INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS), VOL. 6, NOS.1& 2 (2011); P. 1 – 8, 2 TABLES.

Determination of phytochemicals in *Telfairia occidentalis*, *Amaranthus hybridus*, *Phaseolus vulgaris* and *Sphenostylis stenocarpa* inoculated with *Telfairia mosaic virus* (TeMV)

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ABSTRACT

Qualitative and quantitative determinations of phytochemicals in *Telfairia occidentalis* Hook., *Amaranthus hybridus* L., *Phaseolus vulgaris* L. and *Sphenostylis stenocarpa* Hochst. inoculated with *Telfairia mosaic virus* (TeMV) was evaluated. Alkaloids, glycosides, saponins, tannins, flavonoids, steroids, trepenoids, reducing compounds, polyphenols, phlobatanins, anthraquinones and hydroxymethyl anthraquinones were all present in *Telfairia occidentalis* in healthy and infected leaves. Phlobatanins, anthraquinones and hydroxymethyl anthraquinones were absent in *A. hybridus*, in addition steroids and flavonoids were absent in *P. vulgaris.* Phlobatanins and anthraquinones were absent in *S. stenocarpa* in healthy and infected leaves. TeMV caused significant reductions in alkaloids (40.o%) and flavonoids (22.63%) in *T. occidentalis.* The virus also caused marked decreases in flavonoids (88.55%), saponins (70.31%) in *A. hybridus.* Saponins (89.29%) and alkaloids were significantly reduced in infected samples of *P. vulgaris*. However,, there were marked increases in alkaloids and saponins contents by 92.24% and 51.85% respectively in *S. stenocarpa.* Infection of TeMV caused significant reductions in some phytochemicals (alkaloids, flavonoids and tannins) and increases in other polyphenol and saponins in infected and healthy in the two vegetables and legumes studied.

INTRODUCTION

Vegetables are indispensable components of human diets. They supply the body with minerals, vitamins and certain hormone precursors in addition to proteins and energy (Oyenuga and Fetuga, 1975). *Phaseolus. vulgaris* is a legume that is widely consumed throughout the world and it is recognized as the major source of dietary protein in Latin American and African countries. Recently, *Phaseolus vulgaris* (common beans) is gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals such as fibre, polyphenolic compounds, trysin inhibitors, phytic acid among others with potential benefits. *Stenocarpa* another (African yam beans) is a legume that is rich in potassium, phosphorus, magnesium, calcium, iron, zinc but low in sodium and copper (Edem *et al.*, 1990).

Plants serve as food and medicine to the body. The medicinal values of plants and vegetables are dictated by their phytochemical and other chemical constituents (Fallah *et al*., 2005). The most important of these phytochemicals (bioactive constituents) of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). This explains why efforts have been expended in studies aimed at elucidating their levels in many plants both in Nigeria and elsewhere (Edeoga *et al.*, 2005, 2006). *Telfairia. occidentalis, A. hybridus, P .vulgaris* and *S. stenocarpa* have been reported as susceptible host of TeMV (Shoyinka and Thottappilly, 1998). TeMV is a potyvirus characterized by flexuous rod-shaped particles of about 806 nm in length, transmitted by *Aphis spiraecola* in a foregut-borne manner (non-persistent) (Shoyinka *et al*., 1987).

Effect of TeMV on vitamins and amino acids profile in two ecotypes of *T. occidentalis* has been reported by Mofunanya *et al.*, (2007). Report also exists on effect of TeMV on proximate, mineral and anti-nutritive contents of *T. occidentalis* (Fluted pumpkin) (Mofunanya *et al*., 2008). No reports however, exist on the effect of TeMV on phytochemical constituents. The present study is aimed at determining the qualitative and quantitative contents of phytochemicals in *T. occidentalis*, *A. hybridus*, *P. vulgarius* and African yam beans (*S. stenocarpa*) inoculated with TeMV.

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MATERIALS AND METHODS

Qualitative analysis of the phytochemicals

Qualitative analysis of chemical constituents was carried out using the ethanolic extract of healthy and infected plants of each species using standard procedures as described by Sofowora (1993), Trease and Evans (1989), Harbone (1973) and Guilei (1982).

Test for alkaloids

To 2 ml of healthy and infected extracts were added 5 ml of 1% aqueous HCl and stirred in a waterbath, and one ml of the filtrate of the samples was treated with few drops of Dragendoff's reagent and a second 1 ml was treated with Mayer's reagent. Turbidity and precipitation with either of these reagents was taken as evidence for the presence of alkaloids.

Test for glycosides (Sofowora, 1993)

Salkowski test: To test for glycosides, 2 ml of healthy and infected leaf extract of the different plant species were dissolved in 2 ml of chloroform. Sulphuric acid (H2SO4) was carefully added to form a lower layer. A reddish – brown colour at the interface indicates the presence of glycosides.

Test for Saponins (Sofowora, 1993)

Two grams of the healthy and infected powered samples were boiled in 20 ml of distilled water in a waterbath and filtered. To 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was added with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for tannins (Sofowora, 1993)

The test for tannins was carried out using five grams of dried powdered samples of healthy and infected leaves. This was boiled in 20 ml of water in test tube and then filtered. The presence of tannins was determine by adding a few drops of 0.1% ferric chloride to 2 ml of ethanolic extracts of the samples and observed for brownish green or blue-black coloration.

Test for flavonids (Sofowora, 1993; Harbone, 1973)

To determine the presence of flavonoids in healthy and infected plant samples, 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow coloration in each extract indicated the presence of flavonoids.

Test for polyphenols (Guilei, 1982)

To test for polyphenols, 2 ml of ethanolic plant extracts of both healthy and infected samples were treated with 5 ml of distilled water and heated for 30 minutes in a waterbath. To this was added 1 ml of 1% $FeCl_3$ followed by the addition 1 ml of 1% potassium ferrocyanide solution. The mixture was filtered. The formation of green-blue colouration indicates the presence of polyphenols.

Test for phlobatanins (Trease and evans, 1989)

The presence of phlobatanins in infected and healthy samples was tested by adding 5 ml of 1% HCl to 2 ml and boiled for 5 minutes. Deposition of a red precipitate was evidence of the presence of phlobatanins.

Test for anthraquinones (Trease and Evans, 1989)

To 2 ml of each of the extracts was shaken with 10 ml of benezene and filtered. Five ml of 10% NH_3 was added to the filtrate and the mixture was shaken. The presence of pink red or violet coloration in ammonical (lower) phase indicates the presence of free anthraquinones.

Test for hydroxymethyl anthraquinones (Guilei, 1982)

To test for the presence hydroxymethyl anthraquinones, 2 ml of each of the extract was treated with a solution of 5 ml of 5% ammonia. The formation of a red color or precipitate indicates the presence of hydroxymethyl anthraquinones.

Test for steroids

The presence of steroids was tested by adding 2 ml of acetic anhydride to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test)

The test for presence of terpenoids was carried out by carefully adding 5 ml of healthy and infected samples to 2 ml of chloroform and 3 ml of concentrated H_2SO_4. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

Phytochemicals in some infected seeds

Quantitative determination of the effect of TeMV on phytochemicals

Alkaloid determination using Harbone (1973) method

The presence of alkaloid in infected and healthy plant samples was determined by adding 5 g of the samples to 200 ml of 10% acetic acid in ethanol into a 250 ml beaker. The beaker was covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a waterbath to 60 m1. Concentrated ammonium hydroxide was added dropwise to the extract until precipitation was completed. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

Tannin determination by van-Burden and Robinson (1981) Method

To determine the quantity of tannin, 500 mg of infected and healthy samples of each plant species was weighed into a 50 ml plastic bottle. To this was added 50 ml of distilled water and shaken for 1 hour on a mechanical shaker. The solution was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted into a test tube and mixed with 2 ml of 0.1 ml $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 280 nm within 10 min.

Saponin determination

The method used was that of Obdoni and Ochuko (2001). The infected and healthy leaf samples of each plant species were homogenized and 20 g of each were put into a conical flask and 100 m1 of 20% aqueous ethanol were added. The samples were heated in a hot waterbath for 4 hours with continuous stirring at 55^{o}C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over a waterbath at about 90^{o}C. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated after which 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated using the formula:

$$\% \text{ saponin} = \frac{Weight\ of\ residdue}{Weight\ of\ sample} \times 100$$

Flavonoid determination by the method of Boham and Kocipal (1994)

Ten gram of infected and healthy samples of each plant species was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a waterbath to a constant weigh

Total phenol determination by spectrophotometric method

The fat -free sample of each plant species was boiled with 50 ml of ether for 15 min for the extraction of the phenolic component. About 5 ml of the extract pipetted into a 50 ml flask, and 10 ml of distilled water was added, to which 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development. Phenolic component was measured in a spectrophotometer at 505 nm.

RESULTS

Results of qualitative determination of phytochemicals in the four plant species revealed the presence (+) of some chemical constituents in both healthy and infected plant samples and the absence (-) of others. Of the twelve phytochemicals investigated, all were present in *T. occidentalis*, nine present in *A. hybridus*, eight in *P. vulgaris* and ten in *S. stenocarpa* (Table 1).

Results of infection on the percentage of phytochemicals are presented in Table 2. *Telfairia mosaic virus* significant ($P<0.05$) reductions in alkaloids and flavonoids in infected samples of *T. occidentalis*. Mean values for alkaloids and flavonoids in healthy and

infected samples were 3.50 ± 0.03, 2.10 ± 0.012 mg/100 g and 13.70 ± 0.002, 10.60 ± 0.012 mg/100 g respectively. Infection of TeMV led to marked reductions in saponins, tannins, polyphenols, reducing compounds and alkaloids in *A. hybridus*. The mean values for healthy samples were 3.20 ± 0.02, 0.54 ±0.02, 19.22 ±0.01, 46.76 ± 0.2 and 26.20 ± 0.01 mg/100 g respectively. The corresponding values for infected samples were 0.95 ± 0.02, 0.31 ± 0.01, 11.05 ± 0.02, 28.26 ± 0.02 and 17.80 ± 0.02 mg/100 g. The virus also caused significant decreases in saponins, alkaloids, polyphenols, tannins and reducing compounds in infected samples of *P. vulgaris*. The mean values of saponins and alkaloids were 2.80 ± 0.02 and 15.40 ± 0.1 mg/100 g as against values of 0.30 ± 0.1 and 7.02 ± 0.1 mg/100 g in healthy and infected samples. Alkaloids and saponins had mean values of 1.16 ± 0.1 and 1.35 ± 0.02 mg/100 g in healthy controls as against values of 2.23 ± 0.02 and 2.05 mg/100 g for infected samples.

DISCUSSION

Many higher plants accumulate extractable organic substances in quantities sufficient to be economically useful as chemical feed stocks or raw materials for various scientific, technological and pharmaceutical applications. Natural substances are employed, either or direct, by a large number of industries and natural plant products including (phytochemicals) feature prominently in several of these (Prode and Dotty, 1981).

Results of this study revealed significant decreases in alkaloids and flavonoids in *T. occidentalis* TeMV combination. This result revealed the presence of alkaloids and flavonoids in healthy and infected leaves of *T. occidentalis* in contrast to earlier report of phytochemical description of *T. occidentalis* in healthy leaves (Ezugwu and Nwodo, 2000). Infection of TeMV led to marked decreases in alkaloids, saponins, tannins, reducing compounds and phenolic compounds in *A. hybridus* and *P. vulgaris.* Results of this study agrees with previous works by Legrand *et al.* (1976), Rangaraju and Chenulu (1975) recorded a decrease in growth characters and alkaloid content of its host by *Solanum klasianum mosaic virus*, El-Hammandy *et al*. (1982), reported a decrease in alkaloid contents of *Datura stramonium* leaves infected by CMV and PVX, El-Dougdoug *et al.* (2007) reported on decrease in alkaloid contents of medicinal plants infected by *Potato virus Y* and Duarte *et al.* (2008) also reported on decreases in phenol and alkaloid contents in *Datura stramonium* leaves inoculated with *Potato virus X*.

The reductions in these important phytochemical constituents caused by TeMV on these vegetables and beans species is worrisome. Plants form an important part of the human diet and a major source of biologically active substances. Knowledge of complete alkaloids pattern is of interest not only phytochemically, but also in relation to aspects of alkaloid biogenesis metabolism and application in the plant biotechnology (El-Dougdoug *et al*., 2007). Plant metabolites such as flavonoids, phenolics, glucosinolates, carcinogenic glycosides and terpens are essential to plant growth, development, stress adaptation and defense against pathogens, herbivores and insects (Harborne, 1998, Hounsome *et al*., 2008). Besides the importance for the plant itself, a large number of phytochemicals with antioxidant, antimutagenic, cytotoxic, antifungal, antiviral activities have been reported in plants (Goldberg, 2003). These phytochemicals have been linked to many positive effects on human health, including coronary heart diseases, diabetes, high blood pressure, cataracs, degenerative diseases and obesity (Liu and others 2000, Djousse *et al* 2004). Metabolite also determine the nutritional quality of food, color, taste and smell.

Levels of metabolites are strongly affected by genetic and environmental factors as well as transportation and storage condition. Growth factors such as light, temperature, humility, type of soil, application of fertilizers, damage caused by pathogens and insects, stress induced by UV radiation, heavy metals, and pesticides all alter metabolite composition of plants (Orcutt and Nilsern, 2000).

The high levels of phytochemicals in infected African yam bean induced by TeMV may be associated with infection. The accumulation of these phytochemicals in infected samples suggest that their synthesis is stimulated by virus infection. This result correspond to previous report by Uegaki *et al*. (1988) who reported on the accumulation of chemical compounds in leaves of *Nicotiana undulate* inoculated with *Tobacco mosaic virus*.

Table 1. Phytochemical screening of *Amaranthus hybridus, Telfairia occidentalis, Phaseolus valgaris* & *Sphenostylis stenocarpa* indicated with *Telfairia mosaic virus.*

Chemical Constituentes	*Telfairia* occidentails		*Amaranthus hybridus*		*Phaseolus vulgaris*		*Sphenostylis stenocarpa*	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Alkaloids	++	+	+	+	++	+	+	+
Glycosides	++	+	+	+	+	+	+	++
Saponins	++	++	+	++	+	+	++	++
Tannins	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	-	-	+	+
			+	+	-	-		
Reducing compounds	++	++	+	+	++	++	+++	++
Polyphenols	+++	++	+++	+++	+++	++	+++	+++
Phlobatanin	+	+	-	-	-	-	-	-
Anthraquinones	+	+	-	-	-	-	-	-
Hydroxymethyl Anthraquinones	+	+	-	-	-	-	+	+
Trepenoids	+	+	+	+	+	+	+	+

+ = Present

++ = Very much present

+++ = Present in excess

- = Absent

Table 2. Percentage of crude alkaloids, saponins, tannins, flavonoids, reducing compounds and phenols in *Telfairia occidentalis, Amaranthus hybridus, Phaseolus vulgaris and Sphenostylis stenocarpa*

(mg/100 g dry matter)

	Alkaloids		Saponins		Tannins		Flavonoids		Reducing compounds		Phenols	
Plants	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**infected**
Telfairia occidentalis	3.50 ± 0.03	2.10 ± 0.012	2.35 ± 0.012	3.40 ± 0.002	0.33± 0.02	0.26 ± 0.01	13.70 ± 0.002	10.60 ± 0.012	8.74 ± 0.002	9.89 ± 0.003	14. 11 ± 0.001	18.12 ± 0.003
Percentage difference	40.00		44.68		11.54		22.63		13.56		28.42	
Amaranthus hybridus	26.20 ± 0.01	17.80 ± 0.02	3.20 ± 0.2	0.95 ± 0.02	0.54 ± 0.02	0.31± 0.01	0.31± 0.01	2.70 ± 0.02	46.76 ± 0.2	28.26 ± 0.02	19.22 ± 0.001	11.05 ±0.02
Percentage Difference	32.06		70.31		42.59		88.52		39.56		42.51	
Phaseolus vulgaris	15.40± 0.1	7.02 ± 0.01	2.80 ± 0.02	0.30 ± 0.1	0.58 ± 0.01	0.43± 0.01	-	-	47.91 ± 0.01	39.12 ± 0.01	16.32 ± 0.02	10. 14 ± 0.003
Percentage differencence	54.42		89.29		25.86		-	-	18.35		37.87	
Sphenostylis stenocarpa	1.6 ± 0.1	2.23 ± 0.02	1.35 ± 0.02	2.05 ± 0.02	0.18 ± 0.02	0.23 ± 0.01	8.35± 0.02	10.60 ± 0.01	9.80 ± 0.02	10.40 ± 0.2	12.26 ± 0.03	17.61 ± 0.001
Percentage difference	92.24		51.85		27.78		26.95		6.12		43.64	

Mean ± SE, n = 3 replicates, $P < 0.05$. Percentage difference was obtained by expressing the difference between the values for the healthy and the inoculated plant as a percentage of the healthy.

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