

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF THE ETHANOLIC AND AQUEOUS EXTRACTS OF SOLANUM NIGRUM L

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ABSTRACT

Solanum nigrum Linn. belongs to the Solanaceae family and is a widely used as medicinal plant. This herb has been found to possess a diverse array of therapeutic properties, such as its ability to combat tumors, reduce inflammation, provide antioxidant benefits, exhibit antibacterial effects, and offer neuroprotective advantages. Historically, it has been employed for addressing a spectrum of conditions including to treat rheumatic and gouty joints, skin ailments, nervous disorders, nausea, and tuberculosis, acute nephritis, urethritis, eczema, leucorrhea, cancer, sore throat, toothache, dermatitis, carbuncles, and furuncles. The phytochemical components found in *Solanum nigrum* encompass alkaloids, saponins, flavonoids, phenolic compounds, and steroids, among others. In this research, a qualitative examination of the aqueous extract from *Solanum nigrum* indicated the presence of alkaloids, saponins, flavonoids, terpenoids, resins, and cardiac glycosides, while minimal amount of tannins was detected and steroids were not detected. The quantitative phytochemical analysis revealed that the constituents in both the aqueous and ethanolic extracts had higher concentrations of flavonoids, phenols, and ascorbic acid, whereas cardiac glycosides and alkaloids were present in negligible quantities. Additionally, the ethanolic extract of phytate showed minimal amounts of these compounds.

Keywords: *Solanum nigrum* L, Saponins, Alkaloids, Flavonoids, Phenols

1. INTRODUCTION

Plants have been utilised as medicine for many years because they are a rich source of physiologically and therapeutically active compounds (Awah et al., 2016; Awah et al., 2017; Ubaoji et al., 2020). These plants have been linked to the treatment of a variety of diseases, although some of them may have negative effects on the body (Agu et al., 2013; Agu et al., 2014; Adindu et al., 2016). *Solanum nigrum* L, commonly referred to as Black nightshade, is a medicinal plant classified within the Solanaceae family. It is characterized by its smooth, green, semi-climbing stem and typically reaches a height of 10-60 cm as an annual herbaceous plant. *Solanum nigrum* has been recognized for its pharmacological properties, which are attributed to a variety of compounds found within it. The plants contain phytochemical components such as alkaloids, flavonoids, steroids, tannins, and phlobatannins, as reported by Rajathi D Modilal et al., in 2015. Additionally, research by Chinthana and Ananthi in 2012 highlighted the antioxidant, anti-inflammatory, and antipyretic properties of the chloroform extract from *Solanum nigrum*. The plant contains a variety of active constituents, which encompass glycoproteins, glycoalkaloids, and polysaccharides. Moreover, *Solanum nigrum* leaves are abundant in a range of polyphenolic compounds, including catechin, gallic acid, protocatechuic acid (PCA), epicatechin, caffeic acid, rutin, and naringenin, as documented in a study conducted by Ravi et al., in 2009. In traditional medicinal practices, *Solanum nigrum* leaves have been employed to address conditions such as rheumatic and gouty joints, skin disorders, nervous ailments, nausea, and tuberculosis.

Furthermore, the decoction and juice extracted from its berries have been utilized as remedies for cough, diarrhea, inflammation, and various skin conditions, as reported by Dilip et al., in 2012 and Chauhan et al., in 2012. Additionally, research by Rani et al., in 2017 indicated that the ethanol extract of this plant possesses anticancer properties. In the realm of traditional medicine, *Solanum nigrum* leaves have a longstanding history of application in addressing a spectrum of health issues. These include the management of conditions like rheumatic and gouty joints, skin disorders, nervous disorders, nausea, and tuberculosis. Furthermore, decoctions and berry juice derived from this plant have found utility as remedies for ailments such as coughs, diarrhea, inflammation, and various skin conditions, as demonstrated by studies conducted by Dilip et al., 2012 and Chauhan et al., 2012. Notably, the ethanol extract of this plant has been explored for its potential in the realm of anticancer properties, as elucidated in the research conducted by Rani et al., in 2017.

2. MATERIALS AND METHODS

Sample Collection

Solanum nigrum leaves were procured from the Eke Awka market in Awka and identified by a botanist. The leaves were carefully washed, air-dried at room temperature, ground into a powder, and then stored for subsequent analysis

Extraction of Phytochemical Components

Fifty grams (50g) of the dried and powdered plant material were immersed in 500 ml of both water and ethanol and allowed to steep for 24 hours. Afterward, the extracts were filtered using Whatman No. 1 filter paper and then concentrated using a rotary evaporator. The resulting extract was subsequently stored in a refrigerator for further analysis.

Qualitative Analysis of Phytochemical components of *Solanum nigrum*

The qualitative phytochemical analysis of the extract was conducted following established procedures as outlined in the works of Harbonne (1998) and Trease and Evans (1983).

3. ALKALOIDS DETERMINATION

Test for Alkaloids

Reagent: 2% HCl

Procedure for processing/preparation

After pipetting 1.0 ml of the extract into a test tube, 5.0 ml of 2% HCl was added. The test tube was then subjected to heating in a Memmert water bath for 10 minutes, followed by filtration using Whatman No. 1 filter paper. The resulting filtrate was subsequently employed for the following tests.

Wagner's Reagent test

Principle: Alkaloids under acidic condition and at room temperature reacts with iodine and potassium iodide to produce reddish brown precipitate.

Reagent: Wagner's reagent: 2g of iodide and 3g of potassium iodide are weighed, mixed and dissolved in 30ml distilled water and made up to 100ml with distilled water.

Procedure

One millilitre of filtrate was pipetted into a test tube, 1.0ml of Wagner's reagent was added in the test tube mixed properly and observed for colour change. A reddish brown precipitate indicates presence of alkaloids.

Meyer's Reagent test

Principle: Alkaloids under acidic condition and at room temperature reacts with mercuric chloride and potassium iodide to give a cream colour or precipitate.

Procedure

A volume of 1.0 ml of the filtrate was transferred into a test tube using a pipette, and an additional 1.0 ml of Meyer's reagent was similarly pipetted into the same test tube. The contents were thoroughly mixed and then examined for any color change. The formation of a cream-colored precipitate would indicate the presence of alkaloids, in accordance with the method outlined by Trease and Evans (1989)

4. TEST FOR FLAVONOIDS

Ferric chloride test for phenolic nucleus:

Principle: Phenolic nucleus reacts with ferric chloride at room temperature to give greenish brown or black colour or precipitate.

Procedure:

Taking 1.0 ml of the extract, it was carefully transferred into a test tube. Subsequently, 1.0 ml of 10% ferric chloride was introduced into the same test tube, and the contents were thoroughly mixed and observed for any alterations in color.

The development of a greenish-brown or black hue or the formation of a precipitate would signify the presence of a phenolic nucleus."

Lead Acetate Test

Procedure:

Pipette 1.0ml extract into a test tube, 1.0ml of 10% lead acetate solution was pipetted into the same test tube. The contents were mixed properly, and observed for any colour change or precipitate which indicates presence of flavonoids.

Sodium Hydroxide Test

Principle: Flavonoids at room temperature and under alkaline pH forms observable precipitate.

Procedure:

Pipette 1.0ml of extract into a test tube, then 1.0ml of dilute NaOH solution the same test tube, mixed and observed for any change in color. Formation of precipitate indicates presence of flavonoids. (Trease and Evans (1989).

Test for Reducing Sugar:

15mls of a mixture of Fehlings solution A and B (equal parts) was added to 2g of the powdered plant materials in the test tube. These were heated in a boiling water-bath for five minutes. A brick red precipitate indicates a positive result.

Test for Tannins

Acid Test

Principle: phlobotannins under acidic condition reacts with dilute HCl to give a red colour or precipitate.

Procedure:

Three milliliters (3.0 ml) of the extract were introduced into 2.0 ml of 1% HCl, and the presence of a red color or precipitate was observed. This observation would indicate the presence of phlobotannins

Lead Acetate Test

Principle: phlobotannins reacts at room temperature with lead acetate to give dark-blue to black precipitate.

Procedure:

Pipetted into a test tube, 2 ml of extract. Added 3 drops of 5% lead acetate solution to the extract. A dark blue to black precipitate indicated the presence of phlobotannins."

Test for Proteins

To a little portion of the filtrate in a test tube, 2 drops of millon reagents were added. A white precipitate indicates the presence of protein.

Test for Resins

Approximately 0.2 grams of the powdered material were subjected to extraction with 15 ml of 90% ethanol. The resulting alcoholic extract was subsequently combined with 20 ml of distilled water in a beaker. The observation of a precipitate would signify the presence of resins.

5. TEST FOR CARBOHYDRATES

Molisch's test for Glucose- Approximately 1 gram of the powdered samples was boiled with 10 ml of water in test tubes and then filtered. A few drops of Molisch's reagent were added to the filtered liquids in the test tubes, followed by the gentle pouring of 5 ml of concentrated H₂SO₄ down the sides of the tubes to create a lower layer. The formation of a purple-colored ring on the outer surface was considered a positive result

Test for Saponins- Approximately 2 grams of the powdered sample were heated in 20 ml of distilled water using a water bath and subsequently filtered. Ten milliliters of the resulting filtrate were combined with 5 ml of distilled water and vigorously shaken to produce a stable, persistent froth. This frothy mixture was then blended with 3 drops of olive oil and shaken vigorously once more, after which it was observed for the formation of an emulsion.

Test for Cardiac Glycosides- - ne milliliter (1 ml) of the extract was combined with 10 cm³ of 50% H₂SO₄ and subjected to heating in boiling water for 5 minutes. Following this, 10 cm³ of Fehling's solution (5 cm³ each of solutions A and B) was introduced and boiled.

The observation of a brick-red precipitate indicated the presence of glycosides

Test for Terpenoids- Five milliliters (5 ml) of each extract were combined with 2 ml of chloroform (Numex, India), and then 3 ml of concentrated H₂SO₄ was cautiously introduced to create distinct layers. The formation of a reddish-brown coloration on the inner surface indicated a positive result for the presence of terpenoids

6. QUANTITATIVE ANALYSES

Flavonoid Determination- A 0.5 ml of plant ethanolic extract was mixed with 2 ml of water, followed by adding 0.15 ml 5% NaNO₂. After 6 minutes, 0.15 ml 10% AlCl₃ was added and left for 6 minutes. Then, 2 ml 4% NaOH was introduced. The volume became 5 ml with water, standing for 15 more minutes. Absorbance at 510 nm was measured, using water as a blank. A catechin reference standard was prepared. Results were expressed as milligrams of catechin equivalent per 100 grams of the sample and converted to a percentage (Barros et al., 2007).

Total Phenol Content Determination- The colorimetric method from Barros et al., (2007) was adapted. In this method, 1 ml of the sample extract was mixed with Folin and Ciocalteu's phenol reagent, followed by the addition of 1 ml of

saturated sodium carbonate. The volume was adjusted to 10 ml with distilled water, and the mixture was left in darkness for 90 minutes. Afterward, the absorbance was measured at 725 nm.

Terpenoids - An aliquot of the sample was mixed with 3.5ml of iced cold 95% methanol and left at room temperature in dark for 48 hours. The sample was centrifuged at 400g for 15 minutes and the supernatant was collected. 200µl of the supernatant was mixed with 1.5ml of chloroform, mixed and allowed to stand for 3minutes, this was followed by addition of 100µl of concentrated H₂SO₄, allowed to stand in the dark for 90minutes after which the supernatant was carefully decanted and the red precipitate dissolved with 1.5ml of 95% methanol, then absorbance taken at 538nm against 95% methanol as blank.

Saponin Content- A 5 ml sample was mixed with 200 ml 20% aqueous ethanol, shaken for 3 hours, filtered, and reduced to 10 ml at 96°C. This concentrate was combined with 5 ml diethyl ether, shaken, and the aqueous layer kept. Purification was repeated using 15 ml n-butanol, washing twice with 2.5 ml 5% aqueous sodium chloride, and drying in a water bath (Obadoni and Ochuko, 2002).

Alkaloid Content - Alkaloid content was determined using Harbone 1995 method. Five grams of the plant sample were mixed with 200 ml of 20% acetic acid in ethanol, standing at 25°C for 4 hours. After filtration and concentration, ammonium hydroxide was added to form a precipitate. The precipitate was collected, washed with dilute NH₄OH, and filtered. The residue was dried at 80°C, and alkaloid content was calculated as a percentage of the sample weight.

Phytate Determination - Phytate content was determined following Young and Greaves' (1999) method. In 250 ml conical flasks, 2 ml of sample extract mixed with 100 ml of 2% concentrated HCl for 3 hours. The filtrate was transferred to a 250 ml beaker with the addition of 50 ml distilled water. An indicator, 5 ml of 0.3% ammonium thiocyanate solution, was introduced, and titration was conducted using standard iron (III) chloride solution with a concentration of 0.00195 g iron per ml.

Cardiac Glycosides Determination- According to the method described by Osagie in 1998, 1 ml of the extract was combined with 1 ml of a 2% solution of 3, 5-DNS in methanol and 1 ml of a 5% aqueous NaOH solution. This mixture was subjected to boiling for 2 minutes, followed by filtration, and the residue on the filter paper was dried at 50°C. The weight of the filter paper along with the residue was recorded.

7. RESULTS

The qualitative analysis of *Solanum nigrum* results showed the presence of alkaloids, flavonoid, terpenoids, cardiac glycosides, saponin and resins. Alkaloids were confirmed by a faint reddish precipitate, saponin by formation of emulsion, flavonoid by greenish brown colour change, terpenoids by visible colour change, cardiac glycosides by light brick red precipitate, and resin by red colour formation, while the quantitative analysis indicated the presence of The quantitative phytochemical analysis revealed that the constituents in both the aqueous and ethanolic extracts had higher concentrations of flavonoids, phenols, and ascorbic acid, whereas cardiac glycosides and alkaloids were present in negligible quantities, while ethanolic extract of phytate showed minimal amounts of these compounds. As shown in the Tables 1 and 2 below.

Results

Table 1: Qualitative analysis (phytochemical) properties of *Solanum nigrum*

PARAMETER	OBSERVATION	INFERENCE
Alkaloid	Faint reddish brown precipitate	+
Flavonoid	Greenish brown observed	++
Terpenoid	Visible colour change was observed	++
Steroids	No visible colour change was observed	-
Cyanogenic glycoside	Light Brick red ppt formed	+
Saponins	Partial formation of emulsion	+
Resins	Red colour formation	+
Tannins	No precipitate visible	-

++ Denotes very positive

+ Positive

-..... Negative

Table 2: Quantitative analysis (phytochemical) of the *Solanum nigrum* (Anyara leaf)

Parameter	Aqueous Extract of Anyara	Ethanol Extract of Anyara
Ascorbic acid (mgAAE/G)	22.5	24.043478264
Saponin (mg/g)	1.475	0.111
Phenol (mgGAE/g)	34.583333335	54.80769231
Flavonoid (mgCE/g)	69.375	354.0625
Tanin (mgTAE/g)	0.875420875	6.228956229
Alkaloids (mg/g)	0.0725	0.1035
Phytate	6.203896875	0.238850625
Cardiac Glycosides (mg/g)	0.096	0.08
Terpenoids (mgL/g)	0.0	0.1530857145

Mg GAE: Milligram Gallic equivalent

Mg CE: Milligram Catechin equivalent

Mg AAE: Milligram Ascorbic Acid Equivalent

MgL: Milligram Linalool equivalent

8. DISCUSSION

The qualitative screening results indicated the presence of alkaloids, flavonoids, terpenoids, cyanogenic glycosides, saponins, and resins in *Solanum nigrum* L. In terms of quantitative analysis, both aqueous and ethanolic extracts of *Solanum nigrum* leaves were found to contain saponins, phenols, flavonoids, ascorbic acid, tannins, alkaloids, phytate, and cardiac glycosides. Additionally, the ethanolic extract exhibited the presence of terpenoids, while the aqueous extract did not. These findings align with the research conducted by Silivano et al., in 2018, which reported that both aqueous and ethanolic extracts of the leaves contained alkaloids, reducing sugars, saponins, tannins, and terpenoids, with the presence of reducing sugars being specifically noted.

Furthermore, Silivano et al., (2018) documented the presence of steroids and flavonoids in the ethanolic crude extract of the leaves, which contradicted earlier findings. The absence of steroids and flavonoids in the aqueous crude extract of the leaves could be attributed to their limited solubility in aqueous solutions. This observation aligns with a previous study conducted by Pankaj et al. (2016), which reported the presence of saponins, tannins, alkaloids, terpenoids, flavonoids, and glycosides, while noting the absence of steroids and proteins in the upper parts of *Solanum nigrum*, including leaves, stems, and fruits.

Rajathi D Modilal et al., (2015) similarly documented the presence of alkaloids, flavonoids, steroids, and tannins in both aqueous and ethanolic crude leaf extracts of *S. nigrum*. This finding corroborated the statement made by Senjobi et al., (2017), emphasizing that plants from various geographical regions around the world may exhibit different combinations and concentrations of active substances. The identification of tannins in *Solanum nigrum* leaves is significant, as it underlines their astringent properties and their physiological role in wound treatment, as noted by Sodipo et al., (2008). Tannins, as reported by Sodipo et al., (2008), have the capacity to inhibit the growth of microorganisms by causing precipitation when exposed to aqueous extracts of *Solanum nigrum* leaves. Furthermore, the phenolic compounds found in *Solanum nigrum*, as indicated by Rajathi D Modilal et al., (2015), demonstrate a range of properties including antiviral, antimicrobial, hypotensive, and antioxidant effects.

It contains flavonoids that impart antioxidative effects, as reported by Pandey et al., (2012), and has demonstrated hepatoprotective qualities according to Tapas et al., (2008). Additionally, it has been noted for its antibacterial and anticancer properties by Mirsha et al., (2013). Most plants known for their antidiabetic properties are characterized by the presence of secondary metabolites like glycosides, alkaloids, and flavonoids, as mentioned by Craig (1990). The leaf extract has exhibited antioxidant properties, as evidenced by results from DPPH, ABTS, NO, and superoxide radical scavenging assays. These findings imply that *Solanum nigrum* could potentially be employed in the treatment of diseases associated with free radicals, such as diabetes and cancer."

9. CONCLUSION

The analysis of phytochemicals in *Solanum nigrum* has unveiled that its antimicrobial effectiveness can be attributed to the presence of a range of bioactive compounds, including alkaloids, saponins, steroids and flavonoids. These findings

strongly suggest that medicinal plants with established traditional uses could serve as valuable resources for the production of novel antimicrobial agents.

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