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Original Research Article

Chemical diversity, Antimicrobial, Antioxidant and Preservative Potentials of Crude Extracts of *Trametespolyzona*an Endophytic Fungus found in *Thaumatococcusdanielli*Leaves

Collins I. Chimezie[1], Ugochukwu M. Okezie[1*], Mark O. Akpotu[2], Ahamefula A. Ahuchaogu[3], Ifeanyi E. Nwike[1], Ezinne C. Anieto[4], Pass C. Chijindu[5], Malachy C. Ugwu[1]

[1]Department of Pharmaceutical Microbiology and Biotechnology, NnamdiAzikiwe University, Awka, Anambra State, 5025, Nigeria
[2]Department of Pharmaceutical Microbiology, OlabisiOnabanjo University, Sagamu Campus, Ogun State, 2001, Nigeria
[3]Department of Pharmaceutical Chemistry, Abia State University, 2000, Nigeria
[4]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University on the Niger, Umunya, 433101, Nigeria
[5]Department of Biological Sciences, University of Delta, Agbor, Delta State, 2090, Nigeria

ABSTRACT

Endophytic fungi are known to produce diverse bioactive metabolites that offer a promising avenue for drug discovery, with potential applications in developing new preservatives. This study aims to isolate bioactive metabolites from endophytic fungi to discover novel, sustainable preservatives, leveraging their natural ability to produce diverse compounds with potential industrial applications. Axenic culture of *Trametes polyzona*was isolated from *Thaumatococcus daniellii and* fermented on local rice, and the ethyl acetate extract was subjected to preliminary antimicrobial and antioxidant assays and then a preservative test adopting the United State Pharmacopoeia USP-2004 protocol. *Trametes polyzona appeared* creamy-white on potato dextrose agar. The crude extract exhibited a broad-spectrum growth inhibition. Inhibition zones of 14, 12, 7, 4, and, 12 mm were recorded at 1000 µg/mL against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and*Aspergillus niger* respectively. Also, *T. polyzona* crude extract exhibited good radical scavenging activity with inhibition of 69.70 % in comparison with quaercetin 73.40%. The extract exhibited moderate preservative efficacy against *E. coli, P. aeruginosa, S. aureus,* and, *A. niger.* Gas chromatography – Flame ionization Detector and Gas chromatography and mass spectroscopy analyses of the crude extract detected the presence of flavonoids (58.47%) and phenolics (9.22%) and Ethyl Iso-allocholate, 3-octyl-, cis-(Epoxyoleic acid), and Oxiraneoctanoic acid respectively. As a result, *Thaumatococcusdanielli* can be relied upon for fungal secondary metabolites for the development of effective preservatives.

Keywords:Thaumatococcus danielli, Trametes polyzona, Preservative, broad-spectrum, bioactive compounds.

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Introduction

Plants are abundant in microbes and constitute an ecosystem for these tiny, microscopic beings where both have formed a symbiotic relationship, with the microbes producing compounds that offer some protective functions to the plants while the plants protect the microbes from hazardous environmental conditions.[1]Endophytic Fungi are micro-organisms that exhibit a beneficial association with their host plants without any accompanying disease.[2] Reported as a productive group of microorganisms that produce microbial metabolites that exhibit crucial biological actions and act as potential reliable biosynthesizers of natural products for the development of new medicines by pharmaceutical industries.[2] Bioprospecting bioactive secondary metabolites of fungal endophytes with pharmaceutical and biotechnological potentials are basic for the isolation of new chemical actives for use in the development of human medicines including antibiotics, antimalarial, anticarcinogenic, and preservatives.

*Corresponding author. mail: um.okezie@unizik.edu.ng
Tel: +2347064878382

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Acquainted with the reality of acquiring infections by diverse-resistant pathogens as a result of the use or administration of contaminated preparations; side effects caused by some chemical preservatives, and the biosynthetic capacity of microbe-inhibiting molecules by fungal endophytes, it becomes necessary to explore newer antimicrobial molecules that posses novel pathway of action, nontoxic effect and resist microbial degradation from this inexhaustible source.[3] Following the discovery of extended spectrum of microbial resistance to antibiotics such as betalactams antibiotics, and other forms of resistance the exploitation of secondary metabolites produced by endophytic fungi of plant origin was observed to have extended to date and has been investigated by several researchers. [4, 5, 6, 7, 8]
Some of the problems facing healthcare delivery in the world are due to the incompatibilities of conventional (synthetic) preservatives used to store and preserve pharmaceutical products leading to the deterioration of the pharmaceutical preparations.[9] With regards to the antimicrobial activities of fungal secondary metabolites[4,5] their potential to also act as preservatives in pharmaceutical products is possible. Activity against spoilage fungi implicated in food spoilage has also been reported.[10]
Also, the use of leaves of *Thaumatococcus daniellii* to preserve and improve the shelf life of certain foods as well as against fungi implicated in the spoilage of corn Jell-O and orange juice may be due to specific secondary metabolites produced by the endophytic fungi present in such leaves.[11] These calls for an urgent need for the isolation and characterization of new and useful lead compounds to provide novel preservatives useful for the preservation of both pharmaceuticals and food preparations. While Trametes species are known to produce bioactive compounds, and *T. daniellii* has

documented phytochemicals, the unique interaction between them — and whether it leads to enhanced or novel metabolite production — has not been explored. Thus this study was carried out to determine the potential bioactive properties — particularly preservative (antimicrobial) and antioxidant activities — of *Thaumatococcus daniellii –associated Trametes polyzona* metabolites.

Materials and Methods

Collection of Plant Material

Fresh leaves of *Thaumatococcus daniellii* without any obvious disease condition were harvested on 9th of September, 2024, from a local farmland located in Nneogidi-Agulu, Latitude: 6° 06' 1.62" N Longitude: 7° 03' 39.60"in Anambra State, South-Eastern Nigeria. After the identification of the plant sample, it was authenticated by Dr. Mrs Amaka Roseline Onwunyili a plant taxonomist at the Department of Pharmacognosy and Traditional Medicine, NnamdiAzikiwe University, Awka and voucher specimen PCG/474/M/071 deposited at the faculty herbarium (Figure 1).

Figure 1: *Thaumatococcus daniellii plant*

Isolation and Identification of Thaumatococcus daniellii-associated*Fungus*

The selected leaf was thoroughly washed under running tap water thereafter subjected to surface-sterilization by a time-dependent immersion in 70 % ethanol, 2 % sodium hypochlorite solution, and sterile double distilled water for 3, 2, and 5 min respectively. Fragments ranging between 1 – 2 cm of the sterilized leaf were cut aseptically and then each was gently inserted into sterile potato dextrose agar (PDA) supplemented with 500 µg/mL Chloramphenicol contained in a sterile plate. In other to rule out false positives that may be present on the leaf as surface contaminants, the surface rinse solution was plated, which served as the negative control. The Petri dishes were sealed using paraffin and incubated at 28°C for 96 hours while the emergence of visible fungal growths was monitored daily. Fragments of hypha of apparent isolates that emerged from the cultured leaf fragments were subcultured onto sterile PDA plates to obtain axenic cultures. The fungal endophyte observed to have the fastest growth rate and largest diameter was selected for characterization. Fungal identification was achieved through fungal-DNA amplification followed by sequencing of its fungal ITS region as previously reported by,[12] followed by BlastN search in the NCBI database.

Rice Fermentation and Ethyl acetate Extraction of Fungal Secondary Metabolites

Fermentation of endophytic fungus was carried out by adopting a solid-state approach. The fermentation medium comprised of 100g of rice + 100 ml of deionized water, contained in 1L Erlenmeyer flasks and sterilized using the autoclave at 121°C at 15 psi, for 30 min. The fungus was aseptically transferred to the surface of the rice medium, plugged with cotton wool and sealed with masking tape. Each of the fermentation flasks was allowed for 21 days at 28 °C. Following the fermentation process, 500 mL of the extraction solvent ethyl acetate was used to discontinue the process, then filtered and evaporated under a vacuum to dryness at 40°C using a rotary evaporator.

Preliminary Determination of Antimicrobial Activity

The antimicrobial potential of the fungal metabolites was assayed using the agar well diffusion method as presented by Okezie *et al*.[4] The sterile nutrient broth was used to grow the test clinical strains: *Pseudomonas aeruginosa* and *Escherichia coli (Gram negatives)*, *Staphylococcus aureus* (Gram positive), and *Aspergillusniger*(mould), *and Candida albicans*(yeast) provided by the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, NnamdiAzikiwe University, Nigeria. A solution of 5 µg/ml and 50 µg/ml of Ciprofloxacin and Miconazole served as the positive control for bacterial and fungal isolates respectively. Dimethyl sulfoxide at 100% (v/v) was used as the negative control. A working concentration of 1000 µg/mL stock solution of the endophytic fungal extract was made. Thereafter, the stock was subjected to two-fold serial dilution to get other graded concentrations 500, 250, 125, 62.5 µg/mL. Wells/holes having a diameter of 6 mm were made then 50 µL of the prepared extract and controls were carefully dispensed. A pre-diffusion time of 1hr at room temperature was observed for proper diffusion of the agents before incubation of MHA plates for 24 hours at 37°C and the SDA plates for 48 hours at 28°C. The zones of inhibitions indicating antimicrobial action were measured, excluding the diameter of the well/hole. Triplicate measurements were made and the average values for IZD were calculated and recorded.[4]

Determination of Minimum Inhibitory Concentration (MIC)

The method described by *the European Committee for Antimicrobial Susceptibility Testing* (EUCAST)[13] was adopted with slight modifications. The Minimum Inhibitory Concentration (MIC) of the fungal extract was determined for each of the test organisms previously sensitive to the extract in the preliminary antimicrobial evaluation. Dimethyl sulfoxide was used to reconstitute the stock (10000 µg/mL) concentration thereafter, two-fold serial dilutions were made to get other graded (5000, 2500, 1250 and 625 µg/mL) concentrations. This was followed by a 10-fold dilution of each of the concentrations to get the working concentrations of (1000, 500, 250, 125 and 62.5 µg/mL) using 9 mL sterile molten agar and then allowed to solidify. Standardized (standardized to 0.5 McFarland turbidity) microbial inoculums were streaked on the agar appropriately. The plates were incubated at optimum temperatures of 37°C for 24 hr and 25 for 48 hr for the bacterial and fungal plates respectively. The examination of plates for the presence (+) or absence (-) of growth was done immediately after incubation.

Formula for Preparation of Aqueous Cream

The aqueous cream was formulated according to British Pharmacopoeia,[14] with the following excipients: Emulsifying ointment 30 g; Chlorocresol 0.1 g; purified water 69.9 g. Each excipient was weighed out using an analytical scale in a sterile container. The weighed quantity of Chlorocresol was dissolved in purified water and triturated. Next, the emulsifying ointment was fused by heating, and the purified water mixed with chlorocresol was added to the emulsifying ointment in aliquots. The volume of the formulation was reached using 100 mL of purified water. The formulation was then homogenized for ten minutes at a reduced speed using a homogenizer until a semi-solid was formed.

For preservative activity, the chlorocresol preservative was substituted with the fungal crude extract at 1 mg/g to prepare the test product to be inoculated with microorganisms. The Preservative properties were determined according to the United States Pharmacopoeia.[15]

Preparation of Inoculum for Preservative Testing

Each of the test pathogenic fungi (*C. albicans, A. niger*) and bacteria (*E. coli, S. aureus, P. aeruginosa)* was grown on nutrient and saboraud dextrose agar for 24 and 48 hours respectively. After the incubation period, sterile saline solution (0.9% w/v NaCl) was used to prepare each test microbial suspensions that corresponded to 1×10^8CFU/ml.

TestProcedure

The assay for preservative potentials was carried adopting the method described by. [9]

Aqueous Cream (extemporaneous preparation) in the ready-to-use tubes was employed in the challenge test. A 1 mL volume of the standardized inoculum was aseptically transferred into the preparation. Then, a homogeneous solution with an appropriate distribution of the test organism was prepared by thorough mixing and incubation under optimal conditions. After incubation, the preservative potentials of the preparation were assessed by determining the number of CFU/ml of the preparation at predefined time intervals of 0 to 28 days (7 seven days interval), on sterile nutrient and saboraud dextrose agar plates. The log values corresponding to the number of CFU/ml of the aqueous cream were determined and interpreted as per the guidelines of USP.[15] This was carried out with the standard preservative (Chlorocresol), the fungal extract, and without any preservative.

The USP [15] requirements for Topical products containing aqueous bases of vehicles were adopted as the criteria for acceptance of the preservative system.

The effectiveness of a preservative is met if no observed increase was recorded from the initial calculated counts at day14 and 28 for bacteria, molds, and yeast, and, where no increase is defined as CFU/mL not more than 0.5 log10 more than the initial count recorded.

DPPHAntioxidantAssay

The potential of the fungal extract to inactivate free radicals was determined using a standard protocol that assesses the propensity of the fungal metabolites to neutralize free radicals (DPPH: 2,2-diphenyl-1-picrylhydrazyl), as described by Okezie et al.[4] A reaction mixture comprising 0.25 mL of fungal extract and quercetin (positive control) at 100 µg/mL, 0.25 mL of DPPH (0.06 mmol), and 2 mL of methanol was prepared and incubated for 30 minutes at room temperature in the dark. The absorbance, which measures antioxidant potential, was determined at 517 nm using a UV-vis spectrophotometer (Model 721, ANENG, China). Each experiment was performed in triplicate.

The IC_{50} values were calculated by plotting percentage inhibition against log concentration and fitting the data to a nonlinear regression curve. The regression equation for the fungal extract was:

$y = 92.4 – 1.18x$,

with a coefficient of determination of $R^2 = 0.976$, indicating a strong fit and high reliability of the derived IC_{50} value.

DPPH radical scavenging activity (%) = $\frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$

Identification of fungal-molecules produced by Trametes polyzona by Gas chromatography flame ionization detector (GC-FID)

The analysis of fungal-chemical constituents was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector as described by Kelly and Nelson.[16] The ethyl acetate fungal extract was analyzed for the presence of bioactive molecules. A micropipette was used to transfer 0.1 mL of the fungal extract into the gas chromatography machine.

Fungal metabolites were determined by the ratio between the area and mass of internal standard and the area of the identified compounds. The concentrations of the different compounds were expressed in ug/g.[16]

GC-MS Analysis of Secondary Metabolites Produced by Trametespolyzona

Gas Chromatography-Mass spectrophotometry-detection of the produced fungal compounds present in the extracts was done using the Agilent Technologies (Wilmington, Delaware, USA) Agilent 7809A gas chromatograph. The method described by Bruce et al.[17] with moderate modifications was used. A sample of 1µl of the crude fungal extract was injected. For the detection of compounds, an ionization system operated at electron Impact mode with energy of 70 eV was used. A constant flow rate of Helium (99.9995) gas was achieved, and stabilized at 1.1ml/min. The injection and detector temperatures were 2500 C and 3100 C respectively. The oven was warmed at 600 C (isothermal temperature) for 5 min followed by increased from 50 C/min to 1000 C/min and 100 C/min up to 2500C/min for 2 min and 5 min respectively. Mass spectra of compounds detected were interpreted using The National Institute of Standard and Technology (NIST 14) NIST/EPA/NIH Spectral Library.

Statistical Analysis

Triplicate (n = 3) measurements were obtained and analyzed using GraphPad Prism 9.5.1. Descriptive statistics were used, and data are presented as mean ± standard error of the mean (SEM). Microsoft Excel was used to calculate the mean and standard deviation (SD).

Results and Discussion

Molecular Characterization of Trametespolyzona

Trametes polyzona, an endophytic fungus isolated from healthy leaves of *Thaumatococcus daniellii* on the PDA plate appeared to be creamy-white, leathery to touch, and glabrous with a characteristic aromatic smell also with golden-colored liquid produced as droplets on the surface of the fungi (Figure 2). Furthermore, the nucleotide sequence obtained was compared with the NCBI BLAST Database. *Trametes polyzona* was returned with the highest percentage similarity in the NCBI BLAST database. The percentage similarity score and accession numbers are 99.49 % and KC589124.1, respectively.

Figure 2:*Trametes polyzona* axenic culture on Potato Dextrose Agar (PDA

Yield estimate and Chemical analysis of the crude extract ofTrametes polyzona

The Yield estimate of *Trametes polyzona* extract was observed to be 114.9 mg.The GC-FID analysis of the crude ethyl acetate extract of *Trametes polyzona* resulted in the detection of Flavonoids (Proanthocyanins, Naringin, Naringenin, Flavan-3-ol, Flavones, FlavanonesandCatechins) (58.47 %), alkaloids (quinine, ribalinidine, and sparteine) (9.24 %), Phenolics (resveratrol and phenol) (9.22 %) and steroids (22.23 %) based on retention time of each secondary metabolite detected compared to known standards (Table1).

The major secondary metabolites detected were flavanones and proanthocyanin at RT of 21.553 and 0.120 m. Also, the ethyl acetate crude extract was observed to have high concentrations of flavanones

27.5840 µg/mL (13.96 %) and Proanthocyanins 26.3095 µg/mL (13.36 %) (Table 1). Similarly, Adeogun *et al*.,[11] and Oduntan,[18] reported the detection of the presence of flavanones and proanthocyanin in the crude extract of *Thaumatococcus danielli* and the endophytic fungi, *TrametesPolyzona* as well as their antimicrobial, Antioxidant and preservative properties. Other important secondary metabolites include catechins, anthocyanins, and naringenin which are also flavonoids detected at RT 39.900, 8.436, and, 12.450 m (Table 1).

Gas chromatography and mass spectroscopy evaluation of *Trametes polyzona* crude extract identified the presence of six (6) compounds (Table 2), including Ethyl Iso-allocholate, Oxiraneoctanoic acid, 3-octyl-, cis- (Epoxyoleic acid), 6,19-cycloandrostane-3,7-diol,3β-methoxy-, 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis- with reported pharmacological activities and Pregn-4-ene-3,20-dione, 16,17-epoxy-, (16α)- and Hexadecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aα,2α,5β,5aβ,6β,8aα,9α,10aα)]- with no known pharmacological activity.

Table 1: GC-FID Analysis of phytochemical constituents present in *Trametes polyzona* ethyl acetate extract

S/N	Phytoconstituents	Retention	Area	Height	Concentration(µg/ml)	% Composition
1	Proanthocyanin	0.120	2346.8895	138.870	26.3095	
2	Naringin	2.700	2433.8762	69.313	2.4887	
3	Flavan-3-ol	5.793	5827.3383	165.688	4.9716	
4	Anthocyanin	8.436	4718.5262	134.350	16.8713	Flavanoids
5	Naringenin	12.450	4688.6453	134.423	14.4353	
6	Flavanones	21.553	5014.4776	142.908	27.5840	
7	Flavone	34.120	2175.8156	61.926	5.4689	
8	Cathechins	39.900	2200.2156	62.715	17.3023	
9	Ribalinidine	10.793	4755.2150	135.746	4.2561	
10	Quinine	14.040	4552.3342	129.836	10.2096	Alkaloids
11	Spartein	15.076	1007.6931	29.316	3.7972	
12	Phytate	31.363	4201.4042	119.437	0.3825	Anti-Nutrients
13	Oxalate	34.120	661.7672	18.886	5.4689	
14	Steroids	24.580	4958.7882	141.081	43.8881	Steroids
15	Resveratol	41.836	3150.1012	89.812	7.8203	Phenols
16	Phenol	19.756	3923.7622	111.807	10.3918	
	Total		56616.8496		197.5508	

Table 2: Compounds detected from the GC-MS analysis of the ethyl acetate extracts of *Trametes polyzona*

S/N	Chemical compound	Molecular formula	Molecular weight
1	Ethyl Iso-allocholate	$C_{26}H_{44}O_5$	436
2	Oxiraneoctanoic acid, 3-octyl-, cis- (Epoxyoleic acid)	$C_{18}H_{34}O_3$	298
3	6,19-cycloandrostane-3,7-diol,3β-methoxy-	$C_{20}H_{32}O_3$	320
4	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-	$C_{28}H_{44}O_4$	444
5	Pregn-4-ene-3,20-dione, 16,17-epoxy-, (16α)-	$C_{21}H_{28}O_3$	328

Preliminary Antimicrobial Activity

A preliminary investigation of *TrametesPolyzona* crude extract revealed its antimicrobial properties (Table 3). *TrametesPolyzona* crude extract demonstrated a dose-dependent growth inhibition. Also, the activity was observed to be broad-spectrum, with growth inhibitions against two Gram-positive: *S. aureus* and *B. subtilis*, two Gram-negatives: *P. aeruginosa* and *E. coli*, and, a mold: *A. niger*. The inhibition zones produced ranged between 2 – 12 mm (Table 3). The test organisms displayed varying susceptibility patterns to the fungal extract as observed by their different MIC's which ranged from 1,000 to 125 µg/mL (Table 4).

Preservative activity

An effective preservative activity by the crude extract of *TrametesPolyzona*was recorded. *TrametesPolyzona crude* extract inhibited the microbial contaminants: *Staphylococcus aureus*, *Escherichia coli*, and, *Pseudomonas aeruginosa* as there was no increase in CFU/mL above 0.5 log observed at 14th day and after 28days, compared to the standard which was moderately as effective as the fungal extract. Hence, the endophytic fungal extract showed good potential for use as a preservative against commonly implicated microbial contaminants in pharmaceutical preparations (Table 5a - e).

However, the activity showed by *TrametesPolyzona crude* extract when tested against *C. albicans* and *A. niger* was observed to be moderately effective, this is due to the recorded decrease in growth i.e. in log CFU/ml slightly below 0.5 log10 on 14th day and after 28 days below the acceptable limits. The standard preservative showed moderately low preservative effectiveness when compared to the fungal extract (Table 4a - e).

Table 3: Preliminary antimicrobial activities of *TrametesPolyzona* crude extract against the test isolates.

Concentration (µg/mL**)**	**Test organisms / Inhibition zone diameter (mm)**					
	S. aureus	***P. aeruginosa***	***E. coli***	***B. subtilis***	***C. albicans***	***A. niger***
1000	14±0	4±0	7±0	12±2.12	0±0	12±0
500	12±0	2.5±0.7	6±0	8±0	0±0	11.5±0.7
250	10±0	2±0	6±0	6±0	0±0	10±0
125	8±0	0±0	5±0	0±0	0±0	9±0
62.5	2±0	0±0	5±0	0±0	0±0	6.5±2.12
Cp. 5µg/mL	10	13	20	14	*	*
Mc. 50 µg/mL	*	*	*	*	19	0

Cp: Ciprofloxacin; Mc: Miconazole

Table 4: Minimum inhibitory concentration against the test organisms

	Test organisms / minimum inhibitory concentration (µg/mL)					
Extract	***S. aureus***	***P. aeruginosa***	***E. coli***	***B. subtilis***	***C. albicans***	***A. niger***
1	125	500	125	250	>1000	125

Antioxidant activity of secondary metabolites of Trametes Polyzona

The reaction of the fungal extract with DPPH showed *Trametes Polyzona* crude extract had good capacity to scavenge free radicals. The fungal extract produced DPPH radical inhibition of 69.79 % which was observed to be comparable with the standard compound (Quercetin) with inhibition of 73.40 % when tested at 100 mg/mL (Table 6).

In this study, a fungal endophyte identified to be *Trametes polyzona* was isolated from *Thaumatococcus daniellii* leaves. *Trametespolyzona* is a member of the Basidiomycota family characterized by a coriaceous and corky pantropical polypore. *Trametes polyzona* has been previously identified to be responsible for pulmonary infections Alexandre, et al.[19] Previous studies show this fungus to be a producer of metabolites with antibacterial, antioxidant, and preservative properties.[18, 20]

Trametes Polyzona extract was active against all the test bacteria: *P. aeruginosa, S. aureus, E. coli*, and *B. subtilis* while *A. niger*was observed to be the only susceptible fungi. *T. Polyzona* extract demonstrated a broad-spectrum antimicrobial activity. The extracts inhibited all the pathogenic bacteria isolates with an IZD and MIC that varied between 2 – 12 mm and 125 – 1000 µg/mL respectively.

The search for plants that possess antioxidant properties that can be developed into effective medicines for the management/treatment of dangerous diseases associated with oxidative damage informed this analysis. The *TrametesPolyzona* crude extract showed good inhibition of DPPH free radical 69.79 %.

Similarly, Williams & Randolph,[20] reported antioxidant activity by *Trametes polyzona* extract against DPPH free radicals. The Antioxidant effect shown by the fungal extract is an indication that the fungal extract could help prevent not only microbial degradation of pharmaceutical products but also reduce microbial oxidation of products that eventually lead to spoilage. The preservative effectiveness exhibited by *Trametes polyzona* crude extract against *Staphylococcus aureus* produced a reduction in viable count lower than 2.0 log for the initial calculated count after 14 days and 28 days respectively. The preservative activities of the fungal extract were observed to be comparable to the standard (Table 5a).

Against *E. coli*, the extract of *T. polyzona* was also observed to be effective, as there was less than 2.0 log CFU/ml count on the 14th day and no observed increase after 28 days. The capacity to prevent degradation by *E. coli* as observed for the *T. polyzona* extract is comparable with the standard preservative (Table 5b)

However, a moderate preservative activity by the *T. polyzona* extract was recorded against *P.aeruginosa*. No increase (i.e. less than 2.0 log CFU/ml) from the initial inoculum count on the 14th day but was found to be less effective (more than 2.0 log CFU/mL) against *P. aeruginosa* from the 14th day count at 28 days (Table 5c).

When tested against *C. albicans* and *A. niger*, *T. polyzona* extract recorded low-growth inhibitory activities. The endophytic fungal extract was found to be ineffective as there was a 2.0 log increase in CFU/ml after 14 days and an increase in inoculum count after 28 days (Table 5d & e). The observed change was more than the acceptance limit, thus no activity was observed.

Table 5a: Bacterial count (*Staphylococcus aureus)*

Preservative used	**Log CFU/ml (time in days)**			
	0	**7**	**14**	**28**
Standard	1.000	1.125	1.000	0.757
Extract	1.602	1.301	0.824	0.862
Control (no preservative)	0.757	1.010	1.079	1.187

Table 5b: Bacterial count (*Escherichia coli)*

Preservative used	**Log CFU/ml (time in days)**			
	0	**7**	**14**	**28**
Standard	0.824	1.176	1.125	0.778

Extract	1.778	1.000	0.424	0.301
Control (no preservative)	1.602	2.316	2.489	2.986

Table 5c: Bacterial count (*Pseudomonas aeruginosa)*

Preservative used	**Log CFU/ml (time in days)**			
	0	**7**	**14**	**28**
Standard (Chlorocresol)	2.000	1.187	1.000	0.839
Extract	1.187	0. 886	0. 796	0.602
Control (no preservative)	1.602	1.870	2.602	2.783

Table 5d: Fungal count (*Candida albicans)*

Preservative used	**Log CFU/ml (time in days)**			
	0	**7**	**14**	**28**
Standard	3.000	2.699	2.602	1.058
Extract	3.000	2.684	2.602	1.339
Control (no preservative)	1.959	2.398	2.981	3.824

Table 5e: Fungal count (*Aspergillusniger)*

Preservative used	**Log CFU/ml (time in days)**			
	0	**7**	**14**	**28**
Standard	1.535	1.234	2.699	2.301
Extract	2.079	1.079	1.033	1.000
Control (no preservative)	2.079	2.870	2.899	2.902

Table 6: Antioxidant activity of Endophytic fungal extract

Concentration (100 µg/mL)	**Mean Absorbance (521nm)**	**Percentage inhibition (%)**
TrametesPolyzona	0.887	69.79
CONTROL (Quercetin)	0.2463	73.40

The Preservative efficacy test validates that the *Trametes Polyzona* extract passed the criteria for effective Preservative against *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* and*Aspergillus niger*. While *Candida albicans* resisted its effect at the tested concentration. This is similar to the findings of Oduntan,[18] that investigated the effects of *Trametes Polyzona* extract on the preservation of tomato fruits and found it to be effective against spoilage bacteria found on the surface of this fruit. Furthermore, the preservative activity of *TrametesPolyzona* crude extract against the test bacteria isolates showed that it is useful in the prevention of microbial spoilage including multidose preparations.

Bioactive compounds including terpenes, phenolic compounds, and alkaloids from natural sources such as microorganisms have reliable defense potentials against pharmacologically active functional groups forming part of the molecule Baptista *et al.*[21]Endophytic fungi have a high potential as reliable sources of bioactive compounds with preservative properties.

The compounds including flavonoids and phenolic detected in the crude extract of *Trametes polyzona* could be linked with the observed antimicrobial, antioxidant, and preservative activities (Fig. 3a-f). Microbial inhibitory and physiological activities have been observed for phyto-molecules such as flavonoids, alkaloids, and tannins.[22]

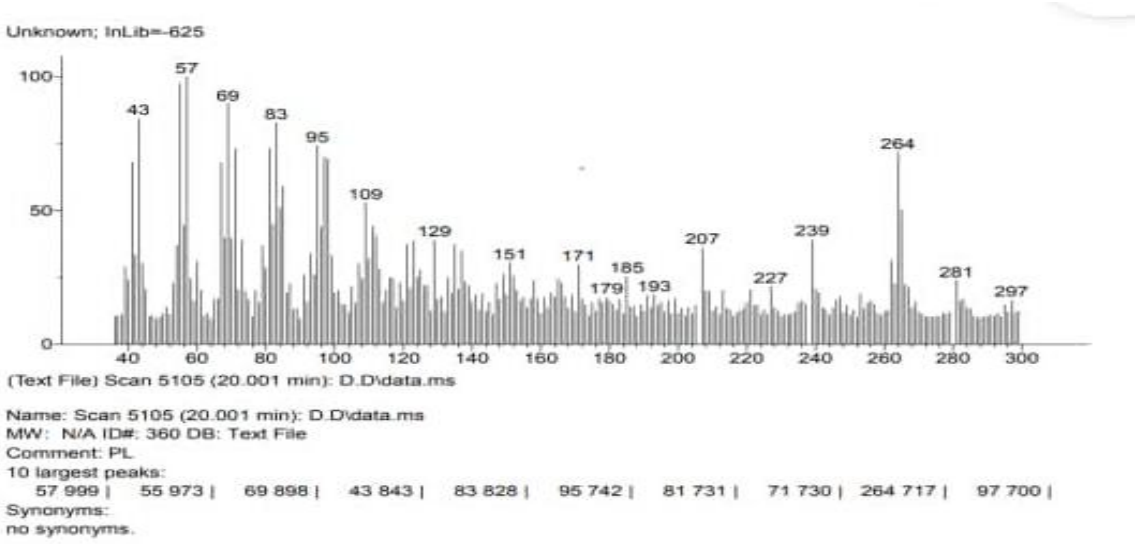

Figure 3a: GC-MS Chromatogram of ethyl aceate crude extract of *Trametespolyzona*

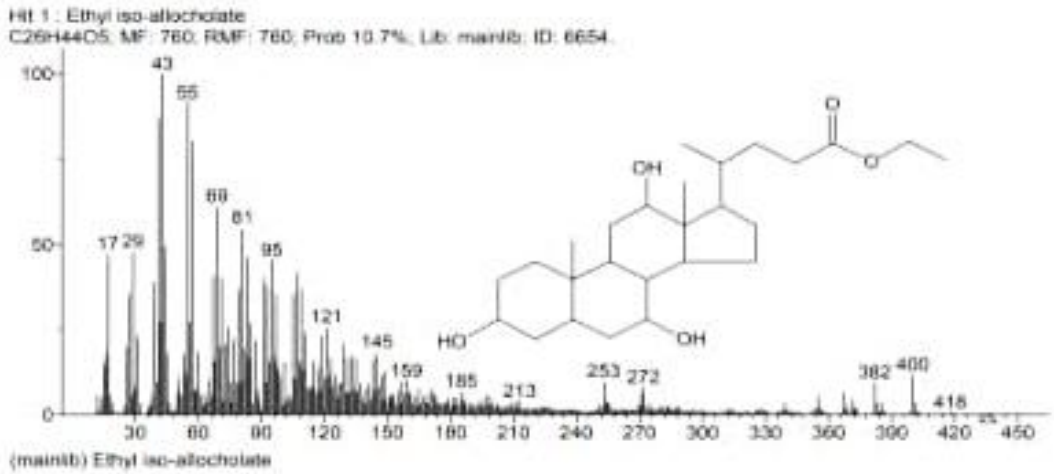

Figure 3b: Structure of Ethyl Iso-allocholate present in the ethyl acetate extract of Trametepolyzona using GC-MS analysis.

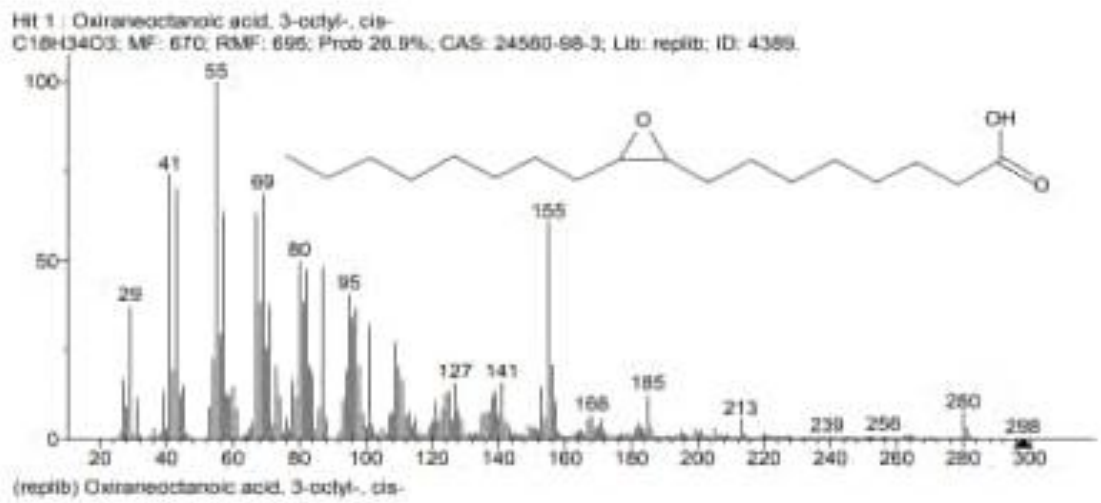

Figure 3c: Structure of Oxiraneoctanoic acid, 3-octyl-, cis- (Epoxyoleic acid)present in the ethyl acetate extract of *Trametes polyzona* using GC-MS analysis

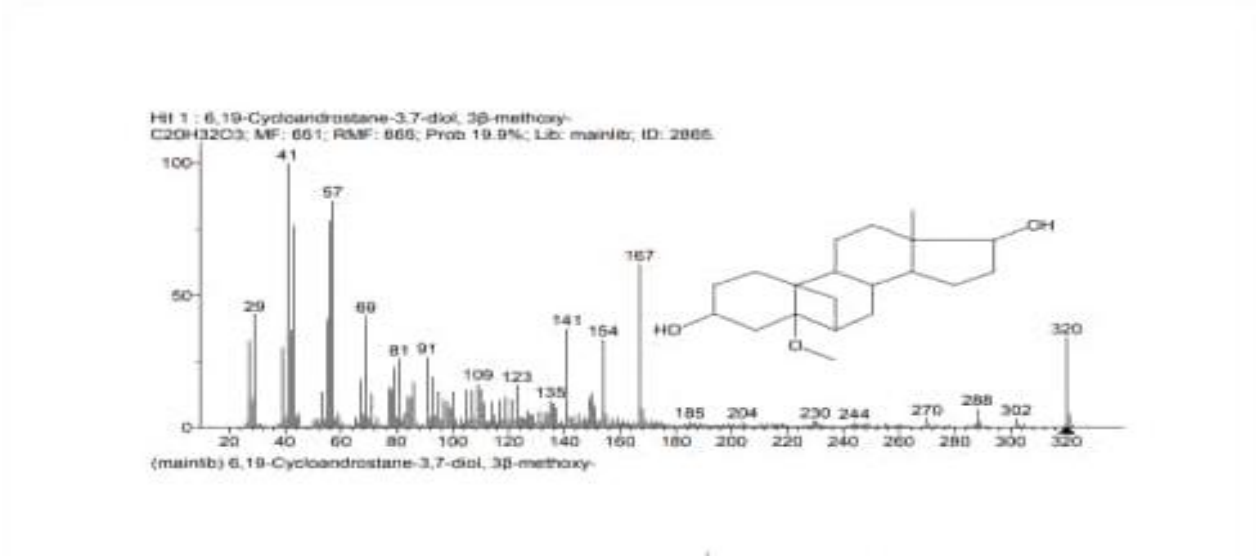

Figure 3d: Structure of 6,19-cycloandrostane-3,7-diol,3β-methoxy-present in the ethyl acetate extract of *Trametes polyzona* using GC-MS analysis

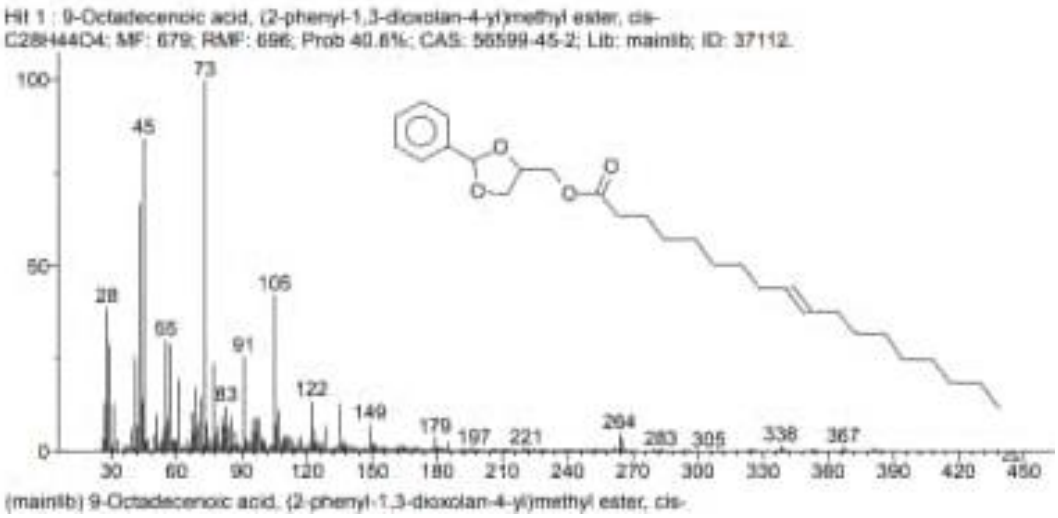

Figure 3e: Structure of 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis- -present in the ethyl acetate extract of *Trametes polyzona* using GC-MS analysis

Flavonoids, a polyphenol present in high concentration in *Trametes Polyzona* extract the major active nutraceutical found in plants that possess antimicrobial, antioxidant, anticarcinogenic, and enzyme modulatory functions (they have been found to modulate Eicosanoid pathway enzymes).[23, 24]

The Antioxidant activities demonstrated by *Trametes Polyzona* extract could be associated with a high concentration of flavonoids in the extract. The presence of high hydroxyl groups in flavonoids affords them the ability to scavenge free radicals, which helps to prevent degradation of pharmaceutical products and extends their shelf-life.[25, 26]Naringin and Narigenin, are flavanones present in grapefruits, sour oranges, tomatoes, and lemons in varying amounts and possess antioxidant activity dependent on their environment[25], they also possess Hypolipidemic, antitumor, and antidiabetic properties,[27] and Inflammatory properties due to their COX-inhibiting Property [28]Naringenin's ability to absorb UV rays have been found helpful in protecting against DNA damage and protect against prostate cancer in humans.[29] The antimicrobial activity of Naringenin has been demonstrated against *S. aureus, E. coli, and E. faecalis* and is very effective against these pathogens.[30] This may have also contributed to the antimicrobial activity of the extract of this endophytic fungus *TrametesPolyzona*. Catechins, a well-known subgroup of flavanols are found in bananas[28], chocolates, green and black tea, red wine, apples, and raspberries have cardiovascular benefits in inducing the release of Nitric Oxide after ingestion and also improve endothelial function.[29, 25] This group of compounds has been shown from previous reports to modulate some biological activities; have potent α-glucosidase and α-amylase inhibitory activity thereby modulating blood-sugar levels in diabetic patients, induce apoptosis in cancer cells, has potent activity against bacterial DNA replication process and hepatoprotective properties. [29, 26]Catechins have also been classified under flavan-3-ol as reported by.[24]Flavanones, the most concentrated flavonoid in this extract, are present in all citrus fruits and are responsible for their bitter tastes e.g include Hesperetin, Naringenin, and eriodictyol. They are known for their characteristic free-radical scavenging properties, anti-oxidant, lowering of blood cholesterol, and anti-inflammatory.[27] Two major flavones have been found as phytochemicals egapigenin and luteolin,[23] and have been found to possess anti-cancer, anti-inflammatory, hepatoprotective, and anti-atherosclerotic properties. [27, 25] Anthocyanins the second most abundant flavonoid in this extract, which exists as colored pigments in plants are also found in honey, vegetables, cocoa, hibiscus sabdariffa,[31] berries, bilberries, cranberries, and red wine. They suppress mRNA downstream process for fatty acid and triglycerol synthesis, possess anti-diabetic properties, anti-cancer, and anti-bacterial properties, and support Rhodopsin development.[25,28] Anthocyanin has been reported to possess anti-diabetic and

antioxidant properties, it can also inhibit the harmful effects of oxidative stress on pancreatic -cells.[32]

The abundance of a variety of flavonoids in *T. polyzona* extract could dictate the activity of this extract and combinatorial biosynthesis using this endophytic fungus as a start-up culture and other precursor substances could help optimize the production of various classes of Flavonoids. Alkaloids which constitute 9.24 % of the endophytic fungal extract are a group of important Nitrogen-containing compounds useful in the pharmaceutical space since medieval times for the treatment of certain ailments, chemical poisoning, and beauty purposes, etc.[33]The armamentarium of medications used in the pharmaceutical industry of today mostly belongs to the class of alkaloids. Alkaloids found in this extract are quinine, ribalinidine, and sparteine in the order of their decreasing concentration in this extract. Ribalinidine, a quinoline alkaloid has been identified to possess free radical scavenging properties,[34] Quinine, another quinoline alkaloid has been identified to possess antipyretic, anti-inflammatory properties and used to treat nocturnal leg cramps and beneficial in arthritis treatment, [35] It is used in the treatment of severe malaria unresponsive to current Artemisinin combination therapy (ACT).[36]Sparteine has been shown to demonstrate antiarrhythmic, hypoglycemic, antimicrobial, diuretic, anti-convulsant, and anti-inflammatory properties.[37] The result of the GC-FID analysis (Fig. 3a-f) on the extracts of *TrametesPolyzona* correlates with the report of Oduntan, [18] who also identified phyto-chemicals including flavonoids, alkaloids, and phenols present in ethyl acetate extracts of *Trametespolyzona*.

Gas chromatography and mass spectroscopy screening carried out on the ethyl acetate crude extract of *TrametesPolyzona* detected four major compounds with established pharmacological activities. These included ethyl iso-allocholate; Oxiraneoctanoic acid, 3-octyl-, methyl ester; 6,19-cycloandrostane-3,7-diol,3β-methoxy; 9-Octadecenoic acid, methyl ester (Fig. 3a-f). Similarly, GC-MS assessment on the ethanol extract of the leaves of Feroniaelephantum detected ethyl iso-allocholate, a steroid with various pharmacological activities such as Antimicrobial, Diuretic, Antiasthma, and Anti-inflammatory activities.[38] Also, ethyl iso-allocholate detected in "Karungkavuni" a medicinal rice variety demonstrated potential as an effective inhibitor of dihydropteroate synthase enzyme in *Escherichia coli*.[39] Furthermore, a study by Idan *et al.*[40] validated the anti-inflammatory and antimicrobial properties of ethyl iso-allocholate (2), an alkaloid.

Oxiraneoctanoic acid, 3-octyl-, methyl ester identified in the alcoholic seed extract of *F. vulgare*and has been reported to possess Antibacterial activity.[41] Also, Oxiraneoctanoic acid, 3-octyl-, methyl ester was identified in the n-hexane fractions of *S. diversifolia* leaf and stem bark has been previously validated to possess a range of antibacterial activity against *Staphylococcus aureus*.[42]

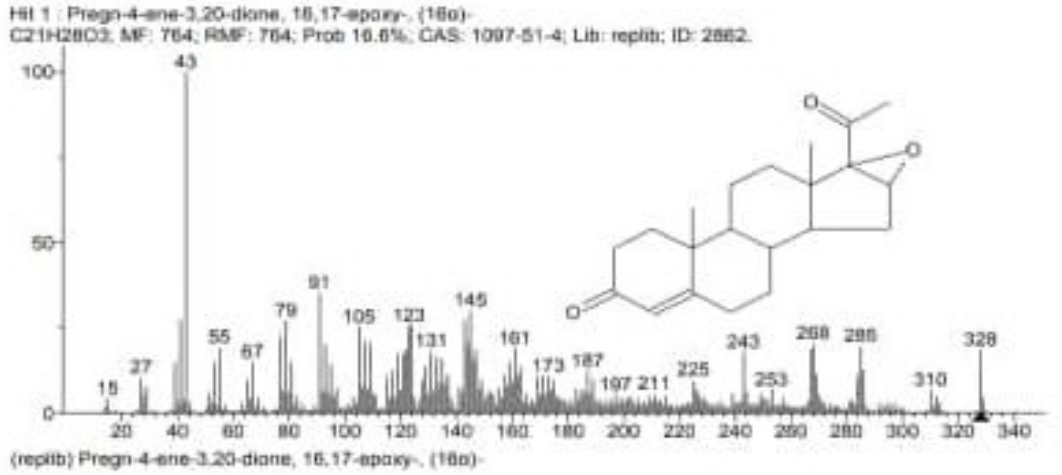

Figure 3f: Structure of Pregn-4-ene-3,20-dione, 16,17-epoxy-, (16α)-methyl ester, cis--present in the ethyl acetate extract of *Trametes polyzona* using GC-MS analysis

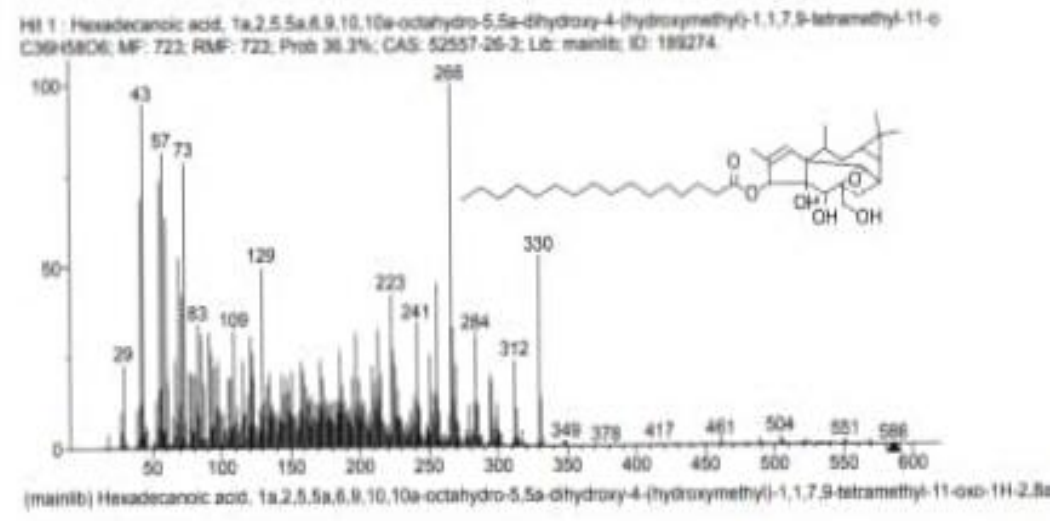

Figure 3g: Structure of Hexadecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aα,2α,5β,5aβ,6β,8aα,9α,10aα)]- present in the ethyl acetate extract of *Trametes polyzona* using GC-MS analysis

Mridha *et al.*[43] detected the presence of 6,19-cycloandrostane-3,7-diol,3β-methoxy through GC-MS chemo profiling as a major bioactive component of *Spirogyra triplicate* methanolic extract which induced anti-proliferative activity specifically against A549 cell.
Also, 9-Octadecenoic acid, methyl ester a bioactive molecule obtained by GC-MS analysis in lupin species was observed to have Antioxidant and antimicrobial activities.[44] 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- possesses antimicrobial and anti-inflammatory activity as reported by Kadhim et al,.[45]

Conclusion

In conclusion for the first time, we reported that *Trametes Polyzona* has a metabolic capacity to biosynthesize bioactive secondary metabolites with promising effects on the prevention of microbial spoilage of pharmaceutical preparations caused by microbial contaminants that is comparable to that of commercialized preservatives. In addition, *Trametes Polyzona* produced compounds with broad-spectrum antimicrobial properties that could be useful in the pharmaceutical industry for the preservation of pharmaceutical products.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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