International Journal of Molecular Medicine and Advance Sciences 2(3): 326-329, 2006

Tannins and Calcium Oxalate Crystals in Lamina of Some *Phyllanthus* Species

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Abstract: Studies on the distribution of tannins and calcium oxalate crystals in the lamina of three species of *Phyllanthus* L. including *P. amarus* (Schum and Thonn.), *P. niruroides* Mull-Arg. and *P. urinaria* L. occurring in Nigeria were carried out and reported for the first time. This is with a view to determine the value of these compounds in traditional medicine. Localization of these compounds in the leaves of these plants differed. The probable reasons for these variations in distribution and the biological significance of these compounds were discussed citing the current available literature. The discussion was also done in relation to the ethnomedicinal uses of these *Phyllanthus* species in Nigeria; thus revealing the need for reappraisal of germplasm exploitation and conservation of these important plant resources in Nigeria.

Key words: Calcium oxalate, euphorbiacere, sp., tannins, traditional medicine

INTRODUCTION

The genus *Phyllanthus* L. is a member of Euphorbiaceae family (Spurge family), which groups over 6500 species in 300 genera. Euphorbiaceae is a large family of upright or prostrate herbs and shrubs, often with milky acrid juice[1] and is mainly a pan tropical family with some species either more of less temperate. Numerous species of this family are native of Southern region of Nigeria and all tropical regions of the world from North Central and South America[2]. The medicinal value of these plants lies on some chemical substances that produce a definite physiological action on the human body. The most important of these substances are alkaloids, glycosides, tannins, oils and many others[3]. According to Rahila *et al.*[4] there is need that the local herbs be evaluated for phytochemistry so as to determine the potential of indigenous source of medicines.

According to Thyagarajan and Philogene[5] plants extracts from *Phyllanthus* have beneficial effects on liver functions. Uander and Blumberg[6] showed, using *in vitro* studies that the *P. amarus* extracts (Polar fractions) also have antiviral activity and are a potential remedy for hepatitis-B viral infection. Since the extract of *Phyllanthus* has a long history of use in tropical countries in indigenous medicine for the treatment of liver ailment, they were examined during several researches.

Phyllanthus can be found around all tropical regions of the world from Africa to Asia, South America and West Indies. *Phyllanthus* contains about 50 species in 10-11 sub-genera[2].

P. amarus can be found in all the tropical regions of the world: through the roads, valleys, on the riverbanks and near lakes. This plant is a common arable weed, which is usually misidentified with the closely related *Phyllanthus nururi* in appearance, phytochemical structures and history of use. *P. nururi* reaches a length of 60 cm, the fruits are larger and the seeds are dark brown and warty[7].

The aim of this study is to investigate and report the presence of tannins and calcium oxalate crystals in the lamina of these taxa. This is because the leaves of these plants are the most frequently used in traditional medicine in most parts of Nigeria and West Africa.

MATERIALS AND METHODS

Mature and fresh leaves used for this investigation were obtained from living specimen of these plants namely *Phyllanthus amarus*, *P. niruroides* and *P. urinaria*. They were collected from different parts of Southern Nigeria. The voucher specimens were deposited at the Michael Okpara University of Agriculture Herbarium, Umudike, Nigeria. At least thirty samples of each specimen were collected and screened for the presence of calcium oxalate and tannins.

Calcium oxalate crystals in the leaves, of these plants were histochemically localized following the method of Silver and Price[8]. This was done with slight modifications such as reducing the staining period from 3 to 1 min[9]. The types of crystals of calcium oxalate localized in different tissues were microscopically examined and their

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morphology and location noted. Photomicrographs were taken from these slides using a Leitz Ortholux microscope fitted with a Vivitar-V335 camera.

For tannin determination 500 mg of the sample was weighed into a 50 mL plastic bottle. Fifty ml of distilled water was added and shaken for 1 hr in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 3 mL of 0.1 m $FeCl_3$ in O.IN H C1 and 0.008 m potassium ferrocynide. The absorbance was measured in a spectrophotometer at 120 nm wavelengths within 10 min[10,11].

RESULTS

The results of the distribution of tannins and calcium oxalate crystals on the lamina of the investigated plants are shown in Table 1 and illustrated in (Fig. 1a-c). In *P. amarus* the presence of tannins was evident in the chloroplasts of the palisade mesophyll. Spots of dark staining of calcium oxalate crystals were also visible around the underlying tissues of the spongy mesophyll (Fig. 1a). There was more tannins in *P. amarus* than observed in the lamina of *P. niruriodes* (Fig. 1b). Conversely, the darkly stained chloroplasts of this taxon showed that there are more calcium oxalate crystals in the lamina than in the other two plants investigated. The lamina however contained few crystals of calcium oxalate (Fig. 1c). The calcium oxalate crystals were found mostly around the region of the midrib.

DISCUSSION

The highly tanninferous nature of *P. amarus* reported in this study (Table 1 and Fig 1a) is significant in view of the role of tannin in animal nutrition. This is because the presence of tannin in plants have been suggested to be the reason why most animals do not graze on plants with large amount of tannins. Thus it is widely believed that tannins have anti-herbivore functions in plants and inhibit pathogenic fungi[11-14].

The presence of hydrolysable tannin which is a condensed tannin in *P. amarus* has proved why it is recommended for a wide range of treatment including inflammation, liver injury, kidney problems, arteriosclerosis, hypertension, stomach disorders, diuretics, antidiarrhea and haemostatic[15,16]. No wonder then that most of the *Phyllanthus* species investigated are put into most of these uses medicinally both in Nigeria and most parts of Africa[17].

Tannins protect the cells of the liver from damage and so the plants are used for the treatment of jaundice

Table 1: Distribution of calcium oxalate and crude tannins in the *Phyllanthus* Sp., studies

Taxa	*P. amarus*	*P. niruroides*	*P. urinaria*
Quantity of tannins (%)	1.26	1.15	1.17
Calcium oxalate crystals	present in chloroplasts and mesophylls	Highly associated with chloroplasts and mesophyll cells	Present in chloroplants and mesophyll cells

which arises from liver damage. Tannins are also haemostatic and is responsible for the protection of red blood cell membrane with enhanced blood volume, hence it is used in treatment of anemia.

The conspcicouces presence of calcium oxalate crystals in *P. niruroides* was also significant. That could be an ecological adaptation acquired to invade grazers including human beings that are always searching for *P. amarus* and related taxa for the treatment of different health disorders in Nigeria. This not with standing, the biological role of calcium related compounds in plants are known but the physiological significant of the association of the calcium oxalate crystals with the chloroplasts of this plant remains an area of further research. Previous reports have shown that calcium is an important component of the cell wall. The middle lamella regions of cells are known to be rich in calcium, hence they are known to be vital in maintaining the strength of the entire cell.

The location of the calcium oxalate crystals around the region of the mid rib in *P. uninaria* was not strange since previous researches have clearly explained the significance of calcium oxalate crystals in the mechanical strength of most plants[9,12-14,18-21]. The present result stands as an additional information but the first as far as these *Phyllanthus* species are concerned.

Similarly, the high level of calcium related compound (oxalate) in these plants was equally important in view of the vital role of calcium in human medicine. This is because calcium is known to be one of the important mineral elements required in building of red blood cells. Hence is not also strange that diabetic and anemic patients in Nigeria often take the aqueous leaf extract of some *Phyllanthus* species in order to reduce the level of blood sugar and at the same time increase the building of red blood cells[17,22].

Calcium oxalate crystals outside the cells will make them easily available to the animals that graze on plants rich in calcium oxalate crystals. It is also possible to use such plants in the treatment of bone fractures and osteoporosis (weak bones).

In view of the useful role of these plants in traditional medicine, the probable utility of *Phyllanthus* species in pharmaceuticals is not in

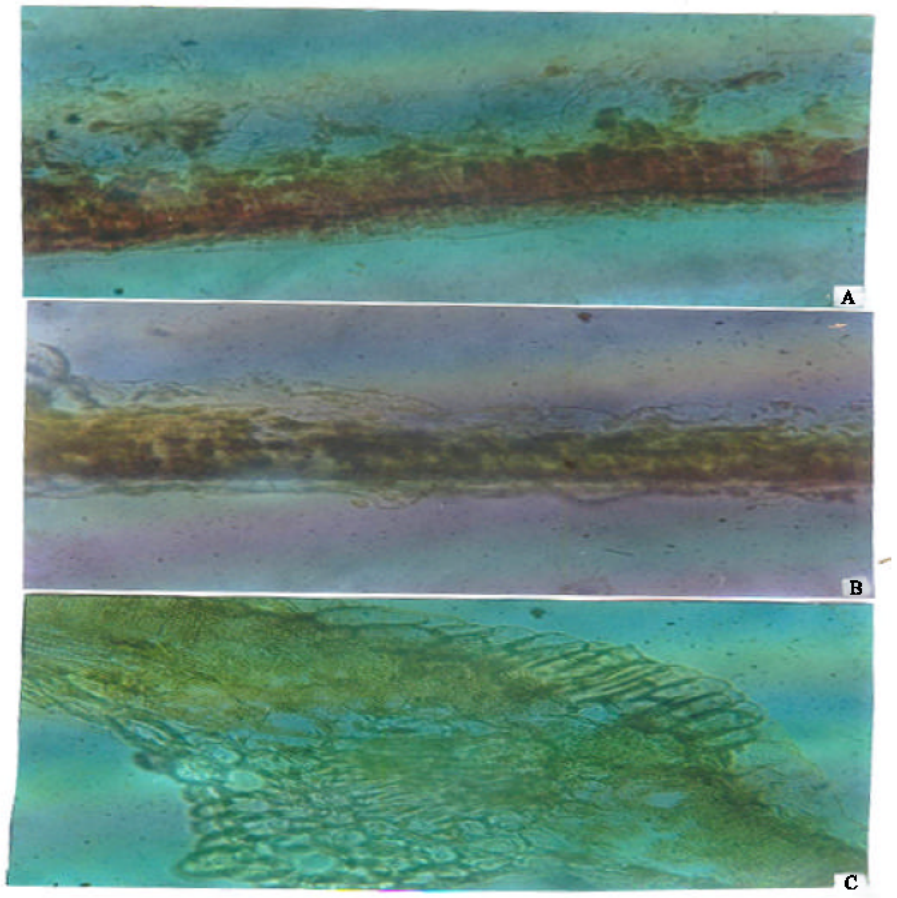

Fig. 1: T.S. of Lamina of: (a) *P. amarus:* Tannins present in chloroplasts of palisade mesophyll. Spots of dark-stained calcium oxalate crystals visible around spongy mesophyll. (X 100). (b) *P. nururoides:* Calcium oxalate crystals darkly stained chloroplasts. Tannins also present. (X 100). *P. urinaria:* Tannins present but sparse. Crystals few but found mostly around midrib region (X 100)

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