Pharmaceutical Sciences, 2024, 30(4), x-x
doi:10.34172/PS.2024.26
https://ps.tbzmed.ac.ir/

Research Article

Cytotoxic Flavonoids from *Lannea egregia* Engl. & K. Krause

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Article Info

Article History:
Received: 13 Apr 2024
Accepted: 3 Aug 2024
ePublished: 7 Sep 2024

Keywords:
-Anacardiaceae
-Cancer cell lines
-Cytotoxicity
-Flavonoids
-*Lannea egregia*
-Myricetin

Abstract

Background: *Lannea egregia* Engl. & K. Krause (family: Anacardiaceae) is a well-known medicinal plant in Nigeria whose various parts have been shown to elicit several biological activities. This study specifically explored the leaf of *L. egregia* for potential cytotoxic compounds.

Methods: *n*-Hexane, dichloromethane and methanolic extracts of the leaf were prepared using the Soxhlet apparatus and concentrated using the rotary evaporator. Compounds were isolated by reversed-phase preparative high-performance liquid chromatography, and the structures were determined by spectroscopic means. The methanolic extract and the isolated compounds were screened for cytotoxicity against HeLa and MCF-7 cancer cell lines, using the MTT assay.

Results: Three flavonoids, myricetin (**1**), myricetin 3-*O*-α-L-rhamnoside (**2**) and quercetin 3-*O*-α-L-rhamnoside (**3**), were isolated from the methanolic extract of the leaf of *L. egregia*. The methanolic extract and compound **3** showed the most potent inhibition profiles against the cells, with IC_{50} values (Mean ± SEM) of 45.3 ± 1.5 µg/mL and 57.5 ± 0.4 µg/mL for the methanolic extract, and 36.5 ± 2.0 µM and 57.9 ± 10.1 µM for compound **3**, against HeLa and MCF-7 cells, respectively.

Conclusion:

Introduction

Global mortality is impacted by the cancer burden, which is a major public health concern. Due to demographic shifts and related risk factors, cancer incidence and death rates are predicted to climb sharply, with a projected 47% increase in cancer cases by 2040, especially in transitioning nations.[1] The bulk of cancer diagnoses and fatalities worldwide today occur in less developed nations, which has led to a change in the cancer burden that exacerbates this trend even more.[2] Several other variables, including dietary exposures, viral infections, particularly by human papillomavirus (HPV), and bacterial infections have also been linked to the prevalence of cancer.[3,4]

Research has been conducted to improve the number of medications available and accessible for cancer prevention, as there are still only a small number of drugs approved for this purpose, despite some achievements.[5] Given that drug resistance in cancer involves complicated mechanisms and heterogeneity of cancer cells, it is a serious challenge that needs more research and development of treatments.[6]

The *Lannea* genus belongs to the family Anacardiaceae and comprises plants that are distributed across tropical and subtropical regions, and have long been used as traditional medicines, particularly in Nigeria.[7] The *Lannea* species such as *L. acida, L. macrocarpa*, *L. welwitschia,* and *L. schimperii* contain phenolic compounds with diverse biological activities.[8,9] *Lannea acida*, which is known as faruhi in Nigeria, has been reported to be an anti-abortifacient and to cure anal haemorrhoids, diarrhoea, dysentery, dysmenorrhea and infertility, while *L. microcarpa*, which is referred to as African grape, has been used locally to treat ulcers, skin diseases, rheumatism, and dysentery, and to regularize blood pressure. The ethyl acetate extract of the bark was reported to prevent hypertension.[10,11]

Previously isolated phytochemicals from this genus include: 5-[12'(*E*)-pentadecenyl]-4,5-dihydroxycyclohex-2-enone, 5-[14'(*E*)-heptadecenyl]-4,5-

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dihydroxycyclohex-2-enone and 5-[16'(*E*)-nonadecenyl]-4,5-dihydroxycyclohex-2-enone from *L. schimperi* and myricetin 3-*O*-α-L-rhamnopyranoside from *L. macrocarpa*.[8,9]

Lannea egregia Engl. & K. Krause, which is synonymous with *Lannea barteri*, is a tree which is referred to as Ekika in Southwest Nigeria and commonly known as fake marula and Ekudan in Yoruba.[12] *L. egregia* has been used traditionally to treat diarrhoea, gastric pains, epilepsy, rheumatism, sores, ulcers and cancer.[13-17] *L. egregia* essential oils, obtained from its leaves, twigs, and stem bark, showed anticancer properties, while the stem bark extracts additionally displayed antioxidant activities.[12,18] Furthermore, a study on the antibacterial properties of *L. egregia's* sesquiterpene-rich essential oils indicated its potential for use in traditional medicine to treat infectious diseases.[7] Ajiboye *et al.*[19] reported that the leaf of this plant, particularly its alkaloid-rich extract, could alter enzyme activity and inhibit digestive enzymes that break down carbohydrates.[19] There are reports on some phytochemical studies and assessment of antioxidant, wound healing and antibacterial activities of *L. egregia*.[15,20] The present work describes, for the first time, the isolation and identification of three flavonoids from the methanolic extract of the leaves of *L. egregia* and their cytotoxic properties against selected cancer cell lines (MCF-7 and HeLa).

Methods

Plant collection and identification

Leaves of *Lannea egregia* Engl. & K. Krause were collected from the Olokemeji Forest Reserve on the outskirts of Ibadan, Nigeria. Mr. Oba of the Forest Research Institute of Nigeria (FRIN) identified the plant. Following the authentication of the plant sample, the herbarium number -FHI 112357 - was assigned. Plant sample was cleaned, shed-dried and pulverized.

Extraction and isolation

The ground plant material (300 g) was extracted using a Soxhlet apparatus, successively, with normal (straight chain) hexane (*n*-hexane), dichloromethane (DCM) and methanol (MeOH), 900 mL each. The extracts obtained were concentrated at 40°C using the BUCHI rotary evaporator (R-100 rotavapor). The extracts were transferred and stored in sample vials. The MeOH extract (1 mg/mL) was subjected to analytical reversed-phase high-performance liquid column chromatography (RP-HPLC) on Agilent 1200 (Infinity Diode Array Detector ± 0.6 µAU/cm noise (60 mm Max-Light cell) or ± 3 µAU/cm noise (10 mm Max-Light cell); 80 Hz; spectra; 8 signals; 190 to 640 nm and Phenomenex Luna column: 150 x 4.6 mm, 5 mm) and compounds were isolated from 100 mg/mL MeOH extract by preparative RP-HPLC using an Infinity Diode Array Detector and preparative Phenomenex Luna C18(2) 100 Ä column (10 mm x 21.2 mm, 10 mm). A standard linear gradient comprising MeOH (0.1% trifluoroacetic acid (TFA)) in water (0.1% TFA) 30% to 100% over 30 min afforded three flavonoids **1**-**3**. Spectroscopic (Ultraviolet and Nuclear Magnetic Resonance) and spectrometric (Liquid Chromatographic-Mass Spectrometry) analyses were carried out to identify the compounds.

Cell lines and cell culture preparation

The human cervical (HeLa) and breast (MCF-7) cancer cell lines used in this study were resurrected from our cell bank, but their stocks were originally obtained from the American Type Culture Collection (ATCC). They were prepared, maintained, and used as previously reported.[21] The cell lines were maintained in an incubator at 37°C in a humidified environment with 5% CO_2 and cultivated in growth media comprising Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% 2 mM L-glutamine, and 1% penicillin-streptomycin solution. To passage the cells, the old medium was removed, the cells were rinsed with 5 mL of PBS, and 5 mL of recombinant trypsin (TrypLE) was added for approximately 60 sec, after which the trypsin was removed and the cultures were incubated for about 3-5 minutes. To get the dissociated cells into a homogenous suspension, the growth media was added to the flask containing the cells, followed by trituration for a few minutes. A plastic hemocytometer (C-Chip NanoEnTek, USA) and microscope-assisted counting were used to calculate the density of the suspension. A 100 µL volume containing 7.5 × 10^3 cells was introduced into every well of transparent 96-well microtitre plates with a flat bottom. Before testing, the plates were incubated for 24 h. An Olympus CKX41 inverted microscope (UK) set to x10 was used to view the cells.

Cytotoxicity assay

The methanolic extract and three purified flavonoids (**1**-**3**) were subjected to cytotoxicity test using the MTT colourimetric assay as previously reported.[21] A cell density of 7.5 × 10^3 cells/well was used for the MCF-7 and HeLa cells. The cells were cultured for 24 h and then treated with *L. egregia* Leaf (LEL). The samples were prepared from a DMSO stock concentration of 100 mg/mL and the concentrations of the methanolic extract used were 1, 10, 50, 100, 200 and 500 µg/mL (the concentrations used for the isolated flavonoids (**1**-**3**) were 12.5, 25, 50, 100, and 200 µM). The working dilutions were prepared using the growth medium, and the final DMSO content was kept to a maximum of 0.1%, which was not toxic to the cells. The dilutions were applied to the cells in triplicate, at 100 µL/well. As a vehicle, negative, and positive controls, DMSO (at the %v/v in the highest sample concentration), growth medium, and the anticancer drug vincristine were employed, respectively. The plates were incubated for 48 h. Then, to assess cytotoxicity, to each well 10 µL of a 5 mg/mL MTT solution was added. The tetrazolium salt was changed into purple formazan crystals by mitochondrial enzymatic action over a 2-hour incubation period. After emptying each well, 100 µL of DMSO was added to dissolve

the purple formazan that had formed. For five minutes, the plates were gently shaken at 95 revolutions per minute. Using a Tecan microplate reader (Tecan, UK), the optical densities (OD) (absorbance values) were measured at 570 nm. Every experiment was carried out two or three independent times (n=2 or n=3).

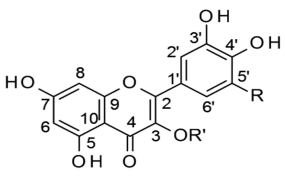
Data presentation and statistical analysis

Values are shown, where relevant, as Mean ± SEM (Standard Error of the Mean). Each treatment was performed either in duplicate or triplicate and the average of the readings was taken. Statistical analyses were done using the GraphPad Prism software (Version 9.3.1) (GraphPad Software, Inc., CA, USA). To determine statistically significant differences between means, Analysis of Variance (ANOVA) followed by a post-hoc test for multiple comparisons was used, with a P-value of less than 0.05 considered statistically significant. The IC_{50} value was determined using GraphPad Prism by fitting the data to the non-linear regression "log [inhibitor] versus response (three parameters)".

Results and Discussion

The dried and ground leaves of *Lannea egregia* were Soxhlet-extracted, successively, using *n*-hexane, DCM and MeOH. The methanolic extract was analysed by reversed-phase analytical HPLC to develop an optimum isolation protocol for the isolation of flavonoids present in the extract. The standard linear gradient comprising 30-100% MeOH in water over 30 min with a flow rate of 1 mL/min was found to be ideal for the separation of the three flavonoids (**1**-**3**) (Figure 1). This method was scaled up for the preparative RP-HPLC-aided isolation of these flavonoids: **1** (2.1 mg; t_R = 13.44 min), **2** (6.8 mg; t_R = 11.09 min) and **3** (3.8 mg; t_R = 15.65 min). While the linear gradient was the same as for the analytical method, the flow rate was 10 mL/min in the preparative run.

The UV-Vis spectra (λ_{max} = 253-258 and 373-377 nm) obtained from the photo-diode-array detector indicated that the purified compounds were flavonoids.[22] It is known that the above absorbance bands are diagnostic for aromatic system (π- π*) and carbonyl (n- π*) as present in flavonols like **1**-**3**. Comprehensive 1D and 2D NMR analyses, including ^{1}H NMR, ^{13}C-DEPT-Q, ^{1}H– ^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC, together with MS data obtained from LC-MS analysis and comparison with respective published data, confirmed the identity of these three known compounds as myricetin (**1**), myricetin 3-*O*-α-rhamnoside (**2**) and quercetin 3-*O*-α-L-rhamnoside (**3**).[22-25] All three flavonoids (**1**-**3**) are reported here from *L. egregia* for the first time. However, myricetin 3-*O*-α-L-rhamnoside (**2**) was previously reported from another species of the genus *Lannea* (*L. macrocarpa*).[9]



Myricetin (**1**) R = OH, R' = H
Myricetin 3-*O*-α-rhamnoside (**2**) R = OH, R' = Rhamnosyl
Quercetin 3-*O*-α-L-rhamnoside (**3**) R = H, R' = Rhamnosyl

Figure 1. Structures of flavonoids isolated from *Lannea egregia*

*Myricetin (**1**):* Yellow crystals; λ_{max} (MeOH-water) 253 nm and 377 nm; ESI-MS (negative ion mode) $[M-1]^-$ *m/z* 317.24 corresponded to the molecular formula of $C_{15}H_{10}O_8$; ^{1}H and ^{13}NMR (Table 1).

*Myricetin 3-O-α-L-rhamnoside (**2**):* Yellow crystals; λ_{max} (MeOH-water) 253 nm and 377 nm; ESI-MS (negative ion mode) $[M-1]^-$ *m/z* 463.08 corresponded to the molecular formula of $C_{21}H_{20}O_{12}$; ^{1}H and ^{13}NMR (Table 1).

*Quercetin 3-O-α-L-rhamnoside (**3**):* Orange amorphous crystals; λ_{max} (MeOH-water) 258 nm and 373 nm; ESI-MS (negative ion mode) $[M-1]^-$ *m/z* 447.77 corresponded to the molecular formula of $C_{21}H_{20}O_{11}$; ^{1}H and ^{13}NMR (Table 1).

The MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay was utilized for assessing the cytotoxicity potential of the MeOH extract of the leaf of *L. egregia* and three flavonoids (**1**-**3**) purified from the extract. The tested samples displayed cytotoxicity against the HeLa and MCF-7 cells (Table 2), which was concentration-dependent (Figure 2 for compounds 1-3). The crude methanolic extract showed a good cytotoxic effect against both HeLa and MCF-7 cells, with IC_{50} values (µg/mL) of 45.3 ± 1.5 and 57.5 ± 0.4, respectively, which was in line with previous studies that reported anticancer potential of this plant.[12,18] Myricetin (**1**; IC_{50} against HeLa and MCF-7 = 63.6 ± 2.5 µM and 97.1 ± 3.7 µM, respectively) and its rhamnoside (**2**; IC_{50} against HeLa and MCF-7 = 63.7 ± 4.5 µM and 91.5 ± 0.5 µM) manifested remarkably similar cytotoxicity, indicating that glycosylation at C-3 of the flavonol skeleton did not produce any significant differential cytotoxicity, albeit a previous report stated that the presence of a rhamnose unit on a triterpenic aglycone could enhance cytotoxicity.[26] However, it should be noted that flavonols and triterpene acids are completely different types of phytochemicals, and any effect of glycosylation on cytotoxicity could usually be different. Previously, it was shown that the cytotoxic property of myricetin (**1**) was mediated through apoptosis, and this compound could enhance chemosensitivity in ovarian cancer cells.[27] Therefore, it can be assumed that in the present study, the cytotoxicity displayed by myricetin (**1**) and its rhamnoside (**2**) against HeLa and MCF-7 cell lines could also have been exerted through apoptosis. However, in a recent publication, it was demonstrated that the anticancer potential of myricetin could be because of its ability to modulate inflammation and signal transduction pathways.[28] Also, myricetin significantly reduced the proliferation of human anaplastic thyroid cancer cells (SNU-80 HATC) by 70% at 100 µM and reduced the cell viability of MCF-7 when used at a maximum concentration of 80 µM.[29-30]

Quercetin rhamnoside (**3**) displayed significant cytotoxicity against both cell lines, with IC_{50} values of 36.5

Table 1. ^{1}H and ^{13}C NMR data (DMSO-d_6) of flavonoids **1-3**, isolated from *Lannea egregia*.

Numbering	^{1}H NMR chemical shifts δ in ppm (coupling constant *J* in Hz)			^{13}C NMR chemical shifts δ in ppm		
	1	**2**	**3**	**1**	**2**	**3**
			Flavonol unit (aglycone)			
2	-	-	-	157.2	157.1	156.9
3	-	-	-	136.2	134.9	134.6
4	-	-	-	176.1	178.2	178.2
5	-	-	-	161.0	161.8	161.7
5-OH	12.5 s	12.4 s	12.4 s	-	-	-
6	6.10 d (2.0)	6.20 d (2.0)	6.20 d (2.0)	98.6	98.3	99.2
7	-	-	-	164.2	164.4	164.6
8	6.30 d (2.0)	6.30 d (2.0)	6.40 d (2.0)	93.7	94.3	94.2
9	-	-	-	156.5	158.0	157.7
10	-	-	-	103.3	104.4	104.5
1'	-	-	-	121.2	120.5	121.2
2'	7.30 s	6.90 s	7.20 d (1.5)	107.6	108.1	115.9
3'	-	-	-	146.6	145.4	145.6
4'	-	-	-	136.2	136.4	148.9
5'	-	-	6.70 d (8.3)	146.6	145.4	116.1
6'	7.30 s	6.90 s	7.1 dd (1.5, 8.3)	107.6	108.1	121.5
			Rhamnosyl unit (glycone)			
Rh-1	-	5.30 d (1.4)	5.41 d (1.3)	-	102.2	102.4
Rh-2	-	4.20 dd (1.6, 3.3)	4.29 dd (1.6, 3.3)	-	71.9	70.5
Rh-3	-	3.80 dd (3.3, 9.6)	3.55 dd (3.3, 9.6)	-	71.6	71.1
Rh-4	-	3.3 d (9.6)	3.10 d (9.6)	-	72.8	71.7
Rh-5	-	3.5 dd (6.3, 9.6)	3.22 dd (6.3, 9.6)	-	70.4	70.9
Rh-6 (CH_3)	-	0.90 d (6.2)	0.85 d (6.0)	-	17.7	17.9

± 2.0 µM against HeLa and 57.9 ± 10.0 µM against MCF-7. It can be mentioned that quercetin, one of the most common flavonols found in plants, was shown to have cytotoxicity against various cancer cell lines, including the breast cancer cell line MCF-7, and it was observed that this flavonol could interact with DNA, arrest the cell cycle and cause tumour regression by activating the mitochondrial pathway of apoptosis.[31] Therefore, it is likely that because of the same structural features present in quercetin and compound **3**, but with the only exception of a rhamnose unit at C-3, flavonoid **3** could also exert its cytotoxicity through the same mechanism as quercetin. The MeOH and the flavonoids (**1**-**3**) were more potent against the HeLa cancer cell line (Table 2).

Conclusion

This is the first report on characterized isolated compounds from the leaf of *Lannea egregia* and the occurrence of flavonols in the leaf. Thus, this plant could be utilized as a potential source of bioactive flavonoids. The methanolic extract as well as the purified flavonoids (**1**-**3**) showed cytotoxicity against HeLa and MCF-7 cell lines, and this finding is in line with the cytotoxicity of plant-derived flavonols reported previously.

Table 2. Cytotoxicity of the methanolic extract and isolated flavonoids (**1**-**3**). Values shown are averages of two or three independent experiments, each run in duplicate or triplicate. The values for the positive control vincristine were 1.4 ± 0.4 µM and 0.5 ± 0.1 µM for HeLa and MCF-7, respectively.[32]

Tested samples	IC_{50} ±SEM (µM, or µg/mL for MeOH)	
	HeLa	MCF-7
MeOH extract	45.3 ± 1.5	57.5 ± 0.4
1	63.6 ± 2.5	97.1 ± 3.7
2	63.7 ± 4.5	91.5 ± 0.5
3	36.5 ± 2.0	57.9 ± 10.1

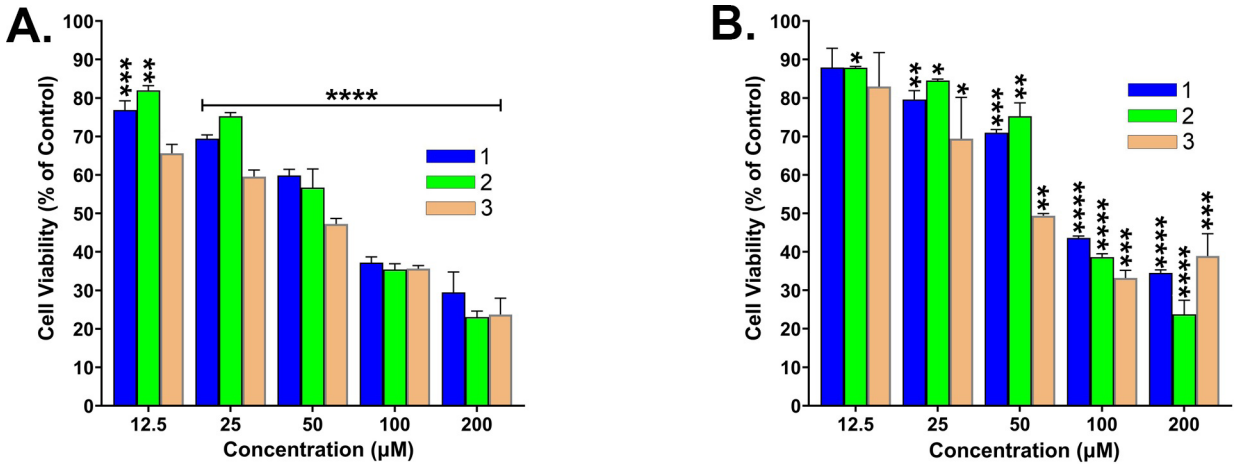


Figure 2. Cytotoxic effects of Compounds 1-3 on (A) the human cervical cancer cell line HeLa, and (B) the human breast cancer cell line MCF-7. Each compound exhibited concentration-dependent toxicity on either cell line. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 with respect to the negative control (the horizontal line on top of Fig. 2A bars from 25-200 μM indicates the value for each bar within its span was significant at ****P<0.0001). Each experiment was run in duplicate or triplicate two or three independent times (n=2-3).

Acknowledgements

Lutfun Nahar gratefully acknowledges the financial support of the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and the Czech Agency Grants - Project 23-05474S and Project 23-05389S.

Author Contributions

Oluwatosin T. Ayodele: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Funding Acquisition, Visualization, Writing – Original Draft, Writing – Review & Editing. Olaoluwa O. Olaoluwa: Conceptualization, Methodology, Resources, Project Administration, Supervision. Saheed O. Benson: Validation, Formal Analysis, Visualization, Writing – Review & Editing. Olapeju O. Aiyelaagbe: Conceptualization, Methodology, Resources, Project Administration, Supervision. Lutfun Nahar: Validation, Formal Analysis, Project Administration, Visualization, Supervision, Writing – Review & Editing. Amos A. Fatokun: Conceptualization, Methodology, Validation, Formal Analysis, Resources, Project Administration, Visualization, Supervision, Writing – Review & Editing. Satyajit D. Sarker: Conceptualization, Methodology, Validation, Formal Analysis, Resources, Project Administration, Visualization, Supervision, Writing – Review & Editing.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Data

Supplementary data can be downloaded at: https://doi.org/10.34172/PS.2024.26.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209-49. doi:10.3322/caac.21660
2. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends- An update. Cancer Epidemiol. Biomarkers Prev. 2016;25(1):16-27. doi: 10.1158/1055-9965.EPI-15-0578
3. Li T, Kung HJ, Mack PC, Gandara DR. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. J Clin Oncol. 2013;31:1039-49. doi:10.1200/jco.2012.45.3753
4. Ikhimiukor OO, Odih EE, Donado-Godoy P, Okeke IN. A bottom-up view of antimicrobial resistance transmission in developing countries. Nat Microbiol. 2022;7(6):757-65. doi:10.1038/s41564-022-01124-w
5. Reynolds AR, Moschetta M, Yohannes AR, Walcott F, Ashford M, Szucs Z, et al. A view on drug development for cancer prevention. Cancer Discov. 2023;13(5):1058-83. doi:10.1158/2159-8290.cd-22-0776
6. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, Sarkar S. Drug resistance in cancer: an overview. Cancers. 2014;6(3):1769-92. doi:10.3390/cancers6031769
7. Ogundajo AL, Ewekeye T, Sharaibi OJ, Owolabi MS, Dosoky NS, Setzer WN. Antimicrobial activities of sesquiterpene-rich essential oils of two medicinal plants, Lannea egregia and Emilia sonchifolia, from Nigeria. Plants. 2021;10:488. doi:10.3390/plants10030488
8. Okoth DA, Koorbanally NA. Cardanols, Long chain cyclohexenones and cyclohexenols from Lannea schimperi (Anacardiaceae). Nat Prod Comm. 2015;10(1):103-6. doi:10.1177/1934578X1501000126
9. Imran M, Saeed F, Hussain G, Imran A, Mehmood Z, Gondal TA, et al. Myricetin: A comprehensive review on its biological potentials. Food Sci Nutr. 2021;9(10):5854-68. doi:10.1002/fsn3.2513
10. Oumarou, MR, Zingue S, Bakam BY, Ateba SB,

Foyet SH, Mbakop FTT. Njamen D. *Lannea acida* A. Rich. (Anacardiaceae) ethanol extract exhibits estrogenic effects and prevents bone loss in an ovariectomized rat model of osteoporosis. Evid Based Complement Alternat Med. 2017;2017:7829059. doi:10.1155/2017/7829059

11. Bazongo P, Bassolé I, Nielsen S, Hilou A, Dicko M, Shukla V. Characteristics, composition and oxidative stability of *Lannea microcarpa* seed and seed oil. Molecules. 2014;19(2):2684-93. doi:10.3390/molecules19022684
12. Ayodele OT, Olaoluwa OO, Aiyelaagbe OO, Flamini G, Ogbole OO, Akinleye TE, et al. Biochemical constituents and anticancer activities of the essential oils of *Lannea egregia* Engl. & K. Krause. J Complement Alt Med Res. 2023;21:33-42. doi:10.9734/jocamr/2023/v21i4446
13. Burkill HM. The useful plants of west tropical Africa. Econ Bot. 1986;40:176 doi:10.1007/BF02859140
14. Koné WM, Atindehou KK, Dossahoua T, Betschart B. Anthelmintic activity of medicinal plants used in northern Côte d'Ivoire against intestinal helminthiasis. Pharm Biol. 2005;43:72-8. doi:10.1080/13880200590903408
15. Rafiu BO, Sonibare AM, Adesanya, EO. Phytochemical screening, antimicrobial and antioxidant studies of *Lannea egregia* Engl. and K. Krause (Anacardiaceae) stem bark. J Med Plants Econ Dev. 2019;3(1):a62. doi:10.4102/JOMPED.V3I1.62
16. Neuwinger HD. African Traditional Medicine: A Dictionary of Plant Use and Applications. Stuttgart: Medpharm Scientific Publishers; 2000.
17. Soladoye MO, Amusa NA, Raji-Esan SO, Chukwuma EC Taiwo AA. Ethnobotanical survey of anti-cancer plants in Ogun State, Nigeria. Ann Biol Res. 2010;1:261-73.
18. Akoro SM, Omotayo MA, Ogundare OC, Akpovwovwo SA, Bello GP. Phytochemical analysis, antioxidant and cytotoxic activity of *Lannea egregia* Engl. & K. Krause stem bark extracts. Pharm Sci Res. 2022;9:149-56. doi:10.7454/psr.v9i3.1277
19. Ajiboye BO, Oyinloye BE, Lawal OE, Oyeleke AS, Balogun BA, Falode JA, et al. Alkaloid extract of *Lannea egregia* inhibits carbohydrate digestive enzymes and modulates gluconeogenesis and nucleotide-hydrolyzing enzyme activity in oxidative isolated psoas muscles injury. Nutrire. 2022;47:29. doi:10.1186/s41110-022-00179-3
20. Idowu P, Ekemezie C, Olaiya C. Phytochemical, antioxidant and antimicrobial studies of *Lannea egregia* Engl. & K. Krause (Anacardiaceae) extracts and chromatographic fractions. J Phytomed Ther. 2020;19:348-63. doi:10.4314/jopat.v19i1.4
21. Omondi RO, Bellam R, Ojwach SO, Jaganyi D, Fatokun AA. Palladium (II) complexes of tridentate bis(benzazole) ligands: Structural, substitution kinetics, DNA interactions and cytotoxicity studies. J Inorg Biochem. 2020;210:111156. doi:10.1016/j.jinorgbio.2020.111156
22. Mabry TJ, Markham KR, Thomas MB. The Systematic identification of flavonoids. Berlin, Heidelberg: Springer; 1970.
23. He D, Gu D, Huang Y, Ayupbek A, Yang Y, Aisa HA, et al. Separation and purification of phenolic acids and myricetin from black currant by high-speed countercurrent chromatography. J Liq Chromatogr Relat Technol. 2009;32:3077-88. doi:10.1080%2F10826070903320756
24. Motlhatlego KE, Abdalla MA, Leonard CM, Eloff JN, McGaw LJ. Inhibitory effect of Newtonia extracts and myricetin 3-O-rhamnoside (myricitrin) on bacterial biofilm formation. BMC Complement Med Therap. 2020;20:358. doi:10.1186/s12906-020-03139-4
25. Zhang Y, Wang D, Yang L, Zhou D, Zhang J. Purification and characterization of flavonoids from the leaves of *Zanthoxylum bungeanum* and correlation between their structure and antioxidant activity. PLoS ONE. 2014;9:e105725. doi:10.1371/journal.pone.0105725
26. Sylla B, Lavoie S, Legault J, Gauthier C, Pichette A. Synthesis, cytotoxicity and anti-inflammatory activity of rhamnose-containing ursolic and betulinic acid saponins. RSC Adv. 2019;9:39743-57. doi:10.1039/c9ra09389c
27. Zheng AW, Chen YQ, Zhao LQ, Feng JG. Myricetin induces apoptosis and enhances chemosensitivity in ovarian cancer cells. Oncol Lett. 2017;13:4974-8. doi:10.3892/ol.2017.6031
28. Rahmani AH, Almatroudi A, Allmailem KS, Alwanian WM, Alharbi BF, Alrumaihi F, et al. Myricetin: A significant emphasis on its anticancer potential via the modulation of inflammation and signal transduction pathways. Int J Mol Sci. 2023;24:9665. doi:10.3390/ijms24119665
29. Jiang M, Zhu M, Wang L, Yu S. Anti-tumor effects and associated molecular mechanisms of myricetin. Biomed Pharmacother. 2019;120:109506. doi:10.1016/j.biopha.2019.109506
30. Jiao D, Zhang XD. Myricetin suppresses p21-activated kinase 1 in human breast cancer MCF-7 cells through downstream signaling of the β-catenin pathway. Oncol Rep. 2016;36:342-8. doi:10.3892/or.2016.4777
31. Srivastava S, Somasangara RR, Hegde M, Nishana M, Tadi SK, Srivastava M, et al. Quercetin, a natural flavonoid interacts with DNA, arrests cell cycle and causes tumor regression by activating mitochondrial pathway of apoptosis. Sci Rep. 2016;6:24049. doi:10.1038/srep24049
32. Adekunle YA, Samuel BB, Nahar L, Fatokun AA, Sarker SD. Cytotoxic triterpenoid saponins from the root of *Olax subscorpioidea* Oliv. (Olacaceae). Phytochemistry. 2023;215:113853. doi:10.1016/j.phytochem.2023.113853