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GC-MS PROFILING AND PATHOGENIC EFFECT OF KHAYA ANTHOTHECA METHANOLIC LEAVES EXTRACT ON SOME SELECTED FUNGI

Isaac John Umaru[1], Philip Shadrach[2], Godwin Baaku Adi[3], Egeonu Stephen Ugoeze[4], Chakfa Nanmar[5], Akafa Andes Tansaba[6], Kerenhappuch Isaac Umaru[7], and Bando Christopher David[8]

[1,2,3,4,5,6]Federal University Wukari, Taraba State, Nigeria
[7]University of Higher Institute Buea, South West Cameroon, Cameroon
[8]National Biotechnology Research and Development Agency, Jalingo, Nigeria
umaruisaac@gmail.com

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Abstract

This research evaluated GC-MS profile, and pathogenic effect of methanolic leaves extracts of Khaya anthotheca on Some Selected fungi. Khaya anthotheca a member of Meliaceae family is traditionally used for treating several ailments. The leaves were harvested in Sukundi road Wukari and air dried for four weeks, the leaves were pulverized into powder using manual blender and stored in an air tight container. Extraction was done with absolute methanol; the extract was filtered using mesh and the filtrate was concentrated at 68oC. Only two grams of the filtrate were used for the GC-MS study. One-way analysis of variance (ANOVA) was used for statistical analysis. The GC-MS result revealed 50 constituents. Result of Methanolic extracts of leaves of

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Khaya anthotheca only showed significant activity against Aspergillus niger at dose100 μg/mL, Candida tropicalis and Fusarium oxysporium at dose 500 μg/mL. This study supports the use of Khaya anthotheca by indigenous herbalists by providing scientific proof of the plant's ability to treat pothegenic diseases. To establish the effectiveness of this plant in the management of fungal effections, more clinical trials at the clinical levels are needed.

Keywords: Antifungal, Khaya Anthotheca, Phytochemicals, Medicinal

INTRODUCTION

The rising prevalence of pathogenic microorganisms which are resistant to the modern antimicrobial drugs that have been produced in the last three decades [1, 4, 5] This has been a treat to health as a result of the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized [20]. Among the pathogenic microorganism fungal diseases is one of the most threatening and major cause of morbidity and mortality worldwide. [2] As a result of the number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antifungal and immunosuppressive agents [3, 4]. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine. [5, 7]

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [8]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. While some of these raw drugs are collected in small quantities by the local communities and traditional healers for local use [19]. Considering the vast potentiality of plant as sources for antimicrobial drugs with reference to antifungal agents, an investigation as regard the plant Khaya anthotheca was undertaken to screen the local flora for its antifungal activity. This situation provided the impetus to the search for new antifungal substances from various sources like Khaya anthotheca. Traditional medicine

has made use of many different plant extracts for treatment of fungal infections and some of these have been tested for in vitro antifungal activity [6].



Figure 1: *Khaya anthotheca* leaf

Khaya anthotheca is a member of the family Meliaceae. Six species make up this little genus; two are found in Madagascar and the Comores and four are found in tropical Africa [8]. The paripinnate leaves and mostly spherical, 4-5 valved, dehiscent woody capsules make the genus easily identifiable [8]. In addition to being frequently used for window frames, paneling, doors and stair cases, the wood is highly prized for use in furniture, cabinetry, veneer and decorative boxes and cases. It works well for light flooring, automotive bodies, musical instruments, sporting goods, toys, plywood, pulpwood, shipbuilding and carving [9].

Research also demonstrated that Khaya anthotheca possesses neuroprotective action [10]. Some important bioactive constituents such as gedunin and limonoids which are of great benefits have been reported to be present in this plant [11]. Yacine et al.[12] also reported the presence of phytochemicals such as sterols and polyterpenes, flavonoids, polyphenols, saponins and tannins in the plant. Elegant research also reported that K. anthotheca possesses high antioxidant and antiradical properties due to the presence of bioactive

constituents such as polyphenols, polyterpenes and in addition to tannins and saponins13. Studies also documented that K. anthotheca possesses antimalarial [7], anti-protozoal [14], anticancer [15] and neuroprotective [16] activities. However, reports on the anti-ulcerogenic and anti-bacterial efficacy of plants are rather rare or speculative. This study therefore evaluates the anti-ulcerogenic and anti-bacterial activities of methanolic crude extract of Khaya anthotheca root on indomethacin treated albino rats

METHODS

Materials

Mortar and pistle, beakers, Conical flask, Spatula, Filter paper (Whatman No.1), Masking tape, Hand gloves, Cotton wool, face mask, Separating funnel, Digital analytical weighing balance (OHAUS: PA-4120), Refrigerator (HTF-146F), Thermostatic Water Cabinet (MODEL: HH-W420), Micro pipette, Test tubes and Test tube racks, and sample bottles, Glucometer (Accu chek)

Chemicals and Reagents

Methanol, distilled water, 75% of alcohol, were purchased from Justlab Nigeria Limited 140 Isolo road Egbe - Okotun, Lagos - Nigeria. All chemicals used were of highest quality.

Plant collection and identification

The *Khaya anthotheca* Leaves was rinsed under running water and air dried. To prevent ultraviolet light from eliminating the active ingredients, the leaves were air-dried under shade [18,19]. Using a mortar and pestle, the dried leaves were ground into a fine powder and kept in an airtight container until required.

Determination of % yield of extract

The percentage yield of the extract of *Khaya anthotheca* was determined by weighing the coarse sample before extraction and the *Khaya anthotheca* ethanol leaves extract after concentration and then calculated using the formula.

GC- MS phytochemial profiling

The GC-MS analysis was performed in a 7890A gas chromatograph that also functions as a mass spectrometer (Agilent 19091-433HP, USA). It is equipped with a triple axis detector and an inert MSD 5675c infection as well as an HP-5 MS fused silica column (5% phenyl

methyl siloxane, 30.0m 250m). Helium gas was set to flow at a rate of 1.0 ml per minute as a carrier gas. Other GC-MS conditions include a 1 μL injector in split mode with a split ratio of 1:50 and an injection temperature of upto 300 °C. Ion source temperature is 250 °C, interface temperature is 300 °C, pressure is 16.2 psi, and out time is 1.8 mm. Over the course of five minutes, the column's temperature rose from 36°C to 150°C at a rate of 4°Cmin-1. At a rate of 20°Cmin-1, the temperature increased to 250°C and maintained there for 5 minutes. Elution lasted for 47.5 minutes in total. By comparing each component's average peak area to the sum of all areas, the corresponding percentage quantity of each component was computed. The supplier's MS solution software was utilized to manage the system and gather data**.** Mass Hunter Library NIST17 was used in comparing and identifying the constituents.

Antifungal potential

The antifungal potential of the plant extract was performed by agar disc diffusion method as reported by [20, 21].

Fungal preparation

The fungi were standardized by extracting fungal spores from a 7-day-old culture on SDA slant and introducing a normal saline solution that was sterile with a 48-hour pure culture. Turbidity was adjusted to match 0.5 McFarland stand standards. With the help of sterilized glass beads, ten milliliters of pure normal saline containing 3% w/v Tween 80 was employed to distribute the spores. A UV spectrophotometer (Spectronic 20D; Milton Roy Company, Pacisa, Madrid, Spain) set to a transmittance of 70–72% of the suspensions was used to standardize the spore suspension to 1.0106 spores/mL. The prepared plates were allowed to incubate for 24 hours at 37°C [22].

RESULTS AND DISCUSSION

Phytochemical constituents of leaves of Khaya anthotheca methanol crude extract

Table 1: Phytochemical profile of *leaves Khaya anthotheca* methanol crude extract

S/N	Name	R.Time	Area	Height
1	Dodecane	15.326	3444740	1271238
2	Tetradecane	18.304	150967299	16523371
3	Undecanal	18.600	2087082	894718
4	1-Undecanol	20.355	2595232	841001
5	Tetradecane	20.848	4828945	1819690
6	Dodecanal	21.378	77701897	15172477
7	(E)-.beta.-Famesene	22.304	1629324	613674
8	1-Dodecanol	23.007	46136232	10172333
9	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-meth	23.182	8868440	3176963
10	Heneicosane	23.444	51540066	13142990
11	.alpha.-Farnesene	23.643	2003129	791455
12	.beta.-Bisabolene	23.825	2834162	1117573
13	Tridecanal	23.936	61459079	13774706
14	(1S,5S)-4-Methylene-1-((R)-6-methylhept-5-	24.263	1923373	697160
15	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (	25.098	5000845	1357406
16	1-Tetradecanol	25.594	58505167	9246835
17	Heneicosane	25.815	2017498	509524
18	Caryophyllene oxide	25.923	7120718	1860590
19	Tetradecanoic acid, 2-oxo-, methyl ester	26.215	1502359	334904
20	Tetradecanal	26.678	416219832	22244814
21	Neointermedeol	26.986	12224325	1199765
22	cis-1-Chloro-9-octadecene	27.861	114555022	13733228
23	Heneicosane	28.140	42557088	6691262
24	Pentadecanal-	28.665	19732438	6276275
25	Dodecanal, O-methyloxime	28.790	2878675	171426
26	1-Tetradecanol	28.992	6256826	864056
27	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedeca	29.291	5442959	890324
28	9,12-Octadecadienoic acid (Z,Z)-	29.440	1449467	347279
29	Tetradecanoic acid	29.955	78259929	7209241
30	Tetradecanoic acid, 2-oxo-, methyl ester	30.225	9026018	1022046
31	cis-9-Hexadecenal	30.415	4910683	727269
32	Eicosanal-	30.874	45458691	10063730
33	2-Pentadecanone, 6,10,14-trimethyl-	31.277	31303258	4888448
34	2-Methyltetracosane	31.590	3024460	351320
35	Pentadecanoic acid	31.862	15252037	2175323
36	n-Pentadecanol	32.171	8432271	2324597
37	Heneicosane	32.371	30492415	5915702
38	5,9,13-Pentadecatrien-2-one, 6,10,14-trimeth	32.762	7336599	1090178
39	Hexadecanoic acid, methyl ester	32.969	6012999	1408015
40	Andrographolide	33.183	8706779	2327089
41	Bicyclo[4.4.0]dec-6-en-9.beta.-ol, 1,7-dimeth	33.363	12182572	1734591

42	n-Hexadecanoic acid	34.428	376367103	11986924
43	Docosanoic acid, 1,2,3-propanetriyl ester	34.865	27302036	1338321
44	Bicyclo[2.2.1]heptan-2-ol, 3-amino-1,7,7-tri	35.296	9873042	1131538
45	Oleic Acid	35.691	15580728	2525909
46	n-Hexadecanoic acid	35.982	24635421	3842420
47	7-Hexadecenal, (Z)-	36.372	27120743	5193798
48	8,11,14-Docosatrienoic acid, methyl ester	36.516	7910817	2105619
49	Phytol	36.804	68746295	10546910
50	9-Octadecenoic acid, 1,2,3-propanetriyl ester	38.163	406333014	10069983

The GC-MS results of methanolic extracts of *leaves Khaya anthotheca* revealed tetradecanal, tetradecane, 1-undecanol, pentadecanoic acid, neointermedeol, 1-dodecanol, heneicosane, docosanoic acid 1,2,3-propanetriyl ester, phytol, 9-octadecenoic acid, 1,2,3-propane triyl ester, 1-tetradecanol and andrographolide etc. as some of the constituents.

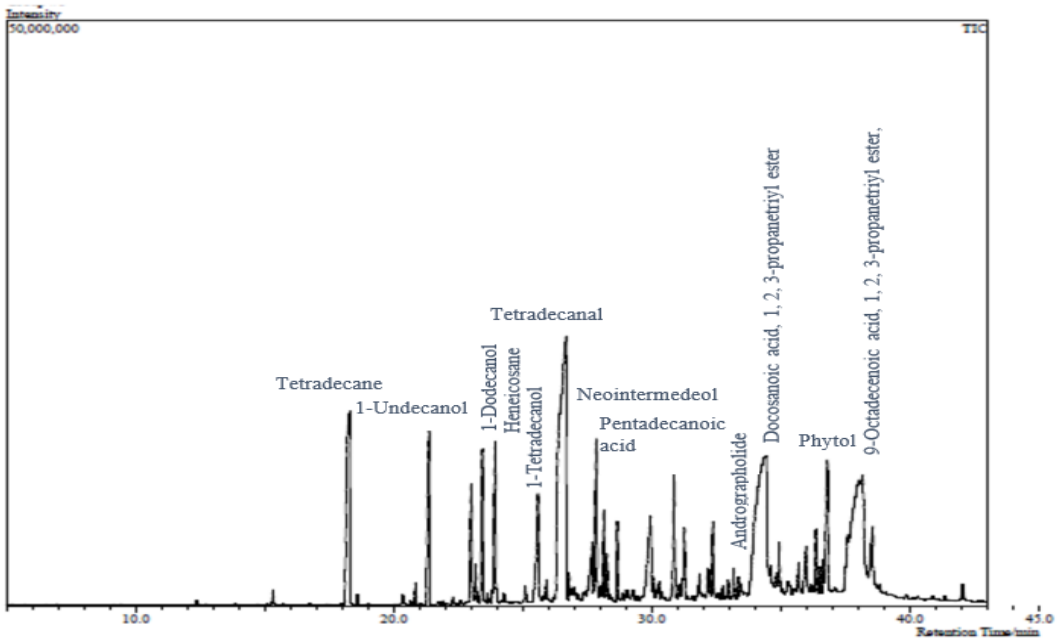


Figure 2: GC-MS chromatogram of *Khaya anthotheca* methanol leaves crude extract

Result obtained from GC-MS of methanolic extracts of *leaves* of *Khaya anthotheca* in table 1. Fifty (50) constituents were identified. Tetradecane is a long-chain fatty aldehyde in which two hydrogen atoms connected to a terminal carbon are changed by an oxo group to form tetradecanal. It serves as a metabolite for both plants and microorganisms. It is both a 2,3-saturated and long-chain fatty aldehyde. It comes from a tetradecane hydride [23]. A common monounsaturated fat in the human diet is oleic acid. Oral consumption of monounsaturated fat is associated with lower levels of LDL cholesterol and maybe higher

levels of HDL cholesterol [24,25]. Oleic acid is a crucial omega-9 monounsaturated fatty acid that is used as an emulsifier. It has also been reported to be hypotensive [26] and to slow the advancement of adrenoleukodystrophy, a dangerous condition that affects the brain and adrenal glands [27,28].The manufacturing of personal care and cosmetic products uses n-hexadecenoic acid, which has also been shown to have antioxidant, hypocholesterolemia, anti-inflammatory, and 5-alpha reductase inhibitor effects [29–30]. Antioxidant, neuroprotective, antibacterial, anticancer, anti-inflammatory and anti-diuretic properties of phytol have been documented [28,31]. The anti-inflammatory properties of pentadecanoic acid are excellent [32].

1-undecanol increases male attractiveness in sex pheromone trap species *Grapholita molesta* (Busck) [33]. 1-Dodecanol is a fatty alcohol that is dodecane with a hydroxy group in place of hydrogen from one of the methyl groups. It is approved for use as Lepidopteran pheromone/sex bait in apple and pear orchards to prevent particular insects, the larvae of which destroy crops, from mating. It serves as a pheromone, pesticide, plant metabolite, insect attractant and cosmetic [23]. Heneicosane is used as an antimicrobial [34]. Tetradecanoic acid has been demonstrated to exhibit antifungal action against the fungi *C. albicans* and *A. niger* when combined with hydrazide-hydrazones [35]. Administration of *Khaya anthotheca* leaves extract to animals or humans may exert some the pharmacological activities discussed on the active phytochemicals present in this extract.

Antifungal activity of *Khaya anthotheca*

Table 2: Antifungal activity of *Khaya anthotheca in* on *Aspergillus niger*, *Aspergillus flavus*, *Candida tropicalis* and *Fusarium oxysporium* in millimetre (mm)

		controls		Extracts millimeter (mm)			
Solvent	Organism	Nystatin+ve	DMSO -ve	50	100	250	500
Methan	*Aspergillus niger*	24.67±0.11	30 + 0.8	12.43±0.12	13.62±0.25*	12.49±0.18	11.43± 0.25
	Aspergillus flavus	23.40±0.05	30 + 0.8	10.40±0.13	11.578±0.18	12.57±0.13	12.63± 0.15

ol	*Candida tropicalis*	23.10±0.08	30 + 0.8	12.17±0.23	12.72±0.12	15.47±0.36	16.55±0.11*
	Fusarium oxysporium	23.20±0.10	30 + 0.8	12.45±0.26	13.20±0.11	14.29±0.18	17.60±0.13*

Result are expressed as mean ± standard deviation (N = 5)

*= significant activity is seen when compared to the control (p<0.05)

Concentration of standard is 500 µg/mL of nystatin, Conc= Concentration, DCM = Dichloromethane, DMSO = Dimethyl sulfoxide.

Result of antifungal activity of methanolic extracts of *leaves Khaya anthotheca* showed that application of all doses of extract showed no significant activity (p> 0.05) against *Aspergillus niger* except 100 µg/mL which showed significant activity (p< 0.05) against *Aspergillus niger* compares to the normal control. Application of all doses of methanolic extracts of *leaves Khaya anthotheca* showed no significant activity against *Aspergillus flavus* when compared to the control. Application of all doses of methanolic extracts of *leaves Khaya anthotheca* showed no significant activity against (p> 0.05) *Candida tropicalis* except 500 µg/mL which showed significant activity (p< 0.05) against *Candida tropicalis*. Application of all doses of methanolic extracts of *leaves Khaya anthotheca* showed no significant activity (p> 0.05) against *Fusarium oxysporium* except 500 µg/mL which showed significant activity (p< 0.05) against *Fusarium oxysporium*. The antifungal activity exhibited by *Khaya anthotheca* leaves could due to the presence of phytoconstituent such as phytol, oleic acid, octadecanoic acid etc. The result of antifungal activity suggests that methanolic extracts of *leaves Khaya anthotheca* may have the potential to be used as an antifungal drug [36].

CONCLUSION

According to the findings in this study, the *Khaya anthotheca* leaves fraction has antifungal activities with less negative effects when administered at low concentrations. *Khaya anthotheca* leaf fraction may be used as a potential effective agent as antifungal which is less expensive, easily accessible, and site-harming because of its antifungal activity displayed by methanolic extract of *Khaya anthotheca* leaves. The outcome of this study show that the plants investigated possess antifungal activities, thus justifying their use in folk medicine for

the treatment of skin and other related infections. long-term treatment with *Khaya anthotheca* methanolic leaves extract may achieve long-term potentials of the extract.

Ethics approval and consent to participate

The study was approved by the Animal Experimentation Ethics Committee of Federal University Wukari, Nigeria. Reference Number AEEC/FUW/014/2023

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Conflict of Interest

All authors declare that they have no conflict of interest

Abbreviations

RBG, random blood glucose; Conc, concentration; DCM, dichloromethane; DMSO, dimethyl sulfoxide; GCMS, gas chromatography mass spectrophotometer, STZ, streptozotocin; Bwt; body weight.

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