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Chemical Characterization of an Ethyl Acetate Extract of *Annickia polycarpa*

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ABSTRACT

Context: Medicinal plants have played an essential role in the health and well-being of human societies. Thanks to their natural active compounds, they help relieve various ailments, strengthen the body, and prevent certain diseases. The aim of this study was to to characterize the chemical compounds *Annickia polycarpa,* a plant used in traditional Ivorian medicine to treat solid tumors. **Methods**: An extraction was performed with ethyl acetate as the solvent and Chemical composition of active extract was analyzed by HPLC-PDA-ESI-MS/MS **Results**: Putative compound identification showed that this extract contained principally acetogenin derivatives.**Conclusion and outlook**: The identified compounds are responsible for the plant's various properties, particularly its anticancer activity.

Keywords: medicinal plants; traditional Ivorian medicine; identification

INTRODUCTION

In Côte d'Ivoire, a multitude of plant species are employed for the treatment of tumours. One such example is *Annickia polycarpa* (DC.) Engl. & Diels (syn. *Annickia polycarpa* (DC.) Setten & Maas ex I.M.Turner). This is a medium sized tree that can reach up to 20 meters in height and belongs to the Annonaceae family. The bark is smooth, somewhat rough or cracked, and varies in color from green to blackish (Ambe *et al*., 2016). The interior of the bark is fibrous and exhibits a bright yellow hue. The pharmacological properties of *A. polycarpa* extracts have been observed to vary depending on the plant organ and the type of extract. As reported by research conducted by Ambe *et al*, (2016) in Côte d'Ivoire, the ethanolic extract of bark displays antifungal activity against *Candida albicans* and cytotoxic activity against human HFF cells. Furthermore, Anosa *et al*. (2014), Ajali (2020) and Kamanzi *et al*., (2004) have demonstrated that it possesses antibacterial (Gram +/-), antiplasmodial and antitrypanosomial properties *in vitro*. The main objective of the present work is the chemical characterization of the compounds present in the ethyl acetate extract of *Annickia polycarpa* leaves.

Materials and methods

Plant material

Leaves of *A. polycarpa* were collected in Daloa, Côte d'Ivoire, during February 2023. The botanical identification was carried out at the National Floristic Center of Abidjan. The herbarium number assigned to the plant is UCJ001189. Following the harvesting process, the leaves were dried in a shade environment at room temperature. The material was then pulverized and meticulously preserved. Subsequently, an extraction was performed by macerating 20 g of Annickia leaf powder in 250 mL of ethyl acetate for 24 hours."

Methods

Identification of the active compounds

HPLC-PDA-HRMS/MS analyses were performed on an HPLC-PDA Agilent 1200 system coupled with an Agilent Accurate Mass QToF 6520 mass spectrometer (Agilent, USA), controlled with a Mass Hunter software. The chromatographic separation was done on a 5 µm particles Uptisphere C18, 250 x 4.6 mm column. Stock solutions of crude extract were prepared in methanol at 10 mg/mL concentration and the injection volume was 10 µL. The mobile phase consisted of 0.1% of formic acid in water (solvent A) and 0.1% of formic acid in acetonitrile (solvent B). The optimized gradient for separation is as follows: the percentage of solvent A % (time in min): 80 % (0-10min), 0 % (40-50 min), 80 % (55-65 min) and solvent B (time in minutes) was as follows: 10 % (0-20 min), 60 % (40 min), 100 % (45-55 min), 10 % (60-65 min). The solvent flow rate was set at 0.8 mL/min, and the injection volume was 0.8 µL (Azi, 2024). Detection wavelengths for chromatograms were set between 190 and 500 nm. HRMS analyses were performed in ESI positive and negative modes with the following inlet conditions for both modes: ESI gas temperature of 340 °C, nebulizer of 30 psig, MS TOF fragmentor of 120 V, skimmet of 68 V and a collision energy of 30 eV. The MS/MS events were performed on the most abundant ions detected in full MS scans. The putative identification of compounds present in each sample was achieved by comparison of chemical formula of isolated compounds from Annonaceae family spp. (Lotus data base www.lotusnaturalproducts.net). MS/MS fragmentation data were faced with putative structures.

Results

The extraction carried out from 20 g of powder using ethyl acetate allowed us to obtain a yield of 5.24 %.

Identification of chemical compounds

In positive mode (more informative chromatogram) were detected a total of 32 major compounds (Table II), principally C35-acetogenin derivatives. The first eluted compound was putatively identified as C35-acetogenin-γ-lactone derivative , which gave a pseudo-molecular ion $[M+Na]^+$ at *m/z* 651 and fragmented at *m/z* 539, corresponding to the loss of the γ-lactone group $[M-C_6H_8O_2+Na]^+$. Same fragmentation pattern was observed for compounds 2-4, 6, 10-13, 14, 17-30. The loss of this group is characteristic of acetogenins (ref 10.1021/np960487i) (Figure 8) derivatives and their presence in the ethyl acetate extract of *A. polycarpa* are consistent with previous phytochemical studies of Annonaceae plants (ref 10.3390/molecules27113462).

Table 1. Compounds identified in the ethyl acetate extract of *Annickia polycarpa*

Code	Temps de rétention (min)	Spectroscopie de masse	Addiction	Formule chimique	MS/MS Fragmentation	Identification Putative
1	34.76	651.4424	M+Na	$C_{35}H_{64}O_9$	394.1769, 540.3675	C35-Acetogenin-γ-lactone derivative I
		629.4604	M+H			(idée: Murihexocin isomer I)
2	35.35	651.443	M+Na	$C_{35}H_{64}O_9$	539.3768	C35-Acetogenin-γ-lactone derivative II
		629.4603	M+H			(idée: Murihexocin isomer I)
3	35.56	651.4438	M+Na	$C_{35}H_{64}O_9$	539.3889	C35-Acetogenin-γ-lactone derivative III
		629.4611	M+H			(idée: Murihexocin isomer I)
4	35.89	651.4441	M+Na	$C_{35}H_{64}O_9$	539.385	C35-Acetogenin-γ-lactone derivative IV
		629.4602	M+H			(idée: Murihexocin isomer I)
5	36.53	651.4424	M+Na	$C_{35}H_{64}O_9$	438.3126, 541.4047	C35-Acetogenin-γ-lactone derivative V
		629.4604	M+H			(idée: Murihexocin isomer I)
6	37.27	635.4484	M+Na	$C_{35}H64O_8$	523.4106	C35-Acetogenin-γ-lactone derivative VI
		613.4681	M+H			
7	38.18	365.1367	M+Na	$C_{20}H_{22}O_5$	n-f	n-d

		343.154	M+H			
		707.2818	2M+Na			
8	38.44	365.136	M+Na	$C_{20}H_{22}O_5$	n-f	n-d
		343.154	M+H			
		707.2817	2M+Na			
		388.2106	+46?			
10	39.35	635.4501	M+Na	$C_{35}H_{64}O_8$	523.3823, 297.2454	C35-Acetogenin-γ-lactone derivative VII
		613.4677	M+H			(Idée: Annomuricin isomer I)
11	39.63	635.4493	M+Na	$C_{35}H_{64}O_8$	523.3823, 297.2454	C35-Acetogenin-γ-lactone derivative VII
		613.4656	M+H			(Idée: Annomuricin isomer II)
		647.4133	+35?			
12	39.91	635.4492	M+Na	$C_{35}H_{64}O_8$	523.4022	C35-Acetogenin-γ-lactone derivative IX
		613.4656	M+H			
		647.4133	+35?			
13	40.65	635.4488	M+Na	$C_{35}H_{64}O_8$	523.4022	C35-Acetogenin-γ-lactone derivativeX
		613.4661	M+H			
		647.4123	+35?			
14	41	635.4433	M+Na	$C_{35}H_{64}O_8$	235.0919, 421.3301, 521.3857	C35-Acetogenin-γ-lactone derivative XI
		613.4662	M+H			
15	41.26	635.4495	M+Na	$C_{35}H_{64}O_8$	401.2821, 523.3932	C35-Acetogenin-γ-lactone derivative XII
		613.4666	M+H			
16	41.56	425.215	M+Na	$C_{20}H_{34}O_8$	n-f	n-d
		403.2324	M+H			
		827.4406	2M+Na			
		448.2899	+46?			
17	41.91	591.4229	M+Na	$C_{33}H_{60}O_7$	479.359	C33-Acetogenin-γ-lactone derivative I
		569.4401	M+H			
18	42.31	633.4338	M+Na	$C_{35}H_{62}O_8$	235.0917, 421.3271, 521.3807	Annoglaxin isomer I
19	42.62	633.4335	M+Na	$C_{35}H_{62}O_8$	235.0859, 421.3264, 521.3500	Annoglaxin isomer II
20	42.94	633.4329	M+Na	$C_{35}H_{62}O_8$	521.3755	C35-Acetogenin-γ-lactone derivative XIII
21	43.23	633.4327	M+Na	$C_{35}H_{62}O_8$	420.3152, 521.3881	Annoglaxin isomer III
		617.4362	-16			
22	43.62	619.4547	M+Na	$C_{35}H_{64}O_7$	507.404	C35-Acetogenin-γ-lactone derivative XVI
		597.4725	M+H			(Idée : Annonacin isomer I)

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23	44.54	617.4385	M+Na	$C_{35}H_{62}O_7$	442.3253, 505.3696	C35-Acetogenin-γ-lactone derivative XV
24	45.12	619.4551	M+Na	$C_{35}H_{64}O_7$	507.4036	C35-Acetogenin-γ-lactone derivative XVI
		597.4727	M+H			(Idée : Annonacin isomer II)
25	45.83	617.4397	M+Na	$C_{35}H_{62}O_7$	421.3190, 505.3893	C35-Acetogenin-γ-lactone derivative XVII
26	47.12	619.4547	M+Na	$C_{35}H_{64}O_7$	507.3877	C35-Acetogenin-γ-lactone derivative XVI
		597.4716	M+H			(Idée : Annonacin isomer III)
27	47.53	619.4547	M+Na	$C_{35}H_{64}O_7$	507.3882	C35-Acetogenin-γ-lactone derivative XVI
		597.4721	M+H			(Idée : Annonacin isomer IV)
28	47.91	617.4384	M+Na	$C_{35}H_{62}O_7$	505.3873	C35-Acetogenin-γ-lactone derivative XX
		595.4578	M+H			
29	48.61	619.4543	M+Na	$C_{35}H_{64}O_7$	507.3967	C35-Acetogenin-γ-lactone derivative XVI
		597.4738	M+H			(Idée : Annonacin isomer V)
30	50.1	617.4396	M+Na	$C_{35}H_{62}O_7$	505.3837	C35-Acetogenin-γ-lactone derivative XXI
31	50.99	601.4429	M+H?	$C_{37}H_{60}O_6$	n-f	n-d
32	52.56	583.4337	M+H?	$C_{37}H_{58}O_5$	n-f	n-d

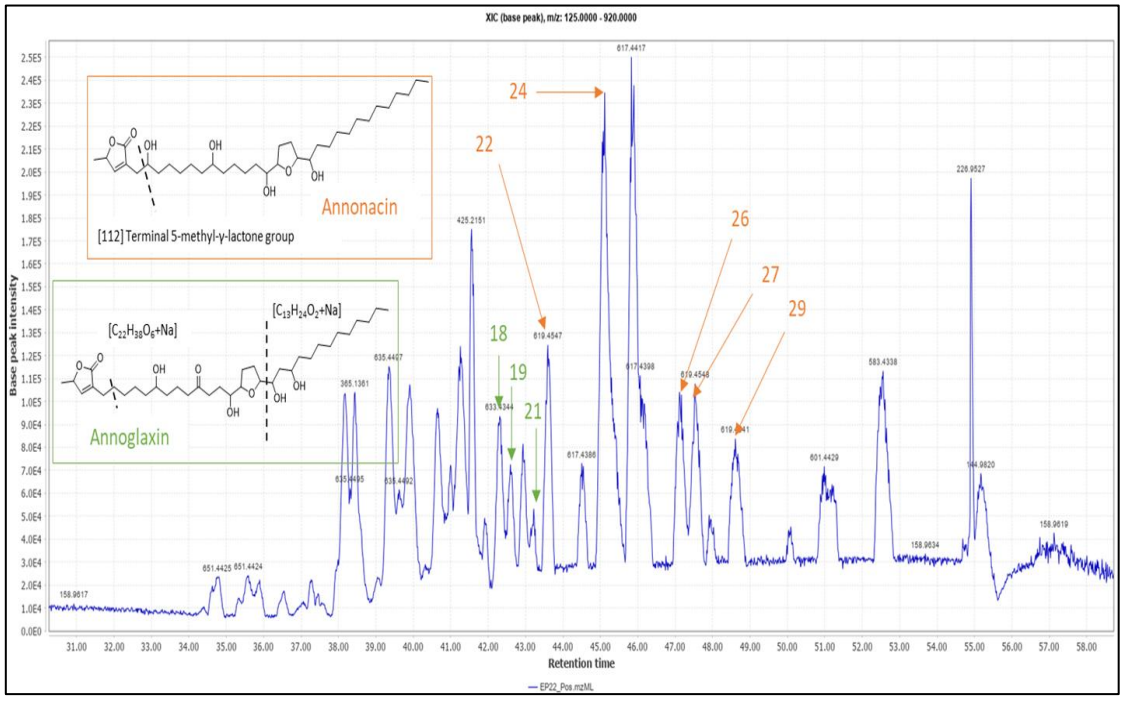

Figure 1: HPLC-HRMS/MS chromatogram of ethyl acetate extract of *A. polycarpa* in positive mode showing the C35-acetogenin-γ-lactone derivatives and characteristic loss of 112 amu

The structures of these compounds identified at different retention times are:

- **Compounds 18, 19 and 21**

The chemical compounds under investigation have the following chemical formula: $C_{35}H_{62}O_8$+Na. Their respective retention times were determined at 42.31; 42.62 and 43.23 minutes. The molecular weight of the compound was calculated to be 633.4327 g/mol (Figure 13).

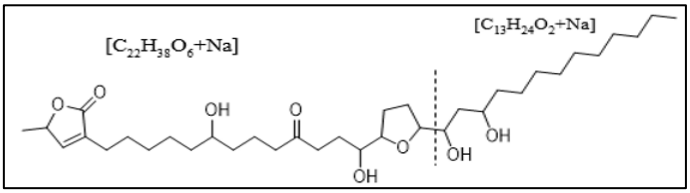

Figure 2: Structure of Annoglaxine isomer

$C_{35}H_{62}O_8$ + Na of molar mass 633.4327

- **Compounds 22, 24, 26, 27 and 29**

The chemical compounds, which possess a chemical formula of $C_{35}H_{64}O_7$ were obtained at times of 43.62; 45.12; 47.12; 47.53 and 48.61 minutes, respectively. Their molecular weight 619.4547 g/mol, and they were isolated from *Annona muricata* (Figure 14).

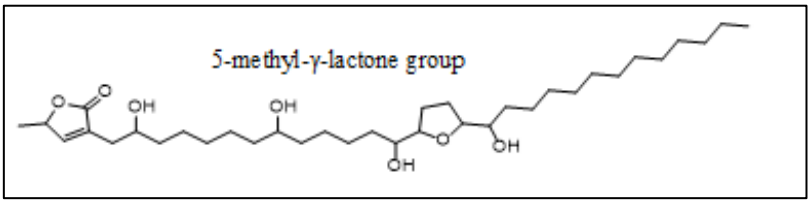

Figure 3: Structure of Annonacin

$C_{35}H_{64}O_7$ + Na with a molar mass of 619.4547 g/mol

DISCUSSION

The compounds identified in the ethyl acetate leaf extract of *A. polycarpa* are believed to be Annonaceous acetogenins, specifically annonacin and annoglaxin. Acetogenins, or polyketides, are metabolites derived from fatty acids. They have a long aliphatic chain of 35–38 carbons linked to a g-lactone ring, terminally substituted by β-unsaturated methyl, with tetrahydrofurans (THF) located along the hydrocarbon chain (Mutakin *et al*., 2022). They represent a key group of compounds found in the Annonaceae family (Shi *et al*., 2020), which consists of plants traditionally used in medicine for the treatment of cancer and various other diseases (Herrera *et al*., 2019). Their diverse properties including antitumor, cytotoxic, antimalarial, vermifuge, pesticide, antiviral, and antimicrobial activities offer a wide array of potentially valuable applications (Alkofahi *et al.,* 1988 ; Li *et al*., 2008 ; Jerry, 2008). In this study, two acetogenins were identified.

First Annonacin, previously isolated from other species, is known to be an inhibitor of mitochondrial complex I (NADH-dehydrogenase) (Potts *et al*., 2012). Numerous studies have demonstrated that annonacin possesses anti-inflammatory, anti-ulcer, wound-healing, hypoglycemic, antitumor, cytotoxic, and antioxidant activities (Roduan *et al.,* 2019 ; Zine, 2018). Furthermore, preliminary studies have shown that this compound, extracted from *Annona muricata* seeds, exhibits antiproliferative effects on EC cell lines (endometrial adenocarcinoma), with an IC_{50} value of 4.62 µg/mL, and induces cell death through apoptosis (Yap *et al*., 2017).

Next, Annoglaxin, for its part, is a molecule that also exhibits cytotoxic activity (Xiang et al., 2010). In the study conducted by Liu *et al*. in 1999, this compound was isolated from the ethanolic leaf extract of *Annona glabra* (Annonaceae). Its identification in both the ethyl acetate leaf extract of *Euphorbia polycarpa* and the ethanolic leaf extract of *Annona glabra* may be explained by the fact

that these two plants belong to the same botanical family. Subsequently, the tests performed by Liu et al. demonstrated that annoglaxin possesses significant cytotoxic activity against the human breast carcinoma cell line (MCF-7) (Liu *et al*., 1999), which could account for the observed toxicity of *A. polycarpa* on this cell line**.**

Conclusion

The annoglaxin and annonacin molecules identified in the extract support these biological activities.

Together, these results demonstrate that *A. polycarpa* is a plant rich in anticancer compounds, supporting its potential as a source of therapeutic agents for cancer treatment.

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Conflict of Interest Statement: All authors have nothing else to disclose.