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"Biochemical Diversity and Elemental Composition of *Irpex lacteus* and *Hexagonia nitida*: Towards Sustainable Exploitation of Fungal Resources"

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ABSTRACT

To carry out Mycochemical analysis of *Irpex lacteus* and *Hexagonia nitida* by conventional and advanced techniques. Extracts of fungal material was prepared using eight different solvents on the basis of their polarity index. Mycochemical analysis was carried out by using different reagents, florescence analysis, percent extractive value, Fourier transform infrared spectroscopy, Atomic absorption spectroscopy and High percent extractive value was recorded for methanolic extract of *Irpex lacteus* (56 %). Mycochemical analysis, FTIR and UV- visible spectroscopy showed the presence of, flavonoids, terpenes, phenolics alkaloids, anthraquinones, and saponins and their presence has been reported in *Irpex lacteus* and *Hexagonia nitida*. Trace elements have shown highest concentration in *Irpex lacteus* followed by *Hexagonia nitida*. Obtained results show the potential of this fungal material to be used in herbal drugs.

INTRODUCTION

Fungi found all over and are well adapted to use a wide range of substrates as their carbon, nitrogen, and energy source (Quiroga *et al.*, 2001). From the olden times wood rotting fungi (Aphyllophorales) have been used in the treatment of many diseases (Yemul *et al.*, 2019). Aphyllophorales have been utilized in traditional medicine by indigenous communities in Asia. Basidiomycetes have been identified as a valuable source of bioactive compounds exhibiting a wide range of biological activities, such as antimicrobial (Nakajima *et al.*, 1976), anticancer (Kobayashi *et al.*, 1994), antioxidant (Yun *et al.*, 2002), and nematocidal effects (Morrison *et al.*, 2002), among others (Stamets *et al.*, 2001). In medicine, biologically active substances are used to cellular and humoral immune factors in the body (Wasser, 2002). *Ganoderma lucidum* have been used in traditional Chinese medicine as a tonic for promoting health, vitability and longevity. It is also used to make toothpaste and tea powder (Thyagarajan *et al.*, 2010).

In the present study, we have carried out chemical analyses (conventional and modern methods) of *Irpex lacteus* and *Hexagonia nitida*. In addition to conventional mycochemical analysis, we have used advanced techniques like Attenuated Total Reflectance- Fourier transform infrared spectroscopy and Atomic absorption spectroscopy. ATR- FTIR have several advantages over conventional chromatographic techniques, as these are non-destructive, fast and require minimum amount of analyte. Medicinal properties of fungi are because of its active constituents and a number of trace elements. Screening of these trace elements is essential because above optimum level these elements show lethal effects. In our study, AAS has been employed to quantify heavy metals from *Irpex lacteus* and *Hexagonia nitida*.

1. Materials and Methods

2.1. Collection of Fungal species and Extract preparation:

Irpex lacteus and *Hexagonia nitida* was collected around Junnar, Purandhar, Mulshi, Ragad area of Pune district during September to December 2022. Collected fungal material was dried at hot air oven.

The dried fungal material were powdered and 10g of powder mixed with 100ml of different solvents. The selection of solvents was done on the basis of polarity index where non-polar (dichloromethane petroleum ether and toluene), mid-polar (ethyl acetate and chloroform) and polar (ethanol, methanol and water) solvents were used. All the extracts were sonicated for 30 min. at 33 kHz followed by cold maceration for 24 hours at 4°C and filtered through Whatmann filter paper 1. The obtained filtrate was evaporated and concentrated on rotary evaporator and temperature and obtained viscous extracts were stored at -20°C in air-tight micro-centrifuge tubes for further analysis.

2.2 Percent extractive value

Percent extractive value was determined as per the guidelines of Indian Pharmacopoeia with minor modifications as described by (Nikalje *et al*.2017)

2.3 Mycochemical analysis

Mycochemical test for phenolics, flavonoids, terpenes, alkaloids (Mayer's, Wagner's, Dragendroff, Hager's), anthraquinones, coumarins and saponins were carried out for fungal extracts of *Irpex lacteus* and *Hexagonia nitida* prepared in different solvents (Table 1).

2.4 Mycochemical analysis using ATR-FTIR

The extract was analyzed with an ATR-FTIR spectrosopy (Shimadzu IRAffinity-1S, Serial No. A221354) over a wavenumber range of 4000–400 cm−1, with 30 scans performed at a resolution of 4 cm−1. Background air spectra were subtracted prior to recording the data, which was then processed using the IR solution software (version 1.60SU1).

2.5. Trace element analysis by AAS

Seven different concentrations of heavy metals were prepared to obtain a calibration curve by diluting stock solution of standard for the respective heavy metals. Heavy metal analysis was done by the method described by (Akram *et al*. 2015). All the samples were analyzed in triplicate.

3. Results and Discussion

3.1. Percent extractive value (PEV)

The determination of the percent extractive value (PEV) is essential for assessing drug quality. A low extractive value may suggest the use of depleted or adulterated material, or highlight om *Ganoderma applanatum*.

problems in the manufacturing process, such as inadequate drying, improper storage, or formulation issues (Nisha *et al*., 2017). Among the extracts studied, the highest extractive value was found in the methanolic extract of Irpex lacteus (56%), followed by Hexagonia nitida (37%). In our study, we observed that the PEV varied depending on the solvent's polarity, with polar solvents showing higher extractive values compared to mid-polar and non-polar solvents. These findings align with the work of (Patil *et al*. 2012), who suggested that differences in PEV across various solvents reflect the presence of polar, medium-polar, or non-polar mycochemicals in the extracts. Based on these observations, it can be inferred that the higher PEV observed in methanol and water extracts is likely due to the increased concentration of polar mycochemicals present in these solvents.

3.2. Mycochemical tests

Extraction of *Irpex lacteus* and *Hexagonia nitida* in polar (ethanol, methanol and water) and mid-polar (ethyl acetate) solvents showed presence of phenolics. Previous report on other fungal species has shown that phenolics are more soluble in polar solvents and commonly preferred for recovering phenolics from fungal materials. Xueming *et al*. (2010) investigated Mycoochemical characteristics and hypoglycaemic activity of fraction from mushroom *Inonotus obliquus*. Samuel *et al*. (2012) reported secondary metabolites anthraquinones, flavonoids and steroids from ethanolic extract of the mushroom *Ganoderma applanatum*.

Table 1 Different mycochemical test of *Irpex lacteus* and *Hexagonia nitida*.

Mycochemical	Reagent	Observation by change in colour/ ppt	Reference
Phenolics	Acetic acid	Red	Boxi *et al*. (2010)
Flavonoids	Conc. HCl + Mg turnings	Pink	Nikalje *et al*. (2012)
Terpene	2 ml chloroform + 3 ml of H_2SO_4	Reddish brown	Obianime and Uche, (2008)
Alkaloids	Mayer's reagent	White/ pale ppt	Harbone, (1973) ; Trease and Evans(2002)
	Wagner's reagent	Reddish brown ppt.	
	Dragendroff reagent	Orange ppt	
	Hager's reagent	Yellow ppt	
Anthaquinones	Benzene + 10 % NH_4OH	Bright pink	Auwal *et al* (2014)
Coumarins	10% NaOH + chloroform	Yellow	VimalKumar *et al*. (2014)
Saponins	Distilled water	Froth	Adegoke et al.(2010)

Table 2 Screening of mycochemicals in *Irpex lacteus* and *Hexagonia nitida* **prepared in different solvents.**

mycochemical	***Irpex lacteus***							
	Non polar			Mid polar		Polar		
	PE	T	DM	C	EA	E	M	W
Phenolics	-	-	-	+ +	+	+	+	+
Flavonoids	-	-	-	+	+	+	+	+
Terpene	+ +	+ +	+ +	+ +	+	+	+	+
Alkaloids								
Mayer's reagent	-	+	+ +	+ +	+ +	+	+	-
Wagner's reagent	-	+	+	+	+	+	+	-
Dragendroff reagent	-	+	+ +	+	+	+	+	-
Hager's reagent	-	+	+ +	+	+	+	+	-
Anthaquinones	+	+	+	+	+	+	+ +	-
Coumarins	+	+ +	+	+	+ +	+ +	+	-
Saponins	+	+	+	+ +	+ +	+ + +	+ + +	+ + +
Mycochemical	***Hexagonia nitida***							
	Non polar			Mid polar		Polar		
	PE	T	DM	C	EA	E	M	W
Phenolics	+	-	-	+	+	+	+	+
Flavonoids	-	-	-	+	+	+	+	+
Terpene	+	+	+	+	+ +	+	+	+
Alkaloids								
Mayer's reagent	-	+	+	+	+	+	+	-
Wagner's reagent	-	+	+	+	+	+	+	-
Dragendroff reagent	-	+	+	+	+	+	+	-
Hager's reagent	-	+	+	+	+	+	+	-

Anthaquinones	-	+	+	+	+	+	+	-
Coumarins	+	+	+	+	+	+	+	-
Saponins	+	+	+	+	+	+	+	+ +

*+ + + very high concentration, + + medium concentration, + low concentration, - absent

*PE- Petroleum ether, T- Toluene, DM- Dichloromethane, C- Chloroform, EA- Ethyl acetate, E- Ethanol, M- Methanol, W- Water (pH- 7).

3.3. Mycochemical analysis using ATR**-FTIR**

Identify the chemical bond in different fungal extracts of *Irpex lacteus* and *Hexagonia nitida* was done using ATR-FTIR technique. *Irpex lacteus* prepared using toluene showed maximum peak values (3307.4, 2821.32, 2750.91, 1625.4, 1363.07, 1278.2, 1160.54, cm^{-1}). While, for *Hexagonia nitida* extracts prepared in ethanol showed maximum peak values. The presence of chemical bond within a range of (3322.43, 3352.32, 2838.68, 2362.9, 1626.36, 1541.49, 1462.49, 1229.67 cm^{-1}). This FTIR investigation shows in these two species of fungi many secondary metabolites are present.

Table 3 Mycochemical analysis of extracts of *Irpex lacteus* and *Hexagonia nitida* **by ATR- FTIR**

No	Functional group assignment	Peak values	
		Irpex	*Hexagonia*
		T	E
1	O-H stretch	3307.4	3322.43
2	N-H stretch	3307.4	3352.32
3	C-H stretch	2821.32, 2750.91	2838.68
4	C=N stretch	-	2362.9
5	C=O stretch	1625.4	1626.36
6	C=O stretch	-	-
7	C=O stretch	-	1541.49
8	C-O stretch	1363.07	1462.49
9	O-H bend	1278.2	-
10	C-N stretch	1160.54	1229.67

* T- Toluene, E- Ethanol

3.4 Trace element analysis by AAS

The results of heavy metals analysis in *Irpex lacteus* and *Hexagonia nitida* are presented. Iron: The observed range of iron (Fe) in the present investigation ranged between 0.001–0.002 $mg/100g^{-1}$. The zinc (Zn) content in *Irpex lacteus* and *Hexagonia nitida* ranged between 0.028–0.035 $mg/100g^{-1}$. Manganese: In the present investigation, the concentration of manganese (Mn) in this material of dried powder is ranged between 0.001–0.002 $mg/100g^{-1}$. Manganese is an important heavy metal and its deficiency can lead to reproductive and skeletal abnormalities, whereas excessive concentration can cause adverse effects on human brain and lungs. Copper: The concentration of copper (Cu) ranged between 0.132–0.152 $mg/100g^{-1}$. Copper is involved in various physiological processes in humans, but consumption of excess levels of copper can cause irritation of respiratory tract, diarrhea and liver damage.

Table 4 AAS analysis of powder of *Irpex* and *Hexagonia*

No.	Fungal powder	Element ($mg/100^{-1}$ g of dried tissue)						
		Ferrous	Zinc	Manganese	Copper	Sulphur	Potassium	Phosphorus
1	*Irpex*	0.002	0.035	0.001	0.152	1.85	1.54	81.00
2	*Hexagonia*	0.001	0.028	0.002	0.132	2.02	1.68	75.08

CONCLUSION

Irpex lacteus and *Hexagonia nitida* is an ethno medicinally important fungus. The present investigations carried out in *Irpex lacteus and Hexagonia nitida* are, to the best of our knowledge, reported for the first time. The results obtained by percent extractive values and fluorescence analysis can be used for authentication of fungal material and for validating adulteration in drug preparation. Mycochemical screening revealed the presence of phenolics, flavonoids, terpenes, alkaloid and saponins in *Irpex lacteus and Hexagonia nitida*. Further, these mycochemicals were confirmed by ATR-FTIR spectroscopy. Heavy metals like iron, zinc, manganese and copper were detected and quantified using AAS. The results showed that *Irpex lacteus* and *Hexagonia nitida* contained all these heavy metals.

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