# Studies on Immunomodulatory and prophylactic properties of some wild Nigerian mushrooms

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**Abstract:** The prophylactic potentials of five Nigerian higher fungi – *Pleurotus tuber-regium, Pleurotus pulmonarius, Termitomyces robustus, Fomes lignosus, Lentinus subnudus* and their combination in equal proportion (mixture extract) were investigated. The ethanolic fungi extracts were tested on sets of albino rats (*Rattus norvegicus*) infected with selected bacteria (*Escherichia coli, Staphylococcus aureus, Salmonela typhi, Pseudomonas aeruginosa, Streptococcus feacalis* and *Bacillus subtilis*. Extracts were orally administered at 1.0ml concentration against 0.1ml/cfu microbial suspension intraperitoneally . Survival rates of the experimental rats were monitored. Mixture of these extracts exhibited significant level of prophylactic effect against all the bacteria in the host organisms.

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## 1. Introduction

*Pleurotus tuber-regium* is a tropical mushroom and an edible higher fungus which produces a sclerotium or underground tuber. A sclerotium is a dark brown, yam-like material specially produced by some specific higher fungi (Oso, 1977; Jonathan *et al.,* 2008). It could be used in combinations with other plants parts to treat headache, stomach ailments, colds , fever, asthma, smallpox and high blood pressure (Oso, 1977). The fruitbodies and sclerotium of this basiomycete have been found to possess antimicrobial potential against some disease causing microorganisms (Jonathan and Fasidi, 2003). Also, *Pleurotus pulmonarius* (oyster mushroom) has been produced using several agricultural residues (Bononi *et al.,* 1995). Banana leaf, mixed with sugarcane bagasse or corn cob are examples of the substrates used for the production of *P. pulmonarius* (Chang *et al*., 1981) while cassava residues with sugarcane bagasse are used for the production of *P. oestreatus* (Felinto, 1999).

*Fomes* is a genus of polypore higher fungus which belongs to the order Aphyllophorales, and family polyporaceae ( Alexopoulous *et al.,* 1996). The basidiocarps of *Fomes* may generally be recognised by conspicuous fruitbodies which are perennial with attractive yellow or brownish colouration. The sporocarp are fleshy when young and becomes woody on aging. *Fomes lignosus* had also been reported to be utilized in Nigeria folk medicine due to its inhibitory potentials against some microbes implicated in the pathogenesis of skin infections, food poisoning, gastro-intestinal tract and urino-genital tract infections (Olawuyi *et al*., 2010; Oluranti *et al.,* 2012).

*Termitomyces* is a genus of basidiomycete fungi belonging to the family Lyophyllaceae (Kirk *et al.,* 2008). They usually grow in close association with termite nests. Despite their spore, transfer is mainly by shedding from higher fungi which protrude from the termite mounds (Mueller *et al.,* 2005). *Termitomyces titanicus* of West Africa is one of the largest higher fungi in the world.

Lentinus is a genus whose plants are tough and plaints, becoming hard when old unless very watery. *Lentinus subnudus* belongs to the family polyporaceae which is the largest and the most diverse group of poroid aphyllophorales (Pegler, 1983). Though, grows naturally on dead woods, logs and trunks, they can also be cultivated in logs of *Sporandias mombin* and unfermented composts (Fasidi and Kadiri, 1993; Gbolagade *et al.,*2006).

Mushrooms have been widely reported for their nutritional and medicinal properties (Mizuno *et. al.*2000; Oluranti *et al*,2012;Aina *et al.,* 2012a and b ;Jonathan *et al.*,2012a).This study was aimed at examining the immunomodulatory and prophylactic potentials of these higher fungi found in Nigeria.

## 2. Materials and methods

### 2.1 Higher fungi and extracts preparation

Fruit bodies of *Pleurotus tuber-regium, P. pulmonarius, Fomes lignosus, Termitomyces robustus* and *Lentinus subnudus* were cut into bits,

dried at $40^0$C and milled into powder using grinding machine. Ethanol was used as solvent for the extraction of the higher fungi using the methods described by Jonathan and Fasidi (2003).

### 2.2 Experimental rats

Albino rats (*Rattus norvegicus*) were used for the study and were about 12 weeks old with average weight of 132 grammes.

### 2.3 Prophylactic experiment

One millilitre (1mL) of the extract was administered orally into each experimental animal for 7 consecutive days. The test bacteria (*E. coli, S. aureus, S. typhi, P. aeniginosa, S feacalis,* and *B. subtilis*) were homogenised in peptone water and 0.1ml of the microbial suspension were intraperitoneally administered into each animal post-higher fungi extract administration. This was done thrice at intervals of 2 days. Infection was then monitored for about 10 days. Survival rate of the experimental rats was based on the number that survived the infection. Dead animals were dissected immediately to remove their livers and kidneys, while the survived ones were later sacrificed. The excised organs were weighted and kept in formalin (10%) for histological studies.

### 2.4 Histological studies

The livers and kidneys kept in 10% formalin were histologically examined for pathological changes (Wiess *et al.,*1984).

### 2.5 Analysis of Data

The results of this study were subjected to the analysis of variance (ANOVA) and Duncan's Multiple Range Tests (DMRT) ($p<0.05$).

## 3. Results and Discussion

The five higher fungi in this study were found to exert prophylactic effects against the infected organisms in the test animals, though at different levels. The mixture extract and *Pleurotus pulmonarius* were most effective against *E. coli*. Survival rates of experimental rats were 100% after the bacterial infection (Table 1).

**Table 1: Survival rates of *Rattus norvegicus* used for prophylactic administration of the higher fungi against *E. coli*.**

| Day | FOM | PT | PS | Term | Lent | Mixt | Control A | Control B |
|---|---|---|---|---|---|---|---|---|
| 1 | 91.67[a] | 75.00[b] | 100.00[a] | 100.00[a] | 100.00[a] | 100.00[a] | 75.00[b] | 100.00[a] |
| 5 | 75.00[b] | 25.00[c] | 91.67[a] | 75.00[b] | 75.00[b] | 100.00[a] | 0.00 | 100.00[a] |
| 10 | 75.00[b] | 16.67[c] | 91.67[a] | 75.00[b] | 75.00[b] | 100.00[a] | 0.00 | 100.00[a] |

Values with the same letter(s) in each row are not significantly different by Duncan's multiple range test ($P < 0.05$). Each is a mean of three replicates.

**KEY**:
FOM = *Fomes lignosus,* PT = *Pleurotus tuber-regium,* PS = *Pleurotus pulmonarius,* Term = *Termitomyces robustus,* Lent = *Lentinus subnudus,* Mix = Mixture extract, Control A = Infected rats without extract, Control B = Uninfected rats.

*Lentinus subnudus* and *Pleurotus tuber-regium* demonstrated the best prophylactic activity against *S. aureus* with survival rates of 100% and 91% respectively (Table 2). *F. lignosus*, *T. robustus* and the mixture extracts were very effective against *S. typhi*. Virtually all the animals survived the infection. *L subnudus* also had an appreciable effect against the organism (Table 3). Both *Pleurotus* species (*Pleurotus tuber-regium* and *P. pulmonarius*) had the same effect. The prophylactic effects against *P.* aeruginosa shown in Table 4 indicates *P. tuber-regium* as the best extract with a 100% animal survival throughout the experimental period. Table 5 shows that all the higher fungi extracts were uniformly active against *S. Feacalis,* while the mixture extract and *F. lignosus* were the most active extracts against *B. subtilis* (Table 6). Survival rates of the animals were 100% and 91% respectively.

**Table 2: Survival rates of *Rattus norvegicus* used for prophylactic administration of the higher fungi against *S. aureus***

| Day | FOM | PT | PS | Term | Lent | Mix | Control A | Control B |
|---|---|---|---|---|---|---|---|---|
| 1 | 83.33[ab] | 100.00[a] | 75.00[b] | 75.00[b] | 100.00[a] | 83.33[ab] | 50.00[c] | 100.00[a] |
| 5 | 66.67[b] | 91.67[a] | 50.00[c] | 75.00[b] | 100.00[a] | 75.00[b] | 0.00 | 100.00[a] |
| 10 | 66.67[b] | 91.67[a] | 25.00[c] | 75.00[b] | 100.00[a] | 75.00[b] | 0.00 | 100.00[a] |

**Table 3: Survival rates of *Rattus norvegicus* used for prophylactic administration of the higher fungi against *S. typhi***

| Day | FOM | PT | PS | Term | Lent | Mix | Control A | Control B |
|---|---|---|---|---|---|---|---|---|
| 1 | 100.00[a] | 75.00[b] | 75.00[b] | 100.00[a] | 100.00[a] | 100.00[a] | 75.00[b] | 100.00[a] |
| 5 | 91.67[a] | 66.67[b] | 66.67[b] | 100.00[a] | 75.00[b] | 100.00[a] | 0.00 | 100.00[a] |
| 10 | 91.67[a] | 66.67[b] | 66.67[b] | 100.00[a] | 75.00[b] | 100.00[a] | 0.00 | 100.00[a] |

**Table 4: Survival rates of *Rattus norvegicus* used for prophylactic administration of the higher fungi against *P. aeruginosa***

| Day | FOM | PT | PS | Term | Lent | Mix | Control A | Control B |
|---|---|---|---|---|---|---|---|---|
| 1 | 100.00[a] | 100.00[a] | 83.33[b] | 83.33[b] | 75.00[b] | 83.33[b] | 75.00[b] | 100.00[a] |
| 5 | 75.00[b] | 100.00[a] | 66.67[b] | 50.00[c] | 50.00[c] | 75.00[b] | 0.00 | 100.00[a] |
| 10 | 66.67[b] | 100.00[a] | 66.67[b] | 50.00[c] | 50.00[c] | 75.00[b] | 0.00 | 100.00[a] |

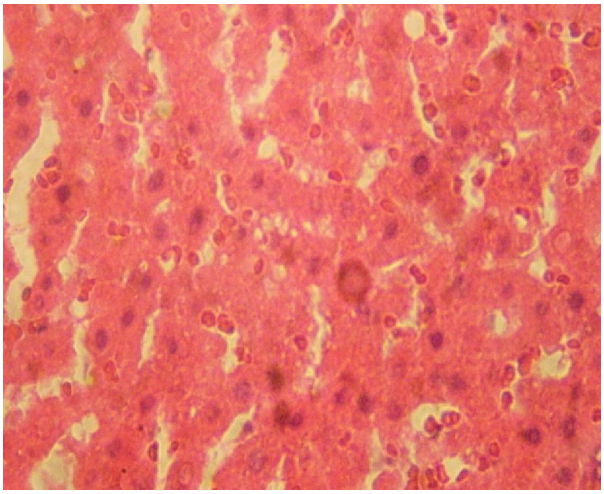
**Table 5: Survival rates of *Rattus norvegicus* used for prophylactic administration of the higher fungi against *S. feacalis***

| Day | FOM | PT | PS | Term | Lent | Mix | Control A | Control B |
|---|---|---|---|---|---|---|---|---|
| 1 | 75.00[b] | 75.00[b] | 100.00[a] | 75.00[b] | 100.00[a] | 83.33[ab] | 50.00[c] | 100.00[a] |
| 5 | 66.67[b] | 75.00[b] | 75.00[b] | 75.00[b] | 75.00[b] | 75.00[b] | 0.00 | 100.00[a] |
| 10 | 66.67[b] | 75.00[b] | 75.00[b] | 75.00[b] | 75.00[b] | 75.00[b] | 0.00 | 100.00[a] |

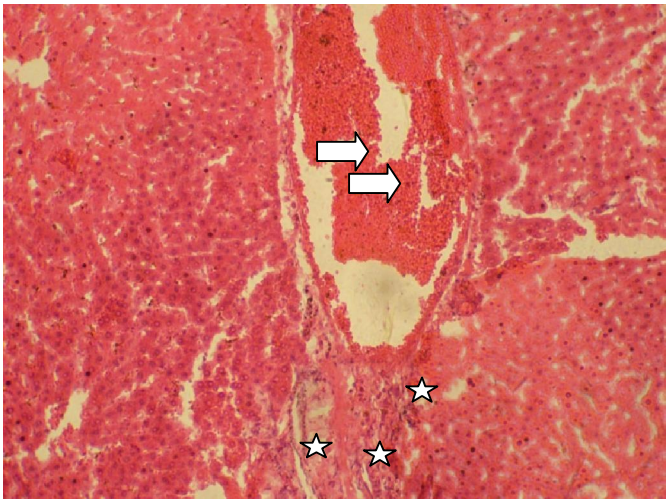
**Table 6: Survival rates of *Rattus norvegicus* used for prophylactic administration of the higher fungi against *Bacillus subtilis***

| Day | FOM | PT | PS | Term | Lent | Mix | Control A | Control B |
|---|---|---|---|---|---|---|---|---|
| 1 | 100.00[a] | 75.00[b] | 100.00[a] | 100.00[a] | 100.00[a] | 100.00[a] | 75.00[b] | 100.00[a] |
| 5 | 91.67[a] | 75.00[b] | 0.00 | 75.00[b] | 75.00[b] | 100.00[a] | 0.00 | 100.00[a] |
| 10 | 91.67[a] | 75.00[b] | 0.00 | 75.00[b] | 75.00[b] | 100.00[a] | 0.00 | 100.00[a] |

The histological findings on the tissues of rats infected post-fungi extract administration are shown in Tables 7 and 8. These further confirmed the prophylactic effect of the higher fungi extracts. No visible lesions were seen on both liver and kidney tissues of rats administered with the extracts 7 days before infection and also the control uninfected rats. The control infected rats (without extracts) showed some abnormalities in the kidney (marked portal congestion, diffuse tubular necrosis and numerous tubules with protein casts in the tubular lumen) and the liver (marked bile duct proliferation, mild cellular infiltration). The pathological changes were prevented in the experimental rats by the protective and preventive potentials of the higher fungi extracts (Plates 1 and 2).

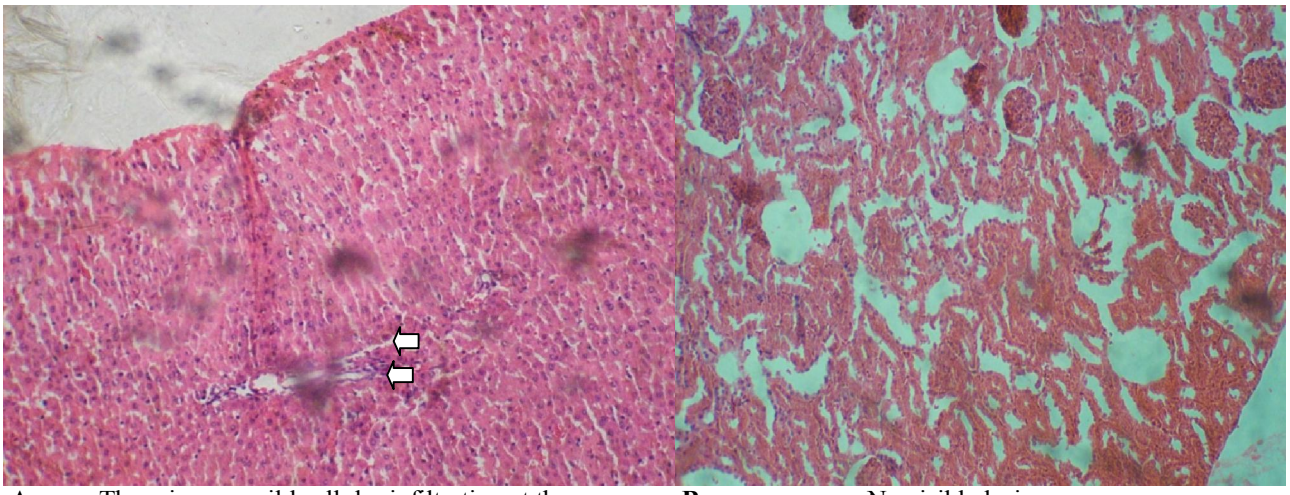


**A** No visible lesions



**B** **X400** There is marked portal congestion (arrows) and mild portal fibrosis (stars).

**Plate 1**: **Photomicrograph of the liver of rats infected with *Escherichia coli* with no visible lesions (A) and the kidney with marked portal congestion (B)**



**A** There is very mild cellular infiltration at the portal area.

**B** No visible lesions seen.

**Plate 2: Photomicrograph of rats' livers infected with *Bacillus subtilis* (A) Mild cellular infiltration (B) The kidney of infected rates with no visible lesions.**

**Table 7: Histological findings on tissues of rats infected with *E. coli*, those treated with *Fomes lignosus* and control**

| Group | Organ | Findings |
|---|---|---|
| Rats administered with *F. lignosus* extract 7 days before infection | Liver | No visible lesions seen |
| | Kidney | No visible lesions seen |
| Control (uninfected) | Liver | No visible lesions seen |
| | Kidney | No visible lesions seen. |
| Control (infected) | Liver | No visible lesions |
| | Kidney | Marked portal congestion |

**Table 8: Histological findings on tissues of rats infected with *Bacillus subtilis*, those treated with Mixture extract and control**

| Group | Organ | Findings |
|---|---|---|
| Rats administered with Mixture extract 7 days before infection | Liver | No visible lesions seen |
| | Kidney | No lesions developed |
| Control ( uninfected) | Liver | No visible lesions seen |
| | Kidney | No visible lesions seen. |
| Control (infected) | Liver | Mild cellular infiltration at the portal area. |
| | Kidney | No visible lesions. |

On the overall, the mixture extract was found to have the best prophylactic effect against the bacterial infections in the experimental rats. The survival rates of the control (uninfected) rats remain the *same* (100%) throughout the period of experiment. For the control infected rates, no animal survived after the 4$^{th}$ day of infection.

Wang *et al*., (2007) obtained similar results when glucans extracted from different polysaccharides were used against *Aeromonas hydrophila* and *Edwardsiella tarda* infections in tilapia. The survival rates of the fish increased significantly. Typically for fungi, the most active components of their immunomodulators are the glucan structures. The immunomodulatory properties of these mushrooms are due to the presence of glucans in thier cell wall (Benedict 1972; Kupra *et al.,* 1979; Suzuki *et al.,* 1989*;* Brandt *et a*l., 2000).

According to Medzhitove *et al.*(1997), the glucan structures are generally indicated as pathogen associated molecular patterns. Betaglucan is one of the most effective immunomodulators and are recommended by Clayton (2004), because of its ability to activate macrophage. An example of betaglucan is lentinan obtained from *Lentinus edodes* which stimulates natural killer cell, T-cell and macrophage dependent responses (Nanba *et al.,* 1987; Mizuno and Zhuang, 2000). A mushroom compound (active hexose ) which is a product of the enzymatic modification of several types of medicinal higher fungi has the ability to increase natural killer cell and macrophage activity. Moreover, several clinical tested health supplements from *Fomes lignosus* have been considered as best natural products that can be consumed to regulate healthy diet and also boost the immune system. *Grifola frondosa* (maitake) enhance the activities of natural killer cells, T-cells and macrophages. In Asia, *Trametes verscolor* is a popular higher fungi that has been used traditionally because it stimulates macrophages. *Agaricus blazei* also has the ability of increasing T-cell activity thereby stimulating the immune system. The beta glucans associated with this mushroom are the active polysaccharides (having 1, 6-betaglucan as the most potent immunomodulator). In one animal study, *Cordyceps sinensis* was discovered to increase antibody thereby enhancing immunity. Also, in an in-vitro study, a cordyceps polysaccharide was found to increase cytokines (Wang *et al.,* 2007; Huang *et al.,*2006).

Stamets (2001), also reported that compounds with antibacterial properties are prepared from mushroom such as *Fomitopsis*, *Piptoporus, Ganoderma, Inonotus* and *Trametes* species. They are also useful in preventing and treating viruses such as poxyiridae and orthopox viruses, flu viruses including bird flu, SARS and hepatitis as well as infections from *Mycobacterium tuberculosis, S. aureus* and *E. coli.*

Generally, mushrooms have been suggested to have immunological properties, giving protection against a number of infectious diseases (Hobbs, 1986; Chilara, 1992; Mizuno *et. al.*1995*;* Jonathan and Awotona, 2010).

The findings of Medzhitov *et al.*(1997) confirmed that the mixture of mushroom polysaccharides gives the maximum response in the immune system by increasing the number and enhancing the activity of killer, T and NK (natural killer) lymphocytes.

## 4.0 Conclusion

It is clear from the study that preventive abilities were exhibited against the test pathogenic bacteria. This could be attributed to the prophylactic effects of the higher fungi extracts. The strength for the prophylactic activity was obtained within the seven days pre-infection fungi-extract administration. This was evidenced in the survival rates (100%) of the experimental rats used for the study. The ability of the extracts to exert prophylactic effect within a week at low concentration has to do with the immune-enhancing properties of the higher fungi. Therefore, they are recommended as good prophylaxis for prevention and protection against infections, especially the blend or mixture of the higher fungi.

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