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Research Article

In Vitro Antifungal Susceptibility Indices of *Tetrapleura tetraptera* on Cucumber Fruits Isolates

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Abstract
The increasing prevalence of fungal pathogens affecting cucumber fruits necessitates the exploration of alternative antifungal agents, particularly from natural sources. This study investigates the in vitro antifungal efficacy of *Tetrapleura tetraptera* extracts against fungal isolates from infected cucumber fruits. The in vitro antifungal susceptibility indices of *Tetrapleura tetraptera* ("Uhiokrihio/Oshosho" in Igbo) on cucumber fruits isolates were determined using standard microbiological methods. A total of one hundred and twenty-five (125) samples were randomly picked from five (5) different markets/ hawking points, transported in ice bags to microbiological laboratory for analysis. The mean fungal count ranged from $1.0 \times 10^3 \pm 0.25/\pm 0.32$ to $5.4 \times 10^3 \pm 0.12$. Four (4) fungal species were isolated and identified to include *Aspergillus* species, *Penicillium* species, *Fusarium* species, and *Mucor* species. The duo (aqueous and ethanolic) extracts of *Tetrapleura tetraptera* (Uhiokrihio/Oshosho) exhibited potency at varying concentrations on the zones of inhibition on the test isolates. Ethanolic extracts were more potent with higher zones of inhibitions than aqueous extracts. *Penicillum* species had a different MIC that is lower in concentration to other isolates. On the reason that these fruits are consumed fresh, and are potential sources and vehicles of transmission of various microbial pathogens, there is need to incorporate *Tetrapleura tereptera* extract as spices/ additives to most fruits preparations, cookings and food formulations. Since cost of affording good health care is high, the incorporation of this spice will help boost immunity, protect unsuspecting consumers from ravaging infections associated with fruits consumption, and avert the imminent health risks caused by synthetic drugs since the extract is of natural origin and plant based. Finally, as a precautionary measure, fruits should be washed with salt-water for disinfection and transit eating of fruits should be discouraged.

Key words: Cucumber; Pathogen; Antifungal; Public health; Extract.

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1.0 Introduction

The global demand for sustainable agricultural practices has fueled interest in natural antifungal agents as alternatives to synthetic chemicals. Cucumber ("Okoko" in Igbo) (*Cucumis sativus*) is a herbaceous vine of the botanical family Cucurbitaceae widely cultivated for its edible fruit. It is a dark green fruit, elongated and cylindrical with rounded ends and a slightly bumpy skin, and inside it is a crisp white flesh surrounding the seeded core. Cucumbers are eaten unripe since ripe specimens lose their crispness and become spongy and yellow. Cucumber provide a lot of nutrients but are low in calories, fat, cholesterol, and sodium. Cucumbers have a mild, refreshing taste and a high-water content which makes it pleasant to eat in hot weather (John & Mullinix, 2008). Cucumber has a lot of dietary and health benefits ranging from weight loss to cancer treatment and even skin care. Cucumber and other green vegetables contain chlorophyll, a pigment that gives plants their green color, and had been identified with strong history in connection with reduced risk of cardiovascular disease as well as cancer diseases, including breast, uterine, ovarian, and prostate cancers (Lee, 2010). Its high-water content promotes hydration which helps the body to flush out toxins and keep the skin healthy. Cucumbers are also rich in vitamin B, which cares for acne and other skin problems. They also contain minerals, amino acids, phytosterols, phenolic acids, fatty acids, cucurbitacins and other vitamins (Abiodun, 2010).

Drug resistance and associated negative health effects of synthetic antimicrobials has been a major challenge in the use of antimicrobials. It has remained a major concern to pharmaceutical microbiologist and herbal medicine scientists. Plants have emerged as potential replacement as suitable alternatives for the synthetic antimicrobials. Some plant materials exhibit antifungal properties. *Tetrapleura tetraptera* ("Uhiokrihio/Oshosho" in Igbo) is one of those medicinal plants ("Osisi ogwu" in Igbo) in Nigeria. The presence of bioactive substances (Kigigha *et al*., 2015) and phytosterols accounts for the therapeutic potentials of such plants. The bioactive substances include saponins, tannins, flavonoids, cyanogenic glycosides, alkaloids, and steroids. The biological or pharmacological activities of *Tetrapleura tetraptera* was used in the management of cardiovascular, neuromuscular, hypotensive, convulsion, ulcerative, hypoglycemia, inflammation, rheumatoid pains and microbial diseases (Enaregha *et al.*, 2021; Achi, 2006). Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although, the statistical rise in precise public health risk and estimated health care cost is a difficult task. The emergent of antimicrobial resistance is a serious global problem as susceptibility testing remains an area of intense interest. Susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome (Berkow *et al*., 2020).

Due to health related issues pertaining to high mortality among the middle aged, there is high rate of fruits consumption in order to maintain a healthy living and shedding of weights unlike what is associated with consumption of conventional foods. Nevertheless, there is serious concern about the food safety and peculiarity of consumed fresh fruits and vegetables, as they are consumed fresh without further heat treatment. This has projected most fruits as potential sources and vehicles of transmission of various microbial/ foodborne pathogens. On this note, there is need to ascertain the potency of selected plant-based antifungals on identified isolates. Therefore, this novel study is targeted at evaluating the in vitro antifungal susceptibility indices of *Tetrapleura tetraptera* (Uhiokrihio/Oshosho) on cucumber fruits isolates.

2.0 Materials and methods

2.1 Study area

The study area is Aba Metropolis, Abia State, in the South-Eastern Nigeria. The Aba town is a major commercial center ("Azuma-ahia" in Igbo) in Eastern Nigeria, of the Igbo tribe and inhabited by Ngwa people. The geographical coordinates are 5.1215^{o}N and 7.3732^{o}E.

2.2 Collection of samples

A total of one hundred and twenty-five (125) samples of cucumber fruits were bought at random from five different markets/ hawking points in Aba metropolis in the Southeastern Nigeria, and were transported in ice bags to microbiology laboratory for analysis.

2.3 Sample preparation

The cucumber samples prior to culturing were all-surface-swapped and disengaged in a test tube containing diluent as stock and serially diluted. Ten-fold serial dilutions of the cucumber samples were done using sterile peptone water as diluent. One milliliter (1 mL) each of the samples was aseptically transferred into a sterile test tube containing nine milliliters (9 mLs) of sterile peptone water, stirred with sterile glass rod and shaken vigorously to ensure adequate disengagement of entrapped microorganisms to obtain 10^{-1} dilution. Serial dilutions of the homogenates were continued and made

step-wisely till the fourth (4th) tube, to obtain dilutions of 10^{-1} to 10^{-4}.

2.4 Microbiological analysis of samples

Spread plate technique of (Cappucino & Sherman, 2010) was used to enumerate fungi in the samples. One milliliter (1 mL) each from the dilution was plated in replicates using fortified Sabouraud's dextrose agar (SDA) for mean fungal count. The plates were incubated at 25±2 °C for 120 hours for mean fungal counts. The purified fungal isolates were identified on the basis of macroscopic and microscopic characteristics by slide culture technique and lactophenol staining. The scheme of Kidd *et al.* (2005) was used for the identification. Isolated test strains for susceptibility test after proper identity were further purified and maintained in fortified SDA prior to use.

2.5 Plant material preparation

Pods of *Tetrapleura tetraptera* (Uhiokrihio/Oshosho in Igbo) were obtained from commercial sources and identified by a botanist in the University. The pods were further dried, cut into smaller pieces, and were grinded to fine powder using a high power electric grinder.

2.6 Bioactive extraction

Fifty (50) grams of milled powder was soaked in 250 mL of distilled water for 72 hours at room temperature to obtain aqueous extract, while another 50 g of the milled powder was soaked in 70 % v/v ethanol for 72 hours at room temperature to obtain ethanolic extract. After soaking, both extracts were filtered using muslin cloth followed by Whatman No.1 filter paper. The solvents were evaporated in an evaporator to obtain dry extracts. The dried extract was re-suspended in dimethyl sulfoxide (DMSO) to obtain various concentrations of both aqueous and ethanolic extracts, which were used in the experiment.

2.7 Qualitative phytochemical screening of *Tetrapleura tetraptera* ("Uhiokrihio/Oshosho" in Igbo) constituents

Extract components were subjected to standard phytochemical analyses for the presence of different constituents (Edeoga *et al.*, 2005; Evans, 2002; Harborne, 1984). The presence of the following constituents; alkaloids, tannins, saponins, glycosides and flavonoid were determined.

2.8 Sensitivity test

The disc diffusion method as described by CLSI (2019) and Hudzicki (2009) was employed. A 16-18 hours culture, which was sub-cultured twice onto Sabouraud's dextrose agar (non-selective medium) was diluted with sterile physiological saline solution (0.85 % (w/v) sodium chloride) with reference to McFarland standard. A 0.5 McFarland standard is equivalent to a bacterial suspension which represented 1.5×10^8 containing between 1×10^8 and 2×10^8 CFU/mL of Escherichia coli. The standard was prepared by adding an aliquot of 0.5 ml of $BaCl_2$ (1.175% w/v $BaCl_2.2H_20$) to 99.5 ml of H_2SO_4 (1% v/v) with constant stirring to achieve an inoculum of approximately 1×10^8 CFU/mL.

Using spread plate technique, a prepared Mueller-Hinton agar with 2% glucose and 0.5 µg/ml methylene blue dye (GMB) for maximum reproducibility of testing from dehydrated media were poured to an approximate depth of 4 mm (approximately 25 ml of liquid agar for 100-mm plates). The addition of glucose supports fungal growth, and the addition of methylene blue dye enhances the zone edge definition. Caution: Plates that are too shallow will produce false susceptible results as the antimicrobial compound will diffuse further than it should, creating larger zones of inhibition. Conversely, plates poured to a depth greater than 4 mm will result in false resistant results.

A sterile swab was dipped into the inoculum tube, rotated at the side of the tube to remove excess fluid, and then inoculated by streaking the swab three times over the entire agar surface of a pre-dried Muller Hinton agar plates. The plates were rotated at approximately 60 degrees each time to ensure even distribution of the inoculum. The inoculated plates were rimed with the swab to pick up any excess liquid, and the swab discarded into appropriate container. The plates had the lid slightly at ajar and allowed to stand at room temperature for at least 3 to 5 minutes for the surface of the agar plate to dry.

Sterile commercial filter paper disc of 6-8 mm were used to absorb the extract samples re-suspended in DMSO. The appropriate antimicrobial-impregnated disks were placed on the surface of the agar, using forceps to dispense each antimicrobial disk one at a time. After allowing for an hour contact time at room temperature for the samples to diffuse across the surface, the plates were incubated at 37 ^oC for 24 hours. The zone of inhibition was measured in millimeter with meter rule or vernier calliper.

2.9 Statistical analyses

All obtained data in this study were analyzed using analysis of variance (ANOVA). Descriptive statistics in form of mean, standard deviation, and Duncan post hoc were also used to assess the data, and analyses were done using SPSS version 20 (Statistical Product and Service Solutions).

3.0 Results

The result of the mean fungal count (MFC) for cucumber fruits sold in Aba metropolis is shown in Figure 1. The highest fungal count was observed in samples obtained from Ariaria market (C5) 5.4 $x10^3$ ±0.12 while the lowest fungal count was observed in samples obtained from Afule market (C5) 1.0 $x10^3$ ±0.25 and Ahiaohuru market (C1) 1.0 $x10^3$ ±0.32. Four (4) fungal isolates were identified with cucumber fruits to include: *Aspergillus* species, *Penicillum* species, *Fusarium* species, and *Mucor* species. All data obtained were statistically analyzed and compared, with values of same alphabets as not significant ($p>0.05$), while those with different alphabets as significant ($p<0.05$).

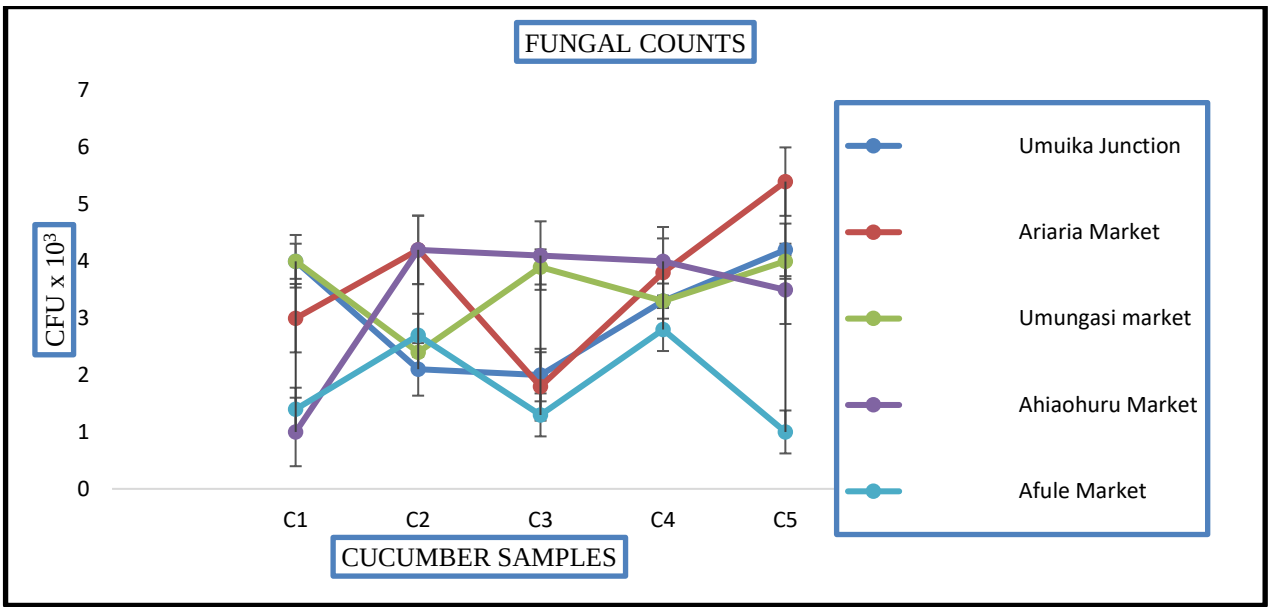


Figure 1. Mean fungal counts of cucumber fruits

Recommended Standard Counts: Fungal count (fc) = ≤ 10^4/g (FSANZ, 2018; PHLS, 2000). Key: C = cucumber sample

The qualitative screening of *Tetrapleura tetraptera* (Uhiokrihio/Oshosho) fruit is shown in Table 1. The phytochemical screening of *Tetrapleura tetraptera* fruit revealed the presence of some bioactives such as saponins, alkaloids, tannins, glycosides and flavonoid (Table 1). These bioactives were present in both extractive solvents (aqueous and ethanol) used.

Table 1. Qualitative screening of *T. tetraptera* fruit

Solvent	Saponins	Alkaloids	Tannins	Glycosides	Flavonoid
Aqueous	+ve	+ve	+ve	+ve	+ve
Ethanol	+ve	+ve	+ve	+ve	+ve

Legend: +ve = present and –ve = absent

The minimum inhibitory concentration (MIC) of both aqueous and ethanolic extracts of *Tetrapleura tetraptera* (Uhiokrihio/Oshosho) is shown in Figure 2. The minimum inhibitory concentration (mg/mL) of extracts of *Tetrapleura tetraptera* for the tested isolates is as follows *Aspergillus* species (200), *Penicillum* species (150), *Fusarium* species (200), and *Mucor* species (200). *Penicillum* species had a different MIC that is lower in concentration to other isolates. Generally, ethanolic extracts were more potent, with higher zones of inhibitions than aqueous extract of same concentration, and Figure 2 has shown that *Tetrapleura tetraptera* extracts has antifungal activities on isolates.

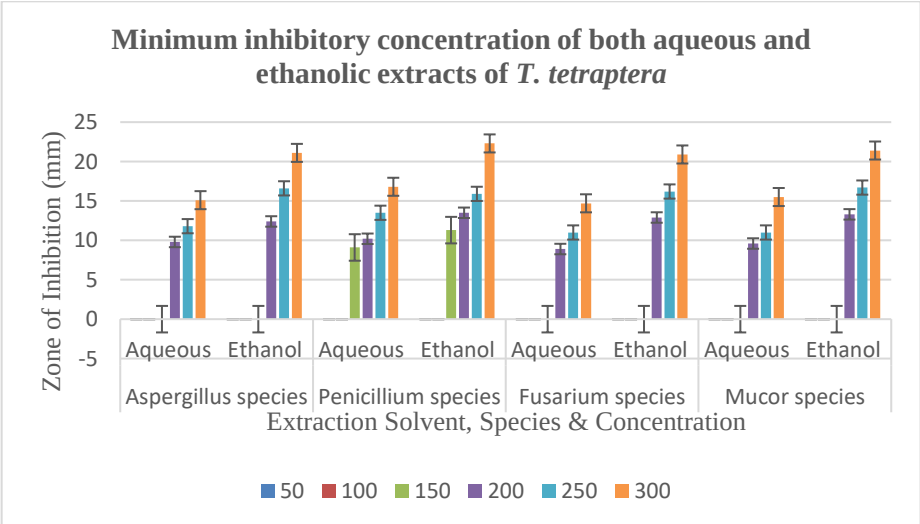


Figure 2. Minimum inhibitory concentration of both aqueous and ethanolic extracts of *T. tetraptera*

4.0 Discussion

Cucumbers is a pleasant fruit that is hawked along the highways and junctions where there are traffics and pot holes that slows vehicular movements, and also sold in the markets. Notwithstanding, its dietary and health benefits, it has its challenges especially as it concerns microbial contamination and deterioration ("orure/ire-ere na mmebi" in Igbo). Most of those that patronizes the hawked fruits especially the travelers, eat this fruit raw while on transit. This is really a public health concern considering the high microbial loads recorded in the obtained results of this study.

The prevailing dirty environments and poor hygiene practices associated with its transportation and storage are majorly the reasons for the high fungal counts recorded in the result. Fungi has been accused to be responsible for the deterioration and spoilage of most fruits. The pH of such fruits were always supporting the activities of these fungi. The presence of the presented fungal isolates is an indication of poor storage conditions. The presence of *Aspergillus*, and *Penicillium* species points to flora of surrounding environment as was reported by Ike *et al*. (2017). Aspergillus have been reported to produce potent mycotoxins responsible for various mycotoxicosis in humans (Abbey, 2006; Efiuvuwevwere, 2000). The presence of the isolated fungal species in this work could be attributed to the poor surrounding environment, hence the obtained results were similar to previous reports of Ike *et al.* (2017; 2015). The reports of Jay *et al*. (2005) that fungi survive in low pH conditions, could attribute to the recorded high fungal counts in the study.

Recently, there is growing concern about safety of consumed fruits, taking a holistic assessment of the retailing environment, and the peculiarity of cucumber fruits, as they are consumed fresh without further treatment. This singular attribute most times makes fruits a major vehicle for transmission of foodborne pathogens. On this note, there is need for consumers to incorporate extracts of *Tetrapleura tetraptera* (Uhiokrihio/Oshosho) as spices into fruits processing or in formulation of most diets/foods as a back-up inhibitory agent to ingested fungal pathogens. This is necessary because of several health effects caused by synthetic drugs which has caused the recent drift in search of natural antimicrobials (Burt, 2004), which is resident in plant materials.

Tetrapleura tetraptera (Uhiokrihio/Oshosho) has been found to possess some bioactive components that exhibited great antimicrobial effect and inhibitory activities on the identified isolates of this study. The bioactive compounds found present in *Tetrapleura tetraptera* extract of this study include saponins, alkaloids, tannins, glycosides and flavonoid. This did not agree with the findings of Achi (2006) that reported the presence of tannins and glycoside and absence of alkaloids, saponin, and flavonoids in both cold water and ethanol extracts of *Tetrapleura tetraptera* whole pod. However, the reported result of bioactive components in Tetrapleura tetraptera (saponin, tannin, alkaloid, cyanogenic glycosides and flavonoids) of this study was in in agreement with the findings of Ekwenye and Okorie (2010).

The antifungal activity of *Tetrapleura tetraptera* (Uhiokrihio/Oshosho) as evidenced in the study is in agreement in pattern with previous work of Ogodo *et al*. (2017), in the study of "antimycotic and antibacterial activity of *Aframomum melegueta* seed extracts against bacteria and fungi species from food sources". Other reports in agreement in potency were the works of Ikegbunam *et al*. (2016) on the "evaluation of the antifungal activity of aqueous and alcoholic extracts of six spices"; and Okigbo and Ogbonnanya (2006) on the "antifungal effects of two tropical plants extracts *Ocimum gratissimum* and *Afromaomum melegueta* on post-harvest yam *Discorea* species rot". According to Okigbo and Ogbonnanya (2006), the two plant extracts in that study inhibited the spores of *Fusarium oxysporum* and *Aspergullus niger* as ethanolic extracts of *Ocimum gratissimum* inhibited more than that of *Aframomum melegueta*, with less inhibition coming from plant extracts of cold water. There are few reports on the potency of *Tetrapleura tetraptera* against bacterial species, but there are none in existence against fungal species. Considering the diverse relationship of fruits with fungi, there is need for more investigations on the activities of plant extracts against many associated fungi species. However, extracts of *Tetrapleura tetraptera* has shown synergy in potency against mycological species when compared with findings from researchers on other plant extracts.

The zones of inhibition in this study are different from the ones previously reported by authors. However, variations in zones of inhibition could be due to the following differences, which is not limited to; type of plant species, type of solvent used, extraction method applied, concentration of the solvent used for extraction, age of plant material, isolates used and other environmental factors. In overall performance, ethanolic extract of *Tetrapleura tereptera* (Uhiokrihio/Oshosho) was more effective than aqueous extract in creating zones of inhibition at same concentration. This is an indication that ethanol has more extracting capacity when compared with aqueous solvent.

The observed antifungal activity can be attributed to the presence of bioactive phytochemicals, corroborating previous studies on *T. tetraptera*'s antimicrobial properties. This aligns with the philosophical doctrine of naturalism, which emphasizes the explanatory power of natural substances in addressing real-world challenges.

Moreover, the study underscores the interplay between empirical data and theoretical frameworks. While empiricism focuses on observable outcomes, a Kantian perspective suggests that the efficacy of *T. tetraptera* extracts is not merely empirical but also a testament to the harmony between nature's design and human ingenuity.

It is advisable that cucumber fruits should be properly washed with salt-water before consuming them as it is a transient host to variety of pathogens. Finally, this study has exposed the importance of incorporating

Tetrapleura tereptera (Uhiokrihio/Oshosho) extracts as spices into diets, deserts and food/fruit formulations, as this practice will not only boost the immunity of unsuspecting consumers but will save the populace from imminent health risks.

5.0 Conclusion

Due to health related issues among the middle aged, there is high rate of fruits consumption in order to maintain healthy living, and shedding of weights that is associated with consumption of conventional foods. Fruits are consumed fresh, and are identified as potential sources and vehicles for transmission of various microbial pathogens as fruit spoilage are majorly caused by fungi, there is need to incorporate *Tetrapleura tereptera* (Uhiokrihio/Oshosho) extract as spices/ additives to fruits preparations, cookings and food formulations since the cost of affording good health care in this part of the world is high. The incorporation of this valuable spice into fruits preparations and formulations will boost body immunity and avert the imminent health effects caused by synthetic drugs since the extract is of natural origin and plant based.

Finally, as a precautionary measure, fruits should be washed with salt-water for disinfection and transit eating of fruits should be discouraged. While future studies should explore the synergistic effects of *T. tetraptera* with other natural antifungal agents and its applicability in field conditions. The philosophical implications of using plant-based solutions for crop protection, challenge the anthropocentric paradigm, advocating for a more holistic view of agricultural ecosystems.

6.0 Acknowledgments

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7.0 Disclosure of conflict of interest

There is no conflict of interest

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