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Study of the Effect of Three Extraction Processes on the Phytoactives of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae) and Evaluation of their Antioxidant and Analgesic Activities

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: An important aspect of the valorization of plants of interest is the search for phytoactives. This work aims to show the effect of extractive processes (maceration, decoction, Soxhlet extraction) both on the phytoconstituents of a plant species and on their intrinsic properties.
Methodology: *Alchornea cordifolia*, a botanical species growing in Côte d'Ivoire, was chosen for this purpose. Phytophenols, flavonoids and condensed tannins were quantified by spectrophotometry. Antioxidant (DPPH and FRAP) and analgesic activity were also evaluated.
Results: The quantitative analysis of total phytophenols, total flavonoids and condensed tannins of macerated, decocted and Soxhlet extracts of the plant organs (leaves, stems, roots), highlighted their respective contents, varying proportionally according to the extraction conditions. The same observation was made at the end of the estimation of the antioxidant (DPPH and FRAP tests) and analgesic activities of these different extraction fractions.
Conclusion: This study has demonstrated the effect of three extraction processes on some phytoactives quantified of *A. cordifolia*.

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Keywords: Alchornea cordifolia; phytoactives; DPPH; FRAP; analgesic activity.

1. INTRODUCTION

Species of the family of Euphorbiaceae, *Alchornea cordifolia* is a plant used in traditional medicine, in decoction or maceration for its analgesic, anti-inflammatory, antimicrobial, antiparasitic, veinotonic, antioxidant, antidiabetic and astringent properties [1,2]. Biological and pharmacological activities of this plant can be attributed to the phytoactives it contains, which prevent or slow down oxidation by neutralizing free radicals [3-5]. Their extraction from a plant matrix by means of a process appropriate is an initial and necessary step before any analysis. It depends on several factors, in particular the nature of the solvent, the temperature, the method and the extraction time [6]. Optimizing the extraction yield of phytoactives is necessary since the effectiveness of extracts depends on their chemical composition [7]. *A. cordifolia* is a perennial shrub, bushy, erect or climbing, which can reach 4 m in height. Its leaves are simple, alternate with the blade cordate at the base and acuminate at the top. Its flowers are greenish-white in color, and its small fruits are capsular with three compartments containing red seeds [8,9]. Our study was initiated with the intention to understand how traditional extraction processes have an impact on phytoactives and their antioxidant and analgesic activities. Thus, we focused our attention on the phytophenols of *A. cordifolia*.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consists of the leaves, stems and roots of *Alchornea cordifolia*, crescent on the NANGUI ABROGOUA University site (Abidjan / Ivory Coast) (5° 20′ 11″ north, 4° 01′ 36″ west). After identification with respect to the species in the herbarium of the Center National de Floristique (CNF) of the Félix HOUPHOUËT-BOIGNY University (Cocody / Abidjan) and authentication (N^0AD 224) [10], the organs were cleaned, dried under permanent air conditioning (20°C, 15 days) and reduced to powder using an electric grinder.

2.2 Methods

2.2.1 Preparations of extracts

Three extraction processes were used: maceration, decoction and Soxhlet extraction. The extracts were filtered using a funnel and a Büchner flask and then reduced with a BÜCHI type EL-131 rotary evaporator.

2.2.2 Quantification of total phytophenols, total flavonoids and condensed tannins

2.2.2.1 Assay of total phytophenols (TP)

The TP contents were determined by adding to 1 ml of alcoholic extract, 1.5 ml of sodium carbonate (Na_2CO_3) (17%, m/v) and 0.5 ml of Folin-Ciocalteu reagent (0.5N). The mixture was incubated for 30 min in the dark and the absorbance read at 760 nm with a UV-visible spectrophometer (Spectro AL 800). Calibration with gallic acid (0-1000 µg /ml) was carried out under the same conditions and the contents were expressed in milligrams of gallic acid equivalent per gram of dry matter (mg EAG/g DM) [11,12].

2.2.2.2 Assay of total flavonoids (TF)

The TF contents were determined by adding to 100 µl of Neu's reagent, 2 ml of alcoholic plant extract or quercetol (0.05 mg/ml). The absorbance of the mixture was read at 404 nm (UV-visible spectrophometer (Spectro AL 800)) and the contents were determined according to formula (1) [13].

$$\mathbf{TF}(\%) = \frac{\mathbf{0.05} \times \frac{\mathbf{A_{ext}}}{\mathbf{A_q}}}{\mathbf{C_{ext}}} \times \mathbf{d} \times \mathbf{100} \qquad \textbf{(1)}$$

A_{ext}: Absorbance of the extract; **Aq**: Absorbance of quercetol; **C_{Ext}**: Concentration of the extract.

The proportions of TF determined were subsequently expressed in mg EAG/g DM relative to the TP contents.

2.2.2.3 Dosage of condensed tannins (CT)

To 400 µl of alcoholic extract were added 3 ml of a methanolic solution of vanillin (4%, m/v) and 1.5 ml of concentrated hydrochloric acid (HCl). After 15 min at room temperature, the absorbance of the mixture was read at 500 nm (UV-visible spectrophometer (Spectro AL 800)). A calibration line is drawn with catechin (0-300 µg/ml), prepared under the same conditions. The CT contents obtained were expressed in milligram catechin equivalent per gram of dry extract (mg EC/g DM) [14].

2.2.3 Evaluation of antioxidant activity

2.2.3.1 DPPH test

To 0.4 ml of alcoholic plant extract were added 1.2 ml of a methanolic solution of DPPH (0.03 mg/ml). The mixture was adjusted with 2.4 ml of methanol (MeOH), and the absorbance read at 517 nm every 3 min for 30 min against a blank. Plant extracts and ascorbic acid (vitamin C, reference antioxidant) were prepared at concentrations (0.128; 0.0357; 0.0014; 0.001 and 0.00071 mg/ml) [15]. The percentage reductions (PR) of DPPH were determined from equation (2):

$$\% \mathbf{PR} = \frac{(\mathbf{Ab}-\mathbf{Ae})}{\mathbf{Ab}} \times \mathbf{100} \qquad \mathbf{(2)}$$

Ae: Absorbance of the extract;
Ab: Absorbance of DPPH

The effectiveness of the samples against DPPH was assessed by graphical determination of the $\mathbf{CR_{50}}$ (concentration that reduces 50% of DPPH) [16,17].

2.2.3.2 FRAP test

The FRAP reagent used was a mixture of sodium acetate buffer (300 mM, pH = 3.6), TPTZ (2, 4, 6-Tri (2-pyridyl)-s-triazine at 10 mM prepared in a solution of HCl at 40 mM) and iron trichloride ($FeCl_3$) (20 mM) in a volume ratio of 10:1:1. The freshly prepared FRAP reagent was previously heated to 37°C in a water bath. 100 µl of Trolox (0.187; 0.375; 0.75 and 1.5 mM) (reference antioxidant) and the samples to be tested were prepared at 0.25 mg/ml, and added to 3 ml of the FRAP reagent. The absorbance of the complex (TPTZ-Fe^{3+}) was read at 593 nm after 4 min. The results were expressed in mM Trolox equivalent with the calibration line constructed from the Trolox [18,19].

2.3.4 Assessment of analgesic activity

The formaldehyde test was used to assess the effectiveness of plant extracts on pain. The control batch of mice received distilled water while the other batches were treated with the extracts (60 and 120 min) of *A. cordifolia* at 100 mg/kg, acetyl salicylic acid (aspirin) (100 mg/kg) and tramadol hydrochloride (trabar) (30 mg/kg). Male and female mice of the species *Mus musculus* of mass between 18 and 22 g, aged 5 to 6 weeks were used. They were obtained from the Physiology, Pharmacology and Phytotherapy laboratory in the Natural Sciences Training and Research Unit (UFR-SN) of the UNA. One hour before the injection of 20 µl of the formaldehyde solution (2.5%) was given to the right hind paw, the mice received the extracts and the reference products orally. Immediately after the injection of the formaldehyde solution, the number of licks of the treated paw was counted during the first five minutes, then between 15-30 min **[**20,21]. The analgesic effect was determined by the formula **(3)**:

$$\% \mathbf{Inhibition} = \frac{\mathbf{Nc}-\mathbf{Nt}}{\mathbf{Nc}} \times \mathbf{100} \qquad \mathbf{\ldots (3)}$$

Nc =number of licks of the paw of the mice of the control group;
Nt control =number of licks of the paw of the mice of the treated group.

2.4 Statistical Analysis

The results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare total phytophenol contents, antioxidant activities, and formaldehyde-induced pain inhibition using Graph Pad Prism 5 software. When a significant difference exists between the means ($P<0.05$), the multiple comparison test of the means was carried out (Tukey's test) to determine the level of difference between the different groups of extracts [22] (Westlake, 1971).

3. RESULTS AND DISCUSSION

3.1 TP Levels

The TP contents of *A. cordifolia* (Table 1) were determined from the linear regression equation for gallic acid (expressed in mg EAG / g DM) taken as standard ($y = 0.0232 x + 0.0002$; $R^2 = 0.9983$). The proportions were variable with significant maximum levels ($p < 0.001$) observed with the Soxhlet extracts of leaves (120 min: 183.57 ± 0.01). The maximum TP content in the stems was obtained by maceration (120 min: 137.28 ± 0.52) and that of the roots by decoction (77.53 ± 0.28). Whatever the organ, TP levels in macerated extracts increase over time (60 min to 120 min). In addition, the roots have significantly ($p < 0.001$) the lowest phytophenol contents for any of the extraction methods.

The results demonstrated that the TP increase when the extraction time increases. Indeed, a high extraction time allows a prolonged contact between the solutes and the solvent, resulting in

Table 1. TP levels in the organs of *A. cordifolia*

Organ	Time (mins)	TP content (mg EAG/g DM)		
		Macerated	Decocted	Soxhlet extract
Leaves	60	108.22 ± 0.15	129.26 ± 0.02	155.12±0.05
	90	109.09 ± 0.01	118.91 ± 0.08	175.03 ± 0.01
	120	111.5±0.15	114.6 ±0.4	183.57 ± 0.01
Stems	60	120.12±1.49	121.50 ± 0.12	112.02 ± 0.15
	90	125.72 ± 0.45	125.12 ± 0.15	133.05 ± 0.15
	120	137.28± 0.52	113.40 ± 0.14	117.45 ± 0.15
Roots	60	64.86 ± 0.26	73.4 ± 0.15	48.66 ± 0.83
	90	71.67 ± 0.15	77.53 ± 0.28	52.53 ± 0.65
	120	72.28 ± 0.14	70.55±0.40	61.41 ± 0.68

a large mass transfer [23]. In addition, there is a decrease in TP content in the decoctions and Soxhlet extracts, probably due to heating, which dehydrates the cell walls allowing easy penetration of the extraction solvent, and the release of solutes [24]. The increase in temperature promotes the extraction of phytophenols by increasing the diffusion coefficient, the solubility of solutes in the extractor and the decrease in its viscosity [25]. Studies have shown that increased extraction time and prolonged exposure to high temperatures potentially increase the loss of phytophenols [26,27].

3.2 TF Content

The TF contents (in mg EAG/g DM) estimated (Table 2) are in variable proportions, depending on the organ, the process and the extraction time. Whatever the extraction process, TF contents in the leaves were significantly higher ($p < 0.001$) than those of the stems and roots.

The TF contents of macerated extracts increase with time (leaves: 33.92 ± 0.22 mg EAG/g DM; stems: 12.22 ± 0.38 mg EAG/g DM; roots: 12.24 ± 0, 30 mg EAG/g DM at 120 mins). Concerning the roots, the TF contents of the decoctions and Soxhlet extracts increase when the extraction time was prolonged. However, in the decoctions of the leaves and stems, we note the influence of the temperature and the extraction time on the different TF contents. Indeed, flavonoids were both thermo and photosensitive [28].

3.3 CT Contents

The catechin calibration line ($y = 0.004 x + 0.006$) was used to determine the CT contents of macerated, decocted and Soxhlet extracts. Table 3 summarizes the results obtained. Levels that vary proportionally were more significant in macerated leaves and stems. The CT of macerated extracts increase when the extraction time was prolonged (Leaves: 60 min 31.67 ± 0.62; 90 min 34.17 ± 0.8 and 120 min 36.25 ± 0.95). Whatever the organ, the macerated CT were more significant ($p < 0.05$) than decoctions and Soxhlet extracts. The leaves and stems contain significantly ($p < 0.001$) more CT than roots. However, in decoctions and Soxhlet extracts, the levels drop when the extraction time was extended. The decreases in CT recorded could also be due to their degradation under the effect of heat. Indeed, some phenolic phytocompounds seem to be heat-sensitive, therefore can be degraded by heating. Heating for a prolonged extraction time can lead to the decomposition of target phytocompounds contained in the extracts [29,30].

3.4 Antioxidant Activity

The antioxidant profile of macerations, decoctions and Soxhlet extracts of *A. cordifolia* was evaluated by spectrophotometry against the DPPH radical and by the FRAP method.

3.4.1 DPPH antioxidant activity

The DPPH antioxidant potential of the extracts evaluated by spectrophotometry made it possible to determine the percentage reductions (PR) of the DPPH radical by the extracts of *A. cordifolia* (Fig. 1). The PR were in variable proportions, and have made it possible to graphically determine the concentrations which reduce 50% of the DPPH (CR_{50}). This quantitative parameter of the effectiveness of an analyte against DPPH is the concentration necessary to reduce 50% of the initial concentration of the DPPH radical. The lower its value, the more pronounced the antioxidant activity [16,17]. Irrespective of the extraction method, the CR_{50} values of the leaf extracts decrease when the extraction time increases up to 120 min; indicating that the

antioxidant activity is enhanced. The prolonged extraction time has a positive impact on the antioxidant activity of the leaf extracts (macerated CR_{50} = 0.0017 at 120 min; Decocted CR_{50} = 0.0012 at 120 min; Soxhlet extract CR_{50} = 0.0012 at 120 min).

The Soxhlet extracts showed greater antioxidant activity than the decoctions, however this difference is not significant. As observed in the leaves, the CR_{50} of the stem and root extracts decrease with an increase in the extraction time. However, beyond 90 min, an increase in the CR_{50} of decoctions and Soxhlet extracts of stems and roots was observed, corresponding to a drop in antioxidant potential. The antioxidant activity of the extracts could be linked to the presence of quantified phenolic phytocompounds. Indeed, most antioxidants of natural origin have in their molecular structures, hydroxyphenolic groups responsible for the antioxidant character [31,32]. The results obtained show the influence of temperature and extraction time on the antioxidant profile of the extracts. The decoctions and Soxhlet extracts showed higher antioxidant activity than the macerated ones.

Several studies have shown that decoctions and Soxhlet extracts of plants show greater antioxidant activity than macerated ones [33-36]. A rise in temperature allows a reinforcement of the antioxidant activity up to a threshold beyond which the antioxidant activity decreases; which would probably be due to the degradation of antioxidant molecules [37,38]. The extraction time has an influence on the antioxidant activity because a prolongation of the contact time of the extractor and the drug would improve the diffusion of the antioxidant compounds [37,39]. On the other hand, a prolonged extraction time can expose antioxidant phytocompounds to degradation, polymerization and/or oxidation [25,39].

3.4.2 FRAP antioxidant activity

The evaluation of the FRAP antioxidant profile was carried out with the extracts of the leaves, stems and roots of *A. cordifolia* by determining their potential for reducing Fe^{3+} to Fe^{2+} (Fig. 2). The presence of Fe^{2+} ions was evaluated by measuring the blue color of the reaction medium [40]. The FRAP activities of the extracts, estimated at 0.25 mg/ml, were expressed in mM TE/g MS from the Trolox calibration line. The FRAP antioxidant activity of vitamin C, under the same conditions, is 6.45 mM TE/g DM. Leaves and stems showed significantly ($p < 0.001$) the

Table 2. TF contents of *A. cordifolia organs*

Organ	Time (mins)	TF content (mg EAG/g DM)		
		Macerated	Decocted	Soxhlet extract
Leaves	60	28.52 ± 0.13	42.05 ± 0.25	44.54 ± 0.35
	90	31.21 ± 0.25	35.82 ± 0.01	56.24 ± 0.22
	120	33.92 ± 0.22	33.75 ± 0.13	64.38± 0.82
Stems	60	10.46 ± 0.14	13.09 ± 0.01	11.7±0.94
	90	10.94 ± 0.152	13.57±0.14	20.13 ± 0.39
	120	12.22 ± 0.38	9.79 ± 0.08	13.29 ± 0.51
Roots	60	10.07±0.08	12.09 ± 0.01	11.27 ± 0.12
	90	11.23 ± 0.14	19.51 ± 0.09	12.83 ± 0.62
	120	12.24 ± 0.30	21.16 ± 0.08	17.88 ± 0.06

Table 3. CT levels in the organs of *A. cordifolia*

Organ	Time (min)	CT content (mgEC /g DM)		
		Macerated	Decocted	Soxhlet extract
Leaves	60	31.67 ± 0.62	22.5 ± 0.63	10.0 ± 0.62
	90	34.17 ± 0.8	14.17 ± 0.01	28.75 ± 0.30
	120	36.25 ± 0.95	12.92 ± 0.36	12.92 ± 0.40
Stems	60	32.13 ± 0.13	22.21 ± 0.19	17.25 ± 0.01
	90	33.88 ± 0.07	21.46 ± 0.31	22.25 ± 0.13
	120	35.88 ± 0.01	21.08 ± 0.07	19.0 ± 0.08
Roots	60	12.67 ± 0.07	7.38 ± 0.03	3.83 ± 0.07
	90	15.88 ± 0.13	8.04 ± 0.14	4.58 ± 0.26
	120	16.42 ± 0.19	7.88 ± 0.01	6.21 ± 0.14

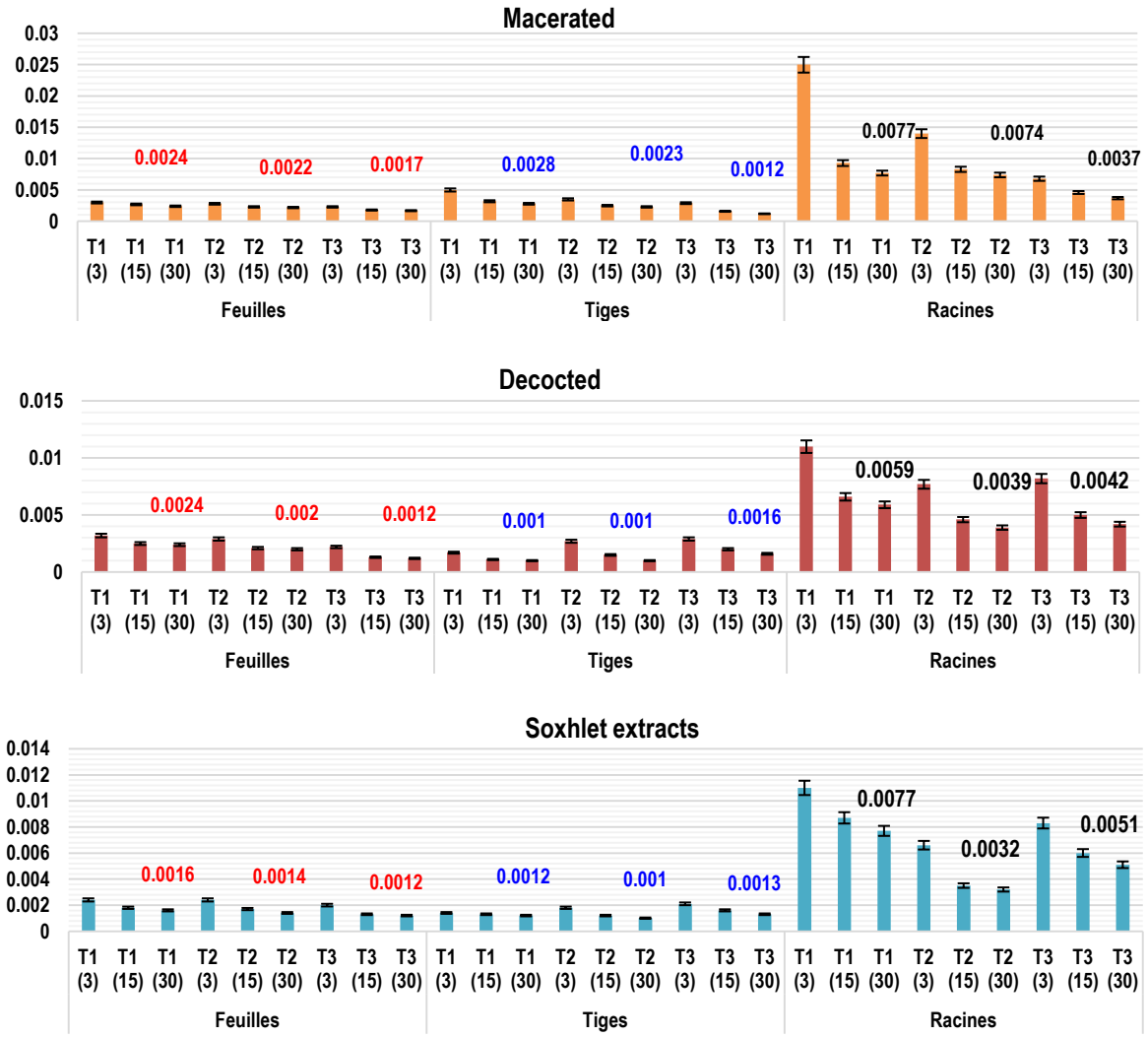

Fig. 1. CR_{50} of *A. cordifolia* organ extracts as a function of time and extraction method

T1 (3): 60 min extract after 3 min incubation with DPPH; T1 (15): 60 min extract after 15 min incubation with DPPH; T1 (30): 60 min extract after 30 min incubation with DPPH
T2 (3): 90 min extract after 3 min incubation with DPPH; T2 (15): 90 min extract after 15 min incubation with DPPH; T2 (30): 90 min extract after 30 min incubation with DPPH;
T3 (3): 120 min extract after 3 min incubation with DPPH; T3 (15): 120 min extract after 15 min incubation with DPPH; T3 (30): 120 min extract after 30 min incubation with DPPH

highest FRAP antioxidant activities. However, for leaves, strong FRAP antioxidant activities were recorded in Soxhlet extracts (5.64 mM TE/g MS). In stems, macerations and decoctions were more active than Soxhlet extracts. The maximum activity of the macerated was observed after 120 min while that of the decoctions and extracts with Soxhlet was perceived at 90 min. Overall, FRAP antioxidant activity varies very little depending on extraction method and time. Statistical analysis reveals a significant difference ($p < 0.001$) between the antioxidant activity of vitamin C and those of stem and root extracts. However, this difference is moderately significant ($p < 0.05$) in macerated leaves and decoctions. There is no significant difference between the Soxhlet extracts of the leaves and that of vitamin C.

The macerated organs reduce the Fe^{3+} ions to Fe^{2+}. Some studies revealed that the methanol maceration of the leaves of *A. cordifolia* obtained in 72 h of extraction, showed a FRAP antioxidant activity of 533.33 µM Fe(II) / g against 1005 µM Fe(II) / g for vitamin C [41]. Antioxidants, for the most part, are good reducers. The reducing power of a compound can therefore serve as a meaningful indicator of its potential antioxidant activity [42,43].

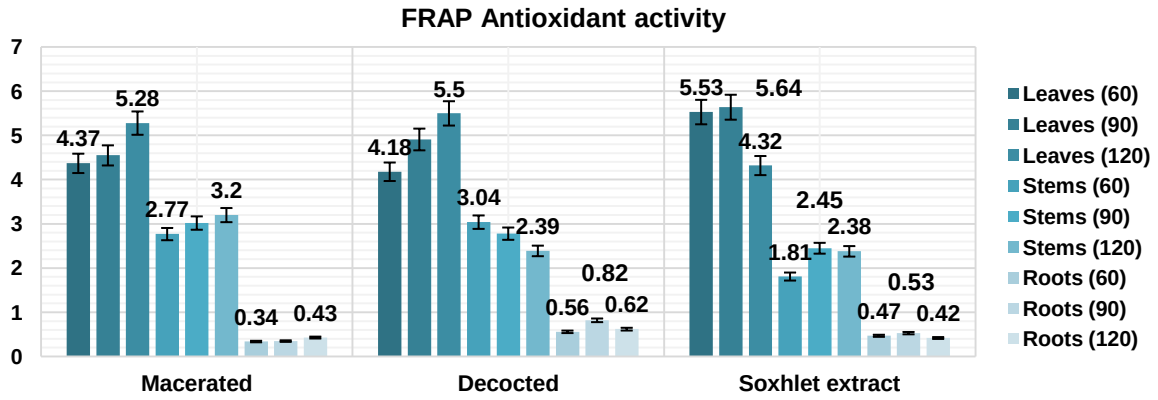

Fig. 2. FRAP antioxidant profiles of *A. cordifolia* organ extracts

3.5 Analgesic Activity

The formaldehyde test was used to evaluate the analgesic activity of macerated, decocted and Soxhlet extracts of *A. cordifolia*. Fig. 3 presents the results translated into percentage inhibition (%). The 60 min and 120 min extracts were administered at a dose of 100 mg/kg of MC, and compared with the effect of aspirin and trabar on the pain caused. All *A. cordifolia* extracts significantly inhibited phases 1 and 2 of formaldehyde-induced pain, with a remarkable effect in the second phase close to the effect of aspirin (82%). The effect of macerated increases when the extraction time increases (FM2 76.85%; TM2 61.57%; RM2 60.19%). However, a decrease in the effect of decoctions of leaves, roots and Soxhlet extracts of roots were observed.

This decrease would be due to the thermal degradation of the phytocompounds, responsible for the activity. Overall, leaf and root decoctions exhibited significantly ($p < 0.001$) more effective analgesic activity; whereas that of the Soxhlet extracts is more significant in the stems ($p < 0.001$). The effect of time and the extraction process on the analgesic activity of the extracts is proven. In phase 2 (Fig. 3), aspirin inhibited the activity of cyclo-oxygenase which leads to the

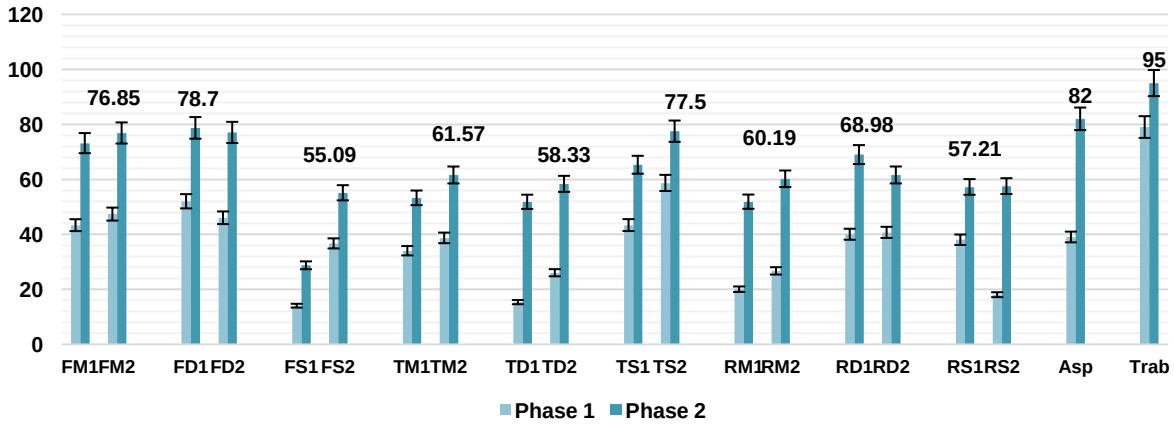

Fig. 3. Analgesic profiles of *A. cordifolia* organ extracts

FM1 and FM2: macerated leaves obtained respectively in 60 min and 120 min; FD1 and FD2: decoctions of the leaves obtained respectively in 60 min and 120 min; FS1 and FS2: Soxhlet extracts of the leaves obtained respectively in 60 min and 120 min

TM1 and TM2: macerated stems obtained respectively in 60 min and 120 min; TD1 and TD2: stem decoctions obtained respectively in 60 min and 120 min; FS1 and FS2: Soxhlet extracts of stems obtained respectively in 60 min and 120 min

RM1 and RM2: macerated roots obtained respectively in 60 min and 120 min; RD1 and RD2: root decoctions obtained respectively in 60 min and 120 min; RS1 and RS2: Soxhlet extracts of the roots obtained respectively in 60 min and 120 min

formation of prostaglandins. These metabolites of arachidonic acid cause inflammation, swelling, pain and fever [44]. In phase 1 (Fig. 3), the trabar highlighted local inflammatory pain, which is influenced by inflammatory mediators (serotonin, histamine and prostaglandins) [21]. Similar to the analgesic effect of aspirin, all extracts inhibited in varying proportions phase 2 of the response to formaldehyde. The analgesic effect of the extracts in phase 1 could be related to the inhibition of cyclo-oxygenase. Several studies have reported the analgesic effects of flavonoids, coumarins, tannins and phenolic acids [45,46]. The presence of phytocompounds in *A. cordifolia*, including phytophenols, total flavonoids and quantified condensed tannins, seems to attest to the observed analgesic properties.

The results of this study show that leaf extracts are more effective than stem and root extracts. Therefore, the use of leaves in decoction for 1 hour should be encouraged.

4. CONCLUSION

The implementation of the present study demonstrated the manifest and palpable effect of three extraction processes (maceration, decoction, Soxhlet extraction) on some quantified phytoactives (polyphenols, flavonoids, condensed tannins) of *A. cordifolia* and their activities. antioxidant (interactions with DPPH and Fe^{3+} ions) and analgesic. The deductions resulting from this study remain dependent on our experimental choices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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