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Article

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Effect of extraction method on Mycochemicals and Proximate composition of *Pyrrhoderma noxium* (Corner) L.W. Zhou & Y.C. Dai, (Hymenochaetales, Basidiomycota)

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Abstract

Several medicines obtained from *Pyrrhoderma* mushrooms are widely used against several diseases throughout the world. So, in preparation of crude drugs/ folk medicines of mushroom i.e. *Pyrrhoderma noxium* collected from Krishna district, Andhra Pradesh, India was studied and identified phenotypically. The present work aims to test the presence of Mycochemicals and proximate composition for standardization of powder of *P. noxium.* The solvents used for extraction and method of extraction played a vital role on mycochemical and proximate composition of *P. noxium* sporophore. The maceration water bath assisted extraction method is the best extraction method compared to maceration and Reflux extraction methods for all parameters studied in this work. The present study shows the extraction method and type of solvent influencing the presence of mycochemical composition like alkaloids, carbohydrates, phenols, flavonoids, tannins, terpenoids, diterpenoids, and anthocyanin in hymenochaetales fungi. The proximate composition evaluation is very much useful for standardisation of *P. noxium* in powder form. That will help to identify the genuine species and check for adulteration of powder available commercially.

Key words – Extraction type – Hymenophore – Phytochemicals – Phenotypical – *Phellinus noxius*

Introduction

Allopathic medicines prepared from pharmaceutical industry are costly, have various side effects, and are not safe for use. So alternative to synthetic drugs, medicines prepared from natural herbal sources are in great demand in these days. Medicines prepared from mushrooms are widely used against several diseases throughout the world (Chenghom et al. 2010). hymenophores of *Pyrrhoderma* is used as folk medicine for curing different diseases since ancient times(Chenghom et al. 2010). *Pyrrhoderma* is reported to contain different bioactive compounds, such as carbohydrates, proteins, phenols, alkaloids, terpenoids, polysaccharides, steroids and fatty acids (Lahiri et al. 2010, Nagadesi et al. 2016). The phytochemicals, physicochemical and mycochemical aspects have not been studied for every *Pyrrhoderma* species. In this study phenotypical, mycochemical, proximate composition of *P. noxium* is carried out for the specimens collected from Krishna district, Andhra Pradesh, India. This research also helps to establish the standards for identity, quality, purity and mycochemical composition of *P. noxium*.

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Materials & Methods

Collection of Hymenochaetales fungi

The sporophores of *Pyrrhoderma noxium* was collected from Andhra Pradesh, India, during the rainy season (July–September) of the years 2014 to 2017. Field characters like habit, host, name of locality, and other macro-morphological characteristics were recorded for sample specimens. Voucher specimen of *P. noxium* (ALC 25) has been deposited at the herbarium of the Museum of Botany Department, Andhra Loyola College, Vijayawada, Andhra Pradesh, India (ALC).

Phenotypical identification

For identification of hymenophore different Macroscopic features like abhymenial, hymenial surfaces, context, and pore tubes of species were examined. Microscopic features like hyphae, basidiospores and pilear crust were observed by preparing crush mounts and free-hand sections in water, 5% KOH solution, and staining was done with cotton blue (1%, in lactophenol), Congo red (1%, in distilled water), phloxine (1%, in distilled water), and Melzer's reagent (Nagadesi et al. 2016).

Preparation of Extraction

The fruiting bodies of *P. noxium* were initially rinsed thrice in distilled water and dried on paper towels and samples was cut into fine pieces and powdered. For preparing the extracts water, methanol, and ethanol were used as solvents.

Maceration

5 grams of sporophore powder were soaked in 100 ml of water, methanol and ethanol, separately. All the samples were left at room temperature for 24 hr. Then the samples were filtered through whatman filter paper No.1 pore size and further used for mycochemical qualitative and quantitative tests.

Maceration Water Bath

5 g of sporophore powder were soaked in 100 ml of water, methanol and ethanol, separately. All the samples were heated in Maceration Water Bath at 600°C for 1 hour. Then the samples were filtered through whatman filter paper No.1 pore size and further used for mycochemical qualitative and quantitative tests.

Reflux apparatus

For every 5 gram of powder, 100 ml of solvent was used and was subjected to extraction using a reflux apparatus. After the completion of extraction, the supernatant was filtered through Whatman No. 1 filter paper. The filtrates were stored at 4°C to perform various assays for determination of bioactivity mycochemicals.

Screening of Bioactive Mycochemical

The screening of bioactive mycochemicals in fresh sporophores of *P. noxium* were tested by using standard methods followed by Evans & Trease 1989, Gokhale 1993, Trease & Evans 1996, Harborne 1973).

Proximate composition

To establish standards for their identity, quality, purity of hymenophore powder the proximate composition was carried out for *P. noxiun* collected from Krishna district, Andhra pradesh, India. The pulverized sporophore of *P. noxium* was used for the standardization of physicochemical parameters in triplicate. Foreign matter, moisture content, extractive values, ash values (Gaithersburg 2000, Indian Pharmacopoeia Commission 2007) dry matter (Kornerup & Waanscher 1978), absorption properties, foaming properties (Aremu et al. 2007), emulsion values

(Yatsumatsu et al. 1972), dispersibility (Kulkarni & Ingle 1991), flow characteristics, swelling index (Prodhan et al. 2015) were determined.

Results

Phenotypical identification

***Pyrrhoderma noxium* (Corner) L.W. Zhou & Y.C. Dai, Mycologia 110 (5): 882 (2018)**

Basidiocarps perennial, imbricate, effused-reflexed, woody hard, light in weight when dry, 18cm wide 10cm broad, 6 cm thick near the base, upper surface black, glabrous, with a hard crust, azonate, frequently nodulose at the center, margin, obtuse, entire, yellowish brown; pore surface reddish brown, tubes distinctly layered, 2 mm deep in each layer, pores small 6-8 per mm, angular, moderately thick-walled entire, darker than the context. Context: 2 cm thick at base, zoned, pale brown, radially fibrous; hyphal system dimitic, setal hyphae present, 6.2 – 15.6 µm wide, up to 4.2 µm long, rare and narrow in context, frequently projecting into the lumen of tubes, dark reddish brown, tips obtuse; hymenial setae absent, Basidia hyaline, 5.2 - 8.3 × 3.1 - 5.2 µm, Basidiospores hyaline, subglobose, smooth, thin-walled, 2.1 – 3.8 x 2.1 µm.

Preparation of Extraction

Three extraction methods were employed in order to obtain the biologically active myco-chemical components in water, methanol, ethanol and 50% hydro-methanol as solvents. The Maceration Water bath extraction shown better mycochemical and proximate composition when compared to Maceration and Reflux apparatus extraction methods.

Effects of extraction method on Mycochemical composition in *P. noxium*

Mycochemical compounds screening of water, methanol, ethanol and 50% Hydro-methanol extracts of *P. noxium* were prepared by using Maceration, Maceration Water bath, Reflux apparatus methods and the results of the effect of extraction methods and solvent on mycochemical compounds are presented in Tables 1. The best extraction method for *P. noxium* is maceration water bath extraction because almost all mycochemicals are extracted. The best solvent for preparation of *P. noxium* extraction in all three methods is methanol because all tests for mycochemical composition shown positive. The methanol extract of *P. noxium* prepared by maceration water bath showed excellent concentration of alkaloids, carbohydrates, phenols, flavonoids, tannins, terpenoids, diterpenoids, and Anthocyanin; the ethanol extract showed excellent concentration of alkaloids, phenols, flavonoids, tannins, terpenoids, diterpenoids, and Anthocyanin; 50% hydromethanol showed phenols, flavonoids, tannins, terpenoids.

Proximate composition

The hymenophore powder of *P. noxium* shows the proximate composition in Table 2. Foreign matter present in sporophore powder of *P. noxium* is 0.03% whereas moisture content in sporophore powder of *P. noxium* is 7.4%. The higher the dry matter in sporophore of *P. noxium* indicate presence of less moisture. The extractive value of powder of *P. noxium* shows higher ethanol soluble value when compared to water soluble content. The ash content of sporophore powder of *P. noxium* shown higher water soluble ash when compared to acid insoluble ash. The absorption of sporophore powder of *P. noxium* shown higher water absorption when compared to oil absorption. The emulsion formation capacity of sporophore of *P. noxium* shown higher dispersibility when compared to emulsion stability. The flowing properties of sporophore powder of *P. noxium* shown tapped density is more when compared to bulks density. The sporophore powder of *P. noxium* shown higher foaming capacity.

Table 1 Mycochemicals screening of different extracts of *P. noxium*

Extraction by maceration	**Alkaloid**	**Carbohydrates**	**Proteins**	**Amino Acids**	**Tannins**	**Flavonoids**	**Phenols**	**Terpenoids**	**Di Terpenoids**	**Anthocyanins**
Water	++	+++	++	-	++	+	++	+	+	+
Methanol	+++	+++	+	+	+++++	+++++	+++	+++++	++++	+++
Ethanol	+++	++	++	+	+++++	+++++	+++	+++++	+++	+++
50% methanol	+++	+++	++	-	+++	+++	+++	+++	++	++
Extraction by Water Bath	**Alkaloid**	**Carbohydrates**	**Proteins**	**Amino Acids**	**Tannins**	**Flavonoids**	**Phenols**	**Terpenoids**	**Di Terpenoids**	**Anthocyanins**
Water	+++	++++	+++	+	+++	++	+++	++	++	++
Methanol	++++	++++	++	++	++++++	+++++	++++	++++++	+++++	++++
Ethanol	++++	+++	+++	++	++++++	++++++	++++	++++++	++++	++++
50% methanol	+++	++	+++	+	++++	++++	++++	++++	+++	+++
Extraction by reflux apparatus	**Alkaloid**	**Carbohydrates**	**Proteins**	**Amino Acids**	**Tannins**	**Flavonoids**	**Phenols**	**Terpenoids**	**Di Terpenoids**	**Anthocyanins**
Water	++	++	+	-	++	++	++	++	++	++
Methanol	++	++	+	-	++++	++++	+++	++++	+++	+++
Ethanol	++	+	+	-	++++	++++	+++	++++	+++	++
50% methanol	+++	+++	+	-	+++	++	++	++	++	++

+= present, ++ (or) +++= moderately present, ++++ (or) +++++= Excellent

Discussion

Mycochemical compounds

P. noxium showed positive reactions to bioactive compounds like Alkaloid, Tannins, Flavonoids, Phenols, Terpenoids, Diterpinoids, and Anthocyanins in both Methanol and Ethanol extracts (Nagadesi et al. 2016). In the present study, the mycochemical composition of water methanol ethanol and 50% Hydromethanol is studied and the methanol is best solvent shown all the compounds tested. For the first time the carbohydrate, proteins, amino acids are shown positive results in all extraction methods and solvents of *P. noxium.*

Proximate composition

Foreign matter present in a sample or drug masks its quality and purity. Therefore it cannot be neglected. A very small amount of foreign matter was noticed i.e. 0.16% in *P. gilvus* and 0.02% in *P. torulosus* respectively (Azeem et al. 2018). In this study, very small amount of foreign matter is shown by *P. noxium* (0.03%). The higher the moisture content, the greater is the degradation of the mushroom sample due to enhanced microbial

growth and hydrolytic enzyme activity. The moisture content in the *P. torulosus* is 11% and *P. gilvus* is 21.33% (Azeem et al. 2018). In the present study, the moisture content of *P. noxium* is less (7.46%) when compared to *P. torulosus P. gilvus*. Dry matter of sporophore powder of *P. gilvus* is 78.67 and *P. torulosus* is 89.1% (Azeem et al. 2018). In the present paper, the dry matter of *P. noxium* is 90%. Extractive values indicate that each tested mushroom had greater alcohol soluble polar constituents i.e. 2.93% in *P. gilvus* and 2.33% in *P. torulosus* than water soluble constituents i.e. 1.73% in *P. gilvus* and 1.83% in *P. torulosus* (Azeem et al. 2018). In the present paper, the extractive values like alcohol soluble polar constituents and water soluble constituents of *P. noxium* is high when compared to *P. gilvus P. torulosus* Ash content provides an indication of earthy material or inorganic compounds in the drug. The ash content of mushrooms was found in the range 1.3–6.3% estimated for *Phellinus linteus* (Gordon 1993). The ash content in sporophore powder of *P. gilvus* is 4.33 % and *P. torulosus* is 3.83% respectively (Azeem et al. 2018). In the present paper, the total ash content in sporophore powder of *P. noxium* is 5.25%. Water soluble ash indicates the amount of inorganic constituents in herbal drugs. Acid insoluble ash gives an idea of silica present and contamination with earthy material. The values for acid insoluble ash for *P. gilvus* is 1.66% and *P. torulosus* is 1.5% and water soluble ash content in *P. torulosus* powder is 1.0% and *P. gilvus* powder is 1.5% (Azeem et al. 2018). In the present paper, the water soluble and acid soluble ash of *P. noxium* is high when compared to *P. gilvus P. torulosus.*

Table 2 Proximate composite evaluation in sporophore of *P. noxium*

Parameters		***P. noxious***
	Foreign matter (%)	0.03
	Moisture content (%)	7.46
	Dry matter (%)	90
Extractive values (%)	**Ethanol soluble extractives**	4.23
	Water soluble extractives	3.4
Ash content (%)	**Total ash**	5.25
	Acid insoluble ash	2.12
	Water soluble ash	4.5
Absorption properties (ml/g)	**Oil absorption capacity**	9.45
	Water absorption capacity	63.63
Emulsion properties (%)	**Emulsifying capacity**	30.65
	Emulsion stability	21.35
	Dispersability (%)	50
Flow properties	**Bulk density (g/ml)**	1.3
	Tapped density (g/ml)	1.8
Foaming properties (%)	**Foaming capacity**	48.80
	Foaming stability	37.63
	Swelling Index (%)	40

Imbibition of water is an important trait in food products such as sausages, custards and doughs. Water absorption depends on amount and type of hydrophilic constituents, pH and nature of the powder (Omimawo & Akubor 2012). Oil absorption capacity indicates the rate at which proteins bind to fats in food and drug formulations (Khan et al. 2011). *Phellinus torulosus* had higher oil absorption capacity and water absorption capacity than *P. gilvus* (Azeem et al. 2018). In the present paper, the absorption of water and oil of *P. noxium* is high when compared to other phellinus samples. Emulsion capacity is the ability of powder to emulsify oil. Emulsions play a crucial role in pharmaceutical preparations like cosmetics, pastes etc. Emulsions have also been used for treating skin disorders, lacerations and for drug delivery etc. (Dickinson 1994). Certain biochemical constituents too help in stabilizing the emulsion (Narayana & Rao 1982). *Phellinus torulosus* showed greater emulsion capacity and emulsion stability (Azeem et al. 2018). In the present paper, the emulsion capacity and emulsion stability is 30.65% and 21.35% respectively.

Bulk density is a measure of heaviness of powder which provides the relative volume of the packaging material required. Dispersibility of powder in water gives an idea of its reconstitutability. Both the studied *P. gilvus* and *P. torulosus* showed good bulk density i.e. 0.14 g/ml and 0.19 g/ml respectively and dispersibility of *P. gilvus* is 83.67 and for *P. torulosus* is 84.33% (Azeem et al. 2018). In the present study, the bulk density and dispersibility of *P. noxium* is 50% and 1.3 g/ml respectively. Flow properties indicate that powder may be utilized as a direct compression excipient. Hausner ratio provides inter particle friction and Carr's index is a measure of compressibility of powder. Hausner ratio and Carr's index of *Phellinus torulosus* was observed lower than 1.25 and l5% respectively indicating good flow properties (Azeem et al. 2018). In the present study, the flow properties like tap density of *P. noxium* is 1.8 g/ml. Foaming capacity is the ability of a powder to form foam. It is related to the amount of solubilised proteins (Nwokolo 1985) and polar and non-polar lipids in the sample (Akubor & Eze 2012). Saponins also play a role in foam formation. Foams are used to improve texture, consistency and appearance of food and drug (Akata et al. 2012). There was no observed foam formation in any of the tested mushroom samples (Azeem et al. 2018). In this work the foam formation is shown by *P. noxium.* The powder of all tested *Phellinus* mushroom did not swell indicating lack of mucilage substances (Azeem et al. 2018). In the present study the sporophore powder of *P. noxium* showed presence of mucilage like substance. The sensory evaluation of powder of *P. noxium* provides useful information which may prove helpful in authentication and detection of adulteration for quality control.

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References

Akata I, Ergönùl B, Kelyoneu F. 2012 – Chemical compositions and antioxidant activities of 16 wild edible mushroom species grown in Anatolia. International Journal of Pharmacology, 8, 134–138.

Akubor PI, Eze JI. 2012 – Quality evaluation and cake making potential of sun and oven dried carrot fruit. International Journal of Biosciences, 2, 19–27.

Aremu MO, Olaofe O, Akintayo ET. 2007 – Functional properties of some Nigerian varieties of legume seed flour and flour concentration effect on foaming and gelation properties. Journal of Food Technology, 5, 109–115.

Azeem U, Dhingra GS, Shri R. 2018 – Evaluation of Taxonomy, Physicochemical Parameters, and Mycochemical Composition of Wood Decaying Indian Fungi *Phellinus gilvus* (Schwein.) Pat. and *Phellinus torulosus* (Pers.) Bourdot & Galzin: A Comparative Study International Journal of Phytopharmacy Research 9(1),17–25.

Chenghom O, Suksringram J, Morakot N. 2010 – Mineral composition and Germanium contents in some *Phellinus* mushrooms in the Northeast of Thailand. Current Research in Chemistry. 2(2), 24–34.

Dickinson E. 1994 – Protein-stabilized emulsions. Journal of Food Engineering, 22, 59–74.

Evans WC, Trease GE. 1989 – Pharmacognosy 13th ed., Bailliere Tindall, London.

Gaithersburg MD. 2000 – AOAC. Official methods of analysis of the Association of Official Analysis Chemists (17th ed.), AOAC international.

Gokhale SB, Kokate CK, Purohit AP. 1993 – A text book of Pharmacognosy. Published by Nirali Prakshan, Pune, India, 1–50.

Gordon MS. 1993 – Simulation tools for the thermal processing of foods. Food Technology, Phytochemical Methods. Chapman and Hall, London.

Harborne JB. 1973 – Phytochemical Methods: A Guide to Modern Techniques of plants Analysis, 3st Edn., Chapman and Hall, Madras, pp 302.
Indian Pharmacopoeia Commission. 2007 – Indian Pharmacopoeia. Vol.1. New Delhi: Government of India Press.
Khan BA, Akhtar N, Khan HMS. 2011 – Basics of pharmaceutical emulsions: a review. African Journal of Pharmacy and Pharmacology, 5, 2715–2725.
Kornerup A, Waanscher JH. 1978 – Metheun's handbook of colors. 3rd ed. Metheun and Co. Ltd. London, pp 252.
Kulkarni DN, Ingle UM. 1991 – Sorghum malt based weaning food formulations: preparation, functional properties, and nutritive value. Journal of Food and Nutrition, 13, 322–323.
Lahiri SK, Gokania RH, Shuklab MD, Modic HA et al. 2010 – Evaluation of Antioxidant Activity of Plant-parasitic Macrofungus: *Phellinus durissimus* (Lloyd) Roy. Eurasian Journal of Analytical Chemistry, 5(1), 32–45.
Nagadesi PK, Aravind G, Kannamba B. 2016 – Taxonomy and Bioactive chemicals from Ganoderma and Phellinus of India. Biological Forum An international Journal, 8(2), 240–246.
Narayana K, Rao NMS. 1982 – Functional properties of raw and heat processed winged bean (*Psophocarpus tetragonolobus*) flour. Journal of Food Science, 47, 1534–1538
Nwokolo E. 1985 – Nutritional quality of the seeds of the African breadfruit (*Treculia africana* Decne). Tropical Science, 27, 39–47.
Omimawo IA, Akubor PI. 2012 – Food Chemistry (Integrated Approach with Biochemcial background). Agbowo, Ibadan, Nigeria
Prodhan UK, Linkon KMdMR, Al-Amin MdF, Alam MdJ. 2015 – Development and quality evaluation of mushroom (*Pleurotus sajor-caju*) enriched biscuits Emirates Journal of Food and Agriculture. 27(7), 542-547, doi: 10.9755/ejfa.2015.04.082
Trease GE, Evans WCA. 1996 – Textbook for pharmacognosy, 14th Eds. Saunders, W.B (Ed), London. pp 13–53.
Yatsumatsu K, Sawuda K, Moritaka S, Miscki M. 1972 – Whipping and emulsifying properties of soybean products. Journal of Agaricultural and Biological Chemistry, 36, 719–726.