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European Journal of Experimental Biology, 2016, 6(3):46-54

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Cultivation and phytochemical analysis of wild mushrooms *Daldinia concentrica and Pheolus schweinitzii* from Tamilnadu, India

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ABSTRACT

Daldinia concentrica and Pheolus schweinitzii were collected from the deep forests of western ghats. Then that collected mushrooms were cultivated. The qualitative chemicals screening were performed for the Methonolic extracts of mushrooms. Then the phytochemicals like alkaloids and flavonoids were quantitatively estimated. The wild mushrooms were collected properly, identified, and classified depend upon the Botanical classification. That classified mushrooms were cultivated. The qualitative chemicals analysis of the mushrooms showed positive results for alkaloids, phenols, flavonoids, tannins and glycosides.

Keywords: Identification, Botanical classification, *Daldinia concentrica, Pheolus schweinitzii,* Phytochemical

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INTRODUCTION

Fungi are among the most diverse, important and omnipresent groups of organisms on earth, though they have received less attention than animals and plants, therefore related studies are mostly inadequate worldwide. These are the members of the family both Ascomycotina and Basidiomycotina. Mushrooms are comprised of around 230 genera and 5000 species. Of these more than 2000 species are reported to be edible throughout the world and about 283 of these are reported to be available in India. Mushrooms have continued to generate a lot of interest particularly in its consumption as food , in the cure of diseases, in bioremediation and as important items of commerce in India, and all over the world. The increased interest in consumption of mushrooms as food items from their nutritional, antioxidant and therapeutic values. Studies have shown that tropical mushrooms are highly rich in proteins, minerals, vitamins, crude fiber and carbohydrate with low fat and oil content. Barros *et al*., [5] reported that the wild mushrooms were richer sources of protein and had a lower amount of fat than commercial mushrooms. 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

1.1 MUSHROOM CULTIVATION

Beginning from Mushroom's maiden artificial cultivation in 1976 on a mixture of soil, sand and maize meal (12: 6: 1) in soil jars, improved production techniques were developed later on by various workers. It is gaining popularity among the potential mushroom growers as well as perspective consumers owing to attractive shape and size, simple growing technique, low capital investment, wide substrate range, sustainable yield, long shelf-life and ability to thrive in a wide range of climatic conditions. Quality and quantity of spawn play an important role in the successful production of any mushroom species .India produces nearly 140 million tonnes of cereals and equal amount of straw is generated by the farmers, which can partly be utilized by the farmers for mushroom cultivation. Land requirement is a minimum and any spare room of the house can be converted into a mushroom growing room, or a hut built on a

piece of land can also be used for the purpose. The raw materials required for crop raising or generated by the farmers on their own fields (paddy/wheat/or any other cereal straw The raw materials requirement for raising a crop of mushroom is recyclable cereal straw/organic waste/organic by products. The main by products used for substrate preparation for mushroom farming are a. Wheat / paddy straw, b. Sugarcane baggage, c. Saw dust, d. Cotton seed meal/soybean meale. Scores of the locally available agro by products and other agro waste materials like banana pseudo stem/ corn cobs/ groundnut hull etc. poultry manure is also used as nitrogen rich supplement for compost making for mushroom cultivation. These raw materials are locally available in all the rural areas of the country.

1.2. PHYTOCHEMICAL ANALYSIS OF MUSHROOMS

Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Also, a mushroom phenolic compound has 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 human. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides. They are capable of attacking the healthy cells of the body, causing them to lose their structure and function.

The present study deals with the cultivation of the varieties of wild mushrooms from Tutucorin District, Tamilnadu, and it's phytochemical analysis were assayed

MATERIALS AND METHODS

2.1. Sample collection

Two different types of wild mushrooms were collected from various places around Tutucorin District. Then it was transported to laboratory using a clean poly ethylene Bag.

2.3. Identification

For confirmation of identification, taxonomic keys and descriptions were consulted. Descriptions of basidiomycetes were made according to their macro, micro and cultural features by using standard manuals such as Manual of soil fungi,. Colony color, morphology, hyphal structure, spore size and spore bearing structures were identified and compared

2.4 Sample Preparation

Mushrooms from the forest were first washed thoroughly to free from mud, ferns and other extraneous maerial, dried on blotting paper. Fresh mushrooms were used for the tissue culture. The mushrooms selected are normally harvested for consumption without division into pileus and stipe Then the mushrooms were destalked, washed and sun-dried by constant exposure to sunlight for 2 – 4 days while turning the mushrooms to avoid fungal growth.(*G. Johnsy et al.,* 2011)

Then it was cut into pieces. Therefore, the whole mushrooms (Pileus + stipe) were dried, later milled to obtain mushroom meals (MRMS) using mortar and pestle and this was stored in a container until needed for analysis.(Egwim ECet al., 2011).

2.5. Macroscopic examination

Macroscopic characters of fresh specimens were noted after the collection. Photographs of the fresh specimens were taken both in the collection place as well as in the laboratory (Atri *et al.,* 2003; Kaviyarasan *et al.,* 2009).

2.6. Microscopical observation

Morphological observations mainly followed by different methods. Lacto phenol cotton blue staining was used as the mounting medium. Microscopic characters were observed using a light microscope.

2.7. Grinding

The sporo caps were cut in to small pieces, dried at 40° C for 48 hours and powdered. In each step, the plant material was dried to remove moisture and overcome the fungal contamination. The air-dried powder was stored in an air tight container for further use.

2.8. MYCELIAL WEIGHT:

Mycelia fresh weight (in grains) was determined as follows:
Weight (wt) of bottle = xg

Wt of bottle and spawn grains = (X + Y)g
Wt of bottle + wt of spawn grains + wt of fresh mycelia = (X + Y + Z)g
Fresh mycelial wt. = (X + Y + Z)g – (X + Y)g = Zg
A Ruler was used to measure the mycelial extension.

2.9. Culture of fungi

Fresh mushrooms were taken and its surface were sterilised. Then a small piece of the mushrooms inner tissue was taken and placed on PDA medium using sterile forceps. The inoculated dishes were incubated at 27 degree C for ten days. Several sub-culturings were produced

Fig:1 Tissue culture

Fig:2 Mycelial production

3. CULTIVATION PROCESS

3.1. Spawn Preparation

250g of red sorghum were washed in clean water three times to remove chaff, dust and other particles. The grains were then soaked in water for 24 hours for maximum absorption of water. Soaked grains were again washed in water drained and put into spawn bottles. 2/3 of each spawn bottle were filled with grains and mixed with 5g of calcium carbonate (CaCO3) after which they were autoclaved at 1210c (at a pressure of 1kg/cm2) for 2 hours each day, for 3 consecutive days. The grains in the bottles were then inoculated with three 9mm mycetial discs per bottle (in triplicate of each grain type) under aseptic condition (Fasidi and Kadiri, 1993). These were incubated at 27 + 10c. (Fritsche., 1978)

Environmental factors such as temperature, O2, Co2, humidity, light and pH have been reported also to affect mycelia growth in the spawn preparation (Nwanze et al, 2005b).

3.2. Mushroom cultivation from spawns

Rice straw was cut into pieces of 5 cm long. Then soaked in water for 2 to 4 hours, then cleaned and. Then it was boiled at 15 minutes for the purpose of sterilization. After the sterilization it was introduced and dumped into 12x24cm polypropylene bags. The spawns were spreaded over the straw. Then the small holes were produced on bags for aeration purpose. Then it was placed into the dark room. And maintained moisture condition for 20-25 days. After 23 days of incubation the cultivated wild mushrooms were harvested. (O.P. Ahlawat et al., 2007)

Fig:3 Spawns

Fig:4, 5:Mushroom cultivation

3.3. Determination of Moisture: The moisture content is determined by measuring the of a material before & after the water removed by evaporation Moisture content was determined by following

%Moisture =initial –dried/initial *100

Here M initial & M dried are the mass of sample before & after drying respectively To obtain an accurate measurement of the moisture content of material evaporation method necessary to remove all water molecules.

3.4. Preliminary qualitative phytochemicals screening

The phytochemical analyses of the mushroom extracts were carried out following the methods of Adebayo and Ishola, and the following phytochemicals were evaluated. *(G. Sathyaprabha et al.,2011)*

The methanol extract of *Daldinia concentrica* and *Pheolus schweinitzii* checked for the presence of the following secondary metabolites such as Alkaloids, Phenols, Flavonoids, Saponins, Steriods, Cardiac glycosides, and Tannins

by standard procedures. The phytochemical analyses of the mushroom extracts were carried out following the methods of Adebayo and Ishola , and the following phytochemicals were evaluated. *(G. Sathyaprabha et al.,2011)*

3.4.1. TEST FOR ALKALOIDS
Dragendroff's test: Sample was dissolved in chloroform. Evaporated chloroform and acidified the reside by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates the presence of alkaloids.

Percentage alkaloids %: = Weight of ppt/ Weight of the sample ×100

3.4.2. TEST FOR FLAVANOIDS
Ferric chloride test: To the alcoholic solution of the sample, drops of neutral ferric chloride solution were added. Appearance of green colour indicates the presence of flavanoids.

3.4.3. ANTHRAQUINONES:
5 g of the extracts was shaken with 10 ml of benzene. The solution was filtered and 5 ml of 10% NH4OH solution was added to the filtrate. A pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones

3.4.4. TEST FOR GLYCOSIDES
Keller-Killiani test: To Sample, 1ml of glacial acetic acid plus few drops of ferric chloride solution + Conc. H2SO4 was added slowly through the sides of the test tube. Appearance of reddish brown ring at the junction of the liquids indicates the presence of de-oxysugars.

3.4.5. TEST FOR PHENOLIC COMPOUNDS AND TANNINS
Ferric chloride test: To 2 ml of sample in a test tube, ferric chloride solution was added drop by drop. Appearance of bluish black precipitate indicates presence of phenolic compounds and tannins.

3.4.6. TEST FOR PHYTOSTEROLS
Salkowski reaction: 0.5 ml sample in chloroform was taken in a test tube and 1ml of Conc.H2SO4 was added from the sides of the test tube. Appearance of reddish brown color in chloroform layer indicates presence of phytosterols.

3.4.7. SAPONINS:
Foam test: A small amount of sample was taken in a test tube with little quantity of water and shaken vigorously. Appearance of foam persisting for 10 minutes indicates presence of saponins

0.1 g of the powdered extract was boiled in 10 ml of distilled water for 5 min and decanted while still hot. The filtrate was used for the following tests.

(a) Frothing test: 1 ml of filtrate was diluted with 4 ml of distilled water and mixture was shaken vigorously and observed for persistent foam which lasted for at least 15 min.

(b) Emulsion test: This was performed by adding 2 drops of olive oil to the frothing solution and shaken vigorously. Formation of an emulsion indicated a positive test

3.4.8. TEST FOR TRITERPENOIDS
Tschugajeu test:
To the sample in chloroform, excess of acetyl chloride and a pinch of zinc chloride were added and kept aside for reaction to subside and then warmed on water bath. Appearance of eosin red colour indicates presence of triterpinoids.

3.4.9TEST FOR CARBOHYDRATES
Benedicts test: 5-8 drops of the sample was heated with Benedict's reagent in a test tube. Change in blue color varying from yellow to brick red indicates the presence of carbohydrates.

Molisch's test: Sample was mixed with Molisch's reagent and Conc. H2SO4 was added along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicates the presence of carbohydrates

3.5. TEST FOR PROTEINS

Ninhydrin test: Few drops of Ninhydrin were added to the sample. Appearance of blue colour indicates positive result.

RESULTS AND DISCUSSION

4.1. IDENTIFICATION:

The collected wild mushrooms were identified according to their macro, micro and cultural features by using standard manuals such as Manual of soil fungi.

According to the manual, Ash coloured, spindle shaped, didn't have the stem, Terricolous in natured fungi is *Daldinia concentrica.* So our Sample A is Confirmed that *Daldinia concentrica*.

Fig: 6 *Daldinia concentrica*

Classification as follows,
Kingdom – Fungi
Family: Xylariaceae
Common name: Cramp balls
Cap colour: Jamun colour later becoming black.
Cap shape: Hemispherical with slightly incurved base.
Cap diameter: 2.5 to 6.0 cm.
Tube length: no tube found.
Spore print: Black.
Spore colour: Black.
Spore size: 4.74 to 5.54 × 2.74 to 3.40 µ
Spore shape: Spindle shaped
Edibility: Not known .

The sample B is flat shaped mushroom,It is in yellow colour surrounded by creamy in nature.terricolous in nature.It didn't contain tubes.Based on it's Structure, the sample B is *Pheolus schweinitzii*, It's classification is as follows,

Pheolus schweinitzii
Kingdom – Fungi
Cap colour- yellow colour surrounded by creamy white colour.
Cap shape- Flatted dish shape
Cap surface- Smooth
Cap diameter- 9 to 12 cm
Cap margin- creamy white colour.
Tube length- no tubes found.
Spore colour- creamy white colour.
Edibility-Non-edible.
The collected samples were *Daldinia concentrica, Pheolus schweinitzii.*

Table: 1 Habit

s. no	Wild mushrooms	c.names	Habitat
1	*D.concentrica*	Carbon balls	Lignicolous
2	*P. schweinitzii*	velvet-top fungus	Lignicolous

4.2. CULTIVATION:

The spawns were produced then it was introduced into the Cultivation process. Prepared.Spawns were germinated. The air dried mushrooms were powdered. In between its moisture level was identified, by using standardized formula.

4.3. Moisture level

Daldinia concentrica

Initial weight-100 g
After dried=11.3g
MOISTURE= initial –dried=100-11.3=89.7g
%Moisture=89.7%

Pheolus schweinitzii

Initial weight-100 g
After dried-11g
MOISTURE= initial –dried=100-11=89g
%Moisture=89%

Table:2 *g-Grams*

s.no	Identification	*D.concentrica*	P.*schweinitzii*
1.	Moisture	89.7%	89%
2.	Mycelial weight	181g	239g

4.4.Mycelial weight

Daldinia concentrica

Weight of bottle = 234 g
Weight of spawn grains =150 g
Weight of fresh mycelia = final weight of bottle- (Wt of bottle + wt of spawn grains)
=565g-(234+150)
Weight of fresh mycelia =181 g

Pheolus schweinitzii

Weight of bottle = 234 g
Weight of spawn grains =150 g
Weight of fresh mycelia = final weight of bottle- (Wt of bottle + wt of spawn grains)
=623g(234+150)
Weight of fresh mycelia =239g

After produced the methanolic extracts. Phytochemical analysis were undertaken.

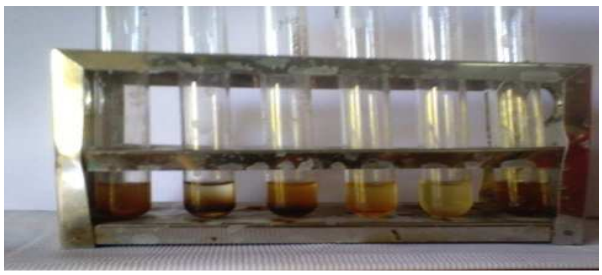

Fig: 7 *Daldinia concentrica*

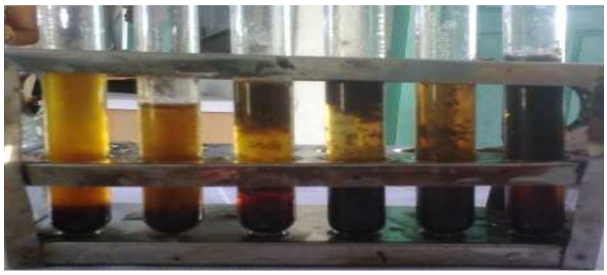

Fig: 8 *Pheolus schweinitzii*

Phytochemical screening of these mushrooms revealed the presence of alkaloids, saponins, steroids, flavonoids, Anthraquinones, glycosides, tannins which varies quantitatively from low to highly present.

Table: 4 Phytochemicals

S.no	Phytochemicals	*D.concentrica*		P. *schweinitzii*	
1	Alkaloids	+	0.103+0.01	+	0.048+0.03
2	Flavonoids	+	1.76+20	+	1.36+0.03
3	Anthraquinones	-	0	+	3.24+0.03
4	Glycosides	+	2.046+0.02	+	5.790+0.01
5	Tannins	-	0	-	0
6	PhytoSterols	+	7.90+0.59	+	16.81+0.01
7	saponins	+	4.07+0.03	+	4.21+0.03
8	Triterphenoids	+	8.65	-	0
9	Carbohydrates	+	8.14+0.03	+	5.42+0.01
10	proteins	-	0	-	0

(+) *Indicates presence of phytochemicals* ***(-)*** *Indicates absence of phytochemicals*

4.4.1. TEST FOR ALKALOIDS

Dragendroff's test: Sample was dissolved in chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). The orange red precipitate was appeared for both samples.

4.4.2. TEST FOR FLAVANOIDS

Ferric chloride test: To the alcoholic solution of the sample, drops of neutral ferric chloride solution were added. Green colour appeared. It indicates presence of flavanoids. Flavonoids presence in the samples. Level of flavonoids were measured.

4.4.3. ANTHRAQUINONES:

5 g of the extracts was shaken with 10 ml of benzene. The solution was filtered and 5 ml of 10% NH4OH solution was added to the filtrate. A pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones. *D.concentrica* did not contains anthraquinones. *Pheolus schweinitzii* contains Anthraquinones. Its level was identified.

4.4.4. TEST FOR GLYCOSIDES

Keller-Killiani test: 1ml of glacial acetic acid plus few drops of ferric chloride solution + Conc. H2SO4 was added slowly through the sides of the sample tube. Reddish brown ring at the junction of the liquids indicates the presence of de-oxysugars Both samples produced positive results.

4.4.5. TEST FOR PHENOLIC COMPOUNDS AND TANNINS

Ferric chloride test: To 2 ml of sample in a test tube, ferric chloride solution was added drop by drop. Appearance of bluish black precipitate indicates presence of phenolic compounds and tannins. In this test both samples produced Negative results.

4.4.6. TEST FOR PHYTOSTEROLS

Salkowski reaction: 0.5 ml sample in chloroform was taken in a test tube and 1ml of Conc.H2SO4 was added from the sides of the test tube. Appearance of reddish brown colour in chloroform layer indicates presence of phytosterols. It indicates positive result.

4.4.7. SAPONINS:

Foam test: A small amount of sample was taken in a test tube with little quantity of water and shaken vigorously. Appearance of foam persisting for 10 minutes indicates presence of saponins. The foam was produced in each samples.

0.1 g of the powdered extract was boiled in 10 ml of distilled water for 5 min and decanted while still hot. The filtrate was used for the following tests.

(a) Frothing test: 1 ml of filtrate was diluted with 4 ml of distilled water and mixture was shaken vigorously and observed for persistent foam which lasted for at least 15 min.

(b) Emulsion test: This was performed by adding 2 drops of olive oil to the frothing solution and shaken vigorously. Formation of an emulsion indicated a positive test. The foam was produced.

4.4.8. TEST FOR TRITERPENOIDS

Tschugajeu test:

To the sample in chloroform, excess of acetyl chloride and a pinch of zinc chloride were added and kept aside for reaction to subside and then warmed on water bath. Sample 1 contains eosin red colour. Sample 2 did not contains colour reaction .It indicates absence of triterpinoids.

4.4.9TEST FOR CARBOHYDRATES

Benedicts test: 5-8 drops of the sample was heated with Benedict's reagent in a test tube. Change in blue color varying from yellow to brick red indicates the presence of carbohydrates. In this test gives the positive result for our samples.

Molisch's test: Sample was mixed with Molisch's reagent and Conc. H2SO4 was added along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicates the presence of carbohydrates.

4.5. TEST FOR PROTEINS Ninhydrin test:

Few drops of Ninhydrin were added to the sample. Appearance of blue colour indicates presence of amino acid where as proteins may rarely give positive result. The samples did not produced blue colour.

CONCLUSION

Since 1970s numerous mushroom fungi have been increasingly used as a source of medicinal compounds and therapeutic adjuvants or health food supplements. It contains phytpochemical 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. Plant-based compounds have been playing an important role in the development of several clinically useful anticancer agents i.e., including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan and etoposide derived from epipodo phyllotoxin.

Different studies have shown many secondary metabolites as a source of bioactive compounds with allelo chemical potential have great chemical diversity and are involved in many metabolic and ecological processes.

In drug discovery, the major secondary metabolites (terpenoids, phenolics and alkaloids) are of potential medicinal interest. The mentioned structure diversity is reflected in a variety of biological activities as, for instance, inhibitors of enzymes and antitumor, immunosuppressive and anti parasitic agents.

Based on the result of these findings, it can be concluded that the six wild mushrooms were cultivated and investigated about its phytochemical analysis. These mushrooms contain the various quantities and chemical components.

Acknowledgement

We thank The Principal Chief Conservator of Forests, Chennai and The Chief Conservator of Forests and Field Director, Kalakad Mundanthurai Tiger Reserve, Tirunelveli. We also thank Dr. M. Kannan, assistant professor, the forest guides, and family members for assisting in collection.

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