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Short Communication

Histochemical studies of some Nigerian species of *Aneilema* R. BR. (Commelinaceae): An under growth of rubber plantation

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Four Nigerian spp of *Aneilema* (*A. aequinoctiale, A. beniniense, A. paludosum* and *A. unbrosum*) common as under growth of rubber plantations were screened for their condensed tannins and saponins. This is because of the importance of tannins and saponins in industries, such as the leather industry. Both chromatographic and spectrophotometric analyses were employed in this study. Procyanidin and saponins related to furostanol saponins were isolated from these spp of *Aneilema* investigated. This report of the presence of these chemical constituents in Nigerian *Aneilema* spp is the first to be documented in literature. Since these *Aneilema* spp serve important medicinal purposes in Nigeria, the need to develop and determine proper methods of producing these active components from these *Aneilema* spp is suggested. Furthermore, the defensive role of tannins and saponins in the ecological survival of these plants as perennial herbs in the thick tropical rain forest of West Africa sub – region is discussed. This is as a result of the importance of these *Aneilema* spp studied both as livestock and weeds of plantation crops in Nigeria.

Key words: *Aneilema* spp. Commelinaceae, Saponins, Tannins, Histochemical study.

INTRODUCTION

Aneilema R. Br. is a herbaceous monocotyledonous plant and is distributed in the tropical and subtropical parts of the world. *Aneilema* is the second largest genus in the family; Commelinaceae with about 100 spp out of which 10 are common in the West African sub – region. They are popularly recognized as weeds but some spp serve as livestock feed and medicinal plants (Hutchinson and Dalziel, 1968; Burkill, 1985; Akobundu and Agyakwa, 1987). The four commonly found in West Africa include *A. aequinoctiale* (P. Beaux) Kunth, *A. beniniense* (P. Beaux) Kunth, *A. paludosum* A. Chev. and *A. unbrosum* (Vahl) Kunth.

Documented reports on the biology of the genus *Aneilema* including those of Salive and Vargas (1984) and Gaullier (1990) who agreed that *A. nudiflora* is a weed with high potential of economic danger to rice and ascertained the biomass of different flora including that of *A. beniniense* industrial oil palm plantations subjected to cattle rearing. Other reports are those of Tomlinson (1969), Edeoga et al. (1996), Edeoga and Ogbebor (1999ab, 2001).

The authors are therefore not aware of any other information relating to the biology or distribution of saponins and tannins on *Aneilema* spp. Tomlinson (1969) did not even suggest or suspect the presence of saponins in these investigated *Aneilema* spp. Other workers have dwelt on the presence of saponins and tannins in different groups of plants excluding *Aneilema* spp (Ding and Zhu, 1993; Liu et al., 1995).

The absence of information specifically on the presence of saponins and tannins in some *Aneilema* spp indicates that this paper is a worthy one. This gap in knowledge is expected to be filled with this study. Attentions are on these *Aneilema* spp due to the different medicinal and economic importance and uses of these plants. The leaves of *A. beniniense* are pounded and use as a strong laxative. The root of *A. aequinoctiale* is used

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Table 1. Qualitative analysis of tannins and saponins in *Aneilema* species investigated.

Taxa	Tannin	Saponin
Aneilema aequinoctiale	+	+
Aneilema beniniense	+	+
Aneilema paludosum	+	+
Aneilema unbrosum	+	+

in different parts of Africa for feeding livestock and treatment of amenorrhoea, skin trouble, eye-diseases, leprosy, constipation and to promote walking in rachitic children.

According to Gaullier (1990) *A. beniniense* is regarded as a weed of high nutritional value and their presence in forest indicates a good cocoa soil. Though many ethno medical uses of some *Aneilema* spp were enumerated including the four species investigated. It was clearly remarked that no active principle had been found or reported in these plants. The aims of this study are to document and determine for the first time the active chemicals (saponins and tannins) found in these four Nigerian *Aneilema* spp.

MATERIALS AND METHODS

Mature and fresh leaves, stems and roots used for these investigations were obtained from the Rubber Research Institute of Nigeria (RRIN) and from the wild living spp of these plants: namely *A. aequinoctiale*, *A. beniniense*, *A. paludosum* and *A. unbrosum.* Thirty different plants were analyzed in each of the *Aneilema* sp studied. These plants were collected from thirty different populations by digging them up with their roots. The voucher specimens (RRIN 042, 043, 044 and 045) were deposited at RRIN herbarium. The experiment was repeated twice.

The whole plants including roots, stems leaves, seeds flowers and fruits were washed and dried under shade. These specimens were in their reproductive stage to ensure that they were studied at fully matured stage and to ensure consistency. Melting points were initially determined on a Yanaco Mp-3 type micromelting point apparatus. Gas chromatography was run on a HP-5890 series II gas chromatography. For isolation, purification and analysis of condensed tannins, dried plant specimens were blended with 75% aqueous acetone containing 0.1% ascorbic acids and the mixture filtered. Acetone was removed by evaporation under reduced pressure. The aqueous residue was then subjected to column chromatograph using Sephadex LH-20 according to the procedure described by Koupai-Abyazani et al. (1992).

The structure of purified tannins was determined using a hydrolytic procedure that employed phloroglucinol as the nucleophic scavenger of the freed monomeric units. Resultant adducts were analyzed by the high-pressure liquid chromatography (HPLC) using a Lichrospher 100RP-18 column (25 x 4 mm i.d.) with monitoring at 280 nm. A linear gradient of 1% aqueous acetic acid and methanol was used for elution (Bohm and Koupai-Abyazani, 1994). ^{13}C-NMR spectra were recorded in water: d6-acetone (1:1 v/v) on a Varian XL (300MHZ) spectrometer. Optical rotations of polymers dissolved in methanol – water (1:1 v/v) were measured in a 10 mm path length cell at 20^{o}C on a Jasco J710 spectrophotometer. For chromatographic analysis of saponins content, the aqueous residue was dissolved in 10 ml methanol and analyzed by thin layer chromatography (TLC) following the methods of Brain and Turner (1975) and Liu et al. (1995). A suspension of the resulting extract was applied to a column of MCL gel CHP20P and MeOH. The resulting fraction (total saponins) that eluded with MeOH was repeated by repeated chromatography on silica gel (1.0 kg) to give different fractions. When two of these fractions were repeatedly rechromatographed on Lobar column, three mixtures of furostanol glycosides were obtained. When one of the fractions was chromatographed repeatedly on the reversed-phase Lobar column, it yielded a mixture of furostanol saponins which were further separated by preparative HPLC to give new furostanol glycosides. The effluent was also analyzed using a Camag TLC scanner II with the following conditions: scanning speed 5.0 mm, deuterium lamp, wavelength 366 nm, mode absorbance and reflection, sens 170, span 7, offset 5% and slit width 6. The mobile solvent was Et0Ac:H_2O (8:2:1). TLC plates were detected with 15% H_2SO_4 followed by heating for 3 min at 105^{O}C. standard samples of saponins were purchased and dissolved in MeOH to make a concentration of 1 mg/µl. A calibration was obtained by varying the amount of standard saponins from 2 to 10 µl. The curve was linear within the range of saponins amounts used.

RESULTS

The quantitative analysis on the powdered tissues of *Aneilema* spp including the estimation of the tannins and saponins are summarized in Table 1. Enzymatic hydrolysis of precipitated mixture of glycosidase with B-glycosidase followed by washing with hot water showed that glucose was present in the filtrate when developed by TLC. Detailed analysis of both the ^{1}H and BC-NMR spectra suggest that the saponins present in the *Aneilema* spp are related to furostanol saponins than any other types of saponins.

Tannins were apparently present in all the *Aneilema* spp studied. The aqueous acetone extraction of the powdered tissues of *Aneilema* spp investigated yielded purified polymer after a combination of liquid partitioning and chromatography on Sephadex LH-20 column. The polymer is a typical of a procyanidin with absorption maximum near 278 nm. TLC analysis and the spectrophotometric measurement of the hydrolysis products revealed that the polymer which indicated the presence of Procyanidin units (Signal 145 ppm). There is no signal observed for prodelphinidin in the spectra. The results from the HPLC analysis also agreed with the results from the BC-NMR spectroscopy. Identical results were obtained for all the collections of the species of *Aneilema* investigated.

DISCUSSION

The present report on the presence of tannins and saponins among some Nigerian *Aneilema* spp are interesting since Burkill (1985) had previously indicated that no active principle has been found in most of West African species of *Aneilema*. This report on the presence of tannins and saponins in these *Aneilema* spp (*A. aequinoctiale, A. beniniense, A. paludosum* and *A. unbrosum*) is therefore the first to be documented among these

Nigerian plants. Similarly, the report on the presence of tannin in the *Aneilema* spp studied is important since this constitutes the first report on the taxa. This is economically significant both at small-scale and commercial level.

This findings will enable farmers know some of the constituents of some of their livestock feds. In the same vain industrialists could exploit these constituents for their various needs relating to tannins and saponins. Although Tomlinson (1969) reported the presence of tannins in unspecialized ground tissues cells in all parts of some members of Commelinaceae, no specified reference were made on the presence of tannins and saponins on *Aneilema* spp at all. This gap in knowledge has been filled by the present report documented in the *Aneilema* spp investigated. The present observations on the chemical properties of these *Aneilema* spp therefore provide certain information vital in understanding the importance of these plants. Closely related to these is the suggestion of Edeoga and Ogbebor (1999b) who agreed that the distribution patterns of calcium oxalate crystals in some Nigerian species of *Aneilema* is significant enough to such extent that its biological relevance is undutiful among scientists of diverse interests.

The condensed tannins identified from the four species of *Aneilema* studied are similar to the one that was earlier reported by other workers in other plants (Czochanska et al., 1980; Bohm and Koupai-Abyazani, 1994). Since tannins and saponins are the main active principles in the *Aneilema* sp that constitute important medicinal plants in Nigeria, it becomes very necessary to develop proper method for determining and producing them from these *Aneilema* spp.

This suggestion is vital since Liu et al. (1995) made similar recommendation concerning the determination and production of saikosaponins from *Bupleurum* plant. Generally our results agreed with previous reports on the distribution of tannins in some flowering plants. Thus it could be suggested that tannins is common among most of Nigerian species of *Aneilema*.

The presence of furostanol saponins reported in this study is not strange since Peng et al. (1995) equally reported that furostanol saponins are obtained from *Allium macrostemon*.

The report on this investigation is therefore suggesting that these types of saponins could be more common in monocotyledonous groups since *Aneilema* and *Allium* belong to this group. The defensive
role of tannins and saponins in plants has been documented (Haslem, 1989). Thus tannins and saponins could be serving as feeding deterrants to grazers, a defense strategy that could be responsible for the continuous survival of the *Aneilema* spp investigated in high rain forest zone of West Africa.

The occurrence of tannins and saponins in the Nigeria species of *Aneilema* may be useful to investigate its relationships with other members of the Nigerian Commelinaceae in future studies.

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