Septième article : *Newbouldia laevis* (Bignoniaceae) and *Zanthoxylum zanthoxyloides* (Rutaceae) used in folk medicine: anatomical features, preliminary phytochemical analysis and anthelmintic activity

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Pages (pp.) 59-69

Bulletin de la Recherche Agronomique du Bénin (BRAB) - Numéro spécial Agronomie, Société, Environnement & Sécurité Alimentaire - Août 2016

Le BRAB est en ligne (on line) sur le site web http://www.slire.net et peut être aussi consulté sur le site web de l'Institut National des Recherches Agricoles du Bénin (INRAB) http://www.inrab.org

ISSN sur papier (on hard copy) : 1025-2355 et ISSN en ligne (on line) : 1840-7099

Bibliothèque Nationale (BN) du Bénin

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Centre de Recherches Agricoles à vocation nationale basé à Agonkanmey (CRA-Agonkanmey)

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Bulletin de la Recherche Agronomique du Bénin (BRAB)
Numéro spécial Agronomie, Société, Environnement & Sécurité Alimentaire - Août 2016
BRAB en ligne (on line) sur le site web http://www.slire.net
ISSN sur papier (on hard copy) : 1025-2355 et ISSN en ligne (on line) : 1840-7099

Newbouldia laevis (Bignoniaceae) and *Zanthoxylum zanthoxyloides* (Rutaceae) used in folk medicine: anatomical features, preliminary phytochemical analysis and anthelmintic activity.

P. A. Olounladé[15], E. V. B. Azando[15], G. C. Akouedegni[15], M. S. Hounzangbé-Adoté[15], T. B. Tam Ha[16]; I. Fourasté[16], C. Moulis[16,18], N. Fabre[16,18], H. Hoste[17] and A. Valentin[18]

Abstract

Newbouldia laevis (P. Beauv.) Seeman ex Bureau is a Bignoniaceae widely distributed in USA, Central Africa and West Africa. *Zanthoxylum zanthoxyloides* (Lam.) Zepernick & Timler is a Rutaceae widely distributed in West Africa. They have been commonly used in folk medicines against human and animal external and gastrointestinal parasites. In this study, the botanical identification criteria as well as a preliminary phytochemical composition and an anthelmintic activity of these species are reported. Extracts of both plants were prepared by use of chloroform, acetone/water (70:30), ethanol/water (70:30) and their anthelmintic activity was measured by the larval migration inhibition assay (LMI) applied on the abomasale species *H. contortus*. Then anatomical and phytochemical examinations were performed. Anatomical study has shown that *N. laevis* presents: lower epidermis with sinuous wall-cells and anomocytic stomata, acicular calcium oxalate crystals, unicellular echinulate covering trichomes, 8-20 cell-head glandular trichomes. Anatomical study has shown that the leaflet of *Z. zanthoxyloides* presents as anatomic features: epidermis with polygonal-cells and wavy striated cuticle, calcium oxalate clusters and sphaerocrystalline masses of flavonoid compounds. The phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, volatile oil, anthocyanins, leucoanthocyanins, reducing compounds and coumarins in the leaves of the studied species. Saponins, cyanogenic and cardiac glycosides were absent in all the studied plants parts. Mucilages were present in *Zanthoxylum zanthoxyloides* while quinones were present in *Newbouldia laevis*. Chloroform, acetone and ethanol extracts were used for anthelmintic studies. The extracts of *N. laevis* and *Z. zanthoxyloides* inhibit *in*

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ISSN sur papier (on hard copy) : 1025-2355 et ISSN en ligne (on line) : 1840-7099

vitro the larval migration of *H. contortus*. This effect is dose depending ($p < 0,001$). Acetonic extracts seem to be more active especially in high doses. These extracts from *N. laevis* and *Z. zanthoxyloides*, identified in the ethno knowledge could be used as improved traditional medicines and may contain compounds with potential anthelmintic activity.

Key words*:* Small ruminants*,* phytochemical*, Haemonchus contortus, extracts of plants, Newbouldia laevis, Zanthoxylum Zanthoxyloides,* Benin

Newbouldia laevis (Bignoniaceae) et *Zanthoxylum zanthoxyloides* (Rutaceae) utilisés en médecine traditionnelle: anatomie, analyse phytochimique et activités anthelminthiques

Résumé

Newbouldia laevis et *Zanthoxylum Zanthoxyloides* sont deux espèces de plantes couramment utilisées en médecine populaire contre les parasites gastro-intestinaux des humains et des animaux. La composition phytochimique préliminaire et l'activité anthelminthique de ces plantes ont été évaluées. Ainsi, des effets des extraits de plantes préparés à l'aide du chloroforme, de l'acétone/eau (70:30) et de l'éthanol/eau (70:30) sur *Haemonchus contortus* des petits ruminants ont été évalués à travers le test d'inhibition de la migration larvaire (LMI). Les observations ont porté sur des examens anatomiques et phytochimiques. L'examen anatomique a montré que *N. laevis* renfermait des épidermes recouverts d'une cuticule striée. Ces épidermes étaient constitués par une assise de cellules plus ou moins isodiamétriques, des poils tecteurs dressés, unicellulaires, à paroi épaissie et échinulée et des poils sécréteurs enfoncés dans l'épiderme, à pied unicellulaire et à tête pluricellulaire composé de 8 à 20 cellules. L'examen anatomique de *Z. zanthoxyloides* a indiqué que cette plante présentait des épidermes avec des cellules polygonales et cuticularisées, des macles d'oxalate de calcium et des masses de sphaerocrystalline de flavonoides. Le criblage phytochimique a révélé au niveau des feuilles des deux plantes, la présence de flavonoïdes, des tanins, des alcaloïdes, des huiles volatiles, des anthocyanes, des leucoanthocyanes, des composés réducteurs et des coumarines. Des Mucilages étaient présents dans *Z. zanthoxyloides* et les quinones étaient présentes dans *N. laevis*. Les extraits de *N. laevis* et de *Z. zanthoxyloides* ont inhibé *in vitro* la migration des larves de *H. contortus*. L'effet des deux plantes sur *H. contortus* était une dose dépendante ($p<0,001$). Les extraits acétoniques en particulier, semblaient être plus efficaces, à des doses élevées. Les extraits de plantes de *N. laevis* et de *Z. zanthoxyloides* peuvent être utilisés comme des anthelminthiques traditionnels améliorés par la population locale. Des travaux ultérieurs doivent permettre d'isoler de ces deux plantes tropicales des métabolites secondaires ayant des activités anthelminthiques potentielles.

Mots clés: Petits ruminants, phytochimie, *Haemonchus contortus*, extraits de plantes, *Newbouldia laevis*, *Zanthoxylum Zanthoxyloides,* Bénin.

INTRODUCTION

Drug chemotherapy still remains one of the major curative options worldwide (Ugbabe *et al.,* 2010). Currently, attention is being given to the use of herbal medicinal products and therapy for the treatment of most ailments whether physiological disorder or of bacterial, viral or parasitic origin. Screening of plant extracts for anthelmintic, antiviral or antiparasitic properties are one of the basic steps in identifying target drugs after extraction (Ugbabe *et al.,* 2010).

The World Health Organization (WHO) reported that more than 80% of the world's population depends mainly on traditional medicine. Traditional treatment involves mainly the use of plant extracts. Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases, this practice being an alternative way to compensate some perceived deficiencies in orthodox pharmacotherapy (Sofowora, 1989). Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. The rationale for the utilization of these remedies rested largely on long term clinical experience. But now, with the upsurge in the use of

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herbal medicine, a thorough scientific investigation of the plants will go a long way in validating their folkloric usage (Sofowora, 1989).

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. It is commonly used in African folk medicine for the treatment of several diseases such as diarrhea and icterus (Tra-Bi, 1997). It is also employed against malaria, sexually transmitted disease, dental caries, arthritis pain, gastroenteritis, dysentery and as vermifuge (Ayensu, 1978; Abbiw, 1990; Eyong *et al.,* 2005). Leaves are used against infertility (Burkill, 1985; Adjanahoun *et al.,* 1991; Igoli *et al.,* 2002; Igoli *et al.,* 2003; Tor-Anyiin *et al.,* 2003).

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 (Griffin *et al.,* 2000). The tree branches are adorned with numerous spines. It has been commonly used against human and animal parasites (Arbonnier, 2004). Leaves have been used as tea against inflammatory diseases and against malaria. Roots and stems have been used to treat jaundice, sore throat, hemorrhoids, gastroenteritis, dysentery, gonorrhea and as vermifuge. Root bark and leaves are used as anti-odontalgic and to treat stomatitis, gingivitis and dental caries. Roots are used for tooth friction (Malgras, 1992; Chaaïb, 2004).

In Benin, stock breeding is an economically important activity because it represents one of the most important sources of jobs and income. It contributes for about 4 to 6 % in the Gross domestic product of Benin. Except the functions of prestige and savings, the animals of breeding intervene to increase the income of the breeders through on one hand, the sale of animals and their by-products and on the other hand through the use of the fertilizer for the fertilization of farms (Savy *et al.,* 2005). However, this breeding is confronted with numerous constraints: the high cost of medicines, state of financial fragility of the producers, disturbing appearance of crossed resistances in the modern molecules, the lack of sanitary frame of the breeders and the traditional system of the managements of the herds. Among the constraints, one of the essential factors of limitation of the animal productions is the animal health. The levying of these constraints requires the implementation of the efficient practices of fight by the use of the available endogenous resources. Among these resources, we can quote healing plants. Helminthes infection is a major threat to small ruminant production leading to enormous economic losses particularly in areas where extensive grazing is practiced (Waller, 1997).

The plants were chosen on the basis of a recent questionnaire survey in Benin which indicated that they were frequently used by small scale farmers against parasitic infections or to treat associated clinical signs (Hounzangbé-Adoté, 2000). *H. contortus* is a gastrointestinal nematode parasite of the small ruminants with which the economic consequences are particularly marked in developing countries. As all the digestive strongyles, their biological cycle is monoxene and divides in an external phase (free life) and in a phase of parasitic life (Craplet et Thibier, 1984) Eggs of the nematodes, rejected with feces by animals, eclose to give larvae of the first age L_1. These last ones undergo a metamorphosis to give larvae of 2nd age L_2 which undergo in turn a metamorphosis to give larvae of 3rd age L_3. L_3 is characterized by its inclusion in the exuviae of the stage L_2 and represents the infective stage. The exit of the larva L_3 from its girdle establishes the passage of the life.

This paper deals with an anatomical identification, a preliminary phytochemical analysis and an anthelmintic evidence of the leaves of *N. laevis* and *Z. zanthoxyloides* found in Benin.

MATERIALS AND METHODS

Plant material and anatomical evaluation

Plants screened were leaves of *Z. zanthoxyloides* and *N. laevis*. The samples of each plant were collected from their natural habitat. The specimens were identified at national herbarium of Benin

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(Abomey-Calavi University) respectively under the numbers: AA 6301/HNB and AA 6302/HNB. The Slices of leaflets were obtained by standard techniques and disaggregated by a 10% solution of sodium hypochlorite during 10 min. Slices of leaflets were stained by an alum-carmine-green mixture called Mirande's reagent (Mirande, 1920). Leaflet transverse sections were mounted on glass slides using glycerin gel. Leaf powder was observed using chloral hydrate solution (80 g/20 ml). All observations were performed using a LEICA DMLB microscope. Pictures were taken with a Digital Camera Power Shot S40CANON photo-micrographic system.

Phytochemical analysis and preparation of plants extracts

For the phytochemical screening, the standard methods of Odebiyi et Sofowora (1990), Trease et Evans (2002), Fadeyi *et al.,* (1989), Potterat (1997) and Banso et Ngbede (2005) were adopted. . Chloroform, acetone and ethanol extracts of the leaves were used for the anthelmintic screening studies. Leaves extracts were prepare by drying leaves indoors at room temperature. Then a large part was reduced into powder for extraction. About 50 grams of leaf powder was refluxed in a water bath under magnetic stirring during one hour in 500 mL of chloroform. The solution was cooled then filtered. The operation was repeated twice and chloroform was removed under reduced pressure at T = 40℃. The residue was freeze-dried; it was weig hed, and then refluxed under magnetic stirring during one hour in 500 mL of acetone-water (70: 30). The solution was cooled then filtered. The operation was repeated twice and acetone and water were removed under reduced pressure. The residue was freeze-dried; it was weighed, and then refluxed under magnetic stirring during one hour in 500 mL of 70 % ethanol. The solution was cooled then filtered. The operation was repeated twice and ethanol-water was removed under reduced pressure. The residue was freeze-dried.

Evaluation of larval migration inhibition

The larval migration inhibition (LMI), the method of bioassay was used as described by Rabel *et al.,* (1994) and adapted for the plant extracts (Jackson et Hoste, 2010), in order to measure inhibiting activity against infective larvae of *H. contortus* (L_3). *H. contortus* L_3 were obtained by fecal culture. Eggs reached the stage L_3 after 10 days. The L_3 were then collected by sedimentation using Baermann's devices. Larvae were incubated for 3 h at 20 ℃ in PBS plant extract solutions, at concentrations of 150,300, 600 or 1200 µg/mL at the rate of 3 repetitions by concentration. The larvae were then washed three times in phosphate buffer (PBS) (pH 7.2, 0.15 M) and centrifuged. After the last washing, 800 µL of larvae at a concentration of 1000 L_3/mL was pipetted onto a 20 µm mesh. The sieve was inserted into a conical tube, so that it just touched the surface of the PBS contained therein. Three replicates were run at room temperature (23℃) for each plant concentration. In addition, negative (larvae incubated in PBS) and positive (larvae incubated in levamisole at concentrations of 62.5, 125, 250 and 500 µg /mL) controls were run in parallel. After 3 h, the L_3 above the sieve were discarded and those which had actively migrated through the mesh into the PBS below, were counted under an optical microscope (at 40 x magnification), based on a 10% aliquot technique. The percentage of LMI was calculated as [((T-M)/T) ×100)] where T is the total number of L_3 deposited in the sieve and M the number of L_3 having migrated through the mesh into the PBS.

Statistical analyses

The experiment was to determine the efficacy of the extracts, based on the inhibition of larval migration (L_3) of *H. contortus*. The percentage (%) of inhibition of larval migration was calculated using the formula (modified according to Coles et al., 1992): Inhibition (%) = 100 $(1-P_{test}/P_{control})$, where P_{test} is the number of L_3 having migrated in test extracts or levamisole and $P_{control}$ is the number of L_3 having migrated in the negative control (PBS). The mean values were calculated using the Excel statistical package. Larval migration test was transformed by the formula: log(x+1) and submitted to analysis of one-way variance and compared by the Tukey test, with 5% significance level, using the Prism 5.04 program. The dose response relationship was determined considering the statistical level of significance at $P < 0.05$

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ISSN sur papier (on hard copy) : 1025-2355 et ISSN en ligne (on line) : 1840-7099

RESULTS

Anatomical characterization of *Newbouldia laevis*

The leaf anatomy of *N. laevis* showed abundant cells containing small acicular calcium oxalate crystals in the centre of the mesophyll. The vascular bundles were surrounded by fibers. Two supernumerary vascular bundles were observed inside the major vascular ring, as well as a small vascular bundle located between the upper epidermis and the major vascular ring. The mesophyll showed the upper epidermis accompanied by a hypodermis, one layer of palisade cells and spongy parenchyma. The lower epidermis showed sinuous wall cells and anomocytic stomata (4-6 guard cells). Unicellular, thick-walled, echinulate covering trichomes were observed. Glandular trichomes were embedded in the epidermis; they had a unicellular foot and a flattened, circular, 8-20-cell head (Figure 1).

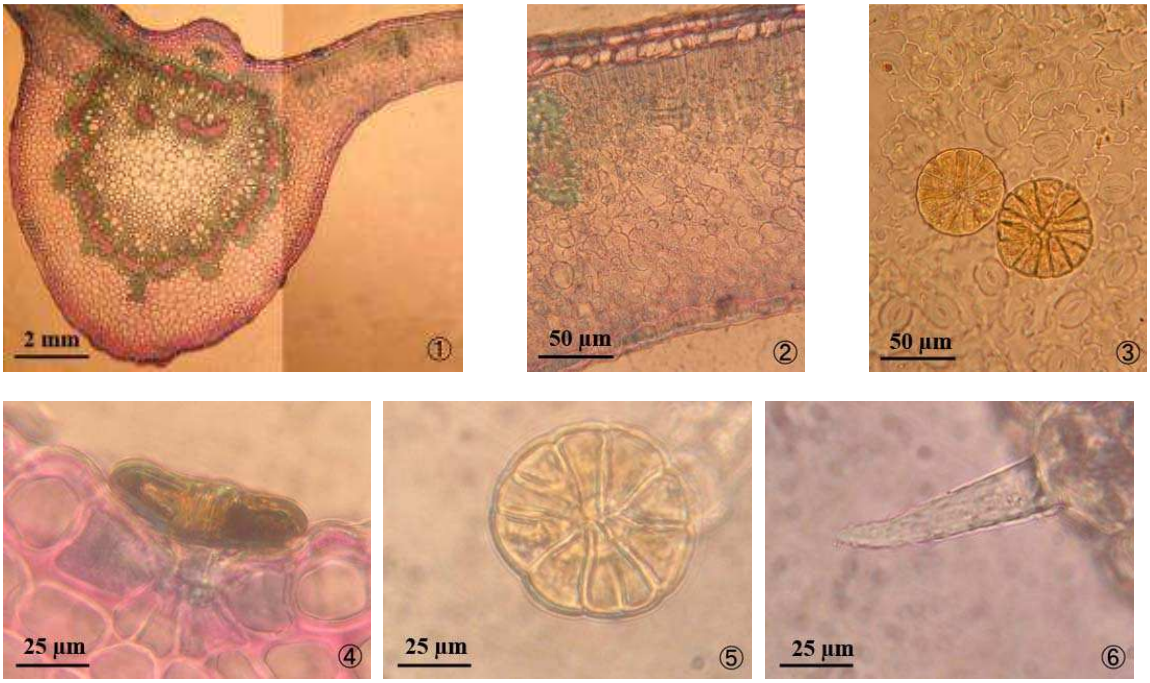

Figure 1. 1-6 Photomicrographs of *N. laevis* leaflet

(1) Leaflet transverse section: supernumerary vascular bundles, groups of fibers, calcium oxalate crystals (scale bar: 2 mm). (2) Mesophyll: upper epidermis, hypodermis, palisade cells, spongy parenchyma, and lower epidermis (scale bar: 50 µm). (3) Upper epidermis in surface view: straight wall cells and glandular trichomes (scale bar: 50 µm). (4) Glandular trichoma in transverse view showing the unicellular foot (scale bar: 25 µm). (5) Glandular trichoma in surface view showing the multicellular head (scale bar: 25 µm). (6) Unicellular echinulate covering trichoma (scale bar: 25 µm).

Anatomical characterization of *Zanthoxylum zanthoxyloides*

In transverse section, the leaf showed abundant cells containing calcium oxalate clusters in the centre of the mesophyll. The vascular bundles in ring shape are surrounded by fibers. The mesophyll showed an upper epidermis with mucilage cells, one layer of palisade cells and spongy parenchyma. Both epidermis have polygonal cells with wavy striated cuticle, the lower epidermis showed anomocytic stomata. Schizogenous oil glands are observed in parenchyma. In the chloral hydrate solution, the upper epidermis showed in several cells sphaerocrystalline masses composed

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of fine needles. In presence of potassium hydroxide (20 g/L in ethanol) those crystals disaggregated (Figure 2).

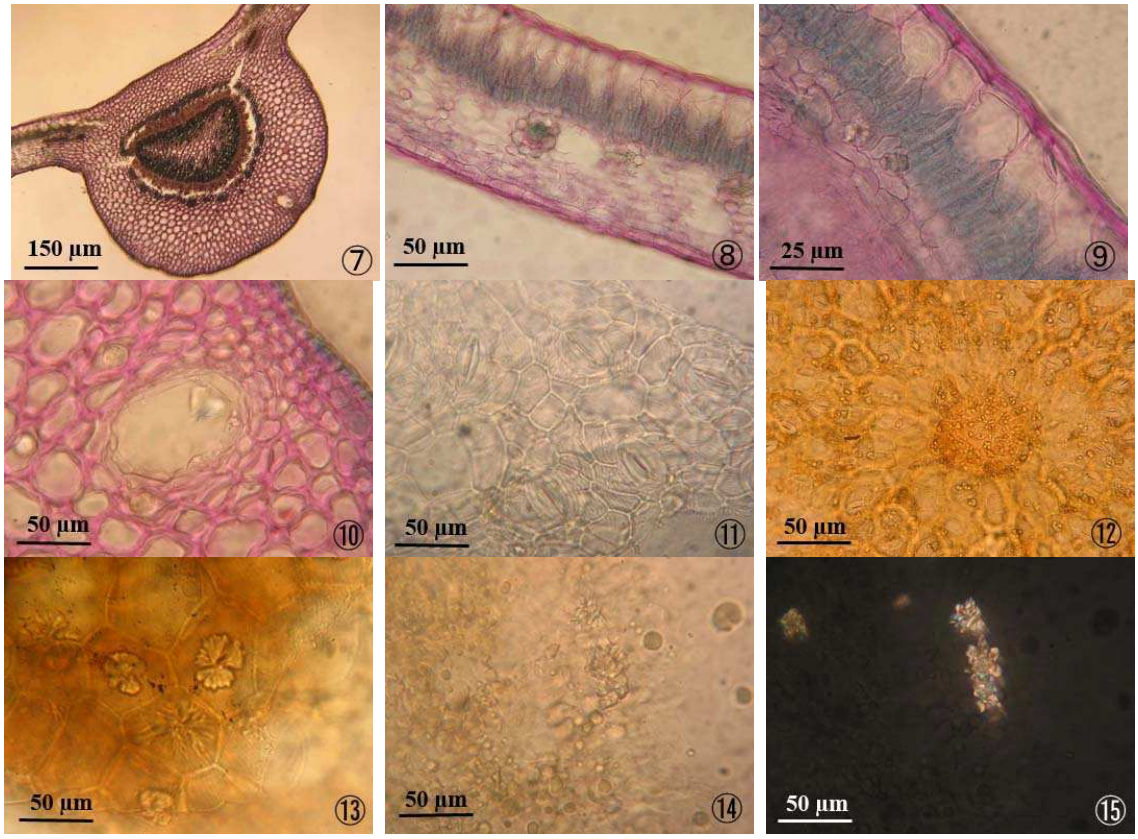

Figure 2. 7-15 Photomicrographs of *Zanthoxylum zanthoxyloides* leaflet

(7) Leaflet transverse section in the midrib: showing the typical ring-shape vascular bundle (scale bar: 150 µm). (8-9) Mesophyll: upper epidermis with mucilage cells, palisade cells, spongy parenchyma with calcium oxalate clusters, lower epidermis (scale bar :50 µm – 25 µm). (10) Schizogenous oil gland in transverse section (scale bar: 50 µm). (11) Lower epidermis with polygonal cells, anomocytic stomata (scale bar: 50 µm). (12) Upper epidermis with underlying schizogenous oil gland (scale bar: 50 µm). (13) Upper epidermis with polygonal cells and sphaerocristalline masses of flavonoid compounds (scale bar: 50 µm). (14) Calcium oxalate cluster in day light (scale bar: 50 µm). (15) Calcium oxalate cluster in polarized light (scale bar: 50 µm).

Phytochemical component of plants

The phytochemical investigation revealed the presence of several components in the leaflet of both plants (Table 1). Besides, mucilages were present in *Z. zanthoxyloides* and quinones were present in *N. laevis*. The ratios drug/extract (mass/mass) for *N. laevis* were 10:1 for chloroform, 20:1 for acetone and 8:1 for ethanol. : This ratios drug/extract (mass/mass) for *Z. zanthoxyloides* were 10:1 for chloroform, 10:1 for acetone and 9:1 for ethanol.

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Table 1. Phytochemical analysis of the plants leaves

Chemical component	*N. laevis*	*Z. zanthoxyloides*
Tannins	+	+
Flavonoids	+	+
Anthocyanins	+	+
Leucoanthocyanins	+	+
Cyanogenic glycosides	-	-
Saponins	-	-
Coumarins	+	+
Mucilages	-	+
Alkaloids	+	+
Reducing compound	+	+
Cardiac glycosides	-	-
Quinones	+	-
Volatile oil	+	+

Key: (+) = Present, (-) = Absent

In vitro anthelmintic activity of plant extracts

The migration rate observed for the larvae of negative control was 87%. The positive control, levamisole was highly effective. The percentage of inhibition of larval migration increased significantly ($p<0.001$) with the increasing concentrations of chloroform, acetone and ethanol extracts (Figures 4 and 5) Overall, the inhibition rates obtained with ethanol and chloroform extracts ($p<0.001$) were lower than those obtained with acetone extract at similar concentrations ($p<0.001$). Levamisole was more effective than the extracts, but the compounds in the extracts have more or less similar dose-response profiles (Figure 3). The *N. laevis* and *Z. zanthoxyloides* extracts appeared to inhibit larval migration.

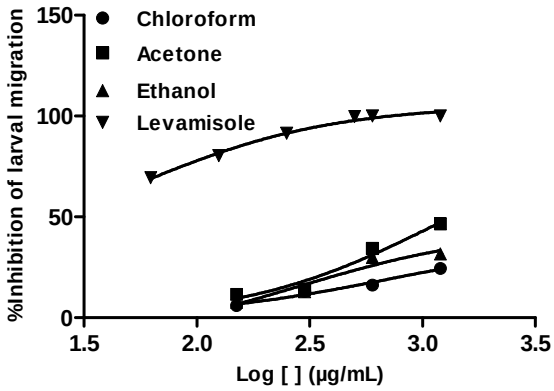

Figure 3. Dose-Reponse profil for inhibition of larval migration (L3) of *Heamochus Contortus* by *Newbouldia laevis* extracts

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ISSN sur papier (on hard copy) : 1025-2355 et ISSN en ligne (on line) : 1840-7099

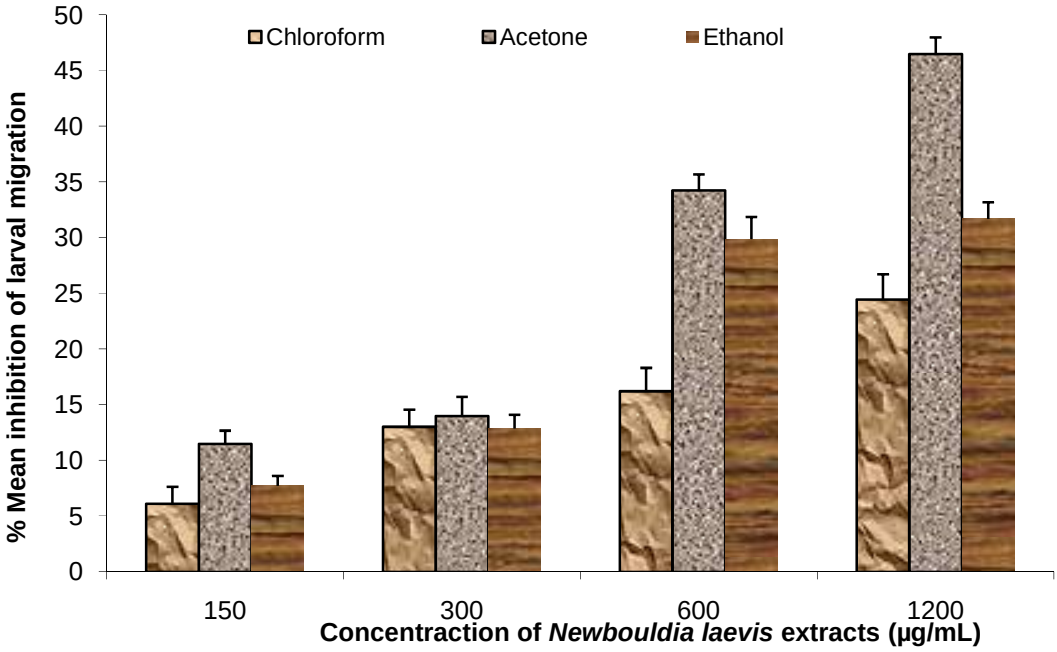

Figure 4. Percentage of inhibition of larval migration (L3) of H. contortus by *N. laevis* extracts.

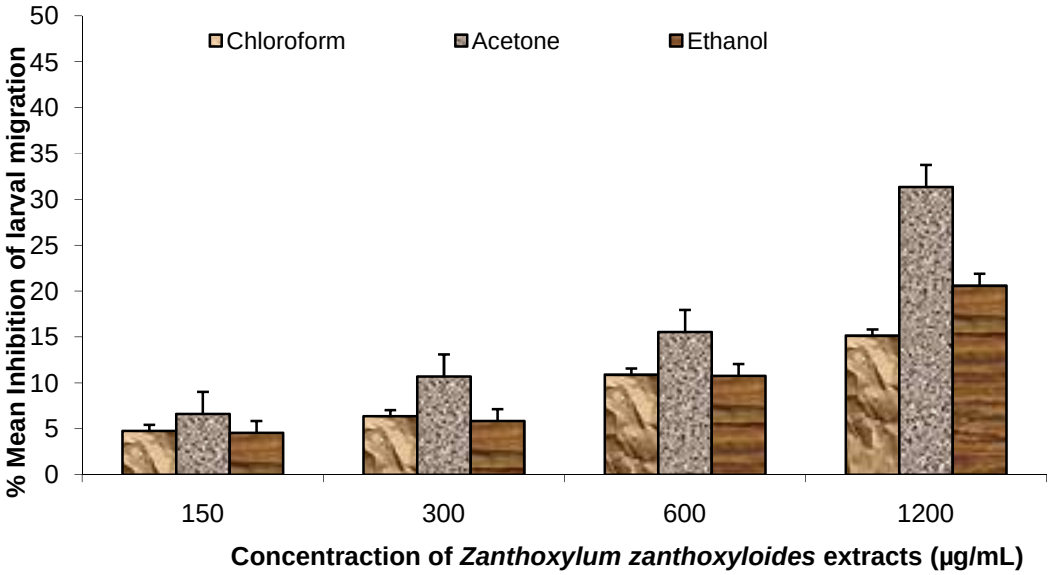

Figure 5. Percentage of inhibition of larval migration (L3) of *H. contortus* by *Z. zanthoxyloides* extracts

DISCUSSION

According to the obtained results, a complete ring of vascular bundles observed in *N. laevis* leaves could be a characteristic of the Bignoniaceae family since generally transverse section of the leaf shows midrib in the form of a continuous arc (Ayodele et Olowokudejo, 1997). *N. laevis* leaves present non-reverse supernumerary bundles inside the vascular ring, this is uncommon in plants.

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The presence of a hypodermis in the upper face of the lamina is rather frequent in tropical plants. The calcium oxalate crystals in fine needles observed in the whole of the parenchyma of *N. laevis leaves*, are rarely found in plants. This feature may be an identification criterion *of N. laevis* leaves in the family of Bignoniaceae. The specific trichomes, either unicellular straight covering trichomes with echinulate wall, located on the rib, or glandular trichomes with unicellular foot and peltate multicellular head are observed. The lower epidermis shows sinuous wall cells. All these characteristics would be a good criterion for identification of *N. laevis* in Bignoniaceae family.

According to our observations, we find in *Z. zanthoxyloïdes* leaflet the characters common to the Rutaceae family such as the presence of a vascular ring at the midrib (genera *Citrus, Jaborandi, Barosma*) (Jackson,1990), the presence of an internal secretor apparatus consisting of schizogenous oil glands, the presence of calcium oxalate crystals. The distinctive characteristics such as the absence of an external secretor apparatus, yet common in many species of the family, the presence of mucilage located in hypertrophied cells in the upper epidermis of the lamina, a similar organization is found in the genus *Barosma*, the presence, in chloral hydrate solution, of flavonoid compounds that crystallize in masses or in fan-shaped fine needles. This phenomenon has already been described in several species such as *Citrus* leaflets (Perrot , 1942) or in species not belonging to the Rutaceae family but known for their high content of flavonoids such as *Sophora japonica* flower (Ph. Eu. 7th edition).

The phytochemical investigation revealed the presence of alkaloids, flavonoids, tannins, volatile oil, anthocyanins, leucoanthocyanins, reducing compounds, coumarins and quinones, and the absence of mucilages, saponins, cyanogenic and cardiac glycosides in the leaf of *Newbouldia laevis. Z. zanthoxyloïdes* leaves gave similar results, except for mucilage which was present and quinones which were absent. For *Newbouldia laevis*, a wide diversity of secondary metabolites has also been described depending on the part of the plant used. In root bark, the presence of naphtoquinones and anthraquinones has been reported (Eyong *et al.,* 2005, 2006) whereas in the leaves, the presence of flavonoids, tannins and terpenes has also been mentioned (Gafner *et al.,* 1997; Samy et Gopalkrishnalkone, 2008). Quaternary alkaloids have also been identified (Kerharo & Adam, 1974). This showed that *N. laevis* contain many secondary metabolites. In similar studies alkaloids (Perrett & Whitfield, 1995), tannins (Banso et Ngbede, 2006), flavonoïds (O'Sullivan, 1983; Ayres et Loike, 1990), coumarins (Gray, 1983) were found in the leaf extracts of *Z. zanthoxyloides*. The presence of such metabolites suggests that the plant might be of great importance in phytomedicine. For instance, the presence of alkaloids, flavonoids and tannins might be responsible for the use of this plant as vermifuge in Benin against parasitosis (Burkill, 1997; Hounzangbé-Adoté, 2000).

The presence of tannins and flavonoids in plant extracts suggest that the plant has anthelmintic activity. The presence of tannins could also mean that it is an astringent, helping in wound healing and an antiparasitic which is in line with its folkloric use. Tannins also contain different type of acids, for example catechic, chlorogenic acids, gallo and ellagic acids (epigallitannins), which are inhibitors of HIV replication (http://en.wikipedia.org/wiki/Tannin).Tannins have shown potential antiviral, antibacterial, antiparasitic effects. Their potential effects against cancer through different mechanisms have been studied (Paolini *et al.,* 2003). Presence of volatile oil in the leaves suggests that it can be used in natural body cosmetic and scents (Paolini *et al.,* 2003).This is even more important since the oil was active on all the test organisms. This shows that it can be topically used to cure skin infections. Since it was also highly antimicrobial, anthelmintic, it can be used to treat infections.

Larvae L_3 establish an important stage of the parasitic cycle of *H. contortus*. They are the infective stage and are at the origin of the losses of production at the host and the contamination of the outside environment (Paolini *et al.,* 2003; Hounzangbé-Adoté *et al.,* 2004). The decrease of migration of the larvae of *H. contortus* may be due to their immobility or their mortality probably because of the effects of *N. laevis* and *Z. zanthoxyloides* extracts. Acetone and ethanol were selected as suitable extractants as they extract compounds of a wide polarity range, are miscible with organic and aqueous solvents, and are non-toxic to test organisms (Eloff, 1998), and indeed organic solvents extract more plant material than water. An analogous method has been used for

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the test of larvicidal effects of plant extracts (Lorimer *et al.,* 1996; Molan *et al.,* 2003) according to the modifications of the World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance (Coles *et al.,* 1992). Besides, chloroform acetone and ethanol extract largest amounts of compounds from plants compared to dichloromethane and hexane (Bizimenyera *et al.,* 2005). The *in vitro* model reported in this study demonstrated larvicidal effects of chloroform, acetone and ethanol extracts of *N. laevis* and *Z. zanthoxyloides* against *H. contortus*. The acetone extracts tannins of plants mainly. The inhibitory effect of the migration of *H. contortus* larva could be assigned to the tannins. The mechanism of the anthelmintic action is yet to be determined. Tannins in plant extracts have anthelmintic activity, attributed to physical astringent action on helminths (Athanasiadou *et al.,* 2001; Molan *et al.,* 2000). The present study showed that the larvicidal actions of the leaf extracts were nearly similar, yet the polyphenol content of the leaves of Tropical plants is much lower than that of the root and bark (Bizimenyera *et al.,* 2005). The mode of action of the anthelmintic compounds could be similar to that of levamisole, judging from the dose-response curves, but more work needs to be done for rational conclusions

The traditional use of the *N. laevis* and *Z. zanthoxyloides* extracts against diarrhea, dysentery and unthriftiness (Hounzangbé-Adoté, 2004), may also be due to their anthelmintic activity, as these signs are consistent with parasitic gastroenteritis. The anthelmintic activity of *N. laevis* and *Z. zanthoxyloides* extracts, in addition to the antibacterial, antioxidant and anti-HIV activities (Hounzangbé-Adoté, 2004) further support the traditional use of the plants. Research work is ongoing for determining better methods of plant extraction, elucidation of the chemical structure of the active compounds, and for *in vivo* tests in suitable target livestock. This work may lead, not only to possible isolation of novel anthelmintics from the plants, but also to identification of better methods of plant extraction which are readily adaptable for use by rural communities against helminthosis.

CONCLUSION

The result of this study on anatomical point of view supports largely the demarcation of the taxonomy of the families of Bignoniaceae and Rutaceae by Hutchinson and Dalziel (1954). A rigorous and relevant approach of determination and botanical identification of *N. laevis and Z. zanthoxyloides* allowed us to raise a real "ID card" to this raw material and to assure the reproducibility and the traceability. The presence of a variety of secondary metabolites and the activity of the extracts on *H. contortus* suggest that the plants can be exploited for phytomedicine development. *N. laevis and Z. zanthoxyloides* extracts may be useful in the control of gastrointestinal nematodes of small ruminants. Further study is necessary for isolation and characterization of the active principles from the acetone fractions obtained from *N. laevis* and *Z. zanthoxyloides* leaves.

ACKNOWLEDGEMENTS

This work was supported by the CORUS Project No. 6040 and Niche Project No. BEN 174. Mr. A.P. OLOUNLADE acknowledges receiving a scholarship from AUF to undergo his PhD studies. Mr. E.V.B. AZANDO is a grateful recipient of a grant provided from ARHES. The authors would like to thank sincerely Valérie CRISTOFOLI for her technical assistance in the treatment of anatomical photos.

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