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Qualitative Analysis of the Pyrrolizidine Alkaloids from 11 Asteraceae and Boraginaceae Used in Traditional Medicine in Cote d'Ivoire

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ABSTRACT

Pyrrolizidine Alkaloids (PAs) are toxic for human and livestock. Worldwide many episodes of human PA intoxications are well reported. This study aimed at assessing the presence of PAs in some Asteraceae and Boraginaceae used in traditional medicine in Cote d'Ivoire to treat various diseases such malaria, infant cure, microbial infections. TLC detection with Ehrlich reaction method of Mattocks was used to screen 11 plant species, *Ageratum conyzoides* L. (Asteraceae), *Aspilia africana* (Pers.) C.D. Adams (Asteraceae), *Bidens pilosa* L. (Asteraceae), *Chromolaena odorata* L. (Asteraceae), *Erigeron floribundus* (Kunth) Sch. Beep (Asteraceae), *Emilia praetermissa* Milne-Redh (Asteraceae), *Heliotropium indicum* L. (Boraginaceae), *Synedrella nodiflora* (L.) Gaertn (Asteraceae), *Tridax procumbens* L. (Asteraceae), *Vernonia cinerea* L. (Asteraceae) and *Vernonia colorata* (Willd) Drake (Asteraceae). All these plants were found to contain at least trace amount of PAs. The richest species were *H. indicum*, *T. procumbens* and *V. colorata*. The presence of PAs in the studied plants is an indication of people exposure to probable toxicity. This warrants assessment of risk related to the consumption of such plants in Cote d'Ivoire as medicinal herbs.

Key words: Medicinal plants, pyrrolizidine alkaloids, Asteraceae, Boraginaceae, Cote d'Ivoire

INTRODUCTION

Since ancient time, plants have been used by human for various needs including food and health care. In Africa, people rely heavily on medicinal plants for curing diseases (Garba *et al.*, 2007). Nowadays, nature still provides more reliable source of medical agents. Almost 40% of currently available drugs are direct or indirect derivatives of natural precursors (Sohail *et al.*, 2011). In some Asian and African countries, the use of herbal remedies containing plant and other materials are an integral part of traditional culture, with approximately 80% of the population (WHO, 2008), relying on such remedies for primary health care. Some consider that medicines based on herbal formulations usually have lesser side effects and better compatibility with human body than modern medicines (Upadhyay *et al.*, 2011). The consumption of natural medicines is relatively safer than that of synthetic drugs (Saputera *et al.*, 2006).

Safety is the most important consideration before administration of herbal products. Contrary to popular perception that medicinal plants are safe, profound toxicity can result from their use (Moss, 1998; Bateman *et al.*, 1998). Despite their growing popularity as naturally safe with

bioactive products, very little information is available on the safety of medicinal herbs (Sulaiman *et al.*, 2010), in particular in West African countries. From time to time, plants are discovered to be toxic for both human and animal (Younis and Adam, 2008). In many countries, medical practitioners and local people are not aware of the toxicity of remedies. They still use medicinal plants without anxiety.

However, some plants that may cause intoxication are Pyrrolizidine alkaloid-containing plants. Alkaloids are natural plant products which are sometimes toxic to animals (Holstege *et al.*, 1995) and human when eaten. For example, cryptolepine, the major alkaloid of the roots of *Cryptolepis sanguinolenta*, a medicinal plant used in Ghana for treating malaria, was shown to provoke toxicity in mammalian cells (Ansah *et al.*, 2008).

Interest in the pyrrolizidine alkaloid (PAs) content of plant species is obviously stimulated by the fact that pyrrolizidine alkaloidosis is a serious problem for livestock production and human (Stegelmeier *et al.*, 1999). Pyrrolizidine alkaloid-containing plants are widely distributed in the world and are probably the most common poisonous plants affecting livestock, wildlife and humans.

These alkaloids and their N-oxides occur as natural components of many herbal preparations, cooking spices and honey and can contaminate food crops and animal-derived food (Edgar, 2003; Cao *et al.*, 2008). Humans can become inadvertently exposed through consumption of contaminated food and herbal remedies. Chronically ingesting low levels of pyrrolizidine alkaloids and/or their N-oxides can be source of intoxication.

The potential toxicity of pyrrolizidine alkaloids containing plants is well documented. The major effect of dietary pyrrolizidine alkaloids and chronic exposure on humans is hepatic veno-occlusive disease, leading to cirrhosis and eventually irreversible liver damage (IPCS, 1988; Zuckerman *et al.*, 2002; Fu *et al.*, 2004; Wiedenfeld *et al.*, 2008).

The widespread use of medicinal plants in many West African countries such as Cote d'Ivoire indicates possible human exposure to hepatotoxic and carcinogenic PA. This situation warranted studies on these plants to assess their possible toxicity.

Most PA-containing plants are found in three plant families, namely Boraginaceae, Asteraceae and Leguminosae (Cheeke, 1988). A current search of the literature does not reveal any detailed report on the pyrrolizidine alkaloid content of medicinal plants from Cote d'Ivoire. We therefore, reported herein the pyrrolizidine alkaloid content of 11 Asteraceae and Boraginaceae used in traditional medicine in Cote d'Ivoire. These plants are *Chromolaena odorata*, *Aspilia africana*, *Vernonia colorata*, *Vernonia cinerea*, *Erigeron floribundus*, *Bidens pilosa*, *Heliotropium indicum*, *Tridax procumbens*, *Emilia praetermissa*, *Synedrella nodiflora* and *Ageratum conyzoides*.

MATERIALS AND METHODS

Selection of studied plant species: The 11 studied plants were selected after an ethnobotanical review on Asteraceae and Boraginaceae used in Cote d'Ivoire and other West African countries for the treatment of various pathological conditions (Watt and Breyer-Brankwijk, 1962; Bouquet and Debray, 1974; Adjanohoun and Ake Assi, 1979; Djeneba, 1982; Adjanohoun and Ake Assi, 1989; PHARMEL, 1992; Hladik *et al.*, 1996; Tra Bi, 1997; Arbonnier, 2002; Kone, 2003; Adoukou, 2007; Idu and Onyibe, 2007; Adou, 2008; Apema *et al.*, 2007).

The selection of these species was based on 3 criteria namely the use in traditional medicine, accessibility and lack of information on the presence of pyrrolizidine alkaloids for most of these

plant species. *H. indicum* (Souza *et al.*, 2005), *C. odorata* (Biller *et al.*, 1994) and *A. conyzoides* (Wiedenfeld, 2011) were included because data on samples from other ecological zones were available on PA content.

Ten of these plants are from the family of Asteraceae and 1 from Boraginaceae (Table 1).

Alkaloid extraction: The leaves, stems and whole plants of selected plants were collected in Abidjan from September 2010. The samples were dried during 2 weeks under air-conditioned room (18°C) and then grounded in a mortar to obtain powders.

For alkaloid extraction, 5 g of powder were moistened with 5 mL of NaOH solution 10%. This mixture was extracted with 50 mL of dichloromethane, under mechanical stirring during 24 h (Fig. 1). The macerates were filtered on Whatman paper. The filtrates obtained were evaporated to dryness and lyophilized to remove all traces of solvent. Extract solutions (10 mg mL⁻¹) were prepared by dissolving 10 mg of extract in dichloromethane and methanol (1/9; v/v).

Detection of pyrrolizidine alkaloids: TLC using Ehrlich reaction method of Mattocks is the most useful colorimetric method for potentially hepatotoxic pyrrolizidine compounds (Mattocks, 1986; Mattocks and Nwude, 1988). It is specific for pyrrolizidine alkaloids and is not useful for any other alkaloids. This is a quick, sure and easy method for a qualitative detection of PAs (Wiedenfeld, 2011). This qualitative test was used for detection of pyrrolizidine alkaloids in organs of the 11 Asteraceae and Boraginaceae species. The presence of alkaloids is important, as it is the first indication that the species may be toxic (Nuhu *et al.*, 2009).

Twenty microhiter of extracts (10 mg mL⁻¹) were applied on aluminum backed Silicagel 60 F254 and developed in mobile phase hexane-ethyl acetate (1:1). After development, chromatograms were dried and then sprayed with Ehrlich reagent (1 g of p-dimethylaminobenzaldehyde/100 mL of ethanol/15 mL of concentrated chlorhydric acid). After heating at 95°C during 15 min, pyrrolizidine alkaloids appear as blue or purple spots (Fig. 1).

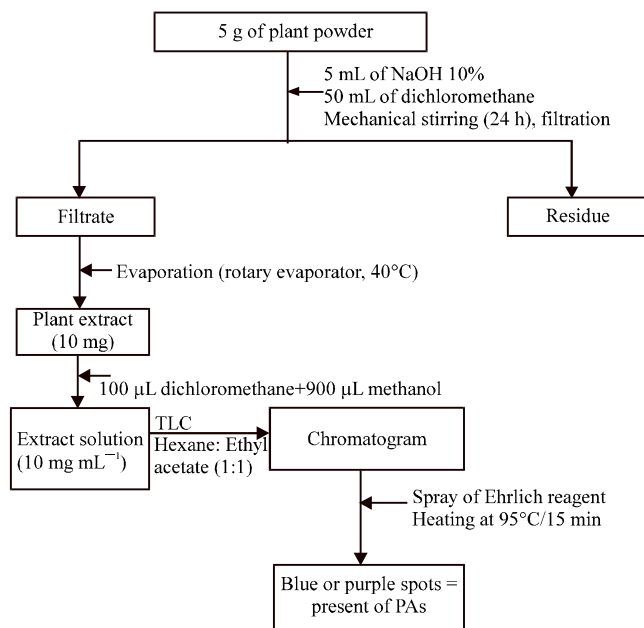


Fig. 1: Alkaloid extraction and analysis

Table 1: Some utilization of studied asteraceae and boraginaceae species in traditional medicine (Source: Literature)

Plant species	Family	Plant part used	Therapeutic indications	Preparation and administration	References
<i>Ageratum conyzoides</i> L.	Asteraceae	Leaves	For facilitating delivery, fresh wound healing, ulcer, headache, dysentery, stomach pain in children	Enema Paste for wounds	Adjahoun and Aké Assi (1979); Watt and Breyer-rankwijk (1962); Idu and Onyibe (2007)
<i>Aspilia africana</i> (Pers.) C.D. Adams	Asteraceae	Leaves	Prevent conception, febrile headache, wounds, sores, stop bleeding	Decoction	Oluyemi <i>et al.</i> (2007); Okwu and Josiah (2006)
<i>Bidens pilosa</i> L.	Asteraceae	Leaves	Fresh wound healing, sedative, anti-diarrheal	Paste for wounds, beverage	Adjahoun and Ake Assi (1989) Bouquet and Debray (1974)
<i>Chromolaena odorata</i> L.	Asteraceae	Leaves	Antibacterial for infected wounds, antiplasmoidal, stop bleeding	Paste for wounds, beverage	Kone (2008); Green (2007)
<i>Emilia praetermissia</i> Milne-Redh	Asteraceae	Leaves	Vision cleaning	Leaves squeezed, eye drop	Green (2007)
<i>Erigeron floribundus</i> (H.B. et K.) Sch. Bip.	Asteraceae	Leaves	Dermatitis	Paste for wounds	Tra Bi (1997)
<i>Heliotropium indicum</i> L.	Boraginaceae	Leaves	Dysmenorrhoe, for facilitates delivery	Paste for enema	Adou (2008)
<i>Synedrella nodiflora</i> L.	Asteraceae	Leaves	Aphrodisiac	Beverage	Hladik <i>et al.</i> (1996)
<i>Tridax procumbens</i> L.	Asteraceae	Leaves	Hypertension	Beverage	Apema <i>et al.</i> (2008)
<i>Vernonia cinerea</i> L.	Asteraceae	Leaves	Dysmenorrhoe	Paste for enema	Bouquet and Debray (1974)
<i>Vernonia colorata</i> (Willd) Drake	Asteraceae	Leaves	Malaria, fresh wound healing	Beverage, paste for wounds	Djeneba (1982)

RESULTS AND DISCUSSION

The TLC screening of studied plants for presence of pyrrolizidine alkaloids revealed that all contained at least trace amount of PAs (Table 2). The richest plant species were *Heliotropium indicum*, *Tridax procumbens* and *Vernonia colorata*. *T. procumbens* leaves showed 3 spots while its stems with flowers indicated 4 spots corresponding to PAs. For *H. indicum*, leaves and roots present 3 spots each.

The remaining plant contained less quantity or trace of pyrrolizidine alkaloids. This was the case of *Ageratum conyzoides*, *Aspilia africana*, *Emilia praetermissa*, *Bidens pilosa*, *Chromolaena odorata*, *Erigeron floribundus*, *Vernonia cinerea* and *Synedrella nodiflora*.

Phytochemicals are non-nutritive plant chemicals that have protective or diseases preventive properties (Karthishwaran *et al.*, 2010). Although, useful and beneficial for health, certain of these phytochemicals such as PAs can be dangerous for human due to their hepatotoxicity and nephrotoxicity. The PAs cause acute and chronic hepatic affections, in particular, the hepatic veno-occlusive affections (Willmot and Robert, 1920; Wiedenfeld *et al.*, 2008). We consequently assessed the presence of PAs in 11 Asteraceae and Boraginaceae species used in traditional

Table 2: Pyrrolizidine alkaloid-containing studied plants

Plant species	Organ studied	PAs detected		
		Presence	Color	Rf
<i>Ageratum conyzoides</i>	Leaves	+	Blue	0.69
<i>Aspilia africana</i>	Leaves	+	Blue	0.27
		+	Purple	0.55
<i>Bidens pilosa</i>	Leaves	+	Purple	0.69
<i>Chromolaena odorata</i>	Leaves	+	Blue	0.30
		+	Purple	0.67
<i>Emilia praetermissa</i>	Leaves	+	Purple	0.62
<i>Erigeron floribundus</i>	Leaves	+	Purple	0.61
<i>Heliotropium indicum</i>	Leaves	++	Blue	0.26
		++	Purple	0.56
		++	Purple	0.66
	Whole plant	++	Blue	0.26
		++	Purple	0.51
		++	Purple	0.55
<i>Synedrella nodiflora</i>	Leaves	+	Blue	0.20
		+	Purple	0.55
<i>Tridax procumbens</i>	Leaves	++	Blue	0.17
		++	Blue	0.26
		++	Purple	0.64
	Stems with flowers	++	Blue	0.06
		++	Purple	0.58
		++	Purple	0.69
		++	Purple	0.81
<i>Vernonia cinerea</i>	Leaves	+	Blue	0.26
		+	Purple	0.62
<i>Vernonia colorata</i>	Leaves	++	Blue	0.29
		++	Purple	0.59
		++	Purple	0.67

+: Present, ++: Abundant

medicine in Cote d'Ivoire. All these plants showed at least trace amount of pyrrolizidine alkaloids. This study is the first report of the pyrrolizidine alkaloid content of the studied plant species apart from *H. indicum*, *C. odorata* and *A. conyzoides*. Previous studies have shown the presence of PAs in these 3 plants. The rationale for selecting samples of *H. indicum*, *C. odorata* and *A. conyzoides* from Cote d'Ivoire for this study arose from the fact that variation of phytochemical content could occur according to ecological and growth conditions. The results obtained in this study for samples from Cote d'Ivoire are in full agreement with the previous reports of the PA content of *H. indicum*, *C. odorata* and *A. conyzoides*.

H. indicum is popular medicinal plant used in many countries such as Cote d'Ivoire (Kamanzi, 2002) and Brazil (Souza *et al.*, 2005) for health care needs. However, in Costa Rica, this species has been implicated as the most probable principal cause of massive mortality in horses due to intoxication by pyrrolizidine alkaloids from the plant (Van Weeren *et al.*, 1999). The presence of PAs in *H. indicum* was also reported for a sample of Brazil (Souza *et al.*, 2005).

Ageratum conyzoides has been found to be responsible for actual incidents with toxic PA problem in Ethiopia (Wiedenfeld, 2011). *A. conyzoides* is widespread and the seeds of this weed can contaminate millet. This plant is medicinal plant, but also a containing toxic PAs. *A. conyzoides* is used in Edo State, Nigeria for treating stomach pain in children, ulcer, headache and dysentery (Idu and Onyibe, 2007). Study on the toxicity of *A. conyzoides* showed that the extract at doses of 500 and 1000 mg kg⁻¹ administered orally and daily for a one month period did not show any toxic effects in rats (Igboasoyi *et al.*, 2007). This study was based on lethal dose determination and evaluation of serum levels of some enzymes and biomolecules. According to results obtained, Igboasoyi *et al.* (2007) have concluded that *A. conyzoides* is safe for use in ethnomedicine. However, the presence of PAs indicates a probable risk which might be assess.

In this study, *C. odorata* leaves present trace of PAs. This tropical weed has been shown to contain the N-oxides of five pyrrolizidine alkaloids. Highest concentration was found to occur in roots and mature flower heads, while leaves and stems are almost devoid of alkaloids and no PAs are present in nectar (Biller *et al.*, 1994). The data on leaves is in agreement with the content of this plant part observed in the present study.

Even considered as toxic compounds, some PAs could have interest in fighting against diseases. PAs are class of secondary plant metabolites that are active in defense against herbivore and microorganism. Interestingly, pure PAs from *C. odorata* have nematicidal effects on root-knot nematode *Meloidogyne incognita* (Thoden *et al.*, 2007). Also, a study has reported the interest of the indicine N-oxide, a PAs, in the treatment of patients with advanced cancer (Ohnuma *et al.*, 1982). Also PAs have possible interest in agriculture. Timbilla *et al.* (2008) have shown that dry chopped roots of *C. odorata* could serve as effective PA-lures for the development of PA-based attracticides for the management of *Zonocerus variegatus*. This variegated grasshopper is a serious pest of agriculture and forestry in sub Saharan Africa.

For the other species, previous phytochemical screening has revealed presence of class of alkaloids. This is the case of *E. floribundus* (Asongalem *et al.*, 2004), *V. colorata* (Gasquet *et al.*, 1985), *T. procumbens* (Jude *et al.*, 2009; Mundada and Shivhare, 2010; Agrawal *et al.*, 2010), *V. cinerea* (Maheshwari *et al.*, 2007), *S. nodiflora* (Bhogaonkar *et al.*, 2011), *B. pilosa* (Geissberger and Sequin, 1991; Deba *et al.*, 2008) and *A. africana* (Okwu and Josiah, 2006; Abii and Onuoha, 2011).

The presence of PAs in studied plants indicates a probable toxicity. We plan to extend this work to a great number of Asteraceae and Boraginaceae used in traditional medicine as medicinal herbs and food, to isolate and elucidate structure of PAs of plants that contain this class of alkaloids.

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