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Synergistic Antibacterial Activity of *Pleurotus* Species (Mushroom) and *Psychotria microphylla* (Herb) against Some Clinical Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Author OEUI designed the study, wrote the protocol and served as the principal investigator. Author OO was the principal supervisor. Author UAI made a conceptual contribution as well as study design. Author AUN made a conceptual contribution and handled data analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigated the synergistic antimicrobial activity of the extracts of *Pleurotus* species and *Psychotria microphylla* against five clinical bacterial isolates: *Escherichia coli, Pseudomonas aeruginosa, Salmonella* Typhi*, Klebsiella pneumoniae* and *Staphylococcus aureus* from Federal Teaching Hospital, Abakaliki (FETHA), Ebonyi State, Nigeria.
Study Design: This was an experimental study.
Place and Duration of Study: This study was carried out in the Department of Applied Microbiology Laboratory, Ebonyi State University, Abakaliki, Nigeria between October, 2014 and December, 2014.
Methodology: Antimicrobial components from the mushroom and herb were extracted with water and ethanol. In this, the antimicrobial activities were examined by agar well diffusion method adapted from Kirby Bauer disk diffusion technique against confirmed isolates of *Escherichia coli, Pseudomonas aeruginosa, Salmonella* Typhi*, Klebsiella pneumoniae* and *Staphylococcus aureus.*

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Mixtures of the different extracts (mushroom and herb) at varying ratios (of 1:1, 1:2, 2:1) were also used to determine their synergistic inhibitory effects at 37ºC and 24 hours incubation. Inhibition zone diameters were measured and recorded.

Results: The result revealed antibacterial activity of the extracts although at varying degrees. All the isolates (except *Klebsiella pneumoniae*) were sensitive to *Psychotria microphylla*. The highest sensitivity (7.0 mm) with ethanol extract of the herb was observed against *Pseudomonas aeruginosa,* closely followed by *Salmonella* Typhi (5.0 mm) and then *Escherichia coli* and *Staphylococcus aureus* (4.0 mm each). Although the herb showed a wider range of antimicrobial activity against the tested isolates than the mushroom, *Klebsiella pneumoniae* was sensitive (6.0 mm). The herb-mushroom extract combination produced a better antimicrobial activity against the isolates with a very high sensitivity of 16.0 mm recorded against *S. aureus* as compared to the highest sensitivity (10.00 mm) produced by Ampiclox against *S.* Typhi. The activity of *Pleurotus* sp was independent of the extract (p-value=0.189), meaning that the difference in zones of inhibition in relation to extract types was not significant (p>0.05). *P. microphylla* activities was dependent on the type of extract (p-value=0.031).); that is, the difference in zones of inhibition produced by *P. microphylla* in relation to extract types was significant (p<0.05). The most efficient ratio of synergy was the 1:2 ratios which involved one part of *Pleurotus* species and two parts of *P. microphylla*.

Conclusion: The synergistic effect produced by *Psychotria microphylla* and *Pleurotus* species holds a good promise in the treatment of infections caused by tested bacteria and should be harnessed and patented.

Keywords: Mushroom; synergy; antimicrobial activity; Psychotria microphylla; Pleurotus sp.; herb; ampiclox.

1. INTRODUCTION

Microbial infections are the major cause of morbidity and mortality worldwide [1]. Infectious diseases have lived with man since antiquity although several natural as well as synthetic drugs have been developed, patented and used to combat the menace over these periods [2]. The prevalence of emerging and re-emerging infectious diseases is of growing public health concern globally. However, of greater public health concern is the high prevalence, spread and continuous threat of antimicrobial resistance among the agents (pathogens) of infectious diseases [3]. This has rendered almost all known and available antimicrobial agents ineffective, hence the incessant need for novel drugs which can adequately curb this challenge [4].

Historically, medicinal plants have been a major source of novel drug compounds and plants-derived products have made significant contributions to human health and well-being [1]. A number of medicinal plants and mushrooms have been screened for antimicrobial activity in recent years and efforts have been made to identify their bioactive constituents [1,4-6]. Herbal plants have been found to have antimicrobial activities for example: *Persea Americana* (Avocado pear), *Didemnum psammathodes* (cowries' cypress spp) to mention but a few [7].

Macro fungi (mushrooms) have been known and used as a valuable source of food and traditional medicines since antiquity [8-10]. About 75% of edible mushroom and herbs has shown reasonable antimicrobial activity [11]. Mushroom and herbs alike has been effective in inhibiting both Gram-positive and Gram-negative bacteria in vitro and hence recently they have been incorporated in chemotherapeutic agents used in treatment of various bacterial diseases.

In comparison to synthetic drugs, natural drugs of plant origin have fewer side effects, inexpensive, show better patients' tolerance and are readily available for low socioeconomic population [12-13].

Although the *In vitro* therapeutic indices or values of many plants, herbs and mushrooms extracts have been widely researched upon and reported [4,14-27], there are almost unavailable reports, especially in Nigeria, regarding the combined or synergistic therapeutic effects of two or more of these plants. In many places in Nigeria, a combination of two or more plants or herbs has been used to treat various infections and diseases.

Therefore, the aim of this study was to determine or investigate the synergistic antibacterial activity of the edible mushroom, *Pleurotus* spp. and

Psychotria microphylla against some clinical isolates in Abakaliki, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

The basidiocarp of the oyster mushroom was grown naturally using (Osu) and the herb was collected in uncultivated forest of Ekoli Edda Ebonyi state in the area where they grow naturally. Macroscopic identification of the sample was performed by a reputable botanist.

2.2 Preparation of Sample Extracts of the Edible Mushroom and Herbs

The mushroom and herb sample was ground into fine powder. Twenty grams of the pulverized sample was soaked separately in 200 ml of various solvents (aqueous and ethanol) at room temperature for three days and filtered with muslin cloth. The extracts were recovered by freeze drying. The residue was then collected and stored for further use.

2.3 Microorganism Tested

The clinical isolates used in the present study are as follow: *Escherichia coli, Pseudomonas aeruginosa, Salmonella* Typhi*, Klebsiella pneumoniae* and *Staphylococcus aureus*. The bacterial isolates were collected from Federal Teaching hospital, Abakaliki, Ebonyi State and were characterized using basic microbiological techniques as described by Cheesbrough [28].

2.4 Screening for Antibacterial Activity of Mushroom and Herbal Extract

The test microorganisms were activated/pre-incubated on Mueller Hinton Broth (at 37ºC for 24 hours). The 0.5 McFarland standards was used to adjust the turbidity to prepare inoculum from overnight grown bacteria. The mushroom and herbal extracts were dissolved in distilled water. The medium (Mueller Hinton agar) was prepared according to the manufacturer's specification (Oxoid Company, UK). The antibacterial activities of the extracts were determined by agar well diffusion by standard technique [29]. In this, holes (known as wells) of about 6mm were aseptically bored using heat-sterile cork borer and sterility test was performed before inoculating the isolates on the media. Petri plates containing 20 ml Muller Hinton agar medium were seeded with 24-hour culture of bacterial strains. Twenty micro litre (20 µl), equivalent to 100 mg/ml of the aqueous and ethanol extracts of both the *Pleurotus* spp. and *Psychotria microphylla* were added to each of the wells. Different ratios of the *Pleurotus* spp. and *Psychotria microphylla* were combined and the combination also added to different wells and noted. Standard antimicrobial agent, Ampiclox (100 mg/ml) was used as positive reference for the bacterial isolates. The plates were then incubated at 37ºC for 24 hours. Antimicrobial activity was determined by measuring the diameter of the clear inhibition zone around each well [9,30] by standard procedures [31]. After incubation the zone of inhibition was measured and the result obtained was compared with the reference antibiotics.

2.5 Statistical Analysis

The data were analyzed and significant difference (at 0.05 level of significance) was determined using Independent Sample T-test. Statistical package used was SPSS (Scientific Package for Social Sciences) version 16.0.

3. RESULTS AND DISCUSSION

The antimicrobial effects of the aqueous and ethanol extracts of edible mushroom *Pleurotus* sp. and *Psychotria microphylla* were tested against five clinical isolates including: *Escherichia coli, Pseudomonas aeruginosa, Salmonella* Typhi*, Klebsiella pneumoniae* and *Staphylococcus aureus*. The results showed that the ethanol extracts of the *Psychotria microphylla* showed inhibitory effects against all the bacterial isolates tested except *Klebsiella pneumoniae*, which showed no sensitivity at all to both the ethanol and aqueous extracts of the herb. This result can be compared with the work of Zhu et al. [32] in which various herbs were tested and they were able to inhibit various bacterial isolates used against them. The highest sensitivity was recorded against *Pseudomonas aeruginosa* with inhibitory zone diameter of 7.0 mm, closely followed by *Salmonella* Typhi (5.0 mm), and then *Escherichia coli* and *Staphylococcus aureus* (4.0 mm each). Details of the result are shown in Table 1. *P. microphylla* activities was dependent on the type of extract (p-value = 0.031), meaning that the difference in zones of inhibition produced by *P. microphylla* in relation to extract type is significant ($p<0.05$).

However, for the edible mushroom, *Pleurotus* sp., virtually all the clinical isolates used were

resistant to both the aqueous and the ethanol extracts except for *Klebsiella pneumoniae*, which was sensitive showing 6 mm inhibition zone diameter with the aqueous extract and 3 mm for the ethanol extract (Table 1). This result is in consonance with Li et al. [33] and Imitiaj [34] which showed that the fruiting bodies of mushroom has antimicrobial activity against various bacterial isolates. Comparatively, the aqueous extract of the mushroom produced more inhibitory effect (6.0 mm) than the antibiotic (5.0 mm) against *Klebsiella pneumoniae*. This is also observed in a similar work performed by Iwalokun et al. [35]. The activity of *Pleurotus* species was independent of extracting solvent (p-value=0.189), meaning that the difference in zones of inhibition in relation to extracting solvent is not significant (p>0.05). The insignificant difference associated with *Pleurotus* spp. may be due to the discrepancies in the zones of inhibition produced by both the aqueous and ethanol extract of the plant. In both extracts, majority of the organisms produced zero zone of inhibition.

From Table 1 below, it was observed that the zones of inhibition (which connote antimicrobial strength) of the ethanol extract of *Psychotria microphylla* was highest against all the test organisms as compared to aqueous extract. The study revealed that the ethanol extracts gave the maximum activity when compared to the aqueous extracts. This might be due to the fact that ethanol is a better solvent for extracting the active ingredients from the herbs than water. This result is in agreement with the findings of Ogunmoyole et al. [36]. There was no significant difference between the aqueous extract of both plant (p>0.05). Also, no significant difference was observed in the activity of the ethanol of both plant (p>0.05). It was also observed that *P. microphylla* extract produced better and comparable zones of inhibition against the isolates in relation to the reference antibiotic used. The most comparable result to the reference antibiotic was that of the ethanol extract of *P. microphylla*. Thus, ethanol extract of *P. microphylla* is the most efficient of all the extracts against the test organisms.

Among the isolates tested *S. aureus* was more susceptible to the combination of the herb and mushroom followed by *S.* Typhi but *P. aeruginosa* had the least susceptibility followed by *Klebsiella* sp (Fig. 1). It was noted also that the ratio of *Psychotria microphylla* and *Pleurotus* sp. that gave the highest antimicrobial activity was the ratio of 2:1 followed by 1:1 which gave 16 mm and 15 mm respectively against *S. aureus* but for *S.* Typhi the ratio of 1:2 gave 10 mm whereas 2:1 gave 9 mm. The herb-mushroom extract combination produced a better antimicrobial activity against the isolates with a very high sensitivity of 16.0 mm recorded against *S. aureus* as compared to the highest sensitivity (10.00 mm) produced by Ampiclox against *S.* Typhi. This might be due to the synergistic multi target effect theory that states that single constituent of a mono-extract or multi-extract combination affect not only single target but several targets and therefore results in an antagonistic or synergistic response [37]. From Fig. 1, the most susceptible organism to the synergistic action of the plant extracts was *S. aureus*. This can be seen from the insignificantly (p>0.05) improved zones of inhibition of the three different ratios of the extracts when compared to the other organisms and the reference antibiotics. *S. aureus* is a gram positive agent while other isolates used in this study where gram negatives. The improved synergistic action of the extracts against gram positive organism supports the hypothesis that the plant extracts contains chemical components which can be harnessed and used in the production of antibiotics against infections caused by gram positive agents.

Table 1. The antibacterial activities of the *Psychotria microphylla* and *Pleurotus* sp. extracts (zone of inhibition in mm)

S/N	Isolate	*Pleurotus* species (mm)		*Psychotria microphylla* (mm)		Ampiclox
		Aqueous	Ethanol	Aqueous	Ethanol	
1.	*Staphylococcus aureus*	0.00	0.00	1.00	4.00	5.00
2.	*Salmonella* Typhi	2.00	0.00	0.00	5.00	10.00
3.	*Pseudomonas aeruginosa*	0.00	0.00	3.00	7.00	5.00
4.	*Escherichia coli*	0.00	0.00	2.00	4.00	4.00
5.	*Klebsiella pneumoniae*	6.00	3.00	0.00	0.00	5.00

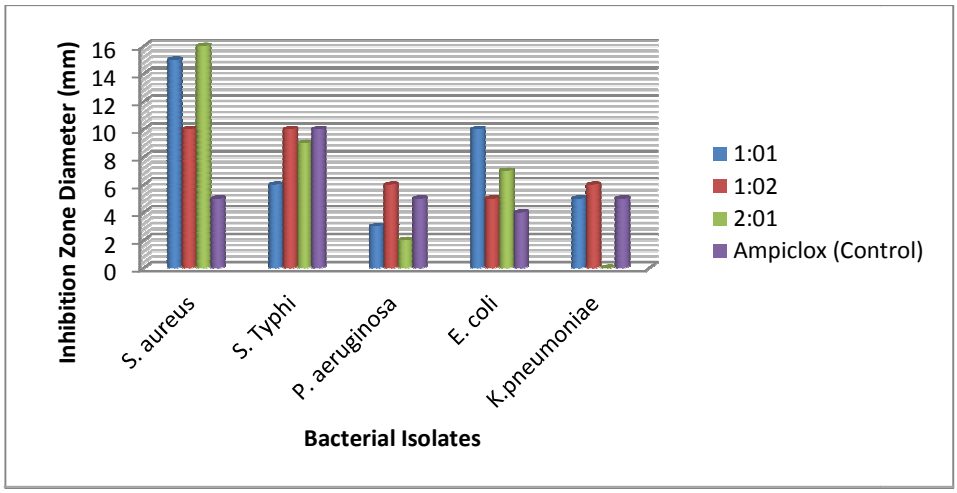

Fig. 1. The synergic activity of the combination of the ethanol extract of *Psychotria microphylla* and *Pleurotus* sp. (Zone of inhibition in mm)

The synergistic property of the extracts can be seen against *S. aureus* where the inhibition zone diameter at varying ratios were higher than that of the control antibiotics used (Fig. 1). Again this might be due to the bio active compounds present in the herbs as well as the mushroom as reported by Jang and Hyung [38]. In their study, they observed the presence of saponin and flavonoid in the extracts. Research has it that the presence of saponin and flavonoid in a given extract promotes synergistic action [39]. The combination of eight Chinese herbs as performed by Yang et al. [40] has confirmed the better effectiveness of the herbs when used in pairs than when used individually. Also, the treatment of various ailments like malaria, HIV/AIDS, even cancer promotes the advantages of combination therapy in such treatments. Toews and Bylund [41] further highlight the importance of combination of drugs either to promote absorption as in pharmacokinetics or availability as in pharmacodynamic. The same principles play out in this work as the herbal sample and the mushroom sample was combined boosting their antibacterial effect against the isolates.

In this study, the most efficient ratio ($p<0.05$) of synergy was the 1:2 ratio which involves one part of *Pleurotus* species and two parts of *P. microphylla*. This can be seen from Fig. 1 in which there were a clear, improved and well defined zone of inhibition against each organism as compared to other ratios and that of the extracts when used singly. A good example is the synergistic action of the extracts in 1:2 combinations against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as compared to the zones of inhibition of the extracts used singly against the organisms.

4. CONCLUSION

The study showed that the antibacterial activity of the plant or mushroom extract was improved when they were combined and suggests that the best way of controlling drug-resistant microorganisms is the use of combination therapy. It further shows that the edible mushroom and the herb have high antibacterial potentials and can serve as good natural antimicrobial agent against infection caused by the test isolates and such properties can be further explored and harnessed in the pharmaceutical industries. The synergistic effects produced by the combination of *Psychotria microphylla* and *Pleurotus* species holds a good promise in the treatment of infections caused by tested bacteria. It is therefore, recommended that these potentials in the two extracts be harnessed to curb the rampant menace of antimicrobial resistance. The study silently revealed that water and ethanol are good solvents each for the full exploitation of the therapeutic ingredients of *Psychotria microphylla* and *Pleurotus* species respectively.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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