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RESEARCH ARTICLE

Ethnotherapy study, phytochemical and antiradical activities of *Agelaea pentagyna* (Lam) Baill and *Dialium dinklagei* Harms. Medicinal plants from Gabon

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Abstract

In Gabon as in the more part of developing countries populations use to the medicinal plants for their needs of health. This work consists to contribute to the medicinal plants knowledge that are used in gabonese pharmacopeia. Ethnotherapy study, phytochemical and antiradical activities of two plants which are used in pharmacopoeia a pygmy South-East Gabon by conventional methods used in laboratory. The results are showed that both species are rich in secondary metabolites. The values in total phenols and proanthocyanidins are respectively from 269.5 ± 0.34 and 56.83 ± 4 mg/100 g of drugs (*Agelaea pentagyna*) and 442 ± 45 and 46.83 ± 3 mg/100 g of drugs (*Dialium dinklagei*). Results also show that two plants have a relatively significant antiradical activity with values of IC_{50} ranging from 177.02 ± 0.42 (*A. pentagyna*) to 279.64 ± 0.40 % drugs (*D. dinklagei*). The abundance of bioactive compounds would explain the therapeutic effects observed and the use of these plants in traditional medicine. For antiradical activity of extracts, these plants could be used like deterrent against the cardiovascular pathologies. Abundance in secondary metabolites and antiradical activity of two plants justifies their use in ethnotherapy by populations Pygmy of Gabon.

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Introduction

In Africa, plants are used in the treatment of several pathologies. Several studies have been shown that plant extracts to possess antioxidants and antimicrobial properties *in vitro* (Sundaram *et al.*, 2011; Nene-Bi *et al.*, 2012). However, in Gabon, phytochemical properties and biologic activities medicinal plants used in traditional pharmacopeia by populations are known little. In order to make a contribution to the knowledge of two medicinal plants (*Dialium dinklagei* and *Agelaea pentagyna*) who are used in ethnotherapy by populations Pygmy of Gabon.

Dialium dinklagei is a tree of Caesalpiniaceae family with trunk cylindrical right. The sheets made up have an acid taste, the flowers are of color yellows and the bark is very thick. *Agelaea pentagyna* is a twining liana or scandens shrub of Connaraceae family. Leaves 3-foliolate, 3-veined from the base. Fruits are ovoid, brown-red when ripe. The roots are used to treat fever (Kokwaro, 1976). This study consists in evaluating therapeutic proprieties of *D. dinklagei* and *A. pentagyna*.

MATERIAL AND METHODS

Ethnobotanical study

The research was approved by the Department of Biology Sciences, Faculty of Science of University of Sciences and Technical of Masuku (USTM). The survey was performed using to interview on selected local people during the academic year 2011–2012. The study was realized during march 2012 area in habited by pygmy people from Benguia village South-East from Gabon at 5 km from Franceville city in Haut-Ogooué (Gabon). The information were collected from the traditional healers, village dwellers, herbalists and the aged and experienced people the herbal medicine practitioners, maydays and their traditional healers following the method (Edwards *et al.*, 2005). Information was collected through questionnaires, bilateral discussion and open ended interviews on plants used by population for treatment of pathologies. A total 30 informants have been interviewed on random basis. Information about the family, botanical name of species, local name, plant parts used, plant crude drug preparation, mode of applications, dosage and duration were documented (Kokwaro, 1976; Walker and Sillans, 1961) and medicinal uses, plant parts that were identified as having use in ethnotherapy were collected. The choice of this two plants species study was based by lack of data on pharmacological.

Plant material

The leaves of *Dialium dinklagei* and *Agelaea pentagyna* were collected in April 2012 in Lékédi Park of Bakoumba, at 80 km of Franceville city (Gabon). The plants were taxonomically authenticated at the National Herbarium of Gabon Pharmacopoeia Institute of Traditional Medicine (IPHAMETRA, Libreville) where voucher specimens were deposited.

Preparation of plant extract

The leaves of *D. dinklagei* and *A. pentagyna* were air-dried at room temperature for a total period six weeks and pulverized to powder using a clean electric blender (Waring® commercial Blender). A 25 g of the powder of every sample was soaked in 300 mL of solvents water (Aq) and ethanol (EtOH) and allowed to stand for 72 h with intermittent stirring. This was filtered through a whatman No. 1 filter paper and the filtrate obtained was evaporated to a dry mass using a rotary rotavaporator at 40 °C. The residues recovered were dried in an oven at a temperature of 65 °C. The extract obtained is stored in vials protected from light until the completion of various tests. The yields of the extracts (%) were calculated (Boulenouar *et al.*, 2009; Salem, 2009).

Phytochemical screening

The extracts of *D. dinklagei* and of *A. pentagyna* were analyzed for their classes of bioactive compounds using standard procedures with small modifications (Culei, 1982; Harbone, 1984; Sofowora, 1993; Trease and Evans, 2002). The extracts were tested qualitatively for the presence of chemical constituents such as tannins, terpenes, saponins, flavonoids, cardiac glycosides, coumarins, alkaloids, anthraquinones and reducing sugar. For gallic tannins, 2 mL of 1% ferric chloride solution was added to 2 mL of the filtrate (Stiasny's test). Dark-greenish coloration indicated their presence. catechic Tannins, 2 mL of a solution of hydrochloric n-butanol are added to 2 mL of filtrate, and then heating in a water bath for 5 to 10 minutes (Bate-Smith's test). Intense red coloration indicated the presence of the catechin tannins. For total flavonoids and anthocyanes, 1 mL of the sulfuric acid was added to 2 mL of the filtrate, then 1 mL NaOH. There shown a dark color after adding acid, indicating the presence of flavonoids, the color changes to purple after addition of NaOH, indicating the presence of anthocyanins. 2 mL of the filtrate were added magnesium strips followed by hydrochloric alcoholic (cyanidine test). A rose-orange effervescence showed the presence of flavones, rose-purplish indicated of flavanones and red denoted of flavonols. We had applied the Folin's test to determine polyphenols contents. 1 mL of the Folin reagent was added to 2 mL of the filtrate, then 1 mL NaOH. Dark green coloration indicated the presence of polyphenols. For coumarins, 2 mL of filtrate combined with 2 mL of NH₄OH, then, lookin at UV lamp (366 nm). The fluorescence presence indicated the presence of coumarins; 2 mL NH₄OH solution was added to 2 mL of the filtrate (Borntrager's test). A rose pink colour in the ammonia layer indicated the presence of anthraquinones. For alkaloids, some drops of sulfuric Dragendorff's reagent were added to 2 mL of the filtrate. Orange precipitate formed had showed the presence of alkaloids. To determine cardiac glycosides and terpenes, test such as Salkowski's and Lieberman's test were applied; 2 mL of concentrated H₂SO₄ were added to 2 mL of filtrate, a reddish-brown ring indicated the presence of steroid, an aglycone part of the cardiac glycoside (Salkowski's test). Another part of the filtrate (2 mL) was added with 2 mL of acetic anhydride and cooled well in ice and concentrated H₂SO₄ (2 mL) was carefully added. A color change from blue to green indicated the presence of terpenes (Lieberman's test). Saponins were determined through frothing test. The filtrate was vigorously shaken. Frothing which persisted on warming for about 15 min indicated the presence of saponins. For reducing sugars, equal volume of Fehling's A and Fehling's B reagents were taken in equal quantities

and were added to filtrate and boiled on water bath (Fehling's test). Appearance of brick red precipitate indicates the presence of reducing sugars. Cardiac glycosides: to 1 mL of filtrate 1 mL of ferric sulfate solution (5 %) and 2 mL of concentrated sulfuric acid gives a color reaction Kiliiani-Keller based structure cardiac glycosides into play, namely (Parekh *et al.*, 2006): digitoxin: dirty red brown, digitoxigenin: red fluorescent, gitoxin: yellow then red blue and gitoxigenine: yellow then red purple.

Phenols and proanthocyanidins content extracts

The Folin-Ciocalteu method was used to measure total amount of total phenols content (Singleton *et al.*, 1999). Aliquots of 0.25 mL of leaf extracts (1 mg/mL) were mixed with 1.25 mL Folin–Ciocalteu reagent (0.2 N diluted in methanol). A reagent blank using methanol instead of sample was prepared. After 5 min incubation at room temperature, 1 mL sodium carbonate solution (7.5 %) was added. Samples were incubated at room temperature for 1 h and the absorbance was measured at 765 nm versus the prepared blank. All tests were carried out in triplicate and total phenols content was expressed as mg of gallic acid equivalents (GAE) per 100 g of drug.

Proanthocyanidins (PAs) were quantified with the hydrolysis test of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavans-3-ols constituting the polymers (Prigent, 2005). The heating step destroys the anthocyanidins pigments generated by flavan-4-ols and eliminates part of the chlorophyll pigments. The routine assay is performed by mixing 0.16 mL (1 mg/mL) of the extract with 2.33 mL of 30 % HCl-butanol solution (v/v). The mixture was put in tightly closed tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, the absorbance was read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as aforementioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE).

Antiradical activity

DPPH spectrophotometric (quantitative) assay was performed with some modifications (Brand-Williams *et al.*, 1995). Method was widely used to test the ability of antioxidant bioactive compounds to activity as free radical scavengers or hydrogen donors. This test is based on the capacity of stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen (H) donors, including phenols. It is used for the quantification of antioxidants in the complex of biological systems (Miliauskas *et al.*, 2004). Each sample of extract was prepared at different concentrations (100, 200, 300 and 400 µg /mL). The reaction mixture contained 1 mL of DPPH prepared at a concentration 20 mg /L in methanol, and 1 mL of test samples. After a 30 min reaction, the absorbance was read at 517 nm and converted into percentage of antiradical activity, using the following the formula (Abdoul-Latif *et al.*, 2010).

$$\% \text{DPPH radical scavenging} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$$

Where, A_{control} is the absorbance of the blank; A_{sample} is the absorbance of the sample.

Control contained 1 mL of DPPH solution and 3 mL of methanol. The measurements of DPPH radical scavenging activity were carried out for three sample replications, and values are an average of three replicates. IC_{50} is defined as the concentration of the test sample leading to a 50 % inhibition of the DPPH free radicals. IC_{50} value was calculated from the separate linear regression of plots of the mean percentage of the antioxidant activity against concentration of the test compounds (mg /mL) obtained from three replicate assays.

RESULTS

Ethnobotanical survey

The results for the listed of twenty plants and their therapeutic indications are shown in table 1. Diverse parts of the plant (root bark and stem, leaves, fruits, twigs) are used in medicinal preparations (friction, plaster, cooking, mixture, bath, fumigation, steeping, eating raw, maceration, infusion). Leaves are the part of the plant the more used (55 %).

Phytochemical screening

Phytochemical analysis is important in the evaluation of bioactive compounds from medicinal plants are shown in table 2. Qualitative analyzes was carried out in both dry extracts of *A. pentagyna* and *D. dingklagei*. The results show that the both the two species are rich in polyphenols, alkaloids, tannins and flavonoids. This shows the high level of its possible medicinal and food values (Oloyed, 2005). Ethanolic extracts of two plants contain tannins and only the aqueous extract of *A. pentagyna* is rich in saponins.

Total polyphenols, proanthocyanidins contents

The yields of different extracts of *A. pentagyna* and *D. dingklagei* were respectively 06 % (Aq), 1.44 % (EtOH), 5.44 % (Aq) and 2.48 (EtOH) (Table 3). Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). The equation of the right and side of the proportioning of total phenolic content by the method of Folin-Ciocalteu gave $Y = 0.0012 X - 0.0004$ with $R^2 = 0.9902$ (Abdoul-latif *et al.*, 2012).

Levels of proanthocyanidins were expressed in terms of apple proanthocyanidins equivalent (APE). The equation of the right-hand side of the proportioning of the proanthocyanidins by the HCl- Butanol method gave $Y = 0.0006 X + 0.0024$ with $R^2 = 0.9869$ (35). Among concentrations in proanthocyanidins are 18.5 mg APE/100 g of drug for aqueous extract and 56.83 mg APE/100 g of drug for ethanolic extract of *A. pentagyna* and 24.33 mg APE/100 g of drug for ethanolic extract and 46.83 mg APE/100 g of drug for aqueous extract of *D. dingklagei* (Table 3).

Antiradical activity

Antioxidant activity using DPPH radical-scavenging assay expressed as IC_{50} value and antiradical capacities of extracts are showed in table 3 and figures.1-2, lower IC_{50} indicating the higher antioxidant activity of extract. The results of DPPH antiradical activity were differed significantly between different plants. *A. pentagyna* has better radical scavenging activity (117.02 ± 0.42 mg /mL) compared to *D. dinklagei* with IC_{50} of two extracts equal to 288.57 ± 0.34 mg /mL (EtOH) and 279.64 ± 0.40 mg /mL (Aq).

Table 1: Some ethnomedicinal plants used in traditherapy by pygmy people of South-East Gabon.

Species name	Family	Local name	Part used	Preparations	Anti-infective use
<i>Nauclea latifolia</i> Smith	Rubiaceae	Moubole	Root	Decoction	Gastro-intestinal tract pains and antimalarial.
<i>Bridelia ferrugenea</i> Benth	Euphorbiaceae	dzana	Stem bark	Decoction	Treatment against poisoning
<i>Alchornea cordifolia</i> Müll. Arg.	Euphorbiaceae	Moubouni	Leaves	Decoction	Stomach pain and epilepsy
<i>Manihot esculenta</i> Crantz	Euphorbiaceae	Tsara	Leaves	Friction	Eye infections
<i>Costus silhouette</i>	zingiberaceae	Moukoussa	Stem	Plaster	Gum inflammation
<i>Dissotis rotundifolia</i> Sm.	Melastomaceae	Ntonki	Leaves	Cooking	Gum inflammation
<i>Cassia manii</i> Oliv.	Scrophulaniaceae	Gari	Leaves	Decoction	Hemoroides
<i>Mangifera indica</i> L.	anacardiaceae	Moumanga	Stem bark	Décoction	Treatment of toothache
<i>Alchornea floribunda</i> Müll. Arg.	Euphorbiaceae	Mounotogo	Leaves	Prepare a mixture	Epilepsy and fever
<i>Harungana madagascariensis</i> Choisy	Hypericaceae	Moussassa	Leaves	Prepare a mixture	Treatment of stomach
<i>Helychrisum mehovianum</i>	Astéraceae	Ipelakaye	Leaves	Bath / fumigation	Tightening the vagina
<i>Solanum torvum</i> Sw.	Solonaceae	Moutouti	Stem bark	Steeping	Urinary tract and Skin infection
<i>Scroporia sp.</i>	Scrophylaniaceae	Essiga	plant	Steeping	Urinary tract infection
<i>Anthocleista nobilis</i> G. Don	Loganiaceae	Moukoro	Root	Steeping	Sexual dysfunction
<i>Millettia versicolor</i> Welw	Papilionaceae	Eboto	Root	eating raw	Sexual dysfunction
<i>Aframomum melegueta</i> K. Schum	Zingiberaceae	Doumou koutou	fruits	eating raw	Sexual dysfunction
<i>Smilax Klausiana</i> Meisn.	Smilacaceae	Mouguila	Leaves	Bath	Epilepsy
<i>Dialium dinklagei</i> Harms	Caesalpiniaceae	Ndoma	leaves	Decoction	Cutaneous disease and fever
<i>Agelaea pentagyna</i> (Lam.) Baill	Connaraceae	Oboki	Leaves /root	Maceration	Antimalarial and diarrhea
<i>Tamarindus Indica</i> L.	Mimosaceae	Dalè	Leaves	Infusion	Stomachache,diarrhea,purgative.

Table 2. Results of phytochemical screening of extracts from *D. dinklagei* and *A. pentagyna*

Chemical constituents	<i>Dialium dinklagei</i>		<i>Ageleae pentagyna</i>	
	EtOH extract	Aqueous extract	EtOH extract	Aqueous extract
Saponins	-	++	-	+++
Tannins	Gallic	+++	-	++
	Catechin	-	+	+++
Alkaloids	++	+++	+++	+++
Triterpenoids	-	-	++	+
Polyphenols	+++	+++	+++	+++
Flavonoids	Flavonols	-	-	-
	Flavones	-	-	+++
	Flavanones	-	-	-
Free anthraquinones	-	-	-	-
Coumarine	++	++	++	+++
Total flavonoids	+++	-	+++	++
Cardiac glycosides	Digitoxine	++	-	-
	Digitoxigenine	-	-	-
	Gitoxine	-	-	-
	Gitoxigenine	-	-	-
Reducing sugars	++	+++	-	+

+++ = High, ++ = Moderate; + = Low; -: negative test.

Table 3. Comparison of total phenolic compounds, proanthocyanidins and antiradical activity of *A. pentagyna* and *Dialium dinklagei* extracts.

Extractions	yields (%)	Total phenols (mg GAE/100 g of drug)	PAs (mg APE/100 g of drug)	Quota of PAs in Total phenols (%)	DPPH : IC ₅₀ (µg/ml)
<i>A. pentagyna</i>					
Aqueux	0.6	186.17 ± 0.20	18.5 ± 2	9.94 ± 0.25	0
Ethanolique	1.44	269.5 ± 0.34	56.83 ± 4	21.09 ± 0.25	177.02 ± 0.42
<i>D. dinklagei</i>					
Aqueux	5.44	442 ± 0.45	46.83 ± 3	10.60 ± 0.26	288.57 ± 0.34
Ethanolique	2.48	227 ± 0.38	24.33 ± 1	10.72 ± 0.24	279.64 ± 0.40

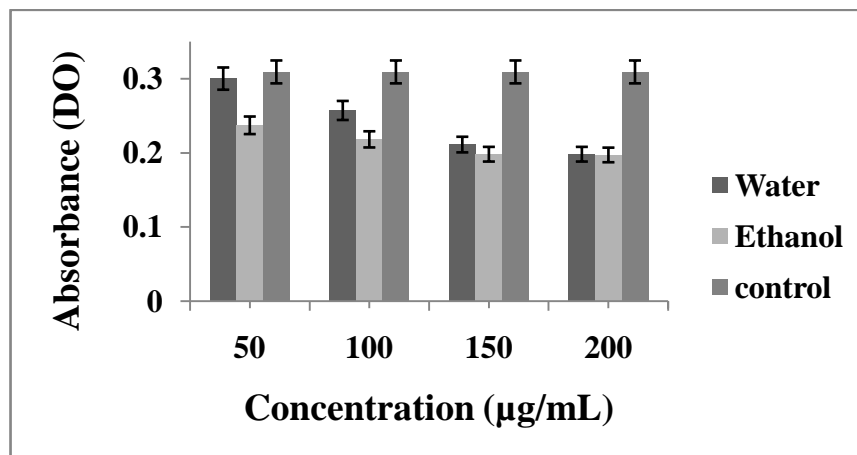


Figure 1. Antiradical activity extracts of *Dialium dingklagei* Beauv.

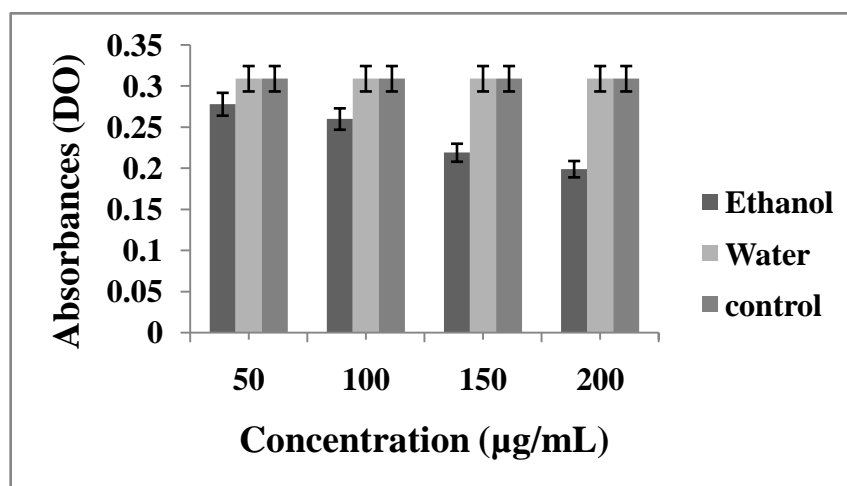


Figure 2. Antiradical activity extracts of *Ageleae pentagyna* (Lam) Bail.

DISCUSSION

Ethnobotanical survey

These resulting are similar those gotten during various investigations ethnomedicinal (Ouattara, 2006; N'Guessan *et al.*, 2009; Nunkoo and Mahomoodally, 2012) at Bétés of Issia (Côte-d'Ivoire). Leaves are used abundantly in 64.49 % of cases (Zirihi, 1991). Other works showed that leaves were solicited in 59.10 % of cases (Adjanohoun and Aké Assi, 1979). The decoction constitutes the fashion of preparation frequently used (30 %) by the aforesaid population. This result is near the one gotten by other authors. The decoction is indicated more in 32.94 % of preparation (Adjanohoun and Aké Assi, 1979; Zirihi, 1991).

Phytochemical screening

The phytochemical analysis shows that the two plants are rich in secondary metabolites. Abundance of compounds phenols, flavonoids and alkaloids justifies the use of these plants in gabonese traditional medicine. Indeed, several studies have shown that compounds such as polyphenols, flavonoids and alkaloids have therapeutic properties such as antimicrobial, antiparasitic, antidiarrhea and healing (Karou *et al.*, 2005, Vyas *et al.*, 2010, Sivananthan and Elamaran, 2013). Several studies have shown that flavonoids and saponins such possess antimotility and antisecretory activity on gastrointestinal tract (Galvez *et al.*, 1991; Agbor *et al.*, 1999; Oben *et al.* 2006). In addition, the abundance of biologic antioxidant compounds such as phenol compounds, flavonoids, alkaloids and tannins suggests that these species may have a preventive effect on diseases like cancer, cardiovascular diseases and

diabetes. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxy radicals, hydroxyl radicals and peroxy nitrile. Antioxidants induce balance between antioxidants and reactive oxygen species results in oxidative stress, protecting to cellular damage (Burlon and Ingold, 1984; Tchiagam *et al.*, 2012). In addition, epidemiological studies have shown those flavonoids and carotenoids intakes are inversely related to mortality from coronary heart diseases and to the incidence of heart attacks (Donald, 2006; Anoosh *et al.*, 2012). The healing properties of plants are correlated with the abundance of tannins. This is the case for example *Lannea acida*, rich in tannins, is used in traditional medicine for wound healing (Sereme *et al.*, 2008). Moreover, concentrations in phenol compounds in the two plants are similar those met in certain medicinal plants used in traditional medicine in certain regions of Gabon (Nsi Akoué *et al.*, 2013; Ondo *et al.* 2013). This abundance in phenol compounds would confirm the therapeutic properties that there are assigned in ethnotherapy. Indeed, several works have demonstrated that phenolic compounds confer to the plant several biologic activities.

Total polyphenols, proanthocyanidins contents

The concentrations of total phenols in the different plants extracts of study are 186.17 and 269.5 mg GAE /100 g of drug (*A. pentagyna*), 442 (Aq) and 227 mg GAE /100 g of drug (EtOH). more significant 442 mg GAE/100 g of drug (*D. dinklagei*) and 269.5 mg GAE/100 g of drug in comparison with those of the cereals (0,481 à 0,896 mg/g of matter) appreciably equal with those of other plants such as *Broccolis* (11.7 mg /g of matter), the gallic ones (9.9 mg/g of matter) and the fruits (23.1 mg/g of matter for the *blackberry*) (Wang and Lin, 2000). *A. pentagyna* is richer in proanthocyanidins (56.83 mg APE/100 g of drug) by contribution to *D. dinklagei* (46.83 mg APE /100 g of drug). The HCl/butanol assay used here for the determination of proanthocyanidins is more specific than many other tests such as the vanillin assay (Makkar, 2000, Santos-Buelga and Scalbert, 2000). The interferences, which might result from flavan-4-ols conversion into proanthocyanidins or from chlorophylls, may have been minimized during the heating step (Prigent, 2005; Catherine *et al.*, 1996). This abundance in phenol compounds would confirm the therapeutic properties that there are assigned in ethnotherapy. Indeed, several works have demonstrated that phenolic compounds confer to the plant several biologic activities.

Antiradical activity

Antiradical activity obtains show that *A. pentagyna* has better radical scavenging activity compared to *D. dinklagei* and that *A. pentagyna* is greater than *D. dinklagei* in addition aqueous extract shows no radical activity. This difference of results must be explained by the fact that an antiradical activity of phenolic compounds depends on their molecular structure, on the availability of phenolic hydrogens and on the possibility for stabilization of the resulting phenoxy radicals formed by hydrogen donation (Sundaram *et al.*, 2011; Catherine *et al.*, 1996; Ramarathnam *et al.*, 1997). The more chemical structure and polarity of the antioxidant are critical to its ability to scavenge free radicals. The observations on the variation in the antiradical activity of different compounds according have showed that synergistic and antagonistic effects of the molecules that make up the extract influenced the antiradical activity of extracts (Popovici *et al.* 2009).

CONCLUSION

The present study was aimed to evaluate the phytochemical and antiradical allow us to infer that the use of *A. pentagyna* and *D. dinklagei* traditherapy by pygmy people against various diseases would depend on its relative wealth in saponins, phenolic compounds (tannins, Coumarins and flavonoids) and nitrogen (alkaloids) endowed of pharmacological properties. This abundance of active plant gives remarkable properties, which could justify its multiple therapeutic indications for which it is used traditherapy. These preliminary results could provide a scientific basis for the research of new therapeutic molecules. Further pharmacological investigations allow us to determine precisely the different biological activities and to evaluate the acute and subacute *A. pentagyna* and *D. dinklagei*.

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