connections and verrucose spores. The unique combination of these morphological characteristics separates *Gomphus* from other genera within the Gomphales [12].

The Nomenclature of *Gomphus clavatus*

The concept of *Gomphus* was established when Persoon [13] first

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Figure 1: (*Gomphus Clavatus*, Source: Wikipedia, google).

Table 1: (*Gomphus Clavatus*, Source: Wikipedia, google).

Gomphus clavatus	
Scientific classification	
Kingdom	Fungi
Division	Basidiomycota
Class	Agaricomycetes
Order	Gomphales
Family	Gomphaceae
Genus	*Gomphus*
Species	*G. clavatus*
Binomial name	
Gomphus clavatus	
(Pers.) Gray [9]	

mentioned the name Merulius. Subsequently, Persoon [14] proposed four new combinations, including *M. clavatus*. Persoon [15] also introduced the name *Gomphus* at the generic level as a segregate from the genera Clavaria, Geoglossum, Mitrula, and Spathularia. The genus, described by Persoon [15], has undergone several taxonomic and nomenclatural modifications over the past 200 years. Persoon did not assign species to his newly created genus. The first citation of a species for *Gomphus* came only when Gray [9] described *Gomphus clavatus* based on *Merulius clavatus* Pers. The genus has frequently been cited in the past as "*Gomphus* (Pers.) Gray" or "*Gomphus* Pers. ex Gray". Currently *Gomphus* has 35 described species. It is close related to other members of the Gomphales and somewhat related to the Phallales, Hysterangiales, Gautieriales, and the families Geastraceae and Sphaerobolaceae [12].

Descriptions of *Gomphus clavatus* (Fungal Biology)

The basidiocarps, or fruit bodies, of immature *Gomphus clavatus* are club-shaped and have one cap or pileus, but later spread out and have a so-called *merismatoid* appearance-several vase-shaped caps rising from a common stem. The fruit body is up to 15cm (6inc) wide and 17cm ($6\frac{3}{4}$ in) tall, fan-shaped with wavy edges. The upper surfaces of the fruit bodies are covered with brown, simple or branched, thin-walled, septate generative hyphae that form small, distinct patches towards the margin, but combine to form a continuous felt-like fine-haired area, or tomentum, over the center of the cap. There are clamp connections present. The color of the upper cap surface is orange-brown to violet but fades to a lighter brown with age. The cap margins of older mushrooms can be quite ragged. The lower spore-bearing surface-the hymenium-is wrinkled, often with folds and pits, and violet to brown in color. Pileus and stipe contexts are interwoven. Hyaline hyphae are 2.5-6 m wide and generally uninflated except adjacent to the clamp connections. Hymenial trama is composed of thin- to thick-walled hyphae and have clamp connections. In subhymenial trama, there are interwoven hyaline hyphae up to 8m wide. The solid stripe is 0.8-3 cm ($\frac{3}{8}$-1 $\frac{1}{8}$ in) wide, 4-10 cm ($1\frac{5}{8}$-$3\frac{7}{8}$ in) tall and covered with fine hairs that become coarser (hispid) towards the base. It is often compound, with several fruit bodies arising from the basal portion. Fruit bodies may bruise reddish-brown where handled. The flesh can be whitish-pink to lilac or cinnamon-buff. Thick under the center of the cap, it thins out towards the margins. It can be crunchy, though it is softer than that of the chanterelle. The taste and odor are mild. The spore print is yellow to orange-yellow. Representatives of the Hymenomycetes have *dolipore septa* with various types of cantharelloid forms (*Gomphus*), coralloid fungi (Lentaria, Ramaria), hydnoid. *Gomphus* has a dolipore septum. The basidiospores are elliptical, wrinkled or slightly warted, and 10-14 by 5-7.5 µm. They are nonamyloid, meaning they have a negative orange color reaction with the iodine in Melzer's reagent, and dark olive color in mass with KOH. Basidiospores usually in discrete, raised patches that give an undulate appearance to the spore surface. The spore-bearing structures, the basidia, are elongated or club-shaped, hyaline (glassy or translucent), and four-spored, with dimensions of 60-90 by 8.5-11.5 µm. Basidia is clavate and collapse after spore discharge. *G. clavatus* does not contain cystidia, the sterile cells associated with basidia in many species. *G. clavatus* has clamp connections at base. The life cycle of mushrooms is as follows. And, the life cycle of Basidiomycota belonging to *Gomphus* is described in the Figure 2.

Habitat and Distribution

Growing on the ground, *Gomphus clavatus* mushrooms appear singly, in clusters or clumps, or even occasionally fairy rings [16]. The species is typically found in coniferous forests, and with a preference for moist, shady areas with deep leaf litter, or rotten wood debris on the ground [17]. It is equally common in older or younger stands of trees. Fruit bodies are easily missed because their colors blend with those of the forest floor. It is more common at elevations of greater than 2,000ft (600m) [18]. *Gomphus clavatus* has been reported as forming mycorrhizal associations with a variety of trees: Abies alba, Abies cephalonica, Picea species, Pseudotsuga menziesii, and Tsuga heterophylla [8].

The Nutritional and Health Benefits of the *Gomphus clavatus* Mushrooms

Mushrooms are fungi with distinctive fruiting bodies, which are enough to be seen with the naked eye and to be picked by hand [19]. Wild mushrooms have been part of the human diet for centuries because of their flavor, texture, nutritional value of the fruiting body. Mushrooms are low in energy and fat but high in protein,

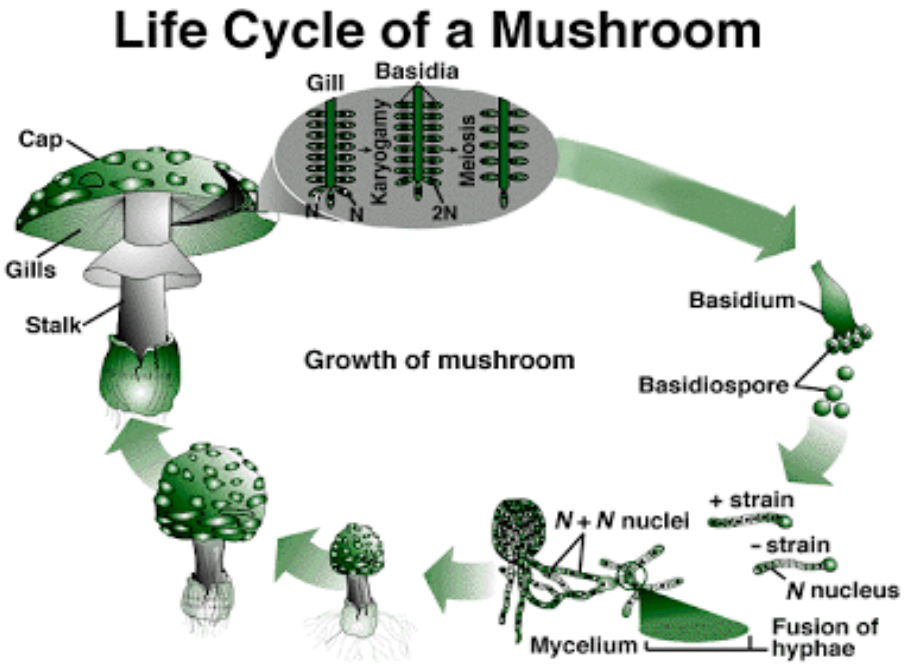

Figure 2: Life Cycle of a Mushroom (Basidiomycete).

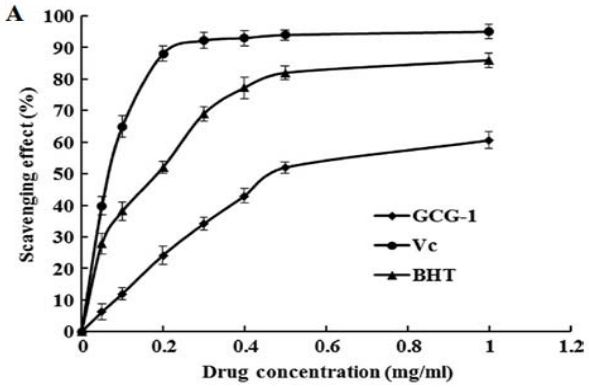

Figure 3: (A) DPPH- radical scavenging effect of GCG-1 from *Gomphus clavatus* Gray [4].

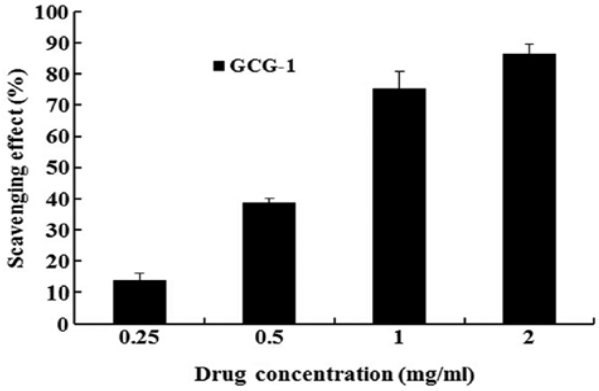

Figure 4: GCG-1 attenuates PC12 cell damage induced by hydrogen peroxide. GCG-1, *Gomphus clavatus* Gray polysaccharide [4].

carbohydrate, and dietary fiber. Carbohydrate is composed of chitin, glycogen, mannitol, and trehalose. In addition, mushrooms contain various minerals and trace elements such as potassium and copper and vitamins such as riboflavin, niacin, and folates [6].

These mushrooms might be not only used for healthy foods but also taking advantage of the additive and synergistic effects of all the bioactive compound present to against oxidative processes as natural potential antioxidants In the last few years, an increasing interest in the antioxidant activity of mushrooms has arisen from researchers and consumers [4-7].

Edibility of *Gomphus clavatus*

Gomphus clavatus is edible. It has an earthy flavor and meaty texture that has been regarded as suiting red meat dishes. Like many edible fungi, consumption may cause gastrointestinal distress in susceptible individuals. The flesh becomes bitter with age, and older specimens may be infested with insects. *Gomphus clavatus* has been used for cooking for some time.

As a New Research, the Medicinal Value of 'Antioxidant Activities' of *Gomphus clavatus* Gray

A novel heteropolysaccharide from the fruiting bodies of *Gomphus clavatus* Gray: Extracts prepared from *Gomphus clavatus* fruiting bodies have a high antioxidant activity, and a high concentration of phenolic and flavonoid compounds. Fungal polysaccharide is a type of active organic compound that is found in medicinal fungi, fruiting bodies. Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages and upon hydrolysis yield the constituent monosaccharides or oligosaccharides. They range in structure from linear to highly branched. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating

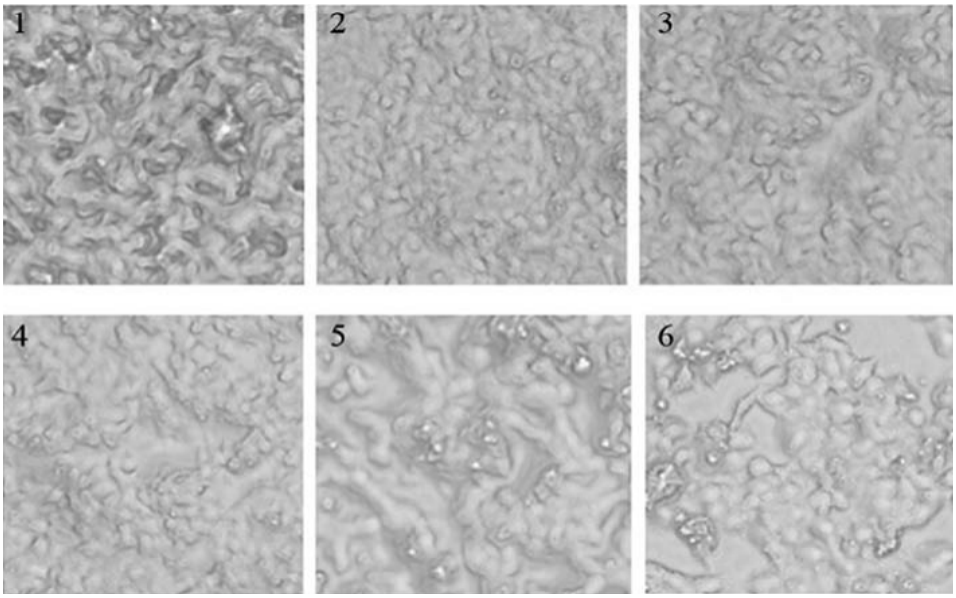

Figure 5: Effect of GCG-1 on the morphology of human hepatoma HepG-2 cells at concentrations of 0, 1.25, 2.5, 5, 10 and 20 μg/ml (images 1-6, respectively). GCG-1, *Gomphus clavatus* Gray polysaccharide [4].

unit [4]. Recently, an increasing number of fungal polysaccharides have been reported to exhibit a variety of biological activities, including immunostimulatory, antitumor and antioxidant properties [4-7].

Collection, Extraction, composition of the polysaccharides from *Gomphus clavatus* Gray: The fruiting bodies of *Gomphus clavatus* Gray were collected in Xiaojing Country of Sichuan Province, China. After extraction with 2,000ml distilled water at 90°C for 6h, the crude polysaccharide, named GCGP, was obtained from the fruiting bodies of *Gomphus clavatus* Gray with a yield of 9.3% [4]. After fractionation with Sephadex G-200 and DEAE-cellulose columns, a novel heteropolysaccharide, named GCG-1, from the fruiting bodies of *Gomphus clavatus* Gray was obtained. Molecular weight of GCG-1, determined by HPGPC was ~50,000Da. By TFA/TLC analysis, it was shown that GCG-1 polysaccharides had a composition of D-glucose and D-galactose [4]. The composition analysis of polysaccharides is an important step to control the quality and obtain basic information about polysaccharides [4].

Determination of 1, 1-diphenyl-2-picrylhydrazyl-free (DPPH-) radical scavenging activity of GCG-1: The DPPH- radical scavenging activity of the polysaccharide sample was measured according to the method described by Braca et al. [20] Ding et al. [4] showed that the decrease in absorbance of the DPPH- radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progress, which results in the scavenging of the radical by hydrogen donation. GCG-1 exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentrations tested. There was a dose-dependent increase in the percentage of antioxidant activity for all concentrations tested (Figure 3). Antioxidation test *in vitro* showed that GCG-1 had a noticeable effect on scavenging the DPPH- radical, which may be comparable to Ac and BHT [4].

Antioxidant activity analysis of GCG-1: PC12 cells were seeded into 96-well plates at a concentration of $5x10^4$ Cells/ml using DMEM. In the CCK-8 experiments, it was shown that the protective effect of GCG-1 on PC12 cells from hydrogen peroxide (H_2O_2)-induced injury. After pretreatment with 0.25, 0.5, 1, 2 mg ml of GCG-1, the PC12 cells were protected from H_2O_2 (300mM) injury in a dose-dependent manner, with cell viability rates of 13.8, 38.5, 75.2 and 86.3%, respectively (Figure 4). In PC12 cells as determined by the antioxidant effect assay, it was confirmed that GCG-1 attenuates the injury on PC12 cells induced by H_2O_2. Through these results obtaining from radical scavenging activity and Antioxidant activity analyses of GCG-1, we assume that *Gomphus clavatus* Gray may be an ideal source for antioxidant agents [4].

Effect of GCG-1 on the morphology of human hepatoma hepG-2 cells: The 96-well plates were placed under an inverted microscope, and images recorded the changes in cell morphology for different concentrations of GCG-1 for measuring the effect of GCG-1. GCG-1 exhibited high anticancer activity as observed from the cell morphology, examples are shown in Figure 5 [4]. These are anticipated that new discoveries of the health benefits in *Gomphus clavatus* Gray will be promising for medicinal products for human diseases in the future.

Conclusion

Mushrooms are valuable resources for many purposes. Wild mushrooms have been consumed by humans as a diet for centuries for their nutritional and medicinal benefits. Mushrooms have low in energy and fat but high in protein, carbohydrate, and a variety of vitamins, minerals for nutrient foods. In addition, some mushrooms are also an important source of biologically active compound with medicinal value. Recently, many researchers pay attention to study antioxidant activities of edible mushrooms species. According to the results of recent studies, it is clearly indicated that the extract from mushroom, fruiting bodies has significant antioxidant activities

against various oxidative damages [4-7]. *Gomphus* clavatus, commonly known as pig's ears, is a species of fungi in the genus *Gomphus*, family Gomphaceae in Basidiomycetes. *Gomphus clavatus* is not only used as a nutritious food but also might be an important source of biologically active compounds with potential additional medical value. In recent research, the fruit bodies of *Gomphus clavatus* Gray were collected in China. A novel heteropolysaccaride, namely 'GCG-1', from the fruit bodies of *Gomphus clavatus* Gray was isolated through Sephadex G-200 and DEAE-cellulose columns. Antioxidation test in GCG-1 *in vitro* showed that it has strong free radical scavenging activity. It suggests that *Gomphus clavatus* can be considered as a medicinal food with antioxidant activities. Given the many mushrooms species that have not yet been studied, it is anticipated that new discoveries of the health benefits in mushrooms will continue and promising mushroom treatments and products for human diseases may be found in the future [21].

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