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PHYTOCHEMICAL SCREENING AND ANTI-RADICAL SCAVENGING ACTIVITY OF THREE ANTIANAMIC PLANTS ACCLIMATED IN BENIN : *Alternanthera brasiliana, Monechma depauperatum* and *Hibiscus acetosella*

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ABSTRACT

Resort to the use of plants for the treatment of human diseases including anemia is common both in Benin's urban and rural areas. The aim of this work is to identify the secondary metabolites and evaluate the anti-radical activity of the crude extracts of *Alternanthera brasiliana*, *Monechma depauperatum* and *Hibiscus acetosella*, three plants used in traditional medicine in Benin for several diseases including anemia. By complexion and/ or precipitation reactions, the different groups of secondary metabolites in these plants were highlighted. The content of polyphenolic compounds was determined by the Folin-Ciocalteu method for phenolic compounds, aluminum trichloride ($AlCl_3$) for total flavonoids and sulfuric vanillin for condensed tannins. The scavenging activity was evaluated by the DPPH method and the hydroxyl radical method. Phytochemical analysis reveal the presence of gallic tannins, saponins, terpens and sterols. The absence of coumarins and alkaloids was noticed. The extract of *H. acetosella* has the highest anti-radical scavenging activity. This activity is correlated with the content of phenolic compounds that are flavonoids, tannins and phenolic acids. The richness in secondary metabolites extracted and the high content of phenolic compounds responsible of anti-radical activity can justify the use of these plants against anemia whose pathophysiology involves oxidative stress.

KEYWORDS: Antianaemic plants - secondary metabolites - scavenging activity

1. INTRODUCTION

Anaemia is considered as a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. All categories of people are exposed, but young children and pregnant women are the most vulnerable [1, 2]. In 2002, Iron Deficiency Anaemia (IDA) was considered to be among the most important contributing factors to the global burden of disease. According to Global Health Observatory data repository, about 59.3% of children under five are affected by anemia in Africa [3]. In Benin, the rate of people with anemia is 3.9 % in 2014 [4]. Correcting anaemia often requires an integrated approach. In order to effectively combat it, plants are also associated [5]. For millennia, mankind has always used plants for various purposes: treatment, food, perfumery, cosmetics, etc [6]. The vegetable kingdom is an inexhaustible alleged potential source of a huge variety of potential drugs and functional foods for humans. World Health Organization (WHO) has estimated that 80% of the population use plants for healing [7]. In Benin, *Alternanthera brasiliana, Monechma depauperatum* and *Hibiscus acetosella* are part of the plants used for the treatment of anemia.

Alternanthera brasiliana or *Gomphrena brasiliana* is a native herb of Brazil being species of Amaranthaceae family. It is a species introduced in Benin [8]. It is used by the rural population as a medicine to cure various diseases such as inflammation, pain infections. It is an analgesic with anti-tumor and immunomodulatory activity [9].

Monechma depauperatum is an herb of savannas and derived woody stem. It is 30-60 cm tall with pale white or yellow flowers and belong to the Acanthaceae family [8]. Antimicrobial and antifungal powers of the seeds of a species of the genus Monechma, *M. ciliatum* were studied in Nigeria [10].

Species of the genus Hibiscus presented antimicrobial activity against *Staphylococcus aureus*, antiviral activity against *Herpes simplex* type II, antifungal activities, antispasmodic. *H. acetosella* called false roselle belongs to the family of Malvaceae. Other uses in traditional medicine have been reported in Mexico Thailand and Indonesia [11]. The extract of their flowers is used in the treatment of epilepsy and leprosy. Its leaves are used against fatigue and skin diseases; the juice of fresh root in the treatment of gonorrhea, powdered root used in the treatment of menorrhagia [11].

Furthermore, to prevent or reduce the oxidative stress induced by free radicals, it is necessary to consume a sufficient amount of antioxidants such as polyphenols, carotenoids etc that can help protect the cellular oxidation and also to lower the risk of chronic diseases including anemia [12].

The traditional and popular use of these plants do not guarantee the safety and efficacy of medicinal preparations [13], it is essential to study and to provide the scientific basis of their use.

The objective of this work is to valorize some medicinal plants of Benin flora through the identification of secondary metabolites, the evaluation of the anti-radical activity of the crude extracts of *A. brasiliana, M. depauperatum* and *H. acetosella*.

2. MATERIALS AND METHODS

2.1. Material

Three plants were used as vegetable material: leafy stems of *M. depauperatum* and *H. acetosella* respectively harvested in September 2015 at Djougou and in October 2015 at Cotonou area (Vedoko). Leaves of *A. brasiliana* were harvested in the grounds of the University of Abomey-Calavi in September 2015. The plants were identified at the National Herbarium of the University of Abomey-Calavi.

The collected plant material was dried at room temperature (25 ° C to 30 ° C) for two weeks and then reduced to powder. Phytochemical screening, the quantification of the phenolic compounds and the anti-radical (DPPH and hydroxyl OH) assay were made upon the powder.

2.2. Methods

2.2.1. Phytochimical Screening

The secondary metabolites present in all three samples were identified by the method described by Koudoro *et al.* and based on the color reactions and / or precipitation [14].

Phenolic compounds: The determination of compounds was made by using ferric chloride.

Tannins: They were highlighted by the Stiasny test.

Flavonoids: The identification of flavonoids was conducted by the Cyanidin test.

Saponins: The saponins were determined by the foam test; degree of dilution of aqueous decoction giving a persistent foam after shaking.

Sterols and terpenes: sterols and terpenes have been highlighted by the Liebermann-Burchard test.

Alkaloids: alkaloids have been identified by the test of Meyer and confirmed by the test Bouchardat.

Anthraquinones: They have been identified by the test Bornträger.

Mucilages: Obtaining a flocculent precipitate of a decoction in ethyl ether indicated the presence of mucilage.

Coumarins: The coumarins have been identified by the fluorescence to the UV at 365 nm.

2.2.2. Preparation of extracts and yield of extraction

The leaves and the leafy stems of the plants were powdered and extracted with the mixture water/ethanol (70/30). The mixture were filtered under Buchner system. The filtrate is condensed by using a rotary evaporator.The yield of the extraction was determined by the following formula:

Formula a :

$$\text{Yield (\%)} = \frac{\text{Extracts weight}}{\text{Powder weight}} \times 100$$

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2.2.3. Determination of phenolic compounds content

Total Phenolics Content: The content of total phenolics was analyzed by spectrophotometry at 765 nm using the Folin-Ciocalteau colorimetric method [15]. Folin-Ciocalteau reagent consists of a mixture of phosphotungstic and phosphomolybdic acids which is reduced during the oxidation of phenols mixture of the blue oxides of tungsten and molybdenum [15]. The absorbance was measured at 765 nm against the blank using a JENWAY 50/60 Hz spectrophotometer. Gallic acid was used as standard and the Total Phenolic Compounds content in the extracts was expressed as mg of gallic acid equivalent per g of dry matter (mg GAE/g DM).

Total Flavonoids Content: The method of the aluminum trichloride ($AlCl_3$) is used to quantify the total flavonoids. This technic is based on the formation of a flavonoid-aluminum complex which has an absorption maximum at 500 nm [16]. Total Flavonoids Content was expressed as mg quercetin equivalent per g of dry matter (mg GAE/g DM)

Condensed Tannins Content: The dosage of the condensed tannins is made by the method using sulfuric vanillin [16]. The principle of this assay is based on the attachment of the aldehyde group of vanillin on the carbon 6 of the cycle A of catechin to form a red colored complex chromophore that absorbs at 510 nm. Condensed Tannins Content was expressed as mg catechin equivalent per g of dry matter (mg GAE/g DM).

2.2.4 Evaluation of the radical-scavenging activity

✓ ***DPPH radical scavenging assay***

The principle of this method is based on measuring the trapping of free radicals by DPPH solution. This trap is shown by the disappearance of the purple color of DPPH. Mixing of reagent (4% of DPPH in ethanol) and extract are left in the dark for an hour and the absorbance was measured at 517 nm [16]. The percentage of scavenge was determined by the formula:

Formula b :

$$P = \frac{(Ab-Ae)}{Ab} \times 100$$

With P: Scavenge percentage; Ab: absorbance of the blank, Ae: Sample Absorbance.

✓ ***Hydroxyl radical scavenging assay***

This test is performed according to the method described by [17]. 2 mL of the aqueous $FeSO_4$ solution (6 mM) were added to 2 mL of extract and 2 ml of H_2O_2 (6 mM) were added there to. After 10 min incubation at room temperature, 2 mL of an aqueous solution of salicylic acid (6 mM) were added to the mixture and the whole was incubated again for 30 min. The absorbance is measured at 510 nm. The radical scavenging activity was calculated as stated above.

3. RESULTS AND DISCUSSION

3.1. Phytochemical results

Table 1 presents the secondary metabolites composition of leafy stems of *M. depauperatum*, leaves of *A. brasiliana* and leafy stems of *H. acetosella.*

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Table 1: Secondary metabolites composition in M. depauperatum, A. brasiliana and H. acetosella extracts

Secondary metabolites		*M. depauperatum*	*A. brasiliana*	*H. acetosella*
Tannins	Catechic type	+	+	-
	Gallic type	+	+	+
Flavonoïds		+	+	-
Anthocyans		+	-	+
Leucoanthocyans		+	-	-
Coumarins		-	-	-
Alcaloïds		-	-	-
Mucilages		+	+	+
Anthraquinones		+	-	-
Saponosides		+	+	+
Reducing compounds		-	-	-
Terpenes and sterols		+	+	+

+: présence; - : absence

The analysis of Table 1 revealed the presence in the three samples of gallic tannins, mucilage, terpenes and sterols. A total absence of alkaloids, coumarins and reducing compounds is noted in all samples.

Phytochemical studies also revealed the presence of phenolic compounds like tannins (gallic and catechin), flavonoids, anthocyanins, leucoanthocyanins, mucilages, saponins and anthraquinones in the leafy stems of *M. depauperatum*. To our knowledge, there are no previous phytochemical data on this plant. However, the presence of flavonoids, tannins, anthraquinones, triterpenes and unsaturated sterols in seeds of a species of the genus, *Monechma ciliatum* has been shown in Nigeria [18]. This study is supposed to be one of the first on the leafy stems of *M. depauperatum* widely used against anemia and fatigue associated with intense physical activity.

The phytochemical composition of *A. brasiliana* revealed the presence of phenolic compounds like tannins (gallic and catechin) and flavonoids, saponins, mucilage, sterols and triterpenes. Barua *et al*. in India found alkaloids, sterols and triterpenes in the leaves of this plant [19]. Similar to our results, some authors have reported the presence of flavonoids, saponins and the absence of alkaloids in *A. brasiliana* of Nigeria [20] and India [21]. Our samples as well as those of India contain tannins unlike those from Nigeria [20]. The different uses of *A. brasiliana* reported in traditional medicine as an antibacterial, anti-inflammatory, analgesic, inhibiting cell proliferation and healing [22] could be explained by the diversity of secondary metabolites in this plant.

Phenolic compounds such as gallic tannins and anthocyanins, mucilage, anthraquinone, saponins, terpenes and sterols are present in the leafy stems of *H. acetosella*. Tsumbo *et al.* have revealed the existence of phenolic compounds like phenolic acids, flavonoids and tannins in the leaves of *H. acetosella* harvested in Congo [23]. The literature has provided very little information on the phytochemical composition of this plant. But other species of the genus *Hibiscus* including *H. sabdariffa* contain flavonoids, anthocyanins, alkaloids and saponins in their chalices [24].

In general, the difference in phytochemical composition between samples of this study and the others in literature may be related to climate, soil conditions of the harvesting areas [25] and plant harvesting age [14].

3.2. Yield of extraction

Two types of extracts were made and three plants extraction yields are given in Table 2.

Table 2: Plants Extraction yield (%)

	Yield (%)		
Type of extacts	*M. depauperatum*	*A. brasiliana*	*H. acetosella*
Hydro-ethanolic	3.2 ± 0.17	3.4 ± 0.78	2.9 ± 0.65

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It appears from this table that the extract of *A. brasiliana* has the highest yield (3.4 ± 0.78 %) followed by the hydroethanolic extract from *M. depauperatum* plant (3.2 ± 0.17%). The extract of *H. acetosella* has the lowest yield 2.9 ± 0.65 %.

3.3. Phenolic compounds content of hydro-ethanol extracts

The total phenolic compounds content (expressed in mg gallic acid equivalent per gram of dry matter), total flavonoïds content (expressed in mg quercetin equivalent per gram of dry matter) and condensed tannins content (expressed in mg catechin equivalents per gram of dry matter) of hydro-ethanolic extracts of the three plants are shown by figure 1.

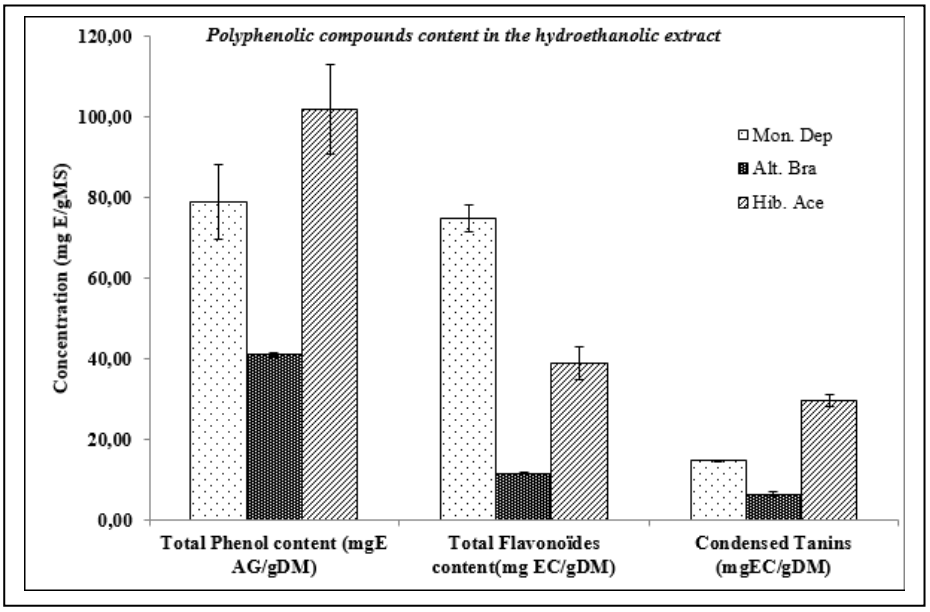

Figure 1 : Phenolic compounds, total flavonoïds, and condensed tannins contents of hydro-ethanolic extracts of the three plants Mon. Dep: M. depauperatum, Alt. Bra: A. brasiliana Hib. Ace: H. acetosella.

This figure shows that the hydro-ethanolic extract of *H. acetosella* is the richest with a total phenolic compounds content of 101.98 ± 11.13 mg EAG / g DM followed by *M. depauperatum* extract 78.84 ± 9.25 mg EAG / g DM. The extract of *A. brasiliana* presents the lowest at 40.98 ± 0.66 mg EAG / g DM. For the total flavonoïds content in mg EQ / g DM, the figure 1 shows that the extract of *M. depauperatum* is the richest with 74.80 ± 3.20, followed by the extract of *H. acetosella* (38.89 ± 4.06) and *A. brasiliana* extract, (11.59 ± 0.06). The dosage of the tannins content showed once again that the extracts of *A. brasiliana* contains a small amount of condensed tannins expressed mg EC / g DM compared to the other two extracts. This content is 6.43 ± 0.53 is lower than 14.74 ± 0.20 and 29.44 ± 1.52 as condensed tannins content respectively for *M. depauperatum* and *H. acetosella*.

Few works from the literature have been devoted to the determination of phenolic compounds in the three plants. However, Tsumbu *et al*. obtained 1.79% (17.9 mg / g DM) of dry matter as phenolic content of an aqueous extract of *H. acetosella* using pyrogallol as standard compound. The same authors showed that the aqueous extract of *H. acetosella* was the most concentrated in flavonoids (775 mg / 100 g) of the four studied leaf vegetables such as *Abelmoschus esculentus* (425 mg / 100 g), *Manihot esculenta* (1.381 mg / 100 g) and *Pteridium aquilinum* (448 mg / 100 g).

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3.4. Radical scavenging activity

✓ *DPPH radical assay*

Table 3 shows the inhibitory concentrations of 50% of DPPH radical (IC_{50}) hydro-ethanolic extracts of the three plants.

Table 3: The IC_{50} (ug / mL) of different extracts.

Plants	IC_{50} (µg/mL)
M. depauperatum	32
A. brasiliana	66000
H. acetosella	44
Quercetin	3

It appears that the aqueous extract of *H. acetosella* and hydro-ethanolic extract of *M. depauperatum* have the lowest IC_{50} (32 mg / mL) followed by hydro-ethanol extract of *H. acetosella* (44 µg / mL). These values are higher than quercetin's (3 µg / mL), that is a standard compound. The least effective extracts of all are aqueous and hydro-ethanolic extracts of *A. brasiliana* with respectively 160 and 66 mg / mL as IC_{50}.

✓ *Correlation between polyphenolic content and radical scavenging activity*

The correlation between the phenolic compounds content and radical-scavenging activity of different extracts is shown in Figure 2.

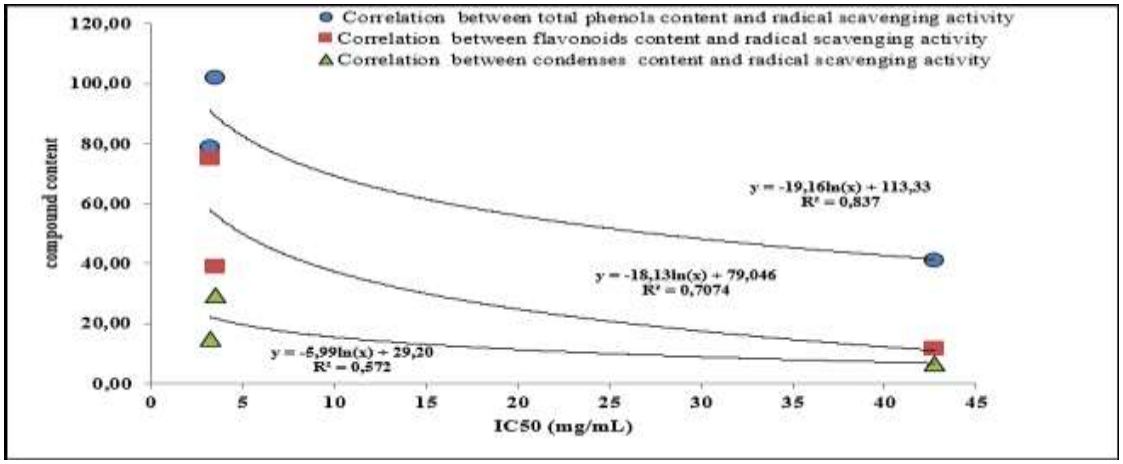

Figure 2: Correlation between polyphenolic compounds content and radical-scavenging activity

Figure 2 reveals that when the phenolic compounds content increases, the IC_{50} decreases. Since, the extract being effective when the IC_{50} is as low as possible, we can conclude that the extracts with low IC_{50} and thus high phenolic compounds content are most effective. The p-values of correlations between IC_{50} and phenolic compounds content are above 5%. Thus, the correlation between anti-radical activity and the phenolic content is positive but not significant at the 5% level (n = 3).

✓ *Hydroxyle radical assay*

The inhibitory concentrations of 50% of the hydroxyl radical (IC_{50}) of aqueous and hydro-ethanolic extracts of the three plants are presented in the Table 4.

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Tableau 4 : The IC 50 (µg/mL) of different extracts.

Plants	IC50 (µg/mL)
M. depauperatum	150
A. brasiliana	ND
H. acetosella	27
Gallic acid	135

Note : ND stands for Non Determined

It appears from this table that the aqueous-ethanolic of *H. acetosella* exhibits lower IC*50* values (25µg / mL) followed by gallic acid (135µg / mL), reference compound. These values are very low as compared to 1230 µg / mL obtained for the aqueous extract of *H. sabdariffa* by Xu and Chang [26].

4. CONCLUSION

The present study aimed to valorize three plants with anti-anemia activity and showed that leafy stems of the three plants studied are rich in secondary metabolites such as polyphenols flavonoids, tannins, anthocyanins, leuco anthocyanin, mucilage, the saponins, sterols and terpenes. The high phenolic content of hydro-ethanolic extracts of *H. acetosella* and *M. depauperatum*, responsible of anti-radical activity may be a reason for using these extracts to reduce oxidative stress invading the anemic body. However, "in vivo" antioxidants tests and antianemic tests are required to justify and provide a scientific basis for their use in traditional medicine in Benin.

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