ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue IX September 2024

Evaluation of the Mineral, Amino Acid and Antimicrobial Properties of Raw and Fermented *Trametes elegans*

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DOI: https://doi.org/10.51244/IJRSI.2024.1109097

Received: 05 September 2024; Accepted: 17 September 2024; Published: 18 October 2024

ABSTRACT

Mushrooms are known to be sources of food and bioactive compounds with medicinal properties. In this study, the mineral, proximate, amino acid and antimicrobial properties of a wild macrofungus, *Trametes elegans* was investigated. Standard methods were used to evaluate the mineral, proximate and amino acid content of raw and fermented *T.elegans.* By soaking in methanol and acetone, extracts from the macrofungus's powdered fruiting bodies were obtained. Using common microbiological methods, the macrofungus extracts' antimicrobial activity was assessed on test isolates. In addition, isolation and identification of microorganisms associated with raw and fermented *T. elegans* was done using standard microbiological techniques. The physicochemical parameters (Temperature, pH and Total Titratable Acid) were also monitored during solid and liquid state fermentation of *Trametes elegans*. The concentration of carbohydrate (70.24±0.33) was higher in the raw sample, while moisture (3.22±0.13), fat (1.85±0.04) and protein contents (22.48±0.09) were higher in sample subjected to solid fermentation. Also, crude fibre (6.68±0.76) and Ash (3.60±1.39) were higher in sample subjected to liquid fermentation. The micro elements analysed are zinc and iron and macro elements are potassium, magnesium and phosphorus and their values increased in fermented *Trametes elegans.* Analyses of both essential and non-essential amino acids were conducted. The most common amino acids found in all of the samples examined were glutamic acid, leucine, arginine, and aspartic acid. Cysteine and methionine are the least abundant. The amino acid scores were determined by ratio of the milligrams of amino acid per gram of test protein to the milligrams of amino acid per gram in the reference pattern. The results showed that methionine had the lowest score in the raw sample (3.14) and tryptophan had the highest score (18.17) in *T. elegans* that had undergone solid fermentation. Methanol and acetone extracts inhibited all test isolates except *Lactobacillus plantarum.* The commercial drug exhibited higher antibacterial activity compared to the extracts, with a significant difference ($P \leq 0.05$). The inhibitory zones ranged from 5.50 mm against *Bacillus subtilis* to 18.13 mm against *E. coli*. The range of the minimum bactericidal concentration (MBC) was 12.5 to 50 mg/mL, while the minimum inhibitory concentration (MIC) was 12.5 to 50 mg/mL. In light of this findings, *T. elegans*, could be considered as potential sources of natural antimicrobial and can also be relevant for use as food supplements in animals and man.

Keywords: Extracts, Fermentation, Raw, Trametes, *Trametes elegans*, Species.

INTRODUCTION

There are currently over 100,000 known species of fungi, and even fewer have been thoroughly studied to determine whether they could yield important therapeutic chemicals. Yet, some of the most potent drugs and fungicides used in farming have been developed utilizing fungal secondary metabolites (De Silva *et al*., 2013). According to Kozlovskii *et al*. (2013), these include drugs that lower cholesterol, such as statin derivatives (mevinolin, lovastatin, and simvastatin), antifungal agents (griseofulvin, strobilurins, and echinocandins), antibiotics (penicillins, cephalosporins, and fusidic acid), and immunosuppressive drugs (cyclosporin).

In addition to being a source of chemicals with pharmacological effects in medicine, mushrooms have long been used as food (Oyetayo and Akingbesote, 2022). They exist in nature, although during the wet season, they are more common in terrestrial settings (Adeniyi *et al*., 2018). In particular, lignin, cellulose, and organic

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components are prevalent in macrofungi. They thus play a significant role in the terrestrial ecology as biodegraders (Adebiyi and Yakubu, 2016; Adeniyi *et al*., 2018).

In traditional medicine, mushrooms are well-known for treating ailments like rheumatism, kwashiorkor, obesity, diarrhea, and as a purgative (Apetorgbor *et al*., 2005; Ejelonu *et al*., 2013).

Trametes elegans, which Fries officially described as *Trametes* in 1835 (Olou *et al.*, 2020), was formerly known as Lenzites (Ediriweera *et al*., 2021). This is because of its ease of recognizing. The endophytic fungus *T. elegans* is typically found in hardwood woods (Mayaka *et al*., 2019). According to Sagar *et al*. (2020), it is a member of the genus Basidiomycota, the family Polyporaceae (Pathania and Chander, 2018), and the phylum Basidiomycete (Mendez *et al*., 2018).

There may be inherent antimicrobial qualities in *T. elegans*. Researchers discovered in a study that *T. elegans* compounds might stop the growth of germs, and that these compounds may have antimicrobial effects on a range of bacteria and fungus Mayaka *et al*. (2019). Mayaka *et al*. (2019) identified many chemicals that are associated with this compound: ergosta-5,7,22-trien-3-ol; 5α,8α-epidioxyergosta-6,9,22-trien-3βol; 5α,8α-epidioxyergosta-6,22-dien-3β-ol; Ergosta -7,22-diene-3β; 5α,6β-triol; lupeol; and 9,19-cycloartan-3,30-diol. The present study is therefore aimed at determining the antimicrobial properties and food value of raw and fermented *Trametes elegans* collected in forest around Federal University of Technology, Akure.

MATERIALS AND METHODS

Collection and identification of *T. elegans*

Samples of *Trametes elegans* were collected from dead woods in forests around FUTA, Akure, Ondo State, Nigeria. The *T. elegans* were identified by a taxonomist in the department of microbiology, FUTA.

Fermentation of *T. elegans* samples: The fermentation took place for four days. On the fourth day, the fermented mushroom was oven dried at 40^{o}C for 48 hours.

Determination of physicochemical parameters during fermentation

The temperature during fermentation was measured using thermometer and also, the pH values during fermentation was measured using a pH-meter at 0, 24, 48, 96 and 120 hours Total titratable acidity was determined by titrating 10 ml of the samples with 0.1 M NaOH using (2 drops) phenolphthalein as indicator. Sodium hydroxide was added slowly into samples until an equivalent pink color appeared. The total titratable acidity was then calculated according to A.O A. C (2019).

Preparation of Extracts from *T. elegans* samples: *T. elegans* samples were split into three parts, according to Oyetayo and Akingbesote (2022). The first part was prepared as a raw sample, and the second and third portions underwent solid and submerged fermentation for four days at room temperature (27 ± 1∘C). 100 g of each sample was extracted by adding it to 750 mL of 95% acetone and methanol in an Erlenmeyer flask after both samples had been dried and processed into powder using a mill machine. For three days, the flasks were let to stand for extraction, with sporadic stirring. Next, the extracts were passed through cotton wool that had been placed inside a funnel. In a rotary evaporator operating at 90 rpm and low pressure, the filtrates were evaporated until they were completely dry at 50°C.

Isolation and identification of microorganisms associated with raw and fermented *T. elegans*:

After cutting and macerating both raw and fermented *T. elegans*, a five-fold serial dilution was made, and 0.1 mL of the 103 and 104 dilutions were inoculated onto sterile Petri dishes. Nutrient and potato dextrose agar, which has been sterilized, was placed onto Petri dishes, swirled, and left to gel. Following that, the solidified plates were incubated for 24 hours at 37 °C and 48 hours at 28 °C for fungus and bacteria, respectively. According to Alves *et al*. (2022), the physical and cultural characteristics of the isolates were noted and monitored. Biochemical tests and Gram staining are two identification techniques.

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Gram Staining

There are four phases in this staining technique: staining with a water-soluble dye called crystal violet, using a mordant called Grams Iodine to bind the dye, decolorizing with acetone or 95% ethyl alcohol, and counterstaining, primarily with diluted (1:10) Carbol fuchsin or Safranin (Tripathi and Sapra, 2023).

Staining Procedure:

The prepared smear was placed on a staining rack over the sink, and it was then covered for one minute with primary stain, namely crystal violet. Gram's Iodine was applied for one minute, and then the smear was rinsed under soft tap water for another minute. After another minute, the smear was cleaned with tepid tap water and a short 10-second addition of decolorizer, such as acetone or 95% ethyl alcohol, was made. Once more, the smear on the slide was gently cleaned with tap water before being stained for less than a minute with either diluted (1:10) carbonyl fuchsin or safranin. The smear was cleaned with mild tap water and allowed to air dry for a minute. The back side of the smear was cleaned with tissue paper and a drop of Cedar wood oil is added and focused on microscope using Oil Immersion lens (100 X).

Assessment of antimicrobial properties of *T. elegans*

The Ogidi *et al*. (2015) Agar well diffusion method was used to evaluate the extracts' antibacterial activity. In a nutshell, the organisms were grown on nutrient broth for 24 hours at 37∘C for bacteria. The size of the inoculum was changed to meet the 0.5 McFarland turbidity limits. The extract was reconstituted in dimethyl sulfoxide (DMSO) at a volume of 30% v/v. An 8 mm cork borer was used to create holes in an aliquot of 0.1 mL of organism that had been aseptically transferred and evenly dispersed onto the dried surface of a sterile Mueller Hinton agar plate. Using a micropipette, 0.1 mL of the extract was aseptically added to the well of Petri dishes that had already been inoculated with isolates after the extract was sterilized through a membrane filter (0.22 μm). A volume of 0.1 mL of ciprofloxacin was used as positive control while sterile distilled water was used as negative control and the plates were incubated at 37 °C for 24 hours. The experiment was carried out in triplicates and inhibition zones measured and recorded in millimeter.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

In test tubes, 0.1 mL of a standardized inoculum (0.5 McFarland turbidity standards) was combined with sterile nutritional broth and extracts ranging in concentration from 12.5 to 50 mg/mL (Ogidi *et al*., 2015). For a whole day, the tubes holding the isolated bacteria were incubated aerobically at 37°C. As a control, the test tubes with the growth medium, sterile distilled water, and each organism's inoculum were kept intact. When compared to the control tubes, the extract concentration that resulted in no discernible growth (no turbidity) was considered the minimum inhibitory concentration (MIC). Mueller Hinton Agar (MHA) was used to quantify subculturing 0.1 mL from a test tube in order to estimate the minimum bactericidal concentration (MBC). At 37°C, these plates were incubated. The MBC is defined as the lowest concentration of extracts that killed the bacteria tested at 99.9 to 100%.

Proximate analysis of raw and fermented *T. elegans*

Using the techniques of AOAC (2019), the samples' moisture, protein, fat, ash, and fiber content were examined. By difference, the percentage of carbohydrates was derived. In compliance with AOAC (2019), this was accomplished by deducting the total organic nitrogen, fat, ash, and fiber from the total dry matter.

Mineral analysis of raw and fermented *T. elegans*

The method outlined by AOAC (2019) for magnesium, iron, and zinc and flame photometer for potassium was used to determine the mineral contents of the samples. Using the modified AOAC technique (2019), the molybdate method was also used to determine the total phosphorus content of the samples. Nitric, sulfuric, and perchloric acids are combined for wet digestion, and an atomic absorption spectrophotometer is used in this process.

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Amino acid analysis of raw and fermented *T. elegans*

The techniques outlined by AOAC (2019) were used to determine the amino acid profile in the samples. The known sample was placed into the Applied Biosystems PTH Amino Acid Analyzer (MODEL:120A), dried to constant weight, defatted, hydrolyzed, and evaporated in a rotary evaporator.

Data analysis

Experiments was carried out in triplicates and data obtained was analyzed by one-way analysis of variance (ANOVA) and means separated by Duncan multiple range test (SPSS 20.0 version). Differences was considered significant at $P \leq 0.05$.

RESULTS

Tables 1 to 3 shows the physicochemical parameters (Temperature, pH and Total Titratable Acid) that were monitored during solid and liquid state fermentation of *Trametes elegans*. There was increase in total titratable acid during fermentation (solid and liquid state fermentation). For solid state fermentation (SSF) it ranged from 1.50 to 3.50 g/l and for liquid state fermentation (LSF) it ranged from 4.50 to 6.75 g/l as shown in Table 1. There was decrease in pH during fermentation (solid and liquid state fermentation). For SSF it ranged from 8.20 to 4.10 and for LSF it ranged from 4.10 to 3.00 as shown in Table 2. Also, there was decrease in temperature during fermentation (solid and liquid state fermentation). For SSF it ranged from 32.0 to 30.0^{o}C and for LSF it ranged from 34.0 to 32.0^{o}C as shown in Table 3.

Tables 4 and 5 shows the bacterial and fungal loads associated with raw and fermented *T. elegans*. There was decrease in bacterial count during solid state fermentation from day 1 to 4 and it ranged from 5.0 to 1.6 (10^3cfu/ml) and 3.7 to 0.8 (10^4cfu/ml). Moreover, there was decrease in bacterial count during liquid state fermentation from day 1 to 4 which ranged from 7.6 to 4.0 (10^3cfu/ml) and 4.0 to 1.6 (10^4cfu/ml). There was increase in fungal count during solid state fermentation from day 1 to 4 and it ranged from 1.5 to 4.3 (10^3cfu/ml) and 1.6 to 3.1 (10^4cfu/ml). And also, there was increase in fungal count during liquid state fermentation from day 1 to 4 which ranged from 0.3 to 5.0 (10^3cfu/ml) and 0.2 to 2.5 (10^4cfu/ml).

The cultural and morphological characteristics of bacterial isolates obtained from raw and fermented samples of *Trametes elegans* are shown in Table 6. The bacteria strains consisted of *Bacillus cereus*, *Microbacterium* sp., *Streptococcus* sp., *Actinomyces* sp., *Macrococcus* sp., *Streptomyces* sp., *Clostridium perfringens* and *Lactobacillus acidophilus.*

The cultural and morphological characteristics of fungal isolates obtained from raw and fermented samples of *Trametes elegans* are shown in Table 7. The fungi consisted of *Candida* sp., *Rhizopus* sp., *Aspergillus niger*, *Mucor* sp. and *Penicillium* sp.

Table 1: Total Titratable Acid (TTA) of *Trametes elegans* During Solid and Liquid State Fermentation

Days	Solid state fermentation(g/l)	Liquid state fermentation(g/l)
0	1.50	4.50
1	1.88	4.88
2	2.21	5.25
3	3.12	6.00
4	3.50	6.75

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Table 2: pH of *Trametes elegans* During Solid and Liquid State Fermentation

Hours	**Solid state fermentation**	**Liquid state fermentation**
0	8.20	4.10
24	7.00	4.00
48	6.10	3.80
96	5.00	3.20
120	4.10	3.00

Table 3: Temperature of *Trametes elegans* During Solid and Liquid State Fermentation

Days	**Solid state fermentation(ºC)**	**Liquid state fermentation(ºC)**
0	32.0	34.0
1	30.0	34.0
2	30.0	34.0
3	30.0	32.0
4	30.0	32.0

Table 4: Bacterial load of *Trametes elegans* During Solid and Liquid State Fermentation

NUTRIENT AGAR (TOTAL COLONY COUNT)

Sample/Days	**10^3(cfu/ml)**	**10^4(cfu/ml)**
Day 1		
Raw	3.2	1.0
SSF	5.0	3.7
LSF	7.6	4.0
Day 2		
SSF	3.5	2.8
LSF	6.4	3.8
Day 3		
SSF	3.5	2.5
LSF	6.0	3.0
Day 4		
SSF	1.6	0.8
LSF	4.0	1.6

*__Key__: **SSF**-Solid State Fermentation; **LSF**-Liquid State fermentation

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Table 5: Fungal load of *Trametes elegans* During Solid and Liquid State Fermentation

POTATO DEXTROSE AGAR (TOTAL COLONY COUNT)

Sample/Days	**10^3(cfu/ml)**	**10^4(cfu/ml)**
Day 1		
Raw	7.4	0.3
SSF	1.5	1.6
LSF	0.3	0.2
Day 2		
SSF	3.0	2.0
LSF	0.4	0.4
Day 3		
SSF	3.2	2.9
LSF	1.3	0.7
Day 4		
SSF	4.3	3.1
LSF	5.0	2.5

*__Key__: **SSF**-Solid State Fermentation; **LSF**-Liquid State fermentation

Table 6: Morphological and Biochemical Characteristics of Bacterial Isolates Obtained from *T. elegans*

Isolates	R1	S1	L1	S2	L2	L3	S3	L4
Colour	Cream	Yellow	Cream	Cream	Cream	White	Cream	White
Opacity	Opaque	Transparent	Translucent	Translucent	Opaque	Opaque	Translucent	Opaque
Shape	Circular	Circular	Circular	Filamentous	Circular	Rhizoid	Circular	Circular
Size	Small	Small	Medium	Small	Large	large	Medium	Small
Elevation	Raised	Raised	Flat	Raised	Raised	Flat	Raised	Raised
Texture	shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny
Morphology								
Gram Stain	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Cell type	Rod	Rod	Cocci	Rod	Cocci	Rod	Rod	Rod
Biochemical								

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Tests								
Coagulase	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	_-Ve
Methyl red	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
V. P	+Ve	+Ve	-Ve	-Ve	+Ve	-Ve	-Ve	-Ve
Indole	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Catalase	+Ve	+Ve	-Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Citrate	+Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Oxidase	-Ve	-Ve	-Ve	-Ve	+Ve	-Ve	-Ve	-Ve
Urease	-Ve	+Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Motility	+Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Sugar Test								
Maltose	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	+Ve
Dextrose	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Sucrose	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Lactose	+Ve	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve	+Ve
Glucose	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Probable	*Bacillus*	*Microbacterium* sp.	*Streptococcus* sp.	*Actinomyces* sp.	*Micrococcus* sp.	*Streptomyces* sp.	*Clostridium*	*Lactobacillus*
Isolates	*cereus*						*perfringens*	*acidophilus*

*__Key__: **R**- Isolates from Raw *T. elegans*

S- Isolates from *T. elegans* subjected to solid fermentation

L- Isolates from *T. elegans* subjected to liquid fermentation

V.P- Voges Proskauer

Table 7: Cultural and Morphological Characteristics of Fungal Isolates Obtained from *T. elegans*

Isolates	**A**	**B**	**C**	**D**	**E**
Cultural characteristics	Cream, smooth colony, raised, entire with yeast smell.	White colony growing to cover plate.	Fluffy colony with reverse side black.	White to dark grey colony.	Flat and thick colony with dirty white reverse coloration.
Nature of hyphae	Non-septate	Coenocytic	Septate	Non-septate	Septate

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Spore type	Conidiospores	Sporangiospores	Conidiospores	Sporangiospores	Conidiospores
Putative identity	*Candida* sp.	*Rhizopus* sp.	*Aspergillus niger*	*Mucor* sp.	*Penicillum* sp.

Figures 1 to 5 and Table 8 shows the antimicrobial activities of *T. elegans* extracts (50mg/ml) and commercial drug (ciprofloxacin) against the test organisms; *Klebsiella pneumoniae* (ATCC 13885), *Pseudomonas aeruginosa* (ATCC 9027), *Clostridium perfringes, E. coli, Bacillus subtilis* and *Lactobacillus plantarum* respectively. Methanol and acetone extracts inhibited all test isolates except *Lactobacillus plantarum.* The activities of the commercial drug when compared to those of the extracts were slightly higher and significantly different ($P \leq 0.05$). The zones of inhibition ranged from 5.50 mm (*Bacillus subtilis*) to 18.13 mm (*E. coli*).

Tables 9 and 10 shows the minimum inhibitory concentration and minimum bactericidal concentration of *T. elegans* extracts. The MIC value ranged from 12.5 to 50mg/mL and MBC ranged from 12.5 to 50mg/mL.

The proximate composition of the raw (unfermented) and fermented (solid and liquid) *T. elegans* is presented in Table 11. The concentration of carbohydrate (70.24±0.33) was higher in the raw sample, while moisture (3.22±0.13), fat (1.85±0.04) and protein contents (22.48±0.09) were higher in solid fermentation. Also, crude fibre (6.68±0.76) and Ash (3.60±1.39) were higher in liquid fermentation.

Table 12 shows the mineral composition of the raw, solid and liquid fermented *Trametes elegans*. The micro elements which are zinc and iron and macro elements which are potassium, magnesium and phosphorus increased in fermented *Trametes elegans.*

The amino acids analyzed includes, essential amino acids (phenylalanine, valine, tryptophan, threonine, isoleucine, methionine, histidine, leucine and lysine) as shown in Table 13 and non-essential amino acids (alanine, glycine, aspartic acid, glutamic acid, cystine, proline, serine, arginine, tyrosine and norleucine) as shown in Table 14. Glutamic acid, leucine, arginine and aspartic acid are the most predominant amino acids in all the samples analysed. While methionine and cystine are the least.

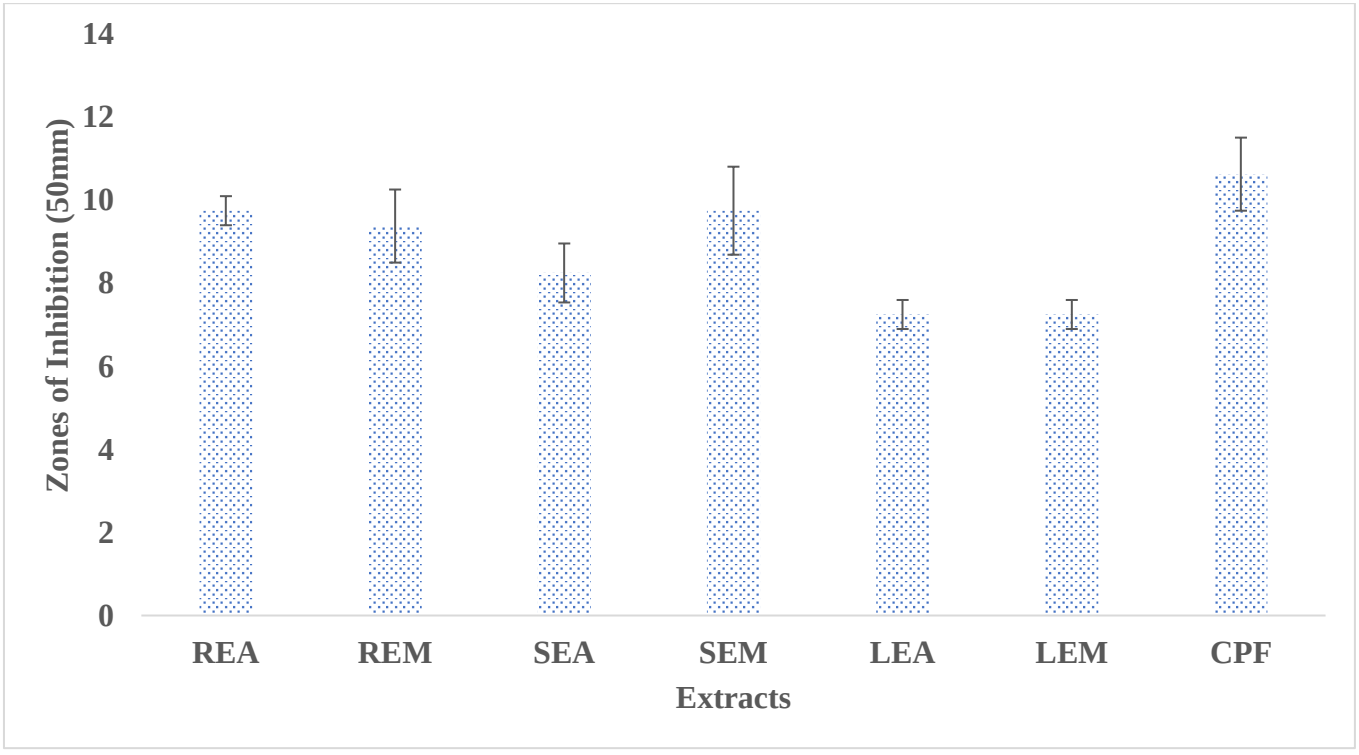

Figure 1: Antimicrobial Activities of Raw and Fermented *T. elegans* Extracts on *Klebsiella pneumoniae* (ATCC 13885)

KEY: REA- Raw extracted with Acetone; **REM**- Raw extracted with methanol; **SEA**- Solidly fermented

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extracted with Acetone; **SEM**- Solidly fermented extracted with Methanol; **LEA**- Liquid fermented extracted with Acetone; **LEM**- Liquid fermented extracted with Acetone; **CPF**- Ciprofloxacin; **ATCC**- American type culture collection.

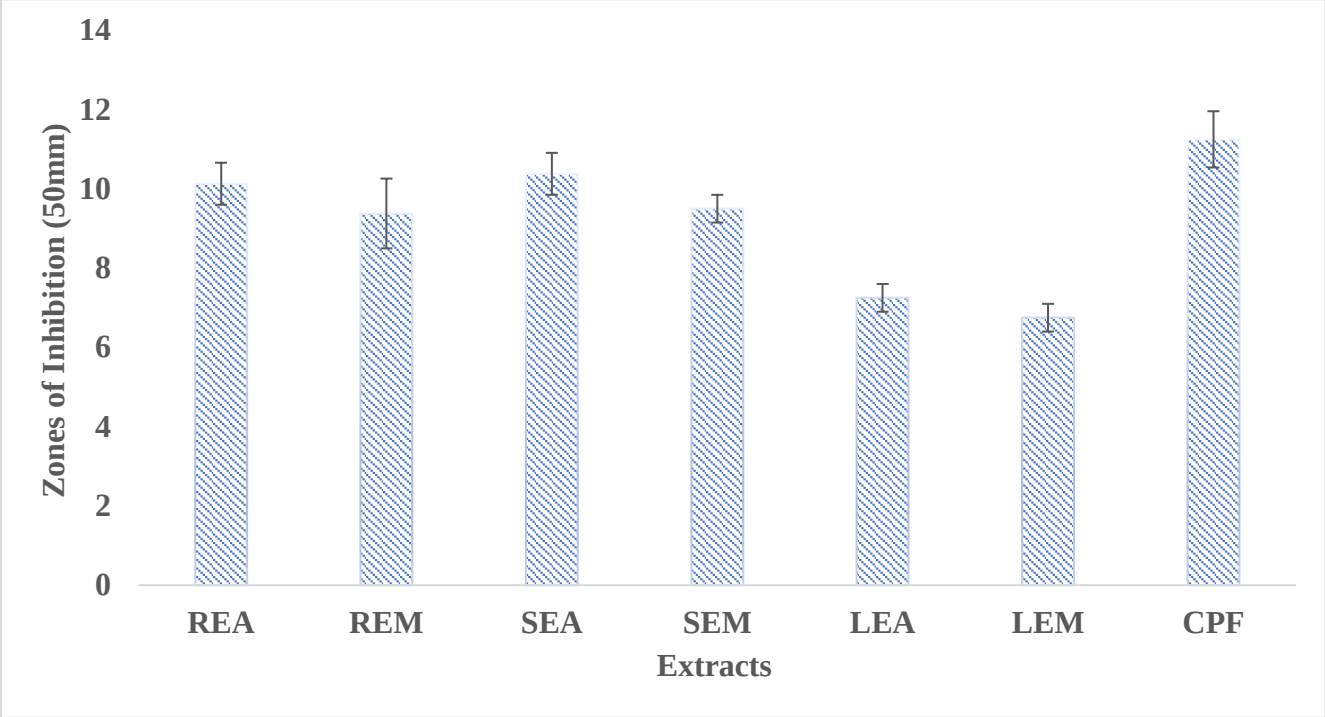

Figure 2: Antimicrobial Activities of Raw and Fermented *T. elegans* Extracts on *Pseudomonas aeruginosa* (ATCC 9027)

KEY: REA- Raw extracted with Acetone; **REM**- Raw extracted with methanol; **SEA**- Solidly fermented extracted with Acetone; **SEM**- Solidly fermented extracted with Methanol; **LEA**- Liquid fermented extracted with Acetone; **LEM**- Liquid fermented extracted with Acetone; **CPF**- Ciprofloxacin; **ATCC**- American type culture collection.

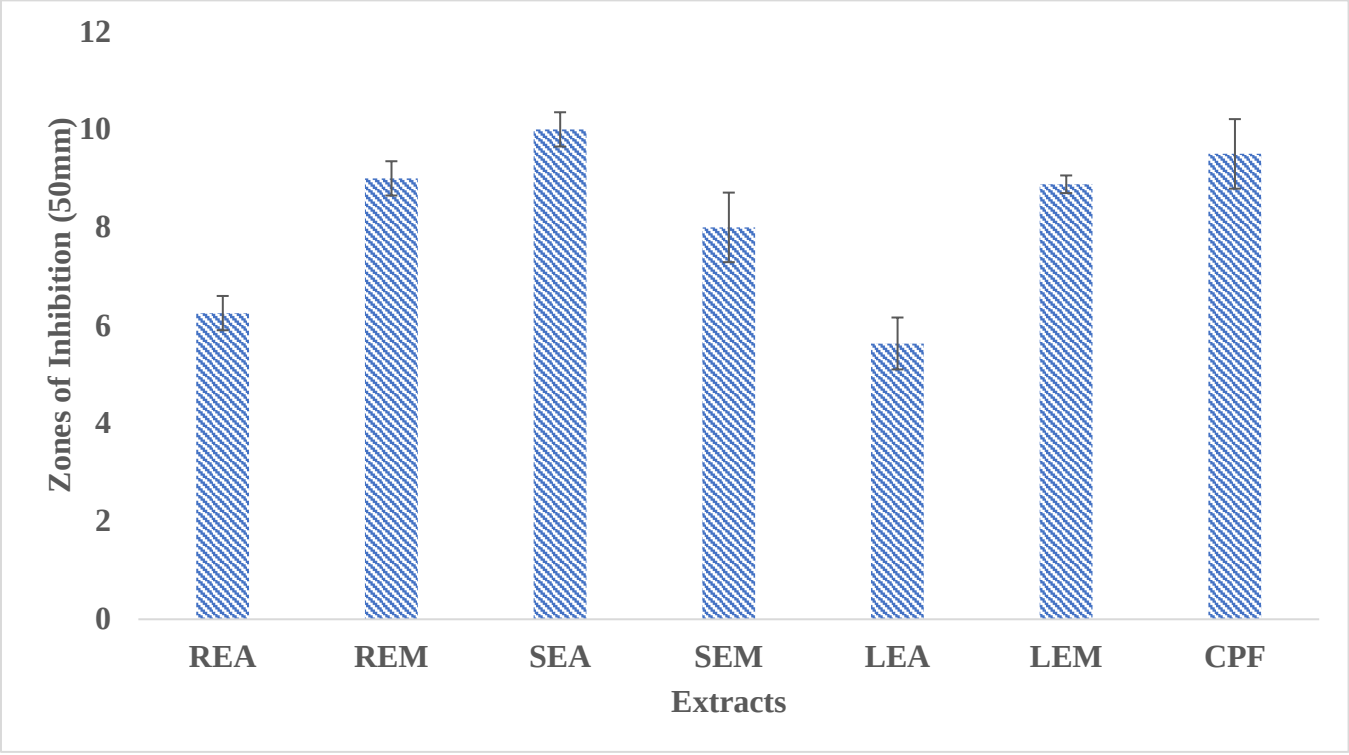

Figure 3: Antimicrobial Activities of Raw and Fermented *T. elegans* Extracts on *Clostridium perfringes*

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KEY: REA- Raw extracted with Acetone; **REM**- Raw extracted with methanol; **SEA**- Solidly fermented extracted with Acetone; **SEM**- Solidly fermented extracted with Methanol; **LEA**- Liquid fermented extracted with Acetone; **LEM**- Liquid fermented extracted with Acetone; **CPF**- Ciprofloxacin.

Table 8: Antimicrobial Activities of Raw and Fermented *T. elegans* Extracts on *Lactobacillus plantarum* Zones of Inhibition of Raw and Fermented Extracts against Organisms at 50mg/ml

ORGANISM	***Lactobacillus plantarum***
REA	0.00 ± 0.00
REM	0.00 ± 0.00
SEA	0.00 ± 0.00
SEM	0.00 ± 0.00
LEA	0.00 ± 0.00
LEM	0.00 ± 0.00
CPF	4.75 ± 0.35

KEY: REA- Raw extracted with Acetone; **REM**- Raw extracted with methanol; **SEA**- Solidly fermented extracted with Acetone; **SEM**- Solidly fermented extracted with Methanol; **LEA**- Liquid fermented extracted with Acetone; **LEM**- Liquid fermented extracted with Acetone; **CPF**- Ciprofloxacin.

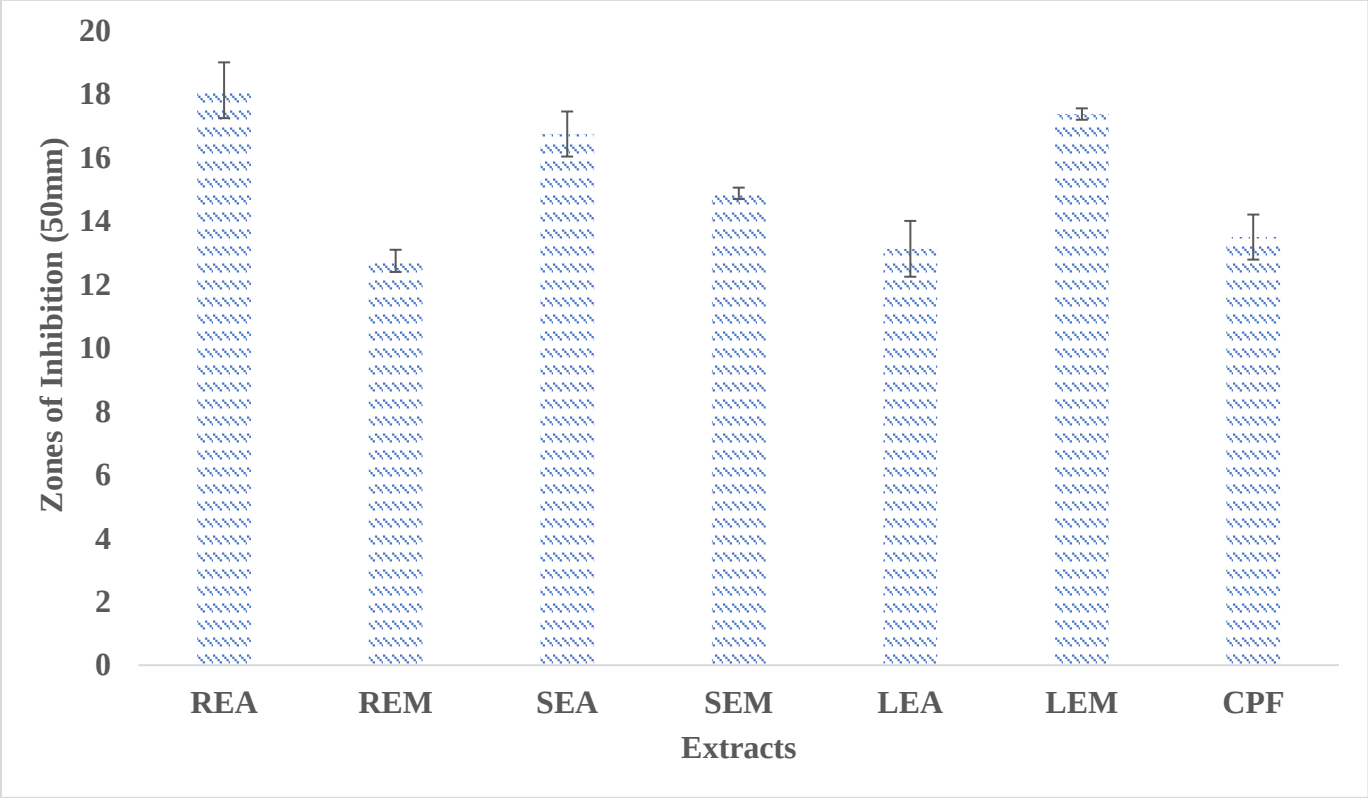

Figure 4: Antimicrobial Activities of Raw and Fermented *T. elegans* Extracts on *Escherichia coli*

KEY: REA- Raw extracted with Acetone; **REM**- Raw extracted with methanol; **SEA**- Solidly fermented extracted with Acetone; **SEM**- Solidly fermented extracted with Methanol; **LEA**- Liquid fermented extracted with Acetone; **LEM**- Liquid fermented extracted with Acetone; **CPF**- Ciprofloxacin.

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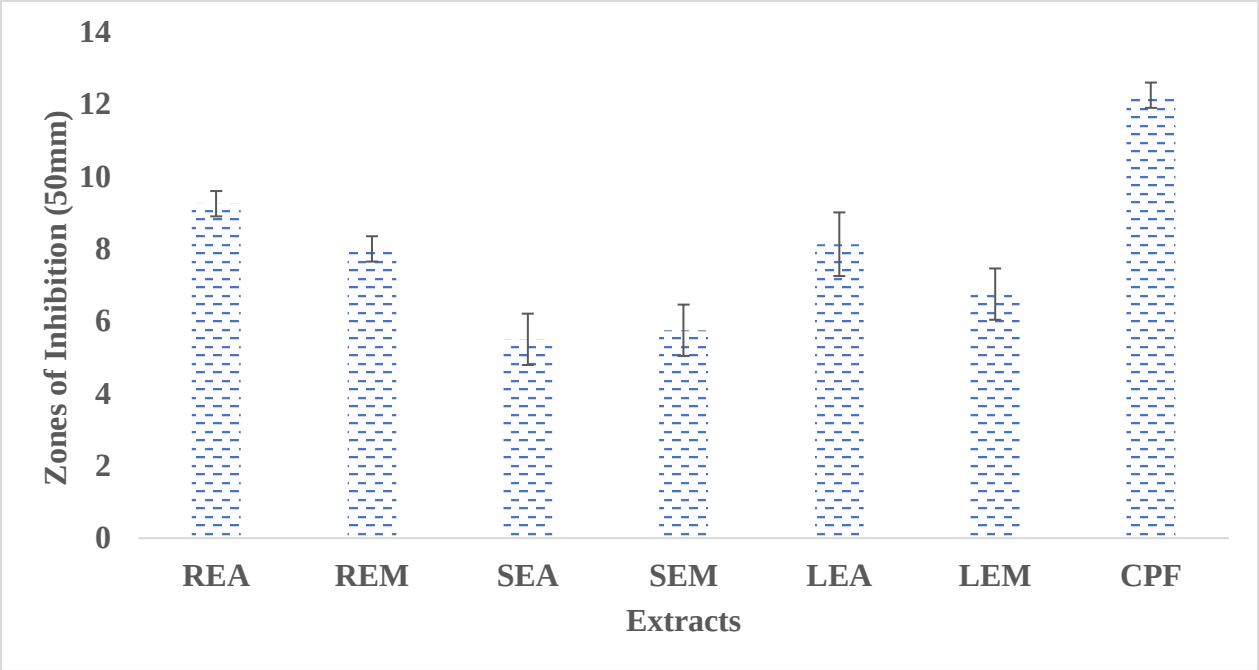

Figure 5: Antimicrobial Activities of Raw and Fermented *T. elegans* Extracts on *Bacillus subtilis*

KEY: REA- Raw extracted with Acetone; **REM**- Raw extracted with methanol; **SEA**- Solidly fermented extracted with Acetone; **SEM**- Solidly fermented extracted with Methanol; **LEA**- Liquid fermented extracted with Acetone; **LEM**- Liquid fermented extracted with Acetone; **CPF**- Ciprofloxacin.

Table 9: Minimum Inhibitory Concentration (MIC) of Raw and Fermented *T. elegans* Extracts on Test Isolates

Organism	**REA**	**REM**	**SEA**	**SEM**	**LEA**	**LEM**
Klebsiella pneumonia (ATCC 13885)	25.0	12.5	12.5	25.0	50.0	12.5
Pseudomonas aeruginosa (ATCC 9027)	25.0	25.0	50.0	25.0	50.0	25.0
Clostridium perfringes	50.0	12.5	12.5	12.5	50.0	25.0
Lactobacillus plantarum	0.0	0.0	0.0	0.0	0.0	0.0
Esherichia coli	50.0	12.5	50.0	12.5	25.0	50.0
Bacillus subtilis	50.0	50.0	12.5	25.0	25.0	25.0

*Each value is mean of triplicates results (n=3)

Table 10: Minimum Bactericidal Concentration (MBC) of Raw and Fermented *T. elegans* Extracts on Test Isolates

Organism	**REA**	**REM**	**SEA**	**SEM**	**LEA**	**LEM**
Klebsiella pneumonia (ATCC 13885)	25.0	12.5	25.0	50.0	50.0	25.0
Pseudomonas aeruginosa (ATCC 9027)	25.0	25.0	50.0	25.0	50.0	50.0
Clostridium perfringes	50.0	12.5	25.0	12.5	50.0	25.0
Lactobacillus plantarum	0.0	0.0	0.0	0.0	0.0	0.0

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Escherichia coli	25.0	12.5	50.0	25.0	12.5	50.0
Bacillus subtilis	50.0	50.0	25.0	12.5	25.0	25.0

*Each value is mean of triplicates results (n=3)

Table 11: Proximate Composition of Raw and Fermented *T. elegans* extracts (g/100g)

Sample	Moisture	Fat	Crude	Ash	Protein	Carbohydrate
A	2.64 ± 0.02^{b}	1.18 ± 0.03^{c}	5.96 ± 0.42^{a}	3.11 ± 0.47^{d}	16.88 ± 0.32^{e}	70.24 ± 0.33^{f}
B	3.22 ± 0.13^{b}	1.85 ± 0.04^{a}	5.77 ± 0.08^{d}	3.38 ± 0.08^{e}	22.48 ± 0.09^{f}	63.30 ± 0.43^{c}
C	2.56 ± 0.06^{c}	1.71 ± 0.12^{d}	6.68 ± 0.76^{e}	3.60 ± 1.39^{f}	21.54 ± 0.36^{a}	63.92 ± 2.56^{b}

Note: Values with different alphabets in same row are not significantly different ($p \geq 0.05$)

Key: A - Raw *T. elegans*

B- *T. elegans* subjected to solid fermentation

C- *T. elegans* subjected to liquid fermentation

Table 12: Mineral Composition of Raw and Fermented *T. elegans* extracts (mg/100g)

Sample	Magnesium	Iron	Zinc	Potassium	Phosphorus
A	465.5 ± 2.12^{a}	17.47 ± 0.66^{b}	4.02 ± 0.16^{c}	2391.5 ± 2.12^{d}	1093.5 ± 4.95^{e}
B	593 ± 1.41^{c}	18.56 ± 0.01^{d}	4.55 ± 0.06^{e}	2531 ± 2.83^{a}	1131 ± 2.83^{b}
C	582 ± 4.24^{b}	18.3 ± 0.08^{e}	4.17 ± 0.07^{c}	2412 ± 1.41^{d}	1152 ± 1.41^{a}

Note: Values with different alphabets in same row are not significantly different ($p \geq 0.05$)

Key: A - Raw *T. elegans*

B- *T. elegans* subjected to solid fermentation

C- *T. elegans* subjected to liquid fermentation

Table 13: Essential Amino Acid Composition of Raw and Fermented *T. elegans*

Amino Acid	**A (g/100g)**	**B (g/100g)**	**C (g/100g)**
Histidine	1.11 ± 0.01^{b}	1.36 ± 0.01^{c}	1.18 ± 0.01^{a}
Isoleucine	2.35 ± 0.03^{c}	3.03 ± 0.03^{b}	2.46 ± 0.05^{a}
Leucine	3.34 ± 0.05^{a}	4.06 ± 0.04^{b}	3.44 ± 0.04^{c}
Lysine	2.20 ± 0.05^{b}	2.59 ± 0.03^{c}	2.34 ± 0.04^{a}
Methionine	0.69 ± 0.04^{a}	0.98 ± 0.01^{b}	0.83 ± 0.04^{c}

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Phenylalanine	2.62 ± 0.06^{a}	2.90 ± 0.05^{b}	2.52 ± 0.06^{c}
Threonine	2.06 ± 0.08^{c}	2.89 ± 0.08^{a}	2.39 ± 0.02^{b}
Tryptophan	0.72 ± 0.03^{b}	1.09 ± 0.01^{c}	0.91 ± 0.02^{a}
Valine	2.86 ± 0.13^{c}	3.38 ± 0.03^{b}	3.13 ± 0.04^{a}

Note: Values with same alphabets in same row are not significantly different ($p \geq 0.05$)

Key: A - Raw *T. elegans*

B- *T. elegans* subjected to solid fermentation

C- *T. elegans* subjected to liquid fermentation

Table 14: Non-Essential Amino Acid Composition of Raw and Fermented *T. elegans*

Amino Acid	**A (g/100g)**	**B (g/100g)**	**C (g/100g)**
Alanine	2.56 ± 0.09^{b}	3.10 ± 0.01^{c}	2.79 ± 0.03^{a}
Arginine	4.25 ± 0.08^{c}	5.04 ± 0.18^{b}	4.95 ± 0.06^{a}
Aspartic acid	4.35 ± 0.09^{a}	5.07 ± 0.06^{b}	4.65 ± 0.04^{c}
Cystine	0.28 ± 0.03^{c}	0.53 ± 0.04^{b}	0.40 ± 0.04^{a}
Glutamic acid	8.68 ± 0.04^{a}	11.07 ± 0.09^{a}	9.64 ± 0.04^{a}
Glycine	1.42 ± 0.05^{a}	2.34 ± 0.11^{b}	1.87 ± 0.05^{c}
Norleucine	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Proline	1.82 ± 0.02^{b}	2.38 ± 0.06c	2.07 ± 0.06a
Serine	1.92 ± 0.06^{c}	2.51 ± 0.03^{b}	2.25 ± 0.06^{a}
Tyrosine	1.39 ± 0.01^{a}	2.08 ± 0.01^{b}	1.59 ± 0.06^{c}

Note: Values with same alphabets in same row are not significantly different ($p \geq 0.05$)

Key**:** A - Raw *T. elegans*

B- *T. elegans* subjected to solid fermentation

C- *T. elegans* subjected to liquid fermentation

Table 15 shows the percentage of total essential amino acids and total non-essential amino acids. Total essential amino acids ranged from 38.86% to 40.21% while, total non-essential amino acids ranged from 59.76% to 61.14%.

From the data on Tables 16 and 17, the amino acid scores were calculated as a ratio of mg amino acid per g of test protein to mg of amino acid per gram in reference pattern multiplied by 100. Table 17 shows tryptophan as the highest scoring essential amino acid, with the highest score (18.17) in solid fermentation and methionine to be the most limiting with a score as low as 3.14 in the raw sample.

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Table 15: Percentage Essential and non-essential amino acids in Raw and Fermented *T. elegans*

Amino Acid	**A**	**B**	**C**
TAA	44.63	56.40	49.41
TEAA	17.95	22.28	19.20
TNEAA	26.67	34.12	30.21
% TEAA	40.21	39.50	38.86
% TNEAA	59.76	60.50	61.14

Key**:** TAA - Total amino acids

TNEAA - Total non-essential amino acids

TEAA - Total essential amino acids

A **-** Raw *T. elegans*

B- *T. elegans* subjected to solid fermentation

C- *T. elegans* subjected to liquid fermentation

Table 16: Provisional amino acid scoring pattern* of Raw and Fermented *T. elegans*

Amino acid	g/100g
Histidine	15
Isoleucine	30
Leucine	59
Lysine	45
Methionine	22
Phenylalanine	38
Threonine	23
Tryptophan	6
Valine	39

* (WHO, 2007)

Table 17: Calculated amino acid scores

Amino Acid	**A**	**B**	**C**
Histidine	7.4	9.1	7.9

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Isoleucine	7.8	10.1	8.2
Leucine	5.66	6.88	5.83
Lysine	4.88	5.75	5.20
Methionine	3.14	4.45	3.77
Phenylalanine	6.89	7.63	6.63
Threonine	8.95	12.56	10.39
Tryptophan	12.00	18.17	15.17
Valine	7.33	8.66	8.03

Key**:** A - Raw *T. elegans*

B- *T. elegans* subjected to solid fermentation

C- *T. elegans* subjected to liquid fermentation

DISCUSSION

As non-timber forest products, mushrooms have been used for food, medicine, enzymes, and as a major source of income for people all around the world (Titilawo *et al*., 2021). But due to human activities including burning of shrubs, deforestation, pesticide and herbicide use, urbanization, and climate change, they are gradually becoming extinct in the wild (Adeniyi *et al*., 2018). The mineral, amino acid and antimicrobial potential of extracts of *T. elegans* indigenous to Nigeria were assessed in this study.

Trametes elegans fermentation was characterized by a rise in TTA and a reduction in pH. In their study on the microbiological, nutritional quality, and antioxidant activity of fermented Delonix regia seeds, Olaniyi (2018) reported a reduction in pH and a rise in TTA during the fermentation process. The presence of organic acids, such as lactic acid produced by LAB involved in the fermentation of the carbohydrate contents of the fermenting substrates, may be the cause of the pH decrease and concomitant rise in TTA (Olaniyi, 2018).

Table 4 shows that the bacterial count decreased following the *Trametes elegans*' exposure to both solid and submerged fermentation from day 1 to day 4. This could be because numerous organisms frequently exhibit preference conditions. According to Zhu *et al*. (2023), these metabolic products (antibiotics) are able to inhibit the growth of other bacteria. This suggests that the decrease in the number of bacteria in relation to the number of fermentation days may be caused by the presence or growth of Bacillus and Streptomyces, which produce metabolic products that may have inhibitory effects on other organisms.

Table 5 illustrates the rise in fungal count that occurred throughout the *Trametes elegans* fermentation process from day 1 to day 4. This increase may have been caused by the low pH values that were reached during fermentation, which may have been favorable for fungal development. Fungi are acid tolerant, as described before by Ali *et al*. (2017).

The occurrence of different bacterial and fungal isolates such as *Bacillus cereus, Microbacterium* sp., *Streptococcus* sp., *Actinomyces* sp., *Macrococcus* sp., *Streptomyces* sp., *Clostridium perfringes, Lactobacillus acidopillus. Candida* sp., *Rhizopus* sp., *Aspergillus niger*., *Mucor* sp. and *Penicillum* sp. during the fermentation of *Trametes elegans* as shown in According to Tables 6 and 7, they might be categorized as either naturally occurring microflora or as contaminating microorganisms that come from the soil, substrates, aerosols, rodents, and the personnel that are involved in the fermentation process (Olaniyi *et al*., 2018).

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The inhibitory effects of *T. elegans* extracts, both raw and fermented, varied, ranging from 5.50 mm against *Bacillus subtilis* to 18.13 mm against *E. coli*. Numerous factors could be responsible for the varying antimicrobial activities. Research indicates that all mushroom extracts have variable antimicrobial activities, depending on the test organisms, the type of environment and media the organism grows in, the genetic makeup of the species of mushrooms, the extraction solvent, and the physical and biochemical makeup of the antimicrobial components (Awala and Oyetayo, 2015).

The results of Awala and Oyetayo (2015), who reported varying antimicrobial activities of *T. elegans* extracts against tested isolates, while studying the phytochemical and antimicrobial properties of the extracts obtained from *Trametes elegans* collected from Osengere in Ibadan, Nigeria, also correlated with the antimicrobial activities of methanol and acetone extracts on tested isolates, as shown in Figures 1 to 5 and Table 8. Extracts from *T. elegans* showed inhibitory effects on cultured cells.

Al- Fatimi *et al*. (2013) also examined a variety of Yemeni medicinal mushrooms against P. aeruginosa (ATCC 27853) and *S. aureus* (ATCC 29213). In light of this, mushrooms include physiologically active substances with antibacterial qualities (Shen *et al*., 2017). The antibacterial action of *T. elegans* extracts is unaffected by the fermentation process, according to this study.

Additionally, the inhibition zones (5.50 mm to 18.13 mm) found in this investigation were consistent with the findings of Chowdhury *et al*. (2015), who reported that various medicinal mushroom extracts have antibacterial properties and that their zones of inhibition ranged from 3.7mm to 20.3mm. As seen in Figure 7, the crude fiber content of the raw and fermented *T. elegans* is comparable to the results obtained by Oluwaniyi *et al* (2020). According to study, edible mushrooms, both wild and commercial, are high in crude fiber. Much of the insoluble dietary fiber found in higher basidiomycetes is attached to chitin, hemicellulose, mannans, glucans, glycogen, and trehalose in their cell walls (Ogidi *et al*., 2018).

Components of the cell walls of mushrooms include fiber and nondigestible carbohydrates, which can be used to increase the nutritional value of some low-fiber diets. According to Liu *et al*. (2018), dietary fiber has several health benefits, such as reducing the risk of colon disease, hemorrhoids, and constipation. It also increases the viscosity of the food matrix, slows down digestion, lowers blood sugar, and boosts immunity with antitumor activity (Ogidi *et al*., 2018).

Therefore, mushrooms are a great way to increase the dietary fiber content of biopharmaceutical products. It was discovered that *Trametes elegans* had a decreased fat content, which is consistent with previous research results indicating that mushrooms typically had low concentrations of crude fat. Therefore, the mushroom sample may be helpful for creating diet plans that restrict food intake.

In this investigation, fermented *T. elegans* showed a significant rise in protein content but a decrease in carbohydrate level. According to Ogidi and Agbaje (2020), the separation of proteins attached to carbohydrates inside the mushroom cell wall and the proteolytic activity of LAB may have contributed to the increase in protein content throughout the fermentation process. According to Ogidi *et al*. (2018), the dissociation of protein bound with polysaccharides at the fungal cell wall resulted in an increase in the protein content of fermented *Lenzites quercina.*

Moreover, microbes consume sugars as a source of energy, which raises the nitrogen concentration in the fermentation medium and increases the fraction of protein (Zadeike *et al*., 2022). Both raw and solid fermented mushrooms, as well as wild and medicinal mushrooms from Ghana, have been found to contain essential elements like zinc, iron, magnesium, and potassium (Obadai *et al*., 2014). In raw, solid, and liquid fermented *Trametes elegans*, phosphorus and potassium were abundant; these elements are known to regulate blood pressure and maintain cellular functions. The researchers emphasized the importance of minerals as constituent of metalloenzymes, which are involved in biochemical processes such as haemoglobin synthesis and catalysis of metabolic growth (Staff, 2023).

Zinc, iron, potassium, magnesium, and phosphorus contents of fermented *Trametes elegans* were increased, indicating a substantial change in the mineral composition of the mushrooms during fermentation improving the

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bioavailability of minerals. Fermentation had played a pivotal role in human food production, nutritional supplementation, and health promotion (Huan *et al*., 2019). The bioavailability of macro and micro elements in medicinal mushrooms could promote their uses for health benefits. Mushrooms are known to possess a very effective mechanism that enable them to readily take up some metals from the ecosystem (Woldemaiam, 2019).

The acidic amino acids may be found in large amounts because they are used as precursors to create the backbone of other amino acids. According to Kubala (2023), arginine ranks third in all the samples analyzed and is a crucial amino acid for a child's development. As can be seen in Table 11, its distribution was such that the sample that underwent solid fermentation had the highest concentration (5.04±0.18), while the raw sample had the lowest concentration (4.25±0.08).

As seen in Table 12, all of the mushroom extracts from the raw, solid, and liquid fermented samples generally included the necessary amino acids, which ranged from 38.86 to 40.21% of the total amino acid (%TEAA) content. These indicate that the amount of essential amino acids in the diet will be considerably increased by *Trametes elegans*. Nutritional value is mostly determined by the amount and balance of necessary amino acids, while overall protein content has a significant role in how proteins are used by the body. The rate of protein synthesis will decrease by the same ratio if the availability of one or more necessary amino acids is insufficient.

There are numerous techniques to assess the quality of dietary proteins, but one common method is to compare the ratio of required to available amino acids in the food (Hayes, 2020). A significant number of essential and non-essential amino acids may be found in raw, solid, and liquid fermented *Trametes elegans*. These amino acids are recognized as essential parts of functional diets and are found in all body cells (Mikstas, 2022).

CONCLUSIONS

In conclusion, this study's findings demonstrated the raw and fermented *Trametes elegans'* mineral, amino acid, and antimicrobial qualities, as well as the possibility of introducing them into animal feed formulations. It was observed that the extracts of *Trametes elegans* were effective against tested bacteria. In general, *Trametes elegans* extracts showed broad spectrum antibiotic activity, making them a viable therapy alternative for common illnesses brought on by pathogenic organisms. Additionally, this study demonstrated that *T. elegans'* antimicrobial quality is unaffected by the fermentation process. Food properties such as proximate, amino acids, and minerals in the examined *Trametes elegans* suggests that it can be exploited as food supplements and for the development of biopharmaceuticals, since it contained some biologically active compounds that are safe.

Conflict of Interests

The authors have no conflict of interests to declare.

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