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Phytochemical screening of Saye, a traditional herbal remedy for malaria

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ABSTRACT

A phytochemical assay was conducted to establish the chemical profile of "Saye", a mixture of leaf of *Cassia alata*, root of *Cochlospermum planchonii* and whole plant of *Phyllantus amarus*, used as antimarial remedy. Water and organic extracts were prepared. Characterization of phytoconstituents using specific chemical reagents was performed in tubes, by thin layer chromatography and by high performance liquid chromatography. Steroids and/or triterpenes, cathechic tannins were identified in the decocted and the macerated water extracts of "Saye". An anthraquinone with a retention time Rt corresponding to 3.34 min was identified by the HPLC analysis.

Keywords: Chemical profile, anthraquinones, steroids, triterpenes, tannins.

INTRODUCTION

The African Region continues to shoulder the heaviest malaria burden with 214 million new cases of malaria and 438 000 deaths (World malaria report, 2015). With the resistance of Anopheles mosquito to

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insecticides and *Plasmodium* species to most of the drugs currently in use (White, 2004), there is a call for a return to natural products as antimalarial drugs or as sources of inspiration for the development of novel drugs (Ginsburg et al., 2011). In Burkina Faso, a traditional preparation named "Saye", manufactured by mixing dried rhizomes of *Cochospermum planchonii*, leaves of *Cassia alata* and whole plant of *Phyllanthus amarus* is used for the treatment of uncomplicated malaria (Dakuyo et al., 2015). Extracts from each individual plant contained in Saye or their 2 x 2 mixture were found to inhibit malaria parasites (Da et al., 2014). In the framework of a clinical study, there was a need of chemical standardisation of "Saye". The objective of the study was to establish the chemical profile of "Saye" and its content plants.

MATERIALS AND METHODS

Plant materials

The leaf powder of *Cassia alata*, the root powder of *Cochlospermum planchonii*, the whole plant powder of *Phyllantus amarus* and "Saye", were all supplied by Phytofla laboratory, with market authorization number: 0497920050N110000/2011.

Extracts preparation

Crude extracts from individual plant powder (15 g) and "Saye" (15 g) were prepared by maceration during 24 h successively with chloroform (150 mL), methanol (150 mL) and distilled water (150 mL). Organic extracts obtained were dried with an evaporator Buchi R110 type MKE 6540/2. Water extracts from individual plant powder (50 g) and "Saye" (50 g) was also prepared by maceration (24 h) or decoction (10 min) with water (1 L). After filtration, all water extracts were frozen at -20 °C and freeze-dried using a FTS Systems Dura-Dry MPII®, Floor model freeze dryer. Aliquots (1 g) of these extracts were re-dissolved in 30 mL of distilled water, acid hydrolyzed under reflux for 30 min and then extracted (liquid-liquid extraction) with chloroform.

Phytochemical analysis of the extracts by qualitative methods

The detection of phytoconstituents such as alkaloids, flavonoids, steroids, tannins and saponins in the extracts was performed in tubes according to Ciulei (1982) method.

Phytochemical analysis of the fractions by thin layer chromatography (TLC)

TLC was carried out on a 60 F254 silica gel plate in glass, Merck (20 cm x 10 cm). Hydrolyzed and non-hydrolysed extracts were spotted on the plate and developed in 2 different eluent systems: (a) chloroform/acetone (20:10), (b) ethyl acetate/formic acid/acetic acid/water (50:5.5:5.5:13). The various spots were visualized by exposure of the plates to Neu, Keddee, Anisaldehyde sulfuric acid, ferric chloride ($FeCl_3$), Liebermann-Burchard, Dragendor'ff and ammonia aqueous solution 25% reagents. A TLC monitoring of the chloroform extract by liquid-liquid extraction was done and the main compounds purified by high performance liquid chromatography (HPLC).

RESULTS

Phytochemical profile of the extracts by the qualitative method

Over all, "Saye" extracts were found to contain esters, glycosides, steroids and /or triterpens, flavonoids, carbohydrates, gallic tannins, carotenoids, saponins, flavonic aglycones, anthocyanosides, cardenolides, anthraquinones and reducing compounds. Emodols were more available in *Cassia alata* leave extracts. Flavone aglycones were present in *Cochlospermum planchonii*. Glycosides and anthocyanosides were present in the hydrolyzed phase of the methanolic and aqueous fractions of each plant. Flavonosides as well as cardenolides were present in *Cochlospermum planchonii*. Anthracenosides were more available in *Cassia alata* leaves.

Gallic tannins, saponins, reducing compounds and carbohydrates were present in the non-hydrolyzed methanolic fraction and in each plant aqueous extract (Table 1).

Phytochemical profile of the extracts by TLC analysis

Steroids and/or triterpenes, cathechic tannins and anthraquinones were identified in the decocted and the macerated water extracts of "Saye". The DCM/MeOH extract of "Saye" has also shown the presence of steroids and/or triterpenes. Steroids and/or triterpenes were found in the decocted and macerated extracts of each plant whereas anthraquinones and cathechic tannins were only found in *Cassia alata* leaves and the whole plant of *Phyllanthus amarus* (Table 2).

Identification of the chemical markers from TLC profiling of the fractions

Three main spots with different Rfs were identified from TLC analysis, one from each plant: Rf = 0.59, (*Cochlospermum Planchonii),* Rf = 0.75 (*Cassia alata)* and Rf = 0.90 (*Phyllanthus amarus*). The compound from *Cassia alata* purified by HPLC was identified as an anthraquinone with a retention time t_R = 3.34 min (Figure 1). The spots (Rf = 0.59 and Rf = 0.90) have the characteristics of steroids and triterpenic compounds.

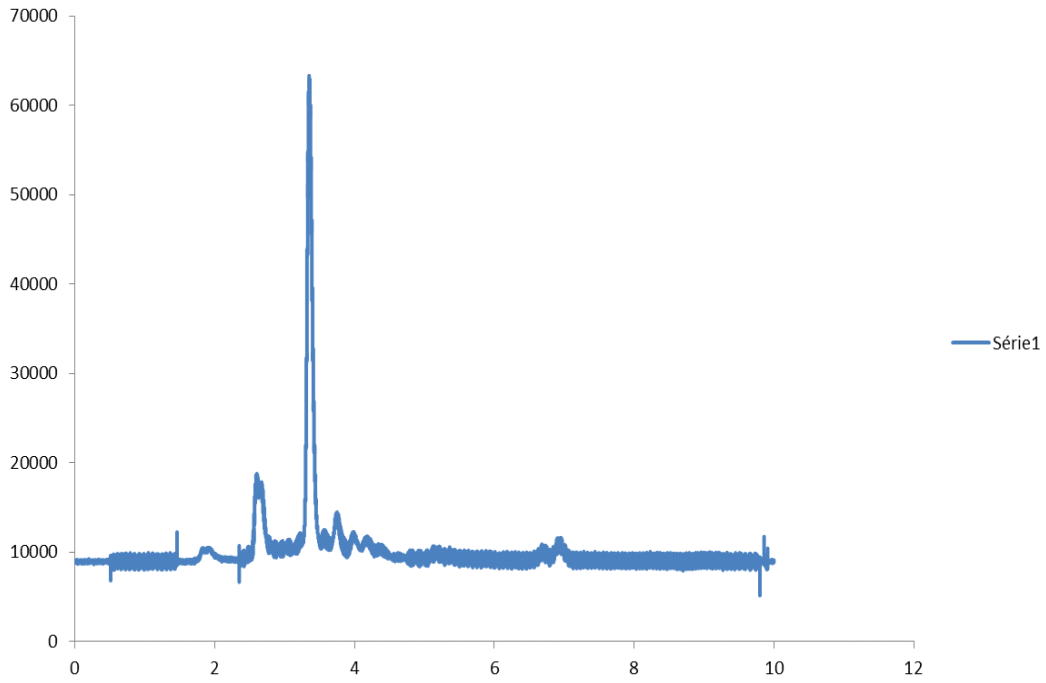

Figure 1: HPLC chromatogram of chloroform extract. Mobile phase in isocratic mode: acetonitrile-0.1% (v/v) formic acid in water (3:2); Flow rate: 1 mL/min; detection wavelength: λ = 250 nm.

Table 1: Compounds identified in the extracts by qualitative phytochemical screening.

Compounds	***Tisane Saye*** *(Decocted extract)*	***Phyllantus amarus***	***Cassia alata***	***Cochlospermum planchonii***
Chloroformic extract				
Steroids and/or triterpenoids	++	++	++	++
Flavone Aglycones	ND	ND	ND	+
Emodols (Anthracenoside and Anthracenoside Aglycone)	++	ND	++	ND
Alkaloids	ND	ND	ND	
Coumarines and /or coumarin derivatives	ND	ND	ND	ND
Carotenoids	+	+	+	++
Hydrolyzed aqueous and methanolic Fraction				
Glycosides	++	++	++	++
Flavonosides	ND	ND	ND	+
Anthracenosides	++	ND	++	ND
Coumarines derivatives	ND	ND	ND	ND
Cardenolides	ND	ND	ND	+
Anthocyanosides	+	++	++	+
Non-hydrolyzed aqueous and methanolic fraction				
Tannins	++	++	+	++
Saponins	++	+	+	++
Alkaloids	ND	ND	ND	ND
Reducing compound	++	++	++	+
Carbohydrates (Oses)	+	+	+	++
Carbohydrate (polyoses)	++	ND	ND	++

ND=Not detected; + = present; ++ = more presence.

Table 2: Chemical profile of Saye established by thin layer chromatography analysis.

Compounds	***Cassia alata***			***Phyllanthus Amarus***			***Cochlospermum planchonii***			**Tisane saye**		
	DCM/MeOH	**M**	**d**	**DCM/MeOH**	**M**	**D**	**DCM/MeOH**	**m**	**d**	**DCM/MeOH**	**m**	**d**
Alkaloid	nd	Nd	nd	nd	Nd	Nd	nd	nd	nd	nd	nd	nd
Flavonoid	nd	Nd	nd	nd	Nd	Nd	nd	nd	nd	nd	nd	nd
Steroid and/or triterpene	++	+	+	++	+	+	+	+	+	++	+	+
Tanin	+	Nd	+	+	++	++	+	nd	nd	+	+	+
Anthraquinone	+	++	+	nd	Nd	Nd	nd	nd	nd	nd	++	++
Cardenolide	nd	Nd	nd	nd	Nd	Nd	nd	nd	nd	nd	nd	nd

nd= not detected; DCM /MeOH = Dichloromethane/Methanol extract; + = present; ++ = more present

DISCUSSION

The main chemical groups found in "Saye" are tannins, anthraquinones, steroids and/or triterpenes. These chemical compounds, which were also found in the extracts of the individual plant, may be supportive of the antiplasmodial activity of the extracts (Da et al., 2014). In most studies, flavonoids were reported in the three plants: *Phyllanthus amarus* (Hemant et al., 2013); *Cochlospermum planchonii* (Nafiu et al., 2011); *Cassia alata* (Matthew et al., 2013). Previous works have also revealed the presence of tannins in the whole plant of *Phyllanthus amarus* (Obianime et al., 2009), *Cochlospermum planchonii* (Ezeja et al., 2010) and in the leaves of *Cassia alata* (Awomukwu et al., 2015). As in previous studies (Matthew et al., 2013) anthraquinones were found in abundance in the leave extracts of *Cassia alata* and in "Saye". Triterpenes were found in *Phyllanthus amarus* (Hemant et al., 2013), *Cochospermum planchonii* (Nafiu et al., 2011) and *Cassia alata* (Kayembe et al., 2012). Steroids were present in *Phyllanthus amarus* (Hemant et al., 2013), *Cochospermum planchonii* (Nafiu et al., 2011) and *Cassia alata* (Matthew et al., 2013). Cochloxanthine and dihydrocochloxanthine were previously identified in *Cochlospermum* species and found to have an antimalarial activity with an IC_{50} estimated to 1 µg/ml (Benoit-Vical et al., 2001).

Conclusion

This study showed that the main chemical components in "Saye" are tannins, anthraquinones, steroids and/or triterpenes. The results allowed establishing in part the chemical profile of "Saye" that will be used for to its quality control during the clinical study. Taking into account all the results from prior works, Cochloxanthine and dihydrocochloxanthine anthraquinones, flavonoids and kaempferol might be the target chemical compounds for the choice of the chemical marker of "Saye".

COMPETING INTERESTS

The other authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

The study was conceived by MTC, JBO, JBN, JCWO, FK and MGM. It was run by IRSS with contributions from OD, RSY, BY, JCWO, FK and BK. IRSS Bobo and IRSS Ouaga: chemical analysis. UPB: academic training of OD under supervision of MTC and GAO. Phytofla laboratory: provide plant materials. The paper was drafted by OD and supervised by JCWO, MTC and GAO. OD, RSY, JBO, MTC and JCWO participated in the overall running of the project. "Saye" and plants samples were provided by ZPD. All the authors have read and approved the final manuscript.

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