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ORIGINAL ARTICLE

Chemical Characterization and Biological Activities of *Newbouldia laevis* and *Pterocarpus Santalinoides* Leaves

Fifa T. D. Bothon^{1*}, Moutcharaf Moustapha¹, G. Sophie Bogninou¹, C. Pascal Agbangnan Dossa¹, Boniface Yehouenou¹, Seindé E. Medoatinsa¹, Jean Pierre Noudogbessi^{1†}, Félicien Avlessi¹ and Dominique C. K. Sohounhloue¹

¹ Laboratoire d'Etude et de Recherche en Chimie appliquée, Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi 01 BP 2009 Cotonou, Bénin

*Email: bothon2006@hotmail.com, fifa.bothon@iut.uac.bj

ABSTRACT

This study was aimed at phytochemical screening, antibacterial and antiradical activities of aqueous and hydro-ethanolic extracts of two medicinal plants leaves, *Newbouldia laevis* and *Pterocarpus santalinoides*, collected in southern Benin. Phytochemical analysis of these plants revealed the presence of coumarins, polyphenols (flavonoids and tannins) and mucilage in both samples. But only *Pterocarpus santalinoides* leaves showed the presence of saponins and leucoanthocyanins. The quantitative analysis of phenolic compounds in both samples showed that the extracts (aqueous and hydro-ethanolic) of *Newbouldia laevis* leaves had the highest levels of total polyphenols (22.18 ± 0.02 et 22.63 ± 0.07 $\mu\text{g GAE}/\text{mg}$ of extract) but lower levels of condensed tannins and flavonoids. While the aqueous-ethanolic extract of *Pterocarpus santalinoides* leaves has the highest tannins content (22.90 ± 0.01 $\mu\text{g CE}/\text{mg}$ of extract) and flavonoid content (3.88 ± 0.01 $\mu\text{g QE}/\text{mg}$ of extract). For antibacterial test *Newbouldia laevis* hydro-ethanolic extract was more effective on *Klebsiellapneumoniae* than the aqueous extract, while the aqueous extract of *Pterocarpus santalinoides* has been active on the same strain. *Escherichia coli*, *Salmonella typhi* and *Shigella* sp were inhibited by higher CMI (1.23 mg/mL) of the four extracts. The antiradical activity was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, the best IC_{50} were: 65 $\mu\text{g}/\text{mL}$ and 125 $\mu\text{g}/\text{mL}$ respectively for *Newbouldia laevis* and *Pterocarpus santalinoides* leaves aqueous extracts.

KEY WORDS: Phytochemical, antiradical, antibacterial, *Newbouldia laevis*, *Pterocarpus santalinoides*.

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INTRODUCTION

According to the World Health Organization, diarrhea is defined by too frequent stools, daily emissions, liquid or very soft (greater than 300 g / day weight). In practice, they have diarrhea when there are at least three very loose stools and liquids per day [1]. It is usually a symptom of an intestinal infection that can be caused by various microorganisms, bacteria, viruses or parasites. The infection is spread through contaminated water or food or from one person to another in case of insufficient hygiene [2]. Diarrheas are among the most common and most widespread diseases in the world [3]. In 2009, the following statistics were recorded by the World Health Organization: 2 billion cases of diarrhea worldwide, 6.9% of all deaths in developing countries, the second cause of death among children fewer than five years after pneumonia, 1500000 children died of diarrhea with 80% before the age of two years. In Benin, the prevalence of diarrhea was 9.0% among children under five years. This prevalence is particularly high (14%) among children aged 6-23 months. Diarrhea is a real public health problem especially in developing countries [3]. Faced with such an alarming picture, high costs and difficulties of access to antibiotics on the one hand and on the other hand the increase in antibiotic-resistant bacteria; So it seems important to direct research towards new avenues such as herbal extracts to serve as a basis for obtaining new antibacterial. The World Health Organization (WHO) estimates that about 80% of the population living in Africa (80-85% in Benin) use traditional medicine for their primary health care [4]. In addition, the 300,000 plant species identified, it is estimated that only 15% of them were studied phytochemical level, 6% for their biological activities [5].

Native to tropical Africa *Newbouldia laevis* (Bignoniaceae) is a small tree about 7-8 meters [6]. The roots and leaves are used in the treatment of dysentery, malaria, elephantiasis migraines and seizures [7]. The bark and twigs are used to treat women pelvic pain, peptic ulcer disease, earache, skin ulcer, epilepsy, hemorrhoids and constipation [8]. The flowers are also known for their anti-inflammatory activities [9].

Pterocarpus santalinoides (Fabaceae) is a tree of about 9 and 12 meters high. The leaves are used in the treatment of skin diseases such as eczema, candidiasis and acne [10]. The bark extracts are used in the treatment of diabetes, cough and sore belly [11, 12, 13].

So, the present work was designated to investigate the antiradical and antibacterial activities of *Newbouldia laevis* and *Pterocarpus santalinoides* to know the scientific basis of the traditional use of those plants.

MATERIALS AND METHODS

Plant material:

Newbouldia laevis(Bignoniaceae) and *Pterocarpus santalinoides*(Fabaceae) leaves were collected respectively from Godomey and Avrankou, in the south of Benin.

Microorganisms used

Diarrheal strains studied: *Klebsiella pneumoniae*, *Salmonella typhi* come from the National Laboratory of the Ministry of Health; those of *Shigella* and *Escherichia coli* were obtained from the Bacteriological Laboratory of the National University Hospital "koutoukou Hubert Maga (CNHU HKM)" of Benin.

Preparation of plant extracts

After collecting and drying in the laboratory (25°-30°C) plant material was ground and extracted with water and aqueous ethanol (70:30). The aqueous extract was prepared by addition of 500mL of distilled water on 50g of powdered plant and subjected to boiling for 60 min. The hydro-ethanolic extract was prepared by adding 1L of ethanol-water mixture (70/30) on 100g plant powder and mechanically stirred for 48 h. The resulting solutions were filtered through Whatman n° 1 paper and then lyophilized. The lyophilized obtained were stored in a freezer (-5°C) until analysis.

Determination of extraction yields

The extraction yields of the samples are calculated by the following formula:

$$\text{yield (\%)} = \frac{\text{massextract (g)}}{\text{massofvegetaldrug (g)}} \times 100$$

Phytochemical screening

A qualitative phytochemical screening was performed by staining and precipitation reactions. Quantitative analysis of phenolic compounds was carried out by spectrophotometry.

Alkaloids: by Mayer reagent: The powdered sample (5g) was extracted with 25 mL of a mixture of ether/chloroform (2:1) after adding 5 mL of ammonia. The suspension was macerate 24h and filtrate was extracted with 5mL of HCL solution. The formation of a yellow precipitate, after adding a few drops of Mayer reagent indicates the presence alkaloids [14].

Anthocyanins: 2 mL of aqueous extract is added to 2 mL of 2N HCl. The appearance of pink-red turn's blue-violet by the addition of ammonia indicates the presence of anthocyanins[15].

C-glycosides by Keller- Kiliani test: 2 mL of filtrates with 1mL of glacial acetic acid, 1mL of ferric chloride and 1mL of concentrated sulfuric acid.Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green indicates the presence of glycoside

Coumarins: The detection of coumarins was carried out by extracting 0.5 g of sample in 10 mL of ether. The suspension was filtrated, evaporated and 2 mL of distilled water were added. The observation of fluorescence was performed by UV light at 365 nm[14].

Flavonoids: by Shinoda test: To 2–3 mL of methanolic extract, a piece of magnesium ribbon and 1 mL of concentrated hydrochloric acid were added. (Pink red or red coloration of the solution indicate the presence of flavonoids in the drug)[14].

Leucoanthocyanins: 5 mL of extract added to 5 mL of isoamyl alcohol. Upper layer appears red color indicates for presence of leucoanthocyanins [15].

Mucilage: were determined by observing the viscosity after addition of absolute ethanol [14].

Saponins: were determined by Frothing test / Foam test: 0.5mL of filtrate with 5mL of distilled water and shake well. (Persistence of frothing indicates the presence of saponins) [15].

Tannins: by Braemer's test: 3 mL of methanolic extract, 10% alcoholic ferric chloride solution was added. (Dark blue or greenish grey coloration of the solution indicate the presence of tannins in the drug) [16].

Determination of total phenolic content: Total phenolic contents were determined using Folin-Denis' reagent method [17]. Gallic acid was used as a reference and for the calibration curve; results were expressed as Gallic Acid Equivalents (GAE, µg gallic acid/mg extract).

Tannins content: Condensed tannins content was determined according to Agbangnan *et al.*, (2013) [18]. The tannins content was calculated from catechin standard curves and expressed as Catechin Equivalents (CE, µg catechin/mg extract).

Total flavonoid content: The total flavonoid content was determined according to Enujiugha *et al.*, (2012) [19] using AlCl₃. The flavonoid content was calculated from a catechin standard curve and expressed as Catechin Equivalents (CE, µg catechin /mg extract).

Antimicrobial assay

Preparation of inoculum: The different strains were streaked on Mueller-Hinton Agar (MHA) plate which were kept for incubation at 37°C for 24 h. Active cultures for experiments were prepared by introducing one colony of bacteria into 5 mL of Mueller-Hinton Broth (MHB). The suspensions were incubated for 18 to 24 h at 37°C without agitation, to achieve the concentration of 10⁶ Colony Forming Units (CFU mL⁻¹).

Antibacterial activity: The activity of four extracts of these organisms was estimated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The microplate dilution method was used to determine the minimum inhibitory concentration (MIC) [20]. The minimum bactericidal concentration (MBC) was determined by isolation of wells greater than MIC concentration on Muller-Hinton agar in boxes kneaded and then incubated at 37 ° C for 24 h.

Antiradical activity

The stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to measure the antiradical activity of the samples according to Molyneux 2004 [21]. 200 µl of extract were added to 2800 µl of ethanol solution of 1 mM DPPH; after 1 hour of incubation in the dark, the absorbance was read at 517 nm using a spectrophotometer (type GenwayGenova) and IC₅₀ (Inhibitory concentration 50%) was determined. IC₅₀ value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The percentage inhibition activity was calculated from:

$$I(\%) = [(A_0 - A_1)/A_0] \times 100$$

Where A₀ was the absorbance of the control reaction and A₁ was the absorbance in the presence of the sample.

RESULTS AND DISCUSSION

Table-1 shows the yields after extraction and lyophilization. For the two plants, aqueous extract presents the best extraction yield.

Table-1: Yields of extracts

Plant	Extracts	Yields (%)
<i>Newbouldia laevis</i>	aqueous	6.03
	hydro- ethanolic	5.23
<i>Pterocarpus santalinoides</i>	aqueous	11.5
	hydro- ethanolic	5.25

Qualitatively, the phytochemical screening of *Newbouldia laevis* and *Pterocarpus santalinoides* leaves revealed the presence of mucilage, tannins (gallic and catechin), coumarins, flavonoids in both samples. But Only *P. santalinoides* leaves contain leucoanthocyanins and saponins. We noted a total absence of anthocyanins and alkaloids in both samples studied (Table- 2).

Table-2: Phytochemical constituent of plants studied

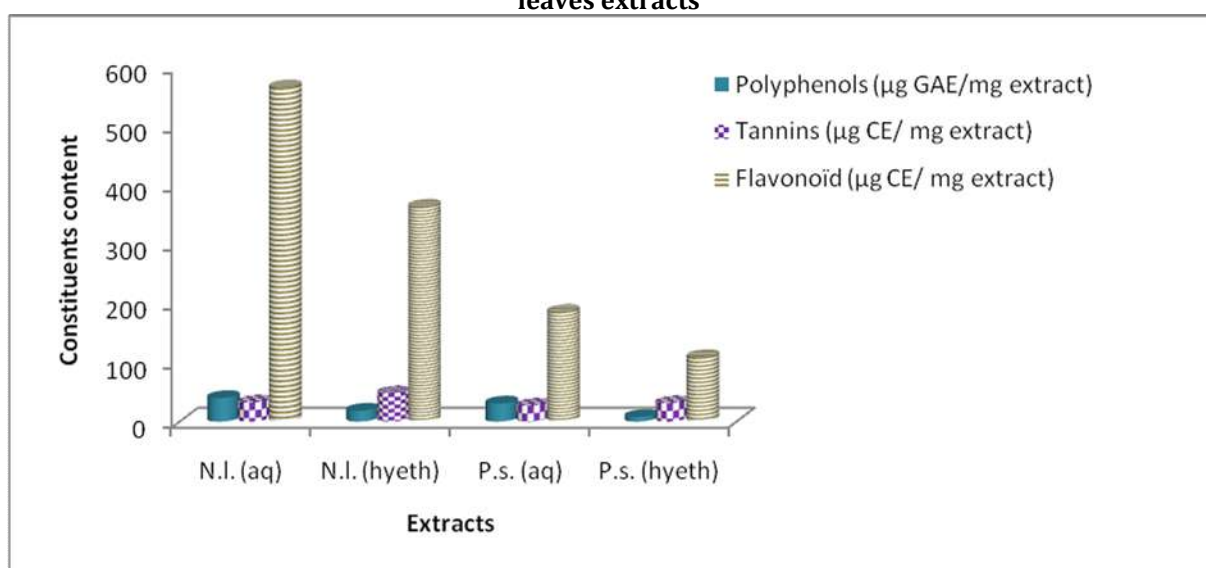
constituents	<i>Newbouldia laevis</i>	<i>Pterocarpus santalinoides</i>
Alkaloids	-	-
Anthocyanins	-	-
Leucoanthocyanins	-	+
c-glycosides	-	-
Coumarins	+	+
Flavonoids	+	+
Mucilage	+	+
Saponins	-	+
Tannins	+	+

“+” indicates presence and “-” indicates absence

Newbouldia laevis screening results are consistent with those of Usman et al. [6] but present differences from those of Adeossi [22] in terms of saponins and anthocyanins. Similarly Hassan et al. [23] in their study, revealed more saponins, anthocyanins and the presence of alkaloids in leaves of *N. laevis*. The results of qualitative screening of *Pterocarpus santalinoides* leaves are different from those of Anowi et al., [24] and Eze et al. [25] who had respectively work on the methanolic and aqueous extract of the same plant from Nigeria and revealed the presence of alkaloids and anthocyanins. According to the literature, environmental factors influence the production of secondary metabolites in plants, the differences observed could be related to the fact that samples were collected in different regions and at different times of the year.

Figure-1 summarizes the quantitative phytochemical screening of samples studied: *Newbouldia laevis* leaves aqueous extract (Nl-(aq)) presented the highest levels of polyphenols ($39.71 \pm 0.05 \mu\text{g GAE/mg extract}$) and flavonoids ($565.43 \pm 0.73 \mu\text{g CE/ mg extract}$) while the important tannins content was found in its hydro-ethanolic extract (Nl-(hyeth)) ($47,90 \pm 0.06 \mu\text{g CE/mg extract}$).

Figure-1 Quantitative phytochemical screening of *Newbouldia laevis* and *Pterocarpus santalinoides* leaves extracts



Nl-(aq) = *Newbouldia laevis* aqueous extract; **Nl-(hyeth)** = *Newbouldia laevis* extract hydro-ethanolic extract; **Ps-(aq)** = *Pterocarpus santalinoides* aqueous extract; **Ps-(hyeth)** = *Pterocarpus santalinoides* hydro-ethanolic extract.

The aqueous extract of *Pterocarpus santalinoides* leaves (Ps-(aq)) compare to its hydro-ethanolic extract (Ps-(hyeth)) has the highest content of polyphenols ($30.59 \pm 0.04 \mu\text{g GAE/mg extract}$) and flavonoids ($185.78 \pm 0.24 \mu\text{g CE / mg extract}$). But the important tannins content was revealed in hydro-ethanolic extract (Ps-(hyeth)) ($27.10 \pm 0.03 \mu\text{g CE/mg extract}$). Any previous phytochemical studies have not performed quantitative screening of these chemical compounds in these two plants extracts; apart Ogunlana [26] who quantified in 2008 the total polyphenol content in methanolic extract of leaves of *N. laevis* from Nigeria and found a lower value than our results (0.007 mg/mL).

Antimicrobial activity

Despite the chemical composition observed, none of the samples show a bactericidal power on the diarrheal strains studied to 5 mg/mL . This could be due to the fact that all four strains investigated are Gram negative with their outer wall consisting of phospholipids and lipopolysaccharides, hydrophobic structure making difficult the penetration of hydrophilic extract [20].

The table-3 shows the values of minimum inhibitory concentrations (MIC) of each plant extract (aqueous (aq) and hydro-ethanolic (hyeth)).

Table-3 MIC(mg/mL) of *Newbouldia laevis* and *Pterocarpus santalinoides* leaves extracts against tested bacteria

Bacteria Extracts	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Shigellasp</i>
<i>N.l.</i> (aq)	1.25	0.62	0.62	0.62
<i>N.l.</i> (hyeth)	1.25	0.31	1.25	1.25
<i>P.s.</i> (aq)	0.62	0.31	0.62	1.25
<i>P.s.</i> (hyeth)	0.62	0.62	1.25	1.25

Klebsiellapneumoniae strain was inhibited by *P. santalinoides* aqueous-ethanolic extract and *N. laevis* aqueous extracts with the lowest extract concentration (0.31 mg/mL). The lowest inhibition concentration of *Shigellasp* strain was with *N. laevis* aqueous extract (MIC = 0.62 mg/mL). For *Salmonella typhi* it was the two extracts of *P. santalinoides* (MIC = 0.62 mg/mL). In the case of *Escherichia coli*, it was the aqueous extracts of the two plants with a MIC = 0.62 mg/mL.

Some previous studies have investigated the antibacterial activities of these two plants. Usman et al., (2007) [6] studied antimicrobial activity of the ethanolic extract of leaves of *Newbouldia laevis* from Nigeria and their results showed respectively with *Escherichia coli*, *Klebsiellapneumoniae* and *Salmonella typhi* a minimum inhibitory concentration of 1.563; 1.563 and 3.125 mg/mL. These MIC compared to those of present study are high, so one could assume that the hydro-ethanolic extract of *N. laevis* is more active than that from ethanol. The same remark was done with *Pterocarpus santalinoides* activity results in this study compared to those of Osuagwu and Akomas [10] work on *Escherichia coli*, *Salmonella typhi*, *Shigella flexnerii* and *Klebsiella pneumoniae*. Their MIC was high and respectively equal to: 4mg/mL; 3.5 mg/mL, 1.25 mg/mL and 1.75 mg/mL; exception to *Shigella flexnerii* which is inhibited by a same concentration like *Shigellasp* in the present study. These observations could be explained by the fact that aqueous and aqueous-ethanolic extracts are richer in bioactive substances (flavonoids, tannins, saponins, phenols) than that of ethanol extract [10, 27].

DPPH is the best, easiest and widely used method for testing preliminary free radical scavenging activity of a compound or a plant extract. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance [28].

The IC₅₀ from DPPH radical scavenging activities of the four samples are summarized in Table 4. Highest scavenging was observed with aqueous extracts, with IC₅₀ value of 65 µg / mL and 125 µg / mL respectively for *N. laevis* and *P. santalinoides*.

The antiradical activity of these two plants extracts are not subject of previous study. Only Ogunlana (2008) [26] work evaluated antioxidant activity of *Newbouldia laevis* stem bark on the basis of its ability to prevent the oxidation of β-carotene and the strength of its ferric reducing capacity. Several studies support the antiradical activity by the presence of total polyphenols [26], [28]. In the present work, this relationship is verified by the fact that aqueous extracts are rich in polyphenols and gave more interesting free radical scavenging activity than hydro-ethanolic extracts. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [28].

Table-4: Extracts Inhibitory concentration (IC₅₀) (µg/mL)

Plants	extracts	IC₅₀ (µg/mL)
<i>Newbouldia laevis</i>	aqueous	65
	hydro-ethanolic	365
<i>Pterocarpus santalinoides</i>	aqueous	125
	hydro-ethanolic	Nd

ND = Not determinate

CONCLUSION

The results obtained in the present study indicate that *Newbouldia laevis* and *Pterocarpus santalinoides* leaves aqueous and hydro-ethanolic extracts content polyphenol; the aqueous extract exhibit potent free radical scavenging. *Klebsiellapneumoniae* were inhibited by lowest MIC of hydro-ethanolic extract of

Pterocarpus santalinoides and aqueous extract of *Newbouldia laevis*. Any CMB was determined for the strains studied. Those plants could have great importance as therapeutic agents in preventing or slowing the progress some diseases.

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