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Phytochemical investigation on the seed of *Sphenostylis stenocarpa* (Hochst ex A. Rich.) Harms (Family Fabaceae)

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ABSTRACT: Phytochemical investigations carried out on the seeds of *Sphenostylis stenocarpa* (Hochst ex A. Rich.) Harms (Family Fabaceae) revealed the presence of the flavonoids (tricin, apigenin, chrysoeriol and genisten), tannin and alkaloids. The three putative varieties previously separated on the basis of seed colour were only distinguished on the amount of tannin present: white = 0.35, brown = 0.55 and red = 0.65. This result if corroborated by other systematic lines of evidence may lead to the delimitation of the three putative varieties into either distinct varieties or species but if otherwise could lead to the recognition of this species as monolithic. @JASEM

Key words: *Sphenostylis stenocarpa*, Fabaceae, phytochemistry, seeds, delimitation.

Sphenostylis stenocarpa (Hochst ex A. Rich) Harms (Family Fabaceae, Sub family Papilionoideae, Tribe Phaseoleae, synonyms*: Dolichos stenocarpus* Hochst. ex A. Rich. and *Vigna ornata* Welw. ex Baker) is one of the three species of *Sphenostylis* E. Meyer in West Africa (Hepper, 1963; Nyananyo and Osuji, 2007). The two other species are *S. schweinfurthii* Harms and *S. holosericea* (Welw. ex Baker) Harms. There are, however, sixteen species endemic to Africa (Airy-Shaw, 1985; Nyananyo and Osuji, 2007). Although the specific and infra-specific boundaries within this genus and some of its species are well defined, the infra specific boundaries in *Sphenostylis stenocarpa* are unclear (Potter, 1992; Potter and Doyle, 1992; Ene-Obong and Okoye, 1993; Adeyeye, 1997; Agunbiade, and Longe, 1999; Nyananyo and Osuji, 2007). This makes characterization of *S. stenocarpa* germplasm to determine infra-specific boundaries difficult even as it is known that this is one of the most under-exploited legumes in Africa (Anon, 1975; Klu *et al.,* 2007).

Phytochemistry is a systematic line of taxonomic evidence dealing with the chemical processes associated with the physiology of plants and the chemical compounds produced by plants. Phytochemistry is concerned with the enormous variety of organic substances that are produced and accumulated by plants. It deals with the chemical structures of these substances, their biosynthesis, metabolism, natural distribution and biological functions (Harborne, 1973).

Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals by using techniques such as chromatography (paper and thin layer) techniques and electrophoresis. The realization that behind the universal occurrence in plants of many vital biochemical pathways, there is an enormous variation between taxa in many other less vital pathways and the current belief that evidence from as many sources as possible should be used in plant classification (Stace, 1980).

Sphenostylis stenocarpa originated from Abyssinia (i.e. present day Ethiopia) and though occurs in the wild, is widely cultivated in west Africa particularly in Cameroon, Cote d'Ivoire, Ghana, Nigeria and Togo (Dalziel, 1937; Marechal *et al*., 1950; Lackey, 1981; Potter,1992; Ene-Obong and Okoye, 1993; Nyananyo and Osuji, 2007) though its cultivation is now spreading to East, Central and Southern Africa for its relatively very proteinous seeds and tubers, especially as a security crop by peasant farmers (Adeyeye, 1997; Agunbiade and Longe, 1999). It faces extinction as its cultivation receives little or no economic support (Klu *et al*., 2001). This species is commonly called 'African yam bean, AYB', owing to its ability to produce edible tubers (Onwuka *et al*., 2009). The plant is an annual with a climbing habit. In many places where it is cultivated, it is inter-cropped with yam, *Dioscorea* spp. with which it shares stakes. The tubers which are the most valuable parts of this plant have the capability to sprout and often serve as secondary propagules of the crop (Anon, 1979).

The plant is slow to set seeds (Ene-Obong and Okoye, 1993). The Nigerian genotypes take an average period of 86 days to produce first flowers and 140 days to produce first ripe fruits and the seeds have high crude protein content (Okigbo, 1973; Anon, 1979). The dry seeds are used in the preparation of special meals during festival and celebration of puberty rites for girls in the avatime traditional area of the HO west district, giving the

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crop a special role in the socio-cultural lives of the people; as animal feed in some localities and as a source of protein in various food preparations.

The tuberous roots of African yam bean are used as a source of carbohydrate in West Africa. *Sphenostylis stenocarpa* features extensively as an intercrop in the traditional farming system. (Okigbo, 1973; Anon, 1979; Ene-Obong and Okafor, 1992; Potter, 1992).

The crop also nodulates profusely and probably has high nitrogen fixing ability, thereby helping to replenish soil nitrogen. Though the African yam bean currently serves as a security crop, it has the potential to meet year round protein requirements if grown in a large scale. Currently only limited quantities are offered for sale in local markets, even though the price per unit of measure is comparable to that of cowpea and groundnut. Large scale production should therefore lead to increases in family income.

The aim of this experiment is to determine the presence of chemicals especially secondary metabolites in the seeds of *Sphenostylis stenocarpa* and to use the data in combination with data from other systematic lines of evidence to distinguish and determine the three putative varieties, presently distinguished only on the basis of colour i.e. white, brown and red variety as either varieties or species if the differences are significant or as a monolithic species if not so.

MATERIALS AND METHODS

Seeds of *Sphenostylis stenocarpa* were obtained from stands growing in the University of Port Harcourt garden. Tests for flavonoids, tannic acids and alkaloids were carried out.

Flavonoid test: The flavonoids were determined by gravimetric method (Harborne, 1973).

Chromatographic paper separation: The chromatographic paper was demarcated into three parts, using a meter rule and a pencil. The first part measured 10cm, the second 22cm and the third 2cm. A pencil was used to create dark spots on the first demarcation of the chromatographic paper, two spots for each putative variety.

Sample preparation: The seeds of the three putative varieties of *Sphenostylis stenocarpa* were crushed separately using a grinder. Relatively small amount of the samples were immersed in three different test tubes containing ethanol. The samples were stirred with a stirring rod for proper mixing and allowed to settle for 10 minutes.

Spotting on chromatographic paper: Spotting was done by using capillary tubes to transfer the solution from the different test tubes to designated spots on the chromatographic paper. Specific capillary tubes were used for different solutions to avoid incorrect results. Spotting was done several times for easy detection of the colours. The chromatographic paper was hung to dry for ten (10) minutes.

Sample separation: The samples were separated using a chromatographic tank with the required solvent in a trough at the top of the chromatographic tank. The end of the chromatographic papers nearest to the sample spots were held in the trough at the top of the chromatographic tank and the rest of the paper made to hang vertically.

The required solvents BAW which is a combination of (butanol-acetic acid-water) in the ratio 4:1:5 and forrestal which is a combination of (acetic acid-conc. HCL-water) in the ratio 30:3:10 were added to the trough differently for each chromatographic paper for the solvent to travel through the chromatographic paper. Separation occurred as the solvent front was obtained. The chromatographic paper was removed from the chromatographic tank and hung to dry.

Colour detection and calculation of retardation factor, R_f values: The dried papers were viewed under visible light and in total darkness under UV light to see the colours they produced as well as their points of separation in order to determine the retardation factor (R_f values) of the different samples. The retardation factor (i.e. R_f value) of each variety of *Sphenostylis stenocarpa* was calculated using this formula:

$$Rf = \frac{D_{sp}}{D_{sf}} \times \frac{100}{1}.$$

Where: D_f = retardation factor; D_{sp} = distance from the origin to centre of solvent front and D_{sf} = distance between origin and the solvent front

Tannic acid determination: Tannic acid was determined by using the follin-denis spectrophotometric method. A relatively small amount (0.1g) of the crushed sample was weighed into a 100ml conical flask. Fifty ml (50 ml) of

distilled water was added to the sample and placed on a hot plate for one hour. The samples were filtered whilst warm through Whatman no 44 filter paper into a 50ml volumetric flask.

A pipette was used to measure 1ml of each sample into three different test tubes and relatively small amounts (0.25ml) of tannic acid and follin-denis reagent respectively were added to the samples. One (1.0) ml of 17% sodium carbonate solution was added to these mixtures, diluted with five (5.0) ml distilled water and stirred for proper mixing. The optical density of the samples were measured at 520nm and recorded.

Alkaloid detection test: A relatively small amount (0.1g) of the crushed sample was weighed into a 100ml conical flask. Ten (10 ml) of 1.0% HCL was added into the flask and heated until the volume reduced to 5ml for 5minutes. The mixture was filtered and the filtrate taken into six different test tubes, two test tubes for each variety. Few drops of Meyer's and Wagnard's reagents were added into the different test tubes containing the filtrate. The samples were observed and the results recorded.

RESULTS

The results are as presented in Tables 1 and 2. The R_f values and the colours detected under uv. light of each sample was used to determine the type of flavonoids present in the three putative varieties of *Sphenostylis stenocarpa* (Harborne, 1973).

Table 1: Parameters investigated on the seeds of *Sphenostylis stenocarpa*

Seed colour	*White*	*Marble/Brown speckled*	*Black/Red*
Flavonoids	Apigenin and Tricin (flavones)	Chrysoeriol (flavone). Genistein (isoflavone)	Apigenin (flavone) Genistein (isoflavone)
Tannin %age	0.35	0.55	0.65
Crude protein (%age)	10.6	11.2	11.7
Amino acids (whole seeds)	Lysine, Glycine, Aspartic acid, Alanine, D-Tyrosine & Leucine	Glycine, Aspartic acid, Alanine, Valine & Proline	Lysine, Glycine, Aspartic acid, Alanine, D-Tyrosine & Leucine
Amino acids (Decoated seeds)	Lysine, Glycine, Aspartic acid, Alanine, D-Tyrosine, Leucine & Proline	Lysine, Glycine, Aspartic acid, Alanine, Proline & Valine	Lysine, Glycine, Aspartic acid, Alanine & D-Tyrosine

Tannic acid determination: The result obtained from the test for tannin of the three putative varieties of *Sphenostylis stenocarpa* were measured at an absorbance of 520nm with the colour bluish green showed that the optical density of the samples are as follows:

Table 2: Optical density of the samples

Colour of the yam bean	*Optical density*
White	0.07
Brown	0.11
Red	0.13

The percentage of tannin present in the three putative varieties of *Sphenostylis stenocarpa* was calculated using the values in table 3 in the formula below:

$$\%\ \text{Soluble Tanin} = \frac{\text{weight(g)} \times \text{T(ml)}}{10 \times \text{A(ml)} \times \text{SW(g)}}.$$

Where: T = total total extract volume (ml); A = aliquot (ml); SW = sample weight (g)

Alkaloid Detection Test: The quantitative determination of alkaloid was carried out by alkaline precipitation through the method described by (Harborne, 1973). The result obtained from the test for alkaloid to determine the presence or absence of alkaloid in the three putative varieties of *Sphenostylis stenocarpa.*

DISCUSSION

The results obtained from the phytochemical investigation carried out on the three putative varieties of *Sphenostylis stenocarpa* (Hochst ex A. Rich) Harms in Nigeria are as shown in Table 1.

The identification of compounds isolated from plants is a practical method (Harborne, 1973). In the case of secondary metabolites, it involves the separation of crude mixture by some form of chromatography and the probable identification of the components in comparison with the patterns of chromatographic behaviour of known substances. The most useful

class of compounds for such study are flavonoids (Harborne, 1973). Surveys of many other classes of compounds, notably, alkaloids, non-protein amino acids, terpenes and sulphur compounds have also yielded potentially useful new information for taxonomic purposes (Swain, 1966). These analyses have been carried out on comparative basis on the three putative varieties of *Sphenostylis stenocarpa.*
The flavonoids recorded in this study in Forrestal and BAW are Tricin (flavone) and Apigenin (flavone), Chrysoeriol (flavone) and Genistein (isoflavone) and Apigenin (flavone) and Genistein (isoflavone) for the white, brown and red putative varieties respectively (Table 1). This result shows that these chemicals cannot be used to distinguish the three putative varieties.

The optical density of the three putative varieties of *Sphenostylis stenocarpa* measured at an absorbance of 520nm. were 0.07, 0.11 and 0.13 for the white, brown and red respectively (Table 1)

Also, the percentage of tannin recorded were 0.35, 0.55 and 0.65 for the white, brown red respectively (Table 1). Although the values obtained are discrete, they cannot possibly be used to classify except in combination with other characters (Stace, 1980). The alkaloids recorded were similar for the white, brown and red putative varieties (Table 1).

This shows that chemical evidence cannot possibly be used as a distinguishing factor among the three putative varieties and so it will be appropriate to carry out more studies especially at the gene level to determine whether there will be adequate additive characters to support or reject the separation recorded in this study. This is in line with the position of some earlier workers (Potter, 1992; Potter and Doyle, 1992; Adeyeye 1997; Agunbiade and Longe, 1999; and Nyananyo and Osuji 2007).

*Conclusion***:** The results of the study carried out on the phytochemistry of *Sphenostylis stenocarpa* showed that flavonoids, tannin and alkaloids are present in the three putative varieties of *Sphenostylis stenocarpa.* Since the three putative varieties have the same chemicals present in them, they cannot be distinguished using their chemical composition. The percentage of tannin in the three varieties differ, and so this could be used in combination with other distinguishing characters for separating the three varieties. An investigation in this direction is advisable. It will therefore be appropriate to carry out further studies on *Sphenostylis stenocarpa* to either discover distinguishing factor(s) among the three putative varieties or not.

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