Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2018, Volume 9, Issue 2, Page 724-729

https://doi.org/10.26872/jmes.2018.9.2.80

http://www.jmaterenvironsci.com

Phytochemical screening and antioxidant profile of leave decoctions of five wild edible plants from Côte d'Ivoire

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Received 01 May 2017,
Revised 07 Jul 2017,
Accepted 11 Jul 2017

Keywords
- ✓ Edible plants,
- ✓ decoction,
- ✓ polyphenols,
- ✓ antioxidant activity,
- ✓ Côte d'Ivoire.

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Abstract

This work is devoted to the phytochemical survey and the assessment of the antioxydant capacity of decoctions of 5 wild edible plants leaves (*Amaranthus hybridus, Ipomea batatas, Basella alba, Talinum triangulare* and *Colocasia esculenta*) from Côte d'Ivoire. Phytochemical characterization tests revealed the coexistence of polyphenols, flavonoids, tannins, saponins, leucoanthocyanes, steroids and terpenoids in the studied decoctions. The quantitative spectrophotometric assay of each decoction showed that the total polyphenol contents are higher with 1/100 (m/v) ratio for an optimum extraction's time of about 60 min. For the similar ratio, *B. alba*, *I. batatas, C. esculenta, A. hybridus* and *T. triangulare* showed maximum contents in total phytophenols at different extraction times. The evaluation of the antioxidant activity of these decoctions for the ABTS test allowed to establish a linear correlation between their antioxidant capacity and their content of total phenolic secondary metabolites: *T. triangulare* (R2 = 0.98), *A. hybridus* (R2 = 0.93), *B. alba* (R2 = 0.83), *I. batatas* (R2 = 0.80) and *C. esculenta* (R2 = 0.42).

1. Introduction

In developing countries, consumption of wild edible plants by populations is recursive for different vital needs (food, health, well-being). Different parts (fruits, leaves, flowers and seeds) of these plant species are used as ingredients, natural dyes or medicinal preparations in traditional gastronomy [1]. Adjanohoun and Aké-Assi [2] have listed more than 5000 plant species in Côte d'Ivoire, including several edible wild species known to populations [3].There is another source of vitamins, minerals and nutrients for rural populations during lean periods [4]. In addition to their nutritional importance, there are of considerable socio-economic interest because of they are not expensive, accessible and easy to prepare. Therefore, wild edible plants could be used as a source of new active principles [5].Oxidative stress seems to be the main cause of several diseases [6]. Oxidative stress results from the increase of free radicals in mitochondrial multiplication [7,8], which potentiates the appearance of multifactorial affections: diabetes, Alzheimer's disease, rheumatism, cardiovascular diseases, etc. [9]. Given the diversity and severity of diseases caused by oxidative stress, several researches have been initiated to discover new antioxidants in order to limit this aggression of the cellular constituents and the associated pathologies. The low purchasing power of the majority of Ivorian populations limits their access to primary health care. Nutritherapy appears as an alternative for these populations. The use of edible wild plants could be substantially improving their resistance to multiple diseases related to oxidative stress. As new perspective, this present work concerned the qualitative and quantitative phytochemical analysis of aqueous decoctions obtained from organs of Amaranthus hybridus, Ipomea batatas, Basella alba, Colocasia esculent and Talinum triangulare, consumed in Côte d'Ivoire.

2. Material and Methods

2.1. Plant material

Plant material consists of leaves of the following plant species: *Amaranthus hybridus* (Amaranthaceae), *Basella alba* (Basellaceae), *Ipomoea batatas* (Convolvulaceae), *Colocasia esculenta* (Araceae) and *Talinum triangulare* (Portulacaceae), purchased at Abobo market (Abidjan, Ivory Coast) in November 2015. After cleaning, the leaves were air-dried (18°C) for one week then pulverized with an electric mill (RETSCH Brand, type SM 100). Analytical grade reagents and solvents were purchased from various suppliers (Carlo-Erba, Fluka, Panreac Quimica).

2.2. Preparation of extracts

5 g of powdered leaves were taken up in 100 mL of H_2O and boiled during x hours. After cooling and filtration, various fractions *Amaranthus hybridus* (AH), *Basella alba* (BA), *Ipomea batatas* (IB), *Colocasia esculenta* (CE) and *Talinum triangulare* (TT) were obtained.

2.3. Phytochemical screening

Phytochemical screening of different decoctions of AH, BA, IB, TT and CE was performed to detect secondary metabolites such as alkaloids, flavonoids, polyphenols, steroids, terpenoids, tannins, sterols and saponins [10] and leucoanthocyanins [11].

Alkaloids

6 mL of liquid of each decoction were evaporated and the residue is taken up in 6 mL of EtOH (60°C) in a test tube. Then, the reagent Dragendorff (2 drops) was incorporated. The formation of an orange precipitate indicates the presence of alkaloids.

Flavonoids

2 mL of liquid of each decoction is concentrated and the residue is taken up in a test tube with 5 mL of hydrochloric EtOH (90°C). 3 small pieces of Mg are added. The appearance of the pink-orange or purplish coloration, intensified by the addition of 3 drops of isoamyl alcohol, indicated the existence of flavonoids.

Polyphenols

To 2 mL of decoction was added a drop of aqueous $FeCl_3$ solution (2% m/v). The appearance of a more or less dark green coloration indicates the presence of phytophenols.

Steroids and terpenoids

5 mL of each decoction were concentrated to provide a residue, dissolved in 1 mL of $(CH_3CO)_2O$ in a test tube to which is slowly poured 0.5 ml of concentrated H_2SO_4. The appearance of a purple or violet color, turning blue to green, indicated the presence of steroids and terpenoids.

Saponins

A test tube containing 10 mL of decoction is vigorously stirred for a few minutes, and the height of the foam formed greater than 1 cm and persistent indicated the existence of saponins.

Tanins

Catechic tanins: 5 mL of each decoction were concentrated to provide a residue who was taken in a test tube to which was added 15 ml of Stiasny reagent (10 mL of formalin (40%) in 5 mL of concentrated HCl). The mixture was held in a water bath at 80°C for 30 min. After cooling under a stream of water, precipitation of flakes indicated the presence of catechuic tannins. Gallic tannins: the solution containing flakes was filtered and the collected filtrate was saturated with CH_3CO_2Na, and 3 drops of $FeCl_3$ (2%, w/v) were added. The appearance of an intense blue-black staining confirms the existence of gallic tannins. Leucoanthocyanins: 5 mL of concentrated HCl and 1 mL of isoamyl alcohol were added to a test tube containing a decoction obtained after concentration of 2 mL of each decoction. The solution was heated to 80°C in a water bath for 30 minutes. The appearance of a red coloring showed the presence of leucoanthocyanins.

2.4. Total phenols determination

The method of Wood et al. [12] was used for total phenols quantification.

First, 4 g of pulverized leaves of each plant matrix were refluxed in 200 mL of distilled H_2O. Every 10 min, 5 mL of decoction were taken, filtered and analyzed to determine the optimal time for extraction of polyphenols in each plant. Secondly, 1, 2, 4, 6 and 8 g respectively of pulverized leaves of each plant matrix were heated to reflux in 100 mL of distilled H_2O to provide 25 decoctions which were tested at each optimal time to determine the extraction ratio. The contents of total phenols were expressed in equivalent mg gallic acid/g dry mass (mg EqAG/g).

2.5. Antioxidant capacity evaluation

Antioxidant capacity was carried out for the ABTS test (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) described by Choong et al. [13] with extract (100 µL) of each plant that signed a better phytophenol content according to optimal time and extraction ratio. The antioxidant capacity was expressed as follows:

% I = [(Abs control - Abs extract) / Abs control] × 100
Abs control = diluted ABTS absorbance
Abs extract = absorbance ABTS diluted + extract

The percentage inhibition of the analyzed extracts corresponds to the graphically obtained Trolox equivalents (TEAC). Thus, the following Trolox concentrations (0.192, 0.288, 0.384, 0.480, 0.576, 0.672, 0.768 µM) were prepared to plot the calibration line. The experiment was repeated 3 times.

3. Results and discussion

3.1. Plants qualitative composition

Phytochemical screening of decoctions indicates a coexistence of flavonoids, polyphenols, steroids, terpenoids, saponins, catechuic tannins and leucoanthocyanins in all plants. Decoctions AH and IB do not contain catechuic tannins and saponins respectively. As for the alkaloids and gallic tannins, they are absent in all extracts (**Table1**).

Table1: Decoctions phytochemical screening

Decoction	Pol	Flav	Tan		Leu-Ant	Ste/ Ter	Alc		Sap
			Cat	Gal			Dra	Bou	
AH	+	+	+	+	+	+	+	+	+
A	+	+	-	-	+	+	-	-	+
IB	+	+	+	-	+	+	-	-	+
CE	+	+	+	-	+	+	-	-	-
TT	+	+	+	-	+	+	-	-	+

(+): presence (-): absence; Pol: polyphénol; Flav: flavonoid; Tan: tanin; Cat: catéchic; Gal: gallic; Leu-Ant; leucoanthocyanin; Alc: alkaloid; Sap: saponin; Ste/Ter: steroid/terpenoid.

The coexistence of polyphenols, steroids, terpenoids, catechic tannins and saponins revealed in the decoctions of the plant matrices, analyzed by phytochemical screening, could justify the consumption of the aerial parts of said plants in the form of vegetables for therapeutic purposes. Indeed, secondary metabolites are bioactive and in this regard, their presence in the human diet would potentiate the immune system. Consequently, for low-income populations in particular, a diet rich in plants seems to be a way of improving their health.

3.2. Total phenols diffusion

The optimal extraction time of polyphenols differs with each plant studied (**Figure 1**). Indeed, the extraction time for the maximum levels of total phytophenols (59.5; 57.5; 22; 17 and 13 mg Eq GA/g DM) *B. alba*, *I. batatas*, *C. esculenta*, *A. hybridus* and *T. triangulare* are respectively 50, 60, 40, 60 and 70 min. From the foregoing, it appears evident that the leaves of *B. alba* are the richest in total phenols with a relatively average extraction time. However, the leaves of *T. triangulare* are less rich in total phenols, which hardly seem extractable decoction with water.

Figure 1 shows that the extracted quantities of total phenols from the leaves of *B. alba* and *I. batatas* are more significant (59.5 and 57.5 mg Eq GA/g DM) than those provided by *C. Esculenta*, *A. hybridus* and *T. triangulare* (22. 17 and 13 mg Eq GA/g DM). Furthermore, graphics (**Figure 1**) that highlight extraction kinetics include 3 periods that are the exponential phase, tray and decline.

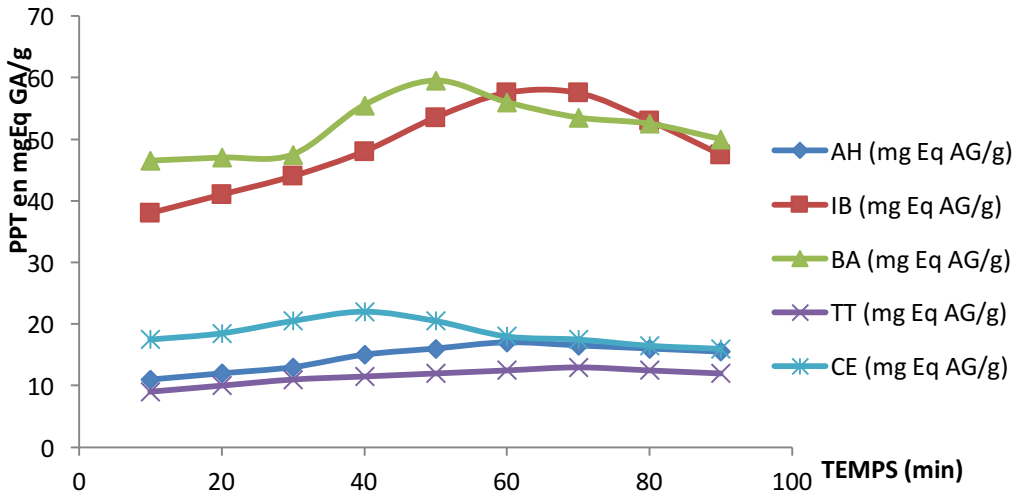

Figure 1: Total phenols diffusion based on the extraction time

The exponential phase suggests a rapid diffusion of phytophenols in the solvent at temperatures ranging from 80 to 100°C, following the break of vacuoles. Thus, high temperatures would further energize the penetration of water into the vegetable matrix by lowering the viscosity of the latter [8]. This would facilitate the solubilization and diffusion of the water-soluble molecules. The tray seems to show a diffusion. Ultimately, the decline phase suggests a degradation of phytophenols when the decoction is done at 100°C for a prolonged period.

Figure 2 shows the results of quantitative analysis of total phenols for each plant extracted studied according to the following ratios: 1/100, 2/100, 4/100, 6/100, 8/100 (w/v). The ratio extraction 1/100 (m/v) signed contents higher total phenols for each plant. However, *C. esculenta* showed the highest total phenol content followed by *I. batatas, B. alba, A. hybridus* and *T. triangulare*. This appears to bind to the saturation of the extraction medium, limiting the plant's area of contact with water.

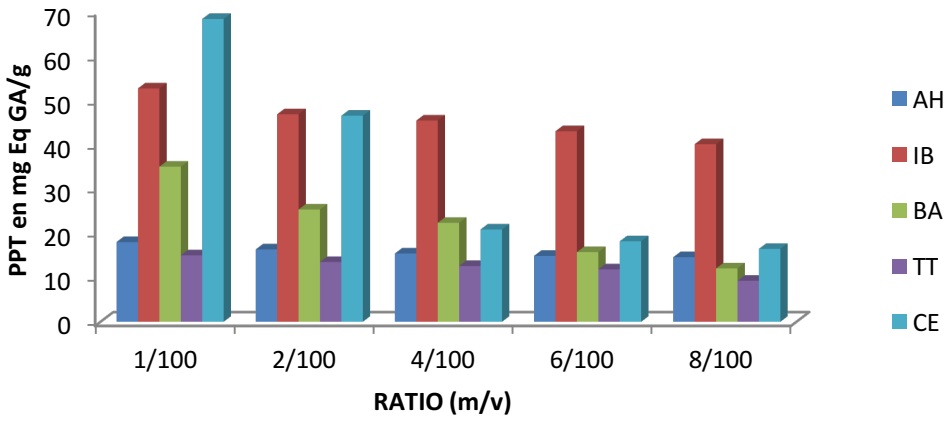

Figure 2: Total phenols diffusion based on the ratio of extraction

3.3. Quantification of antioxidant capacity

The antioxidant capacity of the various extracts for the ABTS test was expressed in µmol TE/L (Trolox Equivalent per Liter) as a function of the Trolox calibration curve (**Figure 3**).

Each extract exhibited maximum reductive activity of ABTS at a defined time during the study of extraction kinetics (**Figure 1**). Indeed, decoctions from *I. batatas* and *B. alba* showed a significant reductive activity of ABTS of 27.24 µmol TE / L at 60 min and 25.32 µmol TE/L at 50 min, respectively; the other extracts showed relatively inefficient reducing activity ABTS: *C. esculenta* (18.42 µmol TE/L to 40 min), *A. hybridus* (11.63 µmol TE/L to 60 min) and *T. triangulare* (7 µmol TE/L at 70 min). **Figure 4** shows the results obtained at the end of the study of the antioxidant capacity of the various extracts as a function of the extraction ratio. For each extract, the 1/100 (w/v) ratio showed a greater reduction in ABTS activity.

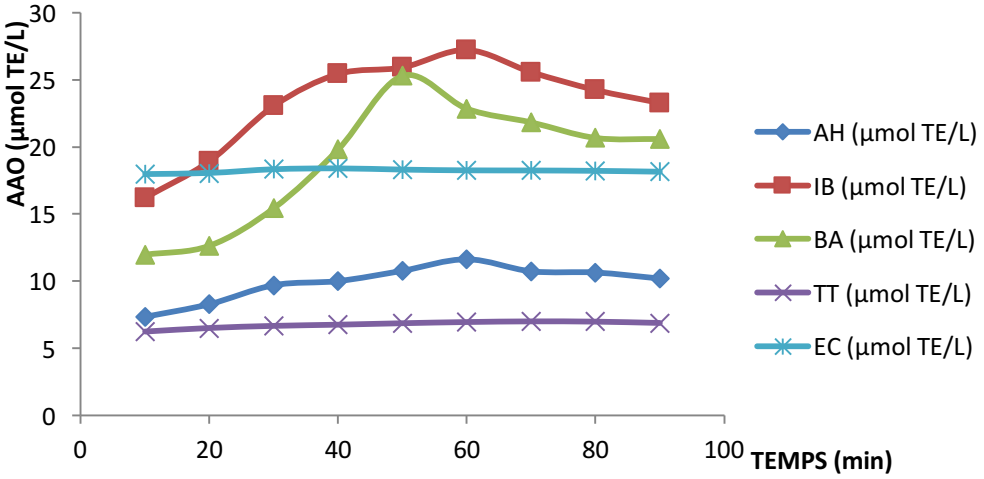

Figure 3: Antioxidant capacity as a function of extraction time

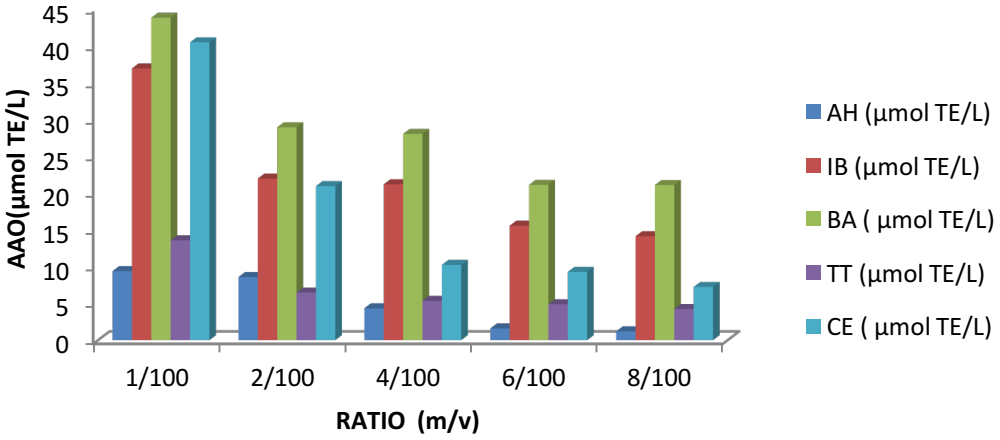

Figure 4: Antioxidant capacity as a function of extraction ratio

In general, the antioxidant capacity depends on the diffusion of phenolic compounds. Quantification of total phenols (**Figure 2**) and the antioxidant capacity (**Figure 4**) of the analyzed decoctions showed that the most significant levels of total phenols and the most important values in relation to the antioxidant capacity, were observed in 1/100 (m/v). In this regard, it is reasonable to think that the antioxidant potential of the studied plants is related to the existence of phenolic secondary metabolites.

3.4. Correlation between total phenol content and antioxidant capacity

Apart *C. esculenta (R2 = 0.42),* the other plant species studied showed a good linear correlation as a function of extraction time: *A. hybridus* (R2 = 0.93), *I. batatas* (R2 = 0, 80), *B. alba* (R2 = 0.83) *and T. triangulare* (R2 = 0.98).

As regards the relationship between the total phenols content and antioxidant activity according to the extraction ratio, an expressive linear correlation was observed in all plant extracts: *A. hybridus* (R2 = 0.86), *I. batatas* (R2 = 0.94), *B. alba* (R2 = 0.93), *T. triangulare* (R2 = 0.64) and *C. esculenta* (R2 = 0.96).

These results could point out that this linear correlation translates a relational dependency between the content of total phenols and antioxidant capacity of different extracts. This does not contradict the results obtained by other authors [15-17].

Conclusion

Phytochemical screening revealed a coexistence of polyphenols, flavonoids, tannins, saponins, leucoanthocyanins, steroids and terpenoids in decoctions obtained from five edible wild plants of Ivory Coast (*A. hybridus*, *I. batatas*, *B. alba*, *T. triangulare* and *C. esculenta*) which have studied. Quantitative investigation of these decoctions revealed an appreciable content of total phytophenols, as a function of time and extraction ratio. Their ABTS radical scavenging activity was also carried out. It has evidently indicated a dependence on the antioxidant capacity of the plant matrices of the phenolic content as a function of time and the extraction ratio. These findings would justify the place of choice of these plants in the diet of the populations in Côte d'Ivoire. In addition, further trials will be carried out with the aim of enhancing the value of food plants derived from the Ivorian wild flora.

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(2018) ; http://www.jmaterenvironsci.com