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Full Length Research Paper

Antimicrobial and anti-inflammatory potential of polysaccharide from *Pleurotus pulmonarius* LAU 09

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The *Pleurotus pulmonarius* LAU 09 was characterized to the species level and registered at the GenBank database (accession number: JF736658). *P. pulmonarius* LAU 09 formed a phylogenetic cluster with *P. pulmonarius* complex with bootstrap value of 77%, which confirmed the close evolutionary lineage with previous characterized *P. pulmonarius* complex. The polysaccharide obtained from *P. pulmonarius* was structurally characterized and tested for antimicrobial and anti-inflammatory activities. The polysaccharide was active against all tested pathogens except *Pseudomonas aeruginosa* with percentage activity of 85.75%. The highest zone of inhibition was obtained against *Staphylococcus aureus* (30 mm), while the lowest zone size obtained against *Escherichia coli* (7 mm). The polysaccharide revealed the potent anti-inflammatory effects against carrageenan and formalin – induced paw edema in rats with higher inhibitory percentage in rats writhing; 83.33% and 92.37% inhibition were obtained in carrageenan and formalin-induced paw edema in rats treated with polysaccharide compared with 33.33 and 84.73% in rats treated with standard (dichlofanac) respectively. The percentage sperm motility were higher in both carrageenan and formalin- induced paw edema in rats treated with polysaccharide (76.25 and 67.95%) while its low in dichlofenac treated rats (47.35 and 63.20%) respectively. The sperm counts were increased considerably in the rats treated with polysaccharide (carrageenan: 44.75 × 10^6, formalin: 44.80 × 10^6) compared with the control, 24.70 × 10^6 (carrageenan) and 42.00 × 10^6 (formalin). The significant ($p < 0.05$) increased obtained in both carrageenan and formalin-induced paw edema in rats treated with polysaccharide in some blood parameters in comparison with dichlofenac treated rats was an evidence of anti-inflammatory potential of the polysaccharide.

Key words: Exopolysaccharide, anti-inflammatory, antimicrobial, carrageenan, formalin.

INTRODUCTION

There are many varieties of mushrooms species; of which *Pleurotus* are characterized by a white spore print, attached to the gills, often with an essentric stip, or no stip at all, and they are commonly known as Oyster mushrooms (Miles and Chang, 1997). *Pleurotus* species have been recognized as mushroom with dual functions to humans; both as food and medicine (Chang and Buswell, 2003). They are nutritive with good quantity of proteins, vitamins and minerals. Medicinally, they are been recommended for obese persons and diabetes patients because of low calorie value (Chang and Buswell, 2003) and very low sugar without starch. Traditionally, extracts from *Pleurotus* species have been reported to be used in treating some ailments (Osemwegie et al., 2010; Idu et al., 2007).

During the past three decades, many polysaccharides and polysaccharides- proteins complexes have been isolated from fungi, algae, lichens and plants, homogeneous and heterogeneous polysaccharides, glycans, and glycan-protein complexes from fungi have been shown to promote good health (Jong, 2002). Many

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Basidiomycetes mushrooms contain biologically active polysaccharides (Yap and Ng, 2001), some of which exhibiting haematological, antiviral, antitumour, antibiotic, antibacterial, and immunomodulating activities.

The effects of extracts from *Pleurotus* species against some pathogenic organisms and inflammation have been widely reported by researchers (Mehmant and Sevda, 2009; Solak et al., 2006; Jose et al., 2002; Hajhashmi et al., 2004). However, no report is available on the antimicrobial and anti-inflammatory effects of polysaccharides from *P. pulmonarius.*

The present study was carried out in an attempt to investigate the potential of polysaccharides from *P. pulmonarius* against some pathogenic organisms and its anti-inflammatory effects on Formalin and Carrageenan-induced paw edema in Rats.

MATERIALS AND METHODS

DNA extraction and internal transcribed spacer (ITS) amplification

Mycelia were grown on potato dextrose agar, harvested using a scalpel, transferred into Epperdorf tubes, small amount of autoclaved refined sand (Sigma) was added and ground to fine paste with pestle-like stick (High Media), 400 µl of DNA Extraction buffer pH 8 (1M Tris-Cl pH 8.0; 1M NaCl; 200 mM EDTA pH 8.0; 10% SDS; 0.1% β- Mercaptoethanol) was added and centrifuged at 4°C (12000 g) for 10 mins. To the collected supernatant 300 µl phenol and 300 µl chloroform: Isoamylalcohol (24:1) were added and mixed gently. This was centrifuged (12000 g, 4°C for 10 mins), and aqueous phase was collected and 500µl chilled Isopropanol was added and incubated at -20°C overnight. After the incubation, it was centrifuged (12000 g, 4°C for 10 mins), and the pellet was washed with chilled 70% ethanol centrifuged for 5 mins. The dried pellet was resuspended in 50 µl of Tris EDTA (10 Mm Tris and 1 mM EDTA, pH 8.0) buffer.

Amplification of the ITS region of the rRNA gene was carried out with a modified method of Gerdes and Bruns (1993), using primers ITS1-F and ITS4-B. The final concentration of 25 µl PCR reaction volume consists of 200 µM each of dATP, dCTP, dGTP and dTTP, 2.5 mM $MgCl_2$, 10X Taq.DNA Polymerase and 20Pico mole of each of the two primers (Banglore Genei). The PCR profiles have initial denaturation step of 94°C for 85 s followed by 25 amplification cycles of denaturation, annealing and extension. The temperature and times for these steps were 95°C for 35 s, 55°C and 55 s and 72°C for 2 mins with further incubation at 72°C for 10 mins. The amplified PCR products were resolved on a 1.2% agarose gel, and stained with Ethidium bromide. A 1 kb ladder DNA marker (GeneRuler^{TM}) was used as a size standard.

Sequencing and phylogenetic analysis

The PCR products were purified using Exonuclease I and Shrimp Alkaline phosphatasa in buffer (EXOSAP Kits). Both strands of the amplified region were sequenced using fluorescent dye terminator chemistry and were run on ABI 3130 (4 capillary) or 3730XI (96 capillary) Automated Sequencer (Perkin Elmer Applied Biosystems, Foster City, CA), following the manufacturer's protocols. Sequencing primers were ITS1-F, 5.8S, 5.8SR and ITS4-B. Oligonucleotide sequences for primers 5.8S and 5.8SR were given in Vilgaly and Hester (1990). Sequence contigs were assembled and edited using Sequencer 3.0 software (Gene codes Corporation, Ann Arbor, MI).

Phylogenetic trees were constructed by using all cloned sequences together with all nonredundant large subunit (nLSU) sequences of named *Pleurotus* species obtained from GenBank. The multiple alignments of all the sequences were performed using CLUSTAL W (http://www.ebi.ac.uk/Tools/msa/clustalw2/), followed by manual adjustments. The phylogenetic analyses was carried out using sequence data of ITS 5.8 and 28 s ribosomal RNA gene from LAU 09 (wild) and LAU 60 (mutant) of *P. pulmonarius* and corresponding GenBank data of related species. The identical sequences were merged into one input sequence when running the computer programs to generate the phylogenetic trees constructed by UPGMA, Neighbor-joining (NJ) and parsimony methods. The boostrap test for estimating the reliability of phylogenetic tree topology was performed using 100 replications by the SEQBOOT program (Felsenstein, 1989). The consensus tree was obtained by running the consense program (Felsenstein, 1989).

Extraction, purification and characterization of polysaccharide

The polysaccharide production was carried out on submerged fermentation culture with compositions (g/l); glucose (25), peptone (2.5), yeast extract (2.5), KH_2PO_4 (2.0), $MgSO_4.7H_2O$ (1.0) and $CaCl_2$. $2H_2O$ (1.0). Two plates (90 mm) of the actively growing of LAU 09 were used to inoculate 1 L, incubated at 25°C for 10 days at 150 rpm. The mycelia mat was harvested from the culture after 10 days by filtration. The filtrate is then centrifuged and polysaccharide precipitated with 95% acetone. Further purification was done using Yap and Ng (2001) method.

Infra-red (IR) spectroscopy of polysaccharide

The IR spectroscopy of the polysaccharide was carried out by the KBr pellet method using FT-IR Spectrometer (Spectrum 100: Perkin Elmer) from 500 to 4000 cm^{-1} (Lin et al., 2008).

Magnetic resonance of polysaccharides

The ^{1}H nuclear magnetic resonance (NMR) spectra of polysaccharides in D_2O were obtained with 300 MHz Bruker NMR spectrometer (DPX-300, Bruker Biospin GmbH, Rheinstetten, Germany) (Lavi et al., 2010).

Experimental animals

Male albino wistar rats (150-230 g weighing) were obtained and acclimatized for one week. Rats were divided into four groups, each containing six animals. One of the groups was negative control (non- induced rats), and the other three were treatment groups. All the animals were kept under controlled conditions of temperature (22±7°C) and humidity (60±5°C). They were given pellet food and drinking water *ad libitum*. A twelve hour day and night cycle was maintained in the animal house.

Test organisms

Clinical isolates of *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella* species and *Klebsiella pneumoniae* were obtained from University of Lagos Teaching hospital. The isolates were tested for viability by resuscitating them in buffered peptone water after which they were subculture into nutrient agar medium and incubated at 37°C for 24 h. The organisms were then stored at 4°C until needed.

Evaluation of antimicrobial activity

Agar well diffusion techniques as described by Adeniyi et al. (1996) was adopted for the study. Mueller Hinton agar plates (MHA oxoid) England, were inoculated with 0.1 ml of an overnight broth culture of each bacteria isolate (Equivalent to 3×10^7 cfu/ml) Mcfarland standard (MF) in sterile Petri-dish. The seeded plates were rocked for uniform distribution of isolates and allowed to set. Holes were bored on the plates by using standard sterile cork borer of 6 mm diameters and equal volumes of the polysaccharides (100 µl of 25% solution in water) were transferred into the well with the aid of micropipette. The experiments were carried out in triplicate. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the polysaccharide. The plates were incubated at 37°C for 24 h until marked decline in the potency of the polysaccharide to inhibit the growth of the test isolates was observed. Zone of inhibitions were measured in millimeter (mm) and the average values were calculated and recorded. Reference discs used for control are as follows; Gentamicin (10 µg), Tetracycline (30 µg), Oxacillin (5 µg), Ampicillin (10 µg) and Penicillin (10 µg) (12).

Formalin- induced paws edema in rats

Animals were divided into 4 groups comprising 6 animals in each group. Except group D, chronic inflammation was produced by injecting 0.02 ml of 4% formalin in the right hind paw of the rats (Ajith and Janardhanan, 2001). Group A was injected the formalin without administered the polysaccharide (positive control), Group B injected the formalin along with 2 ml of 6 mg/kg body weight, and the standard reference drug, dichlofenac (10 mg/kg body weight), 1 h prior formalin injection. The administration of the polysaccharides was continued for 15 days consecutive days. The paw size was measured using vernier calipers before, 2 h after formalin injection and 5 days interval.

Carrageenan- induced paws edema in rats

Animals were divided into 4 groups comprising 6 animals in each group. Except group D, chronic inflammation was produced by injecting 0.02 ml of 4% carrageenan in the right hind paw of the rats (Ajith and Janardhanan, 2001). Group A was injected the carrageenan without administered the polysaccharide (positive control), Group B injected the formalin along with 2 ml of 6 mg/kg body weight, and the standard reference drug, dichlofenac (10 mg/kg body weight), 1 h prior carrageenan injection. The administration of the polysaccharides was continued for 15 days consecutive days. The paw size was measured using vernier calipers before, 2 h after carrageenan injection and 5 days interval and average mean were recorded. The percentage inhibition was calculated by;

$$\% \text{ inhibition} = \frac{(P_t - P_o) \text{ control} - (P_t - P_o) \text{ treated}}{(P_t - P_o) \text{ control}} \times 100$$

Where; P_t = Paw size at Fifteen day, P_o = Paw size at zero day.

Sperm motility assay

The epididymis was minced in prewarmed normal saline (37°C). One drop of sperm suspension was placed on a slide glass to analyse 200 motile sperm in four different fields. Sperm motility was assessed by the method of Morrisey et al. (1988). The motility of epididymal sperm was evaluated microscopically within 2 to 4 min of their isolation from the epididymis and data were expressed as percentages.

Sperm count

Epididymal sperm was obtained by mincing the epididymis in normal saline and filtering through a nylon mesh. The sperm were counted using a haemocytometer following the methodology of Freund and Carol (1964).

Haematological analysis

The blood sample was collected from each animal by cardiac puncture under anesthesia in vials containing EDTA (mg/ml), and following parameters were determined using Auto haematological analyzer ADVIA 60 (Bayer Bayer, Germany); packed cell volume (PCV), haemoglobin (HB), white blood cell (WBC), albumin (ALB), globulin (GLO), while total protein and glucose were determined using Bradford (1976) and Schmidt (1971), respectively.

Statistical analysis

The results were presented as mean ± SEM and statistically analyzed by one- way ANOVA followed by the Duncan test.

RESULTS AND DISCUSSION

The phylogenetic analyses

The phylogenetic analyses using sequence data of ITS 5.8 and 28s ribosomal RNA gene from *P. pulmonarius* (LAU 09) and corresponding GenBank data of related species was shown in Figure 1. The out group arising from the root of the phylogenetic tree for the *P. pulmonarius* (accession number: JF736658) for phylogenetic evaluation is *P. tuberregium* (AY450344) from GenBank database. *P. pulmonarius* LAU 09 formed a phylogenetic cluster with *P. pulmonarius* complex at top of phylogenetic tree with bootstrap value of 77%, which confirmed the close evolutionary lineage of the strain with *P. pulmonarius* complex from GenBank database. The cladistic position of the *P. pulmonarius* (LAU 09) strain in the phylogenetic trees suggested that there was common ancestor, sub ancestors diverged majorly at early stages of evolution (Vilgalys et al., 1996). The phylogenetic analyses based on the PCR-RFLP data of the partial 26srDNA has also reported (Bao et al., 2004) which revealed that 9 of the biological species, the *P. cornucopiae* complex, *P. cystidiosus* complex, *P. salmoneos-tramineus*, complex, *P. dryinus*, *P. nebrodensis*, *P. smithii*, and *P. ulmarius* were congruent with independent phylogenetic lineages.

Structural characterization of polysaccharide

The IR spectrum of the purified polysaccharide for LAU

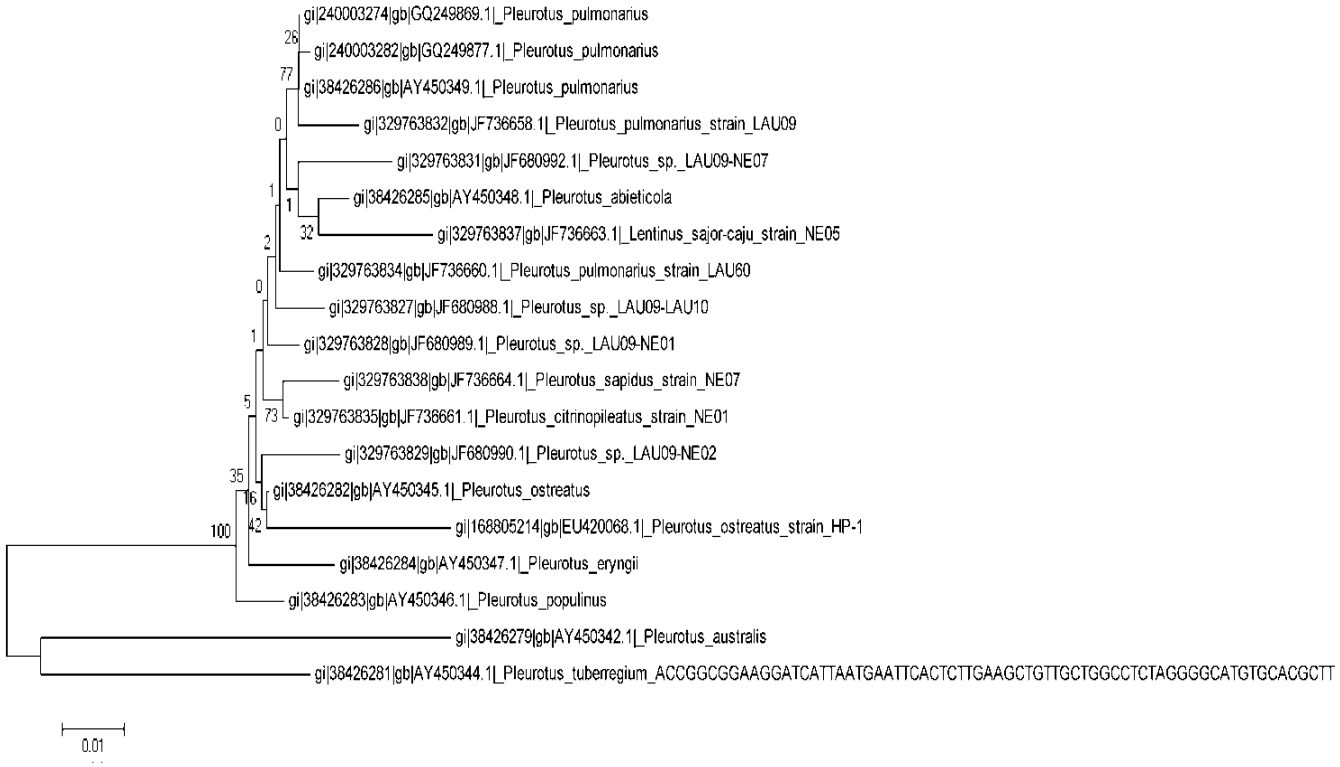

Figure 1. The Phylogenetic trees of LAU 09 (wild) constructed by UPGMA, with boostrap values from 100 replicates showing maximum similarity with the homogeneous strains of *Pleurotus* species from GenBank database.

09 (Figure 2) indicated that it posses characteristic absorption peaks for polysaccharides. The strong peak at 3487.9 cm^{-1} is the absorption of the O-H bond and peak at 2929.2 cm^{-1} is the absorption of the C-H bond. The peak at 1650.6 cm^{-1} is the hydrated water in the polysaccharide. The peaks at 1200-1000 cm^{-1} are the absorption of C-O bond. There was no absorption at 1730 cm^{-1} nearby, indicating that polysaccharide contains no uronic acid.

^{1}H NMR analyses linkage within the LAU 09 was analyzed by ^{1}H NMR. ^{1}H NMR spectrum of the analysed polysaccharide by LAU 09 showed anomeric carbon peaks at 5.10 and 4.51 ppm, which characterized the α and β linkages respectively (Figure 3). The obtained peaks of anomeric carbon obtained by LAU 09 which contain both α and β linkages is an indication of the strong chemical component present in obtained polysaccharide. The preliminary structural analysis shows that the purified polysaccharide in this study is similar to polysaccharides from other fungi, such as PSP, Krestin, Lentinan and Schizophyllan which are all with abundant glucose (Jong and Yang, 1999). These polysaccharides or polysaccharide-peptide complexes have a β-D- glucan core structure, which is the basis for their pharmaceutical activities (Jong, 2002). Since the polysaccharide has α and β-D- type linkages, it may have similar pharmaceutical activities.

Antimicrobial activity of polysaccharide

The polysaccharide shown antagonistic effect against all tested pathogens except *P. aeruginosa,* with percentage activity of 85.75 and 57.14% zone size above 15 mm (Table 1). The highest zone size of 30 mm obtained against *S. aureus*. The percentage zone size above 15 mm is higher than all other tested synthetic antibiotics except Gentamicin and Tetracycline. The ability of the polysaccharide to inhibit all tested organisms except *P. aeruginosa*, suggests that this product contained potential antibacterial agents against infections from these pathogens. Gbolagade et al. (2007) reported that *P. tuber-regium* extract had powerful medicinal importance by inhibiting the growth of *Bacillus cereus, E. coli, K. pneumoniea, S. aureus, Proteus vulgaris*, and *P. aeruginosa*. The antimicrobial activity of mushroom extracts has been reported earlier by several researchers (Gezer et al., 2006; Solak et al., 2006; Mehment and Sevda, 2009). The highest zone size (30 mm) against *S. aureus* suggested that the polysaccharide could be used in the treatment of infections commonly associated with the organisms (Adebayo and Ishola, 2009), and confirmed the traditional use of *Pleurous* extract in

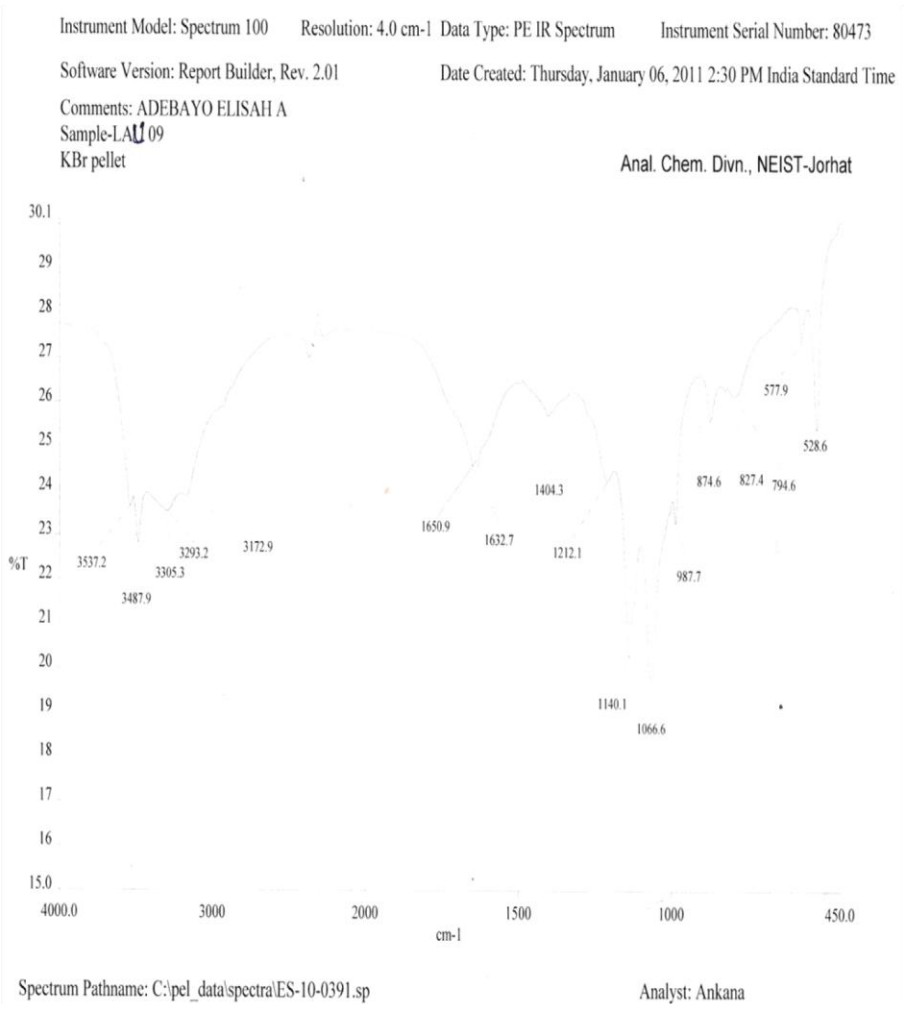

Figure 2. The IR spectra of purified polysaccharide from LAU 09.

treatment of skin diseases (Idu et al., 2007).

Anti- inflammatory activity of polysaccharide

Table 2 shows the effect of oral administration of polysaccharide and standard (dichlofenac) on the carrageenan- induced paw edema on rats. Compared with control, polysaccharide reduced significantly ($p < 0.05$) carrageenan- induced paw edema on the 5th, 10th and 15th days, while dichlofenac significantly ($p < 0.05$) reduced paw edema on 10th. The highest percentage inhibition (83.33%) against carrageenan- induced paw edema was obtained in polysaccharide, while least percentage (33.33%) obtained in dichlofenac. Also, the formalin- induced paw edema in rats was reduced significantly ($p < 0.05$) on 5th, 10th and 15th days by polysaccharide, while dichlofenac have significantly ($p < 0.05$) effect on formalin-paw edema on the 10th day alone. The higher percentage of 92.37% was obtained with oral administration of polysaccharide compared 84.73 obtained for dichlofenac (Table 3). The anti-inflammatory results revealed that polysaccharide has potent anti-inflammatory effects. Jose et al. (2002) reported the anti-inflammatory activity of *Pleurotus* species methanolic extract against the carageenan and formalin- induced in paw edema in mice. A number of *Pleurotus* species have been reported to have immunomodulating, hypoglycemic, hypolipididemic, anti-inflammatory, and antitumour activities (Hajhashmi et al., 2004).

The highest percentage of sperm motility (76.25%) was obtained in carrageenan-induced paw edema in rats treated with exopolysaccharide compared with the rats treated with standard dichlofenac (47.35%), so also the sperm count significantly ($p < 0.05$) increased with the treated rats compared to the control (Table 4).

The percentage sperm motility is higher (67.95%) and sperm count significantly ($p< 0.05$) increased in formalin–induced paw edema rats treated with exopolysaccharide,

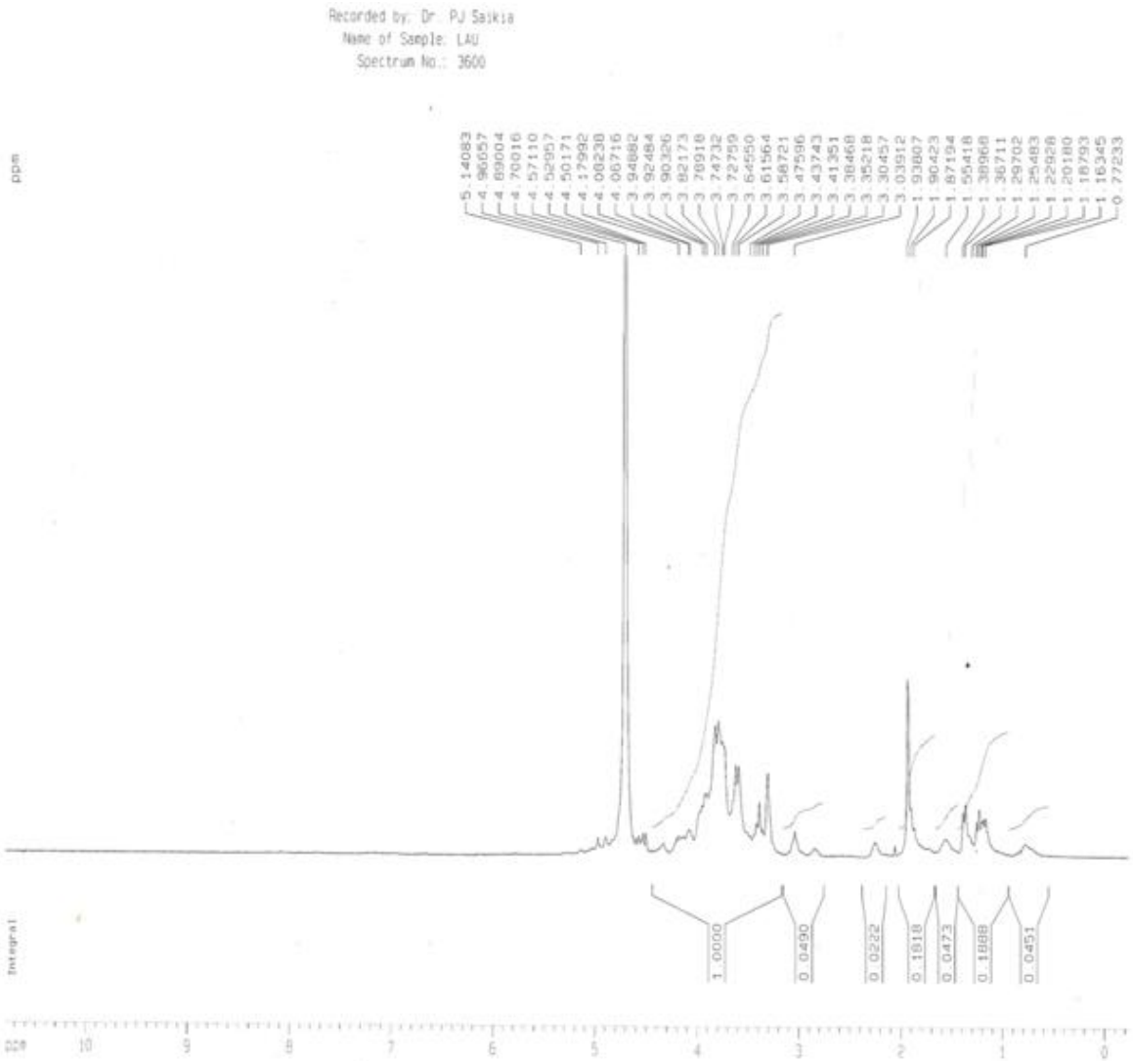

Figure 3. The NMR spectra of purified polysaccharide from LAU 09.

Table 1. Antimicrobial activity of the Exopolysaccharide and antibiotic sensitivity on selected pathogens (zone size in “mm”).

Test organisms	EXP	GEN	TET	OXA	AMP	PEN
P. mirabis	27.0±4.5	18.2±0.2	14.1±2.1	23.4±3.1	21.0±0.7	12.2±0.3
K. pneumoniea	25.2±0.4	14.3± 0.6	22.0±0.5	-	13.4±3.1	10.6± 0.2
S.aureus	30.0±0.6	24.0±0.4	12.0±4.1	18.4±0.2	23.0±5.1	8.0± 0.7
S. typhi	20.0±0.2	27.2± 0.3	31.0±2.1	-	12.0±0.6	27.0±0.3
Shigella spp	10.2±0.3	-	13.0±0.2	17.0±0.3	21.0±0.6	16.0±2.1
E. coli	7.0±4.1	16.2±0.4	19.0±3.1	14.0±3.4	14.4±0.6	14.0±1.4
P. aeruginosa	-	21.0±0.6	24.1±0.2	13.4±0.5	14.0±0.5	21.0±0.3
%activity	85.75	85.75	100	71.42	100	100
% zone size above (15 mm)	57.14	71.42	57.14	42.85	42.85	42.85

- = no zone of inhibition, EXP = Exopolysaccharide, GEN = Gentamicin, OXA= Oxacillin, AMP = Ampicillin, PEN = Penicillin.

while low percentage sperm motility (63.20%) with no significant (p < 0.05) different in sperm count obtained with dichlofenac rats treated compared with the control (Table 5). The increased in sperm motility and sperm

Table 2. Effect of oral administration of polysaccharide on carrageenan- induced writhing in rats.

Groups	0 day	5 days	10 days	15 days	Inhibition (%)
A	2.2±0.3	3.6±1.2	3.0±0.7	2.5± 0.4	---
B	2.4±0.6	3.6±3.2	2.8±4.5[a]	2.6±0.9	33.33
C	2.10±2.1	3.2±1.3[a]	2.3±0.5[a]	2.15±1.7[a]	83.33
D	2.2±0.3	2.2±0.9	2.3±1.0	2.33±1.4	-

[a]$p < 0.05$ significantly different from control (A). A= Carrageenan injection only, B = Carrageenan + dichlofenac, C = Carrageenan + exopolysaccharide, D = Non- Induced rats.

Table 3. Effect of oral administration of polysaccharide on Formalin- induced writhing in rats.

Groups	0 day	5 days	10 days	15 days	Inhibition (%)
A	2.3±0.6	3.3±0.2	3.6±0.7	2.61± 0.4	---
B	2.2±0.7	3.2±4.2	3.0±4.1[a]	2.4±0.3	84.73
C	2.2±0.5	2.6±2.1[a]	2.8±1.5[a]	2.3±0.7[a]	92.37
D	2.4±2.3	2.41±0.3	2.6±0.4	2.61±0.4	-

[a]$p < 0.05$ significantly different from control (A). A= Formalin injection only, B = Formalin + dichlofenac, C = Formalin + exopolysaccharide, D = Non- Induced rats.

Table 4. Effect of carrageenan – induced on rats sperm motility and total epididymal sperm count.

Groups	Total sperm count (10^6)	Sperm motility (%)
A	24.70± 3.5	61.90
B	44.50± 4.1[a]	47.35
C	44.75± 2.7[a]	76.25
D	51.60± 0.4	83.57

[a] $p < 0.05$ significantly different from control (A). A= Carrageenan injection only, B = Carrageenan + dichlofenac, C = Carrageenan + exopolysaccharide, D = Non- Induced rats.

Table 5. Effect of formalin – induced on rats sperm motility and total epididymal sperm count.

Groups	Total sperm count(10^6)	Sperm motility (%)
A	42.00± 0.5	51.30
B	43.50± 2.1	63.20
C	44.80± 0.5[a]	67.95
D	51.60± 0.7	83.57

[a]$p < 0.05$ significantly different from control (A). A= Formalin injection only, B = Formalin+ dichlofenac, C = Formlin + exopolysaccharide, D = Non- Induced rats.

count of the rats, suggests a strong evidence anti- inflammatory activities of the polysaccharide with ability to protect the germ cells and sertoli cells from been damaged, which is in accordance with work of Srivastava et al. (1992).

Table 6 shows the effect of oral administration of exopolysaccharide on blood parameters of carrageenan-induced paw edema in rats with significantly ($p < 0.05$) increased in haemoglobin, glucose and total protein, but no significant effect on other parameters, while packed cell volume, white blood cell, total protein and albumin were significantly ($p < 0.05$) increased with administration of dichlofenac compared with control. The blood haemoglobin and globulin were significantly ($p < 0.05$) increased, while glucose and total protein reduced significantly ($p < 0.05$) with the administration of

Table 6. Assay of blood parameters on carrageenan- induced rats paw edema.

Blood parameter	A	B	C	D
PCV (%)	53.0± 0.3	60.0± 1.5 [a]	58.0± 2.7	56.0± 2.3
HB (g/dl)	3.0± 0.9	3.3± 1.9[a]	3.2± 0.8	3.1± 0.7
WBC (mm^3)	3.1± 0.6	2.9± 0.6	4.4± 0.2 [a]	2.8± 4.0
GLU (g/dl)	2.5± 2.5	2.9± 0.8	5.0± 3.4 [a]	4.6± 0.3
TP (mg/dl)	51.0± 0.4	65.0± 0.10[a]	73.0± 5.1[a]	80.0± 0.6
ALB (mg/dl)	25.0± 3.4	25.0± 0.6	33.0 ± 0.5[a]	22.0± 1.7
GLO (mg/dl)	49.0± 0.9	51.0± 4.3 [a]	26.0± 2.7	58.0± 0.9

[a] $p < 0.05$ significantly different from control (A). A= Carrageenan injection only, B = Carrageenan + exopolysacchiride, C = Carrageenan + dichlofenac, D = Non- Induced rats, PCV = packed cell volume, HB= haemoglobin, WBC = white blood cell, GLU = glucose, TP = total protein, ALB = albumin, GLO = globulin.

Table 7. Assay of blood parameter on formalin - induced rats paw edema.

Blood parameter	A	B	C	D
PCV (%)	57.0± 0.7	59.0± 1.2 [a]	48.0± 0.9	56.0± 0.3
HB (g/dl)	3.1± 0.6	3.3± 1.1[a]	2.7± 1.4	3.1± 0.5
WBC (mm^3)	3.4± 0.3	2.0± 1.6	3.6± 0.8 [a]	2.8± 2.1
GLU (g/dl)	3.2± 3.1	0.9± 0.3	1.9± 0.4	4.6± 0.5
TP (mg/dl)	67.0± 0.2	62.0± 0.7	68.0± 3.1	80.0± 3.4
ALB (mg/dl)	32.0± 0.5	21.0± 0.4	40.0 ± 0.6 [a]	22.0± 0.3
GLO (mg/dl)	22.0± 0.5	39.0± 0.8 [a]	28.0± 3.6	58.0± 1.2

[a] $p < 0.05$ significantly different from control (A). A= Formalin injection only, B = Formalin + exopolysacchiride, C = Formalin + dichlofenac, D = Non-Induced rats, PCV = packed cell volume, HB= haemoglobin, WBC = white blood cell, GLU = glucose, TP = total protein, ALB = albumin, GLO = globulin.

exopolysaccharide to the formalin-induced paw edema rats (Table 7), and administration of dichlofenac increased white blood cell and total protein while reduced packed cell volume and haemoglobin significantly ($p < 0.05$). The high level of HB, PCV, and GLO obtained in some of the experimental rats is an indication that the rats are not anaemic while a lower level is a sign of anaemic condition. This also justified traditional use of extract from *Pleurotus* species (Osemwegie et al., 2010) as anti- aneamic condition. The low level of WBC count in the induced rats treated with exopolysaccharide suggests that it might not enhanced immune capacity against some other infections (Selmanoglu et al., 2001). The high protein content obtained in all treated rats, indicate that the exopolysaccharide could be a good source of dietary supplement (Urbano and Goni, 2002). The increased in glucose and albumin content also suggests its important constituent in source of food material.

Mushroom neutriceuticals are of multi-functional value with concerted effects. These dietary supplements will be useful in prevention and treatment of various human diseases (Wasser et al., 2000). The present investigations indicate that polysaccharide obtained from *P. pulmonarius* possesses significant antimicrobial and anti- inflammatory activities. The findings thus suggest a potential therapeutics of polysaccharide in controlling some diseases and as food supplement.

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