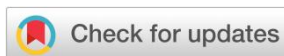
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Research Article

Effect of extraction method on the antioxidant activity of cashew apples collected in northern Côte d'Ivoire

TIEKPA Wawa Justine ¹, KOUANGBE Mani Adrien ^{2*}, DJOUPPO Agnon Prisca ³, TOURE Abdoulaye ⁴

^{1,4} Biotechnology and Valorisation of Agroressources Laboratory, Training and Research Unit in Biological Sciences, Peleforo GON COULIBALY University, Korhogo, 1328 Korhogo, Côte d'Ivoire

² Department of Agroindustrial Sciences and Technologies, Training and Research Unit in Agriculture, Fisheries Resources, and Agro-industry, Polytechnic University of San Pedro, BP 1800 San Pedro, Côte d'Ivoire

³ Laboratory of Clinical Biochemistry, Training and Research Unit of Medical Sciences, Alassane Ouattara University, Bouaké, Côte d'Ivoire, BP V 1801, Bouaké, Côte d'Ivoire

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*For Correspondence:

KOUANGBE Mani Adrien, Department of Agroindustrial Sciences and Technologies, Training and Research Unit in Agriculture, Fisheries Resources, and Agro-industry, Polytechnic University of San Pedro, BP 1800 San Pedro, Côte d'Ivoire

Abstract

The aim of this study was to evaluate the effect of extraction method on the antioxidant activity of cashew apples. The aqueous extracts studied were obtained either by maceration or decoction. Their polyphenol and flavonoid contents were determined by spectrophotometric assay, then their free radical scavenging activity was assessed using the DPPH method. Characterization of the phenolic compounds in the various extracts showed that the DAd, AMd and SId aqueous extracts obtained by decoction contained higher levels of polyphenols at 197.15 ± 2.65 , 183.49 ± 3.55 and 161.16 ± 2.02 mg EQ/g extract respectively, and higher levels of flavonoids at 274.32 ± 2.65 , 196.44 ± 2.65 and 193.55 ± 2.31 mg EQ/g extract respectively. DAM, AMm and Sim, extracts obtained by maceration had the lowest phenolic compound contents of 170.09 ± 2.74 , 168.19 ± 3.45 and 123.38 ± 3.65 mg EQ/g extract respectively, and flavonoid contents of 158.94 ± 3.57 , 147.40 ± 1.45 and 110.86 ± 1.02 mg EQ/g extract respectively. Taking harvest sites into account, extracts from the DAO site were richer in phenolic compounds than extracts from other sites. Determination of antioxidant activity showed that decoctions have better antioxidant activity than macerations. This anti-free radical activity is thought to be correlated with phenolic compound content. The good antioxidant activity would justify its use in traditional diets and therapeutics.

Keywords: *Anacardium occidentale*, cashew apple, antioxydant activity, extraction method, food and therapeutic use

INTRODUCTION

Medicinal plants are a therapeutic resource frequently used by populations. Even today, there is considerable renewed interest in their use. Indeed, the World Health Organization (WHO) estimates that 80% of the world's population uses medicinal plants to alleviate health problems¹. This popularity can be explained by the accessibility and diversity of their floral potential, which includes both medicinal and condiment plants such as *Anacardium occidentale*. Cashew is a tropical plant belonging to the Anacardiaceae family, which comprises around 75 genera and 700 species² whose growth is characterized by morphological diversity. Indeed, the cashew tree can develop into large trees reaching up to 40 meters in height and arboricoles 4 meters in height³. Its flared crown bears oval leaves 10 to 20 cm long and flowers that are generally small and greenish to purplish. After inflorescence, the cashew tree produces a fruit

called the "anacarde", comprising two parts: the cashew apple and the cashew nut⁴. Originally from South America, the cashew tree was introduced to northern Côte d'Ivoire in the 1950s and planted in orchards for cashew production⁵. Côte d'Ivoire has become the world's leading producer, accounting for almost 40% of global supply⁶. This production can generate income to reduce poverty and unemployment in rural areas.

As for apples, they are not used industrially in Côte d'Ivoire because of their astringent taste^{7,8}, but they are used traditionally for therapeutic purposes. Indeed, apple juice is used as an antihyperglycemic, promoting the action of insulin, anti-flu, anti-inflammatory and anti-diarrheal⁹. It also helps reduce the risk of stroke and prevent memory loss. The cashew apple is therefore a genuine and significant therapeutic source, which can be used in the search for natural bioactive substances to combat a number of pathologies.

Despite the many uses of cashew apples in the traditional environment, little scientific data is available to elucidate their use to support and optimize effective therapeutic doses. This study therefore aims at the scientific valorization of cashew apples in order to develop nutraceutical products against several pathologies. Specifically, it will address to:

- quantify the phenolics compounds in the aqueous extracts,
- assess *in vitro* antioxidant activity of extracts using the DPPH free radical scavenging method.

1. MATERIALS AND METHODS

1.1 Plant material

The plant material consisted of *Anacardium occidentale* apples. These fresh and ripe cashew apples were harvested from cashew orchards in three village sites, namely Siendou, Amara, and Dao, in the sub-prefecture of Dabakala, a town in the Hambol region located 479 km from Abidjan.

1.2 Preparation of aqueous extracts of cashew apples

After being cleaned and sliced into little pieces, the recently harvested cashew apples were allowed to air dry at room temperature for two weeks. The powder obtained after grinding this dry plant material was used for the preparation of extracts by maceration and decoction according to the method described by Falleh¹⁰ with slight modifications. For the extracts prepared by maceration, 10 g of dried apple powder were dissolved in 100 mL of distilled water. The mixtures were homogenized for 2 minutes and then left to macerate for 24 hours at room temperature under magnetic stirring. The obtained macerates were successively filtered, twice through hydrophilic cotton and once through Whatman 3 mm filter paper. Then, the obtained filtrates were dried in an oven at 50 °C for 5 days. The brown-colored evaporates obtained constituted the extracts of the apples and were coded SI_m, AM_m, and DA_m respectively for the Siendou, Amara, and Dao sites.

Decoction extracts were prepared by dissolving 10 g of powder in 100 mL of distilled water under vigorous manual agitation. The mixture was boiled for 30 minutes, then cooled for 24 hours. The decocts were successively filtered, twice on absorbent cotton and once on Whatman 3 mm filter paper. The filtrates were then oven-dried at 50 °C for 5 days. The brown-colored dry evaporates obtained constituted the decoction extracts, coded SI_d, AM_d and DA_d respectively for the Siendou, Amara and Dao sites.

1.3. Quantitative determination of polyphenols and flavonoids

1.3.1 Quantitative determination of polyphenols

The determination of the total polyphenol concentrations of the extracts was carried out using the Folin-Ciocalteu method¹¹. To do this, 200 µL of the extracts at 10 mg/mL (decocted and macerated) diluted to 1/10 were taken and then added to 1 mL of Folin-

Ciocalteu reagent. After 5 minutes of incubation at room temperature, 800 µL of aqueous sodium carbonate solution (7.5%) were added to the contents of the previous tubes. The tubes were homogenized and kept in a water bath for 10 minutes, and the absorbance was read at 765 nm using a spectrophotometer. A calibration curve of gallic acid was performed in parallel with different concentrations ranging from 10 to 100 µg/mL. The concentration of total polyphenols was expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g Extract).

1.3.2 Quantitative determination of flavonoids

Flavonoids were determined using the aluminum trichloride (AlCl₃) method¹². One (1) mL of each extract was collected in test tubes each containing 1 mL of 1 % (w/v) AlCl₃. After homogenization and incubation of the tubes for 1 min and 10 min respectively at room temperature, absorbance was determined at 760 nm. Flavonoid content was determined from the regression equation of a quercetin calibration line established with the concentration range from 10 to 80 µg/mL with a step of 10. Flavonoid content was expressed as milligram quercetin equivalent per gram extract (mg QE/g extract).

1.4. Antiradical activity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antiradical effect of the six extracts (SI_m, AM_m, DA_m, SI_d, AM_d, and DA_d) from cashew apples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, for which the solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. For this experiment, 50 µL of each apple extract were added to 1.96 mL of DPPH in a tube and homogenized for 30 seconds. The homogenates were incubated in the dark for 30 minutes and the decolorization was compared to the negative control containing only the DPPH solution. The absorbance is read at 517 nm using a UV/visible spectrophotometer of the Biotech Engineering Management type. The antiradical activity of DPPH was calculated as follows¹³:

$$\text{DPPH Scavenged (\%)} = ((\text{Abs B} - \text{Abs E}) / \text{Abs B}) \times 100$$

Where Abs B is the absorbance of the blank solution and Abs E is DPPH radical + plant extract. The results were expressed as minimum Inhibitory Concentration (IC₅₀).

1.5. Data statistical analysis

Curves and histograms are plotted using Microsoft Excel 2007 and GraphPad software Prism 8.0.2. Test results are expressed as mean ± standard deviation with n = 3. IC₅₀ values (50% inhibitory concentration) are calculated by the linear regression method from the curve [% inhibition = f (concentrations)]. The one-way ANOVA statistical analysis was used to perform the significant differences between the mean values were probability of P < 0.05 was considered as significant.

2. RESULTS

2.1. Extraction yields

The yields of cashew apple extracts by decoction and maceration are shown in **figures 1A** and **1B** respectively.

According to Figure 1A, the extraction percentages by decoction of apples from the Siedou; Dao and Amara sites are 23.13%; 21.92% and 19.02% respectively.

Similarly, Figure 1B shows that the extract by maceration of apples from the Siedou site gives a yield of 22.18%,

that of apples from the Dao site is 17.79% and the yield of extraction by maceration of apples from the Amara site is 14.63%. Extracts extracted by decoction have a higher yield than those extracted by maceration.

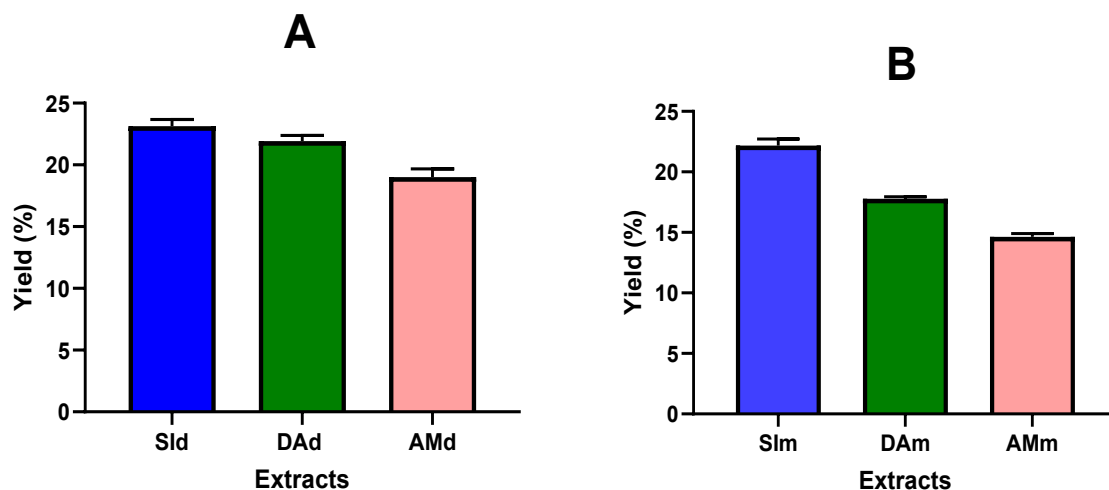


Figure 1: Percentage of extraction of extracts by decoction (A) and by maceration (B)

Sld: Extracted by decoction from apples from the Amara site; **DAd:** Extracted by decoction of apples from the Amara site; **AMd:** Extracted by decoction of apples from the Amara site; **SIm:** Extracted by maceration of apples from the Amara site; **DAm:** Extracted by decoction from apples from the Amara site; **AMm:** Extracted by maceration from apples from the Amara site

2.2. Total phenolic and flavonoids content

The results of the quantification tests of the extracts obtained by decoction from the Siendou (SI), Amara (AM), and Dao (DA) sites are presented in **Figures 2** and **3**. According to these results, the values of polyphenols in the decoction extracts DAd, AMd, and Sld are respectively 197.15 ± 2.65 ; 183.49 ± 3.55 ; and 161.16 ± 2.02 ; mg EQ/g Extract (**Figure 2C**). Similarly, for the extracts obtained

by maceration DAm; AMm and SIm, the polyphenol levels were respectively 170.09 ± 2.74 ; 168.19 ± 3.45 ; 123.38 ± 3.65 mg EQ/g Extract (**Figure 2D**). Moreover, the flavonoid levels of the decoction extracts DAd, AMd, and Sld were respectively 274.32 ± 2.65 ; 196.44 ± 2.65 ; and 193.55 ± 2.31 mg EQ/g Extract (**Figure 3E**). Also, the flavonoid contents of the DAm, AMm, and SIm extracts were respectively 158.94 ± 3.57 ; 147.40 ± 1.45 ; and 110.86 ± 1.02 mg EQ/g Extract (**Figure 3F**).

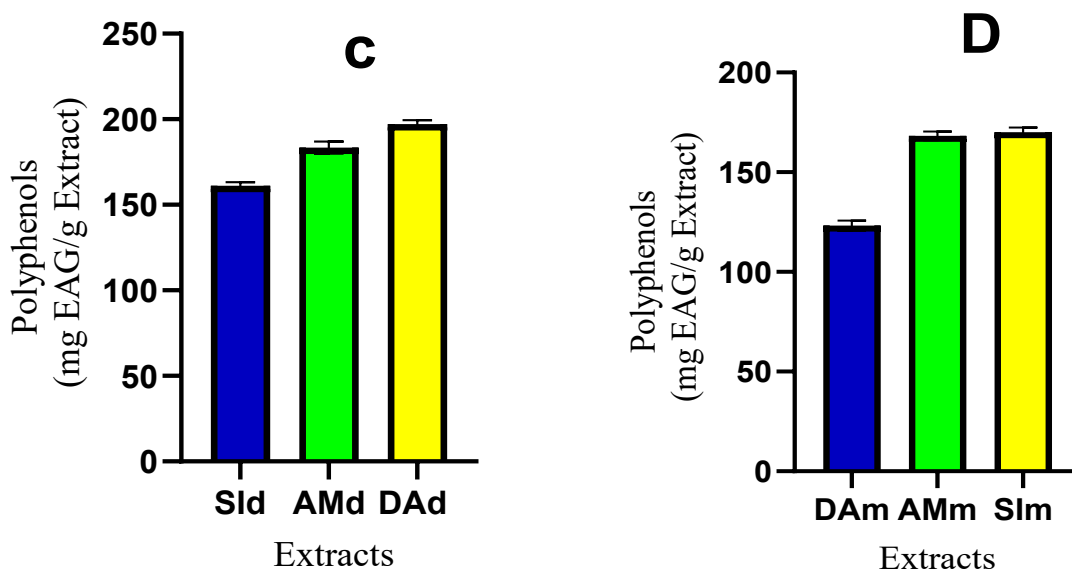


Figure 2: Polyphenols content of decoction (C) and maceration (D) extracts

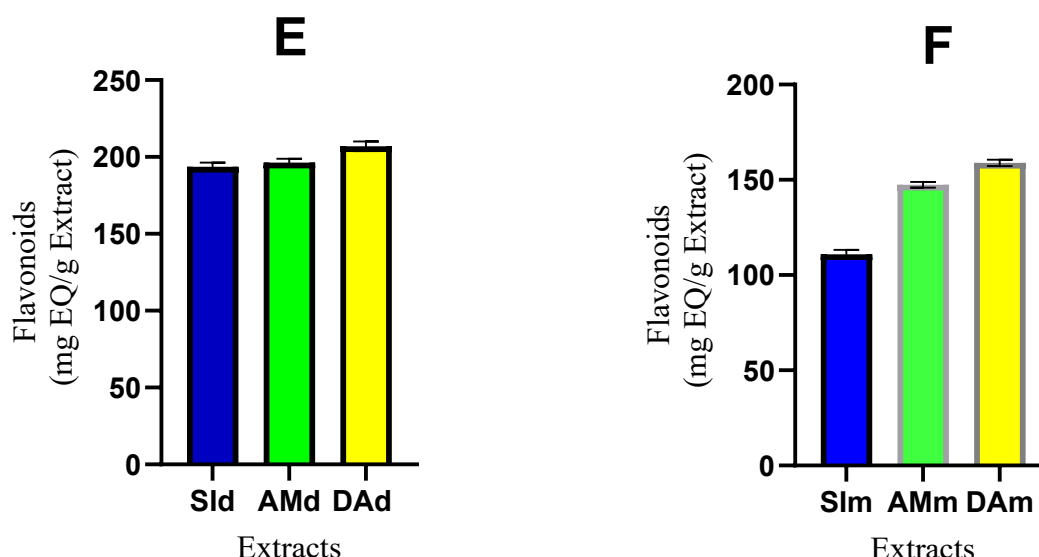


Figure 3: Flavonoids content of decoction (E) and maceration (F) extracts

SId: Extracted by decoction of apples from the Amara site; **DAd:** Extracted by decoction of apples from the Amara site; **AMd:** Extracted by decoction from apples from the Amara site; **SIm:** Extracted by maceration of apples from the Amara site; **DAm:** Extracted by decoction from apples from the Amara site; **AMm:** Extracted by maceration from apples from the Amara site

2.3 DPPH free radical scavenging assay

The reference molecule Vit C gives an IC₅₀ of 0.25±0.05 µg/mL (**Figure 4**). Extract by decoction and by maceration of apples from the same site exhibit the same 50 % inhibitory concentration (IC₅₀) of DPPH. Thus, the IC₅₀ of the DAm and DAd extracts is determined to be

0.55±0.12 µg/mL (**Figure 5**), that of the AMm and AMd extracts is 0.65±0.18 µg/mL (**Figure 6**), and the SId and SIm extracts give an IC₅₀ of 0.95±0.13 µg/mL (**Figure 7**). IC₅₀ value of vitamin C and the different extracts from the same site are reported by the histograms in **Figure 8**. The analysis of Figure 8 shows that the extracts have significantly higher IC₅₀ values than Vit C IC₅₀ (p<0.05).

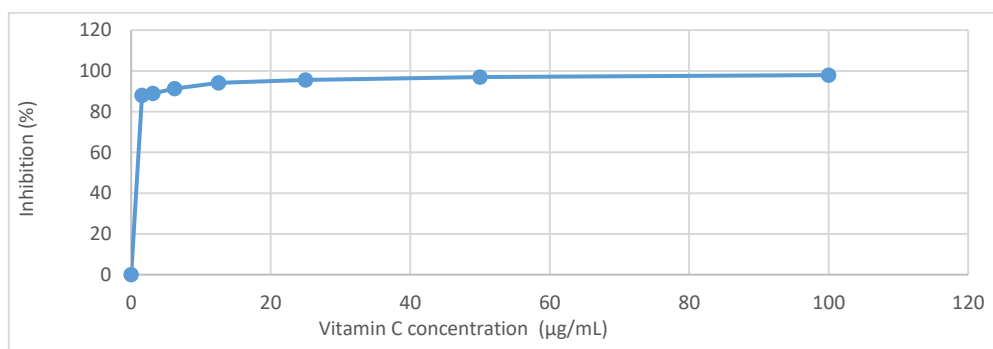


Figure 4 : DPPH radical inhibition by Vitamin C

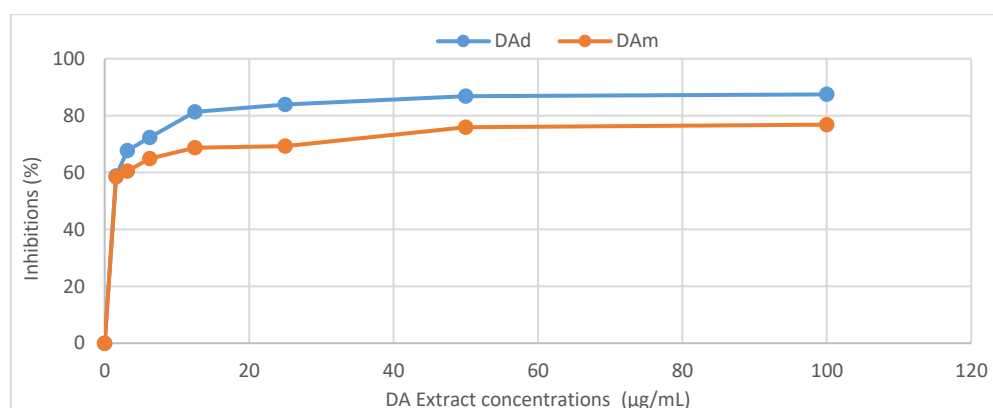


Figure 5 : Percentage of DPPH radical inhibition by DAd and DAm extracts

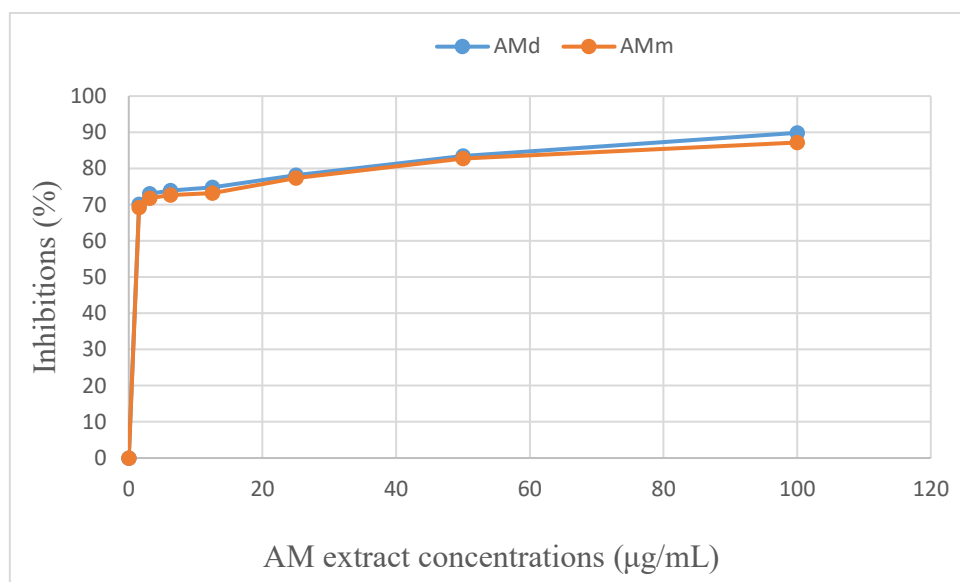


Figure 6 : Percentage of DPPH radical inhibition by AMd and AMm extracts

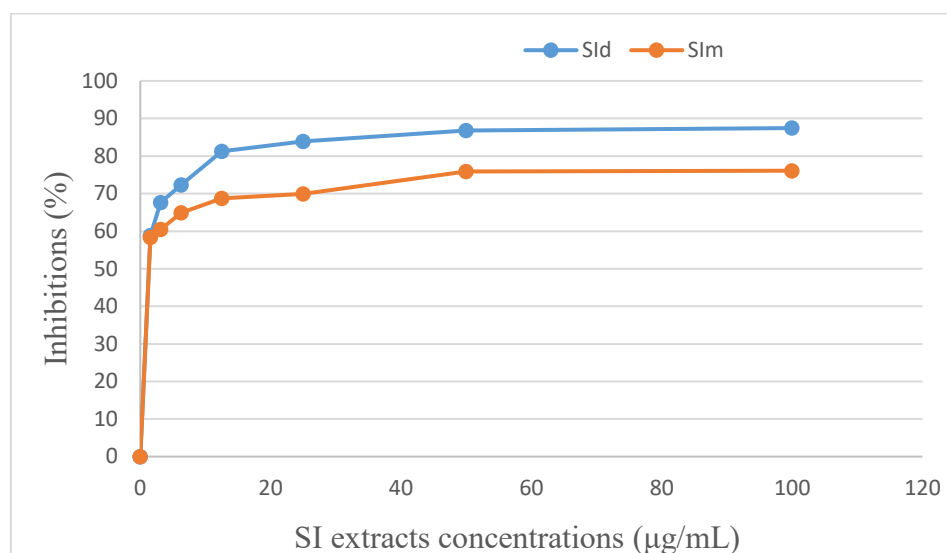


Figure 7 : Percentage of DPPH radical inhibition by SId and SIIm extracts

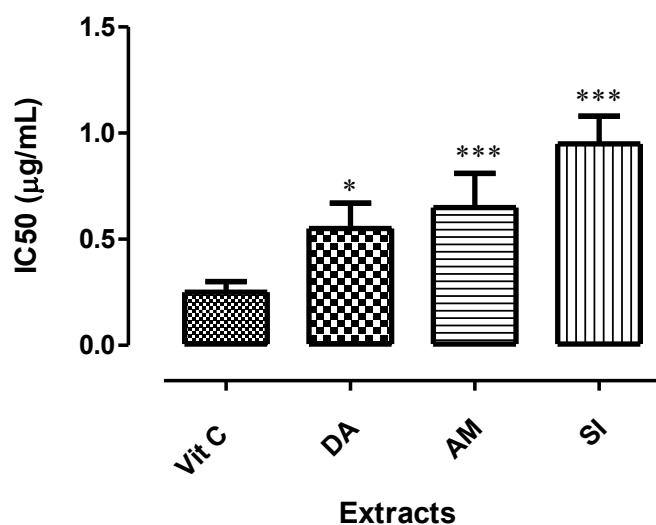


Figure 8: IC50 values of Vitamin C and cashew apple extracts

Vit C : Vitamin C ; **DA :** Extract apples from the site DAO; **AM :** Extract apples from the Amara site; **SIIm :** Extract from the apples on the Siendou site

3. DISCUSSION

One of the biggest concerns facing the world today is the search for novel therapeutic compounds made from medicinal plants¹⁴. Determining the yield and performing a phytochemical analysis of the extracts of the relevant plants is one of the study's initial procedures. These operations allow for the identification of the appropriate extraction method and the identification of the various secondary metabolites of the studied plants in order to better guide the research. The present study has shown that for the same site, extraction by decoction (SId, AMd, and DAd) gives a better yield than extraction by maceration. This difference can be attributed to climatic and geological factors in the apple harvesting areas, as well as to the extraction method used. Furthermore, the determination of total polyphenols and flavonoids in extracts shows that extracts obtained by decoction DAd, AMd and SId contain polyphenols at respective values of 197.15 ± 2.65 ; 183.49 ± 3.55 and 161.16 ± 2.02 mg EQ/g Extract and flavonoids at respective values of 274.32 ± 2.65 ; 196.44 ± 2.65 and 193.55 ± 2.31 mg EQ/g Extract. On the other hand, DAm, AMm and SIm extracts obtained by maceration had polyphenol contents of 170.09 ± 2.74 , 168.19 ± 3.45 and 123.38 ± 3.65 mg EQ/g extract respectively, and flavonoid contents of 158.94 ± 3.57 , 147.40 ± 1.45 and 110.86 ± 1.02 mg EQ/g Extract respectively. These results show that extracts obtained by decoction are richer in phenolic compounds than those obtained by maceration. Therefore, a significant amount of the polyphenols included in the dried apples would have been released during the decoction. This indicates that the content of the extracted secondary metabolites is influenced by the extraction technique. This conclusion had also been proposed by Faye et al.¹⁵, who state in their study on the best method for aqueous extraction of total polyphenols from dried *Combretum Micranthum* leaves that aqueous decoction is the best polyphenol extraction technique. The key factor in this difference is the extraction temperature. Indeed, the work of Kiassos et al.¹⁶ explained that extracting phenolic compounds at high temperatures (above 80°C) affects the stability of the compounds due to chemical and enzymatic degradation or losses through thermal decomposition.

The antiradical study of a plant extract involves determining the ability of this extract to neutralize free radicals. Moreover, the inhibitory concentration IC₅₀ expresses the amount of antioxidant needed to reduce the DPPH free radical by 50%¹⁷. Thus, the smaller its value, the greater the antioxidant activity of the studied extract¹⁸. Our work shows that the macerated and decocted extracts from the AM, DA, and SI sites give respective IC₅₀ values of 0.65 ± 0.18 µg/mL; 0.55 ± 0.12 µg/mL; and 0.95 ± 0.13 µg/mL. This is explained by the fact that these extracts, which are very rich in polyphenols, have a higher antioxidant potential than those from the SI site. This result confirms the correlation between the phenolic compound content and the anti-radical activity as observed by Kagnou et al.¹⁹ in their work. Indeed, the hydroxyl groups of phenolic compounds could serve as electron donors to trap and

neutralize free radicals. The present results are similar to those of Massengo et al.²⁰ who explain that the antiradical power of *Lippia multiflora* leaves would be due to their phenolic compound content. Similarly, Mohammadi²¹ states in his work that the antioxidant potential of the red fruits of *Arbutus unedo* L. would be attributed to polyphenols. Also, Maria et al.²² asserted in their study that phenolic compounds would be the main components responsible for the antioxidant activity of the extracts.

CONCLUSION

Cashew apple extracts obtained by decoction are richer in phenolic compounds than those obtained by maceration. In addition, they have better antioxidant potential, correlated with their high phenolic content. Decoction would therefore be the best extraction method for obtaining phenolic compounds, thus optimizing antioxidant activity..

Declaration of Competing Interest: The authors declare they have no competing financial interests or known personal relationships that could have appeared to influence the work reported in this article.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethical approval: Not applicable.

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