



SCREENING OF *ENTOLOMA SPECULUM* FOR ANTIMICROBIAL PROPERTIES

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ABSTRACT

The study deals with the antimicrobial activity *Entoloma speculum*, a saprophytic, white fleshy fungi. The pileus varies from 5-10 cm in diameter, stalk being 2-10 cm long, gills are light pink. The antimicrobial activity of different solvent extracts of *E speculum* against *Xanthomonas campestris*, *Agrobacterium tumefaciens*, *Klebsiella pneumonia*, *Escherichia coli*, *Candida albicans*, *Penicillium chrysogenum*, *Asperillus flavous* and *Aspergillus niger* was tested in three concentration 100%, 50% and 25%. The methanol extract showed maximum activity in all the concentration, followed by chloroform. The petroleum ether showed least inhibition in 50% (9mm) and 25% (7mm). *Pseudomonas aeruginosa* (37mm), *Klebsiella pneumonia* (34mm) and *Staphylococcus aureus* (15mm) were maximum inhibited pathogens by methanol chloroform and petroleum ether extracts respectively. The *E. speculum* inhibited bacteria greater than fungi. The GCMS analysis of methanol extract showed the presence of 1, 2-Benzenediol, which may be responsible for activity of methanol extract against pathogens.

KEY WORDS: *Entoloma speculum*, antibacterial, human pathogens, *Klebsiella pneumonia*, Gas Chromatography, Mass Spectrometry



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INTRODUCTION

Mushrooms are defined as fungi which bear large, easily observed spore bearing structures found above or below ground¹. These are omnipresent and helpful in decomposing dead and decaying organic matter. Mushrooms are estimated to be 41,000 species of which 850 are reported from India². According to uses, mushrooms can be divided into four groups, edible, medicinal, poisonous and miscellaneous³. Mushrooms are believed to produce antibacterial components/compounds for their survival in nature/environment these may be isolated for welfare of humans⁴. Mushrooms in spite of being functional food they possess high medicinal value, viz, *Ganoderma*⁵. Many mushrooms contain commercially important components⁶. Mushrooms contain pigments which are used as dyes⁷ and has immunomodulatory properties⁸. In mushrooms both mycelia and fruiting bodies are potent to produce secondary metabolites which are antimicrobial in nature⁹. *Ganoderma lucidum* can inhibit the multidrug resistant *Staphylococcus aureus*¹⁰. *Pleurotus florida*¹¹ and *Phellinus gilvus*¹² are antibacterial mushrooms. *Fomitopsis pinicola* and *Lactarius vellereus* are potent to inhibit fungi¹³. *Agaricus bernardii*, *A. arvensis*, *A. bisporus*, *A. porphyrocephalus* and *A. silvicola*, exhibit antimicrobial nature¹⁴. Exploring these antimicrobial components from mushrooms is an important as infectious disease causing pathogens are showing multiple drug resistance for natural and synthetic antimicrobial agents/ medicines used so far. There is a need for such an antimicrobial agents which are broad spectrum in combating bacteria, fungi and viral pathogens of both humans and plants. The present study area is rich in variety of mushrooms and this piece of work is to find out the secondary metabolites from *E. speculum* which can prove to be good antimicrobial components.

MATERIALS AND METHODS

E. speculum was collected from Kuvempu University campus during June-Aug 2015. The taxonomy of *E. speculum* were recorded and identified (*E. speculum* was authenticated by Dr. Syed Abrar and Prof. M. Krishnappa, Mycology lab, Department of Applied Botany, Kuvempu University, Shankaraghatta).

EXTRACTION

The samples were oven dried at 45- 50°C for two hrs. Material was ground to a coarse powder using mixer. Three hundred grams of the same material was subjected to Soxhlet extraction for 24 hrs each using 1000 mL of three solvents, petroleum ether, chloroform and methanol respectively. Secondary metabolites were extracted from *E. speculum* extracts were dried to powder and kept at 4°C¹⁵.³ These extracts were screened against pathogenic fungi and bacterial species. All the three extracts were screened against the test organisms.

Test organisms

The test organisms were collected from the Microbial Type Culture Collection (MTCC), The Institute of Microbial Technology, Sector 39-4, Chandigarh, India.

Bacteria

Xanthomonas campestris [MTCC-2286], *Pseudomonas syringae* [MTCC-1604], *Agrobacterium tumefaciens* [MTCC-431], *Klebsiella pneumonia* [MTCC-7028], *Escherichia coli* [MTCC-1559], *Salmonella typhi* [MTCC-734], *Pseudomonas aeruginosa* [MTCC-1934], *Staphylococcus aureus* [MTCC-4734], *Streptomyces pneumoneae* [MTCC-4734]

Fungi

Candida albicans [MTCC-1637], *Chrysosporium merdarium* [MTCC-4608], *Trichophyton rubrum* [MTCC-3272], *Chrysosporium keratinophilum* [MTCC-1367], *Fusarium solani* [MTCC-1040], *Penicillium chrysogenum* [MTCC-947], *Asperillus flavous* [MTCC-1783], *Aspergillus niger* [MTCC-514]

Preparation of extract:

100% = 400mg in 4ml of DMSO
50% = 200mg in 4ml of DMSO
25% = 100mg in 4ml of DMSO

Agar Well diffusion method

Antibacterial and Antifungal activity of the mushroom extracts were tested using Agar well diffusion method (Sridhar et. al., 2011). The prepared culture plates were prepared by inoculating with different bacteria and fungi. Wells were made with 6mm cork borer. The wells were loaded with extracts which were dissolved in dimethyl sulfoxide (DMSO) of different concentration (100%, 50%, and 25%) using micro pipette. Ciprofloxacin for bacteria, Terbinafine for fungi were used as standard and DMSO was used as control for test microorganisms. The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity (Das et. al., 2010). The zone formation was observed in plates around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around. The reading also includes the well diameter. The readings were taken in 4 replicates and the average values were tabulated in Table 2 and 3. The result of antimicrobial activity of *E. speculum* are tabulated in table 2 and 3. The data obtained were analysed using Microsoft Excel and expressed in terms of Mean ± standard deviation (SD).

RESULTS AND DISCUSSION

TAXONOMY

Entoloma speculum (Fr.) Qué!

Mémoires de la Société d'Émulation de Montbéliard 5: 119. 1872 Mycobank: 221523 Classification: Fungi, Basidiomycota, Agaricomycotina, Agaricomycetes, Agaricomycetidae, Agaricales, Entolomataceae, *Entoloma*
Synonym: *Agaricus speculum* Fr., *Spicilegium* Pl. neglect :4. 1836. *Pileus* 20.0–80.0 mm diam, white (7A1) to pinkish white (7A2), conical or bell-shaped, sometimes flattening with age, smooth, margin involute, glabrous at the centre. *Lamellae* white (7A1) to pinkish white (7A2), attached, almost distant, emarginated, ventricose. *Stipe* 25.0–60.0×2.0–10.0 mm, white (1A1), cylindrical, fistulose, striate of silver lining along the length, fragile and easily splitting. *Basidia* 25.0–

35.5×7.4–15.7 μm, clavate, 4–sterigmate. *Cystidia* absent, clamp connections present. *Spore-print* Pink. *Basidiospores* 8.5–12.5×7.2–11.5 μm. *Habitat and distribution*: Soil. Shankaraghatta. Methanol extract yield was maximum followed by chloroform and petroleum ether. Bioactive compounds obtained in methanol are more in number, compared to other two solvents¹⁷. The result of secondary metabolites tests are tabulated in (Table 1). Methanol extract showed positive result to all test except Saponins and Triterpenoids hence it was subjected to GCMS analysis to know/find out bioactive compounds in it. The methanol fraction of *E speculum*. showed different bioactive compounds (Fig 1). 1, 2-Benzenediol, Octadecanoic acid methyl ester (CAS) methyl stearate, Ergosterol and Ergosta-7, 22-dien-3-ol, are the major compounds. Similar compounds are found from methanol extract of *Termetomyces* spp. from GCMS analysis¹⁹. The minimum inhibitory concentration values of pet ether chloroform and methanol extract varied from 6-15, 7-29 and 11-37 respectively. Petroleum ether extract showed maximum inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, showed minimum inhibition against *Chrysosporium merdarium* and *Fusarium solani*. Whereas petroleum ether extract of *E speculum* did not show any inhibition against *Chrysosporium keratinophilum* and *Penicillium crysoginum*. But *Aspergillus flavous*, *A. niger*, *Candida albicans*, *Salmonella typhi* and *Escherichia coli* were moderately inhibited (Table 2 and 3). Chloroform extract showed maximum inhibition against *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* higher than the standard drug. Chloroform extract of *E speculum* showed minimum effect against *Candida albicans*, and *Trichophyton rubrum* and other test organisms were moderately affected (Table 2 and 3). Methanolic extract exhibited a maximum activity against all the test organisms. The best was 37 mm against *Pseudomonas aeruginosa*, followed by *Klebsiella pneumonia* (36mm), *Staphylococcus aureus* (35mm), *Fusarium solani* (28mm), *Penicillium crysoginum* (27mm) and *Apergillus niger* (26mm) and the least inhibition was observed against *Trichophyton rubrum* (13mm) and all other organisms were inhibited moderately with 13-21mm. Comparatively methanol showed good result this may be due to the presence of

1,2-Benzenediol, (Chetaol: commercial name) being a phenol its antimicrobial in nature¹⁷ and used in manufacture of pesticides¹⁹. Among the solvents used, methanolic extract found to be superior to chloroform and petroleum ether extracts and found to have more potential antimicrobial properties. Methanolic extract exhibited a maximum activity against all the test organisms. The best was 37 mm against *Pseudomonas aeruginosa*, followed by *Klebsiella pneumonia* (36mm), *Staphylococcus aureus* (35mm), *Fusarium solani* (28mm), *Penicillium crysoginum* (27mm) and *Apergillus niger* (26mm) and the least inhibition was observed against *Trichophyton rubrum* (13mm) and all other organisms were inhibited moderately with 13-21mm. Comparatively methanol showed good result this may be due to the presence of 1,2-Benzenediol, (Chetaol: commercial name) being a phenol its antimicrobial in nature¹⁷ and used in manufacture of pesticides¹⁹. Among the solvents used, methanolic extract found to be superior to chloroform and petroleum ether extracts and found to have more potential antimicrobial properties. Methyl alcohol extract of *Pleurotus* sps against *C. albicans* the inhibition zone was 7.5-8.5mm⁸ but in present study the chloroform and methanol extract of *E speculum* showed higher inhibition zone range from 6-20mm. The observed result of effectiveness of extracts, methanol proved best and Petroleum ether showed less inhibition effect. This is favourable with the findings of Ehssan and Saadabi (2012). The antimicrobial activity of ethanol extract of *Rusulla delica* against *E.coli* (9mm), *S. aureus* (10mm), *P. aeruginosa* (7mm)²⁰ and but in present study the chloroform extracts of *E speculum* inhibited *E.coli* (12-19mm), *S. aureus* (21-35mm), *P. aeruginosa* (19-29mm) better than *Rusulla delica*. (Table 2 and 3). It was observed that different concentration of all the three solvents (petroleum ether, chloroform and methanol) extracts of *E speculum* posed different in inhibition level. This may be due to the concentration of mushroom extract used also play crucial role in inhibiting potential of macrofungi²¹. Generally observed the *E speculum* activity against pathogenic bacteria is high compared to fungi supporting the result of (Jonathan and Awotona) from *Lycoperdon* sps.

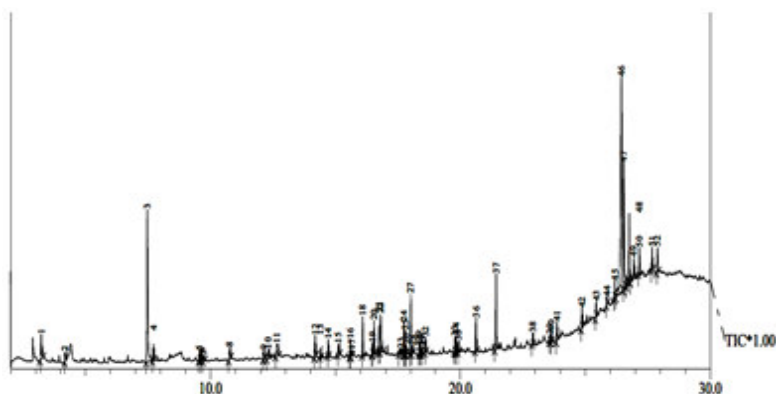


Figure 1
Chromatogram showing the compounds of methanol extract

Table 1
Secondary metabolites of *Entoloma sp. sporocarp*

Tests	Pet. Ether	chloroform	Methanol
Alkaloids	+	+	+
saponins	-	-	-
Tannins	+	+	+
Flavonoids	+	+	+
Steroids	+	-	+
Glycosides	+	+	+
Triterpenoids	-	-	-
Phenols	-	-	+

Table 2
Antibacterial activity of *Entoloma sp.* at different concentration and different solvent

Sl. No	Name of pathogen	Zone of inhibition in mm (mean±sd)									Standard
		Petroleum ether			Chloroform			Methanol			
		100 %	50%	25%	100 %	50%	25%	100 %	50%	25%	
1	<i>Xanthomonas compes tris</i>	15±1	8.66±1.5	7±1	19±1	15±1	12±1	34±1	28±1	26±1	25±1
2	<i>Pseudomonas syringae</i>	13±2	10±2	7.33±2.0 8	18±0	14.66±0.5 7	12.33±1.5 2	30±1	24.66±0.5 7	19±1	24±1
3	<i>Agrobacterium tumefaciens</i>	13±1.73	12±0	9±1	29.33±0.5 7	24±1	13.66±1.5 2	33.66±1.5 2	28.66±1.5 2	25.33±1.5 2	33.66±2.5 1
4	<i>Klebsiella pneumonia</i>	12.33±1.1 5	10.33±0.5 7	10±0	34±1	24±1	20±1	36±1	28.33±1.5 2	26±1	28±1
5	<i>Escherihia coli</i>	10.66±1.1 5	8.66±1.15	7.33±1.1 5	19.33±0.5 7	16±1	12.66±0.5 7	33.66±1.5 2	28.66±1.5 2	25.33±1.5 2	22.66±1.5 2
6	<i>Salmonella typhi</i>	11±1	11±1	8±0	25±6.08	9.66±0.7	12.66±1.1 5	29±1	17±0.7	21.33±1.5 2	25±1
7	<i>Pseudomonas arimginosa</i>	14.33±1.5 2	12±1	12±2	29.66±0.5 7	24.33±0.5 7	19±1	37±1	29.66±1.5 2	24.66±0.5 7	28±1
8	<i>Staphylococcus aureus</i>	15.33±0.5 7	13±2	7.66±0.5 7	37.66±1.5 2	30±1	21±2	35±1	30±1	23.33±2.0 8	28.33±1.5 2
9	<i>Streptomyces pneumoneae</i>	11±1.73	7.33±2.08	7.33±1.1 5	17.66±2.5 1	13±1	11.66±2.0 8	29±1	19±1	11.66±1.5 2	18±1

Mean of 4 replicates for each concentration

Table 3
Antifungal activity of *Entoloma sp.* at different concentration and different solvent

Sl. No.	Name of pathogen	Zone of inhibition (mm)									Standard
		Petroleum ether			Chloroform			Methanol			
		100 %	50%	25%	100 %	50%	25%	100 %	50%	25%	
1	<i>Candida albicans</i>	10.66±0.57	6.33±1.52	0±0	15±1	14±1	11±1	20±1	15±1	12±1	24.66±0.57
2	<i>Chrysosporium merdarium</i>	9±1	0±0	0±0	15.33±2.51	13±1	9.33±0.5 7	21±1	17.33±1.15	14±1	30±1
3	<i>Trichophyton rubrum</i>	0±0	0±0	0±0	11.33±1.52	9±1	7±1	13±1	11±1	8.33±0.5 7	24±1
4	<i>Chrysosporium keratophilum</i>	0±0	0±0	0±0	19.33±0.57	15.66±1.15	12.33±0.57	19.33±1.52	10.66±1.15	0±0	29±1
5	<i>Fusarium solani</i>	8±0	0±0	0±0	19.66±1.52	14.66±0.57	11±1	28.33±1.52	18±1	13±1	29±1
6	<i>Pinicillium crysoginum</i>	0±0	0±0	0±0	11.33±1.52	10±1	7.33±1.5 2	27±1	21±1.73	18.33±0.57	19.66±0.57
7	<i>Asperillus flavous</i>	10.33±0.57	9±1	7±1	19.66±1.52	15±1	11.33±1.52	25.33±1.52	22.33±2.08	15.33±1.52	24±1
8	<i>Apergillus niger</i>	10.33±1.15	10±1	6.66±1.15	19±1	14±1	10±1	26.66±1.52	21.66±2.08	14±1	20.33±1.52

Mean of 4 replicates for each concentration

CONCLUSION

The mushroom under studied has useful bioactive compounds which can be helpful in management of pathogenic organisms of humans. Further work is needed to identify and isolate pure compounds and test its antimicrobial activity against wide range of pathogens which may prove a precursor to development of new and strong antibiotic.

CONFLICT OF INTEREST

Conflict of interest declared none.

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