Asian Journal of
Science, Technology, Engineering, and Art

e-ISSN : 3025-4507
p-ISSN : 3025-5287

Index : Harvard, Boston, Sydney University, Dimensions, Lens, Scilit, Semantic, Google, etc

https://doi.org/10.58578/AJSTEA.v2i3.3185

PHYTOCHEMICAL PROFILING, AND ANTIFUNGAL POTENTIALS OF STEM-BARK EXTRACTS OF EAST AFRICAN (KHAYA ANTHOTHECA)

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Article Info:

Submitted:	Revised:	Accepted:	Published:
May 25, 2024	Jun 5, 2024	Jun 10, 2024	Jun 13 2024

Abstract

Fungal infection is an infectious disease that is commonly implicated as aetiologic agents that contribute to the increasing burden of morbidity and mortality in developing countries as a result misdiagnosis or improper diagnosis. This study determined the profiling and antifungal potentials of Stem-Bark Extracts of East African (Khaya Anthotheca). The methanol solvent was used for disc diffusion assay. The inhibitory concentration of the extract was performed by broth dilution method and zone of inhibition was studied by disc diffusion method at the concentration of 50, 100, 250, and 500 in DMSO. Nystatin was used as the reference control for antifungal study. The extract showed maximum inhibition potential sof zone of inhibition against most of the pathogen (Aspergillus niger, Aspergillus flavus, Candida tropicalis and Fusarium oxysporium) used at concentration 50ppm to 500ppm. The zone of inhibition for 500ppm is shown as (319.12± 0.11, 19.23 ± 0.12, 20.33±0.23* and 18.34±0.21*mm respectively). The extract showed minimum

inhibition potential against Aspergillus flavus in all the concentration when compared with the control as well as to the other pathogens. Candida tropicalis and Fusarium oxysporium was found to be more sensitive to the methanol extract followed by Aspergillus flavus and Aspergillus niger lastly. The extract was found to be rich in phytochemical, with about 20 different chemical constitutes, with 12.14- Pentane, 3-ethyl-2,4-dimethyl-, 14.66-3-Hexen-2-one and 27.23-2-Nonenal, 2-pentyl-as the highest. The Present study indicates the potential usefulness of methanol extract of extract of khaya anthotheca as antifungal agent. Thus, has therefore, contributed to the pool of knowledge already available in this area of research. Considering the effects that both phytochemicals and its potentials as antifungal, it would be expedient to conduct further studies to assess its cancer potentials.

Keywords: Phytochemical Profiling, Antifungal Potentials, Stem-Bark, Extracts, East African, Khaya Anthotheca

INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. They represent a rich source of phytochemicals (such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc.) and antimicrobial agents (Umaru et al., 2018; Teklit and Birhanu, 2018). In different regions of the world particularly Africa and Asia, plant materials have also played an important role in the traditional treatment and management of diabetes mellitus (Eze et al. 2012). The broad-range of bioactive secondary metabolites are present in different plant organs such as fruits, stem, bark, leaves, seeds and roots, and are structurally and functionally diverse, thereby providing various prospects for developing raw drugs by the local communities and traditional healers (Rekha et al., 2014) and novel drugs in the pharmaceutical industry (Idris et al., 2019).

The past two decades have witnessed an incredible increase in acceptance and public interest in natural therapies both in developing and developed countries. This is attributed to several factors including, limited availability and accessibility of the pharmaceutical products, high prices of the drugs, side effects associated with the pharmaceutical drugs and the society's growing general disapproval of the modern pharmaceutical drugs (Oyebode et al., 2023). In developing countries, the limited number of modern health care workers has contributed greatly to the continued usage of traditional medicine, for instance, the ratio of traditional healers to the population in Africa is 1:500 whereas the

ratio of medical doctors to the population is 1:40,000 (Qi, 2018). Due to this, the WHO has advocated for the incorporation of herbal medicine into the public health care systems of the different countries through authentication of the herbal extracts using modern scientific techniques. With the rapidly growing drug resistance especially among microorganisms, plants are potential sources of novel antibiotics and drugs but only 15% of the world's higher plants had been systematically investigated for bioactivity (Newman et al., 2018).

Considering the vast potentiality of plant as sources for antimicrobial drugs, the plant *Khaya anthotheca* will be considered in this study. *Khaya anthotheca*, commonly known as East African Mahogany, is a large evergreen tree species native to tropical Africa (Mariano, 2018). Khaya anthotheca is fast growing and grows up to 60 m in height (30 m in gardens) (Azad et al., 2016). The leaves are spirally arranged, alternate, with 3–7 leaflets and dark-glossy green in color. The bark of the tree is greyish brown. Stipules are absent, and the petiole is 3.5–7 cm long. The inflorescence is axillary with panicle that is 30–40 cm long and flowers are unisexual. The fruits are erect, nearly globose with woody capsules that are 4–10 cm in diameter, dehisce into 4–5 halves with many seeds (Billowira et al., 2023). Seeds are disk-shaped or quadrangular. Khaya anthotheca is found at low to medium altitudes in riverine fringe and evergreen forests in Africa (Danquah et al., 2011).

Statement of Problem

A lot of scientific evidences have emerged to support the trado-medicinal use of plant extracts as herbal remedies for the treatment of fungi, bacteria and diabetes since they contain different classes of natural products or secondary metabolites. Inadequate treatment of diabetes has led many to use herbs and herbal preparations as antihyperglycemic complementary and alternative medicines (CAM) to manage their diabetic state though safety and efficacy.

There have been several studies in Khaya species, mostly focusing on the plant extracts bioactivities and the composition of the constituents in various plant parts. *Khaya anthotheca* have been reported in literature to be useful in the treatment of fevers, several febrile conditions, microbial infections and worm infestations. However, no study have investigated the anti-diabetic potential of the plant. As a result, this study will consider the stem bark extract of *Khaya anthotheca* in order to evaluate its phytochemical constituents, antifungal activity and as well as its diabetic potentials on alloxan-induced diabetic rats.

Justification of Study

Determination of the phytochemical profile of extracts from the stem bark of Khaya anthotheca is key to the development of a monograph for usage in the incorporation of herbal medicine into modern public health care systems.

This study will provide adequate information on the antifungal and antidiabetic potentials of the stem bark of Khaya anthotheca on alloxan-induced diabetic rats.

And lastly, this study will benefit other researchers that aim to understand the role of plan extracts in the traditional treatment and management of diabetes mellitus and other diseases.

Aim of the Study

The aim of this research is to evaluate the phytochemical constituent, antifungal and diabetic potentials of *Khaya Anthotheca* stem-bark extracts on alloxan-induced diabetic rats.

Objectives of the Study

1. To extract and determine phytochemical constituents from the stem bark of *Khaya Anthotheca.*
2. To determine the antifungal potential of the stem bark of the plant Khaya Anthotheca.

Khaya Anthotheca

Khaya anthotheca is a large evergreen tree up to 60 m tall (up to 30 m in the garden) with an elongated or rounded, mu branched crown; the trunk is buttressed in old specimens. The bark on the young branches is smooth and greyish-brow but smooth to sometimes mottled grey and brown, flaking on the older branches and stems.

Fig 1. The tree and bark of *Khaya anthotheca*

Leaves are alternate, evenly compound with 3-7 pairs of leaflets, 150-300 mm long and dark glossy green, base broadly tapering to round and slightly asymmetric, smooth and glossy, veins distinct on the lower surface, margin smooth.

Flowers appear in branched sprays at the tips of branches, are white and sweetly scented, up to 10 mm in diameter, the male and female flowers are separate but on the same tree, and the stamens join to form a tube up to 6 mm long. The flowering period is from September to December. The fruit is a hard, woody, oval, splitting capsule up to 60 mm in diameter, with 4 or 5 valves. Fruiting occurs from March to September.

Origin and Distribution of *Khaya Anthotheca*

Khaya anthotheca occurs at medium to low altitudes in evergreen forests and riverine fringe forests in Africa (Pinheiro 2011). Khaya anthotheca is widespread, from Guinea Bissau east to Uganda and Tanzania, and south to Angola, Zambia, Zimbabwe and Mozambique. It is fairly widely grown in plantations within its natural area of distribution, but also in South Africa, tropical Asia and tropical America (Deguene et al., 2020). In Bangladesh it is planted in homesteads throughout plain districts with alluvial soils. In Tanzania is commonly found in the foothills of mountain ranges, in well-drained soils, and swamp and riverine areas. It has been successfully grown in the eastern parts of South Africa, and in Cuba and Puerto Rico.

Properties of *Khaya Anthotheca*

The heartwood is pinkish brown to deep red and more or less distinctly demarcated from the pale brown, up to 6 cm wide sapwood. The grain is straight or interlocked, texture rather coarse. The wood has an attractive figure with irregular ripple marks (Amadou, et al., 2024).

The wood is medium-weight, with a density of 490–660 kg/m³ at 12% moisture content. It generally air dries easily with little degrade, but the presence of tension wood may cause serious distortion. The rates of shrinkage are moderate, from green to oven dry 2.7–4.1% radial and 5.8–6.4% tangential. Once dry, it is fairly stable in service.

At 12% moisture content, the modulus of rupture is 50–110 N/mm², modulus of elasticity 7800–10,300 N/mm², compression parallel to grain 24–53 N/mm², shear 8–14 N/mm², cleavage 11–12 N/mm radial and 13–16 N/mm tangential, Janka side hardness 3250–5120 N and Janka end hardness 3600–6360 N.

The wood is usually fairly easy to saw and work, although the presence of interlocked grain may cause some difficulties; saws should be kept sharp to prevent a woolly finish and a cutting angle of 20° is recommended (Okimat et al., 2024). The wood can be finished to a smooth surface, but the use of a filler is required before staining and varnishing. The wood holds nails and screws well, but may split upon nailing, and glues satisfactorily. The bending properties are usually poor. The wood peels and slices well, producing an excellent quality of veneer. It turns fairly well. The wood is moderately durable but can be susceptible to termite and pinhole borer attacks. The heartwood is strongly resistant to impregnation, the sapwood moderately resistant. The wood dust may cause irritation to the skin.

Propagation and planting of *Khaya Anthotheca*

Khaya anthotheca is propagated by seed (Oliveira et al., 2012). The 1000-seed weight is 180–280 g. The seeds are often already attacked by insects while they are still on the tree, and undamaged seeds should therefore be selected before sowing or storage. The seeds can be stored for up to 1 year in a cool and dry place; adding ash to reduce insect damage is recommended. Fungi can cause serious losses of stored seed, with seeds stored at –18°C and 5°C showing higher occurrence of fungi and lower germination rates than seeds stored at 15°C. The seeds are best sown in seed beds in the nursery, they should be covered with only a thin layer of soil, or left partially uncovered. Germination takes 8–35 days. The

germination rate of fresh healthy seed is up to 85%, but decreases rapidly under natural circumstances. When seedlings are grown in small containers, they can be planted out when they reach 30 cm and have fully developed compound leaves. Seedlings can also be left in the nursery until they are 1–2 m tall, after which the root system is slightly pruned and leaves stripped off before planting into the field as striplings. In experiments in Indonesia, vegetative propagation by means of cuttings was successful, with a rooting success rate of 75% when growth hormone was applied (oliveira et al., 2012).

In Côte d'Ivoire Khaya anthotheca has been planted in degraded or secondary forests at a distance of 7–25 m between lines and 3–7 m within the line. Pure plantations have also been established with trees planted at 3 m × 3 m.

Diseases and pests of *Khaya Anthotheca*

Plantations of Khaya anthotheca may suffer seriously from Hypsipyla robusta shoot borers that kill the main stem of young trees, causing excessive branching and contributing to mortality. Silvicultural techniques, such as overhead shading of saplings, mixed planting and removal of lateral shoots, can reduce damage by shoot borers. Seeds are commonly attacked by seed-boring beetles and eaten by small rodents (Alam et al., 2012).

Harvesting and Yield of *Khaya Anthotheca*

The minimum bole diameter for harvesting of Khaya anthotheca trees in natural forest is 60 cm in Côte d'Ivoire, 80 cm in Cameroon, Central African Republic and DR Congo, and 110 cm in Ghana (Maroyi, 2008). The boles are occasionally so large that they cannot be sawn with normal equipment. The high buttresses at the base of the bole often necessitate the construction of a platform before felling can take place, or the removal of the buttresses before felling to recover more timber. For plantations at an age of 30 years, the annual production is 2–4 m^3/ha (Alam et al., 2012).

Uses of *Khaya Anthotheca*

The wood of Khaya anthotheca weathers well and is resistant to borers and termites (Alec, 2007). The wood is highly valued for furniture, cabinet work, decorative boxes and cases and veneer, and is also commonly used for window frames, paneling, doors and stair cases. It is suitable for light flooring, ship building, vehicle bodies, sporting goods, musical instruments, toys, novelties, carving, plywood and pulpwood (Olatunji et al., 2021). The bitter bark is widely used in traditional medicine in Africa. It is taken to treat cough,

whereas bark decoctions or infusions are taken to treat fever, cold, pneumonia, abdominal pain, vomiting and gonorrhea, and applied externally to wounds, sores and ulcers. Pulverized bark is taken as aphrodisiac and to treat male impotence.

In Tanzania, root decoctions are drunk to treat anemia, dysentery and rectal prolapse. In this country, the bark has been used by the Shambaa people for reddish brown dyeing. In DR Congo, the leaves are said to be used for making arrow-poison. K. anthotheca is fairly commonly planted as an ornamental shade tree and roadside tree. It is occasionally planted as a shade tree in agroforestry systems (Olatunji et al., 2021).

Pharmacological Activity of *Khaya Anthotheca*

Antimalarial Activity

Malaria, caused by a protozoan parasite of the genus Plasmodium, is a major public health problem globally, especially in tropical and sub-tropical countries where high morbidity and mortality are recorded (Olatunji et al., 2021). According to the WHO report in 2016, over 216 million malaria cases were reported across 91 countries globally, and over 445,000 deaths resulting from malaria infection were recorded yearly (WHO, 2018, Bapela et al., 2022, Obbo et al., 2024). Malaria cases keep rising in the remote and rural areas of sub-Saharan Africa, where cheap drugs and medical centres are not available. As a result, most people rely on herbal medicine for the treatment of malaria (Tepongning et al., 2013). In that regard, several limonoids in different parts of Khaya species have been effective against different Plasmodium strains.

Lee et al. (2008) assessed that the antimalarial efficacy of two limonoids (anthotechol and gedunin) from "whole plant" of K. anthotheca was assessed against the W2- strain of P. falciparum using [3 H]-hypoxanthine and 48- h culture assay in vitro. The results revealed that anthothechol demonstrated potent antimalarial activity in the two assays with IC50 values of 1.4 μM and 0.17 μM. Similarly, gedunin was also reported to be effective against the Plasmodium strain in the two assays with IC50 values of 3.1 and 0.14 μM.

Antimalarial activity of anthothecol, a limonoid of Khaya anthotheca (Meliaceae) against Plasmodium falciparum was tested using a [3 H]-hypoxanthine and 48 h culture assay in vitro (Sung-Eun et al., 2008). Anthotechol showed potent antimalarial activity against malaria parasites with IC50 values of 1.4 and 0.17 mM using two different assays. Also,

gedunin had antimalarial activity with IC50 values of 3.1 and 0.14 mM. However, the citrus limonoids, limonin and obacunone did not show any antimalarial activity. The antimalarial activities were compared with the three currently used antimalarial medicines quinine, chloroquinine and artemisinin.

Antimicrobial Activity

Globally, infectious diseases are among the leading cause of mortality and account for half of the deaths in tropical regions (Paritala et al., 2015). The increasing antibiotic resistance resulting from antibiotics' overuse is a common health challenge, particularly for human infectious diseases (Aboutabl et al., 2015). This has necessitated the search for healthier and efficient natural product alternatives in treating several microorganisms with little or no toxicity to humans. Antimicrobials that are plant-based represent a wide range of underexploited alternatives that can be used to treat infectious diseases with no such side effects associated with synthetic antimicrobials (Paritala et al., 2015).

In the studies carried out by Obbo et al. (2013), in vitro antitrypanosomal and antileishmanial activities of two limonoids (Grandifolione and 7-deacetylkhivorin) isolated from the petroleum ether seed extract of K. anthotheca were evaluated against Trypanosoma brucei brucei, Trypanosoma brucei rhodesiense, Trypanosoma cruzi and Leishmania donovani. Both Grandifolione and 7-deacetylkhivorin showed activity against Trypanosoma brucei rhodesiense (with IC50 values of 10.66, 16.88 µg/ml, respectively), Trypanosoma cruzi (with IC50 values of 20.97, 31.82 µg/ ml, respectively) and Leishmania donovani (with IC50 values of 13.31, 36.71 µg/ml, respectively). The results justify the traditional use of K. anthotheca in treating microbial infections.

Anticancer Activity

Globally, cancer in whichever form is one of the leading causes of mortality. In the next two decades, it is estimated to increase by about 70% worldwide with the most significant impact on low income and developing countries (Aladejana., 2023). Several efforts such as surgery, chemotherapy and radiotherapy have been used in the fight against cancer in the past few decades. However, cancer treatment remains a significant challenge owing to tumour diversity, damage to normal cells, resistance and recurrence of tumours even after years of remission (Raimi, et al., 2020).

Studies have indicated that bioactive compounds in medicinal plant extracts with anticancer potentials are currently attracting researchers' attention in the fight against cancer. Verma et

al. (2015) examined the inhibitory capability and mechanism of action of anthothecol, a limonoid from K. anthotheca and anthothecol encased poly (D,L-lactic-co-glycolic acid) nanoparticles (AnthoNPs) on pancreatic cancer stem cells and cell lines obtained from human and KrasGI2D mice. The study established that anthothecol and antho-NPs repressed the spread of cancer stem cells and cell lines in a dose-dependent form without affecting the normal human pancreatic ductal epithelial cells.

Table 1 Other Reported Bioactivities in the K. anthotheca

Activity	Part	Solvent	Phytochemicals detected	References
Antiplasmodial activity (IC50 0.955 µg/ml) against Plasmodium falciparum and antitrypanosomal activity (IC50 5.72 µg/ml) against Trypanosoma brucei rhodesiense	Seeds	Petroleum ether	N.r.	Obbo et al. (2013)
Antiplatelet activity (EC50 0.97 ± 0.03 µg/ml) in adrenaline (epinephrine) induced platelet aggregation in equine platelets	Leaves	Acetone	N.r.	Suleiman et al. (2010)
Antioxidant (EC50 0.10) for TEAC assay and (EC50 176.40 ± 26.56 µg/ml) for DPPH assay	Leaves	Acetone	N.r.	Suleiman et al. (2010)

METHODS

Materials

Reagents

Detergent, distilled water, formaldehyde, ammonia, ammonium chloride, mercuric chloride, potassium iodide, iodine, copper sulphate, potassium sodium tartrate, sodium hydroxide, ferric chloride, lead acetate, H_2SO_4, ethanol, ethyl acetate, aluminium chloride, HCl, chloroform, acetic anhydride, glacial acetic acid, $NaNO_2$, vanillin-methanol solution, dimethyl sulfoxide (DMSO), Fluconazole (Diflucan), sterile normal saline, alloxan monohydrate, glibenclamide or insulin, isoflurane and blood

Equipment and Apparatus

Mortar and pestle, electric blender, weighing balance, beaker, Whatman filter paper, rotary evaporator, oven, UV lamp, graduated cylinder, glass slide, agar disc, incubator, sterile Petri

plates, sterilized glass beads, UV spectrophotometer, bulbed steel needle, spectrophotometer, plain tubes, ultra-freezer

Media and organisms

Potato dextrose agar media , *Aspergillus niger, Aspergillus flavus, Candida tropicalis, Fusarium oxysporium.*

Procurement of Sample

Collection of Plant Materials

The stem bark of *Khaya Anthotheca* will be collected from the premises of the Federal University Wukari Taraba State, Nigeria. The stem bark will be air dried in the laboratory under room temperature and powdered using mortar and pestle.

Preparation of Plant Material

The freshly dried stem-bark of *Khaya Anthotheca* will be grounded into fine powdered form using laboratory mortar and pestle and electric blender (Umaru et al., 2018). 150mg of the powdered stem-bark will be weighed into a beaker and mixed with distilled water three times the quantity of the sample and allow to stand for two days with continues shacking at time interval for 12hrs. The mixture will be then filtered using Whatman filter paper No.4 and the solvent will be then evaporated using a rotary evaporator (Heldolph Laborato 400). It will then be stored under frozen condition for further use.

Sterilization of Materials Used

All glass wares will be thoroughly washed in water containing detergent and rinsed with distilled water, they will be air dried and sterilized in the oven at 160°C for one hour. Inoculating chamber and growth chamber will be fumigated using formaldehyde and then irradiated on exposure to UV lamp for one hour. Laboratory benches will be cleaned with absolute alcohol while the inoculating loop will be flamed to redness and allowed to cool prior to use.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis:

Phytochemical screening was done in order to detect the presence of plant constituents. 1µL of the crude extract dissolved merhanol GC MS grade was subjected to the GC MS for the profiling of the chemical constituents. The GC-MS analysis was carried out in a

combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer, fitted with an HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m×250 μm, film thickness 0.25 μm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to a column velocity flow of 1.0 mL min–1. Other GC-MS conditions are ion-source temperature, 250°C, interface temperature, 300°C, pressure, 16.2 psi, out time, 1.8 mm and 1 μL injector in split mode with split ratio 1:50 with injection temperature of 300°C. The column temperature started at 36°C for 5 min and changed to 150 V at the rate of 4°C min–1. The temperature was raised to 250°C at the rate of 20°C min–1 and held for 5 min. The total elution was 47.5 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by the supplier was used to control the system and acquire the data.

Determination of the Antifungal Potential of *Khaya Anthotheca* Stem-Bark Extracts

The antifungal potential of the plant extract will be performed by agar disc diffusion method as reported by Umaru et al. (2018). Dimethyl sulfoxide DMSO will be used as a negative control and Fluconazole (Diflucan) will be used as a positive control. The plates will be incubated at 37°C. The fungal activity will be taken on the basis of diameter of zone of inhibition in triplicate, which will be measured before and after 5days of incubation and the mean of three readings is presented. The presence of inhibition of the treated fungus will be calculated using positive control as standard (100% inhibition) (Pandurangi et al., 2023; Aboh et al., 2014). The plant extract and the standard antifungal agents will be dissolved in DMSO, 100% biologically inert substances, with the disc diameter of 6mm. The extracts will be separately dissolved in dimethyl sulphoxide. This (DMSO) solvent served as reference control for the antifungal study. The solvent control (DMSO) will be also maintained throughout the experiment. Potato dextrose agar media will be used for the antifungal study. The molten media will be then inoculated with 200μl of the inoculums (1×10^8 Cfu) and poured into the sterile Petri plates. The disc will be saturated with 20μl of the extracts separately, allowed to dry and will be introduced on the upper layer of the seeded agar plate. The plates will be incubated at 28°C and the zone of inhibition will be measured every after 24h for five days.

Fungal Preparation

The fungi will be standardized by inoculating sterile normal saline solution with a 48h pure culture by adjustment of turbidity to match 0.5 McFarland stand Standardization of the microorganisms included harvesting fungal spores from a 7 days old culture on SDA slant. Ten milliliters of sterile normal saline containing 3% w/v Tween 80 will be used to disperse the spores with the aid of sterilized glass beads (Oladimeji, et al., 2015). Standardization of the spore suspension to 1.0×10^6 spores/mL will be achieved with a UV spectrophotometer (Spectronic 20D; Milton Roy Company, Pacisa, Madrid, Spain) at 530nm (OD at 530) of the suspensions and adjusted to a transmittance of 70-72%. The plates will be incubated at 37°C for 24h (Aberkane et al., 2002).

Statistical Analysis

Values will be expressed as Mean±standard deviation for three determinations of each experiment. The analysis will be done using the software-SPSS one-way ANOVA. Differences between means will be considered significant when a 2-tailed value of P will be less than 0.05.

RESULTS

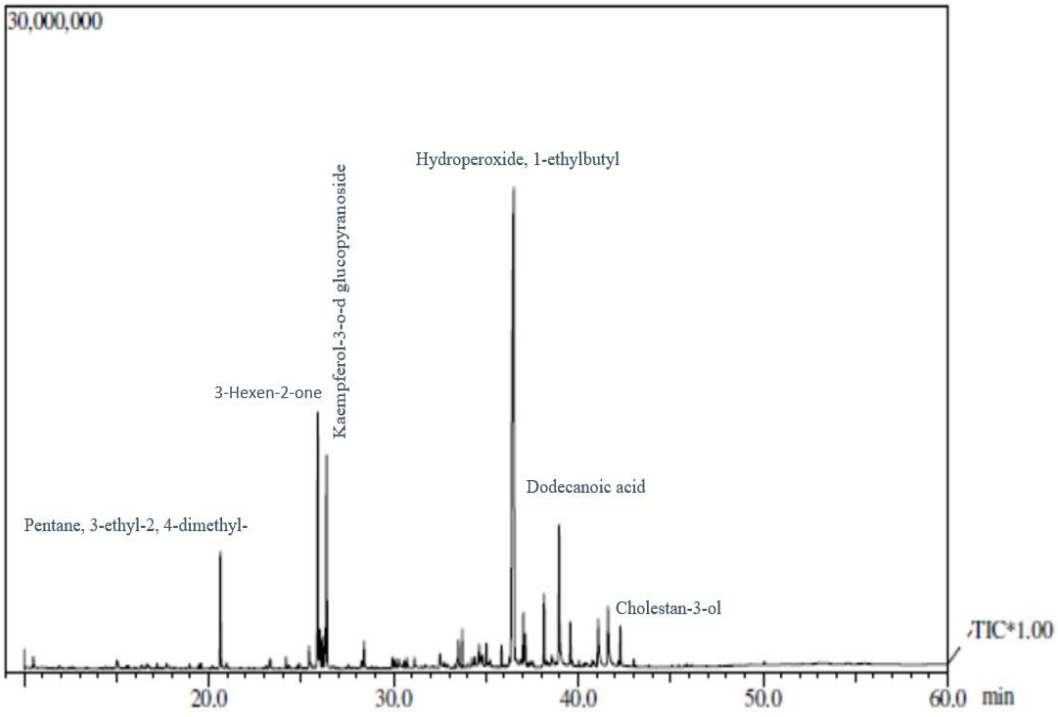

Figure 1: GC-MS chromatogram of *Khaya anthotheca* stem-bark methanol crude extract

Table 2. Phytochemical profile of *stem-bark Khaya anthotheca* methanol crude extract

Peak #	R.Tim	Area	Area %	Height	Height %	Base	Names
1	20.631	1406598	4.26	518673	6.59	57.05	Pentane, 3-ethyl-2,4-dimethyl-
2	25.904	33448808	10.13	11529693	**14.66**	**57.05**	**3-Hexen-2-one**
3	26.028	5439577	1.65	1694953	2.16	105.10	Kaempferol-3-o-d
4	26.181	3692118	1.12	1360720	1.73	93.10	p-Cymene
5	26.373	32345068	9.79	9548118	12.14	191.15	Pentane, 3-ethyl-2,4-dimethyl-
6	28.409	3017708	0.91	1123289	1.43	58.00	Cryptomeridiol
7	33.511	2436262	0.74	1012155	1.29	68.05	Spirostan-3-one
8	33.723	4297719	1.30	1706369	2.17	43.00	Glutaric acid, di(2-fluorophenyl)
9	34.638	2429212	0.74	783959	1.00	43.00	2-Fluoro-3-trifluoromethylbenzoic
10	35.026	2600191	0.79	990923	1.26	58.00	Myrtanol, 2-mercapto-
11	35.847	2636993	0.80	958487	1.22	81.05	5,8,11,14-Eicosatetraenoic acid,
12	36.509	154351239	46.72	21418251	**27.23**	**43.00**	**2-Nonenal, 2-pentyl-**
13	37.031	7295720	2.21	2363155	3.00	91.05	Ethyl trans-4a,cis-4b,trans-8a,cis-
14	37.138	4141291	1.25	1432477	1.82	95.10	Hydroperoxide, 1-ethylbutyl
15	38.153	9415985	2.85	3179399	4.04	95.10	Ethanol, 2-(hexyloxy)-
16	38.961	20181077	6.11	6123526	7.79	71.05	Dodecanoic acid
17	39.568	4993489	1.51	1871375	2.38	84.05	Theobromine
18	41.080	8732238	2.64	2073628	2.64	81.05	Cyclohexanone, 2-(1-mercapto-1-
19	41.607	9831395	2.98	2556545	3.25	81.05	E-3-Pentadecen-2-ol
20	42.279	5003732	1.51	1734869	2.21	81.05	Cholestan-3-ol
		330355804	100.00	78648625	100.00		

Table 3: Antifungal activity of *East African* Stem-Bark *extracts khaya anthotheca in* on *Aspergillus niger*, *Aspergillus flavus*, *Candida tropicalis* and *Fusarium oxysporium* in millimetre (mm)

Solvent	Organism			50	100	250	500
		Nystatin +ve	DMSO -ve				
Methanol	*Aspergillus niger*	24.67 ± 0.11	30 + 0.8	14.43 ± 0.11	15.12±0.12	17.44± 0.10	19.12± 0.11
	Aspergillus flavus	23.40 ± 0.05	30 + 0.8	13.40 ± 0.13	17.13±0.15	17.62 ± 0.11	19.23 ± 0.12
	Candida tropicalis	23.10 ± 0.08	30 + 0.8	14.17 ± 0.16	15.34±0.14	18.43 ± 0.16	20.33±0.23 *
	Fusarium oxysporium	23.20 ± 0.10	30 + 0.8	14.45 ± 0.15	16.44±0.15	18.45 ± 0.13	18.34±0.21 *

Result is Mean ± SD. N = 3

*= significant activity was observed when compared to the control ($p<0.05$)

Concentration of standard is 500 µg/mL of Nystatin, Conc= Concentration, DCM = Dichloromethane

DISCUSSION

The study revealed the antifungal activity of the extract against the tested species of microorganisms between concentration ranges for 50 to 500ppm. The results of this study revealed that the extracts possess antifungal activity in a concentration dependent manner against the test organisms and were comparable with the standard drug.

The inhibition yielded at concentration 50-250ppm was found to be active against all tested strains under study. However, the extract showed higher significant antifungal activity against *Candida tropicalis* and *Fusarium oxysporium* over the other pathogens. Though the extract showed moderate activity at all concentration on the fungal species, the potency exhibited less significant activity against Candida tropicalis as well as *Fusarium oxysporium* strains. Thus, the antimicrobial activities of plant related have also been found registered in various literature (Satish et al., 2007; Bhardwaj, 2012., Gujar and Taknkar, 2012; Dubey and Kumar, 2003; Dubey et al., 2009) found almost similar effect of khaya anthotheca methanol extract on growth of some other fungi.

The result of this study indicating that differential activities of the plant extracts on the growth of different fungi, because many of these extract concentrations has shown significant and aggressively inhibition against the growth of some of this tested fungus.

The antifungal activities of this medicinal plants are attributed due to the presence of the chemical constitutes found in the stem-back extract. (Barnabas and Nagarajan, 1988). The presence of this metabolite in the leaf extract confirm its potential against the selected pathogens. This shown the significant and aggressive inhibition which was observed against the pathogen as it supressed the growth of all the pathogenic fungi. Thus, the potential usefulness of methanol stem-bark khaya anthotheca in the treatment of various pathogenic diseases, as well as helping in the discovery of new chemical classes for antifungal drugs that could serve as selective agents for the maintenance of human health should be considered.

CONCLUSION

The present study shown that methanol extract of khaya anthotheca stem-bark caused significant inhibition in all the fungi with increase in concentration, the presen of enormous

phytochemical will give more room for future research on endemic diseases like cancer and HIV.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Acknowledgment

Appreciation goes to all co-authors for their contribution and unflinching technical and financial support as there was no external sponsorship for this research work.

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