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*Full Length Research Paper*

# Phytochemical analysis of *Phyllanthus niruri* L. (Phyllanthaceae) extracts collected in four geographical areas in the Democratic Republic of the Congo

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**This study aims at investigating the phytochemical analysis or to analyze the secondary metaboliotes of *Phyllanthus niruri* L. plants from four collection sites which University of Kinshasa (Unikin), National Pedagogic University of Kinshasa (UPN), Kimwenza (Kim) and Kisantu (Kis) in the Democratic Republic of the Congo (DRC). This study should give an explanation about the change of antiplasmodial activity of the same plant depending on the location of harvest. The samples of *P. niruri* were analyzed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) for secondary metabolites characterizations such as of flavonoids, saponins and steroidal sapogenins and others phenolic compounds. The results revealed that UPN location presented more peaks (22) than Unikin (20), Kimwenza (15) and Kisantu (12). But Unikin location revealed more peaks (7) corresponding to major compounds than samples from others locations (UPN: 5; Kimwenza: 2 and Kisantu: 4). The peak 1 of UPN is higher (13.73) comparing to all peaks samples. The yellow-colored spots were present at all samples but those of UPN were more accentuated than all. In Kimwenza samples, two others colored spots (violet and blue) were presented. It suggested that *in vitro* antiplasmodial activity would be based on compounds eluted probably at the retention time around 22 min. Sometime the compounds eluted at 4.28 and 7.8 min contribute to *in vitro* antiplasmodial activity. The results revealed again the presence of the saponins or the steroidal sapogenins in *P. niruri,* made for the characterization by HPLC or by TLC probables flavonoids and presence of steroidal sapogenins.**

**Key words:** Thin layer chromatography (TLC), high performance liquid chromatography (HPLC), *Phyllanthus niruri*, callus.

## INTRODUCTION

Plants have been used in traditional medicine since a long time. About 13,000 plant species have been used as drugs throughout the world, and approximately 25% of the current materia medica are derived from plants in

form of teas, extracts, or pure substances (Adjanohoum, 1982; Oksman-Caldentey and Barz, 2002). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (Igbinosa et al., 2009). In the Democratic Republic of Congo (DRC), among the species used in the treatment against malaria, *Phyllanthus niruri* is well positioned for different previous studies on this plant (Pauwels, 1993; Tona et al., 1999; Cimanga et al., 2004). *P. niruri* is one of the most important medicinal plants used in different regions in the world for the treatment of various diseases such as jaundice, asthma, hepatitis, flu, dropsy, diabetes, fever causing by malaria (Kerharo and Adam, 1974; Ishimari et al., 1999; Paranjape, 2001) but its availability is drastically decreasing because of numerous harvests.

The antiplasmodial activity of various parts of *P. niruri* according to its geographical distribution showed that the biological activity of *P. niruri* depending on its environment of harvest (Soh et al., 2009).

The purpose of this study is to characterize the major chemical groups by (Thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) of the aerial parts, roots and calli of *P. niruri* from the four areas of DRC and, so as to give an explanation of the difference on the *in vitro* antiplasmodial activity of *P. niruri* obtained by Soh et al. (2009) according to the geographical distribution and to suggest a study chase on phenolic constituents and steroidal sapogenins respon-sibles for antiplasmodial activity in *P. niruri* plant.

## MATERIALS AND METHODS

### Plant material

For this work, the aerial parts of *P. niruri* were harvested from August to October 2007 respectively in 4 different areas of the DRC whose Kimwenza, Kisantu, Unikin and UPN. The localities Unikin and UPN correspond to the neighborhood of University of Kinshasa and of the neighborhood of National Pedagogic University. The distances between Unikin and Kimwenza, Kisantu and Kimwenza, Unikin and Kisantu, Unikin and UPN are 4, 116, 120 and 7 Km respectively. The geographic situations for those localities were illustrated in Figure 1 and Table 1. Callus was obtained by *in vitro* cell culture (Luyindula et al., 2004). The botanical identification was assured by M. Nlandu of the INERA (Institut National pour l'Etude et la Recherche Agronomiques), Herbarium – University of Kinshasa. The herbarium samples were deposited at the herbarium under the numbers respectively 72 bis (Kisantu), 66 (Kimwenza), 83 (Unikin) and 66 bis (UPN). All plant materials were dried at 40°C in GCA Precision Mechanical Convection Oven (USA) and reduced to powder, crushed in the mill Thomas (USA).

### Callus culture conditions

Fresh apical stems of *P. niruri* water were sterilized in 70% ethanol for 1 min and then in mercuric chlorid 0.125% (w/v) solution for 3 min. They were finally washed four times with sterile distilled water and aseptically cut in 0.5 cm segments. These segments were cultured in sterile polystyrene flasks containing 30 ml of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962; Hall, 1999) according to the nature of growth factors and the time of cultivation. The medium was supplemented with 1 ml of 1-naphtylacetic acid (NAA: 4 mg/ml) and 0.5 ml of 6-benzylaminopurine (BAP: 2 mg/ml) and albumen liquid of *Coco nucifera* L. (100 ml/l) (Ishimari et al., 1999). The initiated callus was routinely subcultured onto a fresh medium 2 to 3 weeks. Callus obtained were dried at 40°C in GCA Precision Mechanical Convection Oven (USA) and reduced to powder. Callus of 3 and 6 month-old were used for our analysis.

### Phytochemical screening: Chromatography

#### *Preparation of crude extracts and sample for TLC*

One of an equal mixture of 5-methoxyflavone $10^{-4}$ M and α-gluconolactone $10^{-4}$ M in methanol 80% at pH 2 is added to each sample (50 mg) followed by vortexing for 30 s. Treatment for 15 min twice at the ultrasonic agitation allows a better solubilization of the samples. At the 15 min interval, the samples were agitated manually to be returned to the ultrasonic agitation. The samples were then centrifuged at 13,400 rpm for 10 min using an Eppendorf centrifuge Minispin.

#### *Thin layer chromatography: Flavonoids and steroidal sapogenins analysis*

The TLC was performed like essentially described elsewhere (El Euch et al., 1998; Hostettmann and Marston, 1995). Each crude extract (0.5 ml) was separately acidified by addition with 0.5 ml of HCl (2N) in test tubes Soda (75×12×0.8 mm; Germany) (Bhatnagar et al., 1985; Zhang et al., 2011). 0.5 ml of distilled water was added in each test tube Soda after heating during 20 min at the bain-marie and then, 0.5 ml of ethyl acetate (99.8%) was also added for the separation of two stages (aqueous and organic phases).The organic phase was subjected to TLC over gel plates eluting with butanol-ethanol-water (40-10-20, v/v) for flavonoids or chloroform / methanol/water (v/v: 65-42.5-10) for steroidal sapogenins (Yung et al., 2005).

The mixture control containing quercetin $10^{-3}$M, kampferol $10^{-3}$ M and 5-Methoxyflavone $10^{-5}$ M in Methanol 80% (pH 2) was used for phenolic compound quantification. The plates were placed in a chromatographic tank containing 3 ml of butanol-ethanol-water for 1 h or in chloroform/methanol/water during 1 h 30 min (Yung et al., 2005).

The TLC plates were dried under fume hood. Observations of phenolic substances (flavonoids) were made first under 254–366 nm UV without application of any reagent and after application of 4-dimethylamino-cinnamaldehyde (DMACA) (2 g in 100 ml ethanol and 100 ml of concentrated HCl 6N). The liquid nitrogen was used for the eventual fluorescence stimulation of compounds. The revelation of spots for steroidal sapogenins was obtained after heating at 110°C under the Memmert Oven during 10 min after spraying triantimony chloride ($SbCl_3$) solution in HCl 12N (1/1; w/v) (Uematsu et al., 2004). Then, the plates were sprayed with solution of $H_2SO_4$ 10% in methanol.

#### *High performance liquid chromatography (HPLC)*

The HPLC analysis was carried out on a HPLC Waters 2695 Alliance (Germany) equipped with Alltech Altima C18 column (5 µm; 250×4.6 mm) and a diode array detector (Waters 2996). 18 µl of

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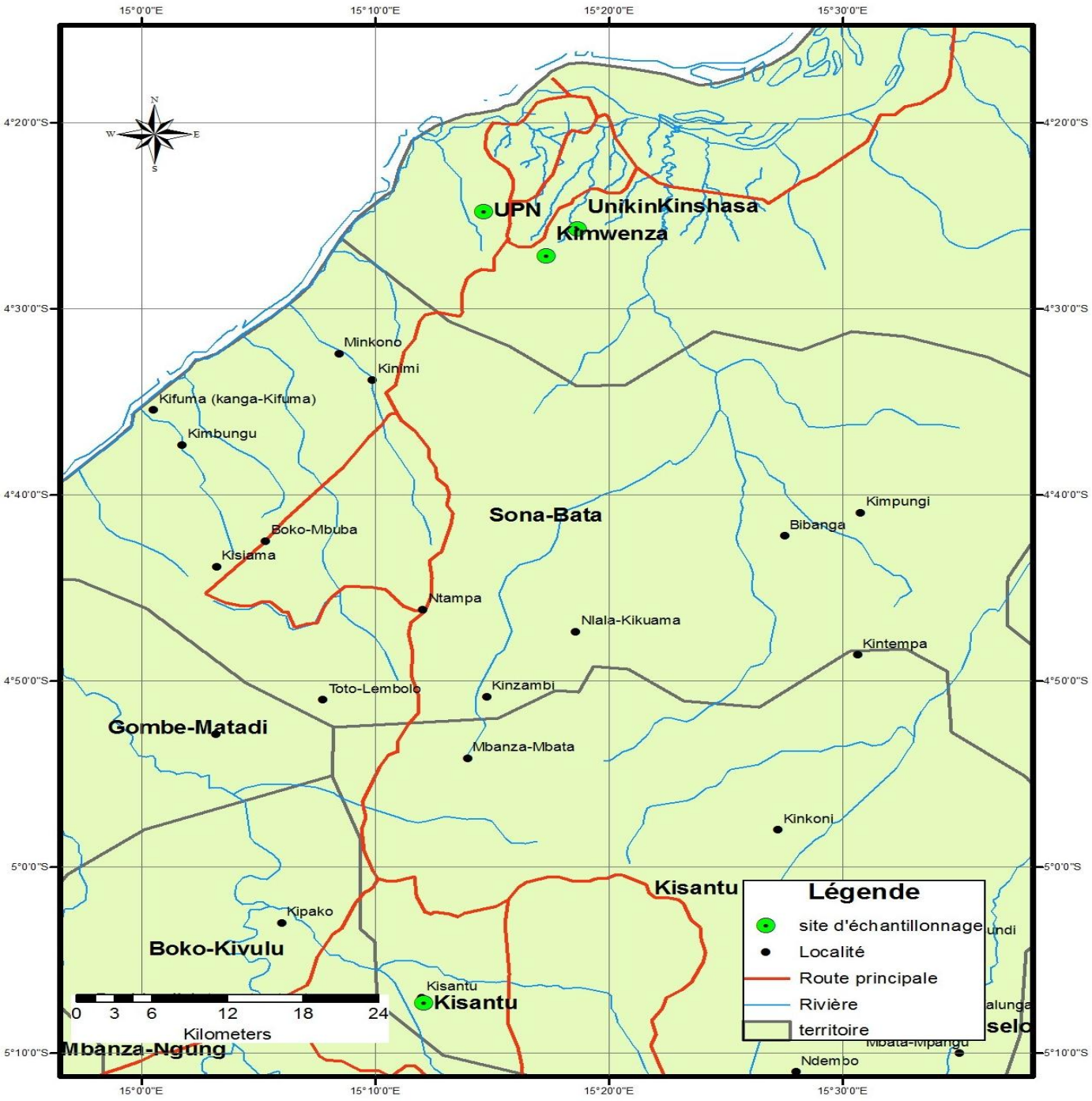


**Figure 1.** Geographical locations of *Phyllanthus niruri* harvested (Map established by M. Nlandu B.J.: Department of Soil and Hydrobiology, Division of Agronomy, CGEA/CRENK).

**Table 1.** The geographical data of *P. niruri* L. harvested sites samples.

| Localities geographical coordinates | UNIKIN | UPN | KIMWENZA | KISANTU |
|---|---|---|---|---|
| Altitude (m) | 471 | 509 | 493 | 409 |
| Latitude | 4° 25' 44.44"(East) | 4° 24' 18" (East) | 4° 27' 11.53"(East) | 5° 7' 48''(East) |
| Longitude | 15° 18' 39.6"(West) | 15° 31' 40.8"(West) | 15° 17' 18.09"(West) | 15°6'(West) |

Spring: M. Nlandu B.J.: Department of Soil and Hydrobiology, Division of Agronomy, CGEA/CRENK.

**Table 2.** Gradient elution HPLC of phenolic compounds in extracts of *P. niruri*.

| Time (min) | Solvant A (%) | Solvant B (%) |
|---|---|---|
| 0 | 22 | 78 |
| 7 | 24 | 76 |
| 17 | 40 | 60 |
| 25 | 100 | 0 |
| 30 | 100 | 0 |
| 35 | 22 | 78 |
| 40 | 22 | 78 |

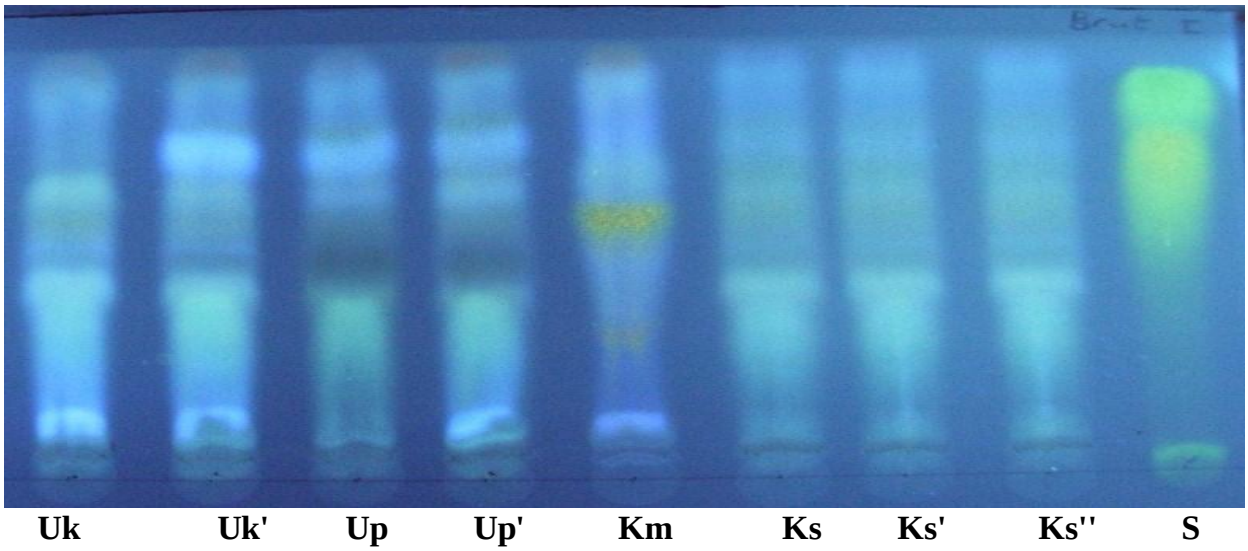


**Figure 2.** TLC at 366 nm in liquid nitrogen of crude extracts from Unikin *P. niruri (*Uk), UPN (Up and Up'), Kimwenza (Km) and, Kisantu's *P. niruri (*Ks, Ks' Ks''); S= control or standard.

each samples (pure or hydrolyzed extracts) were injected in the loop sample. The flow rate was 1 ml/min and the peaks were detected at 275 nm. Gradient elution was performed using acetonitrile (solvent A) and water with 1%HCl (solvent B) as memorized in Table 2 (Von Hoist et al., 2001).

#### *Calculation of relative area of peaks*

The area of each peak is proportional to the concentration of compound which is then dosed at the wavelength selected for analysis by reference to the control compounds. The relative area of each peak was calculated by the relation (Macheix et al., 2005; Audigié et al., 1995):

$A_x$ = area of peak × in the sample/area of peak × in the internal standard

## RESULTS AND DISCUSSION

Results from the chemical analysis of hydrolyzed extracts from *P. niruri* parts or callus extracts are illustrated in Figures 2, 3 and 4.

### Thin layer chromatography

#### *Flavonoids compounds*

The Figures 2, 3 and 4 shown chromatograms of the hydrolyzed extracts of *P. niruri* (stems, leaves, roots and callus respectively). Figures 2 and 3 showed that all aerials part of *P. niruri* from Unikin (Uk), UPN (Up), Kimwenza (Km) and Kisantu (Ks) contain flavonoids revealed in bleu by fluorescence or by UV in liquid nitrogen. *P. niruri* from Km presented again the yellow spot corresponding to control (quercetin) (Mabry et al., 1970). In nitrogen phosphorescence (366 nm) control and *P. niruri* from Kimwenza location no showed coloration or fluorescence.

Figure 4 revealed the presence of flavonoids regarding the hydrolyzed extracts of *P. niruri* from Unikin and UPN. Several of those compounds correspond at same level than control compounds. Figure 5 showed the presence of flavonoids from roots or callus of *P. niruri*. Between root from Unikin and Kisantu and callus obtained with apical


**Figure 3.** TLC of crude extracts at 366 nm in liquid nitrogen phosphorescence of crude extracts from Unikin *P. niruri (*Uk), UPN (Up and Up'), Kimwenza (Km) and, Kisantu's *P. niruri (*Ks, Ks' Ks''); S= control or standard.

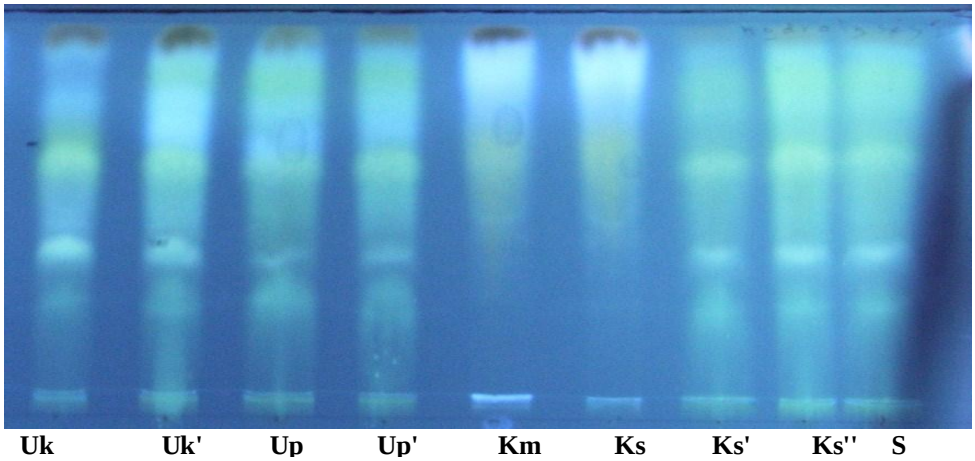


**Figure 4.** TLC at 366 nm in liquid nitrogen of hydrolyzed extracts from Unikin *P. niruri (*Uk), UPN (Up and Up'), Kimwenza (Km) and, Kisantu's *P. niruri (*Ks, Ks' Ks''); S= control or standard.

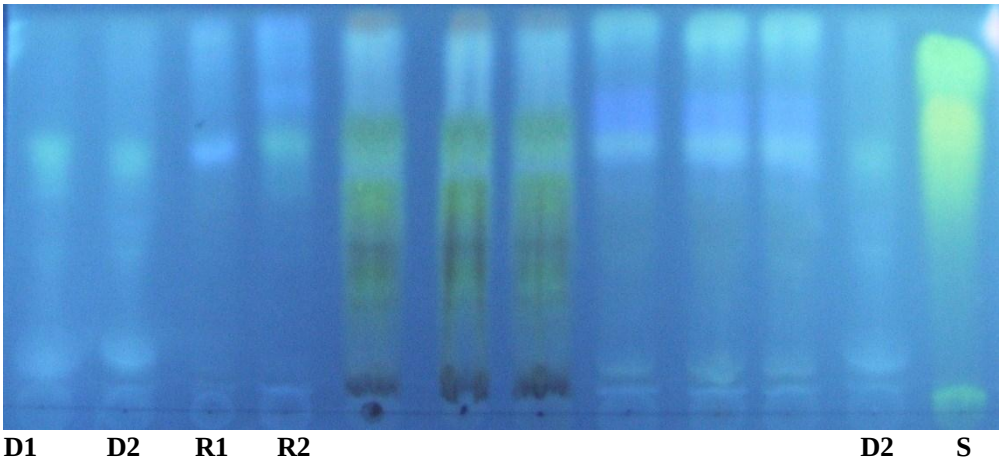


**Figure 5.** TLC at 366 nm in liquid nitrogen of hydrolyzed extracts from Callus of 3 month-old (D1), Callus 6 month-old (D2), Roots Unikin (R1) and Roots Kisantu (R2), S= control or standard.

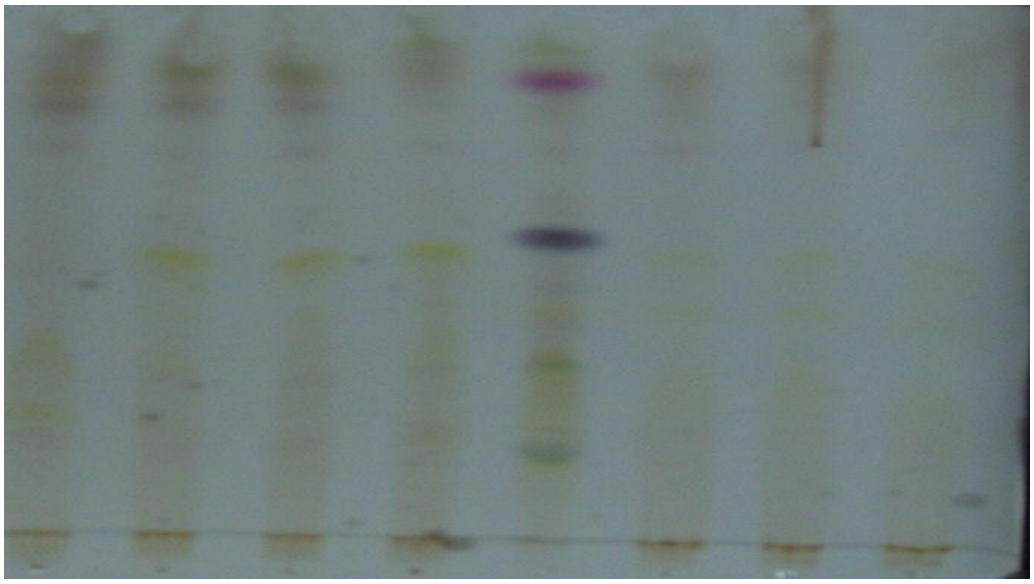


**Figure 6.** TLC of *P. niruri* L. crude extracts from Unikin *(*Uk), UPN (Up and Up'), Kimwenza (Km) and, Kisantu's *P. niruri (*Ks, Ks' Ks'') after spraying Tri-antimony chloride ($SbCl_3$) solution.

stem explants no coloration spots difference was observed.

### *Sapogenins steroids*

Figure 6 illustrated saponins and steroidal sapogenins obtained in *P. niruri* plant using hydrolyzed extracts from stems with their leaves after TLC investigation. All samples of aerials parts of *P. niruri* showed the presence of steroidal sapogenins. These steroidal sapogenins revealed by yellow-colored spots (all samples) and violet, bleu or yellow-green spots depending on that degree of unsaturation of the molecules (Figure 6). In Unikin and UPN samples, yellow spots are more accentuated than of Kimwenza and Kisantu samples. Kimwenza samples presented others colored spots (violet, blue, green and yellow-green). These colored spots are absents in Unikin, UPN and Kisantu samples.

The violet spot in Kimwenza sample may be due to lipids and the blue spot corresponded to that obtained by Uematsu et al. (2004). These authors have identified the blue spot compounds as hydroxymethylfurfral and its ethyl adducts. The same authors reported that yellow-green colored spot obtained with standard sarsasapogenin was characteristic of steroidal sapogenin after application of anisaldehyd reagent. The violet colored spot from Kimwenza's sample was obtained also by Yung et al. (2005) with the butanol extract from aqueous layer of aerials portions of *Pleurospermum kamtschaticum* after several extraction procedures.

In roots and callus obtained from stems, the results revealed the absence of steroidal sapogenins. The TLC plates were not revealed specific coloration by the application of 10% sulfuric acid ($H_2SO_4$) solution on the plate after splaying the tri-antimony chloride ($SbCl_3$). These sapogenins compounds from *P. niruri* as diosgenin, ruscogenin and other triterpenic genins would explain several effects of *P. niruri* on diabetes, anti-inflammatory activity, flu, arthritis, hepatitis treatment (Uematsu et al., 2004; Huang et al., 2008; Kang et al., 2011). It is noted that in general, sapogenins are bio-active principles rather than the saponins themselves (Yung et al., 2005).

### High performance liquid chromatography

The results obtained by high performance liquid chromatography analysis of hydrolyzed extracts of *P. niruri* from 4 agglomerations were presented in Figures 7a to 7h. For comparison of chromatograms of hydrolyzed extracts from *P. niruri* obtained by HPLC, it was revealed that the number of major peaks differs depending on geographical location. If consider as the major peak with a relative area equal to or greater than 2.5: Unikin samples have got more than seven major peaks with their respective relative areas 5.21, 4.82, 4.11, 2.55, 5.65, 2.71 and 3.81 which eluted respectively at 3.83, 4.28, 4.7, 5.59, 7.81, 26.5 and 27.13 min (Figure 7a). Five major peaks for UPN samples with the following relative areas: 7.58, 4.78, 3.24, 3.32 and 4.98 eluted respectively at 3.84, 4.28, 4.7, 5.59 and 7.8 min (Figure 7b). Chromatograms of Kimwenza (Figure 6c) and Kisantu (Figure 7d) samples

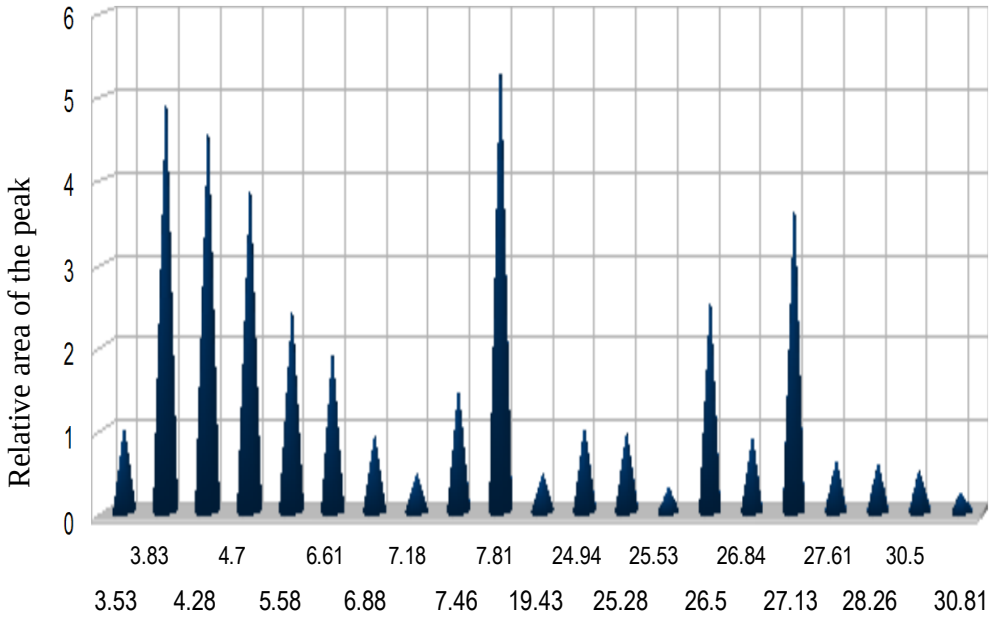


**Figure 7a.** HPLC analysis of some samples from to 4 agglomerations (Unikin, UPN, Kimwenza and Kisantu).HPLC of hydrolyzed extract from aerial parts of *P. niruri* from Unikin.

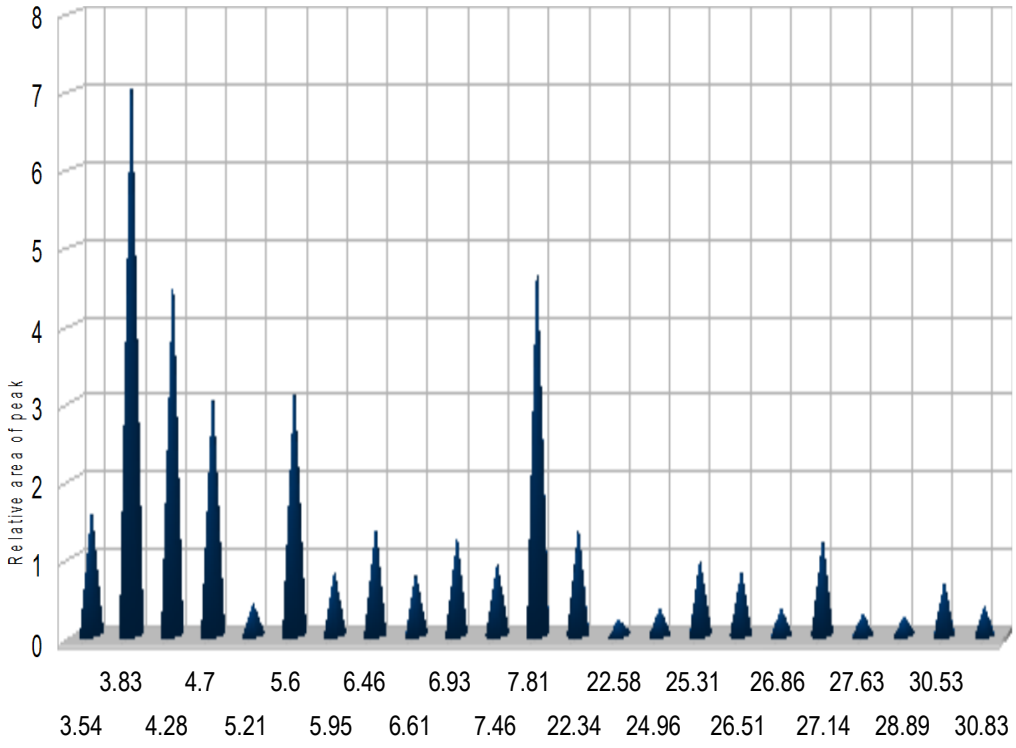


**Figure 7b.** HPLC of hydrolyzed extract from aerial parts of *P. niruri* L. from UPN.

revealed respectively 2 and 4 major peaks. The peaks from Kimwenza have the retention times as 4.28 and 7.8 min with their relative areas as 13.73 and 7.27. The Kisantu samples presented 4 major peaks at the retention times as 6.37, 4.04, 2.5 and 3.89 who have eluted to the following retention times 3.83, 4.28, 5.58

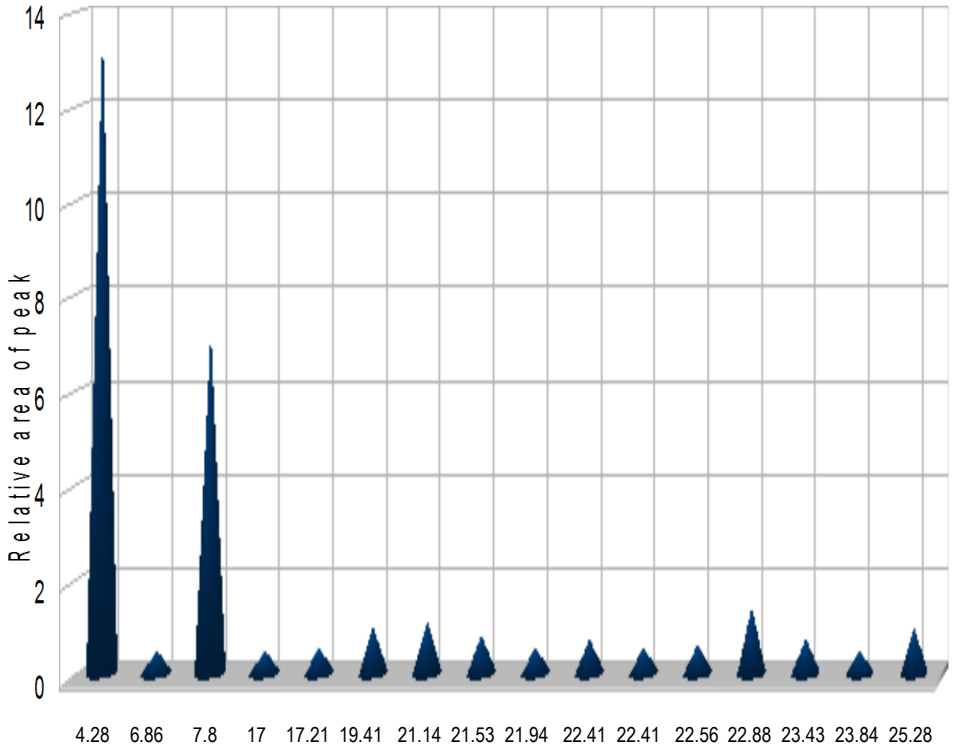


**Figure 7c.** HPLC of hydrolyzed extract from aerial parts of *P. niruri* L. from Kimwenza.

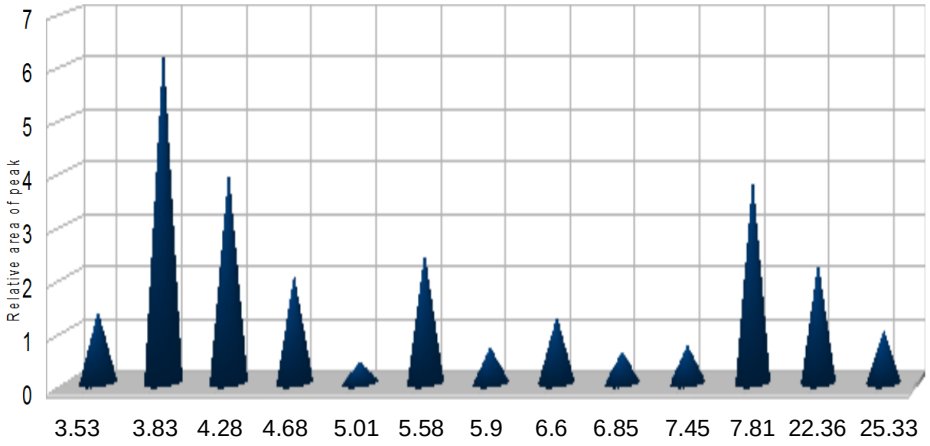


**Figure 7d.** HPLC of hydrolyzed extract from aerial parts of *P. niruri* from Kisantu.

and 7.81 min.

The major peak of which products settled at 4.28 min is present in all samples of *P. niruri* (stems with their leaves, roots and callus) (Figure 7). However, the relative area of those peaks is different. For example, callus of 3 month-old (Figure 7g) showed a major peak at 4.28 min which exceeds all peaks of all samples with the relative area of 10.41. The callus samples showed difference in number of major peaks while the major peaks are the same. The root samples from Unikin (Figure 7e) and Kisantu (Figure 7f) showed difference neither in number of peaks nor in number of major peaks.

This finding is also observed on TLC chromatograms. However, if the spots obtained (Figures 2, 3, 4 and 5) concord, Kimwenza shows yellow spot corresponding to those of quercetin control (Figure 1). That is also believed to differ from the compounds in different samples. This difference for the same species of *P. niruri* can be

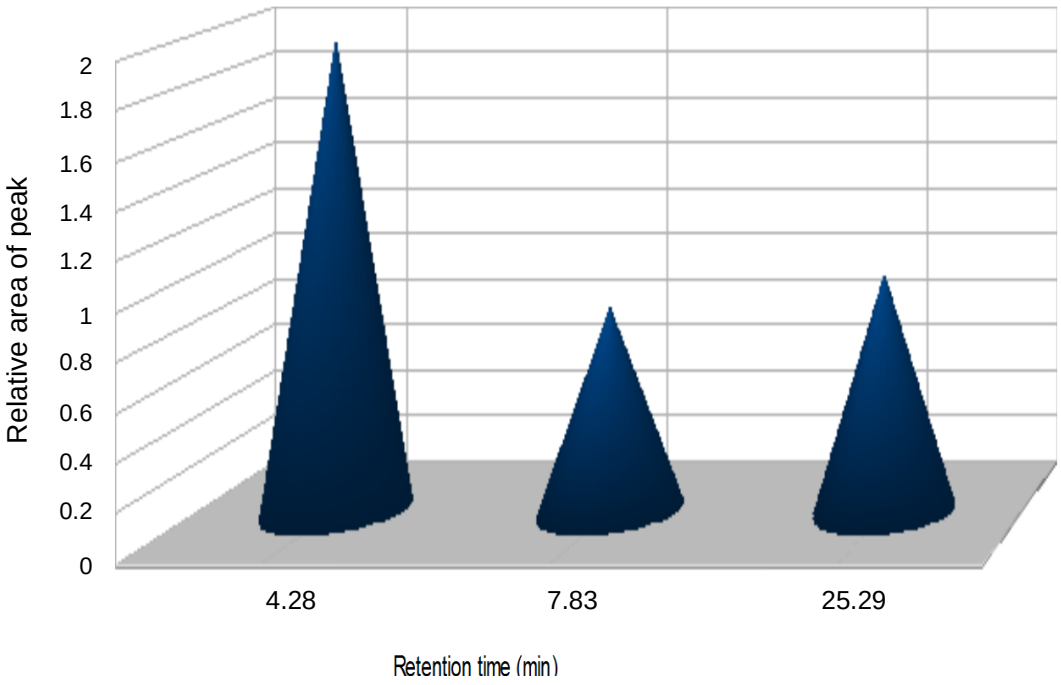


**Figure 7e.** HPLC of hydrolyzed extract from *P. niruri* Roots R1 (Unikin).

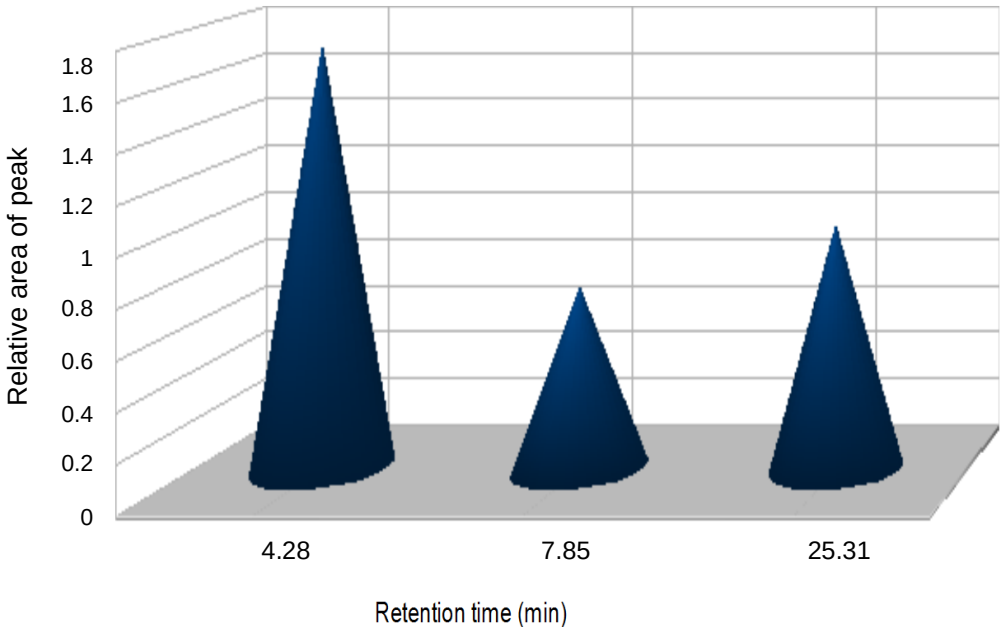


**Figure 7f.** HPLC of hydrolyzed extract from *P. niruri* Roots R2 (Ks).

explained according to the variety and the evolution stage physiology (Macheix et al., 2005; Hess, 1975; Wilkins, 1984).

In the first, Macheix et al. (2005) reported that the absorption spectrum of chlorogenic acid (λmax = 328 nm, marker shoulder around 300 nm) reflects number of natural esters of hydroxycinnamic acid, that of (+)-catechin (maximum at 280 nm) will be found in condensed tannins, that of quercetin (λmax = 375 nm, with a second maximum around 265 nm) reflects number of flavonols and their glycosylates derivatives. On the other hand, Ludwig-Müller et al. (2008) reported that four wavelengths (280,

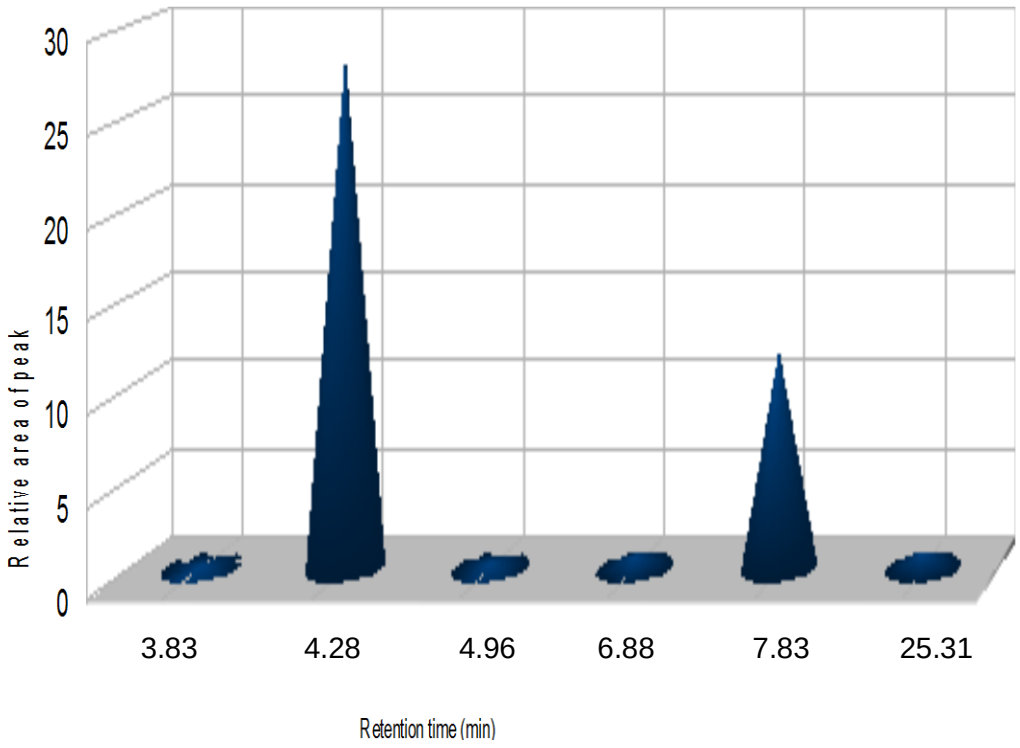


**Figure 7g.** HPLC of hydrolyzed extract from *P. niruri* Callus D1 (3 months).

325, 375 and 450 nm) correspond to the aromatic substances, derivatives of cinnamic acid, flavonoids and many carotenoids and other pigments.

Given our results, we can say that the peaks number 11 (Figure 7a), 13 (Figure 7b) and 12 (Figure 7d) correspond to these phenolic compounds although their change of maxima: $max_1$ = 365.1 nm, $\lambda max_2$ = 266.3 nm for peaks 13 and 12 respectively from UPN (Figure 6b) and Kisantu (Figure 7d) samples; $\lambda max_1$ = 366.3 nm and $\lambda max_2$ = 255.7 nm for peak number 12 from Unikin samples (Figure 7a).

The presence of (+)-catechin or the condensed tannins could be confirmed in the sample content peaks with maximum of wavelength at 280.6 nm (peak n°16 for Unikin sample (Figure 7a) and in the sample containing peak n°3 with λmax = 281.8 nm from Kimwenza (Figure 7c) for further analysis by nuclear magnetic resonance (NMR). Previously, Soh (2008) reported that a polyphenol as ellagic acid was identified in the active fractions of three medicinal plants whose *Chrozophora senegalensis*, *Sebastiania chamaelae* and *P. niruri.* The study of antimalarial properties of ellagic acid has shown high activity *in vitro* against all *P. falciparum* strains used.

The antiplasmodial activity of different *P. niruri* according to their geographical distribution obtained by Soh et al. (2009) showed that crude extracts of *P. niruri* aerial parts from Kisantu presented a higher antiplasmodial activity than to those of Unikin and Kimwenza. In the case of root extract no difference was observed and all samples presented a low antiplasmodial activity. The antiplasmodial activity could be explained by the presence of a particular compound with retention time around 22 min (Figures 7a to 7h). The peak n°12 of Kisantu sample (Figure 7d) has two maxima at 255.3 nm and at 366.3 nm with a relative area of 2.32 and peak exceed all relative area of compounds those settle around 22 min for other samples (UPN and Kimwenza). For Unikin sample (Figure 7a), there is no compound that eluted at 22 min but at 19.43 min (peak 11); compound would be comparing to quercetin with an absorption spectrum containing two maxima (255.7 and 366.3 nm). For UPN sample, there are two compounds those elute around 22 min such as 22.34 min (peak 13) and 22.58 min (peak 14). The peak 13 with an absorption spectrum carrying two maxima (266.3 and 365.1 nm) is similar to the peak 12 of sample from Kisantu but has a lower relative area of 1.45 than this one.

Some effort were made to identified same compounds: Peak 11 Unikin was identified to quercetin, peak 13 UPN was identified to chlorogenic acid and peak 12 Kisantu was identified to catechin by comparing the UV spectra to those of respective reference products. This may also explain the lower antiplasmodial activity of crude extracts from samples of UPN than the crude extracts from samples of Kisantu or Kimwenza obtained by Soh et al. (2009).

These authors illustrated again that roots of *P. niruri* showed a very weak antiplasmodial activity (IC50>50 ug/

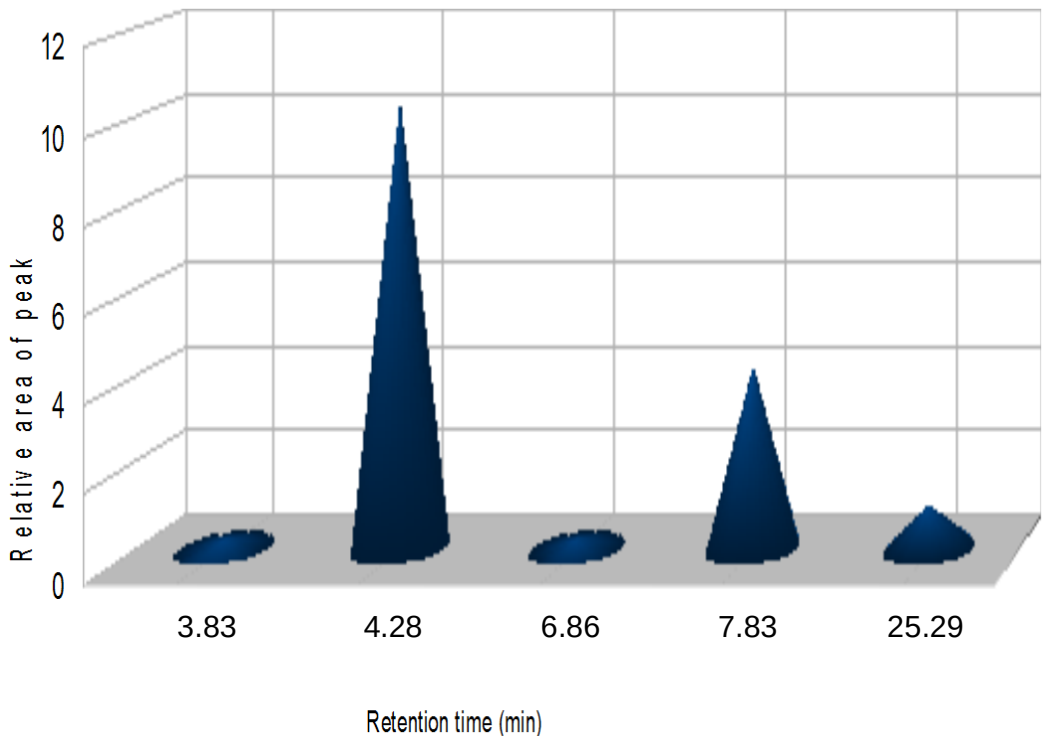


**Figure 7h.** HPLC of hydrolyzed extract from *P. niruri* callus D2 (6 months).

ml) than to those stems with their leaves or than those of whole plants. It is explained that the phenolic compounds in roots are identical, which we have just shown by HPLC of crude extracts from roots. Nevertheless, it can search for compounds which the peaks were eluted at 4.28 and 7.8 min could contain compounds that have effects on *Plasmodium falciparum*. These peaks are found in all aerial parts of *P. niruri*. Considering the profile of the peaks of callus which are almost similar to those of roots, we can suggest that callus would present the same *in vitro* antiplasmodial activity than roots. However, Cimanga et al. (2004) and Luyindula et al. (2004) have shown that *in vitro* antiplasmodial activity of callus varied with the number of months or plant parts used *in vitro* cell culture.

## Conclusion

Through the present work, we found that effectively the metabolites profiles of *P. niruri* change with its geographical distribution certainly because of the change in soil composition or the environmental conditions. These factors can alter yield of the secondary metabolism pathways. All extracts from *P. niruri* aerial parts (stems with leaves) were exhibited in the HPLC profiles of hydrolyzed extracts indicated that *P. niruri* from UPN was able to synthesize more phenolic constituents than *P. niruri* from Unikin, Kisantu or Kimwenza.

Results obtained in the present study confirm the difference of *in vitro* antiplasmodial activity of *P. niruri* according to its geographical location. Roots revealed the same concentration of compounds and the same antiplasmodial activity. Callus showed difference in the secondary metabolites composition and the difference in the *in vitro* antiplasmodial activity. Those results are confirmed by TLC and the extracts showed presence of flavonoids compounds and steroidal sapogenins. These compounds, flavonoids, saponins and steroidal sapogenins, would be explained the many effects which are played by *P. niruri* in the treatments of several diseases. Thus, studies could be continued for the characterization of flavonoids or others compounds responsible of antiplasmodial activity and steroidal sapogenins who would be presents in *P. niruri*.

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**REFERENCES**

Adjanohoum M (1982). Man and the African medicinal plant. In Develop the natural environment. Revue du livre, Afrique-Océan Indien n°Double 66-67, éd. Notre Librairie: 51-58.

Audigié Cl, Dupont G and Zonszain F (1995). Principles methods of biochemical analysis. Tome I, Ed. Doin, Paris p.207.

Bhatnagar S, Dudouet B, Ahond A, Poupat C, Thoison O, Clastres A, Laurent D, Potier P (1985). Marine invertebrates of new Caledonian lagoon IV *. Saponins and sapogenins of cucumber, Actinopyga flammea, Bulletin of the Chemical Society of France, Bull. Chemical Society France 1:124-129.

Cimanga RK, Tona L, Luyindula N, Mesia K, Lusakibanza M, Musuamba CT, Apers S, De Bruyne T, Van Miert S, Hermans N,Totte J, Pieters L, Vlietinck AJ (2004). *In vitro* antiplasmodial activity of callus culture extracts and fractions from fresh apical stems of *Phyllantus niruri* L ( Euphorbiaceae); Part 2. J. Ethnopharmacol. 95:399-404.

El Euch C, Jay-Allemand C, Pastuglia M, Doumas P, Charpentier JP, Capelli P, Jouanin L (1998). Expression of antisens gene chalcome synthase in transformed hybrid Walnut microcuttings: Effect on flavonoid content and rooting percentage. Plant Mol. Biol. 38:467-479.

Hall RD (1999). Plants Cell Culture Protocols, Methods in Molecular Biology. Human Press, Totowa, New Jersey (USA) 111:421.

Hess D (1975). Plant physiology. Molecular, Biochemical, and Physiological fundamentals of Metabolism and Development. Spring-Verlag, New York, p. 333

Hostettmann K, Marston A (1995). Saponins. Chemistry and Pharmacology of Natural products. Cambridge University Press, Great Britain, p. 548.

Huang YL, Kou JP, Ma L, Song JX, Yu BY (2008). Possible mechanism of the anti-inflammatory activity of Ruscogenin: role of Intercellular adhesion molecule-1 and Nuclear Factor-*k*B; J. Pharmacol. Sci. 108: 198-205;

Igbinosa OO, Igbinosa EO, Aiyegoro OA (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). Afr. J. Pharm. Pharmacol. 3(2):058-062.

Ishimari K, Yoshimatsu K, Yamakawa T, Kamada H, Shimomomura K (1999). Genetic Transformation of *Phyllanthus nirui* L. (*P. amarus*). Springer-Verlag Berlin Heidelberg. Biotech. Agric. For. 45:237-248.

Kang TH, Moon E, Na Hong B, Chol SZ, Son M, Park JH, Kim SY (2011). Diosgenin from Dioscorea nippponica ameliorates diabetic neuropathy by inducing nerve growth factor. Biol. Pharm. Bull. 34(9):1493-1498.

Kerharo G, Adam JG (1974). Senegalese traditional pharmacopoeia. Medicinal Plants and Toxic, Vigot Frères pp.427-428.

Ludwig-Müller J, Georgiev M, Bley T (2008). Metabolite and hormonal status of hairy root cultures of Devil's clav (*Harpagophytum procumbens*) in flasks and in a bubble column bioreactor. Process Biochem. 43:15-23.

Luyindula N, Tona L, Lukembila S, Tsakala M, Mesia K, Musuamba CT, Cimanga RK, Apers S, De Bruyne T, Pieters T, Vlientick A (2004): *In vitro* antiplasmodial activity of callus extracts from fresh apical stems of *Phyllantus niruri* L (Euphorbiaceous); Part 1. Pharm. Biol. 42(7):1-7.

Mabry TJ, Markham KR, Thomas MB (1970). The systematic identification of flavonoids. Spring-Verlag, New York; p.354.

Macheix JJ, Fleuriet A, Jay-Allemand C (2005). Phenolic Compounds plants: An example of secondary metabolites of economic importance, Ed. Polytechnic and university romandes presses, Lausanne, Italie p.192

Murashige T, Skoog F (1962). A reviewed medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15: 473-479.

Oksman-Caldentey KM, Barz WH (2002). Plant biotechnology and Transgenic plants. Marcel Dekker, New York, Basel 347-371.

Paranjape P (2001). Indian Medicinal Plants:Forgotten Healers. Chaukhamba Sanskrit Pratisthan, Dheli p.48

Pauwels L (1993). NZAYILU N'ti. Guide trees and shrubs in the area of Kinshasa – Brazzaville. JBNB 4:459.

Soh PN (2008). Search for new compounds with antimalarial activity from different traditional pharmacopoeia (Soh PN, Witkowski B, Olagnier D, Nicolau ML, Garcia-Alvarez MC, Berry A, and Benoit-Vical F: *In vitro* and *in vivo* properties of ellagic acid in malaria), Thèse de Doctorat, Université de Toulouse, France, p.164

Soh PN, Banzouzi JT, Mangombo H, Lusakibanza M, Bulubulu FO, Tona L, Diamuini AN, Luyindula SN, Bénoit-Vical F (2009). Antiplasmodial activity of various parts of *Phyllanthus niruri* according to its geographical distribution. Afr. J. Pharmacol. 3(12):598-601.

Tona L, Ngimbi N, Tsakala M, Messia K, Cimanga RK, Apers S, De Bruyne T, Pieters L, Totté J, Vlietinck AJ (1999). Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. J. Ethnopharmacol. 93:27-32.

Uematsu Y, Hirata K, Suzuki K, Iida K, Kamata K (2004). Investigation of Spectrophotometrically Determined Substances in Yucca Extract by GC/MS, TLC and On-culumn Injection GC. J. Food Hyg. Soc. Jpn. 45(3):141-145.

Von Hoist C, Hammer S, Anklam E (2001). Factorial design approch for optimization of high performance liquid chromatography conditions of tropane alkaloids determination. Deutsche Lebensmittel-Rundschan 97(1):1-7.

Wilkins MB (1984). Advanced Plant Physiology. Pitman press, London p.514

Yung HJ, Kim SG, Nam JH, Park KK, Chung WY, Kim WB, Lee KT, Won JW, Choi JW, Park HJ (2005). Isolation of Saponins with the inhibitory Effect on Nitric Oxide, Prostaglandin E2 and Tumor Necrosis Factor-α Production from *Pleurospermum kamtschaticum*. Biol. Pharm. Bull. 28(9):1668-1671.

Zhang ZQ, Chen JC, Yan J, Qui MH (2011). Three Steroids with Unique Structural Feature of 5β-Spirostan-1β,3β,17α-trihydroxyl from *Reineckia carnea*. Chem. Pharm. Bull. 59(1):56-56.