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Research Article

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Fractionation of the methanolic extract and quantitative analysis of total polyphenols and total flavonoids of the species *Sarcocephalus latifolius* (Sm.) Bruce (MUTUMBI) in the Republic of CONGO.

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Abstract

The ethnobotanical survey conducted on plants with anti-inflammatory, antibiotic, anti-leprosy, analgesic, antimalarial, antibacterial, and antifungal properties in Africa in general and in Congo in particular has led to the selection of the species *Sarcocephalus latifolius* (Sm.) Bruce (Rubiaceae). The study of this species focused on qualitative and quantitative analyses of the methanolic extract as well as those of the fractions obtained after separation on an open silica gel column, which yielded interesting results. The qualitative analyses performed by CCM reveal the presence of polyphenols with a dominance of flavonoids (flavonol and flavones). The results of the quantitative analyses by spectrophotometry of the methanolic extract and the fractions F2, F3, F4, F5, F6, F7, and F8 show that the methanolic extract and the fractions F7, F2, and F4 are quantitatively rich in polyphenolic compounds

compared to the fractions F3, F8, F5, and F6. It is noted that the content of polyphenols and flavonoids in the methanolic extract shows very high values of 3667.868 ±0.027 mgEAG/100 gMS and 207.894 ±0.051 mgEQt/100 gMS, followed by the fractions F7, F2, and F4 with values of 2704.605 ±0.053 ; 2699.232± 0.034 ; and 2344.657± 0.01 mgEAG/100 gMS and 83.700±0.034 ; 273.294±0.015 ; 57.274±0.01 mgEQt/100 gMS, while the fractions F3, F8, F5, and F6 show the lower values of (2194.231±0.036 ; 1963.220±0.051 ; 1898.752±0.036 ; and 812.998±0.053 mgEAG/100 gMS) and (52.320±0.047 ; 40.760±0.015 ; 20.281±0.01 ; and 5.087±0.005 mgEQt/100 gMS), respectively.

Keywords: Extraction; fragmented ; *Sarcocephalus latifolius* (Sm.) Bruce ; total polyphenols ; total flavonoids.

I. Introduction

Human beings have always relied on nature to meet their needs: food, clothing, and health. It is increasingly diverse and plays several roles both nutritionally and health-wise. Indeed, it must, on a daily basis, meet the nutritional needs of the human body and help protect it against external aggressions, particularly diseases and premature aging. It is worth noting that plants today represent an inexhaustible source of new molecules and provide some answers to various questions related to several diseases caused by numerous microbes and other pathogens (viruses, bacteria, fungi, etc.) [1]. *Sarcocephalus latifolius*, Herbaceous annual plant of the Rubiaceae family, native to sub-Saharan Africa, also known as African peach. A species that contains a new pain-relieving molecule from an extract of bark and roots and has been found, after analysis, to be identical to tramadol, a synthetic drug designed by humans widely used as an analgesic around the world. To confirm its discovery, the French team tested different processes to prove the authenticity of this natural origin. These analyses have also been confirmed by three independent laboratories. From a quantitative point of view, the concentration of tramadol in the extracts of dried bark of this plant ranges from 0.4 to 3.9%, indicating high levels of active principles [2]. Twelve compounds including eight (08) tri terpenes and four (04) sterols have also been isolated from the roots [3]. The extraction of high-value active ingredients from plant material, particularly in the case of polyphenols, which are currently attracting a lot of interest due to their analgesic and antioxidant properties, is a very important step in both the isolation and identification of phenolic compounds. The solubility of phenolic compounds depends on their chemical nature in the plant, which varies from simple compounds to highly polymerized ones. Therefore, the best extraction yields are recorded by decoction, averaging 17.34% and 15.64% for maceration; however, water and methanol remain the best extraction solvents. Maceration seems to be better for the extraction of total polyphenols (19.88 mg GAE/g MS on average) and total flavonoids (8.25 mg EQ/g MS on average) [4]. Maceration has been chosen as the extraction method, due to the thermosensitivities of the chemical substances contained in *Sarcocephalus latifolius*. The objective of this study is to evaluate the total polyphenol and total flavonoid content of the roots, and to fractionate the extract by modifying the polarity of the extraction solvent.

II. Materials and Methods

II.1. Materials

The plant material, consisting of the roots of the species *Sarcocephalus latifolius*, was collected in the south of Congo in Niari, specifically in Dolisie. The roots were then dried for 14 days in the open air at an ambient temperature of around 28°C away from light and then ground using a mortar.

II.2. Methods

II.2.1. Preparation of extracts in solvents

II.2.1.1. methanolic extract

The extraction of polyphenolic compounds was carried out by mixing 600 g of plant material in

6000 ml of a 100% methanolic solution. The mixture was allowed to rest for 72 hours. After filtration, the extract was concentrated to dryness and then prepared for analysis.

II.2.2. Fractionation of the methanolic extract (100 %).

17 g of methanolic extract was chromatographed on an open silica gel column with a diameter of 1.5 mm and a length of 50 cm. Elution was carried out using hexane, with the polarity gradually increased by 10% through the addition of a solvent mixture of ethyl acetate and methanol. The monitoring of the different collected fractions was performed by TLC analysis using silica gel on aluminum support. The plates were first visualized under UV (λ = 254 and 366 nm) and then revealed with NEU reagent [5] followed by another visualization under UV-366 nm.

Figure 1. Chromatograpic separation on open column

Stationary phase: Silica gel; Column dimension. Length: 50 cm and Diameter: 1.5 mm

II.2.3. Chemical Analysis

II.2.3.1. The chemical screening;

The secondary metabolites have been highlighted by the protocols recorded in Table I.

Chemical family	Composition of the reagent
Alkaloids	5 ml of the extract + 1 ml of HCl (1N) + three drops of MAYER's reagent
Free flavonoids	5 ml of extract + 5 ml of hydrochloric acid solution + 1 ml of isoamyl alcohol and magnesium shavings
Anthocyanin flavonoids	5 ml of extract + 5 ml of sulfuric acid (H2SO4) at 10% + 5 ml of ammonia (NH3) at 20%
Tannins	5 ml of extract + 1 ml of an aqueous solution of iron(III) chloride.
Catechin tannins	5 ml of extract + 1 ml of HCl * then boiled for 15 minutes, filtered and add 1 ml of isoamyl alcohol.
Leuco-anthocyanin	5 ml of extract + 5 ml of hydrochloric acid solution + 1 ml of isoamyl alcohol, then heat for 5 minutes in a water bath.
Terpenes and sterols	10 ml of ether extract + 1 ml of acetic anhydride + 1 ml of CHCl3 + 1 ml of sulfuric acid (H2SO4)
Composition reducers	5 ml of extract + 1 ml of Fehling's solution
Saponosides	In a series of ten test tubes, successively distribute 1, 2, 3, 4, 5, 6, 7, 9, and then 10 ml of decoction. Adjust the volume of each tube to 10 ml with distilled water, then shake each tube in a horizontal position for fifteen (15) seconds.

II.2.3.2. Thin layer chromatography

The analysis by CCM was carried out with the methanolic extract. The system ethyl acetate/formic acid/water (9/0.5/0.5) then ethyl acetate/formic acid/glacial acetic acid/water (10/1.1/1.1/2.6), followed by a spray with Neu reagent [5], allowed the highlighting of polyphenols (λ = 366 nm).

III. Results and Discussion

III.1. Chemical analyses

Methanolic extract

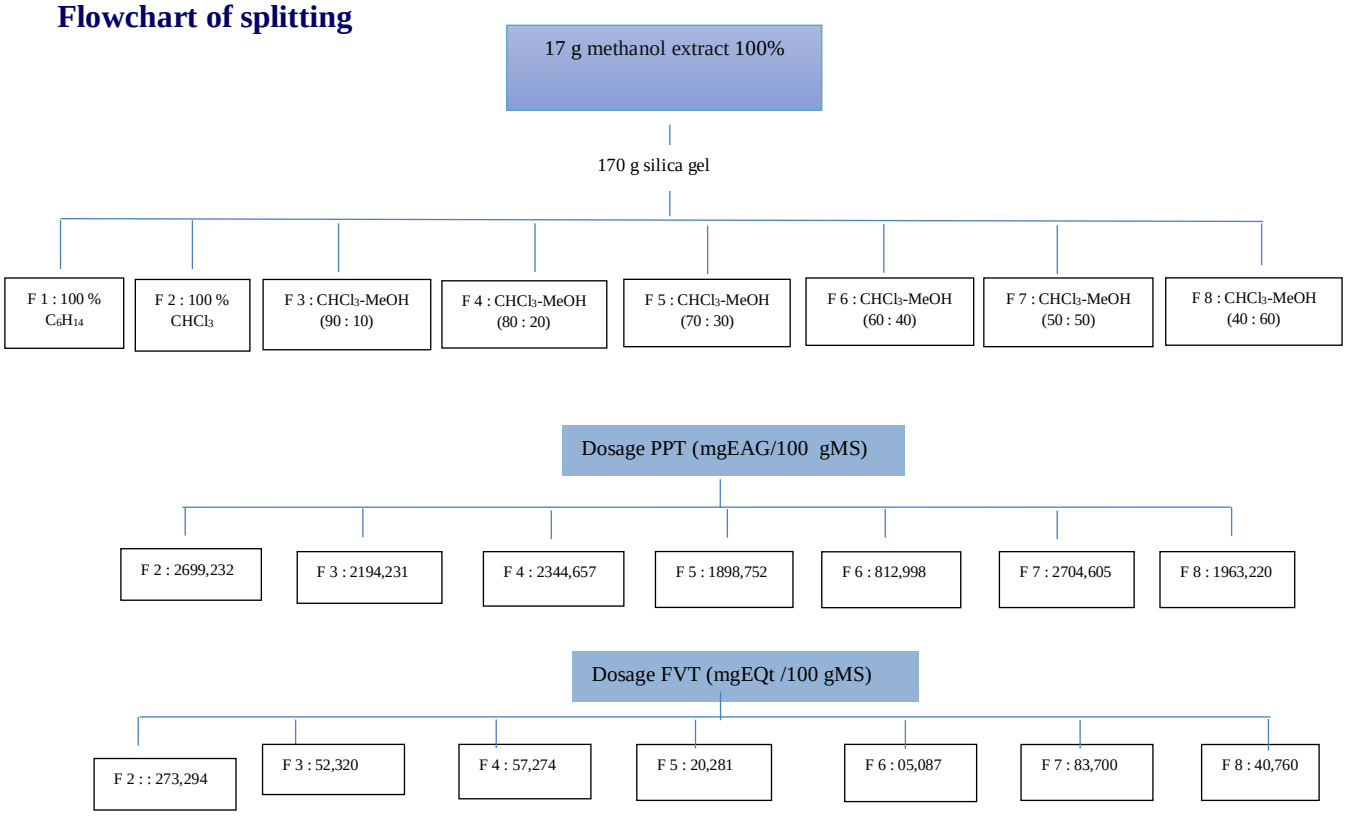
The mass of the macerate after drying is 125.91g with a yield of 21% of dry matter.

III.2. Fractionation

The macerate is fractionated into eight fractions according to an increasing polarity gradient.

Fraction(s)	obtained mass (g)
F1	0,32
F2	1,20
F3	1,61
F4	5,34
F5	2,11
F6	3,23
F7	2,15
F8	0,75

Flowchart of splitting



III.1.1. Chemical analysis of extracts by Thin Layer Chromatography (TLC)

Figure 2 shows the chromatographic profiles of the methanolic extract, the different fractions (F2, F3, F4, F5, F6, F7, and F8) obtained after chromatographic separation. These profiles reveal a series of different fluorescence spots obtained after spraying the plate with Neu and visualizing at 366 nm. These spots could indicate the presence of several chemical families. Based on the literature data provided by Wagner and Blat [6], the following observations can be made:

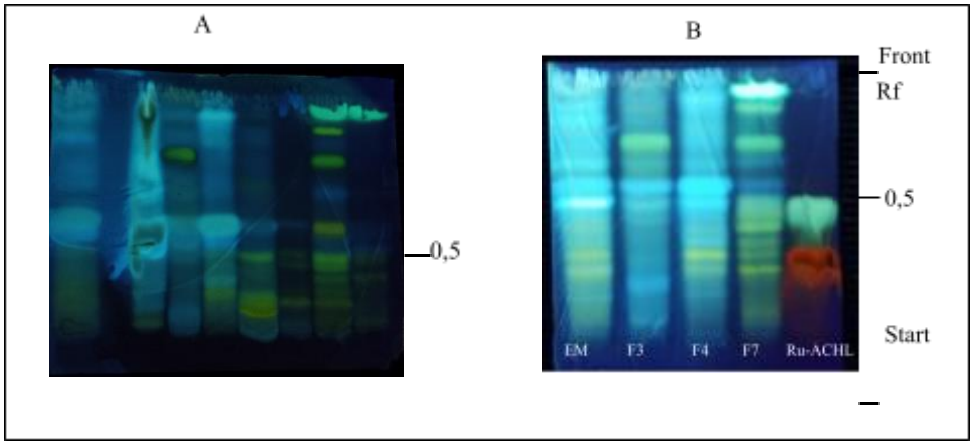


Figure 2: Chromatographic profiles of the methanolic extract and the fractions

Eluent (A): Ethyl acetate/ Formic acid/water (9/0.5/0.5); (B): Ethyl acetate/ Formic acid/Glacial acetic acid/water (10/1.1/1.1/2.6); Stationary phase: (A); (B): Silica gel; Developer: Neu; Observation: UV-366 nm. Reference compound: Ru-Chl: Rutin- Chlorogenic Acid; F1, F2, F3, F4, F5, F6, F7, and F8: Fractions obtained after separations on a silica gel column

The yellow-green fluorescences with frontal retentions varying with the eluents and the stationary phase, clearly highlighted in the extract and fractions (F2, F4, F5, F6, and F7), could be attributed to the derivatives of a hydroxylated flavonol at position 3' [6] (Figure 2A).

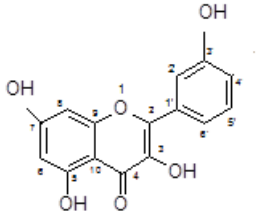


Figure 3: Structure of 3', 5, 7-trihydroxy flavonol: Kaempferol

The bluish-white fluorescence spots with very sharp frontal retentions in the fractions (F2, F3, F4, and F7) highlight the presence of derivatives of an ortho-dihydroxy flavone at positions 3’ and 4’ (luteolin derivative) in the plant. (Figure 2)

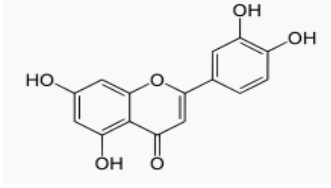


Figure 4: Structure 3’ 4’, 5, 7- trihydroxyisoflavonone: Luteolin

The spots of light green fluorescence in the frontal retentions (Figure 2) highlight the presence of hydroxycinnamic derivatives in the plant. This highlight is very clear in the extract and fractions F3, F5, F7, and F8.

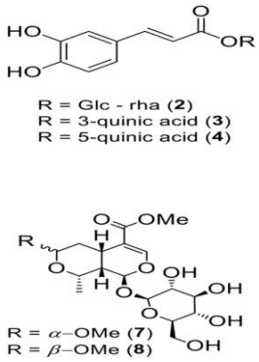


Figure 5: hydroxycinnamic derivatives

R = Glc-rha (2)
R = 3-quinic acid (3)
R =5-quinic acid (4)

These highlighted compounds could be phenolic acid C6-C3 heterosides.

The dark blue fluorescence spots (DBF) visible in the extract and fractions F4, F5, and F7 (Figure 2) highlight the presence of gallic acid derivatives in the plant.

The structures highlighted in this study have been evidenced and quantified in the plant by several authors [7-10]. The co-migration with the rutin standards (Rf 0.4) and chlorogenic acid (figure 2B) confirms the presence of these compounds in the species *Sarcocephalus latifolius.*

These compounds are well-known and their presence in the plant could justify the properties often attributed to the plant. The flavonoids and phenolic acids highlighted in this study are known for their analgesic, anti-inflammatory, antipyretic, and antimalarial properties. [11].

The quantitative analysis of total polyphenols and total flavonoids shows that the methanolic extract and fractions F2, F4, and F7 (figure 6) are quantitatively rich in total polyphenols and total flavonoids compared to fractions F3, F8, F5, and F6. The total polyphenol contents in the extract and fractions F7, F2, and F4 are respectively 3667.868; 2704.605; 2699.232; and 2344.657 mg EAG/100 g Ms, while those of fractions F3, F8, F5, and F6 are respectively: 2194.231; 1963.220; 1898.752; and 812.998 mg EAG/100 g Ms. The low levels of polyphenolic compounds in fraction F1 can be justified by the less distinct chromatographic profiles, whereas those of fractions F2, F3, F4, F5, F6, F7, and F8, which exhibit clearer profiles marked by yellow-green fluorescent compounds (FJV), whitish-blue (FBB), dark blue (FBF), and yellow-green (FJV) evidenced on thin layer chromatography. The levels of flavonoid compounds follow the same order reported below for fractions F3, F8, F5, and F6. It should be noted that this level is negligible for fraction F6 (05.087±0.005 mgEQt/100 gMs). Fraction F2 shows the highest amounts of flavonoid compounds followed by the extract and fractions F7 and F4, at 273.294; 207.894; 83.700; and 57.274 mgEQt/100 gMS, respectively. This representativity in flavonoid compounds could be associated with the presence of the very clear yellow-green fluorescence compounds (figure 2B), highlighted at the front retentions varying according to the nature of the eluents. These compounds are associated with ortho-dihydroxylated flavonol derivatives at position 3' (FJV). While this fluorescence is almost non-

existent infractions (F1 F3) and often appears only as traces in the methanolic extract.

It is noted that the type of stationary phase used (silica gel) in this study allowed for the enrichment of these fractions in polyphenolic compounds. The total polyphenol and total flavonoid contents obtained in this study could be justified by the very clear evidence observed through Thin Layer Chromatography (TLC) and the presence of these metabolites reported by several authors. [7-10].

Samples	PPT (mgEAG/100 gMs)	FVT (mgEQt/100 gMs)
Ext MeOH	3667,868±0.027	207,894±0.051
F2	2699,232± 0.034	273,294±0.015
F3	2194,231±0.036	52,320±0.047
F4	2344,657 ± 0.01	57,274 ±0.01
F5	1898,752±0.036	20,281±0.01
F6	812,998 ±0.053	05,087±0.005
F7	2704,605 ±0.053	83,700±0.034
F8	1963,220±0.051	40,760 ±0.015

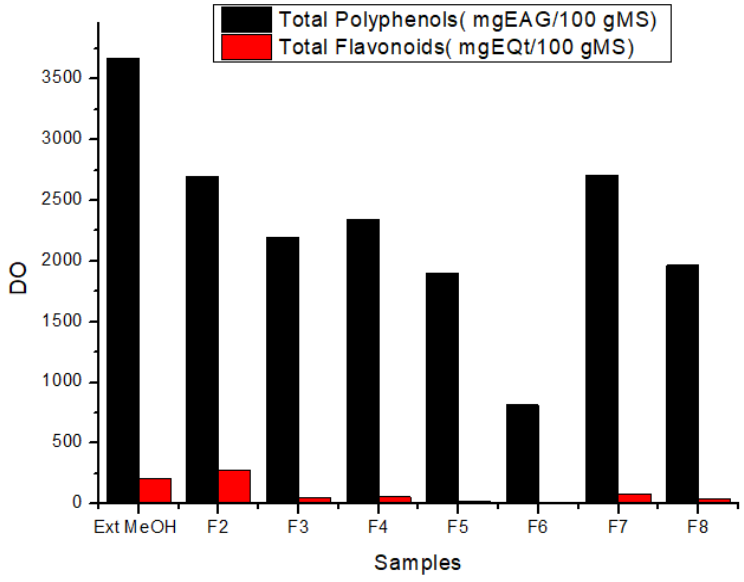


Figure 6 : Total polyphenol content and total flavonoid content of the extract and fractions of *Sarcocephalus latifolius* (Sm.) Bruce

IV. Conclusion

From this study, it appears that the methanolic extract and the fractions obtained after separation on open silica gel column show a chromatographic profile on thin layer dominated by fluorescent White-Blue compounds characterizing the presence of ortho-dihydroxylated flavone derivatives at positions 3' and 4' (a derivative of luteolin) which are very abundant in the plant. Other compounds belonging to the same family have also been identified. These include ortho-dihydroxylated flavonol derivatives at position 3' (Rf 0.8),

hydroxy-cinnamic derivative notably chlorogenic acid and rutin. For the latter compounds, co-migration with standards (AChl-rut) confirms the presence of both compounds in the plant.

She further shows that the levels of appreciable polyphenolic and flavonoid compounds in the extract and fractions F2, F4, and F7 are higher than those in fractions F3, F5, F6, and F8.

These high levels could be attributed to the distinctly highlighted polyphenolic and flavonoid compounds on thin layer chromatography.

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