AJBS Asian Journal of Biological Sciences

OPEN ACCESS

Phytochemical Study and Biochemical Effect of Methanol Exctract of *Khaya anthotheca* in Indomethacin-Treated Albino Wistar Rats

Umaru, Isaac John, Ale, Ebenezer Morayo, Ayo, Victoria Ifeoluwa, Umaru, Kerenhappuch Isaac and Ebuk, Glory Godwin

Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, Taraba State, Nigeria

ABSTRACT

Background and Objective: *Khaya anthotheca* is a medicinal plant native to Africa. It is widely used in traditional medicine to treat a variety of diseases. Herein, phytochemical screening of methanol extract of *Khaya anthotheca* root and its effect on biochemical parameters in indomethacin-treated Wistar rats were investigated. **Materials and Methods:** Mature *Khaya anthotheca* roots were air-dried, pulverized and used for phytochemical study. The 15 rats were distributed into three groups; Group I: Normal control (diet/water), Group II: Negative control (25 mg/kg/b.wt., indomethacin+diet/water) and Group III: Extract treated (25 mg/kg/b.wt., indomethacin+diet/water+400 mg/kg/b.wt., extracts). The treatment lasted for 14 days and animals were sacrificed and blood was collected, centrifuged and serum was separated and used for biochemical analyses. **Results:** Results of phytochemical study revealed the presence of flavonoids, triterpenoids, alkaloids, tannins, saponins and phenolic compounds, while GC-MS analysis revealed 20 compounds with Campesterol, 3-Hexen-2-one, Eicosane, D-Limonene, Z, Z-8, 10-Hexadecadien-1-ol, Neointermedeol, Phytol, 9, 12-Octadecadienoic acid, (Z, Z)-5-Oleic acid and Octadecanoic acid as the major constituents. Furthermore, the results of liver function assay study showed that indomethacin administration led to elevation of both serum hepatic indices (Alkali Phosphatase (ALP), Aspartate Transaminase (AST) and Alanine Transaminase (ALT)) and renal indices (creatine and urea). However, treatment with *Khaya anthotheca* methanol root extract markedly ($p<0.05$) reduced the serum levels of the hepatic parameter and as well normalized the renal indices. **Conclusion:** It is therefore worth suggesting that *Khaya anthotheca* could be a promising therapeutic candidate for hepatic and renal complications. However, further research is needed as limited experimental reports are available on the active ingredients and their pharmacological efficacy in clinical trials.

KEYWORDS

Khaya anthotheca, methanol extract, phytochemicals, hepatic parameters, renal indices

INTRODUCTION

Khaya anthotheca is a medicinal plant native to Africa. It is a member of the Meliaceae family. It is a long, woody tree that is used to produce lumber. Known by many as the "Red Ivory tree," it is highly prized for its remarkable look, sophisticated elegance and height. It has been observed that *Khaya anthotheca* has

ISSN: 1996-3351 (Print)
https://doi.org/10.3923/ajbs.2024.620.626

Received: 06 Apr. 2024
Accepted: 24 Jun. 2024
Published: 31 Dec. 2024

pharmacological effects against malaria and other illnesses[1]. Additionally, studies revealed that *Khaya anthotheca* had neuroprotective properties[2]. This plant is known to contain some significant bioactive components, including limonoids and gedunin, both of which have been shown to have numerous health benefits[3].

In traditional medicine, it is commonly used to treat a wide range of illnesses, such as skin infections, fever, diarrhea and malaria. Additionally, it has been demonstrated that extracts from *Khaya anthotheca* have antibacterial properties against a range of microorganisms, including fungus, *Salmonella typhimurium*, *Escherichia coli* and *Staphylococcus aureus*[4]. *Khaya anthotheca* contains a number of limonoids, which have been shown to have potent antimalarial activity *in vitro* and *in vivo*[3].

In animal models, extracts from *Khaya anthotheca* have also been demonstrated to have anti-inflammatory properties[5]. A study by DonCarlos *et al.*[6] also showed that the bark extract of *Khaya anthotheca* had analgesic properties by successfully lowering rats' pain levels after exposure to acetic acid, which caused them to writhe in their abdomens. Nevertheless, research on the effects of *Khaya anthotheca* extract on biochemical parameters is limited. This forms the basis of this study to investigate the phytochemical composition as well as the effect of methanol extract of *Khaya anthotheca* root on hepatic and renal parameters in indomethacin-treated Wistar rats.

MATERIALS AND METHODS

Study location: The research was conducted at Biochemistry Department of Federal University Wukari, Taraba State, Nigeria between August, 2022 and January, 2023.

Chemicals: Methanol, Indomethacin and other chemicals used in this study were obtained from standard suppliers.

Plant collection and identification: Mature *Khaya anthotheca* roots were gathered from a farm in Taraba State, Nigeria's Wukari Local Government Area. The roots were classified to species at the Federal University Wukari, Taraba State, herbarium section of the biological sciences department. After washing the roots with tap water to remove any dirt particles, they were allowed to air dry in the shade to keep the chemical contents from being inactivated by UV light[7]. Using a mortar and pestle, the dried root was ground into a fine powder and kept in a dry container (Fig. 1).

Extraction of plant material: The 150 g powdered sample was put into a flask and for seven days, while shaking occasionally and letting it stand at room temperature, methanol was used to extract the phyto-constituents. The mixture was concentrated in a rotary evaporator (Gaithersburg, Maryland, United States)

Fig. 1: Powder and roots of *Khaya anthotheca*

after being filtered with Whatman no. 4 filter paper. After being dried, the extract was put in an airtight container, corked and kept cold.

Phytochemical screening: The phytochemical profiling of crude extract of *Khaya anthotheca* was carried out using the method of Umaru *et al.*[8].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: The HP-5 MS fused silica column (5% phenyl methyl siloxane, 30.0 m×250 µm, film thickness 0.25 µm) was used in the GC-MS analysis, which was performed in a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer interfaced with a 5675C Inert MSD with Triple-Axis detector. The column velocity flow of 1.0 mL/min was achieved by adjusting the helium gas utilized as the carrier gas. Additional settings for GC-MS measurements include the following: ion-source temperature of 250°C, interface temperature of 300°C, pressure of 16.2 psi, out time of 1.8 mm and 1 µL injector in split mode with split ratio of 1:50 at 300°C for injection. After 5 min at 36°C, the temperature in the column increased to 150 V at a rate of 4°C per minute. At a rate of 20°C per minute, the temperature was increased to 250°C and maintained for 5 min. The total elution was 47.5 min. By comparing the average peak area of each component to the total areas, the relative percent amount of each component was determined. The supplier's MS solution software was utilized to operate the system and collect the data[9].

Animal procurement: Weighted between 100 and 200 grams, Wistar albino rats were acquired from Animal House in Yola, Taraba State, Nigeria. Rats were housed in a 22±2°C room with 12/12 hrs of light and darkness and adequate ventilation. The rats were given regular feed and unlimited water after a week of acclimatization. The Federal University Wukari, in Taraba State, Nigeria, Faculty of Pure and Applied Sciences set standard laboratory protocols for animal investigations, which were followed.

Animal grouping and administration: Three groups of fifteen albino Wistar rats were formed; there were five individuals (n = 5) in each group. With the exception of the standard control animals, animals were given 400 mg/kg of indomethacin after being allowed to fast for a full day. For 14 days, the plant extract treatment was administered once a day as follows:

- **Group I:** Normal control (diet/water)
- **Group II:** Negative control (25 mg/kg/b.wt., indomethacin+diet/water)
- **Group III:** Extract treated (25 mg/kg/b.wt., indomethacin+diet/water+400 mg/kg/b.wt., extracts)

At the end of 14 days, animals were sacrificed under chloroform anesthesia and blood was collected via cardiac puncture, allowed to clot for 15 min and centrifuged at 4000 rpm for ten minutes. The serum was separated and stored at -20°C for biochemical analyses.

Biochemical analysis: The serum levels of AST, ALT, ALP, blood urea nitrogen and creatinine were assayed using commercial kits.

Statistical analysis: The statistical analysis for biological activities was carried out with the SPSS software, version 20. For each experiment, values were given as Mean±Standard Deviation for three determinations and were considered significant at $p \leq 0.05$. The SPSS one-way ANOVA was the program used for the analysis.

RESULTS

Phytochemical composition of *Khaya anthotheca* root-bark methanol crude extract: The result of phytochemical screening of *Khaya anthotheca* root-bark methanol crude extract (KARB) revealed the presence of chemical constituents such as flavonoids, triterpenoids, alkaloids, tannins, saponins and

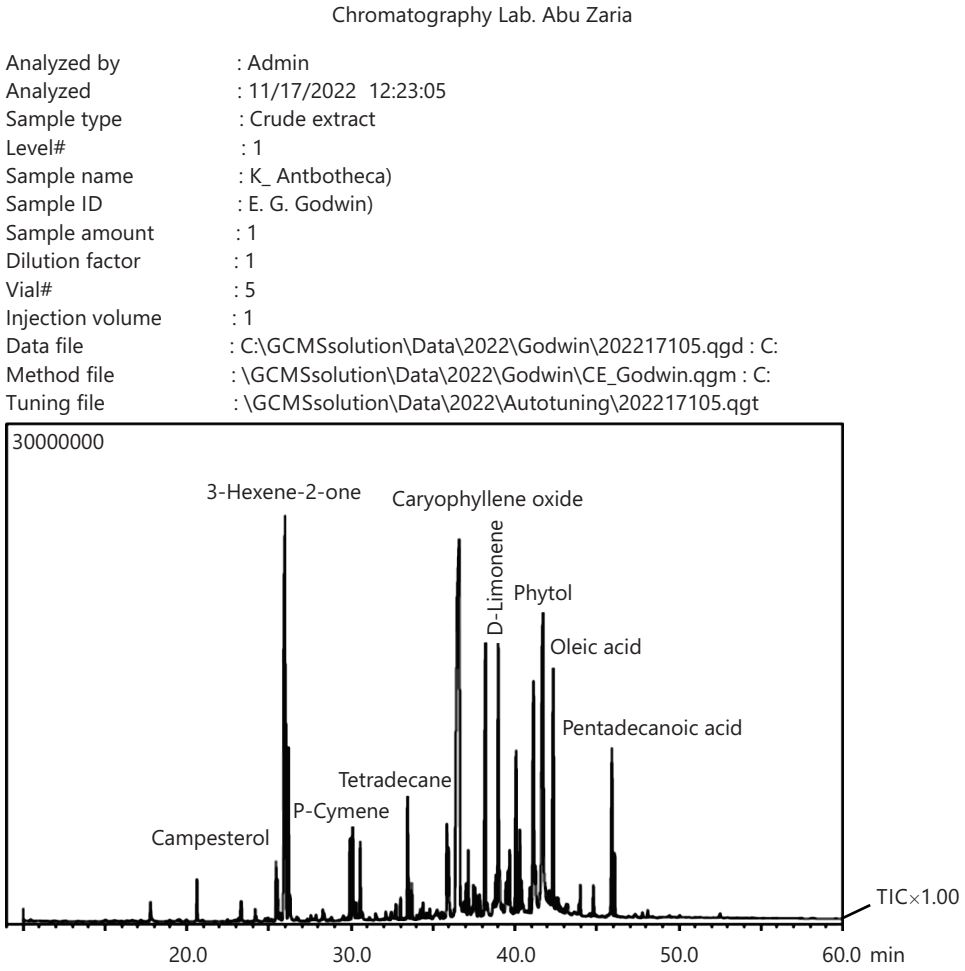

Fig. 2: GC-MS chromatogram of *Khaya anthotheca* root-bark methanol crude extract

phenolic compounds. The GC-MS analysis showed a good number of chemicals such as 3-Hexen-2-one, Alpha-phellandrene, Alpha-pinene, P-Cymene, Bicyclo[3.1.0]hexane, 4-methylene-1-(1-med, Tetradecane, Cryophyllene, Humulene, Caryophyllene oxide, Tridecanoic acid, Eicosane, D-Limonene, Z,Z-8,10-Hexadecadien-1-ol, Neointermedeol, Phytol, 9,12-Octadecadienoic acid (Z,Z)-, Oleic acid, Octadecanoic acid and Pentadecanoic acid (Table 1, Fig. 2)

Effect of *Khaya anthotheca* root-bark methanol crude extract on liver enzymes: The results presented in Table 2 reveal that indomethacin elevated the serum level of AST, ALT and ALP in group II treated with indomethacin only and the increase was significant ($p<0.05$) when compared to the normal control (group I). However, administration of *Khaya anthotheca* extract in group III caused a significant ($p<0.05$) reduction in the level of these enzymes.

Effect of *Khaya anthotheca* root-bark methanol crude extract on renal parameters: Administration of indomethacin led to a marked ($p<0.05$) increase in the level of serum creatinine and blood urea nitrogen (BUN), but extract treatment caused a significant ($p<0.05$) reduction in the level of these renal indices in group III (Table 2).

DISCUSSION

The phytochemical screening of *Khaya Anthotheca* root-bark methanol crude extract revealed the presence of chemical constituents such as flavonoids, triterpenoids, alkaloids, tannins, saponins and phenolic compounds. The result of GC-MS analysis showed a good number of chemicals such as 3-Hexen-

Table 1: Phytochemical profile of *Khaya anthotheca* root-bark methanol crude extract

Peak#	R. Time	Area	Area (%)	Base m/z	Base Int.	Name
1	25.974	143334570	12.15	57.10	5382992	Campesterol
2	26.052	43046901	3.65	105.10	680373	3-Hexen-2-one
3	26.204	33896415	2.87	93.10	650472	Alpha-phellandrene
4	29.942	13442639	1.14	67.05	575954	Alpha-pinene
5	30.106	28309597	2.40	55.05	615410	P-Cymene
6	30.580	12387379	1.05	57.05	943778	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-med)
7	33.463	29824772	2.53	137.15	586206	Tetradecane
8	35.853	18633652	1.58	81.05	325776	Cryophyllene
9	35.954	16612562	1.41	69.05	276733	Humulene
10	36.617	317281037	26.90	43.00	1953776	Caryophyllene oxide
11	37.157	11938116	1.01	95.10	351004	Tridecanoic acid
12	38.212	81224626	6.89	95.10	4080156	Eicosane
13	38.993	55394299	4.70	71.10	3094475	D-Limonene
14	40.068	37878634	3.21	95.10	643124	Z,Z-8,10-Hexadecadien-1-ol
15	40.309	15155785	1.29	95.10	329015	Neointermedeol
16	41.136	69307987	5.88	81.10	1011692	Phytol
17	41.719	131659730	11.16	81.10	1341840	9,12-Octadecadienoic acid (Z,Z)-
18	42.341	66414682	5.63	81.10	1019369	Oleic acid
19	45.926	42287528	3.59	98.05	809779	Octadecanoic acid
20	46.087	11375148	0.96	98.05	313006	Pentadecanoic acid
		1179406059	100.00			

Peak Report TIC

Table 2: Effect of *Khaya anthotheca* root-bark methanol crude extract (KARB) on biochemical parameters in Indomethacin-treated rats

	Treatment group		
Biochemical parameter	0.9% NaCl solution (normal control)	Indomethacin (negative control)	Indomethacin+400 mg/kg KARB
Alkaline phosphatase (mg/dL)	144.19±22.46^{a}	231.75±28.1^{b}	157.10±18.56^{a}
Aspartate transaminase (mg/dL)	139.22±17.06^{a}	223.13±33.27^{b}	164.13±23.67^{c}
Alanine transaminase (mg/dL)	43.18±5.17^{a}	71.18±12.11^{b}	45.07±4.13^{a}
Creatinine	0.14±0.04^{a}	0.28±0.22^{b}	0.19±0.02^{a}
Blood urea nitrogen (mg/dL)	23.44±2.50^{a}	56.23±5.66^{b}	33.83±1.58^{a}

Values are presented as Mean±SD for five rats in each group and Means with different superscript letters are significantly ($p<0.05$) different along the row

2-one, Alpha-phellandrene, Alpha-pinene, P-Cymene, Bicyclo[3.1.0]hexane, 4-methylene-1-(1-med, Tetradecane, Cryophyllene, Humulene, Humulene, Caryophyllene oxide, Tridecanoic acid, Eicosane, D-Limonene, Z,Z-8,10-Hexadecadien-1-ol, Neointermedeol, Phytol, 9,12-Octadecadienoic acid (Z,Z)-, Oleic acid, Octadecanoic acid and Pentadecanoic acid.

The total chemical constituents were found to be 20 compounds (Table 1, Figure 2) with ten : 12.15 Campesterol, 3.65 3-Hexen-2-one, 6.89 Eicosane, 4.70 D-Limonene, 3.21 Z, Z-8, 10-Hexadecadien-1-ol, 1.29 Neointermedeol, 5.88, Phytol, 11.16 9, 12-Octadecadienoic acid (Z, Z)-, 5.63 Oleic acid and 3.59 Octadecanoic acid being the major phytochemical obtained from the profiling of the crude extract.

The result of phytochemical study agreed with that of Amadou *et al.*[4] who demonstrated that ethanolic bark extract of *Khaya anthotheca* contains phytoconstituents such as alkaloids, flavonoids, tannins, saponins and glycosides.

The results hepatic function assay (Table 2) revealed that indomethacin elevated the serum level of AST, ALT and ALP in group II treated with indomethacin only and the increase was significant ($p<0.05$) when compared to the normal control (group I). However, administration of *Khaya anthotheca* extract in group III caused a significant ($p<0.05$) reduction in the level of these enzymes.

Renal function results show that while Indomethacin caused a marked ($p<0.05$) increase in serum creatinine and blood urea nitrogen (BUN), treatment with methanol root extract of *Khaya anthotheca* caused a significant ($p<0.05$) reduction in the level of these renal indices in group III close to normal (Table 2).

Khaya anthotheca is documented to possess numerous pharmacological actions including anticancer[10-12], antimalarial[3,13] and anti-inflammatory[14,15] which are linked to its triterpenoid component, limonoid[13]. It is therefore plausible that the pharmacological activity of *Khaya anthoteca* on the hepatic and renal indices demonstrated herein is associated with the phytochemicals that have been known to be bioactive in the plant.

CONCLUSION

The present study demonstrated that *Khaya anthotheca* methanol root extract contains a substantial number of bioactive phytochemicals and that the extract elicited significant pharmacological effects against hepatic and renal abnormalities induced by indomethacin in albino Wistar rats. We suggest that *Khaya anthotheca* could be a promising therapeutic candidate for hepatic and renal complications. However, further research is needed as limited experimental reports are available on the pharmacological efficacy of the plant.

SIGNIFICANCE STATEMENT

The exploration of the phytochemical composition and biochemical effects of the methanol extract of *Khaya anthotheca* in indomethacin-treated albino Wistar rats holds significant implications for both scientific research and medical practice. This research not only contributes to understanding the benefits of *Khaya anthotheca* in mitigating indomethacin-induced adverse effects but also sheds light on its potential therapeutic properties including its potential protective effects against liver and renal damage. Furthermore, the findings of this study may pave the way for the development of novel treatments for inflammatory conditions, for mitigating drug-induced hepatorenal toxicity, thereby advancing medical knowledge and providing valuable insights into the utilization of natural compounds as alternative and complementary approaches in modern medicine, thereby enhancing overall health outcomes.

REFERENCES

1. Obbo, C.J.D., B. Makanga, D.A. Mulholland, P.H. Coombes and R. Brun, 2013. Antiprotozoal activity of *Khaya anthotheca*, (Welv.) C.D.C. a plant used by chimpanzees for self-medication. J. Ethnopharmacol., 147: 220-223.
2. Franklin, Z.G., D. Sefirin, S.E.P. Faustin, P.C. Anatole and B.A. Yousif *et al*., 2021. Neuroprotective effects of *Khaya anthotheca* (Welw.) C.DC (Meliaceae) decoction on neurodegeneration induced by estrogen depletion in rats. J. Exp. Pathol., 2: 174-181.
3. Lee, S.E., M.R. Kim, J.H. Kim, G.R. Takeoka, T.W. Kim and B.S. Park, 2008. Antimalarial activity of anthothecol derived from *Khaya anthotheca* (Meliaceae). Phytomedicine, 15: 533-535.
4. Amadou, D., H.K. Güvenmez, B. Yilmaz and S. Girey, 2024. Phytochemical screening, antimicrobial and *in-vitro* cytotoxic activities of several extracts of six medicinal plants (*Crossopteryx febrifuga* Benth, *Khaya anthotheca*, *Gardenia ternifolia*, and *Terminalia glaucescens*) from Central Africa Region. Asian J. Biochem. Gen. Mol. Biol., Vol. 16. 10.9734/ajbgmb/2024/v16i7387.
5. Li, R., J. Cui and Y. Shen, 2014. Brain sex matters: Estrogen in cognition and Alzheimer's disease. Mol. Cell. Endocrinol., 389: 13-21.
6. DonCarlos, L.L., I. Azcoitia and L.M. Garcia-Segura, 2009. Neuroprotective actions of selective estrogen receptor modulators. Psychoneuroendocrinology, 34: S113-S122.
7. Das, D. and R.K. Banerjee, 1993. Effect of stress on the antioxidant enzymes and gastric ulceration. Mol. Cell. Biochem., 125: 115-125.

8. Umaru, I.J., F.A. Badruddin and H.A. Umaru, 2018. Phytochemical antifungal and antibacterial potential of *Leptadenia hastata* stem-bark extract. MOJ Toxicol., 4: 263-268.
9. Olivia, N.U., U.C. Goodness and O.M. Obinna, 2021. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. Future J. Pharm. Sci., Vol. 7. 10.1186/s43094-021-00208-4.
10. Fang, X., Y.T. Di and X.J. Hao, 2011. The advances in the limonoid chemistry of the meliaceae family. Curr. Org. Chem., 15: 1363-1391.
11. Akihisa, T., Y. Nishimoto, E. Ogihara, M. Matsumoto, J. Zhang and M. Abe, 2017. Nitric oxide production-inhibitory activity of limonoids from *Azadirachta indica* and *Melia azedarach*. Chem. Biodivers., Vol. 14. 10.1002/cbdv.201600468.
12. Ibrahim, M.A., N.A. Koorbanally and M.S. Islam, 2014. Antioxidative activity and inhibition of key enzymes linked to type-2 diabetes (α-glucosidase and α-amylase) by *Khaya senegalensis*. Acta Pharm., 64: 311-324.
13. Bickii, J., N. Njifutie, J.A. Foyere, L.K. Basco and P. Ringwald, 2000. *In vitro* antimalarial activity of limonoids from *Khaya grandifoliola* C.D.C. (Meliaceae). J. Ethnopharmacol., 69: 27-33.
14. Zhang, W.Y., L. Qiu, Q.P. Lu, M.M. Zhou, J. Luo and Y. Li, 2018. Furan fragment isomerized mexicanolide-type Limonoids from the stem barks of *Khaya senegalensis*. Phytochem. Lett., 24: 110-113.
15. Zhou, M.M., W.Y. Zhang, R.J. Li, C. Guo and S.S. Wei *et al*., 2018. Anti-inflammatory activity of Khayandirobilide A from *Khaya senegalensis* via NF-κB, AP-1 and p38 MAPK/Nrf2/HO-1 signaling pathways in lipopolysaccharide-stimulated RAW 264.7 and BV-2 cells. Phytomedicine, 42: 152-163.