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Qualitative screening of phytoconstituents of *Pleurotus sajor caju* (Fries sing) and comparison between hot and cold – aqueous and silver nanoparticles extracts

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Abstract
The objective of the study is to screen the phytochemicals which are the dependable sources for the treatment of different health problems. The present study aims to investigate the preliminary phytochemical screening using four different extracts of the mushroom *Pleurotus sajor caju* (Fries) Sing. Belonging to the family polyporaceae. Both cold and hot aqueous and silver nanoparticles extracts were subjected to qualitative phytochemical screening and is known to contain certain bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids, vitamins, minerals and has also proved to contain antioxidant and therapeutic properties. The generated data has provided the basis for its wide uses as therapeutics both in traditional and folk medicine. The results obtained in the present study indicate *P. sajor caju* mushroom shows the high percentage of potential to act as a source of useful drug because of the presence of various bioactive compounds.

Keywords: *Pleurotus sajor caju*, aqueous extract, nanoparticles, phytochemical constituents, bioactive compounds.

Introduction
India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value [1, 2]. The scientific search for new drugs from natural products remains a serious task for scientists worldwide. It is a fact that a large segment of the population in tropical countries rely on traditional medicines for their health care needs [3]. Over 80% of population in the developing world makes use of medicinal plant extracts to provide good health [4].

Traditional knowledge of medicine has long been used since ages for curing various human ailments. About 60-80% of world populations still rely on plant based medicines [5]. Though the traditional Indian system of medicine has a long history of use, yet they lack adequate scientific documentation, particularly in light of modern scientific knowledge [6].

The mushrooms are largely used as a foodstuff from historical details as a traditional food and it have greater importance in the diet of mankind most recently. The cultivation and production of edible mushrooms are on the increase, particularly in Europe, America and Asia due to its increased nutritional values. Their increased nutritional importance is almost equals to that of the milk [7]. Edible mushrooms are valuable healthy foods, having rich source of vitamins, proteins and minerals, especially in potassium and phosphorus.

They are also low in calories and fats [8]. Because of the low fat and oil content, they are recommended as good source of food supplement for patients with cardiac problems or at risk with lipid induced disorders. The available literature review indicates that mushrooms have phytochemicals with the compounds of strong antioxidants [9]. The protein content of mushrooms has been reported to be twice that of vegetables and four times that of oranges and significantly higher than that of wheat [10-12]. *Pleurotus* spp. are promising as medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulating activities [13].

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 medicine [14]. Silver is medically considered as one of the most powerful elements due to its activity against mammalian tissues where it acts as an antiseptic agent [15]. The present study is designed to screen and to determine the bioactive compounds present in edible mushroom *Pleurotus sajor caju.*

Scientific Classification

Scientific classification	
Kingdom:	Fungi
Phylum:	Basidiomycota
Class:	Agaricomycetes
Order:	Polyporales
Family:	Polyporaceae
Genus:	Pleurotus
Species:	*P. sajor-caju*

Materials and Methods

1. Sample collection

Fresh fruiting bodies of *Pleurotus sajor caju* mushroom were cultivated during the period from November to February 2014 - 2015 in the mushroom units maintained at Kongunadu Arts and Science College, Coimbatore-641029, Tamil Nadu, India.

2. Mushroom powder

The fresh fruiting bodies of the mushroom were shade dried after washing and powdered in a mixer grinder.

3. Preparation of hot and cold water extract

For hot extract preparation, 10g of mushroom powder was dissolved in 100 ml of distilled water. The extract was boiled for 6 hrs and the supernatant was filtered. The decoction was stored at 4 °C for further usage.

For cold extract preparation, 10g of the powdered the sample was dissolved in 100ml of the distilled water which was continuously shaken for 24 hours in a mechanical shaker at 40 °C. After 24 hours it was filtered and stored at 4 °C.

4. Biosynthesis of silver nanoparticles from hot and cold aqueous extracts

1mM aqueous solution of silver nitrate ($AgNo_3$) was prepared and used for the synthesis of silver nanoparticles. 10ml of *P. sajor caju* was added into 90ml of aqueous solution of 1mM silver nitrate for reduction into Ag+ ions and incubated overnight at room temperature in dark.

5. Qualitative phytochemical screening

The obtained decoctions during extract preparation were subjected to preliminary phytochemical screening. The hot and cold aqueous and nanoparticle extracts of *P. sajor caju* were used for qualitative screening of phytochemicals such as alkaloids, flavonoids, saponins, tannins, phenols, anthraquinones, acid, phlorotannins, resins, coumarins, quinones, thiols, terpenoids, triterpenoids, cardiac glycosides, oxalates, anthracenes, emodins, chalcones, anthocyanosides, gum and mucilages, carbohydrates, proteins, aminoacids, steroids and anthocyanins using standard biochemical procedures. The preliminary tests were performed for both hot and cold aqueous extracts and both hot and cold aqueous nanoparticles extracts to confirm the presence of alkaloids, anthraquinones, anthocyanins, carbohydrates, flavonoids, glycosides, phenols, proteins and amino acids, saponins, steroids, tannins and terpenoids [16].

Results

Traditional knowledge of medicine has long been used since ages for curing various human ailments. So through phytochemical screening one could detect the various important compounds which could be used as the base of modern drugs for curing various diseases.

The result of the preliminary phytochemicals methods are presents of alkaloids, flavanoids, phenols, anthraquinone, glycosides, tannins, saponins, terpenoids, carbohydrates, anthocyanins as well as proteins, amino acids and other phytochemical compounds as found in Table 1. The absence of phlobatannins, anthocyanins and emodins are noted. Phytochemical screenings are highly present in hot water extract and silver nanoparticles.

Discussion

Herbal medicines are free from side effects, adverse effects and they are economical and easily available will be beneficial for the mankind over the centuries. The term “materia medica” which means “Medical Materials” [17] is no longer utilized routinely in Western medicine, the fact remains that the physicians of today continue to use many substances and products derived from natural sources, usually for the same therapeutic benefit as the crude drug. These single chemical entities, i.e., drugs, form the basis for much of our ability to control disease.

Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. Phytochemicals may protect human from a host of diseases. Phytochemicals are non-nutritive plant or mushroom chemicals that have protective or disease preventive properties [18]. There are many phytochemicals in fruits and herbs and each works differently [19]. It contains phytochemical compounds and also the phytotoxicity assay may be accomplished due to the presence of active biological compounds. In drug discovery, the major secondary metabolites are of potential medicinal interest. Drug discovery is the key attempt of our age to overcome many life-threatening diseases like cancer. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics [20] and about 150 phytochemicals have been studied in detail.

Table 1: Qualitative Phytochemical screening of *Pleurotus sajor caju* extracts

S. No	Test	Hot Extract	Cold Extract	Nano Particles Hot Extract	Nano Particles Cold Extract
1.	Alkaloids	+++	+++	++	+++
2.	Flavanoids	++	++	+	+
3.	Saponins	+++	+++	++	++
4.	Acids	+++	++	+++	++
5.	Tannins	++	++	++	++
6.	Phenols	++	+	++	+
7.	Anthraquinones	+	+	-	-
8.	Anthracenoids	+	++	++	-
9.	Phlobatannins	-	-	-	-
10.	Quinones	++	++	+++	++
11.	Triterpenoids	+	-	-	-
12.	Steroids	+	-	-	-
13.	Cardiac glycosides	+++	+	++	+
14.	Oxalates	++	++	+++	++
15.	Anthocyanins	-	-	-	-
16.	Emodins	-	-	-	-
17.	Chalcones	-	-	-	-
18.	Gum & Mucilages	++	+++	-	-
19.	Carbohydrate	+++	++	++	++
20.	Protein	+++	++	++	+++
21.	Resins	++	++	-	++
22.	Coumarins	-	+	+	-
23.	Volatile oils	++	++	++	++
24.	Fatty acids	++	++	++	++
25.	Thiols	-	+ ++	++	-
26.	Anthocyanosides	+	++	-	++

+++ High presence ++ Moderate presence + Presence - Absence

Different aqueous extract have shown many secondary metabolites as a source of bioactive compounds have great chemical diversity and are involved in many metabolic and ecological processes. New scientific strategies are required for the evaluation of natural products with specific biological activities which requires large screening process. Silver nanoparticles have gained much interest among the emerging nanoproducts in the field of nanomedicine due to their unique properties and obvious therapeutic potential in treating variety of diseases, including retinal neovascularization [21, 22]. The nanoparticles can be synthesized by physical, chemical, and biological methods. Silver nanoparticles play a significant role in the field of biology and medicine. Silver nanoparticles were synthesized rapidly within 72 hour of incubation period using *pleurotus sajor caju.* While the mushroom extract incubated with deionized water retained its original color. The silver nitrate treated mushroom extract turned to brown color after 72 hours due to deposition of silver nanoparticles [23].

In the present work the phytochemicals of silver nanoparticles cold and hot water extracted from *Pleurotus sajor caju* might react with free radicals, which are the major propagators of the fat autoxidation chain, there by terminating the chain reaction [24]. The antioxidant activity of natural antioxidants is due to the termination of the free radical reaction [25]. Primary and secondary metabolites are present in cold and hot water extract. The cold water extract of silver nanoparticles possess less phytochemicals when compared to the hot water extract of silver nanoparticles. Cold water extract of *Pleurotus sajor caju* when compared to hot water extracts contain higher amount of bioactive compounds. 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 quantitative estimation and qualitative separation of pharmacologically active chemical compounds. Phlobatannins have been reported for its wound healing properties, these are anti-inflammatory and analgesic [26] and antioxidant [27].

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation [28]. The presence of tannins in diets for livestock have been reported to have anti-nutritional and toxic effects including reduced feed intake, growth, feed efficiency and net metabolizable energy [29]. Saponins have been reported to have antifungal properties [30] as well as serve as an expectorant and emulsifying agent [31]. Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion and progression of tumors [32]. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and [33] cardiac arrhythmia.

Further terpenes or terpenoids are active against bacteria [34-36]. The presence of terpenoids shows that it could be effective against any bacterial infections. Resins are hydrocarbon secretions of many plants valued for their chemical constituent [37]. Coumarin has been reported to exhibit antioxidant, analgesic, anti-inflammatory and anti-mutagenic properties. [38]. 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, anti-congestive, hypotensive, and sedative properties have been assigned to these compounds [39, 40]. Emodins forms the basis of purgative anthraquinones derivatives and from ancient times has also been widely used as a laxative compound [41]. Recent studies have shown that emodin also exhibits numerous other biological activities, which affect the immune system, vasomotor system and the metabolic processes [42-44].

Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections.

Conclusion

The presence of phytoconstituents makes the mushroom extracts useful for treating different ailments and has a potential of providing useful drugs for human use. Herbal medicines are rich in the active ingredients and are safe with the body chemistry of man. The results obtained in the present study indicates *P. sajor caju* mushroom have the potential to act as a source of useful drugs because of presence of various phytochemical components such as carbohydrate, protein, lipids, phenols, flavonoids and tannin. 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.

It is concluded that *P. sajor caju* extract could be a potential source of natural antioxidants, and the consumption of mushrooms might give certain level of health protection against oxidative damages. With the established antioxidant activity of these mushroom extracts, the chemical characteristics of the antioxidative components in the extracts could be further investigated. In the present study most of the phytochemicals are present in the hot aqueous and hot nanoparticles. Since the hot aqueous extract and hot aqueous

nanoparticles extracts of the mushroom contains more constituents it can be considered beneficial for further investigation. Thus, further study can be aimed at establishing the nutraceutical potential of this mushroom. The results are very much encouraging but scientific validation is necessary before being put into practice.

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