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STRUCTURAL IDENTIFICATION OF ISOLATED MOLECULES OF *PARKIA BIGLOBOSA* (MIMOSACEAE) A PLANT USED IN TRADITIONAL MEDICINE IN BENIN

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ABSTRACT

This study was undertaken in the perspective of contributing to the valorization of the plants of the Beninese flora. To do so, we isolated and purified by a series of chromatographic methods two pure compounds M1 and M2 from the fraction of ethyl acetate of the hydro ethanolic extract of the dried leaves of Parkia biglobosa (Mimosaceae). The spectrometric analyses carried out, namely mass spectrometry, magnetic resonance of the proton and carbon, allowed the identification of the two molecules which are all acids. These are 4-methoxybenzoic acid (anisic acid) and 5-O-(3,4-dihydroxycinnamoyl) -L-quinic acid (chlorogenic acid) respectively M1 and M2. The added value of this work lies in the fact that these two molecules have never been identified before in this plant and therefore constitute an avenue for the development of a therapeutic arsenal to fight recurrent microbial infections

KEYWORDS: *Parkia biglobosa* (Mimosaceae), Isolation, Chromatographic, Methods, Spectrometric analysis.

INTRODUCTION

Parkia biglobosa (Mimosaceae) is a tree of 10 m to 15 m high with an umbrella-like habit, with alternate bipinnate leaves traditionally used in febrile states and bronchitis for the leaves in decoction. The crushed leaves applied to the lips would quickly make fever blisters disappear. The slightly roasted leaves, then crushed, are applied to burn wounds. The decoction of the bark used in gargle and in mouthwash also calms the toothaches.

Herbal remedies are an alternative in primary care systems and thus a promising avenue for the development of traditionally improved drugs. The ethyl acetate fraction was found to be active on some bacteria in the hydroethanol extract of Parkia biglobosa (Tokoudagba et al 2022). It is then essential to search for molecules responsible for this microbial activity. It is within this framework that this work was initiated and generally aims at isolating and identifying some bioactive molecules from the ethyl acetate fraction of this plant.

MATERIAL AND METHOD

1- Plant material

The plant material consists of dried leaves of *Parkia biglobosa* harvested in December 2020 in Abomey-Calavi and identified at the National Herbarium of the University of Abomey Calavi. The leaves of the harvested plant were washed and then dried at room temperature in a ventilated room of the Pharmacognosy laboratory for three weeks before being reduced to powder

2- Preparation of the crude extract

The extraction was done from the dried leaves of *Parkia biglobosa* by mixing 50g of powder in 500 ml of hydro ethanolic mixture (40V/60V respectively) for 48 hours. After respective filtration on Whatman paper N°1 the filtrates obtained were evaporated using a rotary evaporator at 40°C. The residues of this filtrate were dried in the oven for 48 hours at 40°C to obtain the dry extractsteria of the hydroethanolic extract of *Parkia biglobosa*. (Hougbeme et al. 2014).

3- Liquid-liquid fractionation

The liquid-liquid extraction is performed by intimate contact of the solvent with the solution in a separating funnel. The separation of the phases is obtained by gravimetric or centrifugal decantation after agitation of the whole. The solution consists of the hydroethanolic crude extract dissolved in 50 mL of distilled water. We used successively during the extraction 500

mL of cyclohexane, dichloromethane, ethyl acetate and butanol. The different fractions (phase resulting from the operation containing the extracted solutes) collected are evaporated in the rotavapor. at 40°C.

4- Purification and Isolation

We performed a fractionation of the ethyl acetate extract and its subfragments by the atmospheric pressure column liquid-solid chromatography (APC) technique. The stationary phases we used were successively R P 18 silica gel (40-63µm); normal silica gel (60 PF 254) and Sephadex LH 20 gel. These different gels were solubilized in Methanol (30g in 150 mL of methanol) and then poured into a glass column. The eluent is under atmospheric pressure, enters at one end and exits at the other. It can be a single solvent for conditioning or a mixture of solvents for the different gradients. The chromatographic partitioning conditions for the ethyl acetate fraction and its subfragments are shown below:

Dichloromethane :1OO% (conditioning of the colonne)

Dichloromethane - Ethyl acetate: 90 - 10

Dichloromethane - Ethyl acetate: 70 - 30

Ethyl acetate - MeOH : 50 - 50

Ethyl acetate - MeOH: 20 - 80

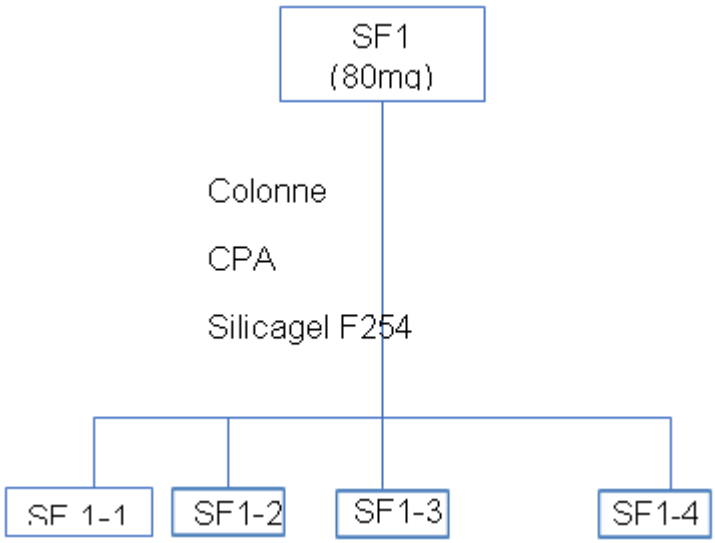
MeOH 100%

Elution: 200 mL of solvent gradients

Collection rate: 5mL/tube at 1 drop / second

Deposition: 200 mg of solubilized fraction in 10 mL MeOH

The analysis of the obtained sub-fractions was carried out by thin layer chromatography with ethyl acetate/MeOH/H2O(v/v/v) 81-11-8 as mobile phase and alcoholic potash at 10% as developer, which revealed the presence of phenolic acids, flavonoids and quinones (Bruneton 1999). The sub-fractions regrouped after the analytical TLC were submitted to further fractionations on CPA. Fractions at the end of purification were run on an SPE column with Sephadex gel for exclusion chromatography that separates compounds based on size and molecular weight (Houngbeme et al, 2015). Pictures 1 and 2 below describe the steps of two major subfragments collected and from which we were able to isolate the molecules.



Picture 1: Diagrams showing the purification steps of the SF1 sub-fraction.

The selected S/F1 fraction was further purified on F 254 silica gel and gave 4 sub-fractions SF-1; SF-2; SF-3; SF-4

The SF-1 sub-fraction reveals two well separated spots at UV 254 nm. Thus we applied preparative TLC to scrape the majority spots. The scraped majority spots are then separated by Exclusion Chromatography (Picture 2)

RESULTS

1. Extraction yield

Table 1: Extraction yield.

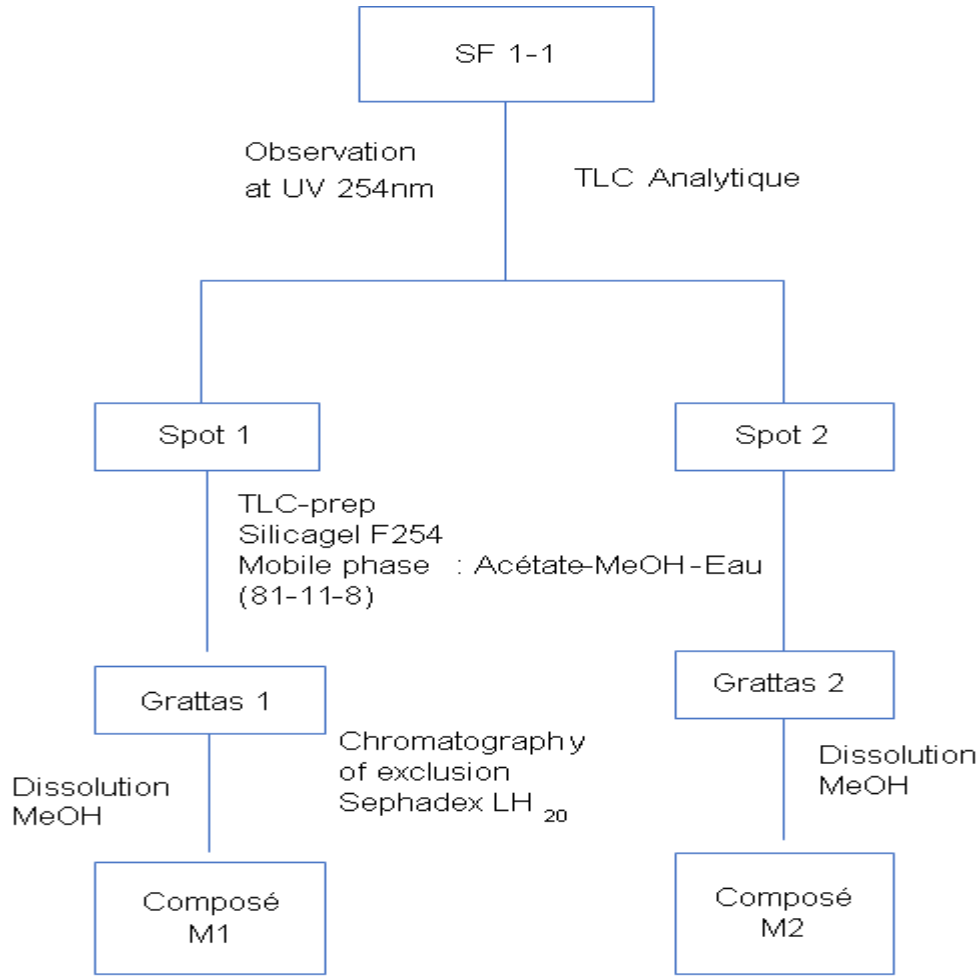
Plants material	Extract	Weight	Yield
Crude extract (20g)	Extract C_6H_{12}	0,25 g	1,25%
	Extract CH_2CL_2	0,29 g	1,45%
	Extract AcOEt	3,58 g	17,9%
	Extract BuOH	3,4 g	17%
	Extract Aqueous	9,25 g	46,2%

2. Collection of sub-fractions

The different sub-fractions are summarized in the following table :

Table 2: Distribution of tubes collected after TLC and Revelation.

Sub - Fractions	Grouped Tubes	Weight(mg)
S/F1	T 1-13	80
S/F2	T 14- 20	50
S/F3	T 21-40	20
S/F4	T 41 -60	14
Total / Yield		119/59,5%



Picture 2: Diagrams showing the purification steps of the SF1-1 sub-fraction.

3. Structure of the isolated molecules

The treatment of information from different spectra, has allowed to elucidate the structure of isolated compounds.

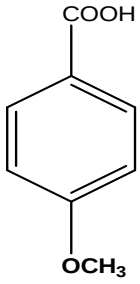
Spectrometric data of compound M1

- **MS (m/z):** 153,157 ($[M+H]^+$); 122,157 ($[M+H]^+$-OCH_3) ; 92,157 ($[M+H]^+$-COOH).
- **1H NMR (CD3OD, 400MHz, δ in ppm)**: δ 6,97 (d, H-aromatique); δ 7,96 (d, H-aromatique); δ 3,84 (S, H-méthoxy); δ 3,34 (dd, H-méthylène); δ 10,56 (S, H-carboxylique).
- **13C NMR (CD3OD, 100MHz, δ in ppm):** δ 170,028 (-COOH); δ 165,177 (O-C aromatique); δ 132,943 (C aromatique); δ 114,814 (C aromatique); δ 56,110 (C-méthoxy); δ 49,313 (C-méthylène)

The mass spectrum of compound M1 shows that the gross formula of compound M1 is C9H10O3 which corresponds to the molecular weight of 152, 157 g/mol. The different major fragments show the presence of methoxy and COOH groups.

The aromatic protons have a chemical shift between 6 and 7 ppm (Houngbeme et al., 2015; Harbone,1993, Fatondji et al., 2010). The H NMR spectrum shows 4 aromatic hydrogens with identical chemical shifts two by two. This means that the aromatic compound is para disubstituted. The 13C NMR spectrum confirms the presence of aromatic carbons, carboxyl group carbon and methoxy carbon.

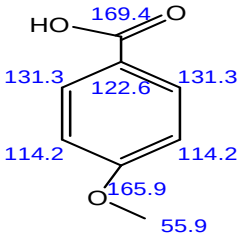
All this information is in agreement with the structure:



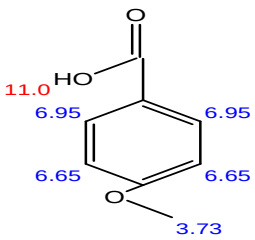
M1

In order to confirm this structure, we performed the Chemdraw simulation of the spectra of M1

ChemNMR C-13 Estimation



ChemNMR H-1 Estimation



We notice that the spectral data obtained by simulation are very close to the recorded experimental data.

The compound M1 is consequently the 4-methoxybenzoic acid (anisic acid). It is a beige colored compound and comes in the form of a powder.

Spectrometric data of compound M2

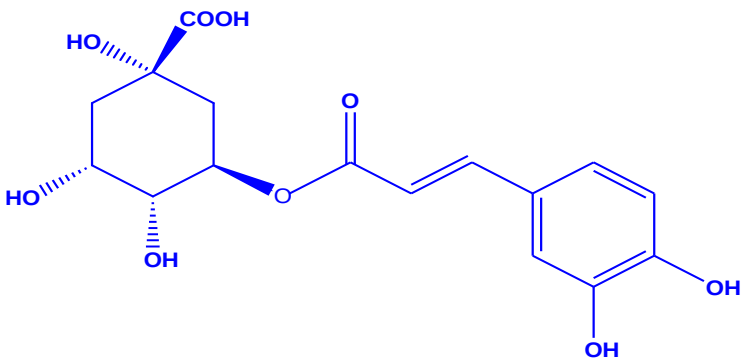
- **MS (m/z):** 355,306 ($[M+H]^+$); 338,306 ($[M+H]^+$-OH); 294,306 ($[M+H]^+$-COOH).
- **1H NMR (CD3OD, 400MHz, δ in ppm):** δ 2,21-2,24 (m, H méthylène cyclohexane substitué); δ 3,30-3,34 (m, H méthyne cyclohexane substitué); δ 4,84 (S, pic du solvant); δ 5,31-5,35 (dd, H-phénolic); δ 6,94-6,96 (d, H-aromatic): δ 7,57 (d, H-éthylénic); δ 10,85 (S, H-carboxyle)
- **13C NMR (CD3OD, 100MHz, δ in ppm):** δ 177,310 (-COOH) ; δ 168,879 (COO) ; δ 146,903-149,689 (C phénolic) ; δ 115,35-127,924 (C aromatic) ; δ 71,46-76,321 (CH-methyne cyclohexane) ; δ 38,34-49,992 (CH2-méthylène cyclohexane)

The mass spectrum of compound M2 shows that the gross formula of compound M2 is C16H18O9 which corresponds to the molecular weight of 354.306 g/mol. The different major fragments show the presence of hydroxy, phenol and carboxylic acid groups.

The H NMR spectrum shows methyne and methylene hydrogens of cyclohexane and aromatic hydrogens.

The 13C NMR spectrum confirms the presence of aromatic, phenolic, carboxylic and ethylenic carbons. (Rajput AP and Rajput T A. 2012 ; Portet, 2007)

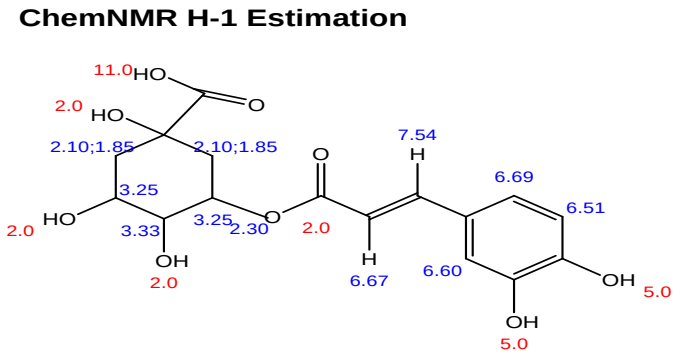
The probable structure that is in agreement with this information is that of chlorogenic acid which is a phenolic acid that reacts with alcoholic KOH.

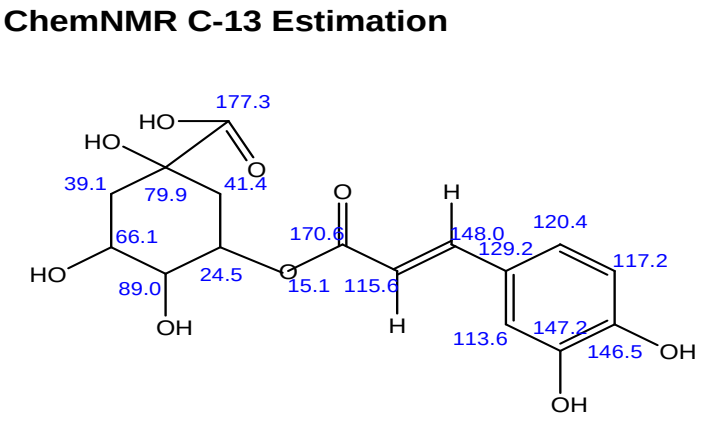


M2

The melting temperature of compound M2 measured on the Kofler bench is (208.35 +/- 0.5). This value is very close to that of the standard chlorogenic acid (207-209).

Following this physical parameter, we performed the Chemdraw simulation of M2 spectra.





We notice that the spectral data obtained by simulation are very close to the experimental data recorded.

The compound M2 is therefore 5-O-(3,4-dihydroxycinnamoyl) -L-quinic acid (chlorogenic acid). This compound has the form of a white powder.

CONCLUSION

We were able to isolate two molecules from the ethyl acetate fraction of *Parkia biglobosa* (Mimosaceae) these are the 4-methoxybenzoic acid (anisic acid). and 5-O-(3,4-dihydroxycinnamoyl) -L-quinic acid (chlorogenic acid).

REFERENCES BIBLIOGRAPHIQUES

1. Kossi Jean Marie D. Tokoudagba, Ayide C. Ahouansou, Fernand A. Gbaguidi [Phytochemical and Antibacterial Activity of Fractions of Hydroethanolic Extracts of

Parkia biglobosa (Mimosaceae) and *Carissa edulis* (Apocynaceae)] *International Journal of Science and Academic Research*, 2021; 2(12): 3241-3245.

2. Fatondji HR, Gbaguidi F, Kpoviéssi S, Sonounameto E, Lagnika L, Ambaliou S, Moudachirou M, Poupaert J, Accrombessi G: Synthèse, caractérisation et études de propriété antimicrobiennes de la semicarmazone et de thiosemicarbazone de la carvone. J.Soc.ouest-Afr.Chim, 2010; 030: 11-17.
3. Harborne JB: *The flavonoids: advances in research since 1986*. Chapman & Hall / CRC, 1993.
4. Houngbèmè AG, Ganfon HMY, Medegan S, Yèhouènou B, Bambola B, Gandonou C, Gbaguidi FA : Antimicrobial activity of compounds from *Acanthospermum hispidum* DC and *Caesalpinia bonduc* (L.) ROXB: Beninese plants used by healers against HIV-associated microbial infections. Journal Applied Pharmaceutical Science, 2015; 5(06).
5. AG Houngbeme. C. Gandonou. B Yehouenou. SDS Kpoviessi D Sohounhloue. M. Moudachirou and F. Gbaguidi Phtochemical analysis, toxicity and antibactérial activity of benin medicinal plants extracts used in the treatement of sexually transmitted infections associated with hiv/aids. Int J Pharm Sci Res, 2014; 5(08): 073-081.
6. Portet B: Recherche bioguidée de molecules antipaludiques d'une plante guyanaise: *Piper hostmanianum var. berbicense*. Thèse de doctorat, 2007. Université de Toulouse.
7. Bruneton J. Pharmacognosie. Phytochimie et Plantes médicinales Edition médicale internationale; Paris, 1999; 4: 364-1043.
8. Rajput AP and Rajput TA: Isolation of stigmasterol and betasitostérol from chloroform extract of leaves of *corchorus fascicularis* Lam. International Journal of biological chemistry, 2012; 6(4): 130-135.