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# Assessment of the antistaphylococcal properties and bioactive compounds of raw and fermented *Trametes polyzona* (Pers.) Justo extracts

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## ABSTRACT

*Trametes* species are polyporoid white rot fungi that are important in medicine as well as serving as bioremediator and biodegrader of cellulosic waste. The present study investigates the bioactive compounds and antistaphylococcal properties of extracts obtained from raw and fermented *Trametes polyzona*. *T. polyzona* was collected from dead woods in farmland around Akungba-Akoko (7°27' 32.4''N 5° 45' 07.8''E) and subjected to submerged and solid-state fermentation. The raw and fermented samples were extracted using acetone and methanol. The mycochemical and antistaphylococcal properties of the extract obtained from raw and fermented *T. polyzona* were assessed using standard methods. Extract obtained from *T. polyzona* subjected to submerged fermentation exhibited higher and better antistaphylococcal activity (28.00 mm) on *Staphylococcus aureus* isolated from blood when compared to other extracts. The minimum inhibitory concentration of the extracts against *Staphylococcus aureus* ranged from 100mg/mL to 400mg/mL. Gas chromatography Mass Spectrophometric (GC-MS) analysis of the sample confirmed the presence of fourteen bioactive compounds of fatty acid group. Results from this study revealed that extracts obtained from *T. polyzona* contain bioactive compounds with potent antistaphylococcal properties that can be exploited by biopharmaceutical companies.

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## Introduction

Mushrooms also known as macro fungi, have been used as source of food and pharmacologically active compounds in medicine over the years. The stated health promoting effects of mushrooms have been attributed to the presence of bioactive compounds that exhibit antioxidant, anticancer, antibacterial, antiviral, anti-inflammatory, immunomodulator and several other health promoting activities (Wasser & Weis 1999; Lindequist et al. 2005; Oyetayo et al. 2012, 2013; Ogidi et al. 2018). These bioactive compounds belong to groups of

polysaccharides, vitamins, terpenes, steroids, amino acids, and trace elements (Lorenze & Anke 1998; Wasser & Weis 1999; Mizuno 1999; Zaidman et al. 2005). Mushrooms have also been found to be a reservoir of new natural bioactive compounds with potential application in medicine due to their ability to grow on various cellulosic wastes and absorb compounds present therein (Oyetayo & Oyetayo 2020). Macro fungi are prolific producers of a variety of secondary metabolites such as phenolic compounds, polyketides, terpenes and steroids (Turkoglu et al. 2007; Wasser 2011).

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*Trametes* species is a common genus of non-gilled mushroom. It is a cosmopolitan polyporoid white rot fungus found on hard wood (Gilbertson & Ryvarden 1987; Tomšovský et al. 2006). This genus possesses pileate basidiocarp, di- to trimitic hyphal systems and smooth, non-dextroid spores (Ryvarden 1991). The genus *Trametes* is known to represent a taxonomic chaos (Ryvarden, 1991). Even the use of ITS-nrDNA region for its species delimitation has resulted in poorly resolved phylogenies and unclear species boundaries, especially in the *T. versicolor* species complex (*T. versicolor* sensu stricto, *T. ochracea*, *T. pubescens*, *T. ectypa* (Pers.) Gilbertson & Ryvarden (Carlson et al. 2014).

Members of the genus are ecologically important as decomposers and economically important as bio-remediator and bio-degrader of cellulosic organic waste in the ecosystem (Gilbertson & Ryvarden 1987). They are widespread in distribution and consist of about fifty species worldwide (Kirk et al. 2008). Recently, Olou et al. (2020) re-established the name *Trametes palisotii* for species previously known as *T. elegans* in tropical Africa and also proposed a novel species, *Trametes parvispora* with its description.

Some species of *Trametes* have been used in medicine in China (Cui et al. 2011). Medicinal properties such as antimicrobial and antioxidant properties of some of the *Trametes* species isolated in Nigeria had been reported (Oyetayo et al. 2013; Adeyelu et al. 2016). The present study was aimed at assessing the bioactive compounds and antistaphylococcal properties of extracts obtained from raw and fermented *Trametes polyzona*.

## Materials and Methods

### Collection, identification and fermentation of *Trametes polyzona*

Samples of *Trametes polyzona* (Fig. 1) were collected from dead woods in farmland around Akungba - Akoko (7° 28'32.4''N 5° 45'07.8''E). The fruit bodies were sorted, cleaned and air-dried. A microscopical study of dried *Trametes polyzona* was carried out as described by Gilbertson and Ryvarden (1987). Free hand section of dry fruit bodies of *Trametes* species were placed on a clean microscope slide and observed. Microscopic features were observed under a Zeiss Axiophot light microscope. The specimens were identified morphologically according to Pegler (1977), Pegler & Pearce (1980), and Callac & Guinberteau (2005). Dried specimens are deposited in the Department of Microbiology, Federal University of Technology, Akure, Nigeria, and duplicates are preserved in the Fungarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).



**Fig. 1.** *Trametes polyzona* collected in Akoko, Ondo State Nigeria.

The dried *Trametes polyzona* were then cut into bits and divided into three portions. The first portion was fermented using solid state fermentation (SSF); the second portion was fermented using submerged fermentation (SMF) while the third portion was not fermented (UFM) (Abiola & Oyetayo 2015). The fermentation took place for four days. On the fourth day, the fermented mushroom was oven dried at 40°C for 48 hours.

### Preparation of Extracts from Mushroom samples

The oven dried mushroom samples above were ground into powder and 100 g of each was extracted by adding into 2000 mL of 95 % acetone and methanol separately in an Erlenmeyer flask. The flasks were allowed to stand for 3 days for extraction with occasional stirring. The extracts were then filtered through cotton wool plugged in a funnel. The filtrates were evaporated to dryness at 50°C in a rotary evaporator (RE - 52A; Union Laboratory, England) at 90 rpm under reduced pressure.

### Sources of *Staphylococcus aureus*

Clinical isolates (*S. aureus*) from blood were collected from Federal Medical Centre Owo, Ondo State. Isolates from soil, water and urine as well as the typed isolates were obtained from Laboratory culture collection of Microbiology Department, Federal University of Technology, Akure.

### Assessment of antistaphylococcal activity of extracts

Antistaphylococcal activity of *T. polyzona* extract was determined by agar well diffusion method as described by Abubakar et al. (2016). *Staphylococcus* species obtained from different sources as stated above were cultivated on nutrient broth at 32 °C for 18 hours. The inoculum size

was adjusted by serial dilution to obtained 0.5 McFarland turbidity standards. The extract was reconstituted in 20 % v/v of dimethyl sulfoxide (DMSO). An aliquot of 0.1 mL containing organism was aseptically transferred and evenly spread onto the dried surface of sterile Mueller Hinton agar plate. A well of 8 mm were bored in the agar plate with sterile cork borer. The extract was sterilized through membrane filter (0.22  $\mu$ m) and 0.1 mL was aseptically introduced into the well in the Petri dishes already inoculated with *Staphylococcus* species with the aid of micro pipette. A volume of 0.1 mL of erythromycin was used as positive control while 20 % of DMSO served as negative control. The plates were incubated at 37 °C for 24 hours. The diameter of the inhibition zones was measured in millimeters.

#### **Determination of minimum inhibitory concentration of the extract**

Agar well diffusion method as described by Ncube et al. (2008) was used to screen the antimicrobial effect of the extracts at different concentrations. The concentrations of the extract ranged from 100 mg/mL to 600 mg/mL. The test was carried out in replicates (n = 3). The MIC was obtained by taking the least concentration of the extract that shows inhibitory effect on the tested organisms.

#### **GCM Spectrophotometric Analysis of Extracts**

Methanolic extract displayed good antistaphylococcal properties at concentrations of 100 mg/mL to 600 mg/mL. The extract was therefore subjected to Gas Chromatography-Mass Spectrophotometric (GC-MS) analysis. The GC-MS analysis was carried out based on comparative retention time, mass and peaks of chemical compounds using computer-aided program for matching of unknown mass spectra of compounds with the known compounds stored in the software database library from the National Institute of Standards (NIS), Washington, USA with more than 62,000 patterns as the reference database.

#### **Statistical analysis**

Experiments were carried out in replicates and data obtained were analyzed by one way analysis of variance (ANOVA) and means were separated by Duncan multiple range test (SPSS 17.0 version). Differences were considered significant at  $P \leq 0.05$ .

#### **Results**

The zone of inhibition of extracts obtained from raw and fermented *T. polyzona* against *Staphylococcus aureus* from different sources ranged from 2.33 mm to 28.00 mm (Tables 1 and 2). Methanolic extract obtained from *T.*

*polyzona* subjected to submerged fermentation displayed the highest zone of inhibition of 28.00 mm at 600 mg/mL. The antimicrobial activity of methanolic extract was concentration dependent (Table 1). Acetonic extracts did not display good antistaphylococcal effect (Table 2). Acetonic extract obtained from *T. polyzona* subjected to submerged fermentation displayed the zone of inhibition of 8.67 mm at 600mg/mL against *Staphylococcus aureus* isolated from blood.

The minimum inhibitory concentration (MIC) of extracts obtained from raw and fermented *T. polyzona* against *Staphylococcus aureus* from different sources ranged from 100 mg/mL to 400 mg/mL (Table 3). Acetonic extract did not exhibit inhibitory effect against *Staphylococcus aureus* sourced from wound and typed strain of *Staphylococcus aureus* at concentrations of 400 mg/mL to 600 mg/mL.

Table 4 showed the various bioactive compounds obtained from the GC-MS analysis of methanolic extract of *Trametes polyzona*. Fourteen (14) bioactive compounds were obtained and these are: Caprylic acid methyl ester, Tridecanoic acid methyl ester, Myristoleic acid methyl ester, Cis- 10 pentadecanoic acid methyl ester, Palmitoleic acid methyl ester, Heptadecanoic acid methyl ester, Stearic acid methyl ester, Elaidic acid methyl ester, Oleic acid methyl ester, Linolelaidic acid methyl ester, g-Linoleic acid methyl ester, x- Linolenic acid methyl ester, Heneicosanoic acid methyl ester and Cis- 11-14-Eicosadienoic acid methyl ester.

#### **Discussion**

Medicinal and edible mushrooms are important sources of food and myco-chemicals of health importance. They are sources of proteins, vitamins, minerals, chitin, essential amino acids as well as low fat and calories (Celal 2018). Therapeutic activities of mushrooms have been linked to the presence of biologically active compounds (Awala & Oyetayo 2015). These bioactive compounds of mushroom origin serve as biological response modifiers in higher animals (Oyetayo & Ogidi 2022). Medicinal mushrooms are obviously sources of antimicrobial agent that can be used to combat pathogens that are resistant to commonly used antibiotics.

*Staphylococcus aureus* is a very important pathogen responsible for bacterial infections in hospitals and communities worldwide. It has been recognized as a versatile microorganism worldwide (Diekema et al. 2001). *Staphylococcus* sp. is a human pathogen and a part of the normal flora of human skin. It can colonize and infect both patients and healthy people with life threatening effects (Daka et al. 2012). *Staphylococcus* species are known to exhibit multiple antibiotic resistances (Jhora & Paul 2011; Julie & Trevor 2013). In this report,

methanolic and acetic extracts obtained from raw and fermented *T. Polyzona* was found to display antistaphylococcal activity against *Staphylococcus aureus* obtained from different sources.

Ethanol extracts of *T. polyzona* displayed higher antistaphylococcal effect (28.00 mm) than acetic extract

(8.67 mm) at concentration of 600 mg/mL against *Staphylococcus aureus* isolated from blood. The extracting solvent and other factors such as the chemical nature of

**Table 1** Zones of inhibition (mm) displayed by methanolic extracts of *Trametes polyzona* against *Staphylococcus aureus* at 600mg/ml

Source of isolate	SMFE	SSFE	UFE
SB	28.00 ± 3.00 <sup>a</sup>	19.33 ± 1.53 <sup>b</sup>	13.33 ± 1.53 <sup>c</sup>
SU	18.33 ± 1.53 <sup>a</sup>	18.33 ± 1.53 <sup>a</sup>	9.33 ± 1.15 <sup>b</sup>
SS	26.33 ± 1.53 <sup>a</sup>	13.67 ± 0.58 <sup>b</sup>	6.33 ± 1.15 <sup>c</sup>
SW	25.67 ± 1.53 <sup>a</sup>	13.67 ± 0.58 <sup>b</sup>	3.00 ± 1.00 <sup>c</sup>
ST	15.67 ± 1.53 <sup>a</sup>	12.33 ± 0.58 <sup>b</sup>	5.00 ± 0.00 <sup>c</sup>

**Note:** Values carrying same alphabets in same row are not significantly different ( $p > .05$ )

Where, SB- *Staphylococcus aureus* isolated from blood

SU- *Staphylococcus aureus* isolated from urine

SS- *Staphylococcus aureus* isolated from soil

SW- *Staphylococcus aureus* isolated from wound

ST- Typed isolate of *Staphylococcus aureus* (ATCC 29213)

SMFE: Extract from *T. polyzona* subjected to Submerged Fermentation

SSFE: Extract from *T. polyzona* subjected to Solid State Fermentation

UFE: Extract from Unfermented *T. polyzona*

**Table 2** Zones of inhibition (mm)\* displayed by Acetic extracts of *Trametes polyzona* against *Staphylococcus aureus* at 600mg/mL

Source of isolate	SMFE	SSFE	UFE
SB	8.67 ± 1.15 <sup>a</sup>	5.67 ± 0.58 <sup>b</sup>	3.00 ± 0.00 <sup>c</sup>
SU	7.67 ± 1.15 <sup>a</sup>	5.00 ± 0.00 <sup>b</sup>	2.67 ± 0.58 <sup>c</sup>
SS	6.00 ± 1.00 <sup>a</sup>	4.00 ± 0.00 <sup>b</sup>	2.33 ± 0.58 <sup>c</sup>
SW	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
ST	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>

Values carrying same alphabets in same row are not significantly different ( $p > .05$ )

**Table 3** Minimum inhibitory concentration (mg/mL) of extracts obtained from raw and fermented *T. polyzona* against *Staphylococcus aureus* obtained from different sources

Source of isolate	Methanolic extract			Acetic extract		
	SMFE	SSFE	UFE	SMFE	SSFE	UFE
SB	100	100	200	200	400	400
SU	100	100	200	200	400	400
SS	100	100	200	200	400	400
SW	400	400	400	NI	NI	NI
ST	400	400	400	NI	NI	NI

NI: No inhibition

phytochemicals, extraction method, sample particle size as well as the presence of interfering substances affects extraction efficiency and efficacy of extracts (Stalikas 2007; Brahmi et al. 2012; Awala & Oyetayo 2015). López et al. (2011) also reported that the nature of the solvent and the chemical properties of the sample are the two most important factors when extraction is subjected to similar conditions of time and temperature. The higher antistaphylococcal activity of methanolic extracts may be due to the ability of the solvent to dissolve endogenous compounds in *T. polyzona*. Awala & Oyetayo (2015) had earlier reported a higher antimicrobial effect for methanolic extract when compared with other extracts. Moreover, *T. polyzona* may contain high levels of polar compounds that are soluble in solvents with high polarity such as methanol (Dieu- Hien et al. 2019).

It was also observed that extracts obtained from fermented *T. polyzona* displayed higher and significantly different ( $p < 0.05$ ) antistaphylococcal effect (28.00 mm) than acetonetic extract (8.67 mm). Fermentation is a process that leads to degradation of macromolecules to micromolecules and some of these molecules are bioactive in nature (Atere et al. 2019). The minimum inhibitory concentration (MIC) of *T. polyzona* extract against *S. aureus* ranged from 100 mg/mL to 400 mg/mL. Adeyelu et al. (2017) had earlier reported higher antifungal activity of methanolic extract of *Trametes elegans* (Spreng.) Fr. against *Candida* spp.

**Table 4** Bioactive compounds present in ethanolic extracts obtained from raw and fermented *T. polyzona*

S/N	Retention Time	Height	Area (%)	Carrier	Name of Compound
1	4.516	43.160	0.7430	Nitrogen	Caprylic acid ethyl ester
2	12.753	145.353	2.3991	Nitrogen	Tridecanoic acid methyl ester
3	14.576	76.234	1.2098	Nitrogen	Myristoleic acid methyl ester
4	16.240	161.290	2.5627	Nitrogen	Cis-10 pentadecanoic acid methyl ester
5	17.840	83.483	1.3090	Nitrogen	Palmitoleic acid methyl ester
6	19.260	211.485	4.0211	Nitrogen	Heptadecanoic acid methyl ester
7	20.660	143.446	2.6906	Nitrogen	Stearic acid methyl ester
8	22.033	275.618	5.6737	Nitrogen	Elaidic acid methyl ester
9	23.216	114.956	2.7858	Nitrogen	Oleic acid methyl ester
10	24.240	523.215	12.4574	Nitrogen	Linolelaidic acid methyl ester
11	26.323	207.108	2.7134	Nitrogen	g-Linoleic acid methyl ester
12	27.943	93.129	1.4846	Nitrogen	x-linolenic acid methyl ester
13	28.840	234.356	5.8626	Nitrogen	Heneicosanoic acid methyl ester
14	30.886	204.230	4.3872	Nitrogen	Cis-11-14-Elcosadienoic acid methyl ester

GC-MS analysis revealed the presence of fourteen bioactive compound namely; Caprylic acid methyl ester, Tridecanoic acid methyl ester, Myristoleic acid methyl ester, Cis- 10 pentadecanoic acid methyl ester, Palmitoleic acid methyl ester, Heptadecanoic acid methyl ester, Stearic acid methyl ester, Elaidic acid methyl ester, Oleic acid methyl ester, Linolelaidic acid methyl ester, g-Linoleic acid methyl ester, x- Linolenic acid methyl ester, Heneicosanoic acid methyl ester and Cis- 11- 14- Elcosadienoic acid methyl ester. In a study, polyunsaturated fatty acids were the main group of fatty acids documented in all the species of coral fungi from Northwestern Himalayas (Sharma & Guatam 2017). These bioactive compounds are known to have potent antimicrobial, antioxidant, anti-inflammatory and antistaphylococcal properties (Ogidi et al. 2015; Falade et al. 2017; Salisu et al. 2019; Akingbesote & Oyetayo 2021). Ogidi (2017) had earlier reported the presence of these groups of fatty acids in bioactive extracts obtained from *Lenzites quercina*. Specifically, omega -6, -7, -9 fatty acids are important dietary nutrients providing various health benefits such as in blood clotting, blood pressure lowering, control of inflammation, maintaining cell membrane structure and lipid metabolism (Wijendran & Hayes, 2004). Most of these bioactive compounds are used in the formulation of cosmetics, pharmaceuticals and nutraceuticals (Mahamed & Farghaly, 2014; Wu et al. 2016).

Conclusively, results from this study revealed the antistaphylococcal properties of extracts obtained from raw and fermented *Trametes polyzona* and the bioactive compounds present in the methanolic extracts. Fermentation was observed to increase the antistaphylococcal properties of the extracts. The bioactive compounds may therefore be harnessed by pharmaceutical industries for the production of effective pharmaceuticals especially against multidrug resistant *Staphylococcus aureus*.

#### Declaration of competing interest

The authors declare that they have no competing interests.

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