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# Preliminary Phytochemical Screening of Healthy and Leaf Curl Virus Infected Tomato (*Solanum Lycopersicum*) Leaves

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**ABSTRACT:** The present investigation deals with the determination of phytochemical constituents of healthy and leaf curl virus infected tomato *(Solanum lycopersicum)* leaves. Specimens were collected from Koraye in Zaria and transported to the Herbarium unit for proper authentication. Healthy and curl leaves of *Solanum lycopersicum* were washed thoroughly three times with running tap water and once with sterile distilled water, air dried at temperature on a sterile blotter. After complete drying, young leaves were pulverized. The powdered material was weighed and kept in air tight container in dark place for further extraction procedure. Extraction was done by methanol method, where 100g each of pulverized powder of both healthy and infected *Solanum lycopersicum* leaves was put in a cornical flask and (1000ml) of measuring cylinder was used to measure 500ml of 70% methanol. The results obtained from the qualitative phytochemical analysis revealed the presence of; Alkaloids, Flavonoids, Tanins, Cardiac glycosides, Phenols and Saponins in both healthy and infected leaves of *Solanum lycopersicum* and the absence of; Carbohydrates, Steroids and Anthroquinone in both healthy and infected leaves of *Solanum lycopersicum.* While the quantitative analysis revealed the presence of 8.2% and 3.8% Alkaloids, 49.6% and 48.2% Flavonoids, 30.6% and 19.99%, Tanins 30.6% and 19.9%, Phenols 13.6% and 7.022% Saponins 1.2% and 0.1% in both healthy and infected leaf curl of *Solanum lycopersicum.* Evidently, from the above investigation there are no reducing sugars in *Solanum lycopersicum* leaves and there are metabolites in some healthy and infected leaf curl of *Solanum lycopersicum* leaves.

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**Keywords:** *Solanum lycopersicum*, Phenols, Saponins, Anthroquinone, Flavonoids

Tomato (*Lycopersicon esculentum L.*) is one of the most widely consumed vegetables and it is a major source of antioxidants contributing to the daily intake of a significant amount of these molecules. It is consumed fresh or as processed products such as canned tomato, sauce, juice ketchup, stews and soup (Lenucci *et al*., 2006). In fact, epidemiological studies have shown that consumption of raw tomato and its tomato based products is associated with a reduced risk of cancer and cardiovascular diseases (Giovannucci *et al*., 2002). Tomato antioxidants include carotenoids such as ß-carotene, a precursor of vitamin A, and mainly lycopene, which is largely responsible for the red color of the fruit, vitamins such as ascorbic acid and *tocopherols*, and phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives (Borguini and Torres, 2009; Vallverdú-Queralt *et al*., 2011). These compounds may play an important role inhibiting reactive oxygen species responsible for many important diseases, through free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways (Crozier *et al*., 2009). Phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack (Chew *et al*., 2009). Plants are rich in a wide variety of secondary metabolites (phytochemicals), such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Therefore, basic phytochemical investigation of plant extracts for their phytoconstituents were also vital. The most

destructive disease of tomato is caused by the *Tomato leaf curl virus* (TLCV). TLCV is the generic name given to a complex of viral species occurring in tropical and subtropical regions that cause severe disease in economically important crops, including tomato, with yield losses of up to 100%. In the Mediterranean basin, based on sequence comparisons, two species of TLCV are present and have been formally recognized as such by the International Committee on Taxonomy of Viruses (ICTV; (Rybicki *et al.*, 2000). They are: *Tomato leaf curl virus*-Israel (TLCV-Isr) (Navot *et al.*, 1991) and *Tomato leaf curl Sardinia virus* (TLCSV) (Kheyr-Pour *et al.*, 1991). Both species cause severe disease in tomato; however, TLCV-Isr is currently the most prevalent species in Europe, also affecting pepper (*capsicum annum*) and probably common bean (Phaseolus vulgaris). Most of the wild tomato species, such as *Lycopersicon chilense, L. hirsutum, L. peruvianum* and *L. pimpinellifolium*, are symptomless carriers (Zakay *et al.*, 1991).

Weeds such as *Datura stramonium* and *cynanchum acutum* present distinct symptoms, while others, such as *Malva parviflora*, are symptomless carriers. With the discovery and characterization of a growing number of viruses, and the growing availability of sequence data, virus taxonomy has become progressively more complex, and this is particularly true for geminiviruses. Therefore, the present investigation deals with the determination of phytochemical constituents of healthy and infected leaf curl virus of *Solanum lycopersicum*

## MATERIALS AND METHODS

*Collection and Identification of Plant Materials:* Healthy and infected *Solanum lycoperscicum* leaves were collected from Koraye in Zaria, Kaduna State. They were brought to the Herbarium unit of Biological sciences for proper authentication. The leaves of *Solanum lycopersicum* were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter.

*Drying:* The plants were air dried for a month in a well-ventilated room in the Department of Botany, Faculty of life Science with constant turning of plants part to prevent them from routing. The purpose of air drying was to prevent ultraviolet rays from destroying the active ingredient in the plant. After complete drying, young leaves were pulverized using laboratory Mortar and pestle to obtain fine powder. The powdered material was weighed and kept in air tight container and stored in a dark place for further extraction procedure.

*Extraction:* Extraction was done by methanol method, where 100g of each pounded powder of both healthy and infected *Solanum lycopersicum* leaves was put in a conical flask. (1000ml) of measuring cylinder was used to measured 500ml of 70% methanol (Mohammed *et al*., 2013)

*Phytochemical Analysis:* Chemical analysis was carried out in the methanol extracts of the leaves of *Solanum lycopersicum* using standard procedures to identify constituents, as described by Trease and Evans (1989), Sofowora (1993), Ushie *et al.*,2016) and Santhi *et al.*,2016).

*Qualitative phytochemical Screening:* Carbohydrate, Cardiac glycoside, Steroids, Tannins, Flavooids, Alkaloids and Phenols

*Test for alkaloids:* Two milliliter (2ml) each of the extracts was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath for 10minutes; 1ml of the extract was treated with a few drops of Mayer's reagent, precipitation with these reagents was seen as evidence for the presence of alkaloids. The method was repeated again to confirm the results (Sofowora, 1993).

*Test for flavonoids:* Three milliliter (3ml) of the aqueous extract was mixed with 4ml 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 phenols:* To 1ml of extract of sample, 2 ml of distilled water was added followed by a few drops of 10% aqueous ferric chloride solutions were added. Formation of blue or green colour indicated the presence of phenols (Suman *et al.*, 2013).

*Test for carbohydrates:* Five milliliter (5ml) of the aqueous extract was treated with the reagent of the starch iodine. Change to blue violet indicates the presence of starch (Sabri *et al.*, 2012).

*Test for tannins:* Two milliliters (2ml) of the aqueous extract was added to 2ml of water, 1 to 2 drops of dilute ferric chloride solution was added. A dark green or blue green coloration indicated the presence of tannins (Sabri *et al.*, 2012).

*Test for steroids:* One milliliter (1ml) each of the extracts was dissolved in 2ml of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish brown colour formed at the interphase indicates the presence of a

steroid ring (Sofowora, 1993). The same procedure was repeated once to confirm the results.

*Test for Cardiac glycosides (Keller-Killiani test:* Five milliliters (5ml) of the extract was mixed with 2ml of glacial acetic acid containing one drop of ferric chloride solution, followed by the addition of 1ml 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 saponins (Foam test):* To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam is observe which indicates the presence of saponins (Sabri, 2015).

*Quantitative Analysis of the Phytochemical Constituents: Alkaloid determination using Harborne (1973) method:* Five grams (5g) of the sample was weighed into a 500ml cornical flask and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. It 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 precipitation is completed. The whole solution was allowed to settle and the precipitates were collected and wash with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973)

*Flavonoid determination by the method of Bohm and Kocipai-Abyazan (1994):* Ten grams (10g) of the plant sample was extracted repeatedly with 100ml 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 into dryness over a water bath and was weighed to a constant weight (Bohm and Kocipai-Abyazan, 1994)

*Saponin determination:* The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples 20g was weighed for each and put in a conical flask and 100ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 55$^{o}$C. The mixture was filtered and the residue was re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90$^{o}$C. The concentrates was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples was dried in the oven to a constant weight; the saponin content was calculated as percentages

*Tannin determination by van- Burden and Robinson (1981) method:* Five hundred grams (500g) of the samples was weighed into a 50ml plastic bottle and 50ml of distilled water was added and shaken for 1hour on a mechanical shaker. This was filtered into 50ml volumetric flasks and was made up to the make. Then 5ml of the filtrates was pipette out into a test tube and mixed with 2ml of 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorption was measured at 720nm within 10 minutes (Robinson, 1981).

*Determination of total phenols by spectrophotometric method:* The fat free samples were boiled with 50ml of ether for the extraction of the phenolic components for 15 minutes. About 5ml of the extracts was pipette into a 50ml flask. Then 10ml of distilled water was also added. This sample was made up to mark to react for about 30 minutes for colour development (Robinson, 1981).

*Data Analysis:* Data obtained from the study were express as mean ± SEM. The difference between the groups were analyzed by Student t. test at 95% level of confidence to compare the means of individual metabolites between healthy and infected *Solanum lycopersicum* leaves, IBM SPSS version 20 was used for the analysis.

## RESULTS AND DISCUSSION

**Qualitative Phytochemical Screening of *Solanum lycopersicum:*** Phytochemical screening result of *Solanum lycopersicum* leaves extract in methanol showed the presence of Alkaloids, Flavonoids, Saponins, Phenols, Tanins, Cardiac glycosides and Carbohydrates, except for Carbohydrate, Anthraquinone and Steroids that were absent. (Table 1) chemical investigation on the tomato leaves plant has resulted in the isolation of a large number of interesting metabolites. Phytochemical Assay of Healthy and Infected Leaves of *Solanum lycopersicum:* The values of the replicates and mean concentration of individual metabolites as well as the comparison of metabolites concentrations between the healthy and infected tomato leaves (Table 2). The result showed no statistical significant difference ($p>0.05$) in the metabolites concentration of healthy and infected tomato leaves. However, the quantity of

phenol and tannin were statistically lower ($p \leq 0.05$) in the infected leaves. Phytochemical assay of healthy and infected tomato leaves curl virus of *Solanum lycopersicum* leaves. The result indicates the presence of alkaloids, flavonoids, phenols, tannin and saponin in varying concentration for both leaves. In infected tomato leaves curl virus, flavonoid was observed to have the highest concentration of 2.48g while saponin was observed to have the least concentration of 0.10g. Similarly, saponin was observed to have the least concentration of 0.07g while flavonoid had the highest concentration of 2.41g in healthy leaves.

**Plate 1:** Healthy leaves of *Solanum lycopersicum*

**Table 1:** Phytochemical screening of healthy and infected leaves of TLCV

| Phytochemicals | Healthy | TLCV infected leaf |
|---|---|---|
| Alkaloids | + | + |
| Flavonoids | + | + |
| Carbohydrates | - | - |
| Tanins | + | + |
| Cardiac glycosides | + | + |
| Phenol | + | + |
| Saponins | + | + |
| Steroids | - | - |
| Anthraquinones | - | - |

*`+`present, `-` absent*

**Plate 2**: TLCV of infected leaves of *Solanum lycopersicum*

**Table 2:** Concentrations of Metabolites in Healthy and infected Leaves of *Solanum lycopersicum*

| **Phytochemicals** | **Rep 1 (g)** | **Rep 2 (g)** | **Rep 3(g)** | **Mean** | **Rep 1 (g)** | **Rep 2 (g)** | **Rep 3 (g)** | **Mean** | **p-value** |
|---|---|---|---|---|---|---|---|---|---|
| **Alkaloid** | 1.52 | 1.02 | 1.45 | 1.33±0.15 | 1.05 | 0.95 | 1.03 | 1.10±0.03 | 0.18 |
| **Flavonoid** | 2.08 | 2.45 | 2.70 | 2.41±0.18 | 2.65 | 2.39 | 2.40 | 2.48±0.08 | 0.75 |
| **Phenols** | 0.95 | 0.67 | 0.81 | 0.81±0.08 | 0.46 | 0.40 | 0.37 | 0.41±0.02 | 0.04 |
| **Tanins** | 2.05 | 1.55 | 1.86 | 1.82±0.14 | 1.23 | 1.16 | 1.18 | 1.19±0.02 | 0.05 |
| **Saponins** | 0.08 | 0.05 | 0.0 | 0.07±0.01 | 0.15 | 0.10 | 0.11 | 0.12±0.01 | 0.07 |

*$P \leq 0.05$ indicates significant difference between the mean of healthy and infected samples.*

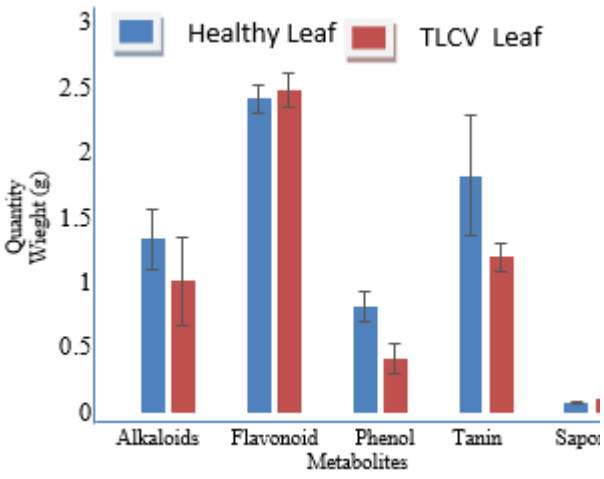


**Fig. 1** Concentrations of metabolites in healthy and infected leaves of TLCV

Flavoniod, Alkaloid and Saponin are present in both healthy and leaf curl virus infected tomato (*Solanum lycopersicum*) leaves. This is in agreement with the work of Mohammed *et al*., (2014). In this study it was observed that the result from table 2 indicated a slight difference in the concentration of Phytochemicals between healthy and infected tomato leaves curl virus of *Solanum lycopersicum*. However result obtained for alkaloid, flavonoids, phenols and saponins in healthy and leaf curl virus infected tomato (*Solanum lycopersicum*) leaves are not statistically different ($p>0.05$), while tanins and phenol are statistically lower in leaf curl virus infected tomato (*Solanum lycopersicum*) leaves with p value of ($p \leq 0.05$). Alkaloids constitute a large group of pharmacologically active nitrogen containing compounds. The slight differences observed for alkaloid could be attributed to increase in the synthesis of these compounds which function as natural defense substance against pathogens and insect. It could also be attributed to higher nitrogen content in the soil where the unhealthy leaves was obtained which may be due to some environmental effects. The increased

concentration of flavonoids in the unhealthy leaves of tomato could be attributed to increase in the production of ROS (reactive oxygen species) by both pathogen and plant as a result of the infection. They are very important in plant resistance against pathogenic bacteria and fungi (Crozier *et al*., 2009). Flavonoid compounds are transported to the site of infection and induce hypersensitivity reaction, which is the earliest defense mechanism employed by infected plants, and programmed cell death (Dai *et al*, 1996; Beckman, 2000).

*Conclusion:* From the result obtained in this study, it can be concluded that both the healthy and infected leaves contained the same constituents with slight increase or decrease in the concentration of some metabolites, the changes in the concentration could be due to the presence of virus or environmental effects.

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