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Qualitative Analysis of Bioactive Components in *Microporus xanthopus* (Fr.) Kuntze

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ABSTRACT: Qualitative analysis has played a crucial role in the identification of new bioactive compounds. The contributions of this study include providing a comprehensive review of the bioactive compounds found in the Polyporales and their pharmacological characteristics as well as identifying several specific compounds using mass spectroscopy. In *Microporus xanthopus* (Fr.) Kuntze compound identification both for positive ion and negative ion mode was done using mass spectroscopy. MS detected presence of several Phenols, Tannin, Sesquiterpenoid, Fatty acid, Macrolide, Steroid like compounds.

Keywords: Polyporales, Bioactive, Mass spectroscopy, Phenols, Tannin.

INTRODUCTION

White-rot basidiomycetes are a group of filamentous fungi that are capable of breaking down lignocellulose in environments rich in lignin and high humidity. These fungi play a crucial role in the degradation of lignin, cellulose, and hemicellulose, and are therefore important in the carbon cycle. Fungi are known to be a significant source of various biological and chemical entities that can have either beneficial or harmful effects (Hyde et al., 2019). In addition, most filamentous fungi produce secondary metabolites that are valuable sources of compounds for various biotechnological and pharmaceutical industries. These metabolites include antibiotics, medicinal products, organic acids, food processing enzymes, and many others that are used in the feed and food industry. Moreover, white and brown-rot fungi that degrade lignocellulose and secrete polysaccharides exhibit a greater diversity of lignin-degrading peroxidases, multicopper oxidases, and glycoside hydrolases. These enzymes work in a sequential pattern to assist in the gradual decay of lignin-rich substrates (Jain et al., 2020; Saini et al., 2020).

Polyporeshas been used in traditional Chinese medicine for thousands of years and is becoming increasingly popular around the world. They exhibit a wide range of bioactivities, including anti-cancer, anti-inflammatory, antiviral, and immunostimulant properties. Many studies have focused on their secondary metabolites, but the importance of Basidiomycetes fungi for bioactivity and metabolite production has not been investigated (Dresch *et al.*, 2015). Bioactive proteins are another essential category of functional components found in mushrooms, which have significant potential for pharmaceutical applications (Xu *et al.*, 2011). fungi consumption, their antioxidant composition attracts the food, cosmetic, and pharmaceutical industries' interest (Quintero-Cabello et al., 2021). Coriolopsis rigida was isolated as an endophytic fungus and two phenolic compounds, tyrosol (1) and a new natural product *p*-hydroxyphenylacetamide (2), were isolated from the extract (Dantas et al., 2022). Medicinal mushrooms also possess several essential biological effects that are beneficial for human health (Bhambri et al., 2022). Ganoderma lucidum main attractive pharmacological characteristics are antitumor and immunomodulatory activities which are chiefly associated with its two principal bioactive compounds, those are polysaccharides and triterpenoids (Ahmad et al., 2022). Fungi bioactive substances are classified into two type's high molecular weight compounds i.e. primarily polysaccharides and proteins and low molecular weight compounds such as steroles, terpenoids, or phenols (Matuszewska et al., 2018). The research problem addressed in this study is the identification of bioactive compounds present in the hot aqueous of Microporus xanthopus (Fr.) Kuntze using mass spectrometry. Specifically, the study aims to identify the different classes of bioactive secondary metabolites, as well as their potential therapeutic properties. This information can provide insight into the bioactivity and metabolite production of these Polypore

good source for developing new antibiotics. Numerous compounds derived from these fungi have antiviral,

cytotoxic, and antineoplastic properties (Zjawiony

2004). Numerous antioxidant compounds have been

identified in Polyporales fungi, including phenolic

compounds, *β*-glucans, ergosterol, ergothioneine,

vitamin C, and tocopherols. Each compound contributes

differently to the antioxidant potential of fungi. Besides

the health benefits for rural communities caused by

An estimated 75% of polypore fungi tested have strong antimicrobial activity, suggesting that they could be a

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fungi, which can potentially lead to the development of new drugs.

MATERIAL AND METHODS

Collection of specimen: The specimen collected from Koyananagar near ozarde waterfall Satara district on fallen angiosperm wood longs. The specimen brought to the laboratory and identify.

Preparation of sample: The specimens washed with distilled water then dry in hot air oven. The basidiocarp is cut into small pieces and grinded to powder. Extracts were prepared by using Soxhlet apparatus. The extract was sent for analysis to the CAMS, Entrepreneurship Development Center, Pune.



Fig. 1. Basidiocarp of *Microporus xanthopus* (Fr.) Kuntze

Sample analysis: The Mass spectrometry was carried out through Agilent TOF/6540B Connected to Agilent

1260 Infinity II HPLC. The Column was Agilent Eclipse XDB –C18, 3X150mm, 3.5 micron. The column temperature is 40°C. Mobile Phase A 0.1% Formic acid in water & Mobile Phase B 0.1% Formic acid in acetonitrile used as in 95:5 percent. The Mobile Phase flow Rate is 0.3 ml/min. Ions source is Dual AJS ESI selected for Positive and Negative polarity. Accurate mass spectra were acquired in the m/z range of 100–1700.Metabolites are identified by matching them to the METLIN database.

RESULT

The sample was analyzed, and the peaks were compared to those of reference chemicals analyzed under the same circumstances. Compounds have some bioactive and pharmacological properties like antifungal, antiviral, anticancer, antitumor, anti-obesity, and anti-inflammatory, etc. This study discovered that compounds from Microporus xanthopus (Fr.) Kuntze contains medicinally and nutritionally significant substances. M. xanthopus (Fr.) Kuntze can be used in effluent treatment, the pulp and paper industry, synthetic chemistry, bio-fuels (Nonate), cosmetics (Lithol Rubine, Polyporusterone F and Hyaluronic acid), agro industry (Dinex, Ethyl(E,Z)-decadienoate, Dinoterb, Malathion and Bromacil), and textile industry produce essential products. Table 1& 2 provides a summary of the bioactive compounds found in Microporus xanthopus (Fr.) Kuntze.

Sr. No.	Compound Name	Retation Time (RT)	Mass	Score	Chemical Nature	Formula	Uses	Reference
1.	Quinoline-3- carboxamides	2.449	359.1056	77.24	-	$\begin{array}{c} C_{21}H_{14}FN_{3}\\ O_{2} \end{array}$	antimalarial, antibacterial, antifungal, antiviral, anticancer, antitumor, anti-obesity, and anti- inflammmatory activities.	Govender <i>et al.</i> (2018), Nilsson <i>et al.</i> (1994).
2.	Quadrigemine A	11.715	690.4143	90.09	Pyrroloindole	$C_{44}H_{50}N_8$	Inhibitors of platelet aggregation	Beretz <i>et al.</i> (1985)
3.	Varanic acid	17.752	466.3278	90.55	Conjugate acid	C ₂₇ H ₄₆ O6	Bile acid metabolite	National Center for Biotechnology Information (2004)
4.	Antimycin A1	19.630	548.2743	91.67	phenols	$C_{28}H_{40}N_2O_9$	Antibiotics use as a fungicide and possibly as an insecticide and miticide	National Center for Biotechnology Information, (2023)
5.	Bisacurone B	19.737	252.1717	96.81	Sesquiterpenoi d	C15 H24 O3	preventing hepatic lipid accumulation	Ashida et al. (2020)
6.	Ethyl(E,Z)- decadienoate	19.926	196.1457	95.93	Ester derivative of geraniol	$C_{12} H_{20} O_2$	Agrochemicals -Pesticides	National Center for Biotechnology Information (2004)
7.	Dinoterb	20.925	240.0741	98.97	Phenols and a C-nitro compound.	C10 H12 N2 O5	Used as a herbicide and a rodenticide	National Center for Biotechnology Information (2004)
8.	Stavudine	21.145	224.0788	93.32	Dideoxynucleo side	$C_{10}H_{12}N_2O_4$	Used in the treatment of HIV infection.	National Institute of Diabetes and Digestive and Kidney Diseases. (2012)
9.	8-(3,3- Dimethylallyl) spatheliachromene	21.586	326.1511	98.36	Polyketides	$C_{20}H_{22}O_4$	Trypanocidal activity	Batista <i>et al.</i> (2008)
10.	Leucomycin V	22.171	701.4002	93.69	Macrolide antibiotic	C35 H59 N O13	Antimicrobial activity against a wide spectrum of pathogens.	National Center for Biotechnology Information (2023)
11.	Dinex	23.753	266.0891	90.80	Alkylbenzene	$C_{12}H_{14}N_2O_5$	Used as an insecticide (control of red mites on citrus) and molluscide	National Center for Biotechnology Information (2004)
12.	Ethyl decanoate	24.013	200.1769	97.42	Fatty acid	$C_{12}H_{24}O_2$	Food additives Flavoring Agents	National Center for Biotechnology

Table 1: Proposed compounds of Microporus xanthopus (Fr.) Kuntze detected by in Negative mode.

								Information
13.	Fungichromin	24.479	670.3908	91.55	Macrolide	C35 H58 O12	Antifungal activity	(2023) Shih et al. (2003)
14.	Scarlet Red	27.531	380.1637	91.06	Bis(azo) compound	$C_{24}H_{20}N_4O$	Role as a histological dye, used in human and veterinary medicine to promote wound healing	National Center for Biotechnology Information (2023)
15.	Bromacil	10.705	260.0163	62.37	Pyrimidone and an organobromine	C9 H ₁₃ Br N ₂ O ₂	Used as an herbicide	National Center for Biotechnology Information (2023)
16.	Contignasterol	20.252	508.3385	93.75	Steroid	C29 H48 O7	Prevention of inflammatory or allergic reactions, or the treatment of cardiovascular or haemodynamic disorders.	Burgoyn <i>et al.</i> (1992)
17.	Polyporusterone F	20.252	462.3330	93.28	Bile acid	C28 H46 O5	Hair Regrowth Substance	Ishida <i>et al.</i> (2010)
18.	Lithol Rubine	17.686	386.0568	94.68	Azo dye	C ₁₈ H ₁₄ N ₂ O ₆ S	In Cosmetics, Used for the coloring of paints, inks, oil colors and watercolors. coloring of rubber, plastic wires, electrospray and daily chemical products.	Lai-Hao <i>et al.</i> 2011
19.	Saussurea lactone	18.907	234.1615	98.67	Lactone	$C_{15} H_{22} O_2$	Anti-inflammatory, antioxidant, hepatoprotective, antiulcer, anticancer, immunomodulatory and pesticidal activities.	Onoja <i>et al.</i> (2019)
20.	Curcumenol	22.464	234.1612	96.71	Natural product	C15 H22 O2	Anti-inflammatory activity antistroke agent with anti-inflammatory and cytotoxic activity for sepsis and leukemia, Anti-proliferative activity against human gastric cancer cells	Gupta <i>et al.</i> (2018); Lee <i>et al.</i> (2019); Jung <i>et al.</i> (2018)
21.	Stigmatellin Y	22.442	484.2810	84.92	Chromones	C29 H40 O6	An Anti-biofilm	Boopathi et al. (2017)
22.	6-gingerol	18.907	294.1826	98.84	Gingerols	$C_{17}H_{26}O_4$	anticancer, anti- inflammation, and anti- oxidation	Wang <i>et al.</i> (2014)
23.	Tanacetol A	18.907	294.1826	98.84	Germacrane sesquiterpenoi d	$C_{17}H_{26}O_4$	antimicrobial and anti- proliferative activities	Ali et al. (2017)
24.	Nordihydrocapsiat e	18.907	294.1826	98.84	Phenols and a member of methoxybenze nes.	$C_{17}H_{26}O_4$	Natural product found in Capsicum annuum	National Center for Biotechnology Information (2023
25.	Annuionone A	16.703	224.1409	92.46	Triterpene saponins	C13 H20 O3	Anti-inflammatory	Onoja <i>et al.</i> (2019)
26.	Malathion	16.161	330.0373	73.89	Organophosph ates	C10 H19 O6 P S2	Biocide, non-systemic broad-spectrum insecticide	Moore <i>et al.</i> (2009)
27.	Nonate	16.161	330.0373	73.89	Dicarboxylic acid	C9 H16 O4	Important Constituent of Pennsylvania petroleum	Mabery et al. (1901)
28.	Rhein	16.161	284.0316	98.72	Anthraquinone	$C_{15}H_8O_6$	Hepatoprotective, nephroprotective, anti- inflammatory, antioxidant, anticancer, and antimicrobial activities	Zhou <i>et al.</i> (2015)
29.	Phytuberin	18.907	294.1826		Sesquiterpenoi d	C17 H26 O4	Antifungal activities	Coxon <i>et al.</i> (1974)
30.	2,3-Di-O- methylellagic acid	16.161	330.0370	98.86	Tannin	C16 H10 O8	antimicrobial and antioxidant activities	Vigbedor <i>et al.</i> (2022)
31.	Hyaluronic acid	2.592	425.1526	98.35	Glycosaminogl ycan	$C_{16}H_{27}NO_{12}$	Ophthalmic, dermal, burns, wound repair, and other health conditions	Gupta <i>et al.</i> (2019)

Sr. No.	Compound Name	RT	Mass	Score	Chemical Nature	Formula	Uses	Ref
1.	Tenuazonic Acid	20.401	197.1048	98.80	Mycotoxin	C ₁₀ H ₁₅ N O ₃	Antibiotic with antiviral and antineoplastic properties	National Center for Biotechnology Information (2023)
2.	Pantothenic Acid	9.248	219.1100	93.47	Vitamin	C ₉ H ₁₇ N O ₅	Antidote to cure poisoning, antioxidant property	National Center for Biotechnology Information (2023)
3.	Ganodosterone	26.392	408.3016	91.77	Ergostanoid	C ₂₈ H ₄₀ O ₂	Liver protectant and stimulates liver	Subramanian (1995)
4.	Irinotecan	28.282	586.2795	99.43	antineoplastic enzyme	C33 H38 N4 O6	Treatment of colorectal cancer	National Center for Biotechnology Information (2023)
5.	Cinnamic acid	2.726	148.0519	94.96	Phenolic compound	C9 H8 O2	Antimicrobial properties	Siddiqui (2018)
6.	Threo- Syringoylglycerol	17.551	244.0943	97.69	Phenolic compound	$C_{11}H_{16}O_{6}$	Anti HSV-1 activities	Tian et al;(2010)
7.	Telithromycin	23.469	811.4710	93.77	Ketolide	C43 H65 N5 O10	Antibiotic treated for pneumonia	Hoofnagle (2013)
8.	Deltaline	22.882	507.2836	98.14	diterpenoid alkaloid	C27 H41 N O8	To treat rheumatic pain, paralysis due to stroke, rheumatoid arthritis	Chen et al. (2015)

Table 2: Proposed compounds of *Microporus xanthopus* detected by in Positive mode.

Compound Spectra (overlaid)







Compound Spectra (overlaid)



Compound Spectra (overlaid) Cpd 880: Bisacurone B; C15 H24 O3; 19.737: - NFE Spectrum (rt: 19.519-19.902 min) 20221114-SAMPLE-M1-Neg_00.d ×10⁴ 311.1856 (M+CH3COO)-7 6 5 4 3 2 312.1887 (H+HCOO) 313.1934 (M+HCOO) 1 0 309.5 310 310.5 311 311.5 312 312.5 313.5 314 314.5 315 315.5 316 316.5 317 317.5 318 313





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Counts vs. Masa-to-Charge (m/z)

Compound Spectra (overlaid)





Fig. 2. Compound Spectra shows Mass to Charge (m/z) in Positive Mode.

Structures of Bioactive Compound Detected in Negative Mode:





Ethyl(E,Z)-decadienoate









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Ethyl decanoate



Scarlet Red



8-(3,3-Dimethylallyl)spathelia













Stavudine



Leucomycin V



Lithol Rubine



2,3-Di-O-methylellagicacid



Structures of Bioactive Compound Detected in Positive Mode:



Deltaline



Pantothenic Acid



Irinotecan



Telithromycin

DISCUSSION

Secondary metabolite study from basidiomycetous fungi which has tremendous commercial potential (Jain *et al.*, 2020). Many industrial products composing of fungal metabolites from basidiomycete source are directly or indirectly benefited to the human health (Shankar and Sharma 2022).

M. xanthopus has been reported to exhibit antibacterial, anticancer, antiangiogenic, and anthelmintic activities. The higher concentrations of these medicinal properties are believed to be a result of the environment and substrate in which the polypore mushroom grows (Chittaragi and Meghalatha 2014; Orango-Bourdette *et al.*, 2018).

The proposed compounds of *Microporus xanthopus* (Fr.) Kuntze detected in negative mode and positive mode, along with their retention times, molecular masses, scores, chemical natures, formulas, and potential uses. These compounds were identified through mass spectrometry, which is a powerful analytical technique used to identify the chemical structure of a compound. The identified compounds have diverse chemical natures and functionalities, ranging from alkaloids, esters, phenols, and steroids to bile acids, macrolides, and sesquiterpenoids.

Among the identified compounds, quinoline-3carboxamides, quadrigemine A, and varanic acid have high scores and diverse pharmacological activities, including antimalarial, antibacterial, antifungal, antiviral, anticancer, anti-obesity, anti-inflammatory, and inhibitors of platelet aggregation. Fransén *et al.* (2018) studied the immunomodulatory activity of Quinoline-3-carboxamides and reported them to have anti-inflammatory properties.

Antimycin A1 and fungi chromin also have significant potential uses as antibiotics, fungicides, and miticides. Maeda *et al.* (2006) reported on the inhibitory activity of Antimycin A1.

Other compounds such as bromacil, bisacurone B, ethyl(E,Z)-decadienoate, dinoterb, stavudine, 8-(3,3dimethylallyl) spatheliachromene, leucomycin V, dinex, ethyl decanoate, and scarlet red have diverse applications in agrochemicals, food additives, insecticides, histological dyes, and wound healing promotion.Molinari et al. (2010) reported olfactory activity of Ethyl (E,Z)-2,4-Decadienoate on oriental fruit moths. According to Sarkar and Khupse (2022) Bromacil is a herbicide utilized for land management purposes as well as selective weed control in pineapple and citrus crops. Moreover, contignasterol, polyporusterone F, lithol rubine, saussurea lactone, curcumenol, stigmatellin Y, 6-gingerol, tanacetol A, and nordihydrocapsiate have potential therapeutic applications, including anti-inflammatory, antioxidant, hepatoprotective, antiulcer, immunomodulatory, anticancer, anti-proliferative, and anti-allergic activities. The 6-gingerol has the potential to enhance the quality of porcine embryos cultured in vitro.

In positive mode eight compounds of *Microporus xanthopus* identified. *M. xanthopus* is significant because it highlights the potential medicinal properties of natural products. Tenuazonic acid, in particular, has

been found to have promising antineoplastic properties and could potentially be developed into a cancer treatment. Pantothenic acid, a common vitamin, has antioxidant properties and is involved in essential metabolic processes. Combs and McClung (2022) importance of Pantothenic reported acid Ganodosterone has potential as a liver protectant, which could be useful in preventing liver damage caused by toxins and alcohol. Irinotecan is an established antineoplastic agent used to treat colorectal cancer, while cinnamic acid and Threo-Syringoylglycerol have antimicrobial and antiviral properties. Shun et al. (2023) conducted a case study to investigate the impact of Irinotecan on induced muscle twitching. Telithromycin is a ketolide antibiotic used to treat pneumonia and Deltaline is a diterpenoid alkaloid used to treat various types of pain and inflammation. Xiong et al. (2001) detected antibacterial and antibiofilm activity of Telithromycin. Overall, the discovery of these compounds highlights the potential for natural products to be developed into effective drugs for a range of conditions.

CONCLUSIONS

This study has identified a range of compounds present in *Microporus xanthopus* using mass spectrometry. These compounds have diverse chemical natures and functionalities, and compounds have promising pharmacological activities with potential uses in medicine, agriculture, and other industries. The identification of these compounds highlights the potential for natural products to be developed into effective drugs for a range of conditions.

FUTURE SCOPE

Further research could focus on isolating and testing the individual compounds to better understand their potential uses and mechanisms of action. Additionally, investigation into the cultivation and optimization of *Microporus xanthopus* could provide a sustainable source of these compounds for future research and development.

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