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Identification of Bioactive Components in Oils from Sprouts and Matured *Coprinopsis lagopus* mushroom

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Abstract
Background: *The potentials of medicinal mushrooms to synthesize different bioactive components has made them prominent for exploration of compounds of therapeutic values. Coprinopsis lagopus is a nonpoisonous member of the corprinoid specie, whose consumption depends on its growth stage.*
Materials and Methods: *Sprouts and matured C. lagopus harvested from decaying empty palm fruit bunches within the University of Port Harcourt Community and identified at the Plant Science and Biotechnology Department of the University of Port Harcourt were separated, washed and homogenized into a fine smooth paste. A quantity of 40 g of each paste placed and sealed in different Whatman no.4 filter papers, was extracted in separate Soxhlet extractors, using dichloromethane as solvent. The oily extracts were separated and concentrated at room temperature and used for GC-MS analyses.*
Results: *The highest peaks in the chromatogram of the oil from the sprout was observed at retention times of 16.088 mins, while that of the matured mushroom was observed at retention times of 33.664 mins. Linoelaidic acid ($C_{18}H_{32}O_2$); Pentadecane,2,6,10,14-tetramethyl ($C_{19}H_{40}$); Eicosane ($C_{20}H_{42}$) and Octadecane ($C_{18}H_{38}$) were predominant amongst the 33 bioactive components in the oil from the sprouts, with a percentage concentration of 20.976, 10.622, 6.235, 5.389, while Dodecanoic acid, 1,2,3-propanetriyl ester ($C_{39}H_{74}O_6$), Phytol ($C_{20}H_{40}O$), Vitamin E ($C_{29}H_{50}O_2$) and Squalene ($C_{30}H_{50}$) were the 4 bioactive components in the oil from the matured mushroom with percentage concentration of 37.228 and 34.681, 18.786 and 9.305 respectively.*
Conclusion: *This study revealed that high concentration of phytol maybe responsible for the avoidance of the matured mushroom. However, the presence of other bioactive components in the sprout and matured mushrooms contributes to its medicinal relevance.*
Key words: *Medicinal properties, Therapeutic potentials, Bioactive components, Mushrooms, Oils.*

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I. Introduction

Mushrooms have been a house hold name in both ancient and modern enriched delicacies to add delightful flavours to food in addition to their numerous nutritional benefits. The consumption of mushrooms is not mainly targeted on its caloric value, but on its medicinal properties. Medicinal mushrooms have established history of use in traditional oriental therapies due to their valuable health benefits. Similar to plants, mushrooms synthesize many chemical constituents that can presently be explored for therapeutic purposes. Antitumor, immunomodulation, antioxidant, radical scavenging, cardiovascular, anti-hypercholesterolemia, antiviral, antibacterial, anti-parasitic, antifungal, detoxification, hepato-protective and anti-diabetic properties are amongst the therapeutic potentials attributed to medicinal mushrooms.[1] Aside their high protein, mineral (iron and calcium) and vitamin contents, mushrooms are generally low in glucose but high in mannitol concentration, which is beneficial to diabetic patients. Their high purine and low sodium concentration make them beneficial against metabolic diseases and high blood pressure.

Only about 2000 of the about 14,000 known species of mushroom are known to be edible. A valid controversy stern from differentiating edible from nonedible mushroom as some of the non-edible mushrooms have striking resemblance with edible ones.[2] Some non-edible mushrooms contain poisonous compounds and the severity of mushroom poisoning may vary depending on the growth conditions, genetic characteristics of the mushroom and the quantity of toxin ingested and absorbed. However, primary processing methods such as boiling, freezing, salting and drying does not significantly alter the toxicity of mushrooms. Variations in clinical

manifestation of mushroom toxicity is a factor of individual's susceptibility and the presence of confounding factors such as contamination and co-ingestion as observed in *Coprinopsis atramentaria*.[3]

Coprinopsis lagopus is a member of the corprinoid specie [4], with a delicate and short-lived fruit body that deliquescence into a black ink within a few hours of its maturity.[5] The process is an autodigestive senescence process enhanced by environmental moisture and humidity. Unlike *C. atramentaria*, whose coprine content makes it synergistically poisonous upon consumption of alcohol, *C. lagopus* is totally a nonpoisonous mushroom[6] and its major bioactive sesquiterpene *"*lagopodin*"* has been report to be a potent antibacterial compound.[7] Davis *et al*., [8], reported *C. lagopus* as been too small to be considered edible. However, its sprout is a traditional celebrated delicacy enjoyed by mostly aged locals of South-Eastern Nigeria. Its preparation entails wrapping a garnished sizable quantity of the sprout in *Alchornea laxiflora* (Okpopia) leaves, cocoyam leaves or plantain leaves and roasting same in a hot ash, mainly generated from palm tree wood. The aim of this present study is to determine the bioactive components in the oil extracted from the sprout and matured *C. lagopus* using GC-MS.

II. Material and Methods

a. *Sample Collection, Preparation and Extraction*

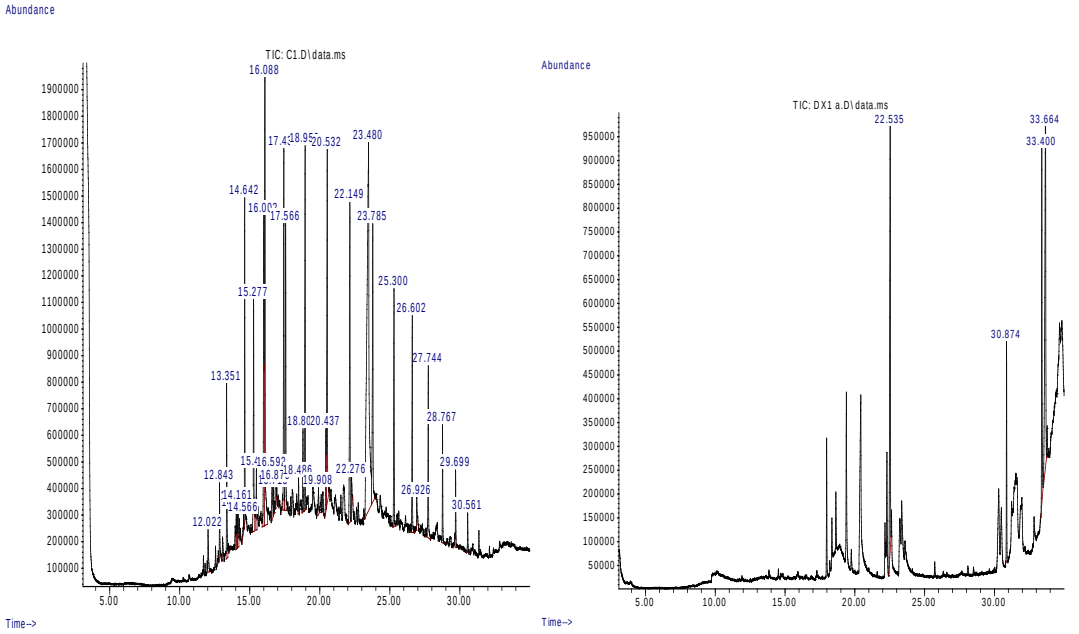
Sprouts and matured *C. lagopus* were harvested from decaying empty palm fruit bunches within the University of Port Harcourt Community and identified at the Plant Science and Biotechnology Department of the University of Port Harcourt. The samples were separated, washed and homogenized immediately into a fine smooth paste using a BLG 450 Binatone electric blender and placed in labeled dry sterile universal sample bottles. A quantity of 40 g of each paste were placed in Whatman no.4 filter paper, staple-sealed and placed into separate Soxhlet extractors, mounted on a dried distillation flask and 50 ml of dichloromethane was introduced into the distillation flask and set up using a retort stand. A continuous jet of cold water was allowed to flow into the condenser and the heated dichloromethane (at 50 ^{o}C) was refluxed. The oily extracts were separated from the solvent and concentrated by evaporation at room temperature.

b. Determination of bioactive components in the oils of sprouts and matured *C. lagopus*

The bioactive components in the oils from the sprouts and matured *C. lagopus* were determined using the procedure described by Ohiri and Bassey [9], where a combined gas chromatograph model HP 6890 and mass spectrometer model 5973 (Agilent Technology) fitted with a capillary column HP-5 MS (5% phenylmethylsiloxane) 30.0 m x 250 µm x 0.25 µm using helium as its carrier gas was used. The initial column temperature was kept at 120°C for 5 minutes and increased at 5°C per minutes to 320°C and held for 5 minutes. A volume of 0.5 ml of each extract was separately diluted with 98% dichloromethane and 2 µl of each diluted sample was automatically injected into Agilent Tech model 5973 mass spectrometer. The compounds present were identified with Chem-Office software attached to the MS library, while their molecular formula, weights and name of the bioactive component were established using the database of National Institute of Standard and Technology.

III. Results

The chromatogram of bioactive components in the oil from the sprout and matured *C. lagopus* are shown in figures 1a and 1b. The highest peaks in the chromatogram of the oil from the sprout (fig. 1a) were observed at retention times of 16.088 mins, 23.480 mins, 18.953 mins, 17.435 mins and 20.480 mins , while retention times of 33.664 mins, 22.535 mins and 33.400 mins had the highest peaks in the chromatogram of bioactive components in the oil from matured *C. lagopus* (fig. 1b).

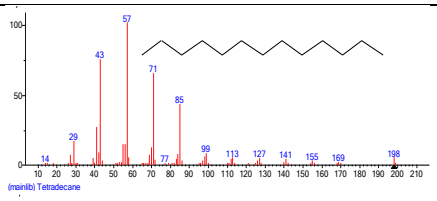
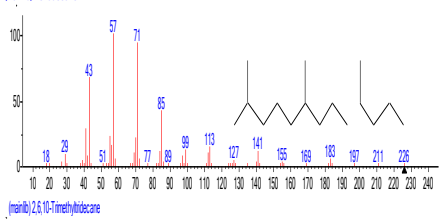
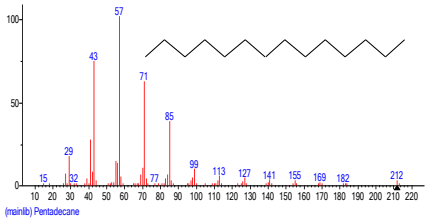


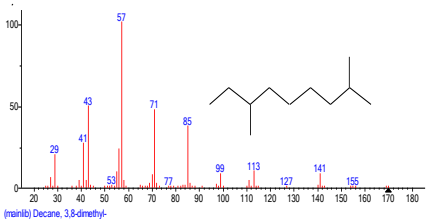
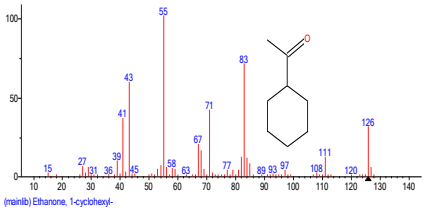
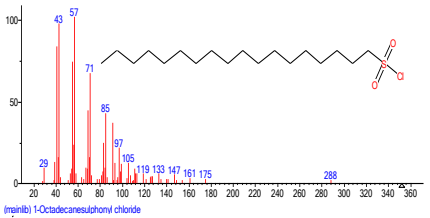
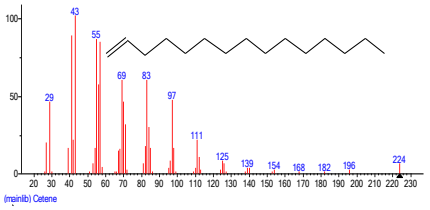
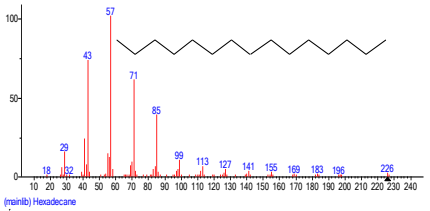
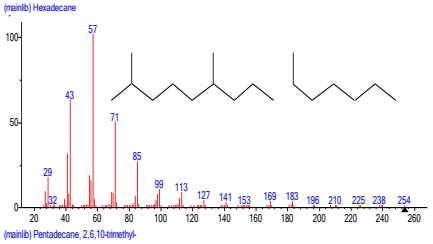
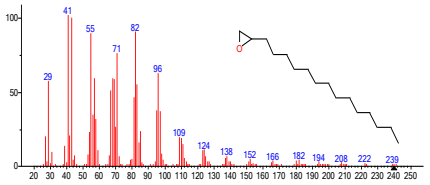
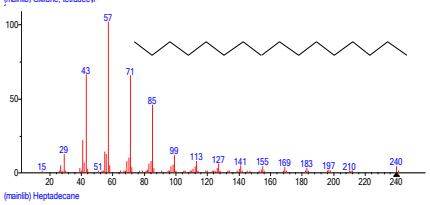
a. b.

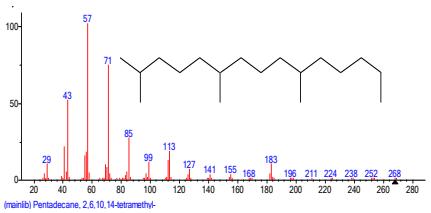
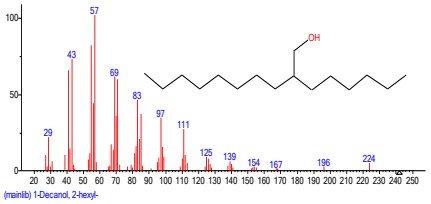
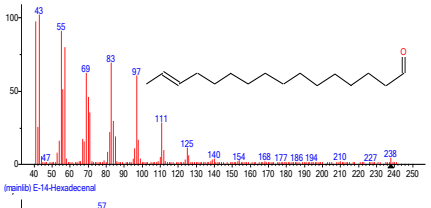
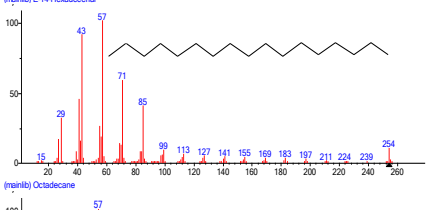
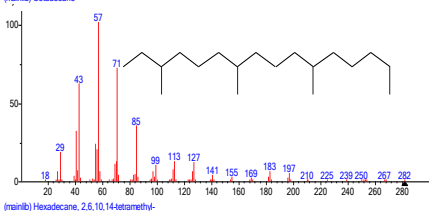
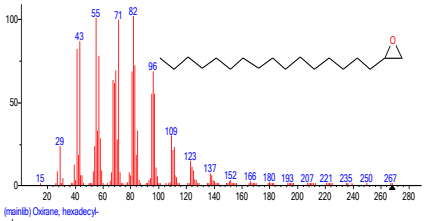
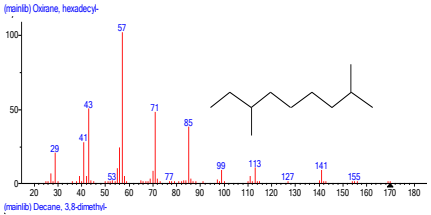
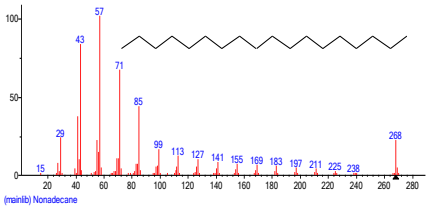
Fig. 1: Chromatogram of bioactive components in the oil from: (a.) Sprouts and (b.) Matured *C. lagopus*

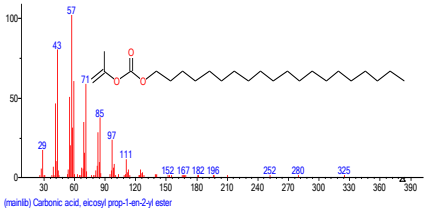
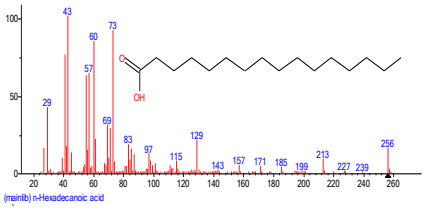
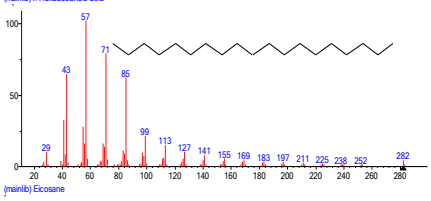
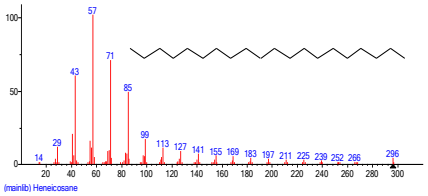
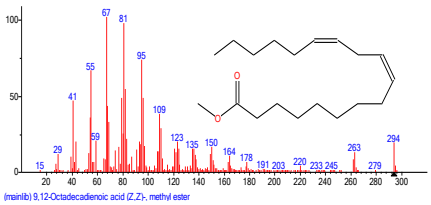
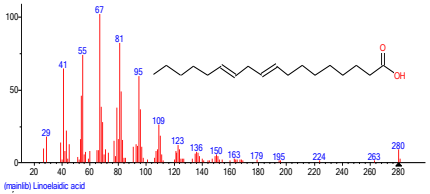
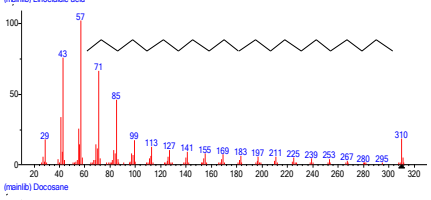
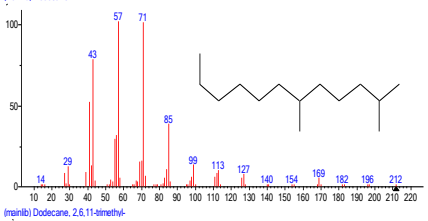
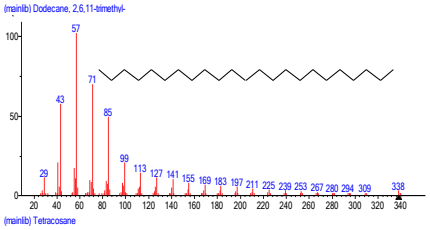
The bioactive components from the GC-MS analyses of oil extracts from sprout and mature *C. lagopus* are presented in tables 1 and 2. Linoelaidic acid ($C_{18}H_{32}O_2$) was predominant amongst the 33 bioactive components in the oil from the sprouts, with a percentage concentration of 20.976, followed by Pentadecane,2,6,10,14-tetramethyl ($C_{19}H_{40}$); Eicosane ($C_{20}H_{42}$); Octadecane ($C_{18}H_{38}$); Nonadecane ($C_{19}H_{40}$); Heptadecane ($C_{17}H_{36}$); Heneicosane ($C_{21}H_{44}$) and Docosane ($C_{22}H_{46}$) with percentage concentrations of 10.622, 6.235, 5.389, 5.338, 5.303, 5.130 and 5.011 respectively.

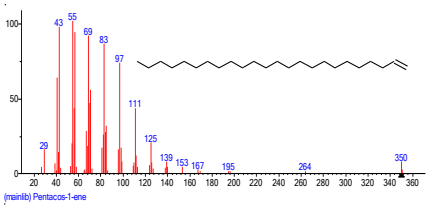
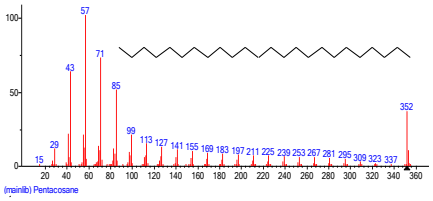
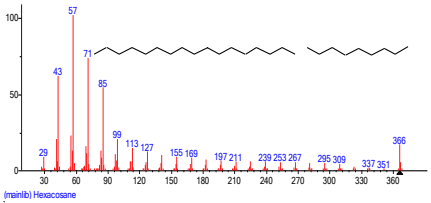
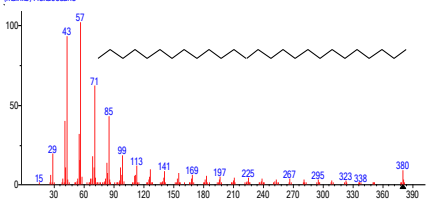
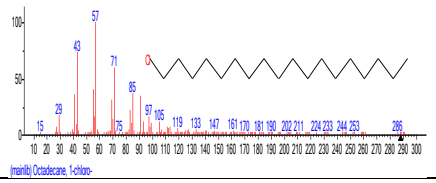
Table 1: Bioactive components in the oil of *C. lagopus* sprouts

S/N	Compound	Retention Time (min)	Percentage concentration	Molecular formula	Molecular weight	Structure
1	Tetradecane	12.022	0.678	$C_{14}H_{30}$	198.3880	
2	2,6,10-Tri methyltridecane	12.843	0.940	$C_{16}H_{34}$	226.4412	
3	Pentadecane	13.351	2.249	$C_{15}H_{32}$	212.4146	

4	Decane, 3,8-dimethyl-	14.036	0.654	$C_{12}H_{26}$	170.3348	
5	Ethanone, 1-cyclohexyl-	14.097	0.471	$C_8H_{14}O$	126.1962	
6	1-Octadecane sulphonyl chloride	14.161	0.857	$C_{18}H_{37}ClO_2S$	353.003	
7	Cetene	14.566	0.266	$C_{16}H_{32}$	224.4253	
8	Hexadecane	14.642	3.433	$C_{16}H_{34}$	226.4412	
9	Pentadecane, 2,6,10-trimethyl-	15.277	3.512	$C_{18}H_{38}$	254.4943	
10	Oxirane, tetradecyl-	15.473	0.920	$C_{16}H_{32}O$	240.4247	
11	Heptadecane	16.002	5.303	$C_{17}H_{36}$	240.4677	

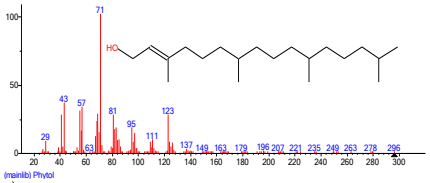
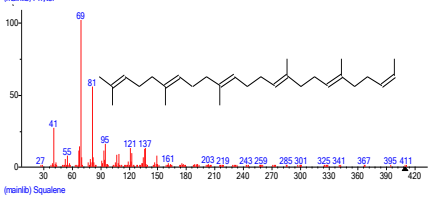
12	Pentadecane, 2,6,10,14-tetramethyl	16.088	10.622	$C_{19}H_{40}$	268.5209	
13	1-Decanol, 2-hexyl-	16.592	0.941	$C_{16}H_{34}O$	242.4406	
14	Carbonic acid, eicosyl vinyl ester	16.713	0.325	$C_{23}H_{44}O_3$	368.5937	
15	E-14-Hexadecenal	16.879	0.185	$C_{16}H_{30}O$	238.4088	
16	Octadecane	17.435	5.389	$C_{18}H_{38}$	254.4943	
17	Hexadecane, 2,6,10,14-tetramethyl-	17.566	4.392	$C_{20}H_{42}$	282.5475	
18	Oxirane, hexadecyl-	18.486	0.466	$C_{18}H_{36}O$	268.4778	
19	Decane, 3,8-dimethyl-	18.802	1.446	$C_{12}H_{26}$	170.3348	
20	Nonadecane	18.953	5.338	$C_{19}H_{40}$	268.5209	

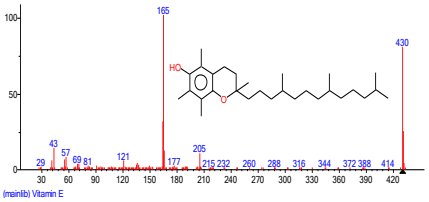
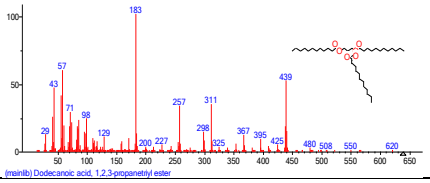
21	Carbonic acid, eicosyl prop-1-en-2-yl ester	19.908	0.459	$C_{24}H_{46}O_3$	382.6202	
22	n-Hexadecanoic acid	20.437	1.316	$C_{16}H_{32}O_2$	256.4241	
23	Eicosane	20.532	6.235	$C_{20}H_{42}$	282.5475	
24	Heneicosane	22.149	5.130	$C_{21}H_{44}$	296.5741	
25	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	22.276	0.421	$C_{19}H_{34}O_2$	294.4721	
26	Linoelaidic acid	23.480	20.976	$C_{18}H_{32}O_2$	280.4455	
27	Docosane	23.785	5.011	$C_{22}H_{46}$	310.6006	
28	Dodecane, 2,6,11-trimethyl-	25.300	3.521	$C_{15}H_{32}$	212.4146	
29	Tetracosane	26.602	2.844	$C_{24}H_{50}$	338.6538	

30	Pentacos-1-ene	26.926	0.339	$C_{25}H_{50}$	350.6645	
31	Pentacosane	27.744	2.486	$C_{25}H_{52}$	352.6804	
32	Hexacosane	28.767	1.457	$C_{26}H_{54}$	366.7070	
33	Heptacosane	29.699	0.892	$C_{27}H_{56}$	380.7335	
34	Heptacosane, 1-chloro-	30.561	0.525	$C_{27}H_{55}Cl$	415.179	

Amongst the 4 bioactive components observed in the GC-MS analyses of the oil from matured *C. lagopus*, Dodecanoic acid, 1,2,3-propanetriyl ester ($C_{39}H_{74}O_6$) was predominant with a percentage concentration of 37.228, followed by Phytol ($C_{20}H_{40}O$), Vitamin E ($C_{29}H_{50}O_2$) and Squalene ($C_{30}H_{50}$) with percentage concentrations of 34.681, 18.786 and 9.305 respectively.

Table 2: Bioactive components in the oil of matured *C. lagopus*

S/N	Compound	Retention Time (min)	Percentage concentration	Molecular formula	Molecular weight	Structure
1	Phytol	22.535	34.681	$C_{20}H_{40}O$	296.539	
2	Squalene	30.874	9.305	$C_{30}H_{50}$	410.718	

3	Vitamin E	33.400	18.786	$C_{29}H_{50}O_2$	430.7100	
4	Dodecanoic acid, 1,2,3-propanetriyl ester	33.664	37.228	$C_{39}H_{74}O_6$	639.0013	

IV. Discussion

The high concentration Linolelaidic acid in the sprout of this mushroom may one of the reseons why the sprout of this mushroom is consumed. Linolelaidic acid (*trans*,*trans*-9,12-Octadecadienoic acid) is an omega-6 trans fatty acid and a cis-trans isomer of linoleic acid. Linoleic acid can be converted to arachidonic acid in vivo in healthy adult humans.[10] Aside serving as a precursor to prostaglandins, Arachidonic acid has been reported to modulate the functions of several receptor proteins, ion channels and enzymes through both activation and inhibition processes.[11]

Pentadecane, 2,6,10,14-tetramethyl present in the sprout of this mushroom not only confers antioxidant potentials to the mushroom. It also suppresses the severity of IRBP-induced inflammation of the inner eye (uveitis). [12] This was demonstrated by the decrease in vasculitis and inflammatory foci in fundus and by a reduction in histological damages and leukocyte infiltration.[12] This observed protective effect of Pentadecane, 2,6,10,14-tetramethyl is associated with a decreased in the activation of peripheral CD4+ and CD8+ T lymphocytes and a decrease in the intensity of the Th1 and Th17 autoimmune response to IRBP.[12] The high concentration of Eicosane also observed in the sprout of this mushroom shows its potential to enhance both topical and abdominal wound healing. The administration of eicosane, naringin, and octacosane to injured diabetic animals has been reported to promote faster healing due to their potent hydroxyproline and glutathione (antioxidant) actions and free radical scavenging ability. [13]

Though n-octadecane has enjoyed industrial relevance as solvent, lubricant, transformer oil, anti-corrosion agents and thermal storage material.[14] Its presence in the sprout of this mushroom may be as a result of its role in chemical signaling as a repellant pheromone has been reported, [15] thus, n-octadecane may aid in the protection of the sprout of this mushroom during its growth period, while n-Nonadecane in the sprout of *C. lagopus* may be responsible for improving its palatability and edibility by enhancing its aroma. n-Nonadecane has been in use as a natural fragrance in both cosmetics and perfumes.[16]

The consumption of the sprout of this mushroom may also be attributed to its heptadecane content. The ability of heptadecane to almost completely block the *de novo* synthesis of fatty acids *in vitro*, [17] may be useful in the prevention of excess fatty acid production and accumulation in the adiposes. This indicates that the sprout of this mushroom may be relevant in the management of excess weight gain. Heptadecane has also been reported to have strong antioxidative effects, which has been proven to be beneficial to the management of renal disease in animal models.[18] Its ability to inhibits the proliferation of human liver cancer cells has also been reported.[19] Heptadecane exerts its anti-inflammatory effect in aged kidney tissues by suppressing age-related increases in pro-inflammatory gene expressions and subsequent reduction of NF-kB activity.[20] The high concentration of Heneicosane in the sprout of this mushroom may be as a defensive shield against microbial attack aimed at allowing sprout maturity and spore formation. Heneicosane has been reported to exhibit excellent antimicrobial activity against *Streptococcus pneumoniae* and *Aspergillus fumigatus* even at moderate concentrations.[21]

The high concentration of Dodecanoic acid, 1,2,3-propanetriyl ester observed in matured *C. lagopus* indicates that the mature mushroom also has some medicinal properties unknown to the local consumers. Therapeutically, Dodecanoic acid, 1,2,3-propanetriyl ester has shown relevant potentials like anti-oxidant, antibacterial, antiviral, antiarthritic hypocholestrolemic and hepatoprotective activities.[22][23][24] Its potentials as a candidicide has also been reported. [24] Industrially, Dodecanoic acid, 1,2,3-propanetriyl ester has enjoyed wide usage as a skin-care additive for skin-conditioning in the production of human body creams. It is also responsible for distracting host-seeking behavior of mosquitoes,[25] thereby acting as a mosquito repellant.

Phytol is an acyclic hydrogenated diterpene alcohol used mainly as a precursor for the production of synthetic forms Vitamins E and K.[26] [27] It is mainly gotten from the hydrolysis of Chlorophyll a, b, d, and f. [28] Therapeutically, pytol has been reported to possess metabolism-modulating, cytotoxic, antioxidant, autophagy and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects.[29] Fermentation of ingested plant materials mainly in ruminants leads to the production of phytol and subsequent

conversion to phytanic acid, which is stored in the adipose tissues.[30] Though the generation of phytanic acid from chlorophyll is impossible in humans, a direct conversion of free phytol to phytanic acid normally takes place. The high concentration of phytol in the matured mushroom may be as a chemical deterrent against insect predators. This is in consonant with the work of Vencl and Morton,[31] which reported the use of phytol and its metabolite (phytanic acid) by some insects as chemical deterrents against predators. High phytol concentration in humans is also responsible for adult Refsum disease, which is an autosomal recessive disorder resulting from the accumulation of phytanic acid in tissues.[32] It clinically manifests as a variable combination of peripheral polyneuropathy, cerebellar ataxia, retinitis pigmentosa, anosmia and hearing loss.[32] This indicates that high concentration of phytol observed in this study is responsible for the avoidance and non-consumption of matured *C. lagopus* mushroom by the locals.

The high concentration of Vitamin E observed in the matured mushroom may be as a protective component against reactive oxygen species targeted at extending the lifespan of this mushroom. Vitamin E is a potent chain-breaking antioxidant that inhibits reactive oxygen species production from free radicals generated through oxidation of fatty acids.[33] However, the absence of Vitamin C in this mushroom must have suppressed the effectiveness of vitamin E to protect this mushroom from its fast autodigestive process. This agrees with the work of Rizvi *et al*.,[34] which reported that optimal effectiveness of vitamin E strongly depends on the availability of vitamin C, vitamin B3, selenium and glutathione. Vitanim E has been reported to have a wide array of therapeutic properties ranging from its ability to prevent and reverse of various disease complications due to its ability function as an antioxidant, anti-inflammatory compound, inhibition of platelet aggregation and its immune-enhancing potentials.[34] These potentials make vitamin E a reliable compound in the management of cancer, ageing, arthritis, cataracts and cardiovascular infractions. However, Howard *et al*.,[35] reported that a combined mixture of different forms of tocopherols poses a stronger inhibitory effect on lipid peroxidation induced in the erythrocytes of humans as compared to alpha-tocopherol alone.

Squalene is a polyunsaturated hydrocarbon, which gained prominence after its characterization in shark liver oil It is considered an important compound because of its nutraceutical and pharmaceutical potentials.[36] Amongst the scientifically determined bioactivities of squalene in both animal models and in-vitro studies are anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient activities.[36]

V. Conclusion

Moderate and non-toxic concentrations of verse bioactive components in the sprout of this mushroom maybe responsible for its preferred consumption, while the high concentration of phytol and its possible accumulation causes the avoidance of the matured mushroom. The finding of the research indicates that the therapeutic properties of this mushroom is not only dependent on its lagopodin content. The presence of other bioactive components as observed in the oils of the sprout and matured mushrooms also contributes to the medicinal relevance of this mushroom. Isolation and purification of these bioactive components may be an importance source of active compounds for production of novel pharmaceuticals.

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