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ASSESSMENT OF CRUDE EXTRACT OF *PLEUROTUS PULMONARIUS* FOR LARVICIDAL EFFICACY AGAINST FEMALE *ANOPHELES* MOSQUITO LARVAE

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ABSTRACT

Anopheles mosquitoes are important vectors that spread diseases such as malaria and lymphatic filariasis. The abundance and distribution of adult Anopheles mosquitoes are based on the presence and productivity of larval breeding habitats. Breeding sites must be eradicated to control mosquito larval development. This study investigated the larvicidal efficacy of *Pleurotus pulmonarius* methanol extract on *Anopheles* mosquito larvae. Five grams of ground *Pleurotus pulmonarius* was extracted with 100 mL of methanol following standard procedures. Phytochemical contents of the crude extract were determined, functional groups in the extract were identified with Fourier transform-infrared spectroscopy, and Gas chromatography-mass spectral. *Anopheles* mosquito larvae were randomly collected from breeding sites and larvicidal efficacy of the extract was determined on 10 larvae exposed to each of 500, 1000, 1500, 2000, and 2500 mL/L (ppm) of the crude extract. A total of 41 compounds were identified in the extract, the most prominent compound being as 7-anti-hydroxy bicycle [2,2,2] oct-5-en-2-one. Other compounds include phenol, flavonoids, saponins, alkaloids, tannins, sterols and steroids, terpenoids, phytate, alcohol, amines, amides, alkenes, amines, aromatic amines, aliphatic amines, amines, and alkyl halides. The 2500 ppm crude extract caused 100% mortality rate in the larvae in 60 hours of exposure, whereas 2000, 1500, 1000, and 500 ppm caused 80%, 60%, 40%, and 10% mortality rates in the larvae respectively. This study suggests that compound 7-anti-hydroxy bicycle [2,2,2] oct-5-en-2-one in combination with other compounds not prominent in the methanol extract may be responsible for the mortality of *Anopheles* larvae, with poytential use in the pharmaceutical industry for production of liquid larvicides and mosquitocides in the tropics.

Keywords: *Anopheles* mosquito, Mosquito larvae, *Pleurotus pulmonariu*s, Fourier transform-infrared spectroscopy, Secondary metabolites, Larvicidal efficacy

INTRODUCTION

Anopheles mosquitoes are important vectors that spread diseases such as malaria and lymphatic filariasis. The abundance and distribution of adult Anopheles mosquitoes are predicated on the presence and productivity of larval breeding habitats (Hinne *et al*., 2021). Species of the *Anopheles gambiae* prefer to breed in shallow water collections that are open to sunlight. Their breeding habitats may include various sizes of water bodies that are natural or man-made, temporary or permanent saline or freshwater. The availability of larval habitats and larval productivity may also be impacted by variations in rainfall patterns or seasonal changes (Hinne *et al*., 2021).

The end of vector-borne diseases like lymphatic filariasis and malaria depends on vector control. Long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS), the two most popular vector control strategies, have decreased malaria transmission in Africa, but they have not been able to completely eradicate the disease due to the emergence and rapid spread of insecticide resistance in mosquitoes (Emidi *et al.,* 2017; Ondiba *et al*., 2019; Hinne *et al.,* 2021). Additionally, the *Anopheles* mosquito's behavior has changed from interior, late-night biting to early biting hours when humans might be unprotected outside as a result of the usage of LLINs and IRS, which target indoor-biting and indoor-resting mosquitoes, respectively. To combat malaria vectors, larval source management or source control could provide an additional valuable tool (Emidi *et al.,* 2017; Ondiba *et al*., 2019; Hinne *et al.,* 2021).

Hence, developing biologically active natural chemical constituents that act as larvicidal and promise to reduce the risk to humans and harmful accumulated residues is essential. It is necessary to develop indigenous vector control methods that are low-risk for the environment, biodegradable, and affordable to the people communities (Kumar *et al,* 2014).

Mushrooms are fungi fruiting bodies bearing spores that grow above the soil or on their substrates, and forms a major group of smaller plant kingdom. Due to their characteristic fruiting bodies and size, mushrooms are large enough to be visible to the naked eye. Some mushrooms are edible due to their nutritional components, while others are heavily used in traditional medicines (Karaman *et al.,* 2012; Nwobodo *et al,* 2021). Mushrooms have medicinal properties especially due to their abundance of physiologically active chemicals with antioxidant and antibacterial qualities that boost the immune system and protect against carcinogens (Nwobodo *et al,* 2021). Mushroom species are known to produce several bioactive compounds like flavonoids, terpenoids, alkaloids, polysaccharides, and tannins (Fakoya *et al.,* 2020; Nwobodo *et al,* 2021). Even with the abundance of bioactive molecules that mushrooms possess, their natural compounds have not been extensively researched. The bioactive compounds found in various cellular components and secondary metabolites have been extracted and identified from the mushroom fruiting bodies as secondary metabolites (Gebreyohannes *et al.,* 2019; Nwobodo *et al.,* 2021).

Pleurotus pulmonarius is a North American macrofungus that can be found worldwide in temperate and subtropical climates. This fungus typically develops on hardwood and conifers in the summertime in the United States. A pileus on *P. pulmonarius* can have a diameter of 5 to 25 cm. The fruit bodies might be light, dark, or gray in color. The stipe often varies in thickness, is white, and is hard. The gills are either cream or white. This fungus often produces white or, on rare occasions, purple spore prints. The stipe of the fungus is thick and short (Gbolagade *et al*., 2020). A study by Wasonga *et al*., (2008) indicates that the extracts of *P. pulmonarius* may reduce replication of cancer cells. *P. pulmonarius* extract was added to the food of mice and was found to delay carcinogenesis, suggesting that the extracts could be used as adjuvants in the treatment of cancer (Gbolagade *et al.,* 2020). Despite the acclaimed medicinal potential of *Pleurotus pulmonarius* as an antibacterial and antifungal agent in literature, determination of larvicidal efficacy of *Pleurotus pulmonarius* would be of great help in the control of larval development. Therefore, the aim of this study was to investigate the larvicidal activity of methanolic extracts of *Pleurotus pulmonarius* on *Anopheles* mosquito larvae.

MATERIALS AND METHODS

Sample Collection

Mushroom (*Pleurotus pulmonarius*) spawn was collected from Ofatedo, Osun State, Nigeria. Sawdust and rice bran used as substrates for mushroom cultivation were obtained from Sawmill, and purchased from market in Oke-Baale Osun State, Nigeria respectively.

Cultivation of *Pleurotus pulmonarius*

The cultivation of mushrooms was done with slight modification to the method of Ogidi *et al.,* (2020); Balaji *et al*., (2020); Dawidowicz (2021); and Ahmed *et al*., (2022). Fine sawdust and rice bran were used as the substrate for the mushroom cultivation. The substrates were pasteurized in hot water for 15 minutes for effective soaking, drained, and allowed to cool. Sawdust (280 g) and 120 g of rice bran (70:30) were mixed evenly. Spawn (100 g) was inoculated into the substrate and carefully mixed to allow even spawn distribution before being packed in a sterile bag. The bag was slightly tied at the top with holes punched around the bag and incubated in a dark covered box until they were fully colonized with the fungal mycelium. The fully colonized substrate was watered twice per day for 23 days to aid mushroom fruit production. The matured fruiting mushroom was harvested for further processing.

EXTRACTION OF METABOLITES

With slight modification to methods of Nwobodo *et al*., (2021); Assemie and Gemeda, (2023), matured fruits of *Pleurotus pulmonarius* were oven-dried at 70˚C for 6 hours. The dried mushrooms were grinded into fine powder and stored in a dry place at room temperature. The mushroom powder (5g) was dissolved in 100mL solvents (methanol) and mixtures were periodically agitated for 72 hours. It was then filtered using Whatman No. 1 filter paper. The collected filtrates (50,000ppm stock solution) were stored in sterile amber screw cap bottles at room temperature.

FT-IR Analysis

Functional groups of compounds present in the stock solution of the *Pleurotus pulmonarius* methanol extracts of *Pleurotus pulmonarius* were determined with infrared spectral analysis using Shimadzu FTIR 8300 instrument. The spectra range was recorded from 400 to 4000 cm^{-1} according to the report of (Vairavan *et al.,* 2018; Baranitharan *et al.,* 2019; Ogidi *et al*., 2020).

Gas Chromatography-Mass Spectrometry (GC-MS) of Extract

The Perkin - Elmer Clarus 680 system (Perkin Elmer Inc. USA) was utilized from the GC-MS analysis of methanol extract of *Pleurotus pulmonarius* which was carried out using equipped with a fused silica column, packed with elite -5MS) capillary column (30m in length *250 nm in diameter *0.25nm in thickness). The carrier gas used was unalloyed helium gas (99.99%) at a constant flow rate of 1ml/min. An electron ionization energy method was used with high ionization energy of 70 eV (electron Volts) with 0.2s of scan time and fragments ranging from 40 to 600m/z for the detection of the GC-MS spectral 1 uL of the extract were injected and the injector temperature was maintained at 250^0C (constant). The column oven temperature was set at 50^0C for 3 min raised at 10 degree per min up to 280^oc and final temperature was increased to 300^0C for 10 min. The phytochemicals present in the methanol extracts were identified by comparing their retention time (min), peak area peak height, and mass spectral patterns with the spectral database of authentic compounds stored in the National Institute of Standards and Technology (NIST) library (NIST Chemistry web book, 2008).

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING

Qualitative and quantitative screening of the phytochemical constituent of methanol extracts of *Pleurotus pulmonarius* were carried out according to the report of Assemie and Gemeda, (2023), to determine the presence of phenol**,** tannins, saponins, flavonoids, sterols and steroids, phytate, terpenoids, alkaloids, cyanogenic glycoside.

Collection of Mosquito Larva

With some modifications to the methods of Kumar *et al.,* (2014); Vairavan *et al.,* (2018); Assemie and Gemeda, (2023), *Anopheles* mosquito larvae were collected from stagnant water in a bucket at Osun State University, Osogbo, Nigeria. The larvae were kept in a plastic container containing sterile water. The larvae were maintained at 25-29°C and 75-85% relative humidity and taken to the laboratory. The larvae were identified with the help of a Zoologist in the Department of Zoology, Osun State University, Osogbo, Nigeria.

Biolarvicidal activity of extract

With slight modification to the method of Kumar *et al*, (2014); Vairavan *et al*, (2018); Assemie and Gemeda (2023), ten (10) healthy *Anopheles* mosquito larvae were distinctly placed in 15 mL of methanol extracts of *Pleurotus pulmonarius* in sterile Petri dishes at varying concentrations of 500, 1000, 1500, 2000, and 2500 mL/L (ppm) for 72 hours. Methanol (99.9%) was used as a positive control, 5% methanol was used as a negative control. The experiment was carried out in triplicate and the mortality rates were calculated and recorded every 6 hours using this formula.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100\%$$

STATISTICAL ANALYSIS

Experimental values are represented as means ± standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA), with significant differences considered at $P<0.05$. Microsoft Excel (2016) and SPSS version 20 software were used for analysis. All experiments were conducted in duplicate.

RESULT

Plate 1: Young fruiting *Pleurotus pulmonarius* mushroom

The extract from *Pleurotus pulmonarius* showed that methanol was able to extract the chemical contents present in the extract. The extract was observed to be odorless and yellow in color (Plate 2).

Fourier-transform infrared spectroscopy (FTIR) of the *Pleurotus pulmonarius* extracts

The FTIR spectrum of the extract was carried out to identify the functional groups. The spectrum is shown in Plate 3. The broad and long peak at 3421 cm^{-1} is due to Dimeric O-H stretch. The peak at 2928 cm^{-1} and 2854 cm^{-1} is due to N-H stretching of amine salt. Also, the peak at 2360 cm^{-1} is due to O=C=O stretching of carbon dioxide. The peak at 1998 cm^{-1} is due to O=C=O stretching of carbon dioxide. The peak at 1635 cm^{-1} is due to C=C stretching of alkene. The peak at 1508 cm^{-1}, and 1458 cm^{-1} is due to N-O stretching of nitro compound. The peak at 1404 cm^{-1}, and 1338 cm^{-1} is due to O-H bending of alcohol. The peak at 1238 cm^{-1} is due to C-O stretching of alkyl aryl ether. The peak at 1076 cm^{-1} is due to C-O stretching of primary alcohol, and the peak at 1037 cm^{-1} is due to S=O stretching of sulfoxide. The peak at 933 cm^{-1}, and 767 cm^{-1} is due to C=C bending of alkene. While the peak at 609 cm^{-1}, 536 cm^{-1}, and 466 cm^{-1} is due to C-Br stretching of halo compound (Table 1).

GCMS result of compounds present in the mushroom methanol extract

The gas chromatography-mass spectroscopy analysis of the compounds present in the mushroom (*Pleurotus pulmonarius*) methanol extract revealed a total of 41 compounds Among the compounds identified, the structure and the characteristics of the six compounds with percentage peak area >5% (Tables 2-5). The most prominent compound in the extract was identified as 7-anti-hydroxy bicycle [2,2,2] oct-5-en-2-one (m/z 139.0678) at a retention time of 7.218 min which account for 46.46% peak area from the total 108.56% peak area. The GCMS spectra (Plate 6) and the structure of some other compounds identified had with percentage peak area <5% (Plate 7, Table 4).

Qualitative and quantitative phytochemical screening of *Pleurotus pulmonarius* extracts

The result of the qualitative phytochemical screening of the methanol extracts of *Pleurotus pulmonarius* revealed the presence of phenol, flavonoid, saponins, alkaloids, tannin, sterols, steroids, terpenoids, and phytate. The quantitative screening showed high concentrations of alkaloids (109.42 mg/g), saponins (89.25 mg/g), phenol (65.32 mgGAE/g), Sterols and Steroids (62.04), terpenoids (58.08mg/g), phytate (30.01 mg/g), and tannin (14.39 mg/g) in the extract. However, low concentration of flavonoid 0.95 mgQE/g was detected in the extract (Table 5).

Biolarvicidal activities and effect of *Pleurotus pulmonarius* methanol extract concentrations

It was observed that at an initial concentration of 2500 ppm of the extract, no mortality of the larval was observed at 0 hours of exposure. As the period of exposure progressed, 20% of mortality was recorded at 12 hours, 50% at 24 hours, 80% at 38 hours, 90% at 48 hours, and 100% at 60 and 72 hours of exposure (Plate 8). The positive control exhibited 100% mortality rate of the larva while no larva mortality was observed in the negative control.

Various concentrations of the extract were tested against *Anopheles* mosquito larvae. The result showed that exposure of the larva to 2000 ppm of the extract, no mortality was seen at 0 hours, 20%, 30%, 50%, 60%, 80%, and 100% mortality rate was recorded at 12, 24, 36, 48, 60, and 72 hours of exposure to the extract. When the larvae were exposed to 1500 ppm of the extract, no larva mortality was observed at 0 and 12 hours of exposure. However, 10% at 24 hours, 30% at 36 hours, 40% at 48 hours, and 60% at 60 and 72 hours of exposure. The extract exhibited no larval mortality at 0, 12, and 24 hours of exposure whereas, 10%, 30%, 40%, and 50% larval mortality was observed at 36, 48, 60, and 72 hours of exposure to 1000 ppm concentration respectively. A weak larva inhibition was observed in exposure to a concentration of 500 ppm as there was no mortality of larva at 0, 12, 24, and 36 hours of exposure to the extract. However, 10% mortality at 48 and 60 hours and 30% mortality was observed at 72 hours of exposure(Plante 9).

Plate 1: Young fruiting *Pleurotus pulmonarius* mushroom

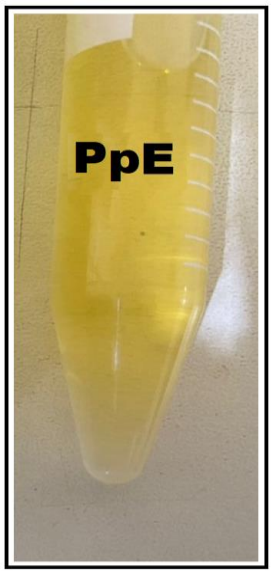

Plate 2: Methanol extract of *Pleurotus pulmonarius* (Mushroom). PpE (*Pleurotus pulmonarius* extract)

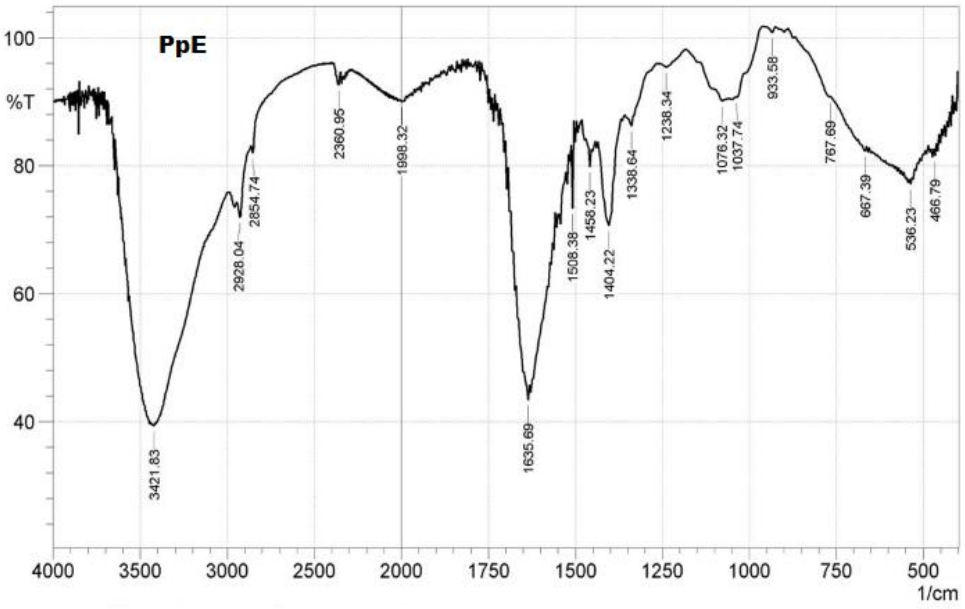

Plate 3: FTIR spectra of *Pleurotus pulmonarius* extract. PpE (*Pleurotus pulmonarius* extract)

Table 1: Spectrum range of FTIR result for *Pleurotus pulmonarius* methanol extract

Absorption (cm^{-1})	Group	Compound Class
600-500	C-I stretching	Halo compound
690-515	C-Br stretching	Halo compound
840-790	C=C bending	Alkene
895-885	C=C bending	Alkene
1070-1030	S=O stretching	Sulfoxide
1085-1050	C-O stretching	Primary alcohol
1275-1200	C-O stretching	Alkyl aryl ether
1420-1330	O-H bending	Alcohol
1450	C-H bending	Alkane
1550-1500	N-O stretching	Nitro compound
1648-1638	C=C stretching	Alkene
2400-2000	O=C=O stretching	Carbon dioxide
3000-2800	N-H stretching	Amine salt
3550-3200	O-H stretching	Alcohol

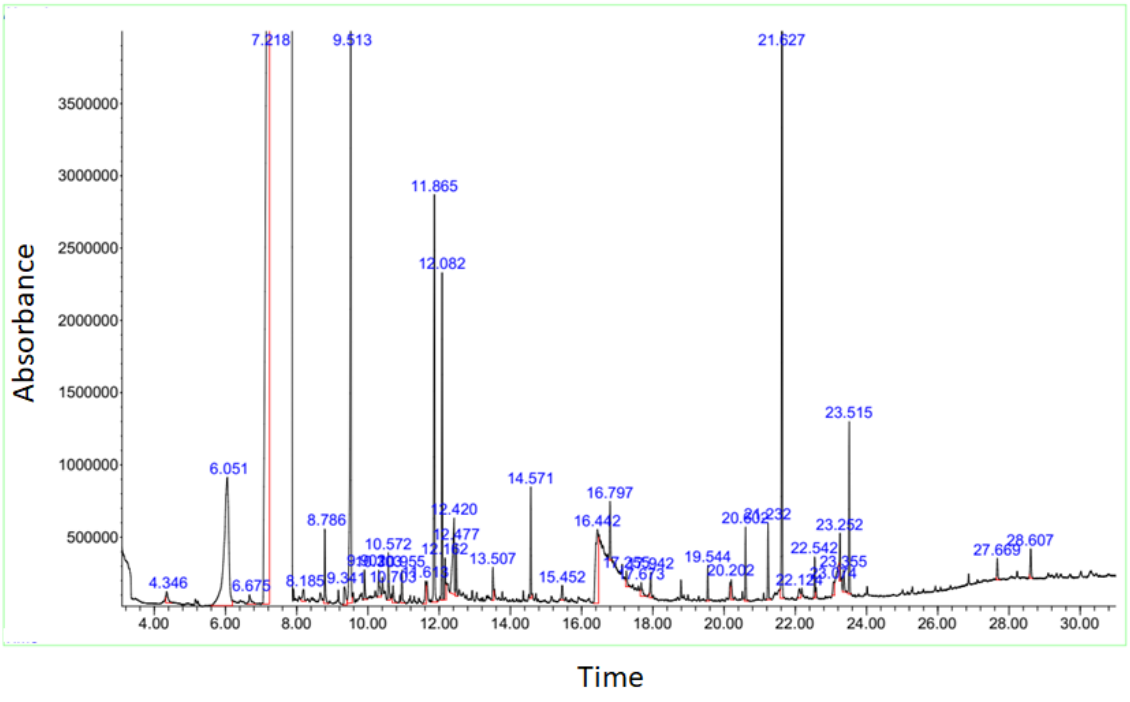

Plate 4: Spectra scan of the compounds identified in the GCMS analysis of the extract

Table 2: Compounds present in the methanol extract of mushroom (*Pleurotus pulmonarius*)

S/N	COMPOUND	RT	AREA (%)
1.	2-pentanethiol	4.346	0.3
2.	Hexamethyl cyclotrisiloxane	6.051	8.56
3.	Arsenous acid	6.051	8.56
4.	1,4-dimethyl pyrazole	6.675	0.3
5.	7-anti-hydroxy bicyclo[2,2,2] oct-5-en-2-one	7.218	46.46
6.	6-(methylamino)phenathren-3-ol	8.185	0.29
7.	N-methyl-2-furan carboxamide	8.786	0.85
8.	5-methyl-2-furan carboxylaldehyde	9.341	0.47
9.	Octamethyl cyclotetrasiloxane	9.513	8.23
10.	5-methyl isothiazole	9.902	0.35
11.	Dimethyl este but-2-enedioic acid	10.303	0.43
12.	Dimethyl-3-oxoadipate	10.572	0.53
13.	2-propenyl cylcopentane	10.703	0.28
14.	4-(phenylsulfanyl)-6-(pyrrolidin-1-yl)-2,1,3-benzoxadiazole	10.955	0.4
15.	1,3-butadiene-1-carboxylic acid	11.613	0.43
16.	2-pentyne	11.865	5.09
17.	Decamethyl cyclopentasiloxane	12.082	2.83
18.	Dimethyl dl-malate	12.162	0.58
19.	Monomethyl ester 2-butenedioic acid (E)	12.42	1.83
20.	Methyl-4-pentynoate	12.477	0.53
21.	1-beta,d-ribofuranosyl-1,2,4-triazole-3-carboxylic acid	13.507	0.43
22.	Dodecamethyl cyclohexasiloxane	14.571	1.05
23.	Methyl ester benzenesulfonic acid	15.452	0.25
24.	5-(hydroxymethyl)-2-pyrrolidinone	16.442	2.97
25.	5-oxo methyl ester L-proline	16.797	0.66
26.	2-piperidinecarboxylic acid	17.255	0.24
27.	2-methyl-3-(1-methylethyl) trans aziridine	17.673	0.29
28.	7-hexadecene	17.942	0.35
29.	Methyl tetradecanoate	19.544	0.34
30.	3-[2-[3-[1-phenyl-1H-tetrazol-5-yl]oxy]propyl]amino] ethyl ester thiosulfuric acid	20.202	0.29
31.	Methyl ester pentadecanoic acid	20.602	0.71
32.	3-(naphthalene-1-ylmethyl0-1-pentyl-1H-indole	21.232	0.83
33.	Methyl ester hexadecanoic acid	21.627	8.66
34.	Dihydropyrimidine-2-methyl thiosulfuric acid	22.124	0.23
35.	4-methoxy-N-(2-phenyl ethyl)-N-pentyl benzamide	22.542	0.44
36.	Dihydropyrimidine-2-methyl thiosulfuric acid	23.074	0.3
37.	Methyl ester-9,12-octadecadienoic acid	23.252	0.55
38.	Methyl ester-11-octadecenoic acid	23.355	0.35
39.	Methyl stearate	23.515	1.64
40.	3,5-bis(1,1-dimethylethyl)-1,2-benzenediol	27.669	0.27

41.	Methyl ester tetracosanoic acid	28.607	0.41
			108.56

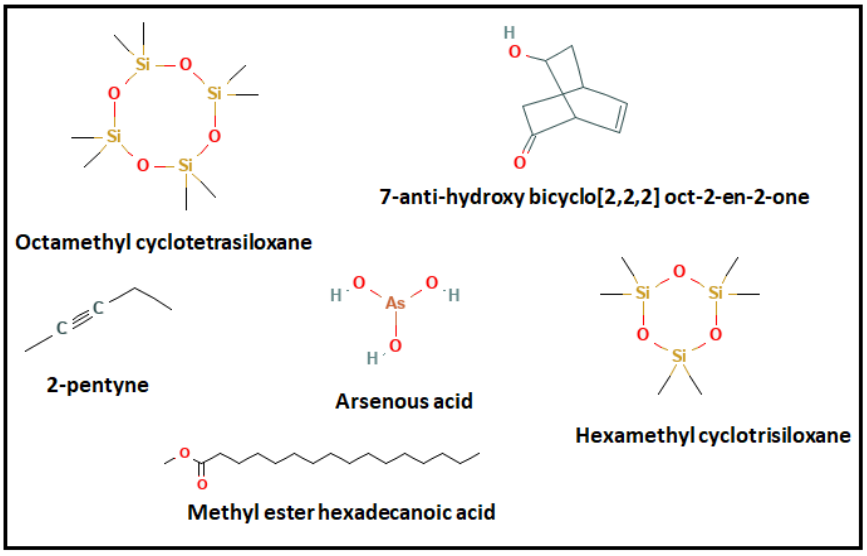

Plate 5: Structure of the compounds with percentage peak area >5%

Table 3: GCMS result of compounds present in the extract with percentage peak area >5%

S/N	Identity	RT	Area %	Molecular weight (g/mol)	Molecular formal
1	7-anti-hydroxy bicyclo[2,2,2] oct-2-en-2-one	7.218	46.46	138.07	$C_8H_{10}O_2$
2	Methyl ester hexadecanoic acid	21.627	8.66	270.5	$C_{17}H_{34}O_2$
3	Hexamethyl cyclotrisiloxane	6.051	8.56	222.46	$C_6H_{18}O_3Si_3$
4	Arsenous acid	6.051	8.56	125.944	AsH_3O_3
5	Octamethyl cyclotetrasiloxane	9.513	8.23	296.61	$C_8H_{24}O_4Si_4$
6	2-pentyne	11.865	5.09	68.12	C_5H_8
Grand total			**85.56**		

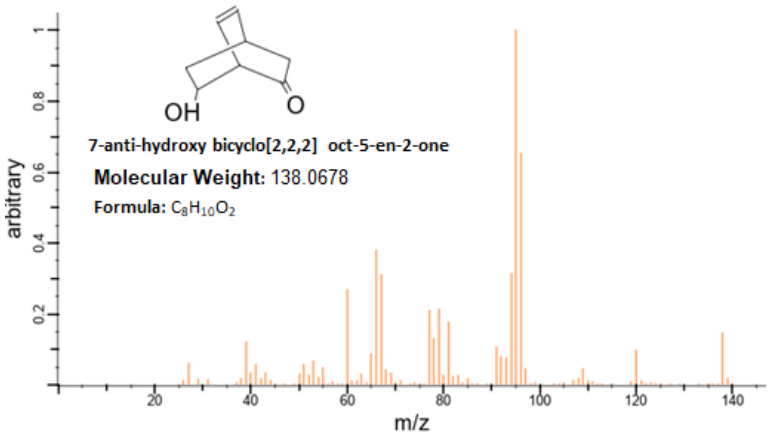

Plate 6: Gas chromatography-mass spectrometry analysis of the most prominent compound (7-anti-hydroxy bicyclo[2,2,2] oct-5-en-2-one)

Table 4: GCMS result of some compounds present in the extract with percentage peak area <5%

S/N	Identity	RT	Area %	Molecular weight (g/mol)	Molecular formal
1	Decamethyl cyclopentasiloxane	12.082	2.83	370.77	$C_{10}H_{30}O_5Si_5$
2	Monomethyl ester 2-butenedioic acid (E)	12.42	1.83	30.1	$C_5H_6O_4$
3	Dodecamethyl cyclohexasiloxane	14.571	1.05	444.92	$C_{12}H_{36}O_6Si_6$
4	5-(hydroxymethyl)-2-pyrrolidinone	16.442	2.97	115.13	$C_5H_9NO_2$
5	Methyl stearate	23.515	1.64	298.5	$C_{19}H_{38}O_2$
Grand total			**10.32**		

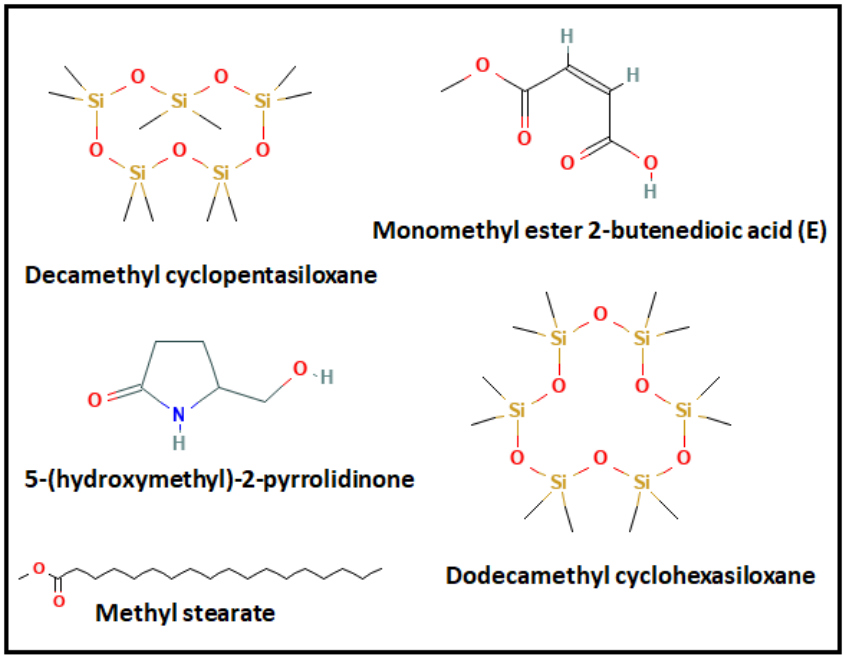

Figure 7: Structure of some compounds percentage peak area <5%

Table 5: Qualitative and quantitative phytochemical composition of methanol extract of *Pleurotus pulmonarius*

Phytochemicals	Qualitative screening	Phytochemicals	Quantitative screening
Phenol	+	Phenol (mg GAE/g)	65.32±0.15
Saponin	+	Saponin (mg/g)	89.25±0.21
Tannin	+	Tannin (mg/g)	14.39±0.10
Flavonoid	+	Flavonoid (mg QE/g)	0.95±0.00
Alkaloids	+	Alkaloid (mg/g)	109.42±0.25
Sterols and Steroids	+	Sterols and Steroids	62.04±0.05
Terpenoid	+	Terpenoid (mg/g)	58.08±0.17
Phytate	+	Phytate (mg/g)	30.01±0.012

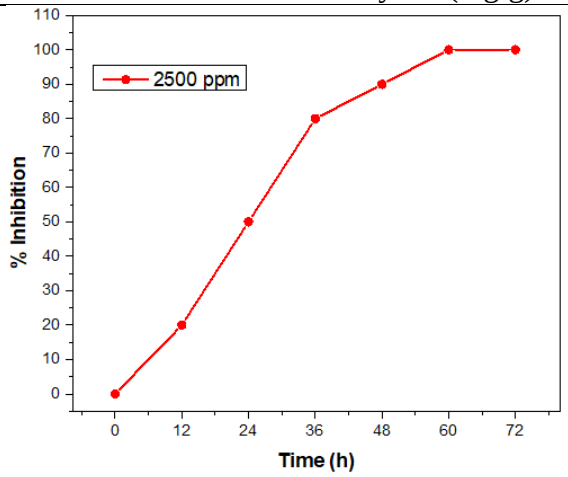

Plate 8: Percentage inhibition of methanol extract of *Pleurotus pulmonarius* against *Anopheles* mosquito larva. ppm (mL/L)

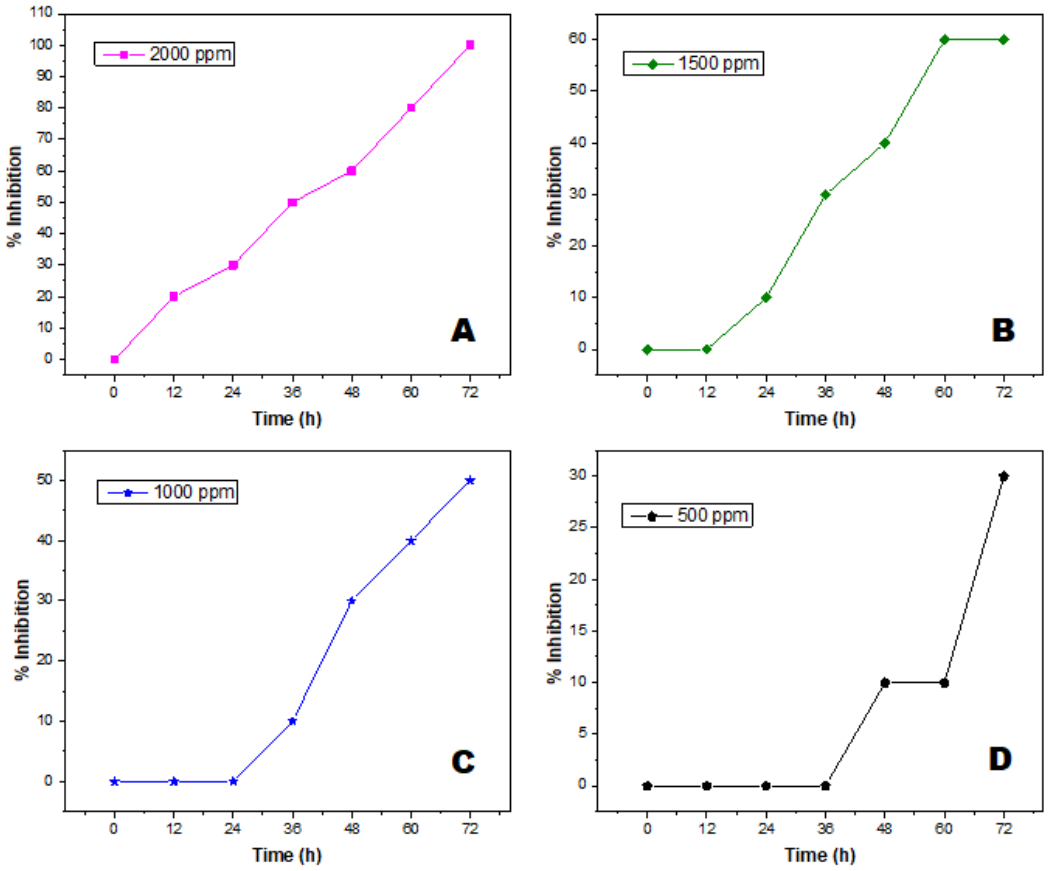

Plate 9: Percentage inhibition of *Anopheles* mosquito larval at different concentrations of *Pleurotus pulmonarius* methanol extract. ppm (mL/L)

DISCUSSION

The obtained mature *Pleurotus pulmonarius* grown on the substrates is as a result of the nutrients present in the substrates required for its growth. This corresponds to the report of Dawidowicz, (2021), on the growth of *Pleurotus pulmonarius* (Fr.) Quel on substrates based on cereal straw and various types of organic waste, including agricultural, horticultural, textile, and forestry in Poland. In another finding by Ahmed *et al*., (2022), used paddy straw as a substrate for the cultivation of oyster mushrooms (*Pleurotus ostreatous).* This showed that the growth of mushrooms could be achieved with the use of waste as substrate.

Our study revealed the presence of phenol, flavonoids, saponins, alkaloids, tannins, sterols and steroids, terpenoids, and phytate in the *Pleurotus pulmonarius* extract. The quantitative screening showed an abundance of alkaloids, saponins, Phenol, Sterols and Steroids, terpenoids, phytate, and tannin in high concentrations respectively. These findings correlate with the report of Nwobodo *et al.,* (2021) who detected the presence of alkaloids, terpenoids, flavonoids, and

glycoside in *Pleurotus ostreatus* and *Agaricus bisporus* and evaluated the antimicrobial activity against pathogenic bacterial strains; *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa*, and the yeast *Candida albicans*. Tannic acid mainly produces maximum damage to the midgut epithelium of some dipterian larvae. Tannins also have various physiological effects like antiparasitic anti-irritant, antiscretolytic, and antimicrobial activities (Vairavan *et al.,* 2018). The alkaloids possess antioxidant activity. Terpenoids and essential oils have membrane disruption characteristics (Vairavan *et al.,* 2018). Quinines and polyphenols inactivate the enzymes, bind to adhesions, and form a complex with cell wall. Flavonoids inhibit gastrointestinal tract releasing acetylcholine. Saponin possesses membrane permeabilizing properties, leading to vacuolization and disintegration of integuments. Some of the characteristics of saponins include formation of foams in distilled water solutions, cholesterol binding properties, and hemolytic activity which affects mosquito larvae (Vairavan *et al.,* 2018). Alkaloids inhibit the metabolic processes in mosquito larvae, interfere with growth hormones, and digest the protein in the larval body and turn it into peptone derivatives (Vairavan *et al.,* 2018).

The FTIR analysis confirmed the presence of phytochemicals belongs to the functional groups such as alkyl halides, aliphatic amines, alcohols, esters, alkanes, nitro, and carbonyl groups. This is in line with the findings of *Vairavan et al.,* 2018, that reported the presence of alkyl halides, aliphatic amines, alcohols, esters, alkanes, nitro group, aromatic hydrocarbons, carbonyl group, imine group and phenols in *Catharanthus roseus* extract accounting for its usefulness as a powerful insecticidal agent. Also, in the findings of Baranitharan *et al.,* 2019, the presence of functional groups such as alcohol, amines, amides, alkenes, amines, aromatic amines, aliphatic amines, amines, and alkyl halides were present in methanol leaf extract of *Erythrina variegata*.

The gas chromatography analysis revealed the presence of 41 compounds with a total of 108.56% peak area. The 7-anti-hydroxy bicyclo[2,2,2] oct-2-en-2-one (46.46%), Methyl ester hexadecanoic acid (8.66%), Hexamethyl cyclotrisiloxane (8.56%), Arsenous acid (8.56%), Octamethyl cyclotetrasiloxane (8.23%), and 2-pentyne (5.09%) were all in abundance in the *Pleurotus pulmonarius* methanol extract. The presence of these compounds in abundance is suggested to be the cause of the high mortality rate of the *Anopheles* mosquito larvae after exposure to the extract. A similar study by Baranitharan *et al*., (2019), on the inhibition of *Anopheles stephensi*, and *Culex quinquefasciatus* larvae development with methanol extract of *Erythrina variegate* with chemical constituents of twenty-five compounds identified in the methanol extract. The major components were 12-octadecenoic acid and methyl ester (37.31%). Shaaban *et al*., (2021), reported the antibacterial activities of Methyl ester hexadecanoic acid from Clove alcoholic extract (CAE) on multi-drug resistant bacteria isolated from diabetic patients. Also, Abubacker and Deepalakshmi, (2013), also reported the effectiveness of Methyl ester hexadecanoic acid extracted from *Annona muricata* Linn. (Soursop) of Annonaceae to inhibit *Alternaria solani* (NCBT-118), *Aspergillus erithrocephalus* (NCBT-124) and *Aspergillus albicans* (NCBT-120) less effective for *Aspergillus fumigatus* (NCBT126) and *Penicillium chrysogenum* (NCBT 162). This result is similar to the result of our study whereby the GCMS analysis revealed the presence of Methyl ester hexadecanoic acid (8.66%) in the mushroom (*Pleurotus pulmonarius*) extract. In addition, Hexamethyl cyclotrisiloxane (8.56%) and Octamethyl

cyclotetrasiloxane (8.23%) were also identified in the extract and was suggested to be responsible for the inhibition of the development of the larvae which is in accordance to Keskın *et al.,* 2012 who reported the presence of cyclotrisiloxane hexamethyl (36.98%), cyclotetrasiloxane octamethyl (15.18%) and cyclopentasiloxane decamethyl (14.59%) being the main components in the aqueous extract of West Anatolian olive (*Olea europaea* L.) leaves which was effective and inhibited the growth of all tested Gram-positive and Gram-negative bacteria except for *Bacillus cereus* CCM 99, *Enterobacter aerogenes* ATCC 13048 and *Enterobacter cloacae* ATCC 13047 in their report. In another study by Keskın *et al*., (2012), GCMS analysis also revealed from aqueous extract of walnut green husks (*Juglans regia*) the bioactive compounds were ethylene oxide (83.67%), cyclotrisiloxane hexamethyl (5.04%), and from walnut leaves were ethylene oxide (14.74%), cyclotrisiloxane hexamethyl (17.89%). The aqueous extract of the walnut leaves was effective against four Gram-positive and one Gram-negative organism while both walnut green husks and leaves extracts exhibited antifungal activity. The combination of these compounds in abundance causes the termination of the developmental stages of the *Anopheles* mosquito larvae.

The initial concentration of 2500 ppm of *Pleurotus pulmonarius* methanol extract exhibited good larvicidal activities following exposure of the larvae beginning from the 12^{th} hour. 100% *Anopheles* mosquito larvae mortality rate was achieved at 60 hours of exposure to the extract. This is similar to the study of Vairavan *et al*, 2018, who achieve 100% mortality rate beginning from 24^{th} hour of exposure of *Culex quinquefasciatus* larvae to 400 ppm of acetone extract of *Catharanthus roseus* leaf. In the exposure to 2000 ppm concentration of the extract, 100% mortality was attained at 72 hours of exposure whereas, 60%, 50%, and 30% mortality were achieved in 1500 ppm, 1000 ppm, and 500 ppm at 72 hours of exposure to the extract. The low mortality rate of the larval could be as a result of the reduction in the concentration of the *Pleurotus pulmonariu*s methanol extract. The lower the concentration, the lower the mortality rate obtained. Baranitharan *et al.,* 2019 exposed mosquito immature third instar larval, Anopheles *stephensi*, and *Culex quinquefasciatus* to different concentrations of 50-250 µg/mL *Erythrina variegate* extract and 98.2% total death rate of the larvae was achieved. In another report, 100% mortality effect of petroleum ether and N-butanol extract of *Cassia occidentalis* (Linn.) was observed at 200 and 300 ppm on late third instar larvae of *Culex quinquefasciatus* (Kumar *et al*., 2014).

CONCLUSION

This study revealed the phytochemical contents and the identity of the compounds present in mushroom (*Pleurotus pulmonarius*) methanol extract. It also help to determine the larvicidal efficacy of the extract on *Anopheles* mosquito larvae. Overall, this study suggests that the methanol extract from the mushroom (*Pleurotus pulmonarius*) can be scaled up in the pharmaceutical industry to produce liquid antilarvicides and mosquitocides to reduce the spread and development of mosquitoes in developing countries.

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