Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(3): 198-202
Received: 15-07-2015
Accepted: 16-08-2015

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Quantitative estimation of primary and secondary metabolites in hot aqueous extract of *Pleurotus sajor caju*

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Abstract
Mushrooms are white rot fungi regarded as one of the well known food and possessing various kinds of biopharmaceuticals compounds. Mushrooms are superior to many vegetables based on their nutritional value and it contains 40-49% of proteins. The present study involves quantification of primary and secondary metabolites like carbohydrates, protein, amino acids, alkaloids, flavanoids, phenol, tannin and vitamins. The presence of biologically active secondary metabolite constituents might be responsible for the bacterial activity observed in the present study. Mushroom *P. sajor caju* bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Medicinal mushrooms have anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammation activities. Phytochemicals are non-nutritive chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life.

Keywords: Primary metabolites and Secondary metabolites, Hot water extract, Medicinal properties and *Pleurotus sajor caju.*

1. Introduction
Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and less side effects [1]. They are part of a very extensive natural health products category in Asia where Traditional Chinese Medicine has utilized herbal preparations for thousands of years. Asia is also the historical site of mushroom cultivation. Shiitake cultivation is reported to have originated in China in the 12th century. Medicinal mushrooms belonging to higher Basidiomycetes reported to have wide range of biological activities [2, 3].

If we are able to evaluate mushroom products on the basis of two or more of the primary active compounds, this would bring a level of consistency and uniformity to an otherwise uncertain and unknown area of product quality [4]. These compounds are classified into primary and secondary metabolites [5]. Primary metabolites are essentially required for growth and development of mushroom such as sugars, proteins, vitamins, lipids and starch whereas chlorophyll, aminoacids, nucleotides and carbohydrates have a key role in metabolic process such as photosynthesis, respiration and nutrient assimilation. Amino acids are building blocks for the synthesis of proteins, including antioxidant enzymes. Some amino acids and small peptides directly scavenge oxygen free radicals.

Secondary metabolites are not necessary for the actual growth or life of the organism. They will accumulate during growth of the fungus and generally speaking do not degrade easily. Some of these metabolites are biologically active [6]. Secondary metabolites are not involved directly and they have been worked as biocatalysts which are synthesized during secondary metabolism of mushroom and are potential source of drugs. The most important secondary metabolites include saponin, tannins, alkaloids, cardiac glycosides [7]. Secondary metabolites are also of interest because of their use as dyes, fibers, glues, oils, waxes, flavoring agents, drugs and perfumes, and they are viewed as potential sources of new natural drugs, antibiotics, insecticides and herbicides [8, 9].

Vitamins are organic compounds required as vital nutrients in tiny amounts by an organism. Vitamins serve as biocatalysts in many chemical reactions as well as precursors to various body factors. They also required for a variety of biological processes such as mental alertness e.g niacin; resistance to infections e.g. vitamin C. Vitamin A is necessary in vision, gene transcription [10,11], immunity, dermatology [12], growth and development [13] and so on.

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

Materials and Method

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 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.

Quantitative determination of primary metabolites
Primary metabolites directly involved in growth and development while secondary metabolites are not involved directly and they have been worked as biocatalysts. Primary metabolites are of prime importance and essentially required for growth of mushrooms. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites of pharmaceutical compounds such as antipsychotic drugs [14, 15].

1. Determination of total soluble carbohydrates
The total soluble carbohydrate content was determined according to the method of Dubois *et al*. [16]. 1.0 ml of sample was mixed with 1.0 ml phenol solution and added 5.0 ml of 96% sulphuric acid to each tube and shake well. Incubated in boiling water bath for 20 minutes, after which the absorbance was read at 490 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.

2. Determination of proteins
Protein content was determined according to the method of Lowry *et al*. [17]. 1 ml of sample was mixed with 0.5 ml of 0.1 N NaOH and 5 ml of alkaline copper reagent, incubated the mixture in room temperature for 30 minutes. Added 0.5 ml of Folin–Ciocalteau reagent and incubated again for 10 minutes at room temperature. Absorbance was read at 660 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed mg/g sample.

3. Determination of total free amino acids
Total free amino acids (ninhydrin method) were determined according to the procedure given by Moore and Stein [18]. 1 ml of the sample was mixed with 1 ml of Ninhydrin in a test tube. Tubes were kept in boiling water bath for 20 minutes and then added 5 ml of diluent (equal volume of water and n-propanol) incubated at room temperature for 15 minutes and absorbance was read at 570 nm against a reagent blank. The analysis was performed in triplicates, and the results were expressed as mg/g sample.

4. Determination of Vitamins
Vitamins in *Pleurotus sajor caju* were estimated by standard methods. Vitamins have diverse biochemical functions and thus the necessity for its analysis.

Quantitative analysis of secondary metabolites
Secondary metabolites are important mediators of ecological interactions between mushrooms and their environment.

1. Determination of Total phenols
Total phenol content were estimated in the ethanolic extract by the procedure given by Bray and Thorpe, 1954 [15], Folin-ciocalteau method. To 1 ml of sample added 0.5 ml of Folin-ciocalteau reagent and incubated at room temperature for three minutes. After three minutes 2 ml of 20% Na_2CO_3 was added, mixed well and incubated the tubes in boiling water bath for 1minute. Cooled rapidly and read absorbance at 650 nm against reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.

2. Determination of Flavonoids
Flavonoids in aqueous extract of *Pleurotus sajor caju* was estimated by the method proposed by Jia *et al*., 1954 [19]. 1 ml of the extract was mixed with 0.075 ml of 5% Sodium nitrite solution and incubated at room temperature for 10 minutes. Then added 10% aluminum chloride and incubated at room temperature for 6 minutes. Then added 1 N NaOH and absorbance was read at 510 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg catechin equivalent/ g sample.

3. Determination of Tannins
Tannins in aqueous extract was done by the procedure given by standard methods of Bray and Thorpe, 1954 [15].1 ml of the sample was mixed with 5 ml of vanillin hydrochloride reagent and incubated at room temperature for 20 minutes. Absorbance was read at 500 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as catechin equivalents.

4. Determination of alkaloid

Estimation of alkaloids in the extract was done by the procedure given by Harborne [20]. 10 mg of mushroom was homogenized in a motor and pestle. Added around 20 ml of methanol: ammonia (68:2). Decanted the ammonicial solution and after 24 hrs added fresh methanolic ammonia. Repeated the procedure thrice and pooled the extracts. The extracts were evaporated using a flash evaporator. Treated the residue with 1 N HCl and kept it overnight. Extracted the acidic solution with 20 ml of chloroform thrice, pooled the organic layers and evaporated to dryness, basic fraction. Basified the acidic layer with concentrated sodium hydroxide to pH 12 and extracted with chloroform (20 ml) thrice, Pooled the chloroform layers, dry over absorbent cotton and evaporated to dryness. The fraction that contains alkaloids was weighed and expressed as mg/100g.

Statistical analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation.

Results

Medicinal mushrooms are of great importance to health of individual and communities. Mushrooms are fungal organisms that are considered health foods, nutritional supplements and nutraceuticals. The medicinal values of a mushroom lie in some chemical substances that produce a definite physiological action on the human body. Phytochemical analysis is of paramount importance in identifying a new source of therapeutically and industrially valuable compounds having medicinal mushroom have been chemically investigated. In the present investigation primary and secondary metabolites and vitamins were qualitatively and quantitatively analyzed using *P. sajor caju* aqueous extract. The results are presented and tabulated in Tables 1, 2 and 3.

Table 1: Primary metabolites in aqueous hot extract of *P. sajor caju* mushroom

Primary metabolites	Quantity mg/g
Carbohydrates	2.53 ± 0.40
Proteins	7.59 ± 0.23
Amino acids	2.89 ± 0.30

Values are expressed by mean ± SD of three samples

Table 2: Secondary metabolites in aqueous hot extract of *P. sajor caju* mushroom

Secondary metabolites	Quantity mg/g
Alkaloids	2.81 ± 0.61
Flavanoids	5.36 ± 0.31
Phenols	3.35 ± 0.20
Tannins	6.84 ± 0.12

Values are expressed by mean±SD of three samples

Table 3: Vitamins in aqueous hot extract of *P. sajor caju* mushroom

Vitamins	Quantity mg/g
Vitamin A	0.87 ± 0.03
Vitamin C	1.13 ± 0.03
Vitamin E	0.52 ± 0.01

Values are expressed by mean±SD of three samples

Discussion

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds having medicinal mushrooms have been chemically investigated [21]. In the present investigation primary and secondary metabolites were quantitatively analyzed using *P. sajor caju*. Quantitative analysis of primary metabolites shows that (Table 1), protein content was found to high (7.593±0.238 mg/g) followed by amino acid (2.89±0.30 mg/g) and then carbohydrate (2.53±0.40 mg/g). Carbohydrates are one such group of carbon compounds, which are essential to life. Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact, exploit their rich supply of potential energy to maintain life. Proteins are essential to maintaining the structure and function of all life and vital for growth and development. The presence of higher protein level in the mushroom points towards their possible increase in food value or that a protein based bioactive compound could also be isolated in future [22].

Different phytochemicals have been found to possess wide range of medicinal properties, which may help in protection against various diseases. The quantitative estimation of primary and secondary metabolites reveals various chemical constituents present in the mushroom.

Secondary metabolites analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. Several studies have indicated that antioxidants prevent the onset of degenerative illness such as certain cancers, cardiovascular and neurodegenerative diseases, contracts, oxidative stress dysfunctions and aging [23]. The hot water extract showed higher level of flavonoids (5.36±0.31 mg/g) than the other secondary metabolites. Flavonoids have been reported to exert wide range of biological activities. These includes: Anti-inflammatory, antibacterial, antiviral, anti-allergic [24-26], cytotoxic anti-tumour, treatment of neurodegenerative diseases, vasodilatory action [27-29]. In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation [30]. These are also reported to inhibit variety of enzymes like hydrolases, hyaluronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase [31].

Polyphenols are the most widely distributed class of secondary metabolites and several thousand different compounds have been identified phenolic compounds are one of the largest and most ubiquitous groups of mushroom metabolites. Many of the phenolics have been shown to contain higher levels of antioxidant activities [32]. The level of phenols (3.35±0.20) number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers, phenols are involved in defense against UV radiation or aggression by pathogens.

Total content of tannins were found to be higher in *P. sajor caju* (6.84±0.12 mg/g), the growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins. A part from these tannins contribute the property of astringent activity i.e. faster the healing of wounds and inflamed mucous membrane [33, 34]. Tannins have also shown potential antibacterial and antiviral effects [35, 36].

Alkaloids protect against chronic diseases [37] and earlier recorded that bitter leaf contains an alkaloid that is capable of reducing headaches associated with hypertension. Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase [38]. The level of alkaloids was (2.81±0.61).

Unlike the traditional vitamins they are not essential for short-term well-being, but there is increasing evidence that modest long-term intakes can have favourable impacts on the incidence of cancers and many chronic diseases, including cardiovascular disease and Type II diabetes, which are occurring in Western populations with increasing frequency. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries and establishment of crude drugs [39]. In addition antioxidants play a key role in these defense mechanisms which are normally vitamin A, vitamin C, vitamin E and polyphenols [40]. The amounts of vitamins are presented in table 3. Vitamin A (0.87±0.03) Vitamin C (1.13±0.03) and Vitamin E (0.52±0.01) are present.

Thus, the results are obtained in the present study indicates the potential to act as a source of useful drugs because of presence of various phytochemical components such as carbohydrate, protein, amino acids, alkaloids, phenols, flavonoids tannin and vitamins. The results are very much encouraging, but scientific validation is necessary before being put into practice.

Conclusion

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. *P. sajor caju* the mushroom has act as a primary and secondary metabolites of protein, carbohydrate, amino acids, phenols, flavanoids, tannins, alkaloids and vitamin A, C and E. In medicinal mushroom play a key role in curing various diseases and antimicrobial, anticancer, antipyretic, astringent, antiviral activities are mushrooms are due to its compound. The results are suggestive of primary and secondary bioactive compounds are commercially and pharmaceutically important. Analysis of mushroom primary metabolites is necessary for knowing the nutritional potential for secondary metabolites for medicinal value.

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