Phytochemical Screening and Biological Activities Evaluation of *Chassalia curviflora*

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

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Approval

The thesis "Phytochemical Screening and Biological Activity Evaluation of *Chassalia curviflora*" submitted by Mahbuba Mohoshina Runa; ID: 15346023 of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 10th October, 2019.

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Ethics Statement

This study includes no animal or human trials.

Abstract

The recent study was done to investigate the presence of phytochemical components and biological activities of the *Chassalia curviflora* whole plant petroleum ether, ethyl acetate and methanol extract. This study was also performed to compare the potency of the plant extract of different solvents as different phytochemical components are found in different solvent extract. The primary objective of the study was to establish this plant as a potential therapeutic agent in traditional practice of medicine in Bangladesh. In this study, disk diffusion method was used to observe antimicrobial activities of the plant extracts of different solvents. Next, DPPH (2,2-diphenyl-1-picrylhydraziyl) free radical scavenging assay, total phenolic content and total flavonoid content were done to determine the in-vitro antioxidant activity of the extract. Finally, the cytotoxic activity was determined by the use of brine shrimp lethality test and calculation of LC_{50} value was done to compare with the standard.

Keywords: *Chassalia curviflora*; Polarity Increasing Order; Phytochemical screening; Antimicrobial activity assay; Antioxidant potential; Cytotoxic activity

Dedication

Dedicated to my family and my honorable supervisor Easin Uddin Syed.

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Firstly, I would like to show my gratitude to the Almighty Allah who helps me throughout the time with His divine blessings, endurance, mental and physical soundness in the completion of this project work and preparation of this paper.

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Mahbuba Mohoshina Runa

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List of Acronyms

WHO	World Health Organization
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent
CNS	Central Nervous System
ULT	Ultra Low Temperature
DMSO	Dimethyl Sulfoxide
ASA	Ascorbic Acid

Chapter 1

Introduction

1.1 General information

As reported by WHO, about 35000 to 70000 species of different plants were found with therapeutic values those are used as medicinal agents till now. Additionally mentioned the presence of about 150 compounds in different plants and defined those plants as ethno medicinal plants and the process of the investigation about those plants as ethno botany (Mamedov, 2012). Ethno pharmacology is the field of research that have significant impacts in the investigation of scientific explanation of using plants as traditional medicines as it includes observation, identification, description and experimental analysis of the components, and the effective activities of the components of plants (Ajayi, 2009). Plants used in herbalism due to having medicinal values and regarded as abundant sources of components that have the possibilities of being used in developing and synthesizing new drugs are known as medicinal plants (Rasool Hassan, 2012). Different parts of the plants including seeds, root, leaves, skin, fruits, flowers or even the whole plant can have importance as medicinal agents because of having different therapeutic activities. The components that exist in the different parts of plant or even the plant as a whole can be proved physiologically efficacious in the treatment of living things (Jamshidi-Kia, Lorigooini, & Amini-Khoei, 2018).

1.2 History of medicinal plants

To survive in this earth dependency between plants and animals is the most important thing that none can avoid and the connection is like one completes another's necessities from the pre-historic time. With time many evolutions occurred and people learned to use and separate

different plants for fulfilling their different needs including shelter, oxygen, foods and even for treating injuries and diseases. Additionally, ancient people also identified those plants that they found therapeutically important in use and developed the traditional methods of treatment such as Ayurveda, Unani etc. systems using those saved information (Mamedov, 2012). From the very ancient time, people used herbs, plant paste, juice to reduce pain, inflammation etc. even without knowing the proper functional explanation. In South Asia above 80% of the people depends on the traditional methods of treatment which is based on the medicinal plants or herbs to treat diseases. This scenario has been so common in developing countries due to the affordable price and less side effects of traditional medicines than the synthetic medicines ("Medicinal Plants: Local Heritage with Global Importance," n.d.). The way we are living in this earth that is only possible for having plants and even before the human identified and started using plants for their regular necessities like for clothing, shelter, food etc. they found out the therapeutic effects of the plants. In several countries like China , India, Greece and Egypt medicinal plants are already recognized as one among the oldest science (Jamshidi-Kia et al., 2018). In Indian culture, people successfully used herbs, plants to cure illness over 3000 years which information should be preserved ("Medicinal Plants: Local Heritage with Global Importance," n.d.). One of the written documents shows that Sumerian clay slab from Nagpur since 5000years ago used medicinal plants to prepare drugs. According to a monument, the ancient people such as Egyptians and Chinese would use medicinal plants from the 27 centuries BC (Jamshidi-Kia et al., 2018).

1.3 History of medicinal plants in Bangladesh:

The people of rural areas of Bangladesh rely on the treatment of traditional curers or healers who use medicinal plants as the primary source of medicines for healing pain, inflammations and treating diseases. This dependency on the traditional methods of treatment grows among

the rural areas population due to having less entrance to the modern treatment facilities (Hossan et al., 2010). Moreover they prefer to take treatment from the local known healers or curers to avoid the uncomfortableness of sharing the problems or diseases with any unknown doctors (Hossan et al., 2010).

Table 01: Traditionally used medicinal plants in different areas of Bangladesh

Sl. No.	**Plant's Local Name**	**Botanical Name**	**Family**	**Traditional Uses**
01	Laal-tarokh	*Barleria lupulina Lindl.*	Acanthaceae	Tonic, dermatitis, sexual disorder, cough,fever.
02	Kalo-bashok	*Justicia aurea Schtldl.*	Acanthaceae	
03	Jaal-chanchi	*Alternanthera paronychioides Hort. ex Regel*	Amaranthaceae	Carminative, dermatitis, poultice.
04	Amra	*Spondias dulcis Sol. ex Parkinson*	Anacardiaceae	Appetizer, toothache, ecbolic, itch.
05	Thankhai	*Centella asiatica (L.) Urb.*	Apiaceae	Dog bite, asthma, carminative, itch, leucorrhea, malaria, tumor, wound.
06	Maan-kochu	*Alocasia macrorrhizos (L.) G. Don*	Araceae	Tiger bite, rheumatoid arthritis, itch.
07	Togor	*Ervatamia divaricata (L.) Burkill*	Apocynaceae	Tonic, tumor, insecticide.
08	Papay	*Carica papaya L.*	Caricaceae	Rheumatoid arthritis, constipation, jaundice, diabetes, dermatitis.
09	Badha-kopi	*Brassica oleracea L.*	Cruciferae	Tonic, insecticide.

10	Tara-grass	*Cyperus amuricus Maxim*	Cyperaceae	Piles, tumor, wound.
11	Shimul	*Bombax ceiba L*	Bombacaceae	Gonorrhea.
12	Korolla		Cucurbitacea	Chicken pox.
13	Haritaki	*Terminalia chebula Retz*	Combretaceae	Stomachic.
14	Sajna	*Moringa oleifera Lam*	Moringaceae	Nasal catarrh, decreased eyesight, bone fractures, sores.
15	Ghritakumari	*Aloe sp*	Asphodelaceae	Anthelmintic, male sexual ailments.
16	Shatamuli	*Asparagus acerosa Sond.*	Rubiaceae	Seminal weakness, stomach troubles, gonorrhea
17	Dholmanik	*Centella asiatica (L.) Urb*	Umbelliferae	
18	Nishinda	*Vitex negundo L.*	Verbenaceae	Rheumatism.
19	Mandar	*Erythrina variegata L.*	Leguminosae-Papilionoideae alt. Fabaceae	Piles.
20	Jiga	*Lannea coromandelica (Houtt.) Merr*	Anacardiaceae	Seminal problems

(Mia, Kadir, Hossan, & Rahmatullah, 2009; Rahmatullah et al., 2010)

1.4 Importance of Phytochemical Investigation and biological activities examination of medicinal plants

Plants are being used as traditional medicines from the pre-historic period of time to heal the pain, inflammations, to treat injuries and different diseases. The ancient people started using plant paste, juice as medicinal agents without knowing the components that a plant contains or even without knowing which component is responsible for curing what type of diseases or injuries. Sometime they would do experiment with plant to treat injuries or diseases, if found effective then the information would pass generation to generation. Thus the way of treatment

with medicinal plants is being used till now in many countries including Bangladesh, India, China etc. So, to ensure patients safety and find out the proper scientific explanation behind using any herb or plant as traditional medicine, phytochemical investigation and biological activities examination of medicinal plants have undoubtable importance.

1.5 Literature review

Chassalia curviflora plant belongs to the family Rubiaceae and traditionally used to treat ear and eye disease, headache, skin diseases, ulcers, phlegm, rheumatism, jaundice, pneumonia, wounds and sour throat (Palayullaparambil et al., 2016). There are several studies done on the biological activities of this plants. Mainly one study is done on the antihypertensive activity, one on the acaricidal activity, one on antibacterial activity and one on antioxidant activity. In the study of antibacterial activity of the plant the investigators used the extract of plant of methanol and hexane and observed the activity on *E. coli, S. aureus, S. typhi, P. vulgaris, P. mirabilis, S. pyogens* bacteria and methanol extract showed activity only against *P. vulgaris* bacteria and hexane extract showed activity against *S. typhi* bacteria at different concentrations.

1.6 Description of the plant: *Chassalia curviflora*

1.6.1 General informations

Chassalia curviflora is a small shrub or tree up to 2 m tall which is distributed throughout different countries like China, Assam, Bangladesh, East Himalaya, Singapore, India, Sri Lanka, Andaman and Nicobar Islands, Peninsular Malaysia, Philippines, Myanmar, Borneo, Vietnam, Thailand, Sumatra, Java and the Lesser Sunda Islands. The lifespan of this plant is perennial in Singapore and it grows well in the hill to lowland forests preferably the zone of having tropical, sub –tropical or monsoonal climate.

Table 02: Description of Plant parts

Plant parts	Description
Branches	Weakly flattened to subterete, glabrous or rarely sparsely puberulent.
Leaves	Leaves are opposite and petiole 1-4 cm. Leaf blades are seen in stalked leaves of the plants which are elliptic to inverted lance-shaped. Colour of leaves are often light, dark or yellowish green. Size of plant is 6-20 by 2.5-7cm.
Flowers	Flowers are sub sessile which indicates absence of any stalk and flowers directly grows from the stem.
Fruits	Fruits are round in shape, black in colour, 5-6mm wide and consist of two seeds in each.

("Chassalia curviflora (Wall.) Thwaites | Species | India Biodiversity Portal," n.d.; "Species Search," n.d.)

1.6.2 Taxonomy

Kingdom: Plantae

Order: Gentianales

Family: Rubiaceae

Genus: Chassalia

Species: *Chassalia curviflora* (Wall.) Thwaites

1.6.3 Synonyms of the *Chassalia curviflora*

- *Chassalia ambigua (*Wight *&* Arn.*)* Alston
- *Chassalia ophioxyloides (*Wall.*)* Craib
- *Psychotria ambigua* Wight *&* Arn.
- *Psychotria ophioxyloides* Wall.

1.6.4 Local names of the plant

- Assamese: Tita-hutuka
- Malayalam: Yamari, Karutha-amalppori,Vellakurinji
- Others: Yamari,Vellakurunji

("Chassalia curviflora longifolia Hook.f. | Species | India Biodiversity Portal," n.d.)

1.6.5 Family Rubiaceae

The Rubiaceae family is one of the largest family which consist of approximately 637 genera and 13000 species in the Mangoliopsida class which has ranked as fourth among Angiosperms in diversity of species. There are about 120 genera and 1400 species found in Brazil which represents one of the most important economic, ornamental and medicinal plant families in the Brazilian families (Martins & Nunez, 2015). Several phytochemical investigations on the plants of Rubiaceae family ensure the presence of bioactive compounds like indole alkaloids, terpenoids and anthraquinones etc. These plants also reveal the antimicrobial, antihypertensive, antimalarial, antidiabetic, antioxidant and anti-inflammatory activities (Chandra Kala, 2015).

According to the findings of recent studies, this family has three sub-families such as Rubioideae, Cinchonoideae and Ixoroideae which shows a large diversity of medicinal

substances. Coffea Arabica which is well known as coffee, is one of the most economically significant species of Coffea genus of Ixoroideae subfamily. This species consists of caffeine which act as CNS (Central Nervous System) stimulant and also as vasoconstrictor, bronchodilator and diuretic besides being one of the components of migraine drugs. Another important genus of Ixoroideae subfamily is Genipa, the Brazilian jenipapo (*Genipa americana*) which have antiangiogenic, anti-inflammatory and antioxidant activities. Cinchona species of Cinchonoideae subfamily which is the source of quinine have the antimalarial activity. Finally, an important genus of Rubioideae subfamily is Cephaelis, especially C. ipecacuanha, a plant which an important source of emetine, an alkaloid that the Brazilian population used for emetic, antihelminthic and expectorant effects(Martins & Nunez, 2015)

1.7 Rationale of the study:

The literature review of *Chassalia curviflora* shows that the activities of the plant have been examined by few numbers of investigators. There is only one research done on the antimicrobial activity of the plant extract using methanol as solvent which is a highly polar solvent. So, here this study is conducted to compare between the recent study of antimicrobial test of methanol extract of the plant and the previously done antimicrobial test of the methanol extract. Additionally, this study also focuses on finding out that the plant has antimicrobial potentials or not while extraction of the plant was done using the different solvents having different polarities. Such as, this study is conducted using three different solvents of different polarities where we have kept methanol in common with the previous study as standard. Then we have done the experiment gradually using less polar to highly polar solvents like petroleum ether, ethyl acetate and lastly methanol.

1.8 Goal of the study:

This study is done to examine the therapeutically essential components present in the *Chassalia curviflora* plant and investigate the presence of some possible biological activities of the plant.

1.9 Objective of the Study:

The objectives of the study include following evaluation:

1. Starting with gradual phytochemical screening of the petroleum ether extract, ethyl acetate extract and methanol extract of the plant *Chassalia curviflora*.
2. Sequential evaluation of biological activities of the petroleum ether extract, ethyl acetate extract and methanol extract of the plant *Chassalia curviflora*. The evaluated biological activities are:
    a. Determination of antimicrobial potentials of the plant extracts.
    b. Determination of Antioxidant activity of the plant extracts.
    c. Determination of Cytotoxic activity of the plant extracts.

Chapter 2

Methodology

2.1 Plant extracts preparation

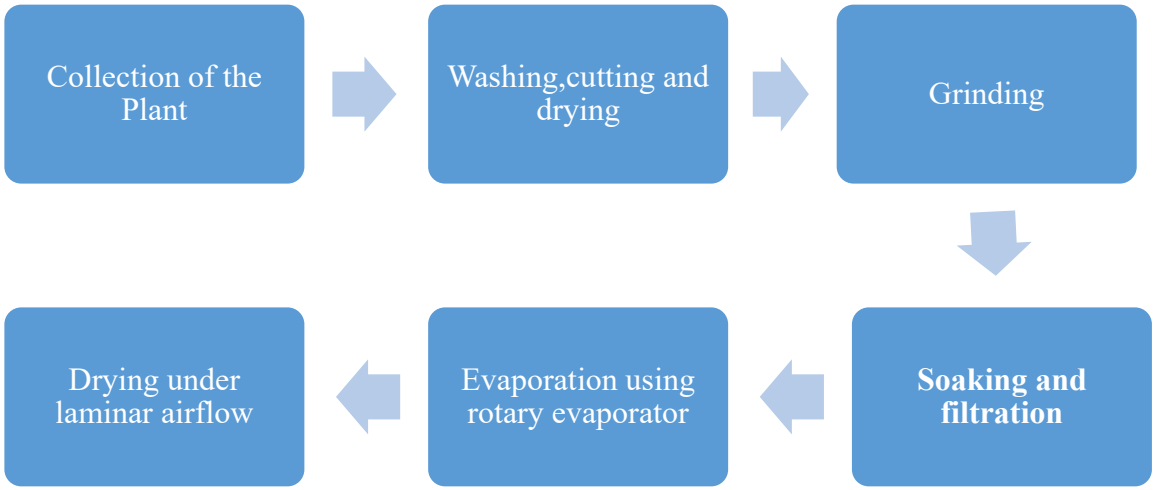

Figure 01: Plant extract preparation

2.1.1 Plant Collection

The collection of the plant *Chassalia curviflora* was done in the month of June of 2019 from division of Sylhet, Bangladesh. After collection of the plant, it was identified by the National Herbarium Bangladesh (NHB), Mirpur, Dhaka (Accession number- 40416).

2.1.2 Plant washing, cutting & drying

The whole plant was dipped into three different bucket with full of water for three times to clean properly. Then there was no dust or any residue of other plants or materials. Next the plant was cut into small pieces and kept inside of 2 bags of net. Then the bags were hanged in

place where the plant can be sun dried properly so that plant don't get burned in extreme sun heat but get the optimum light and heat of the morning. Thus the plant washing and drying was completed.

2.1.3 Grinding

The dried small cut pieces of plant were taken to old Dhaka to grind into powder form and got about 360 g of plant powder. Then the plant powder was packed into an air tight bag and kept in a dry place at room temperature until the further experiments were done.

2.1.4 Soaking and filtration

Firstly, the total quantity of the plant powder was soaked in a clean air tight glass jar using about 1.5 liters of petroleum ether solvent for three days (72 hours). Stirring of the soaked plant powder was done with the interval of 1 hour or sometimes 1 and half hours every day from 9 am to 5 pm. The jar was taken air tight to prevent evaporation of solvent before the desired time period. As the Petroleum ether solvent is very much volatile, I added 200 mL of that solvent more in the jar during the period of 72 hours to ensure proper extraction. Secondly, filtration was performed two times with a clean white colored cloth and cotton respectively to ensure proper filtration after 72 hours of soaking. Thirdly, the filtrate and residue found after filtration was separately collected for further use. As my purpose of doing this study was following Increasing order of polarity, the residue was dissolved in 1.5 litters of ethyl acetate and methanol gradually and the same procedure of soaking and filtration was followed each time.

2.1.5 Evaporation

Stepwise the collected filtrates of three different solvents was concentrated using rotary evaporator (Heidolph, Germany). The evaporation was performed maintaining 100 rpm of speed and 45° C of temperature of the rotary evaporator. The bulk of the crude extracts obtained for petroleum ether, ethyl acetate and methanol was 17 g, 16 g, 20 g respectively. Then the extract was poured in three different petri-dishes. The residual solvent of the extract was dried keeping under laminar air flow to ensure the prevention of any sort of microbial extension in the plant extracts while continuing the evaporation of solvents.

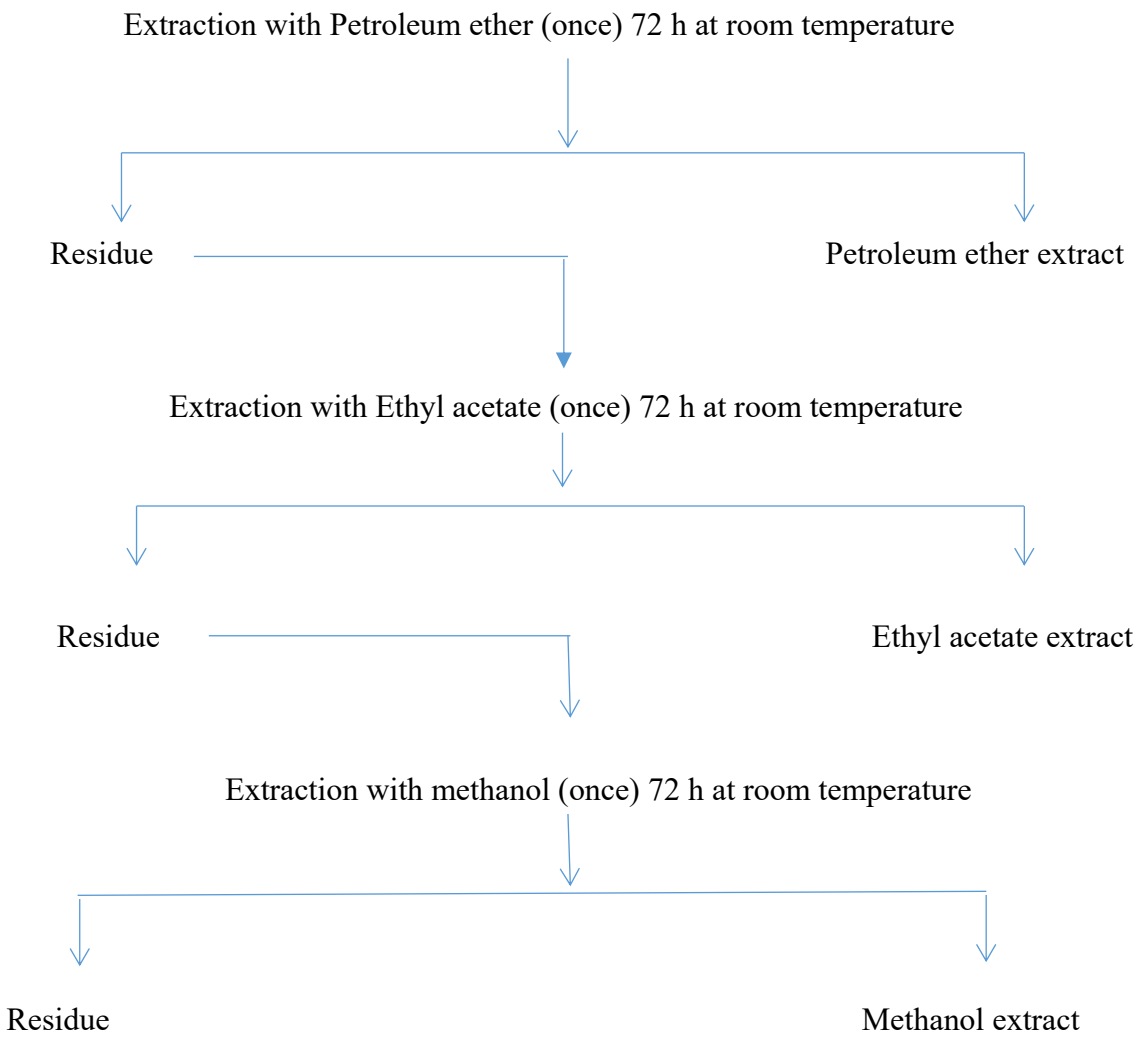

Figure 02: Flowchart of procedure of Preparing of plant extract

2.2 Phytochemicals investigation

Phytochemical investigation was done using the unprocessed extracts of the *Chassalia curviflora* plant of different solvents. Several tests were performed to investigate the presence of different essential components which may have some therapeutic values. These findings reveal the scopes for further study of any plants. The following tests were done:

2.2.1 Solvents used: Petroleum ether, Ethyl acetate and Methanol

2.2.2 Test for alkaloids

- **Mayer's test:** 2 mg of plant extract was added and mixed with few drops of Mayer's reagent (potassium mercuric iodide solution) in a test tube. If the precipitate of reddish brown color forms, it confirms the presence of alkaloids.
- **Hager's test:** 2 mg of extract was taken and mixed with Hager's reagent (saturated picric acid solution). If yellow colored precipitate forms, it reveals the presence of alkaloids.
- **Wagner's test:** 1 mL of plant extract was taken and treated with the Wagner's reagent. Formation of a brown or reddish brown color precipitate indicates the presence of alkaloids.

2.2.3 Tests for flavonoids

- **Lead-acetate test:** 2 mL of extract solution was taken in a test tube. Then a few drops of basic lead acetate solution added in the test tube. If precipitate of reddish brown color form, it confirms the presence of flavonoids.

- **Shinoda's test:** If the addition of 1ml extract solution with the magnesium metal and hydrochloric acid about 0.5 mL shows reddish color, it reveals the existence of flavonoids.
- **FeCl3 test:** 2 mL solution of plant extract was introduced in a test tube. Then the solution of neutral ferric chloride was added in that test tube about 2-3 drops. Deposition of the blackish red color precipitate confirms the presence of flavonoids.

2.2.4 Tests for Phenols /phenolic compound

- **Lead acetate test:** About 50 mg of plant extract was taken and dissolved in distilled water in a test tube. Next 3 mL of 10% lead acetate solution was added in that test-tube. Formation of white bulky precipitate ensures the existence of phenolic compounds.
- **Phenol test:** 0.2 g of extract was taken and dissolved in ferric chloride solution. Formation of a green or dirty green precipitate indicates the existence of phenolic compound.
- **Dilute iodine solution test:** About 2-3 mL solution of plant extract was taken in a test tube. Then 2-3 drops of solution of dilute iodine were introduced in that test tube. If transient red color forms, phenolic compounds are present.

2.2.5 Tests for glycosides

- **Legal's Test**: If cherry red color form upon the addition of pyridine and alkaline sodium nitroprusside in the solution of plant extract, it will designate the presence of glycosides.
- **Keller Killiani test:** 1 mL of plant extract was taken. Then 1ml of glacial acetic acid was introduced and mixed. After that the mixture was kept standing to cool. Next Ferric

chloride about 2-3 drops was added and also 2 mL of concentrated H_2SO_4 was introduced with care in sideways of the test tube's wall. At the junction of two layers, if any ring of reddish color appears, it will confirm the existence of glycosides.

- **Concentrated H2SO4 test:** 1 mL of plant extract solution was taken in a test-tube and then treated with 1 mL of concentrated H_2SO_4 and kept standing about 2 minutes. If reddish colored precipitate forms, it reveals the presence of glycosides.

2.2.6 Test for tannins

- **Ferric chloride test:** If blackish colored precipitate forms upon the addition of 2-3 drops of $FeCl_3$ in the extract of plant, it shows the existence of tannins.
- **Lead acetate test:** 2 mL of plant extract was treated with about 2-3 drops of basic lead acetate. Formation of precipitate of red bulky color points out the existence of tannins.
- **Alkaline reagent test:** In 2 mL of solution of plant extract, a few drops of sodium hydroxide solution was introduced. Appearance of red color confirms the presence of tannins.

2.2.7 Test for Carbohydrates

- **Molish Test:** About 2-3 drops of Molish's reagent was added and mixed with 2 mL of plant extract. If red violet ring form at the junction of the mixture due to addition of 2 mL of concentrated Sulphuric acid to that mixture and disappear the ring upon addition of excess alkali solution, it points out that carbohydrates are present in the extract.
- **Fehling's test:** Both 1 mL of Fehling's A and 1 mL of Fehling's B solutions were introduced in the test tube containing 1 mL of plant extract and then it was heated for

10 minutes keeping in the hot water bath. If any red precipitate forms, reducing sugar is present in the extract. Then again 1 mL of each Fehling's A and B solution were added to the filtrate and heated for 5-10 minutes keeping in a hot water bath. Formation of reddish orange precipitate reveals the presence of carbohydrates.

- **Benedict's test**: Plant extract was taken and dissolved in distilled water and also filtration was done. Then the Benedict's reagent was added and mixed with the filtrate and heated gently. Appearance of orange red precipitate indicates the existence of reducing sugars.

2.2.8 Test for sterols

- **Libermann-Burchard's test:** Extract was treated with chloroform and filtration was also done. Then a few drops of acetic anhydride were added to the filtrate and boiled and the cooled. After that, if ring of brown color forms at the intersection upon addition of concentrated sulphuric acid, it points out the presence of phytosterols.

2.2.9 Test for resins

- **Copper acetate solution test:** About 5 mL extract solution was in a test tube. Then 5 mL of copper acetate solution was added and mixed by vigorously shaking the solution. Next it was left for some time and allowed to separate. Appearance of green colored solution confirms the existence of resins.

- **$CuSO_4$ solution test:** About 3-4 mL of the $CuSO_4$ solution was added and mixed with the extract solution. Then this mixture was strenuously shaken for 1-2 minutes. Then it

was kept standing to be separated. Formation of precipitate of green color is an evidence of presence of resins.

2.2.10 Test for Steroids

- **Acetic anhydride test:** 2 mL of acetic anhydride was added to 0.5 mL crude extract of plant sample with 2 mL H2S04. The change in coloration from violet to blue or green in samples indicates the presence of steroids.
- **Liebermann-Burchard Test:** The extracts were dissolved in 2 mL of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red color from blue to green indicates the presence of steroids.
- **Salkowski's test:** Extract was taken and treated with chloroform and also filtration was done. Then a few drops of conc. sulphuric acid was added to the filtrate and shaken well and allowed to stand. If red color appears in the lower layer, it indicates the presence of steroids.

2.2.11 Test for Saponins

- **Foam test:** About 50 mg of extract was taken and dissolved in distilled water and made up to 20 mL suspension in a graduated cylinder. Then the suspension was shaken for 15 minutes. Formation of foam of 2 cm layer indicates the presence of saponins.
- **Froth test:** To get a stable froth about 5 mL of the plant extract was shaken vigorously taking in a test tube. Then olive oil was added about 5-6 drops in frothing solution, Formation of an emulsion is an evidence of existence of saponins.

2.3 Antimicrobial potential examination using disc diffusion method:

Table 03: Required equipment and reagents

Equipment		**Reagents**
Sterile cotton	Refrigerator	Ethanol
Autoclave	Incubator	Nutrient agar medium
Nose mask and hand gloves	Petri dishes	Molar Hinton Agar
Filter paper discs	Laminar Air Flow hood	
Micropipette	Inoculating loop	
Sterile forceps	Spirit burner	
Screw cap test tubes		

2.3.1 Test organisms

Both the gram negative and gram positive organisms were cultured and used for doing the test. The list of the organisms is shown:

Table 04: List of the organisms

Sl. No.	Gram positive bacteria	Gram negative bacteria
01	*Staphylococcus aureus*	*Escherichia coli*
02	*Bacillus subtilis*	*Shigella dysentarea*
03	*Enterococcus facealis*	*Pseudomonas aeruginosa*

2.3.2 Preparation of the medium

To prepare the medium 3.8 g of Muller Hinton agar was added in 100 mL distilled water and mixed thoroughly till the agar was perfectly dissolved. Then the mixture was autoclaved for 45 minutes at 121℃ and allowed to cool up to 45-50℃. Then the mixture was equally poured into 18 sterilized petri dishes to attain each plate with uniform depth. Each plate approximately got about 25 mL solution of agar and then those plates were kept at room temperature for cooling and solidifying under the laminar airflow.

2.3.3 Culturing of bacterial strains:

At first, broth medium was prepared by adding o.13 g of nutrient broth in 10ml of distilled water and mixed until it was completely dissolved. Collection of desired bacterial strain was done from mediums which were preserved for long term. The mediums contain trypsin, skimmed milk, glycerin and glucose and preservation of the medium was done in (ULT) Ultra Low Temperature. From the medium bacterial strain was moved into the test tube containing

nutrient broth using sterile loop. Then incubation was done to energize the bacteria for 24 hrs. New bacterial colonies were formed at the end of the incubation period.

2.3.4 Preparation of disc:

The disk of 5 mm diameter was prepared of Whatman filter paper and autoclaved in a petri-dish. Then disks were dissolved in the solutions of plant extracts of different solvents with different concentration. Next, those disks were left for soaking all the plant extract for 10-15 minutes.

2.3.5 Procedure:

After solidification of the agar medium in the petri dishes, labeling was done which includes different concentration of plant extract of different solvent, name of standards. Then a cotton swab was immersed into the suspension of bacteria and gently pressed against the test tube to ensure that there is no excess fluid in the swab and then it was used to strain the bacteria in the nutrient agar plate. The straining of the bacteria was done crosswise and cornerwise. After that the disks containing plant extract of different concentration and the disk of standard antibiotics such as Kanamycin30 and Streptomycin10 were placed in the petri dish according to the labeling of the petri dish by using forceps. Sterilization of the forceps were done perfectly by flame after using every time. Same procedure was followed though out the experiment for all the 18 petri dishes. Finally, to attain lawn growth of bacteria, incubation of the petri dishes was done for 24 hr at 37°C.

2.4 Cytotoxic activity:

Table 05: Required apparatus and reagents for cytotoxic activity

Apparatus	Reagents
Small aquarium with oxygen	Dimethyl sulfoxide (DMSO)
Lamp	Sea salt (NaCl)
Micropipette	Vincristine sulphate
Pasteur pipette	*Artemia salina* (brine shrimp egg)
Test tubes	Plant extract
Magnifying glass	

2.4.1 Preparation of sea water

Firstly, 38 g of pure sea salt was measured for per liter of distilled water in a little round shaped aquarium and about 3L of sea water was prepared for performing this test.

2.4.2 Brine shrimps hatching

For hatching, collection of the eggs of *Artemia salina* (Brine shrimp) was done from the Pharmaceutical Microbiology, Physiology and Pharmacology Lab of Brac University. Then those eggs were kept in the previously prepared aquarium containing sea water for 24 hours. In the aquarium, oxygen along with light was provided during that time. After 24 hours, eggs were transformed into nauplii. Then the nauplii were collected into test tubes by using Pasteur pipette. To perform the test, not more than 10±2 live nauplii were put into per test tube containing sea water (around 5 mL).

2.4.3 Test Solution preparation

Firstly, the extract of *Chassalia curviflora* plant was dissolved into dimethyl sulfoxide (DMSO) in test tube. Then serial dilution was done for obtaining several concentrations ranging from 3.90625 µg/mL to 500 µg/mL. Then, in each test tube containing nauplii in 5mL of sea water, 50µL of each diluted plant sample concentration was introduced to perform the experiment.

2.4.4 Preparation of control group

To incline the authenticity of this study and to ensure that the results found were solely from cytotoxic agents, two types of control (positive & negative) were used in the experiment. The controls were:

- Positive control
- Negative control

2.4.5 Positive control group preparation

The often used standard for positive control in the cytotoxicity activity test is Vincristine sulfate. Then the required amount of stock solution of Vincristine sulphate was prepared by using DMSO to get 500 µg/mL concentration from which serial dilution was done to get other concentration. Each test tube containing 10±2 live nauplii in sea water (around 5 mL) was introduced each concentration of positive control.

2.4.6 Negative control group preparation

100 µL of DMSO was added and mixed in each of 3 test tubes containing 5 mL of sea water along with 10±2 live nauplii. These 3 test tubes act as control group for observing that the nauplii died due to other causes except the presence of cytotoxic compound.

2.4.7 Counting of nauplii

About 24 hours later the number of survived nauplii was observed and counted by visual inspection and also using magnifying glass. Next the (%) of mortality was calculated. The calculation was done by using the equation:

$$\left(\frac{\textbf{Total no.of taken nauplii} - \textbf{No.of survived nauplii}}{\textbf{Total no.of taken nauplii}}\right) * 100 = \%\ \text{of mortality}$$

Then the LC_{50} for both standard and sample was calculated.

2.5 Evaluation of antioxidant activity

There are various tests for evaluating the antioxidant activity of plant extract. In this study, three among all the tests are performed to evaluate the antioxidant properties of plant *Chassalia curviflora*. Those are following:

- ✓ Antioxidant potentials determination using DPPH assay
- ✓ Total Phenolic Content (TPC) determination
- ✓ Total Flavonoid Content (TFC) determination

2.5.1 DPPH Free Radical Scavenging Assay:

Table 06: Required materials and reagents

Materials	Reagents
Test tube	Extracts of the selected plant
Light Proof box	Distilled water
Pipette (1 mL and 5 mL)	Ascorbic Acid

Volumetric flask	2,2-Diphenyl-1-Picrylhydrazyl (DPPH)
UV-spectrophotometer	Petroleum ether
	Ethyl acetate
	Methanol

Protocols:

Preparation of sample and Standard

Stock solution of sample is prepared by dissolving about 5 mg of the plant extract in the 10 mL of designated solvent. Then desired sample concentration was prepared through the serial dilution of extract ranging from 500 µg/mL to 0.997 µg/mL. As three different solvents were used to get the plant extract, one stock solution for each solvent of plant extract have to be prepared by following same procedure. Ascorbic acid was selected as positive control for performing the experiment and standard of it also prepared by following the same manner as the sample stock solutions were prepared.

DPPH solution preparation

20 mg of DPPH powder was weighed and then the DPPH solution was prepared by dissolving the powder in the solvent that was used to get the plant extract. The concentration of the solutions was 20 µg/mL.

Preparation of Blank solution

3mL of each solvent and standard solution was used as blank solution for doing this experiment.

Free radical scavenging activity analysis

About 3 mL of a DPPH solution of petroleum ether (20 µg/mL) was mixed with 2 mL of a Petroleum ether solution of the sample extract at different concentrations (500 µg/mL to 0.997 µg/mL). The reaction period was about 30 minutes in the dark place at room temperature. After completion of the reaction period the absorbance was measured at 517 nm against blank using UV spectrophotometer. Here petroleum ether was used as a blank solution. Similar procedure was followed for the plant extracts of ethyl acetate and methanol solvents where DPPH solution of ethyl acetate and methanol was used respectively and blank was also specific with respect to the solvent used to extract plant.

Calculation:

The percentage (%) of DPPH free radical scavenging activity was calculated using the following equation:

$$Free\ radical\ scavenging\ activity\ = \left(\frac{A0-A1}{A1}\right) * 100$$

Where,

The absorbance of the control = A_0

The absorbance of the standard/sample = A_1

Concentration of extract was calculated providing 50% inhibition (IC_{50}) from the graph plotting percentage of inhibition against the concentration of extract.

2.5.2 Total Phenolic Content (TPC) determination

Table 07: Required materials and reagents

Equipment	Reagents
Micropipette (50-200 µL)	Na_2CO_3 solution (7.5 %)
Vial	Folin-Ciocalteu reagent (10 fold diluted)
Test tube	Gallic acid
UV-spectrophotometer	Distilled water

Procedure:

Preparation of Gallic acid solution for standard curve:

Gallic acid is the most commonly used standard for determining the total phenolic content of any plant extract. So, here in this study, Gallic acid is used as standard to determine the total phenolic content. To perform this test, solutions of Gallic acid having different concentration

ranging from 0.5 mg/mL to 0.1 mg/mL were prepared. These solutions were prepared by mixing 2 mL of (7.5% w/v) solution of sodium carbonate, 2.5 mL of Folin-Ciocalteu chemical and 0.5 mL of Gallic acid. Dilution of Folin-Ciocalteu chemical was done using water for 10 times. After completion of the reaction period of 20 minutes in the dark place, absorbance of the mixture was measured at 760 nm by using UV spectrophotometer. By plotting sample concentration against absorbance, a linear equation was obtained.

Preparation of test sample for evaluation:

The desired sample concentration 20 mg/mL was made by dissolving 20 mg of plant extract in 1 mL of distilled water.

Preparation of the blank:

About 2.5 mL FCR, sodium carbonate 2 mL and 0.5 mL of each solvent were used for the blank solution.

Assay of Total phenolic content test:

First, 2.5 mL of 10 times diluted Folin-ciocalteu and half mL of sample solution (20 mg/mL) and 2.0 mL of Na_2CO_3 (7.5% w/v) were mixed together and kept in dark place for 20 minutes. After completion of the 20 min measurement of absorbance was done at 760 nm using UV spectrophotometer. Analysis of standard curve of Gallic acid was done to estimate the total

sample. Sample's Total Phenolic Content was stated as mg of GAE (Gallic acid equivalent)/g of plant extract. The calculation of Total Phenolic Content was done by following equation:

$C = (c \times V) / m$

Where,

C = Total content of phenolic compounds

c = Concentration of Gallic acid obtained from calibration curve (mg/mL)

V = Volume of sample solution (mL)

m = Weight of the sample (g)

2.5.3 Total Flavonoid Content Determination

Table 08: Required chemicals and reagents

Chemicals and reagents required
Petroleum ether
Ethyl acetate
Methanol
Potassium acetate
Aluminium Chloride
Quercetin (Standard)

Preparation of reagent:

- ✓ To prepare 10% Aluminium chloride solution of 100 mL, 10 g of Aluminium chloride was taken into a volumetric flask and dissolved in distilled water and diluted by filling up to the 100 mL mark.

- ✓ To prepare 100 mL of 1M potassium acetate solution, 9.815 g of Potassium acetate was weighed in a 100 mL of volumetric flask. Then distilled water was added to that volumetric flask up to the 100 mL mark.

Procedure:

Aluminium chloride colorimetric assay was used for determination of the Total Flavonoid Content of the extracts of plant. Firstly, standard solution of quercetin (0.1-0.5 mg/mL) and plant extract solution of 5 mg/mL were added with 2 mL of distilled water. Then the mixture was further added and mixed with 0.15 mL of sodium nitrite (5% $NaNO_2$, w/v) solution. Respectively, 0.15 mL of (10% AlCl3, w/v) solution was added about 6 minutes later. Then the solutions were kept standing for 6 minutes more and 2 mL solution of sodium hydroxide (4% NaOH, w/v) was introduced in the mixture. The end volume of the mixture was made 5 mL by adding distilled water immediately and rigorously mixed and kept standing for another 15 minutes. Then the absorbance of each mixture was taken at 510 nm against the same mixture. Using calibration curve of quercetin TFC was determined as mg quercetin equivalent per gram of sample. For each of the fractions The Total Flavonoid Content, C, was expressed as quercetin equivalent (QE). The used equation is as followed:

$C = c \times V/m$

Where,

C = Total content of flavonoid compounds

c = Concentration of Quercetin obtained from calibration curve (mg/mL)

V = Volume of sample solution (mL)

m = Weight of the sample (g)

Chapter 3

Results

3.1 Phytochemical screening of *Chassalia curviflora*

Table 09: Results of Phytochemical Screening

Sl. No.	Components name	Tests name	Petroleum ether extract	Residue (Ethyl acetate)	Residue (Methanol)

01	Alkaloids	Wagner test	-	-	+
		Mayer's test	-	-	-
		Hager's test	-	-	+
02	Flavonoids	Lead acetate test	+	-	+
		Ferric chloride test	+	+	+
		Zinc ribbon test	-	-	+
03	Phenols or phenolic compounds	Lead acetate test	+	-	+
		Ferric chloride test	+	+	+
		Dilute Iodine solution test	-	+	+
04	Tannins	Ferric chloride test	+	+	+
		Lead acetate test	+	-	+
		Alkaline reagent test	-	-	-
05	Glycosides	Keller killiani test	-	+	-
		Conc. H_2SO_4 test	-	+	+
		Borntrager's test	-	+	+
06	Carbohydrates	Fehling's test	-	-	+
		Molish test	+	-	-
		Benedict's test	-	+	+
07	Steroids	Acetic anhydride test	+	-	+
		Liebermann-Burchard's test	-	-	-
		Salkowski's test	-	-	-
08	Phytosterol	Libermann-Burchard's test	-	-	-

09	Saponins	Foam test	-	+	-
		Forth test	-	-	-
10	Resins	Copper acetate solution test	-	-	-
		$CuSO_4$ solution test	-	+	+

(Note: Here "+" indicates presence of the components in the extract and "-" indicates absence of the components in the extract)

3.2 Result of antimicrobial test

Table 10: Antimicrobial activity of petroleum ether extract.

Sl. No.	Name of the Bacteria	Std. Kanamycin	Std. Streptomycin	50 mg/mL	150 mg/mL	250 mg/mL	350 mg/mL
01	*S. aureus*	13	0				
02	*B. subtilis*	18	12				
03	*E. facealis*	14	0				
04	*E. coli*	12	0				
05	*S. dysenteriae*	17	13				
06	*P. aeruginosa*	07	16		08		

Table 11: Antimicrobial activity of ethyl acetate extract

Sl. No.	Name of the Bacteria	Std. Kanamycin	Std. Streptomycin	50 mg/mL	150 mg/mL	250 mg/mL	350 mg/mL
01	*S. aureus*	13	0				
02	*B. subtilis*	18	0				07
03	*E. facealis*	15	0				
04	*E. coli*	18	0				

05	*S. dysenteriae*	15	13				
06	*P. aeruginosa*	07	17		09	08	

Table 12: Antimicrobial activity of methanol extract

Sl. No.	Name of the Bacteria	Std. Kanamycin	Std. Streptomycin	50 mg/mL	150 mg/mL	250 mg/mL	350 mg/mL
01	*S. aureus*	14	0				
02	*B. subtilis*	12	23			07	
03	*E. facealis*	13	0			07	08
04	*E. coli*	12	05	07	06/07	07	
05	*S. dysenteriae*	18	15			07	
06	*P. aeruginosa*	06	10	06	10	09	13

3.3 Result of Antioxidant activity:

3.3.1 DPPH Free Radical Scavenging Analysis

Table 13: IC_{50} value (µg/mL) of Ascorbic Acid (ASA)

Absorbance of the blank	Concentration (µg/mL)	Absorbance of Standard (ASA)	% of inhibition	IC_{50} µg/mL
0.591	500	0.032	94.58544839	89.202

250	0.041	93.06260575	
125	0.067	88.66328257	
62.5	0.181	69.37394247	
31.25	0.283	52.11505922	
15.625	0.379	35.8714044	
7.813	0.421	28.76480541	
3.906	0.47	20.47377327	
1.953	0.483	18.2741117	
0.977	0.498	15.7360406	

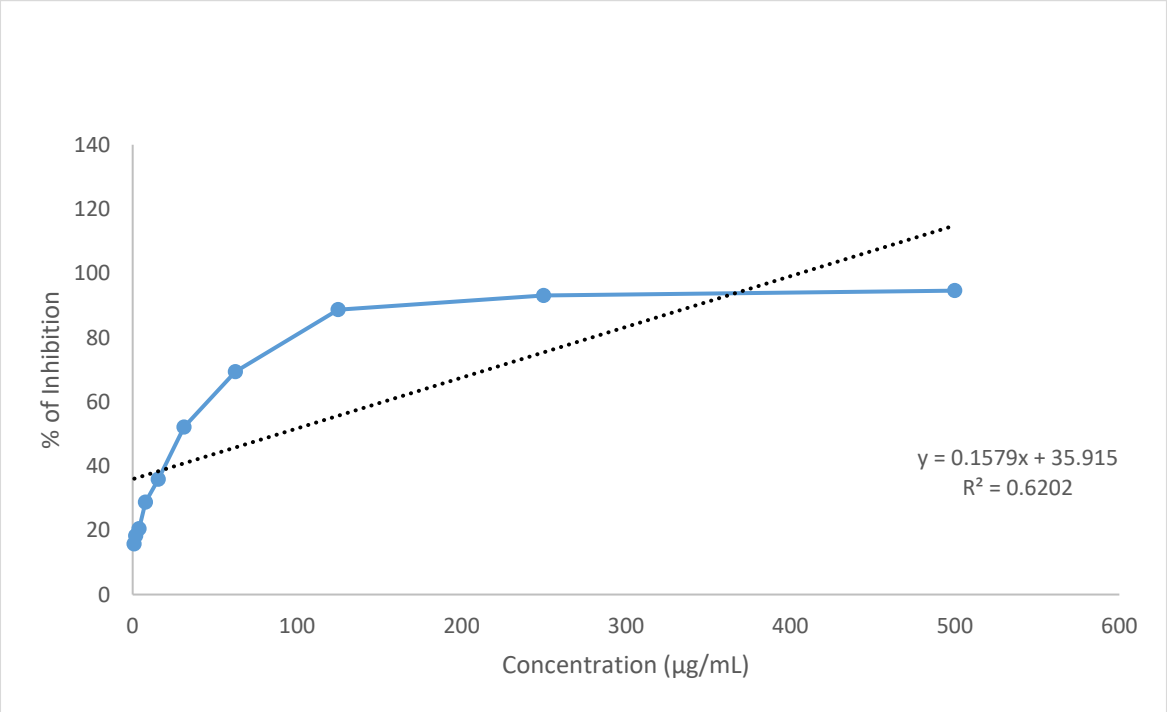

Figure 03: Concentration vs % of inhibition Curve of Ascorbic acid (ASA)

Table 14: IC_{50} value (ug/mL) of Petroleum ether extract

Absorbance of the blank	Concentration (µg/mL)	Absorbance of Petroleum ether extract	% of inhibition	IC_{50} µg/mL
0.991	500	0.113	88.59738	531.144
	250	0.149	84.96468	
	125	0.178	82.03835	
	62.5	0.192	80.62563	

	31.25	0.224	77.39657	
	15.625	0.249	74.87386	
	7.813	0.273	72.45207	
	3.906	0.304	69.32392	
	1.953	0.324	67.3058	
	0.977	0.379	61.7558	

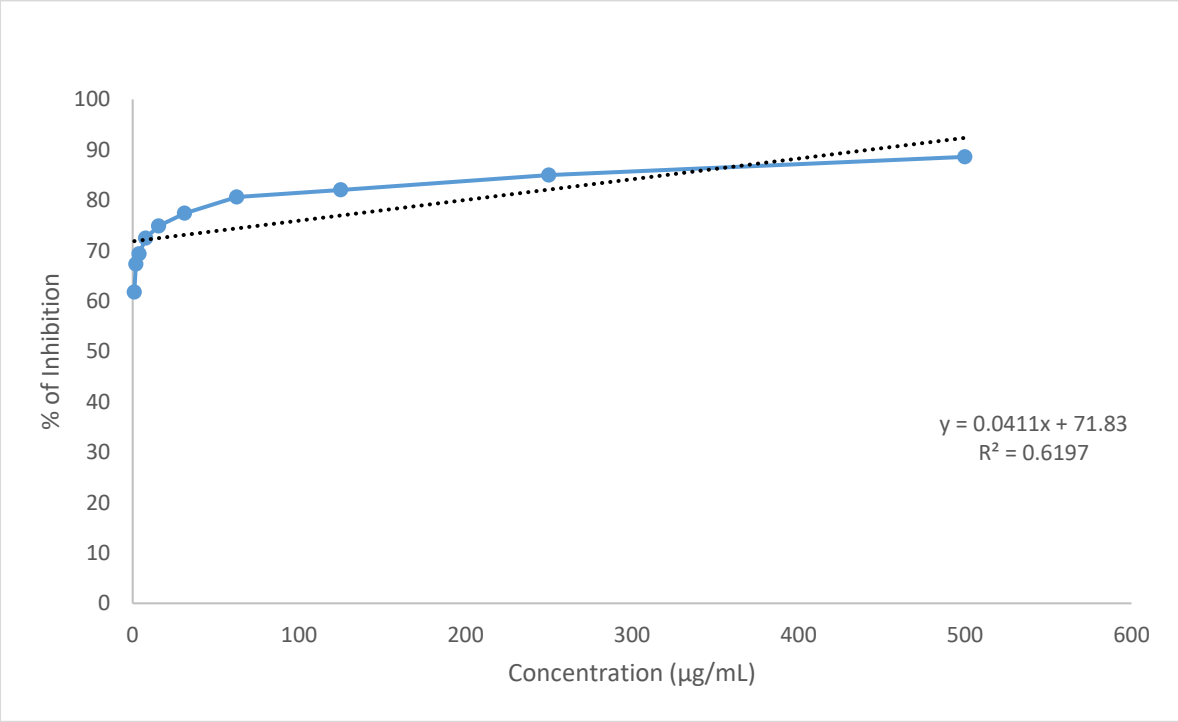

Figure 04: Concentration vs % of Inhibition curve of Petroleum Ether Extract

Table 15: IC_{50} value (ug/mL) of Ethyl acetate (residue) extract

Absorbance of the blank	Concentration (µg/mL)	Absorbance of Ethyl acetate (residue) extract	% of inhibition	IC_{50} µg/mL
0.217	500	0.081	62.67281	321.548
	250	0.103	52.53456	
	125	0.121	44.23963	

	62.5	0.146	32.71889	
	31.25	0.189	12.90323	
	15.625	0.214	1.382488	
	7.813	0.243	-11.9816	
	3.906	0.271	-24.8848	
	1.953	0.293	-35.023	
	0.977	0.311	-43.318	

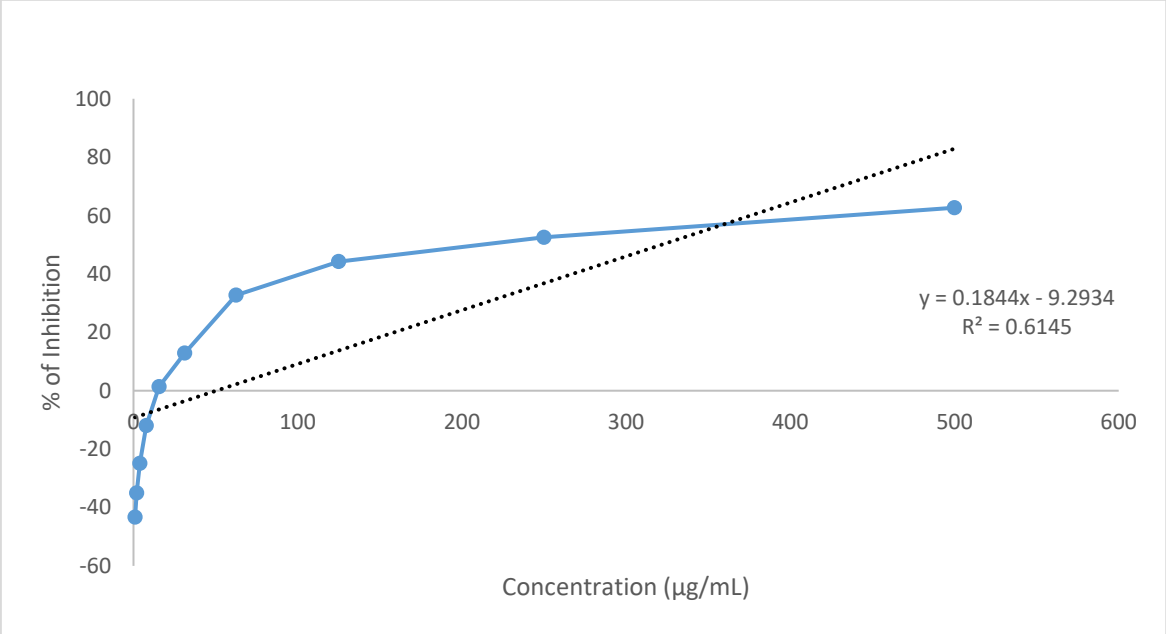

Figure 05: Concentration vs % of Inhibition curve of Ethyl Acetate (residue) Extract

Table 16: IC_{50} value (ug/mL) of methanol (residue) extract.

Absorbance of the blank	Concentration (µg/mL)	Absorbance of methanol (residue) extract	% of inhibition	IC_{50} µg/mL
0.432	500	0.041	90.50926	59.215
	250	0.072	83.33333	

125	0.098	77.31481	
62.5	0.11	74.53704	
31.25	0.141	67.36111	
15.625	0.217	49.76852	
7.813	0.243	43.75	
3.906	0.301	30.32407	
1.953	0.341	21.0648	
0.977	0.379	12.2685	

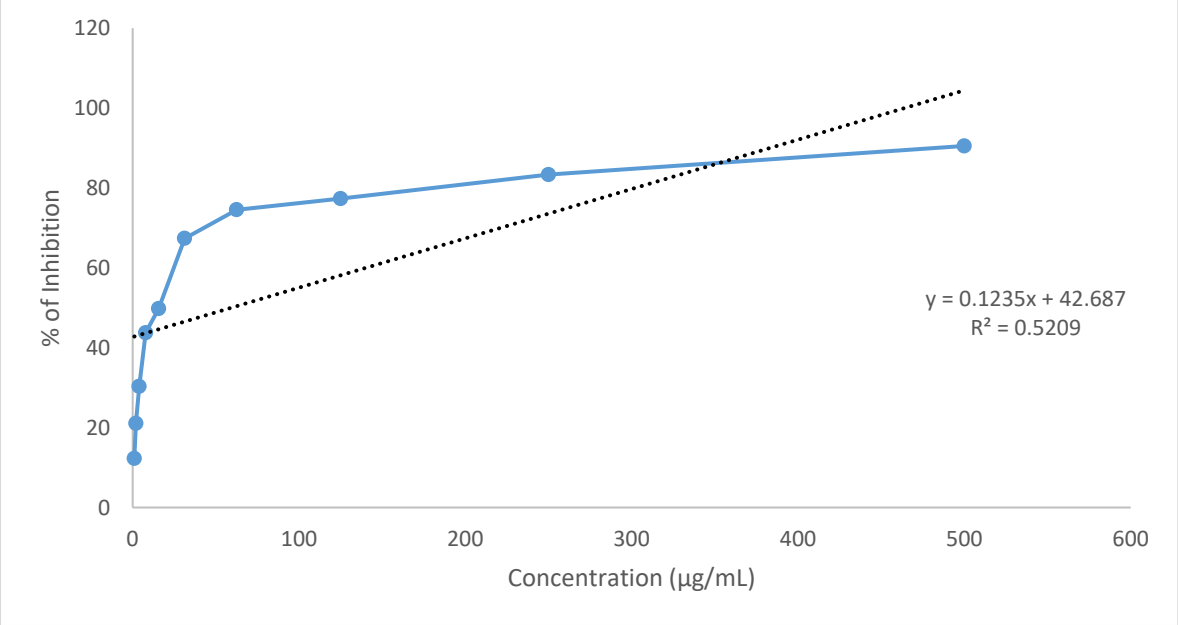

Figure 06: Concentration vs % of Inhibition curve of Methanol Extract

3.3.2 Result of Total Phenolic Content

Table 17: Preparation of Standard curve using Gallic Acid

Sl. No.	Concentration of Gallic acid (µg/mL)	Absorbance	Regression line	Regression coefficient (R^2)
01	0	0		R^2 =0.88

02	100	0.094	y = 0.0002x + 0.1315	
03	500	0.221		
04	1000	0.449		
05	2500	0.979		
06	5000	1.113		

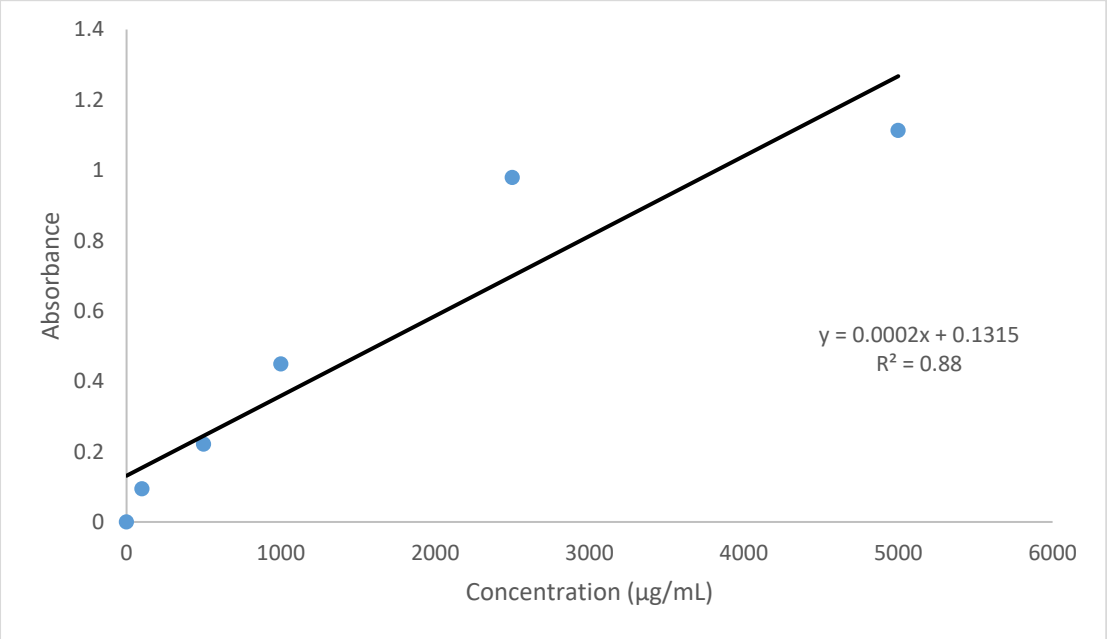

Figure 07: Standard curve of Gallic acid for determination of Total Phenolic Content

Table 18: Total Phenolic Content of Petroleum ether extract of Chassalia curviflora

Sample	Absorbance	TPC (mg of GAE/g of extract)
20mg/mL	1.7	7.84

Table 19: Total Phenolic Content of Ethyl acetate extract of Chassalia curviflora

Sample	Absorbance	TPC (mg of GAE/g of extract)
20mg/mL	2.3	10.84

Table 20: Total Phenolic Content of Methanol extract of Chassalia curviflora

Sample	Absorbance	TPC (mg of GAE/g of extract)
20mg/mL	3.1	14.84

3.3.3 Result of Total Flavonoid content:

Table 21: Preparation of Standard curve using Quercetin

Sl. No.	Concentration of quercetin (mg/mL)	Absorbance	Regression line	Regression coefficient (R^2)
1	0	0		
2	100	0.261	y = 0.0002x + 0.1715	R^2=0.9628
3	500	0.354		
4	1000	0.471		
5	2500	0.779		
6	5000	1.349		

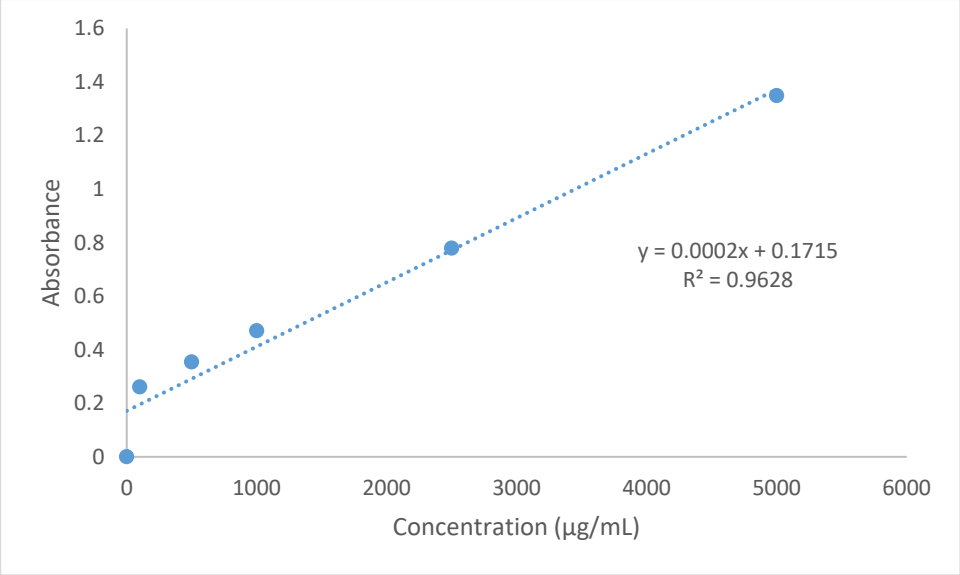

Figure 08: Standard curve of quercetin for determination of Total Flavonoid Content (TFC)

Table 22: Total Flavonoid Content of Petroleum ether extract of Chassalia curviflora

Sample	Absorbance	TFC (mg of QE/g of extract)
20mg/mL	1.7	7.64

Table 23: Total Flavonoid Content of Ethyl acetate (residue) extract of Chassalia curviflora

Sample	Absorbance	TFC (mg of QE/g of extract)
20mg/mL	2.3	10.64

Table 24: Total Flavonoid Content of methanol (residue) extract of Chassalia curviflora

Sample	Absorbance	TFC (mg of QE/g of extract)
20mg/mL	3.1	14.64

3.4 Result of Cytotoxicity activity: *Chassalia curviflora*

Table 25: LC_{50} value of Standard (Vincristine)

Concentration (µg/mL)	No. of Nauplii taken	No. of Nauplii dead	No. of Nauplii alive	% of Mortality	LC_{50} (µg/mL)
3.90625	10	06	04	60	922.44
7.8125	10	08	02	80	
15.625	10	09	01	90	
31.25	10	10	0	100	
62.5	10	10	0	100	
125	10	10	0	100	
250	10	10	0	100	
500	10	10	0	100	

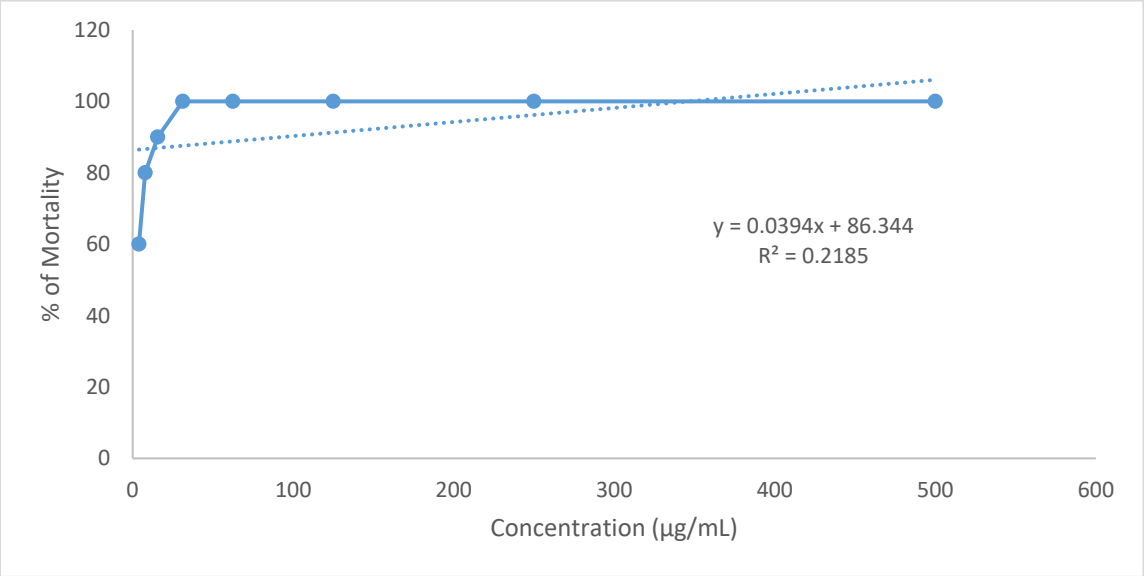

Figure 09: Standard curve for determination of cytotoxic potential of Vincristine

Table 26: LC_{50} value of Petroleum ether extract of Chassalia curviflora

Concentration (µg/mL)	No. of Nauplii taken	No. of Nauplii dead	No. of Nauplii alive	% of Mortality	LC_{50} (µg/mL)
3.90625	10	0	10	0	242.03
7.8125	10	0	10	0	
15.625	10	02	08	20	
31.25	10	02	08	20	
62.5	10	05	05	50	
125	10	06	04	60	
250	10	06	04	60	
500	10	07	03	70	

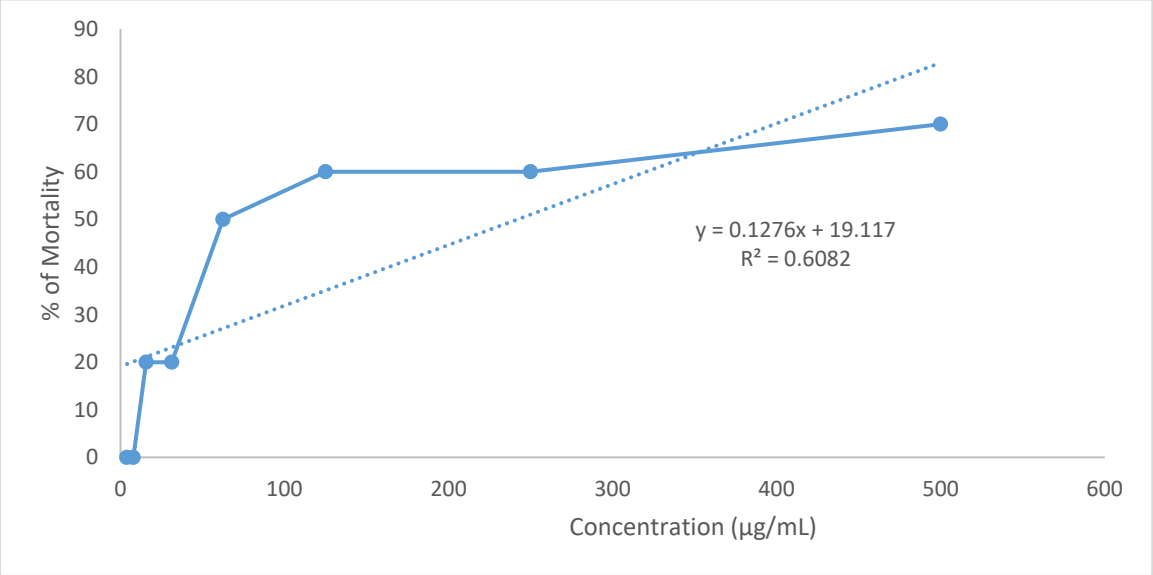

Figure 10: Concentration vs % of mortality of Petroleum ether extract of Chassalia curviflora

Table 27: LC_{50} value of Ethyl acetate extract of Chassalia curviflora

Concentration (µg/mL)	No. of Nauplii taken	No. of Nauplii dead	No. of Nauplii alive	% of Mortality	LC_{50} (µg/mL)
3.90625	10	04	06	40	178.75
7.8125	10	07	03	70	
15.625	10	07	03	70	
31.25	10	06	04	60	
62.5	10	08	02	80	
125	10	10	0	100	
250	10	10	0	100	
500	10	10	0	100	

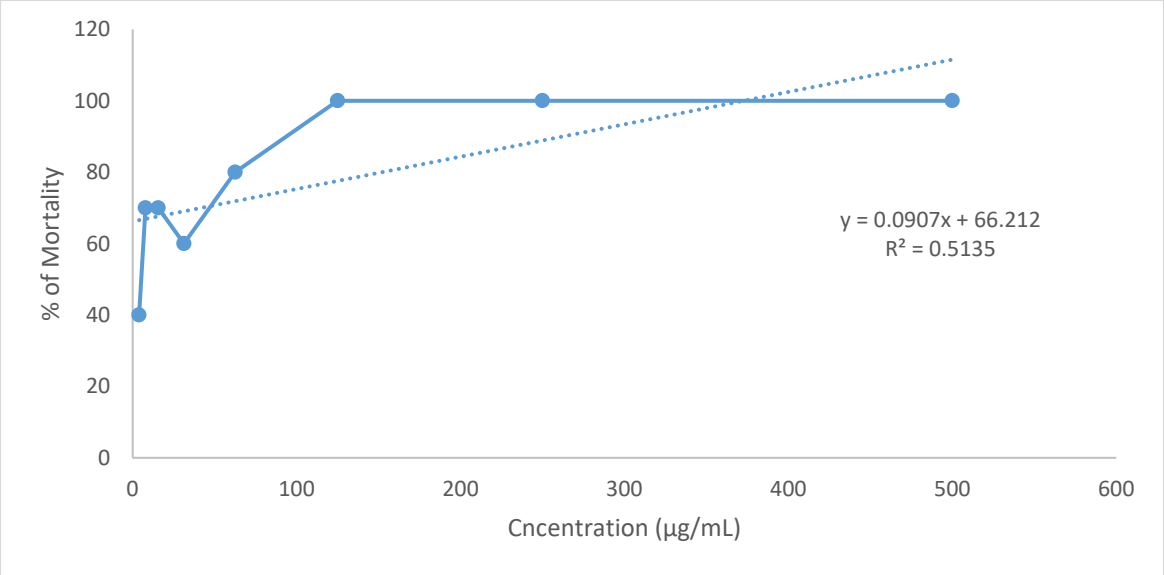

Figure 11: Concentration vs % of mortality of Ethyl acetate extract of Chassalia curviflora

Table 28: LC_{50} value of Methanol extract of Chassalia curviflora

Concentration (µg/mL)	No. of Nauplii taken	No. of Nauplii dead	No. of Nauplii alive	% of Mortality	LC_{50} (µg/mL)
3.90625	10	02	08	20	27.87
7.8125	10	03	07	30	
15.625	10	05	05	50	
31.25	10	05	05	50	
62.5	10	07	03	70	
125	10	09	01	90	
250	10	10	0	100	
500	10	10	0	100	

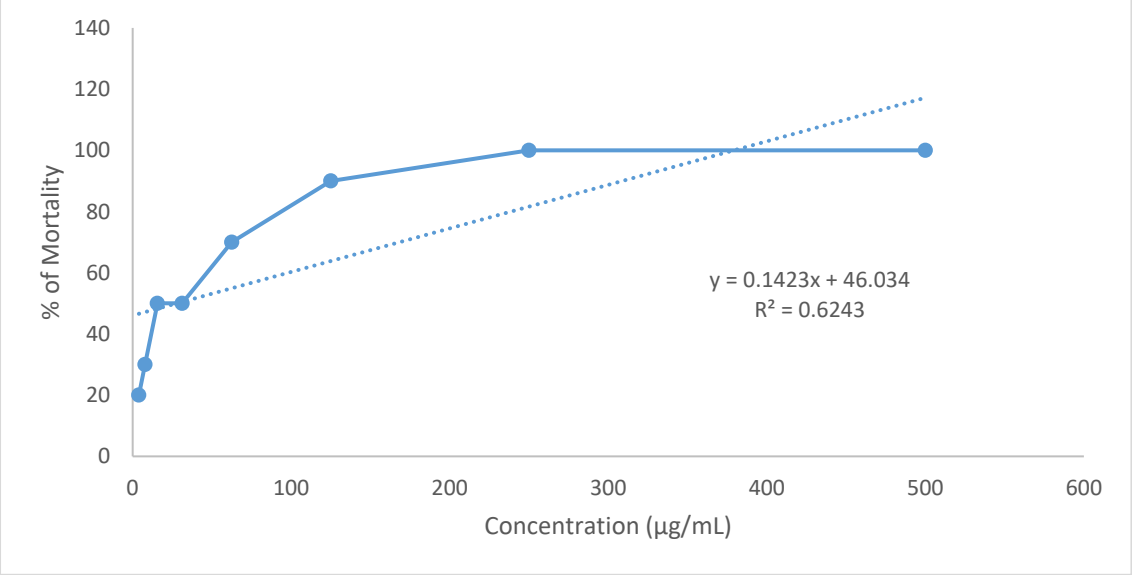

Figure 12: Concentration vs % of mortality of Methanol extract of Chassalia curviflora

Chapter 4

Discussion

This study basically includes phytochemicals investigation and determination of antimicrobial activities, cytotoxic activities and antioxidant potential of the *Chassalia curviflora* plant. Though previously some works are done on this plant, this is the first study which is performed using three different solvent of different polarities to observe that extract of which solvent is more effective and efficient. Here the study was performed following polarity increasing orders using Petroleum ether (Polarity 0.1), Ethyl acetate (Polarity 4.4) and Methanol (Polarity solvents sequentially. This study was also done to observe the effect of these solvents on the extent of the biological activities of the extract of Chassalia curviflora.

Then the phytochemical analysis of the *Chassalia curviflora* plants confirms the presence of alkaloids, flavonoids, phenolic compounds, glycosides, tannins, carbohydrates, steroids, saponins, resins and also shows the absence of phytosterols. However, among three of the solvent extracts, most of the phytochemical compounds are found in the methanol extract of *Chassalia curviflora*. Presence of alkaloids and phenolic compounds confirms the existence of antimicrobial activity of the plant. The plant has antioxidant potentials as flavonoid is also found in the screening of the plant extracts.

Next the antimicrobial text was done for each extract of *Chassalia curviflora* plant by using disk diffusion method. The methanol extract of the plant shows the most antimicrobial activity as alkaloids and phenolic compounds are present in the methanol extract most among the three extract of three different solvents. Then the extract of ethyl acetate shows moderate antimicrobial activities and extract of petroleum ether shows slight antimicrobial activity.

Then the antioxidant potential analysis of each of the three extract of *Chassalia curviflora* was also done by using DPPH free radical scavenging assay, total phenolic content (TPC) and total flavonoid content (TFC). In the DPPH free radical scavenging test, the found IC_{50} values for standard (Ascorbic Acid), petroleum ether extract, ethyl acetate extract and methanol extract were 89.202µg/mL, 531.144µg/mL, 321.548 µg/mL, 59.215µg/mL respectively. Here the found IC_{50} value of methanol extract of *Chassalia curviflora* is even less than the IC_{50} value of the standard ascorbic acid which indicates that the methanol extract of *Chassalia curviflora* has more antioxidant potential than the standard. Next, in TPC test, the results found are 7.84, 10.84, 14.84 mg of GAE/g of extract for petroleum ether extract, ethyl acetate extract and methanol extract of *Chassalia curviflora* respectively. Further, the TFC test result shows 7.64, 10.64 and 14.64 mg of QE/g of extract for petroleum ether extract, ethyl acetate extract and methanol extract of *Chassalia curviflora* sequentially. By observing the results of TPC and TFC tests it can be inferred that extract of methanol has the most total phenolic and flavonoid contents which also reveals that this extract has more antioxidant potential.

Next the test for determination of cytotoxic activity of *Chassalia curviflora* was performed using brine shrimps. In this test the LC_{50} value was found 922.44 µg/mL, 242.03 µg/mL, 178.75µg/mL, 27.87 µg/mL for standard vincristine, petroleum ether extract, ethyl acetate extract and methanol extract of *Chassalia curviflora* respectively. Here the LC_{50} value of methanol extract of the plant is lowest among all including the standard which indicates that this extract will show more cytotoxic activity among all.

Finally, all the results of this study helps to infer that extract of Chassalia curviflora of polar solvent (methanol) will show more biological activities.

Chapter 5

Future work

Traditionally the plant *Chassalia curviflora* is used for treating several diseases such as ear and eye disease, ulcer, rheumatism, pneumonia, skin disease, jaundice, headache, wounds and sour throat. Only few numbers of studies are done on some of the activities. For instance, in vitro antioxidant test, cytotoxicity test, antimicrobial test, acaricidal activity test, anti-hypersensitive tests are done. Moreover, these tests can also be done in future by using different solvents for extraction to compare the results. Additionally, these tests can be done on animal model using rats or mice to ensure more safety as a medicinal agent in the future. So, there are more scopes to work with this plant in future to find out the scientific explanation for the other activities which may help to add a new dimension in the medical field.

Chapter 6

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