PHYTOCHEMICAL SCREENING OF HEALTHY AND SUGARCANE CHLOROTIC STREAK VIRUS-INFECTED LEAVES OF *Saccharum officinarum*

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Adama Yahaya*, Yahaya Salma Shu'aib, Dangora Balarabe Danladi and Rihana Abdullahi Idris
Department of Botany, Ahmadu Bello University, Zaria, Nigeria
Corresponding author: yadamcy@yahoo.com

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Abstract: Sugarcane, *Saccharum officinarum* is a species of grass cultivated in tropical and sub-tropical countries worldwide. It is affected by Sugarcane chlorotic streak virus (SCSV), a leafhopper-transmitted virus which causes chlorotic streaks on leaves and other symptoms. Air-dried healthy and SCSV-infected leaf samples were screened to determine the presence and quantity of various phytochemicals. The methanolic leaf extracts of both samples were used to carry out qualitative and quantitative analysis using standard methods. The results showed that both leaf extracts had alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins, reducing and non-reducing sugars present while antraquinones and steroids were absent. They also showed higher amount of flavonoids (1.45 g), saponins (0.26 g), phenols (0.051 g) and tannins (0.156) in healthy leaf extract while alkaloids (0.23) were higher in SCSV-infected leaf extract. The results also suggest that the differences between mean quantity of phytochemicals for alkaloids, flavonoids and tannins in healthy and SCSV-infected leaf extracts were significant while it was not significant for the phenols and saponins. The studies conducted showed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins, reducing and non-reducing sugars in healthy and SCSV-infected leaf extracts. The healthy leaf extract showed higher quantity for all metabolites except alkaloids.

Keywords: Phytochemicals, *Saccharum officinarum*, sugarcane chlorotic streak virus

Introduction

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicine. Medicinal plants are important for the treatment and management of human diseases due to presence of plant phytochemical constituents. Phytochemicals are naturally occurring compounds which are of significance in the defense and protection of plants from diseases (Doss and Amand, 2012). Phytochemicals are defined as bioactive non-nutrient plant compounds found in plants that have been attributed to reduce the risk of major chronic diseases (Blessy *et al*., 2012). Phytochemicals are basically divided into two groups that are primary and secondary metabolites. The major constituents of phytochemical consists of carbohydrates, amino acids, proteins and chlorophylls, while, secondary metabolites consists of alkaloids, saponins, steroids, flavonoids, tannins etc. Knowing these phytochemicals and their specific uses will go a long way in treating diseases in the medical as well as pharmaceutical field (Enwuru *et al*., 2008).

Saccharum officinarum, sugarcane, is a large, strong-growing species of grass in the genus Saccharum. It originated in Southeast Asia and is now cultivated in tropical and subtropical countries worldwide for the production of sugar and other products. *S. officinarum*, a perennial plant, grows in clumps consisting of a number of strong unbranched stems. A network of rhizomes forms under the soil which sends up secondary shoots near the parent plant. The stems vary in color, being green, pinkish, or purple and can reach 5 m (16 ft) in height. They are jointed, nodes being present at the bases of the alternate leaves. The internodes contain a fibrous white pith immersed in sugary sap. The elongated, linear, green leaves have thick midribs and saw-toothed edges and grow to a length of about 30 to 60 cm (12 to 24 in). The terminal inflorescence is a panicle up to 60 cm (24 in) long, a pinkish plume that is broadest at the base and tapering towards the top. The spikelets are borne on side branches and are about 3 mm (0.12 in) long and are concealed in tufts of long, silky hair. The fruits are dry and each one contains a single seed. Sugarcane harvest typically occurs before the plants flower, as the flowering process causes a reduction in sugar content.

S. officinarum and its hybrids are grown for the production of sugar, ethanol, and other industrial uses in tropical and subtropical regions around the world. The stems and the byproducts of the sugar industry are used for feeding to livestock. Pigs fed on sugarcane juice and a soy-based protein supplement produced stronger piglets that grew faster than those on a more conventional diet. As its specific name (*officinarum*, "of dispensaries") implies, it is also used in traditional medicine both internally and externally (Miraj, 2016). Sugarcane popularly known as noble cane due to its high sucrose content and low fiber content is one of the most important industrial crops of the world. The sugar cane juice contains flavonoids (Abbas *et al*., 2013). The roots and stems of sugar cane are used in medicine to treat skin and urinary tract infections as well as for bronchitis, heart conditions, loss of milk production, cough anaemia, constipation as well as general debility. It is also used to treat jaundice and lowering blood pressure (Mira *et al*., 2011). Characterized Sugarcane chlorotic streak virus (SCSV) is a recently characterized sugarcane-infecting virus arising from a putative interspecific recombination event involving two grass-infecting mastreviruses, Eragrostris streak virus and Uroschloa streak virus ,as putative parental sequences (Yahaya *et al*., 2016). Symptoms of the virus include shortened nodes and chlorotic streaks on leaves. The best management practices for the virus include breeding for resistance, selection of virus-free planting materials and control of vectors (Yahaya *et al*., 2016).

The present study was carried out to evaluate the qualitative and quantitative analysis of phytochemicals in the healthy and sugarcane chlorotic streak virus-infected leaves of *Saccharum officinarum.*

Materials and Methods

Study area

This study was conducted at the Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria and the Department of Pharmacognosy and Drug development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, North-Western Nigeria.

Plant collection

The healthy and SCSV-infected sugarcane leaves were collected from Kubanni ($N11^{0}05.717$ $E007^{0}43.271$), Zaria local government area of Kaduna state, North-Western Nigeria.

Fig. 1: (A) Healthy leaves of *Saccharum officinarum;* (B) A sugarcane chlorotic streak virus infected *Saccharum officinarum* leaves

Preparation of leaf samples

The healthy and sugarcane chlorotic streak virus-infected leaves were air-dried for a month in a well-ventilated laboratory at the Department of Botany. The leaves were constantly turned to prevent rotting. The leaves were air-dried in-house to prevent ultra-violet rays from destroying the active metabolites in the plant. The dried leaves were later pounded using mortar and pestle till it turned to powder.

Extraction

The powdered materials were macerated in 300 ml of 70% aqueous methanol and 40 g of the powdered samples (the healthy and chlorotic streak virus-infected sugarcane leaves) were macerated separately in conical flasks. The set-up was allowed to stand for 2 days after which it was filtered. The filtrate obtained was evaporated to dryness and the extracts obtained were stored till usage.

Phytochemical screening

Chemical tests for the screening and identification of bioactive chemical constituents in healthy and SCSV-infected sugarcane leaves under study were carried out in methanolic extracts as well as powder samples using standard procedures.

Qualitative analysis

Test for phenols: To 1 ml of extracts of sample, 2 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols (Suman *et al*., 2013).

Test for steroids: 0.5 g of the extract was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. Colour change from violet to blue or green in samples indicates the presence of steroids (Suman *et al*., 2013).

Test for saponins: To 1 ml of aqueous extract was added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min (Sabri *et al*., 2012).

Test for tannins: 2 ml of the aqueous extract was added to 2 ml of water, one to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicated the presence of tannins (Sabri *et al*., 2012).

Test for Cardiac glycosides (Keller-Killani test): 5 ml of the extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring was formed at the interface. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer (Suman *et al*., 2013).

Test for flavonoids: 3 ml of the aqueous extract was mixed with 4 ml of 1% potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicated the presence of flavonoids (Suman *et al*., 2013).

Test for alkaloids: 0.5 to 0.6 g of the various extracts was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation (Suman *et al*., 2013).

Test for reducing sugars: 5-8 drops of boiling Fehling's solution was added to 2 ml of the aqueous extract. A red-brick precipitate showed the presence of reducing sugars (Sabri *et al*., 2012).

Test for starch (non-reducing sugar): 5 ml of the aqueous extract was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch (Sabri *et al*., 2012).

Quantitative analysis

Alkaloid determination as described by Harborne (1973)

One gram of the sample was weighed into a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the concentration was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

Flavonoid determination as described by Bohm and Kocipai-Abyazam (1994)

About 5g of the plant sample 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 transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weigh.

Phenol determination by spectrophotometric method

1 g of the sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 minutes. About 5 ml of the extract was pipetted into a 50 ml flask then 10 ml of distilled water was added also. About 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol was added also. The solution was made up to mark and left to react for about 30 minutes for colour development. It was absorbed at 505 nm.

Tannin determination as described by Van-Burden and Robinson (1981)

About 500 mg of the sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.08M potassium ferrocyanide. The absorbance was measured at 720nm within 10 minutes.

Saponin determinationby Obadoni and Ochuko (2001)

Out of the grinded samples will be weighed 20 g for each and put into a conical flask and 100 ml of aqueous ethanol was added. The samples were heated over a hot water bath with continuous stirring at about 55ºC. The mixture is filtered and the residue re-extracted with another 200 ml of ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred to about 250 ml separating funnel and 20 ml of diethyl-ether was added and shaken vigorously. The aqueous layer was recovered while then ether layer was discarded. The purification process was repeated and 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 water bath. After evaporation, the samples were dried in the oven to a constant weight; the Saponin ratio is calculated as percentage.

Statistical analysis

All measurements were done in triplicates and values were expressed as mean ± standard deviation. Student's T-test was used to compare the means between the quantity of metabolites present in healthy and sugarcane chlorotic streak virus-infected sugarcane leaves using MS Excel 2010 version.

Results and Discussion

Phytochemicals are naturally occurring compounds which are of significance in the defense and protection of plants from diseases (Dossand Amand, 2012). The various phytochemical compounds detected in healthy and sugarcane chlorotic streak virus (SCSV)-infected leaves of sugarcane are known to also protect humans against diseases (Narasinga, 2003). Alkaloids, cardiac glycosides, flavonoids, saponins, phenols, saponins, reducing and non-reducing sugars tested positive in the healthy and SCSV-infected leaf extracts of *Saccharum officinarum* (Table 1). These results are in agreement with Palaksha *et al*. (2013) who proposed the presence of these phytochemicals in *Saccharumofficinarum* leaves. It is also in agreement with Eneh *et al*. (2015), who also reported the presence of saponins, tannins and flavonoids in his work.

Table 1: Qualitative phytochemical profile of healthy and SCSV-Infected leaves of *Saccharum officinarum*

S/N	Phytochemicals	Healthy sample	SCSV-infected sample
1	Antraquinones	–	–
2	Alkaloids	+	+
3	Cardiac glycosides	+	+
4	Flavonoids	+	+
5	Phenols	+	+
6	Saponins	+	+
7	Steroids	–	–
8	Tannins	+	+
9	Reducing sugars	+	+
10	Non-reducing sugars	+	+

Key: + = present, - = absent,SCSV = Sugarcane chlorotic streak virus

Table 2: Quantitative phytochemical analysis of healthy and SCSV-infected leaves of *Saccharum officinarum*

S/N	Phytochemicals detected	Healthy Sample (grams)	SCSV-infected sample (grams)	P values
		Mean±SD	Mean±SD	
1	Alkaloids	0.090±0.010	0.230±0.026	0.04
2	Flavonoids	1.450±0.017	0.930±0.040	0.04
3	Phenols	0.051±0.002	0.019±0.004	0.06
4	Saponins	0.26±0.026	0.100±0.020	0.13
5	Tannins	0.156±0.005	0.089±0.006	0.02

SCSV = Sugarcane Chlorotic Streak Virus, SD = Standard Deviation

Quantitative phytochemical analysis of methanolic extracts of both healthy and SCSV-infected leaves were carried out to determine the quantity of alkaloids, flavonoids, phenols, saponins and tannins present in the leaves. Both leaf extracts showed similarity in the presence and absence of metabolites though in varying quantities. The healthy and SCSV-infected leaf extracts showed similarity in the parameters investigated in the quantitative analysis (Table 2). However, the healthy extracts showed higher amounts of flavonoids, saponins, tannins and phenols. Alkaloid concentration detected in quantitative analysis showed higher amount in the SCSV-infected extracts. The statistical analysis (t-test) shows that the difference between the means of the two extracts is not significant ($p > 0.05$) in phenols and saponins while there was significant difference in between the means of the two extracts for alkaloids, flavonoids and tannins ($p < 0.05$). The higher quantity of alkaloids recorded in the SCSV-infected leaf extract could be as a result of the increase in the biosynthesis of the metabolite exhibited by the plant in other to overcome the effect of the viral infection. However, lower quantity of flavonoids, phenols, tannins and saponins recorded in the SCSV-infected leaf extract could be attributed to the adverse effects caused by the viral infection and the plant not being able to cope well in the presence of the virus.

Conclusion

The studies conducted on healthy and sugarcane chlorotic streak virus-infected leaves of *Saccharum officinarum* showed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins, reducing and non-reducing sugars. It also indicated the presence of metabolites in varying quantities with the healthy leaf extract having higher quantity for flavonoids (1.45 g), phenols (0.051 g), saponins (0.26 g) and tannins (0.156 g) while the SCSV-infected leaf extract had higher quantity for alkaloids (0.23 g). Plants are important sources of secondary metabolites which give them therapeutic properties. This phenomenon can be exploited in the medical field to cure a wide range of diseases in humans since these plant based bio-active compounds generally exhibit lesser side effects in treatment. Therefore, healthy (virus-free) sugarcane should be used for extraction of metabolites since a higher quantity of the metabolites are found in healthy samples.

Conflict of Interest

Authors declare that there is no conflict of interest.

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