Molecular Mechanism Research (2024) Volume 2 Issue 1
doi: 10.59429/mmr.v2i1.6742

RESEARCH ARTICLE

Study of Phytochemical Screening and in vitro Antioxidant Activity of Ethanol Extract of *Solanum sisymbriifolium* leaf

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ABSTRACT

Introduction: Plants are rich in biologically active compounds, including morphine, digitalis, quinine, Nicotine and muscarine, used in both natural and synthetic drugs. Recent clinically useful plants include paclitaxel, artemisinin, and vinblastin. Secondary metabolites contribute to modern drugs, antibiotics, vitamins, and hormones. **Materials and Methods:** The study investigates the phytochemical constituents of Solanum sisymbriifolium leaves' ethanol extract using various methods. It identifies secondary metabolites like alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, and anthraquinones. The study also employed a thin layer chromatography (TLC) method for compounddetection and identification. This provides valuable insights into plant extract chemistry. **Results:** The ethanol extract of Sol*anum sisymbriifolium* was subjected to phytochemical screening to identify various phytoconstituents. The extract showed positive results for the presence of alkaloids, flavonoids, steroids, terpenoids, reducing sugars, saponins, tannins, cardiac glycosides, and anthraquinones. The total flavonoid content of the plant extract was 266.34±33.22, while the total phenolic content was 119.69±2.70. The antioxidant test with DPPH showed that the IC50 value of ascorbic acid (AA) increased with the increase of concentration but remained almost constant over time. The reducing power activity test showed that the absorbance of the plant extract increased with different concentrations but remained almost constant with respect to concentration. Thin Layer Chromatography (TLC) was conducted on different solvent systems, showing different polarity and Rf values. The Rf values increased with the increase of polarity, indicating that the separated compounds have more affinity for the comparative polar mobile phase. The Rf values also increased with the increase of polarity, indicating that the compounds have more affinity for the comparative polar mobile phase. **Conclusion:** Medicinal plants, like *Solanum sisymbriifolium* leaves, have antioxidant activity for disease management. Further investigation is needed to isolate and characterize these compounds, assess their preventive role against free radicals.

Keywords: *Solanum sisymbriifolium*; TLC; DPPH; Ascorbic Acid; Phytochemicals

ARTICLE INFO
Received: 1 August 2024 | Accepted: 20 August 2024 | Available online: 30 August 2024

CITATION
Zafreen A, Mohamed MN, Islam S, et al. Study of Phytochemical Screening and in vitro Antioxidant Activity of Ethanol Extract of *Solanum sisymbriifolium* leaf. *Molecular Mechanism Research* 2024; 2(1): 6742. doi: 10.59429/mmr/v2i1.6742

1. Introduction

Plants, particularly medicinal plants, are essential components in Indian medicine and Chinese medicine. These plants contain substances that can be used for therapeutic purposes or as precursors for drug synthesis. Paracelsus (1493-1541) and Chinese saying "Shi Yao San Fei Du" emphasize the importance of dose in determining the potency of drugs[1]. Modern medicines, such as aspirin, are produced indirectly from medicinal plants. These plants naturally synthesize and accumulate secondary metabolites, such as alkaloids, glycosides, tannins, volatile oils, minerals, and vitamins, demonstrating their medicinal properties[2].

Plants have long been used for treating diseases due to their rich source of biologically active compounds or lead compounds. These compounds, such as morphine, digitalis, quinine, nicotine, and muscarine, have been used in both natural and synthetic drugs[3,4]. Recent clinically useful drugs isolated from plants include paclitaxel from the yew tree, artemisinin from Chinese medicinal herb *Artemisia annua*, and vinblastin from *Catharanthus rosesus*[5]. Secondary metabolites of plants have played a significant role in medical care, with around 100 plant species contributing significantly to modern drugs[6]. Many antibiotics, vitamins, and hormones are now used due to the purification and identification of their active principles[7].

Therapeutic purposes are a significant focus for medicinal plants, which contain substances that can be used for various purposes. Approximately 80% of the population in developing countries relies on traditional medicines, primarily plant drugs. Studies show that 74% of 119 plant-derived pharmaceutical medicines or biotechnology medicines are used in modern medicine in ways that correlate with their traditional use[8,9]. Studying medicinal plants helps understand plant toxicity and protects humans and animals from natural poisons[10]. Cultivation and preservation of medicinal plants protect biological diversity, and their potential in the synthetic era is growing. By combining ancient knowledge with scientific principles, medicinal plants can provide powerful remedies to eradicate diseases and reduce drug toxicity[11].

Medicinal plants are crucial for developing new drugs, especially in developing countries where traditional medicine is expensive and resistant. Scientists are searching for inexpensive, long-lasting drugs[12]. About 80% of the world's population relies on herbal remedies, especially in rural areas[13]. A study examining the phytochemical screening and antioxidant activity of ethanol extract from *Solanum sisymbriifolium* leaf aims to identify active chemical constituents and their potential use in disease management and treatment.

1.1. Traditional herbal medicine in Bangladesh

Traditional Medicine is a system of treatment based on the use of plants, animals, natural substances, religious verses, cultural practices, and physical manipulations. It has been used for generations to treat physical and psychological diseases, often influenced by folklore customs, cultural habits, social practices, religious beliefs, and superstitions[14,15]. The earliest mention of traditional medicine is found in Rigveda, the oldest repository of knowledge in the Indian subcontinent[16]. Ayurveda, developed from the Vedic concept of life, is an important source of medical sciences and has become a part of the Indian subcontinent's culture and heritage[17]. Unani medicine consists of whole plants, powders, pastes, extracts, infusions, decoctions, and distillates, with minerals, inorganic chemicals, and animal products also used. Ayurveda remains an important system of medicine and drug therapy in Bangladesh[18], with plant alkaloids being the primary active ingredients.

Some crude drugs used as medicine in Bangladesh are reported in following table.

Table 1: Some crude drugs used as medicine in Bangladesh[13,19]

Common name	Botanical name	Uses
Amla	*Emblica officinalis*	Vitamin - C, Cough, Diabetes, cold, Laxative, hyper acidity.
Ashok	*Saraca asoca*	Menstrual Pain, uterine, disorder, Deiabetes.
Bael / Bilva	*Aegle marmelous*	Diarrrhoea, Dysentry, Constipation.
Chiraita	*Swertia chiraita*	Skin Desease, Burning, censation, fever.
Kalmegh/ Bhui neem	*Andrographis paniculata*	Fever, weekness, release of gas.
Long peeper / Pippali	*Peeper longum*	Appetizer, enlarged spleen , Bronchities, Cold,
		antidote.
Pashan Bheda / Pathar Chur	*Coleus barbatus*	Kidny stone, Calculus.
Sandal Wood	*Santalum album*	Skin disorder, Burning, sensation, Jaundice,Cough.
Satavari	*Asparagus racemosus*	Enhance lactation, general weekness, fatigue,cough.
Senna	*Casia augustifolia*	General debility tonic, aphrodisiac.
Tulsi	*Ocimum sanclum*	Cough, Cold, bronchitis,expectorand
Pippermint	*Mentha pipertia*	Digestive, Pain killer
Henna/Mehd	*Lawsennia iermis*	Burning, Steam, Anti Imflamatary
Gritkumari	*Aloe verra*	Laxative, Wound healing, Skin burns & care,Ulcer
Sada Bahar	*Vincea rosea*	Leaukamia, Hypotensiv, Antispasmodic , Atidot
Vringraj	*Eclipta alba*	Anti-inflamatory, Digestive, hairtonic
Neem	*Azardirchata indica*	Sdedative, analgesic, epilepsy, hypertensive
Anantamool/sariva	*Hemibi smus indicus*	Appetiser, Carminative, aphrodisiac, Astringent
Kantakari	*Solanum xanthocarpum*	Diuretic, Antiinflamatory, Appetiser, Stomachic
Shankhamul	*Geodorum denciflorum*	Antidiabetic

Bangladesh, a South Asian country, has only 20% modern healthcare services, with the rest relying on traditional plant-based systems. Out of 1,900 medicinal plant species, only 500 have been recorded, suggesting many more are undiscovered[20].

1.2. Literature Review

Phytochemical Studies

Phytochemical studies of *Solanum sisymbriifolium* reveal the presence of alkaloids, flavonoids, steroids, and tannins in various parts of the plant[21]. Leaf ultrastructure shows hairs and glandular trichomes, while roots contain alkaloids like cuscohygrine, solacaproine, solamine, solasodiene, and solasodine. Fruits contain ligans (sisymbrifolin) and a C30 sterol (carpesterol), while the aerial part produces spirostane derivatives[22]. A new neolignan and sterols, such as sisymbrifolin and carpesterol, were isolated from the berries. Gibberellin-like substances were detected in methanolic extracts from the vegetative shoot

and inflorescences. The methanolic fraction of the crude contains anthocyanins and a high concentration of derivatives of Luteolin, with chlorogenic acid being the most representative[23].

Table 2: Phytochemical Studies on *S. sisymbriifolium*[18,21]

Part of the plant	Compounds	References
Vegetative shoot and inflorescences	Acidic gibberellin-like substances	Botanic, 1975
Roots	Cuscohygrine	Evans and soman- abandhu, 1980
Roots	Solacaproine	Maldoni, 1984
Roots	Solamine, solasodiene and solasodine	Mazumdar, 1984
Aerial part	Spirostane derivatives	Chakravarty *et al.*,1996.
Fruits	Lignans (sisymbrifolin) and a C_{30} sterol (carpesterol).	Chakravarty *et al.*, 1996
Leafs	Esters of short chain fatty acids and simple sugars such as glucose and sucrose	Steffens, 2000
Roots	Isonuatigenin-3-*O*-β-solatriose, steroidal saponin.	Ferro *et al.*, 2005
Whole plant	Alkaloids, flavonoids, steroids and tannins	Shilpi *et al.*, 2005
Whole plant	Luteolin, chlorogenic acid.	Usai *et al, 2008*
Fruits	Solasodine.	Chauhan *et al.*, 2010

Pharmacological Studies

Solanum sisymbriifolium lamk is a plant with various medicinal properties, including antinociceptive, anticonvulsant, hypotensive, and cardiovascular effects. Its methanolic extract has been found to have antinociceptive activity in Swiss albino mice, while its dried fruits have shown potent anticonvulsant and CNS depressant activities[24]. has been found to induce hypotension activities in anaesthetized normotensive rats. has been found to have antihypertensive effects in experimentally hypertensive rats under chronic administration. The oxalate package in Solanum sisymbriifolium anthers was studied for its development and composition[25]. The crystals originated simultaneously within the vacuoles in association with a paracrystalline protein, with the main elements being calcium, oxygen, calcium oxalate, potassium, magnesium, and silicon. Nuatigenosido, isolated from the root of *Solanum sisymbriifolium*, has been found to have cardiac effects on rats, lowering blood pressure, enhancing contractile force, increasing overshoot amplitude, and increasing action potential durations[26].

Methanolic extracts of 13 Solanum species have been tested for molluscicidal activity against Biomphalaria glabrata[27]. The extract of fruits and aerial parts of *Solanum sisymbriifolium* showed significant positive molluscicidal activity[28]. Argentinean plant extracts have been found to be highly cytotoxic but also have anticancer properties. *Solanum sisymbriifolium* has insecticidal and antifungal properties, with positive inotropic and negative chronotropic effects in frog, guinea pig, and rabbit hearts[21,29]. It also contains steroidal saponins with various biological activities.

Table 3: Pharmacological Studies on *S. sisymbriifolium*[26-29].

Part of the plant	Activity	References
Aerial part	Presents antibacterial activity	Mahato *et al*.1982
Whole plant	Insecticidal properties.	Cesio *et al*. 2000
Root	Hypotensive activity in normotensive rats	Ibarrola *et al.*, 2000
Aerial part	Presents different antifungal activities.	Pragnell, 2003
Aerial part	Positive molluscicidal activity	Silva *et al.*, 2005
Whole plant	The antinociceptive activity of the methanolic extract.	Shilpi *et al.*, 2005
Whole plant	Positive molluscicidal activity.	Silva *et al.*, 2005
Dried fruits	Positive molluscicidal activity.	Silva *et al.*, 2005
Root	Lowered blood pressure, shorten action potential durations. Increase of breathing rate, increase in the gastrointestinal transit.	Derlis *et al.*, 2006
Whole plant	Composition of the crystals :C, O, and Ca were the main elements, and K, Cl, Mg, P, S, and Si, the minor elements.	Burrieza *et al*.,2010
Dried fruits	Potent anticonvulsant and CNS depressant activities.	Chauhan *et al.*, 2010
Root	Reduction of blood pressure, heart rate.	Ibarrola et al., 2011
Flower	Methanolic extract were found highly cytotoxic, and has anti cancer effect.	Mamone et al., 2011
Leafs	The ethanolic extract successfully inhibited the castor oil induced diarrhea, decreases number of stool as well as total weight of fecal output.	Apu et al, 2013

Ethno pharmacological Studies

S. sisymbriifolium is a plant used for treating hysteria, remitting fever, and stomachache. Its roots are used in Paraguay for hypertensive diseases and in Argentina for diuretic, analgesic, contraceptive, antisyphilitic, and hepatoprotective purposes. The fruits are a source of solasodine and a component of oral contraceptives. The leaves are used as febrifuges in Peru and diuretic in Brazil[30]. Aerial parts are used in Argentina for diarrhea and respiratory and urinary tract infections.

Table 4: Ethno pharmacological Studies on *S. sisymbriifolium*[13-17, 30]

Part of the plant	Activity	References
Roots	Treatment of hypertensive diseases in Paraguay As diuretic, analgesic, contraceptive, antisyphilitic and hepatoprotective in Argentine.	Ferro *et al.*, 2005
Flowers	Used in India as analgesic	Ferro *et al.*,2005
Leaf	Used as febrifuge in Peruand. As diuretic in Brazil	Ferro *et al.*, 2005
Aerial parts	Used in Argentine to treat diarrhea, infections of respiratory and urinary tracts	Ferro *et al.*, 2005
Whole plant	Potential trap crop for the control of potato cyst nematodes, species *Globodera rostochiensis* and *G. pallid.* Treatment of hysteria, remitting fever, and stomachache .	Turner *et al.*, 2006 Uddin, 2010
Fruits	Used as a vegetable	Uddin, 2010

2. Materials And Methods

Phytochemical Screening

The plant extract contains a wide range of secondary metabolites are available. For identifying phytochemical constituents in the ethanol extract of *Solanum sisymbriifolium* leaves several methods were used. The freshly prepared ethanol extract of *Solanum sisymbriifolium* were qualitatively tested for the presence of Alkaloids (Mayer test, Wagners test, Hagers test, Tannic acid solution), Flavonoids (Modified Ammonia test), Steroids (Salkowski test), Terpenoids (Modified Salkowski test), Reducing sugars (Fehlings test, benedicts test), Saponins (Frothing test), Tannins ($FeCl_3$ test), Cardiac glycosides (Killer-Killanis test) and Anthraquinones (Chloroform layer test)[31].

Total Flavonoid Compound

The study used a screw cap test tube, conical flask, electric balance, pipette pumper, vortex mixer, and UV-spectrophotometer to determine the flavanoid content in a plant extract. The extract was dissolved in methanol, then diluted with distilled water, 5% sodium nitrite, $NaNO_2$, 10% aluminium trichloride, $AlCl_3$, and 4% sodium hydroxide, NaOH. The reaction mixture was incubated in the dark for 6 minutes, then added to the mixture. The absorbance was measured at 510 nm using a visible spectrophotometer[32].

Total Phenolic Compound

The study focuses on determining the phenolic content in plant extract using various apparatus and reagents. The sample is prepared by dissolving 3 mg of extract in methanol and preparing 10% Folin- Ciocalteu reagent. The reagents are prepared by adding methanol, methanol, 10% F-C reagent, and 700 mM Na_2CO_3 solution. The sample is then transferred to test tubes, centrifuged, and then withdrawn. The absorbance of the total volume is measured in a UV-VIS spectrophotometer at 765 nm. The total phenolic contents are then determined from a standard curve prepared with gallic acid. Different concentrations of gallic acid are used, and the results are expressed as Mean±SD. The study is a valuable tool for understanding the phenolic content of plant extracts [26] .

Antioxidant Test with DPPH

This study used a microscope, electric balance, vortex shaker, and measuring cylinder to conduct an antioxidant test with DPPH. The reagents used were DPPH, methanol, acetone, and distilled water. The extract solution was prepared by dissolved 3.25 mg or 0.00325 g crude ethanol extract of *Solanum sisymbriifolium* leaves with methanol, and then mixed with DPPH reagent. The absorbance was taken at 517 nm using a UV-VIS spectrophotometer. The sample blank was prepared by mixing 4.95 ml methanol with 0.05 ml DPPH. The radical scavenging activity was expressed as the percentage inhibition calculated using the formula: inhibition (%) = {(Ac – As)/Ac} x 100. The IC_{50} values were calculated from the graph and the % inhibitions were plotted against the respective concentrations used[33].

Reducing Power Activity test

The reducing power activity test for *Solanum sisymbriifolium* leaves was conducted using various apparatus and reagents. The test tube used was a pipette and pumper, a sample was prepared using a centrifuge machine, and a hot water bath. The reagents included 1% potassium ferricyanide, $K_3Fe(CN)_6$, distilled water, 10% trichloroacetic acid (TCA), mono basic sodium phosphate, dibasic sodium phosphate, and 0.1% $FeCl_3$. The sample was prepared by dissolved 12 mg of ethanol extract in 10 ml of methanol and mixed with a vortex mixer. The reducing power of the extract was measured using the potassium ferricyanide reduction method.

The test tubes were labeled with different concentrations of the extract sample. The absorbance was measured at 700 nm in a UV-VIS spectrophotometer, with water used as a blank. The test was conducted in a dark place due to the light-sensitive nature of the reaction and reagents[17,33].

Thin Layer Chromatography (TLC)

The study used a thin layer chromatography (TLC) method to analyze the plant extract of *Solanum sisymbriifolium*. The solvent system was prepared using n-haxane 60% and ethyl acetate 40%. Three TLC plates were prepared, each with two spots of plant extract. The plates were marked with three reagents: 10% Sulphuric acid, 0.004% DPPH solution, and Folin-ciocalteu solution. After the TLC, the plates were dried to evaporate the solvents and exposed to reagents for compound detection and identification. The plates were then observed under UV light by naked eye. The study provides valuable insights into the chemistry of plant extracts[34].

3. Results

Screening of Phytochemical Constituents

After preparation of ethanol extract of *Solanum sisymbriifolium,* the extract was run through phytochemical screening. Among different phytoconstituent; Alkaloid, Flavonoid, Steroid, Terpenoid, Reducing Sugar, Saponins, Tannins, Cardiac glycoside and Anthraquinones were tested according to a standard procedure for each. Preliminary phytochemical screening showed the presence or absence of alkaloids, flavonoids, steroids, terpenoids, reducing sugars, saponins, tannins, cardiac glycosides, anthraquinines.

S. sisymbriifolium

Flowers of S. sisymbriifolium

Branchlets of S. sisymbriifolium

Fruits of S. sisymbriifolium

Leaves of S. sisymbriifolium

Figure 1: Different Parts of *S. sisymbriifolium*[3]

Table 5: Phytochemical screening of ethanol extract of *Solanum sisymbriifolium*

Tests		**Observations**	**SSET**
Alkaloids	Mayer‟s reagent	Cream color precipitation	+
	Hager‟s reagent	Yellow color precipitation	+
	Tannic acid solution	White or pink color precipitation	_
	Wagner‟s reagent	Reddish brown color precipitation	+
Flavonoids		Yellow coloration	+++
Steroids		Yellow green fluorescent precipitation	+++
Reducing sugars	Benedict s‟ test	Red precipitation	+
	Fehlings‟ test	Red precipitation	++
Saponins		Emulsion formation	_
Tannins		Brownish green or blue black color	_
Terpenoids		Red violet color	_
Anthraqunones		Rose pink color	_
Cardiac glycosides		Brown ring of the interface	_

SSET represents ethanol extract of *Solanum sisymbriifolium* leaves. „+", „++", „+++" indicate slightly, moderately and highly presence of the compounds respectively. „-„ indicates absence of the compounds.

From the table 5, the extract showed positive result for the presence of alkaloids, flavonoids, steroids and reducing sugars and negative results for saponins, tannins, terpenoids, cardiac glycosides and anthraqunones.

Total Flavanoid Content

Table 6: Total flavonoid contents of ethanol extract of Solanum sisymbriifolium

Sample	**Total flavonoid content (in mg/g cafechin equivalent). Mean ±SEM**
Ethanol extract of *Solanum sisymbriifolium* leaves	266.34±33.22

From the table 6, the flavonoid content of the plant extract was 266.34±33.22. The total phenolic content of the *Solanum sisymbriifolium* ethanol extract was expressed as catechin equivalent.

Total Phenolic Content

Table 7: Total phenolic contents of ethanol extract of *Solanum sisymbriifolium.*

Sample	**Total phenolic content (in mg/g Gallic acid equivalent). Mean ± SEM**
Ethanol extract of *Solanum sisymbriifolium* leaves	119.69±2.70

From the table 7, the phenolic content of the plant extract was 119.69±2.70. The total phenolic content of the *Solanum sisymbriifolium* ethanol extract was expressed as gallic acid equivalent.

Table 8: Absorbance of gallic acid

SL. No.	**Conc. (µg / m)**	**Absorbance**
0	0	0
1	25	0.7
3	50	0.952
4	75	1.215
5	100	1.211

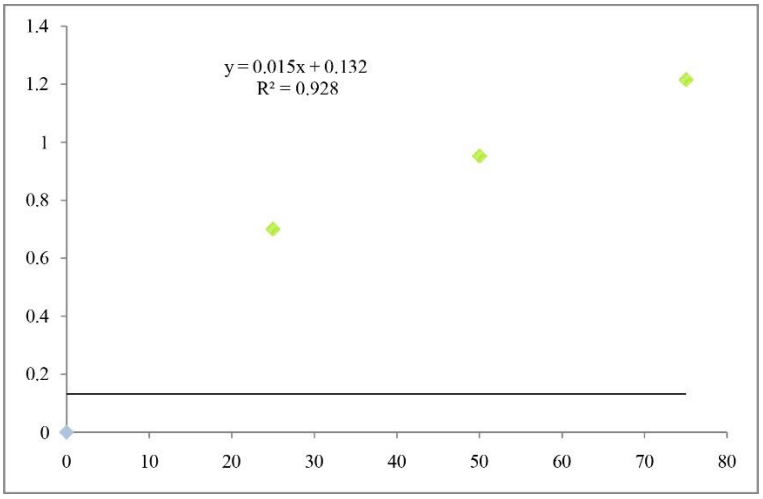

Figure 2: Standard curve of gallic acid.

A linear relationship was observed, when the absorbances in X axis was plotted against the concentration in Y axis. This linear curve was considered as a standard curve and to determine the total phenolic content of the test samples.

Antioxidant Test with DPPH

Table 9: IC50 value of ascorbic acid (AA)

Concentratio ns	**Absorbance of AA**				**Value of IC50**
	Log C		**Absorbance of blank**	**% inhibition**	
0	0.00	0.085	0	0.00	
10	1.00	0.013	0	84.71	
20	1.30	0.011	0	87.06	**5.78**
40	1.60	0.009	0	89.41	
60	1.78	0.008	0	90.59	
80	1.9	0.006	0	92.94	
100	2.00	0.005	0	94.12	

Table 10: IC50 value of leaf of *Solanum sisymbriifolium*

Concentrations	**Absorbance of extract**	**Absorbance of sample blank**	**Absorbance of**	**% inhibition**	**Value of IC50**
0	0.107	0	0.107	0.00	
10	0.068	0.006	0.107	36.45	
20	0.063	0.006	0.107	41.12	**27.87**
40	0.011	0.015	0.107	89.72	
60	0.006	0.018	0.107	94.39	
80	0.003	0.022	0.107	97.20	
100	0.002	0.034	0.107	98.13	

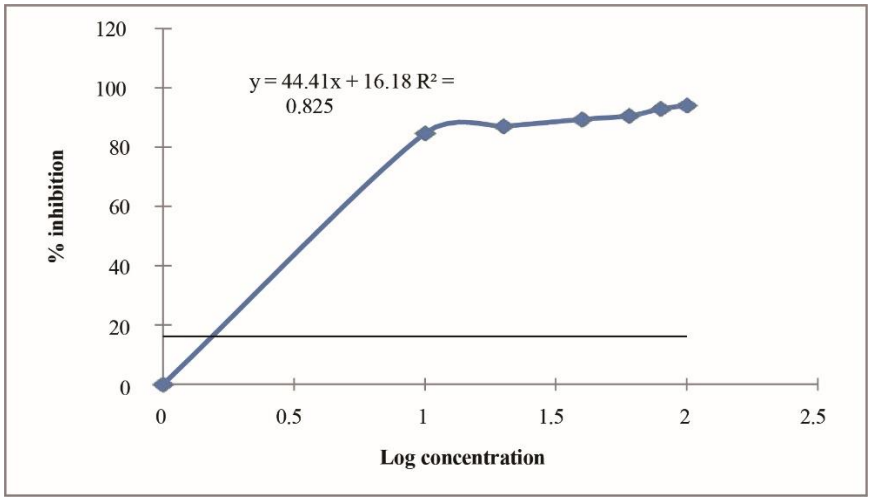

Figure 3: % Inhibition curve of ascorbic acid

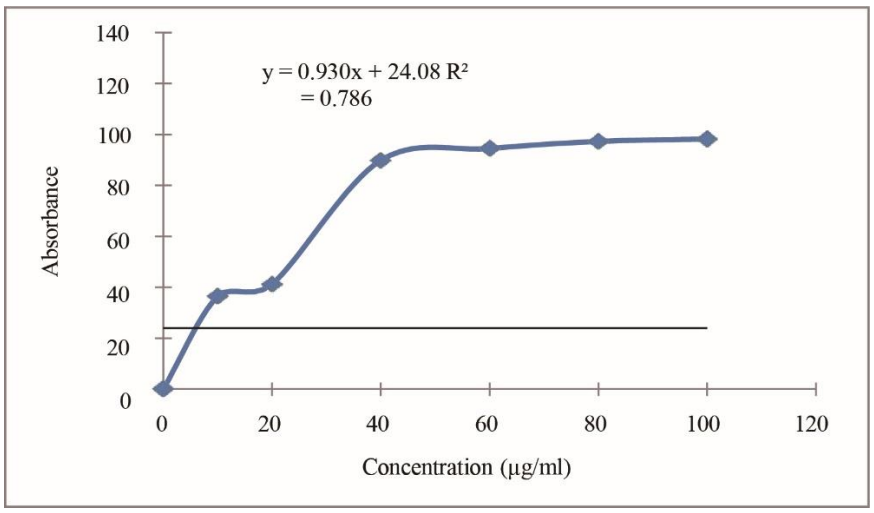

Figure 4: Scavenging effect of the leaf of *Solanum sisymbriifolium*

Reducing Power Activity Test

The absorbance of different concentration of plant extract with reagents was measured at 700 nm in a UV-VIS spectrometer. For different concentration different absorbance was obtained. By using these data, a concentration versus absorbance curve can be ploted.

Table 11: Absorbance of plant extract

Concentration (µg/ml)	Absorbance (nm)
0	0
200	0.044
400	0.046
600	0.047
800	0.045
1000	0.045
1200	0.042

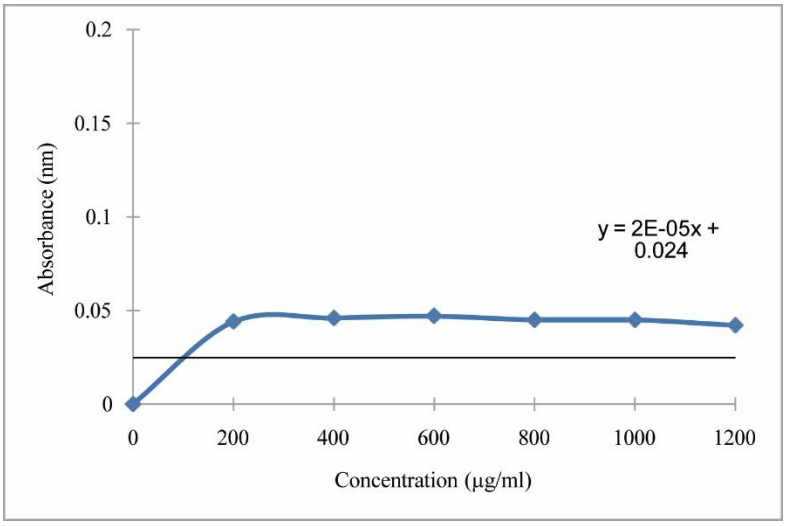

Figure 5: Ethanol extract of *Solanum sisymbriifoliu* in reducing power activity test.

From the figure it was observed that with the increase of concentration the absorbance increases but after a certain time the absorbance remains almost constant with respect to concentration.

Thin Layer Chromatography (TLC)

Different ratio of *n*-hexane with ethyl acetate and *n*-hexane with dichloromethane was run. The ratio of *n*-hexane was from 100% to 0%. At different ration of solvents it shows different polarity. With the polarity the R_f value and the number of spots also changes. Each spots represent pure compounds.

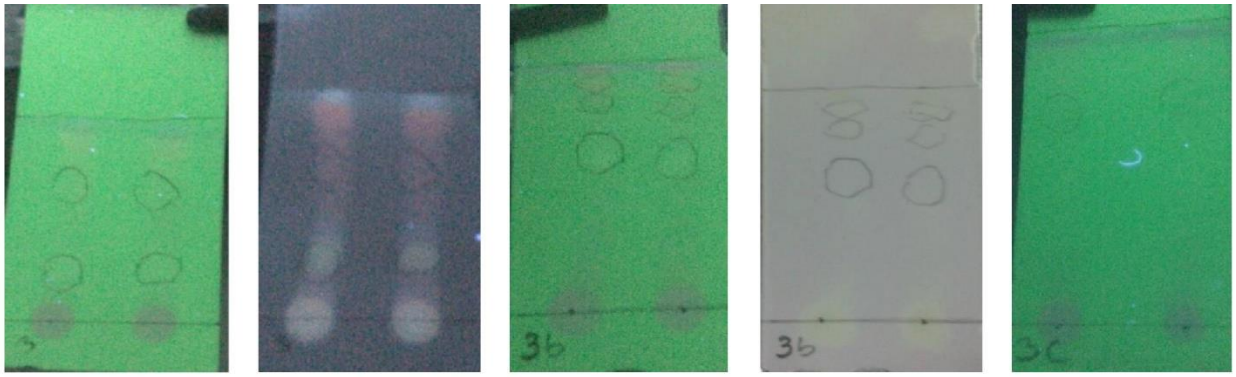

Figure 6: Results for TLC in 60% *n*-hexane and 40% ethyl acetate solvent system

Table 12: Calculation of Rf values of different spots of ethanol extract of *Solanum sisymbriifolium* using *n*-hexane (n-h) and ethyl acetate as mobile phase.

Solvent system	n-h 100%	n-h 90%	n-h 80%	n-h 70%	n-h 60%	n-h 50%	n-h 40%	n-h 30%	n-h 20%	n-h 10%	n-h 0%
Rf value	-	-	0.526 0.394	0.918	0.675 0.575	0.769	0.717	0.875	0.9	0.902	-

From the table 12 it has been found that, with the increase of polarity the R_f value has increased indicating that the separated compounds have more affinity for the comparative polar mobile phase.

Table 13: Calculation of Rf values of different spots of ethanol extract of *Solanum sisymbriifolium* using n-hexane (n-h) and dichloromethane as mobile phase

Solvent system	n-h 100%	n-h 90%	n-h 80%	n-h 70%	n-h 60%	n-h 50%	n-h 40%	n-h 30%	n-h 20%	n-h 10%	n-h 0%
Rf value	-	-	-	-	0.909	-	0.162	0.265	0.388 0.194	0.421 0.184	0.526 0.263

From the table 13 it has been found that, with the increase of polarity the R_f value has increased indicating that the separated compounds have more affinity for the comparative polar mobile phase. With the increase of polarity number of spots also increased.

4. Discussion

Solanum sisymbriifolium is a plant known for its bioactive secondary metabolites, including alkaloids, flavanoids, steroids, and reducing sugars[34]. These compounds are crucial for plant health and can be found in the ethanol extract of the plant. However, some secondary metabolites, such as saponins, tannins, terpenoids, cardiac glycosides, and anthraqunones, were absent in the ethanol extract. The presence of these compounds is believed to be responsible for the significant biological activities of *Solanum sisymbriifolium* leaf extracts, including antioxidant activities, anti-inflammatory effects, and antitumor effects[35].

Solanum sisymbriifolium, a plant with high flavonoids content, was determined using the aluminum chloride colorimetric method. The total flavonoid content in the plant's ethanolic extract was 266.34±33.22 catechin per mg. Flavonoids are crucial in antioxidant systems, scavenging free radicals, chelating metal ions, and inhibiting enzymes. The high flavonoids content in *Solanum sisymbriifolium* may explain its high radical scavenging activity[36].

Solanum sisymbriifolium, a plant known for its flavonoids, anthocyanins, and nonflavonoid phenolic compounds, was tested for its total phenolic content using Folin-Ciocalteau's method. The ethanol extract of *Solanum sisymbriifolium* was found to have a total phenolic content of 119.69±2.70 mg/g Gallic acid equivalent. This study highlights the importance of phenolic compounds in plant extracts, as they have been found to have antioxidant activity, acting as free radical scavengers, hydrogen donors, singlet oxygen quenchers, and metal ion chelators. The antioxidant activity of the plant extract is attributed to the presence of phenolic compounds[37].

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) is a widely used method to evaluate the free radical scavenging capacity of antioxidants. The ethanol extract of *Solanum sisymbriifolium* leaves showed low free radical scavenging activity, with an IC_{50} value of 27.87 mg/ml. The extract's antioxidant properties were compared to the standard (ascorbic acid) at 5.78 mg/ml. The extract contains alkaloids, flavonoids, phytosterols, tannins, and polyphenolic compounds, which are known to be free radical scavengers and super antioxidants. The free radical scavenging activity of the extract may be associated with the presence of these compounds, which have biological functions such as protection against allergies, inflammation, platelet aggregation, and ulcers[38].

The assay measures the reducing power of antioxidant samples by changing the yellow color of the test solution to green and blue. The presence of reductants, such as antioxidant substances, causes the reduction of

the Fe3+/ferricyanide complex to ferrous form. The reducing power ability of the ethanol extract of Solanum sisymbriifolium leaves is determined by plotting the absorbance of various concentrations. The absorbance increases with concentration, but after a certain point, it remains almost constant. Reducing power is associated with antioxidant activity and may reflect the antioxidant activity of compounds. Compounds with reducing power can act as primary and secondary antioxidants[39].

The study found that the separation of compounds increased with polarity, indicating a more affinity towards the mobile phase. The best solvent system for this plant extract was n-hexane 0% and 100% dichloromethane, which gave acceptable Rf values. The best ratios for n-hexane and ethyl acetate were n-hexane 80% and ethyl acetate 20%, n-hexane 60% and ethyl acetate 40%, n-hexane 50% and ethyl acetate 50%, and n-hexane 40% and ethyl acetate 60%. After applying reagents, the plant extract was observed under UV light, showing two spots. After treating with 2,2-diphenyl-1-picrylhydrazyl (DPPH), a pale yellow spot indicated antioxidant and phenolic compounds. Folin- ciocalteu was sprayed on a plate, showing a gray spot after treatment.

5. Conclusion

Medicinal plants offer a wide range of research possibilities, including the development of compounds beneficial to humans. *Solanum sisymbriifolium* leaves, traditionally used in disease management, have antioxidant activity. Further investigation is needed to isolate and characterize these compounds, as their phenolic and flavonoid content can assess their preventive role against free radicals. Further detailed phytochemical investigation is needed to use these plants effectively in disease prevention and treatment.

References

1. Andrew P. (2004). An introduction to the chemistry & therapeutics of herbal medicines.Journal of The Constituents of Medicinal Plants, 2: 155-165.
2. Arvigo R, Balick M, Rainforest Remedies, Lotus Press, Twin Lakes 1993.
3. Atanassova1 M, Georgieva S, Ivancheva K (2011) Total phenolic and total flavonoid content, antioxidant activity and biological contaminants in medicinal herbs. Journal of the University of Chemical Technology and Metallurgy 46(1):81-88
4. Banglapedia (2012). Traditional medicine. Accessed on 3rd May, 2013
5. Brand-Willams W., Cuvelier M. E., & Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft and Technologie, 28:25–30.
6. Burrieza HP, López-Fernández MP, Láinez V, Montenegro T, Maldonado S. On the nature and origin of the oxalate package in Solanum sisymbriifolium anthers. Protoplasma. 2010; 247(1-2): 45-56.
7. CESIO, M.V.; HEINZEN, H.; MONTEIRO DEBARROS, N. And MOYNA, P. Characterization and biological activity towards the soybean caterpillar Anticarsia gemmatallis of new sugar esters from Solanum sisymbrifolium (Lamark). In: IUPAC International Symposium on the Chemistry of Natural Products (22º, 3rd-8th September 2000, Sao Carlos, Brazil) Abstracts 2000 p. 173. Sao Carlos, Editorial de la UFSCAR, 2000
8. Chakravarty AK, Mukhopadhyay S, Saha S, Pakrashi SC. A neolignan and sterols in fruits of Solanum sisymbriifolium. Phytochemistry. 1996; 41(3): 935-939.
9. Da-Silva, J.F.M., De-Souza, M.C., Matta, S.R., De-Andrade, M.R. and Vidal, F.V.N. (2006) Correlation analysis between phenolic levels of brazilian propolis extracts and their antimicrobial and antioxidant activities. Food Chemistry, Volume 99, Pages 431-435.
10. Derlis A. Ibarrola, M. del Carmen Hellion-Ibarrola, Nelson L. Alvarenga, Esteban A. Ferro, Noboru Hatakeyama, and Katsuharu T. Cardiovascular Action of Nuatigenosido from Solanumsisymbriifolium. Pharm. Biol. 2006; 44 (5): 378-381.Evans WC and Somanabandhu A. nitrogen- containing non-steroidal secondary metabolites of Solanum, Cyphomandra, Lycianthes and Margaranthus. Phytochemistry. 1980; 19: 2351-2356.
11. Evans WC and Somanabandhu A. nitrogen-containing non-steroidal secondary metabolites of Solanum, Cyphomandra, Lycianthes and Margaranthus. Phytochemistry. 1980; 19: 2351-2356.

12. Farnsworth NR, Blowster RN, Darmratoski D, Meer WA, Cammarato LV. Studies on Catharanthus alkaloids IV evaluation by means of TLC and ceric ammonium sulphate spray reagent. Lloydia. 1967; 27: 302-314
13. Ferro EA, Alvarenga NL,Ibarrola DA, Hellión-Ibarrola MC, Ravelo AG. A new steroidal saponin from Solanum sisymbriifolium roots. Fitoterapia. 2005; 76(6): 577-9.
14. Ghani A. Medicinal plants of Bangladesh. Chemical constituents and uses. Dhaka: The Asiatic Society of Bangladesh; 2003, p. 500-508.
15. Harborne, Jeffrey B (2001) Twenty-five years of chemical ecology. Natural Products Reports 18(4):361-379.
16. Hoeven VD, Steffens JC (2000). Biosynthesis and elongation of short-and medium-chain-length fatty acids. Plant Physiology 122(1):275-282.
17. Goffreda JC, Mutschler MA; Ave DA, Tingey WM, Steffens JC (1989) Aphid deterrence by glucose esters in glandular trichome exudate of the wild tomato, Lycopersicon pennelli.Journal of Chemical Ecology 15(7):2135-2147.
18. Guerrero RO, Khan MTH, Casanas B, Morales M. Specific bioassays with selected plants of Bangladesh. Rev Cubana Plant Med. 2004; 9(2).
19. Jothy SL, Zuraini Z and Sasidharan S. (2011) Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitiory activities of Cassia fistula seeds extract. Journal of Medicinal Plants Research 5 (10): 1941-1947.
20. Juvik JA, Shapiro JA, Young TE, Mutschler MA (1994) Acylglucoses from wild tomatoes alter behavior and reduce growth and survival of Helicoverpa zea and Spodoptera exigua (Lepidoptera: Nostuidae). Journal of Economic Entomology 87(2):482-492
21. King RR, Calhoun LA, Boucher A, (1990) Sucrose esters associated with glandular trichomes of wild Lycopersicon species. Phytochemistry 29(7):2115-2118.
22. Korolkovas A. Essentials of medicinal chemistry. 2th edition. In: Wiley J, Sons. New York: A wiley-interscience publication; 1988. p. 67.
23. Odukoya OA, Sofidiya MO, Familoni OB, Inya-Agha SI. (2006) Free radical scavenging activity of some Nigerian medicinal plants. Thieme 4:1086-1093.
24. Mamone L, Di Venosa G, Valla JJ, Rodriguez L, Gándara L, Batlle A, Heinrich M, Juarranz A, Sanz-Rodriguez F, Casas A. Cytotoxic effects of Argentinean plant extracts on tumor and normal cell lines. Cell. Mol. Biol. 2011, 57: 1487-99
25. Mazumdar BC. Steroidal sapogenins in two wild species of Solanum. Science and culture. 1984; 50: 122-123.
26. Olckers T, Medal JC, Gandolfo DE (2002) Insect herbivores associated with species of SOLANUM(SOLANACEAE) in northeastern Argentina and southeastern Paraguay, with reference to biological control of weeds in South Africa and United States of America. Florida Entomologist 85(1):254-260.
27. Patrick GL, Spencer J. An introduction to medicinal chemistry. 4th edition. New York: Oxford University PrPCN Control Group. SA-LINK 112 Projects: Introducing Solanum sisymbriifolium as a trap crop for potato cyst nematodes in the UK. Nematode Interaction Unit at Rothamsted Research; 2004.
28. Pereira, J.A., Oliveira, I., Sousa, A., Valentao, P. and Andrade, P.B. (2007) Walnut (Juglans regia L.) leaves: Phenolic com-pounds, antibacterial activity and antioxidant potential of different cultivars. Food and Chemical Toxicology 45:2287-2295.
29. Sarla S, Prakash MA, Apeksha R, Subhash C. Free radical scavenging (DPPH) and ferric reducing ability (FRAP) of Aphanamixis polystachya (Wall) Parker. Int J Drug Dev Res 2011;3(4):271-274.
30. Shilpi JA, Rouf R., Sarker MAM, Uddin SJ.Antinociceptive activity of methanolic extract of
31. Solanum sisymbriifolium Lamk. Pak. J. Biol. Sci.. 2005; 8: 1123-1125.
32. Uddin, S.N., 2006. Traditional Uses of Ethnomedicinal Plants of the Chittagong Hill Tracts. Bangladesh National Herbarium, Dhaka, Bangladesh, 13(9843000007010): 992.
33. UNDP team. United Nations Human Development Report, Oxford University Press, 1999; 57- 72.
34. Usai, Marianna and Foddai, Marzia and Brunu, Antonello and Azara, Emanuela and Camarda, Ignazio (2008) Solanum sisymbrifoliumLamarck: esotica avventizia casuale di Sardegna: diffusione ed aspetti fitochimici. Natural 1, 8 (78): 22-26.
35. WHO. The World Health Report. Mental health: new understanding new hope. Geneva, 2001; pp. 1-15
36. Mahmud MS, Rahman MA, Islam MN, Islam S, Saha SK, Islam MT, Khatun A, Adhikari S. Reduction of Salmonella enterica & Staphylococcus aureus Biofilm Development on Glass Tube by Plant Extracts. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2023 Nov 8;12(4):122-38.
37. Yen, G.C. and Chen, H.Y. (1995) Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenici-ty. Journal of Agricultural and Food Chemistry 43(1):27-32.
38. Yusuf M, Chowdhury U, Wahab A, Begum J. medicinal plant of Bangladesh. Bangladesh Council of Scientific and Industrial ResearcH (BCSIR). 1994: 34.
39. Ainsworth EA, Gillespie KM. (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2 (4): 875-877.