In vivo and *in vitro* evaluation of pharmacological activities of *Solanum sisymbriifolium* fruit extract

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A thesis report, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled

"*In vitro* and *in vivo* evaluation of pharmacological activities of *Solanum sisymbriifolium* fruit extract" is an authentic and genuine research work carried out by me under the guidance of Mr. Apurba Sarker Apu, Senior lecturer, Department of Pharmacy, East West University, Dhaka.

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CERTIFICATE BY THE INVIGILATOR

This is to certify that the dissertation, entitled "*In vitro* and *in vivo* evaluation of pharmacological activities of *Solanum sisymbriifolium* fruit extract" is a bonafide research work done by Maima Matin, in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

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ENDORSEMENT BY THE CHIRPERSON

"*In vitro* and *in vivo* evaluation of pharmacological activities of *Solanum sisymbriifolium* fruit extract" is a bonafide research work done by Maima Matin, under the guidance of Mr. Apurba Sarker Apu, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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ACKNOWLEDGEMENT

I express my profound gratitude and deep respect to Mr. Apurba Sarker Apu, Senior Lecturer, Department of Pharmacy, East West University, for his expert and careful guidance, sincere help, constructive criticism, valuable time and honored suggestion without which I would not have been able to complete this work. He had been very enthusiastic and supportive in my research.

It is my great pleasure and privilege to acknowledge my deepest regards and gratitude to Dr. Sufia Islam, Chairperson and Associate Professor of the Department of Pharmacy, East West University, for her kind words during my troubling moments, and of course for her approval of the topic, constant inspiration and whole hearted cooperation.

I am also grateful to Mr. Apurba Sarker Apu, Senior Lecturer, Department of Pharmacy, East West University, for his professional helps which actually carved the path of our research.

I wish to thank my fellow researchers who had worked with me and special thanks to Monia Mohosina Moni, Shakhawat Hossan Bhuyan and Farah Ahmed for their endless cooperation and inspiration for preparing this work.

I also like to thank my family members for their support and inspiration to complete this work.

Above all, I express my gratitude to Almighty Allah for giving me the strength, energy and patients to carry out this research work.

Dedication

This Research paper is dedicated to
My beloved parents,
Who are my biggest Inspirations…

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ABSTRACT

The present study was carried out to investigate the possible *in vitro* antioxidant, thrombolytic and *in vivo* antidiarrheal, analgesic, cytotoxic and CNS depressant and anxiolytic activities of *Solanum sisymbriifolium* fruits. The analgesic, anti-diarrheal and CNS activities were investigated at the doses of 200 and 400 mg/kg body weight in mice. Analgesic potential of the extract was evaluated for peripheral activity using acetic acid-induced writhing test in which the extract produced a significant ($p < 0.001$) inhibition of writhing response in a dose dependant manner. The percentage of clot lysis for the extract was non-significant when compared to the control. The extract displayed scavenging of NO at the lowest IC_{50} value of 29.43µg/ml. The extract evidenced cytotoxic activity against brine shrimp nauplii and the calculated LC_{50} values were 32.12, 17.66, 103.75, 49.00 and 97.33µg/ml. The extract showed antidiarrheal activity on castor oil induced diarrhea in mice at dose 200 and 400mg/kg and showed a significant reduction in the no. of stool and total weight of fecal output. The extract did not have any effect on the anxiolytic behavior of mice in the Elevated plus maze test. The extract significantly displayed a dose dependent suppression of locomotor activity and exploratory behavior in mice that was carried out by Hole cross and Hole board test. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key Words: *Solanum sisymbriifolium,* antioxidant, thrombolytic, anti-diarrheal, analgesic, cytotoxic, CNS depressant, anxiolytic

Chapter: 1

INTRODUCTION

1. INTRODUCTION

Medicinal plants have been used as a source of medicine in all cultures during the last decades. It is gradually becoming popular throughout the world (Baquar, 1995). Secondary metabolites of plants play an important role in medical care for a good percentage of world population and have been the source of inspiration for several major pharmaceutical drugs. Around 100 plant species have contributed significantly to modern drugs. Approximately half of the worlds 25 best selling pharmaceutical agents are derived from natural products (Abalson, 1990). Thus, emphasis is now given on the standardization of herbal medicines by screening of biological activities of medicinal plants and isolation active principles from them.

1.1. Importance of natural products

The medicinal importance of the plants lies in some chemical substances that produce a distinct physiological action on the body of human. Natural product medicines have come from various sources of materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates. The importance of natural products in modern medicine has been discussed in many recent reviews and reports. The value of natural products in this regard can be assessed using 3 criteria: (1) the rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi-synthetic and total synthetic modification, (2) the number of diseases treated or prevented by these substances, and (3) their frequency of use in the treatment of disease (Newman *et al*., 1981)

Many indigenous plants are used in herbal medicine to cure and heal injuries. Some important chemical substances found in plants are alkaloids, carbon compounds, hydrogen, nitrogen,

glycosides, essential oils, fatty oils, resins, tannins, gums, and others (Pandey, 1980). Most of these are potent bioactive compounds found in medicinal plant parts that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora A, 1993). Alkaloids are very important in medicine and constitute most of the valuable drugs. They have marked physiological activity effect on animals (Edeoga *et al*., 2001). Alkaloids such as solasodine have been indicated as a starting material in the manufacture of steroidal drugs (Maxwell *et al*., 1995). Phenolic compounds are widely distributed in the plant kingdom and presence of phenols is considered to be potentially toxic to the growth and development of pathogen (Singh & Sawhney, 1998). Saponins are glycosides of both triterpenes and sterols and have been detected in over seventy families of plants (Basu & Rastogi, 1967). In medicine it is used to some extent of expectorant and emulsifying agents. Tannins are found in vegetable origin such as tea and many fruits. The oxidation inhibiting activity of tannins have been known for a long time and it is assumed to be due t the presence of gallic acid and digallic acids (Ihekoronye *et al.,* 1985). Flavanoids are 15-carbbon compounds generally distributed throughout the plant kingdom (Harbone J.B, 1998). Some isoflavones is used in insecticides. They may also play a role in disease resistance (Salisbury *et al.,* 1992)

Plants in all aspect of life have served as important material for drug development. Medicinal plants are the foundation of many important drugs of the modern world. Plants are now playing an important role in many medicines like allopathic medicine, herbal medicine, homoeopathy and aromatherapy. Many of these local medicinal plants are used as spices and food items. Many plants are cheaper and more simple to get to most people especially in the developing countries and these plants have lower incidence of side effect after use. Due to this reason they are used

worldwide. Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief and treating skin infections.

The purpose of the recent study is to identify and understand various types of researches which were performed on some plants. It was included phytochemical studies by which various compounds had been isolated from the plant by different researchers at different time period. It was also incorporated pharmacological studies that consisted of performing *in vivo* procedure to identify the biological properties of different extracts of the plant.

1.2. Plant information

Solanum sisymbriifolium is commonly known as Sticky Nightshade, the Fire-and-Ice plant, Litchi Tomato. The small edible fruits are red on the outside and yellow inside. It grows inside a husk which burst open when the fruit ripens. The flavor resembles sour cherries and a little bit like a tomato. This plant has been used as a trap crop to protect potatoes from potato cyst nematode. The stems and leaves contain solasodine which makes the plant very resistant to many pests and diseases, with the exception of potato beetles and tomato worms. It can also be used as a hedge plant to keep animals out of a garden, because it is covered with thorns.

1.2.1. **Common name:** wild tomato, dense thorn bitter apple, litchi tomato, fire and ice plant, kanta begun, dead sea apple, poison berry, sada kantikari, swetrangani etc.

1.2.2. **Taxonomic name:** *Solanum sisymbriifolium* Lam.

1.2.3. **Classification:** *Solanum sisymbriifolium* Lam.

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants

Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Asteridae*
Order	*Solanales*
Family	*Solanaceae* – Potato family
Genus	*Solanum* L – nightshade
Species	*Solanum sisymbriifolium* Lam. – sticky nightshade

1.3. **Botanical description:** *Solanum sisymbriifolium* is an annual or perennial erect, rhizomatous herb about 1 meter in height. The stem and branches are viscid, hairy, and armed with flat, orange-yellow spines up to 15mm in length. The 5-parted flowers are white, light blue, or mauve, about 3cm in diameter, and are subtended by a hairy calyx 5-6mm long. Red, succulent, globular berries are 12-20mm in diameter with pale yellow seeds 2.9-3.2mm long.

Figure: 1.1 *Solanum sisymbriifolium*

1.3.1. **Occurs in:** agricultural areas, disturbed, urban areas

1.3.2. **Fruits:** The small edible fruits of the plant are red on the outside and yellow inside. They grow inside a husk.

1.3.3. **Stems:** The stem and branches are viscid, and are armed with orange yellow spines.

1.3.4. **Leaves:** The leaves are pinnate, and are divided into 4-6 coarse lobes. Leaves are 40 cm long and are 25 cm wide.

1.3.5. **Flowers:** The five parted flowers are white and light blue in color, having a diameter of 3 cm.

Figure 1.2: Plant parts of *Solanum sisymbriifolium*

1.4. **Habitat**

S. sisymbriifolium is found along roadsides and in waste places, landfills, and ploughed fields both in its native South America as well as most of its non-native range. In Australia it is found in shrubby eucalypt woodlands. It is able to succeed in any type of soil and soil pH, but requires moisture and thrives in peat and sandy soils.

1.5. **Distribution***: S. sisymbriifolium* is distributed mainly in the native to the Mediterranean. The plant is native to South America and is currently distributed throughout the world. It is mainly cultivated in Baguio and Manila gardens and is propagated by seeds.

Figure 1.3: Distribution of *S. sisymbriifolium*

1.6. **Cultivation:** *S. sisymbriifolium* can grow anywhere without any care. The plant is resistant to any type of soil and soil pH. All it requires is moisture. It is tolerant of low-light situations and prefers sandy soils. Watering the plant is not required.

1.7. **Health benefits**

Only the fruits are edible. As these plants are grown in the roadsides, they are not so much beneficial for our health. The fruits are consumed by the indigenous birds and rarely by the human beings.

The fruit is a source of solasodine. Solasodine is a glycoalkaloid used in the amalgamation of corticosteroids and sex hormones. The mixture is also used in oral contraceptives.

1.8. Availability

These plants are seen all over the world and their population is increasing day by day. Fruits are picked up from the plants by people casually. These fruits are also sold in the market, especially in the fruit market. The market price is quite cheap.

1.9. Recipes

There are only in a few dishes where these wild tomatoes are used.

- Wild tomato Couscous
- Wild tomato pizza

1.10 Properties and constituents: Fruit of *Solanum sisymbriifolium* is deemed poisonous. A water soluble extract consists of 60-90% solamargine and solasonine, being studied as components in pharmaceutical compositions for inhibiting tumor growth in liver, breast and lung cancer.

Other Plant Chemicals includes (+)- solanocarpine, carpesterol, solanocarpidine, potassium nitrate, fatty acid, diosgenin, sitosterol, isochlorogenic acid, neochronogenic acid, chronogenic acid, caffeic acid, solasodine, solasonine, solamargine, quercetin, apigenin, histamine, acetylcholine.

1.11. Uses: Medicinal uses of *S.sisymbriifolium*

- cystitis
- dermatitis
- ringworn
- pleurisy
- sorethroat
- toothaches and
- infertility.

- hypertension
- fruit and leaves in oil or fat applied to skin rashes
- diuretic and antihypertensive properties.
- hypotensive activity

Solanum sisymbriifolium is best known for its use as a trap crop for potato cyst nematodes (PCN). Using *S. sisymbriifolium* in potato fields helps prevent the potato crop from being infested with PCN, and has been shown to reduce populations of PCN by 50–80%. S. sisymbriifolium is an excellent trap crop because it stimulates the hatching of juvenile PCN from their cysts by root diffusates, yet is completely resistant to infestation by the juveniles once they hatch, preventing reproduction of the pest. The species is also highly resistant to the nematodes Meloidogyne, Trichodorus, and Pratylenchus. Additionally, the roots of *S. sisymbriifolium* are resistant to a number of strains of the bacterica wilt pathogen Pseudomonas solanaceaum. The fruits of *S. sisymbriifolium* are edible and are consumed regularly by indigenous bird and infrequently by the Chorote Indians of Gran Chaco, Argentina. The fruit is also a source of solasodine, a glyco alkaloid used in the synthesis of corticosteroids and sex hormones, and a large component of oral contraceptives. *S. sisymbriifolium* is cultivated as an ornamental in Europe. Used for cystitis, dermatitis, ringworm, pleurisy, sore throat, toothaches and infertility. In the Congo**,** it is used for hypertension. In the Easter Cape, South Africa, poultice of fruit and leaves in oil or fat applied to skin rashes. In Paraguay, it is used for its diuretic and antihypertensive properties. Extract from the plant has been reported to exert hypotensive effects in rats. Nuatigenosido, isolated as one of the prospective active compounds, was shown to lower blood pressure and augment the contractile force in the right atrium.

1.12. Side effects

Some side effects of this vegetable are there that should be accounted.

- Daily consumption can make your digestive tract disturbed.
- Sometime can cause skin infection

1.13. Interesting facts

Being a roadside plant, there are some interesting facts of this species of vegetable.

- *Solanum sisymbriifolium* behaves like a weed.
- Plants of this species invade cattle pastures.
- *Solanum sisymbriifolium* germinates in 2-4 weeks.
- The species reproduce asexually by the growth of its rhizomes.
- The plants are self-incompatible.

1.14. Analgesic activity

To determine the antinociceptive activity following methods can be performed: Acetic acid-induced writhing (chemical stimulus) method, Thermal stimulus by Eddy's hot plate method, Tail flick test, Formalin test, Tail immersion test

The acetic acid induced writhing response is a well known procedure to evaluate peripherally acting analgesics. The test is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like tail flick test (Collier *et al*., 1968; Bentley *et al*., 1981). This invention provides a method of potentiating analgesia in mammals. This method is useful in using lower doses of acetic acid and required to produce

analgesia thereby resulting in fewer undesired side effects, such as physical dependence, tolerance, and respiratory depression.

Writhing, which is characterized by contraction of the abdominal musculature, extension of the hind legs, and rotation of the trunk, is induced in albino mice. The extent to which writhing is reduced following administration of a test compound is an indication of the analgesic activity of that compound (Hynes *et al*., 1982). Increased level of prostanoids, particularly PGE2 and PGF2α (Derardt *et al*., 1980) as well as lipoxygenase products (Levini *et al*., 1984; Dhara *et al*., 2000) have been found in the peritoneal fluid after intraperitoneal injection of acetic acid.

Acetic acid-induced writhing model signifies pain sensation by triggering localized inflammatory response. Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Such pain initiates the release of free arachidonic acid from tissue phospholipids via cyclooxygenase (COX) (Ahmed *et al*., 2001). The response is thought to be reconciled by peritoneal mast cells (Ronaldo *et al*., 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain *et al*., 2006). So, the abdominal constrictions produced after administration of acetic acid is linked to sensitization of nociceptive receptors to prostaglandins. The agents reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis. (Zulfiker *et al*., 2010).

1.15. Thrombolytic activity

Since ancient times, herbal preparations have been used for the treatment of several diseases. The leaves and or twigs, stem, bark and underground parts of plants are most often used for traditional medicines (Gesler, 1992). Considerable efforts have been directed towards the

discovery and development of natural products from various plant and animals which have anti platelet, anticoagulant, antithrombotic, and thrombolytic activity (Demro & Briggs 1995, 2001). This method was done to investigate whether extract from plants possess thrombolytic activity or not.

One of the major causes of blood circulation problem is the formation of blood clots. Thrombo embolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks are the main causes of morbidity and mortality in developed countries. Thrombi can lodge in a blood vessel and block the flow of blood in that location depriving tissues of normal blood flow and oxygen. This can result in damage, destruction or even death of the tissues (necrosis) in that area. Atherothrombotic diseases such as myocardial or cerebral infarction are also serious consequences of the thrombus formed in blood vessels (Thrombus, 2011).

Coagulation or blood clotting involves a complex sequence of steps leading to the conversion of circulating fibrinogen into the insoluble protein fibrin. As the fibrin network grows, it covers the surface of the platelet plug. Passing blood cells and additional platelets are trapped in the fibrous tangle, forming a blood clot that seals off the damaged portion of the vessel.

A blood clot is formed from fibrinogen by thrombin and is lysed by plasmin, which is activated from plasminogen by two enzymes: thrombin, produced by the common pathway, and by tissue plasminogen activator, released by damaged tissue at the site of injury. The activation of plasminogen produces the enzyme plasmin which begins digesting the fibrin strands and eroding the foundation of clot. This process is known as fibrinolysis. Fibrinolytic drugs has been used to dissolve thrombi in acutely occluded coronary arteries thereby to restore blood supply to ischaemic myocardium, to limit necrosis and to improve prognosis (D.R. Laurence, 1992)

Thrombolytic agents such as alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) are used to dissolve clots. All available thrombolytic agents still have significant shortcomings. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs (Nicolini FA *et al*., 1992; Adams DS *et al*., 1991; Lijnen HR *et al*., 1991; Marder VJ, 1993; Wu DH *et al*., 2001).

Streptokinase is a thrombolytic agent used for the treatment of acute myocardial infarction. It reduces mortality as effectively as the nonantigenic alteplase in most infarct patients while having the advantages of being much less expensive. Streptokinase is a protein extracted from cultures of streptococci. It activates plasminogen. Its action is blocked by antibodies which appear about 4days or more after the initial dose. It forms a complex with plasminogen which then converts plasminogen to plasmin. Plasmin breaks down clots as well as fibrinogen and other plasma proteins (Banerjee *et al*., 2004).

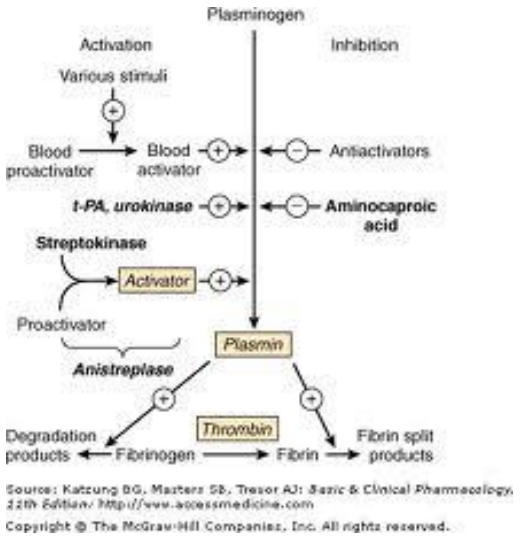


Figure 1.4: Mechanism of anticoagulation of streptokinase

1.16. Anti-oxidant activity

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. Generally, human beings live in a highly oxidative environment and many processes involved in metabolism may result in the production of more oxidants (Rui and Boyer, 2004). It has been estimated that there are more than ten thousand oxidative hits to DNA per cell per day, causes membrane protein damage, a decrease in membrane fluidity and DNA mutation which can further initiate or propagate the developments of many diseases such as cancer, liver injury and cardiovascular disease in humans (Ames *et al*., 1985). For protection, against free radicals, organisms are endowed with endogenous (catalase, superoxide dismutase, glutathione peroxidase/reductase) and exogenous (vitamins C and E, carotene, uric acid) defense systems.

Antioxidants are compounds that inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions. They can interfere with the oxidation process by reacting with fre radicals, chelating metals and also by acting as oxygen scavenger. Antioxidants are radical scavengers may have great revelance to protect the human body against these free radicals. They are of interest for the treatment of many kinds of cellular degeneration. Free radical also induces liver damage. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods require special equipment and technical skills for the analysis. The different types of methods published in the literature for the determinations of antioxidant activity involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the nitric oxide scavenging test, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O2), the hydroxyl radical (OH), or the peroxyl radical (ROO). The

various methods used to measure antioxidant activity of food products can give varying results depending on the specific free radical being used as a reactant. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assays have been used extensively since the 1950's to estimate the peroxidation of lipids in membrane and biological systems. These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix etc. and may not be practical when large numbers of samples are involved. Antioxidant activity methods using free radical traps are relatively straightforward to perform. The ABTS [2,2'- azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics.

A rapid, simple and inexpensive scavenging method to measure antioxidant capacity involves the use of the free radical, nitric oxide test (O'Brian *et al*., 1998). Nitric oxide is implicated for inflammation, cancer and other pathological conditions. Nitric oxide was generated from sodium nitroprusside, which at physiological pH liberates nitric acid. This nitric acid gets converted to nitrous acid and further forms nitrite ions which diazotize with sulphanilic acid and couple with naphthylethylenediamine (Griess reagent), producing pink color which can be measured at 546 nm (Marococci *et al.,* 1994). Hence nitric oxide scavenging capacity may help to arrest the chain of reactions initiated by excess generation of nitric oxide that are detrimental to the human health (Moncada *et al*., 1991).

1.17. Brine shrimp lethality test

Brine shrimp lethality bioassay technique has been applied for the determination of general toxic properties of the plant extracts (Meyer *et al.,* 1982 & McLaughlin *et al.,* 1998). The aim of this study is to use traditional plant drugs for primary threatening diseases like cancer by using rapid screening bioassay. In order to study the toxicity of these medicinal plants brine shrimp lethality bioassay was based on the ability to kill the laboratory cultured brine shrimp. Artemia or brine shrimp is living in salt pane which is more tolerable to any environmental changes. So, while applying the testing compound, if mortality is high even at low dosage means the tested compounds contains highly active principles. The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of toxins, plant extract toxicity, heavy metals, pesticides, and cytotoxicity of dental materials (Harwing *et al*., 1971). The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts. The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the micro well scale (Sam, 1993)

1.18. Anti- diarrheal activity

Diarrhea is characterized by increased frequency of bowel movement, wet stool and abdominal pain. It is a leading cause of malnutrition and death among children in developing countries of the world today. Worldwide distribution of diarrhea accounts for more than 5-8 million deaths each year in infants and children. Many synthetic chemicals like loperamide, antibiotics are available for the treatment of diarrhea but they have some side effects. A range of medicinal plants with anti-diarrheal properties is widely used by traditional healers because these herbs and

plants are readily available, affordable and are an indispensable component of traditional medicine practice.

Anti-diarrheal activity of the extracts can be tested by using the model of castor oil induced diarrhea in mice. Time taken before the first defecation was the 'Latent period'. The total count of stool and latent period of test group can be compared with positive or negative control group. Anti-diarrheal agent would increase the latent period and decrease total stool count.

Castor oil induced diarrhea model is widely used for the evaluation of anti diarrheal property of drugs. Ricinoleic acid, the active metabolites of ricinoleic acid which is present in castor oil is responsible for the diarrhea inducing property of castor oil (Gaginella and Philips, 1975). It stimulates peristaltic activity of the small intestine leading to a change in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the motility and secretion (Pierce *et al*., 1971, Galvez *et al*., 1993).

1.19. CNS depressant activity

To determine CNS depressant activity subsequent experiments such as hole cross, hole board, elevated plus maze, open field test, beam walking test, thiopental sodium induced sedative tests can be performed.

Brain function and nervous system are the most important aspects of physiology that defines the differences between human and other species. Disorders of brain function and nervous system due to improper balance in neurotransmitter levels are a major concern of human society and a field in which pharmacological intervention plays a key role (Katzung, 2004).

Epilepsy, psychosis, insomnia, Parkinsonism, depression, and anxiety are some common clinically important CNS disorders. Among these, anxiety, depressions are the areas of interest in the study. Numerous synthetic CNS depressant drugs are available in the market for the management, control and/or treatment of patients. However most of these synthetic drugs are not only inaccessible and unaffordable, but many of them possess side effects. It is therefore necessary to look inward for the development of inexpensive, effectual and secure CNS depressant agents from plants and other natural resources.

1.20. Hole cross test

To check the neuropharmacological effects or side effects of drugs, hole cross test can be carried out. The purpose of the hole cross method is to determine the stimulatory or depressive effect of test drug. The locomotor activity is a test to appraise the level of excitability of the CNS (Mansur *et al*., 1980) and any decrease of this activity may be narrowly related to sedation resulting from depression of the central nervous system (Ozturk *et al*., 1996). The sedative activity may be related to the interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been defined in certain plants extracts. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS. Different anxiolytic, muscle relaxant and sedative- hypnotic drugs, elucidate their action through GABA. Therefore it is possible that plant extracts may act by potentiating GABAergic inhibition in the CNS via membrane hyper polarization which leads to the decrease in the firing rate of critical neurons in the brain or may be direct activation of GABA receptor by the extract (Kolawole *et al*., 2007) Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the CNS, which led to

the assumption that they can act as benzodiazepines like molecules and produce the activity (Verma *et al*., 2010)

1.21. Hole board

The hole board experiment is a measure of exploratory behavior in animals (File & Wardil, 1975) to an unfamiliar environment and is an accepted parameter for evaluating anxiety, emotionality, and/or responses to stress conditions in animals (Crawley, 1985). Exploration can be defined as a board of behavior, the consequences of which are to provide the organism with information about the environment.

The aim of this test is that a novel situation of open field evokes in the animals a pattern of behavior characterized by exploration (head dipping through holes), locomotion (ambulation past the holes) and emotional defecation. It has been considered that exploration evoked under an unfamiliar environment is modified with physiological factors such a curosity, fear, anxiety and the modulation of these factors after the administration of drugs (Nakama *et al*., 1972)

1.22. Elevated plus maze

Animal tests of anxiety are used to screen for novel compounds for anxiolytic or anxiogenic activity, to investigate the neurobiology of anxiety, and to assess the impact of other occurrences such as exposure to predator odors or early rearing experiences. There is a diversity of animal models of anxiety currently available. These behavioral models involve exposure of animals to stimuli that appear capable of causing anxiety in humans.

Anxiety is an unpleasant feeling of apprehension or fearful concern. It is both a normal emotion and a psychiatric disorder. It is a normal response to stress and only becomes a clinical problem

only if the anxiety becomes severe or persistent, and interferes with everyday performance. A number of pharmacological theories exist which suggest that anxiety is caused by either amine or excitatory amino acid function and anxiolytics have therefore, been developed to target specific brain neurotransmitter systems.

The elevated plus-maze (EPM) is the most popular of all currently available animal models of anxiety, and affords an excellent example of a model based on the study of unconditioned or spontaneous behavior (Carobrez *et al.*, 2005; File, 1992; Handley *et al.*, 1993; Rodgers *et al.*, 1997). It is a simple method for assessing anxiety responses by mice or rodents. The assessment of anxiety behavior of mice can be done by using the ratio of time spent on the open arms to the time spent on the closed arms. Entry into an arm was defined as the animal placing all four paws into the arm. EPM relies upon mice proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces (avoidance) (Walf *et al.*, 2007). However, the focus of this study using the EPM was to assess the anxiolytic effect of the plant extract. Anxiety related behaviors such as rearing, grooming, stretch attend postures, defecation etc can be measured. Protected stretch attend postures were defined as the mouse stretching forward and retracting without moving forward its feet whilst in the closed arm or central platform of the maze whereas unprotected stretch-attend postures were defined as the mouse exhibiting this behavior whilst in the open arms. Rearing can be characterized by the number of times the animal stood on its hind limbs. Self grooming is the number of times the animal scratched various parts of its body. Therefore it can be concluded that this EPM model is an useful model for measuring anxiety and can be assumed that the time spend by the mice in the illuminated side of the box is most useful and consistent parameter of anxiety.

1.23. Purpose of the research

The main objective of the present study are-

- To conduct analgesic effects of fruit of *Solanum sisymbriifolium* by Acetic acid induced test
- To determine pharmacological effects of fruit of *Solanum sisymbriifolium* by Thrombolytic test
- To investigate antioxidant property of fruit of *Solanum sisymbriifolium* by Nitric oxide scavenging test
- To conduct cytotoxic investigation of fruit of *Solanum sissymbriifolium* by Brine shrimp lethality bioassay
- To find out the anti-diarrheal activity of fruit of *Solanum sisymbriifolium* by Castor oil induced method
- To determine neuropharmacological effects of fruit of *Solanum sisymbriifolium* by Hole cross, Hole board and Elevated plus maze test.

Chapter: 2

LITERATURE REVIEW

2. LITERATURE REVIEW

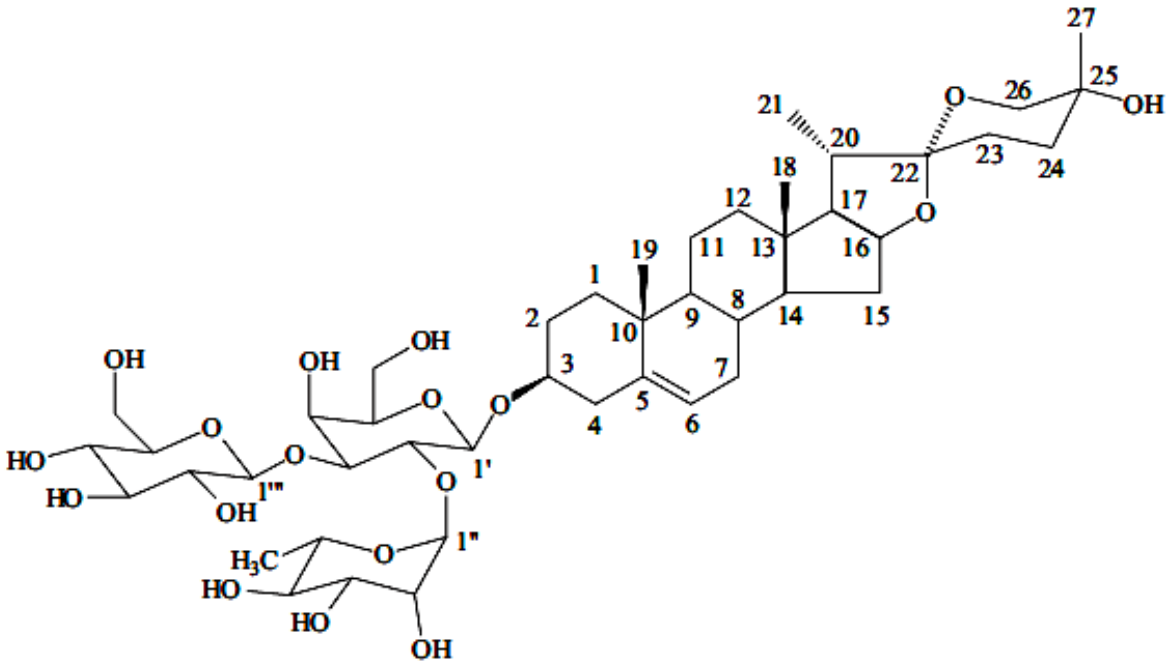
Phytochemical Studies

A neolignan and sterols in fruits of *Solanum sisymbriifolium*

A new neolignan, designated as sisymbrifolin and carpesterol, a rare C_{30} sterol, together with *β*-sitosterol and its *β*-D-glucoside, were isolated from the berries of *Solanum sisymbriifolium* (Chakravarty *et al.*, 1996).

A new steroidal saponin from *Solanum sisymbriifolium* roots.

Isonuatigenin-3-*O*-β-solatriose was isolated from the roots of *Solanum sisymbriifolium*. Its structure was determined by spectroscopic method (Ferro *et al.*, 2005).



Isonuatigenin-3-*O*-β-solatriose

Antinociceptive activity of methanolic extract of *Solanum sisymbriifolium* Lamk.

Phytochemical investigation of the methanolic extract of the whole plant of *Solanum sisymbriifolium* indicated the presence of alkaloid, flavonoids, steroid and tannin (Shilpi *et al.*, 2005).

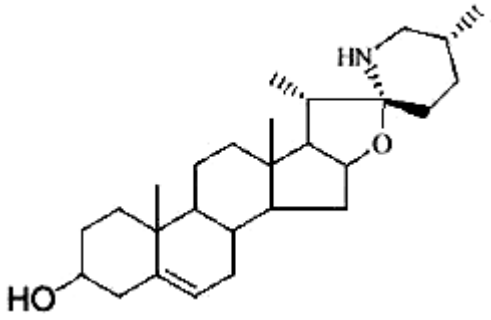
***Table 2.1*:** Summary of the Phytochemical Studies on *S. sisymbriifolium*

Part of the plant	Findings	References
Berries	Isolation of sisymbrifolin (a new lignin) and carpesterol (a C_{30} sterol)	Chakravarty *et al.*, 1996
Roots	Isolation of Isonuatigenin-3-*O*-β-solatriose	Ferro *et al.*, 2005
Whole plant	Presence of alkaloid, flavonoids, steroid and tannin	Shilpi *et al.*, 2005

Pharmacological Studies

Anticonvulsant activity of solasodine isolated from *Solanum sisymbriifolium* fruits in rodents.

Solasodine, a poisonous steroidal glycoalkaloid chemical compound, was isolated from the dried fruits of *Solanum sisymbriifolium* showed potent anticonvulsant and CNS depressant activities (Chauhan *et al.*, 2010).



Solasodine

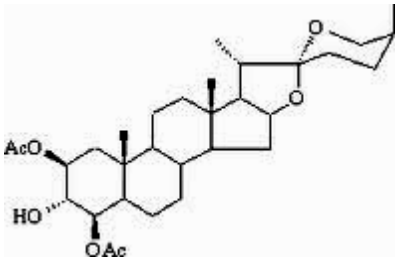
Isolation of hypotensive compounds from *Solanum sisymbriifolium* Lam.

The pharmacological action of fractionation of the crude hydroalcoholic root extract of *Solanum sisymbriifolium* Lam. was examined both in anaesthetized normotensive rats (lbarrola *et al.*, 2000). Depression of locomotion, increase of breathing rate, increase in the gastrointestinal transit and piloerection was observed in anaesthetized normotensive rats. Hexanic and butanolic fractions induced remarkable hypotension activities in anaesthetized normotensive rats. Two compounds isolated from the butanolic fraction induced a significant decrease of the blood pressure, heart rate, amplitude of the ECG and breathing rate both systofic and diastolic, blood pressures were affected in a proportional mode (lbarrola *et al.*, 2000).

Antihypertensive effect of nuatigenin-3-O-β-chacotriose from *Solanum sisymbriifolium* Lam. (Solanaceae) in experimentally hypertensive (ARH+DOCA) rats under chronic administration.

The hydro-ethanolic crude root extract, the n-butanol fraction and nuatigenin-3-O-[beta]-chacotriose, from *Solatium sisymbriifolium* Lam., was assessed in adrenal regeneration hypertension + deoxycorticosterone acetate (ARH+DOCA) rats, following a chronic administration. The oral administration showed a significant reduction of blood pressure value

was induced in hypertensive animals (ARH+DOCA) in comparison to the control group receiving 1% saline solution (Ibarrola *et al.*, 2011). The latest demonstrated that the blood pressure-lowering effect, in clearly hypertensive animals, is reversible and depend upon administration of nuatigenin-3-0-[beta]-chacotriose [(B.sub.3-1]) (Ibarrola *et al.*, 2011).



Nuatigenin-3-O-β-chacotriose

On the nature and origin of the oxalate package in *Solanum sisymbriifolium* anthers. A detailed study was carried out in *Solanum sisymbriifolium* Lam. on the development of the circular cell cluster during crystal deposition, as well as the composition of the crystals (Burrieza *et al.*, 2010). TEM analysis revealed that the crystals originated simultaneously within the vacuoles in association with a paracrystalline protein. Prior to the appearance of protein within vacuoles, protein paracrystals were visible in both rough endoplasmic reticulum and vesicles with ribosomes on their membranes (Burrieza *et al.*, 2010). EDAX revealed that C, O, and Ca were the main elements, and K, Cl, Mg, P, S, and Si, the minor elements. X-ray powder diffraction of crystals detected the predominant presence of calcium oxalate, but also vestiges of calcite, quartz, and sylvite. The calcium oxalate coexisted in the three chemical forms, that is, whewellite, weddellite, and caoxite. Infrared spectrophotometry identified bands that characterize O-C-O, H-O, C-H bonds, all of calcium oxalate, and Si-O-Si, of quartz (Burrieza *et al.*, 2010).

Cardiovascular Action of Nuatigenosido from *Solanum sisymbriifolium*.

The pharmacological actions of nuatigenoside, isolated from the root of the herb *Solanum sisymbriifolium*. Lam (Solanceae), on cardiac system was investigated in rats (Derlis *et al.*, 2006). The study demonstrated the following results: Nuatigenosido lowered blood pressure in rats, augmented the contractile force in the right atrium of a bullfrog, increased the overshoot amplitude in frog atrial myocytes, action potential durations were shortened, the calcium current (I_{Ca}) was increased, and the delayed outward potassium current was increased (Derlis *et al.*, 2006).

Molluscicidal activity of some Brazilian Solanum spp. (Solanaceae) against Biomphalaria glabrata.

Methanolic extracts of 13 Solanum species was tested for molluscicidal activity against *Biomphalaria glabrata*. According to the investigation the extract of fruits and aerial parts of *Solanum sisymbriifolium* showed significant positive molluscicidal activity (Silva *et al.*, 2005).

Cytotoxic effects of Argentinean plant extracts on tumour and normal cell lines.

A study was carried out to investigate the effects of 75 aqueous and methanol extracts from 41 Argentinean plant species on tumour and normal cell lines. From the study methanolic extract of flower of *Solanum* sisymbriifolium were found highly cytotoxic. It inhibited cell growth in a concentration-dependent manner (Mamone *et al.*, 2011).

***Table 2.2*:** Summary of the pharmacological Studies on *S. sisymbriifolium.*

Part of	Findings	References

the plant		
Root	hypotensive activity in anaesthetized normotensive rats	Lbarrola *et al.*, 2000
Whole plant	significant positive molluscicidal activity	Silva *et al.*, 2005
Root	lowered blood pressure, augmented the contractile force in the right atrium, increased the overshoot amplitude, shorten action potential durations	Derlis *et al.*, 2006
Dried fruits	potent anticonvulsant and CNS depressant activities.	Chauhan *et al.*, 2010
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
Root	reduction of blood pressure value was induced in hypertensive animals	Lbarrola *et al.*, 2011
Whole plant	methanolic extract of flower of *S. sisymbriifolium* were found highly cytotoxic	Mamone *et al.*, 2011

Chapter: 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Extraction procedure: During extraction procedure of the experimental plant, following apparatus and solvents were used.

3.1.1 Collection of plant and Identification

Solanum sisymbriifolium was collected from Aftab Nagar, Dhaka, Bangladesh in the month of August, 2011, and the collected plant was identified from Bangladesh National Herbarium of Mirpur, Dhaka on 19th September 2011 where a voucher specimen was conserved under the Accession Number DACB 35894.

3.1.2 Drying, grinding and extraction of fruit

The collected fruit and flower was sun dried. The dried leaves were coarsely powdered by the blender machine to get a homogenized fine powder and stored in air tight container. The amount of powder obtained was 584 gm. In this research, cold extraction process was followed to isolate the ethanol soluble compounds from the fruit. For this purpose, 584gm of powder was transferred to a 1000mL conical flask, and soaked in ethanol for 7 days. The conical flask was properly sealed with aluminum foil for the prevention of evaporation of volatile solvents. Throughout the days the mixture was shaken properly for more interaction between the powdered particles and the solvent. The mixture was then filtered using a filter paper (Double Rings 102- 11cm, Germany). By using the rotary evaporator (IKA, Germany) the filtrate which were soluble in ethanol were poured into 1000 ml round bottomed flask (Borosil, Japan) and were concentrated at low temperature of 45˚C and RPM of 120 to make it dry. When evaporation

was completed, di-ethyl-ether was used for collecting the extract. The dried crude extract was then collected in a beaker, tagged and labeled properly for further use.

Figure 3.1: Rotary evaporator

3.1.3 Solvent-solvent partitioning using Kupchan method

Kupchan and Tsou solvent-solvent partitioning

Ethanolic crude extract (20 g)

↓

Ethanol (90 ml) + water (10 ml)

Aqueous Ethanolic solution

↓

Extraction with *n*-Hexane (100 × 3)

↓

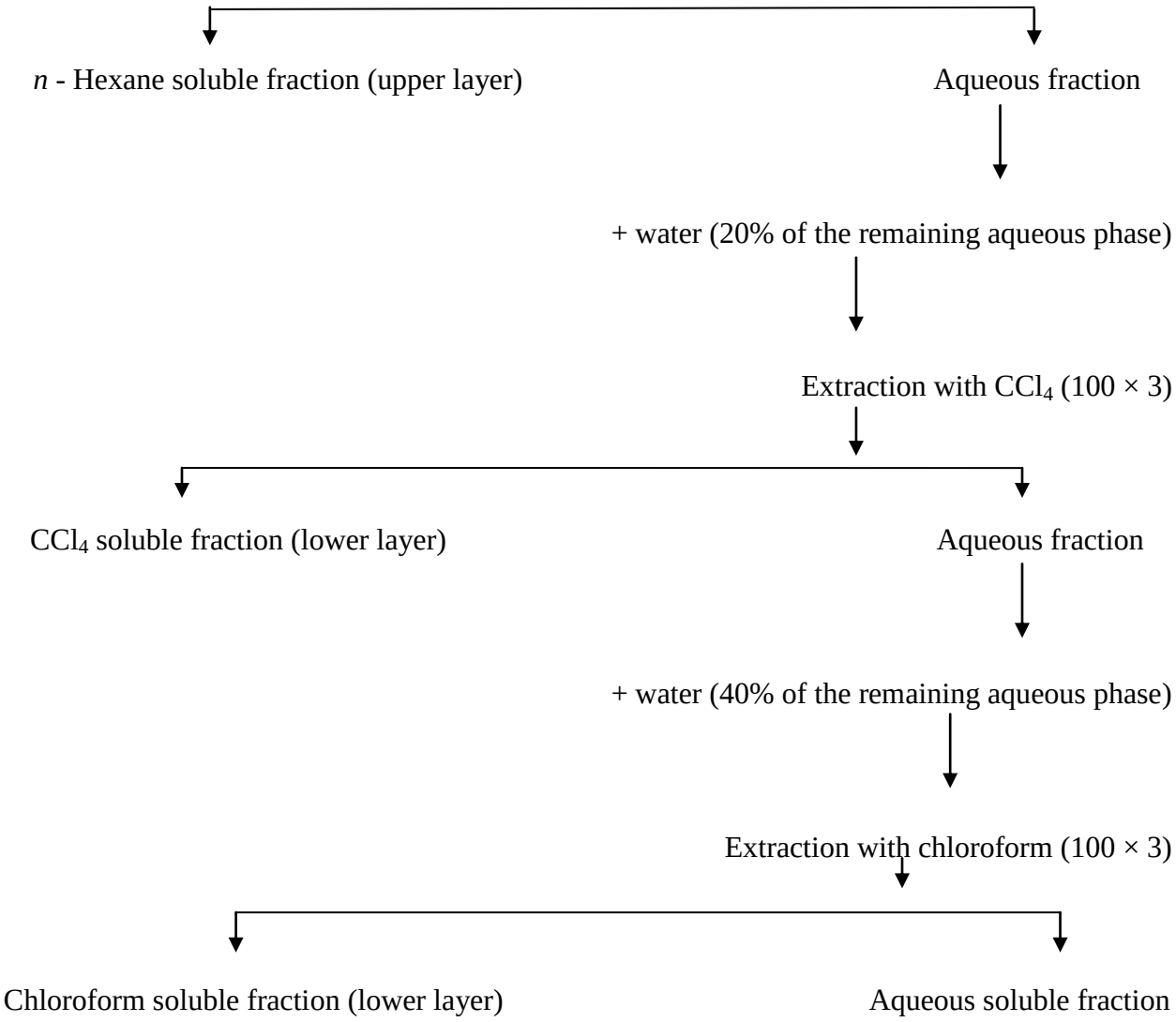


Figure 3.2: Schematic diagram of the method of Fractionation of the ethanol extract

10 gm of ethanolic crude extract of fruit and flower were dissolved in 90mL of ethanol and 10mL of water in a 100mL volumetric flask. The mixture was shaken properly and was transferred to a separating funnel which was clamped by a stand. 100mL n-hexane solvent was measured using a measuring cylinder which was transferred to the clamped separating funnel containing the extract solution. Mild shaking was done. Sometime was given to allow for good separation. Another similar separating funnel was clamped on the stand. Distil water was poured

to the separating funnel to a level of the first funnel which indicates the volume of solution present in the first funnel. The tap was opened to flow the water and the volume of water was measured using a measuring cylinder. Due to low density of n-hexane (0.66g/mL), compounds which were dissolved or soluble in n-hexane were collected from top of the first separating funnel. The volume got was labeled as volume 1. 20% of the volume 1 was calculated to give the value for the volume of water added next. The procedure was repeated for triplicate. The procedure was conducted using other solvents like carbon tetrachloride, chloroform and aqueous. Due to density difference with that of n-hexane, compounds which were soluble in carbon tetrachloride (1.587g/ml) and chloroform (1.483g/ml) were collected from the lower layer of the funnel. For carbon tetrachloride, 100 ml of it were poured in the funnel and were done the same described earlier. The volume got was labeled as volume 2 and 40% of the volume 2 was calculated to give the amount of water added for the next. The experiment was again done for thrice. The process was done thrice similarly with chloroform. After chloroform were collected from lower layer, the last portion left would be aqueous solution which were collected, tagged, and labeled for further use.

3.1.4 Selection of solvent

Ethanol, carbon tetrachloride, chloroform, n-hexane were used for the extraction of *Solanum sisymbriifolium* according to their polarity index.

3.1.5 Properties of the solvents used

Solvent	Molecular weight (g/mol)	Boiling point ˚C	Density (g/ml)	Solubility (g/l)	Polarity

Ethanol	46.07	78.3	0.7851	100	5.2
Carbon tetrachloride	153.82	76.62	1.587	0.785-0.8	1.6
Chloroform	119.38	61.2	1.483	0.815	4.1
n-Hexane	86.18	68.7	0.66	0.0013	0.1

3.1.6 Apparatus and reagents used for plant extraction

Reagents	**Equipments**	**Apparatus**
Acetone (Merck, Germany)	Electric balance (SHIMADZU AY220 & SCALTEC SPB31)	Conical flask 1000 ml
Ethanol (Merck, Germany)	Rotary evaporator (IKA, Germany)	Separating funnel
n-hexane (Merck, Germany)	Blender (Miyako, Japan)	Measuring cylinder
Carbon tetrachloride (Merck, Germany)		Funnel
Chloroform(Merck, Germany)		Beaker
Diethylether(Merck, Germany)		

3.2. Animal

For the experiment Swiss albino mice of either sex of 1-2 weeks of age were collected from the animal research branch of the international center for diarrheal disease and research, Bangladesh (ICDDRB). Animals were maintained under standard environmental conditions (temperature: (23.0 ± 2.0˚), relative humidity: 55 - 65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum.

3.3. Apparatus and Reagents used for writhing test

Reagents	Equipments	Apparatus
0.7% aqueous solution of acetic acid (COO, Germany)	Electronic-balance (SHIMADZU AY220 & SCALTEC SPB31)	Feeding needle
Diclofenac sodium		Injection needle
Normal saline with 1% tween 80		Syringe
		Timer
		mice case

3.3.1. Acetic acid induced writhing test in animal model

3.3.2. Method:

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice. In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. Mice were divided into 4 groups of each case containing five in number. The extract solutions of dose 200mg/kg and 400mg/kg were administered to the test groups. Negative control group received normal saline with 1% tween 80 (0.5ml/mice) and the positive group was treated with diclofenac sodium (10mg/kg) before the intraperitoneal (IP) injection of 0.7% acetic acid was given. After 30 minutes of the administration, 0.7% acetic acid was

induced by intraperitoneal injection to each of the mice. Five minutes after the injection of acetic acid, mice were individually placed in the case to count the number of writhes (painful muscular contraction) or stretching response for a period of 20 minutes. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The mean number of writhes and the percentage inhibition were calculated and the experimental groups were then compared with the control groups.

3.4 Apparatus and Reagents used for *in vitro* thrombolytic activity test

Reagents	**Equipments**	**Apparatus**
Normal saline	Incubater	Eppendorf tubes
Streptokinase(15,00,000 I.U/Ml)		Sterile syringe
		Syringe needle
		Counter.

3.4.1. *In vitro* thrombolytic activity test:

3.4.2. Blood sample

Whole blood (n=10) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 500µl of blood was transferred to the previously weighed eppendorf tubes and was allowed to form clots.

3.4.3. Preparation of stock solution:

5mL normal saline was added to streptokinase vial and was mixed properly.

3.4.4. Procedure:

The thrombolytic activity of the extract was evaluated by using streptokinase as the standard. 3mL venous blood was withdrawn from the human healthy volunteers who have not taken anticoagulant therapy or oral contraceptive for two weeks. Empty eppendorf tubes were weighed as W. 500µL of fresh blood were transferred to the pre weighed eppendorf tubes (500µL/tube) and incubated at 37˚C for 45 minutes for the formation of clot. After the clot has formed, the pale yellow plasma fluid or serum was completely removed by the syringe without the disruption of clot. The tube with the removed serum having the clot was again weighed as W'.

Clot weight= weight of clot containing tube – weight of tube alone

The eppendorf tubes containing clot was properly labeled. 100µL of the ethanolic extract solution was added to the tubes containing pre weighed clot. For positive control, 100µL of streptokinase (1500000 I.U/ml) and for negative control 100µL of saline were added to the tubes. All the tubes were incubated at 37˚C for 90 minutes and observed for the lysis of clot. The tubes were taken out from the incubator after the incubation has completed and the fluid released were withdrawn or removed. Each tubes were again weighed as W" to see the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

$$\% \text{ of clot lysis} = (\text{wt of released clot} / \text{clot wt}) \times 100$$

Clot lysis was expressed as percentage. % of clot lysis can be calculated by a paired T-test analysis or ANOVA.

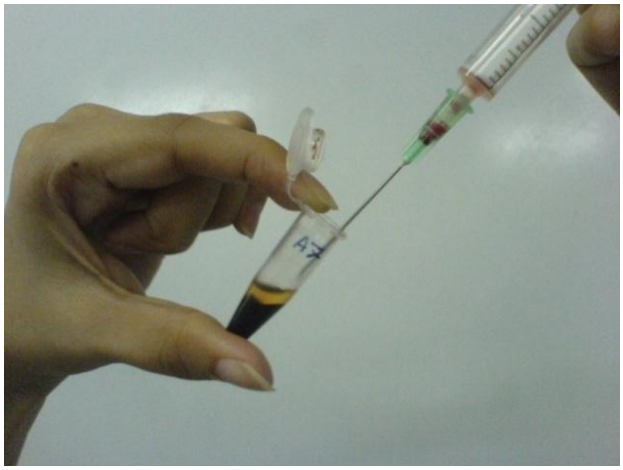


Figure 3.3: Clot withdrawing

3.5 Apparatus and Reagents used in nitric oxide scavenging capacity test

Reagents	**Equipments**	**Apparatus**
Sulfanilamide	Electronic-balance (SHIMADZU AY220 & SCALTEC SPB31)	Volumetric flask 50 ml
Griess reagent	Spectrophotometer	Test tubes
Sodium nitroprusside solution		Test tube rack
Distilled water		

3.5.1 Nitric oxide scavenging capacity assay:

3.5.2 Preparation of 5mM sodium nitroprusside:

5mM solution of sodium nitroprusside for 50 ml was prepared by weighing 0.075 gm of nitroprusside and was dissolved in 50 ml distilled water. The absorbance of this solution was taken by using a spectrophotometer at 546 nm against distilled water as a blank.

3.5.3 Preparation Of Griess Reagent Solution:

0.5 gm of sulfanilamide, 1 ml *o*-phosphoric acid and 0.05 gm of napthyl ethylene diamine di-hydrochloride were weighed in a 50 ml volumetric flask and were dissolved in distilled water upto 50 ml.

3.5.4 Preparation of stock solution

0.006 gm of ethanolic extract was dissolved in 30 ml ethanol.

3.5.5 Procedure:

Nitric oxide radical scavenging activity was measured by Griess reagent. Nitric oxide was generated from sodium nitroprusside, which at physiological pH liberates nitric acid. Five test tubes were taken. Each test tube was labeled as 1, 2, 3, 4, 5 respectively. In the first test tube 10 ml of stock solution was added and concentration of it was 200 µg/ml. 5 ml of stock solution and 5 ml of distilled water were added to the second tube to make the concentration half that is, 100 µg/ml. By dilution, concentration of 50, 30, 10 µg/ml were made to the rest of the three test tubes containing 2.5, 1.5, 0.5 ml of the extract solutions. From each of the tube, 4 ml of the solution were taken and 1 ml of sodium nitroprusside was added and incubated at 30˚C for 2 hours. After incubation, 2 ml solution from each test tube was mixed with 1.2 ml of Griess Reagent. Ascorbic acid was used as a positive control. The absorbancies of the solutions were measured at 546 nm by using spectrophotometer against blank.

3.6 Apparatus and Reagents used for brine shrimp test

Reagents	Equipments	Apparatus
Pure sodium chloride	Electronic-Balance (SHIMADZU AY220 & SCALTEC SPB31)	Measuring cylinder
DMSO (Merck, Germany)		1000 ml beaker
		Vials 5 ml
		Aquarium air pump (SB2488, Sovo)
		Table lamp

3.6.1 Brine shrimp hatching

3.6.2 Procedure

For hatching the brine shrimp nauplii for assay, sea water should be prepared first. 38 gm of pure NaCl were weighed in a 1000 ml beaker and dissolved in distilled water adjusting the volume upto 1000 ml to prepare the sea water. 1 or 2 drops of NaOH solution of 1N were added in the beaker by a dropper to make the pH of the solution 8.4. pH was measured by the pH meter. 0.25 gm of dry eggs of *Artemia salina Leach* was added in the sea water. Oxygen supply was given by an air pump and a table lamp was kept beside the preparation for 24 hrs to ensure survival and

maturity by obtaining the red colored freely swimming nauplii in the sea water. The freshly free swimming nauplii were used for the bioassay.

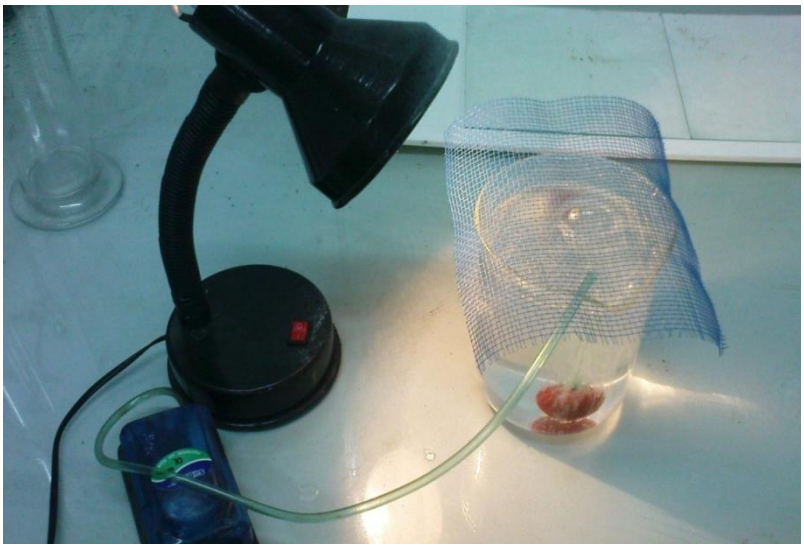


Figure 3.4: Brine shrimp hatching

3.6.3 Preparation of extract solution

0.008 gm of ethanolic solvent fruit extract (crude) and the fractions of n-hexane, carbon tetrachloride , chloroform, aqueous of the crude were weighed in separate vials. Every vial was labeled properly. Then the extracts were dissolved in 400µL of Dimethyl Sulfoxide (DMSO). The mixture was shaken properly until dissolved. 200 µL of the solution was discarded. 4.5 ml of sea water was added in each vial to make the final concentration of the solution 400µg/ml and total volume 5 ml. The extract solutions were then ready for bioassay.

3.6.4 Bioassay procedure

In vitro lethality bioassay of ethanolic extract of *S.sisymbriifolium* was exploited to detect cytotoxicity test. 50 vials were taken and washed thoroughly. 2.5 ml of water was filled in the

vial by using a pipette and the water level was marked. The water was then removed from the vial. All the vials were kept in hot air oven at 50˚C for 20 minutes allowing it to dry. Then the vials were cleaned with acetone and left it for air dried. All the vials were tagged properly according to the concentrations used. Therefore a series of total varying of 10 concentrations were prepared by using serial dilution method. The total concentrations were 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812 µg/ml. After preparing the vials, 10 naupliis were collected from the freshly hatched nauplii using Pasteur pipette and added to all the vials. The sea water level was adjusted till the marked level that is, 2.5 ml. The rest was filled with crude extract solution using 2.5 ml of syringe. In the first pre-marked vial, 2.5 ml of the ethanolic extract solution (400µg/ml) was added from the stock solution. The level of the stock solution was then adjusted by using another fresh syringe with 2.5 ml of sea water. The concentration of the stock solution was 200µg/ml. All the vials were filled in this manner until series of solutions of lower concentration 0.7812 µg/ml were prepared by serial dilution. The vials were kept for 24 hrs under the table lamp to get the result of lethality assay. Each tube containing the nauplii was examined and the surviving nauplii were properly counted. From this, the percentage of mortality was calculated at each concentration.

3.7 Apparatus and Reagents used in anti-diarrheal test

Reagents	**Apparatus**
Castor oil	Mice case
Normal saline 1% tween 80	Counter
Loperamide	Filter paper

3.7.1 Castor oil induced anti-diarrheal activity test

3.7.2 Procedure:

The experimental mice were fasted for 24 hrs before the test with free access to water. The mice were then randomly selected and divided into four groups, each groups containing 5 mice. Of the experimental groups, group-I which was the negative control, received 0.5 ml of 1% tween 80 solution orally. Group-II or positive control received 0.5 ml of the anti-diarrheal drug Loperamide at the dose of 2 mg/kg body weight as oral suspension. The test groups were administered orally with 0.5 ml of ethanolic extract solution at the dose of 200 mg/kg and 400 mg/kg body weight. After 30 minutes, 0.2 ml of castor oil were administered to all the experimental mice. Each mouse was then placed in separate beaker having fresh absorbent filter paper beneath it. Then presence of diarrhea was examined every three hour in three hours duration after the administration of castor oil. Parameters that were to check was, onset of stool, number of wet stool, weight of dry and wet stool and total weight of fecal output. Number of stools that stained the adsorbent paper was counted successively at every hour while conducting the experiment. At every hour, a new filter paper was replaced by the old ones.

3.8 Apparatus and Reagents used in CNS activity test

Reagents	**Apparatus**
Normal saline 1% tween 80	Hole cross instrument
Diazepam	Hole board
	Elevated plus maze

	Feeding needle
	Tissue paper
	Counter

3.8.1 Determination of CNS activity test

3.8.2 Hole cross test:

20 mice were chosen for the experiment. 10 mice were taken as the control groups and the 10 mice were taken for the test groups. A wooden box on which the experiment was carried out was partitioned in the middle having a size of 30×20×14 cm. A hole of 3 cm diameter was made at the center of the box. Negative control group were fed with 0.5 ml of normal saline of 1% tween 80 and the positive were given 0.5 ml of diazepam at the dose of 2 mg/kg. 0.5 ml of ethanolic extract solution at the dose of 200 and 400mg/kg were given to the test groups. Each mouse was placed in the box to observe the spontaneous movement from one chamber to another over a period of 3 minutes. The observation was conducted for 0, 30, 60, 120, 240 minutes.

3.8.3 Hole board test:

The test was conducted to see the emotional behavior of the mice. The experiment was carried out using 20 mice. 10 mice were for test groups and 10 mice were for control groups. Negative control group were fed with 0.5 ml of normal saline of 1% tween 80 and the positive were given 0.5 ml of diazepam at the dose of 2 mg/kg. 0.5 ml of ethanolic extract solution at the dose of 200 and 400mg/kg were given to the test groups. Each of the mice was placed at the center of the board and the number of head dipped by each of the mouse was observed for a period of 5 minutes after 30 minutes of feeding.

3.8.4 Elevated plus maze:

Anxiolytic activity was evaluated using elevated plus maze. Elevated plus maze consisted of two arms crossed with two enclosed arms and two open arms. The arms were connected together with a central square from the floor. The whole set up of the apparatus were placed in a dimly illuminated room. 10 mice were used as test groups which were fed with extract solutions at the dose of 200 and 400mg/kg body weight and the positive control were administered with 0.5 ml of diazepam suspension at the dose of 2 mg/kg and negative control were fed with normal saline 1% tween 80 solution. After 0 minute of feeding individual mice were placed at the centre of the maze facing towards the centre of the open arm. Parameters that were noted are as follows:

1. number of entries and time spent in the open and closed arms,
2. number and duration of rearing,
3. number and duration of grooming and
4. number and duration of stretch attend postures in duration of 5 minutes.

Chapter: 4

RESULTS

4.1. Analgesic activity

Table 4.1: Tabular presentation of the study of acetic acid induced writhing in mice

Group	Dose	No. of writhing	% inhibition
Control (1% tween 80 in saline)	0.5ml/mice; p.o.	78.7±0.22	----
Positive control (Diclofenac sodium)	10mg/kg body weight; p.o.	1.5±0.16**	98.09
SSF 200	200mg/kg body weight, p.o.	15.7±0.41**	80.03
SSF 400	400mg/kg body weight; p.o.	10.5±0.39**	86.64

Values are expressed as Mean±SEM (n=5); **: p <0.001 dunnett t-test as compared to negative control.

Group	Mean	Standard deviation	Standard error mean	95% confidence interval for mean	
				Lower bound	Upper bound
Control	78.72	0.56	0.25	78.02	79.42
Positive	1.50	0.35	0.16	1.06	1.94
SSF 200	15.70	0.91	0.41	14.57	16.83
SSF 400	10.50	0.87	0.39	9.42	11.58

In the acetic-acid induced writhing test, ethanoilc fruit extract of *Solanum sisymbriifolium* demonstrated a statistically significant (p<0.001) analgesic effect at both the dose of 200 and 400mg/kg body weight, inhibiting pain by 80.03% and 86.64% respectively in a dose dependent manner when compared to the control.

4.2 Thrombolytic activity

4.2. Tabular presentation of the study result of *in vitro* Thrombolytic activity

Table 4.2.1: Thrombolysis data of *Solanum sisymbriifolium* fruit extract

No	Wt of clot	Wt of clot (After Applied Sample)	Wt. loss after administration	% Clot lysis	Average of % clot lysis	Standard Deviations
1	0.612	0.591	0.021	3.384		
2	0.428	0.406	0.022	5.232		
3	0.540	0.523	0.016	2.984		
4	0.501	0.470	0.031	6.225		
5	0.370	0.348	0.022	5.952	4.846	1.539
6	0.354	0.329	0.024	6.844		
7	0.282	0.269	0.013	4.720		
8	0.271	0.254	0.017	6.285		
9	0.238	0.233	0.006	2.349		
10	0.252	0.241	0.011	4.484		

Table 4.2.2: Thrombolysis data of positive control (Streptokinase)

No	Wt of clot	Wt of clot (After Applied Sample)	Wt. loss after administration	% Clot lysis	Average of % clot lysis	Standard Deviations
1	0.550	0.308	0.242	43.988		

2	0.498	0.317	0.182	36.431		
3	0.330	0.207	0.123	37.152		
4	0.392	0.213	0.178	45.533		
5	0.356	0.213	0.143	40.146	40.755	5.141
6	0.248	0.127	0.121	48.768		
7	0.247	0.133	0.114	46.007		
8	0.264	0.162	0.103	38.811		
9	0.252	0.155	0.097	38.413		
10	0.348	0.236	0.112	32.299		

Table 4.2.3: Thrombolysis data of negative control (Normal saline)

No	**Wt of clot**	**Wt of clot (After Applied Sample)**	**Wt. loss after administration**	**% Clot lysis**	**Average of % clot lysis**	**Standard Deviations**
1	0.487	0.458	0.029	5.892		
2	0.521	0.498	0.023	4.430		
3	0.394	0.381	0.013	3.398		
4	0.347	0.332	0.015	4.211		
5	0.467	0.443	0.024	5.181	4.712	0.889
6	0.381	0.362	0.020	5.142		
7	0.266	0.254	0.012	4.508		

8	0.260	0.244	0.016	6.147
9	0.250	0.240	0.009	3.647
10	0.239	0.228	0.011	4.559

Group	Mean	Standard deviation	Standard error mean	95% confidence interval for mean	
				Lower bound	Upper bound
Control	4.71	0.89	0.28	4.08	5.35
Positive	40.75	5.14	1.63	37.08	44.43
SSF	4.85	1.54	0.49	3.74	5.95

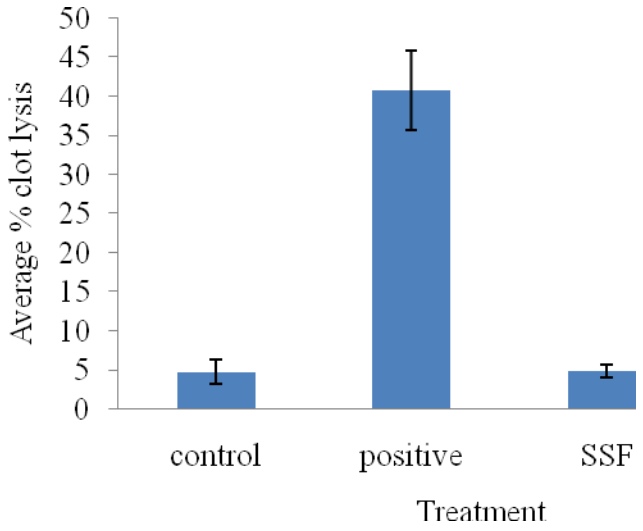


Figure 4.1: Clot lysis of blood samples of normal subjects by extract of *S.sisymbriifolium* fruit, streptokinase and normal saline

When streptokinase (100µl) was added to the clots, maximum clot lysis was visually seen. *In vitro* thrombolytic activity study revealed that ethanolic extract of *S.sisymbriifolium* fruit showed

4.846% clot lysis in average when compared to the negative control. The mean clot lysis was not statistically significant.

4.3 Anti-oxidant activity

4.3 Nitric Oxide Free Radical Scavenging of fruit of *S. sisymbriifolium*

Table 4.3.1: IC_{50} value of ascorbic acid (AA)

Test tube no.	**Conc.(µg/ml)**	**Absorbance of AA**	**Absorbance of blank**	**% inhibition**	**Value of IC_{50}**
1	0	0.096	0.096	0.00	
2	5	0.049	0.096	48.96	
3	25	0.034	0.096	64.58	
4	50	0.026	0.096	72.92	34.06
5	100	0.021	0.096	78.13	
6	200	0.015	0.096	84.38	

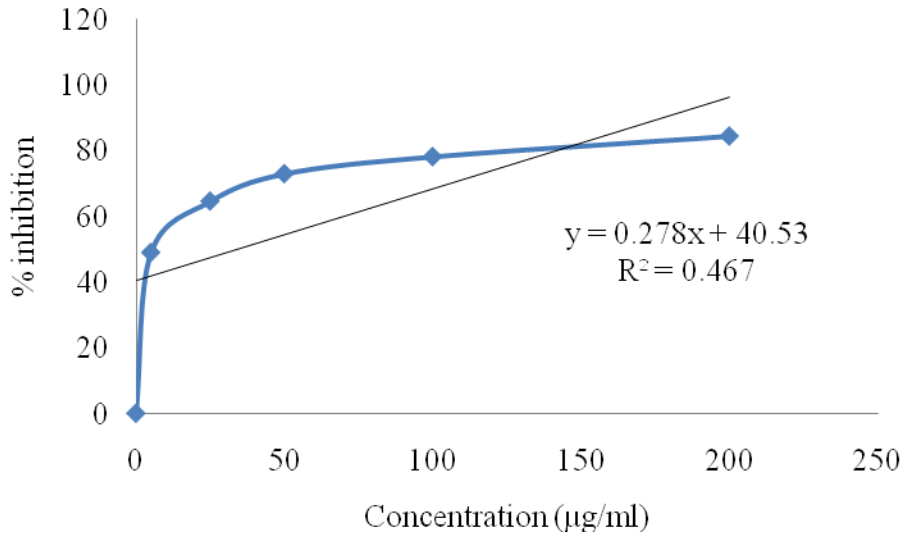


Figure 4.2: Scavenging effect of the Ascorbic acid

Table 4.3.2: IC_{50} value of the SSF

Test tube no.	**Conc.(µg/ml)**	**Absorbance of Sample**	**Absorbance of blank**	**% inhibition**	**Value of IC_{50}**
1	0	0.396	0.396	0.00	
2	5	0.260	0.396	34.34	
3	25	0.171	0.396	56.82	
4	50	0.096	0.396	75.76	43.83
5	100	0.088	0.396	77.78	
6	200	0.014	0.396	96.46	

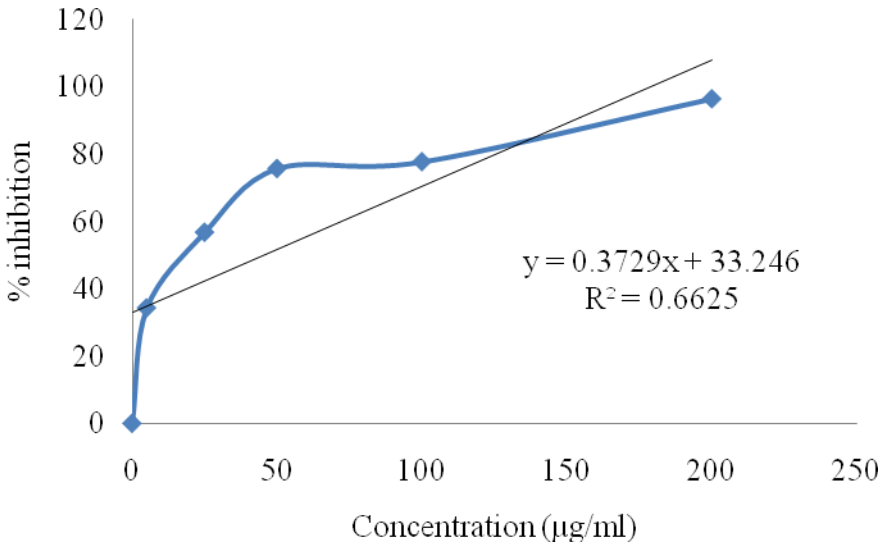


Figure 4.3: Scavenging effect of the SSF

Table 4.3.3: IC_{50} value of the SSF

Test tube	**Conc.(µg/ml)**	**Absorbance of Sample**	**Absorbance of**	**%**	**Value of**

no.			blank	inhibition	IC_{50}
1	0	0.396	0.396	0.00	
2	5	0.221	0.396	44.19	
3	25	0.133	0.396	66.41	
4	50	0.081	0.396	79.55	29.43
5	100	0.062	0.396	84.34	
6	200	0.038	0.396	90.40	

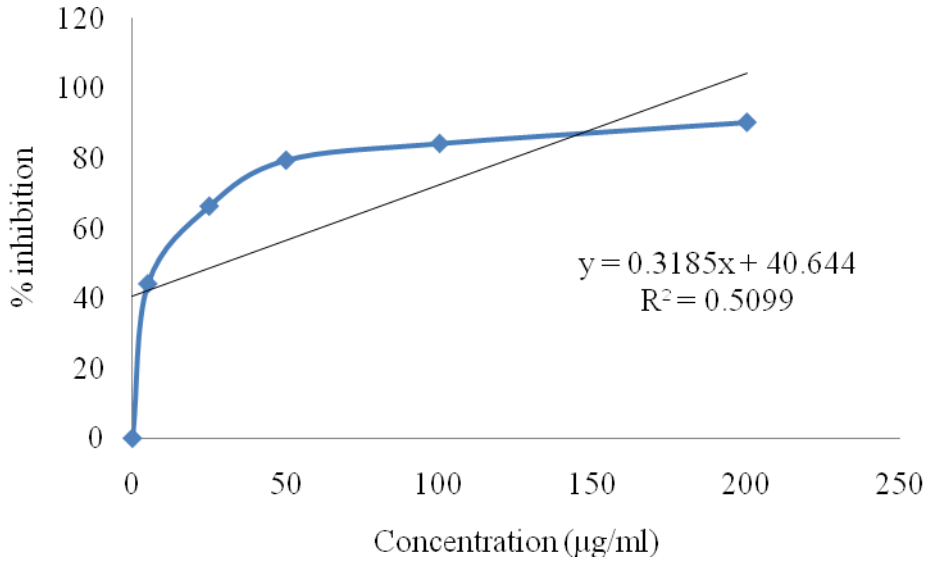


Figure 4.4: Scavenging effect of the SSF

Table 4.3.4: Average % inhibition of *S. sisymbriifolium* extract

Concentration	% inhibition	% inhibition	Average % inhibition
0	**0**	**0**	0
5.00	34.34	44.19	39.27
25.00	56.82	66.41	61.62
50.00	75.76	79.55	77.65

100.00	77.78	84.34	81.06
200.00	96.46	90.40	93.43

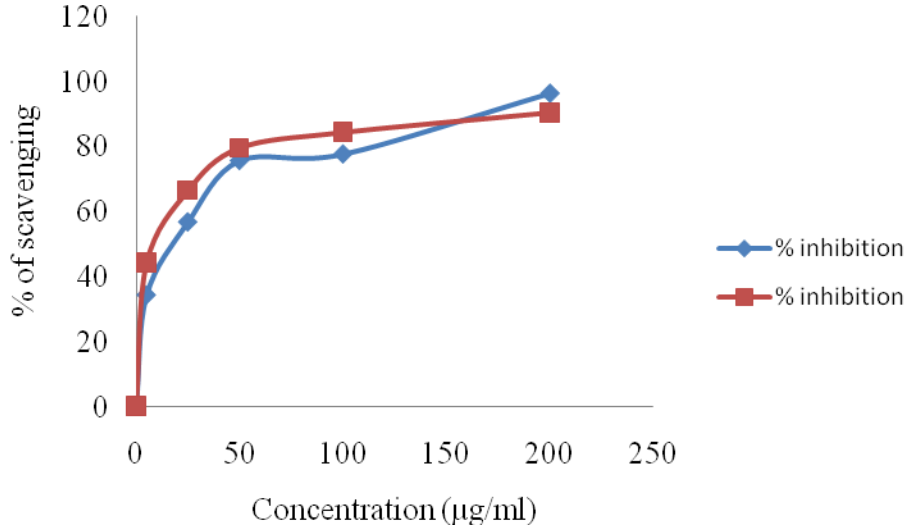


Figure 4.5: NO scavenging activity of AA and the ethanolic extract of *S.sisymbriifolium* fruit

Ethanolic fruit extract of *S.sisymbriifolium* showed the IC_{50} value of 29.43µg/ml whereas ascorbic acid had IC_{50} value 34.06µg/ml. The table (4.3.1) indicates the presence of potential candidates of antioxidant compounds. It appears that SSF has greater free radical scavenging capacity as it has lowest value of IC_{50} 29.43µg/ml.

4.4 Brine shrimp lethality test

Table 4.4: Effect on Brine shrimp lethality test

Test Compound	**LC_{50} (µg/ml)**	**Best Fit Equation**	**R^2**
$KMnO_4$	11.898	y = 5.181x - 0.572	0.851
SSF (ethanol only)	32.12	y = 5.093x - 2.674	0.823
SSF (*n*-hexane fraction)	17.66	y = 2.627x + 1.724	0.844

SSF (chloroform fraction)	103.75	y = 3.816x - 2.693	0.817
SSF (carbon tetrachloride fraction)	49.00	y = 2.482x + 0.805	0.751
SSF (aqueous fraction)	97.33	y = 3.659x - 2.275	0.754

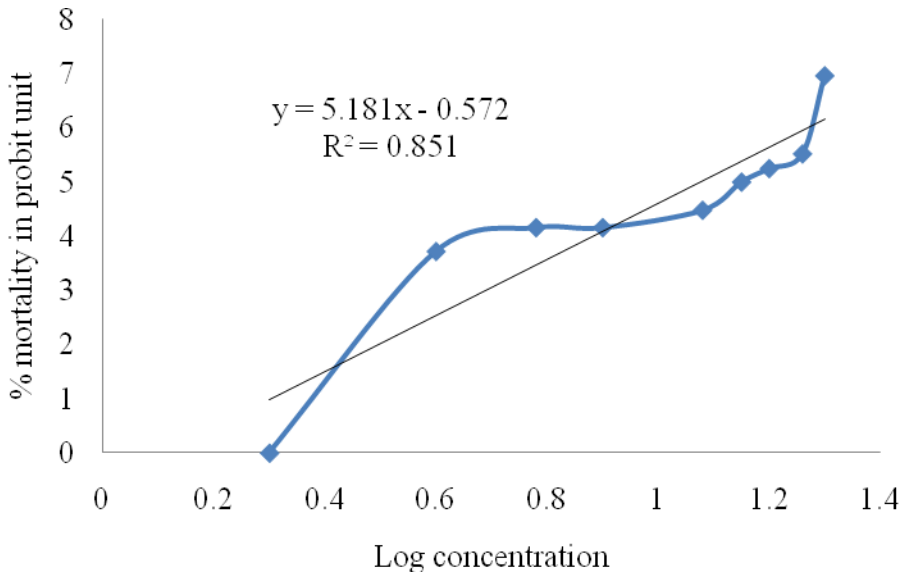


Figure 4.6: Effect of Potassium permanganate on brine shrimp nauplii

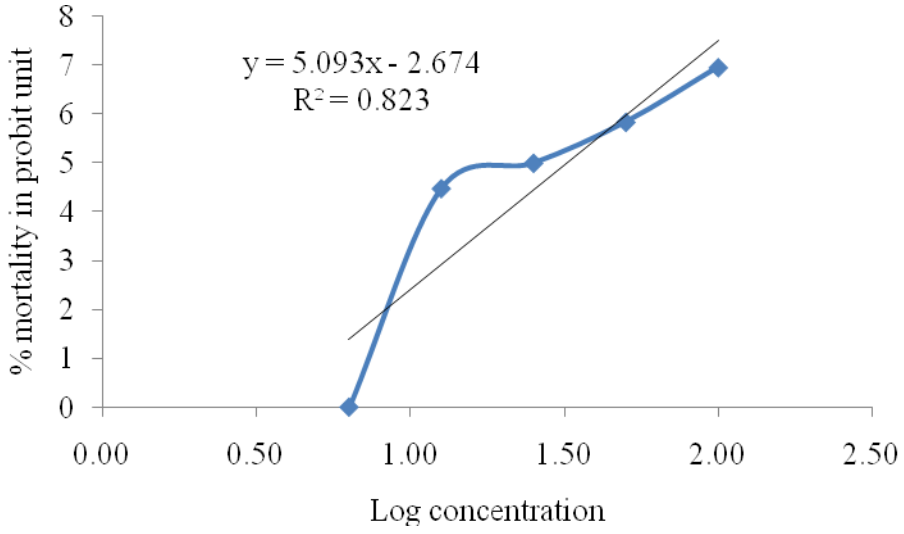


Figure 4.7: Effect of *S.sisymbriifolium* fruit Ethanol extract on brine shrimp nauplii

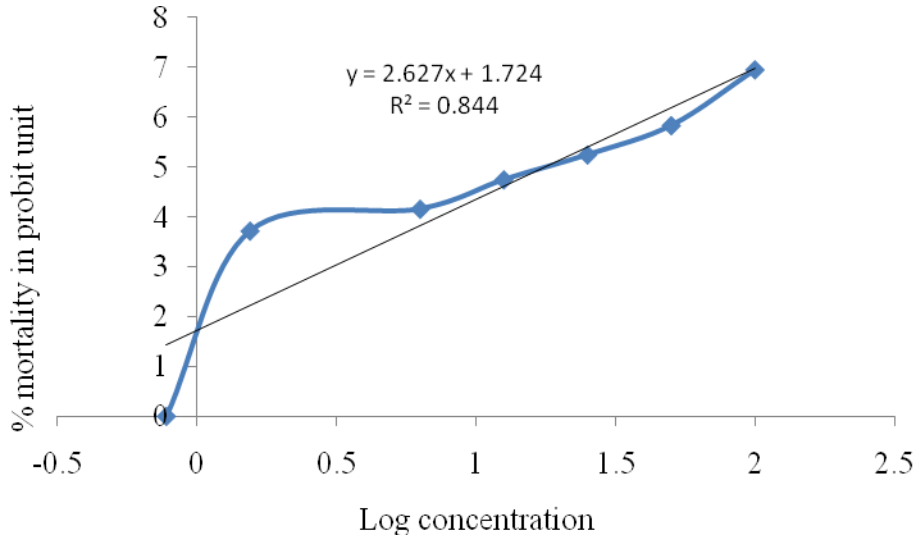


Figure 4.8: Effect of *S. sisymbriifolium* fruit *n*-hexane fraction extract on brine shrimp nauplii

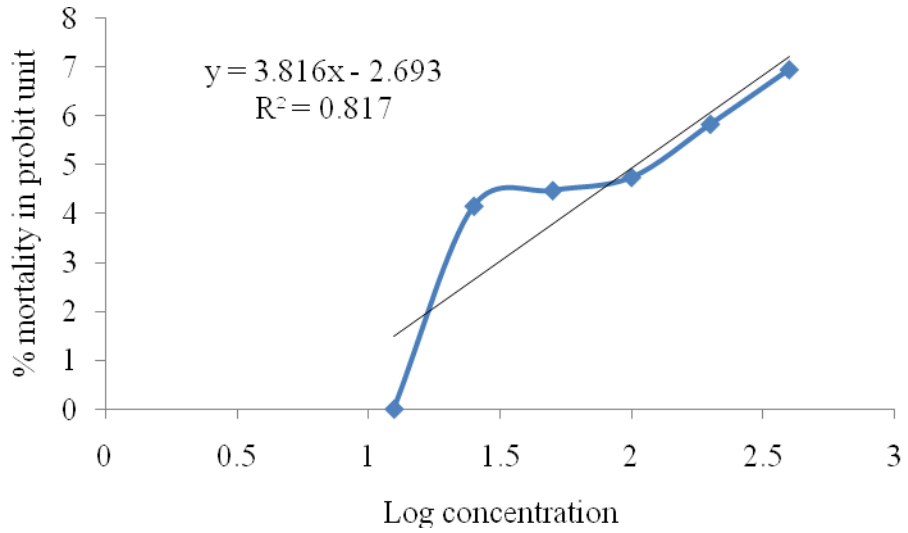


Figure 4.9: Effect of *S. sisymbriifolium* fruit Chloroform fraction extract on brine shrimp nauplii

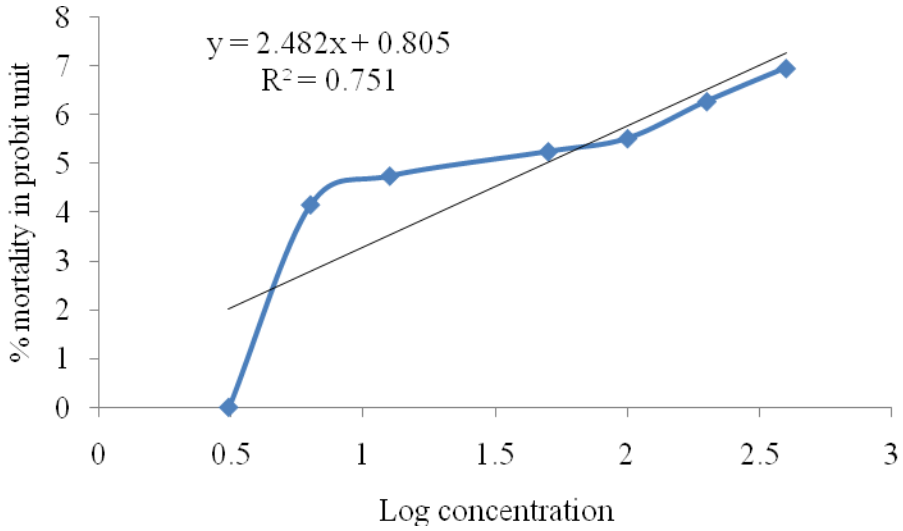


Figure 4.10: Effect of *S. sisymbriifolium* fruit Carbon tetra chloride fraction extract on brine shrimp nauplii

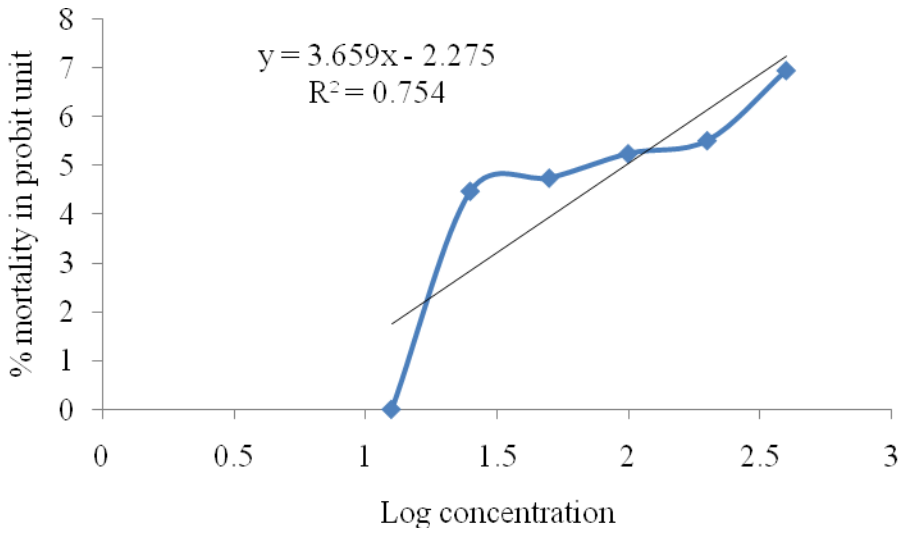


Figure 4.11: Effect of *S. sisymbriifolium* fruit Aqueous fraction extract on brine shrimp nauplii

In case of brine shrimp lethality bioassay, the lethality of ethanolic fruit extract, fruit of n-hexane, chloroform, carbon tetrachloride and aqueous fractions of *S. sisymbriifolium* were evaluated against *A. salina*. The table 4.4 represents the results of brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, potassium per manganate. The LC_{50} values were found to be 32.12, 17.66, 103.75, 49.00 and 97.33µg/ml respectively.

4.5 Anti- diarrheal activity

4.5. Tabular presentation of the study result of castor oil induced diarrhea in mice

Table 4.5.1: Effect on total latent period

Group	**Dose**	**Total latent period (min)**
Negative control (1% tween 80 in saline)	0.5 ml/ mice; p.o.	42.8±1.16
Positive control (Loperamide)	2 mg/ Kg body weight; p.o.	95.2±1.71**
SSF 200	200 mg/ Kg body weight; p.o.	42.6±1.29
SSF 400	400 mg/ kg body weight; p.o.	14.2±0.86**

Values are expressed as Mean±SEM (n=5); **: p <0.001 dunnett t-test as compared to negative control.

Group	**Mean**	**Standard deviation**	**Standard error mean**	**95% confidence interval for mean**	
				Lower bound	**Upper bound**
Control	42.80	2.59	1.16	39.59	46.01
Positive	95.20	3.83	1.71	90.44	99.96
SSF200	42.60	2.88	1.28	39.02	46.18
SSF400	14.20	1.92	0.86	11.81	16.59

Anti-diarrheal activity of the ethanolic fruit extract of *S.sisymbriifolium* was tested by castor oil induced diarrhea in mice. The extract caused a decrease in latent period *i.e* hastened the onset of diarrheal episode at the dose of 400 mg/kg of body weight significantly (p<0.001) which was comparable with the control group. At dose 200mg/kg no change showed in the latent period and the result was non-significant when compared with the control.

Table 4.5.2: Effect on the no. of stool

Groups	**Dose**	**No. of stool**
Control (1% tween 80 in saline)	0.5ml/mice; p.o.	14.4±1.75
Positive Control (Loperamide)	2 mg/kg body weight; p.o.	8.6±0.75*
SSF 200	200mg/kg body weight; p.o.	6.2±0.37**
SSF 400	400mg/kg body weight; p.o.	9.2±0.86*

Values are expressed as Mean±SEM (n=5); *: p <0.05, **: p <0.001 dunnett t-test as compared to negative control.

Group	**Mean**	**Standard deviation**	**Standard error mean**	**95% confidence interval for mean**	
				Lower bound	**Upper bound**
Control	14.40	3.91	1.75	9.54	19.26
Positive	8.60	1.67	0.75	6.52	10.68
SSF200	6.20	0.83	0.37	5.16	7.24
SSF400	9.20	1.92	0.86	6.81	11.59

The effects of ethanolic extract of *S.sisymbriifolium* on the total number of stool of castor oil-induced diarrheal model mice are presented in the table. At dose 200mg/kg the extract showed a marked significant reduction in the total number of stool compared with negative control (p <0.001). At dose 400mg/kg body weight also showed a significant reduction in the total number of stool when compared with the control group (p <0.005).

Table 4.5.3: Effect on the total weight of fecal output

Group	**Dose**	**Total weight of faecal output**
Control (1% tween 80 in saline)	0.5 ml/ mice; o.p.	0.935±0.02
Positive control (Loperamide)	2 mg/ Kg body weight; p.o.	0.731±0.03**
SSF 200	200 mg/ Kg body weight; p.o.	0.396±0.3**
SSF 400	400 mg/ kg body weight; p.o.	0.557±0.03**

Values are expressed as Mean±SEM (n=5); **: p <0.001 dunnett t-test as compared to negative control.

Group	**Mean**	**Standard deviation**	**Standard error mean**	**95% confidence interval for mean**	
				Lower bound	**Upper bound**
Control	0.935	0.044	0.020	0.880	0.990
Positive	0.731	0.063	0.028	0.652	0.809
SSF200	0.396	0.075	0.033	0.303	0.488
SSF400	0.557	0.071	0.031	0.469	0.645

At both doses of 200 and 400 mg/kg body weight there was a reduction in total weight of fecal output which was found to be statistically significant (p <0.001).

4.6 Hole cross test

Table 4.6: Effect of ethanolic fruit extract of *S. sisymbriifolium* on number of movement in hole cross test.

Groups	Dose	Number of movement				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control(1%tween 80 in saline)	0.5ml/mice, p.o	10.0±0.71	8.6±0.60*	7.2±0.58	7.0±0.55	6.2±0.37
Positive Control (Diazepam)	1mg/kg body weight; p.o	7.8±0.74	6.2±0.80*	5.8±0.74	4.2±1.16*	3.6±0.51*
SSF 200	200mg/kg body weight; p.o	12.4±0.12	11.8±0.58*	8.0±0.84	6.0±0.70	4.8±0.20
SSF 400	400mg/kg body weight; p.o	12.4±0.74	11.6±0.50*	6.2±0.66	7.6±0.81	3.8±0.49*

Values are expressed as Mean±SEM (n=5); *: p <0.05 dunnett t-test as compared to negative control.

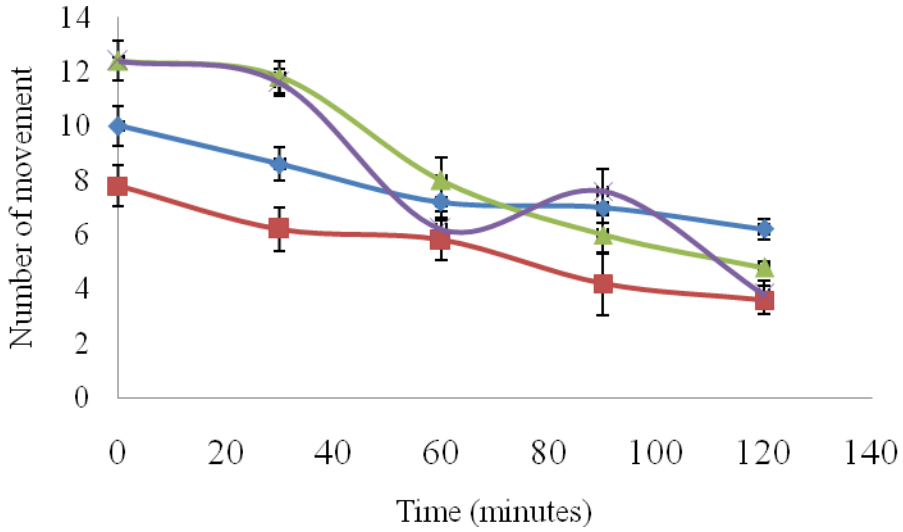


Figure 4.12: No. of movement in hole cross

The number of hole crossed from one chamber to another by mice of the control group was similar from 0 to 120 min. In the hole cross test, ethanolic fruit extract of *S.sisymbriifolium* at both doses of 200 and 400 mg/kg body weight showed a decrease in locomotion in the test animals from second observation period (at 30 min) and was sustained up to 5th observation period (120 min) as evident by the reduction in number of hole crossed by the treated mice compared to the control group. The result was comparable to the control group and was statistically significant (p <0.05) for both the doses at 30 min and for 400 mg/kg body weight at 120 min observation. The CNS was depressed till the completion of the observation period.

4.7 Hole board test

4.7. Tabular presentation of the Hole board test

Table 4.7.1: Effect on the head dipping

Group	**Dose**	**No. of head pocking**

Control (1% tween 80 in saline)	0.5 ml/ mice; p.o	44.8±1.24
Positive control (Diazepam)	1 mg/ Kg body weight; p.o.	29.6±0.93**
SSF 200	200 mg/ Kg body weight; p.o.	52.5±1.16**
SSF 400	400 mg/ kg body weight; p.o.	35.6±0.51**

Values are expressed as Mean±SEM (n=5); **: p <0.001 dunnett t-test as compared to negative control.

Group	**Mean**	**Standard deviation**	**Standard error mean**	**95% confidence interval for mean**	
				Lower bound	**Upper bound**
Control	44.80	2.77	1.24	41.35	48.25
Positive	29.60	2.07	0.93	27.03	32.17
SSF200	52.20	2.59	1.16	48.99	55.41
SSF400	35.60	1.14	0.51	34.18	37.02

At dose 200 mg/kg body weight, ethanolic fruit extract of *S.sisymbriifolium* has increased significantly (p <0.001) the number of head dip responses giving a value of 52.5±1.16 when compared to a value of 44.8±1.24 for control but the dose of 400mg/kg body weight has decreased the activity significantly (p <0.001) giving a value of 35.6±0.51when compared with negative control.

Table 4.7.2: Effect on latency period

Group	**Dose**	**latency until the first entry (second)**
Control	0.5 ml/ mice; p.o	14.8±0.37

(1% tween 80 in saline)		
Positive control (Diazepam)	1 mg/ Kg body weight; p.o.	2.0±0.45**
SSF 200	200 mg/ Kg body weight; p.o.	6.0±0.55**
SSF 400	400 mg/ kg body weight; p.o.	5.2±0.37 **

Values are expressed as Mean±SEM (n=5); **: p <0.001 dunnett t-test as compared to negative control.

Group	**Mean**	**Standard deviation**	**Standard error mean**	**95% confidence interval for mean**	
				Lower bound	**Upper bound**
Control	14.80	0.84	0.37	13.76	15.84
Positive	2.00	1.00	0.45	0.76	3.24
SSF200	6.00	1.22	0.55	4.48	7.52
SSF400	5.20	0.84	0.37	4.16	6.24

In control group, mean number of latency period until the first entry was 14.8. At both doses of 200 and 400 mg/kg body weight showed a significant decrease (p <0.001) in the latency period giving values 6.0±0.55 and 5.2±0.37 respectively.

4.8 Elevated plus-maze test

4.8 Tabular presentation of Elevated plus maze test

Table 4.8.1: Effect on the stretch attend postures

Group	**Dose**	**Mean no. of stretch attend postures**
Control (1% tween 80 in saline)	0.5 ml/ mice; p.o	9.2±1.42

Positive control (Diazepam)	1 mg/ Kg body weight; p.o.	8.6±2.29
SSF 200	200 mg/ Kg body weight; p.o.	5.8±0.66
SSF 400	400 mg/ kg body weight; p.o.	14.2±0.49

Values are expressed as Mean±SEM (n=5); dunnett t-test as compared to negative control.

		Standard	**Standard**	**95% confidence interval for mean**	
Group	**Mean**	**deviation**	**error mean**	**Lower bound**	**Upper bound**
Control	9.20	3.19	1.43	5.23	13.17
Positive	8.60	5.13	2.29	2.23	14.97
SSF 200	5.80	1.48	0.66	3.95	7.64
SSF 400	14.20	1.09	0.49	12.84	15.56

A one-way ANOVA showed that SSF caused increase in the mean number of stretch and attend postures at dose dependent manner when compared to the control. At dose 200mg/kg body weight there was a decrease in mean no. and the mean value was 5.8±0.66. At dose 400mg/kg body weight the mean value increased to 14.2±0.49 when compared to control. The increase was dose dependent and was statistically non significant for all the doses used.

Table 4.8.2: Effect on the no. of entry in open arm

Group	**Dose**	**Mean no. of entry in open arm**
Control (1% tween 80 in saline)	0.5 ml/ mice; p.o	1.2±0.49
Positive control (Diazepam)	1 mg/ Kg body weight; p.o.	1.6±0.81
SSF 200	200 mg/ Kg body weight; p.o.	1.0±0.55
SSF 400	400 mg/ kg body weight; p.o.	1.4±0.75

Values are expressed as Mean±SEM (n=5); dunnett t-test as compared to negative control.

Group	Mean	Standard deviation	Standard error mean	95% confidence interval for mean	
				Lower bound	Upper bound
Control	1.20	1.10	0.49	-0.16	2.56
Positive	1.60	1.82	0.81	-0.66	3.86
SSL 200	1.00	1.22	0.54	-0.52	2.52
SSL 400	1.40	1.67	0.75	-0.67	3.48

Mean no. of entry to the open arm decreased at dose 200mg/kg and the value was 1.0±0.55 but increased the value to 1.4±0.75 at dose 400mg/kg body weight when compared to the control. The increase was dose dependent and was not statistically significant.

Table 4.8.3: Effect on the no. of entry in close arm

Group	Dose	Mean no. of entry in close arm
Control (1% tween 80 in saline)	0.5 ml/ mice; p.o	9.4±1.91
Positive control (Diazepam)	1 mg/ Kg body weight; p.o.	12.0±3.03
SSF 200	200 mg/ Kg body weight; p.o.	7.2±0.73
SSF 400	400 mg/ kg body weight; p.o.	11.2±1.32

Values are expressed as Mean±SEM (n=5); dunnett t-test as compared to negative control.

Group	Mean	Standard deviation	Standard error mean	95% confidence interval for mean	
				Lower bound	Upper bound
Control	9.40	4.28	1.91	4.09	14.71
Positive	12.00	6.78	3.03	3.58	20.42
SSF 200	7.20	1.64	0.73	5.16	14.86
SSF 400	11.20	2.95	1.32	7.54	14.71

The entries into the close arm are expressed as mean no. and the extract at both the doses (200 &400mg/kg body weight) caused a non significant increase in the close arm at dose dependent manner in this parameter. At dose 200mg/kg a decrease in mean value was 7.2±0.73 and at 400mg/kg the mean value increased to 11.2±1.32 when compared to control.

Table 4.8.4: Effect on the no. of entry to center

Group	**Dose**	**Mean no. of entry to center**
Control (1% tween 80 in saline)	0.5 ml/ mice; p.o	4.2±0.20
Positive control (Diazepam)	1 mg/ Kg body weight; p.o.	4.6±0.75
SSF 200	200 mg/ Kg body weight; p.o.	2.4±1.03
SSF 400	400 mg/ kg body weight; p.o.	7.6±1.29

Values are expressed as Mean±SEM (n=5); dunnett t-test as compared to negative control.

Group	**Mean**	**Standard deviation**	**Standard error mean**	**95% confidence interval for mean**	
				Lower bound	**Upper bound**
Control	4.20	0.45	0.20	3.64	4.76
Positive	4.60	1.67	0.75	2.52	6.68
SSF 200	2.40	2.30	1.03	-0.46	5.26
SSF 400	7.60	2.88	1.29	4.02	11.18

The effect was dose dependent and statistically non significant at all doses used. At dose 200mg/kg the mean value of the entry to center decreased to 2.4±1.03 but at dose 400mg/kg the mean value increased to 7.6±1.29 when compared to negative control.

Chapter: 5

DISCUSSION

5.1 Acetic acid induced writhing

The acetic acid induced writhing test has long been used as a screening tool for the assessment of analgesic properties of plant extracts and natural products. It has been suggested that pain sensation is elicited by the release of arachidonic acid from phospholipid of cell membrane via cyclooxygenase (COX) and prostaglandin biosynthesis. It has been reported that acetic acid is used as a writhing syndrome causes analgesia by releasing endogenous mediators that stimulate the pain nerve endings. Acetic acid induced writhing is associated with increased level of PGE2 and PGE2α in peritoneal fluids. The increase in prostaglandin levels within the peritoneal cavity enhances inflammatory pain by increasing capillary permeability. The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agents reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Zulfiker *et al*, 2010). The significant pain reduction of the plant extract at both the doses might be due to the presence of analgesic principles such as flavanoids, alkaloids, tannins and steroids (Chatterjee *et al*., 1970; Ghani, 2003; Jain and Srivastava, 1985) acting with the prostaglandin pathways.

5.2 Thrombolytic activity

Various thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels but these drugs are not above limitations and can lead to serious and sometimes fatal consequences. The present study was carried out to investigate the thrombolytic activity of the *S.sisymbriifolium* fruit. An *in vitro* thrombolytic method was used to investigate the thrombolytic activity of plant extracts in blood sample from healthy human volunteers, along with streptokinase as a positive control and water as a negative control. (Sweta *et al*.2007). SK, a

known thrombolytic drug (Tillet W.S & Garner R.L.1933) is used as a positive control. The comparison of positive and negative control with the extract clearly demonstrated that the clot dissolution does not occur when the extract was added to the clot. On the basis of the result obtained in this present study we can say that *S.sissymbriifolium* extract does not have thrombolytic activity.

5.3 Nitric oxide scavenging activity

On the basis of scavenging ability of the free radicals for NO, the highest antioxidant activity was found from the plant extract. It is may be due to the presence of phenolic compounds such as flavonoids and tannins the antioxidant activity of the extract was observed. The antioxidant property of the ethanolic extract of *S. sisymbriifolium* found in the present study could be owing to the presence of flavanoids and tannins in this plant (Chatterjee *et al*., 1970; Ghani, 2003; Jain and Srivastava, 1985). Therefore the plant could be used as a potential preventive measure against free radical mediated diseases.

5.4 Brine shrimp lethality bioassay

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates well with cytotoxic and anti-tumour properties (Mc.Lauglin *et al*., 1993)

LC_{50} values of different fractions of fruit extracts of the studied plant clearly indicate the presence of bioactive principles in the extracts which might be very useful as antitumor, pesticidal and other bioactive agents. LC_{50} values of the plant extracts were obtained

by a plot of percentage of the shrimp nauplii killed against the concentrations of the extracts and the best-fit line was obtained from the data by means of regression analysis (Meyer *et al*., 1982) In this study, the non-polar fractions of n-hexane of the ethanolic extract showed the most cytotoxic activity, which indicates that the responsible cytotoxic agent could be non-polar in nature.

The significant lethality of several plant extracts to brine shrimp is an indicative of the presence of potent cytotoxic components which warrants further investigation.

5.5 Anti-diarrheal activity

Anti-diarrheal activity of the ethanolic fruit extract of *S.sisymbriifolium* was tested using the model of castor oil induced diarrhea in mice. Castor oil, which is used to induce diarrhea in mice, mixes with bile and pancreatic enzymes and liberates recinoleic acid from the triglycerides upon oral administration. However, it is well evident that castor oil produces diarrhea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins that results in the stimulation of motility and secretion. The recinoleic acid thus liberated readily forms recinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that recinoleate salts stimulates the intestinal epithelial cell's adenyl cyclase (Racusen *et al*., 1979) or release prostaglandin (Awouters *et al*., 1978). Since the ethanolic extract of *S.sisymbriifolium* successfully inhibited the castor oil-induced diarrhea, extract might have exerted its anti-diarrheal action via anti-secretory mechanism which was also the evident from the decreased number of stool as well as the decrease in total weight of fecal output. Again flavonoids present in the plant extract are

reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil (Veiga *et al*., 2001). The anti-diarrheal property of the ethanolic extract of *S. sisymbriifolium* found in the present study could be owing to the presence of alkaloids, flavanoids, tannins in this plant (Chatterjee *et al*., 1970; Ghani, 2003; Jain and Srivastava, 1985). Previous studies showed that antidysentric and antidiarrheal properties of medicinal plants were mostly due to tannins, alkaloids, flavonoids, saponins, triterpenes, sterols (Galvez *et al*., 1991; Longanga *et al*., 2000).On the basis of the result of castor oil induced diarrhea, it can be concluded that the ethanolic fruit extract of *S.sisymbriifolium* might possess anti-diarrheal activity.

5.6 Hole cross test

The extract at lower and higher doses produced a reduction in spontaneous motor activity and this effect may be attributed to CNS depression. The locomotor activity is a test to appraise the level of excitability of the CNS and any decrease of this activity may be narrowly related to sedation resulting from depression of the central nervous system. The sedative effect recorded here may be related to an interaction with benzodiazepines related compounds that binds to receptors in the CNS and have already been identified in certain plant extracts.

Literature review of the plant reveals that *S. sisymbriifolium* contains flavonoids, steroids and tannins (Chatterjee *et al*., 1970; Ghani, 2003; Jain and Srivastava, 1985). Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS. Different anxiolytic, sedative hypnotic drugs elucidate their action through GABAA, therefore it is possible that extract of *S.sisymbriifolim* fruit may act by potentiating GABAergic inhibition in the CNS or may be due to the activation of GABA receptor by the extracts. Earlier investigation on phytoconstituents and plants suggests that many flavonoids, and steroids were found to be the ligands for GABAA

receptors in the CNS which led to the assumption that they can act like benzodiazepine-like molecules (Fernandez *et al*., 2006). So, it is probable that phytoconstituents in this extract is responsible for its CNS activity.

5.7 Hole board test

The whole board experiment is a measure of exploratory behavior in animals and is an accepted parameter for evaluating anxiety conditions in animals. In this test, the test compounds showed dose dependent decrease in the number of nose poking. The extract produced a significant decrease in the nose poking and latency time period at higher doses levels and was more pronounced when compared to a control. This indicates a decrease in the curiosity or exploratory behavior of test animals and also provides evidence in favor of a CNS depressant action (Sonavane *et al*., 2001; Suba *et al*., 2002). Preliminary phytochemical analysis in this study revealed the presence of alkaloids, flavanoids, tannin, solasodine (Chatterjee *et al*., 1970; Ghani, 2003; Jain and Srivastava, 1985). These secondary metabolites, individually or in combination would account for the observed pharmacological effects of this plant in this study.

5.8 Elevated plus maze test

Anxiety, like all emotions has cognitive, neurobiological and behavioral components. Pharmacological knowledge about the plant under study would allow us to evaluate central nervous activity, which could be used to treat anxiety type of disorders. The present work has been done to see the anxiolytic activity by the fruit extract of *S.sisymbriifolium* as assessed by elevated plus maze test.

The EPMT is used to evaluate psychomotor performance and emotional aspects of mice. Results obtained on the EPM after treatment with the extract at dose 400mg/kg body weight revealed anxiolytic activity, since increases in open arm entry parameters are the most representative indices of anxiolytic activity (Lister RG, 1990).

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CONCLUSION

6. CONCLUSION

Phytochemical screening of the extract showed that the *Solanum sisymbriifolium* possess alkaloids, flavonoids, tannins and steroids (Shilpi *et al.*, 2005). The presence of the identified phytochemicals makes the fruit pharmacologically active. The pharmacological profiles of the present investigation of the ethanolic fruit extract of *S. sisymbriifolium* indicate that the extract possess strong analgesic, antioxidant, anti-diarrheal, CNS depressant and cytotoxic properties and might, in part, be due to the presence of such compounds. The results also suggest a rationale for the traditional uses of this plant. However, studies are required on higher animal model and subsequently on human subjects to prove efficacy as an analgesic, antioxidant, anti-diarrheal, CNS depressant and cytotoxic agent.

REFERANCES