VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

PRELIMINARY SCREENING, QUANTITATIVE ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF MUSHROOMS ALONG WITH FTIR ANALYSIS

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ABSTRACT

The present study mainly focused on identifying the efficiency of polypore mushrooms and exploring their medicinal value. In this experiment, the antioxidant and antimicrobial potentials of three mushrooms Trametes pubescens (Schumach.) Pilat, Spongipellis pachyodon (Pers.) Kotl. & Pouzar and Inonotus hispidus (Bull.) P. Karst. were tested along with their qualitative and quantitative analysis. The antioxidant and antimicrobial activities of methanolic crude extracts were determined using the Frap assay (ferric reducing antioxidant power) and agar well diffusion method respectively. The methanolic extracts of all three mushrooms revealed the presence of alkaloids, carbohydrates, proteins, phenolics, flavonoids, saponins, terpenoids, and cardiac glycosides. The antioxidant activity of Trametes pubescens, Spongipellis pachyodon, and Inonotus hispidus found to be 19.83±0.5, 38.5±0.8 and 39.5± 0.9 mg Fe (II)/g of sample, respectively. The antimicrobial activity was tested against Staphylococcus aureus, Bacillus subtilis and Psuedomonas species. The inhibition zone was measured, in which all three species show maximum inhibition against Staphylococcus aureus. The correlation coefficient showed a positive relationship between total flavonoid content and antioxidant activity (r = 0.9). The Fourier Transformed infrared (FTIR) profiling indicates the presence of functional groups in these species for its pharmaceutical activities.

Keywords: Mycochemical screening, antioxidant activity, antimicrobial activity, FTIR

INTRODUCTION

For millennia, mushrooms have been employed as a source of medicinal ingredients. They have been incorporated into traditional medicine practices in various cultures around the world. Additionally, mushrooms are known for their rich nutritional profile, containing essential vitamins, minerals, high proteins and antioxidants that contribute to overall well-being (Quereshi et al., 2010; Chang et al., 1999). Among these, Polypore belongs to the Polyporaceae family and order Aphyllophorales (Barron et al., 1999). They are classified as Basidiomycetes like gilled mushrooms due to their strong, perpetual fruiting bodies (Comandini et al., 2012). The names of this fungus have undergone various modifications to reflect its evolutionary position as a result of improvements to nomenclature and systematics knowledge during the past thirty years (Gilbertson et al., 1986). Polypore has various biological properties such as antimicrobial, antifungal, antiviral, antioxidant, anticancer, cardiovascular, immunostimulating, anti-inflammatory, and nematocidal due to its secondary metabolites (Stamets et al., 2002).

All aerobic life forms on the planet are linked to oxidation processes, which are necessary for their survival (Silva et al., 2013). Reactive oxygen species (ROS) are extremely reactive chemicals produced from oxygen that cause oxidative damage, to living things and animals (Davies, 2000). Surprisingly, the formation of free radicals and other reactive oxygen species by this critical mechanism may cause cell and tissue damage resulting in the aging process (Turkoglu et al., 2007). These radicals are stabilized by interacting with structural and functional

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

cell components such as lipids, proteins, and DNA, thus disrupting the normal functions of cell (Silva et al., 2013). These cellular and tissue abnormalities may cause diabetes, cardiovascular disease, neurodegenerative illnesses, Alzheimer's disease, and malignancies (Petersen et al. 2005). An antioxidant is a natural or artificial chemical substance that can prevent other molecules from oxidizing (Silva et al., 2013). Phenolic compounds act as antioxidant agents in mushrooms by functioning as peroxidase decomposers, metal inactivators, oxygen scavengers, or free radical inhibitors (Dziezak et al., 1986).

The discovery of novel antibiotics from natural compounds was encouraged by the pathogenic resistance against conventional drugs. Since ancient times, mushroom extract has been used as a source of various drugs for the treatment of various microbial diseases. According to a recent biological study of over 200 mushroom species, more than 75% of screened Polypores demonstrated significant antibacterial activity (Suay et al., 2000). Polypores are widely explored for their mycochemicals that have the ability to treat various diseases due to their ethnomedicinal properties. Trametes pubescens (Schumach.) Pilat., Spongipellis pachyodon (Pers.) Kotl. & Pouzar, and Inonotus hispidus (Bull.) P. Karst. have antibacterial, antioxidant, antiviral, and anti-inflammatory properties (Zan et al., 2011). Therefore, this experiment mainly focuses on the mycochemical profiling, including total phenolic and flavonoid content, FTIR screening of T. pubescens, S. pachyodon, and I. hispidus methanolic extract along with their antioxidant and antimicrobial activity.

MATERIAL AND METHODS

Collection and identification

T. pubescens, S. pachyodon and I. hispidus were collected from the nearby Kaneval lake at Tarapur taluka, Anand, Gujarat, India, in December 2021. Fresh samples (fruiting bodies) were collected and washed twice using distilled water to remove soil particles and other dust. The samples were sun dried, oven dried and then ground to a fine powder with the help of a mixer grinder. The powder was kept in an airtight container for further analysis.

Preparation of extract

The maceration extraction method was used to prepare the extract. A 10 gm powder was soaked separately in a100 ml organic solvent like methanol (polar) for 24 hours in an orbital shaker at normal temperature at constant stirring rate of 112 rpm. The extract was filtered through the Whatman No.1 filter paper, and extra solvent was evaporated and stored at 4℃ for further analysis. Finally, the yield value of crude extract was calculated using a standard formula.

$$\% \text{ Yield } = \text{ Weight of dry extract } \times 100 \div \text{Weight of initial extract}$$

Preliminary Myco-chemical Screening

Preliminary mycochemicals were tested by preparing 30 mg extracts of each species in 30 ml of methanol solvent to make a stock solution with a 1 mg/ml concentration. The mycochemical screening of T. pubescens (Schumach.) Pilat., S. pachyodon (Pers.) Kotl. & Pouzar, and I. hispidus (Bull.) P. Karst. The methanolic extracts were used to examine the presence of secondary metabolites such as alkaloids, flavonoids, phenols, proteins, carbohydrates, lipids, saponins, glycosides, terpenoids, and steroids. The preliminary mycochemical screening was conducted using standard methods.

Total phenolic content

The total phenolic content of methanolic extracts of Trametes pubescens, Spongipellis pachyodon and Inonotus hispidus was assessed by the Folin Ciocalteu Reagent Method. The methanolic extract was prepared at a concentration of 1mg/ml. The 0.5 ml of extract with 0.5 ml Folin Ciocalteu reagent was dissolved in 10 ml distilled water. The mixture was incubated for 5 minutes and 2 ml of 20% sodium carbonate was added, then total volume of 25ml made by 12 ml distilled water in each test tube. The test tubes were covered with Aluminium foil and incubated for 30 minutes at room temperature. The gallic acid used as a standard, and the absorbance was read at λ 765nm using spectra against blank. The sample was prepared in triplicates for analysis and the mean absorbance was obtained using spectrophotometer. Total content of the extract was expressed as mg Gallic Acid Equivalents/g of sample.

Total Flavonoid content

The total flavonoid content of T. pubescens, S. pachyodon and I. hispidus methanolic extracts were examined by aluminum chloride colorimetric method. The reaction mixture was prepared by mixing 0.5 ml extract with 50µl 10% $AlCl_3$ and 50µl 1M potassium acetate in 10 ml distillate water. The reaction mixture was incubated for 30 minutes at room temperature. The absorbance was measured at 415nm using spectra in a spectrophotometer. The procedure was also permitted for the quercetin standard. The total flavonoid content of extract was expressed in mg Quercetin equivalent/g of sample.

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Antioxidant activity

The antioxidant activity of methanolic crude extracts of T. pubescens, S. pachyodon and I. hispidus was measured by the FRAP (ferric reducing antioxidant power) antioxidant assay. The FRAP reagent was prepared using 300 mM acetate buffer (pH- 3.6), 10 M TPTZ, and 20 mM Fecl3.6H_2O in a ratio of 10:1:1 in 40mM HCl. The 0.5µl extract and 4 ml frap reagent mix in 10 ml distill water and incubate for 10 minutes. The absorbance was measured at a 593 nm wavelength. The absorbance was compared with the calibration curve formulated by FeCl3.6H_2O as a standard antioxidant agent.

Antimicrobial activity

The antibacterial activity of T. pubescens, S. pachyodon and I. hispidus methanolic extracts was evaluated using agar well diffusion methods. Staphylococcus aureus and Bacillus subtilis were used as Gram positive bacteria, while Pseudomonas sp., was used as Gram negative bacteria for antibacterial activity. The antifungal activity of methanolic extracts was also measured using Saccharomyces cerevisiae. In this study, nutrient agar medium was used to culture bacteria while potato dextrose agar used for growth of fungus. The nutrient agar media plates were inoculated with the desired culture of bacterial inocula using cell density 0.5 McFarland standards. The 1.4% nutrient agar was mixed with 500 ml of distilled water in a conical flask and dissolved the agar. The media flasks were plugged with cotton wool for sterilization in an autoclave at 121° C, 15 psi pressure for 45 minutes. Then sterilized media was poured aseptically into sterilized petri plates and allowed to solidify in a sterile environment for an hour. The petri plates with solidified media were placed in an inverted position to avoid water evaporation within plates and incubated at 37° C for 24 hrs. After the incubation, uncontaminated plates were inoculated with bacteria. The wells were created in the agar plates by sterile cork borer with 5mm diameter. The sample extracts were prepared in DMSO solvent with 1mg/ml concentration. The 100µl sample extracts were poured in to wells and incubated at 37° C for 24 hours in sterile conditions. The Gentamycin drug and DMSO solvent were used as positive and negative controls, respectively. After the incubation, the inhibition zones were measured.

Statistical analysis

The quantitative and graphical data was analyzed through Microsoft Excel. All the tests were carried out in triplicates of each sample and results were expressed as mean value of ± standard error. The test of significance was conducted by one way analysis of variance (ANOVA; $P< 0.05$).

RESULTS

Preliminary Myco-chemical Screening

The preliminary mycochemical screening of T. pubescens methanolic extract of fruiting bodies revealed the presence of alkaloids, carbohydrates, proteins, phenols, flavonoids, saponins, terpenoids, and cardiac glycosides while glycosides, and steroids and fat or oil were absent. However, in S. pachyodon methanolic extract, alkaloids, proteins, phenolics, flavonoids, carbohydrates, saponins, terpenoids, cardiac glycosides and steroids were found, while glycosides and fats were absent. Whereas, I. hispidus methanolic extract of fruiting body showed the presence of alkaloids, carbohydrates, phenols, flavonoids, saponins, terpenoids, and cardiac glycosides while exhibited absence of glycosides, proteins, fats, and steroids.

Table 1: Preliminary mycochemical screening of T. pubescens, S. pachyodon and I. hispidus methanolic extracts of fruiting bodies.

SR.NO.	Myco -chemical	Test	General observations	Results		
				Trametes pubescens	Spongipellis pachyodon	Inonotus hispidus
1.	Alkaloids	**Mayer's Test:** 1 ml filtrate + Mayer's reagent	White creamy ppt.	+	+	+
		Wagner's Test: 1 ml filtrate + Wagner's Reagent	Red brown ppt.	+	+	+
		Hager's Test: 1 ml filtrate + Hager's reagent	Yellow ppt.	+	+	+
		Dragendorff's Test: 1 ml filtrate+Dragendorff's reagent	Orange ppt.	+	+	+
2.	Carbohydrates	**Molisch's Test:** 1 ml	Violet ring	_	_	_

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Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

		filtrate + Molisch's reagent				
		Fehling's Test: 1ml filtrate + Fehling A reagent + Fehling B reagent & boil for 2 min	Red ppt	–	–	–
		Barfoed's Test:1 ml filtrate + Barfoed's reagent, boil for 2 min	Red ppt	+	+	+
		Benedict's Test: 1ml filtrate +Benedict's reagent, boil for 2 min	Colored ppt	–	–	+
3.	**Glycosides**	**Ammonia Test:** 1 ml filtrate + 3 ml Chloroform (shake)+ 10% ammonium solution	Pink Colouration	–	–	–
4.	**Proteins**	**Millon's Test:** 1 ml filtrate+ Millon's reagent	White colour	+	+	–
5.	**Phenolics**	**Folin-ciocalteuTest:**1ml filtrate + Folin-ciocalteu reagent	Blue – green color	+	+	+
6.	**Flavonoids**	**Lead acetate Test:** Extract+ few drops of 10% lead acetate	Yellow ppt	+	+	+
7.	**Saponins**	Foaming Test: 1 ml Extract + 1 ml Dilute water & shake	Presence of Foams	+	+	+
8.	**Fats and Fixed Oils**	Oil stain check: A drop of Extract on Filter paper	Oil stains of Filter paper	–	–	–
9.	**Terpenoids**	**Chloroform Test:** 1 ml Extract + 2 ml Chloroform+ 3 ml Conc. H_2SO_4, forms a layer	Formation of Red brown Coloured Ring	+	+	+
10.	**Cardiac Glycosides**	**Sodium nitroprusside Test:** 2 ml filtrate+ 1 ml pyridine + 1 ml 20% Sodium nitroprusside	Pink or red Colouration	+	+	+
11.	**Steroids**	**Salkowaski's Test:** 2 ml extract + shake with chloroform+ add Conc. H_2SO_4 side by side	Red Colouration	–	+	–

Total phenol and flavonoid content

T. pubescens, S. pachyodon and I. hispidus extracts contain considerable quantities of total phenolic content. T. pubescens extract had a total phenolic content of 12.67±0.5 mg GAE/g of sample, whereas, the flavonoid content was 11.9±0.2mg QE/g of sample. The phenolic content of S. pachyodon extract was found to be 18.83±0.5 mg GAE/g while flavonoid content was 21.33±0.4 mg QE/g of sample. Likewise, I. hispidius extract showed phenolic content of 13.95±0.3 mg GAE/g of sample and total flavonoid content was determined to be 145.5±0.5 mg QE/g of sample.

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ISSN: 2321-1520 **E-ISSN:** 2583-3537

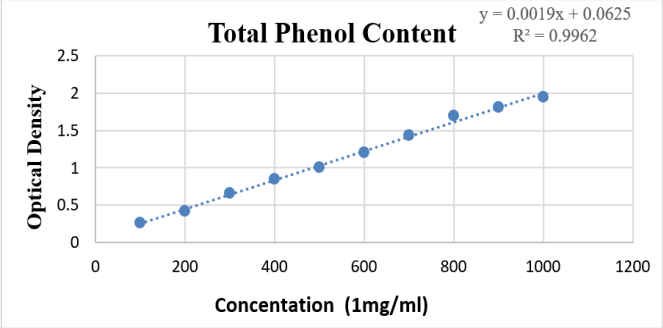

Fig. 1: Standard graph of total phenolic content (Gallic acid)

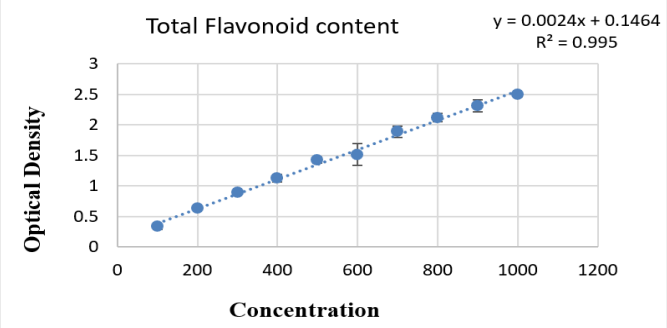

Fig. 2: Standard graph of total flavonoid content (Quercetin)

Antioxidant activity

The antioxidant activity of T. pubescens, S. pachyodon and I. hispidus methanolic extracts was examined by Frap assay (ferric reducing antioxidant power) at 1mg/ml concentration. In this study all of the species showed potent antioxidant value with Trametes pubescens had 19.83±0.5 mg Fe(II)/g of sample, S. pachyodon showed 38.5±0.8 mg Fe(II)/g of sample and I. hispidus had 39.5± 0.9 mg Fe(II)/g of sample.

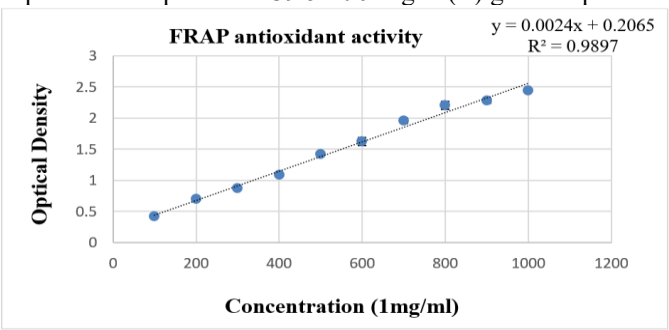

Fig. 3: $FeSO_4.6H_2O$ Standard Graph (FRAP Assay)

Antimicrobial activity

In this study, the antibacterial activity of T. pubescens against S. aureus, showed the highest inhibition zone (15 mm in diameter) followed by S. pachyodon and I. hispidus with inhibition zone 10 mm. Likewise, the inhibition zone of T. pubescens and S. pachyodon against B. subtilis were 10mm and 5mm respectively although I. hispidus did not. There was no effect on pseudomonas species and inhibition zone was absent for all three-sample extracts. Antifungal property was also absent in all three sample extracts which were examined against yeast.

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ISSN: 2321-1520 **E-ISSN:** 2583-3537

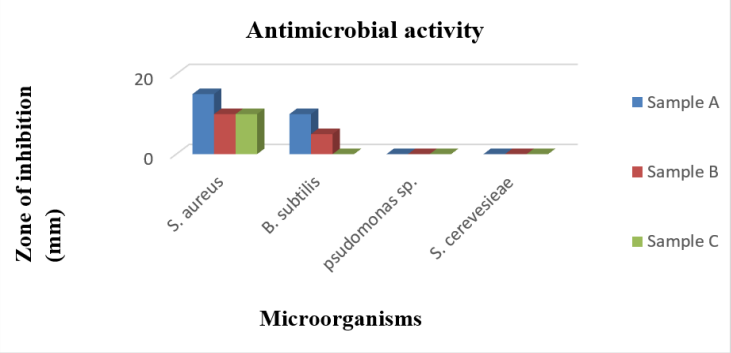

Fig. 4: Antimicrobial activity of sample A, B and C against various microorganism. Sample A indicates T. pubescens, Sample 2 for S. pachyodon and Sample 3 for I. hispidius

Fourier transformed infrared spectra

Table 2: FTIR analysis revealed the presence of functional groups

Sr.no	Species name	Wavenumber (cm^{-1})	Band interaction	Band assignment	Possible compound
1	Trametes pubescens	3339.7	Stretch	O-H	Alcohol
		2944.6	Stretch	C-H	Alkane
		2832.8	Stretch	C-H	Aldehyde
		2538.3	Stretch	O-H	Carboxylic acid
		2344.5	Stretch	O=C=O	Carbon dioxide
		2199.1	Stretch	C≡C	Alkyne
		2098.5	Stretch	N=C=S	Isothiocyanate
		2012.8	Stretch Stretch Stretch	C=C=N C=C=C N=C=S	Ketenimine Allene Isothiocyanate
		1710.8	Stretch	C=O	Conjugated acid or Conjugated aldehyde
		1654.9	Stretch	C=O	δ lactam
		1449.9	Bend	C-H	Alkane
		1408.9	Bend	C-H	Aldehyde
		1207.7	Stretch	C-O C-N	Ester or tertiary alcohol
		1110.7	Stretch	C-O	Secondary alcohol
		1021.3	Stretch	C-N	Amine
2.	Spongipellis pachyodon	3332.2	Stretch	O-H	Alcohol
		2944.6	Stretch	C-H	Alkane
		2832.8	Stretch	C-H	Aldehyde
		2601.7	Stretch	O-H	Carboxylic acid
		2530.9	Stretch	O-H	Carboxylic acid
		2035.1	Stretch	C≡C	Alkyne
		1647.5	Strech	C=O	δ lactam
		1449.9	Bend	C-H	Alkane
		1408.9	Stretch	S=O	Sulfonyl chloride
		1110.7	Stretch	C-O	Secondary alcohol
		1021.3	Stretch	C-N	Amine
3.	Inonotus hispidus	3317.3	Stretch	O-H	Alcohol
		2944.6	Stretch	C-H	Alkane
		2832.8	Stretch	C-H	Aldehyde
		2527.1	Stretch	O-H	Carboxylic acid
		2027.7	Stretch	N=C=S	Isothiocyanate
		2005.3	Stretch	C=C=N	Ketenimine

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Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

			Bend	C=C=C N=C=S C-H	Allene Isothiocynate Aromatic compound
		1654.9	Stretch	C=O	δ lactam
		1449.9	Bend	C-H	Alkane
		1412.7	Stretch	S=O	Sulfate or sulfonyl chloride
		1110.7	Stretch	C-O	Secondary alcohol
		1021.3	Stretch	C-N	Amine
		670.9	Stretch	C-Br C=C	Halo compound Alkane

The FTIR analysis was used to analyze the functional group of bioactive compounds based on the peak's characteristics found in the IR region (Shukla et al., 2020). The FTIR spectrum of the Trametes pubescens methanolic extract revealed the presence of fifteen significant bands between the 650 and 4000 cm^{-1}. The stretching of OH groups produced a strong broad intensity peak at 3339.7 cm^{-1} indicates the presence of alcohol. The band at 2944.6 cm^{-1} and 2832.8 cm^{-1} revealed a medium band of C-H stretching due to aldehyde groups. The strong peak at 2538.3 cm^{-1} recognized as the vibrations of OH groups stretching due to carboxylic acid. Similarly, a peak at 2344.5 cm^{-1} corresponding to the strong O=C=O stretching that verify a presence of carbon dioxide. A weak band at 2199.1 cm^{-1} revealed C≡C stretching of an alkyne group. The strong bands at 2098.5 cm^{-1} and 2012.8 cm^{-1} both are assigned to the stretching of N=C=S suggesting vibration of an isothiocyanate group. The band at 1654.9 cm^{-1} may related to C=O stretching of amide groups as a δ lactam. A peak appeared at 1207.7 cm-1 assigning stretching of C-O and C-N that indicates possible groups like alkyl aryl ether, vinyl ether, ester, tertiary alcohol and amine respectively. A stretching of C-O is detected as band at 1110.7 cm^{-1} suggesting presence of secondary alcohol. The sharp infrared band appeared at 1021.3 cm^{-1} indicates stretching of C-N due to vibration of amine group.

In Spongipellis pachyodon methanolic extract, a band at 3332.2 cm^{-1} indicate the presence of OH stretching of alcohol. Like, Trametes pubescens, two band at 2944.6 cm^{-1} and 2832.8 also detected. Bands at 2601 cm^{-1} and 2530.9 cm-1 produced due to stretching of O-H and S-H verifying carboxylic acid or thiol groups. Likewise, a weak intensity band at 2035.1 cm-1 indicates C≡C stretching of alkyne group. An infrared band at 1647.5 cm^{-1} assigning stretching of C=O and C=C representing to δ lactam and alkene respectively. Similar to the Trametes pubescens a band appeared at 1449.9 cm-1, 1408.9 cm-1, 1110.7 cm-1, and 1021.3 cm-1 detected as C-H bending and stretching of S=O, C-O, C-N may indicate the presence of alkane, sulfonyl chloride, secondary alcohol and amine respectively.

The methanolic extract of Inonotus hispidus shows twelve infrared bands in IR spectrum. Similar to the Trametes pubescens and 2832.8 cm-1 also indicates alcohol, alkane and aldehyde group respectively as stretching of O-H and C-H. A band at 2527.1 cm-1 revealed the presence of OH stretching of carboxylic acid. Furthermore, a band at 2027.7 of N=C=S stretching suggested the isothiocynate group. An infrared band at 2005.3 cm^{-1} may suggesting stretching of C=C=N and C=C=C for ketenimine and allene respectively, while C-H bending of some aromatic compounds. A band appeared at 1654.9 cm^{-1} may assigning as a C=O corresponding to δ lactam. Likewise other two species, the bands also appeared at 1449.9 cm^{-1}, 1408.9 cm^{-1}, 1110.7 cm^{-1}, and 1021.3 cm^{-1} for C-H bending and stretching of S=O, C-O, C-N may indicate groups like alkane, sulfonyl chloride, secondary alcohol and amine respectively. The band at 670.9 cm^{-1} may examined as a C-Br stretching or C=C bending of halo compounds and alkene groups respectively.

DISCUSSION

The medicinal properties of mushrooms are owing to the type of chemical substance that they generate and reserve. The maceration extraction method was used for extract preparation. Extraction is the separation of medicinally active portions of mushrooms using selective solvents through standard procedures. The solvents diffuse into the powdered mushroom and solubilize compounds with similar polarities. The results obtained for the qualitative screening of mycochemicals in methanolic extracts are presented in Table 1. The study examines the mycochemical screening, total phenolic and flavonoid content, and antioxidant and antimicrobial activity of three polypore. Trametes pubescens, Spongipellis pachyodon, and Inonotus hispidus methanolic extracts showed the presence of alkaloids, phenolics, carbohydrates, flavonoids, saponins, terpenoids, and cardiac glycosides. The steroids are present in Trametes pubescens and Inonotus hispidus while absent in Spongipellis pachyodon. However, glycosides, fat, and oil were absent in all the species. The other members of same order, Polyporales also showed the presence of various primary and secondary metabolites in previous studies. The phytochemical screening of Trametes betulina and Trametes cingulata revealed the presence of alkaloids, flavonoids, saponins, steroids and carbohydrates but tannins and phlobatannins were absent in both species

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Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

(Essien, et al., 2014). The other polypore, Ganoderma applanatum, showed the presence of alkaloids, steroids, saponins, phenols, flavonoids, terpenoids, and glycosides in the methanol extract while tannins and anthraquinone were absent (Nagaraj et al., 2013).

The phenolic compounds are regarded as the major contributors to the various pharmacological activities including antioxidant and antimicrobial activities. The methanolic extract of fruiting body of T. pubescens, S. pachayodon and I. hispidius showed a significant amount of total phenolic content. The total phenolic content of T. pubescens methanolic extract was 12.67±0.5 mg GAE/g of sample, while the total flavonoid content was 11.9±0.2mg Quercetin/g of sample. The total phenolic content of S. pachyodon methanolic extract is 18.83±0.5 mg GAE/g while total flavonoid content is 21.33±0.4 mg Que/g of sample. Likewise, the total phenolic content of I. hispidius methanolic extract is 13.95±0.3 mg GAE/g of sample while total flavonoid content is 145.5±0.5 mg Que/g of sample. Among all three polypore species, Spongipellis pachayodon shows highest phenolic content (18.83±0.5 mg GAE/g) while Inonotus hispidus shows highest flavonoid content. The total phenolic content and total flavonoid content of T. versicolor methanolic extract has 25.8 mg GAE/g and 4.3 mg CE/g respectively (Puia et al., 2018). The total phenol content and total flavonoid content of T. gibbosa in similar genus of Trametes show the significant amount of phenol as a 22.22±0.79 mg GAE/g and flavonoid content was 11.65±0.40 mg CE/g of sample (Puia et al., 2018). Thus T. versicolor has higher phenolic content than T. gibbosa and T. pubescens and also it has higher than S. pachyodon. The total flavonoid content was higher in S. pachyodon compare to Trametes species such as T. versicolor, T. gibbosa and T. pubescens. However, this study suggests that T. pubescens has higher flavonoid content than T. versicolor but similar with T. gibbosa.

The antioxidant activity of methanolic extract of T. pubescens, S. pachyodon and I. hispidus (was examined by Frap assay (ferric reducing antioxidant power) at 1mg/ml concentration. In this study all the species show potent antioxidant value with T. pubescens has 19.83±0.5 mg Fe/g of sample, S. pachyodon show 38.5±0.8 mg Fe(II)/g of sample and I. hispidus shows 39.8±0.9 mg Fe(II)/g of sample. In this case, compare to all the species Inonotus hispidus possesses highest (39.8±0.9 mg Fe (II)/g of sample) antioxidant activity. It is interesting to examine the correlation between all three species which show diverse phenol and flavonoid content and thus antioxidant activity. All the species indicates positive correlation of total phenolic and flavonoid with antioxidant activity. The correlation coefficient between total flavonoid content and antioxidant activities is highly significant ($r = 0.99$). The phenol contents were less correlate with antioxidant activities ($r = 0.61$) as compare to flavonoids. The results indicate that high flavonoid content may important factor in determining significant antioxidant activities for all three species.

The antimicrobial activity of DMSO extract of T. pubescens, S. pachyodon and Inonotus hispidus were examine against gram positive bacteria Staphylococcus aureus, and Bacillus subtilis, while gram negative bacteria Psuedomonas species. Trametes pubescens significantly inhibits the zone of S. aureus and B. subtilis with 15mm and 10mm diameter respectively. The antibacterial activity of DMSO extract of S. pachyodon examined against the gram-positive bacteria show the maximum inhibition zone of 10mm and 5mm for S. aureus and B. subtilis respectively. Likewise, I. hispidius also inhibit S. aureus with 10mm inhibition zone but there is no effect on B. subtilis. However, there is no effect on pseudomonas species of all the sample extracts and inhibition zone was absent. The gentamycin drug was used as positive control and DMSO as negative control. The inhibition zone of gentamycin was 20mm for all the bacteria and used as standard. The antifungal properties were also absent in all the three species when examined against yeast. In this study T. pubescens and S. pachyodon have maximum inhibition zone against S. aureus and B. subtilis (Gram positive bacteria). The other polypore Ganoderma applanatum methanolic extract shows effective results when tested against S. aureus (15mm), B. subtilis (12 mm), E. coli (13mm), S. typhi (8mm), K. pneumonia (8mm) and P. aeruginosa (12 mm) with the concentration value 75 mg/ml (Nagara et al., 2013). Trametes pubescens, Spongipellis pachyodon and Inonotus hispidus were more effective for antimicrobial activity with concentration 1mg/ml in DMSO extract than 75mg/ml methanolic extract of Ganoderma applanatum (Nagaraj et al., 2013).

CONCLUSION

The study of T. pubescens (Schumach.) Pilat and S. pachyodon (Pers.) Kotl. & Pouzar and Inonotus hispidus (Bull.) P. Karst. mainly focuses on the screening of various mycochemical screening and their antioxidant and antimicrobial properties. The preliminary mycochemicals of Trametes pubescens (Schumach.) Pilat, Spongipellis pachyodon (Pers.) Kotl. & Pouzar and Inonotus hispidus (Bull.) P. Karst. methanolic extracts revealed the presence of alkaloids, carbohydrates, proteins, phenolics, flavonoids, saponins, terpenoids, and cardiac glycosides. The antioxidant activity was observed using Frap assay (ferric reducing antioxidant power) at 1mg/ml concentration. In this study all the species show potent antioxidant value with Trametes pubescens (Schumach.) Pilat has 19.83±0.5 mg Fe/g of sample, Spongipellis pachyodon (Pers.) Kotl show 38.5±0.8 mg Fe(II)/g of sample while Inonotus hispidus (Bull.) P. Karst has 39.5± 0.9 mg Fe(II)/g of sample. The antimicrobial activity tested against the Staphylococcus aureus, Bacillus subtilis and Psuedomonas species and inhibition zone was measured. All three species show maximum inhibition against Staphylococcus aureus. The

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

Fourier Transformed infrared (FTIR) profiling indicates the presence of functional groups in these species for their biochemical properties. The biomolecules and biological potentiality of T. pubescens (Schumach) Pilat and S. pachyodon (pers.) Kotl. & Pouzar was not efficiently examined by researchers and more research will be needed for the use of it.

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