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Phytochemical Profile and Antioxidant Potential of 12 Antimalarial Recipes Used in Lacustrine Areas In Benin

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***Abstract**: The present work aims to identify the major chemical groups and to evaluate the anti-radical activity of 12 recipes of medicinal plants used in the treatment of malaria among children, pregnant women and adults in two lake villages (Ganvi , Aguegues Daho) of southern Benin. All the recipes contain molecules able to trap the 2,2-diphenyl-1-picrylhydrazyl (DPPH) which is a free radical. This activity would be due to the phenolic compounds identified in the phytochemical screening and whose presence in these recipes has been confirmed by quantitative test performed in a spectrophotometer. Aqueous extract obtain by decoction of recipes 3, 1 and 4 (E3, E1, E4) of the child have the higher contents of total phenolics compounds and good anti-radical activity compared to other extracts. The aqueous extract obtain by decoction of the recipe 3 which has the strongest content of total polyphenols (668.16 mg EAG/g DM) was the most active for trapping DPPH with an IC_{50} of 0.095 mg/mL similar to those of butylated hydroxyanisole (0.090 mg/mL) and quercetin (0.100 mg/mL) that are reference antioxidants used in this study. These results justify the use of these traditional recipes.*
***Keywords**: Malaria, plants, phenolic compound, DPPH*

I. Introduction

Malaria is a parasitic disease due to presence and multiplication in the body of a protozoan of the genus Plasmodium transmitted by the bite of a female mosquito, Anopheles, causing intermittent fevers that determines a erythrocytopathy with hemolysis[1]. The World Health Organization (WHO) estimates the incidence of malaria at 198 million cases. Practically always due to *Plasmodium falciparum* the mortality is 584000 per year and the majority of cases occur in countries of sub-Saharan Africa where we note one child death every minute [2]. In Benin, malaria is the leading cause of hospitalization and death both in the general population, at the level of pregnant women that child under five years [3].

One of the major reasons for the development of anaemia in malaria seems to be oxidative stress [4-6]. The immune system of the body is activated by infections, including malaria, thereby causing the release of reactive oxygen species. In addition to this, the malaria parasite also stimulates certain cells to produce reactive oxygen species thereby resulting in haemoglobin degradation[5,7]. Indeed, depressed level of plasma antioxidants has been shown in *Plasmodium falciparum* infected children and it has been suggested as a possible contributor to the morbidity and mortality of malaria[8]. Malaria accounts for around 20% of cases of illness treated in traditional medicine[9]. The use of plants to treat themselves in Africa is an integral part of the culture. It should be recalled that over 80% of Africa's population is dependent on plants for primaries health needs[10,11], this because of the ease of access and modest cost of these herbal medicines. Unfortunately, these drugs quite often do not benefit from scientific control. So there is a permanent danger regarding the therapeutic doses of the one part and lethal doses of the other part, from where the margin of safety problem.To contribute to the safety of the Beninese population and those living in endemic areas malaria this work was initiated and aims to realize the phytochemical screening of large chemical families and evaluate the anti-radical activity of 12 recipes of medicinal plants used by the lakeside population of southern Benin (Ganvie, Aguegue Daho) to treat malaria a view to pharmacological and toxicological studies to confirm or disprove the use made of the traditional medicinal plants.

II. Material And Methodology

Plant Material

The plant material investigated in this study has been retained after an ethnobotanical survey conducted throughout the South Benin lakeside population to make an inventory of anti-malarial plants for eventual pharmacological and toxicological tests. It is composed of 47 plant species distributed into 12 recipes used to

treat malaria in children, pregnant women and adults at "Ganvie" (Atlantic) and "Aguegue Daho" (Oueme) in Benin. After collection, the plant material has been identified in the National Herbarium of the Department of Botany, Abomey-Calavi University. The samples were dried over laboratory temperature (25-30 °C) until their stabilization and then reduced in powder with an electric grinder (Brand RETSCH, Type SM 100).

Methodology

Identification of secondary metabolites

The determination of metabolites was done by differential coloring reaction and/or precipitation of the major families of chemical compounds contained in plants. So, sterols and terpenes have been identified by the Liebermann-Burchard test[12]. The characterization of the compounds belonging to the group of phenolics compounds was made by the reaction with ferric chloride[13]. Flavonoids identification was carried out by the test of cyanidine[14]. The compounds belonging to the group of tannins have been highlighted by the reaction of Stiasny[15]. The free or combined quinone compounds have been disclosed by the reaction of Borntraeger[13,16]. The saponosides research is based on foam test; degree of aqueous decoction dilution giving a persistent foam after shaking[13,17]. Alkaloids were identified by Mayer test and confirmed by Bouchardat test.

Determination of Phenolic Compounds

The quantification of the phenolic compounds focused on the filtrates hydroethanolic macerated (water/ethanol: 30/70) and aqueous extract obtain by decoction (distilled water) of plant material subject of this study. Method of aluminum trichloride ($AlCl_3$) was used to quantify the total flavonoids[18,19]; condensed tannins were measured by the method to the sulfuric acid vanillin[20,21] and the total polyphenols were measured by Folin-Ciocalteu method[22,23].

Determination of the anti-radical activity

The anti-radical activity is determined by the trapping DPPH test. DPPH was dissolved in ethanol. Different ranges of concentrations in the milligram of each extract were prepared. In dry tubes are introduced 200 µL of extract to analyze and 3800 µL of DPPH solution. After shaking, the tubes are placed in the dark place for 1 hour, and then the absorbance of the mixture is measured at 517 nm in a spectrophotometer against the blanc[24]. The percentage of free radical scavenging DPPH is calculated using the formula:

$$I = \frac{(A \text{ reagent blank} - A\ sample)\ x\ 100}{A \text{ reagent blank}}$$

A: Absorbance

III. Results And Discussion

Phytochemical Screening

Various metabolites have been evidenced in recipes studied by a series of coloring reaction and/or precipitation specific to each class active principles as shown in Table 1.

Table 1: Metabolites identified

		E1	E2	E3	E4	F1	F2	F3	F4	A1	A2	A3	A4
Saponosides		±	±	±	+	+	±	+	+	±	+	+	±
Reducing compound		±	±	+	±	+	±	±	+	+	+	+	+
Phenolics compound		+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids		+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanins		+	+	+	+	±	±	+	-	-	±	+	-
Condensed tannins		+	+	+	+	+	+	+	+	+	+	+	+
Gallic tannins		+	+	+	+	+	-	-	-	±	+	+	±
Leucoanthocyanes		+	+	+	+	-	±	+	+	±	+	+	±
Alkaloids		+	+	+	+	±	±	-	+	-	-	-	-
ANTHRAQUINONE	**Anthraquinones free**	-	-	-	-	-	-	-	-	-	-	-	-
	O-Heterosides	-	-	±	-	-	-	+	-	±	-	+	-
	O-Heterosides in reduced genins	±	+	-	-	-	-	±	±	-	±	±	-
	C- Heterosides	-	+	-	-	-	-	-	-	-	-	-	-

Quinones	-	-	-	-	-	-	-	-	-	-	-	-
Mucilage	-	-	-	-	-	-	-	-	-	-	-	-
Sterols and the terpenes	±	±	±	+	±	-	±	±	-	-	-	+

E: Children; F: Female; A: Adult; +: Presence; ±: Trace; -: Absence

All the recipes investigated are rich in metabolites such as reducing compounds, saponins and phenolic compounds. We note the presence of saponins, reducing sugars, flavonoids and condensed tannins in all recipes. Through against mucilages, quinones and anthraquinones are absent. All the children's recipes investigated as well as recipes 1 and 4 of the pregnant woman; contain alkaloids, sterols and terpenes. The presence of alkaloids, flavonoids, sterols and the terpenes recognized for their antiplasmodial property in recipes would justify their use in the treatment of malaria in Benin [25].

Phenolic compound content

The figures below show the levels of total phenolics compounds, flavonoids and condensed tannins of aqueous extract obtain by decoction and hydroethanolic macerated extract of investigated recipes.

Total phenolics compounds

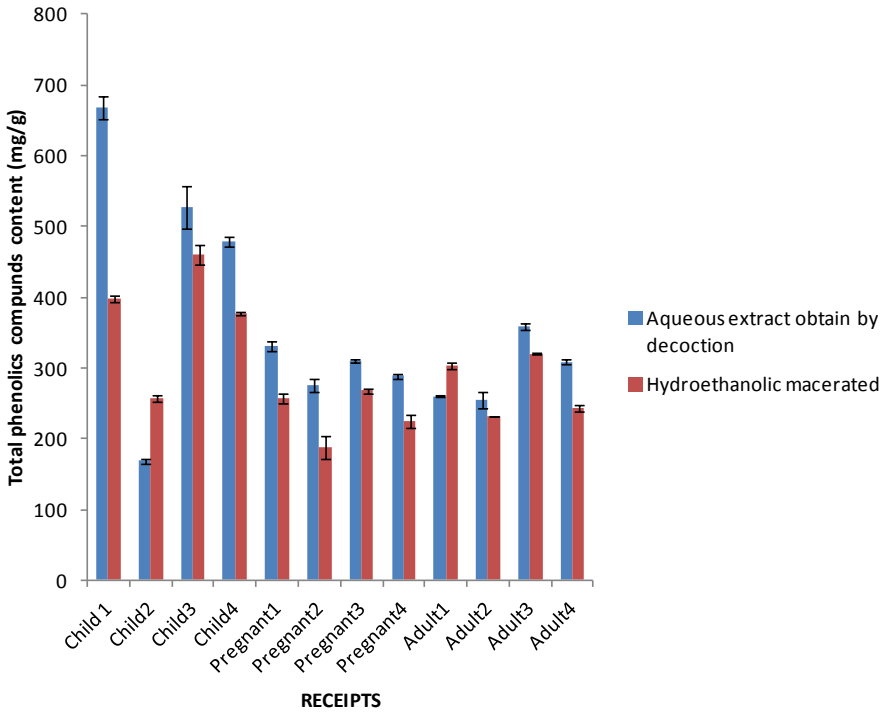

Figure 1: Total phenolic compounds content of extracts studied

The total phenolics compound contents of our extracts range from 668.16 to 168.54 mg/g. The aqueous extract obtained by decoction of the recipe 1 of the child has the strongest content of total polyphenols, while the lowest level was observed in the aqueous decoction of the recipe 2 of the child. The recipes 1 (aqueous decoction: 668.16mg/g; hydroethanolic macerated: 398.34mg/g), 3 (aqueous decoction: 526.77mg/g; hydroethanolic macerated: 460.52mg/g) and 4 (aqueous decoction: 479.24mg/g hydroethanolic macerated 377.10mg/g) of the child are the richest in total phenolics compounds. Among the recipes investigated of the pregnant woman, the aqueous decoction of the recipe 1 (330.56mg/g) has the strongest content of phenolic compounds, while the aqueous decoction of the recipe 3 of adult has the strongest content of this compound in adult. Besides the recipes 2 of the child and 1 of adult, the aqueous extracts of our recipes are richer in total polyphenols than hydroethanolics macerated. These results are consistent with those of Petko *and al.* [26] which show that the extraction rate increases with temperature as well as the team of Agbangnan[27] which showed that the increase in temperature has a positive effect on the extraction of phenolics compounds. In general, the phenolic compound content of the dry extracts not only vary from one plant to another from the same family but also a function of the solid-liquid extraction parameters: the temperature, the extraction solvent, the particle size and solvent diffusion coefficient.

Flavonoids

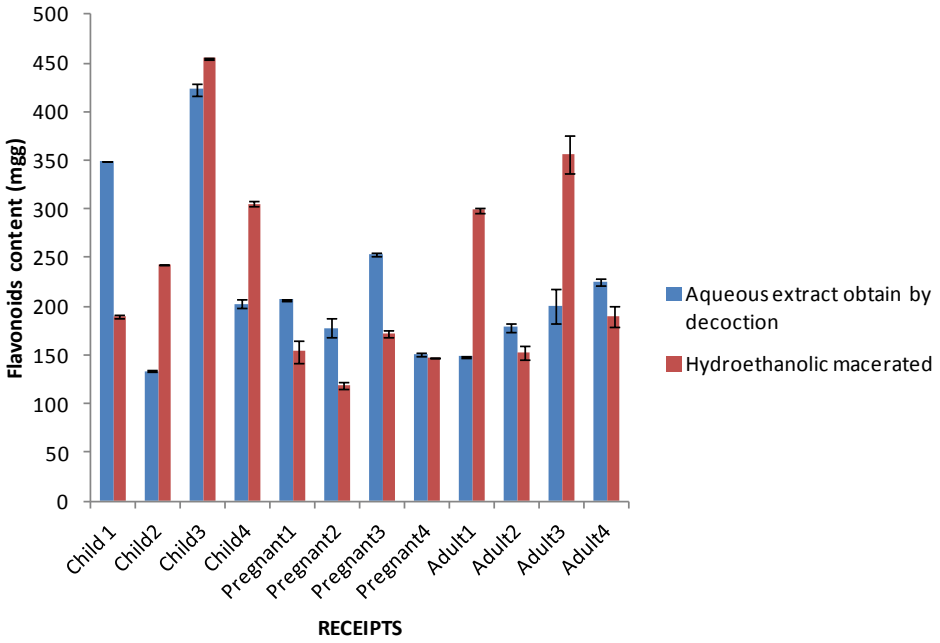

Figure 2: Flavonoid content of aqueous and hydroethanolic extracts

Our recipes are rich in flavonoids with grades ranging from 454.82mg/g to 118.78mg/g. The hydroethanolic macerated of recipe 3 of the child has the highest content followed its aqueous decoction (423.03mg/g) and macerate hydroethanolics recipes 3 (356.55mg/g) of adult and 4 (305.40mg /g) of child while the macerated hydroethanolic recipe 2 of the pregnant woman has the lowest content.

Condensed tannins

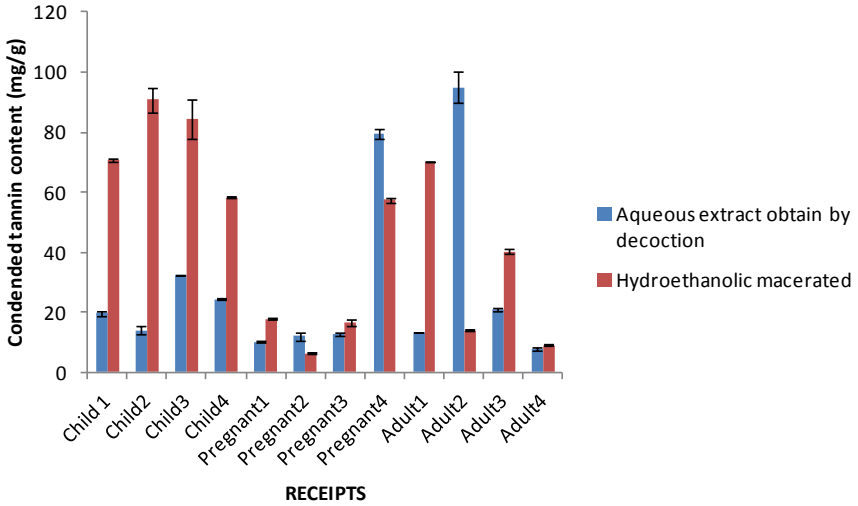

Figure3: Condensed tannins content of the extracts studied

Apart recipes 2 and 4 of the pregnant woman and 2 of adults, macerated hydroethanolics of our recipes are richer in condensed tannins that aqueous extracts. The aqueous extract obtain by decoction of the recipe 2 (94.98mg/g) of the adult has the highest content of condensed tannins followed hydroethanolics macerated of recipes 2 (90.83mg/g) and 3 (84.26mg/g) of child, while the hydroethanolic extract of recipe 2 (6.52mg/g) of the pregnant woman has the lowest content.

Radical scavenging activity

Concentrations for trapping 50% (IC_{50}) free radicals of our extracts are shown in the table 2.

Table2: IC_{50} (mg/mL) DPPH

	Aqueous	Hydroethanolic
Child1	0.150	0.250
Child 2	1.100	0.620
Child 3	0.095	0.350
Child 4	0.160	0.54
Pregnant woman1	0.320	0.400
Pregnant woman 2	0.520	0.480
Pregnant woman3	0.400	0.480
Pregnant woman4	0.520	0.750
Adult1	0.500	0.250
Adult2	0.660	0.800
Adult3	0.270	0.360
Adult4	0.920	0.260
BHA	0.090	
Quercetin	0.100	

The evaluation of the anti-radical property of our extracts shows that the extracts possess constituents which discolor the solution of DPPH. The antioxidant activity of each of the extracts on the free radical DPPH is expressed in substrate concentration which inhibits by 50% the activity of DPPH (IC_{50}). The antioxidant capacity of an extract is considerable when its IC_{50} is low. From the results obtained and as shown in the figure above, aqueous extract recipes 3 (0.090 mg/mL), 1 (0.150 mg/mL) and 4 (0.160 mg/mL) of the child have been very effective with regard to trapping DPPH compared to other extracts and reference antioxidants (quercetin: 0.100 mg/mL; Butylated hydroxyanisole, BHA: 0.090 mg/mL) used in this study. By referring to the contents of phenolic compounds of our extracts, we observe a correlation between their phenolic content and antioxidant activity especially only three extracts which have the highest total polyphenol content showed best activity. These observations corroborate those already made earlier by Chevalley in 2000[28]; Djeridane *et al*., 2006[29] and Wojdylo *et al*., 2007[30].

IV. Conclusion

This work was devoted to the identification of major chemical groups, dosing of phenolic compounds followed by the evaluation of anti-radical activity of 12 recipes of plants used by the population of lacustrine cities in southern Benin for the treatment of malaria. Aqueous extracts obtained by decoction of recipes 1, 3 and 4 of the child which are richer in phenolic compounds, also presented the most interesting anti-radical activity. This work thus makes a significant contribution regarding of knowledge of phytochemical studied receipts and thus enables a better understanding of the pharmacodynamic properties of extracts explaining their use in traditional pharmacopoeia.

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