# Biological Investigation of *Cissus Adnata*

A project submitted

By

Tapu Ghosh

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This research is dedicated to my parents and my elder brother to whom I owe my achievements.

# Certification statement

This is to notify that, this project titled 'Biological Investigation of *Cissus adnata*' submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Ridwan Islam, Senior Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

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Countersigned by the Supervisor

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# Acknowledgement

I would like to begin my gratitude to Almighty GOD for the help in the completion of this research and preparation of this paper.

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# Abstract

This study provides a scientific basis for the use of *Cissus adnata* in traditional medicine for the first time. The plant was powdered and extracted using methanol and different partitionate were obtained using different organic solvents, viz., dichloromethane, petroleum ether, chloroform. All these fractions were subjected to in vitrobiological screening. Aqueous fraction showed maximum total phenolic content. On the other hand, chloroform extract showed the highest activity in the DPPH free radical scavenging assay, whereas, petroleum ether fraction revealed the most potent $LC_{50}$ value among the partitionate used in brine shrimp lethality bioassay. Antimicrobial activity was examined by agar disc diffusion method and methanolic extract was found to be most effective against all of the gram positive and gram negative bacteria. Aqueous soluble fraction showed highest thrombolytic activity and petroleum ether revealed the highest membrane stabilizing effect, establishing the potential of using this plant as a possible source of discovering anti-coagulants and anti-inflammatory agents. Thus, this investigation might lead to the development of new drugs to treat a wide variety of diseases.

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# Abbreviations

DPPH - 1, 1-diphenyl-2-picrylhydrazyl

ASA - Ascorbic Acid

DMSO – Dimethylsulphoxide

GAE - Gallic Acid Extract

VS – Vincristine Sulphate

TPC -Total Phenol Content

WHO – World Health Organization

ME – Methanolic extract

PESF – Petroleum ether soluble fraction

DCMSF – Dichloromethane soluble fraction

CSF – Chloroform soluble fraction

AQSF – Aqueous soluble fraction

SK - Streptokinase

TSP -Tryptic soya broth medium

# Chapter 01: Introduction

## 1.1 Rationale and objective:

The study of phytochemistry has introduced in recent years as definite discipline, somewhere in between natural product organic chemistry and plant biochemistry. It is also related with the different chemicals in those plants and structural description of different chemicals (Harborne, 1998).According to World Health Organization, 20000 common medicinal plants in the different parts of the world used for different diseases. Around 100 botanical enter into regular trades in the pharmaceutical industry which increase the demands of medicinal plant constituents all over the world. As much as 95% of the drug manufacturing use plant source in Africa (Wijesekera, 1991). It is also used for the less side effects and being easily available at reasonable price. Around 70% medicinal plant in India located in tropical forest area. And in the global market medicinal product value is US $ 120 billion per year (Kurian and Shankar, 2007). Treatment of infections from restorative plant goes back an amplified timeframe. Our progenitors were constrained to utilize restorative plants to facilitate the sufferings from sicknesses of ceaseless and intense starting point, physical inconveniences or wounds like injuries and now and again fatal illnesses. Still this custom is somewhat unaltered. In an assortment of creating nations, antiquated pharmaceuticals are as yet utilized as a help of social insurance. Inside the created nations, an ever increasing number of people are taking prepared (home grown) and unani cures nowadays and at a comparable time a large portion of the plant made basic meds are removed from therapeutic plants (Motaleb, 2011). WHO guarantees that roughly 33% of all medications are plant based and if microscopic organisms and growths are additionally included, about 60% of pharmaceuticals are of plant starting point (Ali, 2010). Many plants particularly plants which are being utilized by customary healers, do produces pharmaceutically dynamic intensifies that postures antimicrobial, hostile to helminthic, antifungal, antiviral, mitigating and cancer prevention agent action (Latifou et al., 2011). Bangladesh has advanced herself with a wide assortment of plants. More than 5,700 angiosperm species, 1,700 types of pteridophytes and 3 types of gymnosperms wins in Bangladesh, among which a sum of 24 plants are in different degrees of risk of elimination. (Ali Reza, 2002). Dhaka, Rajshahi, Sylhet and Chittagong division has a rich measure of therapeutic plants (Ghani, 1998). Up until this point, the quantity of restorative plant enrolled as developing in Bangladesh is more than 500 of species (Motaleb, 2011). Expensive imported medications and detachment to western medicinal services office pass on that the conventional method of human services is the main moderate and accessible type

of social insurance. A significant number of definitions have been offered for therapeutic plants. As indicated by the WHO, "A restorative plant is any plant which, in at least one of its organs, contains ingredients that can be utilized for remedial purposes, or which are antecedents for chemo-pharmaceutical semi-blend." When a plant is assigned as 'restorative', it is inferred that the said plant is valuable as a medication or helpful operator or a dynamic element of a therapeutic arrangement. " Medicinal plants may in this manner be characterized as a gathering of plants that have some uncommon properties or ideals that qualify them as articles of medications and restorative operators, and are utilized for therapeutic purposes"(Ghani, 1998).

WHO address a four point plan to establish the promotion of traditional plants (Rafieian-Kopaei M, 2012:

1- Identification of traditional medicine, presentation of a proper policy and plan.
2- Development of research and education, especially in the university level.
3- Establishment of unity and cooperation between the employees of traditional and modern medicine.
4- Development of cultivation of the needed herbs to prevent destruction of natural resources.

Plants constitute a rich sourball for auxiliary metabolites What's more novel restorative exacerbates characteristically will upgrade human wellbeing for controlled unfriendly impact (Jin-Mang et al, 2003). Characteristic results assume a basic part over pharmacological and business industries, process a considerable measure about social insurance What's more medicinal results for example, antimicrobial, anti-tumour agenize What's more hepatotoxic, cardiotonic, cns stimulants, nutraceutical, sweeteners, sustenance additives Furthermore creature encourage ( Gortzi et al, 2008; Verma et al, 2009). Different plants similar to herbs, trees shrubs and climbers are investigated to their Different bioactive exacerbates for human wellbeing (Ezzatzadeh et al, 2012). What's more will that, critical bioactive groups for example, alkaloid, flavonoids, saponin, terpenoids, polysaccharides and tannins display done plants would generally helping will Different living activites for accepted and advanced restorative standards (Doughari

et al, 2012; Mahboobi et al, 2013). The pharmacologically critical need aid differentiated also separated Eventually Tom's perusing utilizing solvents for polarity of different degrees similar to methanol, chloroform, ethyl acetic acid derivation. Investigations directed Toward Williamson On 2011 figures crazy that rough aggregate concentrated exacerbates reveals to noteworthy bring about sickness administration over the individuals of a solitary disengaged animated portion or purified particular constituents. The association of different aggregations from claiming animated metabolites in the extricate might bring amplified the restorative impact more than those absolute element which need an immediate connection in a specific pharmacological movement (Mohammad et al, 2010). Characteristic item, in exact, still fills in as the atomic premise notwithstanding their probability for medication revelation and advancement thinks about. Mixes exhibit in the plant contain atomic skeletons of high enthusiasm for leap forward disclosure. In the present universe of social insurance upset, natural movement guided approach which deal with the phytochemical examination of restorative plant have the colossal plausibility of yielding new compound of striking enthusiasm for the cure, counteractive action and administration of new and rising infections. The presentation of more up to date advances bringing the wellbeing and toxicological issues of utilizing plant separate that is natural medication as solution for the cure and administration of illness into light, broad phytochemical examination, organic action screening and seclusion of immaculate mixes have extended from the position of need to prerequisite for the cure and administration of malady with better medication adequacy with the minimization of reactions. The time of medicinal services transformation has gotten momentous changes the successful and fast phytochemical examination of plant concentrate and these occasions have enhanced the reason for bringing more up to date atoms into light which fills in as the premise of enhanced and more up to date classes of particles. Bantawa P, Rai R, (2009)

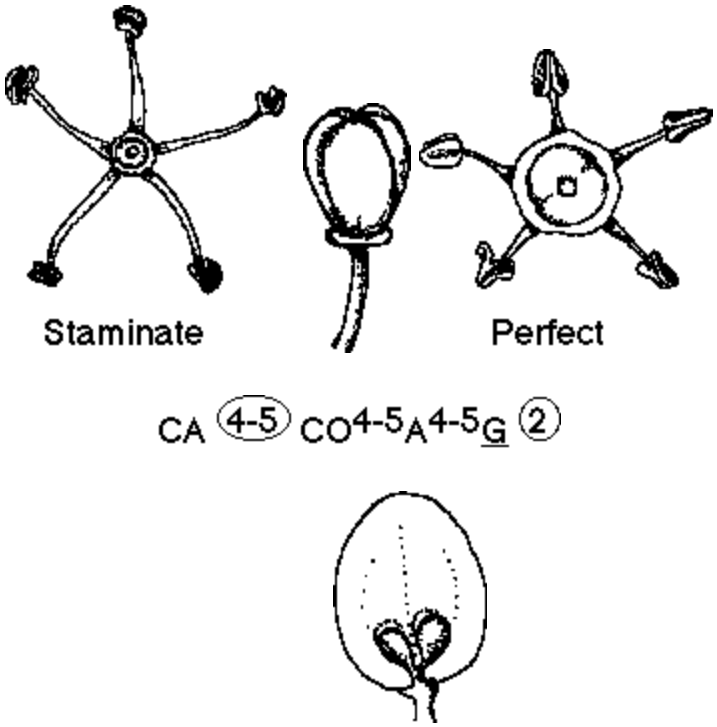
## 1.2 The plant family: Vitaceae

Plants climbing bushes or little trees, leaves compound or basic profoundly lobed; blooms little, bisexual or polygamous – dioecious in spike, racemose, panicles or cymes; sepals 4-5, connate, container formed, petals 4-5, poly or connate at the pinnacle, caducous; stamens 4-5, antipetalous; carpels 2-8, syncarpous, ovary prevalent; organic product a succulent berry.According to Eichler, every ring speaks to the tip of the principle hub which has been pushed aside by the more grounded development of the branch borne in the axil of the juxtaposed leaf. The stretching is cymose scorpioid and the stem is in this manner a sympodium. Some of the time, the stem might be erect.

The hubs are swollen or jointed. Now and again ringlets create glue discs.Alternate or inverse, generally compound, palmate or pinnate, once in a while profoundly lobed and basic (Vitis), leaf bases ventured into membranous stipules. Pellucid puncate specks every now and again present.Bicarpellary syncarpous (infrequently, 3-8 in Leea); ovary-2-celled (Vitis) or 6-celled (Leea), pretty much installed in the roundabout circle, every loculus with 2 rising anatropous ovules; style basic; disgrace terminal, capitate or discoid Yashasvi B(2016).

*Vitis mustangensis* (pubescent)

*Vitis rotundifolia*




## 1.3 The plant genus: Cisssus

The family Cissus comprises of around 350 types of which, no less than, twelve of them utilized internationally in conventional pharmaceutical to treat diverse sicknesses. In Australia, Hedge Prescription Professionals utilize C. hypoglauca to treat sore throat (Lassak and McCarthy, 1997). Many societies in Asia, both East and West Asia, have utilized locally accessible types of Cissus to treat a few medicinal issues. In China and the Far East, C. assamica, is utilized as hostile to snake venom as it abatements endothelin-1 and sarafotoxin 6b (Yang et al., 1998), while in South east Asia including the Indian subcontinent and Sri Lanka, Cissus quadrangularis is utilized for break recuperating (Udupa , Prasad, 1962) and as a hostile to corpulence specialist (Oben et al., 2006). In West Asia, Cissus hamaderohensis, is answered to restrain angiotensin changing over compound (Expert), impartial endopeptidase (NGP) and aminopeptidase N (APN) (Oleski et al., 2006) and also have hostile to viral properties (Mothana et al., 2006). A few nations in Africa utilize diverse types of Cissus in their customary restorative practices: Cameroon customary drug employments C. aralioides as against microbial and toxicological

operator against microorganisms of the gastrointestinal and urogenital tracts (Assob et al., 2011). Alcoholic concentrates of a Gabonese restorative plant – C. debilis - demonstrated antiproliferative action on human CaCo-2 cells (Line-Edwige et al., 2009). In Nigeria, a couple of species like Cissus populnea, Cissus ibuensis and C. quadrangularis are utilized as a part of their local pharmaceutical. Methanolic concentrates of C. populnea expanded multiplication of sertoli cells TM4 in vitro thinks about (Osibote et al., 2011) yet not in people treated for 72 days (Ojekale et al., 2006). What's more, it has against sickling and hostile to bacterial properties (Kone et al., 2004; Cranky et al., 2003) and additionally to treat trypanosomiasis (Atawodi et al., 2002). Generally critically, C. populnea had no antagonistic reactions after long haul organization to Rabbits (Ojekale et al., 2007). While C. ibuensis is utilized to treat gastrointestinal issues (Irvine, 1961), stiffness and joint inflammation (Dalzeil, 1958). In Congo, Cissus rubiginosa, is utilized as against looseness of the bowels and antidiarrhoea operator (Otshudi et al., 2000). Cissus rotundifolia from Africa and Asia demonstrates hostile to diabetic (Onyechi et al., 1998) and also hostile to parasitic properties (Alzoreky and Nakahara, 2003). In the Caribbean islands of Trinidad what's more, Tobago, C. verticillata is utilized as a hostile to diabetic operator and to treat urinary issues (Lans, 2006). Movingon to the terrain of South America, in Brazil, Cissus sycoides is normally utilized as vegetal insulin (Salgado et al., 2009). Of these reports, the most contemplated are C. quadrangularis for corpulence, crack mending and bone infections and C. sycoides as an against diabetic specialist. We have arranged all the related reports for these two species in this audit with an endeavor to decide the probability of utilizing these plants and plant mixes as helpful operators to treat or counteract stoutness, bone related illness and diabetes.

## 1.4 Distribution / Habitat:

Vitaceae or grape group of 11 genera with 600 species conveyed in soggy tropical nations; in Africa and South America numerous succulent xerophytic structures having a place with Cissus is found. C. quadrangularis L. is abundantly found in the fields of Bengal and in different parts of India.

The class Vitis is mild and subtropical happening additionally in northern parts of the globe. Vitis vinifera L., a Mediterranean family is developed in focal and south Europe for wine fabricate. Raisins and currants are dried products of Vitis vinifera L.

## 1.5 Ecology:

*C.Adnata* happens in semi-deciduous thickens near shorelines, rainstorm backwoods and open timberland, as a rule on all around depleted soils, soils are variable however generally sandy to sandy topsoil, here and there lateritic, once in a while calcareous.

## 1.6 Some related species:

*Cissus compressa* Blume

*Cissus latifolia* Vahl

*Cissus pallida* (Wight & Arn.) Steud.

*Cissus simplex* Blanco

*Vitis adnata* (Roxb.) Wall.

*Vitis compressa* (Blume) Backer

*Vitis simplex* (Blanco) Burkill

## 1.7 Description of *Cissus adnata*:

A slim woody climber; stems and inflorescence dressed with orange-red pubescence; ringlets forked. Leaves 7.5-12.5 cm long, extensively praise, unexpectedly and right away taper, swarm serrate, densly dressed with orange-red pubescence underneath. Blooms little, greenish yellow, in tremendously fanned peduncled compound umbellate cymes. Berry 6 mm diam., obovoid or subglobose, dark when ripe.The dried tubers are considered as alterative, blood purifier and diuretic; utilized for cuts and cracks. Decoction of the dried root-stock is utilized as a diuretic and blood purifier. Leaf poultice is utilized to treat bubbles and buboes. Stems are utilized for jaundice in Khagrachari. The plant is additionally utilized as a part of bruises, wounds, syphilis, ulcers and looseness of the bowels.

## 1.8 Taxonomy:

Kingdom: plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: vitales

Family: vitaceae

Genus: cissus

Species: *Cissus adnata*

## 1.9 Plant local names:

**Bangla/Vernacular Name:** Alianga lata, Bhatia-lota.

**Tribal Name:** Isswarmuli (Chakma), Romo-way, La Oye Raa, Oo La Pa (Marma), Mach toi kitab (Tripura) (ethnobotanical data source).

## 1.10 Description of plant:

Vast woody climbers with sulcate branches. Inflorescence and leaves underneath secured with orange red tomentum. Ringlets forked, wooly. Leaves basic, applaud to orbicular. Cymes umbellate on leaf contradicted tomentose peduncle. Berries obovoid, pale blue black.A thin woody climber; stems and inflorescence dressed with orange-red pubescence; rings forked. Leaves 7.5-12.5 cm long, comprehensively applaud, unexpectedly and in a matter of seconds sharpen, swarm serrate, densly dressed with orange-red pubescence underneath. Blooms little, greenish yellow, in greatly spread peduncled compound umbellate cymes. Berry 6 mm diam., obovoid or subglobose, dark when ready (Medicinal plants of south asia) (ethnobotanical data source).

## 1.11 Use of plant:

The smashed leaves of Aristolochia tagala and Cissus adnata are connected to influence territories, and tied set up with a bit of fabric, for the treatment of tumors (Chakma). Leaf poultice is utilized to treat bubbles and buboes. Stems are utilized for jaundice by the Chakma in Khagrachari.The roots [together with 17 different plants (see Typhonium trilobatum)] are utilized to set up a glue which is connected to the influenced zones for the treatment of elephantiasis (Tripura).The dried tubers are considered as alterative, blood purifier and diuretic; utilized for cuts and breaks. Decoction of the dried root-stock is utilized as a diuretic and blood purifier. Leaf poultice is utilized to treat bubbles and buboes. Stems are utilized for jaundice in Khagrachari. The plant is additionally utilized as a part of bruises, wounds, syphilis, ulcers and looseness of the bowels (Medicinal plants of south asia) (ethnobotanical data source).\

# Chapter Two: Methodology

## 2.1 Chemical investigation of the experimental plants

*Cissus adnata*, is the plant of family vitaceae. It was examined for its chemical constituents.

### 2.1.1 Collection and preparation of the plant material

Cissus adnata, were assembled from Dhaka region in February, 2017.After proper washing the whole plants were sun dried for a couple days. The dried plant were then ground to a coarse powder utilizing powerful squashing machine. The coarse powder was then secured in a hermetically fixed holder and stored in cool and dry place.

### 2.1.2 Extraction of the plant material

550gm of the powdered material was taken in two flawless, round bottomed cups (5 liters) and consumed 2.5 liter of methanol. The compartment with its materials was settled and kept for a period of 15 days running with coincidental shaking and blending. The whole mix was then filtered through a perfect cotton plug ultimately with a Whatman No.1 channel paper. The volume of the filtrate was then diminished using a Rotational evaporator at low temperature and weight. The heaviness of the grungy focus was 40gm.

### 2.1.3 Solvent-Solvent partition of crude extract

Dissolvable distributing was finished using the tradition created by Kupchan and changed by Van Wagenen et al. (1993). The grungy think (5gm) was separated in 10% liquid methanol. It was removed with Oil ether, then with Chloroformand finally with Ethyl acidic corrosive inference. The whole allotting technique is schematically showed up in Figure 2.1

All the four divisions were vanished to dryness and were used for further examination

### 2.1.3.1 Partitioning with petroleum ether

The mother arrangement was taken in an isolating channel. 100 ml of the oil ether was added to it and the pipe was shaken and afterward kept undisturbed. The natural bit was gathered. The procedure was rehashed thrice and the portions gathered were dissipated together in revolving evaporator.

### 2.1.3.2 Partitioning with chloroform

To the main subtances that left ensuing to washing with petleum ether, 16 ml of refined water was incorporated and mixed reliably. The main course of action was then taken in a separating channel and removed with trichloromethane (CHCl3) (100 ml X 3). The CHCl3 dissolvable parts were assembled and vanished. The liquid methanolic part was ensured as watery division

### 2.1.3.3 Partitioning with Dichloromethane

To the main arrangement left in the wake of apportioning with oil ether, 12.5 ml of refined water was included and blended. The mother arrangement was then taken in an isolating pipe and removed with dichloromethane (100 ml X 3). The dichloromethane parts were gathered together and vanished. The watery portion was safeguarded for the following stage.

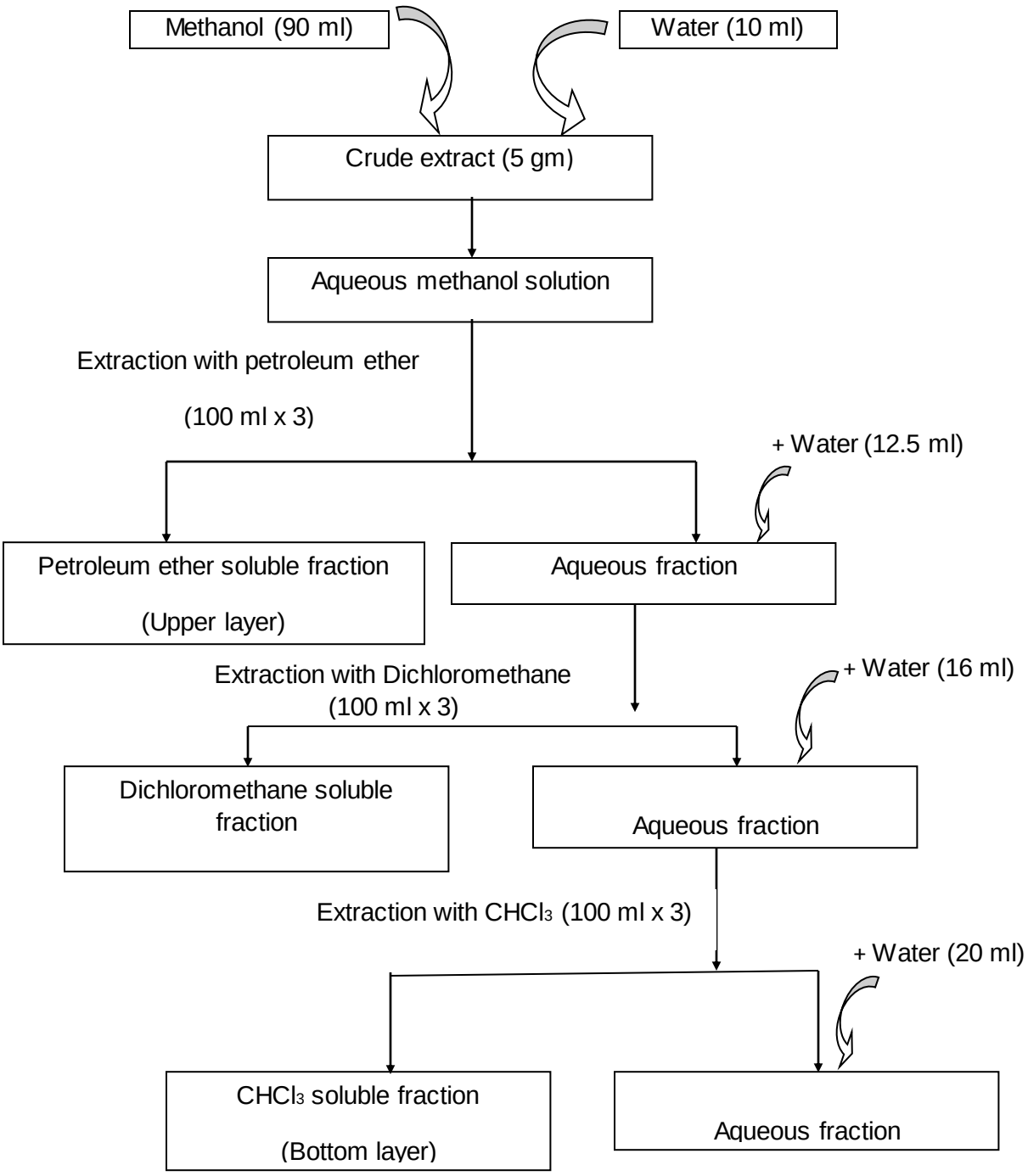



**Figure 2.1: Schematic representation of the modified Kupchan Partitioning of methanolic crude extract of whole plant of *Cissus adnata***

This process was repeated for several times and after evaporation the weight of the different fractions obtained are mentioned in Table 2.1

**Table 2.1: Amount of partitionates obtained from (40 gm) methanolic extract**

| Plant part | Sample code | Fraction | weight (gm) |
|---|---|---|---|
| Whole plant of *Cissus Adnata* | ME | Methanolic extract | 4.0 |
| | PESF | Petroleum ether soluble fraction | 5.5 |
| | DCMSF | Dichloromethane soluble fraction | 4.0 |
| | CSF | Chloroform soluble fraction | 4.5 |
| | AQSF | Aqueous soluble fraction | 14.0 |

## 2.2 Evaluation of Antioxidant activity

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability.

The antioxidant activity assayed in terms of

- Determination of total phenolic content.
- Determination of antioxidant properties: DPPH assay

### 2.2.1 Determination of total phenolic content

The phenolic mixes apply their cancer prevention agent properties by redox response, which can assume an imperative part in retaining and killing free radicals, extinguishing singlet and triplet oxygen, or breaking down peroxides (Osawa, 1994). The antioxidative impact is primarily because of phenolic segments, for example, flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi, Janitha, and Wanasundara, 1992). Numerous phytochemicals have huge cell reinforcement limits that might be related with lower occurrence and lower death rates of malignancy in a few human populaces (Velioglu et al., 1998).

## 2.2.2 Principle:

In the alkaline situation phenols ionize totally. At the point when Folin-Ciocalteu reagent is utilized as a part of this ionized phenolic arrangement the reagent will promptly oxidize the phenols. Common shade of Folin-Ciocalteu reagent is yellow and after the oxidation procedure the arrangement winds up plainly blue. The force of the shading change is measured in a spectrophotometer at 760 nm. The absorbance esteem will mirror the aggregate phenolic substance of the compound (Harbertson and Spayd, 2006).

$$\text{Phenols} + Na_2CO_3 \xrightarrow[\text{ionization}]{\text{Complete}} \text{Ionized phenols}$$

$$\text{Ionized phenols} + \underset{\text{reagent}}{\text{Folin-Ciocalteu}} \xrightarrow{\text{Oxidation}} \underset{\text{(Blue)}}{\text{Folin-Ciocalteu reagent complex}}$$

(Yellow)

## 2.2.3 Materials and Methods

Add up to phenolic substance of Cissus adnata extractives was measured utilizing the technique as portrayed by Skerget et al.,(2005) including Folin-Ciocalteu reagent as oxidizing operator and gallic corrosive as standard (Majhenic et al., 2007).

## 2.2.4 Materials

- Folin-Ciocalteu reagent (10 fold diluted)
- $Na_2CO_3$ solution (7.5 %)
- Distilled water
- Ascorbic acid
- Methanol
- Chloroform
- Ethyl acetate
- Pet ether
- UV-spectrophotometer
- Vial
- Beaker (100 and 200ml)
- Test tube
- Pipette (1ml)
- Pipette (5ml)
- Micropipette (50-200 µl)

### 2.2.5 Composition of Folin-Ciocalteu reagent

| SL. No. | Component | Percent |
|---|---|---|
| 1 | Water | 57.5 |
| 2 | Lithium Sulfate | 15.0 |
| 3 | Sodium Tungstate Dihydrate | 10.0 |
| 4 | Hydrochloric Acid>=25% | 10.0 |
| 5 | Phosphoric Acid 85% solution in water | 5.0 |
| 6 | Molybdic Acid Sodium Dihydrate | 2.5 |

## 2.2.6 Standard curve preparation

Gallic was utilized here as standard. Diverse gallic corrosive arrangement were readied having a fixation going from 100 µg/ml to 0 µg/ml. 2.5 ml of Folin-Ciocalteu reagent (weakened 10 times with water) and 2.0 ml of $Na_2CO_3$ (7.5 % w/v) arrangement was added to 0.5 ml of gallic corrosive arrangement. The blend was brooded for 20 minutes at room temperature. Following 20 minutes the absorbance was measured at 760 nm. In the wake of plotting the absorbance in ordinate against the fixation in abscissa a straight relationship was gotten which is utilized as a standard bend for the assurance of the aggregate phenolic substance of the test tests.

## 2.2.7 Sample preparation

2 mg of the extractives was taken and disintegrated in the refined water to get a specimen centralization of 2 mg/ml for each situation. The specimens alongside their fixation for the aggregate phenolic content estimation are given in the Table 5.2.

**Table 2.2: Test samples for total phenolic content determination**

| Plant part | Sample code | Test Sample | Conc. (mg/ml) |
|---|---|---|---|
| Whole plant of ***Cissus Adnata*** | ME | Methanolic extract | 2.0 |
| | PESF | Petroleum ether soluble fraction | 2.0 |
| | DCMSF | Dichloromethane soluble fraction | 2.0 |
| | CSF | Chloroform soluble fraction | 2.0 |
| | AQSF | Aqueous soluble fraction | 2.0 |

## 2.2.8 Total phenolic compound analysis

To 0.5 ml of concentrate arrangement (conc. 2 mg/ml), 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na2CO3 (7.5 % w/v) arrangement was included. The blend was brooded for 20 minutes at room temperature. Following 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer and utilizing the standard bend arranged from gallic corrosive arrangement with various fixation, the aggregate phenols substance of the example was measured. The phenolic substance of the specimen were communicated as mg of GAE (gallic corrosive equal)/gm of the concentrate.

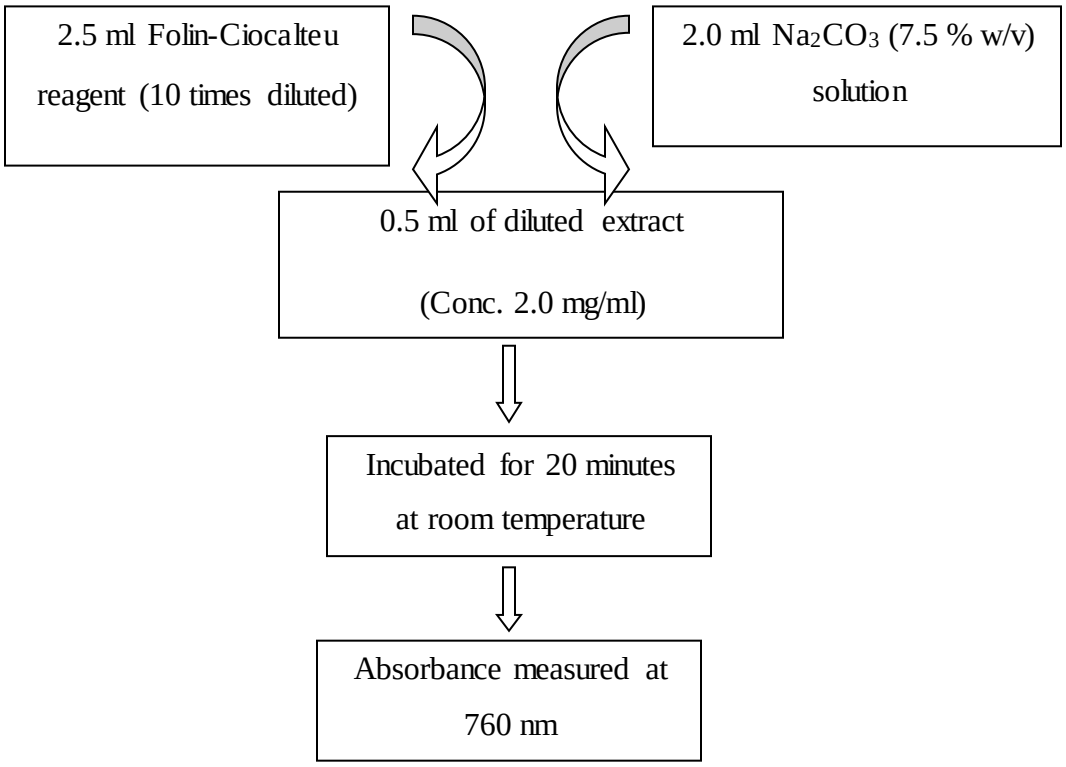
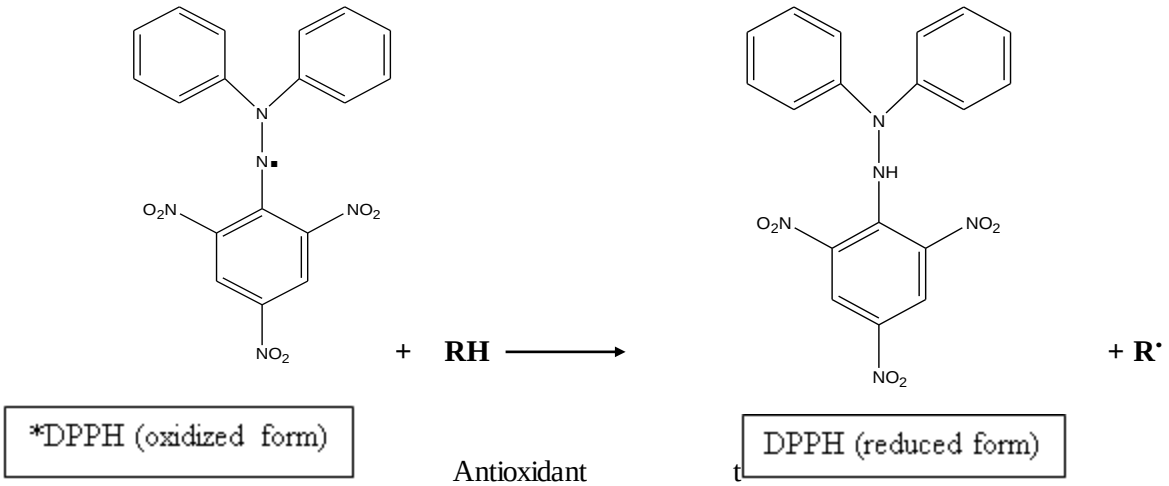



**Figure 2.2: Schematic representation of the total phenolic content determination**

## 2.2.9 Antioxidant activity: DPPH assay

### Principle

The free radical searching exercises (cell reinforcement limit) of the plant separates on the steady radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were assessed by the strategy for Brand-Williams et al., 1995. 2.0 ml of a methanol arrangement of the concentrate at various fixation were blended with 3.0 ml of a DPPH methanol arrangement (20 µg/ml). The cell reinforcement potential was examined from the blanching of purple shaded methanol arrangement of DPPH radical by the plant extricate when contrasted with that of tert-butyl-1-hydroxytoluene (BHT) and ascorbic corrosive (ASA) by UV spectrophotometer.




* DPPH = 1, 1-diphenyl-2-picrylhydrazyl

## 2.2.10 Materials and methods

DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants (Choi et al., 2000; Desmarchelier et al., 1997).

| | |
|---|---|
| 1,1-diphenyl-2-picrylhydrazyl | UV-spectrophotometer |
| Micropipette (50-200 µl) | Beaker (100 and 200ml) |
| Ascorbic acid | Amber reagent bottle |
| Distilled water | Test tube |
| Methanol | Light-proof box |
| Chloroform | Pipette (5ml) |
| Ethyl acetate | |

## 2.2.11 Control preparation for antioxidant activity measurement

Ascorbic acid (ASA) was utilized as positive control. Computed measure of ASA were broken down in methanol to get a mother arrangement having a focus 1000 µg/ml. Serial weakening was made utilizing the mother answer for get distinctive focus going from 500.0 to 0.977 µg/ml.

## 2.2.12 Test sample preparation

Figured measure of various extractives were measured and broken down in methanol to get the mother arrangement (Conc. 1000 µg/ml). Serial weakening of the mother arrangement gave distinctive focus running from 500.0 to 0.977 µg/ml which were kept in the checked flagons.

## 2.2.13 DPPH solution preparation

20 mg DPPH powder was weighed and broken down in methanol to get a DPPH arrangement having a focus 20 µg/ml. The arrangement was set up in the golden reagent bottle and kept in the light proof box.

## 2.2.14 Assay of free radical scavenging activity

. 2.0 ml of a methanol arrangement of the specimen (extractives/control) at various focus (500 µg/ml to 0.977 µg/ml) were blended with 3.0 ml of a DPPH methanol arrangement (20 µg/ml). After 30 min response period at room temperature in dim place the absorbance was measured at 517 nm against methanol as clear by UV spetrophotometer.

Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$(I\%) = (1 - A_{sample}/A_{blank}) \times 100$

Where $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the test material).Extract concentration providing 50% inhibition *($IC_{50}$)* was calculated from the graph plotted inhibition percentage against extract concentration.

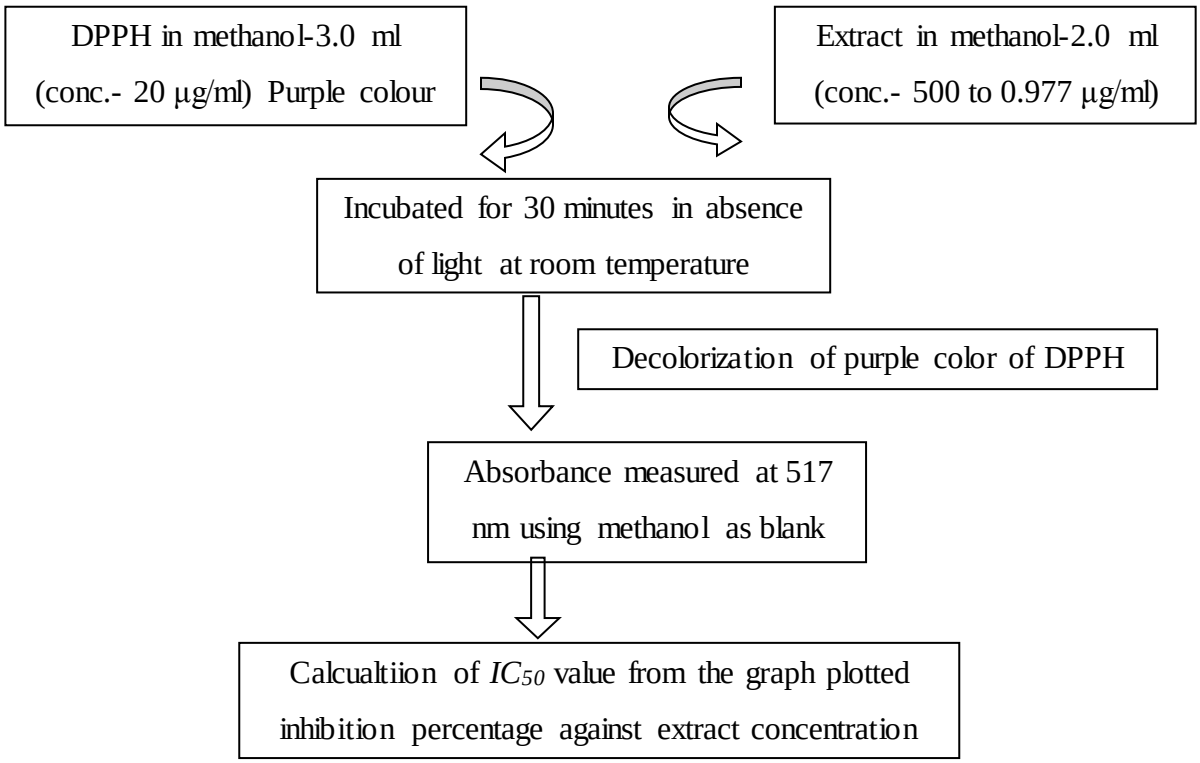



**Figure 2.3: Schematic representation of the method of assaying free radical scavenging activity**

## 2.3 Thrombolytic activity investigation

## Principle

Since antiquated circumstances, home grown arrangements have been utilized for the treatment of a few illnesses. The leaves as well as twigs, stem, bark and underground parts of plants are regularly utilized for conventional medications. Home grown items are frequently seen as sheltered in light of the fact that they seem to be "common" (Gesler, 1992). Cerebral venous sinus thrombosis (CVST) is a typical issue which joined by huge bleakness and mortality (Watson et al., 2002). Heparin, an anticoagulating operator, is the primary line of treatment for CVST, as a result of its viability, wellbeing and attainability (Biousse and Newman, 2004). Thrombolytic drugs like tissue plasminogen activator (t-Dad), urokinase, and streptokinase and so on assume an essential part in the administration of patients with CVST (Baruah, 2006). In this way, the point of the present review was to research the thrombolytic movement of methanolic concentrate and its distinctive portion of entire plant of *Cissus adnata*.

## 2.3.1 Materials and methods

## 2.3.1.1 Preparation of sample

The thrombolytic action of all extractives was assessed by a strategy utilizing streptokinase (SK) as standard substance. The dry unrefined concentrate (100 mg) was suspended in 10 ml of refined water and it was kept overnight. At that point the solvent supernatant was tapped and separated**.**

## 2.3.1.2 Streptokinase (SK)

Monetarily accessible lyophilized altepase (Streptokinase) vial (Beacon pharmaceutical Ltd) of 15, 00,000 I.U., was gathered and 5 ml sterile refined water was included and blended appropriately. This suspension was utilized as a stock from which 100µl (30,000 I.U) was utilized for in vitro thrombolysis.

### 2.3.1.3 Blood Sample

Entire blood (n=10) was drawn from solid human volunteers without a background marked by oral prophylactic or anticoagulant treatment and 1ml of blood was exchanged to the beforehand weighed miniaturized scale rotator tubes and was permitted to shape clusters.

### 2.3.1.4 Thrombolytic activity

Aliquots (5 ml) of venous blood were drawn from sound volunteers which were circulated in five distinctive pre weighed clean smaller scale rotator tube (1 ml/tube) and brooded at 37°C for 45 minutes. After clump development, the serum was totally evacuated without aggravating the coagulation and each tube having cluster was again weighed to decide the coagulation weight (clump weight = weight of cluster containing tube – weight of tube alone).

To each smaller scale axis tube containing pre-measured clump, 100µl watery arrangements of various partitionates alongside the rough concentrate was included independently. As a positive control, 100µl of streptokinase (SK) and as a negative non thrombolytic control, 100µl of refined water were independently added to the control tubes. Every one of the tubes were then hatched at 37°C for a hour and a half and watched for clump lysis. After hatching, the discharged of liquid was evacuated and tubes were again weighed to watch the distinction in weight after clump interruption. Contrast gotten in weight taken previously, then after the fact coagulation lysis was communicated as rate of cluster lysis as demonstrated as follows:

% of clot lysis = (wt of released clot /clot wt) × 100

## 2.4 Brine shrimp lethality bioassay

### Introduction

The mixes which are bioactive are constantly harmful to living body at some higher measurements and it legitimizes the announcement that 'Pharmacology is just toxicology at higher dosages and toxicology is basically pharmacology at lower measurements'. Saline solution shrimp lethality bioassay (McLaughlin, 1998) is a quick and exhaustive bioassay for the bioactive compound of the common and manufactured birthplace. By this strategy, common item extarcts, divisions and additionally the immaculate mixes can be tried for their bioactivity. In this strategy, in vivo lethality in a basic zoological life form (Brackish water shrimp nauplii) is utilized as an ideal screen for screening and fractionation in the revelation of new bioactive characteristic items.

This bioassay demonstrates cytotoxicity and additionally an extensive variety of pharmacological exercises, for example, antimicrobial, antiviral, pesticidal and hostile to tumor and so forth of the mixes (Meyer, 1982; McLaughlin, 1998).

Salt water shrimp lethality bioassay system stands better than other cytotoxicity testing methods since it is fast in process, modest and requires no extraordinary hardware or aseptic strategy. It uses a substantial number of life forms for factual approval and a generally little measure of test. Moreover, not at all like different techniques, it doesn't require creature serum.

### 2.4.1 Principle

Salt water shrimp eggs are incubated in reenacted ocean water to get nauplii. By the expansion of figured measure of dimethylsulphoxide (DMSO), craved centralization of the test is readied. The nauplii are numbered by visual investigation and are taken in vials containing 5 ml of recreated ocean water. At that point tests of various fixations are added to the pre stamped vials through micropipette. The vials are then left for 24 hours. Survivors are tallied following 24 hours.

### 2.4.2 Materials

- Artemia salina leach (brine shrimp egg)
- Sea salt (NaCl)
- Small tank with perforated dividing dam to hatch the shrimp
- Lamp to attract shrimps
- Pipettes
- Micropipette
- Glass vials
- Magnifying glass
- Test tubes
- Test samples of experimental plants

**Table 2.3: Test samples of experimental plants**

| Plant part | Sample code | Test Sample | Calculated amount (mg) |
|---|---|---|---|
| Whole plant of *Cissus Adnata* | ME | Methanolic extract | 4.0 |
| | PESF | Petroleum ether soluble fraction | 4.0 |
| | DCMSF | Dichloromethane soluble fraction | 4.0 |
| | CSF | Chloroform soluble fraction | 4.0 |
| | AQSF | Aqueous soluble fraction | 4.0 |

## 2.4.3 Experimental Procedure

### 2.4.3.1 Preparation of seawater

38 gm ocean salt (immaculate NaCl) was weighed, broken down in one liter of refined water and sifted off to get clear arrangement.

### 2.4.3.2 Hatching of brine shrimps

Artemia salina drain (salt water shrimp eggs) gathered from pet shops was utilized as the test living being. Seawater was taken in the little tank and shrimp eggs were added to the other side of the tank and afterward this side was secured. One day was permitted to incubate the shrimp and to be developed as nauplii. Consistent oxygen supply was helped out through the bring forth time. The brought forth shrimps were pulled in to the light through the punctured dam and they were taken for test.

With the assistance of a pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of seawater.

## 2.4.4 Preparation of test samples of the experimental plant

All the test substances (Table 6.1) were taken in vials and broken up in 100 µl of unadulterated dimethyl sulfoxide (DMSO) to get stock arrangements. At that point 50 µl of arrangement was taken in the main test tube containing 5 ml of recreated seawater and 10 shrimp nauplii. In this way, last convergence of the readied arrangement in the primary test tube was 400 µg/ml. At that point a progression of arrangements of differing focuses were set up from the stock arrangement by serial weakening strategy. For each situation, 50 µl tests were added to test tube and new 50 µl DMSO was added to vial. Along these lines distinctive focuses were found in the diverse test tubes (Table 6.2).

**Table 2.4: Test samples with concentration values after serial dilution**

| Test Tube No. | Concentration (µg/ml) |
|---|---|
| 1 | 400.0 |
| 2 | 200 .0 |
| 3 | 100 .0 |
| 4 | 50 .0 |
| 5 | 25 .0 |
| 6 | 12.5 |
| 7 | 6.25 |
| 8 | 3.125 |
| 9 | 1.5625 |
| 10 | 0.78125 |

## 2.4.5 Preparation of control group

Control gatherings are utilized as a part of cytotoxicity study to approve the test strategy and guarantee that the outcomes got are just because of the movement of the test operator and the impacts of the other conceivable variables are invalidated. Generally two sorts of control gatherings are utilized

i) Positive control
ii) Negative control

## 2.4.6 Preparation of the positive control group

Positive control in a cytotoxicity study is a generally acknowledged cytotoxic specialist and the aftereffect of the test operator is contrasted and the outcome gotten for the positive control. In the present review vincristine sulfate was utilized as the positive control. Measured measure of the vincristine sulfate was broken up in DMSO to get an underlying convergence of 20 µg/ml from which serial dilutions are made utilizing DMSO to get 10 µg/ml, 5 µg/ml, 2.5µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml, 0.0390 µg/ml. At that point the positive control arrangements were add the premarked vials containing ten living saline solution shrimp nauplii in 5 ml reenacted ocean water to gain the positive power gatherings.

## 2.4.7 Preparation of the negative control group

100 µl of DMSO was added to each of three premarked glass vials containing 5 ml of recreated ocean water and 10 shrimp nauplii to use as control gatherings. In the event that the brackish water shrimps in these vials demonstrate a fast death rate, then the test is considered as invalid as the nauplii passed on because of some reason other than the cytotoxicity of the mixes.

## 2.4.8 Counting of nauplii

Following 24 hours, the vials were examined utilizing an amplifying glass and the quantity of survivors were numbered. The percent (%) mortality was ascertained for every weakening. The focus mortality information were dissected measurably by utilizing straight relapse utilizing a basic IBM-PC program. The viability or the fixation mortality relationship of plant item is normally communicated as a middle deadly focus (LC50) esteem. This speaks to the grouping of the substance that produces passing in half of the guineas pigs after a specific presentation period.

## 2.5 Membrane stabilizing activity investigation

In a significant number of the neurotic issue, aggravation is the one of the vital procedures. Incendiary cells create a mind boggling blend of development and separation of cytokines and physiologically dynamic arachidonate metabolites. What's more they have the capacity to create receptive oxygen species (ROS) that can harm cell biomolecules which thus enlarge the condition of aggravation (Cochrane, 1991). Exacerbates that have radical rummaging capacity may in this way hope to have the restorative possibilities for incendiary ailment (Trenam et al., 1992).

The erythrocyte film looks like to lysosomal layer and in that capacity, the impact of medications on the adjustment of erythrocyte could be extrapolated to the adjustment of lysosomal layer (Omale, 2008). In this manner, as film settles that meddle in the discharge as well as activity of go betweens like histamine, serotonin, prostaglandins, leukotrienes and so on. (Shinde et al., 1999). Along these lines, the point of the present review was to research the calming action of methanolic concentrate and its distinctive division of entire plant of Cissus Adnata.

## 2.5.1 Materials and methods

## 2.5.2 Preparation of the extract

**Table 2.5: Preparation of different extracts**

| Plant part | Sample code | Test Sample | Concentration |
|---|---|---|---|
| Whole plant of *Cissus Adnata* | Hypotonic medium | ------ | 50 mM |
| | ME | Methanolic extract | 1 mg/mL |
| | PESF | Petroleum ether soluble fraction | 1 mg/mL |
| | DCMSF | Dichloromethane soluble fraction | 1 mg/mL |
| | CSF | Chloroform soluble fraction | 1 mg/mL |
| | AQSF | Aqueous soluble fraction | 1 mg/mL |
| | ASA | Acetyl salicylic acid | 0.10 mg/mL |

**Solvent used:** Methanol analytical grade

### 2.5.3 Drug

Standard Acetyl Salicylic acid (ASA) or Ibuprofen was utilized as standard medication for examination with various methanolic concentrates of entire plant of *Cissus adnata*.

### 2.5.4 Red Blood Cells (RBC) collection

Human RBCs were gathered for the review. RBCs gathered from the human was male, 70 kg, charge composition and free from infections. The gathered RBCs were kept in a test tube with an anticoagulant EDTA under standard states of temperature 23±2°C and relative moistness 55±10%.

### 2.5.5 Effect on haemolysis

### 2.5.5.1 Erythrocyte suspension

Entire blood was gathered from male human under standard condition. EDTA was utilized to avoid coagulating. The blood was washed three times with isotonic arrangement (154mM NaCl) in 10mM sodium phosphate cushion (pH 7.4) through axis activity for 10min at 3000g. Along these lines the suspension at last gathered was the stock erythrocyte (RBC) suspension.

### 2.5.5.2 Heat induced haemolysis

Aliquots (5ml) of the isotonic cushion containing 1.0mg/mL of various extractives of plant was put into two copy sets of axis tubes (Shinde et al., 1999). The vehicle, in a similar sum, was added to another tube as control. Erythrocyte suspension (30µL) was added to each tube and blended tenderly by reversal. One sets of the tubes was hatched at 540C for 20min in a water shower. The other combine was kept up at 0-50C in an ice shower. The response blend was centrifuged for 3min at 1300g and the absorbance of the supernatant was measured at 540nm.

The rate hindrance or, speeding up of haemolysis in tests was figured by the accompanying condition:

% Inhiition of haemolysis $= 100 \times [1 - (OD_2\text{-}OD_1 / OD_3\text{-}OD_1)]$

Where,

$OD_1$ = test sample unheated, $OD_2$ = test sample heated & $OD_3$ = control sample heated

## 2.6 Antimicrobial screening

Around the world, irresistible malady is one of fundamental driver of death representing roughly one-portion of all passings in tropical nations. Maybe it is not amazing to see these measurements in creating countries, however what might be astounding is that irresistible malady death rates are really expanding in created nations, for example, the Assembled States. Demise from irresistible ailment, positioned fifth in 1981, has turned into the third driving reason for death in 1992, an expansion of 58% .It is assessed that irresistible ailment is the basic reason for death in 8% of the passings happening in the US (Pinner et al., 1996). This is disturbing given that it was once trusted that we would dispense with irresistible infection before the finish of the millenium. The increments are credited to increments in respiratory tract contaminations and HIV/Helps. Other contributing elements are an expansion in anti-toxin resistance in nosicomial and group obtained diseases. Moreover, the most sensational increments are happening in the 25–44 year seniority gathering (Pinner et al., 1996). These negative wellbeing patterns require a restored enthusiasm for irresistible sickness in the restorative and general wellbeing groups and reestablished methodologies on treatment and counteractive action. It is this last arrangement that would envelop the improvement of new antimicrobials (Fauci, 1998). The antimicrobial screening which is the primary phase of antimicrobial medication research is performed to determine the vulnerability of different growths and microorganisms to any operator. This test measures the capacity of each test to restrain the in vitro contagious and bacterial development. This capacity might be evaluated by any of the accompanying three techniques (Ayafor, 1972).

- ✓ Disc diffusion method
- ✓ Serial dilution method
- ✓ Bioautographic method

However, there is no institutionalized strategy for communicating the aftereffects of antimicrobial screening (Ayafor, 1982). A few examiners utilize the width of zone of hindrance as well as the base weight of concentrate to restrain the development of microorganisms. Nonetheless, an extraordinary number of elements viz., the extraction techniques, inoculum

volume, culture medium structure (Bayer et al., 1966), pH, and hatching temperature can impact the outcomes. Among the previously mentioned procedures the plate dissemination (Bayer et al., 1966) is a broadly acknowledged in vitro examination for preparatory screening of test operators which may have antimicrobial action. It is basically a quantitative or subjective test demonstrating the affectability or resistance of the microorganisms to the test materials. Nonetheless, no qualification amongst bacteriostatic and bactericidal movement can be made by this strategy (Roland R, 1982).

### 2.6.1 Principle of disc diffusion method

In this established strategy, anti-microbials diffuse from a restricted source through the supplement agar gel and make a fixation inclination. Dried and cleaned channel paper circles (6 mm breadth) containing the test tests of known sums are set on supplement agar medium consistently seeded with the test microorganisms. Standard anti-infection (kanamycin) plates and clear circles are utilized as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to permit greatest dispersion of the test materials to the encompassing media (Barry, 1976). The plates are then rearranged and hatched at 37°C for 24 hours for ideal development of the creatures. The test materials having antimicrobial property restrain microbial development in the media encompassing the plates and consequently yield an unmistakable, particular range characterized as zone of restraint. The antimicrobial movement of the test operator is then dictated by measuring the distance across of zone of restraint communicated in millimeter (Barry, 1976; Bayer et al., 1966.) In the present review the unrefined concentrates and also divisions were tried for antimicrobial action by plate dispersion strategy. The examination is done more than once and the mean of the readings is required (Bayer et al., 1966).

## 2.6.2 Experimental

### 2.6.2.1 Apparatus and reagents

| | |
|---|---|
| Filter paper discs | Autoclave |
| Nutrient Agar Medium | Laminar air flow hood |
| Petridishes | Spirit burner |
| Sterile cotton | Refrigerator |
| Micropipette | Incubator |
| Inoculating loop | Chloroform |
| Sterile forceps | Ethanol |
| Screw cap test tubes | Nosemask and Hand gloves |

### 2.6.2.2 Test organisms

The bacterial and contagious strains utilized for the test were gathered as fresh cultures from department of pharmacy, State university of Bangladesh (SUB). Both gram positive and gram-negative life forms were taken for the test and they are recorded in the table

**Table 2.6: Different strains used in antimicrobial screening**

| Gram positive Bacteria | Gram negative Bacteria | Fungi |
|---|---|---|
| *Bacillus cereus* | *Escherichia coli* | *Aspergillus niger* |
| *Bacillus megaterium* | *Salmonella paratyphi* | *Candida albicans* |
| *Bacillus subtilis* | *Salmonella typhi* | *Sacharomyces cerevacae* |
| *Sarcina lutea* | *Shigella boydii* | |
| *Staphylococcus aureus* | *Shigella dysenteriae* | |
| | *Pseudomonas aeruginosa* | |
| | *Vibrio mimicus* | |
| | *Vibrio parahemolyticus* | |

**Table 2.7: List of Test materials**

| Plant part | Sample code | Test Sample |
|---|---|---|
| Whole plant of *Cissus Adnata* | ME | Methanolic extract of *Cissus Adnata* |
| | PESF | Petroleum ether soluble fraction |
| | DCMSF | Dichloromethane soluble partitionate |
| | CSF | Chloroform soluble partitionate |
| | AQSF | Aqueous soluble partitionate |

## 2.6.3 Composition of culture medium

The accompanying media was utilized typically to show the antimicrobial action and to make subculture of the test living beings

**a) Nutrient agar medium**

| Ingredients | Amount |
|---|---|
| Bacto peptone | 0.5 gm |
| Sodium chloride | 0.5 gm |
| Bacto yeast extract | 1.0 gm |
| Bacto agar | 2.0 gm |
| Distilled water q.s. | 100 ml |
| pH | 7.2 + 0.1 at 250C |

**b) Nutrient broth medium**

| Ingredients | Amount |
|---|---|
| Bacto beef extract | 0.3 gm |
| Bacto peptone | 0.5 gm |
| Distilled water q.s. | 100 ml |
| pH | 7.2 + 0.1 at 250C |

**c) Muller – Hunton medium**

| Ingredients | Amount |
|---|---|
| Beef infusion | 30 gm |
| Casamino acid | 1.75 gm |
| Starch | 0.15 gm |
| Bacto agar | 1.70 gm |
| Distilled water q.s. | 100 ml |
| pH | 7.3 + 0.2 at 250C |

**d) Tryptic soya broth medium (TSB)**

| Ingredients | Amount |
|---|---|
| Bacto tryptone | 1.70 gm |
| Bacto soytone | 0.30 gm |
| Bacto dextrose | 0.25 gm |
| Sodium chloride | 0.50 gm |
| Di potassium hydrogen Phosphate | 0.25 gm |
| Distilled water q.s | 100 ml |
| pH | 7.3 + 0.2 at 250C |

Supplement agar medium is the most much of the time utilized and furthermore utilized as a part of the present review for testing the affectability of the life forms to the test materials and to get ready crisp societies.

## 2.6.4 Preparation of the medium

To get ready required volume of this medium, computed measure of each of the constituents was taken in a funnel shaped cup and refined water was added to it to make the required volume. The substance were warmed in a water shower to make a reasonable arrangement. The pH (at 250C) was balanced at 7.2-7.6 utilizing NaOH or HCl. 10 ml and 5 ml of the medium was then moved in screw top test tubes to plan plates and inclinations individually. The test tubes were then topped and disinfected via autoclaving at 15-lbs. weight at 1210C for 20 minutes. The inclinations were utilized for making new culture of microbes and organisms that were thus utilized for affectability ponder.

## 2.6.5 Sterilization procedure

To dodge any kind of defilement and cross sullying by the test living beings the antimicrobial screening was done in Laminar Hood and a wide range of precautionary measures were exceptionally kept up. UV light was exchanged on one hour before working in the Laminar Hood. Petridishes and other dish sets were cleaned via autoclaving at a temperature of 1210C and a weight of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, clear plates and so forth were additionally cleaned by UV light.

## 2.6.6 Preparation of subculture

In an aseptic condition under laminar air bureau, the test living beings were exchanged from the unadulterated societies to the agar inclines with the assistance of an exchange circle to have crisp immaculate societies. The immunized strains were then hatched for 24 hours at 370C for their ideal development. These crisp societies were utilized for the affectability test.

## 2.6.7 Preparation of the test plate

The examining organisms were exchanged from the subculture to the test tubes containing around 10 ml of liquefied and disinfected agar medium with the assistance of a sanitized move circle in an aseptic region. The test tubes were shaken by pivot to get a uniform suspension of the living beings. The bacterial and parasitic suspension was promptly exchanged to the sanitized petridishes. The petridishes were turned a few times clockwise and anticlockwise to guarantee homogenous appropriation of the test living beings in the media.

## 2.6.8 Preparation of discs

Test sample was measured and dissolved in specific amount of solvent (Chloroform or methanol) to get the concentration of desire in an aseptic condition. Disinfected metrical (BBL, Cocksville, USA) channel paper plates were taken in a clear petridish under the laminar hood. At that point circles were splashed with arrangements of test tests and dried.

**Table 2.8 Preparation of sample Discs**

| **Plant part** | **Test Sample** | **Dose** μg/disc | **Required amount for 20 disc** (mg) |
|---|---|---|---|
| Whole plant of *Cissus Adnata* | Methanolic extract | 400 | 8.0 |
| | Petroleum ether partitionate | 400 | 8.0 |
| | Dichloromethane soluble partitionate | 400 | 8.0 |
| | Chloroform soluble partitionate | 400 | 8.0 |
| | Aqueous soluble partitionate | 400 | 8.0 |

Standard Kanamycin (30 μg/circle) plates were utilized as positive control to guarantee the movement of standard anti-microbial against the test life forms and also for examination of the reaction delivered by the known antimicrobial specialist with that of created by the test. Clear circles were utilized as negative controls which guarantee that the remaining solvents (left over the plates even after air-drying) and the channel paper were not dynamic themselves.

## 2.6.9 Diffusion and incubation

The example circles, the standard anti-microbial disc0s and the control circles were set tenderly on the already checked zones in the agar plates pre-vaccinated with test microscopic organisms and growths. The plates were then kept in an icebox at 40C for around 24 hour's topsy turvy to permit adequate dissemination of the materials from the circles to the encompassing agar medium. The plates were then rearranged and kept in a hatchery at 370C for 24 hours.

## 2.6.10 Determination of the zone of inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition.After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

# Chapter Three: Result and discussion

## 3.1 Determination of total phenolic content

The methanolic extract (ME) of whole plant of *Cissus adnata* and its distinctive partitionates i.e. petroleum ether (PESF), chloroform (CSF), dichloromethane soluable fraction and aqueous soluble fractions (AQSF) were tried for aggregate phenolic content. Folin-Ciocalteu reagent was utilized for the test. In view of the absorbance estimations of the different concentrate arrangements the colorimetric investigation of the aggregate phenolics of various concentrates were resolved and contrasted and the standard arrangements of gallic corrosive reciprocals. Add up to phenolic substance of the specimens are communicated as mg of GAE (gallic corrosive comparable)/gm of extractives and are given in table.

The measure of aggregate phenolic content varied in various extractives and extended from 11.94mg of GAE/gm of extractives to34.65 mg of GAE/gm of extractives of leaves of *Cissus adnata*. Among all extractives of leaves of *Cissus adnata* the most noteworthy phenolic substance was found in (AQSF – aqueous soluble fraction) 34.65 of GAE/gm of extractives which takes after by 11.94 (PESF – petroleum ether soluble fraction),methanolic extricate (ME) 15.64mg ,18.98 (CSF – chloroform soluble fraction), 13.17 (DCMSF– Dichloromethane soluble fraction)mg of GAE/gm of extractives

**Table 3.1:Standard curve prepration by using gallic acid**

| Sl. No. | Conc. Of the Standard (µg/ml) | Absorbance | Regression line | $R^2$ |
|---|---|---|---|---|
| 1 | 100 | 0.800 | y = 0.0081x - 0.0007 | 0.9975 |
| 2 | 50 | 0.423 | | |
| 3 | 25 | 0.215 | | |
| 4 | 12.5 | 0.123 | | |
| 5 | 6.25 | 0.047 | | |
| 6 | 3.125 | 0.007 | | |
| 7 | 1.5625 | 0.003 | | |
| 8 | 0.78125 | 0.000 | | |
| 9 | 0.3906 | 0.000 | | |
| 10 | 0 | 0.000 | | |

**Figure 3.1: Graphical representation standard curve of gallic acid for total phenolic determination**

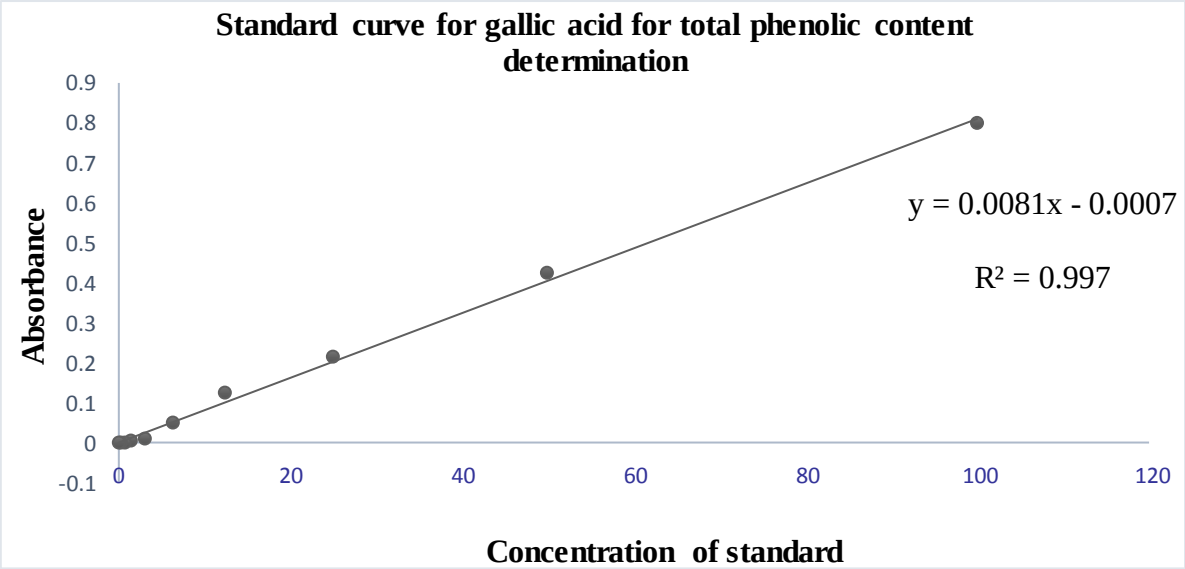


**Table 3.2: Test samples for total phenolic content determination**

| Plant part | Sample code | Test Sample | Total phenolic content (mg of GAE / gm of extractives |
|---|---|---|---|
| **Whole plant of *Cissus Adnata*** | ME | Methanolic extract | 15.64 |
| | PESF | Petroleum ether soluble fraction | 11.94 |
| | DCM | Dichloromethane soluble fraction | 13.17 |
| | CSF | Chloroform soluble fraction | 18.98 |
| | AQSF | Aqueous soluble fraction | 34.65 |

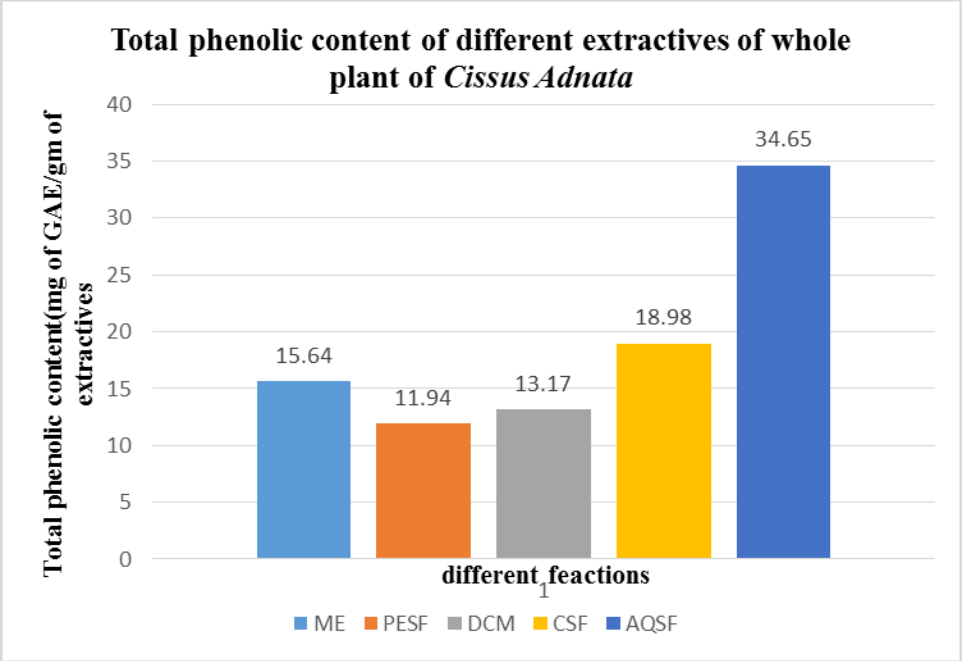



**Figure 3.2: Graphical representation of total phenolic content (mg of gae / gm of extractives) of different extractives of whole plant *Cissus adnata***

## 3.2 DPPH assay

The methanolic concentrate of entire plant of cissus adnata and its diverse partitionates i.e. Pet-ether (PESF), chloroform (CSF), dichloromethane dissolvable fraction(DCMSF) and aqueous (AQSF) solvent divisions of the methanolic concentrate of entire plant of cissus adnata were subjected to free radical searching action utilizing the strategy for brand-williams et al., 1995. Here, ascorbic corrosive (asa) was utilized as reference norms.

In this examination, chloroform soluble fraction CSF demonstrated the prominent free radical searching action with ic50 estimation of 139.11 µg/ml for leaves of *Cissus adnata*. Alternate partionates like MESF, PESF, DCMSF and AQSF showed direct rummaging action having ic50 values 515.10µg/ml, 546.29µg/ml, 343.95µg/ml and 212.66 µg/ml, individually.

**Table 3.3: $IC_{50}$ values of the standard and partitionates of leaves of *Cissus Adnata***

| Plant part | Sample code | Test Sample | $IC_{50}$ (µg/ml) |
|---|---|---|---|
| Whole plant of *Cissus adnata* | ME | Methanolic extract | 515.10 |
| | PESF | Petroleum ether soluble fraction | 546.29 |
| | DCMSF | Dichloromethane soluble fraction | 343.95 |
| | CSF | Chloroform soluble fraction | 139.11 |
| | AQSF | Aqueous soluble fraction | 212.66 |
| ASA (Ascorbic acid) (standard) | | | 2.98 |

**Table 3.4:$IC_{50}$ value of ascorbic acid (ASA)**

| Absorbance of the blank | Conc. (µg/ml) | Absorbance of the extract | % inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| 0.355 | 500 | 0.005 | 98.59 | 2.98 |
| | 250 | 0.006 | 98.31 | |
| | 125 | 0.015 | 95.77 | |
| | 62.5 | 0.024 | 93.24 | |
| | 31.25 | 0.068 | 80.85 | |
| | 15.625 | 0.098 | 72.39 | |
| | 7.813 | 0.139 | 60.85 | |
| | 3.906 | 0.186 | 47.61 | |
| | 1.953 | 0.175 | 50.70 | |
| | 0.977 | 0.193 | 45.63 | |

**Figure 3.3: Graphical representation of $IC_{50}$ value of ascorbic acid**

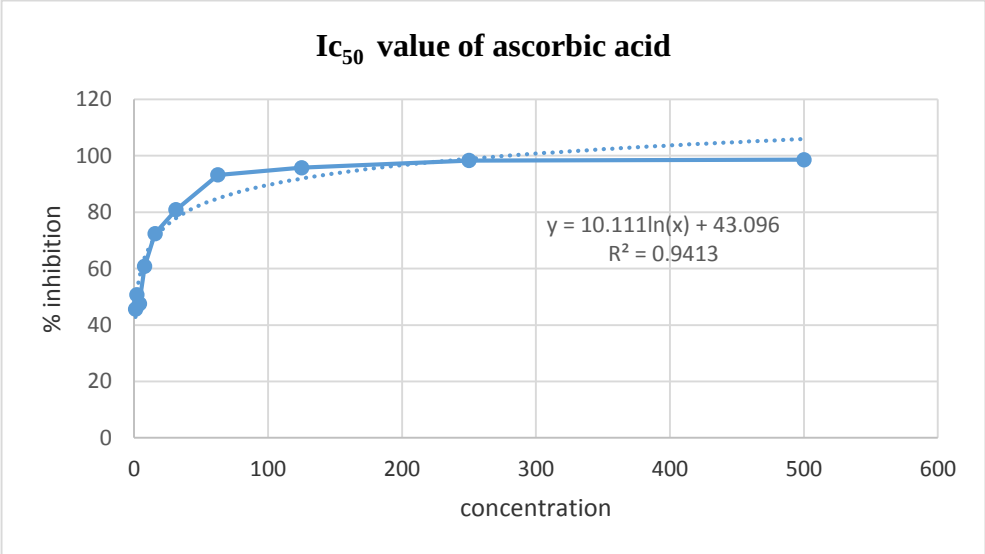



**Table 3.5: $IC_{50}$ value of Methanolic extract (ME)**

| Absorance of the blank | Conc. (µgm/ml) | Absorbance of the extract | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 0.355 | 500 | 0.215 | 39.44 | 515.10 |
| | 250 | 0.195 | 45.07 | |
| | 125 | 0.286 | 19.44 | |
| | 62.5 | 0.296 | 16.62 | |
| | 31.25 | 0.312 | 12.12 | |
| | 15.625 | 0.339 | 4.51 | |
| | 7.813 | 0.329 | 7.32 | |
| | 3.906 | 0.333 | 6.19 | |
| | 1.953 | 0.342 | 3.66 | |
| | 0.977 | 0.344 | 3.09 | |

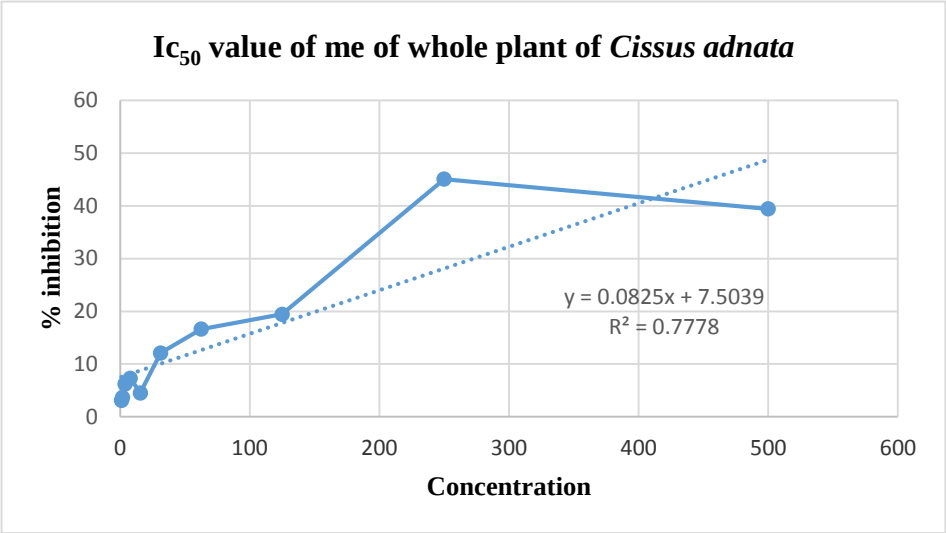



**Figure 3.4: Graphical representation of $IC_{50}$ value of ME of whole plant of *Cissus adnata***

**Table 3.6:$IC_{50}$ value of petroleum ether extract (PESF)**

| Absorbance of the blank | Conc. (µgm/ml) | Absorbance of the extract | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 0.355 | 500 | 0.198 | 44.23 | 546.29 |
| | 250 | 0.259 | 27.04 | |
| | 125 | 0.293 | 17.46 | |
| | 62.5 | 0.309 | 12.96 | |
| | 31.25 | 0.311 | 12.39 | |
| | 15.625 | 0.333 | 6.19 | |
| | 7.813 | 0.334 | 6 | |
| | 3.906 | 0.344 | 3.09 | |
| | 1.953 | 0.348 | 1.97 | |
| | 0.977 | 0.353 | 0.36 | |

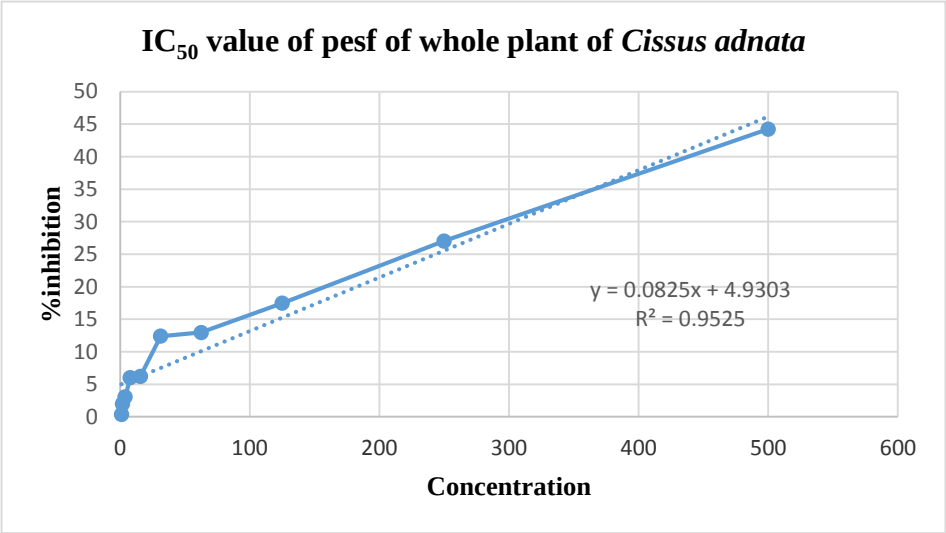



**Figure 3.5: Graphical representation of $IC_{50}$ value of PESF of whole plant of *Cissus adnata***

**Table 3.7: $IC_{50}$ value of dichloromethane extact (DCMSF)**

| Absorbance of the blank | Conc. (µgm/ml) | Absorbance of the extract | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 0.355 | 500 | 0.197 | 44.51 | 343.95 |
| | 250 | 0.096 | 72.96 | |
| | 125 | 0.204 | 42.54 | |
| | 62.5 | 0.235 | 33.80 | |
| | 31.25 | 0.281 | 20.85 | |
| | 15.625 | 0.300 | 15.49 | |
| | 7.813 | 0.333 | 6.19 | |
| | 3.906 | 0.338 | 4.89 | |
| | 1.953 | 0.342 | 3.66 | |
| | 0.977 | 0.352 | 0.85 | |

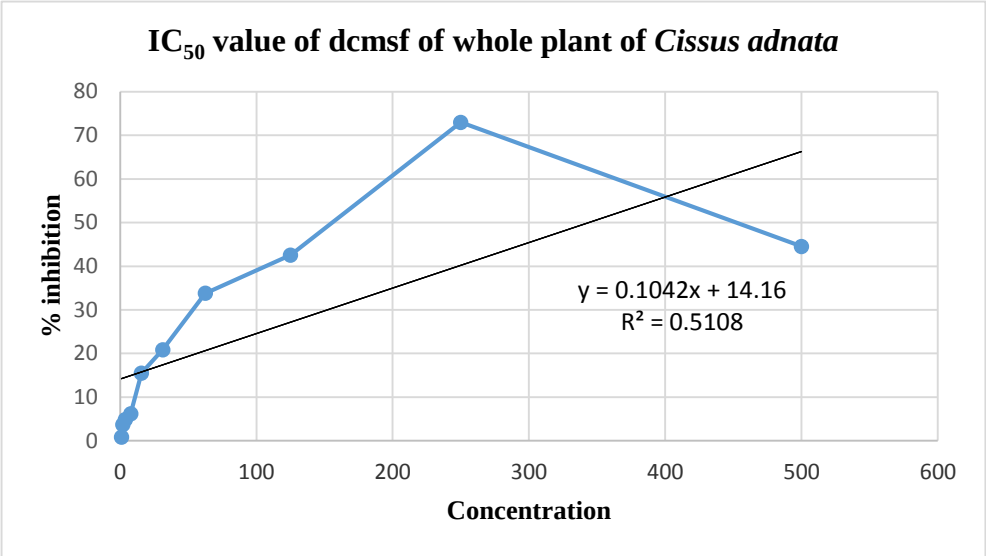



**Figure3.6: Graphical representation of $ic_{50}$ value of DCMSF of whole plant of *Cissus adnata***

**Table 3.8:$IC_{50}$ value of chloroform extract (CSF)**

| Absorbance of the blank | Conc. (µgm/ml) | Absorbance of the extract | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 0.355 | 500 | 0.005 | 98.59 | 139.11 |
| | 250 | 0.010 | 97.18 | |
| | 125 | 0.079 | 77.75 | |
| | 62.5 | 0.152 | 57.18 | |
| | 31.25 | 0.231 | 34.93 | |
| | 15.625 | 0.273 | 23.19 | |
| | 7.813 | 0.323 | 9.01 | |
| | 3.906 | 0.324 | 8.73 | |
| | 1.953 | 0.326 | 8.16 | |
| | 0.977 | 0.330 | 7.64 | |

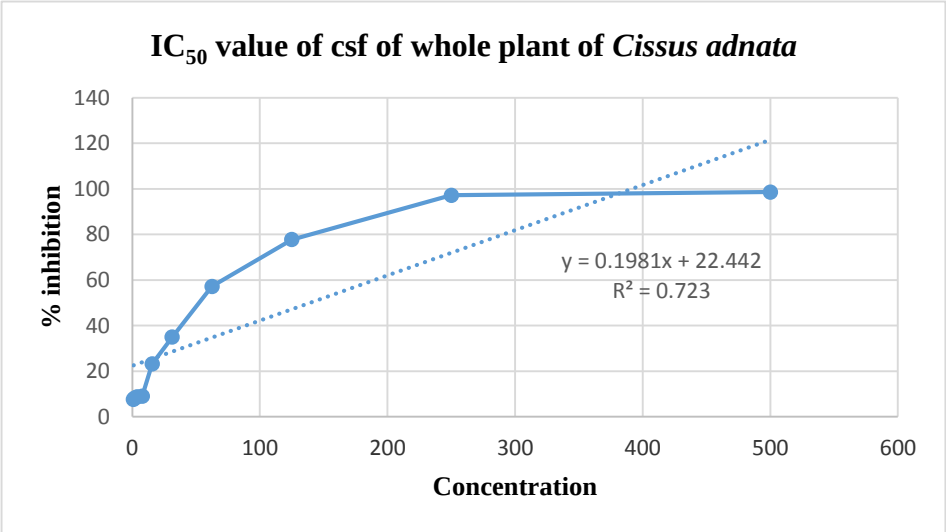



**Figure 3.7: Graphical representation of $IC_{50}$ value of CSF of whole plant of *Cissus adnata***

**Table 3.9:$IC_{50}$ value of aqueous soluble fraction**

| Absorbance of the blank | Conc. (µgm/ml) | Absorbance of the extract | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| 0.355 | 500 | 0.024 | 93.24 | 212.66 |
| | 250 | 0.109 | 69.29 | |
| | 125 | 0.201 | 43.38 | |
| | 62.5 | 0.249 | 29.86 | |
| | 31.25 | 0.294 | 17.18 | |
| | 15.625 | 0.321 | 9.58 | |
| | 7.813 | 0.322 | 9.29 | |
| | 3.906 | 0.24 | 8.73 | |
| | 1.953 | 0.329 | 7.32 | |
| | 0.977 | 0.334 | 6 | |

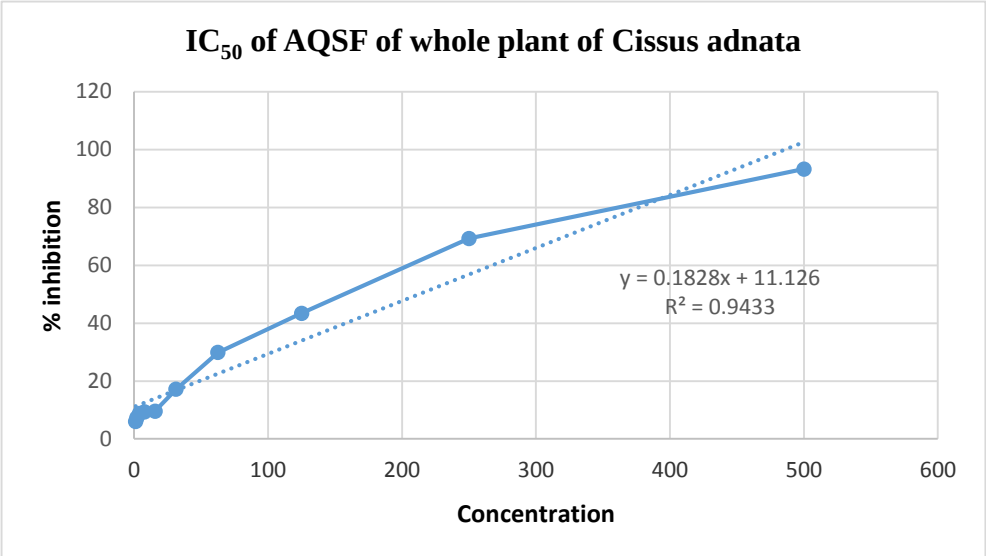



**Figure 3.8: Graphical representation of $IC_{50}$ value of AQSF of whole plant of *Cissus adnata***

## 3.3 Thrombolytic activity investigation

To discover cardio defensive medications from regular sources the extractives of cissus adnata were evaluated for thrombolytic movement and the outcomes are displayed in table 3.9. Expansion of 100µl sk, a positive control (30,000 i.u.), to the coagulations and ensuing brooding for an hour and a half at 37°c, indicated 66.77% haemolysis of cluster. Then again, refined water was dealt with as negative control which showed an unimportant rate of lysis of clump (36.09%). The mean distinction in cluster lysis rate amongst positive and negative control was discovered exceptionally critical. In this experiment, aqueous soluble fraction demonstrated most astounding thrombolytic action (42.32%). In any case, alternate partitionates of cissus adnata i.e.petrolium ether soluble fraction (pesf), chloroform soluble fraction (csf), dichloromethane soluble fraction (dcmsf) demonstrated gentle to direct thrombolytic action.

**Table 3.10: Thrombolytic Activity (in terms of % of clot lysis) of leaves of *Cissus Adnata***

| Fractions | Weight of empty eppendorf tube | Weight of clot containing eppendorf tube before clot disruption | Weight of clot containing eppendorf tube after clot disruption | % of clot lysis |
|---|---|---|---|---|
| MESF | 4800.9 | 5819.4 | 5672.0 | 14.47 |
| PESF | 4816.5 | 5792.8 | 5573.8 | 22.43 |
| DCMSF | 4795.4 | 5675.0 | 5411.2 | 29.9 |
| CSF | 4876.0 | 5701.9 | 5434.4 | 32.39 |
| AQSF | 4674.6 | 5534.5 | 5170.6 | 42.32 |
| Blank | 0.919 | 1.476 | 1.275 | 36.09 |
| SK | 0.905 | 1.913 | 1.24 | 66.77 |

W1 = Weight of eppendorf blank; W2 = Weight of clot containing eppendorf; W3 = Weight of clot containing eppendorf after clot disruption; SK =Streptokinase

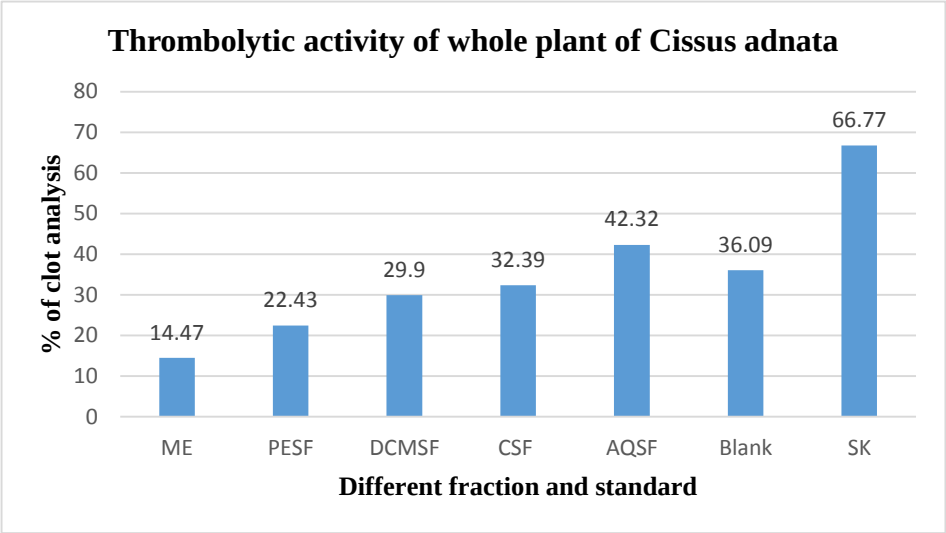



**Figure 3.9: Graphical representation of thrombolytic activity of whole plant of *Cissus adnata***

From this examination, it can be reasoned that few of the extractives of *Cissus Adnata* demonstrated gentle to direct cluster lysis movement. When found these home grown arrangements might be fused as a thrombolytic specialist for the change of the patients experiencing Atherothrombotic infections. This is just a preparatory review and to make last remark the concentrate ought to altogether examined phytochemically and pharmacologically to misuse their restorative and pharmaceutical possibilities.

## 3.4 Brine shrimp lethality bioassay

The methanolic separate (me) of entire plant of cissus adnata and its distinctive partitionates i.e. Petroleum ether (pesf), dichloromethane (dcmsf), chloroform (csf) and aqueous soluble portions (aqsf) were tried for brackish water shrimp lethality bioassay taking after the strategy of meyer et al., (1982). The lethality of the extractives to saline solution shrimp was resolved and the outcomes are given in.

The lethal concentration (lc50) of the test tests following 24 hours was acquired by a plot of rate of the shrimps kicked the bucket against the logarithm of the specimen focus (toxicant focus) and the best-fit line was gotten from the bend information by methods for relapse examination.

Vincristine sulfate was utilized as positive control and the lc50 was observed to be 0.45 □g/ml

Among the extractive pesf showed the most potent lethality with value of 6.79 µg/ml. The $lc_{50}$ values of me, petsf, csf, and aqsf were found to be 4.4 µg/ml, 1.87 µg/ml, 3.69 and 4.33 µg/ml, respectively

**Table 3.11: $LC_{50}$ values of the test samples of whole plants of *Cissus Adnata***

| Test samples | Regression line | $R^2$ | $LC_{50}$ (µg/ml) |
|---|---|---|---|
| VS | y = 30.799x + 60.653 | 0.973 | 0.45 |
| ME | Y = 7.4545x +17.091 | 0.8533 | 4.4 |
| PESF | Y =6.8182x + 37.273 | 0.6114 | 1.87 |
| DCMSF | Y= 11.455x – 27.818 | 0.9439 | 6.79 |
| CSF | Y =9.8182x +13.818 | 0.8022 | 3.69 |
| AQSF | Y = 8.7273x + 12.182 | 0.8845 | 4.33 |

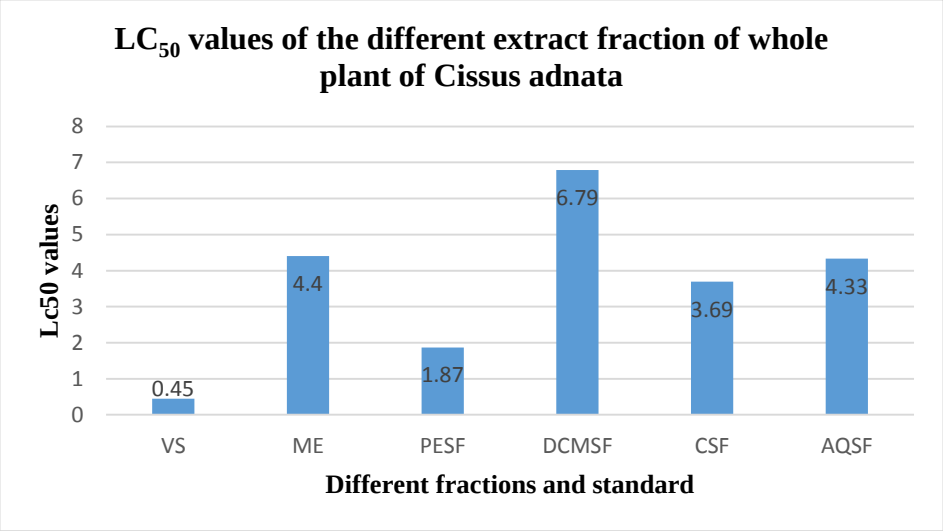



**Figure 3.10: Graphical representation of $LC_{50}$ values of the different extract fraction of whole plant of *Cissus adnata***

**Table 3.12: Effect of Vincristine sulfate (positive control) on shrimp nauplii**

| Conc. (µg/mL) | $Log_{10}$ Conc. | % Mortality | $LC_{50}$ (µg/mL) |
|---|---|---|---|
| 0 | - | 0 | 0.45 |
| 0.039 | -1.4089 | 20 | |
| 0.078125 | -1.1072 | 30 | |
| 0.15625 | -0.8061 | 30 | |
| 0.3125 | -0.5051 | 40 | |
| 0.625 | -0.2014 | 50 | |
| 1.25 | 0.09691 | 70 | |
| 2.5 | 0.39794 | 80 | |
| 5 | 0.6989 | 80 | |
| 10 | 1.00 | 90 | |
| 20 | 1.30102 | 100 | |

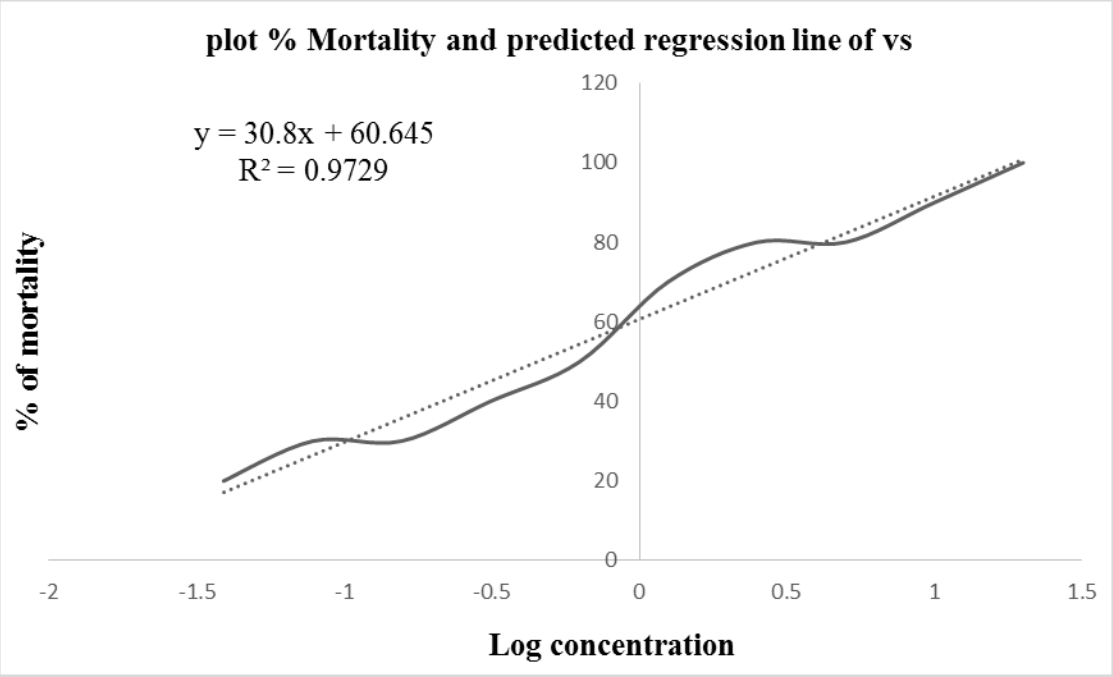



**Figure 3.11: Graphical representation of Plot of % mortality and predicted regression line of VS**

**Table 3.13: Effect of the methanolic extract (ME) of whole plant of *Cissus adnata* shrimp naupli**

| Conc. (µg/mL) | $Log_{10\ conc.}$ | % of mortality | $LC_{50}$ |
|---|---|---|---|
| 0 | - | 0 | 4.4 |
| 0.78125 | -1.1072 | 40 | |
| 1.5625 | 0.19382 | 50 | |
| 3.125 | 0.49485 | 60 | |
| 6.25 | 0.79588 | 60 | |
| 12.5 | 1.09691 | 60 | |
| 25 | 1.39794 | 70 | |
| 50 | 1.69897 | 70 | |
| 100 | 2 | 80 | |
| 200 | 2.30103 | 90 | |
| 400 | 2.60206 | 100 | |

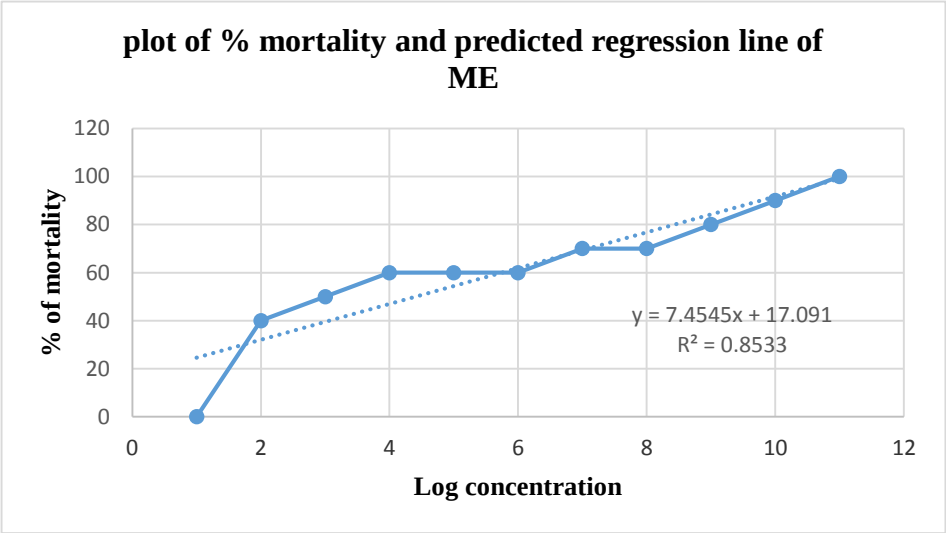



**Figure 3.12: Graphical representation of Plot of % mortality and predicted regression line of ME**

**Table 3.14: Effect of the petroleum extract (PESF) of whole plant of *Cissus adnata* shrimp nauplii**

| Conc. (µg/mL) | $Log_{10}$ conc. | % of mortality | $LC_{50}$ |
|---|---|---|---|
| 0 | - | 0 | 1.87 |
| 0.78125 | -1.1072 | 60 | |
| 1.5625 | 0.19382 | 80 | |
| 3.125 | 0.49485 | 70 | |
| 6.25 | 0.79588 | 80 | |
| 12.5 | 1.09691 | 90 | |
| 25 | 1.39794 | 90 | |
| 50 | 1.69897 | 100 | |
| 100 | 2 | 100 | |
| 200 | 2.30103 | 90 | |
| 400 | 2.60206 | 100 | |

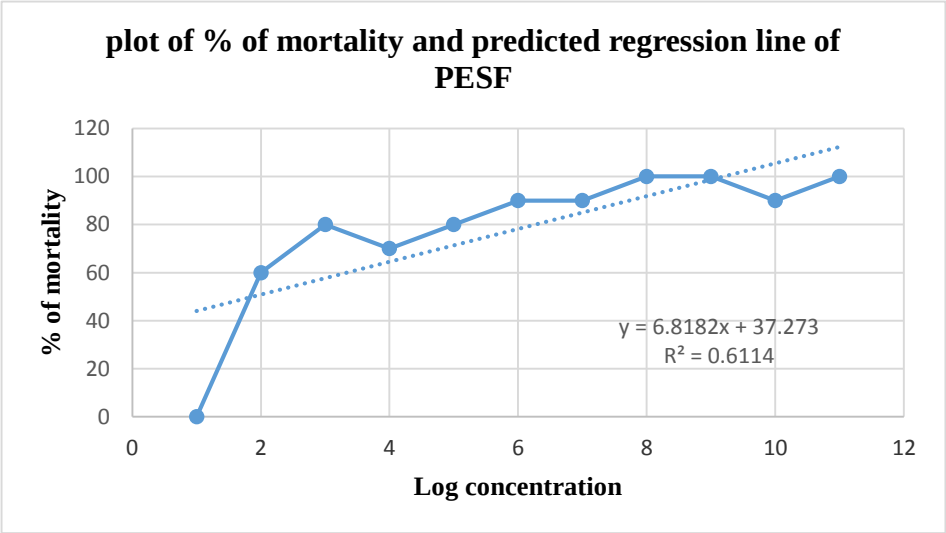



**Figure 3.13: Graphical representation of Plot of % mortality and predicted regression line of PESF**

**Table 3.15: Effect of the dichloromethane extract (DCMSF) of whole plant of *Cissus adnata* shrimp nauplii**

| Conc. (µg/mL) | $Log_{10\ conc.}$ | % of mortality | $LC_{50}$ |
|---|---|---|---|
| 0 | - | 0 | 6.79 |
| 0.78125 | -1.1072 | 0 | |
| 1.5625 | 0.19382 | 0 | |
| 3.125 | 0.49485 | 0 | |
| 6.25 | 0.79588 | 20 | |
| 12.5 | 1.09691 | 40 | |
| 25 | 1.39794 | 60 | |
| 50 | 1.69897 | 60 | |
| 100 | 2 | 80 | |
| 200 | 2.30103 | 90 | |
| 400 | 2.60206 | 100 | |

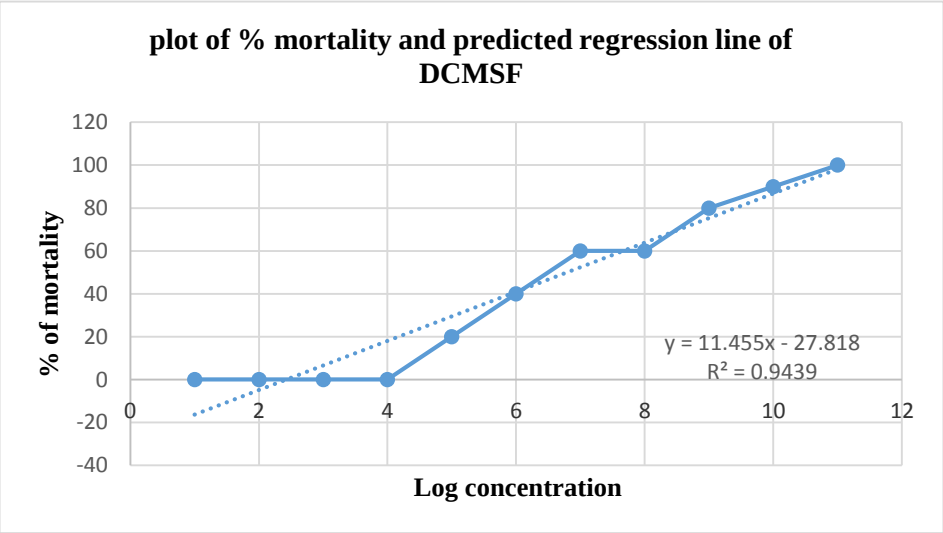



**Figure 3.14: Graphical representation of Plot of % mortality and predicted regression line of DCMSF**

**Table 3.16: Effect of the chloroform extract (CSF) of whole plant of *Cissus adnata* Shrimp nauplii**

| Conc. (µg/mL) | $Log_{10\ conc.}$ | % of mortality | $LC_{50}$ |
|---|---|---|---|
| 0 | - | 0 | 3.69 |
| 0.78125 | -1.1072 | 20 | |
| 1.5625 | 0.19382 | 40 | |
| 3.125 | 0.49485 | 70 | |
| 6.25 | 0.79588 | 80 | |
| 12.5 | 1.09691 | 90 | |
| 25 | 1.39794 | 100 | |
| 50 | 1.69897 | 100 | |
| 100 | 2 | 100 | |
| 200 | 2.30103 | 100 | |
| 400 | 2.60206 | 100 | |

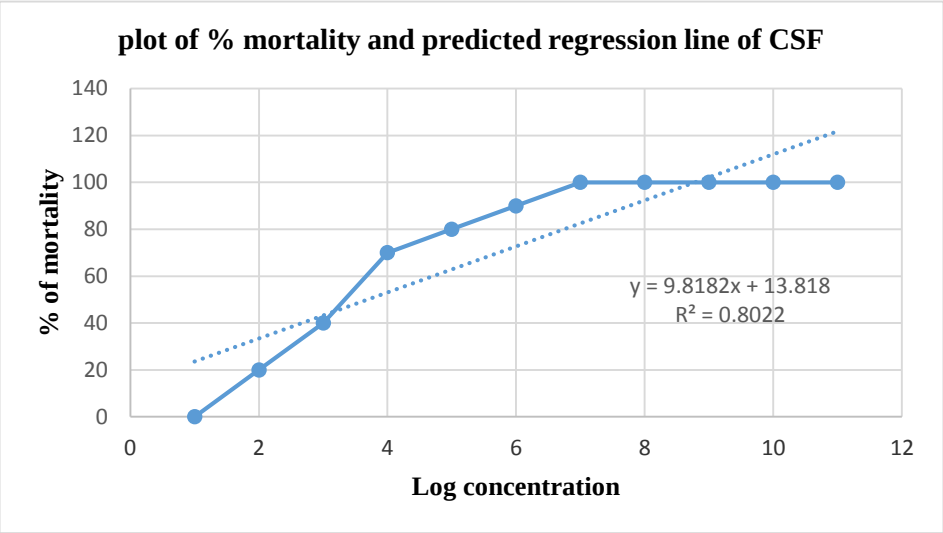



**Figure 3.15: Graphical representation of Plot of % mortality and predicted regression line of CSF**

**Table 3.17: Effect of the aqueous extract (AQSF) of whole plant of *Cissus adnata* Shrimp nauplii**

| Conc. (µg/mL) | $Log_{10\ conc.}$ | % of mortality | $LC_{50}$ |
|---|---|---|---|
| 0 | - | 0 | 4.33 |
| 0.78125 | -1.1072 | 30 | |
| 1.5625 | 0.19382 | 40 | |
| 3.125 | 0.49485 | 60 | |
| 6.25 | 0.79588 | 70 | |
| 12.5 | 1.09691 | 70 | |
| 25 | 1.39794 | 80 | |
| 50 | 1.69897 | 70 | |
| 100 | 2 | 90 | |
| 200 | 2.30103 | 100 | |
| 400 | 2.60206 | 100 | |

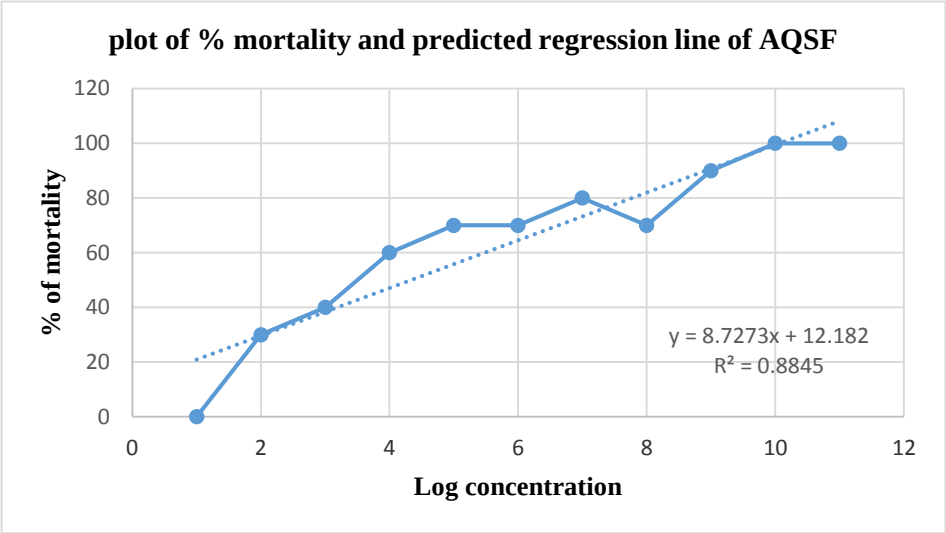



**Figure 3.16: Graphical representation of Plot of % mortality and predicted regression line of AQSF**

## 3.5Membrane Stabilizing Activity Investigation

### 3.5.1Results and discussion of Heat induced haemolysis

The different concentrates of whole plant of *Cissus Adnata* at fixation 1.0 mg/mL were tried to assess the movement against lysis of human erythrocyte film prompted by warmth, when contrasted with the standard acetyl salicylic corrosive (0.10 mg/mL) (Table-8.3). At a grouping of 1.0 mg/mL and in warmth incited condition the petroleum ether soluble fraction portion restrained most potent rate of haemolysis of RBC which is around 51.10% when contrasted with 42.20% hindered by acetyl salicylic corrosive (0.10 mg/mL).

**Table 3.18: Effect of different extractives of leaves of *Cissus Adnata* on heat induced haemolysis of erythrocyte membrane**

| Sample code | Concentration | % inhibition of haemolysis (Heat induced) |
|---|---|---|
| ME | 1 mg/mL | 67.17 |
| PESF | 1 mg/mL | 351 |
| DCMSF | 1 mg/mL | 76.7 |
| CSF | 1 mg/mL | 30.9 |
| AQSF | 1 mg/mL | 10.12 |
| ASA | 0.10 mg/mL | 42.20 |

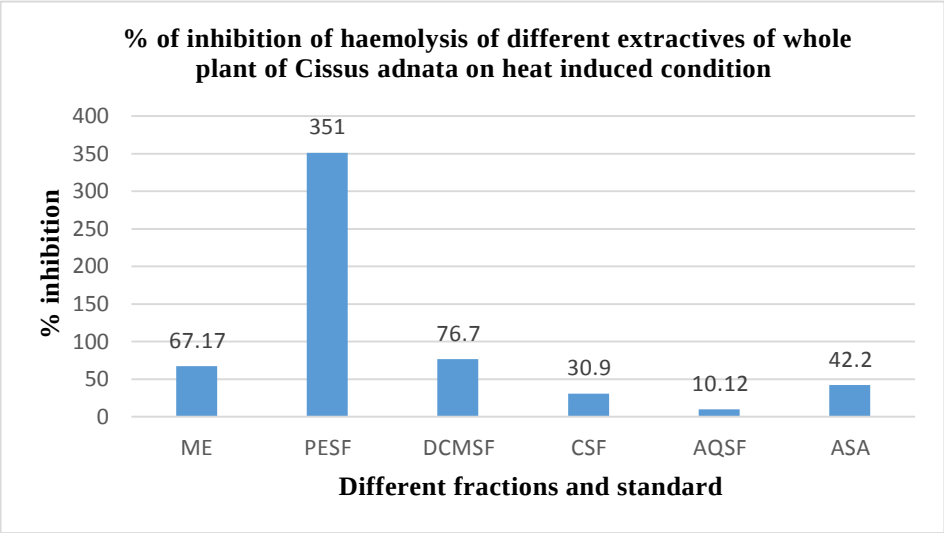



**Figure 3.17: Graphical representation of % inhibition of haemolysis of different extractives of whole plant of *cissus adnata* on heat induced condition.**

## 3.6 Results and discussion of In vitro antimicrobial screening

The methanolic separate (ME) of whole plant of *Cissus adnata* and its diverse partitionates i.e. Petroleum ether (PESF), dichloromethane(DCMSF), chloroform (CSF) and aqueous fraction (AQSF) dissolvable portions of whole plant of *Cissus adnata* were subjected to antimicrobial screening with a convergence of 400 µg/circle for each situation. Among the extractives, ME, PESF, DCMSF, CSF displayed serious antimicrobial movement and AQSF indicated gentle to direct action against various tried microorganisms. The outcomes are given in the table 5.4.

The methalonic extract solvent division displayed the most astounding hindrance against microbial development having zone of restraint zone from 36.0±0.3 mm to 40.0 mm. The most extreme zone of hindrance created by me was observed to be 40.0±0.9 mm against escherichia *coli*, *vibrio mimicus*, *vibrio parahemolyticus*, *sacharomyces cerevacae*. This partitionate additionally indicated direct antifungal movement against *candida albicans* (having zone of restraint of 37±0.8mm) and sacharomyces cerevacae (having zone of hindrance of 40±0.2mm) and antibacterial action against s. Aureus (35.0±0.1 mm), s. Lutea (35.0±0.5 mm), s. Typhi (37.0 ±0.4mm), v. Parahemolyticus (40.0±1.3 mm), b. Cereus (36.0±0.3 mm), b. Megaterium (36.0±0.8 mm), e. Coli (40.0±0.9mm), *bacillus subtilis*(38±0.1mm), *pseudomonas aeruginosa*(35±0.6mm),*salmonella paratyphi*(36±0.7mm), *shigella boydii*( 36±1.1mm) *shigella dysenteriae*(37±0.7mm)(table-3.19).

So only ME shows the significant zone of inhibition against microbial growth. Other than that no other solvent extract shows the minimum zone of inhibition against microbial growth.

**Table 3.19: Antimicrobial activity of test samples of bark of *Cissus Adnata***

| Test microorganisms | Diameter of zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | **ME** | **PESF** | **DCMSF** | **CSF** | **AQSF** | **Kanamycin** |
| **Gram positive bacteria** | | | | | | |
| *Bacillus cereus* | 36±0.3 | 0 | - | - | - | 37.6 ±1 |
| *Bacillus megaterium* | 36±0.8 | - | - | - | - | 38.3±1.1 |
| *Bacillus subtilis* | 38±0.1 | - | - | 7±0.5 | - | 35.0±0.6 |
| *Staphylococcus aureus* | 35±0.1 | - | - | - | - | 35.0±0.8 |
| *Sarcina lutea* | 35±0.5 | - | - | - | - | 37.3±0.9 |
| **Gram negative bacteria** | | | | | | |
| *Escherichia coli* | 40±0.9 | - | 10±1.1 | - | - | 37.0±0.2 |
| *Pseudomonas aeruginosa* | 35±0.6 | - | - | - | - | 35.6±0.4 |
| *Salmonella paratyphi* | 36±0.7 | - | - | - | - | 35.1±0.3 |
| *Salmonella typhi* | 37±0.4 | - | 13±0.7 | 12±0.9 | - | 37.3±0.5 |
| *Shigella boydii* | 36±1.1 | - | - | 7±0.2 | - | 38.0±0.7 |
| *Shigella dysenteriae* | 37±0.7 | - | - | - | - | 35.6±0.6 |
| *Vibrio mimicus* | 40±0.2 | - | - | - | - | 36.0±1.3 |
| *Vibrio parahemolyticus* | 40±1.3 | - | - | - | - | 37.1±0.8 |
| **Fungi** | | | | | | |
| *Candida albicans* | 37±0.8 | - | - | - | - | 38.3±0.9 |
| *Aspergillus niger* | 35±0.5 | - | - | - | - | 37.0±0.5 |
| *Sacharomyces cerevacae* | 40±0.2 | - | - | - | - | 38.6±0.2 |

# Chapter Four: Conclusion

The whole plant of *Cissus adnata* was used for the investigation to find out the different biological activity. The methanolic extract of the plant was fractioned by petroleum ether, dichloromethane, and chloroform. In the thrombolytic study some of the solvent showed the good % of clot lysis establishing good thrombolytic activity. Brine shrimp lethality bioassay showed good cytotoxicity for some partitionates. In the heat induced haemolysis of membrane stabilizing study inhibition of haemolysis of some extractives were close enough to the standard value. So the plant can be effective as an anti-inflammatory agent. Antimicrobial study was done with the extractives and only methalonic extract showed the significant antimicrobial activity against different gram positive and gram negative bacteria. This plant is used traditionally in some region of Asia but its usage is not wide spread although there was no scientific basis of the use of this plant as healing agent. This primary data can provide the proof of the plant's effectiveness in treating different maladies, and can be used by the researchers to discover potential drugs which may be effective against various diseases.

# Reference


Alzoreky, N.S., Nakahara, K., (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int. J. Food Microbiol. 80(3): 223-230.

Ali Reza, A. H. M., Barua, Shuvashish, P., Huq, S. M. Imamul, Khan, A. S., Moniruzzaman, and Nishat, Ainun. (2002). Bio-ecological zones of Bangladesh. *IUCN Publication*. ISBN: 984-31-1090-0

Ali, M., (2010). Textbook of Pharmacognosy. 2nd edition. CBS publishers and distributors. Page 20.

Assob, J.C., Kamga HL, Nsagha DS, Njunda AL, Nde PF, Asongalem EA, Njouendou AJ, Sandjon B, Penlap V.B., (2011). Antimicrobial and toxicological activities of five medicinal plant species from Cameroon Traditional Medicine. BMC Complement. Altern. Med., 11: 70.

Atawodi S.E., Ameh D.A., Ibrahim S, Andrew J.N., Nzelibe H.C., Onyike E.O., Anigo K.M., Abu E.A., James D.B., Njoku G.C., Sallau A.B., (2002). Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. J. Ethnopharmacol., 79(2): 279-282.

Ayafor, J.F., 1972. Limonoids and phytol derivatives from Cedrelasinensis. Tetrahedron. 28, 9343.

Bantawa, P., Rai, R., (2009). Studies on ethnomedicinal plants used by traditional practitioner, Jhanki, Bijwa and Phedangma in Darjeeling Himalaya. Nat Prod RAd 8(5):537-541.

Barry, A.L., 1976. Principle & practice of Microbiology. 3rd Ed., Lea &Fabager, Philadelphia.

Baruah, D.B., R.N., Dash, M.R. Chaudhari and S.S. Kadam,. Plasminogen activators: A Due comparison. Vascular Pharmacol., 44: 1-9,2006.

Bayer, A.W., Kirby, W.M.M., Sherris J.C., and Turck, M., 1966. Antibiotic susceptibility Testing by a standardized single disc method. Am. J. Clin. Pathol. 45: 493-496.

Biousse, and Newman, N.J., Venous disease of the central nervous system. Seminars in Cardiovascular Diseases and Stroke, 4: 2-17'2004

Choi, H.Y., Jhun E.J., Lim B.O., Chung I.M., Kyung S.H. and Park D.K., 2000. Application of flow injection-chemilumineacence to the study of radical scavenging Activity in plants. Phytother Res. 14, 250-253.

Dalzeil, J., (1958). Flora of West Tropical Africa, a Crown Agent for Oversea Publishing.

Ezzatzadeh, E., Farjam, M.H., Rustaiyan A, 2012. Comparative evaluation of antioxidant and antimicrobial activity of crude extract and secondary metabolites isolated from *Artemisia kulbadica*. Asian Pac J Trop Dis, 2:S431-S434.

Fauci, A., 1998. New and reemerging diseases: The importance of biomedical research. Flora & Fauna, Indian Medicinal Plant Article.

Gesler, W.M., Therapeutic landscape: medicinal issue in light of the new cultural Geography. SocSci Med, 34, 735-46.1992

Ghani, A., (1998). Medicinal plants of Bangladesh: Chemical constituents and uses. Dhaka: Asiatic society of Bangladesh. 2nd edition. Page 184.

Gortzi, O., Lalas, S., Chinou, I., Tsaknis, J., 2008. Reevalution of bioactivity and antioxidant activity of *Myrtuscommunis*extract before and after encapsulation in liposomes. Eur. Food Res. Technol 226: 583-590.

Harbertson, J., and Spayd, S., 2006. Measuring phenolics in the winery. Am. J. Enol. Vitic. 57, 280-288.

Harbore, J.B., (1998). Phytochemical methods. 3rd edition. Published by Chapman and Hall. Page 01.

Irvine, F.R., (1961). Woody Plants of Ghana, Oxford University Press, pp. 300-301.

Jin-Mang, K., Ngoh-Khang, G., Lian-Sai, C, Tei-Fatt, C., 2003. Recent advances in traditional plant drugs and orchids. ActaPharmacol, Sin. 24:7-21

Kone, W.M., Atindehou, K.K., Terreaux, C., Hostettmann, K., Traore, D., Dosso, M., (2004). Traditional medicine in north Cote-d'Ivoire: screening of 50 medicinal plants for antibacterial

activity. J. Ethnopharmacol., monitoring the proliferation effects of cissuspopulnea extracts on Sertoli cells. Reprod. Biol. Endocrinol., 9: 65.

Kurian, A., and Shankar. A.M., (2007). Volume 2. Medicinal plants. New india publishing Agency. Chapter 1

Lans, C.A., (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J. Ethnobiol. Ethnomed., 2: 45.

Latifou, L., Eugenie, A., Menonve, A., Brice, A., Karim, D., and Ambaliou, S., (2011). Antimicrobial activity of *Crataevareligiosa* Frost against bacteria isolated from *Thryonomysswinderianus*Remminck. African Journal of Biotechnology, 10(49), 10034-10039. ISSN 1684-5315

Lassak, E.V., McCarthy, T., (1997). Australian Medicinal Plants. Reed Books, Victoria, Australia, P. 230.

Line-Edwige, M., Raymond, F.G., Francois, E., Edouard, N.E., (2009). Antiproliferative effect of alcoholic extracts of some Gabonese medicinal plants on human colonic cancer cells. Afr. J. Tradit. Complement. Altern. Med., 6(2): 112-117.

Maclaughlin, J. L., Anderson, J. E., Rogers, and Lingling, L., 1998. Drug Info Journal, 32: 513-524.

Mahboubi, M., Haghi, G., Kazempour, N., Hatemi, A.R., 2013. Total phenolic content, antioxidant and antimicrobial activites of *Blepharisedulis*extract, Songklanakarin J SciTechnol 35:11-16

Majhenik, et, al., 2007. Antioxidant and antimicrobial activity of guarana seed extracts, Food chemistry. 10, 1016.

Meyer, B.N., Ferringni, N.R., Puam, J.E., Lacobsen, L.B., Nichols, D.E., 1982. Drug Info Journal, 31, 516-554.

Mohammad, A.A., Khalil, A.A., El-Beltagi, H., 2010. Antioxidant and antimicrobial properties of kaff Maryam (*Anastaticahierochuntica*) and doum palm (*Hyphaenethebaica*) GrasasAceites 61: 67-75.

Motaleb, A. M., (2011). Selected Medicinal Plants of Chittagong Hill Tracts. IUCN (International Union for Conservation of Nature) Bangladesh Country Office.

Mothana, R.A., Mentel, R., Reiss, C., Lindequist, U., (2006). Phytochemical screening and antiviral activity of some medicinal plants from the island Soqotra. Phytother. Res., 20(4): 298-302.

Oben, J., Kuate, D., Agbor, G., Momo, C., Talla, X., (2006). The use of a Cissusquadrangularis formulation in the management of weight loss and metabolic syndrome. Lipids Health Dis., 5: 24.

Oleski, A., Lindequist, U., Mothana, R.A., Melzig, M.F., (2006). Screening of selected Arabian medicinal plant extracts for inhibitory activity against peptidases. Pharmazie, 61(4): 359-361.

Osibote, E., Noah, N., Sadik. O., McGee, D., Ogunlesi, M., (2011). Electrochemical sensors, MTT and immunofluorescence assays for monitoring the proliferation effects of cissuspopulnea extracts on Sertoli cells. Reprod. Biol. Endocrinol., 9: 65.

Ojekale, A.B., Lawal, O.A., Lasisi, A.K., Adeleke, T.I., (2006). Phytochemisty and spermatogenic potentials of aqueous extract of Cissuspopulnea (Guill. and per) stem bark. Sci. World J., 6: 2140-2146.

Ojekale, A.B., Ojiako, O.A., Saibu, G.M., Lala, A., Olodude, O.A., (2007). Long term effects of aqueous stem bark extract of Cissuspopulnea (Guill. and Per.) on some biochemical parameters in normal rabbits. Afr. J. Biotechnol., 6(3): 247-251.

Onyechi, U.A., Judd, P.A., Ellis, P.R., (1998). African plant foods rich in nonstarch polysaccharides reduce postprandial blood glucose and insulin concentrations in healthy human subjects. Br. J. Nutr., 80(5): 419- 428

Otshudi, A.L., Foriers, A., Vercruysse, A., Van, Zeebroeck, A., Lauwers, S., (2000). In vitro antimicrobial activity of six medicinal plants traditionally used for the treatment of dysentery and diarrhoea in Democratic Republic of Congo (DRC). Phytomedicine, 7(2): 167-172.

Osawa, T., 1994. Novel natural antioxidants for utilization in food and biological systems.

Omale, J., and. Okafor, P.N., (2008). Comparative antioxidant capacity, membrane.

Pinner, R., Teutsch, S., Simonsen, L., Klug, L., Graber, J., Clarke, M., Berkelman, R., 1996.

Pietta, A., Sionetti, P. and Mauri, P., 1998. Antioxidant activity of selected medicinal plants. J Agric Food Chem. 46, 4487-4490.

Rafieian-Kopaei, M., (2012). Medicinal plants and the human needs. J HerbMedPlarmacol. 1(1): 1–2.

Ronald, R., 1982. Antbiotics-An Introduction, F. Hoffman La Roche & Co. Basle, Switzerland: 70-71

Salgado, JM, Mansi D.N., Gagliardi, A., (2009). Cissussicyoides: Analysis of glycemic control in diabetic rats through biomarkers. J. Med. Food, 12(4): 722-727.

Shahidi, F., Janitha, P.K., and Wanasundara, P.D., 1992. Phenolic antioxidants. Critical Reviews of Food Science & Nutrition, 32, 67–103.

Skerget, M., Kotnik, P., Hadolin, M., Hras, A., Simonic, M. and Knez, Z., 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant Activities. Food chemistry, 89, 191-198.

Shinde, J., Taldone, T., Barletta, M., Kunaparaju, N., Hu B., Kuma, S., Placido, J., Zito, S.W., 2008. Alpha-glucosidase inhibitory activity of Syzygiumcumini (Linn.) Skeels seed kernel in vitro and in Goto-Kakizaki (GK) rats.Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439, United States.

Trends in infectious diseases mortality in the United States- J. Am. Med. Assoc. 275:189–193.

Udupa, K.N., Prasad, G.C., (1962). Cissusquadrangularis in healing of fractures. A clinical study. J. Indian Med. Assoc., 38: 590-593.

Van Wagenen, B.C., Larsen, R., Cardellina, J.H. II, Ran dazzo, D., Lidert, Z.C. and Swithenbank, C., (1993). Ulosantoin, a potent insecticide from the sponge Ulosaruetzleri. J. Org. Chem. 58, 335-337.

Velioglu, Y.S., Mazza, G., Gao, Y.L. and Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem. 46, 4113- 4117.

Watson, R.D., B.S. Chin and G.Y. Lip. . Antithrombotic therapy in acute coronary Syndromes. B. Med. J., 325: 1348-1351, 2002.

Wijesekera, R.O.B. (1991). 1st edition. The medicinal plant industry. Published by CRC press. Chapter 04

Yashasvi, B., (2016). Vitaceae: Characters. Biologydiscussion.com

Yang, L.C., Wang, F., Liu, M., (1998). A study of an endothelin antagonist from a Chinese anti-snake venom medicinal herb. J. Cardiovasc. Pharmacol., 31 (Suppl 1): S249-250.