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Exopolysacharides From White Rot Fungi And Their Antibacterial Studies

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ABSTRACT

In current investigation exopolysacharides are produced from the *Trametes* sp. and antibacterial activity of exopolysaccharides (EPS) was studied. The antibacterial effect of EPS was observed that they were most effective against gram-positive bacteria, especially *B. subtilis* and *S.aureaus* with a zone of inhibition 24 mm and 28 mm, respectively, at a concentration of 80 mg/ml. Moreover, with the increasing concentration, the EPS showed significant increase in antibacterial activity. The activity was lowest in the inhibition of gram-negative bacterium, *E. coli* and *P. aerogenosa* at a low concentration whose inhibition zones are between 5 to 15 mm.

INTRODUCTION

During the past several decades, much interest has been generated in extracellular polysaccharides (EPS) produced by submerged cultures of numerous mushrooms because they have biological and pharmacological activities including anti-tumor, antioxidant, hypoglycemic activities, etc. (Han et al., 2006; Li et al., 2010; Song et al., 2008; Zhao et al., 2012). Polysaccharides include a large and diverse group of substances that play an important role in the structure and function of fungal cell walls, which is the main polysaccharide source. However, it should be mentioned that, depending on the culture conditions, some fungal species also effectively produce fractions of extracellular polysaccharides (Monika et al., 2014). The fungal exopolysaccharides represent a wide range of chemical structures and properties, are rich in high molecular weight polysaccharides and mostly have heteropolymeric composition. Owing to this, the fungal exopolysaccharides have found multifarious applications in the food, pharmaceutical and other industries (Kanchan Lata et al., 2012) The extracellular polysaccharide production by microbes was first reported in 1861 as a “viscous fermentation” by Pasteur. The organism that produces this polysaccharide was a bacterium identified as *Leuconostoc mesenteroides* by Van Tieghem (1878). Among the three major classes of microbial polysaccharides, exopolysaccharides (EPSs) had several advantages over intracellular and cell wall polysaccharides including huge production in short time, easy isolation, and purification. EPSs of microbial origin might represent a valid alternative to plant and algal products considering that their properties are almost identical to those currently used gums (Sutherland 1996; Mahapatra and Banerjee 2013) A number of polysaccharides or exopolysaccharides (EPS) from the fruiting body or the culture filtrate of mushrooms, such as *Ganoderma applanatum, Cordyceps* sp, *Lentinus edodes* and *Grifola frondosa* have been reported with some potential pharmaceutical applications. Some kinds of mushroom polysaccharides such as lentinan, schizophyllan, krestin and grifron-D have now commercial applications. Mushrooms in submerged culture are used to produce EPS gradually substitute for solid culture in recent years. It takes several months for the solid culture mushrooms to grow into the fruiting bodies on solid substrates. Submerged culture gave rise to many potential advantages of higher mycelial biomass or EPS production in a compact space and shorter time with less chances of contamination. In fact food manufactures have directly employed EPS of mushrooms by fermentation to prepare drinks and capsules for sale (Mahendran et al., 2013). The main purpose of this study is to produce EPS by *Trametes* sp and to study their antibacterial activity.

MATERIALS AND METHODS

Microorganism and media

Fruit body of *Trametes* sp was collected from Eturnagaram forest, Warangal, Telangana, India (18020' 20" N, 800 25' 45" E). A small piece of fruiting body was dipped in 0.01% mercuric chloride to remove surface contamination and washed several times with distilled water to remove the traces of mercuric chloride and transferred aseptically on to 3% malt extract agar slants and was incubated for 5– 7 days. The mycelium collected from the growing edge of those slants was transferred on to newmalt extract agar slants and incubated further 5–7 days to obtain pure culture. Pure cultures were subcultured on malt extract agar slants and plates every fortnight (Krishna et al., 2015). Based upon macroscopic features namely, size, shape, sporocarp nature, color, spore print, margin of pileus, characters of lamella, spacing of gills, characters of the stipe, and the presence or absence of veils on stipes, the fungi were identified.

Medium for exopolysaccahrides production(g/l)

Common production medium, was used (Peptone 1.0; yeast extract 2.0; K_2HPO_4 1.0; $MgSO_4.7H_2O$ 0.2; $(NH_4)2SO_4$ 5.0; glucose 39.0; pH 6.0). This medium was selected in preliminary studies as adequate for exopolysaccharide production by Basidiomycetes (Cavazzoni and Adami 1992).

Exopolysacharides production

Erlenmeyer flasks containing 100 ml of sterilized culture medium were inoculated with two 8 mm discs of fungal mycelium and kept for incubation. After seven days of incubation the mycelia biomass was separated from the liquid medium by centrifugation (4000 rpm, 15 min) and the supernatant was filtered through a Whatman filter paper No.1. The supernatant was collected and Isopropanol was added to the culture filtrate (1:1 v/v) and after 24 h at 4°C the precipitated biopolymer was separated by centrifugation (8,000 rpm for 10 minutes) the precipitate was collected as the crude EPS fraction.

Bacterial Cultures

Staphylococcus aureus, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the, IMTECH, Chandigargh, India culture collection centre.

Estimation of Carbohydrate and Protein Content of Crude

EPS: The total carbohydrate content was estimated by phenol sulphuric acid method proposed by Dubois et al.,(1956). The amount of protein present in Trametes sp extract for both EPS basal medium and malt medium was estimated by the Lowry's method (1951).

FTIR spectroscopy analysis of EPS

The EPS was characterized by using a Fourier Transform Infrared Spectrometer (PerkinElmer Spectrum Version 10.03.06). The dried EPS was ground with KBr powder and passed into pellet for FTIRtroscopy between frequency range 5 to 4000 cm-1 with resolution 4 cm-1 and 2 scans.

FTIR spectroscopy between frequency range 5 to 4000 cm^{-1} with resolution 4 cm-1 and 2 scans.

Anti bacterial activity of exopolysacharides

Exopolysaccahrides produced by *Trametes* sp were tested for antimicrobial activity. This involved using 2 Gram-positive and 2 Gram-negative bacteria by the agar well-diffusion method. Approximately 25 ml of nutrient agar medium was poured into sterilized petri dishes. The bacterial test organisms were grown in nutrient broth for 24 h. A 100 µl nutrient broth culture of each bacterial organism (1×10^5 CFU/ml) was used to prepare bacterial lawns. Agar wells of 8 mm diameter were prepared with the help of a sterilized stainless steel cork borer. The wells were loaded with various concentrations of exopolysacharides crude extract ie., 20 mg/ml, to 80 mg/ml and 60 µl of streptomycin (10 mg in 100 ml of distilled water). The plates were incubated at 37 °C for 24 h and then were examined for the presence of zones of inhibition. The diameter of such zones of inhibition was measured and the mean value for each organism was recorded and expressed in millimeters.

RESULTS AND DISCUSSION

Fungal species belonging to genus *Trametes* sp are known for their ability to produce a number of substances with promising biomedical properties. The fruiting bodies of *Trametes* sp are very often used in traditional Chinese medicinal therapies. Here carbohydrate and protein estimation was done for the *Trametes* sp crude EPS and the optical density for carbohydrate was 0.81+ 0.10mg/100ml respectively. Protein content was about 0.11+ 0.007mg/100ml.Functional group was detected by using FTIR spectrum from crude EPS. The IR range of polymer has actually shown a broad extreme band at 4000 cm-500 cm-1 with resolution 4 cm^{-1}. The spectrum displayed in Figure. 1, sharp band at 3437.89 cm^{-1} highly recommends a visibility of extensive stretching (O-H, carboxylic acid and H- bound) teams. The optimal at 1038.92 cm-1 corresponds to extending of (C-O, alcohol, ether as well as phenol) teams. Polysaccharides possessing carboxyl team is reported formerly Jindal et al., 2013. The IR spectrum of polymer showed the presence of carboxyl group which may act as a binding site for divalent cations. This carboxyl team might additionally function as a useful moiety to generate a cutting-edge new or changed polymer, by using various novel methods like synthetic polymers. The crude concentrated exopolysaccharide filtrate of mushrooms under study showed a wide range of antibacterial in vitro activity showed in (Table 1). Among all microorganisms tested the exopolysaccharides was found to be most effective against gram-positive bacterium, especially *B. subtilis* and *S.aureaus* with a zone of inhibition 24 mm and 28 mm, respectively, at a concentration of 80 mg/ml. Moreover, with the increasing concentration, the EPS showed significant antibacterial activity against all selected microorganisms by a dose-dependent manner. The activity was lowest in the inhibition of gram-negative bacterium, *E. coli* and *P. aerogenosa* at a low concentration whose inhibition zones are between 5 to 15 mm (Figure 2). The antimicrobial activity of polysaccharides depends on a variety of factors, including its type (e.g., plain or derivative), molecular weight, composition and even the chelating activities (Rabea et al., 2003). Different theories have been put forward to explain polysaccharides antimicrobial mode of action, especially molecular weight. The molecules of chitosan larger than 10 kDa were responsible for its antimicrobial activity (Raafat et al., 2008). Sadik and Barakat (2014) reported that the filtrate of *Pleurotus ostreatus* is very effective against *E. coli* and *S.aureus*. Ishikawa et al (2001), showed that the mycelial culture filtrate of Lentinula edodes inhibited the growth of *B. subtilis*. According to Demir (2008) exopolysaccharides of *Cerrena unicolor*, *Ganoderma carnosum*, *Lenzites betulina* and *Polyporus arcularius* showed activity against some microorganisms. The activities of *Ganoderma carnosum* exopolysaccharides were higher than positive control (vancomycine or fluconazole) against *Micrococcus luteus*, *Enterococcus faecium* and *Candida albicans*. For instance, Suay et al. (2000) reported that two different *C. unicolor* strains showed the highest activities against *B. subtilis*. However, basidiomycetes may be a source of new and useful bioactive compounds.

CONCLUSION

In the research described here, we determined the exopolysacharides production from the *Trametes* sp. and antibacterial activity of exopolysaccharides (EPS) was studied. Furthermore, the result indicated that the exopolysaccharides produced by *Trametes* sp. showed antibacterial activity against gram-positive bacterium, especially *B. subtilis* and *S.aureaus*. This could provide more data for application of Trametes sp. as a biological agent which is useful in industries

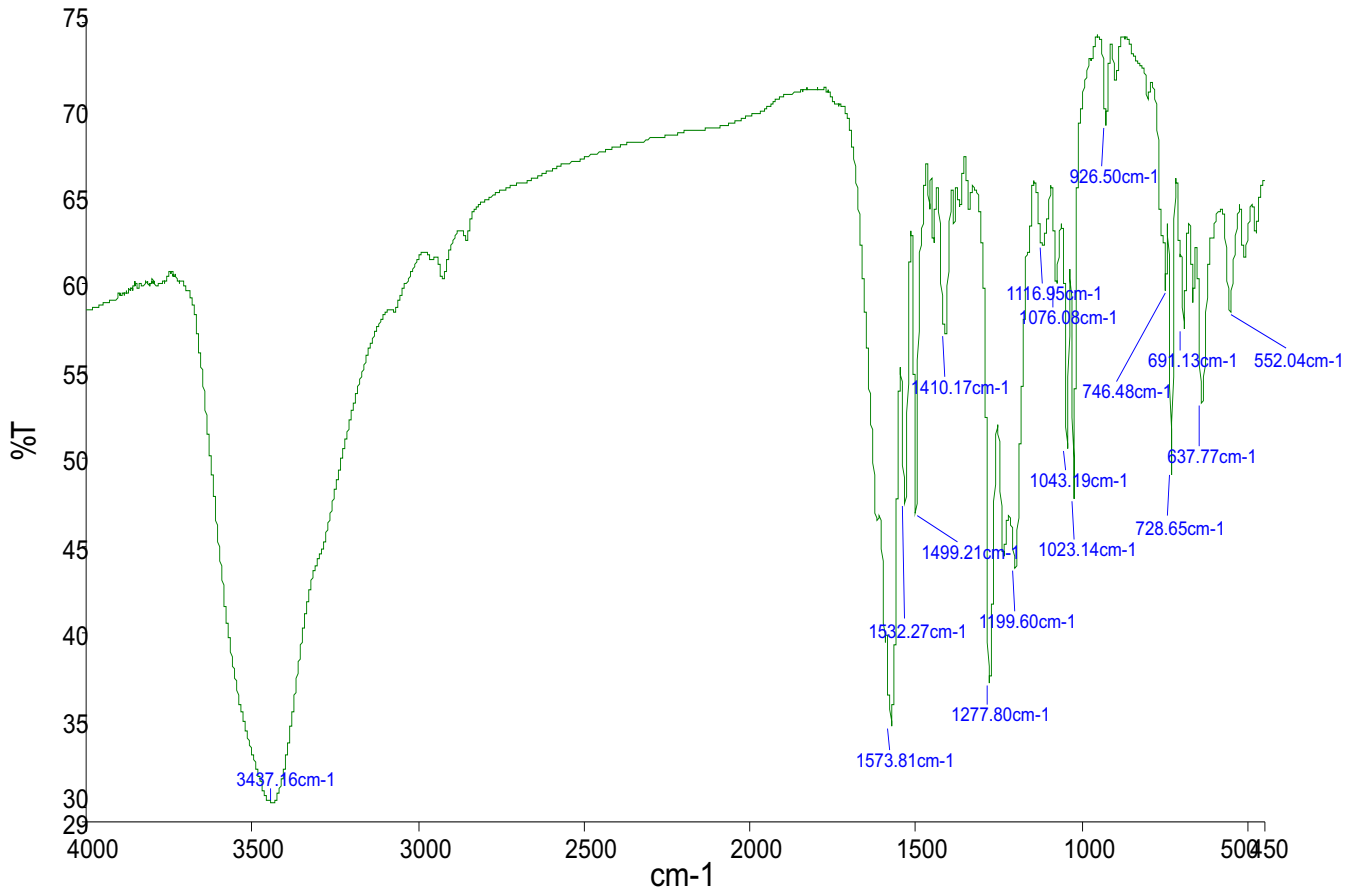

Figure 1- Fourier Transform Infrared Spectrometer of EPS produced by strain *Trametes* sp.

Table: 1: Antibacterial activity of exopolysacharides against bacterial pathogens

Organisms	**Concentrations** (mg/ml)							
	20 mg/mL	30 mg/mL	40 mg/mL	50 mg/mL	60 mg/mL	70 mg/mL	80 mg/mL	Std
Escherichia coli	05	08	09	09	10	11	16	30
Staphylococcus aureus	10	12	14	14	16	14	20	29
Bacillus subtilis	12	16	19	20	23	24	28	32
Pseudomonas aeruginosa	12	16	20	22	22	25	28	30

Std: Standard Streptomycin

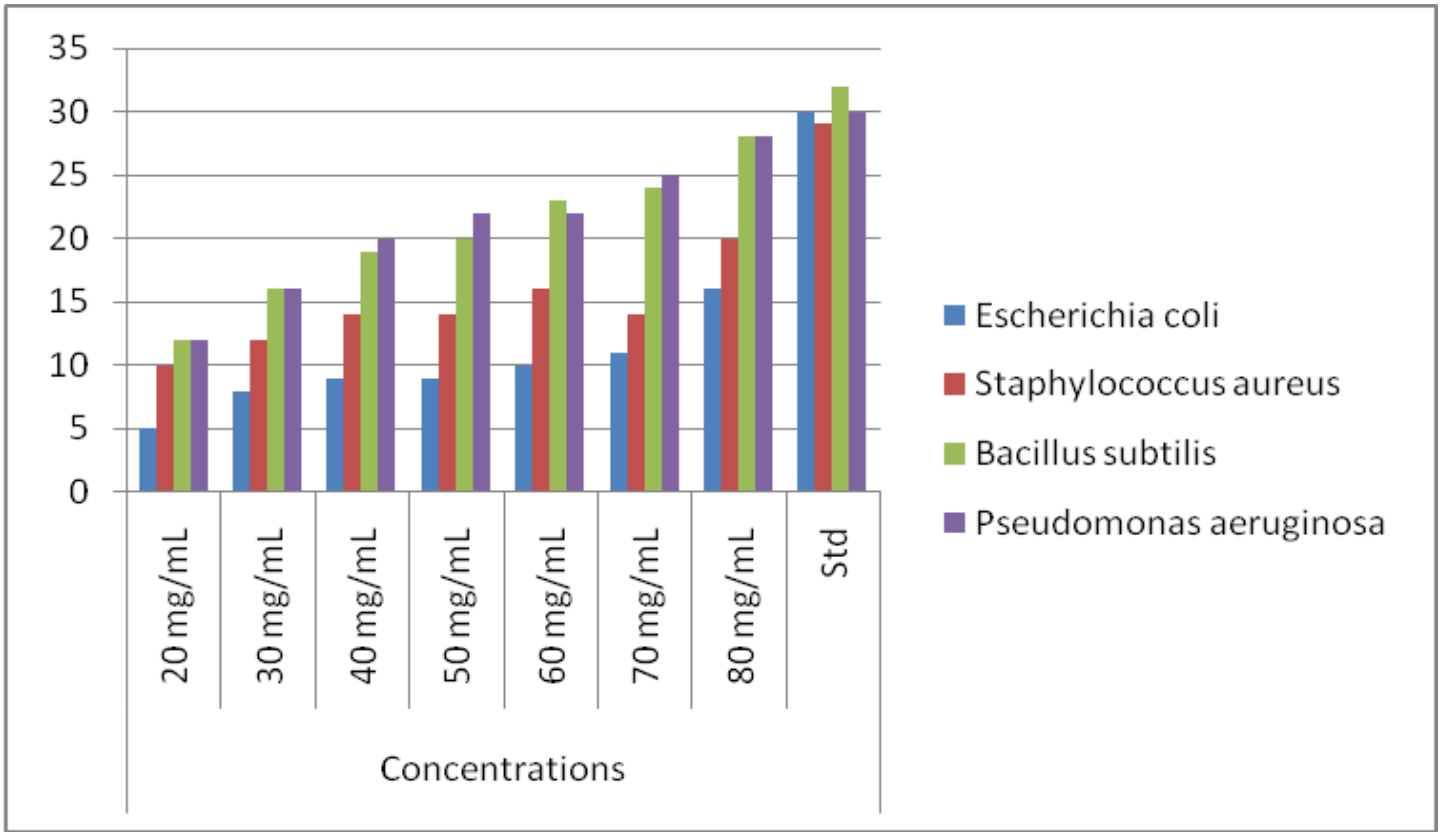

Figure: 2 Graphical representations of exopolysacharides activity against bacterial pathogens

Disclosure statement

No potential conflict of interest was reported by the author.

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