

Short Communication

***Amanita cokeri* (a Poisonous Suspect Mushroom) Contains Chemical Compounds of Industrial Importance**Mobolaji Adenike Titilawo^{1*}, Temitope Fasunloye Ajani¹, Oladapo Mojisola Aderonke²¹Department of Microbiology, Osun State University, Osogbo, Osun State, Nigeria²Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria**Abstract**

Globally, little research effort is devoted to investigating the potential benefits of toxic or poisonous suspect mushrooms. In this study, we profiled *Amanita cokeri* metabolites using GC-MS to identify compounds of industrial value. The mushroom was collected at a forest reserve in Ilesa, Southwest Nigeria, and identified by comparing it with literature. Extraction of the metabolite from *A. cokeri* was done using methanol and characterized using GC-MS. Compound identification was achieved using the NIST 11 mass spectral library. Morphologically, the mushroom was identified as *A. cokeri*. GC-MS profile revealed that *A. cokeri* metabolite contained 20 fractions belonging to three functional groups namely, terpene, fatty acid, and its derivatives and sterol. Cis-9-hexadecenal was the most abundant compound (45.07%). 1-(+)-Ascorbic acid 2,6-dihexadecanoate and D-limonene were noted for the first time in the mushroom. This study provided background information into the chemical composition of *A. cokeri* metabolite for possible exploration and exploitation for humankind.

Keywords: *Amanita cokeri*, substrate, metabolite, Gas chromatography-mass spectrometry, compound identification

Резюме

В световен мащаб малко изследователски усилия са посветени на проучването на потенциалните ползи от токсични или съмнително отровни гъби. Морфологично обекта на изследване е идентифициран чрез сравняване с литературата като *Amanita cokeri*. Гъбата е събрана в горски резерват в Илеса, югозападна Нигерия. Чрез GC-MS е установен метаболитния профил на *A. cokeri*, за да се идентифицират съединения с индустриална стойност. Екстракцията на метаболита от *A. cokeri* се извършва с помощта на метанол. Идентифицирането на съединението е постигнато с помощта на NIST 11 маспектрална библиотека. GC-MS профил доказва, че метаболитът на *A. cokeri* съдържа 20 фракции, принадлежащи към три функционални групи, а именно терпен, мастна киселина и нейните производни и стерол. Цис-9-хексадеценалът е най-разпространеното съединение (45.07%). Аскорбинова киселина 2,6-дихексадеканоеат и D-лимонен са установени за първи път в гъби. Това проучване предостави основна информация за химичния състав на метаболита от *A. cokeri* за изследване и възможно използване.

Introduction

Mushrooms constitute the earliest form of fungi known to humankind (Okhuoya *et al.*, 2010). In the natural environment, they exist as conspicuous fruiting bodies on soils or lignocellulose-rich substrates (Adeniyi *et al.*, 2018a, b), and offer diverse advantages as a food source, nutraceutical, drugs development, and economic empowerment (Titilawo *et al.*, 2020). At the time of this study, the

literature reveals that research efforts focused more on the exploration of edible mushrooms for numerous human benefits, with little or no attention on toxic species. This may be due to poisonous macrofungi possessing neurotoxic capability, triggering hallucinations, nervousness, bronchospasm, confusion, nausea, abdominal pain, diarrhea, nausea vomiting, altered sense of time and space, and even

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death (Fiedziukiewicz, 2013).

The genus *Amanita* is widely known to be toxic, although some edible species exist within the group (Codja *et al.*, 2020). Globally, it is estimated that 900 - 1000 *Amanita* species exist, with only about 500 currently described (Vargas *et al.*, 2017). In Africa, especially Nigeria, mycodiversity is underexplored and many *Amanita* species remain unidentified (Fraiture *et al.*, 2019). Previous works on *Amanita* focused on the identification of species including *A. phalloides* (Oso, 1985), *A. caesarea* and *Amanita* sp. (Adejumo *et al.*, 2015), *A. muscaria*, *A. novinupta* and *A. rubescens* (Ali *et al.*, 2018) and *A. caesarea* (Keta *et al.*, 2019) from different states in the country.

Amanita cokeri, a poisonous suspect mushroom has been earlier reported. In North California, Drehmel and Chilton (2002) profiled amino acids in *A. cokeri* using thin-layer chromatography while Adeniyi *et al.* (2018a) and Titilawo *et al.* (2020) reported the occurrence, and proximate, vitamins and elemental composition of *A. cokeri* from ENPOST forest reserve, Ilesa, Southwestern region of Nigeria. To unveil the possible industrial importance of this species, the present study investigated the chemical composition of *A. cokeri* metabolite using gas-chromatography mass spectrometry.

Materials and Methods

Sample collection and phenotypic identification of mushroom

The mushroom fruitbody was obtained from ENPOST forest reserve in Ilesa, Osun State, Nigeria (Latitude 4°42'30"E to 4°42'45"E longitude 7°36'55"N to 7°37'10"N) in 2019. The sample was carefully uprooted from the soil with a sterilized hand trowel, placed in a paper bag, and transported immediately to the laboratory for proper morphological description. Cap morphology including size, colour, shape, margin, the shape of margin; the stem length and diameter, location, shape, colour, surface texture, texture; and gill shape, attachment, colour were observed and documented. In the months the mushroom was observed, its habitat, substrate, and habit were noted and recorded. Identification was done by comparing described morphology with published literature (Odeyemi and Adeniyi, 2015).

Preparation and extraction of the metabolite from the mushroom sample

The sample was air-dried and ground into powder using an electronic grinder. Extraction was done according to Gonmori and Yoshioka (2005).

A 100 mg aliquot of the mushroom powder was mixed with 9 ml methanol, extracted by sonication for 120 min, and then filtered through Whatman filter paper No 1 to remove any particulate matter. The filtrate was analyzed using gas chromatography-mass spectrometry (Shimadzu, QP-2010 Ultra, Japan).

Gas chromatography-Mass spectrometry (GC-MS) instrumentation and conditions

The GC was fitted with a DB-5MS capillary column (30 m x 0.25 µm, film thickness 0.25 µm). The injection temperature was 250°C with an inlet pressure of 29.2 kPa. The column flow rate was 0.6 ml/min (total flow rate 4.4 ml/min) with a linear velocity of 30 cm/sec. Purge flow was 3 ml/minute. Injections were performed in splitless mode, with a ratio of 1.0. The column oven temperature was set at 60°C then increased at a rate of 10°C/min to 300°C and held for 16 min. Ion source and interface temperatures were 230 and 280°C with a solvent cut time of 3.50 min. All mass spectra were acquired in electron impact mode. The scanned mass ranged from 50 to 700 m/z with a scan speed of 2500 scan/sec and an event time of 0.30 sec. Analysis was performed in full scan mode with an injection volume of exactly 1 µl.

Identification of the chemical compounds present in the extracted metabolite

The compounds were identified using the National Institute of Standard Technology (NIST) 11 mass spectral library. The compounds present in the extracted metabolites were identified by comparing the spectra with those of the reference compound in NIST 11 mass spectral library.

Results

A. cokeri was found growing on soil debris under tree canopies and exhibiting scattered habitats over three months, from March to May 2019. The mushroom possesses a large cap ranging from 7.8 to 15 cm, yellow and white in colour, fleshy, with sticky brown and yellow scales. Interestingly, the cap displayed different morphology within its 4 days lifespan. On appearance in the field, the cap is parabolic in shape and alternates between convex and plane as it matures. The margin is appendiculate and the shape of the margin is either straight or uplifted. The dimension of the stem is 149 to 182 mm x 22 to 28 mm, located centrally, in equal shape, and white in colour. The surface is scabrous, and the texture is firm. The spore-bearing surface is gill having an unequal shape and the attachment is adnex. The gill is white but changes to yellow

Table 1. Morphological description of *A. cokeri*

Month of growth		March to May, 2019
Cap	Size	7.8 to 15 cm
	Colour	Yellow and white in colour, fleshy, with sticky brown and yellow scales
	Shape	Parabolic in shape and alternate between convex and plane as it matures
	Margin	Appendiculate
	Shape of margin	Straight or uplift
Stipe	Length / diameter	149 to 182 mm / 22 to 28 mm
	Location	Central
	Shape	Equal
	Colour	White
	Surface	Scabrous
	Texture	Firm
Gill	Shape	Unequal
	Attachment	Adnex
	Colour	White but changes to yellow after harvest
Habitat / substrate		Terrestrial / soil debris
Habit		Scattered
Odour		Mild
Edibility		Poisonous / suspect
Presumptive identification		<i>A. cokeri</i>

after harvest (Table 1). The physical appearance of the mushroom suggests its poisonous or a suspect. Phenotypically, it was identified as *A. cokeri*. The picture of the mushroom is in Fig. 1.

The GC-MS profile showed that *A. cokeri* metabolite contained 20 chemical constituents. The retention time of the fractions was between 5.978 and 28.874 min. The percentage area of the com-

ponents was between 0.32 to 45.07%. The identified compounds belong to three functional groups namely, terpene, fatty acids, its esters, aldehyde and amide, and sterol (Table 2). Amongst these, fatty acid derivatives *cis*-9-hexadecenal and 1-(+)-ascorbic acid 2,6-dihexadecanoate, had a relative abundance of 45.07% and 14.86% respectively, followed by terpene (D-limonene), 12.86%. Fractions hav-

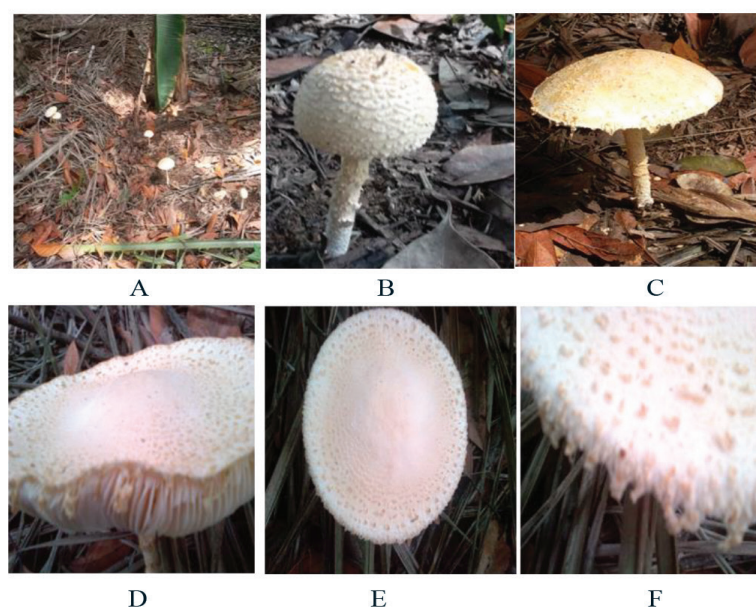


Fig. 1. *A. cokeri* (A) wide view of sample growing on soil debris; (B) lateral view of young sample; (C) lateral view of matured sample with convex cap shape; (D) lateral view of matured sample with plane cap shape and uplift margin; (E) top view; (F) cap margin.

Table 2. Constituents of *A. cokeri* metabolite

Peak	R.T (Min)	Area (%)	Chemical constituents	Functional groups
1	5.978	12.86	D-Limonene	Terpenes
2	15.354	0.41	Myristic acid	Fatty acid
3	16.415	0.32	Pentadecanoic acid	Fatty acid
4	17.070	0.39	Palmitic acid, methyl ester	Fatty acid ester
5	17.269	1.20	Palmitoleic acid	Fatty acid
6	17.549	14.86	1-(+)-Ascorbic acid 2,6-dihexadecanoate	Fatty acid ester
7	17.620	0.66	2,5-Di-benzyloxy-nitrobenzene	Aromatic nitro compound
8	17.740	0.38	Palmitic acid, ethyl ester	Fatty acid ester
9	18.312	0.72	Cis-10-heptadecenoic acid	Fatty acid
10	18.718	0.89	Linoleic acid, methyl ester	Fatty acid ester
11	18.776	1.67	Oleic acid, methyl ester	Fatty acid ester
12	19.338	45.07	Cis-9-hexadecenal	Fatty aldehyde
13	19.397	7.73	Ethyl oleate	Fatty acid ester
14	19.477	6.32	Stearic acid	Fatty acid
15	19.640	1.36	Oleamide	Fatty acid amide
16	21.075	0.42	8,11-Octadecadienoic acid, methyl ester	Fatty acid ester
17	21.121	0.82	Nonadecanamide	Fatty acid amide
18	26.124	0.48	9(11)-Dehydroergosteryl benzoate	Sterol
19	27.644	0.86	Cyclohexanecarboxylic acid, 2-phenylethyl ester	Acid ester
20	28.874	2.59	Ergosterol	Sterol

ing a percentage area between 1 and 9% were ethyl oleate (7.73%)>stearic acid (6.32%)>ergosterol (2.59%)>oleic acid, methyl ester (1.67%)>oleamide (1.36%)>palmitoleic acid (1.20%). Other components had an area >1% (Table 2). The chromatogram showing the peak of each component is in

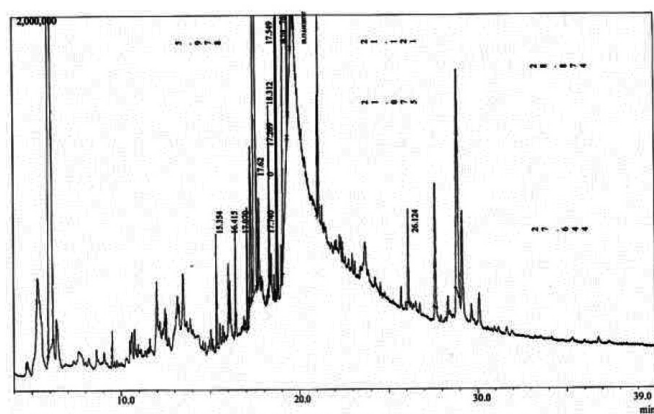
**Fig. 2.**

Fig. 2. Gas chromatography–mass spectrometry (GC-MS) spectrum of *A. cokeri* methanolic extract

Discussion

In the wild, the availability and suitability of substrate are crucial for the development and growth of mushrooms (Adeniyi *et al.*, 2018a).

This study reveals that *A. cokeri* grows on soil debris with possible mutualistic association with the roots of trees, with the tree canopy providing a cool environment that supported its development. This concurs with Sanmee *et al.* (2008) who stated that *Amanita* species are ectomycorrhizal fungi.

Gas chromatography-mass spectrometry is an ideal technique for both quantitative and qualitative analyses of volatile and semi-volatile compounds (Mensah-Agyei *et al.*, 2020). The current study identified 20 major compounds in *A. cokeri* metabolite (Table 2). This agrees with Wekesa *et al.* (2016) who also identified 20 compounds from *P. ostreatus*. On the other hand, Chaudhary and Tripathy (2015) reported 14 compounds from *Irpex lacteus* while Petrova *et al.* (2007) noted 34 and 44 compounds from *Agaricus pseudopratinensis* and *Agaricus placomyces* respectively. Solvent type plays a key role in the number of compounds that will be obtained following extraction (Mensah-Agyei *et al.*, 2020).

Our findings showed cis-9-hexadecenal as the most abundant compound having 45.07% of the total constituent (Table 2). The dominance of cis-9-hexadecenal (23.44%) was earlier reported in ethanolic extract of *Daedalea elegans* (Men-

sah-Agyei *et al.*, 2020). Other works observed dimethylbenzene (32.803%) in *P. ostreatus* (Wekesa *et al.*, 2016), and phenol, 80%, and 40% in *A. pseudoprattensis* and *A. placomyces* respectively (Petrova *et al.*, 2007). Cis-9-hexadecenal is categorized as an agrochemical of the attractant category used as a pesticide for preventing, destroying, or mitigating pests (Mensah-Agyei *et al.*, 2020). The other main compounds, L- (+)-ascorbic acid 2,6-dihexadecanoate and D-limonene, are rarely reported in mushrooms. Whereas L-(+)-ascorbic acid 2,6-dihexadecanoate earlier identified in oil fraction from the stem barks of *Dacryodes edulis* possesses antioxidant, anti-inflammatory, and anti-nociceptive properties (Okwu and Ighodaro, 2009), D-limonene, a major constituent in several citrus oils (orange, lemon, mandarin, lime, and grapefruit) is used as a flavouring agent and considered to have low toxicity and chemopreventive activity against many types of cancer (Sun, 2007).

Fatty acids and their derivatives are a major component of volatile fractions of mushrooms (Petrova *et al.*, 2007). The current study revealed the presence of 15 fatty acids and their esters, amides and aldehyde (Table 2), some of which are reported elsewhere (Afieroho and Ugoeze, 2014). According to Petrova *et al.* (2007), ethyl esters in the mushroom extract may be artifacts. Nevertheless, fatty acid and their esters are responsible for the characteristic aroma of mushrooms (Wekesa *et al.*, 2016) and possess medicinal properties (Phan *et al.*, 2013).

Fungisterol is a vital component of mushroom cell membranes (Joffrion and Cushion, 2010). In this study, ergosterol was more (2.59%) than 9(11)-dehydroergosteryl benzoate (0.48%) (Table 2). In another study, 17.91% ergosterol was noted in *Lentinus tuber-regium* (Afieroho and Ugoeze, 2014). Ergosterol stimulates the growth and development of fungi and is also significant in fungal adaptation to stress. It acts as a biological precursor of vitamin D, and steroid hormone drugs and exhibits a level of antitumor and anti-HIV properties (Huang *et al.*, 2016). Similarly, 9(11)- dehydroergosteryl benzoate is used as a food preservative, manufacture of benzoates and benzoyl compounds dyes, in calico printing, for curing tobacco, plasticizers, benzoyl chloride, alkyd resins, seasonings, flavours, perfumes, dentifrices, and standard in analytical chemistry (O'Neil, 2013).

Conclusion

GC-MS profiling of the *A. cokeri* metabolite revealed 20 chemical fractions with cis-9-hexade-

cenal being the most abundant. The present study noted L-(+)-ascorbic acid 2,6-dihexadecanoate and D-limonene for the first time in mushrooms. Although *A. cokeri* is a poisonous suspect, its methanol extract contains fractions having potential usefulness in the food, pharmaceutical, medical, and industrial sector. Further investigation on extraction, toxicity, and application of the mushroom fractions is highly recommended.

Acknowledgments

The authors appreciate the management of ENPOST farm, Ilesa, Southwest Nigeria for granting access to collect the mushroom sample for this study.

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