International Journal of Microbiological Research 8 (2): 48-58, 2017
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2017.48.58

Biological Activities of Exopolysaccharide from *Fomitopsis feei*

S.V.S.S.S.L. Hima Bindu N. and M.A. Singara Charya

Department of Microbiology, Kakatiya University, Warangal – 506 009, Telangana State, India

Abstract: Different biological activities such as antibacterial, antifungal, antioxidant and 5-lipoxygenase, alpha glucosidase and tyrosinase enzyme inhibitory activities were studied using exopolysaccharide of *Fomitopsis feei*. Exopolysaccharide was found to be most effective against gram-negative bacteria, especially *Proteus vulgaris* and *Pseudomonas aeruginosa* among the 12 bacteria tested. Antifungal activity was tested against four fungi by spore germination inhibition method resulting above 85 percentage of inhibition of all except *A. fumigatus.*5-lipoxygenase, glucosidase and tyrosinase enzyme inhibitory activities with exopolysaccharide showed less effective compared to standards but were not negligible.

Key words: Fomitopsis feei · Antibacterial · Antifungal · Antioxidant · Tyrosinase inhibitory · Lipoxygenase inhibitory · Alpha glucosidase inhibitory · Biological activities

INTRODUCTION

Many natural polysaccharides participate in a variety of *in vivo* biochemical reactions. However, it is quite difficult to elucidate the mechanism of their biological activity because of the complex geometry and chemical structure of natural saccharide chains, as well as due to impurities difficult to be removed. In order to understand better the relationship between the biological activity and the chemical structure of saccharide chains, chemical synthesis of polysaccharides has been attempted [1]. There has been an intensified effort in recent years in identifying the biological functions of polysaccharides as related to potential biomedical applications. Similar types of polysaccharides have also been shown to have dissimilar biological activities.

Polysaccharides from medicinal mushrooms shows different biological activities including antitumor, immunomodulation, antioxidant, radical scavenging, cardiovascular, antihypercholesterolemia, antiviral, antibacterial, antiparasitic, antifungal, detoxification, hepatoprotective and antidiabetic effects. Wide varieties of applications of â-glucan have been reported, including thickening and stabilizing agents in chemical industries and immuno-stimulating and antitumor agents in clinical uses [2-4]. Apart from these applications, beta glucan has been used as a substance that enhances the skin's natural ability to heal and protect itself against infection [5]. Recently published research study [6] predicted that the future of biomass-based polymers is bright, with polysaccharides and polysaccharide-derived polymers being in the forefront.

Different biological applications such as antibacterial, antifungal, antioxidant and enzyme inhibitory activities of exopolysaccharides from *Fomitopsis feei* were studied.

MATERIALS AND METHODS

Isolation of Exopolysaccharide: The wood decaying fungus *F. feei* was collected from the wood logs at Pakhal forest, Warangal district, Telangana State, India, during rainy season and pure mycelial culture was developed and was identified at molecular level by 28S rDNA analysis [7]. Exopolysaccharide was produced under still condition after 14 days of incubation at 28°C after the inoculation of production broth medium of 25 mL with single 6 mm disc of *Fomitopsis feei*. After filtration using whatman No. 1 filter paper and centrifugation of culture broth, filtrate was precipitated with isopropyl alcohol in 1:4 ratio and incubated overnight at 4°C. After centrifugation the pellet was concentrated and dried and used for the biological activities.

Antibacterial Activity: Bacteria causing infectious diseases to both animals and humans were tested for antibacterial activity. Antibacterial activity was done by

Corresponding Author: S.V.S.S.S.L. Hima Bindu N., Department of Microbiology, Kakatiya University, Warangal – 506 009, Telangana State, India.

agar well method. Bacterial cultures viz., *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Micrococcus luteus* (KUCCC 4), *Bacillus cereus* (KUCCC 7), *Bacillus megaterium* (KUCCC 9), *Escherichia coli* (MTCC 443), *Enterobacter aerogens* (KUCCC 12), *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhimurium* (MTCC 98), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 424) and *Salmonella paratyphi* (KUCCC 15) were obtained from the IMTECH, Chandigarh and Kakatiya University Culture Collection Centre (KUCCC), Warangal and were maintained on nutrient agar and were inoculated in nutrient broth tubes and incubated overnight. Inocula of these cultures were standardized using 0.5 Mc Farland solution [8] to get the concentration of $1.5x10^8$ bacteria/mL. Nutrient agar plates were spread with 100 µL of each inoculum by using cotton swab and 100 µL of polysaccharide samples in different concentrations (50, 100, 150 mg/mL) were added to 6 mm wells, which were made by using sterile borer. All plates were incubated at 37°C for 18 hrs. and zone of inhibition was noted.

Antifungal Activity: Plant pathogenic fungal strains were used for the antifungal activity. Spore germination inhibition method [9] was followed for antifungal activity against *Aspergillus fumigatus* (KUCCC 24), *Aspergillus tereus* (KUCCC 25), *Curvularia lunata* (KUCCC 27) and *Drechslera sp*. (KUCCC 29) which were obtained from Kakatiya University Culture Collection Centre (KUCCC), Warangal and were maintained on potato dextrose agar slants. One mL of conidial suspension made in distilled water of each type of pathogenic fungal culture was placed on a slide and mixed thoroughly with 1 mL of polysaccharide sample. Slides were placed in a moist chamber at 25°C and were observed under microscope after 24 hrs. of incubation and number of germinating spores were recorded. Control was prepared using distilled water instead of polysaccharide sample. Percentage of germination inhibition was calculated using the following formula:

$$\text{Percentage of germination inhibition} = 100\frac{\text{No. of germinated spores in treated}}{\text{No. of germinated spores in control}} \times 100$$

Antioxidant Activity: Antioxidant activity was performed [10] by mixing 1 mL of 0.5 mM 2, 2 – diphenyl – 1 – picrylhydrazyl (DPPH) radical solution in methanol with 3 mL of polysaccharide sample in different concentrations ranging from 10 mg/mL to 80 mg/mL along with positive control, ascorbic acid. After incubation for 30 min. in the dark, absorbance was read at 517 nm. The control contained 1 mL of DPPH solution mixed with 3 mL methanol. DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{A0 - A1}}{\text{A0}} \times 100$$

where,
A0 = The absorbance of control
A1 = The absorbance of sample

Tyrosinase Inhibitory Activity: Tyrosinase inhibition assay was carried out by following the modified method [11] using an assay mixture contained 250µL of tyrosinase enzyme, 50µL of exopolysacharide solution and 1250 µL of 8 mM L-Dopa. The reaction was started by the addition of substrate L - Dopa. Then incubated for 1 min. and the activity is measured at 475 nm. Assay was performed in triplicate and given the result in mean values.

$$\text{Percentage of inhibition} = \frac{\text{Control O. D. } - \text{ Test O. D.}}{\text{Control O.D.}} \times 100$$

where,
O. D. = Optical Density

Alpha Glucosidase Inhibitory Activity: It is carried out by following the modified method of [12]. 50 µL of α – glucosidase enzyme (0.4 U/mL) was added to 90 µL of 100 mM phosphate buffer pH 7.0 and 10 µL of exopolysaccharide solution in microtitre plate and incubated at room temperature for 5 min. and added 50 µL of p-nitrophenyl α – D – glucopyranose (20 mM) as substrate, mixed well and incubated for 15 min. at room temperature. The reaction was stopped by the addition of 30 µL of sodium carbonate solution (200 mM). The absorbance was measured at 405 nm using microplate reader. Control and test blank optical density were obtained by replacing the enzyme with buffer and the test was done in triplicate.

$$\text{Percentage of inhibition} = \frac{\text{Control O. D. } - \text{ Test O. D.}}{\text{Control O.D.}} \times 100$$

where,
O.D. = Optical Density

5-Lipoxygenase Inhibitory Activity: 5-lipoxygenase inhibitory activity was carried out by following the modified method [13]. The assay mixture contained 80 mM linoleic acid and sufficient amount of potao 5 –

lipoxygenase enzyme in 50 mM phosophate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mix to substrate (linoleic acid) and the activity was monitored as an increase in absorbance at 234 nm. The reaction was monitored for 120 sec. using UV kinetic mode on Varian Cary – 50 UV – Vis spectrophotometer. In the inhibition studies, the activities were measured by incubating various concentrations of exopolysaccharide solution with enzyme buffer mix for 2 minutes before the addition of the substrate. The assay was performed in triplicate and mean values were used for the calculation. Percentage of inhibition was calculated by comparing slope or increase in absorbance of test substance with that of control.

RESULTS AND DISCUSSION

More than 200 species of fungi called as "medicinal mushrooms" containing biologically active substances, are currently known. The greatest interest in mycochemistry is focused on polysaccharides due to their different biological activities.

Antibacterial Activity: *In vitro* antibacterial activity of exopolysaccharide from *F. feei* is showed in Table 1. Exopolysaccharide from *Fomitopsis feei* tested in our study shown the broad spectrum of antibacterial activity, covering both Gram-positive and Gram-negative strains. The ability of the polysaccharide to inhibit all tested organisms suggests that the power of this activity is moderate. Among all microorganisms tested, the exopolysaccharide was found to be most effective against gram-negative bacteria, especially *Proteus vulgaris* (MTCC 426) and *Pseudomonas aeruginosa* (MTCC 424) with a zone of inhibition 34 and 22 mm, respectively, at a concentration of 150 mg/mL. Usually, *Pseudomonas aeruginosa* show high resistance to antibiotics and other environmental factors [14, 15] but in our present research it was inhibited by exopolysaccharide of *F.feei* which suggests that this polysaccharide could be used in the treatment of infections commonly caused by these bacteria.

The dichloromethane extract of *Fomitopsis pinicola* (Swartz ex Fr.) Karst, Polyporaceae showed antibacterial activity against *Bacillus subtilis* in a TLC bioassay [16]. In our present study also *B.subtilis* (MTCC 441) was inhibited which indicates that the genus *Fomitopsis* could exhibit the similar activity even though species is varied. Our results are in agreement with other studies reporting the moderate activity and the broad antimicrobial spectrum of the different extracts from *G. lucidum* [17, 18]. Its polysaccharides are confirmed to be the bioactive components, which showed the significant antibacterial activity. Polysaccharide from *F.feei* definitely gets powerful medicinal importance since it inhibited the growth of *Salmonella typhimurium* (MTCC 98) and *S.paratyphi* (KUCCC 15).

Generally, the gram positive bacteria are more susceptible than the gram negative bacteria. There are reports that gram positive bacteria developing resistance to virtually every clinically available drug [19]. This observation is in line with the work of earlier report [20] the antimicrobial activity of some wild mushrooms from Korea. In contrast to this, both the Gram negative and Gram positive bacteria were affected by polysaccharide of *F.feei*, it was more effective against Gram negative bacteria in our study. Moreover, with the increasing concentration, the EPS showed significant antibacterial activity against all selected microorganisms by a dose-dependent manner. The antimicrobial activity of polysaccharides depends on a variety of factors, including its type, molecular weight, composition and even the chelating activities [21].

E. coli (MTCC 443) was inhibited at 50 mg/mL concentration with a zone of inhibition 7 mm. This result also supported the earlier report [22], which discloses the inhibition zone of 6.14 mm on *E.coli* by polysaccharide of *Hirsutella* sp. with a concentration of 50 mg/mL. It also showed that *S.aureus* and *B.subtilis* also inhibited at this concentration but it was not in agreement with our present result since different polysaccharides from different sources may act on different bacteria variably. The activity was lowest in the inhibition of gram-positive bacteria, *Micrococcus luteus* (KUCCC 4) (8 mm) and *Bacillus megaterium* (KUCCC 9) (10 mm) even at high concentrations (150 mg/mL). Recently, polysaccharides of white rot fungi were given good antimicrobial activity against pathogenic microorganisms [23, 24]. Our results indicated that the EPS had a significant antibacterial activity (Figure 1).

Antifungal Activity: Polysaccharide from *F.feei* exhibited moderate to good antifungal activity (Figure 2) against the fungal pathogens tested. *Drechslera sp*. (KUCCC 29) was inhibited 96.8% followed by *Cuvularia lunata* (KUCCC 27) (95.2%), *Aspergillus terreus* (KUCCC 25) (89.7%) and *Aspergillus fumigatus* (KUCCC 24) (69.5%). In this present study almost all tested fungi were inhibited showing the percentage of inhibition (Figure 3) above 85% except *A. fugimatus* (KUCCC 24) (69.5%).

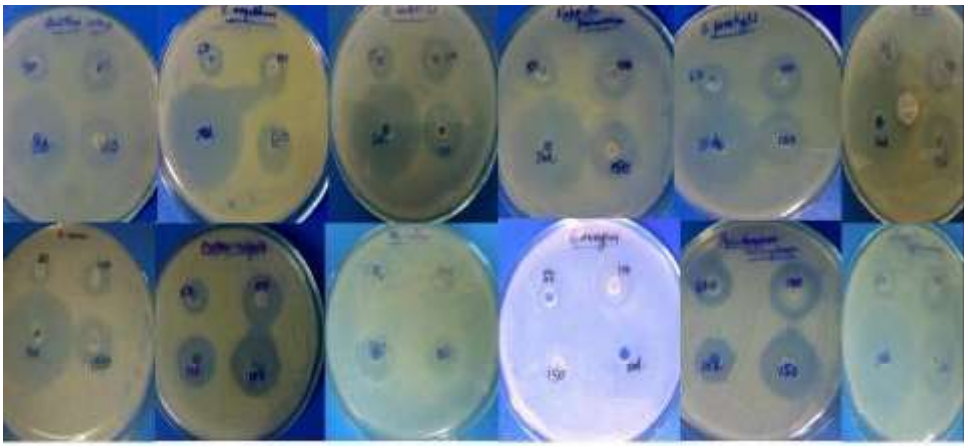

Fig. 1: Antibacterial activity of crude exopolysaccharide from *Fomitopsis feei*

Table 1: Antibacterial activity of exopolysaccharide from *Fomitopsis feei* (Inhibition zones in mm)

S.No.	Pathogenic organism	50 mg/mL	100 mg/mL	150 mg/mL	Standard Streptomycin (mg /mL)
Gram	Positive:				
1.	*Bacillus subtilis* (MTCC 441)	7	10	14	24
2.	*Staphylococcus aureus* (MTCC 96)	5	9	12	29
3.	*Micrococcus luteus* (KUCCC 4)	6	7	8	12
4.	*Bacillus cereus* (KUCCC 7)	6	10	12	30
5.	*Bacillus megaterium* (KUCCC 9)	6	7	10	28
Gram	Negative:				
6.	*Escherichia coli* (MTCC 443)	7	10	12	26
7.	*Enterobacter aerogens* (KUCCC 12)	6	12	13	23
8.	*Klebsiella pneumoniae* (MTCC 109)	7	11	13	19
9.	*Salmonella typhimurium* (MTCC 98)	8	14	16	31
10.	*Proteus vulgaris* (MTCC 426)	10	20	34	14
11.	*Pseudomonas aeruginosa* (MTCC 424)	14	19	22	9
12.	*Salmonella paratyphi* (KUCCC 15)	9	12	16	22

This result indicates that the polysaccharide isolated from *F. feei* is also good antifungal agent. Spore germination inhibition test was also followed for the inhibition of *B. cinerea*, *P. oryzae, F. graminearum* and *G. cingulata* on micro slides with the grifolin isolated from *Albatrellus dispansus* and grifolin inhibited the mycelial growth of *Fusarium graminearum* upto 80.9% [25]. Among the different carbon sources tested, 90% inhibition of spore germination was observed from the medium containing glucose as carbon source when *Gymnopilus Spectabilis* grown under submerged fermentation for antifungal activity [26]. Ethanol and chloroform extracts of *Fomitopsis pinicola* (Sw.:Fr) Karst and *Lactarius vellereus* (Pers.) Fr. showed the antifungal activity against *F. inflexum* and *F. heterosporium* [27].

Our work also supported the antifungal activity of *Fomitopsis* spp. against pathogenic fungi. The mushroom culture filtrates also showed antifungal activity against the two fungi (*Aspergillus flavus* and *Penicillum notatum*) and mycelial weight of *A. flavus* was more inhibited by the culture filtrates of the four mushrooms [28]. Although the method tested for the antifungal activity was differing, these reports also supported that mushrooms having the compounds which inhibits fungal pathogens.

Antioxidant Activity: Reactive oxygen species (ROS) such as hydroxyl and superoxide radicals produced by sunlight, ultraviolet, chemical reactions and metabolic processes have a wide variety of pathological effects on cellular processes [29]. Antioxidants in diet are of great importance as possible protective agents to help human body to reduce oxidative damage. Human diet containing medicinal mushrooms possessing antioxidative properties would be potentially useful to help human body to reduce oxidative damage.

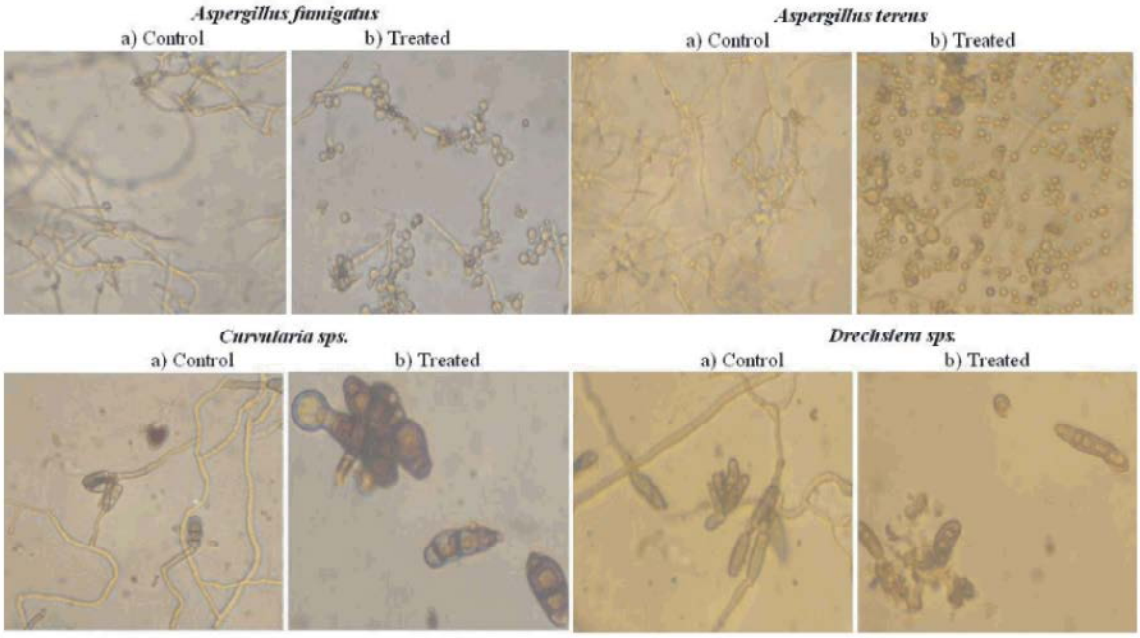

Fig. 2: Antifungal activity of exopolysaccharides from *Fomitopsis feei* in spore germination inhibition method

Fig. 3: Percentage of inhibition of spore germination by exopolysaccharide of *Fomitopsis feei*

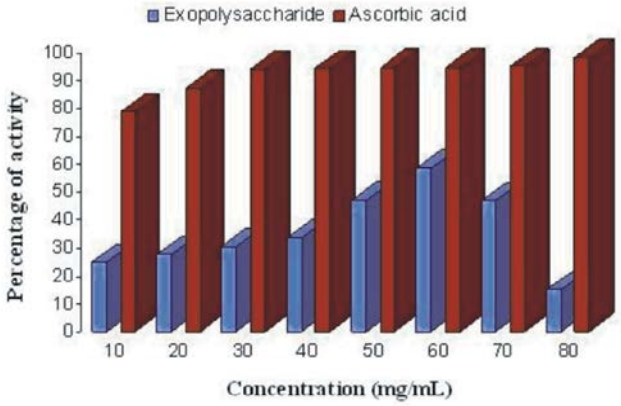

Fig. 4: Antioxidant activity of exopolysaccharide of *Fomitopsis feei*

Figure 4 depicts the antioxidant activity of polysaccharide at different concentration. The antioxidant activity of polysaccharide of *F.feei* is increased in the order of 10 mg/mL (24.97%) > 20 mg/mL (27.49%) > 30 mg/mL (30.50%) > 40 mg/mL (33.51%) > 50 mg/mL (47.19%) > 60 mg/mL (58.68%) but decreased at 70 mg/mL (46.88%) and 80 mg/mL (15.03%). A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517 nm. This purple color generally fades/disappears when an antioxidant is

present. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/ bleached product (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract.

Five groups of polysaccharides from *Grifolia frondosa* had antioxidant and free radical scavenging activities after UV irradiation in addition to other biological activities [30]. Earlier report [31] showed that the reducing power of the tested polysaccharides isolated from the fruit bodies steadily increased with increasing sample concentration. *Fomitopsis pinicola* gave the best results in the three antioxidant activity assays, with EC50 values ? 0.13 mg/mL, with the highest levels of phenolics (388 mg GAE/g extract). Moreover, the reducing power EC50 value (0.06 mg/mL) was similar to the one obtained for the standard Trolox® [32]. The antioxidant scavenging activities of the aqueous extract and polysaccharides of *Cordyceps taii* against various free radicals revealed *in vitro* in a D-Galactose induced aging mouse model [33]. Recently, the phenolic and flavonoid contents of extracts of exo and endopolysaccharide fractions obtained from submerged mycelia cultures of 7 edible or medicinal mushroom species also showed the presence of antioxidant activity [34, 35].

Tyrosinase Inhibitory Activity: Melanin is principally responsible for skin color and plays an important role in the prevention of sunburned skin injury. Tyrosinase (EC 1.14.18.1), an enzyme catalyzes the rate-limiting step for the melanin biosynthesis [36]. Therefore, tyrosinase inhibitors have been established as important constituents of cosmetic materials and depigmenting agents for hyperpigmentation [37]. Significant inhibitions on oxidase activities of mushroom tyrosinase shown by resveratrol suggested that it is a potential candidate of skin-whitening agents [38]. Inhibitory effects on tyrosinase by resveratrol and some related hydroxystilbene analogs were helpful for medical treatement of local hyperpigmentations such as melasma, ephelide and lentigo.

In this present research mushroom polysaccharide is used for the inhibition of tyrosinase enzyme. Recently, a report [39] showed that polysaccharides show a good inhibition activity of tyrosinase, which might be helpful to extend the use of polysaccharides in modern medicine and cosmetics. Ultrasonic-extracted polysaccharides from longan fruit pericarp (PLFP) acted as a noncompetitive inhibitor of tyrosinase [40]. At 25, 50 and 100 mg/mL, the tyrosinase inhibition of polysaccharide ranged from 2.13 to 4.89 and 8.84%, respectively (Figure 5). IC_{50} value for the polysaccharide is >100 μg/mL. However, at 5, 10 and 25 mg/mL, resveratrol showed excellent tyrosinase inhibitory activities of 30.44 to 49.55 and 77.37%. However, there are no reports presently on *Fomitopsis* being the potential source of tyrosinase inhibitors and this could be the first report that polysaccharide of *F.feei* contain tyrosinase-inhibiting activities. The inhibition of tyrosinase activity might depend on the hydroxyl groups of the phenolic compounds of the mushroom extracts that could form a hydrogen bond to the active site of the enzyme, leading to a lower enzymatic activity. Some tyrosinase inhibitors act through hydroxyl groups that bind to the active site on tyrosinase, resulting to steric hindrance or changed conformation [41].

The antioxidant activity may also be one of the important mechanisms for tyrosinase inhibitory activity. Polysaccharides from acetonic extract showed good tyrosinase activity than methanolic and hot water extracts from the fruiting bodies of *P. nebrodensis* [42]. Recent reports also showed that extracts from mushrooms having tyrosinase inhibitory activity [43-45]. High tyrosinase inhibitory activity was detected in *Inonotus mikadoi* (IMI), *Coriolus versicolor* (CVE), *Volvariella volvacea*(VVO), *Panellus serotinus* (PSE), *Auricularia auricula* (AAU) and *Fomitopsis* sp. [46].

Alpha Glucosidase Inhibitory Activity: Diabetes mellitus is a common disease with many complications. α-glucosidase (EC 3.2.1.20) catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can retard the uptake of dietary carbohydrates. The α – glucosidase inhibitory activity was determined by measuring the release of p-nitrophenol from p-nitrophenyl- α – D – glucopyranose. Its inhibitors such as acarbose, miglitol and voglibose can retard the uptake of dietary carbohydrates and suppress post-prandial hyperglycemia and could be useful for treating diabetic and/or obese patients [47].

The α-glucosidase inhibitory activity of exopolysaccharide of *F.feei* is shown in Figure 6. The effectiveness of enzymatic inhibition was determined by calculating IC50. The lower value indicates the higher quality of enzymatic inhibition. According to the result obtained from α – glucosidase inhibitory assay from the polysaccharide of *F. feei*, an inhibition occurred

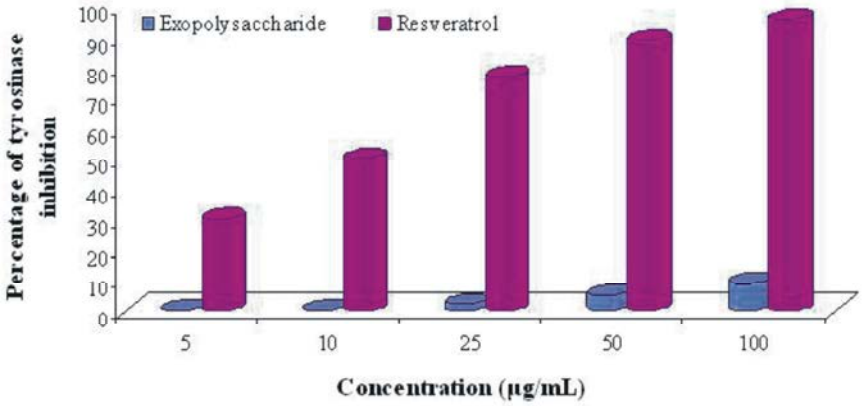

Fig. 5: Tyrosinase inhibitory activity of polysaccharide of *Fomitopsis feei*

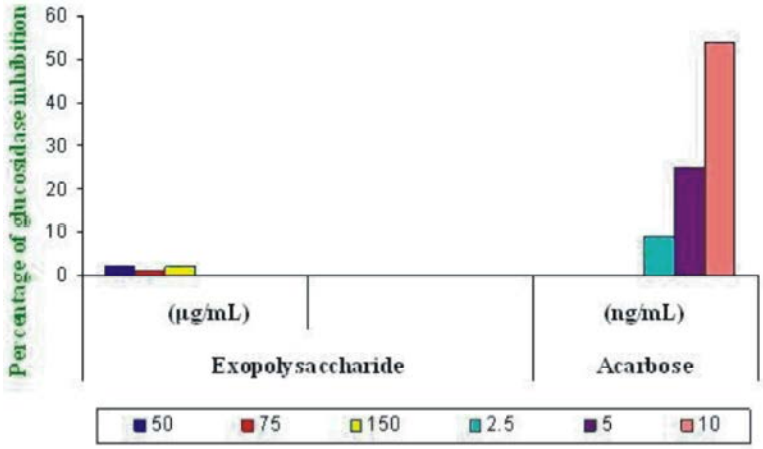

Fig. 6: Alpha glucosidase inhibitory activity of polysaccharide of *Fomitopsis feei*

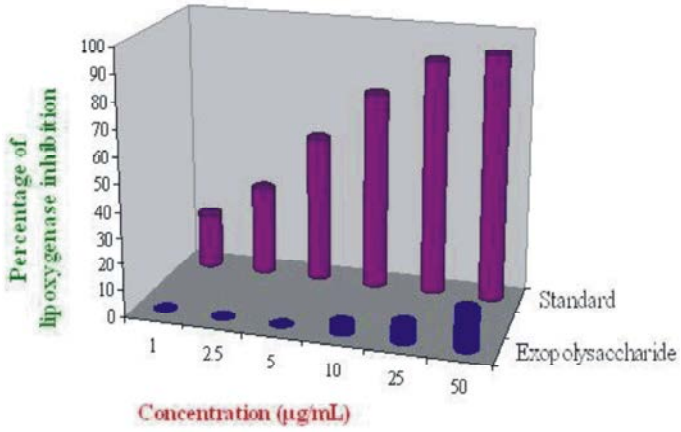

Fig. 7: 5-lipoxygenase inhibitory activity of polysaccharide of *Fomitopsis feei*

at concentration of 150 µg/mL. The positive control against α-glucosidase inhibitory activity was acarbose (IC50 = 9.30 µg/mL). Aqueous extracts of seven varieties of edible mushrooms were tested for α – glucosidase inhibitory activity among them *Flammulina velutipes, Grifolia frondosa, Hypsizigus marmoreus* showed inhibitory activities of more than 70% when the portion of 100 mg equivalent fr.wt. used [48].

The IC50 of positive control for alpha-glucosidase inhibitor (acarbose) is found much higher in the present assay which is similar to many previous literatures [49, 50]. Hispidin, hispolon and inotilone isolated from the fruiting body *Phellinus merrillii* exhibited potent against α - glucosidase inhibitory activity [51]. High inhibitory activity of *Coprinus* polysaccharides (both EPS and IPS) on non-enzymatic glycosylation (NEG) was demonstrated. A 98% NEG inhibition level was reached when =30 mg/mL of EPS or =40 mg/mL of IPS was used [52]. Recent research also revealed that fractions from the 80% ethanol extracts of *Limonium bicolor* possessed strong α - glucosidase inhibitory activity [53].

5-Lipoxygenase Inhibitory Activity: 5-Lipoxygenase (5-LOX) catalyzes the first steps in the conversion of arachidonic acid (AA) into leukotrienes (LTs) that are mediators of inflammatory and allergic reactions. Inhibitors of lipoxygenases (linoleate: oxygen oxidoreductase E.C. 1.13.1.13) have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases. Lipoxygenase catalyzes the oxidation of methylene-interrupted unsaturated fatty acids and their esters such as linoleic and linolenic acids.

The effect of polysaccharide from *Fomitopsis feei* on the inhibition of lipoxygenase activity is shown in Figure 7. Lipoxygenase inhibitory activity of *Fomitopsis feei* was found to be increased dose dependently. A high lipoxygenase inhibition (14.7%) was seen in 50 µg/mL extract concentration. IC_{50} values were found 50 µg/mL. So far, in the literature no information is available concerning the lipoxygenase inhibition assay in *Fomitopsis feei*. 0.32% rosmarinic acid and caffeic acid from *Prunella vulgaris* also reported to have lipoxygenase activity [54]. Sesquiterpenoids, namely warburganal and mukaadial reported to contribute 5-lipoxygenase inhibitory activity. Warburganal displays moderate 5-lipoxygenase inhibitory activity with an IC50 of 61.86 ppm. Mukaadial displayed an IC50 value > 100 ppm in the 5-lipxoygenase assay. LI and LC fractions mycelium of *Ganoderma lucidum* from Central Italy significantly inhibited the oxidation of linoleic acid catalysed by lipoxygenase in a dose dependent manner. At 0.4 and 1.2 mg/mL both extracts inhibited about 40% and 70% of lipoxygenase activity *in vitro*, respectively [55].

Kiss *et al.* [56] have demonstrated that *Epilobium* species inhibited the activity of lipoxoygenase with IC50 = 16 to 28 µg/mL. A recent report [56] showed that *E. angustifolium* water extract had higher lipoxygenase inhibitory activity (0.57 µg/mL). Five extracts of *Indigofera* was evaluated by measuring the inhibition of LOX showing inhibition percentage above 39% at 100 µg/mL [57].

CONCLUSION

The crude extract of exopolysaccharide showed high antimicrobial activities which indicate that the used extracts contain potential antimicrobial compounds to evaluate the inhibitory effects against several disease causing bacteria and fungi. Mostly, gram negative bacteria were inhibited compared to gram positive bacteria. Therefore, it could be suggested that the obtained results could be helpful for evaluating substances of interest compound produced by this fungi. The crude polysaccharide might be not effective compared to pure standard compounds for antioxidant, 5-lipoxygenase, alpha glucosidase and tyrosinase enzyme inhibitory activities, even then their activity was considerable.

REFERENCES

1. Yoshida, T., 2001. Synthesis of polysaccharides having specific biological activities. Prog. Polym. Sci., 26: 379-441.
2. Yanaki, T., W. Ito and T. Kojima, 1981. Ultrasonic degradation of schizophyllan, an antitumor polysaccharide produced by *Schizophyllum commune*. (Fries) Carbohydr. Res., 89: 121-35.
3. Yanaki, T., W. Ito and T. Kojima, 1983. Correlation between the antitumor activity of a polysaccharides schizophyllan and its triple-helical conformation in dilute aqueous solution. Biophys. Chem., 17: 337-42.
4. Czop, J.K. and J. Kay, 1991. Isolation and characterization of β-glucan receptors on human mononuclear phagocytes. J. Exp. Med., 173: 1511-20.
5. Mansell, P.W.A., 1994. Polysaccharides in skin care. Cosmet Toil, 109: 67-72.
6. Persin, Z., K. Stana-Kleinschek, T.J. Foster, J.E.G. Van Dam, C.G. Boeriu and P. Navard. 2011. Challenges and opportunities in polysaccharides research and technology: The EPNOE views for the next decade in the areas of materials, food and health care. Carbohydr. Poly., 84: 22-32.
7. SVSSSL Hima Bindu N. and M.A. Singara Charya, 2017. Growth pattern, molecular identification and bio molecules analysis of *Fomitopsis feei*. Invention J. Res. Technol. Eng. & Management, 1(9): 16-26.

8. Chapin, K. and T. Lauderdale, 2003. Reagents, stains and media:bacteriology In P.R. Murray, E.J. Baron, J.H.Jorgensen, M.A. Pfaller and R.H. Yolken (ed.) Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C. pp: 358.
9. Song, R. and H. Ji, 2006. Inhibitory effects of 8 toxic mushroom strains on growth and germination of *Alternaria alternata.* J. Agric. Technol., 2: 39-48.
10. Yigit, D., N. Yigit and A. Mavi, 2009. Antioxidant and antimicrobial activities of bitter and sweet apricot (*Prunas armeniana* L.) Kernels. Braz. J. Med. Biol. Res., 42: 346-352.
11. Kenji, O., T. Tanaka, I. Iliya, T. Ito, M. Iinuma, K. Matsumoto, Y. Akao and Y. Nozawa, 2003. Gnetol as a potent tyrosinase inhibitor from genus *Gnetum*. Biosci. Biotechnol. Biochem., 67: 663-665.
12. Fan, J.F., Z.Y. Yan, Z.L. Na, L.Z. Gui, Z.B. In, S. Masayoshi and W.X. Nan, 2011. Nutritional composition and α - glucosidase inhibitory activity of five chiniese vinegars. Japan Agri. Res.Quarterly, 45: 445-456.
13. Reddanna, P., J. Ehelan, K.R. Maddipati and Reddy, 1990. Purification of arachidonate 5 -Lox from potato tubers. Methods Enzymol., 187: 268-277.
14. Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo and S. Mitsuhashi, 1977. Frequency of heavy-metal resistance in bacteria from inpatients in Japan. Nature, 266: 165-167.
15. Marques, A.M., F. Congregado and D.M. Simon-Pujol, 1979. Antibiotic and heavy metal resistance of *Pseudomonas aeruginosa* isolated from soils. J. Appl. Bacteriol., 41: 341-350.
16. Hamburger, M.O. and G.A. Cordell, 1987. A direct bioautographic TLC assay for compounds possessing antibacterial activity. J. Nat. Prod., 50: 19-22.
17. Yoon, S.Y., S.K. Eo, Y.S. Kim, C.K. Lee and S.S. Han, 1994. Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibiotics. Arch. Pharm. Res., 17: 438-442.
18. Keypour, S., H. Riahi, M.F. Moradali and H. Rafati, 2008. Investigation of the antibacterial activity of a chloroform extract of Ling Zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae), from Iran. Int. J. Med. Mushrooms, 10: 345-349.
19. Donadio, S., L. Carrano, L. Brandi, S. Serina, A. Offientini, E. Raimondi, N. Montanini, M. Sosio and C.O. Gualerzi, 2002. Targets and asays for discovering novel antibacterial agents. J. Biotechnol., 99: 175-185.
20. Imtiaj, A. and T. Lee, 2007. Screening of antibacterial and antifungal activities from Korean wild mushrooms. World J. Agri. Sci., 3: 316-321.
21. Rabea, E.I., M.E.T. Badawy, C.V. Stevens, G. Smagghe and W. Steurbaut, 200. Chitosan as antimicrobial agent: applications and mode of action. Biomacromol., 4: 1457-1465.
22. Rong, L., X.L. Jiang and H.S.Guan, 201. Optimization of mycelium biomass and exopolysaccharides production by *Hirsutella* sp. in submerged fermentation and evaluation of exopolysaccharides antibacterial activity. Afr. J. Biotechnol., 9: 195-202.
23. Haoran, C., S. Feng, S. Shen, L. Zhang, R. Yang, Y. Zhou and C. Ding. 201. Extraction, antioxidant and antimicrobial activities of *Epimedium acuminatum* Franch. polysaccharide. Carbohydr. Poly., 96: 101-108.
24. Harsimran, K., S. Sharma, P.K. Khanna and S. Kapoor, 2015. Evaluation of *Ganoderma lucidum* strains for the production of bioactive components and their potential use as antimicrobial agents. J Applied and Nal Sci., 7(1): 298-303.
25. Dhiangra, O.D. and J.B. Sinclair, 1986. Chemical control in basic plant pathology methods. CRC Press, Boca Raton, FL, USA, pp: 227-243.
26. Hossain, S.K.M. and N. Anantharaman, 2006. Activity enhancement of ligninolytic enzyme of *Trametes versicolor* with bagasse powder. Afr. J. Biotechnol., 5: 89-194.
27. Guler, P., I. Akata and F. Kutluer, 2009. Antifungal activities of *Fomitopsis pinicola* (Sw.:Fr) Karst and *Lactarius vellereus* (Pers.) Fr. Afr. J. Biotech., 8: 3811-3813.
28. Ayodele, S.M. and M.E. Idoko, 2011. Antimicrobial activities of four wild edible mushrooms in Nigeria. Int. J. Sci. Nat., 2: 55-58.
29. Marx, J.L., 1987. Oxygen free radicals linked to many diseases. Science, 235: 529-31.
30. Bum, C.L., J.T. Bae, H.B. Pyo, T.B. Choe, S.W. Kim, H.J. Hwang and J.W. Yun, 2003. Biological activities of the polysaccharides produced from submerged culture of the edible basidiomycete *Grifola frondosa.* Enz. Microb. Technol., 32: 574-581.
31. Zhong, W.S., L.X. Zhang, B. Zhang and T.G. Niu, 2010. Structural characterization and antioxidant properties of polysaccharides from the fruiting bodies of *Russula virescens*. Food Chem., 118: 675-680.

32. Filipa, S.R., E. Pereira, L. Barros, M.J. Sousa, A. Martins and I.C.F.R. Ferreira, 2011. Biomolecule profiles in inedible wild mushrooms with antioxidant value. Molecules, 16: 4328-4338.
33. Jian, H.X., D.M. Xiao, D.X. Chen, Y. Xiao, Z.Q. Liang and J.J. Zhong, 2012. Polysaccharides from the medicinal mushroom *Cordyceps taii* show antioxidant and immunoenhancing activities in a D-Galactose induced aging mouse model. Evidence Based Complementary and Alternative Medicine, pp: 1-15.
34. Jeong, S.C., S.R. Koyyalamudi, J. Hughes, C. Khoo, T. Bailey, K. Marripudi, J.P. Park, J.H. Kim and C.H. Song, 2013. Antioxidant and immunomodulating activities of exo-and endopolysaccharide fractions from submerged mycelia cultures of culinary-medicinal mushrooms. Int. J. Med. Mush., 15: 251-66.
35. Cornelia M, O. Cioancã, C. Iancu, G. Tãtãrîngã, M. Hãncianu, 2015. *In vitro* antioxidant activity of some extracts obtained from *Agaricus bisporus* brown, *Pleurotus ostreatus* and *Fomes fomentarius*. Farmacia., 63: 927-933.
36. Sanchez, F.A., J.N.R. Lopez, F.G. Canovas and F.G. Carmona. 1995. Tyrosinase: A comprehensive review of its mechanism. Biochim. Biophys. Acta, 1247: 1- 11.
37. Seo, S.Y., V.K. Sharma and N. Sharma, 2003. Mushroom tyrosinase: Recent prospects. J. Agric. Food Chem., 51: 2837-2853.
38. Dong, H.K., J.H. Kim, S.H. Baek, J.H. Seo, Y.H. Kho, T.K. Oh and C.H. Lee, 2004. Enhancement of tyrosinase inhibition of the extract of *Veratrum patulum* using cellulase. Biotechnol. Bioeng., 87: 849-854.
39. Rout, S. and R. Banerjee, 2007. Free radical scavenging, anti-glycation and tyrosinase inhibition properties of a polysaccharide fraction isolated from the rind from *Punica granatum*. Biores. Technol., 98: 3159-3163.
40. Bao, Y., M. Zhao and Y. Jiang, 2008. Optimization of tyrosinase inhibition activity of ultrasonic-extracted polysaccharides from longan fruit pericarp. Food Chem., 110: 294-300.
41. Baek, H.S., H.S. Rho, J.W. Yoo, S.M. Ahn, J.Y. Lee, J. Lee, M.K. Kim, D.H. Kim and I.S. Chang, 2008. The inhibitory effect of new hydroxamic acid derivatives on melanogenesis. Bull. Korean Chem. Soc., 29: 43-46.
42. Nuhu, A., K.N. Yoon and T.S. Lee, 2011. Evaluation of the antioxidant and antityrosinase activities of three extracts from *Pleurotus nebrodensis* fruiting bodies. Afr. J.Biotechnol., 10: 2978-2986.
43. Hae, J.K. and Ki N. Lee, 2008. Anti-oxidization effect of extracts from oriental medicine and cereal medium where *Tricholoma matsutake* mycelia were cultured. Korean J. Oriental Physiol.Pathol, 22: 672-677.
44. Hulya, C.O., A. Yusufoglu, G. Turker and R. Yanardag, 2012. Elastase, tyrosinase and lipoxygenase inhibition and antioxidant activity of an aqueous extract from *Epilobium angustifolium* L. leaves. J. Med. Plants Res., 6: 716-726.
45. Cheng, H., L.Y. Heng, L.X. Ying, L.G. Rong, H.C. Fen and L. Li, 2013. Separation and purification as well as whitening efficacy of polysaccharide from *Tricholoma matsutake* Sing. China Surfactant Detergent & Cosmetics., 2: 134-138.
46. Ki M.P., K. Kwon and S.H. Lee, 2015. Evaluation of the antioxidant activities and tyrosinase inhibitory property from mycelium culture extracts. Evidence-Based Complementary and Alternative Medicine ID 616298, pp: 7, http://dx.doi.org/10.1155/2015/ 616298.
47. Toeller, M., 1994. α-Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. Eur. J. Clin. Invest., 24: 3l-35.
48. Hideyuki, M., C. Asakawa, M. Kurimoto and J. Mizutani. 2002. A - glucosidase inhibitor from the seeds of balsam pear (*Momordica charantia*) and the fruit bodies of *Grifola frondosa.* Biosci. Biotechnol. Biochem., 66: 1576-1578.
49. Youn, J.Y., H.Y. Park and K.H. Cho, 2004. Anti-hyperglycemic activity of *Commelina communis* L. inhibition of a-glucosidase. *Diabetes Res.* Clin. Pract., 66S: S149-S155.
50. Shinde, J., T. Taldone, M. Barletta, N. Kunaparaju, B. Hu, S. Kumar, J. Placido and S.W. Zito, 2008. α-Glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel *in vitro* and in Goto-Kakizaki (GK) rats. Carbohydr. Res., 343: 1278-1281.
51. Guan, J.H., W.T. Hsieh, H.Y. Chang, S.S. Huang, Y.C. Lin and Y.H. Kuo, 2011. r-Glucosidase and aldose reductase inhibitory activities from the fruiting body of *Phellinus merrillii.* J. Agric. Food Chem., 59: 5702-5706.
52. Zhongyang, D., L. Peng, Y. Chen, L. Zhang, Z.G. Shi and K. Zhang, 2012. Production and characterization of thermostable laccase from the mushroom, *Ganoderma lucidum*, using submerged fermentation. Afr. J. Microbiol. Res., 6: 1147-1157.

53. Jiehui, T., L. Ma, W. Li and S. Yao, 2013. Study on the alpha glucosidase inhibitory acitivity of *Limonium bicolor* Kuntze. Adv. Mat. Res., 641-642: 955-958.
54. Psotova, J., M. Kolar, J. Sousek, Z. Svagera, J. Vicar and J. Ulrichova, 2003. Biological activities of *Prunella vulgaris* extract. Phytother. Res., 17: 1082-1087.
55. Roberta, S., P. Ceccaroli, M. Iotti, A. Zambonelli, M. Buffalini, L. Casadei, L. Vallorani and V. Stocchi, 2009. Biochemical characterisation and antioxidant activity of mycelium of *Ganoderma lucidum* from Central Italy. Food Chemistry, 116: 143-151.
56. Kiss, A.K., A. Bazylko, A. Filipek, S. Granica, E. Jaszewska, U. Kiarszys, A. Koœmider and J. Piwowarski. 2011. Oenothein B's contribution to the anti-inflammatory and antioxidant activity of Epilobium sp. Phytomed., 18: 557-560.
57. Bakasso, S., A. Lamien-meda, C.E. Lamien, M. Kiendrebeogo, A.Y. C oulibaly, M. Compaoré, N.R. Meda and O.G. Nacoulma, 2013. *In vitro* inhibition of acetyl cholinesterase, lipoxygenase, xanthine oxidase and antibacterial activities of five *Indigofera* (Fabaceae) aqueous acetone extracts from Burkina Faso. Current Res. J. Biol. Sci., 5: 115-122.