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Phytochemical Composition, Biological Activities of *Croton lobatus L.* Leaves, Hydrolysis Effect on Activities and Chemical Composition

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Abstract: *Croton lobatus L.* is medicinal plant widely used in traditional medicine, but little known in literature for its biological properties. Aims were to study its phytochemical composition and some biological properties and influence of acid hydrolysis on biological activities and chemical composition of *Croton lobatus L.* extracts. Successive extractions with solvents of increasing polarity (cyclohexane, ethyl acetate, and methanol) were carried out on powder of *Croton lobatus L.* leaves. Evaluation of xanthine oxidase, acetylcholinesterase, 5-lipoxygenase and α-amylase inhibitions, antioxidant activity was performed. Acid hydrolysis effect was evaluated by comparison of chemical composition and xanthine oxidase, α-amylase inhibition activities of hydrolyzed and unhydrolyzed extracts. Results showed that at 50 mg/L *Croton lobatus L.* has very small antioxidant activity (3.4 - 4.9%), small inhibitory activity of 5-lipoxygenase (6.3-6.9%), inhibitory activity of acetylcholinesterase between (2.7 - 31.3%), moderate inhibitory activity of α-amylase (52.8 - 64.3%), moderate inhibitory activity of xanthine oxidase (22.2 - 62.6%). *Croton lobatus L.* could be used in diabetes and gout treatments, given results of α-amylase inhibition and xanthine oxidase. Acid hydrolysis has negative effect on process of xanthine oxidase inhibition and on chemical composition.

Keywords: *Croton lobatus L.*, Antioxidant, Acetylcholinesterase, Xanthine Oxidase, α-amylase, 5-Lipoxygenase, Inhibition, Acid Hydrolysis

1. Introduction

Croton lobatus L. called "alovi Aton" by people of Benin southern was an annual herbaceous plant 30-60 cm tall belonging to Euphorbiaceae family. Iwast met from Senegal to Cameroon, in Egypt, Ethiopia, Sudan and Arabia [1]. It was widely used in traditional medicine in treating spasms, threats, abortion and hypertension [2]. *Croton lobatus L.* is one of plants few studied for their biological properties. Previous studies on plant led to isolation of alkaloids [3] and evaluation of antimicrobial activity [4-6]. The objective of this study was to evaluate its phytochemical composition, antioxidant activity, inhibitory activities of α-amylase, xanthine oxidase, 5-lipoxygenase, acetylcholinesterase and then to study influence of acid hydrolysis on some properties and on chemical composition.

2. Materials and Methods

2.1. Plant Material

Plant material consisted in Croton lobatus L. leaves harvested at Abomey-Calavi in the southwest of Republic of Benin. Plant was identified at National Herbarium of University of Abomey-Calavi under number AA 6542/HNB where voucher specimen was conserved.

2.2. Chemicals and Reagents

Chemicals and solvents used were analytical grade and purchased from Sigma-Aldrich-Fluka (Saint-Quentin, France).

2.3. Extracts Preparation

Cyclohexane, dichloromethane, ethyl acetate and methanol extracts were prepared using method cited in Chodaton-Zinsou [7].

2.4. Chemical Composition

Chemical composition (phenolic compounds, flavonoids, tannins, anthocyanins) was determined following methods cited in Chodaton-Zinsou [7].

2.5. Biological Activities

Antioxidant, anti-inflammatory activities, acetylcholinesterase, xanthine oxidase, α-amylase inhibition activities essays were cited in Chodaton-Zinsou [7].

2.6. Extract Hydrolysis

Extract hydrolysis was performed according to method developed by Wach [8]**.** 20 mg of each extract were dissolved in 1 mL of suitable solvent and heated for 30 minutes at 100°C. 200 µL of that solution were collected and evaporated to dryness. Then extract obtained was dissolved in 100 µL of 3 M HCl-methanol mixture (60:40, v/v), heated at 100°C for 20 minutes and evaporated to dryness.

2.7. Derivatization and GC-MS Analysis

Derivatization and GC analysis were carried out following methods cited in Chodaton-Zinsou [7].

3. Results and Discussion

3.1. Phenolic Compounds, Flavonoids, Tannins, and Anthocyanins Contents

Results of quantification of phenolic compounds, flavonoids, tannins and anthocyanins (table 1) showed that these extracts contain few phenolic compounds. Methanolic extract contains more polyphenols than the others and cyclohexane extract contains less. These levels would influence biological activities, in particular antioxidant activity.

Table 1. *Phytochemical composition of Croton lobatus L. leaves.*

Extracts (50 mg/L)	Polyphenols (mg eq AG/g)	Flavonoids (mg EQ/g)	Tannins (mg ECat/g)	Anthocyanins (mg Eq C3G/kg)
Cyclohexane	2.3 ± 0.1	Nd	Nd	0.0015 ± 0
Dichloromethane	15.8 ± 0.6	Nd	Nd	0.0025 ± 0
Ethyl acetate	17.3 ± 0.3	6.3 ± 0.2	Nd	0.0005 ± 0
Methanol	20.2 ± 0.7	0.8 ± 0.1	Nd	Nd

Nd: no determined

3.2. Antioxidant Activity

Results obtained (table 2) show that plant has a very weak antioxidant activity compared to ascorbic acid activity which is reference used. Antioxidant activity of leaves varies from 3.4 to 4.9% while ascorbic acid at 5.3 mg/L was active at 53.01%. Plant therefore contains very few antioxidants responsible for activity. This would be confirmed by low content of polyphenols (table 1) which are antioxidants. This weak antioxidant activity seems to be specific to species of genus *Croton*. Thus, antioxidant activity of *Croton caudatum* was 83.29% with a concentration of 40 mg/mL (40000 mg/L) [9]. That of *Croton bonplandianum*, at 60 µg/mL was 19.06 ± 0.12% [10]. Another *Croton bonplandianum* species, at 0.5 mg/mL (5000 mg/L) exhibited 59.62% activity [11].

3.3. Lipoxygenase Inhibition

Croton lobatus L. showed a very small 5-lipoxygenase inhibitory activity compared to reference which was NDGA (table 2). This activity of *Croton lobatus L.* varies between 6.3% and 6.9%. To our knowledge, there is no investigation in literature on its ability to inhibit 5-lipoxygenase activity. On other hand, *in vivo* studies have been carried out with *Croton argyrophyllus* [12] and *Croton leptostachyus* Kunth [13]. These studies focused on edema inhibition on laboratory's rat and showed its anti-inflammatory activity. Our *Croton lobatus L.* was therefore not a potential inhibitor of 5-lipoxygenase.

3.4. Acetylcholinesterase Inhibition

Table 2 which presented, among others things, acetylcholinesterase inhibition results showed that apolar extracts had a very small activity. The most polar extracts, on the other hand, had moderate activity. Other *Croton* species were more active than *Croton lobatus L.* Thus, leaves of *Croton zambesicus* (42.5 µg/mL) have 51.29 ± 3.86% of inhibition [14]. Extraction solvents do not influence activity within *Croton* genus. Indeed, activity of dichloromethane extract of *Croton lobatus L.* (2.7 ± 0.3%) was much lower than that of dichloromethane extract of *Croton socotramus* bark (0.05 mg/ml) which is 40.61% and methanolic extract at the same concentration has zero activity on this enzyme [15]. Edapho-climatic conditions, extraction technic could explain these differences. To our knowledge, this study on acetylcholinesterase inhibition was the first for *Croton lobatus L.*

3.5. α-amylase Inhibition

Evaluation of α-amylase inhibition activity of *Croton lobatus L.* extracts was performed. Results obtained (table 2) showed that all extracts (50 mg/L) exhibit α-amylase inhibition activity with percentages greater than 50%. The lower percentage was obtained with cyclohexane extract (52.8 ± 0.4%) followed by methanol extract (61.4 ± 1.0%),

dichloromethane extract (63.1 ± 2.5%) and ethyl acetate extract (64.3 ± 2.4%). Acarbose (50 mg/L) which was standard drug of α- amylase inhibition used presented 56,4 ± 2,6% of inhibition. The two most polar extracts were more effective than positive control used: acarbose. Results showed that *Croton lobatus L.* contains α-amylase inhibitors. These results could be attributed to an abundance of inhibitors such as, saponins, steroids, alkaloids, terpenoids [16, 17] responsible of this property.

Note that no data on *Croton lobatus L.* property to inhibit α-amylase in literature that can allow comparison exists. To our knowledge, results of our work represent the first report.

3.6. Xanthine Oxidase Inhibition

Croton lobatus L. extracts were tested for their activities on xanthine oxidase. Results of that evaluation (table 2) showed that only two more polar extracts (50 mg/L) had higher inhibition percentages than 50%. The highest inhibition was achieved with methanol extract (62.6 ± 1.8%) while the lowest inhibition was obtained with cyclohexane extract (22.2 ± 1.1%). Ethyl acetate extract presented 50.5 ± 0.9% of inhibition. Percentages obtained with ethyl acetate and methanol extracts could be explained by abundance or xanthine oxidase inhibitors quality such as polyphenols, flavonoids or saponins which are xanthine oxidase inhibitors [17, 18].

Table 2. *Croton lobatus L. biological activities.*

Extracts (50 mg/L)	Activities (%)				
	Antioxidant	acetylcholinesterase inhibition	5-lipoxygenase inhibition	Xanthine oxidase inhibition	α-Amylase inhibition
Cyclohexane	3.4 ± 0.2	6.0 ± 0.3	6.7 ± 0.3	22.2 ±1.1	52.8 ± 0.4
Dichloromethane	4.2 ± 0.2	2.7 ± 0.3	6.3 ± 0.2	19.3 ± 0.8	63.1 ± 2.5
Ethyl acetate	4.4 ± 0.3	31.2 ± 1.5	6.9± 0.3	50.5 ± 0.9	64.3 ± 2.4
Methanol	4.9 ± 0.1	31.3 ± 1.0	6.6 ± 0.2	62.6 ± 1.8	61.4 ± 1.0
Ascorbic acid (5, 3 mg/L)	53.01 ± 0.4	-	-	-	-
Galanthamine (1, 5 mg/L)	-	96.2 ± 1.8	-	-	-
NDGA (4 mg/L)	-	-	81.9 ± 0.2	-	-
Allopurinol (1 mg/L)	-	-	-	52 ± 1.0	-
Acarbose (50 mg/L)	-	-	-	-	56.4 ± 2.6

NDGA = Nordihydroguaiaretic acid

3.7. α-amylase Inhibition by Hydrolysed Extracts

Cyclohexane, ethyl acetate, methanol hydrolysed extracts were tested for their properties to inhibit α-amylase. Results were presented on table 3. Results showed that all hydrolysed extracts showed activity. Methanol extract was most active with 73.8 ± 2.5% inhibition and cyclohexane extract was least active 35 ± 4.5%. A comparison of extracts activity with acarbose activity showed that ethyl acetate extract and methanol extracts inhibited more than acarbose which is positive control used. Hydrolysed extracts like unhydrolysed extracts contain α-amylase inhibitors.

Table 3. *Results of α- amylase inhibition by hydrolysed and non-hydrolysed extracts of Croton lobatus L. leaves.*

Extracts (50 mg/L)	α- amylase inhibition percentage	
	Before hydrolysis	After hydrolysis
Cyclohexane	52.8 ± 0.4	35 ± 4.5
Ethyl acetate	64.3 ± 2.4	68.9 ± 1
Methanol	61.4 ± 1.0	73.8 ± 2.5
Acarbose (50 mg/L)	56,4 ± 2,6	-

3.8. Xanthine Oxidase Inhibition by Hydrolysed Extracts

Croton lobatus L. hydrolysed extracts were tested for their ability to inhibit xanthine oxidase. Table 4 showed results. Methanol extract showed the best inhibition (31.02 ± 0.4%). Cyclohexane extract presented the weakest inhibition activity with 9.8 ± 0.9%. These results showed that hydrolysed extracts like unhydrolysed extracts have inhibitory properties on xanthine oxidase. This property could be explained by phenolics which, about literature [19] were inhibitors of this enzyme.

Table 4. *Results of xanthine oxidase inhibition by hydrolysed and non-hydrolysed extracts of Croton lobatus L. leaves.*

Extracts (50 µg/mL) and reference	Xanthine oxidase inhibition percentage	
	Before hydrolysis	After hydrolysis
Cyclohexane	22.2 ± 1.1	23.3 ± 0.9
Ethyl acetate	50.5 ± 0.9	46.2 ± 3.7
Methanol	62.6 ± 1.8	54.62 ± 0.4
Allopurinol (1, 2 µg/mL)	52 ± 1,0	-

3.9. Acid Hydrolysis Effect on α-amylase Inhibition and on Xanthine Oxidase Inhibition

A Comparison of results of table 3 showed a slight increase of inhibitory activity after hydrolysis of ethyl acetate and methanol extracts, and a decrease of inhibitory activity of cyclohexane extract. This increase can be attributed to compounds action released after hydrolysis, so an increase inhibitors α-amylase. Decrease in inhibitory activity of cyclohexane extract may be the result of inactivity of novel compounds obtained from hydrolysis. Hydrolysis has positive effect on α-amylase inhibition.

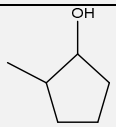
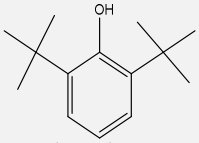
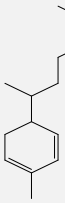
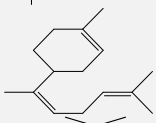
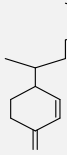
Results of table 4 revealed that ethyl acetate and methanol hydrolysed extracts contain less inhibitors while cyclohexane extract contains a little more. Acid hydrolysis has resulted in the decrease of hydrolysed extracts activities. This decrease in activity could be attributed to disappearance of some compounds responsible for activity present in extract before hydrolysis and also to inactivity of novel compounds derived

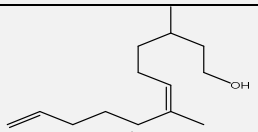
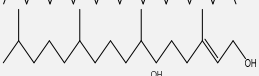
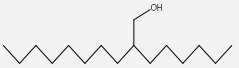
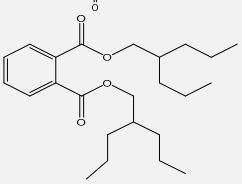
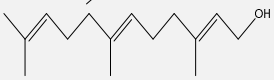
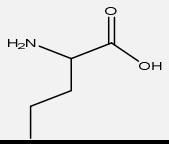
from hydrolysis.

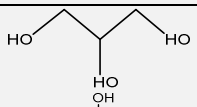
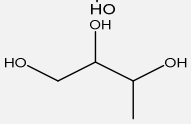
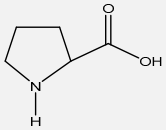
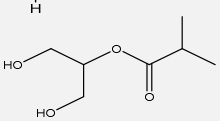
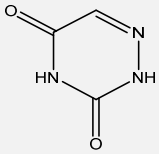
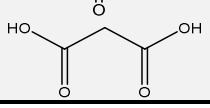
3.10. Acid Hydrolysis Effect on Chemical Composition of Extracts

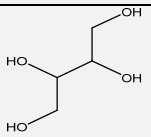
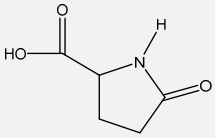
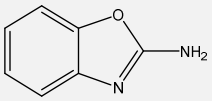
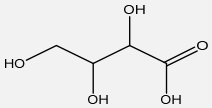
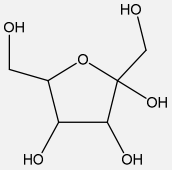
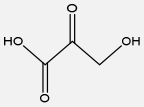
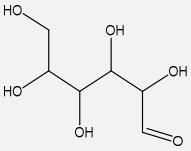
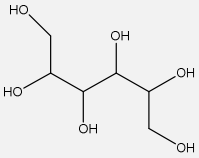
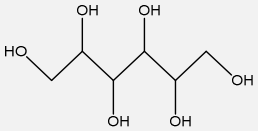
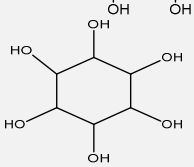
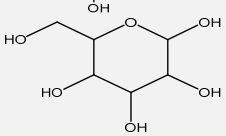
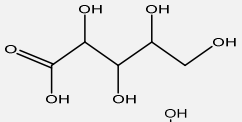
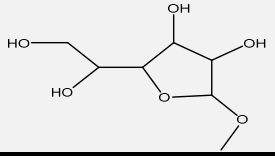
Unhydrolysed and hydrolysed extracts of *Croton lobatus L.* are analyzed by GC-MS before and after derivatization to evaluate hydrolysis effect. Compounds identified in both cases were in tables 5 and 6. Tables showed that before derivatization, 20 compounds (table 5) were identified in unhydrolysed extracts and no compound (table 6) was identified in hydrolysed extracts. After derivatization, 32 compounds (table 5) were identified in unhydrolysed extracts and 19 compounds (table 6) in hydrolysed extracts. Comparison of results before and after acid hydrolysis showed that hydrolysis process has an effect on extracts composition. Hydrolysis causes degradation of many bioactive compounds. The proof is that no compound has been identified in hydrolyzed extracts before derivatisation. Temperature (100°C) heating could be responsible of this degradation.

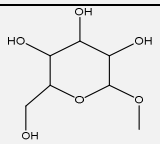
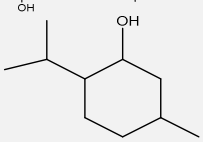
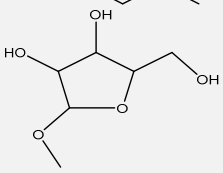
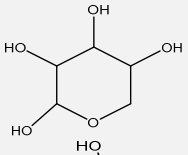
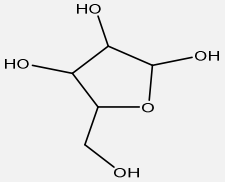
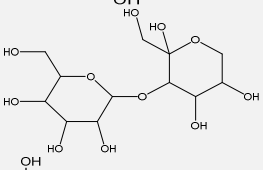
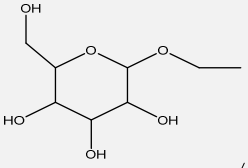
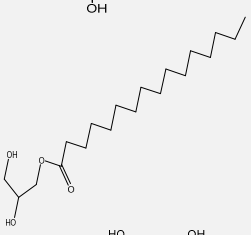
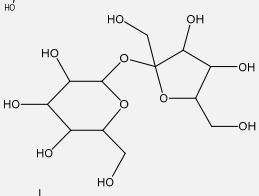
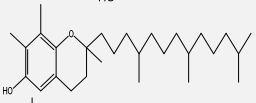
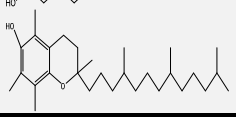
Table 5. *Compounds identified in Croton lobatus L. extracts.*

N°	Tr (min)	Compounds identified	Structure	Cyclo hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract
Before derivatization							
1	6.13	2-Methyl Cyclopentanol		+			
2	10.73	2-Dodecene (E)-			+		
3	12.73	5-Tetradecen				+	
4	12.75	Undecanol			+		
5	13.96	2, 6-bis (1, 1-dimethylethyl)-Phenol			+		
6	13.72	(-)-Zingiberene		+++			
7	13.85	Cis α-bizabolene		+			
8	13.98	β-Sesquiphellandrene		+			
9	14.45	7-Hexadecene (Z)-			++	+	
10	15.96	1-Hexadecanol			++	+	
11	16.02	Phytol acetate					++++
12	16.25	3, 7, 11, 15-Tetramethyl-2-hexadecen—1-ol		++++	++++	++++	+
13	16.43	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol isomere 2		+	+		+

N°	Tr (min)	Compounds identified	Structure	Cyclo hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract
Before derivatization							
14	16.56	3, 7-Dimethyldodeca-6-11-dien-1-ol		+			
15	17.33	10-Heneicosene (c, t)			++	+	
16	18.15	Phytol			+	+	
17	19.82	1-Decanol 2-hexyl			+		
18	19.91	Hexanedioic acid, dioctyl ester			+		
19	21.24	Phthalic acid di (2-propylpentyl) ester			++		
20	24.40	Trans-farnesol		+			
After derivatization							
21	10.72	Norvaline					+

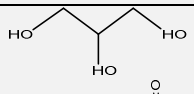
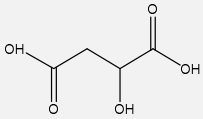
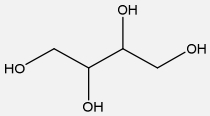
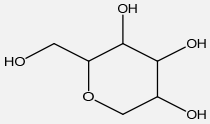
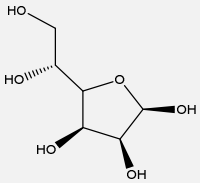
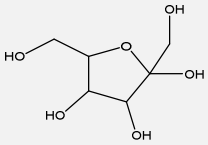
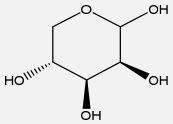
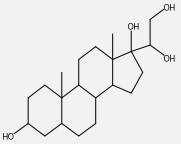
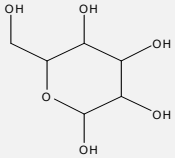
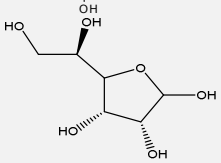
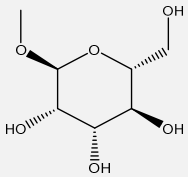
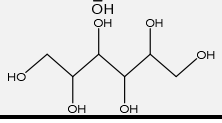
N°	Tr (min)	Compounds identified	Structure	Cyclo hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract
After derivatization							
22	11.33	Glycerol		+	+	++++	+
23	11.53	1, 2, 3-Butanetriol				+	
24	11.61	Proline					++..
25	11.64	2-mono-isobutyrin				+	
26	11.76	3, 6, 9, 12, 15-pentaoxaheptadecanedioic acid				+	
27	12.56	6-Azauracyl				+	
28	13.34	Malonic acid		+	+		+

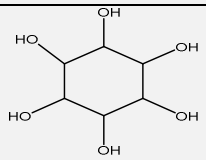
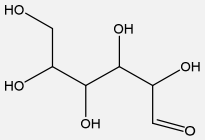
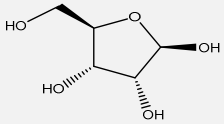
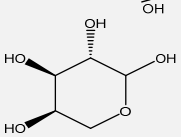
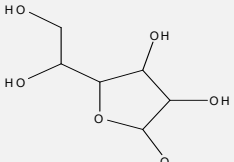
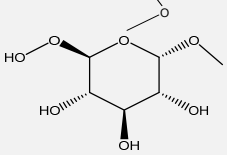
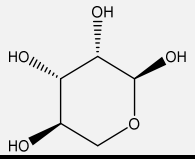
N°	Tr (min)	Compounds identified	Structure	Cyclo hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract
After derivatization							
29	13.48	Erythritol				+	
30	13.71	Pyroglutamic acid ou proline 5-oxo					+
31	13.83	1, 3-Benzoxazol-2-amine			+		
32	13.94	2, 3, 4-Trihydroxybutyric acid				+	
33	15.81	D-(-)-Tagatofuranose				+	+
34	16.24	β-Hydroxypyruvic acid					+
35	16.47	D-Glucose					+
36	16.76	Mannitol			+		+
37	16.76	Glucitol					
38	16.90	Myo-inositol					+++
39	17.01	Glucopyranose					+
40	17.22	Ribonic acid			+		+
41	17.67	Methyl glucofuranoside				+	

N°	Tr (min)	Compounds identified	Structure	Cyclo hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract
After derivatization							
42	18.05	Methyl mannopyranoside				+	
43	18.16	2-isopropyl-5-methylcyclohexan-1-ol		+			
44	18.31	Methyl arabinofuranoside				+	
45	18.80	Arabinopyranose				+	
46	19.89	Ribofuranose			+	++	
47	20.52	Turanose			+		
48	20.63	Ethyl D-glycopyranoside					++
49	20.86	Glycerol 1-palmitate		+			
50	21.67	Sucrose					++++
51	26.01	Tocopherol derivative		+			
52	27.56	Tocopherol		+			

+ = very small presence ++ = small presence +++ = presence ++++ = strong presence

Table 6. *Compounds identified in Croton lobatus L. hydrolysed extracts.*

N°	Tr (min)	Compounds identified	Structure	Cyclo hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract
After derivatization							
Before derivatization							
		Nothingness					
1	11.33	Glycerol		+	++++	++	+
2	13.31	Malic acid					+
3	13.48	Meso-erythritol				+	
4	15.78	1, 5-Anhydro-sorbitol				+	
5	15.89	D-(+)-Talofuranose			+		+
6	15.95	D-(-)-Fructofuranose					+
7	16.12	α-DL-Lyxopyranose			+	+	
8	16.21	17-(1, 2-dihydroxyethyl)-10, 13-dimethylhexadecahydro-1H-cyclopenta [a] phenanthrene-3, 17-diol					+
9	16.44	Mannopyranose					++++
10	16.50	Allofuranose			++	+	
11	16.55	Methyl α-D-mannopyranoside			+	+	
12	16.73	Mannitol					+

N°	Tr (min)	Compounds identified	Structure	Cyclo hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract
After derivatization							
13	16.87	Myo-inositol					++
14	16.99	Glucose					++
15	17.67	D-(-)-ribofuranose				+	
16	17.76	α-DL-arabinopyranose					+
17	19.86	Glucofuranoside methyl				+	
18	20.58	α-Methyl glucoside					+
19	21.67	Methyl lyxopyranoside					+

+ = very small presence ++ = small presence +++ = presence ++++ = strong presence

All compounds were not present in all samples: all solvents do not extract the same compounds. Some compounds were extracted by a single solvent which explains their presence in one extract. Others were present in several extracts. Presence of the same compound in several previews may be explained by the fact that these compounds could not be completely removed by the lower polarity solvent. Some compounds were present in extracts unhydrolysed and hydrolysed extracts, indicating that these compounds do not undergo hydrolysis process. This is case of D-glucose, mannitol, myo-inositol, methyl glucofuranoside, methyl mannopyranoside, arabinopyranose, ribofuranose.

4. Conclusion

This study on in vitro evaluation of antioxidant activity, inhibitory activities on acetylcholinesterase, xanthine oxidase, α-amylase, 5-lipoxygenase of *Croton lobatus L*, and on evaluation of acid hydrolysis effect on biological activities and on chemical composition is the first. The results show that *Croton lobatus L*. has very small antioxidant activity, small inhibitory activity of 5-lipoxygenase, moderate inhibitory activity of acetylcholinesterase. Plant could be very effective in diabetes, gout treatment. Acid hydrolysis sometimes has a negative effect on biological properties and on chemical composition.

Conflict of Interest

We declare that there is no conflict of interest

Acknowledgements

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