Mantarlar, Lenzites Betulinus ve Coriolopsis Gallica Ekstrelerinin Antimikrobiyal Etkinlikleri ve Fitokimyasal Analizi.

[Antimicrobial Efficacy and Phytochemical Screening of Mushrooms, Lenzites Betulinus, and Coriolopsis Gallica Extracts.]

INTRODUCTION

There is a constant search for new and effective drugs, which has been driven by the number of pathogenic organisms reported to have multi resistance against many of the therapeutic products that are available in the market. Fungi from the division Basidiomycota have been of interest recently due to the numbers of biological active compounds that have been isolated from them (1). Basidiomycete fungi of the genera Trametes, Lenzites, and Laetiporus are recognized worldwide as major wood decay fungi (2). Mushroom nutriceuticals are bioactive compounds that are extractable from mushrooms, and they have nutritional and medicinal features that may be used in the prevention and...
treatment of diseases (3). There have been a number of reviews published on the bioactive substances found in mushrooms and their medicinal properties.

In general, the evidence supporting the medicinal use of mushrooms is based on traditional healing paradigms, the popularity of mushroom adjuvant therapy in China and Japan (4). Mushrooms are unique, stationary like a plant, yet built from chitin, understanding the properties of chitin is critical to understanding how to choose an effective, high-quality medicinal mushroom product (5). Only a heated liquid solution can break down the indigestible chitin and release the active compounds into a concentrated, bio-available form (6). Medicinal mushrooms are never used in the un-extracted form (as mycelium bio-mass powder or dried mushroom powder), and rarely prepared as a tincture (soaked in alcohol and water in the absence of heat) (7).

_C. gallica_ is a fungus found growing on decaying wood. It has not been associated to any plant disease; therefore it is not considered pathogenic. _C. gallica_ is downright hairy and disheveled. It is a widespread decomposer of the deadwood of willows and cottonwoods, characterized by its hairy cap, its gray to grayish brown pore surface, its rusty brown flesh, and microscopic features (8). _L. betulinus_ grows scattered or clustered on deciduous wood such as birch, beech and oak, and less frequently on coniferous woods. Viewed from the top, it is similar in appearance to _Trametes versicolor_ or _Trametes hirsuta_, sometimes with a greenish tinge from algal growth (9). Therefore the objective of this research work is to determine the phytochemical components and compare the antimicrobial activities of _L. betulinus_ and _C. gallica_ with some standard antibiotics.

**MATERIAL and METHOD**

**Collection and identification of mushroom samples**

Mushroom samples were collected from a forest near Joseph Ayo Babalola University, Ile-Ife, Osun State. They were resuscitated by streaking on Nutrient agar slant and maintained at 4°C as stock culture.

**Preparation of mushroom extracts**

Mushroom samples were sundried for 3 days and then oven dried for 2 hr at 45°C, dried samples were pulverized using an industrial blender (Excella). Twenty five gram of each mushroom samples was soaked into 300 ml of each solvents (petroleum ether, ethanol and water) for 48 hr. After 48 hr, the soaked samples were filtered using muslin cloth and Whatman no.1 filter paper, the filtrate were concentrated and dried according to the method of (10).

**Collection of bacteria isolates**

Pure bacterial isolates: _Klebsiella pneumonia_, _Escherichia coli_, _Proteus vulgaris_, _Staphylococcus aureus_ and _Pseudomonas aeruginosa_ were collected at Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun State. They were resuscitated by streaking on Nutrient agar slant and maintained at 4°C as stock culture.

**Phytochemical screening of C. gallica and L. betulinus**

Mushroom extracts were obtained using the method of, while the following active ingredients in form of phytochemicals were assayed for: Tannins, Phenolic, Saponin, Flavonoids and Steroids (11).

**Antimicrobial susceptibility assay**

Mushroom extracts were assayed for antimicrobial activity using Agar well diffusion technique using the method of (12).

**Statistical analysis**

Quantitative data were expressed as mean ± standard deviation. Statistical evaluation of the data was performed using one-way analysis of variance followed by Duncan’s multiple range test at 5% level of significance i.e. p ≤ 0.05 (13).

**RESULTS**

The phytochemical screening of _L. betulinus_ and _C. gallica_ revealed the presence of flavonoids, phenolic and steroid in the ethanolic extract of _C. gallica_, presence of tannin in aqueous extract of _C. gallica_. The presence of phenolic and steroid in ethanolic extract of _L. betulinus_, presence of saponin in aqueous extract of _L. betulinus_ and presence of flavonoids, steroids and tannin in petroleum ether of _L. betulinus_ as shown in Table 1.

The zones of inhibition in ethanolic extract and petroleum ether of _C. gallica_ against _P. vulgaris_ was 22.0 mm and 26.0 mm respectively. The aqueous extract of _C. gallica_ against _K. pneumoniae_ was recorded as 24.0 mm. The zone of inhibition in aqueous extract of _L. betulinus_ against _S. aureus_, _P. aeruginosa_ and _E. coli_ were 19.0 mm, 20.0 mm and 15.0 mm respectively while ethanolic extract of _L. betulinus_ inhibited _P. vulgaris_, _E. coli_ and _S. aureus_...
with inhibition zones of 22.0 mm, 10.0 mm, and 26.0 mm respectively. Petroleum ether extract of *L. betulinus* had a zone of inhibition of 20.0 mm and 23.0 mm against *P. vulgaris* and *P. aeruginosa* respectively.

However, Table 2 shows a comparative result of the antimicrobial activities of *L. betulinus* and *C. gallica* and some standard antibiotics. The results showed that the ethanolic extract of *L. betulinum* compared favourably with all the standard antibiotics used against *E. coli*.

### DISCUSSION

Phytochemical analysis revealed the presence of flavonoid, phenolic and steroids in ethanolic extract, tannin in aqueous extract of *Coriolopsis gallica*, presence of phenolic, steroids in ethanolic extract, saponin in aqueous extract, flavonoids, steroids and tannins in petroleum ether extracts of *Lenzites betulinus*. Previous report on flavonoids showed that they are known to be synthesized by plants in response to microbial infection (14). Hence, it is not surprising that they have been found to be effective as antibacterial substances against a wide array of infectious agents (15), which can be the reason why ethanolic extract of *Coriolopsis gallica* inhibits the growth of *Proteus vulgaris* due to the presence of flavonoid. Tannins (commonly referred to as tannic acid) are also known as antimicrobial agents. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein. In recent years, a large number of studies have been done on the antibacterial activity of phenolic compounds of natural origin (16). The growth of many fungi, yeasts, bacteria, and viruses were inhibited by this compound (17). The tannin present in aqueous extract of *C. gallica* can be responsible for its antimicrobial efficacy against the growth of *K. pneumoniae*.

The results of the antimicrobial activity of the extracts against the test organisms revealed that the ethanolic extract of *L. betulinus* and the petroleum ether extract of *C. gallica* have the highest zone of inhibition against *E. coli* and *P. vulgaris* respectively which implies that these extracts can be used in the treatment of both gastrointestinal and urinary tract infections.

### Table 1: Phytochemical screening of the mushroom extracts using three different solvents.

<table>
<thead>
<tr>
<th>Solvent for extraction</th>
<th>Samples</th>
<th>Saponin</th>
<th>Flavonoids</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td><em>C. gallica</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>L. betulinus</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous</td>
<td><em>C. gallica</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>L. betulinus</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td><em>C. gallica</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>L. betulinus</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- = Absent, + = Present

### Table 2: Antimicrobial activities of *C. gallica*, *L. betulinus* and standard antibiotics against bacterial isolates.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Standard antibiotics</th>
<th>Mushroom extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ampicillin</td>
<td>Trimethoprim</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>32.0 ± 0.01^d</td>
<td>25.0 ± 0.02^c</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>20.0 ± 0.02^b</td>
<td>31.0 ± 0.03^c</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>23.0 ± 0.02^d</td>
<td>14.0 ± 0.02^d</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>30.0 ± 0.03^d</td>
<td>25.0 ± 0.01^e</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>15.0 ± 0.02^d</td>
<td>24.0 ± 0.02^d</td>
</tr>
</tbody>
</table>

Values are means of triplicates ± SD. Samples carrying the same superscripts in the same row are not significantly different at (P ≤ 0.05). Aqueous extract of *Coriolopsis gallica* = S2A = Aqueous extract of Lenzites betulinus S1P = Petroleum ether extract of *C. gallica* = S2P = Petroleum ether extract of *L. betulinus*
The petroleum ether and ethanolic extract of *L. betulinus* and *C. gallica* inhibited the growth of *P. vulgaris*, this implies that these extract could be used in the treatment of urinary tract infection. Thus the antimicrobial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components. In the antibacterial susceptibility testing using the agar well diffusion method, absence of any clear zones of inhibition by the extract could be due to low concentration of diffusible compounds, time of collection of the mushroom samples and climate which might in turn affect the amount of constituents of the mushrooms. The high activity of the ethanolic extracts verifies the use of the ethanolic extraction method by local herbalists (18).

**CONCLUSION**

All the mushroom extracts have antimicrobial activities and thus confirmed the historical use of *C. gallica* and *L. betulinus* as antibacterial agent (19). The results of this study therefore form a good basis for selection of *C. gallica* and *L. betulinus* for further phytochemical and pharmacological investigation for their use as possible antimicrobial agents in the treatment of gastrointestinal, urinary tract and skin infections. With ever increasing momentum in the quest for newer antimicrobial agents, to counteract the rise in bacterial drug resistance, mushrooms are being increasingly explored in many parts of the world (20). These mushrooms may offer a new source of potential activity against infective microorganisms. Further research is needed for the identification of bioactive molecule present in the two mushroom samples and in vivo efficacy of the mushroom before it is used for commercialization.

**REFERENCES**