

Assessment of Antioxidant and Mycochemical Properties of Raw and Fermented *Trametes Polyzona* Extracts

Elizabeth T. Akingbesote¹, Victor O. Oyetayo²

Microbiology Department, Federal University of Technology Akure, Nigeria

Abstract: This Study was conducted to assess the antioxidant and mycochemical properties of *Trametes polyzona*. *Trametes polyzona* is a cosmopolitan mushroom. This mushroom was collected from dead woods in farmland around Akungba - Akoko. The mushroom was divided into three portions. The first portion was fermented using solid state fermentation, the second portion was fermented using liquid state fermentation while the third portion was not fermented and the mycochemical constituents were extracted using methanol and acetone. Mycochemical analysis revealed the presence of saponin, tannin, flavonoid, terpenoid, alkaloid, cardiac glycosides in the mushroom extract. The flavonoid (23.91 mg/g), total phenol (282.99 mg/g), iron chelation (25.44), DPPH (37.50%) and ferric reducing property (35.25 %) of raw acetone was the highest when compared with extracts obtained from submerged and solid state fermented *T. Polyzona*. while the ABTS (3.01%) of solid state methanol was the highest when compared with extracts obtained from raw and submerged *T. polyzona*. Data obtained from this study revealed that extracts of raw and fermented *T. Polyzona* contain useful phytochemicals. Moreover, the extracts also exhibit good antioxidant property.

Keywords: Extracts, Ferric reducing antioxidant power (FRAP), Mycochemicals, *Trametes polyzona*

1. Introduction

Fungi have been described as the second most varied organisms, with a diversity (up to 3 to 5 million species) estimated to be an order of magnitude more than that of terrestrial plants (Awala and Oyetayo, 2015).

Only roughly 100, 000 fungal species have been reported so far, and even fewer have been investigated for the synthesis of significant pharmaceutical compounds.

Nonetheless, fungal secondary metabolites have been used to generate some of the most successful medications and agrochemical fungicides on the market (De Silva et al., 2013). Antibiotics (penicillins, cephalosporins, and fusidic acid), antifungal agents (griseofulvin, strobilurins, and echinocandins), cholesterol - lowering agents such as statin derivatives (mevinolin, lovastatin, and simvastatin), and cholesterol - lowering agents such as statin derivatives (mevinolin, lovastatin, and simvastatin), and cholesterol - lowering agents such as Immunosuppressive medications (cyclosporin) and statin derivatives (mevinolin, lovastatin, and simvastatin) (Awala and Oyetayo, 2015). As a result, the proportion of economically useful fungal metabolites is still quite low.

Mushrooms are macrofungi that have a distinct fruiting body that can be hypogeous or epigeous, large enough to be seen with the naked eye and plucked by hand. They've long been thought to be cures for a variety of ailments, and they're known to create a lot of bioactive metabolites (Oyetayo, 2011). Mushrooms have been used as a popular medication in Asia for ages to prevent or treat many ailments (De Silva et al., 2012). The Yoruba tribe of Nigeria in Africa is credited with being the first to use mushroom as a hallucinogenic agent (Oyetayo, 2011).

The present study therefore aims to assess the mycochemical components and determine the antioxidant properties of extracts from *Trametes polyzona*.

2. Materials and Method

2.1 Collection of Samples

Samples of *Trametes polyzona* were collected from dead woods in farmland around Akungba - Akoko (7° 28'32.4''N 5° 45'07.8''E).

2.2 Sources of *Staphylococcus aureus*

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

2.3 Preparation of Mushrooms Extracts

Fresh *Trametes. polyzona* fruit bodies were collected from Akungba - Akoko. The fruit bodies were sorted, cleaned and air - dried. They were then cut into bits and divided into three portions. The first portion was fermented using solid state fermentation, the second portion was fermented using submerged fermentation while the third portion was not fermented. The fermentation took place for four days. On the fourth day, the fermented mushroom was oven dried at 40°C for 48 hours.

2.4 Preparation of Extracts from Mushroom samples

The powdered mushroom sample (100 g) 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.

2.5 Extraction of the Mushroom

The filtrates were evaporated to dryness at 50°C in a rotary evaporator (RE - 52A; Union Laboratory, England) at 90 rpm under reduced pressure. The obtained concentrated extracts were stored in dark at 4°C until further analysis according to the method of Awala and Oyetayo (2015).

2.6 Mycochemical Analysis of *Trametes* Species Extracts

A qualitative and quantitative mycochemical analysis of the crude mushroom extracts was performed using standard procedures described by Odebiyi and Sofowora (1978), Trease and Evans (2005), Harborne (2005).

2.6.1 Qualitative Analysis of *Trametes* Species Extracts

2.6.1.1 Alkaloid Determination

About 5mL of 1% aqueous HCl on a steam water bath, 1mL of the filtrate treated with a few drops of Dragendorff reagent, and blue black turbidity was obtained as early evidence for the presence of alkaloid.

2.6.1.2 Saponin Determination

The ability of Saponin to produce frothing in aqueous solution was used as screening test for Saponin. About 0.5g of extract was shaken with distilled water in a test tube frothing which persist on warming was taken as preliminary evidence for the presence of Saponin.

2.6.1.3 Tannin Determination

About 0.5g of the extract was stirred with 100mL of distilled water, filtered and ferric chloride reagent was added to the filtrate a blue black green or blue green precipitate was taken as evidence for presence of tannin.

2.6.1.4 Phlobatannin Determination

Deposition of red precipitate when 0.5g of the extract was boiled with 1% aqueous HCl was taken as evidence for the presence of phlobatannin.

2.6.1.5 Anthraquinone Determination

Borntrager's test was used for the detection of Anthraquinone 0.5g of the extract was shaken with 10mL of benzene, filtered and 5mL of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of pink red or violet colour in the ammonia layer indicate the presence of free Anthraquinone.

2.6.1.6 Flavonoid Determination

About 0.5g of the extract was stirred with 20mL of dilute ammonia solution a yellow colouration was observed, the disappearance of the yellow colour after the addition of 1mL conc. H₂SO₄ indicate the presence of flavonoid.

2.6.1.7 Steroid Determination

20mL of acetic anhydride was added to 0.5g of the extract and filter, 2mL of conc. H₂SO₄ was added to the filtrate. There was a colour change from violet to blue or green which indicate the presence of steroid.

2.6.1.8 Terpenoid Determination

0.5g of the extract was mixed with 20mL of chloroform and filtered 3mL of conc. H₂SO₄ was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of terpenoid.

2.6.1.9 Cardiac Glycosides

The following were carried out to test for cardiac glycosides

2.6.1.9.1 Legal's test

The extract was dissolve in pyridine and a few drops of 2% sodium nitroprusside with few drops of 20% NaOH were added. A deep red colouration which faded to a brownish yellow indicates the presence of cardenolides.

2.6.1.9.2 Lieberman's test

20mL of acetic anhydride was added to 0.5g of the extract and filter, 2mL of conc. H₂SO₄ was added to the filtrate. There was a colour change from violet to blue or green which indicate the presence of steroids nucleous. (i. e. aglycone portion of the cardiac glycosides.)

2.6.1.9.3 Salkowski's test

0.5g of the extract was mixed with 20mL of chloroform and filtered 3mL of conc. H₂SO₄ was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of steroidal ring.

2.6.1.9.4 Keller - killiani's test

0.5g of the extract was dissolved in 2mL of glacial acetic acid containing one drop of ferric chloride solution. This was then under layer with 1mL of conc. H₂SO₄ a brown obtain at the interface indicate the presence of a deoxy sugar.

Cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer; a green ring may form just above the brown ring and gradually spread throughout this layer.

2.7 Quantitative Analysis of *Trametes* Species Extracts

2.7.1 Tannin Determination

About 0.2g of finely ground sample was weighed into a 50mL sample bottle. 10mL of 70% aqueous acetone was added and properly covered. The bottle were put in an ice bath shaker and shaken for 2hours at 30°C. Each solution was then centrifuged and the supernatant stored in ice. 0.2mL of each solution was pipetted into the test tube and 0.8mL of distilled water was added. Standard tannic acid solutions were prepared from a 0.5mg/mL of the stock and the solution made up to 1mL with distilled water. 0.5mL of Folin-ciocateau reagent was added to both sample and standard followed by 2.5mL of 20% Na₂CO₃ the solution were then vortexed and allowed to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared.

2.7.2 Determination of Total Flavonoid

The total flavonoid content of the extract was determined using a colourimeter assay developed by (Bao, 2005). 0.2mL of the extract was added to 0.3mL of 5% NaNO₃ at zero time. After 5min, 0.6mL of 10% AlCl₃ was added and after 6min, 2mL of 1M NaOH was added to the mixture followed

by the addition of 2.1mL of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

2.7.3 Determination of Saponin

The spectrophotometric method of Brunner (1994) was used for Saponin determination. 2g of the finely grinded sample was weighed into a 250mL beaker and 100mL of Isobutyl alcohol or (But - 2 - ol) was added. Shaker was used to shake the mixture for 5 hours to ensure uniform mixing. The mixture was now filtered with No 1 Whatman filter paper into 100 mL beaker containing 20mL of 40% saturated solution of magnesium carbonate (MgCO₃). The mixture obtained again was filtered through No 1 Whatman filter paper to obtain a clean colourless solution. 1mL of the colourless solution was taken into 50mL volumetric flask using pipette, 2ml of 5% iron (iii) chloride (FeCl₃) solution was added and made up to the mark with distilled water. It was allowed to stand for 30min for the colour to develop. The absorbance is read against the blank at 380nm.

2.7.4 Determination of Cardiac Glycosides

The procedure described by Sofowora (1995) was used, 10mL of the extract was pipetted into a 250mL conical flask. 50ml chloroform was added and shaken on vortex mixer for 1 hour. The mixture was filtered into 100mL conical flask. 10mL of pyridine and 2mL of 29% of sodium nitroprusside were added and shaken thoroughly for 10min. 3mL of 20% NaOH was added to develop a brownish yellow colour, Glycosides standard (Digitoxin). A concentration which range from 0 – 50mg/mL were prepared from stock solution the absorbance was read[at]510nm.

2.7.5 Determination of Terpenoid

The procedure described by Sofowora (1995) was used. 0.5g of finely grounded sample was weighed into a 50mL conical flask 20mL of chloroform: methanol (2: 1) was added, the mixture was shaken thoroughly and allowed to stand for 15min at room temp. The suspension was centrifuged at 3000rpm. The supernatant was discarded and the precipitate was re - washed with 20mL chloroform: methanol (2: 1) and then re - centrifuge again and the precipitate was dissolved in 40mL of 10% SDS solution. 1mL of 0.01M ferric chloride was added and allowed to stand for 30min before taken the absorbance[at]510nm. The STD Terpenoid (alpaterpineol). Concentration ranging from 0 - 5mg/mL from the stock solution.

2.7.6 Determination of Steroid

A quantitative determination of steroid was determined by weighing 5grms of the finely powdered sample into 100mL conical flask and 50mL of pyridine was added to it, and shaken for 30min at room temperature, 3mL of 250mg/mL metallic copper powder or copper (1) oxide and allowed to incubated for 1hr in the dark and the absorbance was measured[at]350nm against reagent blank. Sofowora (1995).

2.7.7 Determination of Alkaloid

200ml of 10% acetic acid in ethanol were added to 5g powdered extract covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to one fourth of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the

precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content can be determined using the formula:

$$\text{Alkaloid (\%)} = \frac{\text{Final weight of sample} \times 100}{\text{Initial weight of Extract}}$$

2.8 Antioxidants

2.8.1 Determination of Total Phenol

The total phenol content of the extract was determined by the method of (Singleton *et al.*, 1999). 0.2 mL of the extract was mixed with 2.5 mL of 10% Folin-ciocalteu's reagent and 2 mL of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45°C for 40mins, and the absorbance was measured at 700nm in the spectrophotometer, gallic acid was used as standard phenol.

2.7.8 Determination of Total Flavonoid

The total flavonoid content of the extract was determined using a colourimeter assay developed by (Bao, 2005). 0.2 mL of the extract was added to 0.3 mL of 5% NaNO₃ at zero time. After 5min, 0.6 mL of 10% AlCl₃ was added and after 6min, 2 mL of 1M NaOH was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

2.8.2 Determination of Ferric Reducing Property

The reducing property of the extract was determined by (Pulido *et al.*, 2000), 0.25 mL of the extract was mixed with 0.25 mL of 200mM of Sodium phosphate buffer pH 6.6 and 0.25 mL of 1% KFC. The mixture was incubated at 50°C for 20min, thereafter 0.25 mL of 10% TCA was also added and centrifuged at 2000 rpm for 10min, 1mL of the supernatant was mixed with 1 mL of distilled water and 0.1 % of FeCl₃ and the absorbance was measured at 700nm.

2.8.3 Determination of Free Radical Scavenging Ability

The free radical scavenging ability of the extract against DPPH (1, 1 - diphenyl - 2 - picrylhydrazyl) was determined using (Gyamfi *et al.* 1999) method. 1 mL of the extract was mixed with 1 mL of the 0.4M methanolic solution of the DPPH. The mixture was left in the dark for 30min before measuring the absorbance at 516nm.

2.8.4 Determination of Iron Chelation property

The ability of the extract to chelate Fe²⁺ was determined using a modified method of Minotti & Aust (1987) by Puntelet *et al.* (2005). Briefly, 150mM FeSO₄ was added to a reaction mixture containing 168mL of 0.1M Tris - HCl pH 7.4, 218 mL saline and extract and the volume is made up 1 mL with distilled water. The reaction mixture was incubated for 5min, before the additional of 13 mL of 1, 10 - phenantroline the absorbance was read at 510nm.

2.8.5 ABTS Scavenging Ability

2, 2 - azino - bis (3 - ethylbenzothiazoline - 6 - sulphonic acid) (ABTS) scavenging ability. The ABTS scavenging ability of the extract was determined according to the method described by Re *et al.*, (1999). The ABTS was generated by reacting an (7mM). ABTS aqueous solution with K₂S₂O₈

(2.45 m/ml, final concentration) in the dark for 16 hours and adjusting the absorbance at 734nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0ml of ABTS solution and the absorbance was read at 732nm after 15mins. The TROLOX equivalent antioxidant capacity was subsequently calculated.

2.9 Statistical Analysis

Data obtained were subjected to statistical analysis using statistical package for social science version 20. Mean were separated using Duncan’s New Multiple Range Test at p< 0.05.

3. Results

Trametes polyzona extract contains saponins, tannins, flavonoids, terpenoids, alkaloids, cardiac glycosides, steroids, phylobatannins, and anthraquinone were absent (Table 1). Figures 1 and 2 showed quantitative antioxidant compounds in the raw and fermented *T. polyzona*. Raw acetic extract had the highest phenolic content (282.99 mg/g) and highest flavonoid content (23.91 mg/g). The antioxidant results of the extracts is also presented in Figures 3 - 5. Raw acetic extracts exhibited highest antioxidant properties with values of 37.50 % for DPPH, 35.25 % for ferric reducing property and 25.44 % for iron chelation. Moreover, acetic extract (0.02%) also had the highest ABTS except methanolic extract (3.01 %) obtained from *T. polyzona* subjected to solid state fermentation (Table 2).

Table 1: Qualitative Phytochemical Properties of *Trametes polyzona*

Phytochemicals	1	2	3
Saponins	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Steroids	-	-	-
Alkaloids	+	+	+
Phylobatannins	-	-	-
Cardiac glycosides	+	+	+
Anthraquinone	-	-	-

KEY

- 1) Raw *Trametes polyzona*.
- 2) Fermented *T. polyzona* (submerged).
- 3) Fermented *T. polyzona* (solid state).
- + Present
- Absent

Table 2: 2, 2’ - azino - bis (3 - ethylbenthiazoline - 6 - sulphonic acid) (ABTS) of Methanolic and Acetic Extracts of *Trametes polyzona*

Extracts	Raw (%)	Submerged (%)	Solid state (%)	Acetone	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.01 ± 0.00 ^c
Methanol	0.00±0.00 ^a	0.01±0.00 ^a	3.01±4.24 ^b	Values are means of replicate n=3). Values along rows with different superscript are significantly different (p ≤ 0.05).			

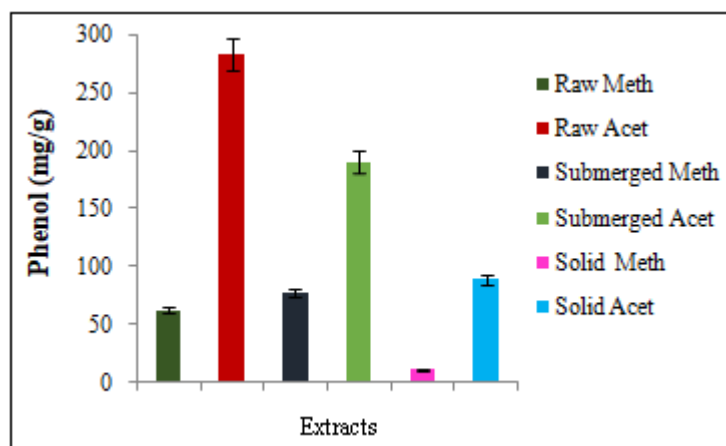


Figure 1: Phenolic Content of Raw and Fermented Extracts of *T. polyzona*

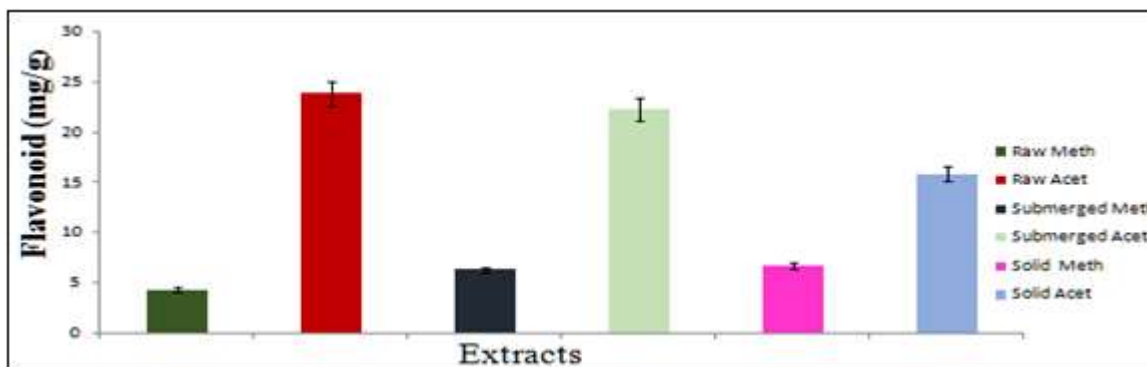


Figure 2: Flavonoid content of Raw and Fermented Extracts of *T. polyzona*

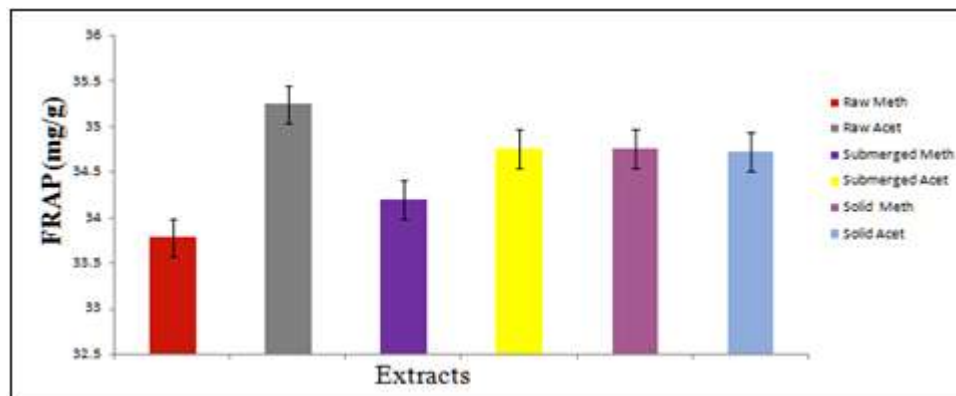


Figure 3: Ferric Reducing Properties of Raw and Fermented Extracts of *T. polyzona*

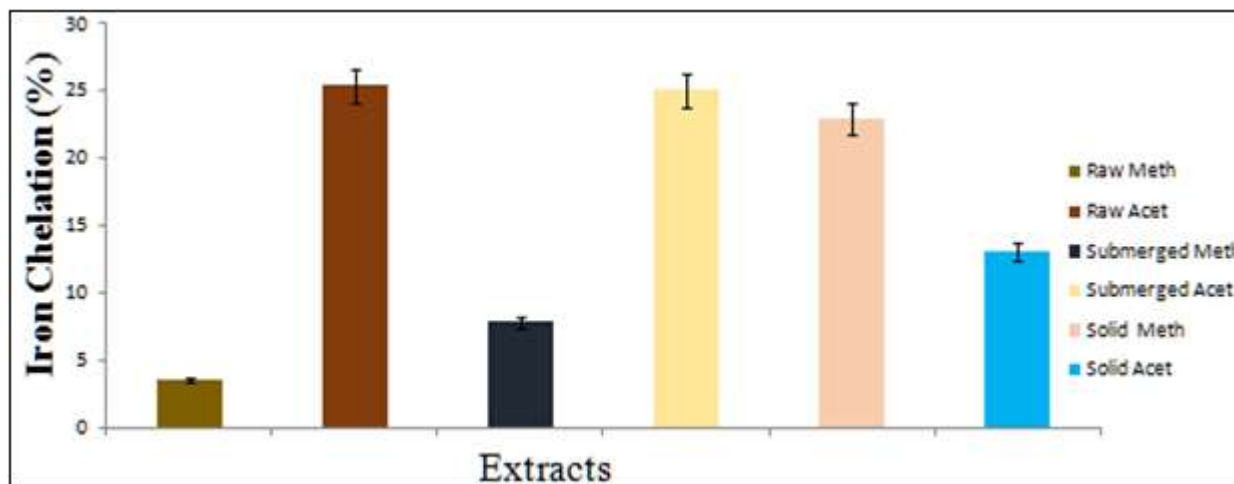


Figure 4: Iron Chelation Capacity of Raw and Fermented Extracts of *T. polyzona*

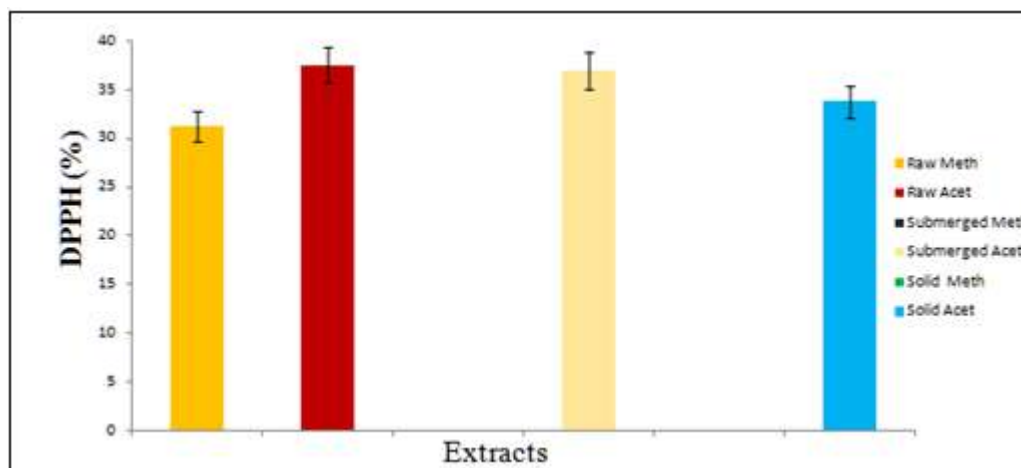


Figure 5: DPPH Scavenging Effect of Raw and Fermented Extracts of *T. polyzona*

4. Discussion

Mushrooms are known all over the world for their high protein, vitamin, mineral, chitin, and vital amino acid content, as well as their low fat and calorie content (Celal, 2018). Medicinal mushrooms include a number of biologically active chemicals, with proven therapeutic effects (Awala and Oyetayo, 2015).

Saponin, tannin, flavonoid, terpenoid, alkaloid and cardiac glycosides were present in *T. Polyzona* methanolic and acetic extracts in this study. These mycochemicals are responsible for the mushroom's antibacterial and antifungal

characteristics, and can thus be employed as a novel mycotherapeutic agent to improve human health (Abubakar et al., 2016).

Raw acetic extract of *T. Polyzona* had highest total phenol. Phenol has been reported to have antimicrobial properties (Awala and Oyetayo, 2015; Deep and Amrita, 2017). Also, phenols are known to be biologically active and serve as a defence mechanism for plants against predation by many microorganisms, insects and other herbivores (Awala and Oyetayo, 2015; Bonjar et al., 2004).

The phenolic compounds and flavonoid detected in *T. Polyzona* extract can easily penetrate the cell wall, interfere with cytoplasm, inhibit the production of enzymes and protein synthesis (Abubakar *et al.*, 2016).

T. polyzona extracts also displayed good antioxidant properties (Figures 3 - 5 and Table 2). The variation observed in the amount of antioxidant present could be as a result of the differences in the solubility of the different antioxidant compound in the extract (Awala and Oyetayo, 2015)

5. Conclusion

The study revealed that *Trametes polyzona* contain useful mycochemical and antioxidant properties. These compounds can therefore be harnessed by pharmaceutical industries for the production of effective pharmaceuticals.

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Author Profile

Akingbesote Elizabeth Tosin, B. Tech, Federal University of Technology Akure, Nigeria, ajeolutosin[at]gmail.com (Correspondence author)

Oyetayo, Victor Olusegun, PhD, Federal University of Technology Akure, Nigeria, ovonew67[at]gmail.com