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EVALUATION OF ANTIMICROBIAL ACTIVITY OF MYCELIA AND SPOROCARP OF *ENTOLOMA SPECULUM*

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Keywords:

Entoloma speculum, Mycelia, Sporocarp, *Trichophyton rubrum*

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ABSTRACT: Antimicrobial activity of mycelia and Sporocarps has been adequately worked out but comparative studies of both has not done in detail. The present work is to compare the antimicrobial activity of mycelia and sporocarps of *Entoloma speculum*. It is a saprophytic, white fleshy fungi, belongs to Entolomataceae. The collected fruiting bodies were dried, powdered and subjected for secondary metabolites extraction. Mycelia was cultured by loading the spore loading the spore to potato dextrose agar plates. Mass culture of mycelia was done on patato dextrose broth (Shittu 2005) and was extracted by methanol. Both extracts were tested against pathogens, *viz*., *Xanthomonas campestris, Pseudomonas syringae, Agrobacterium tumefaciens, Klebsiella pneumonia, Escherihia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Trichophyton rubrum, Chrysosporium keratinophilum, Fusarium solani, Penicillium chrysogenum, Aspergillus niger*. Mycelial extract inhibited *T. rubrum* maximum (36 mm), Sporocarp inhibited *F. solani* at 28mm maximum, the mycelial extract inhibited pathogenic fungi and sporocarps extract inhibited bacterial pathogens.

INTRODUCTION: Mushroom word is used to define fruiting body of macrofungi. Mainly basidiomycetes but also some species of ascomycetes are sporocarp forming fungi. Wild and cultivated mushrooms contain a huge diversity of biomolecules with nutritional [1]. Mushrooms are balanced food stuff that provide definite nutrition and health benefits for human and are appreciated for their taste with pharmacological properties. Natural resources have been exploited in the last years and among them, mushrooms could be an alternative source of new antimicrobials because bioactive compounds associated with mycelia cell wall that help in boosting the immune system [2].

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Mushrooms are used as antimicrobial, anticancerous hepatoprotective antitumor, antiviral and anti-allergants [3, 4, 5, 6]. Mushrooms have good flavours and health promoting constituents with vitamins I, which are good alternatives foodstuff with balanced healthy nutrition. Sporocarp and mycelia both are able to produce secondary metabolites [7] and antioxidant Compounds [8].

Entoloma nubigenum proved potent antimicrobial mushroom [9]. *Entoloma lividoalbum* contains good antioxidant compounds and also it is antimicrobial in nature [10, 11]. Many researchers have carried out on antimicrobial activity of sporocarp. Since last two decades researchers are working on culturing the mushroom mycelium mycelium in large scale for industrially important important components. Therefore the present work deals with comparison the present work deals with comparison of sporocarp and mycelia of *Entoloma speculum* screened against the pathogenic microorganisms.

MATERIALS AND METHODS: *Entoloma speculum* was collected from the Kuvempu University campus during June - Aug 2015. The major field characters were recorded. The sporocarps were brought to laboratory and morphological characters were recorded. The samples were oven dried at 45 - 50 °C for 4 hrs Material was ground to a course powder using mixer. Three hundred grams of the same material was subjected to Soxhlet extraction using 1000 ml of methanol. The extract obtained from *E. speculum* were dried and kept at for further use.

Mycelial Culture and Extraction: The fresh *E. speculum* was collected from field, washed with distilled water, kept for spore print under aseptic condition. Petri plates containing subourdous dextrose agar medium was inoculated with spore spore collected from spore print and inoculated for 7 days at ambient temperature. Several times the procedure is repeated to get pure culture thus obtained mycelia was used for further culture [12]. From the pure culture, plugs of agar medium (6mm diameter) covered with mycelia, were inoculated to 250 ml conical flask containing 100 ml potato dextrose broth of pH - 5 [13], and kept in rotatory shaker at 120 rpm, at 25 ^{o}C for 10 days [14]. Culture filtrate was filtered by whatman paper no 1, and dried at 80 ^{o}C for 30 mins.

Mycelia was weighed before and after drying. Dried mycelia powered 100 gm. was extracted with 150 ml of methanol at 150 rpm and filtered in whatman no1. The residue was then extracted twice with another 150 ml methanol. The total extract was then evaporated to dryness [15]. These extracts were screened against pathogenic fungi and bacterial species.

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-902], *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], *Aspergillus flavus* [MTCC-1783], *Aspergillus niger* [MTCC-514].

Agar Well Diffusion Method: Antibacterial and Antifungal activity of the mushroom extracts were tested using Agar well diffusion method [16]. The test pathogens were maintained on separate nutrient plates. 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.

Amoxycillin for bacteria, Terbinafine for fungi were used as standard and DMSO was used as control for test microorganisms. The plates were incubated at 27±2 °C for 24 h for bacteria and 4 days for fungal activity. 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, data obtained were analysed using Microsoft Excel software and expressed in terms of Mean ± standard deviation (SD).

RESULTS AND DISCUSSION: Phytochemical tests of sporocarp and mycelial extracts both showed the presence of phenols, steroids, terpenoids and alkaloids in qualitative chemical screening. Methanol extracts of mushroom and mycelia showed maximum inhibition of test organism. In the present study the antimicrobial activity of mycelia and sporocarps of *E. speculum* is studied, fruiting bodies and mycelia showed significant activity against test organisms. Mycelia inhibited *T. rubrum* maximum (36mm), mushroom inhibited *F. solani* (28mm) maximum, whereas mushroom extract least inhibited *T. rubrum* (13mm). *C. keratinophilum* and *P. chrysogenum* were inhibited by mycelial extract equaly with (29mm) but *P. chrysogenum* was inhibited with 27mm by mushroom extract. *F. solani* and *A. niger* was inhibited with 30mm inhibition zone by mycelial extract. *A. tumefaciens* inhibited by mycelia extract with 32mm zone.

Mushroom extract showed 26mm inhibition zone against *A niger*. 25% concentration of mushroom

extract did not show any activity on *C. keratinophilum*, but all the three concentration (100% 50% 25%) of mycelia showed inhibition on *C. keratinophilum* range from 29 - 24 mm. whereas *P. chrysogenum* was inhibited by mycelia and mushroom extract both. *C. albicans* was significantly inhibited by mycelium but moderately inhibited by mushroom.

TABLE 1: ANTIFUNGAL ACTIVITY OF MYCELIAL AND SPOROCARP EXTRACT OF *ENTOLOMA SPECULUM* IN DIFFERENT CONCENTRATION

Organisms	Mycelial extract in different concentration in mm			Mushroom extract in different concentration in mm			Standard Terbinafine
	100 % conc	50 % conc	25 % conc	100 % conc	50 % conc	25 % conc	
Ca	31±1	28.33±1.52	25.66±1.15	20±1	15±1	12±1	24.66±0.57
Cm	34±2	29±1	25±1	21±1	17.33±1.15	14±1	30±1
Tr	36.66±1.52	31±3.6	26.33±1.52	13±1	11±1	8.33±0.57	24±1
Ck	29.33±1.15	27±1	24.66±1.52	19.33±1.52	10.66±1.15	0±0	29±1
Fs	30.66±1.15	27±1.73	21.33±1.52	28.33±1.52	18±1	13±1	29±1
Pc	29.33±1.52	24.66±0.57	21.33±1.52	27±1	21±1.73	18.33±0.57	19.66±0.57
Af	32±2	27.66±0.57	25.33±1.52	25.33±1.52	22.33±2.08	15.33±1.52	24±1
An	30.66±1.15	27.33±1.15	23.66±1.52	26.66±1.52	21.66±2.08	14±1	20.33±1.52

Candida albicans=Ca, Chrysosporium merdarium=Cm, Trichophyton rubrum=Tr, Chrysosporium keratinophilum=Ck, Fusarium solani=Fs, Penicillium chrysogenum=Pc, Aspergillus flavus=Af and *Aspergillus niger=An*

Pathogenic bacteria were inhibited by Sporocarp extract than the mycelia. Sporocarp suppressed the growth of all bacterial pathogens. *X campestris* was susceptible to mycelial extract greater than the standard amoxycilin. *E. coli* inhibited with 28 mm zone while *K. pneumonia* and *P. aeruginosa* inhibited with 26 mm zone. *S aureus* was least inhibited with 19 mm. *S typhi* showed 23 mm inhibition by mycelia extract but mushroom extracts inhibited *S. typhi* by 29 mm greater than standard (25 mm). *P. aeruginosa* showed 37 mm zone highest of all the other organisms. Mushroom extract inhibited *X. campestris* equal to standard (33.66), but *A. tumefaciens* inhibition was equal to standard: **Table 2**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF MYCELIAL AND SPOROCARP EXTRACT OF *ENTOLOMA SPECULUM* IN DIFFERENT CONCENTRATION

Organisms	Mycelial extract in different concentration in mm			Mushroom extract in different concentration in mm			Standard Amoxycilin
	100% conc	50 % conc	25 % conc	100% conc	50 % conc	25 % conc	
Kp	26.66±1.52	20.66±5.13	17.66±6.8	36±1	28.33±1.52	26±1	28±1
St	23.33±2.88	21.66±3.21	19.33±3.05	29±1	17±0.7	21.33±1.52	25±1
Sa	19.66±1.52	16.33±1.52	10.66±1.15	35±1	30±1	23.33±2.08	28.33±1.52
Pa	26.33±1.15	15±2	10.33±0.57	37±1	29.66±1.52	24.66±0.57	28±1
At	33.33±1.15	30.33±0.57	26±1	33.66±1.52	28.66±1.52	25.33±1.52	33.66±2.51
Ps	21±3.6	18.66±4.04	16.33±4.5	30±1	24.66±0.57	19±1	24±1
Xc	31.33±1.15	28.33±2.88	24.66±4.16	34±1	28±1	26±1	25±1
Ec	28.66±1.52	21.66±2.08	13.66±1.15	33.66±1.52	28.66±1.52	25.33±1.52	22.66±1.52

Klebsiella pneumonia = Kp, *Salmonella typhi=St, Staphylococcus aureus =Sa, Pseudomonas aeruginosa= Pa, Agrobacterium tumefaciens=At, Pseudomonas syringae=Ps, Xanthomonas campestris=Xc* and *Escherihia coli*=Ec

Mushroom inhibited bacteria greater than fungi, this is in agreement with the result of Jonathan [18]. Mycelia inhibited fungi greater than bacteria, this is in favourable with Osman and Kalyoncu [15, 19]. Pranita [20], investigated the antimicrobial activity of *Trametes versicolar*, against *P. aeruginosa is* 20mm inhibition zone, *S. aureus* 20 mm *Peniillium sp.* 12 mm *A. flavus* 13 m, *A. niger* 14 mm, *E. coli* 18mm, in present study the methanol extract of *E. speculum* inhibited *A flavus at* 32 mm, *A. niger* 30 mm, *S. aureus* 19 mm, *P aeruginosa* 26 mm, higher than *Trametes versiolar*, Omar [21] revealed that mycelia of *Lentinus squarrosulus* possess the antioxidant activity and antitumor potential. Cho [22] investigated hypoglycaemic of exopolysaccharides from mycelial culture of *Tremella fuciformis* and *Phellinus boumni* found excellent activity on mice.

Hatvani [23] investigated the antibacterial effect of culture fluid of *Lentinus edodes* mycelium showed poor activity against *S. aureus* and *S. pyogens* and *Candida albicans E. coli* and *K. pneumonia*, but in present study mycelium extract inhibited *E. coli*, *K. pneumonia*, *C. Albicans* and *S. aureus* significantly. Manjunathan [24] worked on the antimicrobial activity of *Lentinus tuberregium* mycelium extracts on *S aureus* (9 mm) *P aeruginosa* (8 mm) *E. coli* (11 mm) *S. typhi* (12 mm) *C. albicans* (10 mm). Rai [25] investigated the antimicrobial effect of the extract of *Entoloma lividoalbum* fruiting body against *C. albicans* which exhibited complete resistant, and *E. coli* showed 10 mm and *P. aeruginosa* 8mm inhibition zone, but *E. speculum* fruiting body and mycelia both inhibited pathogens significantly **Table 1** and **2**. Ishikawa [26] studies on antimicrobial activity of *Lentinula edodes* mycelium extract *K. pneumonia* showed 4 mm and *P. aeruginosa* 0 mm and *S. aureus* 13 mm inhibition zone in disc diffusion method.

In the present investigation the maximum antifungal activity is showed by mycelia and maximum antibacterial activity shown by sporocarp.

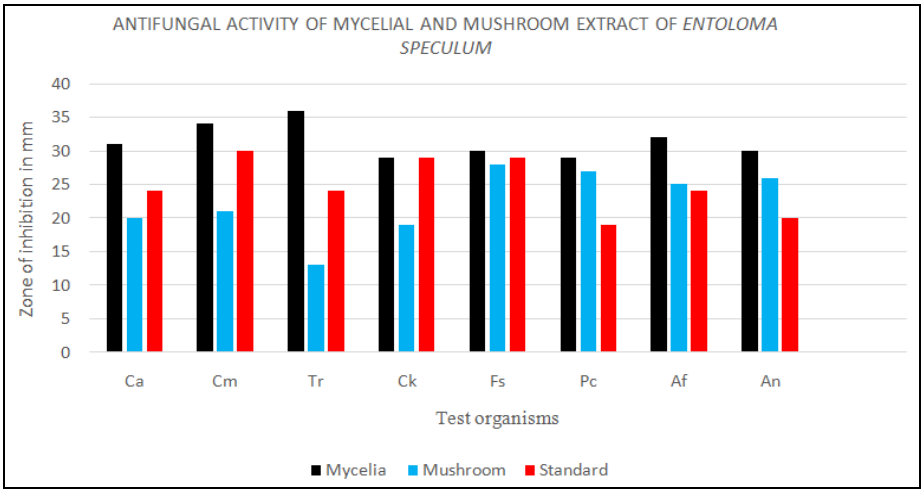

FIG. 1: ANTIFUNGAL ACTIVITY OF MYCELIA AND MUSHROOM EXTRACT OF *ENTOLOMA SPECULUM*

Candida albicans=Ca, Chrysosporium merdarium=Cm, Trichophyton rubrum=Tr, Chrysosporium keratinophilum=Ck, Fusarium solani=Fs, Penicillium chrysogenum=Pc, Aspergillus flavus=Af, Aspergillus niger=An

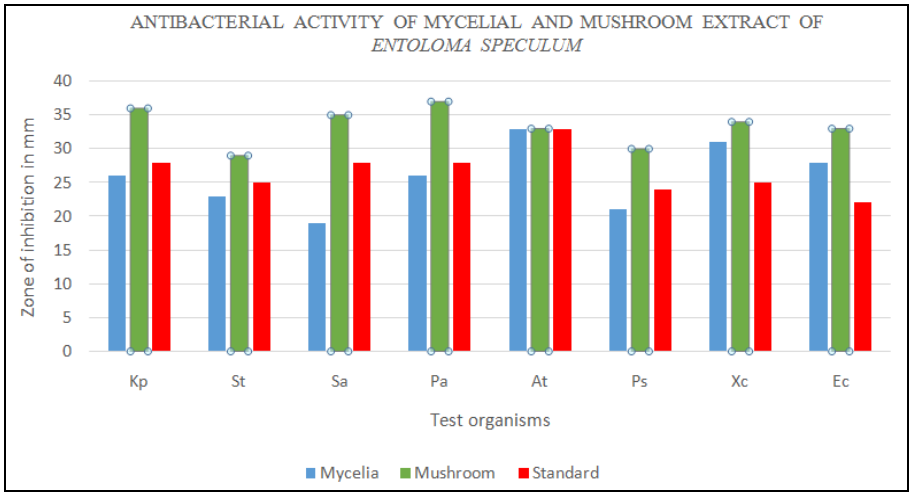

FIG. 2: ANTIBACTERIAL ACTIVITY OF MYCELIA AND MUSHROOM EXTRACT OF *ENTOLOMA SPECULUM*

Klebsiella pneumonia = Kp, *Salmonella typhi=St, Staphylococcus aureus=Sa, Pseudomonas aeruginosa=Pa, Agrobacterium tumefaciens=At, Pseudomonas syringae=Ps, Xanthomonas campestris=Xc and Escherihia coli*=Ec

CONCLUSION: Mushroom are seasonal and limited. As they are rich in metabolites we can get them by culturing their mycelia in large scale and extract the metabolites from mycelia and also By culture filtrate without disturbing the environment by harvesting mushroom in large scale from nature.

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CONFLICT OF INTEREST: Nil

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