



COMPARATIVE STUDY OF PHYTOCHEMICALS IN AQUEOUS AND SILVER NANOPARTICLES EXTRACTS OF *AGROCYBE AEGERITA*, (V. BRIG.) SINGER BLACK POPLAR MUSHROOM

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ABSTRACT

The objective of the present study is to evaluate the phytochemical constituents of the mushroom *Agrocybe aegerita* (V. Brig.) Singer using four different extracts. Preliminary phytochemical screenings for various secondary metabolites were carried out. All the extracts both aqueous and silver nanoparticles were subjected to qualitative phytochemical screening and were analyzed for the presence of active constituents such as alkaloids, flavonoids, phenols, glycosides and triterpenoids. The generated data has provided the basis for its wide uses as a therapeutic both in traditional and folk medicine. The observed phytochemical constituents in the mushroom extract may be associated with its high bioactive constituents that may serve as candidates to new drugs in the treatment and prevention of various human ailments. The result obtained in the present study indicates *Agrocybe aegerita* mushroom have the potential to act as a source of useful drugs because of the presence of various primary and secondary metabolites.

KEY WORDS: *Agrocybe aegerita*, qualitative analysis, silver nanoparticles, primary metabolites, secondary metabolites.

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INTRODUCTION

Since thousands of years, mushrooms are favoured due to their delicious taste and their nutritive and healthy values^{1, 2}. Mushrooms have been widely used as food and food supplements for millennia. It is an important food item concerning human health, nutrition and disease prevention³. There is a common saying that "medicines and foods have a common origin"⁴. Mushrooms are rich in protein, minerals and vitamins and they contain an abundance of essential aminoacids⁵. Mushrooms have long been used as a valuable food source and as traditional medicines around the world, especially in Japan and China⁶. In the recent years, much attention has been paid to the investigation of nutraceuticals from various edible mushrooms⁷. Terpenes or terpenoids are amongst the compounds responsible for the medicinal, culinary and fragrance uses of aromatic and medicinal plants⁸. The herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries;

more than 65% of the global population uses medicinal plants as a primary health care modality⁹. The most predominantly studied about nanoparticles today are those made from noble metals, in particular Ag, Pt, Au and Pd. Among the four, silver nanoparticles play a significant role in the field of biology and medicine¹⁰. The field nanoscience and technology has gained great importance because of their potential applications in various areas such as chemicals, textile industries, material industry, medical diagnostics (future nanobots), drug and gene delivery and electronics, diagnosis, artificial implants, tissues engineering¹¹, computing, biochemical sensors¹², medical imaging¹³ and so on. Silver is medically considered as one of the most powerful elements due to its activity against mammalian tissues where it acts as an antiseptic agent¹⁴. Edible mushrooms are consumed by humans for their nutritional and medicinal values. Mushrooms consumed for health reasons are known as medicinal mushrooms.

SCIENTIFIC CLASSIFICATION OF *AGROCYBE AEGERITA* (V.Brig.)Singer

Kingdom: Fungi
Division: Basidiomycota
Class: Basidiomycetes
Order: Agaricales
Family: Strophariaceae
Genus: *Agrocybe*
Species: *A.aegerita*



In addition to a dietary as well as medicinal point of view, higher fungi may provide potent beneficial effects on human health either directly as antioxidant or through prevention of and metabolic syndrome. Alterations underlying major pathological states such as cancer, diabetes neurodegenerative diseases, cardiovascular diseases and metabolic syndrome¹⁵. Mushrooms have long been appreciated for their flavor and texture. Now they are recognized as not only a nutritious food but also as an important source of biologically active compounds possessing medicinal value¹⁶. This situation has prompted the search for potential antioxidant from natural sources. The phenolic compounds in mushrooms have been found to be an excellent antioxidant and synergist that is not mutagenic. Antioxidant compounds prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis. Mushrooms that contain antioxidants or increase antioxidant enzyme activity may be used to reduce oxidative damage in humans¹⁷. Mushroom species have been shown to possess antioxidant capacity *in vitro* systems¹⁸. Like other matrices containing antioxidant compounds, e.g. phenolics¹⁹, organic acids²⁰ and alkaloids²¹ from mushrooms can be used both as a food supplement and in the pharmaceutical industry. The public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. Worldwide spending on finding new anti-infective agents is increasing. The use of other alternative forms of medical treatments is being investigated by researchers. It has been estimated that phytochemical intake may be related to a reduction in cancer risk upwards of 20%. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value²². For example, saponins have hypotensive and cardio depressant properties²³. The present study evaluates the quantitative phytochemical analysis of mushroom extracts of *Agrocybe aegerita*; so that it's unexplored nutraceutical potential can further be exploited.

MATERIALS AND METHODS

1. Sample collection

Mushroom of *Agrocybe aegerita* was cultivated in mushroom units, maintained in Kongunadu Arts and Science College, Coimbatore-29.

2. Mushroom powder

The mushrooms after washing were shade dried and was powdered in a mixer grinder.

3. Preparation of extracts

(a) Cold water decoction

10g of the powdered sample was dissolved in 100ml of distilled water, which was continuously shaken for 24 hours in a mechanical shaker at 40°C. After 24 hours, it was filtered and used. The decoction was stored at 4°C for further usage.

(b) Hot water decoction

10g of the powdered sample was dissolved in 100ml of distilled water, which was boiled for one and half hours and filtered. The decoction was stored at 4°C for further usage.

4. Biosynthesis of silver nanoparticles from both hot and cold aqueous extract of *Agrocybe aegerita*

10ml of the extract was added into 90ml of an aqueous solution of 1mM silver nitrate of Ag⁺ ions and stirred at room temperature for 24- 48 hours.

5. Qualitative phytochemical screening

Aqueous extracts of *Agrocybe aegerita* were used for qualitative screening of phytochemicals as per standard biochemical procedures. The preliminary tests for both hot and cold aqueous extracts and both hot and cold aqueous nanoparticles extracts were performed to confirm the presence of alkaloids, anthraquinones, anthocyanins, carbohydrates, flavonoids, glycosides, phenols, proteins and amino acids, saponins, steroids, tannins and terpenoids²⁴. The obtained stock solutions were subjected to preliminary phytochemical screening. Tests for alkaloids, flavonoids, saponins, tannins, phenols, anthraquinones,

acid, phlobtannins, resins, coumarins, quinones, thiols, terpenoids, triterpenoids, cardiac glycosides, oxalates, anthracenoids, emodins, chalcones, anthocyanosides, gum and mucilages, carbohydrates, proteins, aminoacids, steroids and anthocyanins were conducted using standard methods.

RESULTS AND DISCUSSION

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. Long practiced outside of conventional medicine, herbalist is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show their value in the treatment and prevention of disease²⁵. Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drug has been isolated from natural sources²⁶. Medicinal values of mushroom have assumed an important dimension in the past few decades. Results of

work had indicated that phytochemicals were responsible for medicinal effects of this mushroom. The medicinal value of mushroom lies in some chemical substances that have a definite physiological action on the human body. The study of mushroom continues principally for the discovery of novel secondary metabolites. Qualitative phytochemical analysis of *Agrocybe aegerita* extracts showed the presence of alkaloids, anthraquinone, glycosides, saponins, flavonoids, terpenoids, phenolics, carbohydrates, anthocyanins as well as proteins and amino acids and steroids (Table 1). Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in each extract and powder. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their qualitative estimation and separation of pharmacologically active chemical compounds.

Table 1
Qualitative phytochemical screening of *Agrocybe aegerita* extracts

Constituents	Hot Aqueous	Cold Aqueous	Hot Aqueous Nanoparticles	Cold Aqueous Nanoparticles
Alkaloids	++	++	+++	+++
Flavonoids	++	++	+	+
Saponins	+++	+++	+++	+++
Tannins	++	++	++	++
Phenols	++	+++	+	+
Anthraquinones	+	+	--	+
Acid	+++	++	+++	+++
Phlobtannins	+	+	--	--
Resins	+	--	--	+
Coumarins	++	+	++	--
Quinones	+++	++	+++	+++
Thiols	++	++	--	++
Terpenoids	+	++	--	--
Triterpenoids	++	++	--	--
Cardiac Glycosides	+	+++	+	+
Oxalates	+++	++	+++	+++
Anthracenoids	+	++	+	+
Emodins	+	++	--	--
Chalcones	+	++	--	--
Anthocyanosides	+	--	--	--
Gum and Mucilages	++	++	--	--
Carbohydrates	+++	+++	++	++
Proteins	++	++	++	++
Aminoacids	++	++	++	++
Steroids	++	++	--	--
Anthocyanins	--	--	--	--

•KEY: + = Presence, - = Absence, ++=Plenty, +++ = Abundance

A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers^{27, 28; 29}. It is now widely accepted that dietary polyphenolics may play an important role in protecting the body against chronic diseases, such as cancer, cardiovascular diseases³⁰ and diabetes mellitus³¹. Flavonoids have been reported to possess many useful properties, including anti-inflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumor activity^{32, 33}. Flavonoids are reported to regenerate the damaged pancreatic beta cells³⁴. The alkaloids are well known to have medicinal properties in animals³⁵. It should be noted that steroids compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones³⁶. Anthraquinones are a class of natural products encompassing several hundreds of compounds, differing in the nature and positions of substituent groups. Many anthraquinones have potential therapeutic value, since antimicrobial, insecticidal, antitumor, anticongestive, hypotensive and sedative properties have been assigned to these compounds^{37, 38}. Terpenoids play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical functions^{39, 40}. Also, terpenoids are attributed for analgesic and anti-inflammatory activities. From clinical studies, it is shown that terpenoids can increase the concentration of antioxidants⁴¹. Resins are hydrocarbon secretions of many plants valued for their chemical constituent⁴². Coumarin has been reported to exhibit antioxidant, analgesic, anti-inflammatory and anti-mutagenic properties⁴³. Chalcones and its derivatives have attracted increasing attention due to numerous pharmacological applications. They have displayed a broad spectrum of pharmacological activities, among which antimalarial⁴⁴, anticancer^{45, 46}, antiprotozoal⁴⁷, antifungal⁴⁸, antimicrobial⁴⁹, larvicidal⁵⁰, anticonvulsant⁵¹ and antioxidant⁵² activities have been reported. Terpenoids and tannins are attributed for

analgesic and anti-inflammatory activities. Apart from this tannins contribute property of astringency i.e. faster the healing of wounds and inflamed mucous membrane⁵³. Emodin forms the basis of purgative anthraquinones derivatives and from ancient times has also been widely used as a laxative compound⁵⁴. Recent studies have shown that emodin also exhibits numerous other biological activities which affect the immune system, vasomotor system and the metabolic processes⁵⁵⁻⁵⁷.

CONCLUSION

The presence of phytoconstituents marks the mushroom useful for treating different ailments and has a potential of providing useful drugs for human use. In the present study, we have found that most of the biologically active phytochemicals were present in the hot aqueous and hot aqueous nanoparticles extracts of *Agrocybe aegerita*. Since the hot aqueous extract and hot aqueous nanoparticles extracts of the mushroom contains more constituents it can be considered beneficial for further investigation. The medicinal value of the plant lies, in some chemical substance that produces a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobtannins. Most of the researchers suggest that the medicinal mushroom contain many of the phyto constituents, could be used for therapeutic purposes as they often exhibit a huge amount of medicinal properties such as antioxidant, anticarcinogenic, antitumor, antidiabetic, anti-inflammatory activities that are non-lethal and most valuable to the living system. In this study *Agrocybe aegerita* mushroom screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various primary metabolites and secondary metabolites that are vital for

good health. The finding of this study suggests that this mushroom could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing various diseases. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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