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Physico-chemical composition and radical-scavenging activity evaluation of the extracts of *Aristolochia albida* Duch. (Aristolochiaceae) of Benin

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ABSTRACT

Objective: To do the phytochemical screening and then to measure total phenols, condensed tannins, flavonoids of aqueous, hydroethanolic and ethanolic extracts of *Aristolochia albida* Duch. in order to identify the best preparation technique and extraction solvent. The antiradical activity of the different extracts of this plant were evaluated to determine the extract that has the best anti-radical activity.

Methodology and Results: Aristolochia albida Duch. is a medicinal plant from the Aristolochiaceae family, widely used in traditional medicine in Benin. In this work ethanolic, hydroethanolic and aqueous extract were prepared from the leaves of this plant. The quantitative estimation of total phenols, tannins and flavonoids by the colorimetric method showed that the extracts are rich in these compounds. Evaluation of antioxidant power was performed using the method of DPPH free radical trapping. The result indicated that The ethanolic extract of *Aristolochia albida* (IC50 = 0.23 mg/ml, IC50 = Concentration inhibiting 50% of reaction) showed more antioxidant and anti-radical capacity compared to hydroethanolic extract (IC50 = 0, 62 mg/ml) and the aqueous extract (IC50 = 0. 65 mg/ml).

Conclusion and application of results: Overall, there is a correlation between anti-radical powers and phenolic contents phytoconstituents (polyphenols, flavonoids, tannins) extracts of the plant studied.

KEYWORDS: *Aristolochia albida* Duch, Medicinal plant, total phenols, flavonoids, tannins, DPPH, antioxidant activity

INTRODUCTION

Medicinal plants are an inexhaustible source of bioactive natural compounds and are largely untapped in consideration of the number of species of higher plants (angiosperms and gymnosperms) in the world estimated at 250,000 (Kong *et al*., 2003). Indeed, of those, only 6% were tested for biological activity and 15% were evaluated on the phytochemical plane (Verpoorte 2000). The

Beninese flora, of which approximately three thousand species have been recorded (Akoègninou *et al*., 2006), appears as an emergency exit in the treatment of specific diseases endemic to tropical countries. In addition, the use of synthetic antioxidant molecules is currently questioned because of potential toxicological risks. Now, new plant sources of natural antioxidants are sought (Suhaj *et al*., 2006 Tadhani *et al*., 2007). Indeed, polyphenols are widespread natural compounds in vegetal kingdom that are increasingly important thanks to their beneficial effects on health (Koechlin. Ramanotxo *et al*., 2006). The role of natural antioxidants attracting more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular disease (Varban *et al*., 2009). They are also used as additives in food, pharmaceutical and cosmetics (Suhaj *et al*., 2006).Scientific research has been developed for the extraction, identification and quantification of these compounds from different sources, such as agricultural and horticultural crops or medicinal plants (Sanchez-Moreno, 2002; Huang *et al*., 2005). This study focuses on *Aristolochia albida* Duch. *(Dutchman's* pipe), a plant of the Beninese pharmacopoeia and is part of the search and recovery of biologically active substances such as natural substances with biological activity of interest in the field of biopharmaceuticals. The main objective of this work is to make the phytochemical screening to evaluate the phenolic composition and anti-radical activity of various extracts of *Aristolochia albida* Duch. that will explain his therapeutic effects. It will characterize the chemical groups, to identify the best solvent extraction of total polyphenols, flavonoids and condensed tannins by testing and modifying the extraction solvents and evaluate in vitro antioxidant activity of aqueous, hydroethanolic, ethanolic extracts from *Aristolochia albida* trapping method according to the free radical DPPH.

METHODOLOGY

Vegetal material: The leaves of *Aristolochia albida* Duch. were used in this study. They were ground in a blender to obtain a powder, which is subsequently stored in desiccators until use.

Chemical reagents: The reagents used were: hydrochloric acid, Meyer's reagent, ammonia 5%, chloroform Ether, ferric chloride 1%, reagent of STIASNY, sodium Acetate, powder magnesium, hydrochloric alcohol, chloroform, ethyl alcohol, lead Acetate, aqueous Solution of sodium phosphate, anhydrous sodium sulphate, acetic acid, sulphuric acid, the Folin-Ciocalteu reagent, Gallic acid, $AlCl_3$ (aluminium trichloride), catechin, the solvent is ethanol.

Methods

Phytochemical screening: The phytochemical screening was based on the characterization of reactions (coloration and precipitation) differentials of major chemical compounds of groups contained in the plants according to the method of HOUGHTON and Raman (1998). This analysis has different physico-chemical characterization of reactions of chemical groups, which are summarized in the following table.

Preparation of hydroethanolic and ethanolic extract The previously dried and ground leaves are macerated in ethanol and ethanol/water mixture (5:5 V/V), under gentle agitation for one night at room temperature) forming a decoction. Hydroethanolic and ethanol extract is recovered in each case after filtration using a paper filter; ethanol is eliminated from the filtrate by evaporation under reduced in a rota-evapour pressure.

Preparation of aqueous extract: A quantity of 50 g of the powder of the leaves is soaked in 250 ml of water at room temperature overnight. It also conducts a decoction of this powder in water. The aqueous extract is recovered initially after filtration of the mixture with a paper filter allowing obtaining a relevant extract as the crude extract.

Determination of polyphenols

Phenols totals: 1 ml of Folin reagent (10-fold diluted) is added to 200 µl of sample or standard (prepared in methanol) with suitable dilutions. After 4 min, 800 µl of sodium carbonate solution (75 mg/ml) are added to the reaction medium. After 2 h of incubation at room temperature, the absorbance is measured at 760 nm. The concentration of total polyphenols is calculated from the regression equation of the calibration range with Gallic acid and it is expressed as mg of equivalent of Gallic acid per gram of extract (mg EAG/g of extract).

Flavonoids: The method of aluminium trichloride is used to quantify the flavonoïdes in extracts of *Aristolochia albida*. 1 ml of the solution of $AlCl_3$ (2% in methanol) is added to 1 ml of sample or standard (prepared in methanol). After 10 minutes of reaction, absorbance is

read at 415 nm. The concentration of flavonoids is derived from a range of calibration with the catechin and is expressed in milligrams of catechins equivalent per gram of extract (mgEC/g of extract).

Condensed tannins: Condensed tannins are determined by the method of vanillin in acidic medium described by Ba *et al*. (2010). Vanillin reagent was prepared by mixing equal volume: 8%, methanol at 37% and 4% of vanillin in methanol. The mixture was maintained at 30 ° C before the assay. Two hundred (200) µl of each extract to be analyzed were added to 1 000 µl of reagent of vanillin; the mixture was stirred and incubated in darkness at 30 ° C for 20 min. The absorbance was measured at 500 nm by a spectrophotometer UV (Perkin Elmer) against white consisting of a mixture of methanol (37%) and HCl (8%) with equal volume.

Radical scavenging activity: The DPPH radical scavenging capacity (2, 2-diphenyl-1-picrylhydrazyle) was evaluated and. expressed as a percentage by using the following method: I = [(Ab-Ae) /Ab] × 100; I: percentage inhibition; AB: Absorbance of the negative control; AE: Absorbance of the specimen.

Table 1: Summary of specific reactions of each compound class

Compound class	Specific reagents and reactions
Alkaloids	-Dragendroff (potassium iodobismuthate) → Red precipitate - Mayer (potassium iodomercurate) → yellow precipitate
Tannins catechiques Gallic tannins	- stiasny reagent → precipitate pink - Saturation of acetate of Na + a few $FeCl_3$ drops to 1% → dark blue, green or black
Flavonoids	Shinoda (cyaniding reaction) → Coloration: orange (flavones); red (flavonols) or purple (flavanones)
Anthocyanins	red colouring of filtrate increased in acid medium and blue-violet in alkaline medium
Leucoanthocyanin	Shinoda (hydrochloric alcohol) → Cherry Red
Quinone derivatives	Born-Träger (reaction between Quinone cycles in HN_3 medium) → pink to purplish red colouring
Saponosides	Determination of foam index (positive if IM> 100)
triterpenoids steroids Cardenolides	-Liberman-Buchard (sulphuric acid-acetic anhydride) violet colour with blue or green - Kedde (Dinitrobenzoic acid in ethanol + 2% NaOH (1N) → purple red wine stain or wheel) -Dinitrobenzene 1% in ethanol + 20% NaOH blue colour
Cyanogenic derivatives	Gugnard (paper soaked in picric acid) orange to brown colouring)
Mucilages	→ study of the viscosity of the infused or decocted
Reducing compounds	Hot Liqueur of Fehling → brick-red precipitate
coumarin	Ammoniac 25% → intense fluorescence
Anthracenic derivatives	Chloroform + ammoniac → intense red coloration
o-heterosides	Hydrolyzed + $FeCl_3$ + Chloroform + ammoniac → red colour
C-heterosides	Aqueous phase + $FeCl_3$ + Chloroform + ammoniac → red colour

RESULTS AND DISCUSSION

Phytochemical Screening: The phytochemical studies highlight the richness of polyphenolic compounds in the leaves of *Aristolochia albida* Duch. (flavonoids, tannins catechiques, anthocyanins, leuconthocyanes, steroids, triterpernoides, mucilage, coumarins) and reducing compounds (table 1). Compounds such as tannins Gallic,

Quinone derivatives, cardenolides, saponins, glycosides, Antraceniques free O. glycosides, C. glycosides were absent in the leaves of *Aristolochia albida* Duch.). Triterpenes have hepatoprotective properties (German *et al*., 2001) and may fight inflammation (Ahmed, 2008). Flavonoids are known for their hepatoprotective activities (Krishna al. 2010). The tannins have power of protein binding with a tendency to the impermeability of the outer layers and the protection of the underlying layers and properties of renewal of tissues; Indeed the Polyphenolic compounds are substances known for their antioxidant property (Yètè *et al*. 2014). Antioxidant and hepatoprotective properties of *Aristolochia albida* Duch. may therefore be mainly due to the presence of flavonoids with a synergy of action of these various polyphenolic compounds.

Table 2: Results of the reactions of characterization

secondary metabolites		***Arisholochia Albida***
Alkaloids		-
Tannins	Cathechic	+
	Gallic	-
Flavonoids		+
Anthocyanins		+
Leucoanthocyanins		+
Quinone derivatives		-
Steroids		+
triterpernoides		+
cardenolides		-
Saponosides		-
cyanogenic		-
Mucilages		+
Coumarins		+
Reducing compounds		+
Free Antraceniques		-
O. Heterosides		-
C. Heterosides		-

+ Metabolite Presence - Absence metabolite

Performance of Extraction: Figure 1 shows the yields of aqueous, hydroethanolic and ethanolic extracts of *Aristolochia albida* Duch. by decoction and maceration. Ethanolic and hydrethanolic extracts gave the highest yields of extraction respectively by maceration and decoction. Ethanol and ethanol binary-water favored the extraction of metabolites in leaves of *Aristolochia albida* Duch.

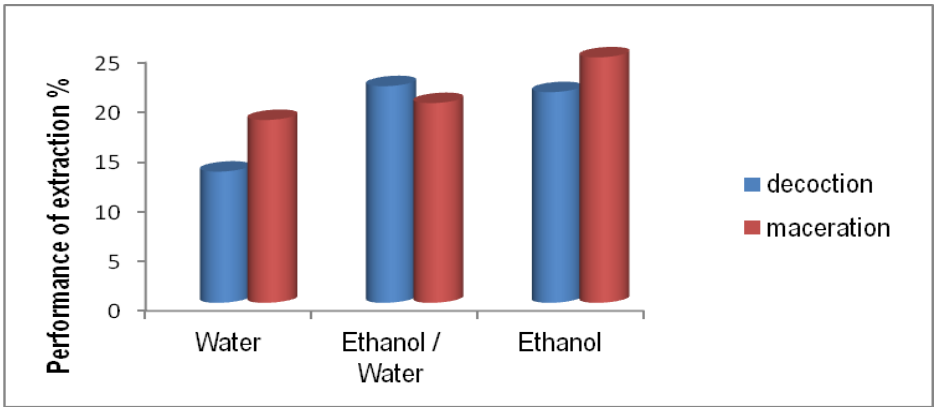


Figure 1: Yields of different extracts of *Aristolochia albida* Duch. by decoction and maceration

Spectrophotometric quantification of total polyphenols, totals flavonoids, tannins condensed in crude extracts of *Aristolochia albida* Duch.

Total polyphenols content: The total polyphenols content obtained by two extraction methods are summarized in table 3 and represented on figure 3. The phenolic content is expressed in milligram equivalent of Gallic acid per gram of dry matter (mgEAG/gMS). The values represent averages of three measures ± standard deviation. The amount of total phenolics would have varied considerably across the various extracts and spread of 0.94 to1.50 mgEAG/gMS by decoction and 1.11 to 1.88 mgEAG/gMS by maceration. Maceration seems to be the best method for extraction of total polyphenols or in average 1.48 against 1.25 mgEAG/gMS for the decoction. Extraction of polyphenols by maceration, although usually long, requires organic solvents that are expensive and sometimes dangerous to health (García *et al*., 2010). The values of total phenols in different extracts are comparable to those obtained with the dates considered rich in phenolic compounds, 5660 µg/g (Cristina *et al*., 2009), and those of the Grape seed, 7500 to 40400 µg/g (Beckman *et al*., 2000). These results are similar to those of Yété *et al*. 2015 making a comparative study of phenolic compounds from extracts of the seeds of *Garcinia kola* (Guttifferaea) and *Cucumeropsis edulis* (Cucurbitaceae) of Benin. Total phenolics are secondary metabolites synthesised by plants during their development but also as a response to stress conditions such as infections, injuries, radiation UV (Laurence *et al*., 2008). The therapeutic action of these on health and their notable content in plants would justify their many traditional uses (Ahmed *et al*.,) 2008. The gallic acid calibration curve is shown on Figure 2.

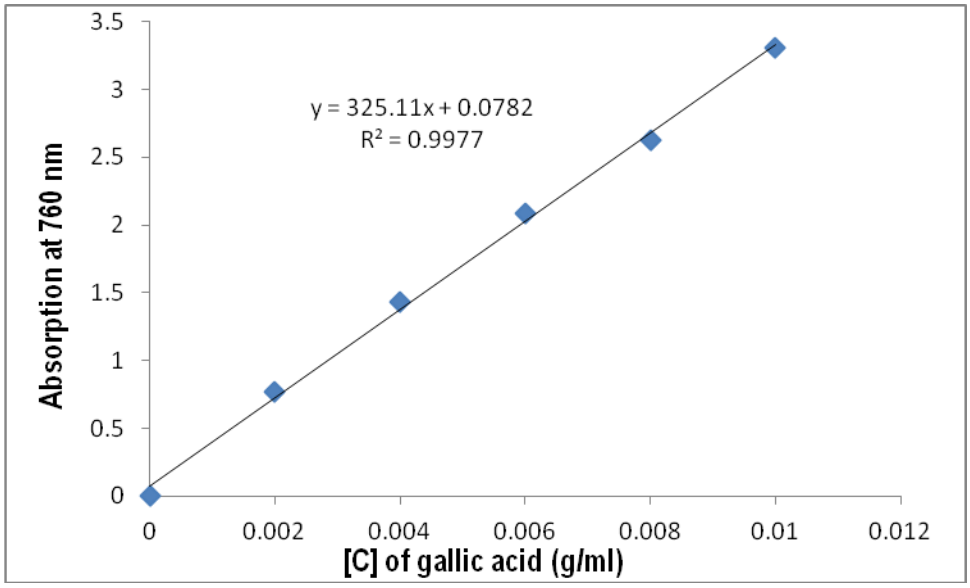


Figure 2: Calibration curve of Gallic acid

Table 3: Content of total polyphenols in different extracts of leaves of *Aristolochia albida* Duch. by decoction and maceration (EAG mg / gMS).

Plant	**extraction Solvent**	**Content of total polyphenols** (mg EAG/g MS)	
		decoction	maceration
Aristolochia alida	Water	**0,94 ± 0,00**	**1,45 ± 0,04**
	Ethanol / Water	**1,31 ± 0,01**	**1,11 ± 0,01**
	Ethanol	**1,50 ± 0,02**	**1,88 ± 0,00**

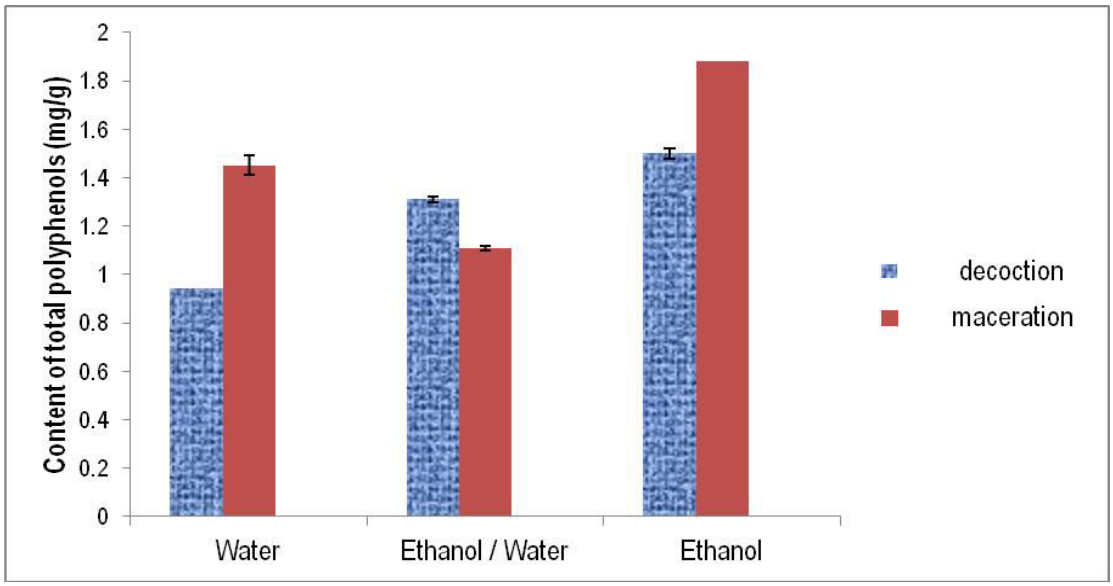


Figure 3: Determination of total phenols

Flavonoids content: The levels of flavonoids of different extracts of the decoctes and macerates of leaves of Aristolochia albida are summarized in table 4, shown in figure 5. The flavonoids content is expressed in equivalent mg of catechins per gram of dry matter (mgEC/gMS). The values represent averages of three measures ± standard deviation. The Analysis of these results shows that the amount of total phenolics would have varied considerably across the various extracts and spread of 0.94 to 1.59 mgEC/gMS by decoction with 1.09 to 1.91 mgEC/gMS by maceration. Maceration is preferable to extract flavonoids with an average of 1.37 against 1.09 mgEC/gMS for the decoction. Moreover, previous work has shown that flavonoids are present almost in all organs of the plants and may be recognized as pigments responsible for the therapeutic properties of these (De oliveira, 1972). Catechin calibration curve is shown in Figure 4.

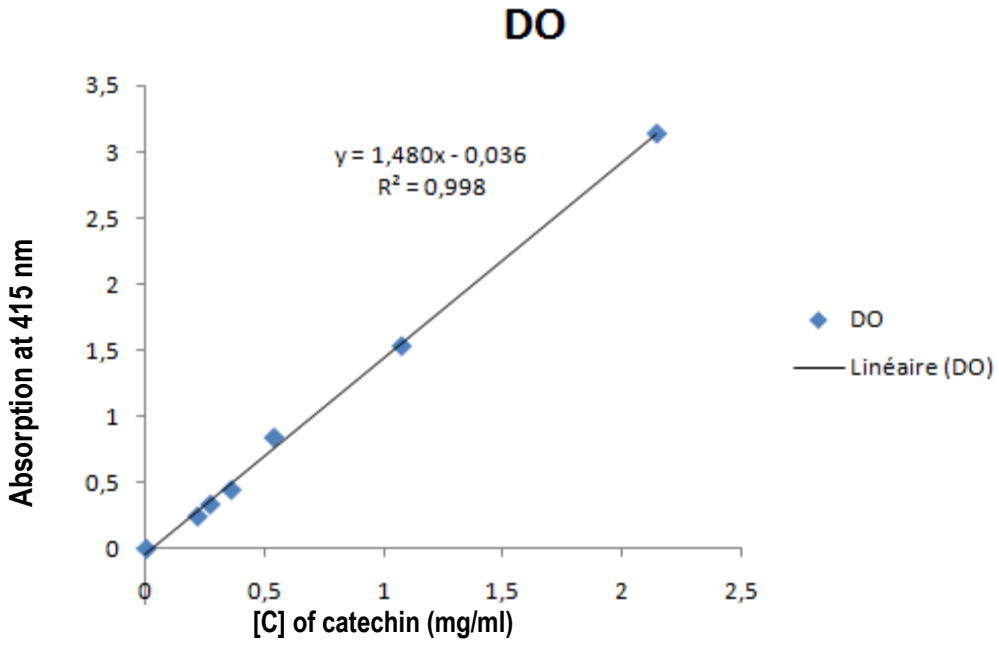


Figure 4: The calibration curve catechin

Table 4: flavonoids content in different extracts of leaves of *Aristolochia albida* by decoction and maceration (MgEC / gMS).

Plant	Extraction Solvent	Content of Flavonoids (mgEC/g MS)	
		decoction	maceration
Aristolochia alida	Water	0.94±0,01	1,09±0,01
	Ethanol / Water	0.98±0,03	1,12±0,02
	Ethanol	1.59±0,02	1,91±0,00

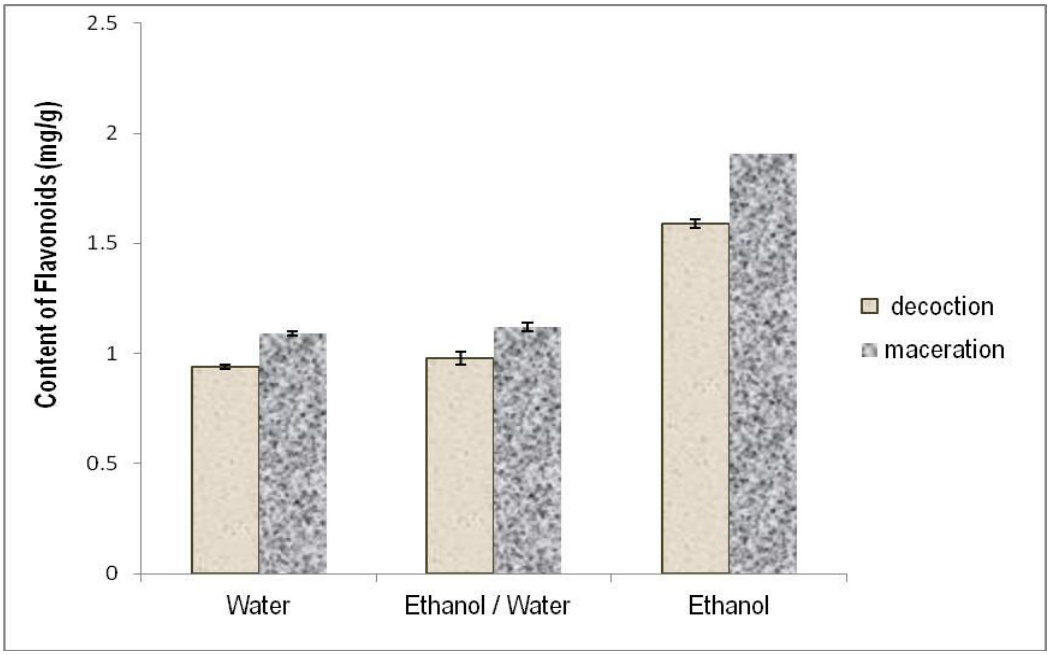


Figure 5: Determination of Flavonoids

Content of condensed tannins: The tannins content condensed from various extracts of the decoctes and macerates leaves of *Aristolochia albida* Duch. are summarized in table 5 and represented in figure 6. The condensed tannin content is expressed in equivalent mg of catechins per gram of dry matter (mgEC/gMS). Analysis of the results of the content condensed tannins contained in table 4 reveals that the decoction is more efficient for the extraction of tannins (0.34 mgEC/gMS on average) than maceration (0.24 mgEC/gMS on average). The increase in temperature on the one hand promotes the diffusion and solubility of substances extracted, on the other hand it destroys substances fragile (Leo *et al*.) 2010. This increase in the levels of tannins condensed in the decoctes can be explained by the destruction by heat of polyphenol oxidase (PPO) falling the polyphenol content; Thus, the breaking of bonds between polyphenols and other substances (proteins, polysaccharides...) leading to the accessibility of these active principles can explain this abundance (Lutz *et al*., 2011). Water recorded the highest levels of condensed tannins, regardless of the mode of extraction or 0.43 mgEC/gMS by decoction and 0.34 mgEC/gMS by maceration. However, ethanol extracted a little low tannin (0.25 mgEC/gMS by decoction) and 0.17 mgEC/gMS by maceration. However, the problem according to Rosales and al., 1999 and Leo *et al*. 2010 is that water, especially at high temperatures, extracted also undesirable substances such as proteins, lipids and non-phenolic dyes that cause interference during determination of tannins. Extraction of condensed tannins depends on the chemical nature of the solvent used and operating conditions (Chavan *et al*..., 2001).

Table 5: Condensed tannins content in different extracts of leaves of *Aristolochia albida* Duch. by decoction and maceration (mg EC / gMS).

Plant	Extraction Solvent	Condensed tannins content (mgEC/g MS)	
		decoction	maceration
Aristolochia alida Duch.	Water	0,43 ±0,05	0,34 ±0,04
	Ethanol / Water	0,35 ±0,00	0,21 ±0,01
	Ethanol	0,25 ±0,03	0,17 ±0,00

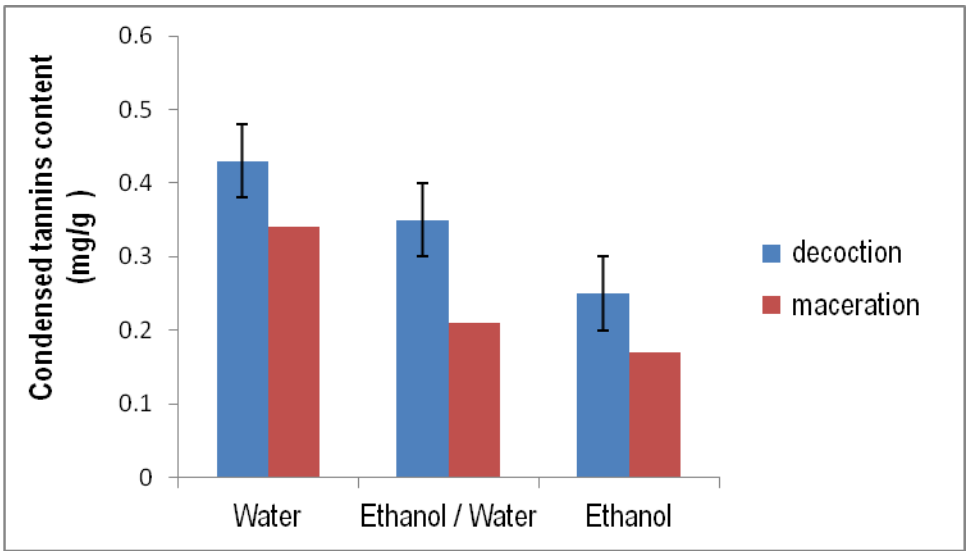


Figure 6: Determination of condensed tannins

Radical scavenging activity of extracts from leaves of *Aristolochia albida* Duch.:

Tests, in vitro the DPPH free radical : The antioxidant aqueous extracts hydroethanolic, ethanolic activity of *Aristolochia albida* and standard antioxidants (quercetin, BHA, Gallic acid) towards the DPPH radical was evaluated spectrophotometrically by following the reduction of this radical which is accompanied by its passage of the purple colour (DPPH•) to the colour yellow (DPPH-H) measurable to 517nm (Figure 7). This capacity reduction is determined by a decrease in absorbance induced anti-radical substances.

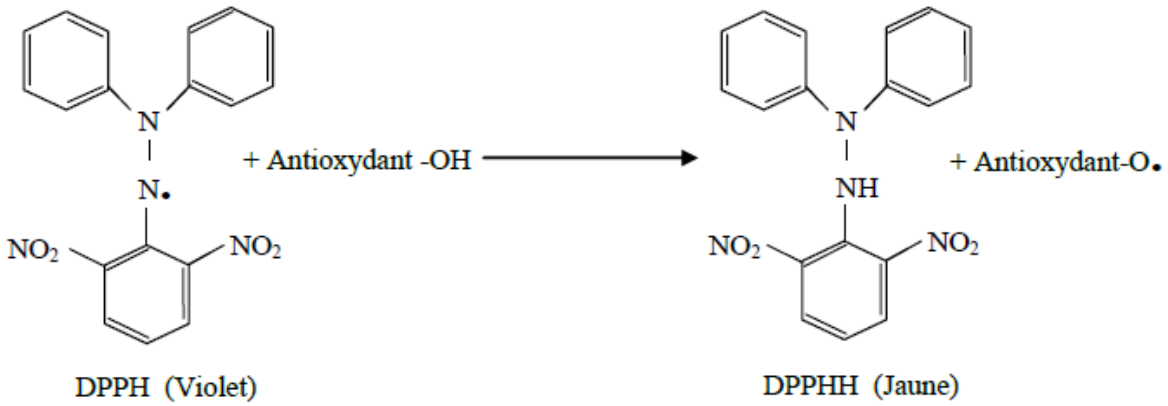
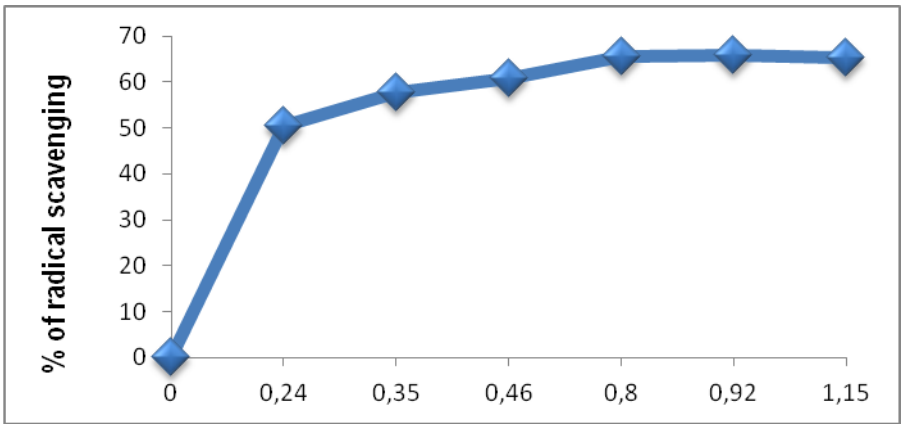
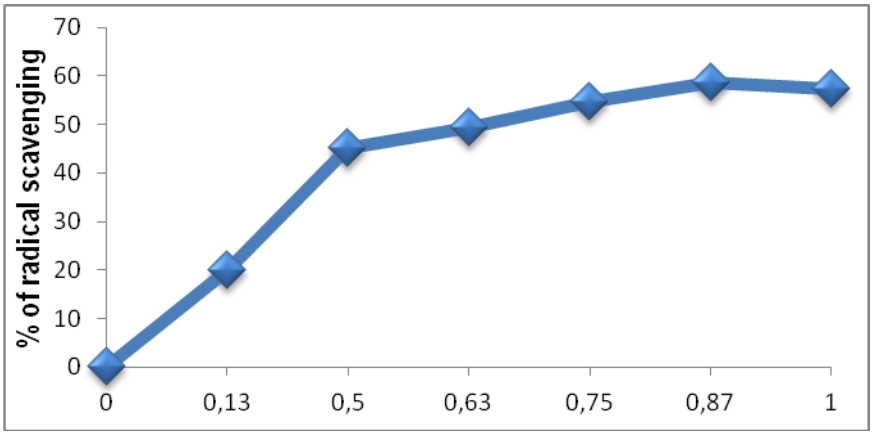


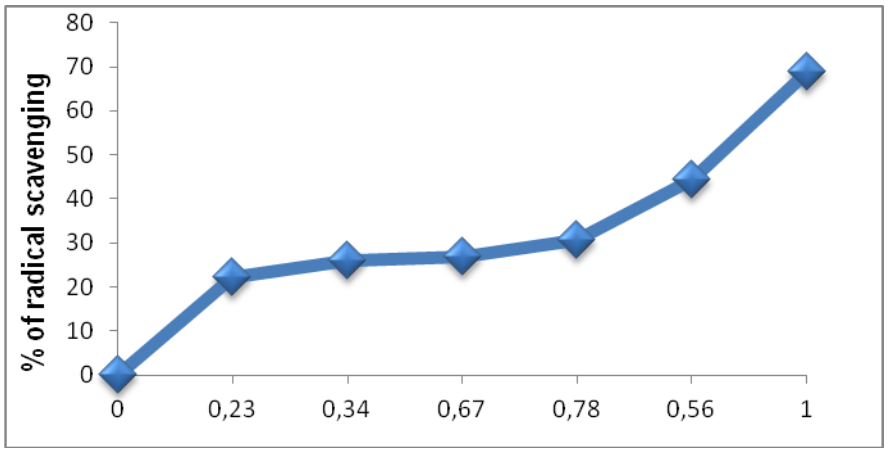
Figure7: reaction of an antioxidant with the DPPH radical



Ethanolic extract



Hydroethanolic extract



Aqueous extract

Figure 8: Antioxidant activity of the extracts

The rate of free radical scavenging depending on the concentrations of the extracts of *Aristolochia albida* Duch. is indicated by the curves of figure 8. The ethanolic extract of *Aristolochia albida* (IC50 = 0.23 mg/ml, IC50 = Concentration inhibiting 50% of reaction) manifest the greatest anti-radical capacity compared to extract hydroethanolic (IC50 = 0, 62 mg/ml) and the aqueous extract (IC50 = 0. 65 mg/ml). This great anti-radical capacity is due to the significant presence of phenolic compounds identified by phytochemical screening. Polyphenols contained in extracts of *Nigella sativa* are probably responsible for the antioxidant activity of these extracts as the antioxidant activity of ethanolic extract is always greater than that of the aqueous extract (Fabienne, 2013). The same studies show that *Nigella sativa* is a species rich in phenolic compounds, which are responsible for many biological activities including the antioxidant, anticancer and antimicrobial activity (Fabienne *et al*., 2013). The Comparison of each extract IC50 to that three references compounds show that the radical-scavenging activity of ethanolic extracts (IC50 = 0.23 mg/ml), hydroethanolic (IC50 = 0, 62 mg/ml) and aqueous (IC50 = 0. 65 mg/ml) is less important than that of quercetin (IC50 = 3µg/ml), the BHA (IC50 = 4, 8µg/ml) and Gallic acid (IC50 = 0 9µg/ml) used as standard. It has been shown that the antioxidant molecules such as Ascorbic acid, flavonoids and tannins reduce and discoloured DPPH due to their ability to yield hydrogen, (De Pooter *et al*. 1986). Whatever the nature of the radical-scavenging power of our plant extracts, it is to see that there is a correlation between the anti-radical powers and levels of phenolic phytoconstituants (polyphenols, flavonoids, anthocyanins, tannins) as eloquently attest to this work (Waghorn, 2013; Garg *et al* 2016).

CONCLUSION

The polyphenolic compounds extraction is a crucial step for the recovery of active ingredients, it depends on the method used and the appropriate solvent that preserve their biological properties. Of this study, it appears that the maceration by ethanol is the best technique for the extraction of total polyphenols and flavonoids while aqueous decoction is preferable for extraction of condensed tannins. The results of this study indicate that the recurring use of the species *Aristolochia albida* Duch. in alternative medicine in the treatment of liver diseases, especially hepatitis in Benin may be justified partly by its radical-scavenging activity, which depends on its relative wealth of Polyphenolic constituents.

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