Original Research Article **pathology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

SCREENING OF *ENTOLOMA SPECULUM* FOR ANTIMICROBIAL PROPERTIES

JAYASHREE K.K. AND KRISHNAPPA M*

Department of P.G. Studies and Research in Applied Botany,Kuvempu University, Shankaraghatta-577451,Shimoga dist., Karnataka, India

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, Escherihia 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**

KRISHNAPPA M*
Department of P.G. Studies and Research in Applied Botany,Kuvempu University, Shankaraghatta-577451,Shimoga dist., Karnataka, India

***Corresponding Author**

Received on : 26-07-2016
Revised and Accepted on : 25-11-2016
DOI: http://dx.doi.org/10.22376/ijpbs.b289-293

INTRODUCTION

Mushrooms are defined as fungi which bare large, easily observed spore bearing structures found above or below ground[1]. 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 India[2]. According to uses, mushrooms can be divided into four groups, edible, medicinal, poisonous and miscellaneous[3]. Mushrooms are believed to produce antibacterial components/compounds for their survival in nature/environment these may be isolated for welfare of humans[4]. Mushrooms in spite of being functional food they possess high medicinal value, *viz, Ganoderma*[5]. Many mushrooms contain commercially important components[6]. Mushrooms contains pigments which are used as dyes[7] and has immunomodulatory properties[8]. In mushrooms both mycelia and fruiting bodies are potent to produce secondary metabolites which are antimicrobial in nature [9]. *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 fungi [13]. *Agaricus bernardii, A. arvensis, A. bisporus, A. porphyrocephalus* and *A. silvicola,* exhibit antimicrobial nature[14]. 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 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 course 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°C[15, 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], *Escherihia 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^{0}C for 24 hours for bacterial activity an48 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 2and 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.) Quél

Mémoires de la Société d'Émulation de Montbéliard 5: 119. 1872 Mycobank: 221523Classification**:** 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 solvents[17]. The result of secondary metabolites tests are tabulated in (Table 1).Methanol extract showed positive result to all test except Saponins and Triterpinoids 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 *Termetomyes* spp. from GCMS analysis[19].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 nature[17] and used in manufacture of pesticides[19]. Among the solvents used, methanolic extract found to be superior to chloroform and petroleum ether extracts and found to have more potential antimicrobial propertiesMethanolic 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 nature[17] and used in manufacture of pesticides[19]. 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 7.5-8.5mm[8] 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 macrofungi[21]. Generally observed the *E speculum* activity against pathogenic bacteria is high compared to fungi supporting the result of (Jonathan and Awotona) from *Lycoperdon* sps.

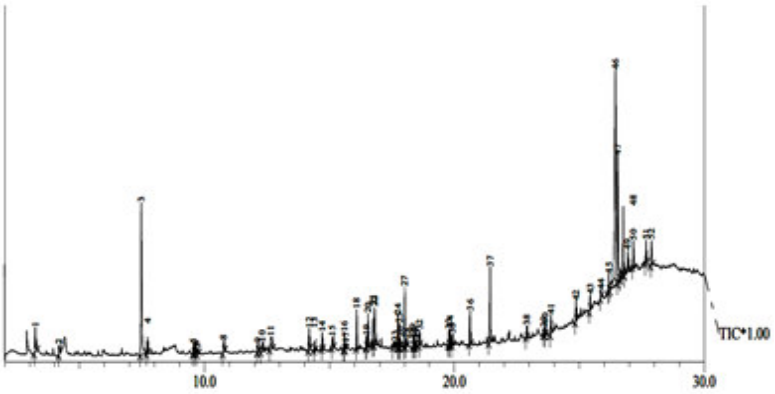

Figure 1
Chromatogram showing the compounds of methanol extract

Table 1
Secondary metabolites of Entoloma sp. sporocarp

Tests	Pet. Ether	chloroform	Methanol
Alkoloids	+	+	+
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	*Xanthomonascompestris*	15±1	8.66±1.5	7±1	19±1	15±1	12±1	34±1	28±1	26±1	25±1
2	*Pseudomonas syringae*	13±2	10±2	7.33±2.08	18±0	14.66±0.57	12.33±1.52	30±1	24.66±0.57	19±1	24±1
3	*Agrobacterium tumefaciens*	13±1.73	12±0	9±1	29.33±0.57	24±1	13.66±1.52	33.66±1.52	28.66±1.52	25.33±1.52	33.66±2.51
4	*Klebsiella pneumonia*	12.33±1.15	10.33±0.57	10±0	34±1	24±1	20±1	36±1	28.33±1.52	26±1	28±1
5	*Escherihia coli*	10.66±1.15	8.66±1.15	7.33±1.15	19.33±0.57	16±1	12.66±0.57	33.66±1.52	28.66±1.52	25.33±1.52	22.66±1.52
6	*Salmonella typhi*	11±1	11±1	8±0	25±6.08	9.66±0.7	12.66±1.15	29±1	17±0.7	21.33±1.52	25±1
7	*Pseudomonas arimginosa*	14.33±1.52	12±1	12±2	29.66±0.57	24.33±0.57	19±1	37±1	29.66±1.52	24.66±0.57	28±1
8	*Staphylococcus aureus*	15.33±0.57	13±2	7.66±0.57	37.66±1.52	30±1	21±2	35±1	30±1	23.33±2.08	28.33±1.52
9	*Streptomyces pneumoneae*	11±1.73	7.33±2.08	7.33±1.15	17.66±2.51	13±1	11.66±2.08	29±1	19±1	11.66±1.52	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	*Candida albicans*	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	*Chrysosporiummerdarium*	9±1	0±0	0±0	15.33±2.51	13±1	9.33±0.57	21±1	17.33±1.15	14±1	30±1
3	Trichophyton rubrum	0±0	0±0	0±0	11.33±1.52	9±1	7±1	13±1	11±1	8.33±0.57	24±1
4	*Chrysosporiumkeratinophilum*	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	*Fusarium solani*	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	*Pinicilliumcrysoginum*	0±0	0±0	0±0	11.33±1.52	10±1	7.33±1.52	27±1	21±1.73	18.33±0.57	19.66±0.57
7	*Asperillusflavous*	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	*Apergillusniger*	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.

Acknowledgment
Authors are thankful to The Chairman, Department of Applied Botany, Kuvempu University Shankaraghatta for providing lab facilities. We are thankful to Dr. Syed Abrar, Research Associate, DST-SERB, Department of Applied Botany, Kuvempu University for identification of the species. We are also thankful to Nandan Patel K.J., Research Scholar, Department of Applied Botany, Kuvempu University for the help and support.

CONFLICT OF INTEREST
Conflict of interest declared none.

REFERENCES

1. Mueller GM, Schmit JP, Leacock PR, Buyck B, Cifuentes J, Desjardin DE, Halling RE, Hjortstam K, Iturriaga T, Larsson KH, Lodge DJ, May TW, Minter D, Rajchenberg M, Redhead SA, Ryvarden L, Trappe JM, Watling R, Wu Q. Global diversity and distribution of macrofungi. Biodivers. Conserv, 2007; 16:37–48.
2. Manoharachary C, Sridhar K, Singh R, Adholeya A, Suryanarayanan TS, Rawat S, Johri BN, Fungal biodiversity: Distribution, conservation and prospecting of fungi from India. Curr. Sci., 2005; 89:58-71.
3. Sridhar S, Sivaprakasam E, Balakumar R, Kavitha D. Evaluation of antibacterial and antifungal activity of *Ganoderma lucidum* (Curtis) P. Karst fruit bodies extracts. World J. Sci. Technol., 2011; 1:08-11.
4. Manjunathan J, Kaviyarasan V. Solvent based effectiveness of antibacterial activity of edible Mushroom *Lentinus tuberregium* (Fr.). Int. J. Pharm Technol Res., 2010; I: 1910-12.
5. Sonawane H, Bhosle S, Garad S. Antimicrobial activity of some species of *Phellinus* and *Ganoderma* sample from Western Ghats of India. Int J. Pharma. Sci Res, 2012; 3:1795-9.
6. Jha SK, Tripati NN, Recent scenario in diversity and applied value of macrofungi: A review. Inter. J. Univ. Pharm. Life Sci., 2012; 2:102-25.
7. Velisek J, Cejpek, Pigments of higher fungi: A review. Czech J. Food Sci., 2011; 29:87-102.
8. Akyuz M, Onganer AN, Erecevit P, Kirbag S. Antimicrobial activity of edible mushroom in the Eastern and Southeast Anatolia region of Turkey, Gazi Univ. J Sci., 2010; 23:125-30.
9. Yamac M, Bilgili F. Antimicrobial activities of fruit bodies and/ or mycelial cultures of some mushroom isolates. Pharm. Biol., 2006; 44:660-7.
10. Prasad Y, Wesely WEG, Antibacterial activity of the bio multidrug (*Ganoderma lucidum*) on multidrug resistance *Staphylococcus aureus* (MRSA). Adv. Biotech., 2008; 3:9-16.
11. Bhat R, Deshpande R, Ganachari SV, Huh DS, Venkataraman A. Photo-irradiated biosynthesis of silver nanoparticles using edible mushroom *Pleurotus florida* and their antibacterial activity studies, Bioinorg. Chem. Appl., 2011; 1-7.
12. Sittiwet C, Puangpronpitag D. Antibaterial activity of *Phellinus gilvus* aqueous extract. Int. J. Pharm., 2008; 4:500-2.
13. Guler P, Akata I, Kutluer F, Antifungal activity of *Fomitopsis pinicola* (Sw.:Fr) Karst and *Lactarious vellereus* (Pers). Fr. Afr. J Biotech, 2009; 8:3811-13.
14. Ehssan MHO, Saadabi AM. Screening of antimicrobial activity of wild mushroom from Khartoum State of Sudan. Microbiol J., 2012; 2:64-9.
15. Dulger B, Gonuz A, Gucin F. Antimicrobial activity of the macrofungus *Cantharellus cibarius*. Pak J. Biol. Sci., 2004; 7:1535-39.
16. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plants products as antimicrobial Agents: Current Methods and Recent Trends. J Med. Plants Res., 2010; 4:104-11.
17. Tiwari P, Kumar B, Kour M, Kour G, Kour H. Phytochemical screening and extraction: A review, Int. Pharm. Sci., 2011; 1:98-106.
18. (https://en.wikipedia.org/wiki/Catechol#Uses).
19. Nakalembe I, Kabasa JD, Antimirobial activity and biochemical constituents of two edible and medicinal mushrooms of Mid-Western, Uganda. Res. J. Pharm., 2012; 6:4-11.
20. Yaitirak T, Aslim B, Ozturk S, Alli H. Antimicrobial and antioxidant activities of *Rusulla delica* Fr. Food Chem. Toxicol., 2010; 47:2052-6.
21. Jonathan SG, Awotona FE. Studies on antimicrobial potential of three *Ganoderma* species. Afr. J. Biomed. Res., 2010; 13:133-9
22. Jonathan SG, Fasidi IO. Antimicrobial activity of two Nigerian edible macrofungi-*Lycoperdon pusilum* (Bat. Ex) and *Lyocoperdon giganteum* (Pers.). Afr. J Biomed. Res., 2003; 6:85-90.