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Phytochemical constituents of the leaves of three bamboo (Poaceae) species in Ghana.

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ABSTRACT
Although herbivores feed on various plant parts (e.g. leaves), inherent phytochemicals present varying degrees of antagonism. Bamboo leaves have therapeutic and nutritional properties, making them a good source of fodder for livestock. To be sure of their safety or otherwise, phytochemical analysis was undertaken on three bamboo species [*Bambusa vulgaris* Schrad. ex J. C. Wendl., *Bambusa ventricosa* McClure, and *Oxytenanthera abyssinica* (A. Rich.)]. The leaves were tested in their wet and dry states. Leaves of all the species contained saponins, general glycosides, coumarins and cyanogenic glycosides. Those of *B. ventricosa* and *O. abyssinica* contained polyphenols and flavonoids as well. However, there were no alkaloids, carotenoids, triterpenoids and steroids, anthraquinones and anthracene glycosides in any of the species/varieties. Generally, air-drying of bamboo leaves does not affect presence of inherent phytochemicals and equally makes them suitable as hay just like fodder in the fresh succulent state. *B. vulgaris* is the safest of the three species analysed as it contained four classes of phytochemicals.

Keywords: Phytochemicals, bamboo leaves, coumarins, saponins, polyphenols.

1. Introduction

Bamboo (Poaceae) is an important grass that has significant uses in many parts of the world especially as a raw material for construction. In Ghana, bamboo is used for rural construction, basketry and decoration-uses restricted to the culms and not the leaves [1]. To increase this already extensive usefulness of bamboo, however, is the need to investigate the chemistry of the less utilised parts of the plant i.e. the leaves.

Bambusa vulgaris Schrad. ex J. C. Wendl. is considered the commonest in Ghana, constituting almost 95% of the total bamboo resources of the country. It is present in forest reserves, community lands, fallow farm fields and can be regarded native [1]. Three other species of bamboo have been recorded as native: *Oxytenanthera abyssinica* (A. Rich.) Munro, *Bambusa arundinacea* McClure and *Dendrocalamus strictus* (Roxb) Nees. Two distinct varieties of *Bambusa vulgaris* are found in Ghana; the more common green variety, *Bambusa vulgaris var.* vulgaris and the yellow variety which is often cultivated- *Bambusa vulgaris var.* vittata. *Oxytenanthera abyssinica* is more drought resistant as it occurs predominantly in the savannah parts of the country [1]. Other species of bamboo such as *Bambusa ventricosa* McClure albeit not native, are cultivated in the country for various purposes.

Phytochemicals are non-nutritive plant chemicals that have protective properties [2]. Phytochemicals, also known as secondary metabolites or extractives, serve plants in their interactions with the animal world in the same manner as spines and thorns do. In most cases, the ecological function of phytochemicals is to protect plants from being fed on by herbivores [3].

Investigations reveal that the main chemical constituents of the leaves of most bamboo leaves are flavonoids, lactones and certain phenolic acids [4].

The objective of this study is 'to establish the chemical suitability of the leaves of bamboo as fodder or feed for livestock in Ghana' by assessing the presence of phytochemicals in the leaves of *Bambusa ventricosa, Oxytenanthera abyssinica* and two varieties of *Bambusa vulgaris.* This is the first time the chemistry of the leaves of the three species has been investigated into in Ghana.

2. Methodology

2.1. Collection of samples

Leaf samples of *Bambusa ventricosa*, *Bambusa vulgaris* (both varieties) and *Oxytenanthera* *abyssinica* were collected at the herbarium of the Forestry Research Institute of Ghana (FORIG) at the Bobiri Forest Reserve. The Moist Semi-Deciduous forest has 1250-1500 mm of rainfall per annum and the soil type is the Forest Ochrosol [5].

Green healthy-looking bamboo leaves were plucked from three and a half years old stands at random from different levels of the plant and placed in black polythene bags. The leaves were plucked at least 1.5 metres above ground level. The collection of leaf samples was done before the end of April at a time non-structural carbohydrate content (TNC) levels were expected to be at a critical low and when the plants were most likely to be hostile to herbivory [6].

The bamboo leaves were divided into two parts; one placed in cold storage while the other part was air dried for 4 hours a day for two weeks. The dried leaves were crushed, and then milled using a laboratory blender into fine powder. The wet samples were crushed and extracted with ethanol before being filtered and the filtrate concentrated in a rotary evaporator (Buchi). The extract was evaporated to dryness over a water bath. The above procedure was separately carried out for the four sets of samples.

2.2. Phytochemical screening

The leaf samples were individually subjected to phytochemical analysis to establish the presence or absence of 11 classes of phytochemicals; using prescribed techniques [7, 8]. The following tests were conducted: froth for saponins, thin layer chromatography (TLC) for carotenoids, Schinoda for flavonoids, NaOH and picrate paper tests for general and cyanogenic glycosides respectively, Liebermann-Burchard for triterpenoids and steroids, Borntrager's for anthraquinones and anthracene glycosides, ferric chloride for tannins and polyphenols, fluorescence test for coumarins and HCl and Dragendorff's for alkaloids.

Data obtained were analysed using the student t-test and the Mann-Whitney statistic with values for $p<0.05$ considered statistically different.

3. Results

The results of the phytochemical analyses for the three species are represented in Tables 1. Generally, the results for the wet alcohol extracted samples are similar to those for the dry powdered samples in any given species/variety. This relation is very strong with a R^2 value (correlation constant) of 0.962. There is no significant statistical difference between the results for the wet alcohol extracted samples and the dry powdered samples using the student t-test ($t = 1.000$, $P \geq 0.05$). When the means of the two groups of results were compared using the Mann-Whitney test, the results were statistically not significant ($\chi^2 = 9.0$, $p \geq 0.05$).

All the samples indicated the presence of coumarins, cyanogenic glycosides and general glycosides; nearly all indicating the presence of saponins, apart from *Bambusa vulgaris*, flavonoids, tannins and polyphenols are present in the other two species. The results showed the absence of alkaloids; anthraquinones; their glycosides (anthracene glycosides); carotenoids and triterpenoids or steroids in all the species investigated.

Table 1: Phytochemical constituents of the leaves of *Oxytenanthera abyssinica, Bambusa ventricosa and* the two varieties of *Bambusa vulgaris*

	B . vulgaris vittata		***B. vulgaris vulgaris***		***B. ventricosa***		***O. abyssinica***	
	Wet	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**
Tannins & polyphenols	-	-	-	-	+	+	++	+
Saponins	+	+	+	-	+	+	++	+
General glycosides	+	+	+	+	++	++	+	++
Cyanogenic glycosides	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-
Carotenoids	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	+	+	+	+
Triterpenoids & steroids	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-
Anthracene glycosides	-	-	-	-	-	-	-	-
Coumarins	+	+	+	+	+	+	+	+

Legend: **+ =** present; **++ =** highly present; **- =** not present

The dry and wet alcohol extracted leaf samples of *Oxytenanthera abyssinica* contain the following classes of phytochemicals: tannins and polyphenols, saponins, general and cyanogenic glycosides, flavonoids and coumarins (Table 1). Tests for alkaloids, triterpenoids and steroids, anthraquinones, carotenoids and anthracene glycosides gave negative results, in both the dry and wet ethanol extracted leaf samples.

The following classes of phytochemicals were detected in both the dry powdered and the ethanolic extracts of the leaves of *Bambusa ventricosa*: tannins and polyphenols, general and cyanogenic glycosides, saponins, flavonoids and coumarins (Table 1). The leaves of *Bambusa ventricosa* gave negative results for the tests for alkaloids, carotenoids, triterpenoids and steroids, anthraquinones and anthracene glycosides.

For the green variety of *Bambusa vulgaris*, coumarins and general and cyanogenic glycosides were detected in both the dry powdered and the wet ethanolic leaf samples. Interestingly the presence of saponins was evident only in the wet ethanol extracted sample and not the dry powdered sample (Table 1).

The yellow variety of *Bambusa vulgaris* showed the same results as the green variety with the absence of saponins in the dry powdered samples of the latter being the only difference. The phytochemical spectrum of *Bambusa vulgaris* compares with that of *Bambusa ventricosa* (Table 1) but for the presence of flavonoids and tannins and polyphenols in the later.

The total number of classes of phytochemicals detected in the four varieties of bamboo examined is represented in Figure 1. *Oxytenanthera abyssinica* and *Bambusa ventricosa* with six different classes of phytochemicals present in both the wet ethanolic extracts and the dried powdered samples have the highest number of classes of phytochemicals. The green variety of *Bambusa vulgaris* had four classes in the wet ethanolic sample and three in the dried samples due to the absence of saponins in the later. The yellow variety of *Bambusa vulgaris* had four classes in both the wet and dry samples.

There were saponins in all the samples except the dry powdered samples of the green variety of *Bambusa vulgaris.* All the samples of *Oxytenanthera abyssinica* and *Bambusa ventricosa* tested gave positive results for flavonoids, tannins and polyphenols. There are no alkaloids, anthraquinones, carotenoids or anthracene glycosides in any of the tested samples.

Although the tests were essentially qualitative, the samples of *Oxytenanthera abyssinica*, by ocular observation, gave highly positive results for tannins and polyphenols, saponins and general glycosides (indicated by ++ in the table). The presence of general glycosides in *Bambusa ventricosa* was very similarly conspicuous.

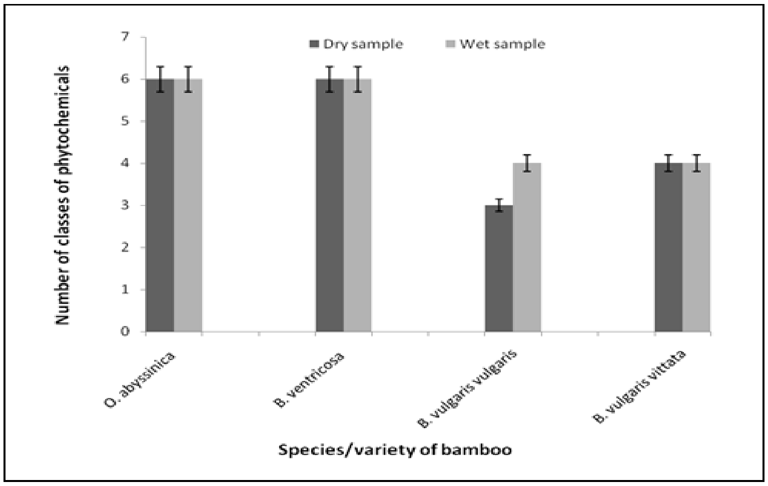

Fig 1: Classes of phytochemicals present in the bamboo varieties tested.

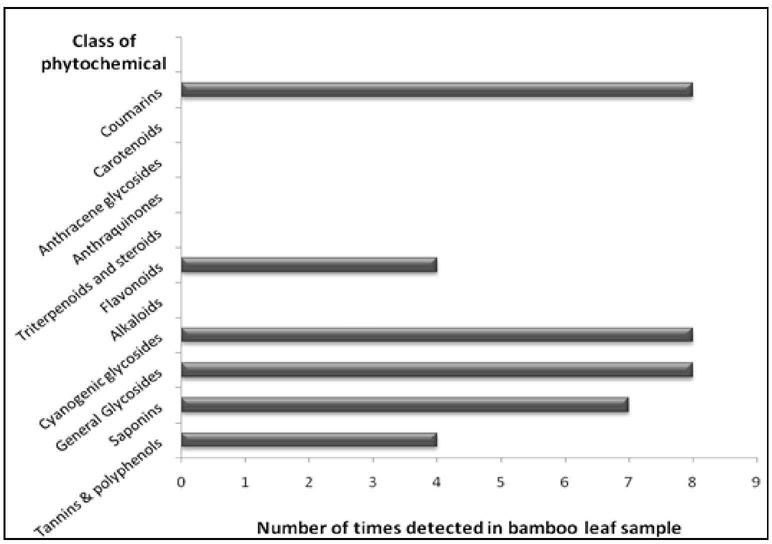

Fig 2: The occurrence of eleven classes of phytochemicals detected in the wet and dry leaves of four bamboo varieties.

In Figure 2, the frequency of occurrence of the 11 classes of phytochemicals tested for is shown. From the diagram, the predominant classes of phytochemicals present in the bamboo leaves analysed are: coumarins, cyanogenic glycosides and general glycosides. Saponins are also widely present in the bamboo leaves analysed. While flavonoids and tannins and polyphenols were present in half the number of tested samples (4), carotenoids, anthracene glycosides, triterpenoids and steroids, anthraquinones and alkaloids were not detected in any of the tested samples.

A statistical analysis of the results in Figure 2 indicates a mean of 3.55 and standard deviation of 3.67 and using a null hypothesis of 'each class of phytochemical occurs in less than 5 leaf samples', there was no significant statistical difference in the treatment means ($\chi^2 = 3.182$, $p \geq 0.05$). Of the 11 classes of phytochemicals tested for in the various leaf samples, five were not detected at all indicating total absence while the remaining six were detected in at least four samples each.

4. Discussion

From the results of the phytochemical analysis, generally the results for the wet ethanol extracted samples are comparable to those for the dried powdered samples in any given species/variety ($R^2 = 0.962$; t=1.000, P>0.05 and $\chi^2 = 9.0$, P>0.05). It suffices to say, therefore, that extracting wet bamboo leaf samples with ethanol is equally efficient as air-drying the leaf samples before extraction.

The absence of saponins (or their glycosides) in the dried powdered leaf samples of *B. vulgaris* may be due to the evaporation of the volatile phytochemicals, and/or the decomposition of the saponins, as they are amphipathic, into other glycosides probably cyanogenic in a chemical reaction which is not certain. This assertion is further supported by the observation that in *O. abyssinica* and *B.*

ventricosa, the presence of saponins was less noticed in the dry samples compared to the wet ethanol extracted ones (Table 1). Saponins have been proven to have an antimicrobial effect [9, 10] and that perhaps explains why there is apparently more in the fresh leaves, as they are more prone to deterioration than the dry ones.

The large number of phytochemical classes present in *O. abyssinica* could be a chemical defence mechanism for a plant that is particularly vulnerable as it is not typically highly foliated and therefore needs its leaves for photosynthesis and optimally nothing else. This is further evidenced by the large presence of haemolytic phytochemicals such as saponins in the wet samples of the plant.

The phytochemical spectrum of *B. ventricosa* is similar to that of *O. abyssinica* but for the greater presence of saponins and polyphenols in the wet samples while *B. vulgaris* leaves contain fewer classes of phytochemicals than in the other two species analysed. Apart from the absence of saponins in the dry powdered samples of the green variety, the phytochemical spectrum of *B. vulgaris* is the same for both the yellow (vittata) and green varieties. Unlike *B. ventricosa* and *O. abyssinica*, *B. vulgaris* is a morphologically robust plant with several anti-herbivory features such as long woody culms with leaves far away from the browsing range of most ungulates. The plant genetically therefore may not be in need of so many chemical defence tools as the other two, hence the fewer number of phytochemical classes.

Glycosides are abundant in green plant parts and it is believed they help keep birds and insects from eating seeds and fruits before they are fully-grown [11, 12]. Glycosides have a therapeutic effect and the medicinal properties of bamboo leaves could be due to their presence [13].

Saponins are highly toxic to ectotherms because of their haemolytic properties [14]. The trend of occurrence of saponins in the species analysed is indicative of some volatility or decomposition of the saponins present. *B. vulgaris* leaves may be the safest for the consumption of haemolymph-containing organisms because its leaves contain relatively less saponins. Saponins can lyse red blood cells of larger herbivores and that explains why plant extracts are not directly injected into human beings [11]. From the results of the phytochemical analysis, bamboo leaves seem to be devoid of saponins upon drying, therefore feed rations meant for haemolymph-containing organisms should be formulated from dry bamboo leaves not fresh ones.

Flavonoids are probably the most important class of phytochemical relative to herbivores [12]. Although flavonoids in plants like tea have beneficial antioxidant properties in humans, their tolerance in smaller mammals is limited [15]. Flavonoids are present only in the leaves of *O. abyssinica* and *B. ventricosa* indicating higher toxicity for small non-specialist herbivores than large mammalian herbivores. Flavonoids present in the leaves of *Olax manni* Olive. and other plants in the *Olacaceae* family are responsible for the toxic effects observed when the plant is consumed by animals including humans [15, 16]. Monkeys however are able to feed on the same plant without problem in various parts of Nigeria, Sierra Leone and Ghana-a clear case of co-evolution [16]. *O. abyssinica* and *B. ventricosa* leaves therefore should be fed on with care as flavonoids inherent can cause poisoning in the herbivores concerned.

Coumarins get fermented to produce a strong anti-coagulant known as dicoumarol [4]. Dicoumarol forms the base of rat poisons which create haemorrhage in the rodents. The above assertion is a source of worry for herbivores that may feed on bamboo leaves. Coumarins are also believed to function against prothrombin, the precursor of thrombin-the substance that facilitates blood clotting [11]. This belief has been strengthened by the fact that vitamin K acts against the effects of coumarins [8, 11].

Chemically, tannins are oligomeric compounds with free phenolic groups [17]. The tannins present in the leaves of *O. abyssinica* and *B. ventricosa* are condensed tannins (proanthocyanidins) as hydrolysable tannins are present only in dicotyledonous plants (Class Magnoliopsida) [15]. The leaves of *Artemisia princeps* PAMP (also a monocotyledonous plant) contains polyphenols, including condensed tannins-substances which have an inhibitory effect on protein fragmentation damage [18]. The absence of tannins and polyphenols in *B. vulgaris* is ample evidence of the absence of flavonoids as well; as the latter is a phenylpropanoid-a group of polyphenols.

Cyanogenic glycosides are stored in inactive forms in plant vacuoles [15]. They become toxic when herbivores eat the plant and break cell membranes allowing the glycosides to come into contact with enzymes in the cytoplasm releasing hydrogen cyanide which blocks cellular respiration and leads to tissue damage [19]. There are approximately 25 cyanogenic glycosides known, produced by at least 2650 plant species [19], and they include amygdalin (almonds); dhurrin (sorghum); linamarin (cassava, lima beans); prunasin (stone fruit); and taxiphyllin (bamboo shoots). Furthermore there is good evidence that cyanogenesis provides partial, if not complete, protection from predation by a wide spectrum of animal species [15]. Taxiphyllin which was isolated from bamboo shoots is likely to be the cyanogenic glycoside present in the leaves but its deleterious effects on herbivores is not established [20].

5. Conclusion

From the results of this study it can be concluded that the phytochemicals present in bamboo leaves are not significantly ($p \geq 0.05$) affected by air-drying of the leaves. Bamboo leaves can therefore be fed to livestock either in the fresh state or in the dry state as hay. Of the species analysed, *B. vulgaris* is the safest of the three.

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