East West University

Phytochemical and biological investigation
of the rind of *Citrullus lanatus* available in Bangladesh

A thesis report submitted to the department of Pharmacy, East West University, Bangladesh, in partial fulfilment of the requirements for the degree of B. Pharm.

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This is to certify that the dissertation, entitled **"Phytochemical and biological investigation of the rind of *Citrullus lanatus* available in Bangladesh"** Is a thesis work done by Kamrun Nahar (ID: 2012-1-70-034) in partial fulfilment of the requirements for the degree of B. Pharm. We further certify that all the sources of information and laboratory facilities availed in this connection is duly acknowledged.

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I, Kamrun Nahar (ID:2012-1-70-034), hereby declare that the dissertation entitled **"Phytochemical and biological investigation of the rind of *Citrullus lanatus* available in Bangladesh"**, submitted by me to the Department of Pharmacy, East West University, in the partial fulfilment of the requirement for the degree of B. Pharm is a genuine & authentic thesis work carried out by me during Spring 2015- Fall 2015 under the supervision and guidance of Dr. Repon Kumer Saha, Assistant Professor, Department of Pharmacy, East West University.

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ABSTRACT

Objective: *Citrullus lanatus* is a tropical fruit in Bangladesh. The objective of this study is phytochemical and biological investigation of the rind of *Citrullus lanatus* available in Bangladesh.

Methods: Thin layer chormatogarphy were used to detect the presence of various types of compound in rind. In vitro antioxidant effects were measured by DPPH scavenging assay. In vitro anti- diabetic assay was carried out by Glucose uptake in Yeast cells. Disc diffusion assay was performed to show the antibacterial effect using gram positive, gram negative strains of bacteria and fungi. The anti-inflammatory activities of different fraction of *Citrullus lanatus* rind were investigated for In-vitro anti-inflammatory activity by human red blood cell membrane stabilization (HRBC) method. Receptor binding activities was performed by hemagglutination inhibition assay.

Result: some fraction of the *Citrullus lanatus* rind contains flavonoids and other biologically active compounds. The extract showed poor antioxidant. DCM fraction of stripe variety showed antibacterial activities against several strains of bacteria. Xylene fraction of stripe variety showed anti-diabetic activity. DCM fraction of deep green variety showed anti-inflammatory activity. DCM fraction of light green variety showed potential binding capacity to human erythrocytes.

Conclusion: Therefore, all varieties of *Citrullus lanatus* rind contain medicinal active components in different ratios.

RATIONALE AND OBJECTIVE OF THE WORK

Tropical fruits are important constituents in the daily diets of billions of people; and many such fruits either harvested from a wide range of minor species-from wild trees or locally cultivated ones. Many such fruit trees have multi-purpose uses and their plant products satisfy a variety of local non-food purposes ranging from timber to forest .It has been estimated that in developed countries such as United States, plant drugs constitute 25% of total drugs which in fast developing countries such as China and India, the contribution is as much as 80% (Narasimhan and Mohan., 2012). Bangladesh is also a major country where people use a high percentage of medicinal plants for various therapeutic activities. Use of volatile and penetrating plant extracts in therapeutic applications for psychological and physical well-being was in practice from ancient times. Bangladesh is gifted by extraordinary natural resources which continuously help us in many ways. One of the most beneficial natural resources is the plant resource which provides us with food, shelter and medicine. According to the World Health Organization more than 80% of the world population in developing countries depends on plant-based medicines for basic healthcare needs.

The Cucurbitaceae, also called cucurbits and the gourd family, are a plant family consisting of around a hundred genera, the most important of which are:

Cucurbita – squash, pumpkin, zucchini, some gourds

Lagenaria – mostly inedible gourds

Citrullus – watermelon (C. lanatus, C. colocynthis) and others

Cucumis – cucumber (C. sativus), various melons

Luffa – the common name is also luffa, sometimes spelled loofah. Watermelon (Citrullus lanatus var. lanatus, family Cucurbitaceae) is a vine-like (scrambler and trailer) flowering plant originally from southern Africa. It is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and white to yellow flowers. It is grown for its edible fruit, also known as a watermelon, which is a special kind of berry botanically called a pepo. The fruit has a smooth hard rind, usually green with dark green stripes or yellow spots, and a juicy, sweet interior flesh, usually deep red to pink, but sometimes orange, yellow, or white, with

many seeds. It is an important medicinal plant in the Ayurveda and Indian traditional system of medicine . The plant is rich in flavonoids, alkaloids, saponins, glycoside, tannins and phenols. Its nutritive values are also useful to the human health. The plant has been extensively studied by various scientist and researchers for its pharmacological activities and therapeutic approaches such as antibacterial, antifungal, antimicrobial, antiulcer, antioxidant, anti- inflammatory, gastroprotective, analgesic, laxative, antigiardial, hepatoprotective and against prosthetic hyperplasia and atherosclerosis. Fruit is used in cooling, strengthening, aphrodisiac and astringent to the bowels, indigestible, expectorant, diuretic, and stomachic, blood purifier. It also allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic to the brain. (Deshmukh, Jain and Tambe, 2015)

The aim of this research project was to carry out the Pharmacological Activities Investigation and comparison among different varieties of *Citrullus lanatus* rind available in Bangladesh.

CHAPTER ONE

INTRODUCTION

Natural product:

Natural products have been a source of medicinal agents since thousands of year and remarkable numbers of modern drugs have been derived from natural sources, predominantly based on their knowledge available in traditional medicine. The past century, however, has seen an increasing role played by microorganisms in the production of the antibiotics and other drugs for the treatment of serious diseases, and more recently, marine organisms have proved to be a rich source of novel bioactive agents. Natural products will continue to play a crucial role in meeting this demand through the expanded investigation of the world's biodiversity, much of which remains unexplored. (Farnsworth et al., 1985)

It has been projected by the World Health Organization (WHO) that approximately 80% of the world's population relies mainly on traditional medicines for their primary health care. Plant products also play an imperative role in the health care systems of the remaining 20% of the population, mainly residing in developed countries, and at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs at present in use in one or more countries. Of these 119 drugs, 74% were discovered as a result of chemical studies aimed at the isolation of the active substances from plants used in traditional medicine . (Pawar, 2014)

History of natural product:

For thousands of year's natural products have played a very important role in health care and prevention of diseases. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases. The earliest known written document is a 4000 year old Sumerian clay tablet that records remedies for various illnesses. For instance, mandrake was prescribed for pain relief, turmeric possesses blood clotting properties, roots of the endive plant were used for treatment of gall bladder disorders, and raw garlic was prescribed for circulatory disorders. These are still being used in several countries as alternative medicines.

However, it was not until the nineteenth century that scientists isolated active components from various medicinal plants. Friedrich Sertürner isolated morphine from Papaver somniferum in 1806, and since then natural products have been extensively screened for

their medicinal purposes. Atropine obtained from Atropa belladonna, strychnine, a CNS stimulant, ziconotide identified from a cone snail, Conus magus, and Taxol® obtained from the bark of the Pacific yew tree are a few examples of active components isolated from natural sources.

Natural Product as a Source of Lead to the Design of New Drugs:

The debt owed by combinatorial chemistry to organic synthesis is obvious and widely recognized by practitioners of either field. As combinatorial chemistry matures, it is becoming clear that combinatorial chemistry is also fuelling major improvements in organic synthesis. The scope and limitations of a number of venerable reactions is now much better understood with their incorporation into combinatorial sequences for the generation of compound libraries. Many of the techniques developed by combinatorial chemists for parallel synthesis and purification can be profitably employed even when making one compound at a time. Combinatorial chemistry will also have a growing impact on natural product total synthesis, an area that has primarily focused on a target molecule as an end in itself rather than an exciting avenue for discovering new compounds with new properties. (Mitsche et al.,1986)

Novel natural product derivatives can be optimized on the basis of their biological activities using modern sophisticated biosynthetic, combinatorial and high throughput techniques to yield effective chemotherapeutic and other bioactive agents. The phytochemists have always been impressed by the fact that compounds found in nature exhibit an almost incredible range of diversity in terms of their physicochemical and biological properties. Most of these compounds are secondary metabolites with functions in plants, fungi, and marine organisms that are still not extensively understood. At present, it is assumed that many of these compounds act in defense of the harmful effects of toxins, carcinogens, or mutagens found in the plant or attack by external predators (Williams et al.,1989)

The modern natural product research is undergoing a revolution due to recent advancements in combinatorial biosynthesis, microbial genomics and screening processes. Moreover, access to hyphenated techniques like Liquid Chromatography-Mass Spectrometry, Liquid Chromatography-Nuclear Magnetic Resonance have raised the hope of drastically reducing the time and cost involved in natural product research by using

dereplication processes that are combination of techniques to avoid the already reported compounds. The developments of antimalarial and anticancer drugs using some of the lead compounds (Phytoconstituent) isolated from natural source are discussed below. (Woodbury et al.,1961)

Use of plant waste:

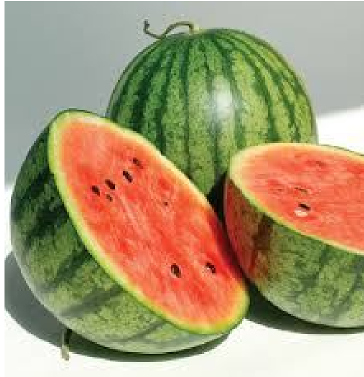
Recently, it has taken a boom the use of fruit and vegetable waste to reduce environmental pollution. Taking into account that these residues are important sources of polyphenols. Agricultural and industrial residues are attractive sources of natural antioxidants and dietary fiber. In Egypt, there are many sources of fruit wastes but there is lake of information about their content and activity of antioxidant compounds. (Karkare, 2007)

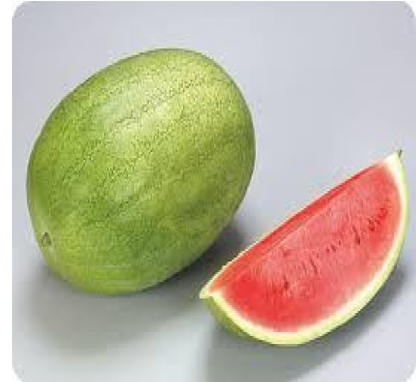
Scientist investigates the extraction and identification of antioxidant compounds in some vegetable and fruit wastes. New by-products applications should be investigated to have a positive environmental impact or to turn them into useful products. Accordingly, the functional properties of some peel components such as, pectin, flavonoids, carotenoids, limonene and polymethoxy flavones should be considered.(Ahmed and Al-Sayeda, 2013)

Medicinal plants represent a rich source of antimicrobial agents. There is also an urgent need to search for a new antimicrobial compounds with novel mechanisms of action because there have been an alarming increase in the incidence of new infections diseases, as well as the development of resistance to the antibiotics in current clinical trials.(Sekar et al., 2014)

Watermelon

Watermelon (Citrullus lanatus var. lanatus, family Cucurbitaceae) is a vine-like (scrambler and trailer) flowering plant originally from southern Africa. It is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and white to yellow flowers. It is grown for its edible fruit, also known as a watermelon, which is a special kind of berry referred to by botanists as a pepo. The fruit has a smooth hard rind, usually green with dark green stripes or yellow spots, and a juicy, sweet interior flesh, usually deep red to pink, but sometimes orange, yellow, or white, with many seed.





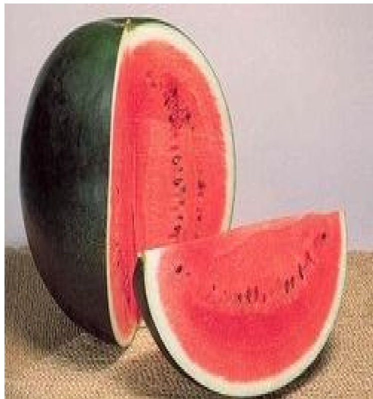


Figure 1: watermelon

Origin and geographic distribution

The wild watermelon is widely distributed in Africa and Asia, but originates from southern Africa occurring naturally in South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Zambia and Malawi. It is cultivated and adventive in warmer parts of the world. Watermelon is thought to have been domesticated at least 4,000 years ago, and the plant was grown as a crop in the Nile valley . The indigenous people of the Kalahari, in their search for water-containing foods, selected varieties with low glycoside content. From there followed the spread to the Mediterranean areas and in an eastern direction to India. Watermelons were developed as a crop in Egypt in ancient times and according to Encyclopaedia Britannica " The history of watermelons is a long one; there is a Sanskrit word for watermelon, and fruits are depicted by early Egyptian artists, indicating an antiquity in agriculture of more than 4,000 years". Purseglove (1968) indicates that it is of ancient cultivation in the Mediterranean and reached India in prehistoric times, but did not reach China until the eleventh century AD. Some websites suggest an introduction to India in 800

AD. C. lanatus var. fistulosus, the "tinda", which has small, apple sized fruit, is grown as a vegetable in India. Modern day cultivated varieties are a popular crop that can be cultivated in any climate that has warm summer, and are best suited to those climates that have long hot summer.(victor, n.d.)

Description of watermelon:

Watermelon plant:

The watermelon is an annual plant with long, weak, trailing or climbing stems which are five-angled and up to 3 m (10 feet) long. Young growth is densely woolly with yellowish-brown hairs which disappear as the plant ages.

Figure 2: watermelon whole plant

Leaves:

The leaves are stemmed and are alternate, large and pinnately-lobed, stiff and rough when old. The plant has branching tendrils.

1 2

Figure 3: young leaf (1), old leaf (2)

Flowers:

The flowers grow singly in the leaf axils and the corolla is white or yellow inside and greenish-yellow on the outside. The flowers are unisexual, with male and female flowers occurring on the same plant (monoecious). The male flowers predominate at the beginning of the season and the female flowers, which develop later, have inferior ovaries.

Figure 4: watermelon flower

Fruit:

The large fruit is a kind of modified berry called a pepo. This has a thick rind (exocarp) and fleshy center (mesocarp and endocarp).Wild plants have fruits up to 20 cm (8 in) in diameter while cultivated varieties may exceed 60 cm (24 in). The rind of this fruit is mid- to dark green and usually mottled or striped, and the flesh contains numerous pips and is red, orange, pink, yellow, green or white.

Figure 5: watermelon

Varieties of Watermelon

All varieties of watermelon share a distinct mouth-watering, thirst quenching, sugary flesh encased by a solid rind. Some watermelon types have higher sugar content and are, thus, sweeter; and some varieties have different colored rind and flesh. Most of us are familiar with the oblong dark green watermelon with vibrant ruby red pulp, but melons may also be light pink, yellow and even orange. Size can vary amongst watermelons from small 5 pounders to a monstrous 200 pounds.

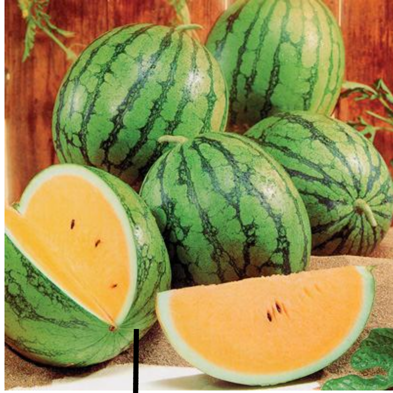
There are four basic types of watermelon: seedless, picnic, icebox, and yellow/orange fleshed.

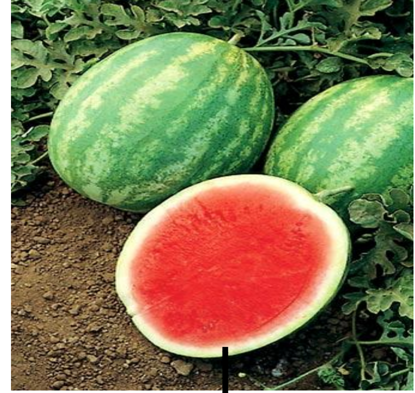
Seedless Watermelons

Seedless watermelons were created in the 1990's. Growing seedless types is a bit more complex than simply planting a seed and letting it sprout. The seed must be kept at a constant 90 degrees F. (32 C.) until emergence. Seedless melons include:

1. Queen of Hearts
2. King of Hearts
3. Jack of Hearts
4. Millionaire
5. Crimson
6. Trio
7. Nova

They do have tiny underdeveloped seeds, despite the name, which are easily consumed. They usually weigh from 10-20 pounds and mature in about 85 days.





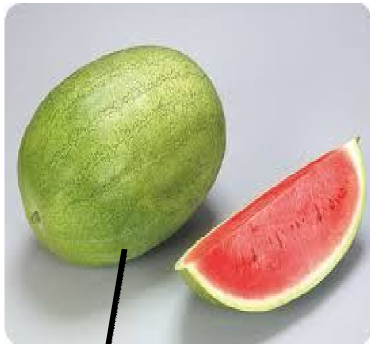
Queen of Hearts **King of Hearts** **Millionaire**

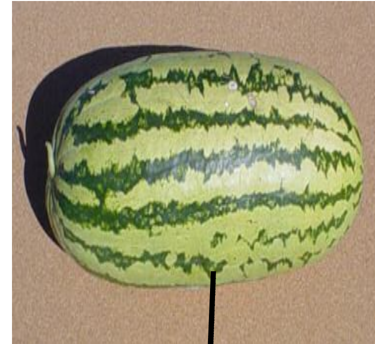
Nova **Crimson**

Picnic Watermelons

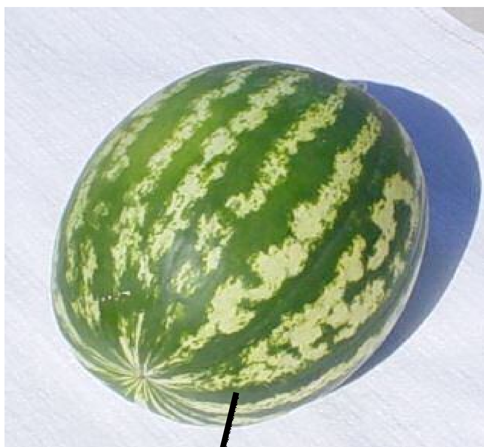
Another watermelon type, the Picnic, tends to be larger, from 16-45 pounds or even larger, perfect for a picnic gathering. These are the traditional oblong or round melons with a green rind and sweet, red flesh – which mature at around 85 days or so. Some varieties here include:

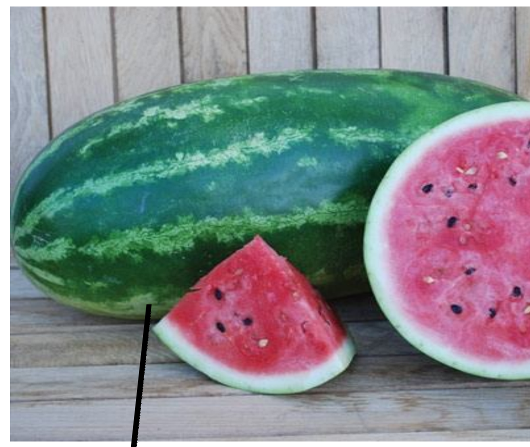
1. Charleston Gray
2. Black Diamond
3. Jubilee
4. All sweet
5. Crimson Sweet





Charleston Gray **Black Diamond** **Jubilee**





Crimson Sweet **All sweet**

Icebox Watermelons

Icebox watermelons are bred to feed one person or a small family and, as such, are much smaller than their counterparts at 5-15 pounds. Watermelon plant varieties in this genre include

1. Sugar Baby 2. Tiger Baby.

Sugar Babies are sweet pulped with dark green rinds and were first introduced in 1956, while Tiger Babies are gray-green with dark green stripes.

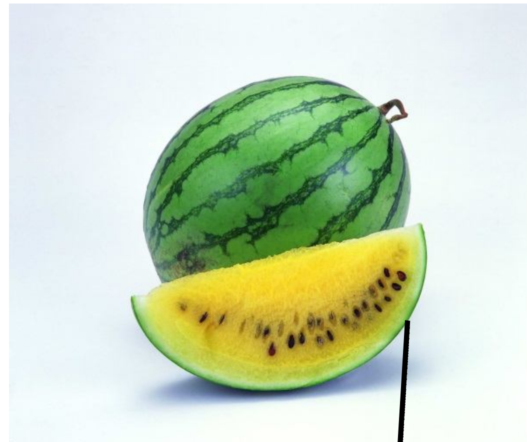
Sugar Baby **Tiger Baby**

Yellow/Orange Watermelons

Lastly, the yellow/orange fleshed watermelon plant varieties, which are typically round and can be both seedless and seeded. Seeded varieties include:

1. Desert King 2. Tender gold

3. Yellow Baby 4. Yellow Doll



Yellow doll

Yellow baby

Seedless varieties include

1. Chiffon 2. Honey heart.

These melons mature in about 75 day. (Gardening Know How, 2015)

Watermelon requirement to grow

Watermelon is one of summer's favorite fruit. It is sweet and delicious and easy to grow. Growing watermelon requires a so much space in the garden and at least three months of reliably hot, sunny weather to grow and ripen a watermelon. During that time average daily maximum temperature should be at least about 20-25°C or 70-80F. Warmer is even better. In cooler climates, watermelon varieties that mature faster may grow.(Tropicalpermaculture.com, n.d.)

Climate

- The plants are susceptible to frost, and low temperatures can cause growth abnormalities, poor setting and hollow fruit.

- Sunburn can be a problem for the fruit, particularly in the 'ground spot' area if the fruit are placed upside down after harvest.

- Light green and grey-green melons are less likely to sunburn than the darker green or striped varieties.(Queensland Government, 2010)

Soil

- Watermelon grows best in soil that has plenty of manure or compost and soil that drains well.

- Watermelons prefer a soil pH between 6 and 6.8.

Figure 6: Quality of soil for watermelon

Planting

- Make mounds of dirt about 3 feet wide and 1foot high.
- Allow 4-5 feet of space between mounds and plant 1 inch deep.

Figure 7: planting strategy of watermelon

Watering

- Watermelon does not take drought.
- So Watermelon requires water frequently (water under the leaves not on top).
- Once it starts producing fruit, cut down on watering.(Instructables.com, n.d.)

Scientific Classification

Following is the scientific classification of watermelon

Kingdom – Plantae

Division – Magnoliophyta

Class – Magnoliopsida

Order – Cucurbitales

Family – Cucurbitaceae

Genus - Citrullus

Species – C. lanatus

Binomial name - Citrullus lanatus

(Fruitdirectory.com, n.d.)

Local name of watermelon

Table 4: Local name of watermelon in different countries

Common name: Watermelon, Wild Watermelon

Local name: Tarbooz

English: Watermelon

Marathi: Tarbooz, Kalingad

Bengali: Tormuz

Malayalam: Thannimathan

Kanada: Kallagadi

Assamese: Tarmuj

Telugu: Pendalam

Tamil: Kizhangu

(Deshmukh, Jain and Tambe, 2015)

Nutritional Value

See the table below for in depth analysis of nutrients:

Watermelon (Citrullus lanatus), fresh, Nutritive Value per 100 g,

(Source: USDA National Nutrient data base)

Principle	Nutrient value	Percentage of RDA (Recommended daily allowance)
Energy	30 Kcal	1.5%
Carbohydrate	7.6 g	6%
Protein	0.6g	1%
Total fat	0.15 g	0.5%
Cholesterol	0 g	0 %
Dietary fiber	0.4 g	1 %
Vitamins		
Folates	3 µg	1 %
Niacin	0.178 mg	1 %
Pantothenic acid	0.221 mg	4.5 %
Pyridoxine	0.045 mg	3.5 %
Thiamin	o.o33 mg	3 %

Vitamin A	569 IU	19 %
Vitamin C	8.1 mg	13.5 %
Vitamin E	0.05 mg	0.5 %
Electrolytes		
Sodium	1 mg	0 %
Potassium	112 mg	2.5 %
Minerals		
Calcium	7 mg	0.7 %
Copper	42 µg	4.5 %
Iron	0.24 mg	3 %
Magnesium	10 mg	2.5 %
Manganese	0.038 mg	1.5 %
Zinc	0.10 mg	1 %
Phyto – nutrients		
Carotene – alpha	303 µg	-
Crypto –xanthin –beta	78 µg	-
Lutein – zeaxanthin	8 µg	-
Lycopene	4532 µg	-

(Krans, 2015)

CHAPTER TWO

LITERATURE REVIEW

Chemical constitute:

Watermelon Composition

Watermelon biomass can be categorized as three main components which are the flesh, seed, and rind. As shown in Figure 2.1, the flesh constitutes approximately 68% of the total weight, the rind approximately 30%, and the seeds approximately 2%.

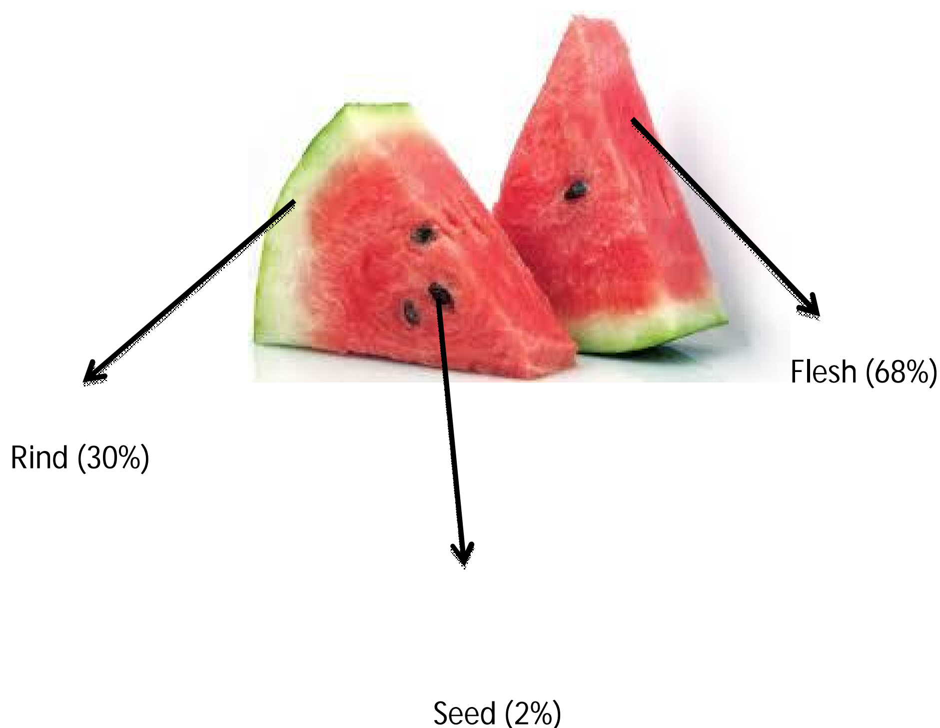


Figure 8: Different parts of watermelon

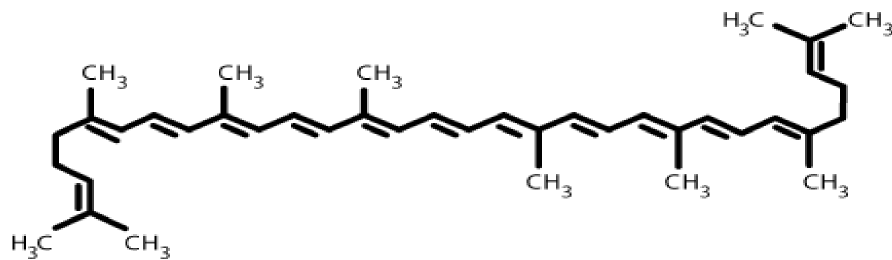
The composition of the flesh, seed, and rind vary considerably. One hundred grams of watermelon flesh was analyzed and found to contain 92.6g water, 0.5 g protein, 0.2 g fat, 6.4 g total carbohydrate, 0.3 g fiber, 0.3 g ash, and a number of vitamins and minerals including 0.7 mg calcium, 590 international units (IU) vitamin A, 0.03 mg thiamine, 0.03 mg riboflavin, 0.2 mg niacin, and 7 mg ascorbic acid. The seed is approximately 42% kernel and 58% hull. Approximately 4.36% of the rind is peel and the other is the inside whitish

portion. One study states that the rind is 93.8% moisture, 0.49% ash, 0.1% nitrogen, and 2.1% sugars. The rind is higher in percent fresh weight, dietary fiber, and potassium but lower in total sugar than the flesh. (campbell, 2015)

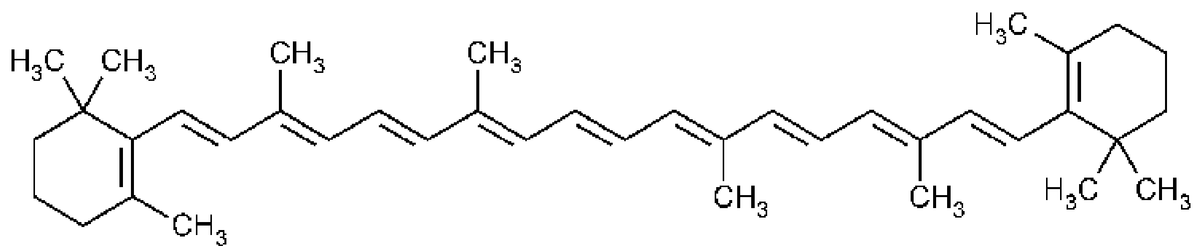
Flesh contain

- Vitamin-A
- Lycopene,
- Beta-carotene,
- Lutein,
- zeaxanthin
- cryptoxanthin
- Potassium
- Vitamin-B6 (pyridoxine),
- Thiamin (vitamin B-1),
- Vitamin-C,
- Manganese
- Pantothenic acid
- Copper
- Biotin

(Rudrappa, n.d.)



Lycopene

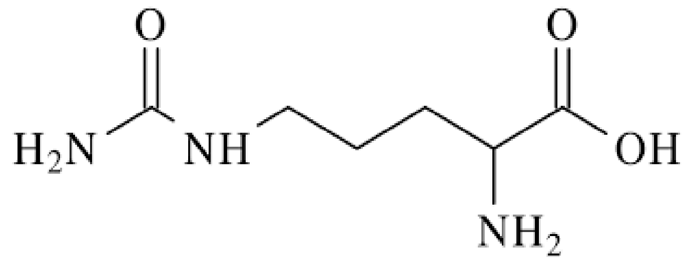


Beta carotene

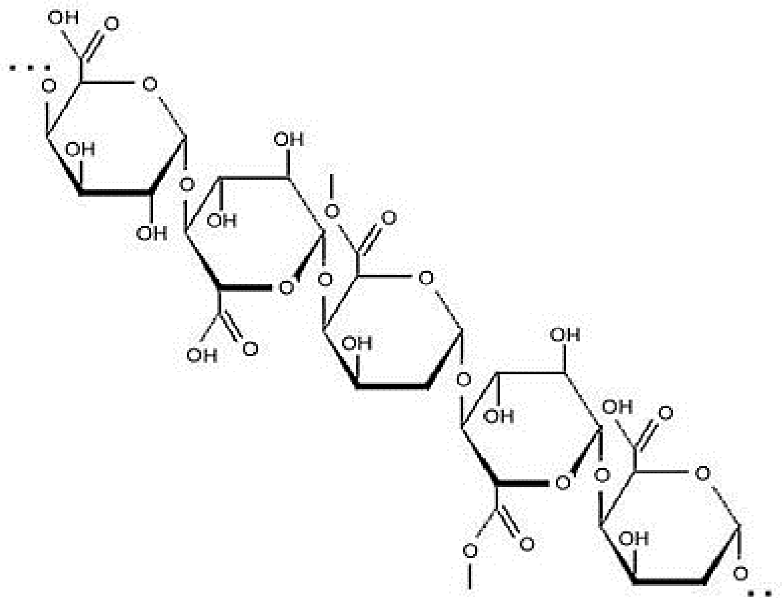
Rind contain

- Citrulline,
- Cellulose,
- Hemicellulose,
- Lignin,

- Pectin,
- Silica,
- Silica free minerals.



citrulline



Pectin

Seed contain

- Protein,
- Melon oil,
- Fiber
- Iron,

- Zinc

Table: Qualitative phytochemical composition of various parts of watermelon

Phytochemicals	Samples			
	Mesocarp	Juice	Seeds	Whole fruit
Saponnin	+	+	+	+
Tannin	-	-	-	-
Anthraquinone	+	+	-	+
Flavonoids	+	+	+	+
Steroids	-	-	-	-
Terpenoids	+	+	+	+
Alkaloids	+	-	+	+
Phlobatannin	-	+	-	+
Cardiac glycosides				
Keller killiani	+	+	+	+
Legal test	+	+	+	+
Steroid	-	-	-	-
Flavonoid	+	+	+	+

(Oseni and Okoye, 2013)

Pharmacological Action:

Citrullus lanatus has been reportedly used widely in traditional herbal medicine.

Fruits

The fruits of *Citrullus lanatus* are eaten as a febrifuge when fully ripe or even when almost putrid. The fruit is also diuretic and is effective in the treatment of dropsy and renal stones. Preliminary research indicates that the consumption of watermelon may have antihypertensive effects. The fruits are used as a drastic purgative in Senegal; they are also used to treat diarrhoea and gonorrhoea in Nigeria.

Root

The root is purgative and in high dose it can also serve as emetic.

Seed

The seed is demulcent, pectoral and tonic. It is sometimes used in the treatment of the urinary tract infections as well as bed wetting. The seed is also a good vermifuge and has a hypotensive action. Fatty oil in the seed, as well as aqueous or alcoholic extracts, had been reported to paralyze tapeworms and roundworms. Tar is extracted from the seeds and used for the treatment of scabies and for skin tanning. The seed oil has an anthelmintic action which is better than that of pumpkin seed oil.

Rind

The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes. *Citrullus lanatus* is used in Northern Sudan for burns, swellings, rheumatism, gout and as laxative. (Erhirhie and Ekene, 2013)

Pharmacological effect of rind of watermelon

Most people discard the rind of the juicy watermelon, but don't be so hasty – the watermelon rind has many benefits. Whether you eat the rind or use it topically, this often-wasted food can do good things for your body. Finding a use for it also helps cut down on the amount of garbage you produce, so it's good for the environment as well.

Figure 9: Rind of watermelon

Nutritional Benefits

The rind may not be as juicy as the flesh of a watermelon, but you can eat it. A 1-inch cube of watermelon rind contains 1.8 calories. The majority of the calories come from carbohydrates, with 0.32 g per serving. While you will not derive a tremendous amount of macronutrients from eating watermelon rind, this food does contain some vitamins. One serving provides 2 percent of the daily recommended intake of vitamin C and 1 percent of the vitamin B-6 your body requires every day. This makes watermelon rind good for your skin and immunity, as well as the health of your nervous system.(Wolf, 2015)

Health benefits of eating watermelon rind

1. Fights free radicals

According to the Journal of the Science of Food and Agriculture, citrulline is a compound found in watermelon rind that plays a role in banishing free radicals. It's converted to an amino acid that ultimately provides heart and immune system protection and keeps harmful toxins from residing in the body.

2. May boost libido

Researchers from Texas A&M's Fruit and Vegetable Improvement Center have noted that citrulline helps blood vessels relax and can even help with erectile dysfunction. In fact, its ability to boost blood flow have led to watermelon's citrulline to be referred to as "nature's Viagra."

3.Weight loss

Once again, citrulline comes into play. Studies have shown that consuming citrulline leads to 30 percent less weight gain. The thought is that the citrulline in the rind leads to less muscle fatigue, which therefore allows people to extend the duration of their workouts and, in turn, better manage their weight.(Michelle, 2014)

4. It Can Reduce Your Blood Pressure

Some research has shown that watermelon extract supplements are able to help obese adults control their blood pressure. Watermelon is also a potential diuretic, which often is prescribed for people with high blood pressure.

5. It Can Help Your Prostate

Watermelon is a great source of lycopene, an antioxidant that could possibly help ward off prostate cancer. More research is needed to establish a concrete relationship between lypocene and cancer prevention, but preliminary studies are promising. Watermelon's diuretic properties can also help keep your urinary tract in healthy working order.(Krans, 2015)

Anti-oxidant activity:

In Vitro Antioxidant Activity (DPPH Based) Assay

The in vitro antioxidant activity of the sample was quantitated according to the traditional method of Blois(1958).To 1ml of plant extract, 1ml of methanolic solution of 2,2-diphenyl -1-picryl –hydrazyl (DPPH)(0.2mM) was added.The mixture was incubated in the dark for 30min.The absorbance of the yellow colour solution was read at 517nm on a spectrophotometer using distilled as blank.

$$\text{DPPH scavenged}(\%) = (A_{DPPH} - A_{sample}) / A_{DPPH} \times 100$$

The order of DPPH radical scavenging activity in vitro for the aqueous extract of water melon plant parts was : Mesocarp >Endocarp >Epicarp for all the concentrations investigated(2- 10%).There was a concentration dependent decrease in antioxidant activity as the concentration of the extract increased.The aqueous mesocarp extract demonstrated high antioxidant activity in vitro(74.25%) at 2% concentration , while the aqueous extracts of epicarp and endocarp exhibited weak antioxidant activities in vitro(5.32% at 10% concentration and 15.96% at 10% concentration ,respectively.(Olabinri et al., 2013)

Nitric oxide radical (NO.) scavenging assay

Nitric oxide (NO.) generated from sodium nitroprusside (SNP). Briefly, the reaction mixture (5.0ml) containing SNP (5mM) in phosphate buffered saline (pH 7.3), with or without the seed extract at different concentrations, was incubated at 25 oC for 180min in front of a visible polychromatic light source (25 Watt tungsten lamp). The NO radical thus generated interacted with oxygen to produce the nitrite ion (NO 2–) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% N- naphthylethylenediamine dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was measured at 546nm. The nitrite generated in the presence or absence of the seed extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations.

Scavenging Effect of Water Melon Seed Extract on Nitric Oxide (NO.) Production The nitric oxide level in all the assay media increased with time as depicted by the increase in nitrite levels. In the 5 mM sodium nitroprusside (SNP) only medium the levels of nitrite and hence nitric oxide (NO .) was significantly higher ($p<0.05$) at every time interval compared to the observed levels in the presence of the water melon seed extract. (Etim, Ekanem and Sam, 2015)

Antioxidant screening of Methanolic extract of Cirullus lanatus (MECL) leaves was done by various in vitro methods.

Procedure: A stock solution of 0.5mg/ml concentration of Methanolic extract of C. lanatus was prepared. To 1ml of various concentrations of test samples, 4ml of DPPH was added. Control was prepared without sample in an identical manner. DPPH was replaced by Ethanol in case of blank. The reaction was allowed to be completed in the dark for about 30min. Then the absorbance was measured at 517nm. Vitamin C was used as standard. The percentage scavenging was calculated using the formula [(Control- Test)/Control] x 100. A graph was constructed by plotting concentration versus percentage inhibition and a linear regression equation calculated. The concentration of the sample required for 50% reduction in absorbance (IC50) was calculated using linear regression analysis. A triplicate reading was taken and average was calculated. Percentage inhibition of MECL was 78.16 % at the concentration of 80 µg/ml and IC50 was 27.29 µg/ml. (Aruna, Vijayalakshmi and Karthikeyan, 2014)

Anti - diabetic Activity:

The anti-diabetic potential of watermelon (*Citrullus vulgaris* Schrad) was evaluated in vivo. ICR mice were fed experimental diet containing none, 10% watermelon flesh powder (WM-P) or 1% watermelon rind ethanol extract (WM-E). At the end of 4 weeks, mice were administrated with streptozotocin (40 mg/kg, i.p.) for 5 consecutive days to induce diabetes. Supplementation with WM-E significantly decreased blood glucose level and increased serum insulin levels. Feeding of WM-P also induced moderate changes but those were not statistically significant. Immuno-histochemical analysis showed watermelon that effectively protected pancreatic cells death, which suggest that watermelon has a beneficial effect on diabetes.(Erthirhie and Ekene, 2013)

Anti-inflammatory activity:

Anti-Inflammatory Activity In-vivo and in-vitro anti-inflammatory activity of Citrullus lanatus seed oil (CLSO) in carrageenan- induced paw edema in rat model and In-vitro anti-inflammatory activity was carried out. The potency of the oil compared with standard diclofenac (10 mg/kg) showed significant reduction of edema in carrageenan induced rat paw edema model maximum at 3 hr (percentage reduction in paw volume 44.44%, 55.56% and 63.11% for CLSO(50 mg/kg), CLSO(100mg/kg) and diclofenec(10mg/kg) respectably and CLSO at concentration of 100, 250 and 500 mcg/ml showed 42.35%, 68.48% and 78.50% protection of

HRBC in hypotonic solution respectably. All the results were compared with standard diclofenac at 50, 100 and 200 mcg/ml which showed 43.74%, 63.93% and 86.73% protection of HRBC in hypotonic solution respectabl . (Erhirhie and Ekene, 2013)

Antibacterial activity:

The antibacterial activity of the four (4) extracts of the C.lanatus seeds were tested using the Agar well diffusion techniques standardized inocula culture of the respective test organisms was spread evenly on the surface of nutrient agar plates. Wells of 6mm were aseptically punched on the agar using a sterile cork borer allowing at least 30mm between adjacent wells and the Petri dish. Different concentrations of the 4 different extracts (1000, 500, 125and 62.5mg) of *C. lanatus* seeds were then introduced into the wells. Each extract was screened separately. The plates were incubated at 370C for 24hours. Activity was determined by measuring the diameter of the zone of inhibition produced by the extracts against the test organisms. The different concentrations were used for determine the minimum inhibitory concentration using Mueller Hinton Agar.

The result of antibacterial activity of the extract against selected human pathogens indicated that the plant sample was active against a wide variety of human pathogenic bacteria. Ethanol extracts exhibited the highest inhibitory effect followed by methanol, hot water and cold water in that trend.(Nwankwo, Onwuakor and Nwosu, 2014)

Adsorbent property:

Watermelon rind (WR) an agro waste was evaluated as an adsorbent for the removal of Cr3+ ions from aqueous solution. Batch mode adsorption studies were performed by varying parameters such as pH, contact time, adsorbent dose and initial metal ion concentration. Maximum loading capacity of WR was found to be 172.6 mg g−1 for Cr3+ ions at pH 3. Kinetic studies show that removal of Cr3+ ions is rapid and follows a pseudo second order model. Equilibrium data fit better with the Langmuir isotherm than the Freundlich isotherm. Thermodynamic parameters such as $\Delta G°$, $\Delta H°$ and $\Delta S°$ were derived for the current system. The negative values of $\Delta G°$ show that the nature of sorption process is spontaneous. FTIR studies

reveal that hydroxyl and carboxyl groups were responsible for uptake of metal ions. Successive desorption of Cr^{3+} ions reveals that WR can be an economical sorbent for the removal of heavy metals from aqueous solution. The results conclude that watermelon rind an agro waste can effectively be used for the removal of chromium ions from aqueous solution.(Reddy, Lakshmipathi and Saradr ,2014)

Ameliorative activity:

The present study investigated the effects of the methanolic extract of the rind of Citrullus lanatus on lead acetate induced toxicity on semen parameters, reproductive hormone assay and testicular histology in male albino Wistar rats. Results obtained showed that, compared to control rats, administration of the methanolic extract of the rind Citrullus lanatus significantly enhanced sperm count and all reproductive hormone levels ($P<0.05$); and also caused non-significant increases in sperm motility, percentage of spermatocytes with normal morphology and percentage of live spermatocytes, but decreased percentage of dead spermatocytes ($P>0.05$). Treatment with lead acetate caused a significant reduction in levels of all reproductive hormones and significant diminution of sperm motility, morphology, viability; with increases in percentage of dead spermatocytes ($P<0.05$). Expectedly, co-administration of the methanolic extract of the rind of Citrullus lanatus with lead acetate ameliorated the deleterious effects of lead acetate resulting in significant increases in sperm count and all reproductive hormones ($P<0.05$) and non-significant increases in motility, morphology and live spermatocytes ($P>0.05$): however, the percentage of spermatocytes with abnormal heads were significantly increased. The results suggest that the methanolic extract of the rind of Citrullus lanatus exerts a possible ameliorative effect on lead acetate induced toxicity on some reproductive parameters in male albino Wistar rats.(Kolawole , Dapper and Ojeka ,2014)

CHAPTER THREE

METHODS AND MATERIALS

Identification and collection of *Citrullus lanatus* (watermelon rind)

Citrullus lanatus(watermelon rind) collection:

Three varieties of watermelons are available throughout the country during the summer season. The watermelons were collected from Tongi Bazar, Gazipur, Bangladesh. 15 watermelon of stripe, 15 watermelon of deep green, 15 watermelon of light green were collected during April 2015.

Citrullus lanatus (watermelon rind) Identification:

It is advisable to attempt field identification of sample collected. To aid taxonomoic experts in confirming the field identification of sample collected. To aid taxonomic experts in confirming the field identification and to get a permanent scientific record (accession number) a voucher specimen was prepared on 21 June, 2015. In the voucher specimen the dried leaf were attached and some information like local name, botanical name, family, location of the sample plant were also written on that voucher specimen. Finally, from BNH (Bangladesh National Herbarium) I got the identification or accession number of collected sample on 21 June, 2015, and the accession number is 41636 with *Citrullus lanatus* and Cucurbitaceae scientific name and family name of the plant respectively.

Citrullus lanatus (watermelon rind) peeling, maceration and preservation:

1. The melons were washed with fresh water so that any dust particles are not attached to the sample.

2. Then the melons were dried so that no water remains on the melon surface.

3. The outer most skin of the exocarp of samples was peeled with a peeler.

4. The rinds were pieced into small pieces.

Figure 10: pieces of the collected rind of *Citrullus lanatus* and maceration with blender

3. Then the rind pieces were macerated with a blender.

4. The liquids were collected in a beaker. It is the crude extract.

5. The crude extract were labeled and. All the samples were preserved in deep fridge in -180 c.

The crude extract of three varieties were collected and preserved in the same way.

Filtration of the collected liquid

After the maceration process the liquid was filtered with sterilized cotton filter. The cotton was folded four times and fitted in a funnel. The filtrate was collected in a beaker. Then again it was filtered and this time Whatman's filter (Double Rings Filter Paper, Source- China) was used for getting more clear extract.

Figure: Whatman's filter paper

Principle of liquid-liquid extraction

Liquid–liquid extraction is also known as solvent extraction and partitioning. It is a method to separate compounds from two different immiscible liquids based on their relative solubilities. The liquids are usually water and an organic solvent. It is an extraction of a substance from one liquid into another liquid phase. Liquid–liquid extraction is a basic technique in chemical laboratories, where it is performed using a separating funnel.

Figure 11: Separating funnel

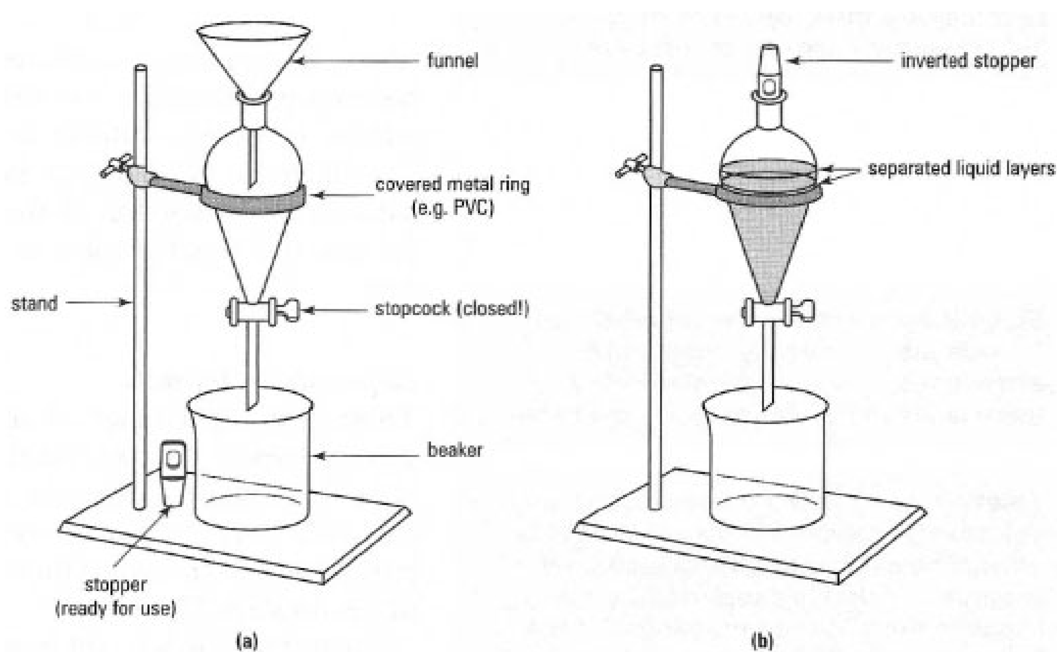


Figure 12: (a) separating funnel ready to use (b) separating funnel in use

The term partitioning is the chemical and physical processes that are involved in liquid–liquid extraction. A popular aphorism used for solubility is "like dissolves like". This indicates that a substant will dissolve best in a solvent which has chemical structures that is similar to the

solvent. The solvation power of a solvent depends primarily on its polarity. For example, urea which is a very polar (hydrophilic) solute is very soluble in water which is highly polar, less soluble in methanol which is fairly polar, and practically insoluble in solvents such as benzene which is non-polar. In contrast, a non-polar or lipophilic solute such as naphthalene is insoluble in water, fairly soluble in methanol, and highly soluble in non-polar benzene. The solubility of a substance fundamentally depends on the physical and chemical properties of the solute and solvent as well as on temperature, pressure and the pH of the solution.

Preparation of pet ether extract

1. 150 ml of crude rind extract was poured in a 250 ml separating funnel.

2. 50 ml of pet ether was added to the separating funnel.

3. The crude extract and pet ether containing separating funnel was mildly inverted back and forth twenty times stopping every three times to allow gas to escape.

4. It was kept stand still for 5 minutes for separation to occur.

5. As the density of pet ether is lower than water, the bottom layer was the organic layer. The organic layer in the funnel was released into a beaker.

6. Then the aqueous layer was collected in another beaker.

7. Another 150 ml crude extract was poured in the separating funnel and the released 50 ml pet ether was added. The organic layer and aqueous layer were collected in the same way.

8. The process of separation was repeated until all the rind extract was extracted.

9. Component of crude extract soluble in pet ether was retained in the organic layer.

10. At last the organic layer containing beaker was labeled properly and it was kept under the laminar flow hood where the organic solvent was evaporated.

11. After solvent evaporation the sample remaining in the beaker was collected and labeled

Same procedure was followed for three varieties of watermelon. Pet ether extract was found only for light green varieties.

Preparation of Cyclohexane extract

1. 150 ml of crude rind extract was poured in a 250 ml separating funnel.

2. 50 ml of cyclohexane was added to the separating funnel.

3. The crude extract and cyclohexane containing separating funnel was mildly inverted back and forth twenty times stopping every three times to allow gas to escape.

4. It was kept stand still for 5 minutes for separation to occur.

5. As the density of cyclohexane is lower than water, the bottom layer was the organic layer. The organic layer in the funnel was released into a beaker.

6. Then the aqueous layer was collected in another beaker.

7. Another 150 ml crude extract was poured in the separating funnel and the released 50 ml pet ether was added. The organic layer and aqueous layer were collected in the same way.

8. The process of separation was repeated until all the rind extract was extracted.

9. Component of crude extract soluble in cyclohexane was retained in the organic layer.

10. At last the organic layer containing beaker was labeled properly and it was kept under the laminar flow hood where the organic solvent was evaporated.

11. After solvent evaporation the sample remaining in the beaker was collected and labeled.

Same procedure was followed for three varieties of watermelon. Cyclohexane extract was found for all varieties.

Preparation of xylene extract

1. 150 ml of crude rind extract was poured in a 250 ml separating funnel.

2. 50 ml of xylene was added to the separating funnel.

3. The crude extract and xylene containing separating funnel was mildly inverted back and forth twenty times stopping every three times to allow gas to escape.

4. It was kept stand still for 5 minutes for separation to occur.

5. As the density of xylene is lower than water, the bottom layer was the organic layer. The organic layer in the funnel was released into a beaker.

6. Then the aqueous layer was collected in another beaker.

7. Another 150 ml crude extract was poured in the separating funnel and the released 50 ml pet ether was added. The organic layer and aqueous layer were collected in the same way.

8. The process of separation was repeated until all the rind extract was extracted.

9. Component of crude extract soluble in xylene was retained in the organic layer.

10. At last the organic layer containing beaker was labeled properly and it was kept under the laminar flow hood where the organic solvent was evaporated.

11. After solvent evaporation the sample remaining in the beaker was collected and labeled.

Same procedure was followed for three varieties of watermelon. Xylene extract was found for all varieties.

Preparation of dichloromethane extract

1. 150 ml of crude rind extract was poured in a 250 ml separating funnel.

2. 50 ml of dichloromethane was added to the separating funnel.

3. The crude extract and dichloromethane containing separating funnel was mildly inverted back and forth twenty times stopping every three times to allow gas to escape.

4. It was kept stand still for 5 minutes for separation to occur.

5. As the density of dichloromethane is higher than water, the upper layer was the organic layer. The aqueous layer in the funnel was released into a beaker.

6. Then the organic layer was collected in another beaker.

7. Another 150 ml crude extract was poured in the separating funnel and the released 50 ml dichloromethane was added. The organic layer and aqueous layer were collected in the same way.

8. The process of separation was repeated until all the rind extract was extracted.

9. Component of crude extract soluble in dichloromethane was retained in the organic layer.

10. At last the organic layer containing beaker was labeled properly and it was kept under the laminar flow hood where the organic solvent was evaporated.

11. After solvent evaporation the sample remaining in the beaker was collected and labeled

Same procedure was followed for three varieties of watermelon. Dichloromethane extract was found for all varieties.

Preparation of chloroform extract

1. 150 ml of crude rind extract was poured in a 250 ml separating funnel.

2. 50 ml of chloroform was added to the separating funnel.

3. The crude extract and chloroform containing separating funnel was mildly inverted back and forth twenty times stopping every three times to allow gas to escape.

4. It was kept stand still for 5 minutes for separation to occur.

5. As the density of chloroform is higher than water, the upper layer was the organic layer. The aqueous layer in the funnel was released into a beaker.

6. Then the organic layer was collected in another beaker.

7. Another 150 ml crude extract was poured in the separating funnel and the released 50 ml pet ether was added. The organic layer and aqueous layer were collected in the same way.

8. The process of separation was repeated until all the rind extract was extracted.

9. Component of crude extract soluble in chloroform was retained in the organic layer.

10. At last the organic layer containing beaker was labeled properly and it was kept under the laminar flow hood where the organic solvent was evaporated.

11. After solvent evaporation the sample remaining in the beaker was collected and labeled

Same procedure was followed for three varieties of watermelon. Chloroform extract was found for all varieties.

Thin Layer Chromatography:

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation .TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC is to obtain well defined, well separated spots.

Principle of Thin Layer Chromatography:

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. (The plate itself contains a fluorescent dye which glows everywhere except where an organic compound is on the plate.)(Orgchem.colorado.edu, 2015)

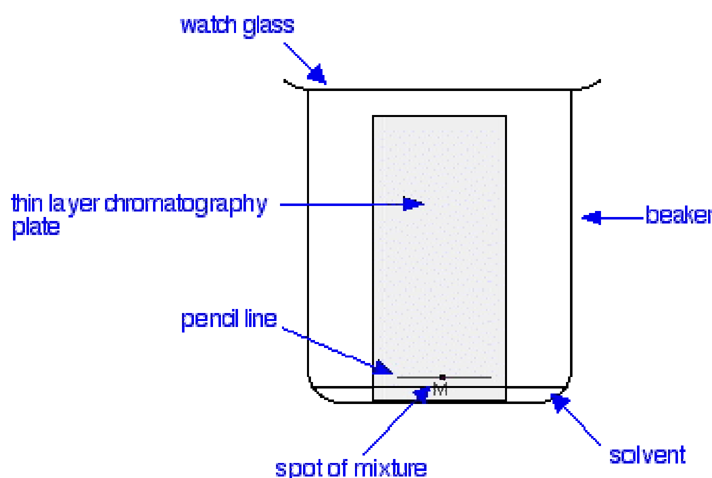


Figure 13: Thin layer chromatography instruments

Solvent Systems of Thin Layer Chromatography

Determining the optimum solvent mixture for your TLC experiment can be challenging, as there are no steadfast rules governing this procedure. It is almost entirely a matter of building experience through trial and error. However, understanding how chromatography works can make your guesswork a bit more educated.

While we have discussed a number of different interactions that occur between the mobile phase, the stationary phase, and the accompanying analyte, the factor that is most important here is polarity.The analyte exists in equilibrium between the stationary and mobile phases. If there exists a greater polar attraction between the solvent and the analyte than the silica and the anaylte, then the analyte will spend more time traveling in the mobile phase along the plate than it will being attached to the stationary phase. Thus, the greater the polarity of a solvent, the greater the elution. However, this is all relative to the polarity of the analyte itself. If the analyte has a greater polarity than the solvent, it will remain more easily attached to the silica. Thus, different analytes will behave differently to the polarity of a solvent, depending on their own polarities. By experimenting with solvent mixtures and keeping in mind this idea of polarity, one can achieve a desirable separation.(Umich.edu,n.d.)

Solvent Systems of TLC

Nonpolar basic solvent

Benzene 9 ml

Ethanol 1 ml

Ammonium hydroxide 0.1 ml

Intermediate polar solvent

Chloroform 5 ml

Ethyl acetate 4 ml

Acetic acid 1 ml

Polar Basic solvent

Ethyl acetate 8 ml

Ethanol 1.2 ml

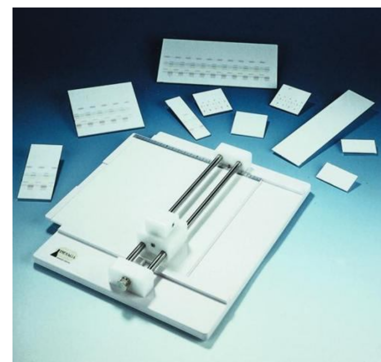
Water 0.8 ml

Apparatus used for Thin Layer Chromatography

1. TLC tank
2. Watch glass
3. TLC plate
4. Spray bottle
5. Pencil
6. Hot plate
7. Scale
8. UV lamp
9. Pipette
10. Capillary tube
11. Pumper
12. Tweezers
13. TLC plate cutter

1

2



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4 5 6

Figure 14: TLC tank (1), TLC Plate (2), TLC plate cutter (3), Spray bottle (4), Hot plate (5), Tweezers (6)

Chemicals Needed for Thin Layer Chromatography:

List of chemicals for TLC

TLC solvent:

Solvent system-1: non polar basic solvent

-Benzene: Ethanol: Ammonium hydroxide (9:1:0.1)

Solvent system-2: polar basic solvent

-Ethyl acetate: Ethanol: water (8:1.2:0.8)

Solvent system-3: semi polar solvent

- Benzene: Chloroform (6:4)

Procedure for Thin Layer Chromatography:

1. A spatula tip amount of extracted sample of three varieties were taken in different watch glass and dissolved in their respective solvent, at last labeled properly.

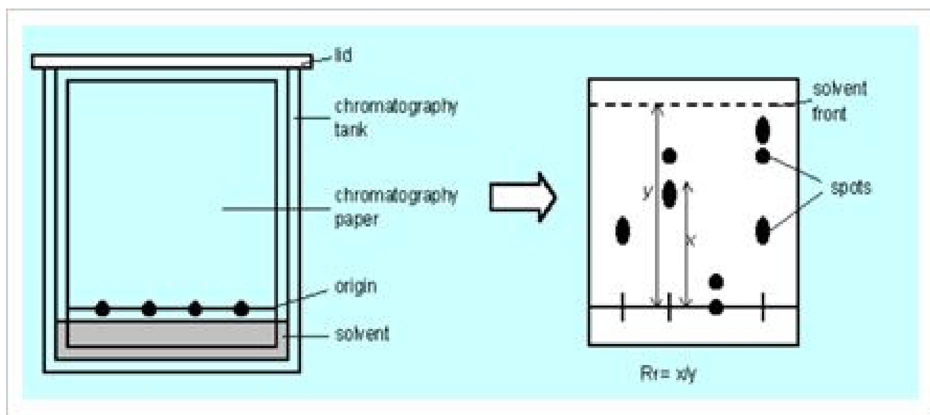
2.2 TLC plates were taken. The plates were marked as plate-e1, plate-2. A pencil was used to lightly mark a straight line about 0.5 cm from both end of the plates.

3. With a capillary micropipette a small spot of the extracted sample was spotted on the plates. Separate capillary micropipette was used for each sample. To avoid confusion, the spots were lightly labeled below the pencil line.

4. The TLC plates were developed by placing them in the TLC chamber that has been filled with the developing solvent. The solvent was allowed to migrate up the TLC plates until it reached the marked top end line.

5. The plates were then removed immediately and the solvent was allowed to evaporate. After that it was visualized under UV light and all the spots were marked.

6. The distance that the solvent moved was measured for the distances of all spots.



X =distance travels by mobile phase

Y= distance travels by spot

Fig: Distance of spot for Rf value

7. The Rf value was then calculated. It can be calculated as:

Rf = Distance spot travels / Distance solvent travels

Charring with H2SO4:

Then charring of 2 plates with 10% H_2SO_4 solution was carried out for further confirmation.

Staining with DPPH (2,2-diphenyl-1-picrylhydrazyl):

0.4% DPPH solution was prepared with methanol as solvent and labeled as stock solution. From the stock solution 10% DPPH solution was taken in a petri dish and the TLC plates were dipped in it. The plate was visually observed for a color change after sometime. All the processes were carried out in dark place. The antioxidant active regions became yellow in color.

Staining with FC (Folin-Ciocalteu) reagent:

Staining with FC reagent was carried out as the same procedure above with plates. 10% FC solution was prepared with water as the solvent. The antioxidant active regions became yellow/ white in color.

Anti-oxidant test:

Principle of DPPH test:

This is known as a standard 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure below, shows the mechanism by which DPPH• accepts hydrogen from an antioxidant. DPPH• is one of the few stable and commercially available organic nitrogen radicals (1). The antioxidant effect is proportional to the disappearance of DPPH• in test samples. Monitoring DPPH• with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH• shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This

reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.

(J. Lewis, 2012)

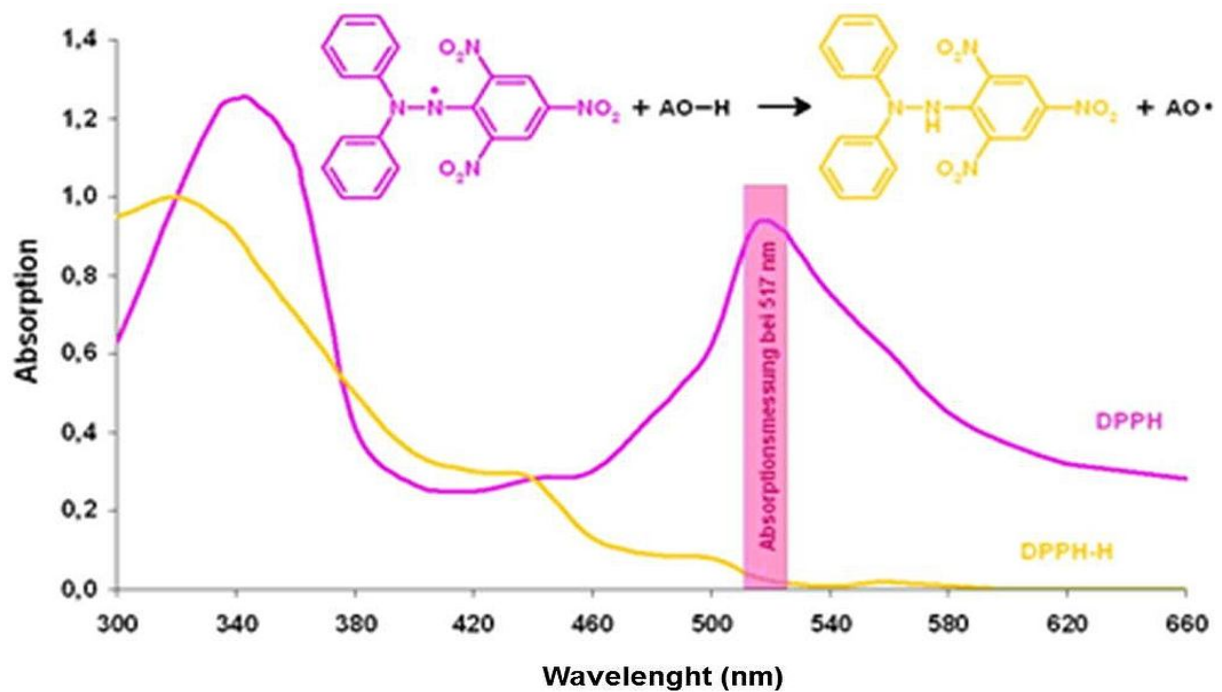


Figure: Graph showing the color change of DPPH from purple to yellow when it is exposed to an antioxidant substance. (Pérez and Aguilar, 2013)

Equipment and Materials Required

Test tube

- Foil paper
- Micro pipette
- UV Spectometer
- Mortar & pestle
- Filter paper
- Funnel

- Beaker
- DPPH
- Methanol
- Distill water

Procedure

a) The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts. Different concentrations of the plant extract were added to methanol solution of DPPH .

b) Different concentrations of L-Ascorbic acid (2-10 mg/ml) were used as the standard antioxidant.

c) After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the equation,

DPPH antiradical scavenging capacity (%) = [(Ab. of control – Ab. of sample)/Ab. of control] × 100.

d) Different concentrations of plant extract solution was used as a blank, while DPPH solution plus methanol was used as a control.

e) The IC50 values were calculated by the sigmoid non-linear regression model using plots, where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of scavenging capacity. IC50values denote the concentration of the sample required to scavenge 50% of DPPH radicals.

Standard Preparation:

1) 250 mg Ceevit Tablet (Square) was crushed in mortar and pestle.

2) 100 ml distilled water was added and the solution was filtered.

3) It was then diluted by 10 times (2 ml of the filtered solution was taken and 18 ml water added).

4) The solution was taken in 5 test tubes to prepare 5 different concentrations.

5) 1ml, 2ml, 2ml, 4ml and 5ml solution were taken in 5 different test tubes and the volume adjusted to 5 ml with water in all the test tubes.

Sample Preparation:

1) 100 µL of extracted sample was added in to first test tube which contain 2 ml methanol.

2) From the first test tube 1ml solution was taken in to second test tube which contain 1 ml methanol.

3) The procedure was continued for other 3 test tube for having total five different concentration.

4) 1ml solution was discarded from the last test tube.

5) The volume was adjusted to 4 ml with methanol in all the test tubes.

Blank Preparation:

Blank was prepared by adding 4 ml methanol in a test tube. In all the test tubes 100 µL DPPH solution was added in dark and left for 30 minutes. After that UV absorbance was measured in UV machine at 517 nm.

Antibacterial test (Disc diffusion method)

Principle of Disc diffusion method:

When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a “zone of inhibition”.

Equipment and Materials Required

- Petri dish
- Disc
- Watch glass
- Tweezers
- Micro pipette
- Autoclave machine
- Laminar air flow
- Incubator
- Spreader
- Spatula
- Ethanol
- Nutrient agar
- Distill water
- Sodium chloride
- Antibiotic (Ciprofloxacin)

Procedure of Disc diffusion method:

1. Six different bacterial strains of gram positive, Six different strains of gram negative bacteria used to carry out this assay.

2. Nutrient agar was used as the culture media. Stocks of these bacterial solutions were revived in nutrient agar by incubating at 370C for 24 hrs.

3. A single disk diffusion method was used to assess the presence of antimicrobial activities of the extract.

4. Whatman's filter paper was punched, and 0.6 cm disks were collected in a beaker. The beaker with petri dishes, forceps, tips, spreader were covered with foil paper and autoclaved.

5.5 µl of extracted sample were loaded per disc with the help of micropipette.

Figure 15: Micropipette, pipette tips

6. The revived test organisms were spread onto nutrient agar plates by spreader.

7. The disc were then placed all plates. Standard disc of Ciprofloxacin (25 µg/disc) was used as positive control.

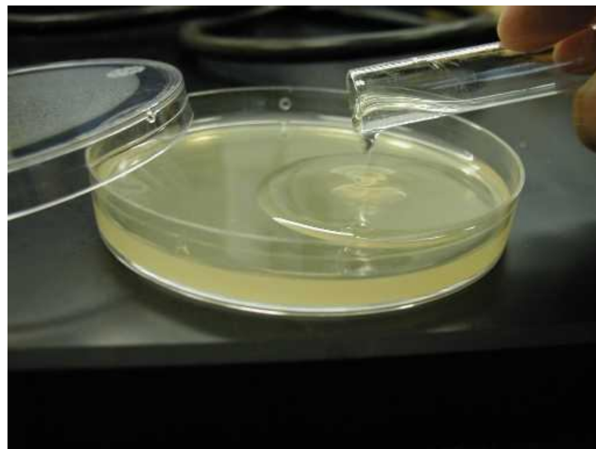


Figure 16: Agar media in plate.

8. After incubation at 370C for 24 hours, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

Figure 17: Incubator (1) of microbiology lab and laminar air flow (2).

In vitro anti-diabetic test

Glucose uptake in Yeast cells

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts were added to 1mL of glucose solution and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min.

After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated in the supernatant (Cirillo, 1962). The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.(Mishra, Kavrekar and Nair, 2013)

Equipment and Materials Required

- Test tube
- Beaker
- Funnel
- Micropipette
- Filter paper
- Incubator
- Centrifuge machine
- UV spectrometer
- Distill water
- Glucose
- Yeast
- Metformin

Preparation of glucose solution:

1) 2gm glucose was added to 5 ml distilled water. Required amount of glucose solution was prepared by this procedure.

Standard preparation:

1) 500 mg metformin Tablet was crushed in mortar and pestle.

2) 100 ml distilled water was added and the solution was filtered.

3) 500 µl filtered solution was taken in the first test tube which contain 1.5 ml glucose solution.

4) Then serial dilution was done to have 5 different concentrations.

Sample preparation:

1) 100 µL of extracted sample was added in to first test tube which contains 2 ml glucose solution.

2) Serial dilution was done to have 5 different concentrations.

Blank preparation:

1) 1ml glucose solution without sample and standard was used as blank.

After standard, sample and blank preparation

Various concentrations of plant extracts, standard and blank were incubated together for 10 min at 37 °C.

After that 100 µL of yeast suspension was added to all and further incubated at 37 °C for 60 min.

Then 3 ml distilled water was added to each test tube.

Then the absorbance was taken in 334 nm

In vitro anti-inflammatory test

Preparation of Human Red Blood Cells (HRBC) Suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized phosphate buffer saline. The blood was centrifuged at 1000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.

Heat Induced Hemolysis

The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 100 μl HRBC suspension [10 % v/v] with 100 μl of plant extracts and standard drug diclofenac sodium of various concentrations and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37 oC for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.(Chippada et al., 2011)

$$\text{Percentage Stabilization} = \frac{\text{Absorbance of control} - \text{Absorbance of Test sample}}{\text{Absorbance of control}} \times 100$$

(Kar et al., 2012)

Equipment and Materials Required

- Test tube
- Beaker
- Funnel
- Filter paper
- Micro pipette
- Measuring cylinder
- Heparin tube
- UV spectrometer
- Incubator
- Centrifuge machine
- Na_2HPO_4
- NaH_2PO_4
- NaCl
- Diclofenac sodium
- Distill water

Standard preparation:

1) 50 mg diclofenac sodium tablet was crushed in mortar and pestle.

2) 10 ml distilled water was added and the solution was filtered.

3) 100 µl filtered solution was taken in the first test tube which contain 2 ml phosphate buffer saline.

4) Then serial dilution was done to have 5 different concentrations.

Sample preparation:

1) 100 µl of extracted sample was added in to first test tube which contains 2 ml phosphate buffer saline

2) Serial dilution was done to have 5 different concentrations.

Blank preparation:

1) 1ml phosphate buffer saline without sample and standard was used as blank.

After standard, sample and blank preparation

2ml hyposaline was added to all test tubes (standard, sample and blank).

Then 100 µl red blood cell suspensions were added to all.

After that all were incubated at 37°C for 30 min and centrifuged respectively.

After centrifugation supernatant was collected.

At last absorbance was taken using spectrophotometer at 560 nm.

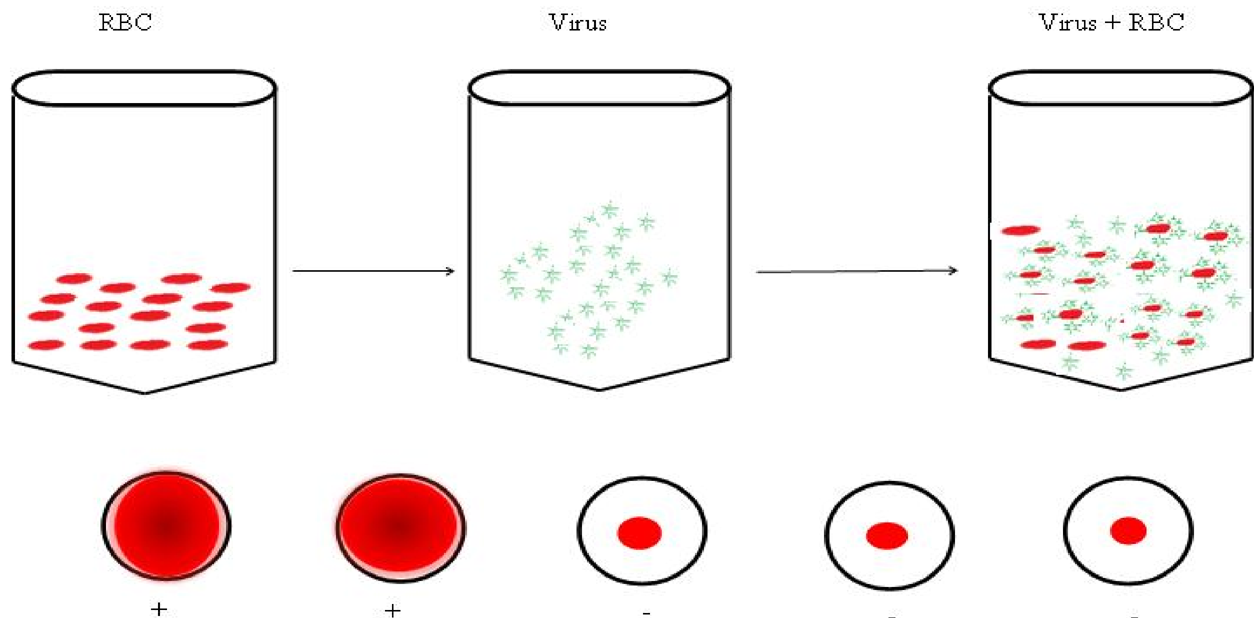
Hemagglutination (HA) Assay

Principle:

The hemagglutination assay is a method for titering influenza viruses based on their ability to attach to molecules present on the surface of red blood cells. A viral suspension may

agglutinate the red blood cells, thus preventing them from settling out of suspension. By serially diluting a virus in a 96-well plate and adding a consistent amount of red blood cells, an estimation of the amount of virus present can be made.

(Hemagglutination (HA) Assay Protocol, n.d.)



Equipment and Materials Required

- U- or V-bottom microtiter plates
- Micropipette with tips
- Phosphate buffered saline (0.01M, pH 7.2)
- Washed human erythrocytes (0.5%)
- Centrifuge machine

(Hemagglutination Assay (HA) and Hemagglutination Inhibition Assay (HI): AI and NDV, 2006)

RBC preparation:

1. 4 ml of human blood was pipetted into a 14 ml test tube and topped off with PBS.

2. Centrifuged at 1000 rpm for 10 minutes.

3. The supernatant was aspirated without disturbing the blood cells.

4. 12 ml PBS was added and mixed by inverting.

5. It was centrifuged again at 1000 rpm for 5 minutes and washed two more times.

6. Supernatant was aspirated after final wash and enough PBS was added to make a 10% solution of red blood cells.

7. Final working solution of 0.5% RBCs was made in PBS.

Procedure:

1. A round-bottomed 96-well dish is preferred for this assay. Flat-bottomed plates will also work, but need to be placed at an incline to develop.

2. To each 50 µl PBS was added.

3. In the first column, 50 µl of sample was added.

4. After mixing each well and transfered 50 µl to the next well on its right. Repeated mixing and transferring 50 µl down the length of the plate. From the last well 50 µl was discarded.

5. 50 µl of 0.5% red blood cell was added to each well. Mixed gently.

6. The plate was kept at room temperature for 30-60 minutes to develop.

(Pankaj, 2015)

CHAPTER FOUR

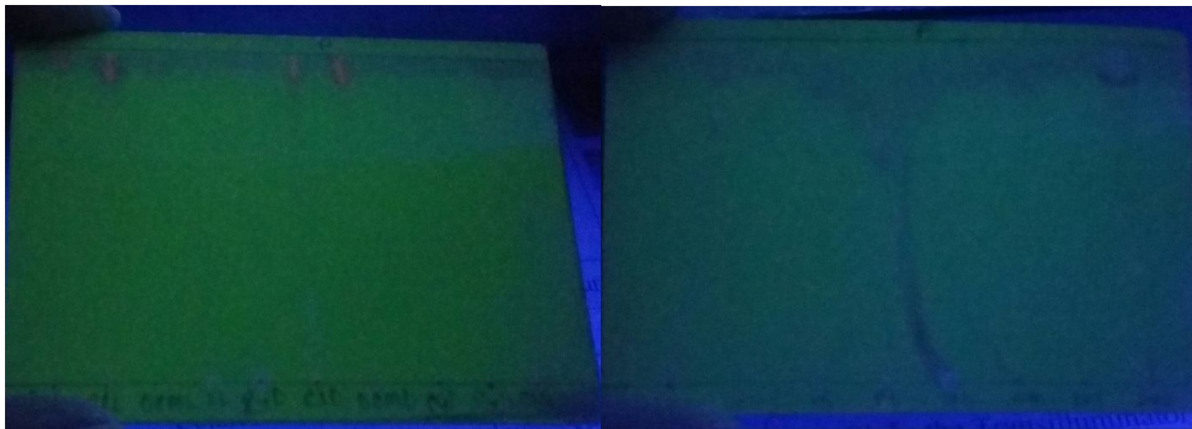
RESULT

Thin layer chromatographic analysis using different fractions of *citrullus lanatus* rinds

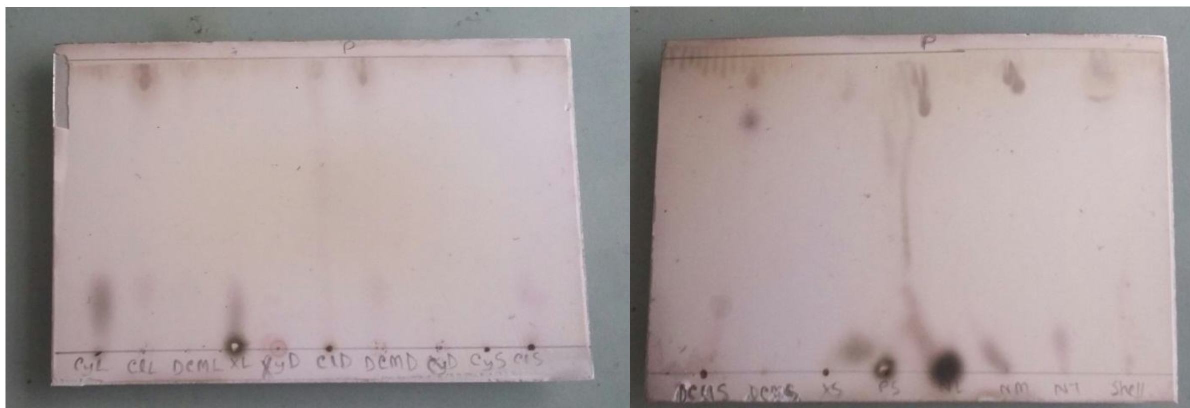
TLC was conducted on different fraction of *Citrullus lanatus* rinds by using all the three types of solvent system. The pictures of the plates were developed are displayed below:

Polar basic solvent:

Here chromatograms developed using the polar basic solvent system containing ethyl acetate, ethanol & water in the ratios of 8:1.2:0.8.



(1)



(2)

Figure 1.1: Results for TLC in polar basic solvent (1 = UV light view; 2 = after Charring)

The naked eye view of the TLC was not mentioned which showed no clear spot. Then the plate was observed under UV which is shown in the plate 1. Charring process done in 10% conc. Sulfuric acid on plate showed spot which indicates the presence of different compounds in light green (cyclohexane ,chloroform) fraction; deep green (chloroform, DCM)fraction; stripe (DCM)fraction.

Calculation of Rf value:

The Rf value was calculated as:

Rf = Distance spot travels / Distance solvent travels.

Light green

Rf value of cyclohexane fraction: 0.25

Rf value of chloroform fraction: 0.95

Rf value of xylene fraction: 0.25

Deep green

Rf value of chloroform fraction: 0.95

Rf value of DCM fraction: 0.95

Stripe

Rf value of chloroform fraction: 0.95

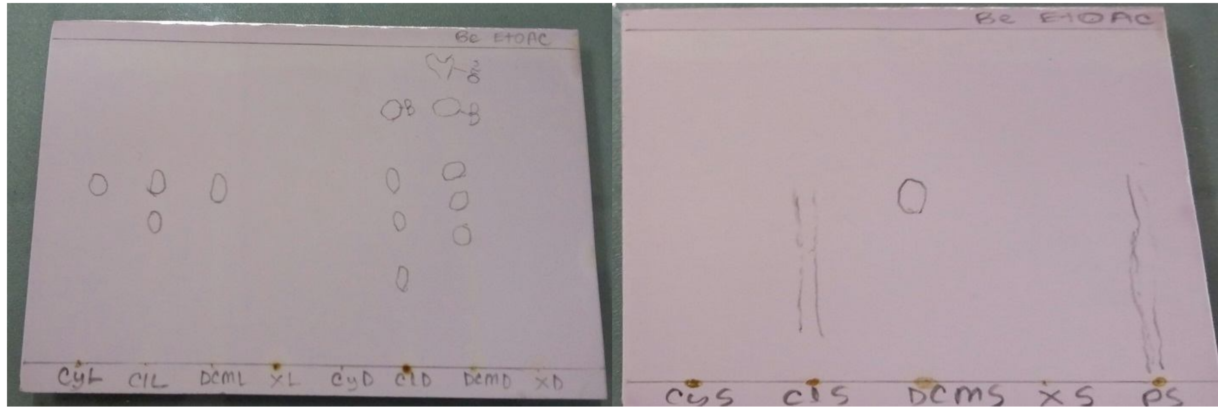
Rf value of DCM fraction: 1st spot: 0.25

2nd spot: 0.75

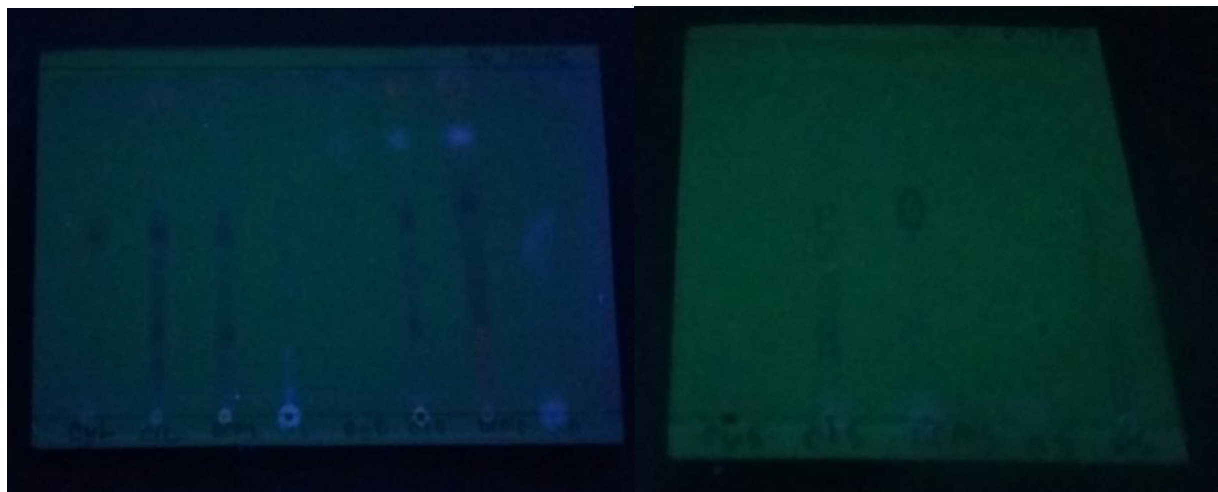
3rd spot: 0.95

Semi polar solvent system:

Here chromatograms developed using the semi polar solvent system containing benzene, ethyl acetate in the ratio of 6:4.



(1)



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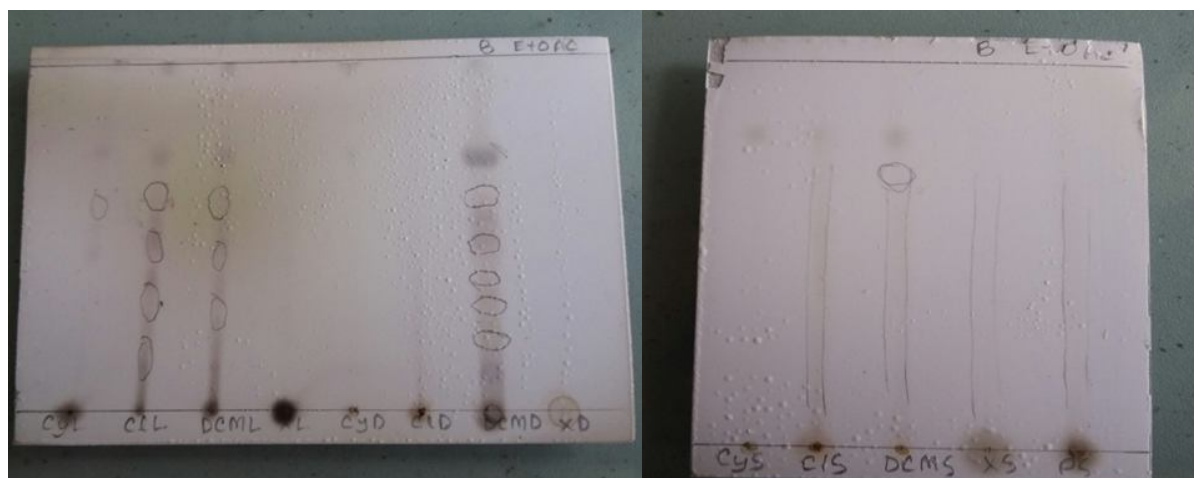


(3)

Figure 1.2: Results for TLC in semi polar solvent (1= naked eye view; 2 = UV light view; 3 = after Charring)

The naked eye view of the TLC (1) showed no clear spot. Then the plate was observed under UV which is shown in the plate (2). It showed some spots which indicate the presence of different compounds in that sample of various fraction such as light green(cyclohexane, chloroform, DCM) fraction; deep green (chloroform, DCM) fraction; stripe (DCM) fraction. Charring process done in 10% conc. Sulfuric acid which is shown in plate (3).After charring the plate showed extra spot which indicates the presence of compounds in stripe (cyclohexane, chloroform) fraction.

Calculation of Rf value:

Light green

Rf value of cyclohexane fraction: 0.77

Rf value of chloroform fraction: 0.77

Rf value of DCM fraction: 0.77

Deep green

Rf value of DCM fraction: 1st spot: 0.11

2nd spot: 0.62

3rd spot: 0.77

Rf value of chloroform fraction: 1st spot: 0.11

2nd spot: 0.62

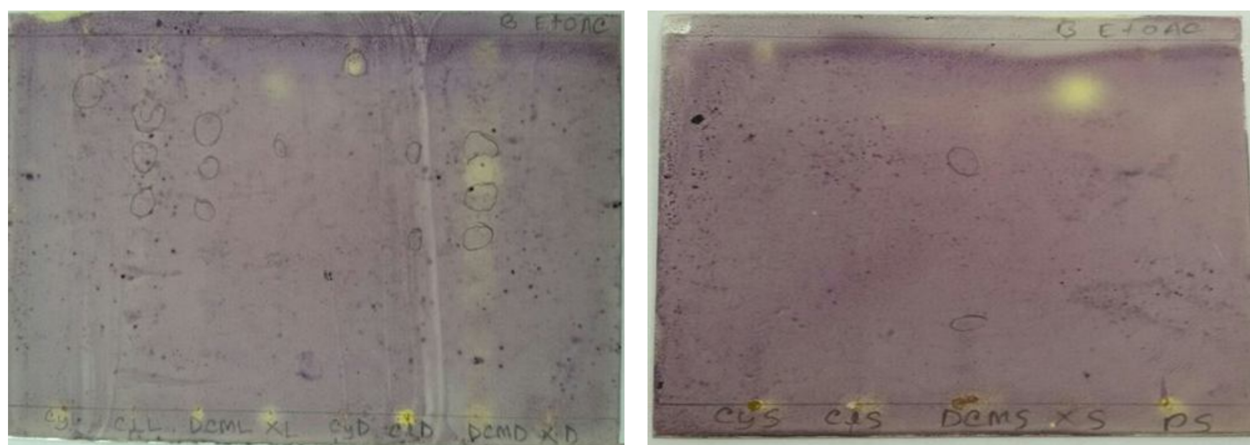
3rd spot: 0.77

Stripe

Rf value of cyclohexane fraction: 0.8

Rf value of chloroform fraction: 0.8

Rf value of DCM fraction: 0.8

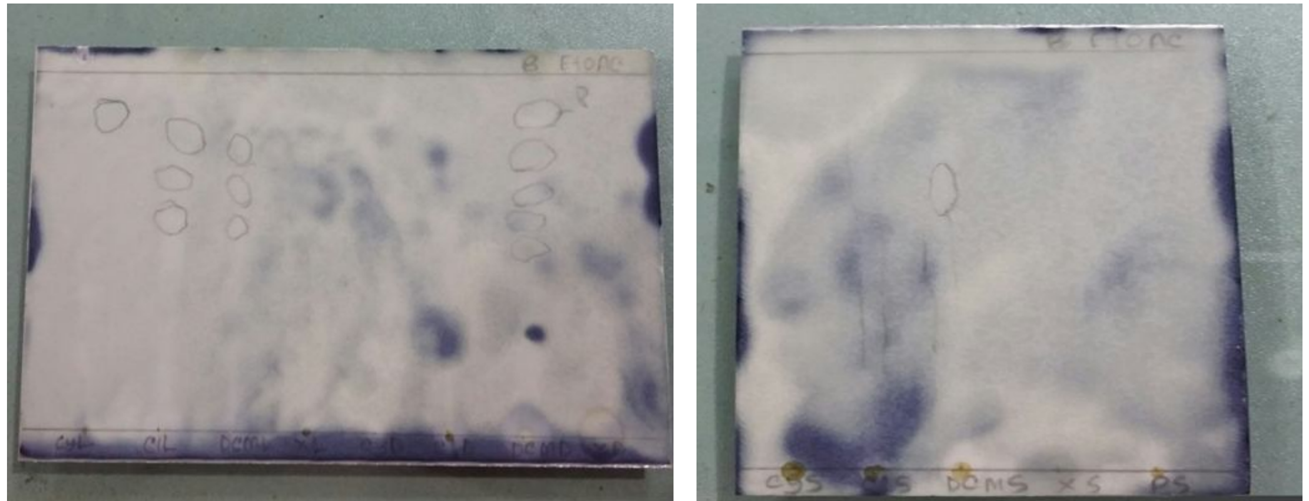


(4)

Figure 1.3: (4) Plate after being soaked in 0.4% DPPH solution.

The formation of yellow colour on the plate after DPPH solution was indicates that light green (chloroform, xylene) fraction; deep green (cyclohexane, DCM) fraction; Stripe (xylene) fraction

of *Citrullus lanatus* rinds contains antioxidant compounds that contain free radical scavenging properties



(5)

Figure 1.4 :(5) plate after being soaked in 10% FC (Folin-Ciocalteu) solution.

Non polar

Here chromatograms developed using the non-polar solvent system containing benzene, ethanol and ammonium hydroxide in the ratio of 9: 1: 0.1.

(1)

Figure 1.5: Results for TLC in non-polar solvent (1 = after Charring)

The naked eye view and under the UV of the TLC were not mentioned which showed no clear spot. Even after charring no clear spot were found. It indicates that none of the sample runs to the plate in non-polar solvent system.

2. Anti-oxidant activity

The result of antioxidant test of different fraction of *Citrullus lanatus* rinds are given below-

Table 2.1: Antioxidant activity of standard Ascorbic acid.

Sample Name	**Dose (mg)**	**Absorbance (517 nm)**	**% of inhibition**
	1	0.027	77.2287 ± 1.45

Ascorbic acid	0.8	0.033	72.09615 ± 1.26
	0.6	0.0375	68.29622 ± 1.04
	0.4	0.0415	64.93107 ± 1.20
	0.2	0.0455	61.5942 ± 1.17

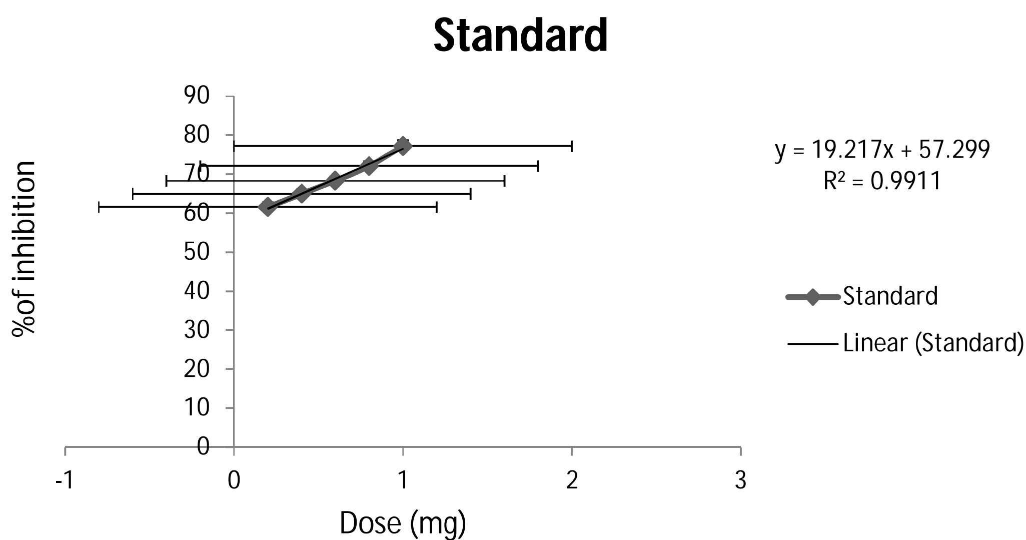


Figure 2.1: % Free radical scavenging activity of ascorbic acid

Table 2.2: Antioxidant activity of cyclohexane fraction of *Citrullus lanatus* rinds light green variety.

Sample	**Dose (mg)**	**Absorbance**	**% of inhibition**

Name		(517 nm)	
Cyclohexane fraction of light green(LG)	77.9	0.17	0± 1.42
	38.95	0.1945	0± 1.04
	19.47	0.223	0 ± 3.67
	9.73	0.282	0± 1.53
	4.865	0.315	0 ± 1.59

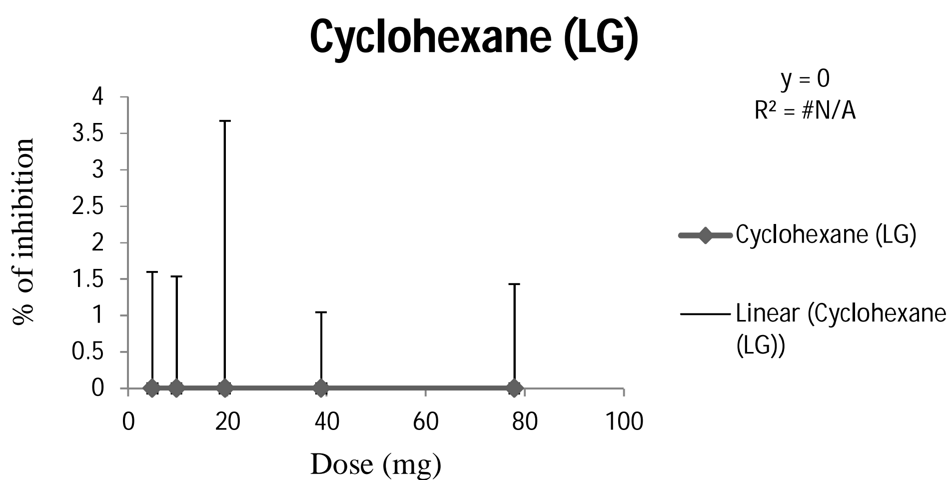


Figure 2.2: % Free radical scavenging activity of cyclohexane fraction of light green

Table 2.3: Antioxidant activity of chcloroform fraction of *Citrullus lanatus* rinds light green variety.

Sample Name	Dose (mg)	Absorbance (517 nm)	% of inhibition
Chcloroform fraction of light green(LG)	149.2	0.2705	0 ± 0.86
	74.6	0.279	0 ± 1.49
	37.3	0.297	0 ± 1.53
	18.6	0.315	0 ± 1.38
	9.3	0.349	0 ± 2.20

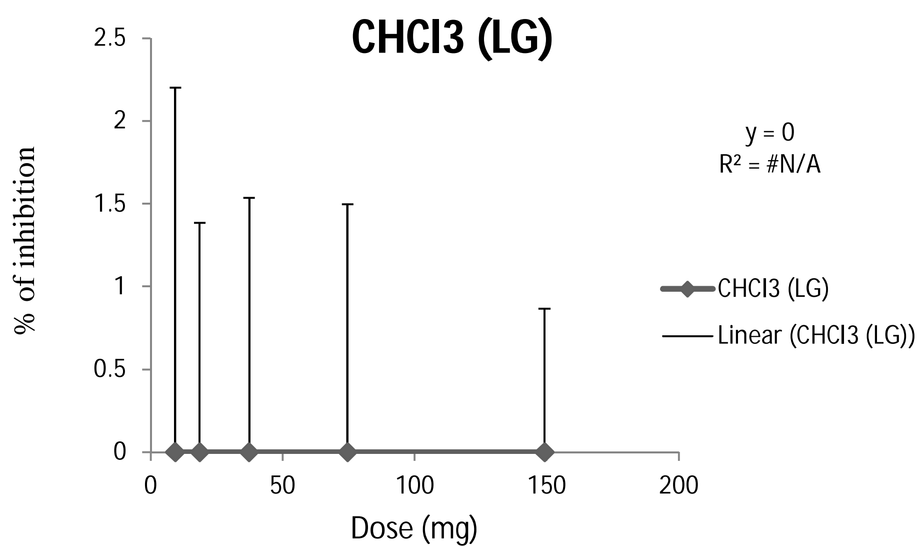


Figure 2.3: % Free radical scavenging activity of chcloroform fraction of light green

Table 2.4: Antioxidant activity of Dichcloromethane fraction of *Citrullus lanatus* rinds light green variety.

Sample Name	**Dose (mg)**	**Absorbance (517 nm)**	**% of inhibition**
Dichcloro-methane fraction of light green(LG)	132.6	0.1095	8.06292 ± 1.35
	66.6	0.1135	4.669495 ± 2
	33.15	0.122	0 ± 0.63
	16.5	0.1305	0 ± 1.27
	8.25	0.1475	0 ± 0.78

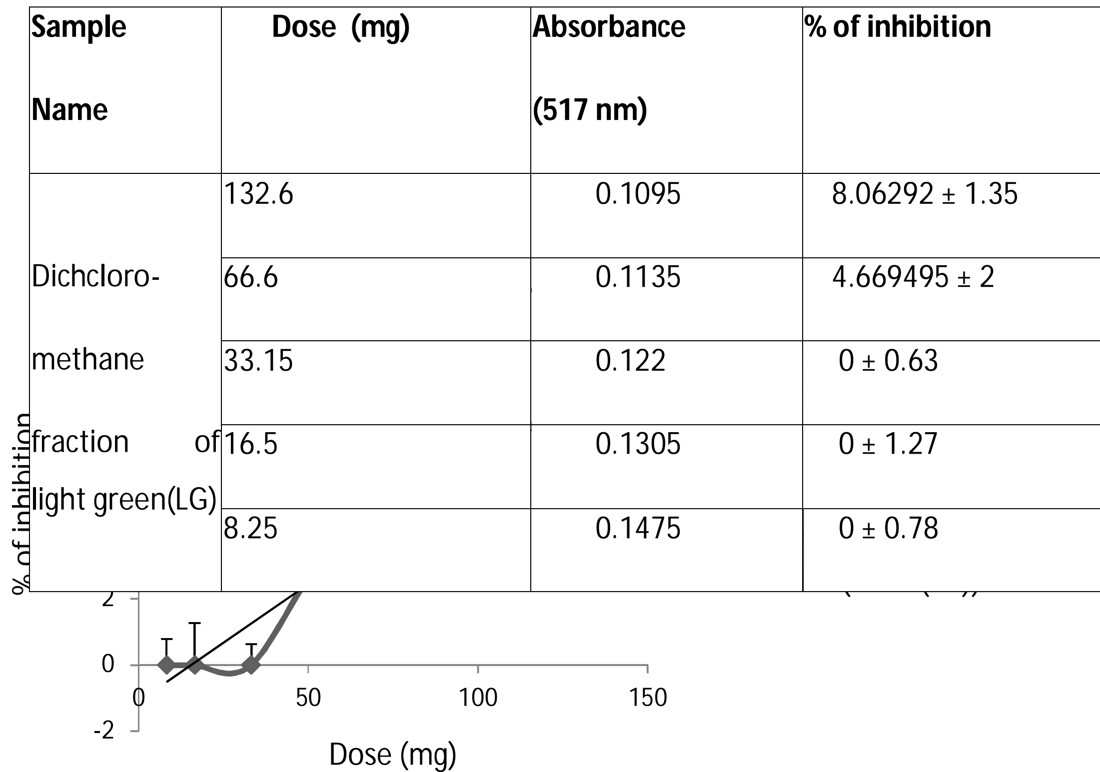


Figure 2.4: % Free radical scavenging activity of dichcloromethane fraction of light green

Table 2.5: Antioxidant activity of xylene fraction of *Citrullus lanatus* rinds light green variety.

Sample Name	**Dose (mg)**	**Absorbance (517 nm)**	**% of inhibition**
	86	0.096	19.43443 ± 1.48
Xylene	43	0.1015	14.70838 ± 0.10

fraction of light green(LG)	21.5	0.116	2.693531 ± 2.25
	10.75	0.1335	0 ± 1.29
	5.375	0.141	0 ± 1.39

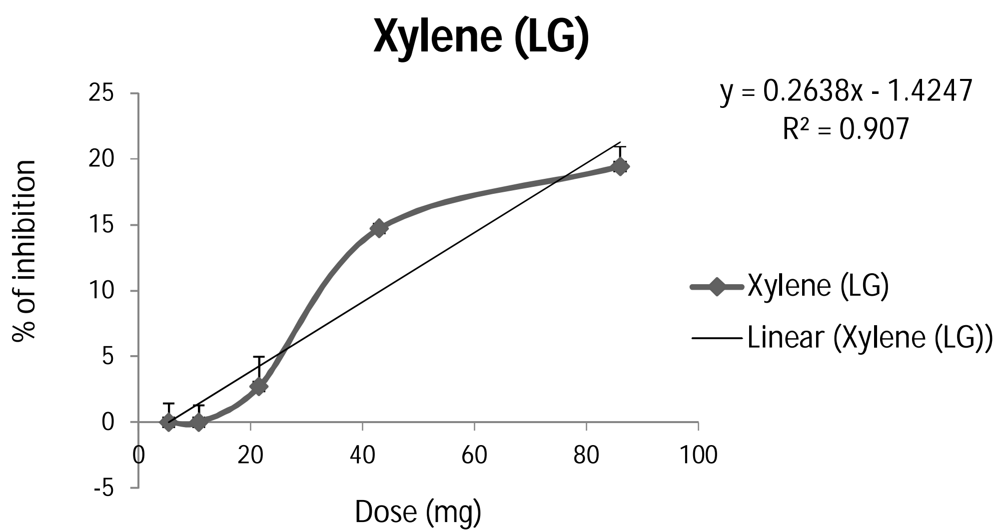


Figure 2.5: % Free radical scavenging activity of xylene fraction of light green

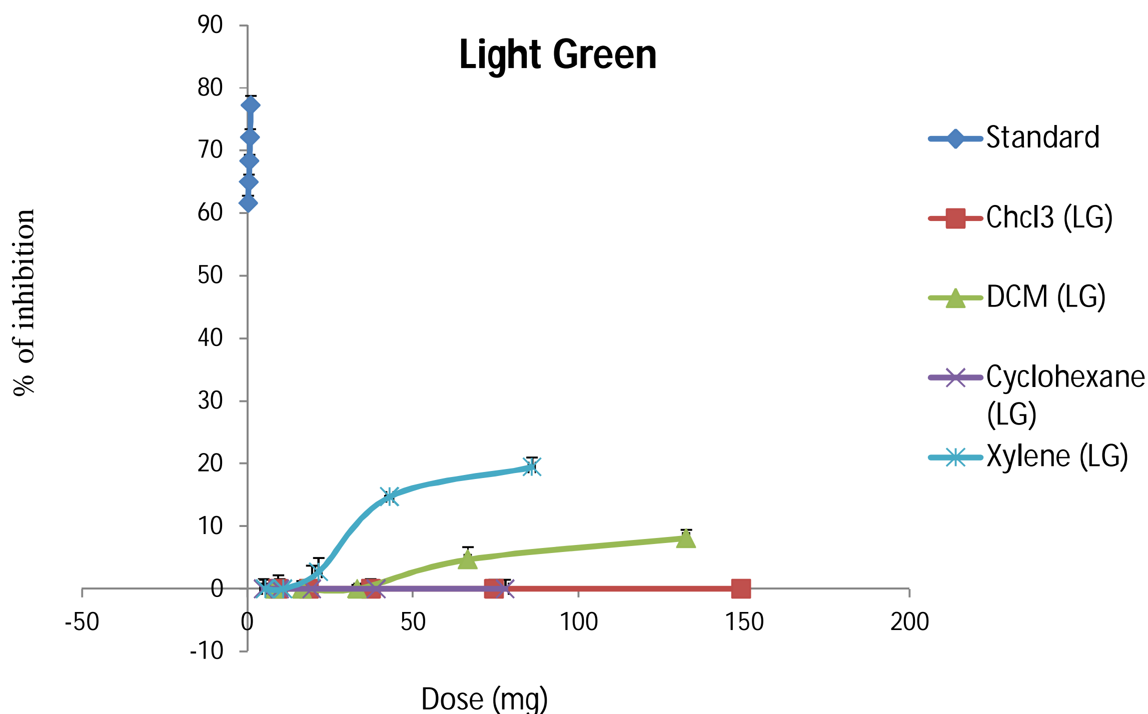


Figure 2.6: % Free radical scavenging activity of chcloroform, dichcloromethane, cyclohexane, xylene fraction of *Citrullus lanatus* rinds light green variety and ascorbic acid.

Table 2.6: Antioxidant activity of xylene fraction of *Citrullus lanatus* rinds stripe variety.

Sample Name	Dose (mg)	Absorbance (517 nm)	% of inhibition
Xylene fraction of Stripe (S)	86	0.088	26.16472 ± 0.80
	43	0.1045	12.41075 ± 1.50
	21.5	0.1105	7.27819 ± 0.69
	10.75	0.12	0 ± 1.33
	5.375	0.1295	0 ± 0.93

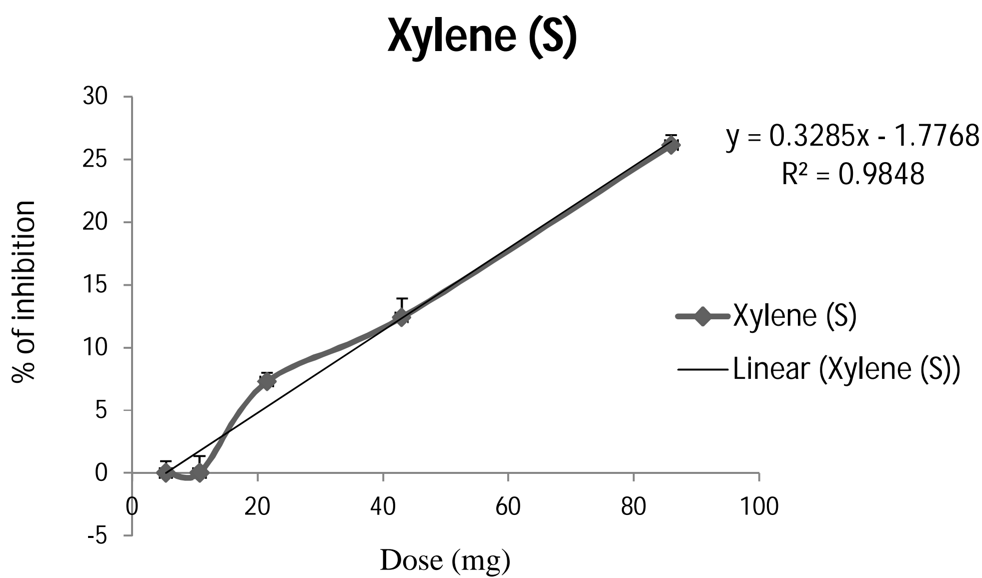


Figure 2.7: % Free radical scavenging activity of xylene fraction of stripe

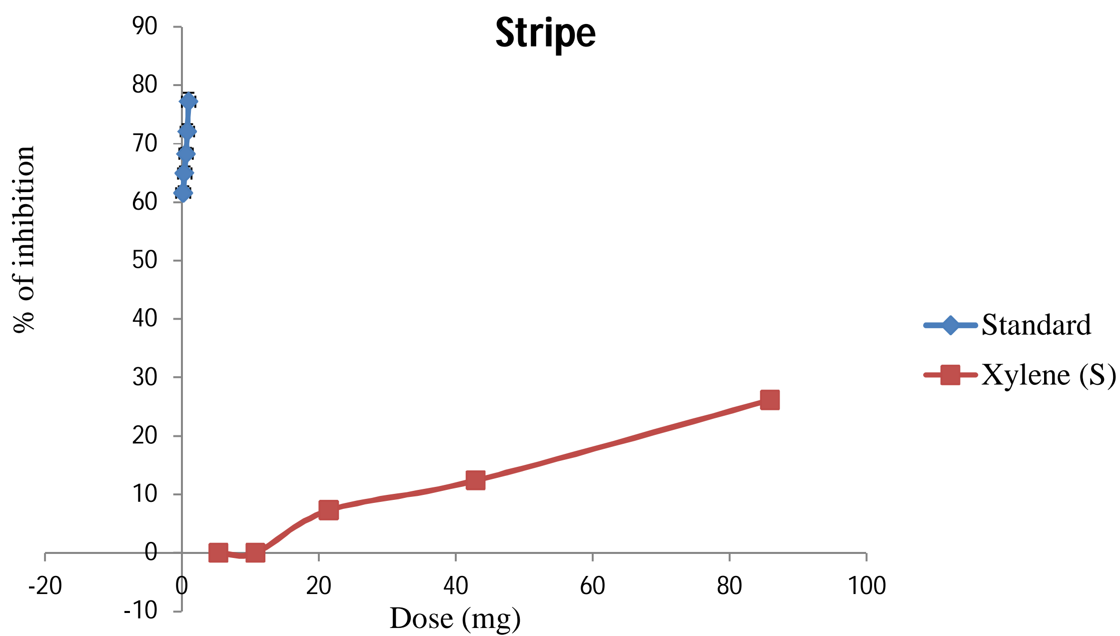


Figure 2.8: % Free radical scavenging activity of xylene fraction of *Citrullus lanatus* rinds stripe variety and ascorbic acid.

Table 2.7: Antioxidant activity of cyclohexane fraction of *Citrullus lanatus* rinds deep green variety.

Sample Name	**Dose (mg)**	**Absorbance (517 nm)**	**% of inhibition**
Cyclohexane fraction of deep green (DG)	77.9	0.1005	15.69106 ± 1.09
	38.95	0.1205	0 ± 1.81
	19.47	0.131	0 ± 2.03
	9.73	0.1385	0 ± 0.95
	4.865	0.145	0 ± 0.71

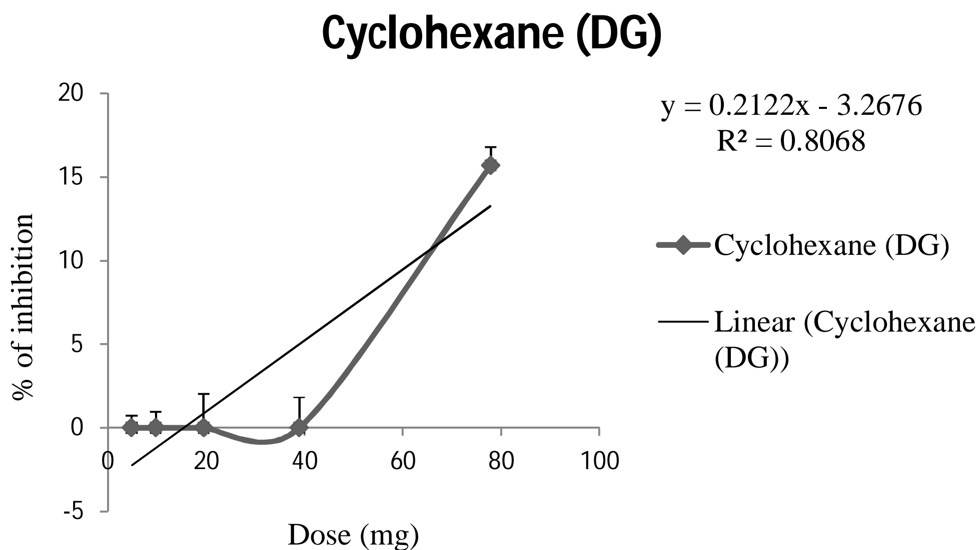


Figure 2.9: % Free radical scavenging activity of cyclohexane fraction of deep green

Table 2.8: Antioxidant activity of Dichloromethane fraction of *Citrullus lanatus* rinds deep green variety.

Sample Name	**Dose (mg)**	**Absorbance (517 nm)**	**% of inhibition**
Dichloro-methane fraction of deep green (DG)	132.6	0.077	35.41888 ± 1.24
	66.6	0.099	17.08024 ± 1.50
	33.15	0.1135	4.95228 ± 0.90
	16.5	0.125	0 ± 2.08
	8.25	0.136	0 ± 1.69

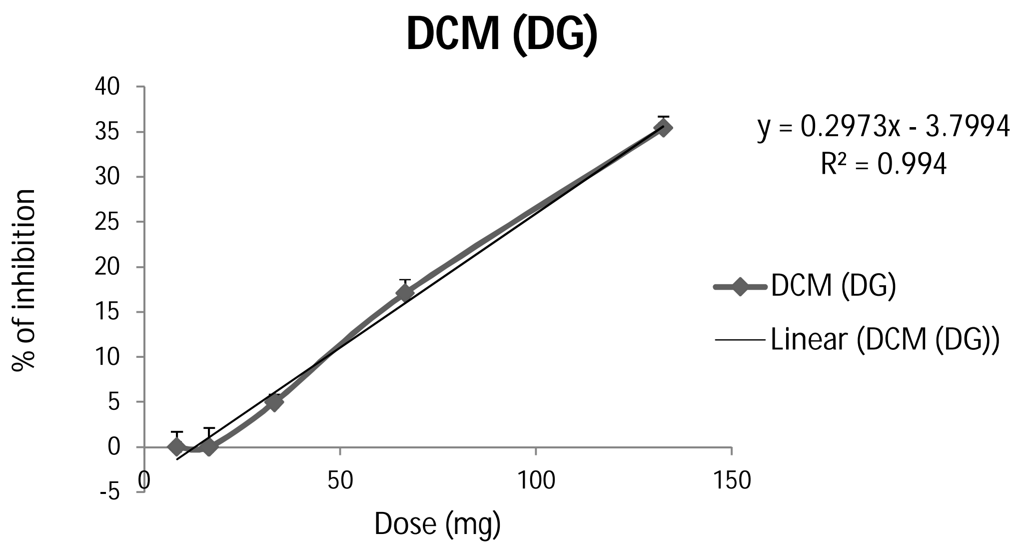


Figure 2.10: % Free radical scavenging activity of dichloromethane fraction of deep green

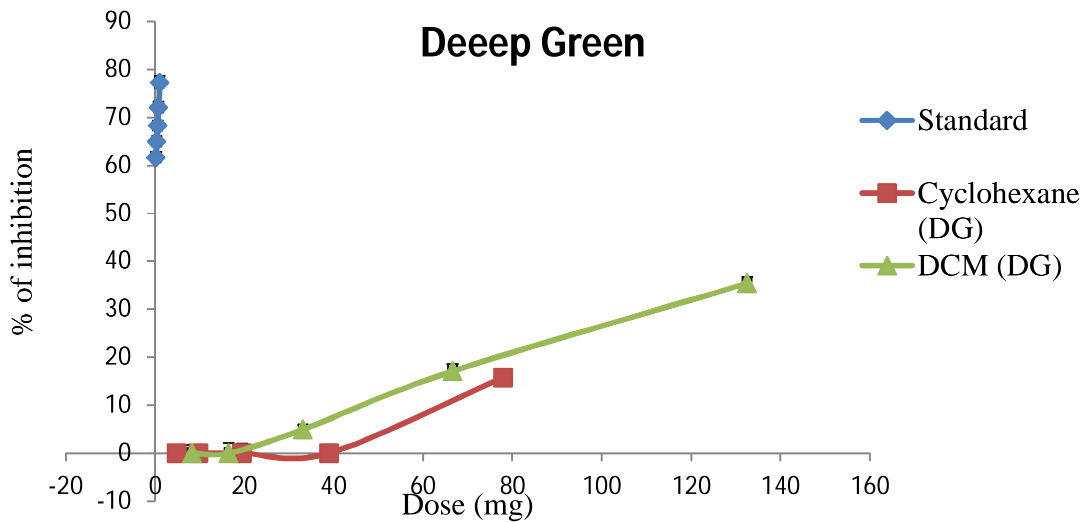


Figure 2.11: % Free radical scavenging activity of cyclohexane, dichloromethane fraction of *Citrullus lanatus* rinds deep green variety and ascorbic acid.

3. Hypoglycemic test

The result of hypoglycemic test of different fraction of *Citrullus lanatus* rinds are given below-

Table 3.1 : Antidiabetic activity of standard Ascorbic acid .

Sample Name	Dose (mg)	Absorbance (334 nm)	% of inhibition
	2.5	0.732	11.67013 ± 1.60
	1.2	0.7075	8.628617 ± 0.96

Metformin	0.624	0.6895	6.413319 ± 1.01
	0.312	0.671	3.989987 ± 1.56
	0.156	0.639	0 ± 1.24

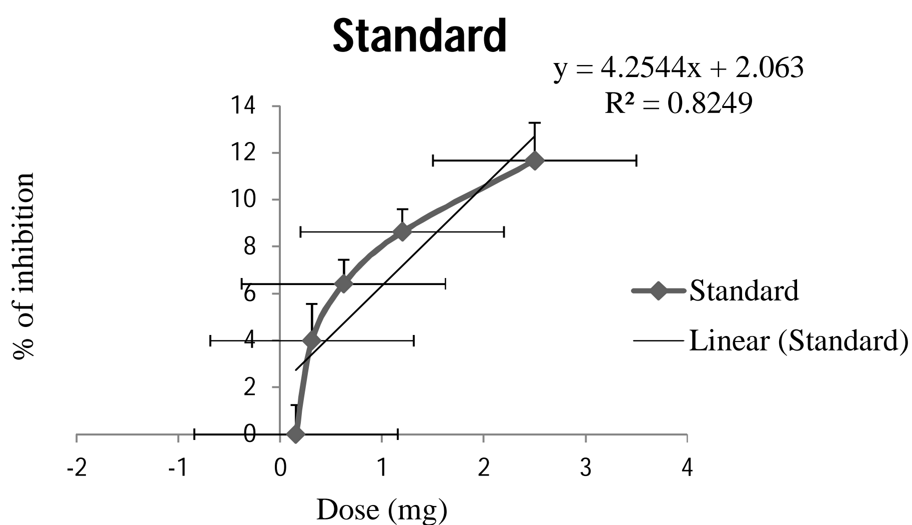


Figure 3.1: Anti-diabetic activity of Metformin

Table 3.2 : Anti - diabetic activity of chcloroform fraction of *Citrullus lanatus* rinds light green variety.

Sample Name	**Dose (mg)**	**Absorbance (334 nm)**	**% of inhibition**
Chcloroform fraction of light green	149.2	0.716	8.668461 ± 1.58
	74.6	0.689	3.828292 ± 1.70
	37.3	0.62	0 ± 1.93

(LG)	18.6	0.5955	0 ± 2.51
	9.3	0.591	0 ± 1.37

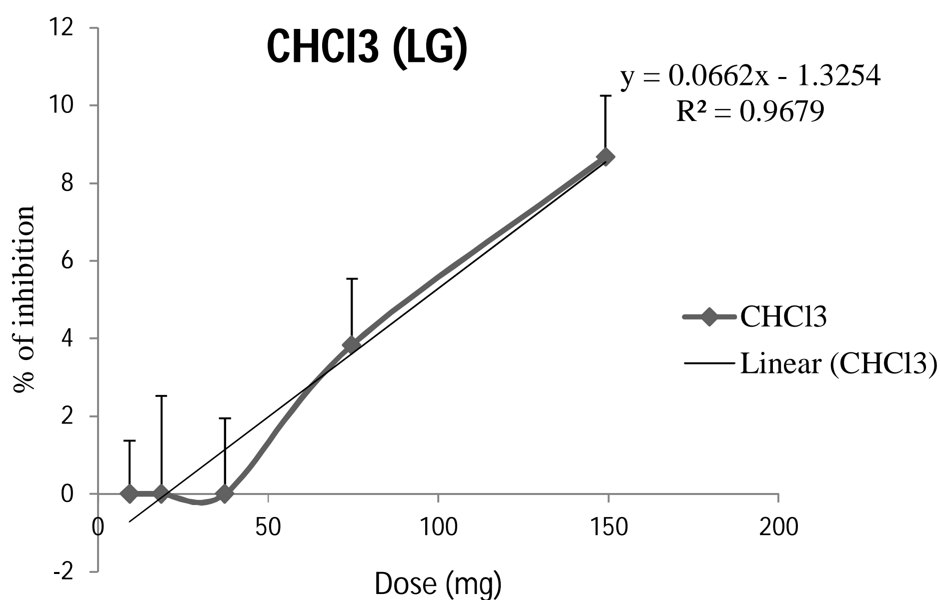


Figure 3.2: Anti - diabetic activity of chloroform fraction of light green

Table 3.3: Anti - diabetic activity of dichloromethane fraction of *Citrullus lanatus* rinds light green variety.

Sample Name	Dose (mg)	Absorbance (334 nm)	% of inhibition
Dichcloro - methane	132.6	0.6755	0.151671 ± 1.48
	66.6	0.6275	0 ± 0.86

fraction of light green (LG)	33.15	0.6345	0 ± 0.59
	16.5	0.644	0 ± 1.49
	8.25	0.616	0 ± 1.10

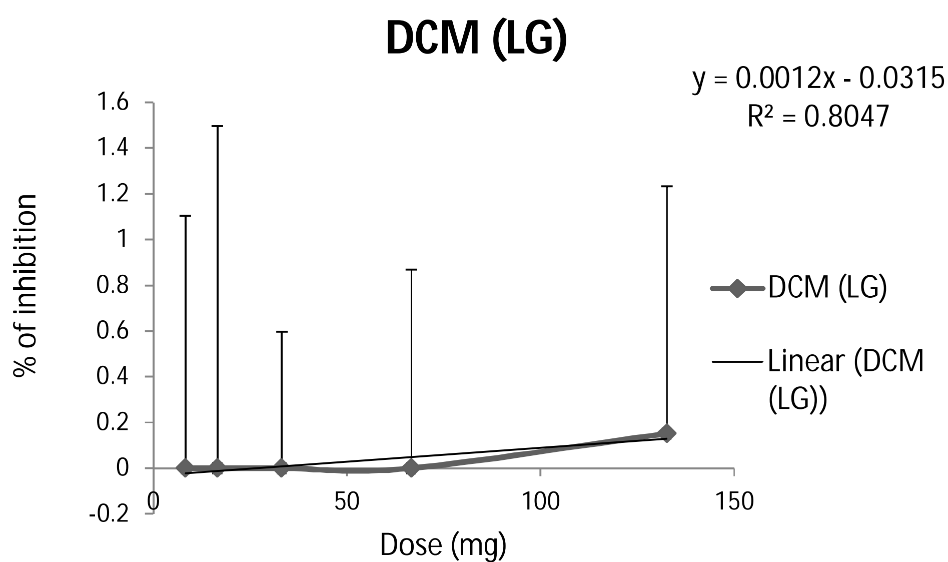


Figure 3.3: Anti - diabetic activity of dichloromethane fraction of light green

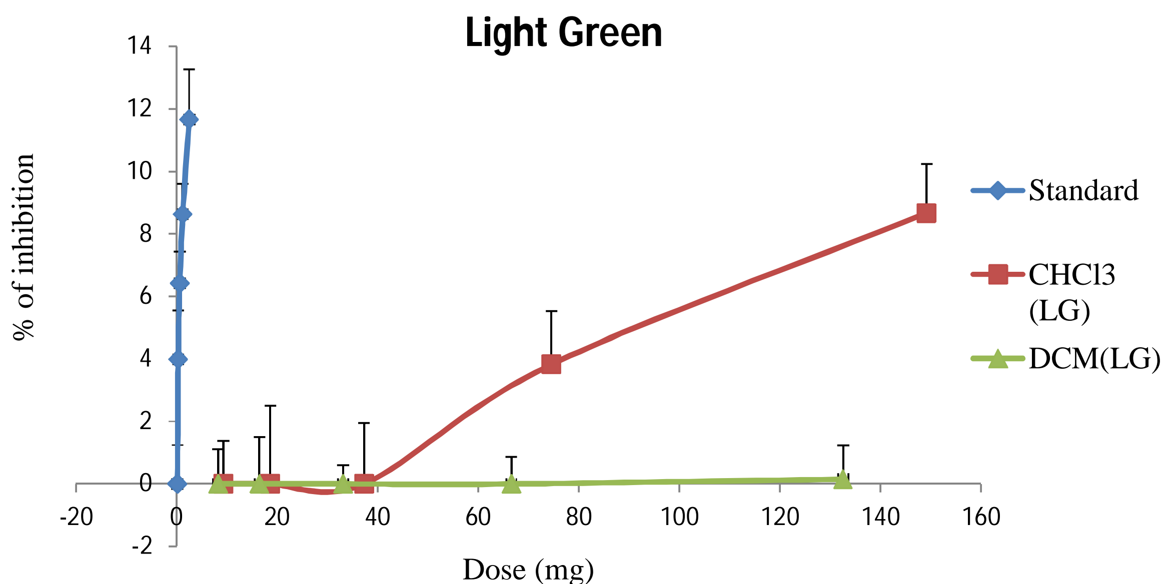


Figure 3.4: Anti - diabetic activity of chloroform, dichloromethane fraction of *Citrullus lanatus* rinds light green variety and metformin.

Table 3.5: Anti - diabetic activity of cyclohexane fraction of *Citrullus lanatus* rinds stripe variety.

Sample Name	**Dose (mg)**	**Absorbance (334 nm)**	**% of inhibition**
Cyclohexane fraction of stripe (S)	77.9	0.6685	0 ± 0.94
	38.95	0.648	0 ± 2.00
	19.47	0.6675	0 ± 1.06
	9.73	0.644	0 ± 1.72
	4.865	0.624	0 ± 2.14

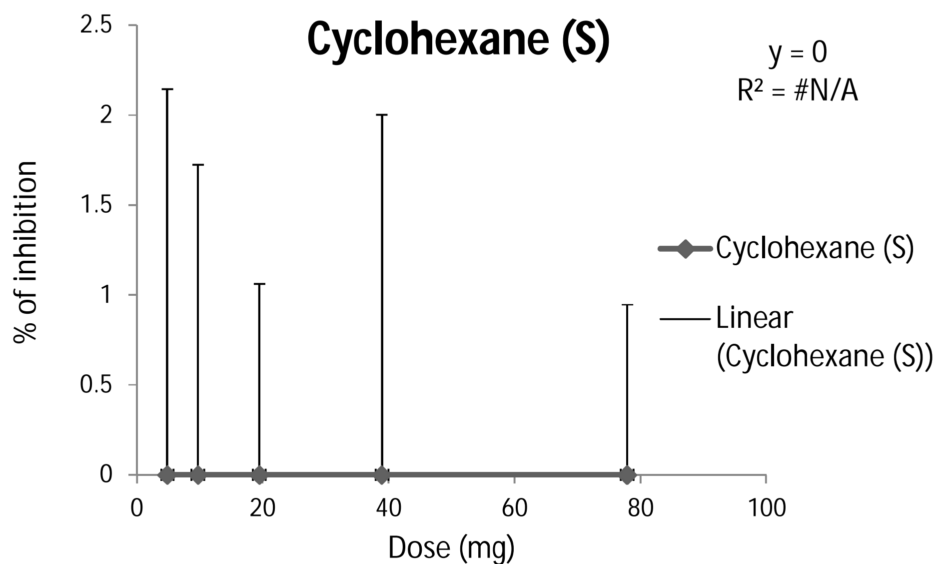


Figure 3.6: Anti - diabetic activity of cyclohexane fraction of stripe

Table 3.6: Anti - diabetic activity of chloroform fraction of *Citrullus lanatus* rinds stripe variety.

Sample Name	**Dose (mg)**	**Absorbance (334 nm)**	**% of inhibition**
Chcloroform fraction of stripe (S)	149.2	1.798	64.16348 ± 0.96
	74.6	1.16	38.75434 ± 1.32
	37.3	0.9045	26.18112 ± 1.11
	18.6	0.7705	14.30191 ± 1.57
	9.3	0.7245	7.081963 ± 1.88

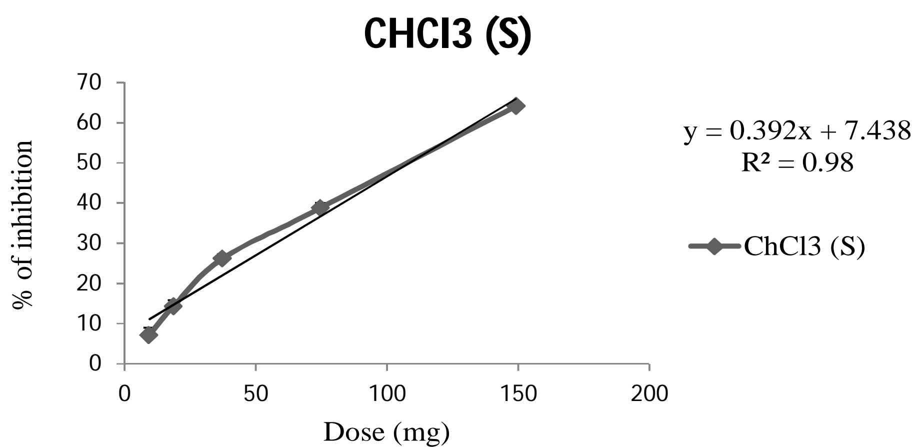


Figure 3.7: Anti - diabetic activity of chloroform fraction of stripe

Table 3.7: Anti - diabetic activity of dichloromethane fraction of *Citrullus lanatus* rinds stripe variety.

Sample Name	**Dose (mg)**	**Absorbance (334 nm)**	**% of inhibition**
Dichcloro-methane fraction of stripe (S)	132.6	0.7065	0 ± 1.75
	66.6	0.698	0 ± 1.79
	33.15	0.6575	0 ± 2.47
	16.5	0.6855	0 ± 1.73
	8.25	0.6435	0 ± 1.85

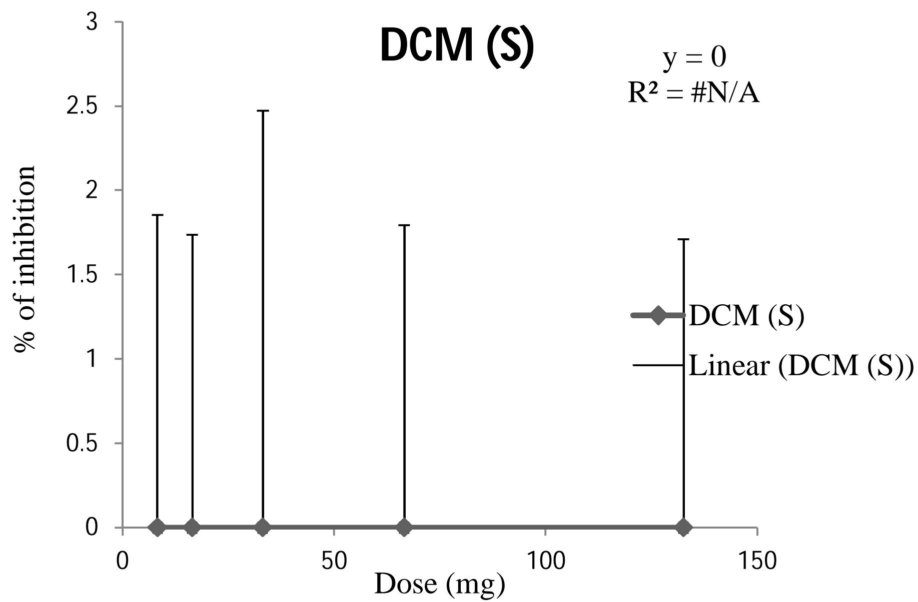


Figure 3.8: Anti - diabetic activity of dichloromethane fraction of stripe

Table 3.8: Anti - diabetic activity of xylene fraction of *Citrullus lanatus* rinds stripe variety.

Sample Name	Dose (mg)	Absorbance (334 nm)	% of inhibition
Xylene fraction of stripe (S)	86	0.9535	31.4915 ± 1.76
	43	0.835	22.48365 ± 2.13
	21.5	0.7845	17.77351 ± 1.40
	10.75	0.7725	16.44662 ± 1.88
	5.375	0.7685	16.01568 ± 1.97

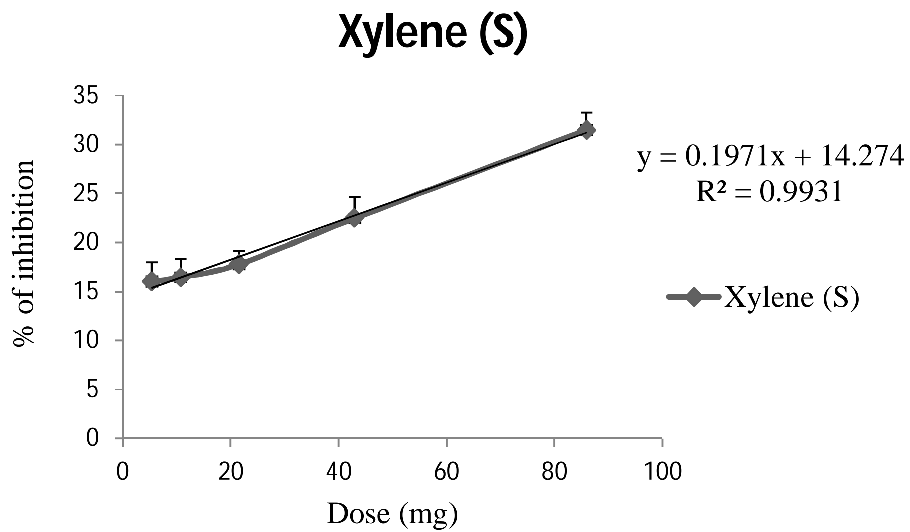


Figure 3.9: Anti - diabetic activity of xylene fraction of stripe

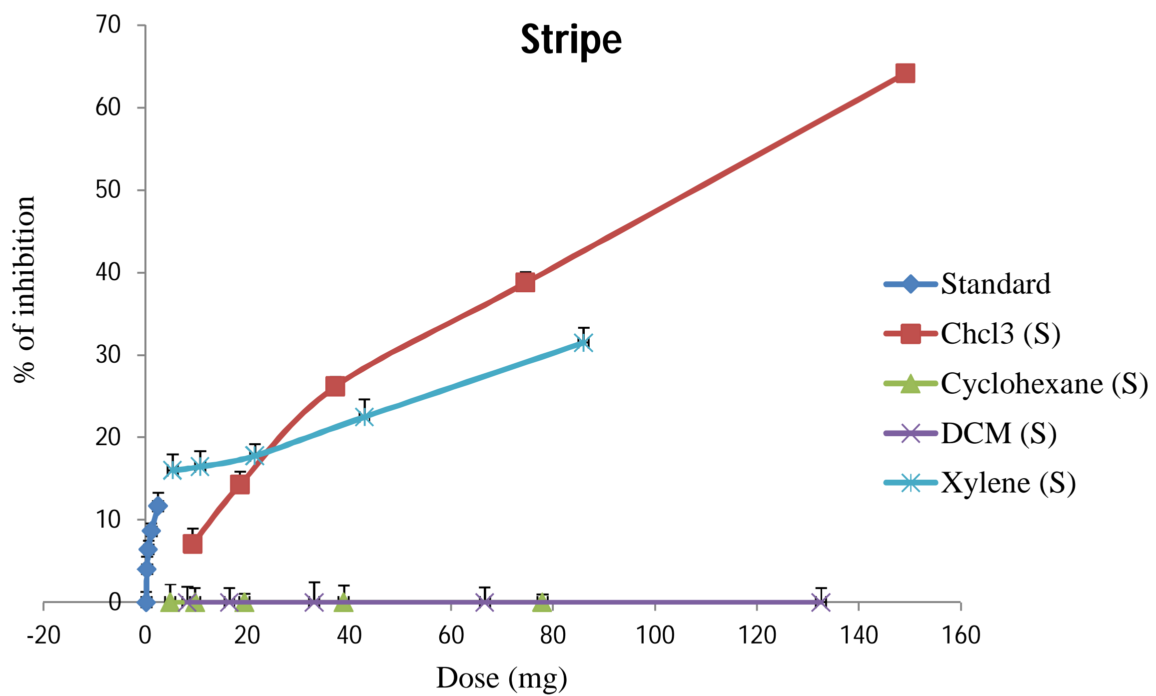


Figure 3.10: Anti- diabetic activity of chloroform, dichloromethane, cyclohexane, xylene fraction of *Citrullus lanatus* rinds stripe variety and metformin.

Table 3.9: Anti - diabetic activity of chloroform fraction of *Citrullus lanatus* rinds deep green variety.

Sample Name	**Concentrations (mg)**	**Absorbance (334 nm)**	**% of inhibition**
Chcloroform fraction of deep green (DG)	149.2	0.8085	19.36902 ± 2.07
	74.6	0.7705	15.9765 ± 1.32
	37.3	0.758	14.29895 ± 2.08
	18.6	0.744	12.66214 ± 1.95
	9.3	0.7	7.440476 ± 1.12

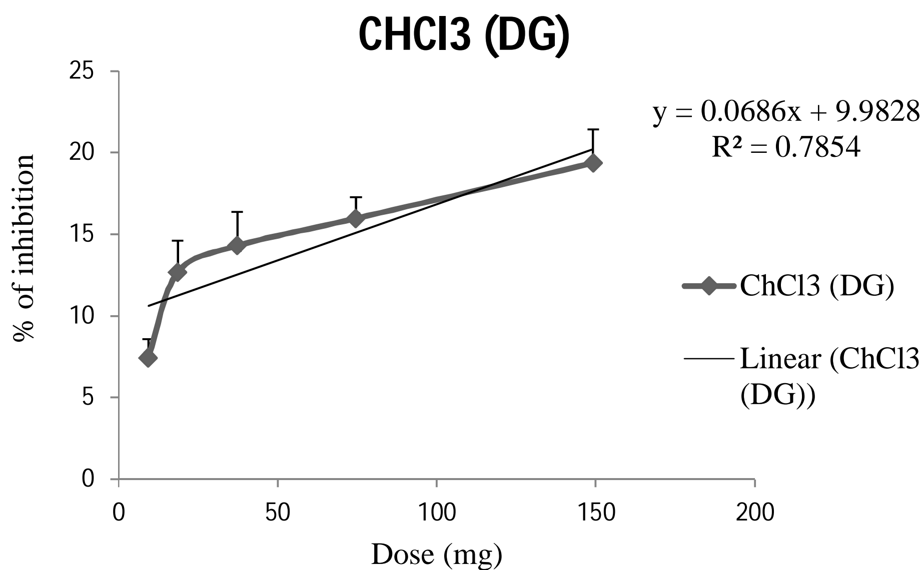


Figure 3.11: Anti - diabetic activity of chloroform fraction of deep green.

Table 3.10: Anti - diabetic activity of dichloromethane fraction of *Citrullus lanatus* rinds deep green variety.

Sample Name	**Dose (mg)**	**Absorbance (334 nm)**	**% of inhibition**
Dichcloro - methane fraction of deep green (DG)	132.6	0.7725	16.62321 ± 1.52
	66.6	0.7605	15.22386 ± 1.26
	33.15	0.741	12.75477 ± 1.64
	16.5	0.7155	9.603104 ± 0.43
	8.25	0.6975	6.986508 ± 1.16

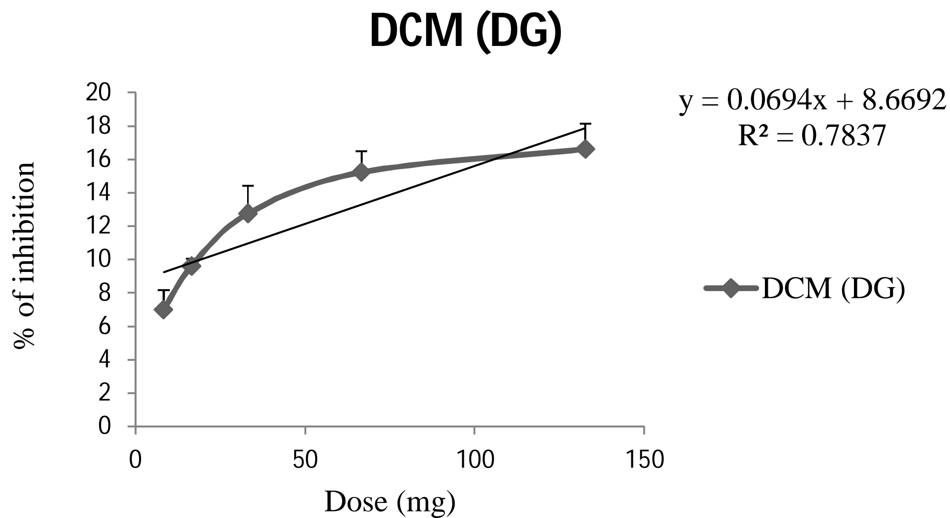


Figure 3.12: Anti - diabetic activity of dichloromethane fraction of deep green.

Table 3.11: Anti - diabetic activity of xylene fraction of *Citrullus lanatus* rinds deep green variety.

Sample Name	**Dose (mg)**	**Absorbance (334 nm)**	**% of inhibition**
xylene fraction of deep green (DG)	86	1.4575	55.86302 ± 1.78
	43	1.028	36.81199 ± 1.07
	21.5	0.856	24.72838 ± 1.69
	10.75	0.782	17.68833 ± 0.60
	5.375	0.739	12.62515 ± 1.80

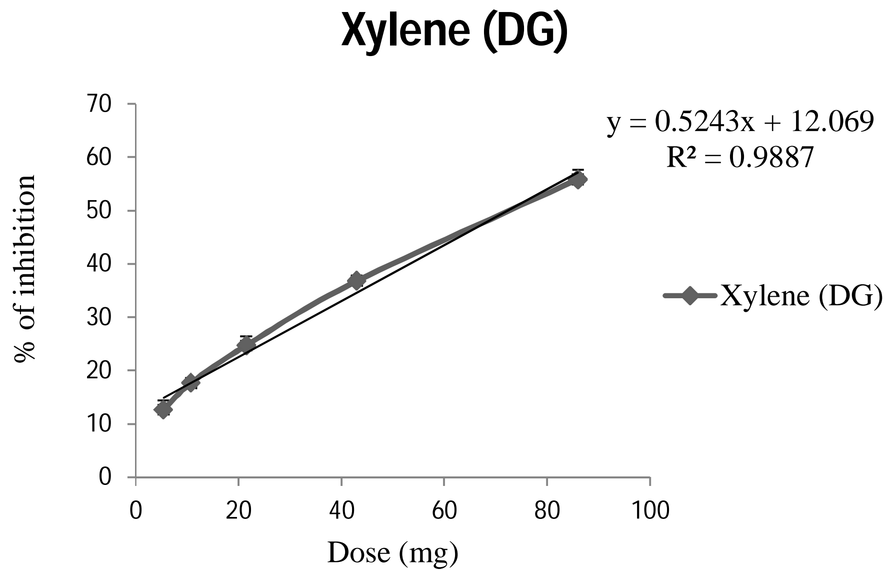


Figure 3.13: Anti - diabetic activity of xylene fraction of deep green.

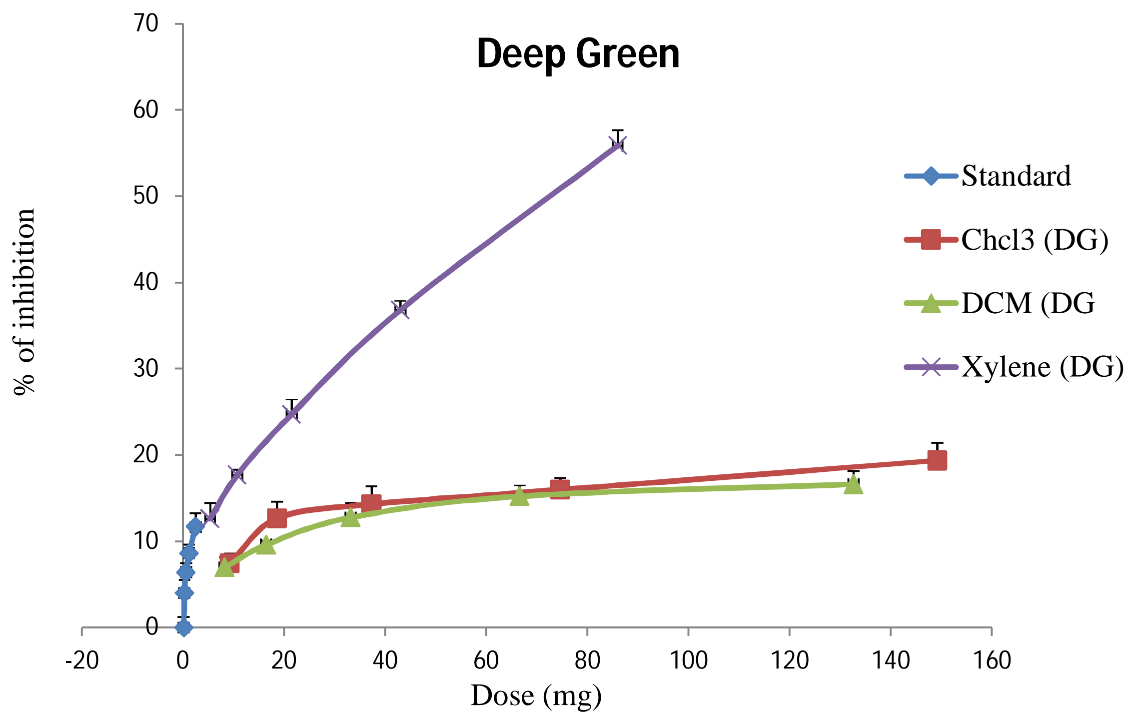


Figure 3.14: Anti- diabetic activity of chcloroform, dichcloromethane, xylene fraction of *Citrullus lanatus* rinds deep green variety and metformin.

4. Antibacterial Activity

Positive control: Ciprofloxacin

4.1: Gram positive bacteria:

Bacteria		*Bacillus cereus*	*Bacillus megaterium*	*Bacillus subtilis*	*Staphylococcus aureus*	*Sarcina lutea*	*Candida albicans*
Code		1	2	3	8	12	15
Sample		Zone of inhibition (cm)					
Light gren	Cyclohexane	----	----	6.5 ± 0.3	----	----	10 ± 0.5
	Chloroform	----	----	6 ± 0.1	----	----	7.5 ± 0.2
	Dichloro-methane	----	----	----	----	----	----
	xylene	----	----	----	6.5 ± 0.4	----	5 ± 0.1
	Cyclohexane	----	----	----	6 ± 0.2	----	----
	Pet ether	----	----	----	----	----	----
	Chloroform	----	----	6.5 ± 0.23	----	----	7 ± 0.12

Stripe	Dichcloro-methane	11 ± 0.1	12 ± 0.05	18 ± 0.3	17 ± 0.2	20 ± 0.25	19 ± 0.125
	Pet ether	----	----	----	----	----	----
Deep green	Cyclohexane	----	----	-----	-----	----	----
	Chloroform	----	----	----	5 ± 0.6	----	----
	Dichloro-methane	----	----	----	----	6 ± 0.1	6 ± 0.2
	xylene	----	----	----	----	----	----
Standard		30 ± 0.1	31 ± 0.1	29.5 ± 0.5	29.5 ± 0.5	28.3 ± 0.75	30.5 ± 0.5

4.2: Gram negative bacteria:

Bacteria		*S. paratyphi*	*S. typhi*	*V. parahemolyticus*	*V. mimicus*	*E. coli*	*S. dysenteriae*
Code		4	5	6	7	9	10
Sample		Zone of inhibition (cm)					
	Cyclohexa	----	----	----	----	----	----

Light gren	ne						
	Chloroform	----	----	----	----	----	----
	Dichloro-methane	----	----	----	----	----	----
	xylene	----	----	----	----	----	----
Stripe	Cyclohexane	----	----	----		----	----
	Pet ether	----	----	----	----	----	----
	Chloroform	----	----	----	----	----	----
	Dichcloro-methane	12.3 ± 0.25	17 ± 0.1	19 ± 0.3	13.5 ± 0.5	12 ± 0.1	12.5 ± 0.5
	Pet ether	----	----	----	----	----	----
Deep green	Cyclohexane	----	----	-----	-----	----	----
	Chloroform	----	----	----		----	----
	Dichloro-methane	----	----	----	----	----	----
	xylene	----	----	----	----	----	----

Standard	30 ± 0.4	33.5 ± 0.5	33 ± 0.1	33 ± 0.1	27.5 ± 0.15	30.5 ± 0.5

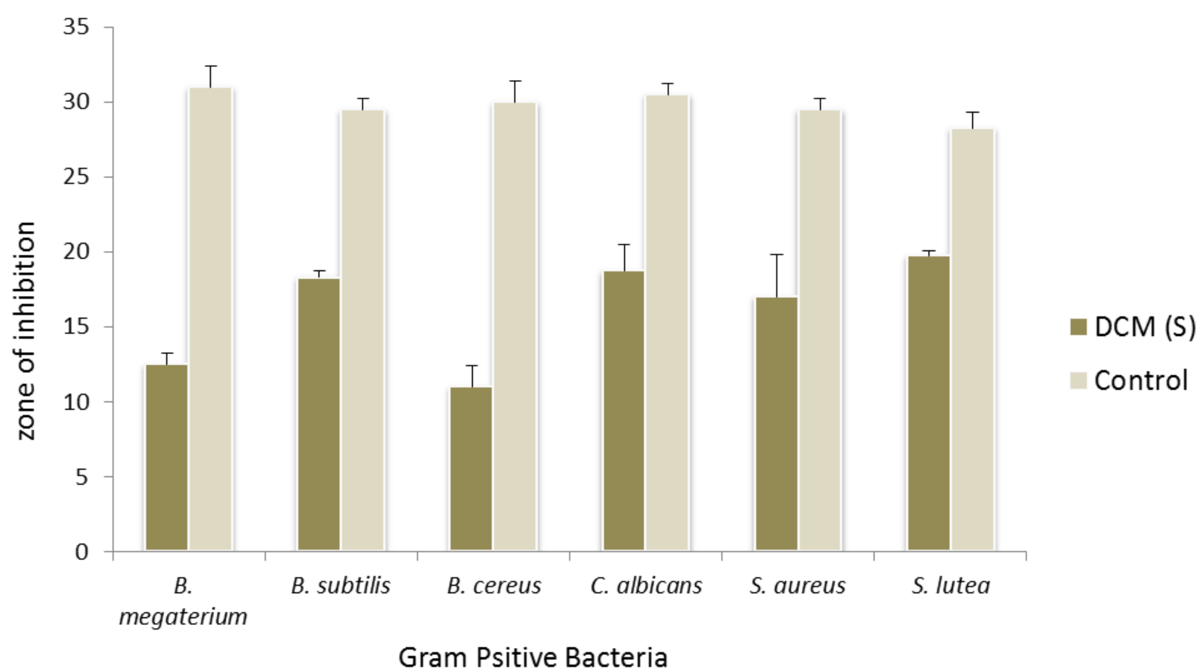


Figure 4.1: Antibacterial activity of dichloromethane fraction of *Citrullus lanatus* rinds stripe variety and ciprofloxacin on gram positive bacteria.

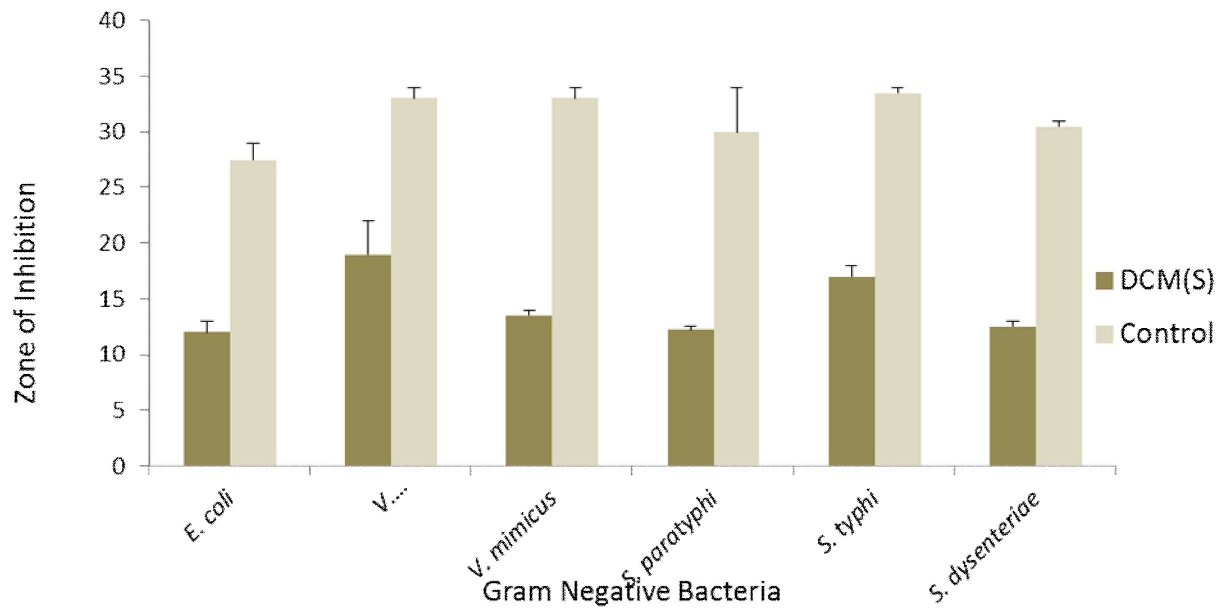
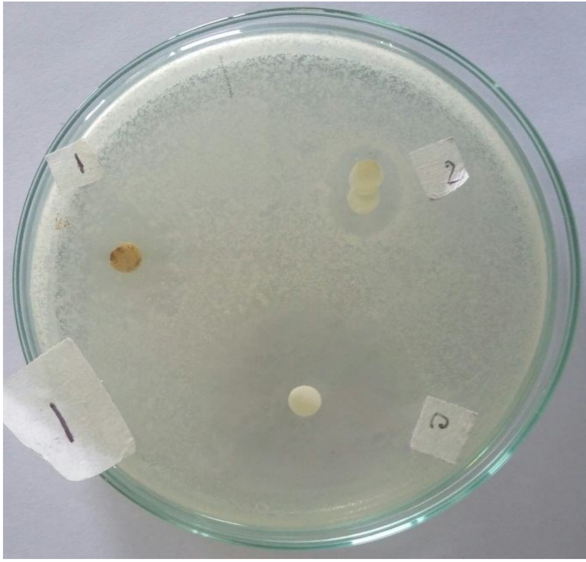
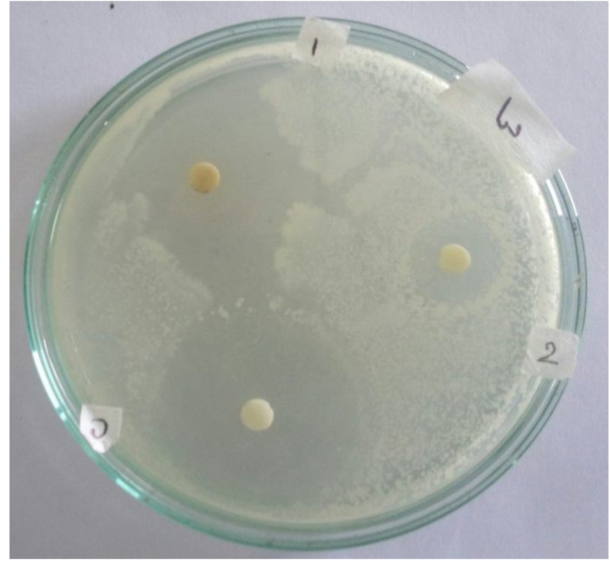
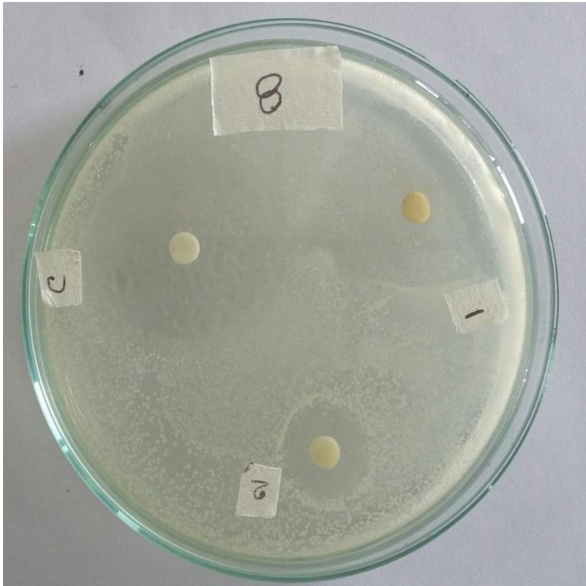


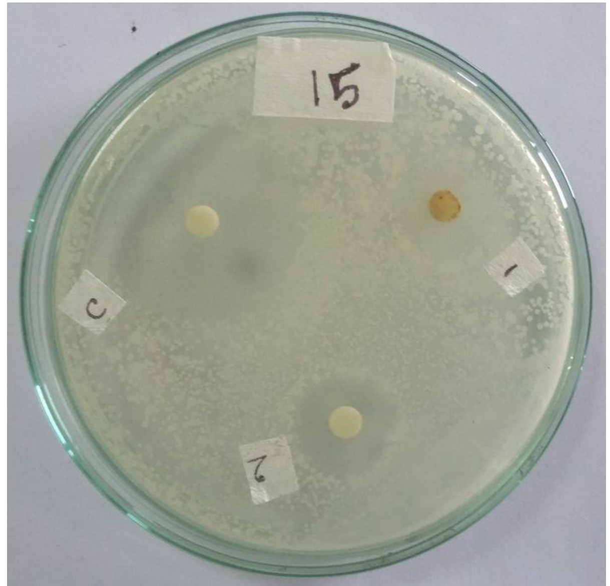
Figure 4.2: Antibacterial activity of dichloromethane fraction of *Citrullus lanatus* rinds stripe variety and ciprofloxacin on gram negative bacteria.

Image of zone of inhibition of gram positive bacteria by dichloromethane fraction of *Citrullus lanatus* rinds stripe variety:









Note,

2 = Dichloromethane (Stripe)

C= Positive control

Dicloromethane(Stripe)

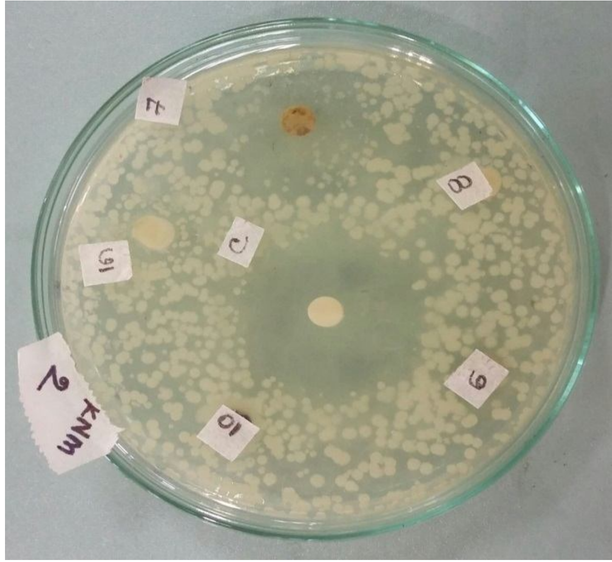
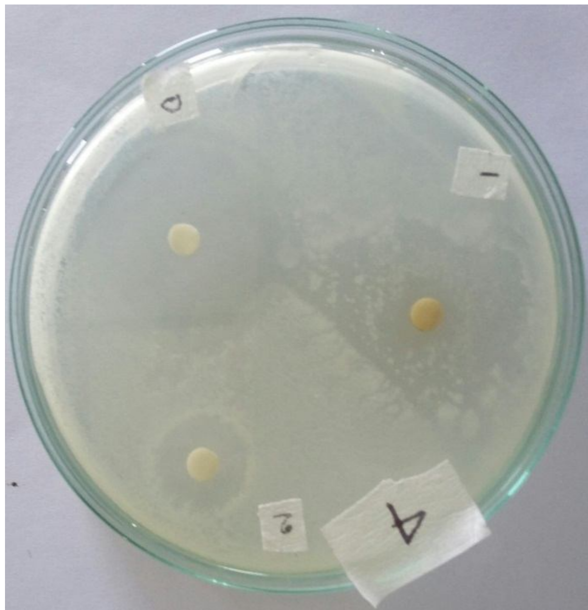
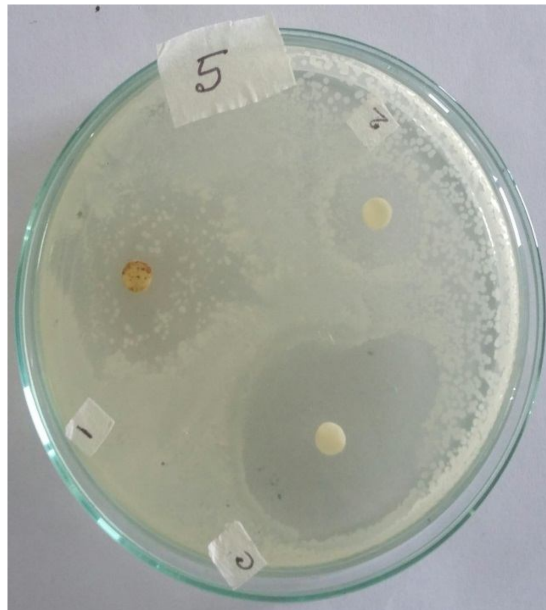
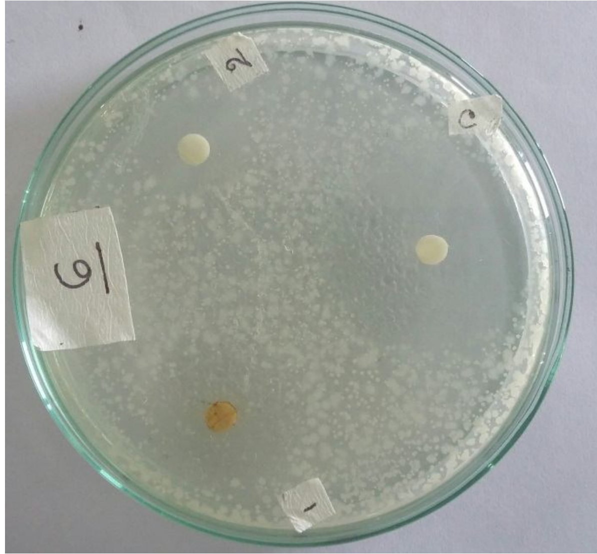


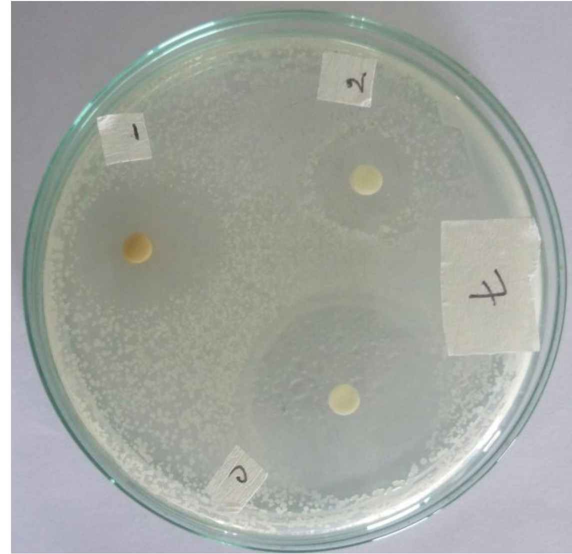
Figure 4.3: Antibacterial activity of dichloromethane fraction of *Citrullus lanatus* rinds stripe variety and ciprofloxacin on gram positive bacteria.

Image of zone of inhibition of gram negative bacteria by dichloromethane fraction of *Citrullus lanatus* rinds stripe variety:









Note,

2 = Dichloromethane (Stripe)

C= Positive control

Figure 4.4: Antibacterial activity of dichloromethane fraction of *Citrullus lanatus* rinds stripe variety and ciprofloxacin on gram positive bacteria.

5. **Anti-inflammatory activity**

The result of anti-inflammatory test of different fraction of *Citrullus lanatus* rinds are given below-

Table 5.1: Anti- inflammatory activity of standard Diclofenac.

Sample Name	Dose (mg)	Absorbance (560nm)	% of stabilization
Diclofenac	0.5	0.092	59.18309 ± 0.74
	0.25	0.097	57.06305 ± 0.11
	0.125	0.099	56.23427 ± 0.26
	0.0625	0.1215	46.06892 ± 1.15
	0.03125	0.129	43.03313 ± 0.76

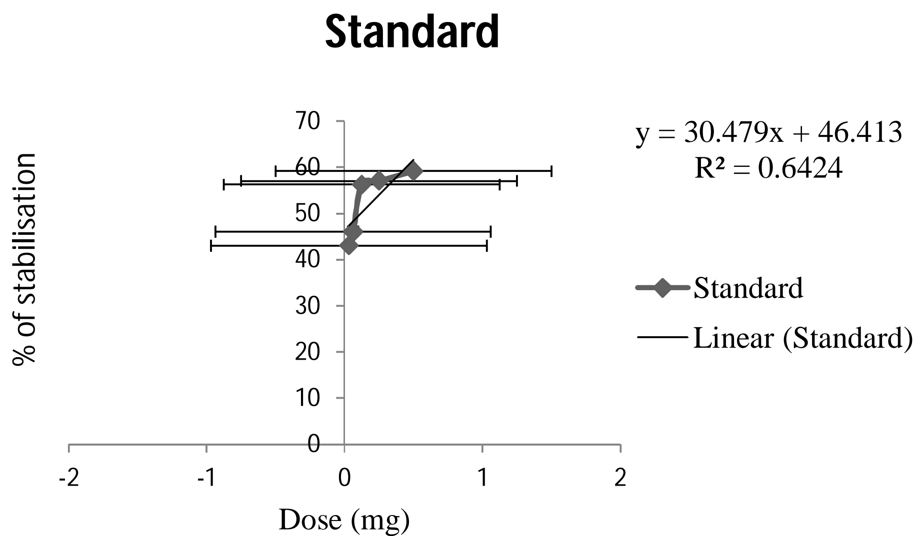


Figure 5.1: Anti-inflammatory activity of Diclofenac

Table 5.2: Anti - inflammatory activity of chloroform fraction of *Citrullus lanatus* rinds light green variety.

Sample Name	**Dose (mg)**	**Absorbance (560nm)**	**% of stabilization**
Chloroform fraction light green (LG)	149.2	0.159	29.3511 ± 2.005
	74.6	0.1775	21.32421 ± 0.924
	37.3	0.177	22.27679 ± 1.048
	18.6	0.195	13.66362 ± 0.361
	9.3	0.2235	1.300469 ± 1.321

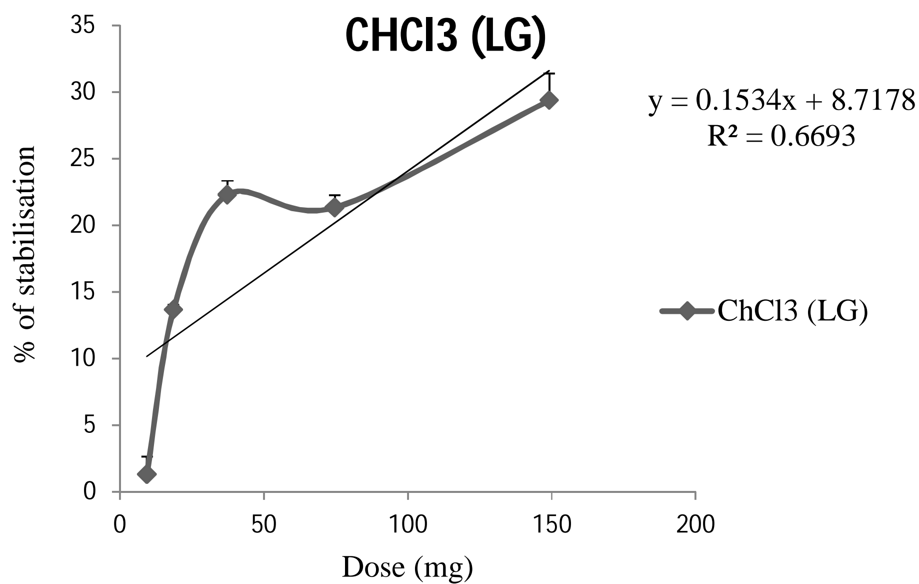


Figure 5.2: anti- inflammatory activity of chloroform fraction of light green

Table 5.3: Anti - inflammatory activity of DCM fraction of *Citrullus lanatus* rinds light green variety.

Sample Name	**Dose (mg)**	**Absorbance (560nm)**	**% of stabilization**
DCM fraction of light green (LG)	132.6	0.391	0 ± 2.21
	66.6	0.296	0 ± 1.44
	33.15	0.267	0 ± 2.08
	16.5	0.258	0 ± 1.82
	8.25	0.3035	0 ± 1.916

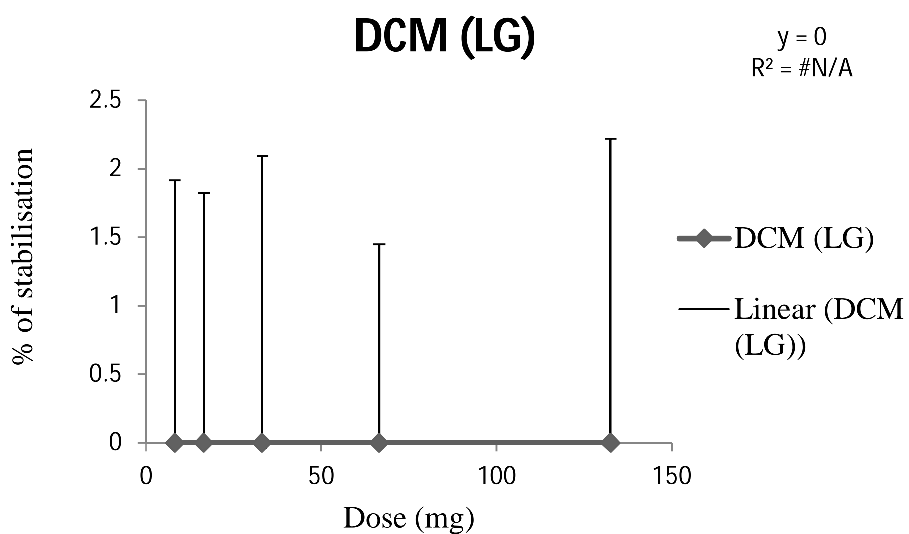


Figure 5.3: anti- inflammatory activity of DCM fraction of light green

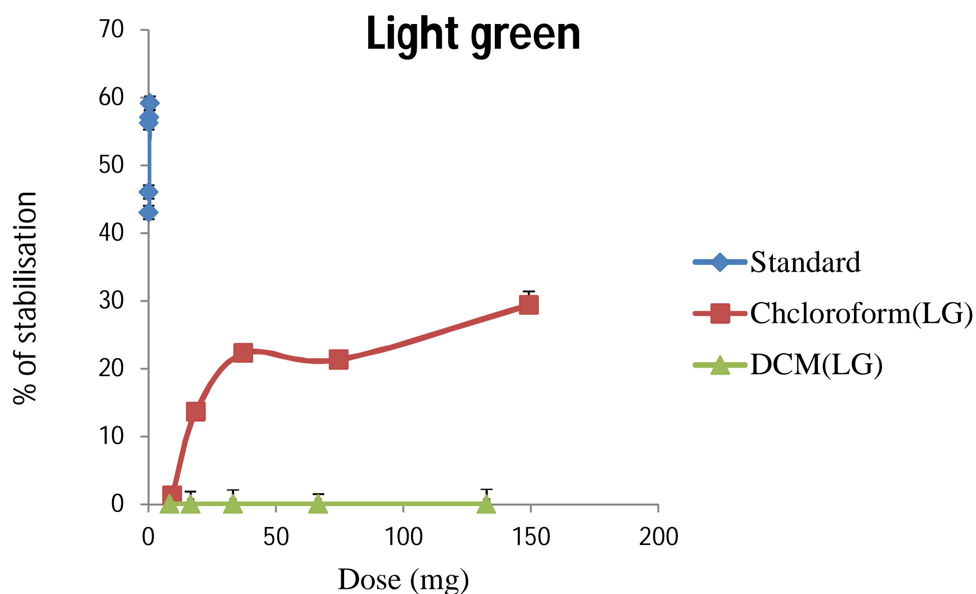


Figure 5.4: Anti- inflammatory activity of chloroform, dichloromethane fraction of *Citrullus lanatus* rinds light green variety and diclofenac.

Table 5.4: Anti - inflammatory activity of Xylene fraction of *Citrullus lanatus* rinds stripe variety.

Sample Name	Dose (mg)	Absorbance (560nm)	% of stabilization
Xylene fraction of stripe (s)	86	0.2775	0 ± 1.40
	43	0.295	0 ± 1.62
	21.5	0.29	0 ± 1.19
	10.75	0.3055	0 ± 1.52

	5.375	0.2935	0 ± 2.42

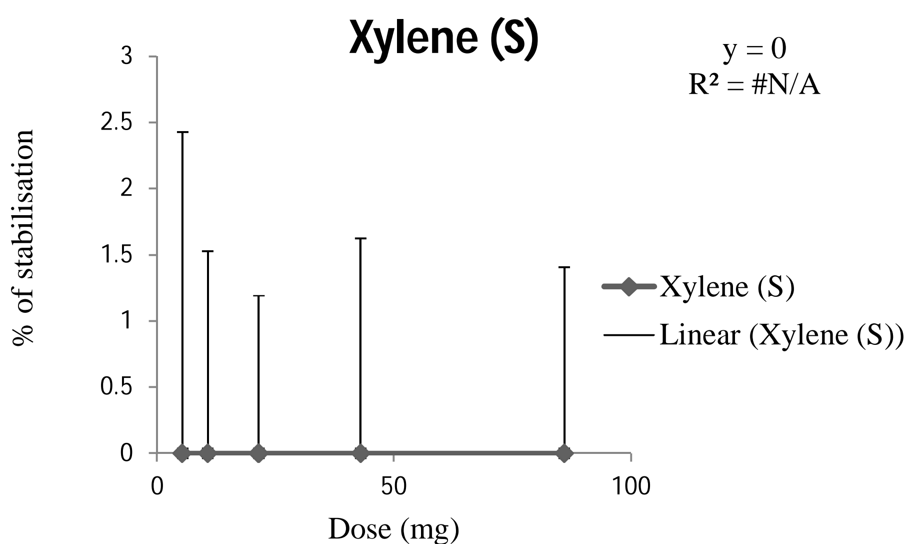


Figure 5.5: anti- inflammatory activity of Xylene fraction of Stripe.

Table 5.5: Anti - inflammatory activity of Cyclohexane fraction of *Citrullus lanatus* rinds stripe variety.

Sample Name	**Dose (mg)**	**Absorbance (560nm)**	**% of stabilization**
	77.9	0.1185	48.03344 ± 3.17

Cyclohexane fraction of stripe(S)	38.95	0.1645	27.5288 ± 2.15
	19.47	0.193	14.20386 ± 2.70
	9.73	0.228	0 ± 0.38
	4.865	0.2515	0 ± 3.79

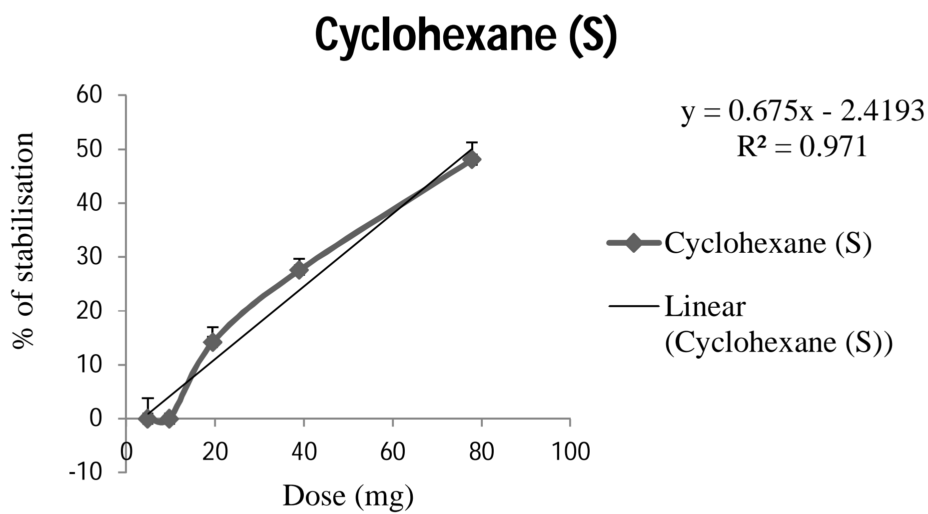


Figure 5.6: anti- inflammatory activity of Cyclohexane fraction of Stripe.

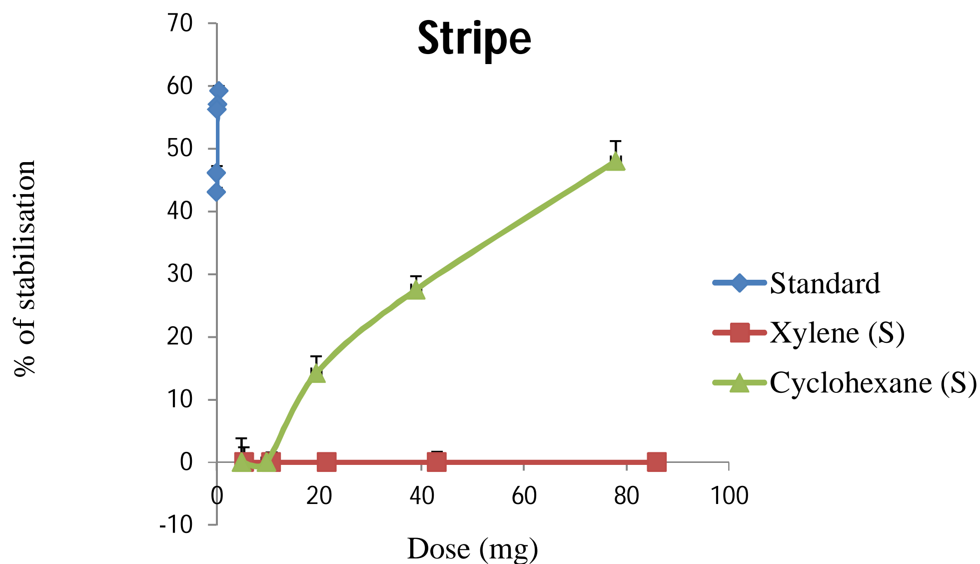


Figure 5.7: Anti- inflammatory activity of xylene, cyclohexane fraction of *Citrullus lanatus* rinds stripe variety and diclofenac.

Table 5.6: Anti - inflammatory activity of Xylene fraction of *Citrullus lanatus* rinds deep green variety.

Sample Name	**Dose (mg)**	**Absorbance (560nm)**	**% of stabilization**
Xylene fraction of Deep green (DG)	86	0.1895	16.4477 ± 2.02
	43	0.2155	5.385025 ± 1.02
	21.5	0.2305	0 ± 0.90
	10.75	0.2385	0 ± 1.85
	5.375	0.2465	0 ± 1.11

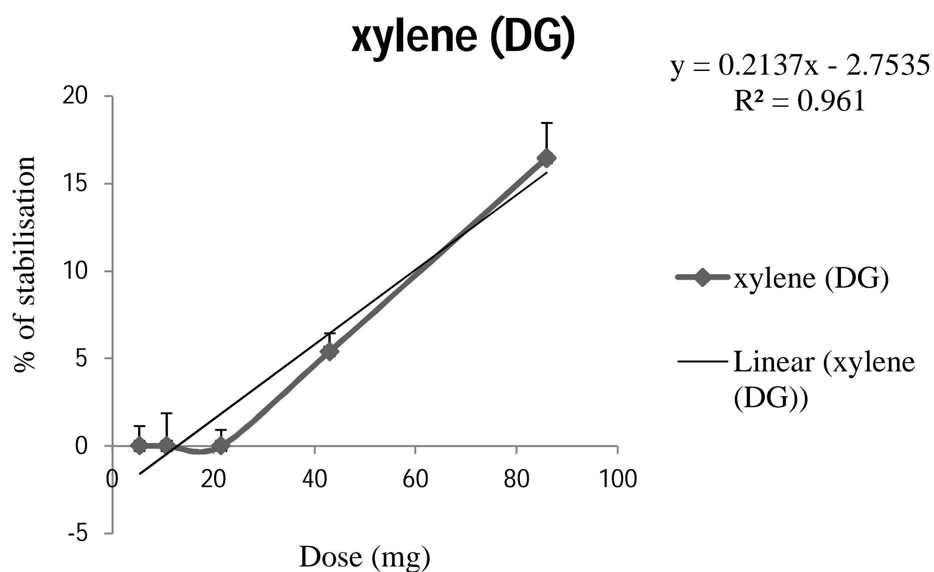


Figure 5.8 : anti- inflammatory activity of Xylene fraction of deep green (DG).

Table 5.7: Anti - inflammatory activity of DCM fraction of *Citrullus lanatus* rinds deep green variety.

Sample Name	**Dose (mg)**	**Absorbance (560nm)**	**% of stabilization**
DCM fraction of	132.6	0.096	57.33317 ± 1.28
	66.6	0.114	49.29707 ± 1.77
	33.15	0.136	39.41105 ± 2.80

deep green (DG)	16.5	0.154	31.47114 ± 2.63
	8.25	0.162	27.96366 ± 2.41

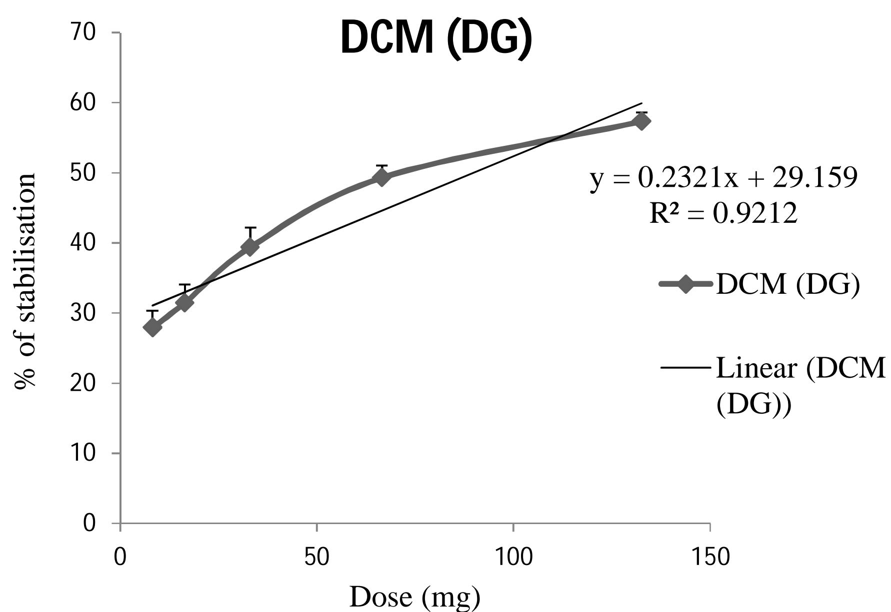


Figure 5.8: anti- inflammatory activity of DCM fraction of deep green (DG).

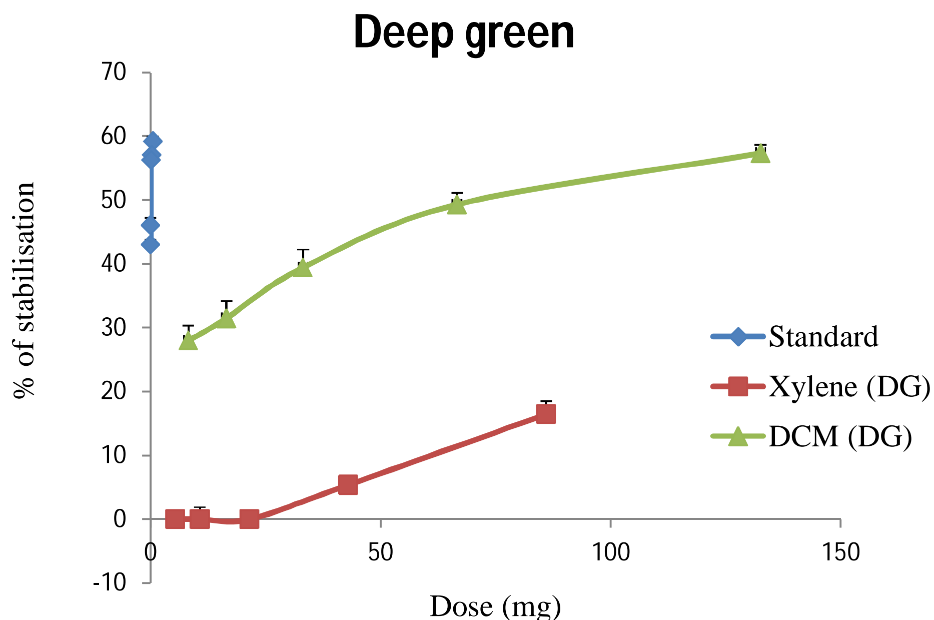


Figure 5.9: Anti- inflammatory activity of Xylene, DCM fraction of *Citrullus lanatus* rinds stripe variety and Diclofenac.

6. Hemagglutination assay

The result of hemagglutination test of different fraction of *Citrullus lanatus* rinds are given below-

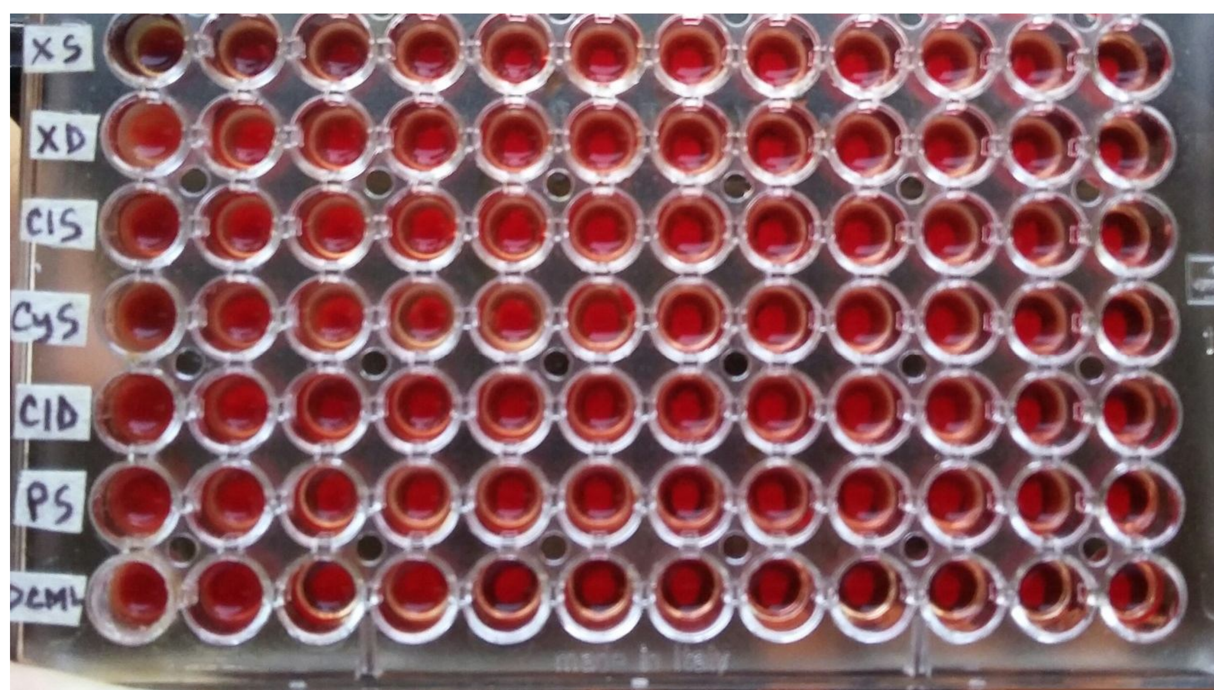
Table 6.1

Concentration (µl)	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09	0.04
Sample name	Cell receptor binding										
Xylene (S)	+	+	–	–	–	–	–	–	–	–	–
Xylene (DG)	+	–	–	–	–	–	–	–	–	–	–
Chloroform(S)	+	+	+	–	–	–	–	–	–	–	–

Cyclohexane(S)	+	+	+	–	–	–	–	–	–	–	–
Chloroform(DG)	+	+	–	–	–	–	–	–	–	–	–
Pet ether (S)	+	+	–	–	–	–	–	–	–	–	–
DCM (LG)	++	+	+	+	–	–	–	–	–	–	–

Note,

\+ = Agglutinated - = Not agglutinated



Colum 12 is control

Figure 6.1: Hemagglutination test of different fraction of *Citrullus lanatus* rinds

CHAPTER FIVE

DISCUSSION

Thin layer chromatography

TLC plates were developed with petroleum ether, cyclohexane, xylene, dichloromethane, trichloromethane fraction of all variety of *Citrullus lanatus* rind using solvent system-1 polar basic solvent (ethyl acetate, ethanol, water in the ratio of 8:1.2:0.8) solvent system- 2 semi polar solvent (benzene, ethyl acetate in the ratio of 6:4) and solvent system-3 non polar basic solvent (Benzene, ethanol, ammonium hydroxide in the ratio of 9:1:0.1). The best result was found using solvent system-2 semi polar solvent (benzene, ethyl acetate in the ratio of 6:4).

In case of polar basic solvent nothing is found in naked eye view, under UV lamp it is found that the sample only run on the plate no spot is found, after charring the plate showed spot in light green (cyclohexane, chloroform, xylene fraction with Rf value of 0.25,0.95,0.25 respectively) ; deep green (chloroform, DCM fraction with Rf value of 0.95,0.95 respectively); stripe (chloroform, DCM fraction).chloroform fraction showed spot with the Rf value of 0.95 and DCM fraction showed three spot with Rf value of 0.25,0.75,0.95 respectively.

In case of non-polar basic solvent the naked eye view and under the UV the TLC plate showed no clear spot. Even after charring no clear spot were found. It indicates that none of the sample runs to the plate in non-polar solvent system.

In case of semi polar solvent the naked eye view of the TLC showed no clear spot, under UV lamp it showed sky blue color spots in light green(cyclohexane, chloroform, DCM fraction with Rf value of 0.77); sky blue and purple color spot in deep green (chloroform, DCM) fraction; stripe (DCM fraction with Rf value of 0.8). After charring the plate showed extra spot in stripe (cyclohexane, chloroform fraction with Rf value of 0.8). Spraying of 0.04% DPPH solution on the TLC plate has shown significant formation of yellow color which assured the presence of flavonoids. Further extractions and purifications from these crude samples may lead to the possible isolation of these compounds from the extracts.

DPPH radical scavenging activity:

Free radical scavenging potential of the different fraction of *Citrullus lanatus* rind was evaluated in vitro by using DPPH assay. In this method the antioxidants present in the plant extracts reacted with DPPH, which is a stable free radical and converted it to 1,1-diphenyl-1,2-picryl, hydrazine which is measured at 517 nm. The previous study also reported the weak antioxidant activities of *Citrullus lanatus* rind in vitro (Olabinri et al., 2013) which is similar to the present results. DCM and Xylene fraction of light green showed 8.06% in 132.6 mg and 19.43 % in 86 mg respectively. Xylene fraction of deep green showed 26.16% in 86 mg. Cyclohexane and DCM fraction of deep green showed 15.69% in 77.9 mg and 35.41% in 132.6 mg respectively. DCM fraction of deep green variety of *Citrullus lanatus* rind showed highest activity.

Anti - diabetic activity:

In Yeast (*Saccharomyces cerevisiae*) glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterised by the deficiency of insulin causing increased amount of glucose in blood. (Sujanya et al.,2014). After the treatment of the yeast cells with these extracts, the glucose uptake was found to increase in a dose dependent manner. The rate of glucose transport across cell membrane in yeast cells system for chloroform and Xylene fraction of stripe variety of *Citrullus lanatus* rind is 64.16% in 149.2 mg and 31.49% in 86 mg respectively. Chloroform fraction of light green variety showed 8.66% in 149.2 mg. Xylene fraction of deep green variety showed 55.86 % in 86mg.The in vitro assays of the present study indicated that chloroform fraction of stripe variety of *Citrullus lanatus* rind possesses good anti diabetic activity. Results also indicated that chloroform fraction of stripe variety of *Citrullus lanatus* rind had greater efficiency in increasing glucose uptake by yeast cell as compared to standard drug metformin.

Anti-bacterial activity:

Various strains of Gram positive, Gram negative bacteria and fungi were used in this test. The positive control used was ciprofloxacin (30µg/disc).Petroleum ether, cyclohexane, xylene, dichloromethane, trichloromethane fraction of all variety of *Citrullus lanatus* rind was used to evaluate the activity against different types of microorganism. The zones of inhibition for the microbes were measured in millimeters using a vernier caliper after 24hrs of incubation. DCM fraction of stripe variety was showed zone of inhibition up to 20 mm antibacterial activity at the concentrations used against *S. aureus, B. subtilis, S. lutea, C. albicans, V. parahemolyticus* than other strain. A previous study showed that the seeds possessing antimicrobial activity can be employed against human pathogens. (Nwankwo, Onwuakor and Nwosu, 2014). The result of antibacterial activity of the DCM fraction of stripe variety against selected bacteria indicated that the plant sample was active against a wide variety of human pathogenic bacteria. Therefore, the dichloromethane fraction of stripe variety of *Citrullus lanatus* rind may be considered as a useful source for discovering a safe and novel antimicrobial compound.

Anti-inflammatory activity:

The inhibition of hypotonicity induced HRBC membrane lysis i.e, stabilization of HRBC membrane was taken as a measure of the anti-inflammatory activity. The percentage of membrane stabilization for Petroleum ether, cyclohexane, xylene, dichloromethane, trichloromethane fraction of all variety of *Citrullus lanatus* rind and Diclofenac sodium were done. Cyclohexane fraction of stripe variety and DCM fraction of deep green variety showed the maximum inhibition 48.03 % at 77.9 mg and 57.33 % at 132.6mg.With the increasing concentration the membrane hemolysis is decreased and membrane stabilization / protection is increased. Hence anti-inflammatory activity of the fraction was concentration dependent.

Hemagglutination Inhibition Assay:

DCM fraction of light green variety of *Citrullus lanatus* rind exhibited hemagglutination inhibition activity potentially from highest concentration 66.3 mg/ml to 8.25 mg/ml i.e. it has potential binding capacity with human erythrocytes.

Conclusion:

This review had shown that rind of *Citrullus lanatus* possesses numerous bioactivities from natural source which is of better advantage than conventional therapies. It provided anti-bacterial and anti-diabetic, anti- inflammatory activity and showed potential binding capacity with human erythrocytes; however, it is not known that which component of the extract is responsible for this effect. Further studies using isolated constituents instead of the whole extract should be carried out.

CHAPTER SIX

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