



Identification of New Molecules in the Trunk Bark of *Sarcocephalus Pobeguinii*

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Abstract :

Background

Medicinal plants are commonly used in both traditional and modern medicine. *Sarcocephalus pobeguunii* is a plant used in the treatment of several diseases in Africa. Because of their uses, these plants contain therapeutic virtues often due to the different molecules present.

Objective

The purpose of this study is to identify new molecules of trunk bark of *Sarcocephalus pobeguunii*.

Method

100 g of crushed bark from the trunk of *Sarcocephalus pobeguunii* were introduced into 1000 ml of distilled water and then kept for 30 minutes at boiling point. The extract is injected into the Ultra Performance Liquid Chromatography coupled with a mass spectrometer. Detection of the extract results in the appearance of a peak whose retention time and surface area are then recorded. The molecules to be analyzed are bombarded by a laser beam and the positive, charged (+ze) molecular fragments are then accelerated in an electric field. Measuring the mass/charge ratios (m/z) of the atoms or molecules present in a given sample has made it possible to obtain their molecular mass, identify them and determine their raw formula by comparison with a database.

Results

The analysis by UPLC made it possible to detect 50 peaks of which 4 were majority peaks with retention times of 4.27; 4.37; 4.52 and 5.78 respectively. The percentage of peaks 20, 21, 22 and 40 are 15%, 9%, 7% and 12% respectively. On the other hand, the molecular fragmentation of the mass spectra of peaks 35, 37 and 40 of the detected compounds made it possible to identify tetrahydrodeoxycordifoline, quercetin and magniflorine with the respective raw formulae $C_{28}H_{36}O_{11}N_2$, $C_{15}H_{10}O_7$ and $C_{20}H_{20}O_3N_2$

Conclusion

This study identified quercetin, magniflorine and tetrahydrodeoxycordifoline in the bark of the trunk of *Sarcocephalus pobeguunii* allowing the identification of other molecules.

Keywords : *Sarcocephalus pobeguunii*, molecules, HPLC/MS

1. INTRODUCTION

Sarcocephalus pobeguunii (syn. *Nauclea pobeguunii*) is a tree 4-25 m high and 8-60 cm in diameter. It is common in backwater and riverbanks. *Sarcocephalus pobeguunii*, because of these virtues, is used both in food and in therapy [1].

The work noted in the literature concerns both total extracts or individual organs and isolated compounds demonstrating antimalarial [2,3,4], antibacterial, antibiotic and antioxidant properties [5,6].

Numerous scientific works on the genus *Nauclea* highlight monoterpenes, saponins, flavonoids, alkaloids [7,8,9,10,10,11,12], steroids and glycosides [13,14]. The structures of the characteristic compounds isolated from the various organs belong mostly to the alkaloid family. For example, (5S)-5-carboxystrictosidine, 19-O-methylangustoline, 3-O- β -fucosylquinovic acid, 3-ketoquinovic acid and strictosamide have been detected in the trunk bark [20]. While other alkaloids such as angustine, naufoline, angustoline, nauclefine, O-acetyl-angustoline, 3,14-dihydroangustine as well as two quinovic acid glycosides have been identified in the roots [15,16,17,18]. Naucleamides, nauclefolinin, angustin, angustifolin, nauclefin and naucletin, cadambin and naufolin, naucleidinal and epinauclédinal, strictosamide, 10-hydroxystictosamide, nauclefolin and nauclechin were also isolated from stem bark and various other organs [7,12,19,20].

2. MATERIAL AND METHODS

2.1. Plant Material and Preparation

The trunk bark of *Sarcocephalus pobeguini* Hua ex Pellegr. harvested in Lambaréné (Gabon) in January 2010 and dried at room temperature for one week in the shade and protected from light and humidity. 100 g of crushed trunk bark of *Sarcocephalus pobeguini* were introduced into 1000 mL of distilled water and kept boiling for 30 minutes.

2.2. Analysis of the Extract by UPLC/SM/TUV

UPLC (Ultra Performance Liquid Chromatography) is performed in RP (reverse phase) mode, and the chromophore detection mode is followed by the TUV detector for UPLC, and a time-of-flight (TOF) mass spectrometer is coupled to a tandem quadrupole (TQ) detector. Thus, the molecules to be analyzed are bombarded by a laser beam. The positive, (+ze) charged molecular fragments are then accelerated in an electric field. The ions formed will reflect the most stable ions that the molecule can form. The highest molecular weight peak in the spectrum will represent the parent molecule with one electron less which is called the molecular ion (M⁺).

Mass spectrometry is a chemical characterization technique that consists of measuring the mass/charge ratios (m/z) of atoms or molecules present in a given sample in order to obtain their molecular weight, identify them and determine their raw formula by comparing it with an online database.

The identification and quantification of the compounds in the extract and sub-fractions were performed using a UPLC-TUV-SM system.

3. RESULTS AND DISCUSSION

3.1. High Performance Liquid Chromatography of the Extract

Figure 1 shows the chromatogram of the extract.

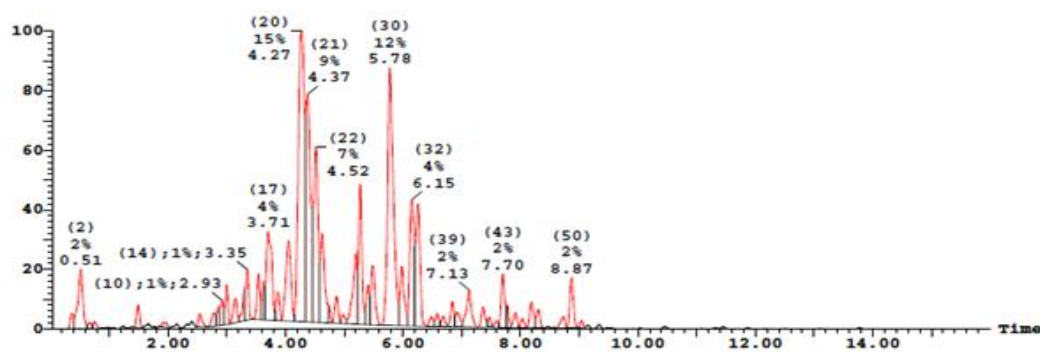


Figure1. Chromatogram of crude extract by UPLC

A total of about 50 peaks were detected, of which four were majority peaks above 7%. These are peaks 20 (15%), 21 (9%), 22 (7%) and 30 (12%) which elute at retention times 4.27; 4.37; 4.52 and 5.78 respectively as shown in the chromatographic profile in Figure 2.

3.2. High Performance Liquid Chromatography Coupled with Mass Spectroscopy

Molecular fragmentations of the peak mass spectra obtained by high-performance liquid chromatography have been listed in Table I.

Table1. Molecular fragmentations of the mass spectra of the different peaks of the detected compounds

Peak no	Retention time	Percentage	[M] ⁺ m/z	Molecular ion
1	0,36	0,37	214.9	214.9(100), 198.9(64), 336.8 (45), 122.9(42), 290.9(29), 458.8(25), 344.9(15), (542.7(13), 672.7(7), 862.6(3), 908.6(3), 1046.5(2)
2	0.51	2	381.1	381.1(100), 362.1(35), 351.1(21.5), 543.1, 258.1(13.5), 443.1(11), 144.1(5), 191.0(5), 614.2(5), 857.3(1.5), 704.2(3), 739.2(3)
3	0.66	0,16	543.1	543.1(100), 226.1(86), 272.1(73), 527.2(65), 330.1(48), 524.2(45), 182.1(39), 434.1(36), 544.1(20), 641.1(19), 124.1(12), 686.2(10), 776.2(9), 776.7(4), 938.3(3), 1028.3(1)
4	0.74	0,1	182.1 M- CH ₃	182.1(100), 136.1(24), 272.1(20), 515.1(16), 365.1(12.5), 543.1(9), 735.1(4), 641.1(3) m/z 197 [M] ⁺ = □-carbolinium Specter UV = melinonine F
5	1.49	0,58	224.1	224.1(100), 224.1(12),226.1(2), 138.1(2),358.1(4), 386.2(1), 920.3(1), 1001.3(1), 1163.4(1), 1244.4(1)
6	1.92	--	407.1	407.1(100), 374.1(72), 423.1(45), 358.1(44), 212.1(35), 247.1(28), 439.1(25), 197.1(17), 136.1(10), 291.1(9), 551.2(9), 728.3(6), 559.7(3), 763.2(2), 85.0(1)
7	2.54	0,21	521.2	521.2(100), 500.0(96), 1003.4(35), 537.7(26), 984.4(20), 1019.3(16), 743.3(16), 171.1(10), 538.1(6), 226.1(11), 743.8(9), 1020.3(6), 502.2(5), 1226.0(6), 744.3(4), 871.3(2), 683.2(2), 291.0(4), 111.1(3), 1467.1(1)
9	2.87	0,73	513.2	513.2(100), 593.2(68), 500.0(36), 692.2(41), 471.1(35), 468.1(30), 244.1(25), 692.7(20), 594.2(19), 233.1(16), 251.1(15), 700.2(12), 204.1(10), 287.1(5), 951.3(5), 916.3(5), 154.1(3)
10	2.93	1	529.2	529.2(100), 530.2(21), 530.2(21), 513.2(17), 692.2(12), 471.1(11), 179.1(6), 672.7(6), 701.2(1), 916.3(1), 950.2(1.5)
11	2.99	0,93	575.2	575.2(100), 576.2(28), 529.2(15), 609.2(2), 355.1(5), 151.0(4), 208.1(4), 609.2(3), 402.2(2) Pic 11 = 5-carboxystrictosidine, C ₂₈ H ₃₅ O ₁₁ N ₂ ; [M+H] ⁺ ; Mesia et al, 2010 isomer 1
12	3.15	0,79	185.1	185.1(100), 163.0(78), 487.2(99), 501.2(60), 737.2(57), 281.1(41), 737.7(38), 716.3(30), 519.1(29), 484.2(28), 745.2(21), 153.1(20), 746.2(16), 533.1(10), 366.1(11), 948.4(9), 135.1(8), 1010.3(13), 1012.3(4), 747.2(4)
13	3.30	1,47	533.2	533.2(100), 182.1(55), 534.2(29), 179.1(20), 531.2(19), 211.1(17), 803.3(14), 542.2(11), 545.2(5), 811.3(5), 281.1(4), 439.1(3), 705.3(2), 1064.4(3), 1100.3(3), 1326.0(1)
14	3.35	1	533.2	533.2(100), 534.2(27), 182.1(17), 211.1(11.5), 542.2(8), 803.3(7), 281.1(4), 1064.4(2)
15	3.54	1,24	543.2	543.2(100), 559.2(27), 325.1(11), 181.1(9), 560.2(6), 219.1(5), 327.2(2), 681.3(1)
16	3.62	0,66	533.2	533.2(100), 227.1(67), 501.2(26), 209.1(24), 534.2(24), 199.1(17), 275.1(10), 181.1(7), 578.2(6), 375.1(5), 111.1(3), 663.2(2), 798.8(2), 1040.9(1)
17	3.71	4	561.2	561.2(100), 577.2(91), 578.2(24), 533.2(21), 327.2(10), 373.2(11), 227.2(9), 501.2(4), 579.2(4),

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				209.1(3), 275.1(2), 751.3(2)
18	3.87	0,35	517.2	517.2(100), 343.2(56), 309.1(46), 518.2(26), 607.2(19), 383.2(12), 246.1(6), 399.2(4), 610.2(3), 176.1(1), 1061.4(4)
19	4.05	1,73	589.2	589.2(100), 527.2(69), 401.2(55), 737.3(36), 590.2(31), 309.1(15), 402.2(13), 738.3(12), 415.2(3), 739.3(3), 199.1(1), 591.2(1), 899.3(1)
20	4.27	15	575.2	575.2(100), 576.2(55), 577.2(22), 413.2(7), 558.2(6), 323.1(4), 578.2(3), 168.1(1), 1149.4(1) Pic 20 = 5-carboxystrictosidine ; C ₂₈ H ₃₅ O ₁₁ N ₂ ; [M+H] ⁺ = 575.2 ; Mesia et al, 2010 ; isomer 2
21	4.37	9	513.2	513.2(100), 575.2(87), 509.2(43), 576.2(30), 1025.4(18), 351.1(15), 591.2(14), 1026.4(11), 635.3(5), 281.1(2), 1027.4(1), 185.1(1) Pic 21 = acid 3-cetoquinovic, C ₃₀ H ₄₄ O ₅ [M+K] ⁺ = 513 ; 1149 [Dimer+H] ⁺ Mesia, 2010
22	4.52	7	555.2	555.2(100), 556.2(43), 571.2(27), 576.2(30), 367.1(2), 293.1(1) Pic 22 = strictosidine, C ₂₇ H ₃₄ O ₉ N ₂ ; [M+Na] ⁺ = 555 Xu et al, 2012 ;
23	4.63	3,21	494.2	494.2(100), 513.2(84), 464.2(22), 555.2(21), 575.2(9), 589.3(3), 191.1(1), 293.1(2) 322.1(1), 1007.3(1)
24	4.87	0,83	429.2	429.2(100), 349.1(97), 566.2(74), 569.2(32), 617.2(25), 476.2(25), 346.1(17), 332.1(12), 618.2(7), 827.3(3), 867.3(3), 943.3(1), 163.0(1), 259.1(3), 667.2(2)
25	4.98	0,28	349.1	349.1(100), 508.2(59), 513.2(51), 407.2(18), 566.2(16), 346.2(11), 573.2(5), 665.2(4), 163.0(2), 315.1(2), 797.4(1)
26	5.19	2,64	522.2	522.2(100), 523.2(30), 411.2(24), 418.2(14), 397.2(11), 419.2(4), 603.3(4), 524.2(4), 419.2(4), 264.1(1)351.1(1), 725.3(1)
27	5.27	4,01	543.2	543.2(100), 544.2(34), 381.1(27), 522.2(22), 411.2(21), 661.3(7), 1085.4(3), 662.3(3), 379.1(2), 169.1(1), 291.1(1), 833.8(1)
28	5.41	1,23	411.2	411.2(100),412.2(23), 390.1(20), 522.2(17), 302.1(8), 543.2(5), 851.3(5), 661.3(2), 185.1(1), 925.3(1), 1173.4(1)
29	5.48	0,88	332.1	332.1(100), 302.1(74), 438.1(36), 375.1(16), 288.1(11), 462.2(11), 522.2(5), 851.3(5), 210.1(1), 631.3(1), 947.3(1) 499.2(100), 997.4(93), 998.4(65), 337.2(56),500.2 (46), 999.4(21), 338.2(18), 267.1(10),171.1(9), 501.2(9), 1000.4(4), 521.2(2), 1266.0(2), 172.1(1), 767.8(1) Pic 30 = Strictosamide ; C ₂₆ H ₃₀ O ₈ N ₂
30	5.78	12	499.2	499.2(100), 997.4(93), 998.4(65), 337.2(56),500.2 (46), 999.4(21), 338.2(18), 267.1(10),171.1(9), 501.2(9), 1000.4(4), 521.2(2), 1266.0(2), 172.1(1), 767.8(1)
31	5.98	1,26	469.3	469.3(100), 499.2(59), 500.2(15), 337.2(12), 307.7(8), 817.4(5), 583.3(3), 679.3(2), 289.1(1), 1108.6(1)
32	6.15	4	469.3	469.3(100), 470.3(33), 423.3(26), 487.3(14), 341.2(14), 817.4(5), 316.1(5), 488.4(5),191.2(1), 671.4(1), 769.4(1), 958.5(1), 1212.2(1)
33	6.29	5,04	499.2	499.2(100),543.2(93), 381.1(30), 544.2(25), 496.2(11), 334.1(10), 382.1(6), 545.2(4), 1085.4(4), 307.1(2), 907.3(2), 171.1(1) 817.3(1)
34	6.49	0,1	379.2	379.2(100), 599.2(60), 339.2(48), 391.1(23), 600.2(18), 480.2(17), 305.1(13), 290.1(5), 571.8(3),

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				899.3(3), 601.2(2), 1120.6(2), 221.1(1), 776.3(1)
35	6.58	0,20	599.2	599.2(100), 541.2(73), 321.2(68), 413.2(60), 265.1(53), 859.3(22), 223.1(9), 524.2(13), 906.4(6), 1186.6(5), 1023.5(3), 152.0(2), 830.9(1), 1469.3(1) Peak 35 = tetrahydrodeoxycordifoline ; [M+Na] ⁺ = 599; C ₂₈ H ₃₆ O ₁₁ N ₂
36	6.68	0,17	321.1	321.1(100), 452.2(71), 307.1(28), 453.2(20), 322.1(19), 446.2(14), 293.1(9), 935.4(7), 859.3(5), 681.4(4), 195.1(3), 1097.6(2)
37	6.87	0,62	303.1	303.1(100), 331.1(42), 335.1(23), 787.3(8), 336.1(4), 788.3(4), 519.2(4) Peak 37 = Quercetin ; [M+H] ⁺ = 303 C ₁₅ H ₁₀ O ₇
38	6.92	0,48	321.2	321.2(100), 335.1(54), 303.1(39), 787.3(25), 355.1(12), 788.3(12), 437.3(5), 555.2(4), 585.2(3), 186.0(3), 789.3(1)
39	7.13	2	360.2	360.2(100), 425.4(15), 337.2(13), 930.5(14), 476.2(6), 856.3(4), 936.5(6), 817.4(2), 288.3(1), 477.2(1), 617.3(1), 953.5(1)
40	7.36	0,54	337.2	337.2(100), 361.2(38), 362.2(4), 468.1(3), 606.3(2), 208.6(1), 288.3(1), 861.3(1) Peak 40 = magniflorine ; [M+H] ⁺ = 377; C ₂₀ H ₂₀ O ₃ N ₂
41	7.47	0,27	383.2	383.2(100), 361.2(38), 393.1(23), 861.3(13), 448.2(8), 348.2(5), 862.3(5), 547.2(4), 935.5(2), 273.1(1), 684.7(1), 817.3(1)
42	7.60	1,5	831.3	831.3(100), 832.3(48), 330.1(40), 315.1(25), 359.1(11), 451.3(9), 313.1(3), 833,3(7), 497.3(2), 728.3(2), 834.3(1)
43	7.70	2	361.2	361.2(100), 362.1(22), 316.1(6), 363.2(2), 508.2(1), 575.2(1), 831.3(1), 859.3(1)
44	7.77	0,41	361.2	361.2(100), 469.3(95), 316.1(65), 470.3(29), 589.2(27), 362.2(22), 423.3(16), 487.3(10), 728.3(7), 1287.8(3), 159.0(1), 200.0(1), 772.4(1), 925.1(1), 969.1(1), 1173.7(1)
45	7.92	0,37	362.2	362.2(100), 383.1(20), 363.2(18), 1108.6(3), 364.2(2), 291.1(1), 439.1(1), 588.2(1)
46	8.04	0,21	425.4	425.4(100), 363.1(59), 469.3(49), 635.2(38), 773.4(26), 485.1(23), 335.1(18), 636.2(14), 774.5(10), 486.1(6), 790.9(3), 273.2(3), 191.2(2), 1362.8(1)
47	8.19	0,83	331.1	331.1(100), 332.1(20), 333.1(13), 335.1(8), 353.1(3), 307.1(2)
48	8.31	0,55	331.1	331.1(100), 332.1(21), 305.1(5), 619.2(2), 347.1(1), 551.1(1)
49	8.73	0,43	315.1	315.1(100), 314.1(30), 316.1(19), 319.1(5), 177.0(1), 454.2(1), 625.3(1)
50	8.87	2	319.1	319.1(100), 361.2(23), 362.2(6), 303.1(4), 485.3(2), 617.2(2)
51	9.04	0,18	361.2	361.2(100), 362.2(22), 425.4(10), 773.5(4), 426.4(3), 287.2(1), 633.2(1)

The coupling of UPLC to ionization mode mass spectrometry allowed the identification of quercetin (peak no. 30 with [M+H]⁺ = 303; C₁₅H₁₀O₇), magniflorine (peak no. 40 = with [M+H]⁺ = 377 ; C₂₀H₂₀O₃N₂) and tetrahydrodeoxycordifoline (Peak no. 35 =; [M+Na]⁺ = 599; C₂₈H₃₆O₁₁N₂) in the trunk bark of *Sarcocephalus pobeguinii*.

Analysis of trunk bark of *Sarcocephalus pobeguinii* by HPLC coupled with mass spectroscopy allowed the identification and isolation of quercetin, magniflorin and tetrahydrodeoxycordifoline. Magnlorin and tetrahydrodeoxycordifoline are alkaloid compounds and quercetin are a tannin. These results corroborate those of other researchers who had identified in trunk bark in addition to (5S)-5-carboxystrictosidine, 19-O-methylangustoline and strictosamide, 3-O-β-fucosylquinovic acid and 3-

ketoquinovic acid [20]. Further work has shown that some alkaloids and other compounds have been found in other plant organs. Thus, other alkaloids such as angustine, naufoline, angustoline, nauclefine, O-acetyl-angustoline, 3,14-dihydroangustine as well as two quinovic acid glycosides have been identified in the roots by authors [16,17,18,21,22]. Similarly, naucleamides A, B, C, D, E and F, nauclefolinin, angustin, angustifolin, nauclefin and nauclestin, naulafin, 3--dihydrocadambin, cadambin and naufolin, naucleidinal and epinauléidinal, isovincoside lactam (strictosidine lactam or strictosamide), 10-hydroxystictosamide, nauclefoline and nauclechine have also been isolated from stem bark and various other organs [7,12,19,20,23].

4. CONCLUSION

Quercetin, magniflorin and tetrahydrodeoxycordifoline were detected in the trunk bark of *Sarcocephalus pobeguini* by HPLC coupled with mass spectroscopy. These results require further fractionation in order to isolate the active ingredients.

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