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PHYTOCHEMISTRY AND ACTIVITY VASODILATOR OF SHEETS OF *SPONDIAS MOMBIN* L. (ANACARDIACEAE)

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SUMMARY

The physiological study of the vasodilator activity of the ethanol-water extract of dried powdered leaves of the *Spondias mombin* L. showed vasorelaxant activity (EC_{50} of 90 mg / L) endothelium-dependent of NO and EDHF vasoactive factors. The liquid - liquid extraction of the extract of *Spondias mombin* gave polar fractions which are richer in total polyphenols than the apolar fractions. The study showed a good vasodilator activity for apolar fractions. The cyclohexane fraction is more active than dichloromethane fraction. The chemical study of the most active fraction of *Spondias mombin* L. (Anacardiaceae) allowed isolation of two molecules belonging to the family of terpenes: 3- [3-methyl-2- (1-methylhexyl) octyl] phthalic acid and 2-hydroxy-6 - [(8'E, 11'e, 14'E) -2- hydroxydocosa-8 ', 11', 14'-trienyl] benzoic acid. These molecules are involved in vasodilating activity endothelium-dependent of the cyclohexane extract fraction of *Spondias mombin.*

KEYWORDS: *Spondias mombin*, vasodilator activity, medicinal plants.

INTRODUCTION

Spondias mombin is a tropical tree grown for its aromatic ovoid yellow fruits similar to plums, called mombins, which are consumed fresh or as jam. The fruits are ovoid or obovoid drupes, yellow at maturity, 25 to 35mm long, wide 15 to 20mm with a pleasant tangy pulp. This is a very common tree in tropical countries because of its food virtues.

Its traditional therapeutic use is immense. The kernel is edible and is considered an anti-diarrheal. The hot vapor nuclei would calm the pain of gout. The flowers are used in infusions against the affections of the throat. Buds and young twigs give a decoction for diseases of the eyes. Those buds are also put in hot baths, as astringents, skin firming. The decoction of the leaves and buds are used to wash wounds and ulcers.

The leaves in decoction are used in drink, as anti-dysentery. In infusion, they are a remedy against cough. A leaf infusion is also given against fever and hypertension, while a decoction is a remedy against gonorrhea. The young and fresh leaves juice, heated and pressed, is given to children for stomach disorders, and a decoction of pounded and macerated leaves are used in lotions for the eyes. In some areas the infusion of young leaves is given to women in childbirth, like beverage, along as hot astringent lotion.

The decoction of the leaves is given in mouthwash as anti-odontalgique. The plant is also used in bleeding after childbirth. The bark contains a certain amount of tannin. Boiled, it is used as a mouthwash against toothache and, intern use, as an anthelmintic while in decoction it fights against violent cough with inflammatory symptoms: she works by bringing relief from vomiting effect. Dried and pulverized, it applies in dressing the wounds of circumcision (Adjanohoun et *al.* 1989; Adjanohoun et *al.* 2002).

The traditional therapeutic use of this plant especially regarding hypertension prompted us to conduct a phytochemical study and evaluate the vasodilator activity of the fractions.

MATERIALS AND METHODS

1-Preparation of extract of *Spondias mombin*

A sample sheets of Spondias mombin harvested in the region of Abomey-Calavi (Atlantic County) was identified in the National Herbarium of Benin under No. AA6334 / HNB. These sheets are then dried powdered.

One hundred grams of powder are macerated in ethanol - water (6/4, v / v) for 72 hours under continuous stirring. The supernatant is filtered and then evaporated to dryness under reduced pressure using a rotary evaporator Buchi RE-type 11. For physiological tests a stock solution (100 mg / mL) of the crude extract was prepared and dilutions were performed: 10 mg / mL; 1 mg / mL; 0.1 mg / mL; 0.01 mg / mL; 0.001 mg / mL; 0.0001 mg / mL. The vasorelaxant tests were carried out from these dilutions.

2- Determination of Total Polyphenols

The determination of total polyphenols is performed by the Folin Ciocalteu reagent using the method described by Singleton et al. (1999). The assays were performed in triplicate and results are expressed in terms of equivalents of gallic acid (GAE) per mg of extract. The basic medium used is 20% Na2CO3 (2 g in 10 mL of distilled water), the gallic acid solution is at a concentration of 1mg / mL. Samples to be assayed are at a concentration of 10 mg / mL. The reading of absorbance was done at 730 nm.

3- Fractionation of crude extract of *Spondias mombin*

The solution in the separatory funnel is formed of ethanol - water crude extract dissolved in 50 mL of distilled water. We have successively used during the fractionation, 500 ml of cyclohexane, dichloromethane, ethyl acetate and butanol. The different collected fractions are evaporated in a rotary evaporator.

4. Purification of the active cyclohexane extract

The cyclohexane was fractionated with the technique of solid phase extraction (SPE) using a cartridge containing 50 g of silica (silica gel 60 TLC) packed with 300ml of cyclohexane / ethyl acetate (95: 2). Elution is made with the following solvents:

- Cyclohexane - ethyl acetate (95: 5) 60 mL
- Dichloromethane 100 mL
- Dichloromethane - methanol (95: 5) 300 ml
- Dichloromethane - methanol (90: 10) 100 ml
- Dichloromethane - methanol (80: 20) 200 ml
- Dichloromethane - methanol (70: 30) 100 ml

5. Isolation of the active compounds extracted of cyclohexane

The selected fractions S3 and S4 solubilized in cyclohexane (suitable solvent) are deposited on the glass plate preparative chromatography (20 x 20cm) containing silica. It is then placed

in a vessel containing saturated eluent that is dichloromethane (100%). After making the development we examine the spots under UV light (366 nm) where it reveals a section of plate with the spray of vanillin sulfuric followed by heating to 105 ° C. Using a spatula we recover the spots of interest fixed to the silica.

6- Physiological Tests

The circumflex arteries taken from pig hearts are cut into rings. The rings are mounted between two hooks and placed in tanks in isolated organ containing 10 ml of Krebs-bicarbonate solution at 37 ° C and oxygenated with a mixture of carbogen (95% O_2, 5% CO_2 Coronary artery rings were subjected to 5g voltage and are then left to stand for 45 minutes stabilization phase. The rings are then contracted with KCl solution (pH 7.4; composition in mM: NaCl 43.7, KCl 80; 1.18 KH_2PO_4, $MgSO_4$ 1.17; 1.25 $CaCl_2$), $NaHCO_3$ 25, glucose 11). After obtaining the maximum effect, three successive washes are performed.

To test the integrity of the endothelium, the rings are contracted with the thromboxane U 46619 (10^{-8} M) and the contraction plateau of bradykinin (3.10^{-8} M) is applied. After three successive washes, a 45 minutes stabilization phase is observed in which the inhibitors are added in appropriate tanks before contracting again with thromboxane U46619 (10^{-8} M). Finally, applied the increasing range and cumulative (10^{-4} to 10^{-1} 3. mcg / ml) of the various plant extracts obtained.[7]

7- Statistical analysis of results

Statistical analysis was made by the Student t test. Results are given as average value +/- SEM. P values <0.05 were considered statistically different.

RESULTS

1- Extraction and Fractionation

The yield of extraction and fractionation of the crude extract is contained in the following (Table 1).

2- Concentration of total polyphenols in fractions of the crude extract of *Spondias mombin*.

The polar fractions are richer in total polyphenols than other non polar fractions (Table 2).

3- Effect of crude extract *Spondias mombin* on vascular reactivity

The crude extract causes endothelium-dependent relaxation and NO and EDHF vasoactive factors are equivalently involved (Picture 1) and (Picture 2).

4-Effect of fractions of crude extract *spondias mombin*

The cyclohexane dichloromethane fractions, and water are more active than the other fractions of the crude extract (Picture 3).

5- Fractionation of cyclohexane extract

We collected fractions 7 S1 (70.5 mg), S2 (22 mg), S3 (107 mg), S4 (63 mg), S5 (640 mg), S6 (96 mg), S7 (170 mg) The Analysis of the fractions obtained was effected by thin layer chromatography with as mobile phase solvents Cyclohexane / ethyl acetate (95/5) and dichloromethane / methanol (95/5) and as developer the sulfuric vanillin.

S3 and S4 selected fractions were purified again and gave respectively the $S3_1$ compounds (2.6 mg), $S3_2$ (21.7 mg), $S3_3$ (3.4 mg), $S3_4$ (80 mg), $S4_1$ (6 mg), S42 (0.2 mg). The majority fraction in $S3_4$ was purified to give the compounds $S3_{4a}$ (10 mg), $S3_{4b}$ (10 mg), $S3_{4c}$ (5 mg), $S3_{4d}$ (12 mg), $S3_{4e}$ (20 mg).

The analysis of these fractions by thin layer chromatography as a developer with sulfuric vanillin gave us an unique spot.

6- Structural Elucidation of isolated compounds

The mass yield and TLC profile of the compounds obtained have allowed us to make the structural elucidation of compounds and $S3_2$ $S3_{4e}$.

6.1 Structural Elucidation of the compound $S3_2$

6.1.1 NMR ^{1}H of $S3_2$

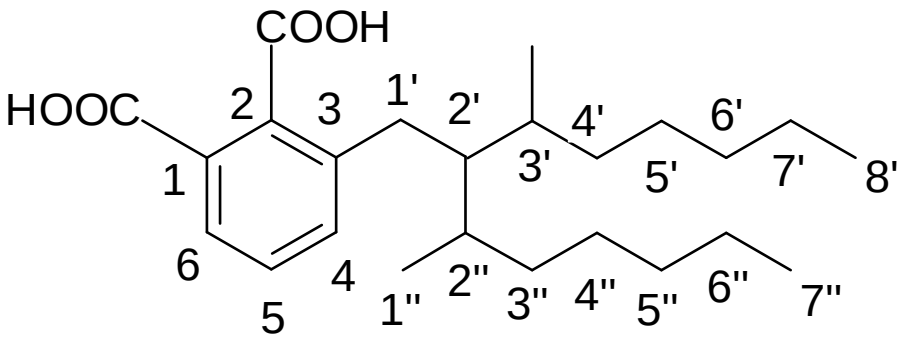
The ^{1}H NMR chemical shifts and signal functions are shown in Table 4. We observed two doublets split around 7.71 ppm and 7.53 ppm corresponding to the protons of the aromatic ring and a multiplet at around 1.8 ppm which could be the protons of carbons linked to the aromatic ring. We also observed a signal in the form of quadruplet around 1.53 ppm. The chemical shifts of the protons type CH_2 are observed around 2.13 ppm. (Picture 4)

6.1.2 NMR ^{13}C of $S3_2$

The ^{13}C NMR chemical shifts and signal functions are shown in Table 3. We observed in FIG June 6 peaks ranging from 117.58 to 131.087 ppm which are the peaks of the aromatic ring bearing two carboxylic groups 145.778 and 144.757 ppm, respectively. Chemical shifts lying between 40 and 11ppm for the saturated carbons are carried by the aromatic ring. We have chemical shifts of methyl groups are 4 14.267; 12.430; 11.992; and 11.496 ppm. Considering the chemical shifts of saturated alkanes we see many similar chemical shifts of 40 to 20 ppm this could well correspond to a linear chain carried by the aromatic ring (Picture 4).

6.1.3 Mass Spectrum

The mass spectrum made of electron impact ionization (ESI = electron spray ionization) in positive mode shown the peak of the molecular ion at m / z 413.26, corresponding to the ion [M + Na] + Another signal obtained at m / z 803.54, corresponding to the same molecule as dimer (413.26 to 23) x 2 characteristic acids which leads to an empirical formula of C_{24} H_{38} 0_4 and to the developed following formula:



Acid 3- [3'-methyl-2 '- (1' '- methylhexyl) octyl] phthalic

6.2 Structural Elucidation of $S3_{4e}$ compound

6.2.1 NM R ^{1}H of $S3_{4e}$

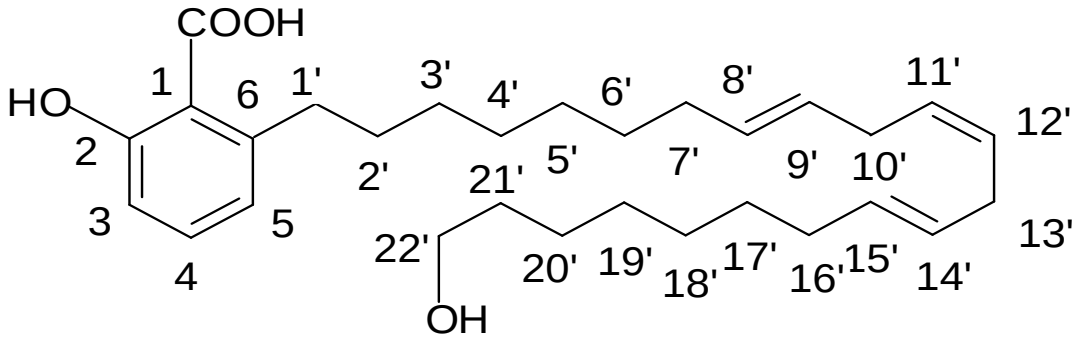
The ^{1}H NMR chemical shifts and signal functions are shown in (Table 6). We observed a chemical shift at 5.35 ppm corresponding to the proton of the OH group. The chemical shifts of protons of the aromatic ring observed between 7.519 and 7.382 ppm. The signals obtained between 6,612 and 7,260 ppm and 6.457 ppm respectively correspond to the phenol proton and protons of alkenes. (Picture 5).

6.2.2 NM R ^{13} C of $S3_{4e}$

We have a chemical shift (Table 5) at 174.12 ppm which corresponds to the carbon of the carboxylic acid. The chemical shifts observed between 128.5 and 127.33 ppm correspond to 6 carbon atoms of the aromatic ring and those between 132 and 130 ppm correspond to alkene carbons. We observed signals for CH_2 and CH_3 groups respectively between 34.623 and 22.906 ppm and between 14.471 and 14.325 ppm on the other. (Picture 5).

6.2.3 Mass spectrum of $S3_{4e}$

The mass spectrum made of electron impact ionization (ESI = electron spray ionization) in positive mode shows the peak of the molecular ion at m / z 457.34, which corresponds to the [M + ion H] + and an empirical formula of C29 H44 O4 and to the following structure:



Acid 2-hydro-6 - [(8'E, 11'e, 14'E) -22'-hydroxydocasa-8 ', 11', 14'-trienyl] benzoic

Table 1: Yield of extraction and fractionation of the crude extract.

Vegetal materiel	Extract	Weight	Yield
Leaves powder(100g)	Crude Extract	19g	19%
Crude extract (17g)	C_6H_6 Ectract	0,19g	1,12%
	CH_2CL_2 Extract	0,25g	1,47%
	AcOEt Extract	3,58g	21,06%
	Buol Extract	3,4g	20%
	Aqueux Extract	7,5g	44,12%

Table 2: Total concentration of polyphenols fractions of the crude extract.

Extract	Total concentration of polyphenols(mgEAG/L)
Crude extract	275,6 +/- 2,2
C_6H_6 Extract	83,4 +/- 1,3
CH_2CL_2 Extract	57,8 +/- 2,0
AcOEt Extract	304,8 +/- 3,3
Buol Extract	247 +/- 1,5
Aqueous extract	210,4+/- 21,8

Table 3: Data of RMN ^{13}C de $S3_2$.

Carbone	δ (ppm)
1	121,215
2	129,031
3	131,087
4	122,841
5	117,584
6	118,671
1'	33,033
2'	38,975
3'	39,610
4'	32,939
5'	28,207
6'	31,787
7'	22,848
8'	14,267
1''	40,054
2''	25,028
3''	24,678
4''	24,09
5''	23,213
6''	12,430
1'''	11,992
2'''	11,496
A	145,778
B	144,757

Table 4: Data of RMN ^{1}H de $S3_2$.

Protons	δ (ppm)	SIGNAL
H-4	7,71	dd
H-5	7,53	dd
H-6	7,8	
H-1'	2,16	d
H-2'	1,53	Q
H-3'	1,8	M
H-4'	2,16	D
H-5'	2,16	D
H-6'	2,16	D
H-7'	2,16	D
H-8'	0,87	D
H-1''	1,8	M

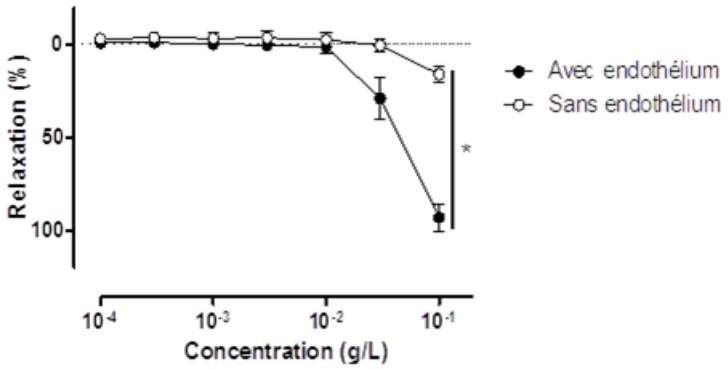
H-2''	2,16	D
H-3''	2,16	D
H-4''	2,16	D
H-5''	2,16	D
H-6''	0,858	D
H-1'''	0,849	D
H-2'''	0,842	D
H-α	11,0	
H-β	11,0	

Table 5: Data of RMN^{13}C de S3-4e.

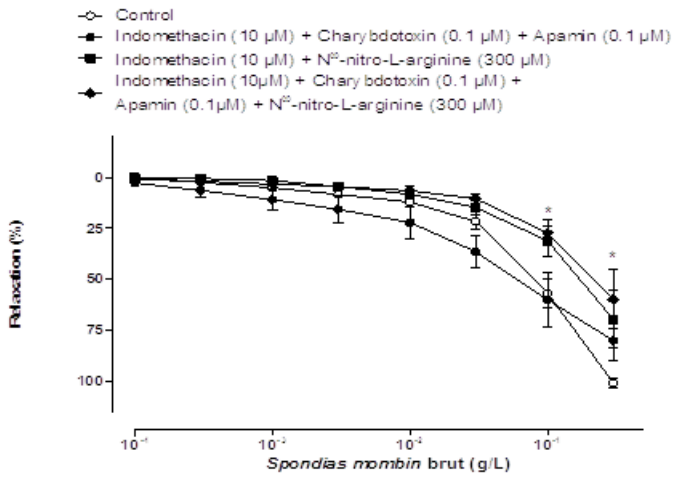
CARBONE	δ (ppm)
α	174,124
1	128,499
2	128,470
3	128,258
4	128,127
5	127,937
6	127,332
1'	34,623
2'	34,608
3'	32,144
4'	29,906
5'	29,862
6'	29,811
7'	29,789
8'	132,020
9'	130,489
10'	29,570
11'	130,423
12'	130,270
13'	29,483
14'	130,205
15'	130,205
16'	29,373
17'	29,330
18'	27,427
19'	25,837
20'	25,218
21'	25,196
22'	22,906

Table 6: Data of ^{1}H de S3-4e.

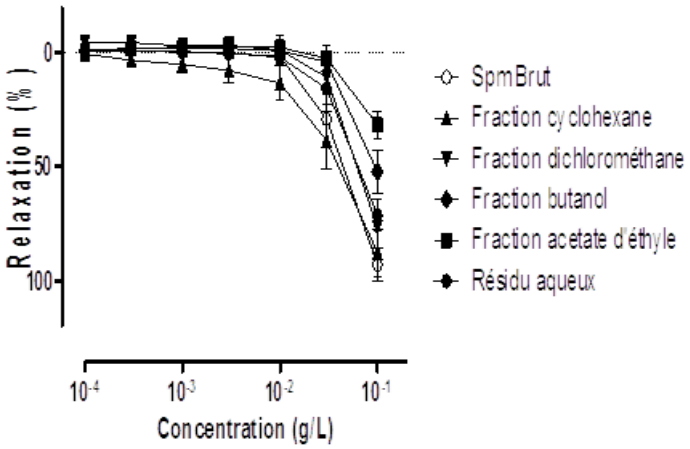
Protons	δ (ppm)	SIGNAL
H-3	7,519	S
H-4	7,437	S
H-5	7,382	S
H-1'	2,820	T
H-2'	2,805	T
H-3'	2,790	T
H-4'	2,301	T
H-5'	2,282	T
H-6'	2,263	T
H-7'	2,076	T
H-8'	7,260	
H-9'	6,996	
H-10'	2,058	T
H-11'	6,612	
H-12'	6,590	
H-13'	2,040	T
H-14'	6,478	
H-15'	6,457	
H-16'	1,631	T
H-17'	1,614	T
H-18'	1,596	T
H-19'	1,308	T
H-20'	1,282	T
H-21'	1,251	T
H-22'	1,233	T
H-α	5,35	T
H-β	7,260	
H-γ	5,35	T



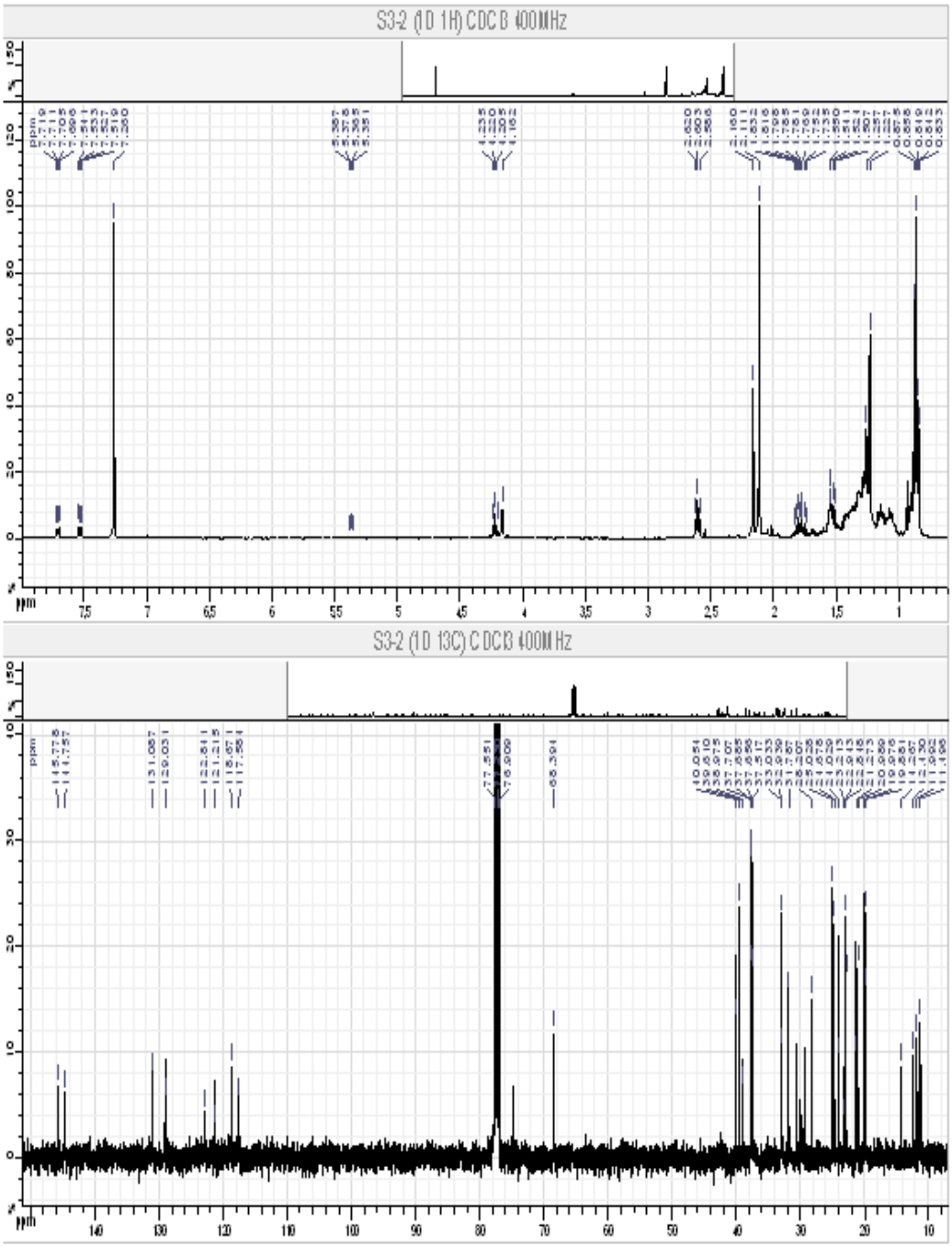
Picture 1: Effect of crude extract of *Spondias mombin* on cornary artery pork filled and devoid of endothélium. The results are given average value ± SEM for n=5 *p< 0.05 vs. contrôle.



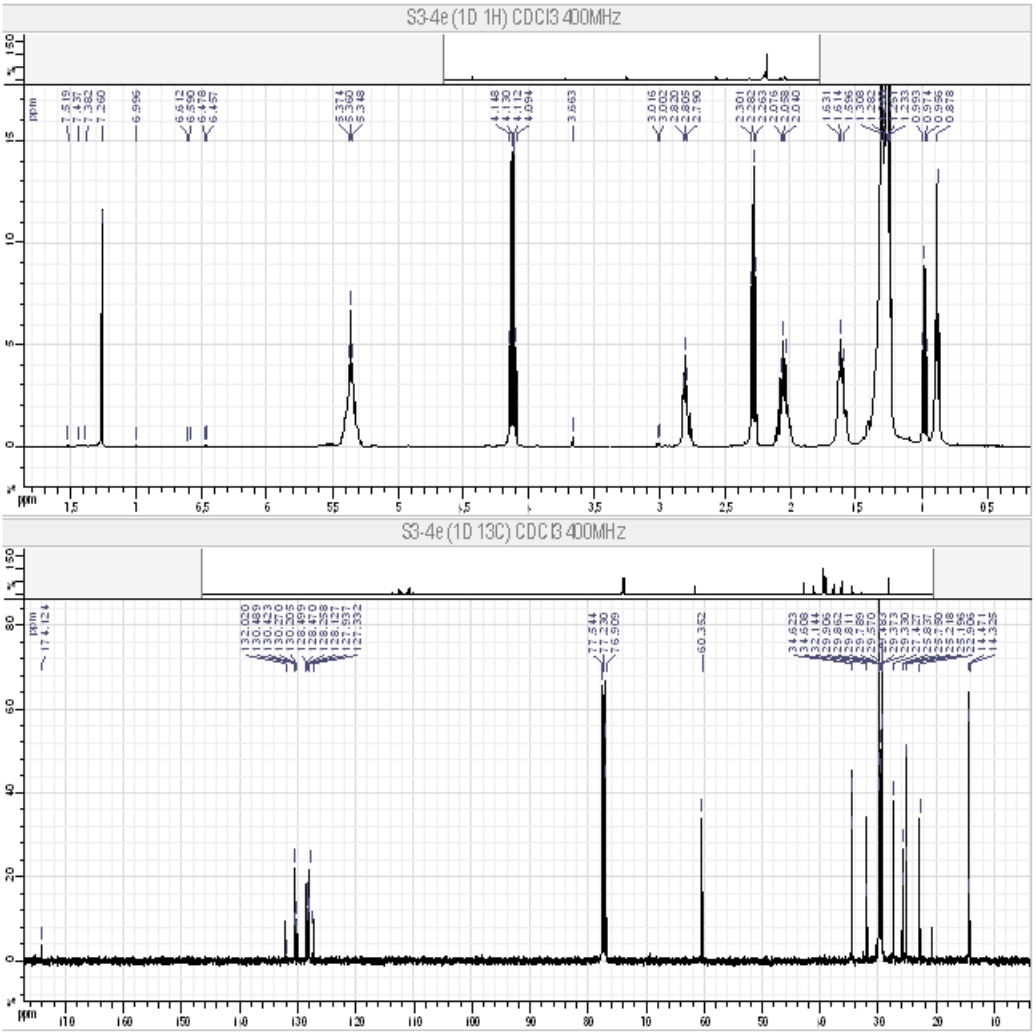
Picture 2: Effect of crude extract of *Spondias mombin* on coronary artery of pork filled of endothélium and with inhibitors. The results are given as average value ± SEM for n=5 *p< 0.05 vs.contrôle.



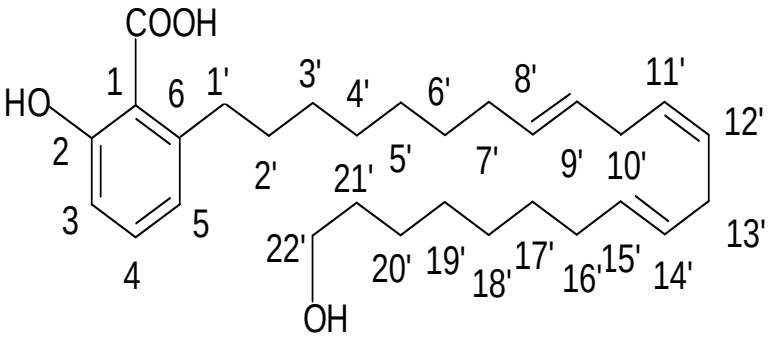
Picture 3: Effect of fractions of the crude extract of *Spondias mombin* on porcine coronary arteries provided with endothélium. The results are given as mean ± SEM for n=5 *p< 0.05 vs.contrôle



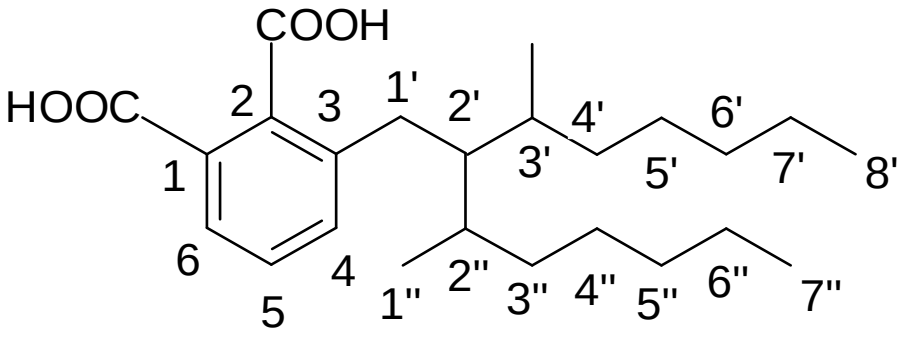
Picture 4: Spectre for NMR ^{1}H and ^{13}C of $S3_2$.



Picture 5: Spectre NMR ^{1}H and ^{13}C of $S3_{-4e}$.



Picture 6: Acid 2-hydro-6-[(8'E, 11'E, 14'E)-22'-hydroxydocasa-8',11',14'-trienyl] benzoic.



Picture 7: Acid 3-[3'-méthyl-2'-(1''-méthylhexyl) octyl] phtalic.

DISCUSSION

The ethanol - water extract of *Spondias mombin* has a less powerful endothelium-dependent vasorelaxant activity (EC50 of 90 mg / L) than that of the leaves of Parkia biglobosa (EC50 of 30 mg / L) on the porcine coronary artery model.[12] The two main vasoactive factors NO and EDHF are involved in this vasorelaxant activity. These are the non polar fractions of hydro ethanol extract of *Spondias mombin* are active compared to hydro ethanol extract of *Parkia biglobosa* whose polar fractions are active. These fractions contain molecules responsible for the vasodilator activity compared to polar fractions which have no vasorelaxant activity. The determination of total polyphenols confirmed their presence in fractions of ethanol - water extract of *Spondias mombin*. The richest polar fractions in total polyphenol comparatively to the non polar fractions have no vasorelaxant activity.

The chemical study of active fractions *Spondias mombin* gave two acids. These are: 3- [3-methyl-2- (1-methylhexyl) octyl] phthalic acid and 2-hydroxy-6 - [(8'E, 11'e, 14'E) -22'-hydroxydocosa-8 ', 11', 14'-trienyl] benzoic acid.

They are molecules that share in their formula benzoic acid that we find in the formula of gallic acid. This may explain the origin of their endothelium independent vasodilator activity which is due to gallic acid.[3]

These molecules have been identified in studies on the chemical composition of the leaves of *Spondias mombin*.[9 10]

CONCLUSION

The study of the vasodilatory hydro activity of ethanol extract powder of dried leaves of Spondias mombin L. showed vasorelaxant endothelium-dependent activity and NO and EDHF vasoactive factors.

The cyclohexane and dichloromethane fractions Spondias mombin L. (Anacardiaceae) have a greater activity than other fractions. The cyclohexane fraction is more active than dichloromethane fraction.

The chemical study of the most active fraction of Spondias mombin L. (Anacardiaceae) allowed isolation of two molecules belonging to the family of terpenes: 3- [3-methyl-2- (1-methylhexyl) octyl] phthalic acid and 2-hydroxy-6 - [(8'E, 11'e, 14'E) -2- hydroxydocosa-8 ', 11',14'-trienyl] benzoic acid. These molecules are involved in endothelium-dependent vasodilating activity of the cyclohexane extract fraction Spondias mombin.

The results obtained in our research, offer a contribution to the enhancement of plant resources of traditional medicine Benin. These results confirm the relevance of the traditional use of some of them, mainly the most active in the pharmacopoeia of Benin.

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