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Research Article

IN-VITRO ASSESSMENT OF ANTIBACTERIAL POTENTIAL OF SOME WILD MUSHROOMS AGAINST PATHOGENIC BACTERIA

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A B S T R A C T

Despite the huge diversity of antibacterial compounds, bacterial resistance to first choice antibiotics has been drastically increasing. Moreover, the association between multi-resistant microorganisms and nosocomial infections highlight the problem, and the urgent need for solutions. Natural resources have been exploited in the last years and among them mushrooms could be an alternative as source of new antimicrobials. In present course of investigation the antibacterial potentiality of wild mushrooms collected from local region has been assessed against $Gram^{-ve}$ and $Gram^{+ve}$ pathogenic bacteria. Out of several collected wild mushrooms six was identified and antibacterial ability of the most frequent thee mushrooms - *Gonoderma lucidum, Xerocomus chryentron* and *Termitomyces tyleranus* were assessed against *E. coli* - ATCC10536 and *Staphylococcus aureus* - ATCC 25923. Ethanol, Methanol and Hot water extracts were employed in 25%, 50%, 75% and 100% concentration by measuring ZOI of inhibition employing well diffusion method. All three wild mushrooms exhibited growth inhibitory talent, however the highest potentiality was found in ethanol extracts of *Xerocomus chryentron,* nearer to the potential of standard commercial antibiotics; whereas *Gonoderma lucidum* showed slightly lesser while very less ability expressed by *Termitomyces tyleranus.* Findings of the present study suggest that *Gonoderma lucidum, Xerocomus chryentron* frequently found in central India, especially in Chhattisgarh and M.P. state, exhibited significant antimicrobial activity that may effectively be used for preparation of drugs and medicines.

INTRODUCTION

Diversity of fungi and their natural beauty occupy prime place in the biological world and India has been a cradle for these species. Only a fraction of total fungal wealth has been subjected to scientific scrutiny and mycologists continue to unravel the unexplored and hidden wealth one third of fungal diversity of the globe exists in India and of this only are characterized until Mushrooms have been extensively studied in the western countries, while tropical countries like India especially in central India it is not much explored. The man has been consuming mushrooms as food, medicine and even as intoxicant, since time immemorial by collecting them from the wild region. Mushrooms alone are represented by about 41,000 species, of which approximately 850 species are recorded from India (Manoharachary *et al.,* 2005). More than 2000 species of edible species are reported in the literature from different parts of the world. As a matter of fact, macro- fungi need antibacterial and antifungal compounds to survive in their natural environment.

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Therefore, antimicrobial compounds could be isolated from many mushroom species and could be beneficial for humans (Yang, Lin, & Mau, 2002; Oyetayo, 2009; Kalac, 2009, 2013).

Bioactive molecules have been isolated not only from edible, but also from inedible species. Bioactivities of mushrooms include antibacterial, antifungal, antioxidant, antiviral, anti-tumor, cytostatic, immunosuppressive, anti-allergic, hypoglycemic, anti-inflammatory, cholesterol lowering and hepatoprotective has been reported by several workers (Cosgrove, 2006, Barros *et al*., 2007; Ozen *et al.*, 2011).

The responsible bioactive compounds belong to several chemical groups which are often polysaccharides or triterpenes (Kim *et al*., 2000; Sun and Liu, 2009; Lee *et al*., 2010). Phenolic acids including benzoic and cinnamic acid derivatives have been pointed out as the most common. Among benzoic acid derivatives, p-hydroxybenzoic, protocatechuic, gallic, vanillic and syringic acids were identified in different mushroom species (Puttaraju *et al*. 2006; Kim *et al*. 2008; Barros *et al.*, 2008; Heleno *et al*., 2011, 2012; Reis *et al*., 2011). Antimicrobial resistance increases morbidity and mortality, while there is a

significant increase in costs for health care institutions. Because of that, a huge effort has been directed towards controlling antibiotic use and raising public awareness of the need for prudent use of antibiotics (Dancer 2001; Coutinho *et al*., 2005; Zepeda, 2016)

Data on chemical composition and notional value of European edible mushroom species were (Bernas *et al.*, 2001, Kalac, 2009) geographically and industrially diverse regions of Poland in 2000-2008. Mercury concentrations Contamination of food resources with Hg from environmental releases of this hazardous metal is 35 a continuous threat to food safety (Olivero *et al*., 2002) weight in 2010 (Jecefa, 2010). A few earlier studies have shown that wild mushrooms can accumulate mercury from the soil to a considerable amount (Melgar *et al*., 2009; Chudzyński *et al.,* 2009 and 2011; Falandysz *et al*., 2007 and 2012)

There are many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain types of cancer in which polyphenol is linked to the antioxidant properties (Barros *et al*., 2007; Jagadish *et al*., 2009; Vaz *et al.,* 2011). Keeping in view the facts as mentioned above, the present course of work has been planned to assess the antibacterial properties of wild mushroom collected from different sites of local region of Chhattisgarh state and investigation have been performed for the same to achieve the objectives.

MATERIALS AND METHODS

An exhaustive survey was performed and samples were collected from different sampling sites, i.e. agriculture fields, road side garbage and forest / plantation area following the sampling method. Organisms were collected in separate polythene bags in air tight condition. Out of thirteen samples of wild mushrooms collected, only seven were identified (Plate -1) with help of text and Department of Rural Technology, G. G. University, Bilaspur (C.G.) and three mushrooms, most frequently available, were selected for extraction and toxicity assessment.

Preparation of crude extract

Fresh fruiting body of selected mushrooms was surface sterilized treated by alcoholic spray tap, air dried then oven dried at 40^{o}c for 24hours and grinded to form as fine powder for crude extract separately under aseptic condition and stored in airtight bottles. Two separate samples of the mushroom powder (1g of each sample) were extracted with hot water (2ml), ethanol 100% (2 ml) and methanol 100% (2 ml) dissolved at aseptic condition. In this way, three different crude extracts were obtained - hot water extract (HE), ethanol extract (EE) and methanol extract (ME) that was graded into 25%, 50%, 75%, 100% concentration.

Assessment of toxic nature of wild Mushroom

In-vitro antibacterial efficacy of wild mushroom crude extract was evaluated using two pathogenic bacteria (*Escherichia coli* ATCC10536 and *Staphylococcus aureus* ATCC 2592*)* as test system for the characterization of its toxin producing nature. Characterization of toxin producing wild mushroom was performed with the help of measurement ZOI.

Antibacterial bioassay

The antibacterial activity was screened by Zone of Inhibition. Overnight cultures (at 37^{o}C for 24 h) of each bacterial strain (*E. coli* - ATCC10536 and *Staphylococcus aureus* - ATCC 25923) were spread with glass rod on the surface of Nutrient Agar plates. The antimicrobial activity was screened using the cark borer well (4mm in diameter) diffusion method, well were saturated with 50µl (1gm /2ml) of the mushrooms extracts under laminar air flow. Agar well diffusion method was used for determining antibacterial activity. Petri plates were prepared by pouring 25 ml of seeded nutrient agar and allowed to solidify. The plates were placed in incubator for 24 hours. After 24hours culture with spread on agar plates were taken. A standard cork borer of 4mm diameter was used to cut uniform wells on the surface of the agar plate and 2ml extracts of each dilution (25%, 50%, 75% &100%) were introduced into wells. The plates were incubated at 37^{o}C for 24hours. After incubation, the diameter of clear zones around each well is measured and compared against zone of inhibition produced by solution of known concentration of standard antibiotic Kanamycin (30 mcg) and Amoxicyllin (30 mcg). Different concentrations (25%, 50%, 75% &100%) of extracts were used and results were observed.

RESULTS AND DISCUSSION

Out of thirteen samples of wild mushrooms collected, only seven were identified (Plate -1) and antibacterial potential three selected mushrooms *(Gonoderma lucidum, Xerocomus chryentron* and *Termitomyces tyleranus),*on the basis of their most frequent occurrence, was examined. The extracts of these wild mushroom was found to restrict the growth of the bacteria on nutrient media around the well. The inhibition zones at different concentration of the extracts of *Gonoderma lucidum* against both pathogenic bacteria, as the test organism, were measured as mentioned in Table 1 and pattern of growth inhibition has mentioned in Plate 2: Fig. 1.

Table 1 Antibacterial effect of crude extracts of *Ganoderma lucidum* on *E. coli & Staphylococcus aureus* and its comparison with standard antibiotics (Mean ± SD)

Crude Extracts of Mushrooms & Standard Antibiotics		Concentrations	Zone of inhibition (mm.) Mean ± SD	
			Escherichia coli ATCC10536	*Staphylococcus aureus* ATCC 25923
		0 %	00	00
Ganoderma lucidum	Ethanol	25%	6.2 ±0.42	6.7 ±0.23
		50%	8.6 ±0.84	8.2± 0.92
		75%	11.2 ±0.51	12.6 ±0.68
		100%	16.2 ±0.48	17.0 ±0.52
	Methanol	25%	5.8 ±0.56	6.3 ±0.23
		50%	8.0 ±0.31	8.8± 0.92
		75%	10.5 ±0.71	11.2 ±0.78
		100%	15.4 ±0.45	16.2 ±0.52
	Hot water	25%	5.20 ±0.37	6.2 ±0.64
		50%	7.5 ±0.48	8.1 ±0.68
		75%	10.6 ±0.52	10.8 ±0.65
		100%	13.5 ± 0.83	13.9 ±0.33
Antibiotics	Kanamycin	30 mcg. / well	16.5 ± 0.40	17.5 ±0.65
	Amoxycillin	30 mcg. / well	15.91 ± 1.73	24.0 ±1.27

Plate 1

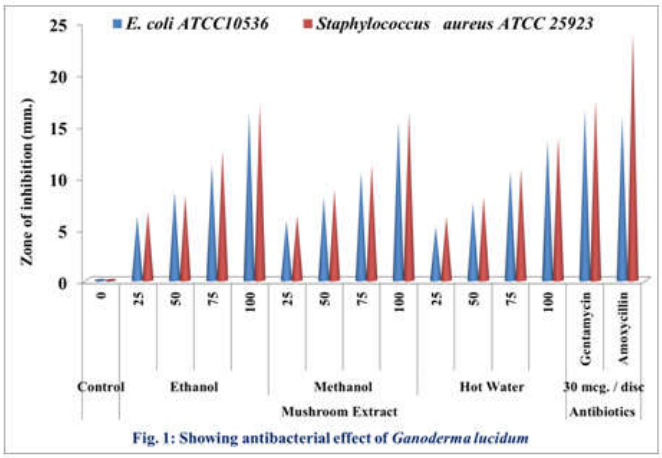

Fig. 1: Showing antibacterial effect of *Ganoderma lucidum*

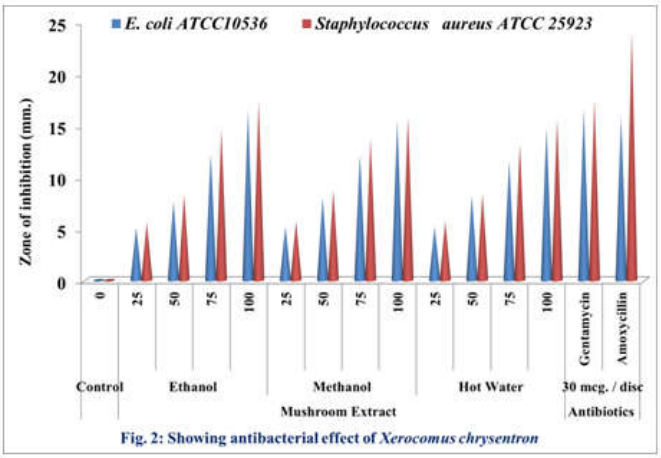

Fig. 2: Showing antibacterial effect of *Xerocomus chrysentron*

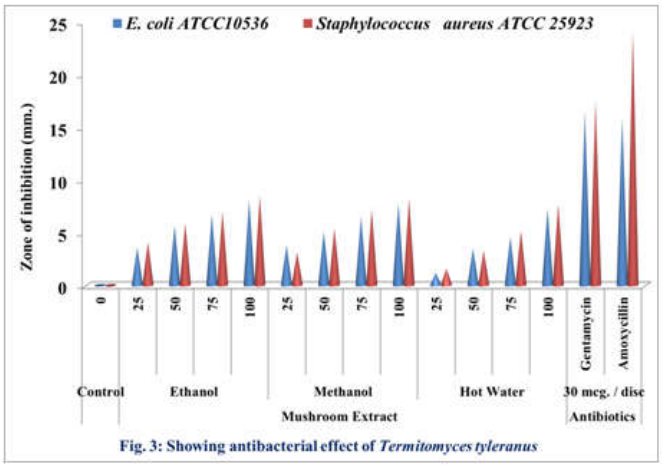

Fig. 3: Showing antibacterial effect of *Termitomyces tyleranus*

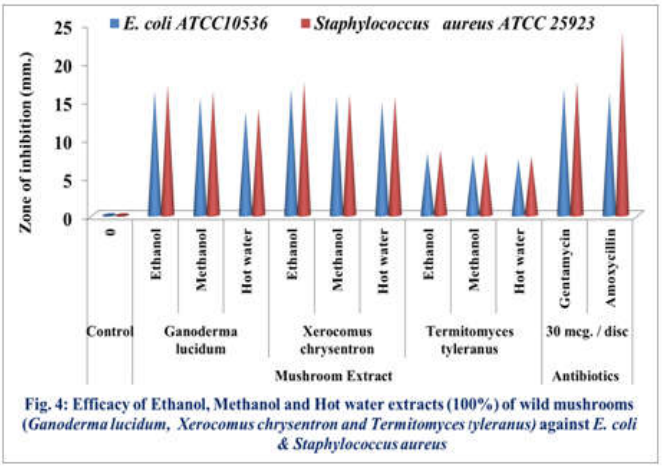

Fig. 4: Efficacy of Ethanol, Methanol and Hot water extracts (100%) of wild mushrooms (*Ganoderma lucidum, Xerocomus chrysentron and Termitomyces tyleranus*) against *E. coli & Staphylococcus aureus*

Plate 2 Fig. 1 to 4

Similarly the ZOI of all three solvent extracts of *Xerocomus chryentron* and *Termitomyces tyleranus* were observed/ measured and found significant inhibitory potential (Table 2 & 3; Plate 2: Fig. 2 & 3). The ZOI ranges 7.3mm to 8.5 mm in case of crude extracts of *Termitomyces tyleranus,* 14.8 mm – 17.4mm in case of *Xerocomus chryentron* and 13.5mm to 17.0 mm in case *Gonoderma lucidum.*

The inhibitory effect was lees in concentration while at 100% the inhibitory ability was found greater in case of *Xerocomus chryentron* and *Gonoderma lucidum* that was nearest to standard commercial antibiotic (ZOI – 15.9mm - 24.0mm). (Table 4; Plate 2: Fig. 4).

Table 2 Antibacterial effect of crude extracts of *Xerocomus chrysentron* on *E. coli & Staphylococcus aureus* and its comparison with standard antibiotics (Mean ± SD)

Crude Extracts of Mushrooms & Standard Antibiotics		Concentrations	Zone of inhibition (mm.) Mean ± SD	
			Escherichia coli ATCC10536	*Staphylococcus aureus* ATCC 25923
		0 %	00	00
Xerocomus chrysentron	Ethanol	25%	5.1 ±0.12	5.6 ±26
		50%	7.7 ±0.89	8.3 ±0.63
		75%	12.3 ±0.51	14.6 ±1.38
		100%	16.5 ±0.65	17.4 ±1.02
	Methanol	25%	5.2 ±0.43	5.7 ±0.62
		50%	8.0 ±0.61	8.7 ±0.48
		75%	12.2 ±0.62	13.7 ± 0.55
		100%	15.5 ±0.83	15.8 ±0.56
	Hot water	25%	5.2 ±0.43	5.7 ±0.62
		50%	8.2 ±0.61	8.4 ±0.48
		75%	11.6 ±0.62	13.2 ± 0.55
		100%	14.8 ±0.83	15.6 ±0.56
Antibiotics	Kanamycin	30 mcg. / well	16.5 ± 0.48	17.5 ±0.65
	Amoxycillin	30 mcg. / well	15.91 ± 1.73	24.0 ±1.27

Table 3 Antibacterial effect of crude extracts of *Termitomyces tyleranus* on *E. coli & Staphylococcus aureus* and its comparison with standard antibiotics (Mean ± SD).

Crude Extracts of Mushrooms & Standard Antibiotics		Concentrations	Zone of inhibition (mm.) Mean ± SD	
			Escherichia coli ATCC10536	*Staphylococcus aureus* ATCC 25923
		0 %	00	00
Termitomyces tyleranus	Ethanol	25%	3.6 ±0.34	4.1 ±0.21
		50%	5.7 ±0.59	5.9± 0.62
		75%	6.7 ±0.41	7.0± 0.38
		100%	8.1 ±0.30	8.5± 0.41
	Methanol	25%	3.8 ±0.64	3.1 ±0.36
		50%	5.1 ±0.55	5.4± 0.65
		75%	6.5 ±0.48	7.2± 0.58
		100%	7.8 ±0.54	8.2± 0.45
	Hot water	25%	1.2 ± 0.47	1.6± 0.62
		50%	3.5 ±0.51	3.3 ±0.40
		75%	4.6 ±0.72	5.2 ± 0.62
		100%	7.3 ± 0.93	7.7 ± 0.43
Antibiotics	Kanamycin	30 mcg. / well	16.5 ± 0.40	17.5 ±0.65
	Amoxycillin	30 mcg. / well	15.91 ± 1.73	24.0 ±1.27

Table 4 Efficacy of Ethanol, Methanol and Hot water extracts (100%) of wild mushrooms (*Ganoderma lucidum, Xerocomus chrysentron and Termitomyces tyleranus)* against *E. coli & Staphylococcus aureus* and its comparison with standard antibiotics (Mean ± SD).

Crude Extracts of Mushrooms & Standard Antibiotics		Extract Solvents	Zone of inhibition (mm.) Mean ± SD	
			Escherichia coli ATCC10536	*Staphylococcus aureus* ATCC 25923
		Nil	00	00
Mushroom Extract	*Ganoderma lucidum*	Ethanol	16.2 ±0.48	17.0 ±0.52
		Methanol	15.4 ±0.45	16.2 ±0.52
		Hot water	13.5 ± 0.83	13.9 ±0.33
	Xerocomus chrysentron	Ethanol	16.5 ±0.65	17.4 ±1.02
		Methanol	15.5 ±0.83	15.8 ±0.56
		Hot water	14.8 ±0.83	15.6 ±0.56
	Termitomyces tyleranus	Ethanol	8.1 ±0.30	8.5± 0.41
		Methanol	7.8 ±0.54	8.2± 0.45
		Hot water	7.3 ± 0.93	7.7 ± 0.43
Antibiotics	Kanamycin	30 mcg. / well	16.5 ± 0.40	17.5 ±0.65
	Amoxycillin	30 mcg. / well	15.91 ± 1.73	24.0 ±1.27

Preliminary antibacterial testing of above mushrooms produced zone of growth inhibition of (13.5mm - 16.2mm) for the gram positive bacteria and (13.9mm-17.0mm) for the gram negative bacteria. In both types of bacteria, inhibition zones due to petroleum ethanol extract were higher than methanol extract. Methanol extract also showed highest activity than Hot water extract in all cases. In general, petroleum ether extracts exhibited stronger inhibition compared to the methanolic extract; with slight variation ethanol extract of *Xerocomus chrysentron Gonoderma lucidum* showed good result for growth inhibition and which offered highest zone of inhibition for *Staphylococcus aureus* and *Escherichia coli.* It was observed that in case of *Staphylococcus aureus*, the highest zone of inhibition showed by *Xerocomus chrysentron.*

Regarding antimicrobial activity, *Xerocomus chrysentron* was proved to be the most potent to kill the gram positive bacteria and gram negative bacteria. Mushrooms store a number of active secondary metabolites including phenolic compounds and steroids (Keles *et al*., 2011). Among these compounds, polyphenols have gained utmost importance due to their large array of bioactivities including free radical scavenging, metal chelating, and immune modulating activities as well asmodulation of enzyme activities, inhibition of lipid oxidation etc. (Teissedre and Landrault, 2000; Mallavadhani *et al*., 2006; Rodrigo and Bosco, 2006). Findings of the present investigation are supported by the result of other wild mushrooms investigated by different workers.

CONCLUSION

On the basis of ongoing investigation, it can be concluded that as the test mushrooms possess a good amount of different antioxidant compounds and as they exhibited significant antimicrobial activity, they may effectively be used for preparation of drugs and medicines. Not only that mushrooms got nutritional values containing good amount of carbohydrate, proteins, minerals, vitamins, fibres etc, and as because they also have some medicinal importance starting from antibacterial, antifungal and antioxidant properties as evident from present

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