Antigiardial Activity of Fruits of Selected Solanum Plants

By

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Dedication

- my father, mother,

- my supervisor

- and all people who are always in my heart.
Acknowledgements

My praise and thanks to Allah, who gave me the strength and confidence to pursue this investigation.

I am glad to express my gratitude and appreciation to my supervisor Dr. Sakina Yagi tirelessly gave me advice, guidance and patience throughout this work.

Thank and gratitude to Dr. Waleed Koko and Mr. Mohammed Garbe at the National Center for Medicinal and Aromatic Plants for their valuable assistance and making available all the laboratory facilities needed during the experimental phase of this study.

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Abstract

The present study was carried on fruits of *Solanum* plants namely; *Datura stramonium*, *Lycium persicum*, *Solanum dubium* and *S. incanum*. Fruits extracts were assessed for their anti-giardial activity and phytochemical constituents.

The anti-giardial activity test was performed for the methanol extracts. The flagellated protozoa *Giardia lamblia* used in this test were taken from patients of Ibrahim Malik Hospital (Khartoum).

All the extracts showed varying degrees of activity on the *G. lamblia* parasite. The highest effective concentration against was obtained from methanolic extract of *S. dubium* at 250 μg/mL with mortality of 60.5% after 24 h and from the methanolic extract of *D. stramonium* at 250 μg/mL with mortality of 64.4% after 72 h.

Preliminary screening of extracts was carried out using thin layer chromatography technique. Results showed that all extracts were rich in secondary metabolites.

In conclusion, the results of the present study paves the way for further research to identify the active compounds for the development of new anti-giardial agents capable of decreasing the burden of drug resistance and cost of management of diseases of clinical and public health importance in the Sudan.
ملخص الدراسة

الحريت هذه الدراسة على ثمار نباتات البذنجانية وهي

* Datura stramonium, Lycium persicum, Solanum dubium and S. incanum.*

وتم تقييم مستخلصات الفواكه لنشاط القاردية والمركبات الكيميائية.

تم الحصول على طفين القاردية المثمرة المستخدمة في هذا الاختبار من مرضى مستشفى إبراهيم مالك (الخرطوم).

أظهرت جميع المستخلصات درجات متفاوتة من النشاط ضد طفين القاردية المثمرة. وتتم الحصول على فعالية ضد المستخلصات البيئية من 250 ميكروغرام/مل مع معدل وفيات 60.5% بعد 24 ساعة. ومن مستخلص الميثانولي في 250 ميكروغرام/مل مع معدل وفيات 64.4% بعد 73 ساعة.

اجري فحص أولي للمستخلصات باستخدام تقنيه القيمة لل chóي الكروماتوغرافي واظهرت النتائج أن جميع المستخلصات كانت غنية بالمركبات الثانوية.

ووفي الختام فإن نتائج هذه الدراسة تمهد الطريق لأجراء مزيد من البحوث لعزل المركبات النشطة لتنمية مركبات جديدة ضد القاردية قادرة على خفض عبء مقاومة الأدوية وتكلفة إدارة الأمراض ذات الهمية للصحة العامة والسريرية في السودان.
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CHAPTER ONE
Introduction and literature review

1.1 General introduction

Medicines form the second most essential need for mankind after water and food. Plants have been used for medicinal purpose from the dawn of history and for many centuries. In the last few decades, the study of medicinal plants and their traditional use in different parts of the World has increased (Lev, 2006). Hundreds of plants have been used as herbal remedies in indigenous medicine systems (Hussain et al., 2008). While herbal medicines are assumed to be of great importance in the primary healthcare of individuals in rural communities (Sheldon et al., 1997; Tene et al., 2007), plant-based traditional knowledge coupled with the high cost involved in the development of patentable chemicals and drugs (Hack, 2006) are recognized as essential tools in search for new sources of drugs and nutraceuticals (Sharma and Mujundar, 2003). Thus, antimicrobial activity of crude and semi-purified extracts of many plants has been widely reported (Cos et al., 2002; Muschietti et al., 2005; Wannissorn et al., 2005; Olajuyigbe et al., 2011). The increasing use of traditional therapies which the laypeople considered as a part of their heritage now requires more scientifically sound evidence for the principles behind plants' therapeutic effectiveness in complementary and alternative medicines (Patwardhan et al., 2005).

The unique geographical position of Sudan and its interaction with different cultures have undoubtedly left its influence on different aspects of the Sudanese traditional medicine. With this unique history and varied climate, terrain, flora and fauna, the people of Sudan have developed their own traditional medical culture. Medicinal and aromatic plants are not only used
to meet healthcare needs but also for cosmetics and perfumery purposes. Traditional medicine is both popular and important as a medical system has been integrated into the national healthcare schemes. There is vast experience in the use of herbs in medical treatments. Many families specialize in herbal medicines and this knowledge is conveyed from generation to generation. Patients travel from urban to rural areas to consult herbalists, especially for chronic diseases.

There are more than 3,132 vascular plant species in Sudan. The Sudan Atlas of Medicinal Plants has records of more than 2,000 medicinal plants collected from different parts of the country. Several native plant species are in use in traditional medicine (Abdalla and Nour, 2001). Moreover, the Medicinal and Aromatic Herbs Research Institute has trained a considerable number of specialists in the various fields required for research in medicinal plants. Legislation is in force for the registration of herbal preparations and herbal products (MAPRI, 1997).

1.2 Protozoan Diseases
Diarrhea is a very common illness, especially in the developing world, and is frequently experienced by travelers. Cryptosporidium, Cyclospora, Isospora, Giardia, amoeba, and Sarcocystis are pathogenic protozoan parasites that can cause these gastrointestinal illnesses. Commensal parasites are also relatively common in developing countries and less frequently identified in the developed world. Worldwide, Giardia is the most common protozoan infection in the gastrointestinal tract of humans. It was probably first seen by Anton van Leuwenhoek in the late seventeenth century. In Tennessee, Giardia cysts have been identified in human feces from about 600 BC. Cryptosporidium and Giardia have also been reported from samples 500 to
3000 years old from the Andean regions in Peru, and from 4300- to 1100-year-old samples from the coastal regions of Peru. *Cryptosporidium* became much more relevant to public health in the early 1980s with the emergence of the AIDS epidemic. Opportunistic and emerging parasitic infections also include *Isospora*, particularly in HIV and AIDS patients. *Cyclospora* has been observed in certain regions of the developing world; however, globalization of the food supply and increase in international travel has revealed that parasitic infections can also cause epidemics in the developed world.

1.2.1 Flagellated Protozoa

1.2.1.1 *Giardia*

*Giardia intestinalis* (also known as *G. lamblia*) is a flagellated protozoa that inhabits the small intestine of man and other animals, including monkeys, rodents, dogs, cats, horses, goats, cattle, birds, reptiles, and fish (Newman et al., 2001). Like many other protozoa, *G. intestinalis* has a trophozoite and a cyst stage. The trophozoite is oblong, pear-, or kidney-shaped, rounded anteriorly, and pointed posteriorly. The trophozoite is flattened laterally, being convex dorsally and concave ventrally; much of the ventral surface comprises the sucking disk that the organism uses to attach firmly to the intestinal mucosa. The trophozoite is microscopic in size, averaging 10 to 20 μm long by 5 to 15 μm in breadth; a prominent pair of nuclei on each side of the organism near the anterior end gives a facelike appearance. There are four pairs of flagella, one pair arising near the anterior and posterior end, respectively, and two pairs arising near mid-body. Rapid movement of the flagella allows the trophozoite to move from place to place. Trophozoites divide by a complicated process of longitudinal binary fission that results in two daughter trophozoites. Transmission from one host to another is
accomplished by viable cysts (Fig. 1). As trophozoites transit down the colon, they prepare for encystation by retracting their flagella. The cytoplasm becomes condensed, and a thin, tough hyaline membrane (cyst wall) is secreted. The cysts are oval in shape and measure 8 to 12 μm in length by 7 to 10 μm in breadth. Mature cysts have four nuclei located at one end of the cyst. As the cyst matures, internal structures and the sucking disk are doubled. When excystation occurs in a new host, division results in two identical trophozoites, which grow flagella and initiate infection.

Diagnosis of infection is typically by microscopic detection of cysts in freshly collected stool (or trophozoites in diarrheic stools). Organisms can occasionally be seen in direct exams, but a concentration procedure is recommended. Because of their distinctive shape, appearance of the nuclei, and other features, the diagnosis can often be made on wet, unstained samples. However, staining may enhance detection and confirmation of infection. In addition to direct or stained specimens, commercial direct fluorescent antibody (DFA) assays are available and often used as the gold standard for diagnosis. Enzyme-linked immunosorbent assay (ELISA) formatted tests are commercially available and are extremely useful for screening large numbers of samples.

Infection with *Giardia* in an appreciable number of cases results in irritation of the duodenum with excess secretion of mucus and dehydration, accompanied by epigastric pain, flatulence, and chronic diarrhea with steatorrheic-type stool containing a large amount of mucus and fat but typically with no blood. It is recognized that giardiasis can cause stunting and interference with growth, particularly in children in developing countries where repeated infections are the norm.
Metronidazole or tinidazole is the recommended drug of choice for treating giardiasis. Nitazoxanide, furazolidone, and paromomycin are alternatives. Paromomycin is not absorbed from the gastrointestinal tract and is often used during pregnancy but it is less efficacious than the other agents.

Transmission of *Giardia* is by viable cysts that are swallowed (Fig. 1). Contaminated food and water are the most common source of exposure although intimate contact with an infected individual may represent a common mechanism. Giardiasis is typically more common in children than in adults, especially in a crowded setting such as day care centers. However, in the United States and other developed countries, outbreaks of giardiasis are also observed in adults. These are often linked to contaminated food or drink, or are associated with recreational water venues. Cats and dogs are also recognized to harbor *Giardia*. Despite the morphologic similarity of the organisms infecting humans and animals, molecular analysis has shown distinct clades or assemblages that seem to suggest some degree of host specificity, with certain assemblages being more restricted in their host preference than others. Further differences in virulence between isolates have also been proposed, but evidence to date has been inconsistent. *Giardia* cysts have a relatively high resistance to routine water treatment procedures, including chlorination, which has led to numerous waterborne outbreaks. Surface water can be widely contaminated, and as a result, giardiasis is one of the most common intestinal parasitic infections. This implies that to provide potable water, surface water should be treated by flocculation, sedimentation, filtration, and finally, chlorination. Use of chlorine alone at levels normally used in municipal treatment facilities does not rapidly inactivate cysts, especially at lower water temperatures, so other
measures must be in place. Purifying water for use when camping or traveling overseas can include boiling, filtration through filters with pore size of less than 1 μm, or treatment with chlorine or iodine preparations (some recommend iodine preparations to be more effective than chlorine preparations).

Giardiasis can occur year-round in all settings, temperate as well as tropical. However, there is strong evidence that some seasonality occurs in temperate regions with increased incidence in the summer months, peaking in early fall (Hill and Nash, 2005).
(www.dpd.cdc.gov/DPDx/HTML/ImageLibrary/Giardiasis_il.htm).

Fig.1: Life cycle of Giardia
1.3 Plant under study

1.3.1 Family Solanaceae

Solanaceae is a medium-sized family with approximately 96 genera and 3000 – 4000 species occurring all over the world (D’Arcy 1991). Members of Solanaceae provide a variety of culinary, medicinal, and ornamental values. In terms of culinary value, one of the most important species of this family for the global diet is the potato or *Solanum tuberosum*, whose carbohydrate-rich tubers have been a staple food in many times and places, and which is one of the most grown crops today. Also, tomatoes, tomatillos, eggplants, uchuva, and peppers which belong to this family are the desirable items.

Medicinally, as well as in terms of poisoning and psychotropic effects, members of Solanaceae have been prized for their alkaloid content and used throughout history (NHM 2008). The members of this family are being used for medicinal purposes as early as 37A.D (Hill, 1952). Important drug plants include deadly nightshade or belladonna (*Atropa belladonna*), jimson weed (*Datura stramonium*), henbane (*Hyoscyamus niger*), and tobacco (*Nicotiana tabacum*) (NHM 2008). In fact, this family is an important source of almost 300 different kinds of alkaloids (Friedman and McDonald, 1997). Solanine, scopolamine, atropine and hyoscymine are the key of this family (Stanker et al., 1994). Presence of these alkaloids makes this family medicinally important.

In Sudan, there are more than six genera comprise 30 species. Most of them are known to be poisonous and are used traditionally to treat different illnesses in Sudan.

The plants chosen in this study were:
1. *Datura stramonium*
2. *Lycium intricatum*
3. *Solanum dubium*
4. *Solanum incanum*

1.3.1.1 *Datura stramonium*

**Synonyms**

*Datura inermis juss.* Ex jacc
*Datura stramonium* var. chalybea w.d.j.koch, nom. illeg
*Datura stramonium* var. tatula (L.) Torr.
*Datura tatula* L.,

*Datura stramonium*, known by the common names Jimson weed or datura is believed to have originated in the Americas, but is now found around the world. *D. stramonium* is a foul-smelling, erect annual, freely-branching herb that forms a bush up to 2–5 ft (1–1.5 m) tall.

All parts of *Datura* plants contain dangerous levels of the tropane alkaloids: atropine, hyoscyamine and scopolamine which are classified as deliriants, or anticholinergics (*Preissel et al.*, 2002). *Datura* has long been used as an extremely effective treatment for asthma symptoms. The active anti-asthmatic agent is atropine, which causes paralysis of the pulmonary branches of the lungs, eliminating the spasms that cause the asthma attacks. The leaves are generally smoked either in a cigarette or a pipe. This practice of smoking *datura* to relieve asthma has its origins in traditional Ayurvedic medicine in India. After this was discovered during the late 18th century by James Anderson, the English Physician-General of the East India Company, the practice quickly became popular in Europe. Other medicinal uses for datura included stimulating abortions, providing relief from sore throat or
toothache, and getting rid of parasites (Pennachio et al., 2010). In Sudan, datura is used as anti-spasmodic, hypnotic and narcotic medicinal plant (Broun and Massey, 1929).

1.3.1.2 Lycium intricatum (Dunal) Hayek

Synonyms: Lycium mediterraneum Dunal var. ramulosum Dunal

The plant has been known to European herbalists since ancient times and was traded from the Far East to Europe by the Romans. The plant is used for the treatment for sore eyes and inflammation. In Sudan, the plant is used for the treatment of headache, abdominal pain, dilated pupils, vomiting, diarrhea (Schmeltzer, 2008).

1.3.1.3 Solanum incanum

Solanum incanum is a species of nightshade that is native to Sub-Saharan Africa and the Middle East, eastwards to India. Common names include Thorn Apple and Bitter Apple. The plant is used in the traditional medicine as anti-asmtic, for snake bite, dysentery, toothache, throat and chest complaints (EL Ghazali, 1986; Chevallier (1996).

1.3.1.4 Solanum dubium Fresen

Vernacular name: Gubbain

Solanum dubium is a weed plant that has milk-clotting ability. The plant is grown widely in Northern, Central and Western Sudan. It is used for the treatment of kidney dysfunction (Barri et al., 1983).
Fig. 2: Fruits of studied *Solanum* plants.
1.4 Research problem
The research problem of the present work was to determine the \textit{in vitro} antigiardial potential of some selected \textit{Solanum} plants. This selection was guided in the first place by ethnomedical claim in traditional medicine associated with digestive system troubles and secondly by lack of information in literature on their chemical constituent and biological activity.

1.5 Objective of the study
The present study was conducted to:

- Determine the secondary metabolites present in the fruits by using thin layer chromatographic technique.
CHAPTER TWO
Material and Methods

2.1 Collection and preparation of plant material
Fruits of Solanum plants were collected in February 2012 from East Sudan, Arkweet region. Voucher specimen was deposited at the Herbarium of Botany Department, University of Khartoum. Fruits were cut into small pieces, dried under shade and ground into a soft powder with a grinding mill. The powdered fruits (50 g from each plant) were stored in glass storage containers in the dark in the research laboratory.

2.2 Extraction
All chemicals used were of Analar grade.

The dried powder of each fruits was extracted separately with methanol (MeOH) (3 X 300 mL). Extracts were filtered, concentrated under reduced pressure, weighed and kept in a desicator.

2.3 In vitro anti diarrheal activity

2.3.1 Parasite isolate
Gajridia lamblia used in all experiments were taken from patients of Ibrahim Malik Hospital (Khartoum). All positive samples were examined by wet mount preparation. Then the positive sample was transported to the laboratory in nutrient broth medium. Trophozoites of G lamblia were maintained in RPMI 1640 medium containing 5% bovine serum at 37 ± 1°C. The trophozoites were maintained for the assays and were employed in the log phase of growth.
2.3.2 *In vitro* susceptibility assays

*In vitro* susceptibility assay using the sub-culture method of Cedilla *et al.* (2002) was adopted, this method being described as highly stringent and sensitive for assessing the anti-protozoal effects (gold standard) particularly in *Entamoba histolytica*, *Gairdia intestinalis* and *Trichomonas vaginalis* (Arguello *et al.*, 2004). 5 mg from each extract was dissolved in 50 μL of (DMSO) at Eppendorf tube containing 950 μL distilled water in order to reach concentration of 5 mg/mL (5000 ppm). The concentrates were stored at -20°C for further analysis. Sterile 96-well microtitre plate was used for different plant extracts, positive control and negative control.

Three out of 8 columns of microtitre plate wells (8 columns × 12 rows) were chosen for each extract, 40 μL of an extract solution (5 mg/mL) were added to the first column wells C-1: On the other hand, 20 μL of complete RPMI medium were added to the other wells the second column and third column (C-2 and C-3). Serial dilutions of the extract were obtained by taking 20 μL of extract to the second column wells and taking 20 μL out of the complete solution in C-2 wells to C-3 wells and discarding 20 μL from the total solution of C-3 to the remaining 20 μL serial solutions in the successive columns. 80 μL of culture medium was complemented with parasite and added to all wells. The final volume in the wells was 100 μL.

In each test metronidazole (a trichomonocide) pure compound [(1-(2-hydroxyethyl)-2-methyl-5 nitroimidazole], was used as a positive control in concentration 312.5 μg/mL, whereas untreated cells were used as a negative controls (culture medium plus trophozoites). For counting, the samples were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer three times for counting after 0, 24, 48
and 72 h. The mortality % of parasite for each extracts activity was carried out according to the following formula:

\[
\text{Mortality of parasite (\%) } = \frac{(\text{Control negative} - \text{tested sample}) \times 100}{\text{Control negative}}
\]

2.4 Phytochemistry

The fruits methanolic extracts were subjected to phytochemical screening using thin layer chromatography technique.

2.4.1 Preparation of thin layer chromatography plates (TLC)

The coating materials were usually applied as aqueous slurries. Slurry was made by mixing 30 g of silica gel G with 50 mL of distilled water in a motor until it was of uniform consistency and free of air bubbles. The slurry was spread using a DESGA spreader at 0.25 mm thick layer on five glass plates (20 x 20 cm). The coated plates were dried at room temperature, then placed vertically in an oven and activated by heating to 110 °C for 30 minutes.

2.4.2 Solvent systems

Twenty mL solvent always freshly prepared mixtures were introduced into the tank one hour prior the chromatography. The tanks were lined with filter paper and were closed tightly by greasing the lid. This was used to assure the saturation of the atmosphere with the solvent vapors. Samples were applied on a starting line about (1-2 cm) from the bottom of the plate, (1-1.5 cm) apart using a tipped dropper.

The chromatographs were developed by the ascending method at room temperature. The most commonly used solvent systems in the present work were:

Chloroform: Hexane (8:2 v/v)
Chloroform: Ethylacetate (8:2 v/v)

Ethylacetate: Hexane (8:2 v/v)

Chloroform: Methanol (17:3 v/v)

Hexane: Chloroform (8:2 v/v)

2.4.3 Detection of spots on TLC

TLC plates were viewed under UV light at 254 and 366 nm for fluorescence or quenching spots. Then sprayed with the appropriate reagent.

R_f values were calculated as follows:

$$R_f \text{ value} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

2.4.4 Preparation of the spray reagents

- Vanillin/H_2SO_4

Six grams of vanillin mixed in 250 mL ethanol and then 2.5 mL concentrated sulphuric acid was added.
CHAPTER THREE
Results and Discussion

3.1 In vitro anti- giardial activity against *Giardia lamblia*

Antigiardial activity test was performed for the methanolic extracts of fruits of Solanaceae plants namely; *Datura stramonium*, *Lycium persicum*, *Solanum dubium* and *S. incanum*. In fact, *G. lamblia* is one of the most common intestinal pathogenic protozoan parasites (Newman et al., 2001). It is becoming increasingly important among HIV/AIDS patients. There are reports that some cases of acute and chronic diarrhea in AIDS patients may be associated with giardial infection (Merchant and Shroff, 1996). However, metronidazole, the common drug of choice, can cause mutagenicity in bacteria (Legator et al., 1975) and is carcinogenic in rodents (Rustia and Shubik, 1972). It also possesses undesirable side effects and treatment failures have been reported (Llibre et al., 1989).

The activity of different tested plants against *G. lambelia* was investigated using three different concentrations and results are presented on Figures 2-5. Extract is considered active with mortality value ≥ 50%.

3.1.1 Antigiardial activity of *Solanum dubium* fruits

The fruits methanolic extract of *S. dubium* showed mortality of 61.9% and 59.9% after 24 h at concentrations 125 and 250 μg/mL respectively. Concentration 500 μg/mL was inactive as it showed weak mortality (≤ 50%) of *G. lambelia*. After 48 h, all extract concentrations used were inactive whereas, after 72 only extract at concentration 125 μg/mL was active with mortality value of 56.5% (Fig. 3).
Fig. 3: Antigiardial activity of *Solanum dubium* fruit methanolic against *Giardia lamblia*.
3.1.2 Antigiardial activity of *Datura stramonium* fruits

The fruits methanolic extract of *D. stramonium* only showed mortality of 64.1% and 52.3% after 72 h at concentrations 125 and 250 μg/mL respectively. Extracts at all concentrations did not antigiardial activity after 24 and 48 h. In contrast, at concentrations 500 and 250 μg/mL an increase of number of *G. lamblia* was observed indicative of the presence of growth stimulators (Fig. 4).

3.1.3 Antigiardial activity of *Solanum incanum* fruits

Antigiardial activity of *S. incanum* fruits was obtained at all concentrations, 125 μg/mL (51.2%), 250 μg/mL (50.0%) and 500 μg/mL (60%) after 72 h. No antigiardial activity was observed for all concentrations after 24 and 48 h (Fig. 5).

3.1.4 Antigiardial activity of *Lycium intricatum* fruits

As observed for *S. incanum* fruit extract, the antigiardial activity of *L. intricatum* fruits methanolic was obtained at all concentrations, 125 μg/mL (55.6%), 250 μg/mL (53.2%) and 500 μg/mL (56.9%) after 72 h. No antigiardial activity was observed for all concentrations after 24 and 48 h (Fig. 6).
Fig. 4: Antigiardial activity of *Datura stramonium* fruit methanolic extract against *Giardia lamblia*.
Fig. 5: Antigiardial activity of *Solanum incanum* fruits methanolic extract against *Giardia lamblia*.
Thus from the above results, the highest effective concentration against *G. lambilia* was obtained from methanolic extract of *S. dubium* at 250 μg/mL with mortality of 60.5% after 24 h and from the methanolic extract of *D. stramonium* at 250 μg/mL with mortality of 64.4% after 72 h. Moreover, the activity of these two extracts was comparable with the positive control (metronidazole).

In this study an interesting observation was noted; an increase in number of parasite in some plant extracts (*D. stramonium* and *S. incanum*) was observed which might reflect the presence of nutritive ingredients for the parasite in these extracts as well. In general, no much work was carried out on the Sudanese medicinal plants for their anti- giardial activity. Elsir *et al.* (2011) investigated the anti- giardial activity of *Citrullus lanatus* var. *citroides* extracts and cucurbitacins isolated compounds. They found that all extracts and isolated compounds showed high anti- giardial activity.
Fig. 6: Antigiardial activity of *Lycium intricatum* fruits methanolic extract against *Giardia lamblia*
3-2 Preliminary phytochemical screening by thin layer chromatography (TLC)

Preliminary phytochemical screening of methanolic extracts from fruits of *S. dubium*, *D. stramonium*, *S. incanum* and *L. intricatum* was carried out using TLC technique. TLC plates of all extracts were developed using vanillin/H$_2$SO$_4$ reagent and results are presented in Figure 7. Several spots with different $R_f$ values were observed in the four extracts indicating that the fruits were rich in secondary metabolites. Interestingly, *S. dubium* and *S. incanum* displayed the same TLC pattern supporting their closer taxonomical relationship. All extracts gave spots with the same colour reaction when sprayed with vanillin reagent (Fig. 7). In fact, *Solanum* plants accumulate, especially green parts, high levels of steroidal alkaloids. Two groups of steroidal alkaloids are distinguished; a spirosolane type with soldulcidine and tomatidine and a solanidine type with solanine and chaconine (Wink and Wyk, 2008). Thus, the observed anti- giardial activity of extracts may be due to the presence of these components. Okwute (1992) reported that alkaloids, saponins, phenolic compounds and cardiac glycosides are known to possess antimicrobial and antiplasmodial activity and pesticide properties.
Fig. 7: Thin layer chromatography of the fruits methanol extract from *Solanum* plants.

Solvent system: Toluene: EtOAc: Formic acid (5:4:1 v/v/v), 1, under UV 254 nm; 2, spray reagent: vanillin/H₂SO₄

CHAPTER Four
Conclusion

Antigiardial activity test was performed for the methanolic extracts of fruits of *Datura stramonium*, *Lycium persicum*, *Solanum dubium* and *S. ineanum*. All the extracts showed varying degrees of activity on the *G. lamblia* parasite. *S. dubium* at 250 µg/mL was the most effective extract against *G. lamblia* with mortality of 60.5% after 24 h whereas, the methanolic extract of *D. stramonium* was effective at 250 µg/mL with mortality of 64.4% after 72 h.

Phytochemical screening revealed that the methanolic extracts of the fruits were rich in secondary metabolites.
Suggestions for future work

- Since plants produce a diverse range of bioactive molecules making them rich sources of different types of medicines, further pharmacological and toxicological of these *Solanum* plants will be necessary to establish their use and safety in the traditional medicine.

- Isolation and identification of pure compounds and evaluation of their biological activity as new source of natural drugs are also recommended.
References


