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VARIABILITY OF CHEMICAL COMPOSITION OF *NEWBOULDIA LAEVIS* AND *ZANTHOXYLUM ZANTHOXYLOIDES* RELATED TO ENVIRONMENTAL FACTORS

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ABSTRACT

N. laevis and *Z. zanthoxyloides* leaves from departments of Atlantic, Oueme, Couffo and Zou Area of South Benin were screened for the presence of chemical compounds by standard methods and compared. The results revealed the presence of the following compounds: alkaloids, tannins, flavonoids, quinones, and essential oils in the plant samples regardless of the area and the harvest season. Saponins, cardiac glycosides, cyanogenic glycosides were found to be absent in the leaves of *Z. zanthoxyloides* and *N. laevis* regardless of the area and the harvest season. The significance of the plants in traditional medicine and the importance of the distribution of the chemical constituents justified the traditional use of these plants for the treatment of tropical diseases. We recommend further research work on these plants leaves for possible use as a source of raw material for industrial and medical science utilization.

Keywords : Chemical Composition, *Newbouldia laevis*, *Zanthoxylum zanthoxyloides,* environmental factors, Benin

VARIABILITÉ LIÉE AUX FACTEURS ENVIRONNEMENTAUX SUR LA COMPOSITION CHIMIQUE DE *NEWBOULDIA LAEVIS* et *ZANTHOXYLUM ZANTHOXYLOIDES*

RÉSUMÉ

La composition chimique des feuilles de *Newbouldia laevis* et de *Zanthoxylum zanthoxyloïdes* des départements de l'Atlantique, de l'Ouemé, du Couffo et du Zou du sud du Bénin a été déterminée par des méthodes standard. Les résultats de cette étude comparée, ont montré que *N. laevis* et *Z. zanthoxyloïdes,* deux plantes tropicales étudiées, renferment des substances appartenant globalement aux mêmes familles de composés chimiques telles que : les alcaloïdes, les tannins, les flavonoïdes, les quinones et les huiles essentielles, dans les différentes zones agro écologiques quelles que soient la saison et la zone de récolte. Les cardénolides, les glycosides *cyanogéniques*, les anthracéniques libres, les O- hétérosides et les hétérosides cardiotoniques ne sont pas présents dans la poudre des feuilles des deux plantes tropicales étudiées. Les mucilages sont également présents dans la poudre de *Z. zanthoxyloïdes* et absents dans la poudre de *N. laevis* alors que les dérivés quinoniques sont présents dans la poudre de *N. laevis* et absents

dans la poudre de *Z. zanthoxyloïdes*. La présence des alcaloïdes, des tannins, des flavonoïdes, des quinones et des huiles essentielles quelle que soient la zone et la saison de récolte justifierait l'utilisation de ces deux plantes en médecine traditionnelle pour traiter de nombreuses pathologies. Des recherches complémentaires sont nécessaires pour purifier les composés chimiques présents dans les deux plantes étudiées (*Z. zanthoxyloïdes* et *N. laevis*) en relation avec les facteurs environnementaux.

Mots-clés : Composition Chimique, *Newbouldia laevis*, *Zanthoxylum zanthoxyloïdes*, facteurs environnementaux, Bénin

INTRODUCTION

Nearly 80 % of natural substances used in the biomedical field come from tropical plants Campa (2005). This is the case of vincristin and vinblastin, two dimeric alkaloids sought after for their anti-tumor activity. Extracted from *Catharanthus roseus*, periwinkle from Madagascar, these compounds are very weakly accumulated in the plant (values less than 0.001 % of dry matter). Also, large crops are needed (50 tons per year) for the active ingredient essential to the therapeutic needs worldwide. In addition, the level of production of secondary metabolites varies with the organ, individuals, places of harvest or culture conditions, climatic factors but also with stage of plant development Manolaraki (2011).

However, the plant related to the content and nature of plant secondary metabolites is probably one of the main sources of the variation. Based on the bibliography the quantity and the nature of tannins as well as other secondary metabolites, depend not only on the plant species, the variety (cultivar), the stage of development but also on the environmental conditions of culture (soil, season, climate, mode of culture) and the mode of conservation Marais *et al.* (2000) ; Mueller-Harvey (2006) ; Heckendorn *et al.* (2006) ; Haring (2007) ; Manolaraki (2011). The secondary metabolites content of a plant depends on several intrinsic factors, such the species and variety, plant part or stage, and extrinsic factors, such as climatic, soil, or the stress of predation Mueller-Harvey & Mark Allan (1992) ; Jean-Blain (1998) ; Norton (1999) ; Waterman (1999) ; Manolaraki (2011).

Man has earlier discovered within his environment the wealth of importance of plants as therapeutic agents. Of the 300.000 plant species acclaimed world wide only about 5 % have been investigated scientifically for their medicinal purposes Sanusi & Rabo (2004) ; Olusola (2011). Researchers have reported that developing countries rely mainly on plants for the treatment of their prevailing ailments especially in areas where hospitals are not accessible Olusola (2011). In industrialized countries it is known that over 30 % of all prescription drugs are from plant origin Iwu *et al.* (1999).

The genus *Newbouldia* (Bignoniaceae) comprises a unique species *Newbouldia laevis*, which occurs in several tropical zones of America, Central

and West Africa. *Newbouldia laevis*, also called «fetish tree» can grow to a height of about 12 m but is most often a shrub or a bush Tra-Bi (1997).

The genus *Zanthoxylum* (Rutaceae) comprises a large number of species, which occur in several tropical and temperate zones of America, Africa and Asia. In Africa, many species are used in folk medicine. *Zanthoxylum zanthoxyloides* is a scandent shrub or a small tree; it has a fragrant bark and grows in coastal areas of West Africa Guffin *et al.* (2000). The tree branches are adorned with numerous spines.

N. laevis and *Z. zanthoxyloïdes* are extensively used in herbal medicine in South Benin. Their various uses in traditional medicine are reviewed in Table 1. The active principles of many drugs found in plants are secondary metabolites Ghani (1990) ; Doelis (1993). Therefore, basic phytochemical investigation of these plants for major phytoconstituents is also vital.

This research was aimed at investigating the possible variability related to environmental factors (Origin and season of harvest) of the chemical composition by quantifying the percentage of crude phytochemical constituents in *N. laevis* and *Z. zanthoxyloides*, in order to support or refute the claims by traditional herbalists in Benin. Benin is divided into twelve departments which are: Alibori, Atacora, Atlantic, Borgou, Collines, Couffo, Donga, Littoral, Mono, Oueme, Plateau and Zou. Benin, like all of Africa except North Africa and some countries of South Africa is in an area of tropical climate characterized by high temperatures and more or less regular rainfall. In the departments of Oueme, Atlantic, Zou and Mono, soils are ferralitic. The vegetation is staggered and degrades when moving from south to north. These environmental factors can influence the chemical composition of medicinal plants from one region to another. Work reported in the South of Benin, in the departments of Atlantic, Oueme, Mono and Zou young plants of *N. laevis* and *Z. zanthoxyloides* are abundant and dendrometric characters are significant. These plants are not found in other departments in the North Benin Orou *et al.* (2009). The departments targeted for this study are: Atlantic, Couffo, Oueme and Zou.

Zou has a transition sub-equatorial climate, characterized by two rainy seasons (April to June and September-November) and two dry seasons (July-August and December-March). The vegetation is also a very important variable of the environment. Vegetation of equatorial Sudanian type consists of trees with coriaceous, varnishing leaves resistant to heat. Alternating dry season and wet season causes a phrenological rate in plants. The soil is made of “terre de barre” (laterite) and appears as the result of an intense and deep

weathering. It consists of a vast homogeneous sandy clay plateau. Almost everywhere, the ground shows a physical homogeneity. Of agronomic, it is low in organic matter, and is characterized by its great depth and high permeability.

Mono is characterized by a sub-equatorial climate with two (02) dry seasons (July to September and November to February or March) and two (02) rainy seasons, one short (September to November) and the other more long (April-July). The Aplahouecity has a variety of soils. The substrate is mainly composed of sediments of the Terminal Continental. These sediments are generally sandy clay.

The humid tropical climate of Oueme is called sub-equatorial climate. This climate is characterized by high humidity and temperatures between 21.9° C and 32.8° C. This department has three types of soil: ferralitic soils, lightbrown colored soils, and hydromorphic clay soils rich in organic matter.

The climate of the Atlantic department is sub-equatorial marked by two rainy seasons and two dry seasons. Most of the territory of the Atlantic department is occupied by tropical ferruginous soils and sandy soils.

Table 1. Review of the various medicinal uses of the studied plants

Species, Family, Local plant name	Traditional use	Reference
Z. zanthoxyloides Rutaceae Fon-Goun : Hê Yoruba-Nagot : Iguiata	It has been commonly used against human and animal parasites	Arbonier (2004)
	Leaves have been used as tea against inflammatory diseases, anaemia, contraceptive and against malaria.	Ogunwolu *et al.* (1996); Ogwal-Okeng (2003); Chaaïb (2004); Igoli *et al.* (2005)
	Roots and stems have been used to treat jaundice, sore throat, hemorrhoids, gastroenteritis, dysentery, gonorrhea and as vermifuge	Malgras (1992); Chaaïb (2004); Mass (1991) ; Hounzangbé-Adoté (2004)
	Root bark and leaves are used as anti-odontalgic , to treat stomatitis, gingivitis, dental caries and for tooth friction	Malgras (1992); Chaaïb (2004)
	Diarrhoea, Gastrointestinal disorders	Roig (1988); Hounzangbé-Adoté (2000)
	antifungal	Diéguez-Hurtado *et al.* (2003)
N. laevis	Leaves are used against cough, infertility,	Burkill (1985); Adjanahoun *et al.* (1991); Igoli *et al.* (2002); Igoli *et al.* (2003); Tor-Anyiin *et al.* (2003)
	Treatement of diarrhoea, icterus, malaria Remedy for dysentery,	Burkill (1985) ; Gbeassor *et al.* (1990) ; Tra-Bi (1997)
	Employed against sexually transmitted disease, dental caries, arthritis pain, gastroenteritis, dysentery and as vermifuge	Ayensu (1978); Abbiw (1990); Mass (1991); Hounzangbé-Adoté (2004); Eyong *et al.* (2005)
	Sedative in rats , antifungal , antibacterial properties, in the fight against	Gafner *et al.* (1996) ; Amos *et al.* (2002)

Species, Family, Local plant name	Traditional use	Reference
Bignoniaceae Goun-Mina: Kpatima, Desretin Yoruba-Nagot: IgiAkôkô Tcha de Bantè: Akôkô Adja: Desre, Aflama, Avenyon Waci: Hwenma Bariba: Deebu Kotafon: Desre	trypanosomiasis	
	Hepatoprotective, antioxidantproperties	Hassan *et al.* (2010)
	Treatment of breast cancers	Burkill (1985) ; Azuine *et al.* (1995)
	Gastro-intestinaldisorders	Hounzangbé-Adoté (2000)
	Antibacterial, antineoplasticactivities	Ogunlana & Ramstard (1975) ; Le Grand *et al.* (1988)
	Exudates from scrapings of inner root bark squeezed onto wound dressing and circumcision	Burkill (1985) ; Adjanahoun *et al.* (1991) ; Igoli *et al.* (2002) ; Tor-Anyiin *et al.* (2003) ; Igoli *et al.* (2003)

MATERIAL AND METHODS

Collection and identification of Plant materials

Fresh sample of *N. laevis* and *Z. zanthoxyloides* were collected from departments of Atlantic, Oueme, Couffo and Zou from South of Benin and verification of identity was done by Professor Akpovi AKOEGNINOU, Curator of the National Herbarium of Abomey-Calavi University. Classification of the species was performed by means of the key according to Cronquist (1988). Voucher specimens were deposited at the Herbarium of Abomey-Calavi University. Plants materials were harvested during rainy season and dry season. The Plants were dried indoors at room temperature and a large part is reduced into powder for extraction.

Plant extracts preparation

The aqueous extract of each sample was prepared by soaking 50 g of dried powdered samples in 500 mL of distilled water for 24 h. The extracts were filtered using Whatman filter papers No 1001 125 (125 mm).

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Harborne (1973) ; Sofowara (1993) ; Williamson *et al.* (1996) ; Trease & Evans (2002) et Banso & Ngbede (2006).

Test for tannins: About 0.5 g of dried sample powder was boiled in 20 mL of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added, the mixture was observed for brownish green or a blue-black coloration.

Test for alkaloids : 3 g of extract was stirred with ethanol containing 3% tartaric acid. The filtrate was shared into 3 beakers and tested for alkaloids as follows: Into the first beaker, Hagar's reagent was added; into the second beaker, Mayer's reagent was added; and into the third beaker Marquin's reagent was added. Precipitations in any of the 3 tests indicate the presence of alkaloid.

Test for saponins : About 2 g of sample powder was boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample Harbrone (1973) ; Sofowara (1993). 5 mL of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminum solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the plant powder was in each case heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

Test for steroids : 2 mL of acetic anhydride was added to 0.5 g ethanol extract of each sample with 2 mL H_2S0_4. The color changed from violet to blue or green indicating the presence of steroids.

Test for cardiac glycosides (Keller-Killani test) : 5 mL of each extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid (H_2SO_4). A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer

Test for cyanogenic glycosides

5 mL of each extract is placed in an Erlenmeyer flask that we recover immediately with a paper soaked in picric acid. After heating at 95 °C for 15 minutes, a brown coloration indicates the presence of cyanogenic derivatives.

Test for quinones (Borntrager test)

In an Erlenmeyer flask, 2 mL of 5 % hydrochloric acid is mixed with 2 g of each plant powder. 20 mL of chloroform are added to this mixture and the whole is stirred continuously for 24 hours. After the maceration, 5 mL of ammonia was added. A purplish red color indicates a positive response.

Test for volatile oil

Dried leaves of *N. laevis* and seeds of *Z. zanthoxyloides* were hydrodistilled for 4 hours to obtain an essential oil from yellow to green trend by using the Clevenger-type apparatus.

Quantitative determination of the chemical constituents

Preparation of fat free sample: 2 g of the sample were defatted with 100 mL of diethyl ether using a Soxhlet apparatus for 2 h.

Determination of total phenols by spectrophotometric method: The fat free sample was boiled with 50 mL of ether for the extraction of phenolic components for 15 min. 5 mL of the extract was pipetted into a 50 mL flask, then 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development. This was measured at 505 nm.

Alkaloids determination using Harborne (1973) method: 5 g of the sample was weighed into a 250 mL beaker and 200 mL of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue of alkaloids was dried and weighed.

Tannins determination by Van-Burden & Robinson (1981) method

500 mg of the sample was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and the bottle was shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Flavonoids determination by the method of Bohm & Kocipai-Abyazan (1994): 10 g of the plant sample was extracted repeatedly with 100 mL of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 1001 125 (125 mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and to a constant weight.

Volatile oil determination

Dried leaves of *N. laevis* and seeds of *Z. zanthoxyloides* were hydrodistilled for 4 hours to obtain an essential oil from yellow to green trend by using the Clevenger-type apparatus. Their percentage contents were calculated on dry weight basis of the plant material.

RESULTS

The present study carried out on the plant samples revealed the presence of the main classes of secondary metabolites: alkaloids, tannins, flavonoids, quinones, and essential oils. The phytochemical characters of the two medicinal plants investigated are summarized in Tables 2 and 3. As shown by the results reported in Tables 2 and 3, the plants in different areas (Couffo, Oueme, Atlantic and Zou) contain substances belonging to the same families of compounds regardless of the harvest season. Saponins, cardiac glycosides, cyanogenic glycosides were found to be absent in the leaves of *Z. zanthoxyloides* and *N. laevis* regardless of the area and the harvest season. Also note that quinones are present in the leaves of *N. laevis* and absent in those of *Z. zanthoxyloides* regardless of the area and the harvest season.

Table 2. Qualitative phytochemical analysis of the medicinal plants for rainy season

Rainy season	COUFFO (Azove)		OUEME (Adjarra)		ATLANTIC (Abomey-Calavi)		ZOU (Bohicon)	
	Nb	Zz	Nb	Zz	Nb	Zz	Nb	Zz
Tannins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-
Cyanogenic glycosides	-	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-	-	-
Quinones	+	-	+	-	+	-	+	-
Volatile oil	-	-	+	+	+	+	+	+

Key : + = Presence of constituent ; - = Absence of constituent;
Nb : *N. laevis* ; Zz : *Z. zanthoxyloides*

Table 3. Qualitative phytochemical analysis of the medicinal plants for dry season

Dry season	COUFFO (Azove)		OUEME (Adjarra)		ATLANTIC (Abomey-Calavi)		ZOU (Bohicon)	
	Nb	Zz	Nb	Zz	Nb	Zz	Nb	Zz
Tannins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-
Cyanogenic glycosides	-	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+	+	+
Cardiac glycosides	+	-	+	-	+	-	+	-
Quinones	+	-	+	-	+	-	+	-
Volatile oil	-	-	+	+	+	+	+	+

Key: + = Presence of constituent; - = Absence of constituent;
Nb: *N. laevis* ; Zz : *Z. zanthoxyloides*

Table 4 and Table 5 present the results of a quantitative analysis, the percentage of crude chemical constituents in these medicinal plants are estimated for the four areas and for two harvest seasons. Tannins are more concentrated in the leaves of both plants and a concentration of tannins equal to 14.77 % is obtained in the leaves of *Zanthoxylum zanthoxyloides* of Oueme harvested during the rainy season. Volatil oils were obtained in the plants but the yields recorded were low: (0.01-0.52 %) during the dry season and (0.04-0.67 %) during the rainy season.

Table 4. Percentage of crude phenols, tannins, flavonoids, alkaloids and essential oil in the medicinal plants investigated during rainy seasons

Area and season	Rainy seasons					
	Plants	P (%)	T (%)	F (%)	A (%)	Eo (%)
COUFFO	Nb	0.25±0.04	01.00±0.07	0.11±0.10	0.12±0.07	0.00±0.01
	Zz	0.15±0.04	01.14±0.08	0.07±0.06	0.11±0.08	0.00±0.01
OUEME	Nb	0.13±0.05	12.08±0,20	0.70±0.14	3.03±0.05	0,04±0,02
	Zz	0.20±0.02	14.77±0.26	1.47±0.11	3.64±0.30	0.59±0.05
ATLANTIC	Nb	0.06±0.01	10.02±0.19	0.72±0.05	1.24±0.19	0.08±0.02
	Zz	0.27±0.02	12.72±0.28	0.84±0.03	2.24±0.19	0.67±0.15
ZOU	Nb	0.05±0.01	12,03±0,02	0.58±0.02	3.11±0.11	0.05±0.01
	Zz	0.44±0.20	11.75±0.17	0.73±0.07	4.14±0.20	0.63±0.04

Nb : *N. laevis* ; Zz : *Z. zanthoxyloides* ; A : Alkaloids ; F : Flavonoids ; P : Phenols ; T : Tannins ; Eo : Essential oil

Table 5. Percentage of crude phenols, tannins, flavonoids, alkaloids and volatile oil in the medicinal plants investigated during dry seasons

Area and season	Dry seasons					
	Plants	P (%)	T (%)	F (%)	A (%)	Eo (%)
COUFFO	Nb	0.25±0.04	0.13±0.12	0.07±0.11	0.12±0.07	0.00±0.01
	Zz	0.13±0.08	0.01±0.02	0.07±0.11	0.06±0.05	0.00±0.01
OUEME	Nb	0.11±0.02	7.97±0.10	0.67±0.08	2.16±0.07	0.01±0.02
	Zz	0.15±0.02	9.74±0.28	0.79±0.15	2.69±0.31	0.46±0.20
ATLANTIC	Nb	0.08±0.06	6.49±0.08	0.42±0.05	0.24±0.09	0.04±0.02
	Zz	0.22±0.15	10.16±0.33	0.61±0.03	5.37±0.31	0.33±0.01
ZOU	Nb	0.04±0.02	9.97±0.11	0.50±0.05	2.12±0.10	0 .05±0.1
	Zz	0 .44±0.11	8.93±0.28	0.67±0.11	3.38±0.39	0.52±0.08

Nb : *N. laevis* ; Zz : *Z. zanthoxyloides* ; A : Alkaloids ; F : Flavonoids ; P : Phenols ; T : Tannins ; Eo : Essential oil

DISCUSSIONS

The various phytochemical compounds detected are known to have beneficial use in industries and medical sciences Sofowara (1993) ; Ngbede *et al.* (2008).

For many decades, Benin medicine has documented the effectiveness of plant therapies. These plants species have now been categorized according to their medicinal properties. The basic principles in formulation of their remedies have also been developed by these herbal therapy enthusiasts. Also, many of these plant extracts have been employed in formulation of modern drugs, chemicals and food. Therefore, plant extracts have now been used in industries and in medical sciences Ngbede *et al.* (2008). Among the Benin and African herbal therapies, a principal herb denoted as emperor herb is chosen as the main therapeutic herb and minister herbs are selected to assist carrying the treatment to the specific channels and organs. The results of these formulations in many ways have proved a better alternative to some of the modern potentially toxic agents that are used in the treatment of infectious diseases. Most herbalists, especially the ancient ones knew more than we do today, at least about the benefits of using various wild plant species Ngbede *et al.* (2008). Their connection with nature was much more profound.

The classes of compounds found in the leaves of *N. laevis* and *Z. zanthoxyloides* are known to show curative activity against several pathogens (*Strogyloides ratti*, *Haemonchus contortus*, *Strogyloides stercoralis*…) and therefore could explain their traditional use for treatment of wide array of illnesses Hassan *et al.* (2004); Usman *et al.* (2005); Usman & Osuji (2007). Tables 2 and 3 showed that the two plants contain different families of compounds (alkaloids, tannins, flavonoids, quinones, and essential oils), but all the samples show the same results irrespective of the origin and the harvest season. Moreover alkaloids Diéguez-Hurtado *et al.* (2003) ; Tringali (2001) ; Couillerot *et al.* (1994), flavonoids, terpenoids and coumarins Mara *et*

al. (1992) were isolated from different species of the genus *Zanthoxylum*. According to Kerharo & Adam (1994), samples of *Fagara zanthoxyloides* collected in West Africa (Senegal, Togo, Ivory Coast and Nigeria) have revealed the presence of tertiary and quaternary alkaloids and a flavone glycoside in the leaves. Traces of tannins in *Z. zanthoxyloides* were reported by Paris & Moyse-Mignon (1947). So far no one has quantified the tannins in the leaves of *N. laevis* and *Zanthoxylum zanthoxyloides*. Tables 4 and 5 are interesting; the results show a fluctuation in the chemical composition, by region and by season. *Zanthoxylum* and *Newbouldia* harvested in Oueme contain more tannins than plants collected in other regions and especially in the rainy seasons, therefore according to the effect that one seeks, if the effect is due to the action of tannins, the plants harvested in Oueme the rainy season will be more active. However variations in results have been observed. For example with regard to the quantity of condensed tannins, the influence of the cultivation location on polyphenol concentration was demonstrated by Tiemann *et al.* (2010). This seemed to be more related to the pH values Kraus *et al.* (2004) than the soil fertilization Tiemann *et al.* (2010). Caygill & Mueller-Harvey (1999) have noticed that the synthesis of tannins in plants is a response to stress conditions, such as low fertility, water deficits or high temperature. The positive effect of a hot dried period on the flavan-3-ols concentrations in plants have also been described in several studies Donelly (1959) ; Lees *et al.* (1994) ; Feutch *et al.* (1997); Vitti *et al.* (2005) ; Assefa *et al.* (2008) ; Manolaraki (2011). The quantity and the nature of tannins as well as other secondary metabolites, depend not only on the plant species, the variety (cultivar), the stage of development but also on the environmental conditions of culture (soil, season, climate, mode of culture) and the mode of conservation Marais *et al.* (2000); Mueller-Harvey (2006) ; Heckendorn *et al.* (2006) ; Haring (2007). In general, the highest concentrations are found in the fruits, flowers and leaves, and the lowest in the stems. For example, the leaves, flowers and stems of sainfoin contain respectively 0.31 %, 0.30 % and 0.07 % of tannin Borreani *et al.* (2003). For a given species, the vegetative stage also influences the content of tannins Jean-Blain (1998). Generally, during the growth of vegetative, a dilution of tannins is observed. In the leaves, the quantity and quality of tannins change during maturation. Similarly, the tannin content generally decreases during fruit ripening. For example, the maturation of oak leaves from April to September is accompanied by changes in the content and an increase in the degree of polymerization of tannins Makkar *et al.* (1991). A similar process was observed in legumes. Thus, the content of sainfoin condensed tannins varies from 27 to 16 g/Kg of dry matter during the growth phase Borreani *et al.* (2003). The quality of condensed tannins varies depending on the vegetative stage Koupi-Abyazani *et al.* (1993), Marais *et al.* (2000). During the maturation of the leaves of sainfoin, variations in the degree of

polymerization (5 to 8.5) and the percentage of prodelphinidols (60 to 95%) were observed Koupi-Abyazani *et al.* (1993). The synthesis of tannins is generally increased in response to stress environmental whatever its origin, such as water stress, depletion of soil or too much sunlight Bennick (2002) ; Manolaraki (2011). In addition, the proportion of condensed tannins in free form or associated with fiber or protein is also under the "influence of climatic conditions and nutritional stress Frutos *et al.* (2002). So after a long period of drought, Feucht *et al.* (1997) observed an increase of 7.4 times of the amount of flavan-3-ols in yellowing leaves, compared to green leaves. Recently, a study on grapes also showed that the content of flavan-3-ols and distribution of TCs in the tissue depended on the exposure to light and weather conditions Cadot *et al.* (2006) ; Manolaraki (2011). The soil also affects the quality of condensed tannins as the grapes grown on sandy soil with little reserve water showed a lower proportion of DPs than those grown on a richer soil Cadot *et al.* (2006). Finally, a stress from the aggression of plants by herbivores or pathogens induces an increased synthesis of secondary metabolites and a large storage of tannins particularly condensed tannins, in the area attacked Woodward & Coppock (1995), Feucht *et al.* (1997) ; Manolaraki (2011). Tannins are inhibitors of enzymes Zimmer & Cordesse (1996). Other work has highlighted the antioxidant activities of tannins Bruneton (1999) ; Treutter & Feucht (1999) ; Hässig *et al.* (1999), Lim *et al.* (2007). Antimutagenic and anticancer activities have been attributed to some tannins because of their antioxidant properties Chung *et al.* (1998) ; Jung & Ellis (2001), Richelle *et al.* (2001). The antiseptic activity of tannins has been widely described Chung *et al.* (1998); Bruneton (1999). Moreover tannins show antimicrobial Chung *et al.* (1998), Hatano *et al.* (2005) ; Song *et al.* (2006), antifungal (Baba-Moussa *et al.* (1999) ; Bruneton (1999) or antiviral effects Chung *et al.* (1998), Yamaguchi *et al.* (2002), Song *et al.* (2005). However, current therapeutic applications are limited Bruneton (1999). It is interesting to recall that the tannins from the grape juice and wine have a preventive effect against cardiovascular disease Bruneton (1999). Tannins were reported to exhibit anthelmintic, antiviral, antibacterial and anti-tumor activities. It was also reported that some tannins are able to inhibit selectively HIV replication and is also used as diuretic Haslem (1989) ; Ngbede *et al.* (2008). Plant tannins have been widely recognized for their pharmacological properties and are known to make trees and shrubs a difficult meal for many caterpillars Ngbede *et al.* (2008).

As far as alkaloids are concerned, the best results have been obtained with *Zanthoxylum* Atlantic harvested during the dry season and *Newbouldia* Zou harvested during the rainy season. Extracts of *N. laevis* collected in Nigeria near Anambra state are rich in alkaloids Gafner *et al.* (1996, 1997). Indeed, *Daturainnoxia* Mill. a plant of the *Solanaceae* family synthesizing secondary

metabolites, including the tropane alkaloids, was used as a model for crops in hydroponics. Environmental parameters (biotic and abiotic) have improved growth and production of secondary metabolites. The response of the plant depended on the oxygen supply of the nutrient solution, temperature level as seen from the aerial parts at root level. An increase in light intensity did not improve alkaloid content of the plant but the use of orange light causes a change in growth parameters and levels of alkaloids. Vu (2008) also reported that the presence of microflora in the culture medium causes a degradation of molecules of interest and alters their accumulation in the plant. Campa (2005) shows the importance of genetic factors over environmental factors on the alkaloid content of different organs of *Canavalia rosea*, a tropical plant, commonly known as pea-edge-de-mer. Most of the known functions of alkaloids are related to protection. Alkaloids show various properties : antiarrhythmic, anticholinergic, antitumor, vasodilating, antihypertensive, cough medicine, anesthetic, remedy for gout, analgesic, antihypertensive, muscle relaxant ,inhibitor of acetylcholinesterase , antiarrhythmic, antipyretics, antimalarial, antiprotozoal agent , sympathomimetic, vasodilator, antihypertensive Bruneton, (2009).

Regarding essential oils, only *Z. zanthoxyloides* harvested in Oueme, Atlantic and Zou show significant results. For the same plant, the composition of essential oil can vary widely according to the organs (leaf, flower, fruit, wood), to the season, to growing conditions (sunshine, humidity, day length, soil fertility). For a species like thyme, we know seven chemical races El Kalamoun (2010).The composition of essential oil of *Z. zanthoxyloides* collected in Benin is very different from *Z. zanthoxyloides* collected in Monteverde, Costa Rica (leaves: Setzer *et al.* (2005)), Cameroon (fruit : Ngassoum *et al.* (2003)). This difference can be explained by the existence of several chemotypes according to climatic, geographical and origin of the plant. Essential oils have various biological effects. Effects and multiple targets are due to each chemical component, and to their multiplicity. Essential oils have the particular effects of antiseptics, antibiotics or anti-infective, anti-inflammatory drugs, antihypertensives, antihistamines, vasopressors, analgesics, sedatives, anxiolytic, anti-insomnia, anti-spasmodic, diuretic, laxative Bruneton, (2009).

Regarding flavonoids, the best results are obtained with *Z. zanthoxyloides* from Oueme and *N. laevis* from Atlantic collected during the rainy season. Note also that the content of flavonoids of both plants regardless of the harvest season in the department of Oueme, Atlantic and Zou is not negligible. Indeed the secondary metabolites such as flavonoids are involved in many plant defense responses. Environmental factors, such as the supply of nutrient substrates, temperature, light or the concentration of carbon dioxide in the atmosphere can affect the flavonoid content of plant tissues

Gayle (2010). Today, the properties of flavonoids are widely studied in the medical field : anti-viral, anti-tumor, anti-inflammatory, anti-allergic, anti-cancerous activities are highlighted. The animal world is also very concerned by flavonoids. Bees instinctively put such implement the antifungal and antibacterial properties of polyphenols to sanitize their hive and seal the cracks. (http://membres.multimania.fr/mourad/flavonoides.html). Flavonoid has been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activities Bruneton (2009). Flavonoids have vasculoprotective and veinotonicproperties, reducing the permeability of blood vessels. They would thus be beneficial for the heart, arteries, liver, and immune system or to muscle tissue. Flavonoids are particularly used to treat hemorrhoid crises, heavy legs and abnormal capillary fragility (http://complement - alimentaire. Comprendre choisir. com /comprendre / flavonoides-complements-alimentaires).

Regarding phenols, no significant difference is observed between the plants collected from different areas and in different seasons.

N. laevis and *Z. zanthoxyloides* are commonly used in Benin to treat human and animal diseases. According to our results, one must keep in mind that the chemical composition of these two plants varies with the geographical origin and with the harvest season. The choice of the plant depends on the properties expected, the users should consider the content of the corresponding active components to take the right plant (origin and harvest season) in order to obtain the best activities and less side effects.

CONCLUSION

The studied plants can be seen as a potential source of useful drugs. Given the wealth of secondary metabolites, *N. laevis* and *Z. zanthoxyloides* could have the chance to be considered as food additives. Further studies on these plants are underway in order to isolate the bioactive compounds. The anthelmintic activities of these plants, as claimed by traditional healers, are also being investigated.

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