



PHYTOTOXIC ASSESSMENT OF PYRROLIZIDINE ALKALOIDS OF SOME EDIBLE AND MEDICINAL LEAFY VEGETABLES IN NIGERIA



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Abstract: Pyrrolizidine alkaloids (PAs) are toxic for humans and animal. Many episodes of human PAs intoxication are reported worldwide. This research investigated the presence of PAs in some plants used as vegetables and herbs (to treat various diseases like malaria, fever, diabetes, microbial infections among others) in Nigeria. Thin Layer Chromatography (TLC) detection with Ehrlich reaction method of Mattocks was used to screen 25 plant species of edible and non edible types. Results revealed that most of these plants contain traces (indicated by +) of PAs; however, PAs was abundant (indicated by ++ sign) in *Moringa oleifera*, *Synedrella nodifolia*, *Velvet tamarind*, *Emillia cocceinea* and *Bombax costatum* while few like *piper guinense*, *Ocimum gratissimum*, *Hibiscus sabdariffa*, *Abelmoschus esculentus*, *Telfairia occidentalis*, and *Spinaciaoleracea* which are usually used as soup, the alkaloid was absence. The presence of PAs in these edible and medicinal plants is an indication that people consuming them are exposing to toxicity. It is therefore, imperative to consume these vegetables with caution and for the tradomedical practitioners, to administer these plants/herbs with verifiable dose to avoid organ toxicity.

Key words: Medicinal plants, Pyrrolizidine alkaloids, Toxicity, Nigeria

Introduction

Since ancient times, plants have been used by human for various needs including food and health care. In African, people rely heavily on medicinal plants for curing diseases (Garba et al. 2007). Till date the world still rely on vegetation as sources of medicinal agents; almost 40% of currently available drugs are direct or indirect derivatives of natural precursors (Sohail et al. 2011). In most African and Asian countries, the use of herbal plants and other natural materials are an integral part of their traditional culture, such that about 80% of the population (WHO, 2008), uses phytomedicine as their primary health care. The reason for this practice may be cost effective, while others consider natural medicines on the ground that it is relatively safe than synthetic drugs (Saputera et al. 2006). Contrary to the popular perception that medicinal plants are safe, profound toxicity can result from their use (Fatima and Martins, 2014). However, not very much information is provided on the safety of these products especially in West African countries where traditional medical practitioners used herbal plants without anxiety.

Alkaloids are nitrogenous bases containing one or more basic nitrogen atoms in a heterocyclic ring. More than 16000 are known and most of these molecules are found distributed in various plant species throughout the world (Qurrat, et al., 2016). They are believed to be naturally synthesized and novelized in time as a result of the evolution of life on earth. Nowadays many of these chemical molecules can be synthesized by chemists and engineers using natural models and high-level technology (Aniszewski, 2015). Several alkaloids are known for their remarkable biological properties, which can be either marked toxicity (hepatotoxicity and nephrotoxicity) or potent pharmacological capacity (Wiedenfeld et al., 2008; Rujjanawate, 2003). Thus, contrary to the popular perception that medicinal plants are safe, research has shown that many plants could cause intoxication especially

those that contain indole class of alkaloids called pyrrolizidine alkaloids (Rute et al., 2018).

PAs are a group of alkaloids derived from ornithine. They are usually found as esters (mono-, di- or macrocyclic diesters) formed by a necine base (amino alcohols) and one or more necic acids (mono- or dicarboxylic aliphatic acids) (Bruneton, 2008). Pyrrolizidine alkaloids and their N-oxides occur as natural components of some herbal preparations, cooking spices, honey and can contaminate food crops and animal derived food (Edgar, 2003; Cao et al. 2008).

The wide use of plant and plant base products for food and medicine can predispose humans to hepatic and carcinogenic PAs. Steenkamp et al., (2000), reported four cases of PAs poisoning from *Senecio* and *Crotalaria* species in South Africa. Dharmanda, (2002), published an extensive review of herbal intoxication, which leads to liver damage and even death in human. These intoxications were due to the presence of PAs in these herbs. These therefore necessitated this investigation for the presence of pyrrolizidine alkaloids in some of the commonly used edible and medicinal plants in Nigeria.

Materials and Methods

Collection of Studied Plants

The plants were harvested from different environments around Gboko Benue State, Nigeria. They include: *Jastropa tajorensis*, *Emilia cocceinea* (yellow tassel: English and Aninge :Tiv), *Chorchorous olitorus*, *Velvet tamarind* (Tsamea; Hausa), *Cyperus esculentus*, *Alchornea cordifolia*, *Crossopteryx febrifuga* (Ikwar; Tiv), *Synedrella nodifolia*, *Moringa oleifera* (jegelegede in Tiv & Hausa), *Annona senegalensis*, *Bombax costatum* (Genger in Tiv), *Phyllanthus amarus* and *Vernonia amygdalina*, *Magnifera indica*, *Carica papaya*, *Gmelina arborea*,

Anacardium accidentata, *Piper guinense*, *Ocimum gratissimum*, *Hibiscus sabdariffa*, *Abelmoschus esculentus*, *Talinum fruticosum*, *Telfairia occidentalis*, *Gnetum africanum* and *Spinacia oleracea*. The plants were identified and authenticated in the Biological Sciences Department, University of Mkar, Mkar and the specimen deposited in the herbarium unit. These plants were selected based on their used in traditional medicine and consumption, accessibility and lack of information on the presence of pyrrolizidine alkaloids in them.

Extraction of Alkaloids

The plants materials were air dried at room temperature (27°C) in two weeks. The samples were then pulverized using mortar and pestle. Each sample (10g) was moistened with 10% NaOH solution (10ml) and macerated in 100ml of dichloromethane for 24hrs. The mixtures were decanted and filtered via Whatman No. 1 filter paper and the filtrates evaporated to dryness at room temperature (27°C).

Detection of Pyrrolizidine

Solutions of the extract (10mg/ml) were prepared by dissolving 10mg of extract in 1 ml dichloromethane and methanol (1/9% v/v). The presence of Pyrrolizidine alkaloids were detected qualitatively using the method adopted by Mattocks (1986). It is a colorimetric method that employed Thin Layer Chromatography (TLC) technique and Ehrlich reaction. Twenty micro liter (20µl) of the sample extracts (i.e 10mg/ml) were applied on pre-coated TLC plates and developed in mobile phase hexane-ethyl acetate (1:1). After the development, the chromatograms were dried and then sprayed with freshly prepared Ehrlich reagent. The appearance of blue or purple spots after heating the plates in an oven at 95°C for 15 mins, indicated the presence of PAs.

Result and Discussion

Result

The TLC screening of studied plants revealed that some of the plants screened have traces of pyrrolizidine alkaloids. Those in which PAs was abundant showed intense color spots on the TLC plates, as indicated with (++) . Detail of the result is as presented in Table 1 below.

Table 1: Pyrrolizidine Constituents of the Studied Plant Species

Plant species	Organ studied	Pyrrolizidine Alkaloids Detected		Retention factor
		Result	Colour	
<i>Jatropha tanjorensis</i>	Leaves	+	Purple	0.62
<i>Emillia coccinea</i>	Leaves	++	Blue	0.65
<i>Chorchorous olitorus</i>	Leaves	+	Purple	0.58
<i>Gmelina arborea</i>	Leaves	-	-	-
<i>Velvet tamarind</i>	Leaves	++	Blue	0.60
<i>Cyperus esculentus</i>	Seeds	+	Purple	0.54
<i>Alchornea cordifolia</i>	Leaves	+	Purple	0.57
<i>Talinum fruticosum</i>	Leaves	+	Purple	0.80
<i>Mangifera indica</i>	Leaves	-	-	-
<i>Crossopteryx febrifuga</i>	Stem bark	+	Purple	0.66
<i>Synedrella nodiflora</i>	Leaves	++	Purple	0.61
<i>Piper guinense</i>	Leaves	-	-	-
<i>Anacardium occidentale</i>	Leaves	+	Purple	0.8
<i>Ocimum gratissimum</i>	Leaves	-	-	-
<i>Carica papaya</i>	Leaves	+	Purple	0.90
<i>Moringa oleifera</i>	Leaves	++	Purple	0.58
<i>Hibiscus sabdariffa</i>	Leaves	-	-	0.80
<i>Abelmoschus esculentus</i>	Leaves	-	-	-
<i>Annona senegalensis</i>	Leaves	+	Purple	0.57
<i>Bombax costatum</i>	Leaves	++	Blue	0.61
<i>Spinacia oleracea</i>	Leaves	-	-	-
<i>Telfaira occidentalis</i>	Leaves	-	-	-
<i>Phyllanthus amarus</i>	Leaves	+	Purple	0.65
<i>Vernonia amygdalina</i>	Leaves	+	Blue	0.59

+: Present; ++: Abundant; - Absence

Discussion

The concept that medicinal plant products are safe may not be generally true. This is because plants products may contain some phytochemicals that in addition to having beneficial effects are physiologically harmful. Notable among these are alkaloids particularly the indole containing type, (pyrrolizidine alkaloids). Pyrrolizidine alkaloids have

been implicated in cases of hepatotoxic and nephrotoxic effect on both human and animals (Dharmanda, 2002; Steenkamp et al, 2000). These may be difficult to curb as human exposure to PAs is likely to increase as alternative herbal remedies becomes more popular (Steenkamp et al., 2000) and most of these leafy vegetables like *Vernonia amygdalina* *Emilia coccinea*, *Chorchorous olitorus*,

Bombax costatum etc are widely consume as soup/vegetable especially in the traditional African society (Achigan-Dako et al., 2010., Unegbu, et al., 2017., Ujah et al., 2019), and these are found to contain high proportions of PAs.

Velvet tamarind is a popular medicinal plant used in treatment of diabetes (Izzo et al., 2005). The fruits of the plant are used as additive in the preparation of pap and the small stems are used as chewing sticks among the Hausas and Tiv tribes of Nigeria. Velvet tamarind roots extract has been reported to have potent antibacterial activities against pathogens (Eze et al., 2018). Despite all these, there is every need to be cautious for its use base on this finding.

Moringa oleifera leaves are usually consumed as vegetable and the seeds as source of vegetable oil (Richa, et al., 2005). The plant is used to treat ulcer, cold, diarrhea and other disease like inflammation, cardiovascular and liver diseases (Richa, et al., 2005). Studies have shown that *M. oleifera* is safe for consumption both as food and as medicinal plant (Asare and Nyarko, 2012), but the presence of pyrrolizidine alkaloids as detected in the present study indicated a probable risk which should be considered.

Bombax costatum a family of Malvaceae, is a pan tropical genus comprising of eight species. It contains white floss that is used for stuffing mattresses, pillow and cushions. The wood is used for making sound box for drums and xylophones, the bark is rich in tannins and the calyx is cooked and eaten as vegetable (Achigan-Dako et al., 2010). *Bombax costatum* is a very rich source of nutrients and phytochemicals and this provides the rational for its use as feed and tradomedicine for various illnesses (Ujah, et al., 2019). Although there is no report so far on the toxicity of this plant however, the result of the present work calls for caution.

Chorchorous oltorus (*C. oltorius*) is a culinary and medicinal herb, used as edible vegetable in several countries in Asia and Africa. The leaves are demulcent, diuretic, febrifuge, and tonic. It is medicinally used in treatment of chronic cystitis, gonorrhoea, and dysuria. A cold infusion of the plant was reported to restore appetite and strength but the extracts of the leaf and seed was however reported to have toxic effects on the multiple myeloma-derived ARH-77 cells with IC₅₀ values of 151 µg/mL and 17 µg/mL, respectively (Iseri et al., 2013). A lot of literatures have reported that the seed of *C. oltorus* is toxic (Negm, et al., 1980; Johnson and Toleman, 1982; Mckenzie, et al., 2008; Egu, et al., 2014). Although, acute toxicity studies on the leaf extract revealed that the extract was safe on animal model at low doses but at higher dose for a prolong period it may pose threat of toxicity (Orieke, et al., 2018); which may be attributed to the presence of PAs as indicated in this research.

Synedrela nodiflora (*S. nodiflora*) is of the family *Asteraceae*. It is an annual herb that grows to about 60-120 cm high. The plant is native to tropical America but widely distributed throughout West Africa. *S. nodiflora* is used as foliage in Ghana and vegetable in Indonesia. In Nigeria, it is used to treat cardiac problems, heal wound and stop bleeding (Idu and Onyibe, 2007). It is also used to treat

headache, stomachache, rheumatism and epilepsy (Bukhill, 1985). The whole plant has been scientifically shown to possess potent antioxidant, analgesic and anticonvulsant effect (Amoateng, et al., 2011; Amoateng, et al., 2012). There are conflicting reports on the safety of this plant; Belmain et al., (2001) and Ramaiah, (2007) reported that *S. nodiflora* extract has no toxicity effect within a therapeutic doses of 100, 300 and 1000mg/kg but the aqueous extract of the leaves has been reported to be toxic (Dutta et al 2012) and could caused cellular degeneration of the seminiferous tubules in wistar rats at dose of 100mgKg (Olukunle and Abatan, 2008). The result of this present research/report is tandem with the work of Witabouna and Brahima, (2012) who previously reported the presence of PAs in the leaves of this plant.

Emilla coccinea known as yellow tassel (English) and Aninge (Tiv), is an edible plant and is used for treatment of eye, ear, fever problem and birth control. *Emilla coccinea* is edible vegetable among the Tiv people of middle belt-Nigeria. The plant has been screened to have high concentration of tannins but moderate concentration of other phytochemicals and showed good potentials for pharmaceutical formulations (Unegbu, et al., 2017). Furthermore, acute toxicity study on methanol leaf extract of *Emilla coccinea*, in mice revealed it was harmless. However, LC-HRFTMS analysis showed 9 putatively identified compounds with pyrrolizidine alkaloids occurring at the higher peak (Uloma, et al., 2016). This result was tandem with the present work that indicated high content of PAs in the plant.

It is worth reporting that this investigation revealed the absence of PAs in some of the edible leafy vegetables used mostly as soup. This includes *piper guinense*, *Ocimuro gratissimum*, *Hibiscus sabdariffa*, *Abelmoschus esculentus*, *Telfairia occidentalis*, and *Spinacia oleracea*; therefore could be consumed without anxiety.

Conclusion

Plants constitute a reservoir of secondary substances with significant potential benefits; however, some (like PAs) are remarkably poisonous. This research qualitatively investigated the presence of PAs in the affected vegetables and herbs. Results of this investigation revealed that all except *piper guinense*, *Ocimuro gratissimum*, *Hibiscus sabdariffa*, *Abelmoschus esculentus*, *Telfairia occidentalis*, and *Spinacia oleracea*; contained traces of PAs and since PAs constitute harm to man (an even animal), these plants should be consumed cautiously. Finally work to quantify PAs in the affected plants is ongoing.

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