International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Qualitative and quantitative phytochemical screening of *Solanum torvum*

Patience Abidemi Omololu *, Victor Olusola Omololu and Ayosunkanmi Temitope Ogunrinde

Department of Animal Nutrition, Peace House Agricultural Training Institute, Isarun, Ondo State, Nigeria.

International Journal of Science and Research Archive, 2025, 14(02), 973-979

Publication history: Received on 29 December 2024; revised on 10 February 2025; accepted on 13 February 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.14.2.0397

Abstract

Solanum torvum is intensively used worldwide in herbal medicine in the treatment of different diseases condition. This study was carried out to investigate the presence of phytochemicals and to determine the percentage of phytochemical constituent in *S. torvum* leaf and fruit.

Qualitative phytochemical screening carried out on *S. torvum* leaf and fruit shows that phenols, tannins, flavonoids, saponins, cardiac glycosides, steroids, terpenoids, anthraquinones and alkaloids were present.

The quantitative phytochemical screening reveals that there is no significant difference in the tannin, terpenoids and phenol contents of *S. torvum* fruits and leaves but there is a significant difference in the alkaloids, flavonoids and saponins contents of *S. torvum* fruits and leaves. The alkaloids contents of *S. torvum* leaves is significantly higher than the fruit while the flavonoids and saponins contents of *S. torvum* fruits is significantly higher than that of the leaves.

The leaves and fruits of *S. torvum* were screened for elemental iron and the results reveals the presence iron in an adequate concentration.

From this study, it could be concluded that extracts from *S. torvum* fruits and leaves are potential source of useful drugs both for human and animal health.

Keywords: *Solanum torvum*; Phytochemical analysis; Iron; Alkaloids and Flavonoids

1. Introduction

Medicinal plants are the greatest source to obtain an array of drugs [1]. The use of medicinal plants in mainly developing countries as remedial agents for the maintenance of health has been broadly observed [2].

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds [3,4]. These compounds are known as secondary plant metabolites (alkaloids, tannins, flavonoids, steroids, terpenoids, phenolics, etc.) and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property.

Solanum torvum (Solanaceae), commonly known as Turkey berry is native and cultivated in Africa [5] and Asia [6]. *S. torvum* is an erect spiny shrub that is usually 2 or 3 m in height and 2 cm in basal diameter, but may reach 5 m in height and 8 cm in basal diameter [7]. The fruits are berries that resemble green peas and grow in clusters of small green

* Corresponding author: Patience Abidemi Omololu

spheres, each measuring about one centimetre in diameter. They become yellow when fully ripe, they are thin fleshed and contain numerous flat, round, brown seeds.

It has been extensively used for both food and pharmaceutical purposes. It is also used horticulturally as a rootstock for eggplant [8]. It is intensively used worldwide in the traditional medicine as poison antidote and for the treatment of fever, wounds, tooth decay, cough, pain, liver problems, reproductive problems and arterial hypertension [9,10].

This study was carried out to elucidate the qualitative and quantitative phytochemical constituents of *S. torvum* leaf and fruit.

2. Material and methods

2.1. Sample Collection and Preparation

Solanum torvum leaves and fruits were collected from the Peace House Agricultural Training Institute. The obtained herbs materials were cleansed and washed with water and air-dried. Extraction was done using digestion method [11].

2.2. Qualitative Phytochemical screening methodology

Phytochemical screening was performed using standard procedures [12,13].

2.2.1. Test for anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

2.2.2. Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish-brown colouration of the interface indicates the presence of terpenoids.

2.2.3. Test for flavonoids

Three methods were used to test for flavonoids.

First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids.

Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids.

Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

2.2.4. Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.2.5. Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.2.6. Test for Steroids

2ml of extract with 2ml of chloroform and 2ml of concentrated H_2SO_4 are added, the appearance of red colour and yellowish green fluorescence indicates the presence of steroids.

2.2.7. Test for Phenol

5ml of extract was added to 2ml of 1% solution of gelatine containing 10% of NaCl. Appearance of white precipitate indicates the presence of phenol

2.2.8. Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

2.2.9. Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

2.3. Quantitative Phytochemical screening methodology

Total alkaloids, flavonoids and saponins would be quantitatively determined using the method described by [14].

2.3.1. Determination of alkaloids

0.2g of the plant extract was placed in a 250ml beaker and 200ml of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4hours. It was then filtered and the filtrate concentrated on a water bath until it reaches a quarter of its original volume. Concentrated NH_4OH was added until precipitation is complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute NH_4OH. The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was then calculated by difference.

2.3.2. Determination of Total flavonoid content

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed in terms of % w/w.

2.3.3. Determination of terpenoids

2g of the extract was placed in a 250ml beaker and 200ml of 100% ethanol was added.

The mixture was covered and allowed to stand for 4hours. It was then filtered and the filtrate concentrated on a water bath until it reaches a quarter of its original volume. Petroleum ether was to d concentrate in a separating funnel, shook, allowed to partition. The petroleum ether layer was collected dried and weighed. The percentage terpenoids was then calculated by difference.

2.3.4. Determination of saponins

0.2g of extract was weighed into a 250ml conical flask. 100ml of 20% C_2H_5OH was added. The mixture was then heated over a hot water bath for 4hours with continuous stirring at about 55°C and filtered with a filter paper. The residue was re-extracted with another 200ml of 20% C_2H_5OH. The combined extract was then reduced to 40ml over a water bath at about 90°C. The concentrated extract was transferred into a 250ml separator funnel and 20ml of $(CH_3CH_2)_2O$ was added to the extract and shaken vigorously. The aqueous layer was recovered while the $(CH_3CH_2)_2O$ layer discarded. This purification process was repeated. 60ml of n-butanol added and the combined n-butanol extract was washed twice with 10ml of 5% NaCl solution. The remaining solution was then heated on a water-bath in a pre-weighed 250ml beaker. After evaporation, the residue was dried in an oven to a constant weight. The % saponin was then calculated by difference.

2.3.5. Determination of total phenol content

Total phenolic compound contents were determined by the Folin-Ciocalteau method [15]

The extract samples (0.5ml of different dilutions) were mixed with Folin-Ciocalteu reagent (5ml,1:10diluted with distilled water) for 5min and aqueous Na_2CO_3 (4ml,1M) were then added. The mixture was allowed to stand for 15min and the phenols were determined by colorimetric method at 765nm. The standard curve was prepared and solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

2.3.6. Determination of tannin Content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of % w/w.

3. Results

Table 1 Qualitative Phytochemical Screening of *Solanum torvum*

Test	Fruit	Leaf
Saponins	++ve	+ve
Tannins	++ve	++ve
Flavonoids	+ve	+ve
cardiac glycosides	+ve	+ve
Anthraquinones	++ve	+ve
Terpenoids	+ve	+ve
Steroids	+ve	++ve
Alkaloids	+ve	++ve
Phenol	++ve	++ve

Note: +ve: present, ++ve: abundant, -ve: absent

Table 2 Quantitative Phytochemical Screening of *Solanum torvum*

Sample	Alkaloids	Flavonoids	Saponins	Tannin	Terpenoids	Phenol
Fruit	2.95±0.001	1.39±0.05	3.60±0.004	1.15±0.009	0.80±0.001	0.79±0.004
Leaf	3.65±0.004	0.14±0.13	1.30±0.001	1.19±0.005	0.50±0.001	0.74±0.026

Values are expressed in mean±SEM. All values are in % w/w.

Table 3 Iron content of *Solanum torvum*

Sample	Fe (mg/l)
Leaf	2.856
Fruit	1.910

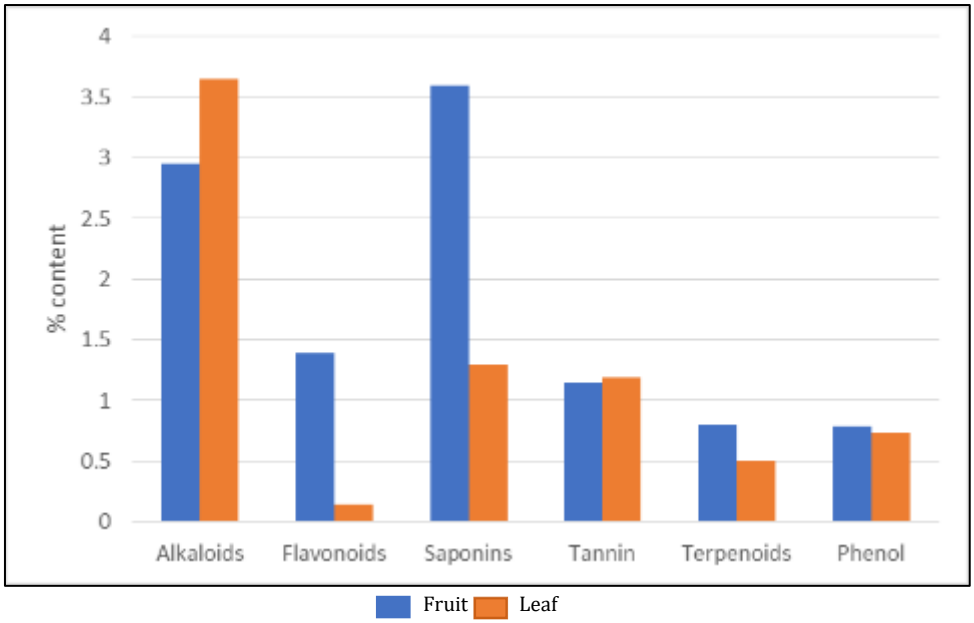

Figure 1 Quantitative Phytochemical Screening of *Solanum torvum*

4. Discussion

Phytochemical screening conducted on *S. torvum* fruits and leaves extracts revealed the presence of constituents which are known to exhibit medicinal activities. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, cardiac glycosides, steroids, terpenoids, anthraquinones and alkaloids.

There is no significant difference in the tannin, terpenoids and phenol contents of *S. torvum* fruits and leaves but there is a significant difference in the alkaloids, flavonoids and saponins contents of *S. torvum* fruits and leaves. The alkaloids contents of *S. torvum* leaves is significantly higher than the fruit while the flavonoids and saponins contents of *S. torvum* fruits is significantly higher than that of the leaves.

The abundance of phenol in the leaves and fruits suggests that *S. torvum* possess biological properties such as anti-apoptosis, antiaging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [16].

Tannins inhibit the growth of bacteria by binding to proteins which inhibit bacterial metabolism. Therefore, the abundance of tannin in *S. torvum* fruits and leaves shows that plant could be a source of a strong antibacterial agent.

Terpenoids is one of the main bioactive compounds of essential oils. Terpenoids has a significant role in treating various types of diseases, in many studies in vitro and in vivo using as anticancer agents, antimicrobial, anti-inflammatory, antioxidants, antiallergic, neuroprotective, anti-aggregator, anti-coagulation, sedative and analgesic [17]. The presence of terpenoids in *S. torvum* fruits and leaves reveals that it has a potent medicinal effect on human health.

Several researches shows that anthraquinones present in plants has antimicrobial, anti-inflammatory and antioxidant properties [18]. The abundance of anthraquinones in the *S. torvum* fruits makes the plant extract to be a potent source of antioxidant, anti-inflammatory, antimicrobial agents.

Flavonoids are hydroxylated phenolic substances which are present in the fruits and leaves of *S. torvum*. Flavonoids are synthesized in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [19]. They also are effective antioxidant and show strong anticancer activities [20,21,22].

Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids [1]. The presence and abundance of alkaloids in the fruits and leaves of *S. torvum* shows that *S. torvum* has antibacterial and antispasmodic properties.

The abundance of steroids in the leaves of *S. torvum* signifies that the plant could be a source of steroidal agents such as steroidal anti-inflammatory agents.

Saponins possess various biological activities such as anti-cancerous activity, hepatoprotective activity, anti-oxidant activity, etc. and are involved in the treatment of various diseases such as osteoporosis, obesity, diabetes, etc. [23]. Saponins prevent the excessive intestinal absorption of this cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension [24]. Saponin is abundant is *S. torvum* fruits and also present in the leaves and this makes it a potent medicinal plant.

The lowering of blood pressure is associated with cardiac glycosides and this phytochemical constituent is present in *S. torvum* fruits and leaves [1].

Iron is an essential mineral for human life which is utilized for production of haemoglobin. The crude extract of *S. torvum* leaf and fruit shows the presence iron (fe) which implies that *S. torvum* can be used a blood booster.

5. Conclusion

The phytochemical screening of the fruits and leaves of *S. torvum* reveals that this plant contains numerous phytochemical constituents and it is a potent medicinal plant and hematinic agent. Therefore, extracts from this plant (fruits and leaves) is a potential source of useful drugs both for human and animal health.

Compliance with ethical standards

Acknowledgments

We like to acknowledge Bro Gbile Akanni, the Chairman Governing Council, Peace House Agricultural Training Institute, Isarun, Ondo State for his support towards the research and Dr [Mrs] Sade Akanni of Peace House, Gboko for her support towards the research.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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