Research Square

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Preliminary Study on the Detection of Toxic Principles from Vegetables

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Research Article

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Abstract

An attempt was made to detect the presence of various toxic principles in Solanum tuberosum, Solanum lycopersicum, Solanum melongena, and Manihot esculenta. The study involved the alcoholic extraction of these toxic constituents from the respective vegetable samples. Phytochemical analysis was conducted using colorimetric tests, microcrystalline tests, and thin-layer chromatography (TLC). The colorimetric assays were employed to detect alkaloids, glycosides, and other phytochemicals. Microcrystalline tests were carried out on glass slides using specific reagents, and the resulting crystal formations were examined microscopically. Following the confirmation of alkaloids, glycosides, and additional phytochemicals through these preliminary tests, TLC was performed using an appropriate solvent system and suitable visualization techniques. This study highlights the application of microcrystalline testing and TLC as innovative and reliable approaches for the detection of naturally occurring toxins. The findings also provide a foundation for future research aimed at exploring toxic principles in vegetables using simple, accessible, and laboratory-friendly analytical methods.

1. Introduction

Natural poisons present in fruits and vegetables provide a defense mechanism for the plants to protect them against predators, fungus, and insects. Alkaloids, glycosides, terpenoids, flavonoids etc. are the examples of such natural toxins. But it causes a wide range of side effects from mild itching, vomiting, and nausea to harmful side effects like psychosis, teratogenicity and arrhythmias[23]. Even though the consumers can handle these food items safely, hence the consumption of these foods are not prohibited [8, 9].

Solanine is an alkaloid poison that can be found in plants in the nightshade family, including belladonna, potatoes, eggplants and tomatoes as a part of its defense mechanism. The studies were conducted to identify novel applications for conventional plants with appropriate chemical and pharmacological profiles[7]. Also there were studies conducted on the determination of the acute toxicity effect of tomato when it was given orally in the form of dried pomace extract[16]. Other studies were done for the extraction and characterization of bioactive compounds from brinjal peel using ultrasound. Using three different solvent, the study tracked the extraction of bioactive components and the antioxidant activity of the brinjal peel[6].

The linamarin is a cyanogenic glycoside present in the leaves and roots of Cassava, Flax and Lima beans. Consumption of food from insufficiently processed plant material containing linamarin produces dietary toxicity called konzo disease [23]. Studies of linamarin derived from cassava have drawn more interest during the past three decades as a result of the high levels of cyanogenic chemicals found in the extracts. Later, a novel cryocooled extraction technique was created to separate linamarin from the peel of Cassava roots[19].

In potato peels, α-solanine levels range from 143 to 1273 mg/kg dry weight, often accompanied by equal or higher levels of α-chaconine. Some commercial varieties like Amandine and Rudolph can contain up

to 3000 mg/kg total glycoalkaloids in the peel[11, 15]. Potato protein isolates may also contain up to 932 mg/kg α-solanine and 120 mg/kg α-chaconine. Solanine is primarily concentrated in sprouts and peels, with sprouts having up to 80 times more than tubers. The recommended safety limit for total glycoalkaloids in potatoes is 150 mg/kg fresh weight[12, 15]

In cassava, linamarin content in wild-type leaves ranges from 2.4 to 3.1 g/kg fresh weight, while gene-edited varieties reduce this to 0.5–0.7 g/kg[1, 10]. Cassava roots and processed foods show variable cyanide levels due to linamarin, with ready-to-eat cassava chips averaging 48.4 ppm and some imported products reaching up to 95.9 ppm. Processing lowers cyanide levels significantly, and food safety guidelines recommend a maximum of 10 ppm cyanide in ready-to-eat cassava products[18].

The objective of the study was to detect the presence of different toxic principles like alkaloids and glycosides from vegetables like *Solanum tuberosum, Solanum lycoperiscum, Solanum melongena*, and *Manihot esculenta*. This study focused on the extraction of toxic principles from the respective vegetables and further analysis and detection of the same using color tests, microcrystalline tests, and thin-layer chromatography. This study helps generate some information about the commonly used potent vegetable toxins and their extraction and detection using basic chemical tests. Thus, it generates an understanding of the chances of such vegetable poisoning cases and helps with their detection.

2. Materials and Methods

In total, four samples of vegetables were collected from the Thrissur district of Kerala: *Solanum tuberosum, Solanum lycoperiscum, Solanum melongena, and Manihot esculenta.* Ripe or sprouted vegetables were selected as the samples.

A mortar and pestle were used to homogenize the chosen vegetable samples. After homogenization, 10 g of each sample was precisely weighed before being extracted using 100 mL of methanol. Methanol was used to do a complete alcoholic extraction, and the mixture was then filtered through Whatman filter paper. To get a concentrated extract, the filtrate was evaporated in a water bath at 50°C for two hours. After that, the concentrated extract was kept and used for additional analytical processes. Colorimetric tests, microcrystalline tests, and thin-layer chromatography (TLC) were employed to facilitate the detection of the vegetable toxins. Each test was performed in triplicate for each sample to assess analytical reproducibility. Reagent blanks containing all reagents except the plant extract were used as negative controls to monitor nonspecific colour development, background TLC spots, and spontaneous crystal formation.

Colour tests were conducted using standard phytochemical reagents[24]. Each reaction was performed in triplicate alongside positive and negative controls, and colour intensity was assessed visually within 5 minutes and observations were recorded.(Table 1)

1. Test for Alkaloids:

a. Dragendroff's test: To 1 ml of extract, add 1 ml of Dragendroff's reagent. An orange-red precipitate indicates the presence of alkaloids.
b. Mayer's test: To 1 ml of extract, add 1 ml of Mayer's reagent. Whitish or cream colored precipitate indicates the presence of alkaloids.
c. Hager's test: To 1 ml of extract, add 3 ml of Hager's reagent (saturated aqueous solution of picric acid). Yellow colored precipitate indicates the presence of alkaloids
d. Wagner's test: To 1 ml of extract, add 2 ml of Wagner's reagent (iodine in potassium iodide). Reddish brown colored precipitate indicates the presence of alkaloids[21]

2. Test for Glycoside

Tests for free sugars: The extract is hydrolyzed with minerals

a. Legal's test: Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red color appears.
b. Bromine water test: Test solution when treated with bromine water gives yellow precipitate.
c. Libermann test: To a little of the extract add 2 ml of chloroform and 2 ml acetic acid. Violet to blue green color indicates positive result.

3. Test for Cardiac Glycosides:

a. Keller killiani test [test for Deoxy sugars]: To a little of the extract add 0.4ml of glacial acetic acid containing a trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube
b. Baljet test: To a little of the extract add a few drops of reagent (95ml 1% picric acid and 5ml 10% NaOH). Yellow orange color indicates the presence of cardiac glycosides.

4. Test for steroidal glycoalkaloid (Solanine)

a. Test with Marquis reagent- To a little of the extract add a few drops of marquis reagent. Violet red color indicates the presence of Solanine.
b. Liebermann Burchard Test- To a little of the extract add a few drops of Liebermann Burchard Reagent. Cherry red color indicates positive result

5. Test for Steroids

Salkowski test – To a little of the extract add a few drops of chloroform and add a few drops of conc. H_2SO_4 along the sides of the test tube. Red ring indicates the presence of steroids

6. Test for Saponin

Foam test- To a little of the extract add a few drops of water and heated.Appearance of creamy miss of small bubbles indicates the presence of Saponin.

7. Test for Carbohydrate

Molisch's test -To 2 ml extract add 2 drops of alpha- naphthol and 1 ml conc. H_2SO_4 along the side of the test tube. Violet ring indicates the presence of carbohydrates.

8. Test for phenol

a. Lead Acetate Test- To a little of the extract add 0.5ml of 1% lead acetate. Precipitation indicates the presence of phenolic compounds.
b. Ferric chloride test- To a little of the extract add a few drops of 5% ferric chloride solution. Dark green or bluish black color indicates the presence of phenolic compound

Microcrystalline Test

A drop of vegetable extract was kept on a separate microscopic slide and one drop from each reagent was put adjacent to extract on separate slides. A cover slip was placed on it, so that extract and reagent meet together at a junction. Slides were kept for 15 minutes for the crystallization process.The crystallization products were examined under a microscope.(Table 2) The similar procedure was repeated for all other extract.[17]

Thin-Layer Chromatography

The reaction was tracked using thin-layer chromatography on TLC Silica gel 60 plates. The plate was spotted with the samples using a capillary tube. A beaker was taken with a suitable solvent system for each sample. The plate was placed in the beaker, and it was covered with a watch glass. The solvent was allowed to run over the plate. After the development of the TLC plate, the solvent front was marked and allowed to dry. The spots were visualized using suitable reagents or methods, and Rf was calculated using the equation:

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

Solvent system and visualization method for each sample is mentioned below:

a) TLC for *Solanum tuberosum, Solanum lycopersicum, Solanum melongena*

Solvent system – Ammonia: Chloroform: Acetic acid:: 7.3:0.5

Visualization was done under short wave UV(254 nm)

b) TLC for *Manihot esculenta*

Solvent system- Chloroform: hexane ::1:4

Visualization was done under short wave UV(254 nm)

The TLC solvent system and ratios were selected after optimization studies, as they yielded optimal separation with clearly distinguishable Rf values. Reference standards were co-spotted as positive controls, while solvent blanks served as negative controls. Each sample was analyzed in triplicate.

3. Results

Table 1
Color test for the detection of phytochemicals

Phytochemicals	**Name of the color test**	***Solanum tuberosum***	***Solanum lycoperiscum***	***Solanum melongena***	***Manihot esculenta***
Alkaloid	Dragendorff's test	+	-	+	-
	Mayer's test	+	-	-	-
	Hager's test	+	-	+	+
	Wagner's test	+	+	+	+
Glycosides	Legal's test	-	-	-	+
	Bromine water test	-	-	-	+
	Libermann test	-	-	-	-
Cardiac glycosides	Keller killiani test	-	-	-	+
	Baljet test	-	-	+	-
Solanine	Marquis test	+	+	-	-
	Libermann Burchard test	+	+	+	+
Steroids	Salkowski test	+	+	+	+
Saponins	Foam test	+	+	+	-
Carbohydrates	Molisch's test	+	+	+	+
Phenol	Lead Acetate test	+	+	+	+
	Ferric chloride test	+	+	+	-

After conducting the color test, some of the tests were positive for all the samples. On the other hand, some of the tests were negative for all the samples or for some of the samples. All the samples showed positive results for the color tests of alkaloid, glycoalkaloid, steroid, saponin, carbohydrate, and phenol. In addition, *Solanum melongena* shows positive results for cardiac glycosides, and *Manihot esculenta* shows positive results for both glycosides and cardiac glycosides.(Table 1)

Table 2
Microcrystalline tests and observation

Reagents	**Types of Crystals formed**			
	Solanum tuberosum	***Solanum lycoperiscum***	***Solanum melongena***	***Manihot esculenta***
Cobalt acetate	Rectangular crystal	Rhombic crystal	Square shaped crystal	-
Ferric chloride	Rectangular crystal	Needle shaped crystal	Square shaped crystal	Rod shaped crystal
Marquis reagent	Hexagonal crystal	-	Square shaped crystal	Rod shaped crystal
Picric acid	Rectangular crystal	Leaf shaped crystal	Rhombic crystal	Rod shaped crystal
Potassium chloride	Rectangular crystal	Rhombic crystal	Broken rod shaped	-
Potassium dichromate	Hexagonal crystal	-	-	Square shaped crystal

Figure 2

Rhombic crystal obtained with picric acid for *Solanum melongena*

In the microcrystalline test, ferric chloride and picric acid formed crystals in all the samples.(Fig. 1&2) In addition, *Solanum tuberosum* forms crystals with cobalt acetate, Marquis reagent, potassium chloride, and potassium dichromate. *Solanum lycoperiscum* forms crystals with cobalt acetate and potassium chloride. While Marquis reagent and potassium dichromate give *Manihot esculenta*.(Table 2) [Insert Fig. 1 and Fig. 2 here]

Table 3
TLC result for *Solanum tuberosum*, *Solanum lycoperiscum* and *Solanum melongena*

Sample	Distance traveled by the component (A)	Distance traveled by the solvent (B)	Rf = A/B
Solanum tuberosum	3.9	5.4	0.72
Solanum lycoperiscum	5.1	5.4	0.94
Solanum melongena	1.3	5.4	0.24

Table 4
TLC result for *Manihot esculenta* (Fig. 3)

Sample	Distance traveled by the component (A)	Distance traveled by the solvent (B)	Rf = A/B
Manihot esculenta	3.6	5.7	0.63

Figure 3

TLC plate viewed under UV for *Manihot esculenta*

After the conduction of TLC, *Solanum tuberosum*, *Solanum lycoperiscum*, *Solanum melongena* gives Rf 0.72, 0.94 and 0.24 respectively in chloroform:methanol:ammonia (7:3:0.5) solvent system (Table 3). While *Manihot esculenta* gives Rf value of 0.63 with chloroform:methanol(9.5:0.5) (Table 4) The Rf values for α-solanine and α-chaconine in TLC typically range from 0.07–0.34, with other glycoalkaloid derivatives showing higher Rf values. These values are essential for toxin identification in potato and related plant samples using TLC[4, 5, 22]. Rf values of sample spots corresponded closely with the reference standard, and no extraneous spots were obsernved in negative controls. [Insert Fig. 3 here]

4. Discussion

From this study, it has been found that some of the phytochemicals present in the vegetables are harmful when consumed. Toxins were successfully extracted from *Solanum tuberosum*, *Solanum lycoperiscum*, *Solanum melongena*, and *Manihot esculenta* using a standardized procedure, and the extracted samples were subjected to color tests, microcrystalline tests, and thin-layer chromatography. All these analyses indicated that glycoalkaloids are present in the vegetables taken from the Solanaceae family and cardiac glycosides in Manihot Esculenta. The conventional method used for the detection of toxic principles is the color test. Here it is found that microcrystalline tests and TLC may be used as alternative screening methods for the detection of toxic principles in vegetables, which are more reliable than color tests. Colour tests and microcrystalline tests were conducted in triplicate. As all observations showed complete agreement across trials, categorical data analysis using Fisher's Exact/Freeman–Halton test revealed no variability, and therefore no statistically significant differences were detected.

It is hoped that this study will be able to generate some information about the commonly used potent vegetable toxins and the extraction and detection of the toxic principles using basic chemical tests. I also hope that this study will generate some knowledge about vegetable poisons and their safe use. It is suggested that more focus and efforts should be made to conduct such studies for the detection of potential toxic principles in vegetables to study their forensic toxicological significance. And also, the novel idea of using microcrystalline tests and thin-layer chromatography for the analysis may have future scope for research.

5. Limitations

The results may vary depending on factors such as ripeness, differences in storage conditions, and other environmental parameters. The study also has qualitative limitations and may be susceptible to potential false positives due to the absence of advanced analytical confirmation. Microcrystalline testing was employed for the first time in the detection of these vegetable toxins, which also represents a limitation of the study. Therefore, this work is considered a preliminary investigation into the detection of toxins in vegetables.

Declarations

1. All the four vegetables are cultivated and collected as per guidelines. Relevant guidelines and regulations were followed while conducting the study for the use of plants.

2. All data generated or analysed during this study are included in this published articles and its supplementary information files.

Author Contribution

Everything done by the author Reni Mary Varghese

Acknowledgement

Ethics, Consent to Participate, and Consent to Publish declarations: not applicable. All the vegetables are cultivated and followed the guidelines. All data generated or analysed during this study are included in this published articles and its supplementary information files.

Data Availability

All data generated or analysed during this study are included in this published articles and its supplementary information files

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Figures

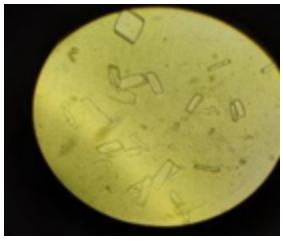


Figure 1

Rectangular crystals with picric Acid *(Solanum tuberosum)*

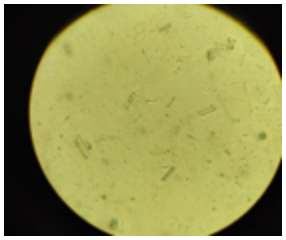


Figure 2

Rhombic crystal obtained with picric acid for *Solanum melongena*



Figure 3

TLC plate viewed under UV for *Manihot esculenta*