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Antimicrobial and phytochemical properties of methanol and hexane extract of non- gilled mushrooms collected from North-Western Himalayas

Shaveta Singh and Astha Tripathi*

Department of Biotechnology, Shoolini University, Solan-173212, Himachal Pradesh, India

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ABSTRACT

The objective of this study was to evaluate the antimicrobial and phytochemical properties of four non-gilled mushrooms collected from the forest of North Western Himalayas. The antimicrobial activity of four non-gilled mushrooms was performed against three pathogenic bacteria (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*) and one fungal strain (*Cryptococcus neoformans*). Quantitative and qualitative analysis of phytochemical properties was carried out using standard methods. The obtained results revealed that *Auricularia polytricha* showed maximum antimicrobial activity against all selected pathogenic strains in methanolic and hexane extract however in hexane extract *Ganoderma lucidum* showed good antimicrobial activity against *Staphylococcus aureus* (24.6±0.47) and *Klebsiella pneumoniae* (13.3±0.47). Last activity was observed in the methanolic extract by *Trametes elegans* (10.3±0.47) and no activity was observed in hexane extract by *Trametes elegans* and *Auricularia auricular-judae* against *Cryptococcus neoformans*. In comparison to hexane extract methanolic extract showed good antimicrobial activity against all pathogenic microorganisms. However, in all selected non-gilled mushrooms, *Auricularia polytricha* showed maximum activity. Methanolic extract showed higher phytochemicals then hexane extract. The antimicrobial and phytochemical analysis of non -gilled mushrooms shown to be a good source of antibacterial and phytoconstituents that warrant further studies as a future dietary supplement to improve health and well being.

* Corresponding Author

Name: Dr. Astha Tripathi
Phone: +91-8219312928
Email: asthatripathi4u@gmail.com

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INTRODUCTION

Fungi are the second most diverse group of organisms, yet only a fraction of all fungal species have been described so far and an even smaller number explored for the production of pharmacologically important metabolites (Silva *et al.,* 2013). Less intensively investigated organisms such as the macrofungi seem to have great promise in terms of compounds with potential biological activities. In recent times mushrooms have been used as food as well as medicine for their popularity. Mushrooms are attracting universal immersion as a valuable herb. There are about 140,000 species that have been reported, out which 10% are known and 5% are undiscovered and more than 3000 mushrooms are edible (Dutta and Acharya, 2014). Non-gilled mushrooms: macrofungi without gills such as *Morchella, Ganoderma, Cordyceps* and *Auricularia* are among the most common and widely distributed members of macrofungi, which generally occur as saprophytes on wood, logs, branch and twigs causing severe degrees of white rotting of forest trees. Among hundreds of known mushroom varieties have been studied for their ability to enhance the human immune system and infections. In the early days proved to be successful, mushrooms may represent potential sources of antibacterial drugs,

screening for antibiotics started with mushrooms (Enman *et al.,* 2007). Some mushrooms have been used in traditional medicine while some mushrooms serve as food because of their nutrient contents (Lindequist *et al.,* 2005). For example, *Ganoderma lucidum* is one of the most famous traditional medicinal fungi, being used as functional food and in preventive medicine (Pala and Wani, 2011). Many scientists have been reported that medicinal values associated with mushrooms. Mushrooms species have been shown to possess antagonistic effects against bacteria, fungi, viruses, and cancer (Jonathan and Fasidi, 2003). For a long time, these mushrooms had provided compounds that have nutritional, medicinal, and biological importance (Klaus *et al.,* 2009). Mushrooms have an important role in antimicrobial and antifungal activity for survival in their natural environment. So that for the benefit of humans' antimicrobial compounds can be isolated from many mushroom species. A large number of metabolites are produced by macrofungi that show antibacterial, antifungal, antiviral, antitumor, hypoglycaemic, antiallergic, immunomodulating, anti-inflammatory, hypolipidemic and hepatoprotective activity (Ashok *et al.,* 2014). From the above reports, it showed that mushrooms are vital sources of medicinal compounds that can be used to cure different disorders and prevent from pathogenic microorganisms. The objective of this study was to measure the antimicrobial potential of various phytochemicals compounds of methanol and hexane extracts from mycelial cultures of four non-gilled mushrooms having a certain biological activity which can be used in medicinal purposes by guiding new drug discovery in future.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used in this work were purchased from Hi-media Pvt. Ltd, Bombay, India.

Collection and identification of fruiting bodies of non-gilled mushrooms

The fruiting bodies of four non -gilled mushrooms were collected from an area of Morny hills in District Panchkula and Chail the area of District Solan of North-Western Himalayas (labelled as 6/15, 32/15, 2/15 and 132/15). These fruiting bodies were brought to the laboratory in sterile poly-bags. Each sample after cleaning with sterile water was cut across the pileus region with the help of sterilized blade to obtain bits (1-2 mm) of tissue, which were dipped in 0.1% mercuric chloride solution by using sterile forceps for 10 to 15 seconds. Then bits were transferred on to the plates of malt extract agar and mycelial cultures grow. These cultures were maintained at room temperature of 25°C.

Source of the test organism and their maintenance

Three bacterial strains and one fungal strain (*Staphylococcus aureus* MTCC 73, *Klebsiella pneumoniae* MTCC 109, *Escherichia coli* MTCC 739 and *Cryptococcus neoformans* ATCC 32045) were procured from Microbial Type Culture Collection (MTCC) from Institute of Microbial Technology (IMTECH), Chandigarh (India) and stored at 4˚C in refrigerator and subcultured at regular intervals of 48 hour until use.

Extract preparation

The liquid medium (pH 6.6) containing glucose 2%, peptone 1 % and yeast extract 2% in a conical flask was inoculated with eight days old mycelia grown on malt extract agar (MEA) plates. Flasks were incubated under shaking condition at 25˚C. The liquid culture was centrifuged along with mycelium at 4000 rpm for 15 mins. The supernatant was lyophilized and then dry powder was used for further study. After that lyophilized samples were extracted with methanol and hexane solvent for 3 days. The extract was then concentrated to dryness using rotary evaporator. At 4˚C all extracts from mycelia cultures were stored. Then the dry extract was dissolved with 10% dimethyl sulfoxide (DMSO) for further use (Mohi *et al.,* 2006)

Antibacterial activity

Muller Hinton agar (MHA) medium supplemented with 4% sodium chloride (NaCl) was prepared. Dipped sterilized swabs in standardized bacterial suspension with inoculums size of 1.5 $\times 10^8$ CFU/ml prepared above. By turning the swab against the side of the tube, the excess culture was removed and the entire surface of Muller Hinton agar Petri plates was spread by inoculums. At least for 15 minutes, Petri plates were allowed to dry and by using sterile cork borer (6 mm diameter) well were made on Petri plates. By using sterile dropping pipette about 25 µl extract was introduced into bore agar wells and kept plates at 4˚C for 6 hours inside the refrigerator to allow proper diffusion of extracts into the medium. After 24 hours of incubation at 37˚C, the plates were examined for antimicrobial activities. After incubation, the diameter of the zone of inhibition in millimetre (mm) was measured to determine the antibacterial activity (Barros *et al.,* 2008).

Antifungal activity

Sabouraud dextrose agar (SDA) medium was prepared. Dipped sterilized swabs in standardized bacterial suspension with inoculums size of 1.5 $\times 10^8$ CFU/ml prepared above. By turning the swab

against the side of the tube, the excess culture was removed and the entire surface of sabouraud dextrose agar (SDA) Petri plates was spread by inoculums. At least 15 minutes Petri plates were allowed to dry and by using sterile cork borer (6 mm diameter) well were made on Petri plates. By using sterile dropping pipette about 25 µl extract was introduced into bore agar wells and Petri plates were kept at 4˚C for 6 hours inside the refrigerator to allow proper diffusion of extracts into the medium. After 72 hours of incubation at 25˚C, the Petri plates were examined for antifungal activities. After incubation, the diameter of the zone of inhibition in millimetre (mm) was measured to determine the antifungal activity (Barros *et al.,* 2008).

Minimal inhibitory concentration (MIC) for estimating antibacterial and antifungal activity

96-well microtitre plate each well was dispensed with 50 µl of Muller Hinton broth (MHB) for bacteria and sabouraud dextrose broth (SDB) for fungus; 12th well (sterility control) was added with 100µl of Ciprofloxacin antibiotic as a positive control for bacteria and Fluconazole for fungus. 11th well (growth control) was added with MHB for bacteria and SDB for fungus with 10% DMSO. 50 µl of mycelia extract initially dissolved in 10% DMSO to the concentration of 100 mg/ml was added into the first well and a serial-fold dilution was performed by transferring 50 µl of the suspension to the subsequent well up till the 10th well; the final 50µl of the suspension was discarded. After that 5 µl, the bacterial suspension was added to each well and incubated at 37˚C for 24 hours for bacteria/27˚C for 72 hours for fungus. Then 5µl of resazurin dye was added to each well after 24 hours incubation. For one hour plates were incubated at 37˚C for bacteria/27˚C for fungus and after incubation, the plates were read to check color change from blue to purple /pink. A blue coloured solution showed the growth inhibition in the test wells, while pink to colourless solution indicated microbial growth or absence of inhibition (Chuah *et al.,* 2014).

Determination of phytochemical compounds

Determination of phytochemical compounds was done for methanol and hexane extracts. The freshly prepared extracts were subjected to phytochemical analysis in order to ensure the existence of phytochemical constituents. Mayer's test and Wagner's test were done for the identification of alkaloids. Molisch's test and Benedict's test were done for the identification of carbohydrates. Alkaline reagent test was done to check the presence of flavonoids. Salkowski test was performed to check the presence of terpenoids. Ferric chloride test and lead acetate test was done to identify the presence of phenolic compounds and tannins respectively (Bains and Tripathi, 2016).

Estimation of phenolic compounds

Phenolic Compound estimation was done by taking 100 µl of extract solution was mixed with 1.5 ml of folin-ciocalteu reagent and incubated at room temperature for one minute followed by the addition of 1.5 ml sodium carbonate (60g/l). The tubes were then vortex-mixed for 15 s and allowed to stand for 90 min in the dark at room temperature. Absorbance was then measured at 725 nm. Gallic acid was used as a standard curve with concentration ranges from 1 to 100 µg/ml (R^2=0.996) and the results were expressed as milligram of gallic acid equivalent (GAE) per gram of extract. All experiments were performed in triplicates (Singleton *et al.,* 1999).

Flavonoid contents

Flavonoid contents were done by taking 250 µl extract of mushrooms was mixed with 1.25 ml of distilled water and 75 µl of a 5% sodium nitrite ($NaNO_2$) solution. 150 µl of 10% aluminium chloride hydrate ($AlCl_3.H_2O$) of was added after 5 min. Then 275 µl of distilled water and 500 µl of 1 mol sodium hydroxide (NaOH) were added to the mixture after 6 min. The solution was then mixed well and the intensity of the pink colour was measured at 510 nm. The flavonoid compounds estimation was carried out in triplicate. Quercetin was used for the calibration curve with a concentration range of 20-100 µg/ml (R^2=0.9938). The results were mean values ± standard deviations and expressed as milligrams of (+) quercetin equivalents (QEs) per gram of extract (Jia *et al.,* 1999).

Ascorbic acid determination

The ascorbic acid determination was done with100 mg of dried extracts were extracted with 10 ml of 1% met phosphoric acid for 45 min at room temperature and filtered through What man No.4 filter paper. The filtrate 1 ml were mixed with 9 ml of 2, 6-dichloroindophenol and then absorbance was measured within 30 min at 515 nm against a blank. The content of ascorbic acid would be calculated on the basis of the calibration curve of authentic L-ascorbic with a concentration range of 2- 125 µg /ml (R^2 =0.9929). All the experiments were performed in triplicates (Klien *et al.,* 1982).

β-carotene and lycopene determination

The dried extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through what man No.4 filter paper. The absorbance of the filtrate was

Table 1: Antimicrobial activities of methanol extracts of mushroom cultures against various bacterial and fungal pathogens

Mushrooms	Test microorganisms and concentration of extract used (100mg/ml)			
	S. aureus	*K. pneumoniae*	*E. coli*	*C. neoformans*
A. polyticha	28.6 ± 0.94^{c}	30.3±0.47^{a}	24.3±0.47^{a}	24±0^{a}
G. lucidum	26±0^{a}	28.3±0.47^{a}	22±0^{b}	19±0.82^{b}
A. auricula-judae	25.3±0.39^{b}	24.3±3.29^{b}	23.3±0.26^{b}	17±0.42^{b}
T. elegans	24.6±0.47^{b}	22±0.63^{c}	21±0^{c}	10.3±0.47^{c}

In each row different letters ($^{a\ b\ c\ d\ e\ f}$): significance at p<0.05. Values are Mean± SEM (n=3) *A. Polytricha*: *Auricularia polytricha*, *G. lucidum*: *Ganoderma lucidum*, *A. auricula-judae*: *Auricularia auricula judae*, *T. elegans*: *Trametes elegans. S. aureus*: *Staphylococcus aureus*, *K. pneumoniae*: *Klebsiella pneumoniae*, *E. coli: Escherichia coli, C. neoformans*: *Cryptococcus neoformans.*

Table 2: Antimicrobial activities of hexane extracts of mushroom cultures against various bacterial and fungal pathogens

Mushrooms	Test microorganisms and concentration of extract used (100mg/ml)			
	S. aureus	*K. pneumoniae*	*E. coli*	*C. neoformans*
A. polyticha	25.3± 0.47^{a}	24.6±0.47^{a}	24.3 ±0.47^{b}	12±0^{b}
G. lucidum	24.6±0.47 ^{ab}	13.3±0.47^{c}	14.6±0.47^{a}	15.3±0.47^{a}
A. auricula-judae	22±1.63^{b}	24.3±0.47^{bc}	21.3±1.89^{b}	--
T. elegans	14±0^{c}	17±1.41^{b}	15.3±0.47^{c}	--

In each row different letters ($^{a\ b\ c\ d\ e\ f}$) means significant at p<0.05. Values are Mean± SEM (n=3). *A.Polytricha*: *Auricularia polytricha*, *G. lucidum*: *Ganoderma lucidum*, *A. auricula-judae*: *Auricularia auricula judae*, *T. elegans*: *Trametes elegans*. *S. aureus*: *Staphylococcus aureus*, *K. pneumoniae*: *Klebsiella pneumoniae*, *E. coli: Escherichia coli, C. neoformans*: *Cryptococcus neoformans*. (-)= indicates the absence of growth.

Table 3: MIC of methanol extracts of mushroom cultures against bacterial and fungal strains

Mushrooms	Minimal inhibitory concentration (mg/ml)			
	S. aureus	*K. pneumoniae*	*E. coli*	*C. neoformans*
A. polyticha	0.78	0.78	1.56	1.56
G. lucidum	0.78	1.56	6.25	6.25
A. auricula-judae	3.125	3.125	12.5	12.5
T. elegans	3.125	3.125	1.56	12.5

A.Polytricha: *Auricularia polytricha*, *G. lucidum*: *Ganoderma lucidum*, *A. auricula-judae*: *Auricularia auricula judae*, *T. elegans*: *Trametes elegans*. *Staphylococcus aureus*: *S. aureus*, *K. pneumoniae*: *Klebsiella pneumoniae*, *E. coli: Escherichia coli, C. neoformans*: *Cryptococcus neoformans*.

Table 4: MIC of hexane extracts of mushroom cultures against bacterial and fungal strains

Mushrooms	Minimal inhibitory concentration (mg/ml)			
	S. aureus	*K. pneumoniae*	*E. coli*	*C. neoformans*
A. polyticha	0.78	0.78	1.56	1.56
G. lucidum	1.56	3.125	6.25	6.25
A. auricula-judae	6.25	0.78	1.56	--
T. elegans	12.5	12.5	6.25	--

A.Polytricha: Auricularia polytricha, G. lucidum: Ganoderma lucidum, A. auricula-judae: Auricularia auricula judae, T. elegans: Trametes elegans. Staphylococcus aureus: S. aureus, K. pneumoniae: Klebsiella pneumoniae, E. coli: Escherichia coli, C. neoformans: Cryptococcus neoformans, (-)= indicates the absence of growth.

measured at 453, 505, 645 and 663 nm (Nagata and Yamashita, 1992). The content of β-carotene and Lycopene was calculated by using the following equations. Lycopene (mg/100µg) = -0.0458 A_{663}+ 0.372 A_{505} -0.0806 A_{453}. β- Carotene (mg/100µg) = 0.216A_{663}-0.304 A_{505}+ 0.452 A_{453}

Statistical analysis

All the experimental was carried out in triplicates. The results are expressed as mean value and standard deviation. The results were analysed by using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test using SAV v 9.13 program. The difference at p<0.05 were considered to be statistically significant.

Table 5: Phyto-chemical analysis of methanol and hexane extracts of four isolates

Mushrooms	Alkaloids	Tannins	Terpenoids	Carbohydrates	Polyphenols	Flavonoids
A.polytricha	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
G. lucidum	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
A.auricula-judae	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
T. elegans	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

+ve =Presence

Table 6: Estimation of phytochemicals compound present in methanol extracts

Mushrooms	Phenols (mg/g)	Flavonoids (mg/g)	β- carotene (µg/g)	Lycopene (µg/g)	Ascorbic acid (mg/g)
A.polytricha	63.66±0.34^{a}	19.16±0.59^{b}	18.13±0.09^{d}	12.03±0.16^{b}	16.65±0.19^{c}
G. lucidum	57.96±0.18^{a}	16.65±0.25^{b}	1.80±0.45^{e}	6.61±0.26^{c}	9.58±0.29^{d}
A. auricula-judae	48.84±2.06^{a}	13.70±0.46^{b}	9.55±0.22^{d}	5.47±0.12^{c}	7.42±0.36^{cd}
T. elegans	43.35±0.12^{a}	10.35±0.23^{b}	8.44±0.13^{e}	4.12±0.22^{c}	4.75±0.19^{d}

In each row different letters ($^{a\ b\ c\ d\ e\ f}$) means significant at P<0.05. Values are Mean± SEM (n=3).
A.Polytricha: *Auricularia polytricha*, *G. lucidum*: *Ganoderma lucidum*, *A. auricula-judae*: *Auricularia auricula judae*, *T. elegans*: *Trametes elegans*.

Table 7: Estimation of phytochemicals compound present in hexane extracts

Mushrooms	Phenols (mg/g)	Flavonoids (mg/g)	β- carotene (µg/g)	Lycopene (µg/g)	Ascorbic acid (mg/g)
A.polytricha	61.25±0.6^{a}	12.95±0.14^{c}	14.68±0.21^{b}	9.51±0.44^{d}	13.04±0.34^{c}
G. lucidum	52.21±0.34^{a}	15.77±0.14^{b}	10.42±0.17^{c}	6.25±0.33^{e}	8.32±0.20^{d}
A. auricula-judae	45.38±0.31^{a}	10.95±0.38^{b}	9.12±0.15^{c}	4.61±0.36^{e}	6.26±0.17^{d}
T. elegans	38.44±0.32^{a}	6.88±0.38^{b}	6.33±0.22^{b}	3.86±0.55^{c}	3.05±0.07^{c}

In each row different letters ($^{a\ b\ c\ d\ e\ f}$) means significant at P<0.05. Values are Mean± SEM (n=3).
A.Polytricha: *Auricularia polytricha*, *G. lucidum*: *Ganoderma lucidum*, *A. auricula-judae*: *Auricularia auricula judae*, *T. elegans*: *Trametes elegans*.

RESULTS AND DISCUSSIONS

Identification of non-gilled mushrooms

The four non -gilled mushrooms were identified on the basis of morphological features and molecular characterization; *Auricularia polytricha* (6/15); *Ganoderma lucidum* (32/15); *Auricularia auricula-judae* (2/15); *Trametes elegans* (132/15). These samples were identified and submitted to National Centre for Biotechnology Information (NCBI) with accession number *Auricularia polytricha* (MF774107), *Ganoderma lucidum* (MF770158), *Auricularia auricula-judae* (MF770159) and *Trametes elegans* (MF770160).

Antimicrobial activity

The antimicrobial activity assessed by agar well diffusion method. The screening for antimicrobial activity focused on four non-gilled mushrooms against three pathogenic bacteria (*Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli*) and one fungal strain (*Cryptococcus neoformans*). In addition, ciprofloxacin was considered positive, whereas dimethyl sulfoxide (DMSO) was preferred as a negative control. The methanolic and hexane extracts were prepared where methanolic extracts showed higher antimicrobial activity against all the pathogenic strains, in comparison to the hexane extracts in (Figure 1 to 4). The methanolic extracts exhibited a larger zone of inhibition (30.3±0.47) in case of *Auricularia polyticha* against *Klebsiella pneumoniae,* followed by *Ganoderma lucidum* (28.3±0.47) against the *Klebsiella pneumoniae.* On the other hand hexane extract also showed higher antimicrobial activity in *Auricularia polytricha* (25.3± 0.47) but against *Staphylococcus aureus,* followed by *Ganoderma lucidum* (24.6±0.47). The studies also revealed that no activity was observed in *Auricularia auricular-judae* and *Trametes elegans* against *Cryptococcus neoformans* in the hexane extracts. It can be concluded the antimicrobial activity was higher in the case of methanolic extracts against all pathogenic strain in comparison to the prepared hexane extracts (Table 1 and 2).

Minimal inhibitory concentration (MIC) of methanolic and hexane extract

Minimal inhibitory concentration result has shown that all methanol and hexane extract of all four mushrooms cultures exhibit a good antibacterial spectrum activity.The methanol extract of *Auricularia polytricha, Ganoderma lucidum* was best to potentiate the antibacterial activity of *Staphylococcus aureus* and *Klebsiella pneumoniae* with MIC

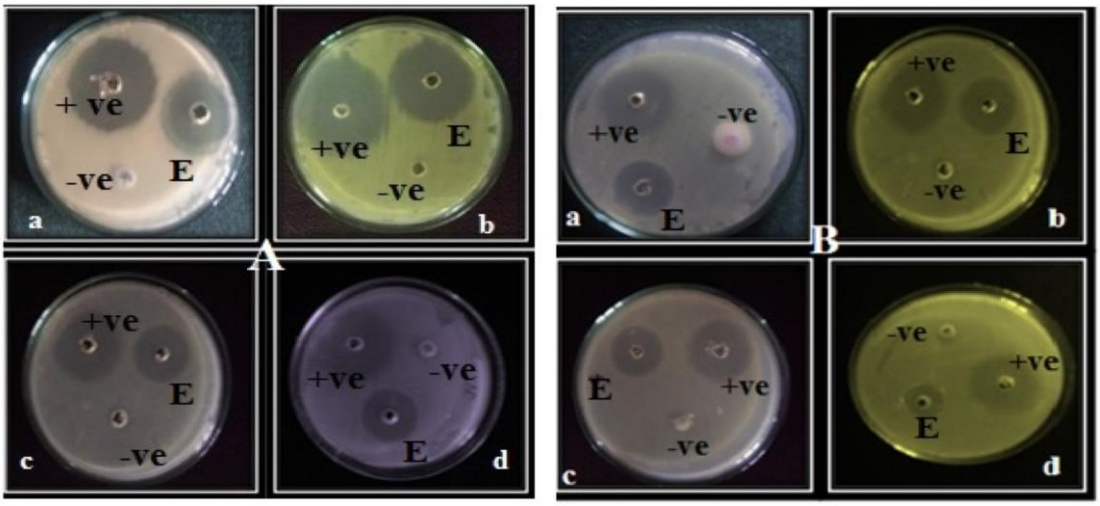

Figure 1: Antimicrobial activity of (A) methanol and (B) hexane extracts of sample *Auricularia polytricha* against (a) *Staphylococcus aureus* , (b) *Klebsiella pneumoniae,* (c) *Escherichia coli and* (d) *Cryptococcus neoformans*, E=extract -ve control=ciprofloxacin/fluconazole , -ve control=dimethyl sulfoxide (DMSO)

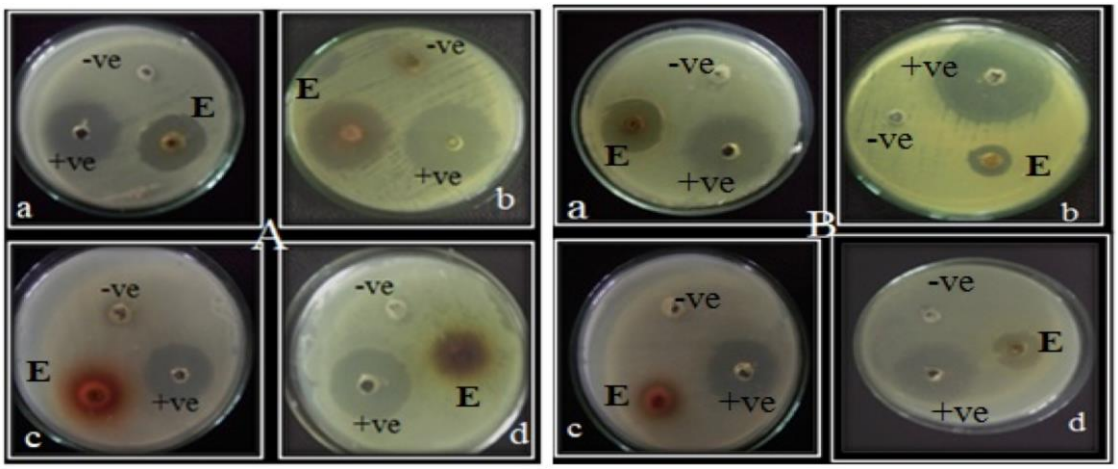

Figure 2: Antimicrobial activity of (A) methanol and (B) hexane extracts of sample *Ganoderma lucidum* against (a) *Staphylococcus aureus*, (b) *Klebsiella pneumoniae* (c) *Escherichia coli and (d) Cryptococcus neoformans*, E=extract, -ve control=ciprofloxacin/fluconazole, -ve control=dimethyl sulfoxide (DMSO)

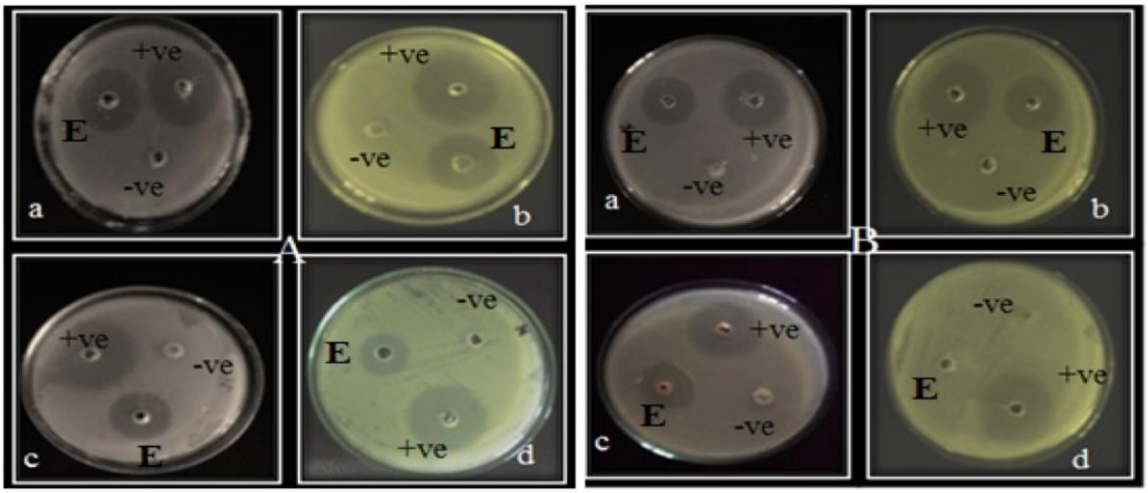

Figure 3: Antimicrobial activity of (A) methanol and (B) hexane extracts of sample *Auricularia auricula-judae* against (a) *Staphylococcus aureus*, (b) *Klebsiella pneumoniae,* (c) *Escherichia coli and* (d) *Cryptococcus neoformans*, -ve control=ciprofloxacin/fluconazole, E= extract, -ve control=dimethyl sulfoxide (DMSO)

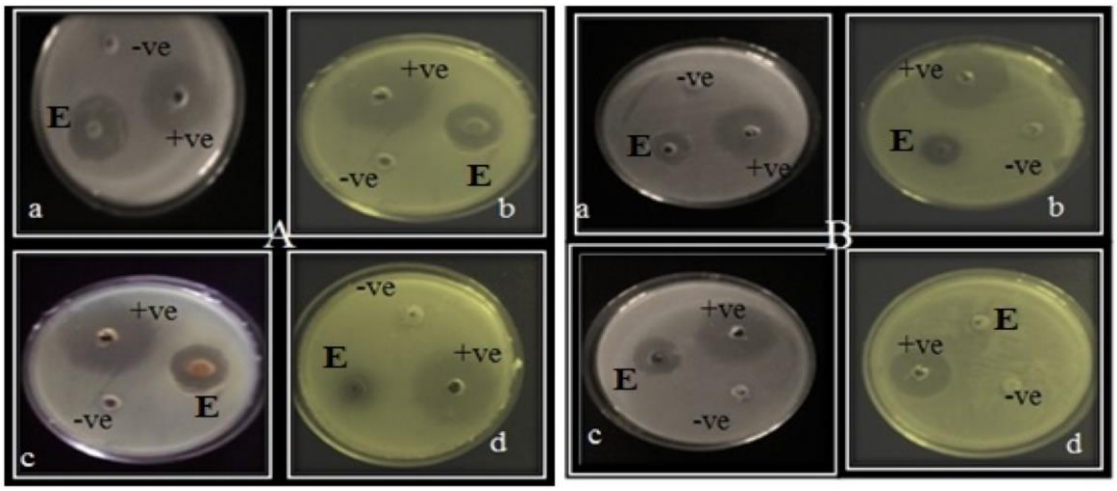

Figure 4: Antimicrobial activity of (A) methanol and (B) hexane extracts of sample *Trametes elegans* against (a) *Staphylococcus aureus* , (b) *K. pneumoniae* , (c) *Escherichia coli* and (d) *Cryptococcus neoformans*, E=extract, -ve control=ciprofloxacin/fluconazole, -ve control=dimethyl sulfoxide (DMSO)

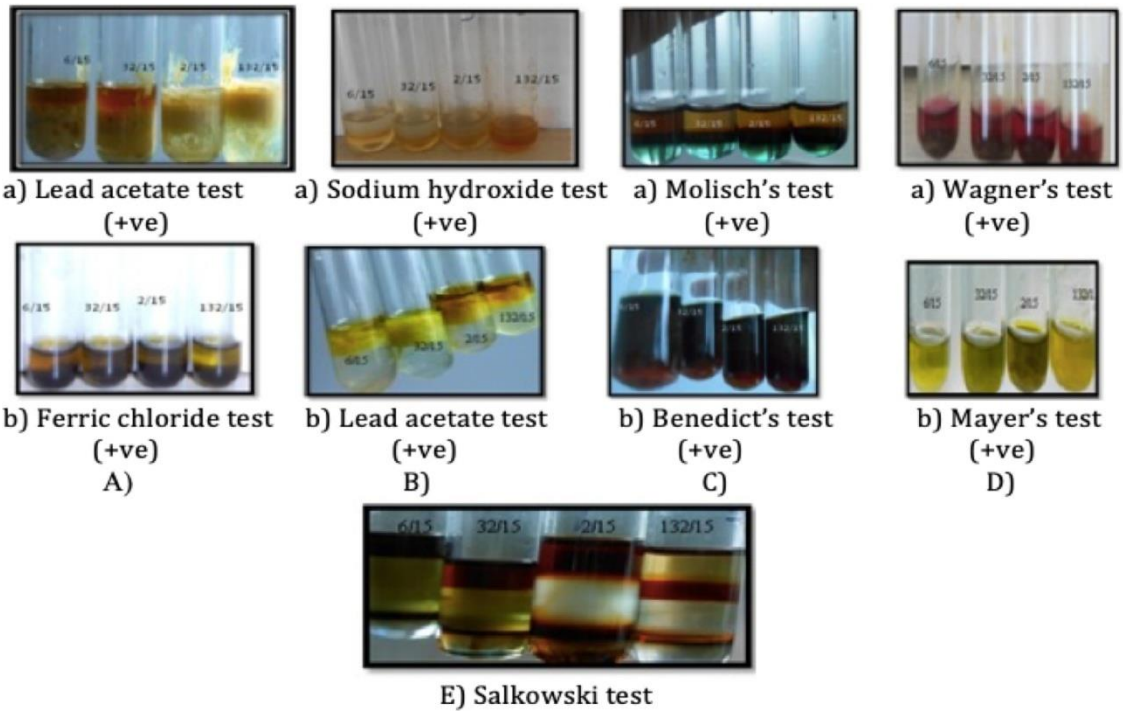

Figure 5: Results of (A) Phenols/ Tannins (B) Flavonoids (C) Carbohydrates (D) Alkaloids (E) Terpenoids, (6/15: *Auricularia polytricha,* 32/15: *Ganoderma lucidum*, 2/15: *Auricularia auricular-judae*, 132/15: *Trametes elegans*)

0.78 mg/ml against *Klebsiella pneumoniae*, *Escherichia coli* followed by *Auricularia polytricha, Ganoderma lucidum and Trametes elegans* showed good antibacterial activity with MIC 1.56 mg/ml followed by *Auricularia auricula-judae* and *Trametes elgans* showed high antibacterial potential against *Staphylococcus aureus* and *Klebsiella pneumoniae* with MIC 3.12 mg/ml. The hexane extract of *Auricularia polytricha* and *Ganoderma lucidum* showed antimicrobial potential against *Staphylococcus aureus* and *Klebsiella pneumoniae* with MIC 0.78 mg/ml against *Staphylococcus aureus* and *Escherichia coli* followed by *Auricularia polytricha* and *Ganoderma lucidum* with MIC 1.56 mg/ml. The results of the MIC of methanolic and hexane extract were shown in Table 3 and 4.

Preliminary phytochemical analysis

On the basis of initial screening, four mushroom cultures have been selected for phytochemical analysis. The preliminary phytoconstituents analysis of all isolates (Figure 5) revealed that all the four extracts contain alkaloids, tannins, terpenoids, carbohydrates, polyphenols and flavonoids (Table 5).

Quantitative analysis of phytochemicals

The quantitative analysis of phytochemical revealed that *Auricularia polytricha, Ganoderma lucidum, Auricularia auricula-judae and Trametes elegans* showed the presence of phenols, flavonoids, β-carotene and lycopene present in the above in specific quantities were shown in Table 4. The maximum amount of phenols were found to be present in the *Auricularia polytricha* (63.66±0.34) of methanolic extract which was similar in case of hexane extracts (61.25±0). The studies revealed that the higher amount of phytochemicals were detected in methanolic extracts in comparison to the hexane extract. Results of phytochemicals are shown in Table 6 and 7.

DISCUSSION

Antimicrobial activity of extracts of mushrooms species (*Auricularia polytricha, Ganoderma lucidum, Auricularia auricula-judae and Trametes elegans*) as well as the phytochemical characteristics were studied. The antibacterial studies presently conducted with the two extract methanol and hexane showed wide variation with respect to their effect. Mushrooms require antibacterial and antifungal compounds in order to survive in their natural habitat (Lindequist *et al.,* 2005; Thillaimaharan *et al.,* 2016). The extracts of concentration 100 mg/ml were found to be effective against gram positive and gram negative bacteria from the present investigation it was proved that methanolic extract of four non-gilled mushrooms shows maximum antimicrobial activity then hexane extract. It has already been reported in various studies that polar solvents to be more effective in extracting organic and inorganic materials from biological sources (Brahmi *et al.,* 2012). Furthermore, the study revealed that the bacterial isolates showed more sensitivity to extracts from mushrooms than fungal isolate. This is in congruence with the finding of (Gbologade and Fasidi, 2005). These attributes might make them rich sources of natural antibiotics.

The phytochemical analysis of four non-gilled mushrooms revealed that both the extracts contained phenolic compounds, carbohydrates, glycosides, and terpenoids. The total phenolic content in methanol was higher than hexane extract. The results are in accordance with the previous literature reported (Menaga *et al.,* 2012). The estimation of phenol was done under the absorbance at 725nm by folin –ciocalteu's reagent. Estimation of flavonoids was done by ferric chloride, estimation of ascorbic by met phosphoric method and estimation of beta-carotene and lycopene was done by Bains and Tripathi (Bains and Tripathi, 2016). These phytoconstituents play a significant role in the medicinal properties of many plants. Major components of plants and mushrooms are phenols (Barros *et al.,* 2007). Phenolic compounds are antioxidants that exhibit a wide range spectrum of medicinal properties such as anti-cancer, inflammatory and diabetic effects (Hamzah *et al.,* 2013; Nagavani *et al.,* 2010). The presence of alkaloids in a various system in the body like an autonomic nervous system, blood vessels, respiratory system, gastrointestinal tract, uterus are responsible for pharmacological properties of many mushrooms (Trease and Evans, 1989). Flavonoids are known to be the most diverse group of natural polyphenolic compounds having the broad spectrum of chemical and biological activities that include antiviral, radical scavenging activity, anti-inflammatory and vasodilating action (Parajuli *et al.,* 2012). Terpenoids show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti-cancer effects among others also been reported (Roslin and Anupam, 2011).

In the present study, it was revealed that methanolic extract and hexane extract for quantitative analysis constitutes phenolic compounds in good amount. The overall methanolic extract showed higher total phenols, flavonoid, carotenoid and ascorbic acid content then hexane extract.

CONCLUSION

The present study was focused on the antimicrobial and phytochemical analysis of four non-gilled mushrooms and determination of their chemical composition in terms of phytochemical compounds. It can be concluded that the methanolic and hexane extracts of non-gilled mushrooms can be successfully applied in the development of a more dynamic and competent antimicrobial agent. This study strongly suggests that increasing number of bacteria that have developed resistance to commercial antibiotics, hence extracts and derivatives from mushrooms hold great promise for novel medicines.

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