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Wild Mushrooms: A Hidden Treasure of Novel Bioactive Compounds

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Abstract: Mushrooms are unexploited treasures of secondary metabolites. Analysis of the chemical constituents of these mushrooms would be necessary for the assessment of their pharmacological and biological activities. This study aimed at profiling of mycochemical constituents of five wild mushroom extracts thereby understanding their biological and pharmacological properties. Mushrooms were collected from Arabuko-Sokoke and Kakamega National Reserved Forests, Kenya. Specimens were identified by both morphological and molecular methods. Bioactive compounds were extracted using chloroform, 70% ethanol, and hot water solvents. Chloroform, 70% ethanol, and hot water extracts of Auricularia auricula-judae, Microporus xanthopus, Termitomyces umkowaani, Trametes elegans, and Trametes versicolor were determined using gas chromatography and mass spectrometry (GC-MS). From all extracts, a total of fifty-one (51) compounds were identified and grouped into carboxylic acids, esters, phenols, fatty acids, alcohol, epoxides, aldehyde, fatty aldehyde, isoprenoid lipids, and steroid. Of the total compounds, Oleic acid (72.90%) from Trametes elegans was detected abundantly. Most of the compounds obtained from the chloroform extract of Trametes elegans and 70% ethanol extract of T. umkowaani are fatty acids. The identified compounds have revealed many biological and pharmacological activities such as antimicrobial, antioxidant, antimalarial, anti-inflammatory, insecticidal, anti-helminthic, larvicidal, vasodilator, antihypertensive, hepatoprotective, anticancer, antidiabetic, antifertility anti-diuretic, antiasthma, antifouling, anti-dermatophytic, antispasmodic, anti-hypocholesterolemic, nematicide, pesticide, immunostimulant, antiarthritic, and antihistaminic. These fatty acids are particularly playing important roles in the anti-inflammatory, hypocholesterolemic anticancer, and anti-biofilm formation activities. The presence of these bioactive components suggests that the extracts of five wild mushrooms could be good sources of secondary metabolites for drug discovery.

Keywords: antioxidant; biological activity; gc-ms; mycochemicals; pharmacological activity; wild mushrooms

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1. Introduction

Macrofungi are vital sources of nutritious and functional food for humankind [1,2]. They are widely reported as reservoirs of highly varied biologically active compounds [2]. Although many higher fungi are sources of many bioactive compounds, yet to be fully harnessed [3,4]. The growing interest in searching for fungi that can contain many bioactive compounds is a growing line of research [5]. The continuous search for new lead compounds of therapeutic importance has become necessary in the face of treatment failures and as the multidrug resistance plaguing the world [3].

Mushrooms are opening numerous opportunities for bioprospecting and downstream applications [6]. Systematic investigation and evaluation of natural compounds obtained from mushrooms can have enormous benefits to tackle infectious and non-infectious diseases [7,8]. Mushrooms are large sources of bioactive compounds which can be exploited for the development of novel drugs [9–13]. Medicinal mushrooms and fungi are thought to possess around 130 medicinal functions, including antitumor, immunomodulator, antioxidant, cardiovascular, anti-hypercholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, antidiabetic, anticancer, antimicrobials, anti-inflammatory, anti-allergic, antibacterial, antifungal, anti-inflammatory, antioxidative, antiviral, cytotoxic, anti-depressive, antihyperlipidemic, antidiabetic, digestive, hepatoprotective, neuroprotective, nephroprotective, osteoprotective, hypotensive, effects, etc. [5,10,14–16]. The stated health benefits of mushrooms have been attributed to the presence of many bioactive compounds such as carbohydrates, proteins, essential amino acids, unsaturated fatty acids, vitamins, and minerals [17,18].

Auricularia auricular-judge (Bull.) is classified as Phylum-Basidiomycota, Class-Agaricomycetes, Order-Auriculariales, Family-Auriculariaceae, and genus-*Auricularia*. *A. auricula-judae* (aka black fungi, wood ear, Jew's ear, or jelly ear) is a highly nutritious edible mushroom with many pharmacological properties [19,20]. It contains amino acids, carbohydrates, vitamins, trace elements, and various health-promoting compounds such as polysaccharides, melanin, polyphenols, and flavonoids. It also has a large number of chemical compositions that possess antioxidant, anticoagulant, and antitumor activity [21].

Microporus xanthopus (Fr.) Kuntze belongs to Phylum-Basidiomycota, Class-Agaricomycetes, Order-Polyporales, Family-Polyporaceae, Genus-*Microporus*. It is a polypore inedible medicinal mushroom. It has diverse chemical compounds such as alkaloids, flavonoids, steroids, triterpenoids, and coumarin which are promising for pharmacological activities with potential uses in medicine, agriculture, and other industries [22]. The identification of these compounds highlights the potential for natural products to be developed into effective drugs for a range of conditions. It has been reported to exhibit antibacterial, anticancer, antiangiogenic, and anthelmintic activities. The higher concentrations of these medicinal properties are believed to be a result of the environment and substrate in which the polypore mushroom grows [23].

Termitomyces umkowaani belongs to the order Agaricales (Agaricomycetes), family Lyophyllaceae and subfamily *Macrotermitinae*. *Termitomyces* species are economically valuable edible mushrooms, that grow in an obligate mutualistic association with fungal-growing termites belonging to the subfamily Macrotermitinae (Isoptera) [24]. The termites provide the ambient microclimatic condition suitable for the growth and propagation of the fungi and the latter provide enzymatic supplement to aid digestion of the divergent termite food [25]. Their geographical distribution coincides with the distribution of termites exclusively found in Africa and some parts of South East Asia [26,27]. *Termitomyces* have indicated that their bioactive compounds have the potential to fight against certain human diseases such as cancer, hyperlipidemia, gastroduodenal diseases, and Alzheimer's [24,28] *Termitomyces* mushrooms also provide digestive enzymes and vitamins to their hosts [27].

Trametes elegans belongs to the phylum of Basidiomycota and the family Polyporaceae. It is both a saprotrophic and endophytic fungus that causes white rot during the decay of woody substrates found generally in hardwood forests [29]. *Trametes versicolor* (aka Turkey tail) has gained remarkable popularity due to its broad spectrum utilization in the food and pharmaceutical industries [30]. It is widely distributed in various biotopes and has been the subject of many physiological and biochemical studies. It has biological and metabolic diversity as a result of its ability to decompose dead organic matter and utilize several substrates [31]. This organism is from the It is also famous for its medicinal values and industrial uses (food production industry) and is commonly used for the restoration of soil, and wastewater treatment, as well as lignin biodegradation as a result it serves as bioremediation and biodegrades of cellulosic waste [17,32]

Trametes versicolor (L) Lloyd (family Polyporaceae) is common in temperate Asia, North America, and Europe, including the UK. Its medicinal value dates back at least 2000 years and includes general health-promoting effects (e.g. endurance and longevity) [33]. It possesses a variety of biologically active polysaccharides used to promote immune function, antivirus, antitumor, anti-diabetes, infections of the respiratory, urinary, and digestive tracts, chronic hepatitis, and rheumatoid arthritis [34]. It consists of 18 different amino acids viz aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, and leucine, and many other compounds, such as proteins, fatty acids, polysaccharides, polysaccharopeptides, glucans, amino acids, vitamins, and a variety of inorganic salts [33,35]. All these amino acids contribute to several potential applications [36,37]. Its fruiting bodies have antiviral and antioxidant activity and increase memory and improvement of mental functions [38,39].

Very few Kenyan wild mushrooms have been reported to have therapeutic potentials, but little/no study has reported the structurally elucidated and identified bioactive compounds conferring these therapeutic properties. Therefore, it is important to determine the bioactive compounds present in the wild mushroom extracts which are responsible for their medicinal values. This study therefore aimed to explore the bioactive compounds present in the chloroform, ethanol, and hot water extracts of five wild mushrooms and to determine their biological and pharmacological therapeutic properties which may provide insight in its use in traditional and modern medicine.

2. Results and Discussion

2.1. GC-MS Analysis of Wild Mushroom Extracts

GC-MS analysis of five wild mushroom extracts revealed the presence of fifty-one (51) compounds. From the extracts, many important compounds such as acyclic monoterpenoids, alcohol, aldehyde, alkene, alkyl benzene, aromatic organic heterocyclic, benzoic acid ester, cycloalkane methanol, cyclohexane, epoxides, ester, fatty acid, fatty acid ester, fatty alcohol, fatty aldehyde, isoprenoid lipid, organosiloxane, phenol, phthalate, pyrrolidines, siloxane, steroid, and β-carotene were obtained. These different compounds and their pharmacological and biological activities are described below (Tables 1, 2, 3, 4, & 5).

2.1.1. GC-MS Analysis of Auricularia auricula-judae

The HWE of AAJ revealed the presence of fourteen (14) bioactive compounds (Figure 1A, Table 1). These compounds have demonstrated many biological and pharmacological activities. Phenol, 2,6-bis (1,1-dimethyl ethyl)-4-methyl-, methylcarbamate (14.21%), 2-nonanol, 5-ethyl- (11.34%), octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (10.65%), 2-methyl-6-methylene-octa-1,7-dien-3-ol (7.98%), 2-methyl-1-ethylpyrrolidine (7.23%), and silicic acid, diethyl bis (trimethylsilyl) ester (7.12%) were identified as major compounds (Table 1). These compounds were classified into alcohol, alkene, siloxane, ester, phthalic acid, and phthalate. The fruiting body of AAJ contains proteins, carbohydrates, fats, and enormous quantities of fibers, carotenes, minerals (calcium, phosphorous, iron), and vitamins [40]. Moreover, AAJ contains some bioactive constituents represented by polysaccharides, melanin, and polyphenols that are vital groups of secondary metabolites and are synthesized in response to biotic (pathogens) and abiotic stresses (salinity, water, and climatic stress) [19]. A study indicates that siloxanes were generally reported to exhibit significant antimicrobial and antioxidant properties [41]. Thus, the compounds found in the HWE of AAJ could prevent diseases such as aging, cancer, cardiovascular disease, inflammation, and other disorders that are dangerous to human health occurred due to the overabundance of free radicals in our body [42]. Phenolic compounds can also affect anti-proliferation, cell cycle regulation, induction of apoptosis, and other biological activities which are mostly mediated by receptor-ligand interactions [43].

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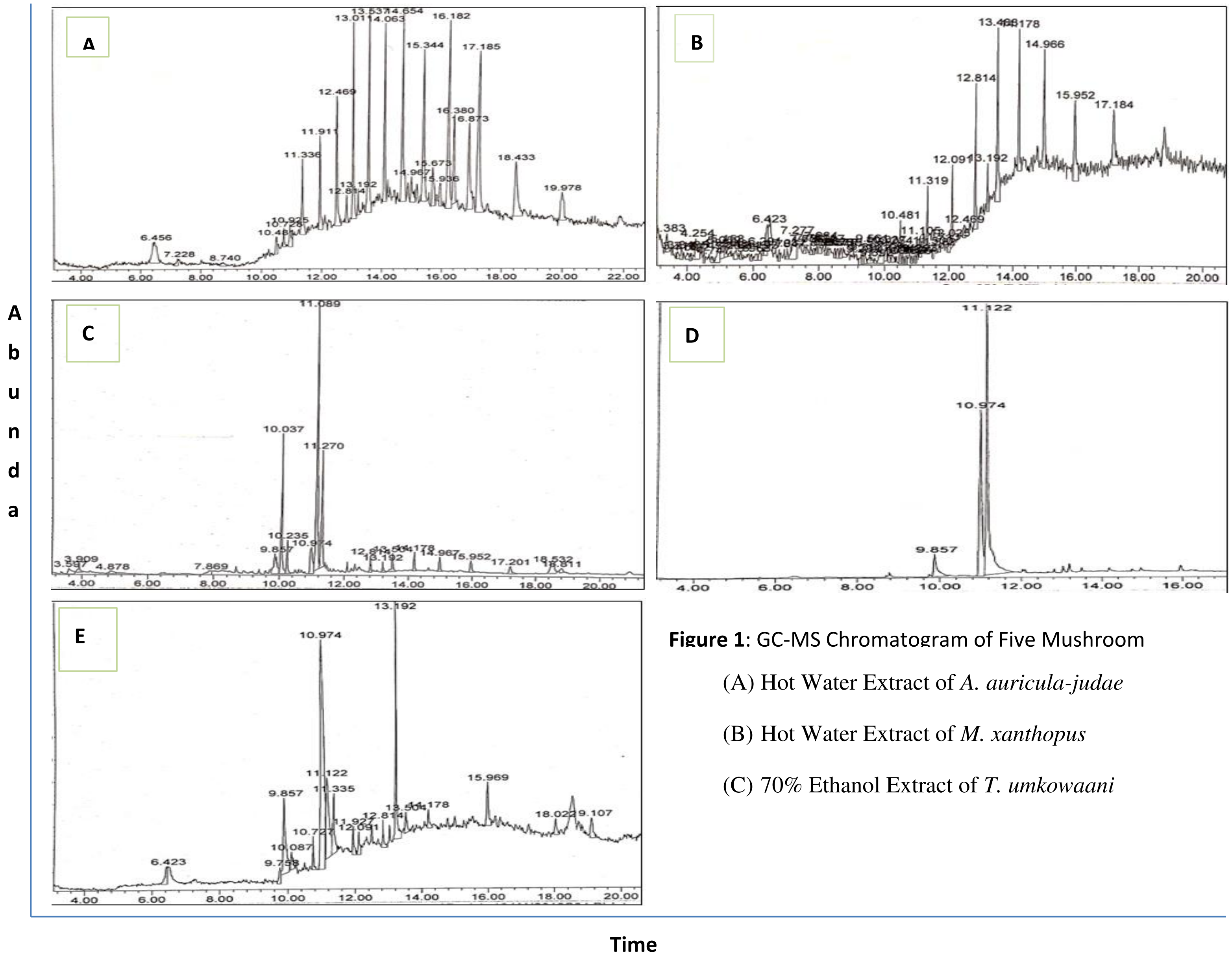

Figure 1: GC-MS Chromatogram of Five Mushroom

(A) Hot Water Extract of *A. auricula-judae*

(B) Hot Water Extract of *M. xanthopus*

(C) 70% Ethanol Extract of *T. umkowaani*

The HWE of AAJ has shown many biological and pharmacological activities such as antidepressant, antimicrobial, antioxidant, antimalarial, anti-inflammatory, insecticidal, hepatoprotective, anti-helminthic, larvicidal, anticholinesterase, antihypertensive, anticancer, antidiabetic, cholesterol-lowering, anti-urolithiasis, and antifertility. Previous studies also confirmed that AAJ extracts exhibit several biological and pharmacological properties such as anticoagulant, anti-diabetic, antioxidant, anticancer, hypolipidemic, anti-obesity, anti-inflammatory, anti-radiation, immunomodulatory, and antimicrobial activities [40,44,45]. A study reported that the HWE of AAJ also contains several phenolic compounds (e.g. epicatechin, catechin, chlorogenic acid, quercetin, and rutin). These phenolic compounds exhibited significant scavenging activity against DPPH free radicals, superoxide anions, and hydroxyl radicals. Crude AAJ extracts exhibit higher antioxidant activities, regulate blood pressure, and lowers cholesterol and lipid levels in the blood [40].

Crude polysaccharides obtained from AAJ have previously exhibited antimicrobial activity against Escherichia coli, Staphylococcus aureus, Bacillus cereus, Salmonella typhi, Proteus mirabilis, Klebsiella pneumonia, Candida albicans, Pseudomonas aeruginosa, and Candida parapsilosis [40,46]. Several in vitro and in vivo studies have shown the presence of many secondary metabolites such as β-glucans, chitin, and ergosterol derivatives. These metabolites exhibit potential anti-inflammatory activities and inhibit the production of pro-inflammatory cytokines [21,40]. The protective mechanisms of AAJ secondary metabolites against inflammatory activities could be by preventing the production of pro-inflammatory cytokines, stimulating the anti-inflammatory cytokines, and averting immune response as well as cancer cell formation in the body [21,40,45,47]. They also defend our body by reducing cholesterol in the blood, supporting the immune system of our body, inhibiting inflammatory diseases, and hindering the onset of cancer [40,47,48]. The cholesterol-lowering properties of the Ergosterol derivatives are mainly because of their structural similarity with the cholesterol, whereas β-glucans and chitin may be due to their binding abilities to cholesterol receptors [49].

Mushroom polysaccharides have proven anti-diabetic activities by maintaining blood glucose homeostasis via the regulation of pancreatic insulin secretion [50]. A previous study also asserted that polysaccharides obtained from AAJ extracts exhibited significant anti-diabetic activity in streptozotocin-induced diabetic rats. Low-density lipoprotein and total cholesterol levels in the blood were significantly reduced after the administration of AAJ polysaccharides to streptozotocin and high-fat diet-induced diabetic rats [51]. Moreover, diabetes-induced rats treated with AAJ polysaccharides led to a reduction of blood glucose levels by altering glucose metabolism, increasing insulin levels, and improving the insulin resistance islet damage in streptozotocin-induced diabetic mice [52,53]. These findings strongly suggested that AAJ-derived polysaccharides can be used as potential therapeutic agents against diabetes via modulation of blood glucose levels [40].

The HWE of AAJ contains high levels of insoluble fibers. These fibers are crucial to give potential health-promoting benefits through the modulation of gut microbiota [54,55]. The insoluble fibers act as prebiotics and are important factors to regulate the environment of the gut microbiota and to mediate their metabolic activities [56,57]. Beneficial gut microbiota plays a key role in protecting our body from various disease-causing pathogenic microbes by competing for food and by preventing attachment to the wall of the gut [58]. During their digestion and fermentation activities, these gut microbiota also help in the production of short-chain fatty acids (e.g. acetate, propionate, and butyrate) for our epithelial cells [59,60]. β-glucans obtained from HWE of AAJ have multiple health-promoting effects by maintaining a healthy gut environment and by serving exclusive carbon sources for intestinal bacteria during fermentation. Furthermore, they increased the number of beneficial bacteria (e.g. *Bifidobacteria* and *Lactobacillus*), which help in the production of short-chain fatty acids in our intestine [61]. They have also increased levels of serum IgA and IgG during the oral treatment of mice [62]. Moreover, they prevented unhealthy microbial growth in our gut, which can eventually protect our body from various gut-associated diseases [63,64].

Edible mushrooms have biological activities against cardiovascular disease. Species of *Auricularia* have been reported to contain cholesterol-lowering compounds [65]. Low-density lipoprotein cholesterol (the culprit of cardiovascular disease) levels were reported to be reduced by

AAJ extracts [56]. Using mice with hyperlipidemia as a model, AP obtained from AAJ extract significantly reduced serum and liver total cholesterol (TC), total triglyceride (TG), and serum Lactate dehydrogenase C (LDH-c) levels in mice. It can also protect the liver by enhancing antioxidant effects as a blood lipid-lowering agent [66].

Medicinal mushrooms are an important source of natural immuno-modulators. They contain diverse immune-regulatory compounds such as terpenes, lectins, immunomodulatory proteins, and polysaccharides. Immunomodulators can be immune-suppressants, immune-stimulants, and immune-adjuvants [67]. For example, an active compound AF1 β-1,3-d-glucan main chain with two β-1,6-d-glucosyl residues isolated from AAJ has induced apoptosis of cancer cells [68].

Table 1. GC-MS Analysis of *A. auricula-judae* Hot Water Extract.

Peaks	RT (min)	PA (%)	IUPAC Name and MF of Compounds	Nature of Compounds	Pharmacological and Biological Activities	Ref.
1	19.98	10.65	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (**$C_{16}H_{50}O_7Si_8$**)	Siloxane	Antidepressant, antimicrobial	[41,69]
2	18.43	7.12	Silicic acid, diethyl bis(trimethylsilyl) ester (**$C_{10}H_{28}O_4Si_3$**)	Ester	Antioxidant, antimicrobial, antimalarial, anti-inflammatory	[70,71]
3	17.19	4.43	Di-n-octyl phthalate (**$C_{24}H_{38}O_4$**)	Phthalic acid	Antimicrobial, insecticidal	[72,73]
4	16.87	6.12	Di-n-decylsulfone (**$C_{20}H_{42}O_2S$**)	Phthalate	Antimicrobial, anticancer, anti-helminthic, antagonistic, larvicidal	[74,75]
5	16.38	7.98	2-Methyl-6-methylene-octa-1,7-dien-3-ol (**$C_{10}H_{16}O$**)	acyclic monoterpenoids	No activity reported	
6	16.18	5.65	1-Heptanol, 2,4-dimethyl- (R, R)- (+)- (**$C_9H_{20}O$**)	Alcohol	Antifungal, antioxidant, anticholinesterase	[76–78]
7	15.34	4.31	Cyclohexano, 2,4-dimethyl- (**$C_8H_{16}O$**)	Cyclohexane	Anticancer	[79]
8	14.65	3.43	Carbonic acid, methyl octyl ester (**$C_{10}H_{20}O_3$**)	Ester	Hepatoprotective, antihypertensive, antioxidant, antimicrobial, antidiabetic, cholesterol-lowering, anti-urolithiasis, antifertility	[80]
9	14.06	5.25	1-Allylcyclopropyl) methanol (**$C_7H_{12}O$**)	Cycloalkane methanol	No activity reported	
10	13.64	7.23	2-Methyl-1-ethylpyrrolidine (**$C_7H_{15}N$**)	Pyrrolidines	Anti-tumor	[81]
11	13.01	6.33	Oxirane, 2,2′-(1,4-dibutanediyl) bis- (**$C_8H_{14}O_2$**)	Epoxides	Antibacterial	[82]
12	12.47	11.34	2-Nonanol, 5-ethyl- (**$C_{11}H_{24}O$**)	Fatty alcohol	Anticancer	[83]
13	11.91	5.86	1-Hexene, 4, 5-dimethyl- (**C_8H_{16}**)	Alkene	Antimicrobial	[84]
14	11.34	14.21	Phenol, 2,6-bis (1,1-dimethylethyl)-4-methyl-, methyl carbamate (**$C_{17}H_{27}NO_2$**)	Alkyl benzene	Antioxidant, antibacterial, anti-inflammatory, temporarily treat pharyngitis	[85,86]

MF: Molecular formula; RT: Retention time; PA: Peak area.

2.1.2. GC-MS Analysis of Hot Water Extract of Microporus xanthopus

From hot water extract (HWE) of *Microporus xanthopus* (MX), twelve (12) compounds were identified (Figure 1B, Table 2). The 1-mono-linoleoyl glycerol trimethylsilyl ether (16.32%), trans-1, 1′-bibenzoindanylidene (14.18%), 2, 2′-divinylbenzophenone (13.76%), and didodecyl phthalate (11.39%) are among the abundant compounds. These compounds were classified into alcohol, epoxides, aldehyde, fatty aldehyde, isoprenoid lipid, n-alkanes, and steroid. These compounds have shown antioxidant, antimicrobial, nematicidal, antimalarial, anti-diuretic, antiasthma, vasodilator, antifouling, anti-dermatophytic, antihypertensive, uric acid excretion stimulant and diuretic, reducing depressive symptoms, and anti-inflammatory activities. Moreover, 1-monolinoleoylglycerol trimethylsilyl ether (steroid) has anti-diuretic, anti-diabetic, anti-inflammatory, antimicrobial antioxidant, anti-arthritic, and antiasthma activities.

Like the present findings, HWE of MX, many mushrooms extracts such as *Agaricus bisporus, Cyclocybe aegerita, Cyclocybe cylindracea,* and *Tremella fuciformis* were studied for the treatment or prophylaxis of type-2 diabetes–occurred when imbalanced insulin is producing due to the dysfunctions of insulin-secreting beta cells in the pancreas [87,88]. As mushrooms contain the least amount of digestible carbohydrates in the diet, they help patients to avoid high levels of glucose in the blood [89]. Bioactive metabolites isolated from medical mushrooms act as anti-hyperglycemic agents in diabetes treatment [90,91]. *Inocutis levis* and *Antrodia cinnamomea* extracts have been reported as a remedy for diabetes by increasing insulin resistance, insulin sensitivity, and glucose uptake in tissues and hence help to control blood glucose levels [88,92]. *Grifola frondosa* has been used

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as medicine for type 2 diabetes, and its extracts can lessen both hyperglycemia and hyperinsulinemia [93]. Moreover, SX-Fraction, ReishiMax capsules, and *Tremella* obtained from *Ophiocordyceps sinensis* and *Tremella fuciformis*, respectively are some examples of anti-diabetic products. These products enhance insulin sensitivity, decrease blood glucose levels, cholesterol levels, blood pressure, and body weight [88,94,95].

In the present findings, most of the compounds identified from the HWE of MX proved antimicrobial activity. A previous study also corroborated that oligosaccharides, polysaccharides, and polyphenols originating from HWE of MX showed antibacterial activity against Shiga-toxin-producing *E. coli* and methicillin-resistant *Staphylococcus aureus* [96]. Likewise, CE of MX has also resulted in higher antibacterial activities against *S. aureus* (ATCC 25923), MRSA (ATCC 33591), and *K. pneumoniae* (ATCC 13883) [97].

In this study, the HWE of MX illustrated the presence of anti-arthritic compounds. Most mushrooms are known to produce certain bioactive substances which are used as potential treatment strategies against cardiovascular diseases [98,99]. Yet, the mechanism of action/treatment of these bioactive substances remains obscure it might be due to the reduction in serum lipid, increase in bile acid secretion and LDL receptor expression, and change in phospholipid metabolism [100]. Other studies also recognized that mushrooms have molecules that can modify cholesterol absorption, metabolism, and also modulate the gene expression related to cholesterol homeostasis [99,101]. For instance, molecules extracted from *Grifola frondosa, Hypsizigus marmoreus,* and *Pleurotus ostreatus* were able to modulate the gene expression patterns of mice livers [88,102].

Table 2. GC-MS Analysis of *M. xanthopus* Hot Water Extract.

Peaks	RT (min)	PA (%)	IUPAC Name and MF of Compounds	Nature of Compounds	Pharmacological and Biological Activities	Ref.
1	6.42	8.11	1-Heptanol, 2,4-dimethyl-, (2S, 4R) -(-)- (**$C_9H_{20}O$**)	Alcohol	Antifungal	[76,77]
2	7.28	4.34	Oxirane, 2,2′-(1,4-butanediyl) bias- (**$C_8H_{14}O_2$**)	Epoxides	No activity reported	
3	10.48	3.67	3-Methyl-2-(2-oxopropyl) furan (**$C_8H_{10}O_2$**)	Aldehyde	Antioxidant, antimicrobial	[103,104]
4	11.32	5.50	7-Hexadecenal, (Z)- (**$C_{16}H_{30}O$**)	Fatty aldehyde	Antiviral, antibacterial	[105,106]
5	12.09	7.87	1,2,3,3a-Tetrahydro-7-methyl-10-4-methylphenyl) benzo [c] cyclopenta [f] -1,2-diazepine (**$C_{20}H_{20}N_2$**)	Aromatic organic heterocyclic	No activity reported	
6	12.81	4.41	Tetradecane, 2,6,10-trimethyl- (**$C_{17}H_{36}$**)	Isoprenoid lipid	Antifungal, antibacterial, and nematicidal	[107]
7	13.19	4.19	Heptacosane (**$C_{27}H_{56}$**)	N-Alkanes	Antibacterial, antifungal, antioxidant, antimalarial, antidermatophytic	[108,109]
8	13.47	11.39	Didodecyl phthalate (**$C_{32}H_{54}O_4$**)	Phthalate	Vasodilator, antihypertensive, uric acid excretion stimulant and diuretic, antimicrobial, antifouling	[110,111]
9	14.18	1.17	Acetamide, N-[3-(10,11-dihydro-5H-dibenzo [a, d] cyclohepten-5-ylidene)propyl] -2,2,2-triflouro-N-methyl (**$C_{21}H_{20}F_3NO$**)	Unknown	Reducing depressive symptoms	[112]
10	14.97	13.76	2,2′-Divinylbenzophenone (**$C_{17}H_{14}O$**)	Unknown	Antimicrobial, anti-inflammatory, antioxidant	[113]
11	15.95	14.18	Trans-1, 1′-Bibenzoindanylidene (**$C_{18}H_{16}$**)	Unknown	No activity reported	
12	17.18	16.32	1-Monolinoleoylglycerol trimethylsilyl ether (**$C_{27}H_{54}O_4Si_2$**)	Steroid	Anti-diuretic, anti-inflammatory, anti-diabetic, antimicrobial antioxidant, anti-arthritic, antiasthma	[114,115]

MF: Molecular formula; RT: Retention time; PA: Peak area.

2.1.3. GC-MS Analysis of 70% ethanol extract of Termitomyces umkowaani

Fourteen (14) compounds were distinguished from 70% ethanol extract (EE) of *Termitomyces umkowaani* (TU) (Figure 1C, Table 3). These compounds were grouped into acids, alcohols, esters, ethers, ketones, aldehydes, and others. Of the 14 compounds, Tetracosamethyl-cyclododecasiloxane

(18.90%), 12-methyl-E, E-2, 13-octadecadien-1-ol (15.90%), and 9, 12-octadecadienoic acid, ethyl ester (13.43%) were noticed abundantly (Table 3).

Many fatty acids (FAs) such as linolenic acid, butanedioic acid diethyl ester, octadecanoic acid, ethyl ester, h-hexadecanoic acid, hexadecanoic acid, ethyl ester, i-propyl hexadecanoate, 9, 12-octadecadienoic acid (Z, Z)-, 9, 12-octadecadienoic acid, ethyl ester, and 7-hexadecenal, (Z)- were noticed in the EE of TU. These FAs showed antimicrobial, antioxidant, antispasmodic, antitumor, anti-hypocholesterolemic, anti-inflammatory, nematicide, pesticide, anti-androgenic, immunostimulant, anti-acne, inhibitor, insecticide, antiarthritic, anti-eczemic hepatoprotective, antihistaminic, and anti-coronary [116–118]. Besides FAs, the EE of TU revealed other bioactive compounds including isopropyl linoleate (β-carotene), 1-monolinoleoylglycerol trimethylsilyl ether (steroid), and 12-Methyl-E, E-2, 13-octadecadien-1-ol (alcohol). These compounds also have antimicrobial, antioxidant, antiasthma, anti-diuretic, anti-inflammatory, and anti-diabetic properties. Linoleic acid and oleic acid exhibited an antimicrobial effect against *Staphylococcus aureus*, by inhibiting its cell growth and biofilm formation [119].

Hexadecanoic acid, ethyl ester (palmitic acid ester) found in the EE of TU has antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, antibacterial, anti-inflammatory, antitumor, immunostimulant, hemolytic 5-α reductase inhibitor, lipooxygenase inhibitor activities. Palmitic acid (PLA) is ubiquitously present in dietary fat guaranteeing an average intake of about 20 g/d. The relatively high requirement in the human body (20–30% of total fatty acids), is justified by its relevant nutritional role [120]. Transcriptomic analysis revealed that palmitic acid impacted several signaling pathways including lipid metabolism in neurons. By contrast, overconsumption of palmitic acid could cause neurodegenerative diseases, including Parkinson's disease [121,122]. However, at low doses, PLA causes mild stress that can activate the stress response pathway to counteract deleterious damages such as oxidative stress and has a key role in the regulation of the longevity pathway [121]. Moreover, PLA is reported to possess antibacterial and anti-cholesterolaemic effects [123].

The 9, 12-Octadecadienoic acid (Z, Z)- (aka conjugated linoleic acid) was found in the EE of TU. Linolenic acid (LA) contains omega-3 and omega-6 fatty acids. LA helps to reduce body inflammation and can also lower risk factors related to heart disease and arthritis. Omega-3 fatty acid transforms into prostaglandin E1 which has blood cholesterol-reducing properties and increases immunity [124,125]. Omega-3 fatty acid has a beneficial effect on cardiovascular health and reduces risk factors associated with strokes, heart attacks, and high blood pressure [125,126]. Unsaturated fatty acid levels are generally higher than saturated ones in mushrooms [127]. This polyunsaturated acid ensures the production of bile acids in the liver, prevents hormonal imbalance, and influences the production of prostaglandins [128].

Currently, LA has shown antimicrobial activity. In corroborative to the present findings, methanol and ethanol extracts of *Termitomyces* species revealed potent antimicrobial activity against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Candida albicans* of pathogenic microbes [129]. The dichloromethane extract of *Termitomyces striatus* also showed antimicrobial activity against bacteria (*P. aeruginosa E. coli*, *B. subtilis*, and *S. aureus*) and fungi (*C. albicans* and *S. cerevisiae*) [130]. Many *Termitomyces* species showed significant antimicrobial activity against different pathogenic microorganisms, for example, water extract of *T. clypeatus*, (*Candida albicans, Escherichia coli, Salmonella typhi*, and *Staphylococcus aureus*), water extract of *T. heimii*, (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas* sp., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Ralstonia* sp., *Salmonella* sp., and *Streptococcus* sp.) [24].

Fatty acids such as octadecanoic acid, ethyl ester, h-hexadecanoic acid, 9, 12-octadecadienoic acid (Z, Z)-, and 9, 12-octadecadienoic acid, ethyl ester obtained from the EE of TU have shown hypocholesterolemic activity. Edible mushrooms possess high dietary fiber levels and other components such as eritadenine, guanylic acid, and ergosterol which play a significant role in the prevention of nutrition-related diseases (e.g. atherosclerosis) by lowering hypocholesterolemic levels [131,132]. Dietary intake of TU was reported to lower serum levels of total cholesterol and LDL-cholesterol [133]. Fed diets mixed with mushrooms reduced levels of total cholesterol, LDL-cholesterol, and triglycerides in rats [128]. Polysaccharides and fibers obtained from water extract of edible mushrooms also lowered the serum triglyceride concentration in hypertensive and hyperlipidaemic rats by altering lipid metabolism and by inhibiting both the accumulation of liver lipids and the elevation of serum lipids [134].

Table 3. GC-MS Analysis of *T. umkowaani* 70% Ethanol Extract.

Peaks	RT (min)	PA (%)	IUPAC Name and MF of Compounds	Nature of Compounds	Pharmacological and Biological Activities	Ref.
1	4.88	5.68	Butanedioic acid diethyl ester ($C_8H_{14}O_4$)	Fatty acid	Antimicrobial, antispasmodic, and anti-inflammatory	[135]
2	7.87	4.11	Octadecanoic acid, ethyl ester ($C_{20}H_{40}O_2$)	Fatty acid esters	Hypocholesterolemic 5-alpha reductase inhibitor, lubricant, and antimicrobial	[11,136]
3	9.86	2.45	h-Hexadecanoic acid ($C_{16}H_{32}O_2$)	Fatty acid (aka palmitic acid)	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, antibacterial, anti-inflammatory, antitumor, immunostimulant, hemolytic 5-α reductase inhibitor, lipooxygenase inhibitor	[3,137]
4	10.04	7.90	Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$)	Fatty acid ester (aka palmitic acid ester)	Antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic, hemolytic 5-α reductase inhibitor	[3]
5	10.24	8.78	i-Propyl hexadecanoate ($C_{19}H_{38}O_2$)	Fatty acid	No activity reported	
6	10.97	9.98	9,12-Octadecadienoic acid (Z, Z)- ($C_{18}H_{32}O_2$)	Fatty acid (aka conjugated linoleic acid)	Anti-inflammatory, antioxidant, hypocholesterolemic, antimicrobial, antitumor, insecticide, antiarthritic, antieczemic hepatoprotective, antiandrogenic, nematicide, antihistaminic, antiacne, hemolytic 5-α reductase inhibitor, anti-coronary	[3,80,137–139]
7	11.09	13.43	9,12-Octadecadienoic acid, ethyl ester ($C_{20}H_{36}O_2$)	Fatty acid ester (aka omega-6)	Hypocholesterolemic, nematicide, antiacne, antiarthritic, hepatoprotective, antimicrobial, antiandrogenic, hemolytic 5-α reductase inhibitor, antihistaminic, anti-coronary, insecticide, antieczemic	[3,70,80]
8	11.27	0.89	Isopropyl linoleate ($C_{21}H_{38}O_2$)	β-carotene	Antimicrobial, antioxidant	[22,43,140,141]
9	13.19	1.50	1-Monolinoleoylglycerol trimethylsilyl ether ($C_{27}H_{54}O_4Si_2$)	Steroid	Antimicrobial, antiasthma, anti-diuretic, antioxidant, anti-inflammatory and anti-diabetic	[114]
10	14.18	15.90	12-Methyl-E, E-2, 13-Octadecadien-1-ol ($C_{19}H_{36}O$)	Alcohol	Antimicrobial	[142]
11	14.97	1.12	7-Hexadecenal, (Z)- ($C_{16}H_{30}O$)	Fatty aldehyde	Antiviral, antibacterial	[105,106]
12	15.95	3.60	1, 2-Benzenedicarboxylic acid, diisooctyl ester ($C_{24}H_{38}O_4$)	Ester	Antimicrobial, antifouling	[143]
13	17.20	18.90	Tetracosamethyl-cyclododeca siloxane ($C_{24}H_{72}O_{12}Si_{12}$)	Siloxane	No activity reported	
14	18.53	5.76	Heptasiloxane hexadecamethyl ($C_{16}H_{48}O_6Si_7$)	Organosiloxane	No activity reported	

MF: Molecular formula; RT: Retention time; PA: Peak area.

2.1.4. GC-MS Analysis of chloroform extract of Trametes elegans

In the chloroform extract (CE) of *Trametes elegans* (TRE), the presence of three (3) compounds was detected (Figure 1D, Table 4). The identified compounds include n-hexadecanoic acid (16.89%), oleic acid (72.90%), and octadecanoic acid (10.21%). These compounds are grouped under essential fatty acids which are playing important roles in the anti-inflammatory, antioxidant, and hypocholesterolemic activities. The deficiency of linoleic acid, typical essential fatty acid, in the diet, causes mild skin scaling, hair loss [21], and poor wound healing in rats [22].

The majority of the identified compounds were reported to have antimicrobial, antioxidant, anticancer, anti-androgenic, hypocholesterolemic, nematicide, pesticide, and anti-biofilm formation properties (Table 4). These comprehensive activities might be correlated with the presence of many compounds such as tocopherols, flavonoids, polyphenols, tannins, and lignins in the extract [144]. The antioxidant activity of the TRE extract is acting by blocking the reactions of the oxidizing chain of free radicals in the molecules and by reducing the oxidative damage caused by oxidative stress

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[145]. Antioxidants protect our bodies from diabetes, cancer, aging, atherosclerosis, and other severe health issues [146].

Three essential fatty acids isolated from the CE of TRE have shown anti-biofilm formation activity. Fungal metabolites have promising anti-quorum-sensing activities for the reduction of drug resistance by inhibiting the biofilm formation of pathogenic microbes. Previous studies also confirmed that many edible mushrooms are sources of many secondary metabolites which have biofilm inhibitory activities. For instance, coprinuslactone, roussoellenic acid, and microporenic acid A derived from *Coprinus comatus, Roussoella* sp, and Kenyan basidiomycete, respectively have shown active anti-biofilm inhibitory activity against *Pseudomonas, Staphylococcus aureus* and *Candida albicans* [147,148]. Biofilm inhibitors enhance the activity of the antibiotics by increasing their ability to penetrate the biofilms [149].

The CE of TRE possesses anticancer activity. Several promising anticancer drugs derived from fungi are currently in the preclinical and clinical developmental stages [150]. For example, irofulven is a semi-synthetic drug derived from illudin S, a natural toxin isolated from *Omphalotus illudens* [151]. Irofulven has been evaluated in phase I and II clinical trials with promising results against the brain and central nervous system, breast, blood, colon, sarcoma, prostate, lungs, ovarian, and pancreas cancers [152,153]. Aphidicolin is also another anticancer compound isolated from *Akanthomyces muscarius* and *Nigrospora sphaerica* fungal species. Although, aphidicolin targets the specific binding site on DNA polymerase α, δ, and ε enzymes, it has not yet been marketed as an anticancer drug [88].

The n-hexadecanoic acid, one of the fatty acids, identified from CE of TRE revealed nematicidal activity (Table 4). Although effective chemical nematicides (e.g. methyl bromide) have been marketed, they can cause serious problems to the environment by killing all life forms in the soil and contributing to the depletion of the ozone layer. Recently there have been great efforts in both academia and industry to find ecologically viable alternatives [88]. Several nematotoxic compounds such as fatty acids, alkaloids, peptide compounds, terpenes, condensed tannins, phenolic compounds, and proteases have been identified in edible mushrooms [154]. Linoleic acid is one of the nematicidal compounds that have been isolated from *Arthrobotrys* species and other fungi [155]. On the other hand, *Pleurotus pulmonarius* and *Hericium coralloides* are two basidiomycetes that have exhibited strong nematicidal effects against *Caenorhabditis elegans* [156]. Metabolites (3, 14′-bihispidinyl and hispidin and phelligridin L) with moderate nematicidal activity against *Caenorhabditis elegans* have been reported from a *Sanghuangporus* sp. collected in Kenya [157]. Chaetoglobosin A and its derivate 19-O-acetylchaetoglobosin A isolated from *Ijuhya vitellina* are recently demonstrated nematicidal activity against eggs of *Heterodera filipjevi* [158].

Table 4. GC-MS Analysis of *T. elegans* Chloroform Extract**.**

Peaks	RT (min)	PA (%)	IUPAC Name and MF of Compounds	Nature of compounds	Pharmacological and Biological Activities	Ref.
1	9.86	16.89	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	Fatty Acid	Antioxidant, antiandrogenic, hypocholesterolemic, nematicide, pesticide, antibiofilm formation	[137,159]
2	10.97	72.90	Oleic acid ($C_{18}H_{34}O_2$)	Fatty Acid	Antioxidant, apoptotic activity in tumor cells, anticancer, antibiofilm formation	[159,160]
3	11.12	10.21	Octadecanoic acid ($C_{18}H_{36}O_2$)	Fatty Acid	Antimicrobial, antibiofilm formation	[161][159]

MF: Molecular formula; RT: Retention time; PA: Peak area.

2.1.5. GC-MS Analysis of hot water extract of Trametes versicolor

Eight (8) compounds were identified from hot water extract (HWE) of *Trametes versicolor* (TRV) (Figure 1E, Table 5). The most dominant compounds were phenol, 2, 6-bis (1, 1-dimethyl ethyl)-4-methyl, methylcarbamate (26.56%), 1-mono-linoleoyl glycerol trimethyl silyl ether (22.40%), and 1, 2-benzene dicarboxylic acid, diisooctyl ester (19.10%).

9, 12-Octadecadienoic (Z, Z)-, a polyunsaturated fatty acid, found in the TRV has shown anticancer activity. The TRV extract contains anticancer and immuno-stimulatory compounds including polysaccharides, β-glucans, lignins, and ergosta-7, 22-dien-3 beta-ol [162]. polysaccharides isolated from TRV extract demonstrated cytotoxic activity against cancer cells [36]. Polysaccharides containing peptides not only greatly uplift the quality of life of cancer patients undergoing chemotherapy or radiotherapy but also contribute to prolonging survival and bettering the quality of life in patients afflicted with hepatitis, hyperlipidemia, and other chronic diseases [36,162]. An aqueous extract of TRV prohibited migration and invasion of 4T1 breast cancer cells and downregulated the activities of tumor necrosis factor-α, interferon-γ, interleukin-2, interleukin-6, and interleukin-12) inducing roles in xenograft-bearing mice [163]. The TRV protein-bound polysaccharides exhibited tumor necrosis factor-α-dependent anti-proliferative activity toward MCF-7 cells and augmented the proliferative response of blood lymphocytes which was associated with interleukin-6 and interleukin-1β mRNA up-regulation [164].

Table 5. GC-MS Analysis of *T. versicolor* Hot Water Extract.

Peaks	RT (min)	PA (%)	IUPAC Name and MF of Compounds	Nature of compounds	Pharmacological and Biological Activities	Ref.
1	6.42	26.56	Phenol, 2,6-bis (1,1-dimethyl ethyl)-4- methyl, methylcarbamate ($C_{17}H_{27}NO_2$)	Phenol	Antioxidant, antibacterial, anti-inflammatory, oral anesthetic/analgesic, temporarily treat pharyngitis	[85,86]
2	9.86	2.20	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	Palmitic acid	Antioxidant, nematicide, pesticide, hypocholesterolemic, antiandrogenic	[165]
3	10.73	3.40	Nonadecane ($C_{19}H_{40}$)	Hydrocarbon	No activity reported	
4	11.12	8.41	9,12-Octadecadienoic (Z, Z)- ($C_{18}H_{32}O_2$)	Polyunsaturated fatty acid	Anti-inflammatory, hypocholesterolemic, antitumor, hepatoprotective, nematicide, insecticide, antibiofilm formation, antihistaminic, antieczemic, antiacne, hemolytic 5-α reductase inhibitor, antiandrogenic, antiarthritic, anti-coronary, antimicrobial	[114,159,166–168]
5	11.34	5.73	7-Hexadecenal, (Z)- ($C_{16}H_{30}O$)	Fatty aldehyde	Antiviral, antibacterial	[105,106]
6	13.19	12.20	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl) oxy] methyl] ethyl ester (Z, Z, Z)- ($C_{27}H_{52}O_4Si_2$)	polyunsaturated fatty acid	Antimicrobial, antioxidant	[169,170]
7	15.97	22.40	1-Momolinoleoylglycerol trimethylsilyl ether ($C_{27}H_{54}O_4Si_2$)		Antimicrobial, antiasthma, anti-diuretic, antioxidant, anti-inflammatory and anti-diabetic	[114]
8	18.11	19.10	1,2-Benzenedicarboxylic acid, diisooctyl ester ($C_{24}H_{38}O_4$)	Benzoic acid ester	Biopesticides, antibacterial	[171,172]

MF: Molecular formula; RT: Retention time; PA: Peak area.

3. Materials and Methods

3.1. Wild Mushrooms Collection and Identification

Mushrooms were collected from Arabuko-Sokoke and Kakamega National Reserved Forests. They were randomly collected from tree barks or other substrates (wood, soil, or leaf litter). They were wrapped in aluminum foil and placed in an ice box to maintain their structure and moisture content. Then, they were identified by both morphological and molecular methods. Specimens were identified using spore print color (white, black, brown, pink, purple, etc.), macroscopic, and microscopic (shape and size of basidiospores, basidia, cystidia, and generative hyphae) methods [173]. Moreover, the morphological characteristics of the specimens were compared to *Species Fungorum* and related literature [174].

Specimens were dried in an electric drying oven at 50 °C for 168 h [175,176]. After drying, gDNA was extracted from the dried fruiting body of mushrooms using the Cetyl Trimethyl Ammonium Bromide (CTAB) method [12]. By designing specific markers, highly conserved regions of the mushroom rDNA genes (i.e., ITS1 and ITS4) were amplified using the PCR amplification method [177]. Amplified PCR products were separated using gel electrophoresis and visualized under UV

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light. The presence and the amount of each PCR product were estimated by comparing it against the control (1kb DNA ladder).

3.2. Extraction of Bioactive Compounds

Bioactive compounds were extracted using chloroform, 70% ethanol, and hot water solvents as per the previous studies with some modifications [178–181]. A 100 g of powdered mushroom was mixed with each 1L of 99.8% chloroform (Sigma Aldrich, USA), 70% ethanol (99.9%) (ECP Ltd, New Zealand), and distilled hot water (heated at 60 °C for 2 h.) separately in an Erlenmeyer flask at 25 °C and shaken using an incubator shaker (SK-727, Amerex instruments, inc., USA) at 150 rpm for 72 h. The extracts were centrifuged at 3000 rpm (Eppendorf centrifuge 5810 R, Germany) for 15 min, filtered with Whatman No. 1 filter paper, and concentrated and dried by a rotary evaporator (EV311, Lab Tech Co., LTD, UK) at 50 °C. The extracts were kept in a –80 °C deep freezer and freeze-dried (mrc freeze dryer, Model, FDL-10N-50-8M). Finally, crude extracts were stored in a 4 °C refrigerator in amber-colored bottles for further analyses.

3.3. GC-MS Analysis of Extracts

The GC-MS analysis was conducted using a silica capillary column (30×0.25 mm ID×1 µm, composed of 100% Dimethylpolysiloxane) and operated in an electron impact mode at 70 eV (Agilent Scientific, Palo Alto, CA). Helium (99.999%) was a carrier gas at a constant flow of 1 mL/min. Extracts were dissolved in dichloromethane and 1 µL was injected into the column at 250 °C and ion-source temperature 280 °C. The oven temperature was programmed at 110 °C for 2 min. The temperature was increased from 110 °C to 200 °C (10 °C/min) then to 280 °C (5 °C/min) and finally ended at 280 °C for 9 min. The total run time was 28 min. The compounds were identified from the MS data, by comparing the spectra of known compounds stored in the National Institute of Standards and Technology (NIST) library with the mass spectrometry (MS) of unknown compounds. The relative % amount of each compound was calculated by comparing its average peak area to the total areas. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software [182].

3.4. Data Analysis

All the tests, experiments, and measurements were carried out in triplicate. Microsoft Excel Package was used to analyze quantitative data.

4. Conclusions

These mushroom metabolites have many bioactive compounds that possess antioxidant, anti-inflammatory, anti-microbial, anticancer, hypocholesterolemic, anti-hypertensive, nematicide, pesticide, and anti-biofilm formation properties. The wild mushroom extracts are rich in essential fatty acids and other many bioactive compounds which could have high potential industrial and biological activities. These compounds can be deployed to discover novel drugs against various cancers. It is recommended that the active ingredients are isolated and subjected to further tests to compare their usefulness in the prevention and treatment of various conditions. More research is necessary to determine which mushroom extracts are most beneficial in treating various cancers. The mechanisms of action for active ingredients in many extract from medicinal mushrooms, rigorous chemical analyses as well an understanding of the in vivo pharmacokinetics and pharmacodynamics of individual compounds is needed. Future investigation is needed to clarify the long-term effects of taking medicinal mushroom products with other drugs.

Supplementary Materials: Not applicable.

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Abbreviations/Acronyms.

AAJ	*Auricularia auricula-judae*
CE	Chloroform extract
EE	70% ethanol extract
FAs	Fatty acids
GC-MS	Gas Chromatography Mass Spectrometry
HWE	Hot water extract
LA	Linolenic acid
LDL	Low-density lipoprotein
MF	Molecular formula
MX	*Microporus xanthopus*
PA	Peak area
PLA	Palmitic acid
RT	Retention time
TRE	*Trametes elegans*
TRV	*Trametes versicolor*
TU	*Termitomyces umkowaani*

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