# Phytochemical Investigation and Antioxidant Properties of Petrolium Ether Fruit Extract of *Ampelocissus barbata* (Vitaceae)

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# DEDICATION

**Dedicated To**

**My**

**Reverend Parents**

**Dhaka International University**

**Department of Pharmacy**

# CERTIFICATE

This is to certify that the thesis entitled **"Phytochemical Investigation and Antioxidant Properties of Petrolium Ether Fruit Extract of *Ampelocissus barbata* (Vitaceae)"** is an original research work carried out for the partial fulfillment of the requirement for the Bachelor of Pharmacy (B. Pharm). None of the parts of this work has been submitted for any other degrees elsewhere. This original research work has been carried out and completed by Badrunnahar Binty, Registration No. PH-24-19-111215, Session: 2019-2020 (24$^{th}$ batch) under my supervision.

**Approved as to the style and content.**

**Supervisor**

Shahenul Islam

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# Approval

This is to certify that the project work has been submitted by Badrunnahar Binty, entitled **"Phytochemical Investigation and Antioxidant Properties of Petrolium Ether Fruit Extract of *Ampelocissus barbata* (Vitaceae)"** has been authorized by the Examination Committee for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.) in the Department of Pharmacy, Dhaka International University, Bangladesh.

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The Author

# ABSTRACT

This dissertation describes the biological investigations of *Ampelocissus barbata*, a plant belonging to the family Vitaceae. The fruits of the *Ampelocissus barbata* were extracted with methanol. The fraction of extract of Pet ether from *Ampelocissus barbata* fruits extract was used for the observation of Phytochemical evaluation, Antioxidant Activity Test. Phytochemical Test such as; Reducing sugar (Benedict's), Reducing sugar (Fehling's), Combined reducing sugar, Tannins (Ferric Chloride), Tannins (Potassium dichromate), Flavonoids, Saponin, Carbohydrates & Gums, Alkaloids (Dragendroff's), Glycoside, Proteins & Xanthoprotein. According to the Phytochemical study of the Fraction of extract of Pet ether from *Ampelocissus barbata*, the presence Tannins (Ferric Chloride) & Proteins & Xanthoprotein was revealed. The Antioxidant Tests such as; DPPH Scavenging Assay, Total Phenolic Content, Nitric Oxide (No) Scavenging Assay, Total Flavonoid Content, Total Tannin Content, Hydrogen Peroxide Scavenging Assay & Hydroxyl Radical Scavenging Assay. Antioxidant Test was revealed that the Fraction of extract of Pet ether from Ampelocissus barbata, extract exhibited high Total Phenolic Content and low Tannin Content and Flavonoid Content.

Total Tannin, Phenol and Flavonoid Content of fraction of extract of Pet ether from *Ampelocissus barbata* in Fruits Extract were found 21.36 mg GAE/g, 1817.5 mg GAE/g (gallic acid equivalent) and 3.5079 mg QE/g (quercetin equivalent) respectively. DPPH Scavenging Assay, Nitric Oxide (No) Scavenging Assay, Hydrogen Peroxide Scavenging Assay & Hydroxyl Radical Scavenging Assay were found $IC_{50}$ 23.87 µg/ml, 24.4608 µg/ml, $SC_{50}$ 29.2 µg/ml and $SC_{50}$ 39.17 µg/ml.

The results suggest that the extract contains a diverse range of phytochemicals with potential antioxidant properties. As the plant remains an integral part in terms of the discovery of medicine thus the aim of the current work was to explore the Phytochemical profiles of the plant *Ampelocissus barbata* for Antioxidant activity.

# Chapter One
# Introduction

## 1.1 Overview :

The practice of diagnosis, the promotion of health, and the treatment and prevention of disease are all included in the definition of medicine as the science of healing [1] . It also refers to plant-based compounds, pharmaceuticals, and drugs that are used to treat a variety of illnesses and advance good health. According to the medical dictionary, medicine is both a drug and a method of illness prevention. Additionally, it is described as the investigation and management of general disorders or those affecting the body, particularly those that typically do not require surgical intervention [2] . In the 21st century, people are totally depended on medicine for various kinds of lifestyle diseases. There are different kinds of medical field such as Allopathy, Ayurveda and Homeopathy currently in from all over the world. People depend on those medical fields, according to their beliefs. Medicine is not a pure science, but is a science of probability. The progress of medicine mainly focuses on the science of healing and the development of science in the medical field. Its history is from prehistoric times to at present.

According to Gillian R Bentley medicines can be classified as [3]

a) Traditional medicine.

b) Modern medicine.

Traditional medicines are purely based on a natural way of treatment for all illness. These medicines are derived from plants and animals, besides that they are not usually processed. Modern medicines are being spread on biomedical science, genetics and medical technology, which are used to diagnose, treat and prevent injury and illness. One of the main medical processes in modern medicines is surgery. There are many new biological treatments being developed by using medicine and opened a new career for both women and men as nurses/compounders and physicians. These advances along with the growth in chemistry, genetics and lab technology led to modern medicine. [3]

## 1.2 History of Medicine:

The invention of medicine is undoubtedly considered the most precious and beneficial in human civilization. The history of medicine shows a remarkable journey of how we humans have approached different illnesses and diseases from the early periods till date. Some of the early medicine traditions come from Babylon, China, Egypt, and India. Though, there isn't much record of when and how plants were used as a healing agent. But, from ancient drawings discovered worldwide, it is believed that early humans used medicinal plants as healing agents [4].

### Prehistoric Medicine

Early humans were unaware of the various diseases and their medications. Early humans used the trial and error method to discover the medicinal benefits of plants and herbs. They considered common cold and constipation as a part of human existence and treated it with various herbs. The unidentified diseases were believed to be supernatural or cast of spells. There's a common belief that in the world, the first doctors were sorcerers and magicians [5].

Magic and religious prayers played an essential role in prehistoric medicine. In ancient Mesopotamia, the people were not able to distinguish between magic and medicine. And if a person suffering from illness came to a doctor, the doctors would prescribe medicinal treatment and magical words to be recited. In the ancient era, the Babylonians, along with Egyptians, implemented diagnosis, physical examinations, and treatments. Early Egyptians were considered the healthiest with a notable healthcare system. [6]

## Traditional Medicine in India, China, and Japan

The Indian civilization was well developed in medicine with herbal treatments. The Atharvaveda, which belongs to the early iron age, throws light on the fact that early Indians used medications and ailments from herbs and other medicinal plants. [7]

The period from 800 BCE till 1000 BCE is considered the golden age of medicine in India with the introduction of medical treaties by Chakra, a physician, and Sushruta, a surgeon of ancient India. The knowledge of anatomy in ancient India was minimal as the Hindus were not allowed to cut dead bodies. The Indian physicians used all five senses in the diagnosis of diseases. The Indians were known to have identified around 700-800 medicinal plants in ancient times. They were also known for using animal parts for remedies. [8]

On the other hand, Chinese medicine was of great importance in history. The Chinese considered the human body to be made up of five elements: wood, fire, earth, metal, and water. As the Chinese's religious beliefs forbade them from tearing the dead bodies, therefore their anatomy knowledge was based on assumptions. According to the ancient Chinese Anatomy, the body contained five organs: heart, lungs, liver, spleen, and kidney, and the blood vessels contained blood and air. [8]

Japanese medicine, on the other hand, is considered interesting for its slow start and rapid modernization. Japanese medicine took a turn in 608 CE when few Japanese physicians were sent to China for study. The Chinese have a significant influence on the Japanese medical system. [9]

The oldest Japanese medical work dates back to 983 CE, which Tamba Yasuyori wrote. In his works, he has discussed different kinds of diseases and their treatment. These diseases and treatments are classified according to body parts. [9]

## Introduction

Speaking of ancient medicine, you must have thought of the world's first doctor. The first doctor in the world was from the Egyptian civilization. According to the medical information given in the Edwin Smith Papyrus, which dates back to 3000 BC, Imhotep is credited with being the first doctor in the world. [10]

## Modern Medicine

The Renaissance brought about a significant change in the history of medicine. The period between the 16th and 18th centuries was remarkable for medicine. This period saw a rapid increase in experimental investigations and advanced anatomy.

Even the first medicine was discovered during this period. Modern medicine started to emerge after the Industrial Revolution in the 18th century. At this time, there was rapid growth in economic activity in Western Europe and the Americas. Hippocrates is credited with being the man who invented medicine. He was a Greek physician who wrote the Hippocratic Corpus, a collection of seventy medical works. He is also accredited with the invention of the Hippocratic Oath for physicians. [11]

During the 19th century, economic and industrial growth continued to develop, and people made many scientific discoveries and inventions.

Scientists made rapid progress in identifying and preventing illnesses and in understanding how bacteria and viruses work.

However, they still had a long way to go regarding the treatment and cures of infectious diseases. [11]

## 1.3 Medicinal plant

Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. The annual global export value of the thousands of types of plants with suspected medicinal properties was estimated to be US$2.2 billion in 2012. In 2017, the potential global market for botanical extracts and medicines was estimated at several hundred billion dollars [12]. Medicinal plants face both general threats, such as climate change and habitat destruction, and the specific threat of over-collection to meet market demand . Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety [12]

The earliest historical records of herbs are found from the Sumerian civilization, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from ancient Egypt, c. 1550 BC, describes over 850 plant medicines. The Greek physician dioscorides, who worked in the Roman army, documented over 1000 recipes for medicines using over 600 medicinal plants in De materia medica, c. 60 AD; this formed the basis of pharmacopoeias for some 1500 years. Drug research makes use of ethnobotany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes: alkaloids, glycosides, polyphenols, and terpenes. [13]

**Fig 1.1 :** Medicinal Plants

## 1.3.1 Characteristics of Medicinal Plants

Medicinal plants have many characteristics when used as a treatment, as follow:

**Synergic medicine :** The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

**Support of official medicine :** In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

**Preventive medicine :** It has been proven that the component of the plants also characterized by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. [14]

## 1.3.2 Basis of modern medicine

Botanical drugs which form the basis for herbal remedies or phytomedicines include, for example: the herb of St John's wort (Hypericum perforatum), used in the treatment of mild to moderate depression, the leaves of Ginkgo biloba, used for cognitive deficiencies (often in the elderly), including impairment of memory and affective symptoms such as anxiety, the flower heads of chamomile (Chamomilla recutita) used for mild gastrointestinal complaints and as an anti-inflammatory agent the leaves and pods of Senna (Cassia spp.) used for constipation. From the perspective of Pharmacognosy and rational phyto-therapy, such products lie alongside, and in some cases are, conventional pharmaceutical medicines. Herbal medicines are often considered to be part of complementary and alternative medicine (CAM), and the use of herbal medicine products (HMPs) and of CAM has increased across the developed world. [15]

Isolated compound from the plants are pure chemical entities, often used in the form of licensed medicines. They are sometimes produced synthetically and referred to as 'nature identical' (if that is the case), but were originally discovered from plant drugs. Examples include: morphine, from opium poppy (Papaver somniferum), used as an analgesic digoxin and other digitalis glycosides, from foxglove (Digitalis spp.), used to treat heart failure taxol, from the Pacific yew (Taxus brevifolia), used as an anticancer treatment quinine, from Cinchona bark (Cinchona spp.), used in the treatment of malaria galanthamine from Galanthus and Leucojum species, used in the management of cognitive disorders. [16]

## 1.3.3 Exploration of medicinal properties of plants

Since prehistoric times, the fields of botany and medicine have enjoyed an enduring and fruitful relationship. Whether in the sophisticated setting of a modern pharmaceutical laboratory or an herbalist's hut on the banks of the Amazon River, plants provide a critical source of treatments for the myriad diseases that afflict humans. In either setting, the principal challenge of the medical practitioner is to distinguish plants that possess pharmaceutical properties from those that are toxic or medicinally inert. Although methods used to screen the plant kingdom for bioactive compounds have changed considerably over the course of human history, the invaluable selection of cures and therapeutics available to modern medicine is the product of a long history of pharmaceutical experimentation. [16]

**The Past -** We can only conjecture as to when and where the search for herbal remedies began. Archeological remains dating back sixty thousand years reveal that Neanderthals laid their dead to rest with plants that later became staples of ancient pharmacopoeias, such as millefoil (Achillea), St. Barnaby's thistle (Centaurea), and joint fir (Ephedra). Whether these plants were actually used as medicines or simply served as a farewell gesture to the deceased may never be established with certainty. Preliterate cultures, whose lifestyles closely mirror those of our distant ancestors, maintain oral traditions of medical practice that depend primarily on native vegetation. By the time literacy developed into a basic means of human communication, the application of botanical lore to the practice of medicine was firmly established and systematized. Sumerian clay tablets and Egyptian papyri (2000 B.C.) describe

ancient prescriptions and pharmacopeias in considerable detail. Many of the plants that appear in these early records are now known to possess highly bioactive constituents, evidence for which can be found in our own medicine, cabinets, Codeine, derived from the opium poppy and used as a narcotic analgesic in Nyquil, appears in medical traditions that predate modern pharmacology by thousands of years. Similarly, salicylate (aspirin) was originally extracted from willow bark, and ephedrine, the flu remedy in Vicks was derived from Ephedra. Approximately twenty-five percent of all modern prescriptions contain natural plant extracts, most of which were used in traditional medicine Moreover; a significant number of synthetic medicines are derived from plant products whose therapeutic qualities have only recently been improved by chemical tinkering. [16]

**The Present -** over the course of the following two centuries, the science of medicine became more sophisticated and specialized than any Renaissance herbalist could have imagined. Indeed, recent developments in the use of laser beams, ultrasonic waves, and genetic engineering continue to challenge the imagination of modern innovators. Surprisingly, this rapid progress in the medical sciences has also reaffirmed the continuing relevance of botany to medicine. Technical innovations have accelerated the search for medicinal substances in natural products by providing increasingly simple and economical methods for screening massive quantities of plant samples. During the last few decades, discoveries of plant products with antitumor, anti-malarial, antibiotic, and immune stimulating properties have demonstrated that we are far from exhausting the medicinal potential of botanical resources. Since the establishment of NCI (National Cancer Institute) in 1937, modern methods of medical research have isolated a number of plant products that have been successful in treating different types of human cancers. Examples include the anti-leukemic agent vincristine, derived from a tropical periwinkle (Catharanthus roseus), and the ovarian cancer therapeutic, Taxol, derived from the yew plant of the Pacific Northwest (Taxus brevifolia). Pharmacologically active compounds like these are identified by exposing crude extracts of plant tissues to living cultures of cancerous or HIV infected cells. If an extract exhibits an effect on diseased cells, the active constituent of the sample is isolated, chemically characterized, and subjected to clinical analysis. Up to ten years of study and hundreds of millions of dollars are required to demonstrate that a promising chemical agent is effective,

safe to use, and economically producible. Since 1986 over 50,000 plant extractions have been screened at the NCI, of which fewer than ten have been identified as potentially useful drugs. (Cragg et al., 1995). Success rates have been particularly low in the search for antitumor agents. By comparison, the more recently initiated anti-AIDS research has been more promising: after only a decade, a number of potential anti-HIV compounds have been extracted from plants collected in distant continents (Ancistrocladus in Africa; Calophyllum in Malesia; Conospermum in Australia; Homolanthus in Samoa. All of these promising discoveries are presently under study by clinical physicians and toxicologists. Although modern medicine has yet to identify cures for AIDS, many cancers, and a host of other human maladies-arthritis, obesity, schizophrenia, parkinsonism, depression, to name just a few-potential pharmaceutical treatments for many of these conditions undoubtedly reside in the rich chemical diversity of the plant kingdom. [16]


**Fig 1.2 :** medicinal properties of plants

## 1.3.4 Role of medicinal plants in modern medicines

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs. Primitive peoples throughout the world made use of their indigenous flora as a source of medicines. Through the process of trial and error, these cultures examined and discovered many plants that produce unique molecular entities with valuable biological properties such as aspirin from willow bark (Salix alba), the anticancer alkaloid vincristine from the Madagascar periwinkle (Catharanthus roseus), and cardiac glycoside digitoxin from Digitalis species. [17]

Galantamine is a natural product discovered through an ethnobotanical lead and first isolated from Galanthus woronowii L. (Amaryllidaceae) and approved for the treatment of Alzheimer's disease. Tiotroprium is an inhaled anticholinergic bronchodilator, based on ipratropium, a derivative of atropine that has been isolated from Atropa belladonna L. (Solanaceae) and recommended for treatment of chronic obstructive pulmonary disease [17].

Exate can is an analog of camptothecin from Camptotheca acuminate Decne. (Nyssaceae) and developed as an anticancer agent. Galegine, an active anti-hyperglycemic agent isolated from the plant Galega officinalis L. provided the template for the synthesis of metformin and opened up interest in the synthesis of other biguanide-type ant diabetic drugs. [17]

## 1.4 The Importance of Medicinal Plants

It is estimated that 80 % of the people in Pakistan depend on plants to cure themselves, a 40% in China. In technologically advanced countries as the United States, it is estimated that 60 % of the population use medicinal plants habitually to fight certain ailments. In Japan there is more demand of medicinal plants than of "official" medicines. Modern medicine, through clinical tests, has been able to validate those plants that the tradition had used with the method of test and error. Many turned out to be been worth; others demonstrated to be innocuous; others, potentially dangerous. Biochemical tests have been the ones that determined the main components of the medicinal plants- the active principles. The capacity of the modern chemical industry to produce these principles without the aid of the plants does not suppose deny the importance that these have and will still have in the future. [18]

Among the main arguments in defense of the medicinal plants we have to mention the

following:

**Synergic medicine:** It has been verified that in many cases the application of an isolated component has not had the wished effect, because it does not have the same curative power that it has when it is taken altogether with the rest of components, or because it has turned out to be toxic.

**Backup of formal medicine:** The treatment of very complex diseases can require in some cases the support of the medicinal properties of the plants or the derivatives that they provide. The importance of taxol, an obtained derivative of the yew of the Pacific (Taxus brevifolia)in the treatment of cancer and especially concerning breast cancer, has been approved by the same American F.D.

**Preventive medicine:** Finally, we do not have to forget the preventive character that the plants have regarding the appearance of diseases. In this sense the plants are better than the chemical remedies that are applied essentially when the disease has already appeared. It has been verified that natural food ingestion can prevent many pathologies. It has been admitted that the ingestion of vegetables with antioxidant properties, especially those that belong to the Brassicaceae group, like cabbages, radishes, etc. [19]

## 1.4.1 The medicinal plants of Bangladesh

South Asian countries have a large number of valuable medicinal plants naturally growing, mostly in fragile ecosystems that are predominantly inhibited by rural poor and indigenous community. In Bangladesh, 5,000species of angiosperm are reported to occur. The number of medicinal plants included in the “material medica” of traditional medicine in this subcontinent at present stands at about 2,000 More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh. Dhaka, Rajshahi, shylet and Chittagong division is rich in medicinal plants. [19]

## 1.5 Contribution of Medicinal Plants in Modern Medicine

The modern medicine refers to those medicinal preparations, which are scientifically by using modern technology and know how and which are in current use in modern pharmacopoeias for cure and management for disease.

**Table 1.1:** Plant derived medicinal substance occurring used in modern medicine. [20]

| Drugs/Chemical | Action/Clinical use | Plant source |
|---|---|---|
| Atropine | Anticholinergic | *Atropa belladonna* |
| Arecoline | Anthelmintic | *Areca catechu* |
| Berberine | Bacillary dysentry | *Berberis vulgaris* |
| Bergenin | Antitussive | *Berberis vulgaris* |
| Caffeine | CNS stimulant | *Camellia sinensis* |
| Camphor | Rubefacient | *Cinnamomum acemphor* |
| Camptothecin | Anticancerous | *Camtotheca acuminate* |
| Calanolide A<br>Calanollide B | AIDS | *Calophyllum lanigerum* |
| Codeine | Analgesic, sedative, antitussive | *Papaver somniferum Linn.* |
| Colchicine | Antiarthritic, antiinflammatory | *Colchicum autumnale Linn.* |
| Digitoxin, Digoxin | Cardiotonic | *Digitalis, purpura, Linn.*<br>*Digitalis lanata Her* |
| Diosgenin | Expectorant, anti-inflammatory | *Dioscorea spp* |
| Hyoscine<br>Hyoscyamine | Parasympatholytic, mydriatic,<br>Antispasmodic | *Datura, hyoscyamus, scopolia,*<br>*dubosia spp.* |
| Morphine | Sedative, narcotic, analgesic | *Papaver somniferum Linn.* |

## 1.6 Phytochemical Screening

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. Nearly 50% of drugs used in medicine are of plant origin, and only a small fraction of plants with medicinal activity has been assayed. There is therefore much current research devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them. The phytochemicals isolated are then screened for different types of biological activity (Harborne et. al., 1998). [21]

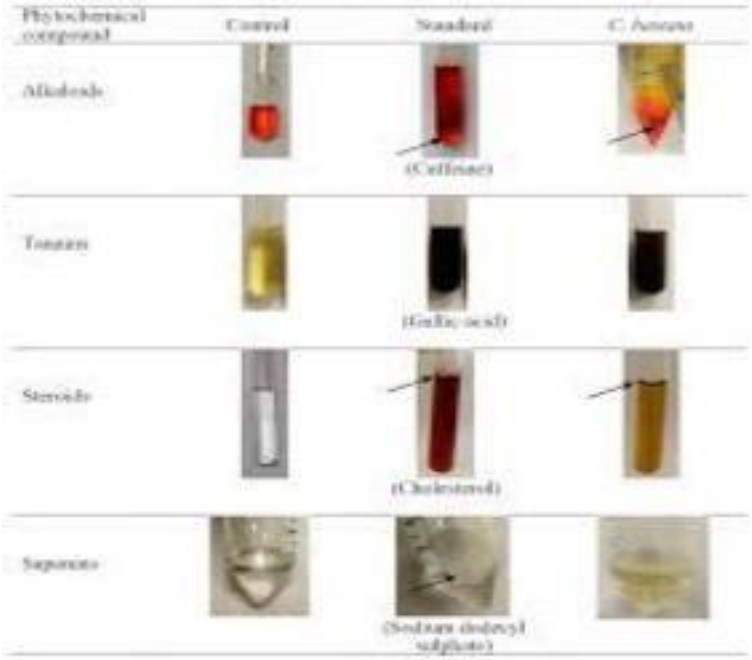


**Fig 1.3 :** Phytochemical Screening

## 1.7 Antioxidant Activity of Plant Extracts

Antioxidants are molecules that inhibit oxidation, a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. They neutralize free radicals by donating electrons, thus preventing them from causing oxidative damage to cells and tissues. Antioxidants are found in various foods, particularly fruits, vegetables, nuts, and seeds, and they are also synthesized by the body. Examples of antioxidants include vitamins C and E, beta-carotene, flavonoids, and polyphenols. Consuming antioxidant-rich foods is associated with numerous health benefits, including reduced risk of chronic diseases such as heart disease, cancer, and neurodegenerative disorders. [22]

The antioxidant activity of plant extracts is often attributed to their rich content of phytochemicals such as flavonoids, phenolic compounds, carotenoids, and vitamin C. These compounds can scavenge free radicals, neutralizing their damaging effects on cells and tissues. Various assays, like DPPH (2,2-diphenyl-1-picrylhydrazyl) or ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assays, are commonly used to measure the antioxidant capacity of plant extracts. [23]

## 1.8 Determination of Total Phenolic Content

Phenolic compounds or polyphenols are the secondary plant metabolites that are all over present in plants and plant products. Many of them are responsible for the reduction of risk of evolving chronic diseases (cardiovascular disease, cancer, diabetes, etc.), due to their antioxidant activities. Phenolic compounds pay to the overall antioxidant activities of plants mainly for their redox activities. Generally, the mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals. [24]

In the present study, the total phenolic content of ethanol extract of Ampelocissus barbata was determined by using Folin-Ciocalteu (FC) reagent with analytical grade Gallic acid as the standard (Marinova et al., 2005). Extract or standard solution (15.62-500 mg/L) of 1 ml was added to distilled water (9 mL). Then 1 ml FC reagent (10 times diluted with distilled

water) was added. After 5 minutes; 10 mL 7% Na2CO3 was added to the mixture. Then it is kept for 30 minutes at room temperature. Then absorbance was measured against blank at 750 nm using UV spectrophotometer. Total phenolic content of the extract was determined from the standard curve and expressed as mg Gallic acid equivalent (GAE)/100 g dried plant extract. (Javanmardi et al., 2003). [25]

## 1.9 Determination of Total Tannin Content

Tannin is natural organic biomolecule. It is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds. (Marinova et. al., 2005).

In the present study, the total tannin content of ethanol leaf extract of was determined by *Ampelocissus barbata* using Folin-Ciocalteu (FC) reagent with analytical grade Gallic acid as the standard. Total tannin content of the extract was determined from the standard curve and expressed as mg Gallic acid equivalent (GAE)/100 g dried plant extract. (Vernon et. al., 1999). [26]

## 1.10 Determination of Total Flavonoid Content

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavanols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, and antitumor and antioxidant activities. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species

results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). Flavonoids may help provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body.

In the present study, the total flavonoid content of ethanol leaf extract of Ampelocissus barbata was determined by analytical grade Quercetin as the standard. Total tannin content of the extract was determined from the standard curve and expressed as mg Quercetin equivalent (QE)/100 g dried plant extract. (Meda et. al., 2005). [27]

## 1.11 Determination of DPPH Scavenging Assay

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a popular, quick, easy, and affordable approach for the measurement of antioxidant properties that includes the use of the free radicals used for assessing the potential of substances to serve as hydrogen providers or free-radical scavengers (FRS).The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Contreras-Guzman and Srong 1982). [28]

DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, based on the measurement of the scavenging capacity of antioxidants towards it. [28]

The ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated using the following equation: The results based on the measurement of the scavenging capacity of antioxidants towards it.where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the sample.

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams et al., 1995. [29]

In the present study, the DPPH of ethanol leaf extract of Ampelocissus barbata was determined by 2.0 ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20µg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radicalby the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) by UV spectrophotometer. [29]

## 1.12 Determination of Nitric Oxide (No) Scavenging Assay

Nitric oxide, also known as nitrogen monoxide, is a molecule with chemical formula No. It is a free radical and is an important intermediate in the chemical industry Nitric oxide is a by-product of combustion of substances in the air, as in automobile engines, fossil fuel power plants, and is produced naturally during the electrical discharges of lightning in thunderstorms. Nitric oxide is a molecule that has a single nitrogen and oxygen atom. This gas is produced when an enzyme group known as Nitric oxide Synthase (NOS), breaks down L-arginine (an amino acid) into L-citrulline (Hou et al., 1999). [30]

Nitric oxide is a reactive gas and cause respiratory damage. Nitric acid and nitrous acid are formed with the presence of moisture in air and in the lungs. This is highly corrosive to the pulmonary system of the body.

NO is an important bio regulatory molecule, which has a number of physiological effects including control of blood pressure. neural signal transduction, platelet function, antimicrobial and antitumor activity. Low concentrations of No are sufficient. in most cases, to effect these beneficial functions. However, during infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects (Marcocci et al., 1994 a, b). [30]

The NO does not interact with the bioorganic macromolecules such as the DNA or proteins directly. However, in the aerobic conditions, the NO molecule is very unstable and reacts with the oxygen to produce intermediates such as NO2. N2O4. N3O4 the stable products nitrate and nitrite (Marcocci et al., 1994 a, b) and peroxynitrite when reacted with superoxide (Wink et al., 1991). These products progenitors are highly genotoxic, the deamination of guanine, cytosine and adenine is mediated primarily by the $N_2O_3$. In addition to the formation of nitrosoamines and deamination of the DNA bases, recent studies indicate that the NO may also act by affecting the enzymatic activities of several thiols rich DNA repair proteins like DNA alkyl transferase, formamopyrimidine-DNA glycosaisse and the DNA ligase that play a critical role in the maintenance of the genetic integrity (Wink et al., 1991). [31]

The formation of carcinogenic N-nitrose compounds, deamination, and oxidation of the DNA bases and inhibition of the critical DNA repair protein leads to mutagenesis and an initiation towards the process of carcinogenesis. There is now increasing evidence to suggest that NO and its derivatives produced by the activated phagocytes may have a genotoxic effect and may contribute in the multistage&inogenesis process (Wink et al., 1991). Direct link between chronic inflammation and induction of chlangiocarcinoma has been found in the infections by the parasites such as opisthrchis viverrini (liver fluke). The continuous exposure to free radicals generated from the chronic inflammation has been found to cause more cancers than environmental chemicals (Ames and Gold, 1990). [31]

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described previously. Sodium nitroprusside in aqueous solution at physiological při spontaneously generates nitric oxide (Green et al., 1982; Marcocci ef al., 1994a, b), which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci et al., 1994 a. b). [32]

## 1.13 Determination of Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide itself is not a free radical as it does not contain any unpaired electrons. However it is a precursor to certain radical species such as peroxyl radical hydroxyl radical, superoxide, Since it is a superb oxidizing agent it can also react with certain other molecules and convert them into free radicals. These hydroxyl radicals in turn readily react with and damage vital cellular components especially those of the mitochondria (Giorgio et al: 2007). [33]

Antioxidants act as peroxide decomposer. Both enzymatic and nonenzymatic antioxidants exist in the intercellular and extracellular environment to decompose hydrogen peroxide (Frie B et al: 1988). The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Roch. The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged (H2O2) = [(Ao-A1)/Ao] x 100 where Ao is the absorbance of the control and A, is the absorbance in the presence of the sample of extract and standard. [33]

## 1.14 Determination of Hydroxyl Radical Scavenging Assay

The hydroxyl radical. HO, is the neutral form of the hydroxide ion (HO), Hydroxyl radicals are highly reactive and consequent. short lived: most notably hydroxyl radicals are produced from the decomposition of hydro peroxides (ROHO) Mechanisms for scavenging peroxyl radicals for the protection of cellular structures includes endogenous antioxidants such as melatonin and glutathione and dietary antioxidants such as mannitol and vitamin E. [34]

The hydroxyl radical scavenging activity can be measured by studying the competition between deoxyribose and the plant extracts for hydroxyl radicals generated with Fe" /ascorbate/EDTA/$H_2O$: system. This can be determined spectrophotometrically at 530nm. [34]

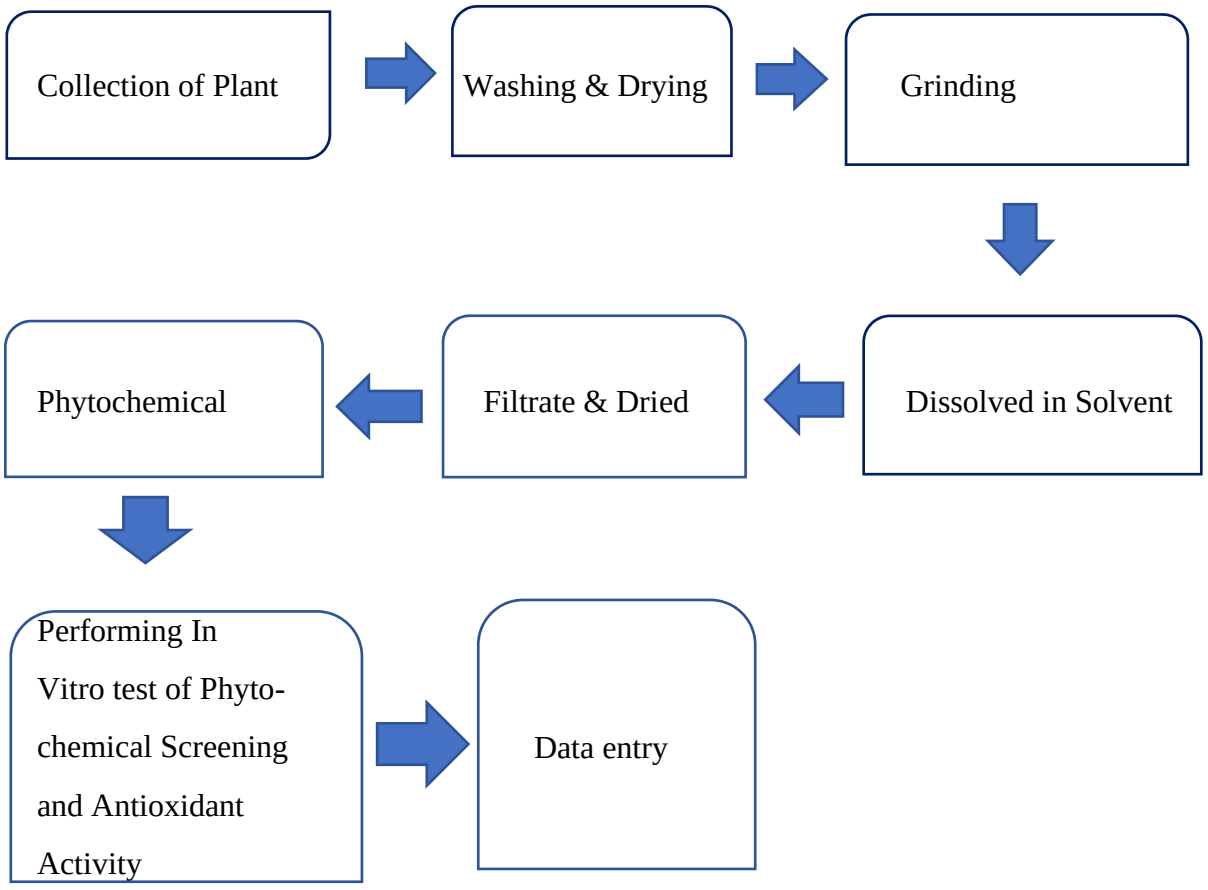
## 1. 15 Data Collection

All the relevant data has been collected from two types of sources:

➢ Primary sources: direct personal contact and observations of the experiments carried out in the laboratory.

➢ Secondary sources: various publications like journals, papers, documents and websites.

## 1.16 Research Protocol



# Chapter Two
# Plant Review
# &
# Literature Review

**Figure 2.1:** *Ampelocissus barbata* plant

## 2.1 Scientific name

- *Vitis barbata* [35]

## 2.2 Synonym

- Ampelocissus barbata wall. Planch
- Vitis barbata wall. Planch
- Vitis latifolia- Ham. ex Wall. [35]

## 2.3 Information about *Ampelocissus barbata*

*Ampelocissus barbata*, a species of liana in the grape family Vitaceae, is a fascinating subject of study in the realm of botany. This plant species was originally described from Sylhet, presently in Bangladesh, by Nathaniel Wallich and was formerly classified in the genus Vitis. In 1884, the species was reassigned to the Ampelocissus genus by Jules Émile Planchon. [36]

## 2.4 Common name

- Lepcha - Mikrum-rik
- Nepali - Jarila laha [37]

## 2.5 Taxonomy

| ***Ampelocissus barbata*** | |
|---|---|
| **Scientific classification** | |
| Kingdom: | Plantae |
| *Clade*: | Tracheophytes |
| *Clade*: | Angiosperms |
| *Clade*: | Eudicots |
| *Clade*: | Rosids |
| Order: | Vitales |
| Family: | Vitaceae |
| Genus: | *Ampelocissus* |
| Species: | ***A. barbata*** |
| **Binomial name** | |
| ***Ampelocissus barbata*** (Wall.) Planch. | |

## 2.6 Location

Within Bangladesh, the plant grows widely in the forests of Chittagong, Chittagong Hill Tracts, Tangail and Sylhet (Flora of Bangladesh, 2019). [37]

## 2.7 Distribution Area

- Global Distribution

India, Bhutan, Bangladesh, Myanmar, Thailand, Laos and Vietnam.

- Indian Distribution

Assam, Uttar Pradesh, West Bengal, Arunachal Pradesh, Nagaland, Mizoram, Tripura, Meghalaya, Sikkim, Andaman Isl. (Middle Andaman Isl., South Andaman Isl.), Nicobar Isl. (North Nicobar Isl.) [38]

## 2.8 Morphology

Trees up to 15 m tall; bark smooth, grey; branches stiff and stout; young shoots, petioles and main nerves strigose. Leaves simple, alternate distichous; stipular spines 1 or 2, ca. 0.4-0.6 cm, erect, caducous; petiole ca. 0.5-1.1 cm, brownish pilose; lamina ca. 5.5-14 x 2-6 cm, ovate or ovate-oblong, rounded or acute at base, acute to acuminate at apex, margin serrulate with hard brown point, unequal, glabrous, shining above, hairy on nerves beneath, membranous, black on drying; secondary nerves 5 pairs. Inflorescences in dichotomous cymes with 5-10 flowers; peduncles ca. 1.5 cm long; Flowers ca. 0.5 cm across; pedicels ca. 0.2 cm long; bract ovate; calyx lobes ca. 0.2-0.25 cm long, deltoid, keeled up to the middle, acute, rusty tomentose without, glabrous within; petals ca. 0.1-0.15 cm long, subcucullate, convex at distal end; stamens equal to petals; anthers dorsifixed; disc 10-lobed, glabrous; ovary bicarpellary; stylar arms straight. Drupe ca. 1-1.2 × 0.8-1.1 cm, red-brown at maturity, subglobose or globose-ellipsoid, glabrous, with persistent calyx tube and disk at base; fruiting pedicel ca. 0.4-1.1 cm, pilose; mesocarp thin; endocarp ca. 0.3 cm, thickly cartilaginous, 2-loculed. Seeds 1-2, black-brown, shiny, smooth, compressed. [39]

Branches petioles and peduncles covered with numerous long spreading glandular capitate hairs, leaves cordate-ovate membranous, peduncle flattened 4-8 in . bearing a long forked slender tendril above its middle, cymes regularly paniculate as long as the peduncle. Branches stoutish, hollow, dark brown or nearly black. Leaves 8 in.-1 ft. or more, roundly cordate-ovate, sinuate-dentate, sometimes sublobed, at length glabrous above, puberulous or densely tomentose beneath. Flowers sessile in large lax ovate paniculate cymes. Fruit the size of a large currant, black, shortly pedicellate. Seed 2/5 by 1/4 in., elliptic, the back flattish and shallowly grooved, the face rather sharply ridged, nearly smooth. --- A very distinct species, known at once by the long spreading black hairs. Wallich describes the flowers as being 4-cleft, but although they may be so sometimes it is certain that they are generally pentamerous. [39]

## 2.9 Natural history

Flowering & Fruiting: March-September. [39]

## 2.10 Traditional uses

*Ampelocissus barbata* (Wall.) Planch. Climber. Vitaceae. Jarila lahara (Li). Plant: Juice given to treat sores in mouth and tongue of milk sucking baby. *Ampelocissus barbata* is used as a medicinal plant by the tribes of the Island. [40]

## 2.11 Habitat

*Ampelocissus barbata* is native to eastern North America, from southern Quebec to Florida and west to Texas. It can be found in a range of environments, including woodlands, forests, thickets, and riverbanks. [40]

## 2.12 Literature Review

The Phytotoxic potential of plants and their constituents against other plants is being increasingly investigated as a possible alternative to synthetic herbicides to control weeds in crop fields.

(Md Monirul Islam et al., 2020) shows that *Ampelocissus Barbata (Wall.)* that the methanolic extract of the plant has DPPH Radical Scavenging, Reducing Power and Antioxidant activity as compared to standard Ascorbic acid. Phenolic compounds are present in plants, have antioxidant activity due to their redox properties, and therefore play a vital role in counterbalancing the free radicals.

With regards to reducing capacity, higher reducing powers might be attributed to higher amounts of Total Phenolic and low Flavonoid, and the Reducing Power of a compound may reflect its Antioxidant potential.

Further comprehensive Phytochemical study for the isolation and characterization of the specific compound is required to get a more potent agent with significant activity. Since the Polyphenol compounds as well as other components with potent Antioxidant activity are not known, thus advanced level of work should be performed for the isolation and identification of the Antioxidant components in *Ampelocissus barbata.*

Stress and pertaining counterbalance mechanism are actively working in the living organisms. The overproduction of Reactive Oxygen Species (ROS) in the ongoing equipoising process requires to be compensated by strong Antioxidants. Plants as a rich source of Antioxidants not only reduce oxidative stress but also possess cytotoxic, thrombolytic and phytochemical potentials. Assessment of the in vitro Antioxidant activity of extract was carried out using DPPH Radical Scavenging Assay, determination of Reducing Power Capacity and Total Phenolic Content. Phytochemical Reagent test for alkaloids; Dragendroff's test, test for Reducing Sugar; Benedict's Reagent, Fehling's Solution A & B reagent, test for carbohydrates; Ferric Chloride (5%) Solution, Potassium Dichromate (10%) Solution, test for

Flavonoids; Froth tests, test for Saponins, test for Carbohydrates/ Gums and test for Proteins-Xanthoprotein.

Statistical analysis used: The statistical analysis was carried out using GraphPad Prism and Microsoft excel. Appreciable DPPH radical scavenging activity of the extract was observed with the $IC_{50}$ value of 23.87 µg/ml. A significant correlation was found between the standard ascorbic acid (AA) and the plant extracts at the $p<0.05$ for the reducing power assay where, the activity increased with the concentration of the extracts and the highest absorbance value was 3.025±0.15 and 1.826±0.006 for the AA and the extracts respectively. The plant also accommodates a considerable amount of Polyphenols, reflected in the value of gallic acid equivalent 277.397±0.419 mg/ml. The study revealed the presence of Phytochemicals namely Tannins (Ferric Chloride) & Proteins & Xanthoprotein.

The study disclosed the promising in vitro activity of the *Ampelocissus barbata* plant, which necessitates the further analysis for the isolation and evaluation of the active principles.

# Chapter Three

# Aims and Objectives

## 3.1 Aims and Objectives of the Study

*Ampelocissus barbata*, often known as the bearded creeper, is a species of plant in the Vitaceae family. It is endemic to numerous places across the globe, including parts of Asia and North America. The plant is recognized for its climbing habit, with tendrils that allow it to mount diverse surfaces. The 'barbata' in its name, meaning 'bearded', alludes to the hairy underside of its leaves, a trait that separates it from other species in the Ampelopsis genus.

For instance, extracts from *Ampelocissus barbata* have been used in traditional medicine to treat conditions such as inflammation and liver problems. However, the scientific validation of these medicinal characteristics is still in its early phases, demanding further inquiry and study.

*Ampelocissus barbata* is used in folklore medicine for many years. This study aimed to investigate the Antioxidant & Phytochemical activities of the methanolic extract of *Ampelocissus barbata* fruits. There are many studies on its bark, root, and leaves and rhizome, but less study on its fruits. It also lacks studies on Antioxidant & Phytochemicals properties of *Ampelocissus barbata* fruits. Because of this, I am more eager to choose *Ampelocissus barbata* fruits for research on potential medical applications. *Ampelocissus barbata*, studying its botanical traits, geographical distribution, potential medicinal effects, and more.

Nature always stands as a golden mark and it has provided the natural products from plant, animal and minerals to cure all aliments of mankind. Medicinal plants in this respect play a significant role. “A medicinal plant is any plant which, in one or more of its organs, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs.” The value of a medicinal plant lies in different chemical substances which

produce different physiological effect within human body. The most important bioactive compounds of these include Alkaloids, Resin, Tannin, Flavonoids, etc. [41]

As the plant remains an integral part in terms of the discovery of medicine thus the aim of the current work was to explore the Phytochemical profiles of the plant *Ampelocissus barbata* for Antioxidant activity.

This study was designed fulfill the following Phytochemical and Antioxidant evaluations

- Evaluation of Phytochemical group test
- Antioxidant properties.

# Chapter Four

# (Materials and Methods)

## 4.1 Extraction

Phytochemistry or plant chemistry subject has been developed recently as a distinct discipline, wherever in between natural product organic chemistry and biochemistry and is thoroughly related to the both. It is apprehensive with the enormous diversity of organic constituents that are expounded and amassed by the plant and deals with chemical structures of these constituents and others like biosynthesis, turnover, metabolism, biological function and natural distribution. For all of these operations, methods are essential for purification, separation and identification of many diverse constituents presents in the plants.

Extraction means solid-liquid extraction (leaching) which is the process of removing a solute or solutes from a solid by using of liquid solvent. The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on the type of substance that is being isolated. Generally, two types of procedure are used for obtaining organic constituents-

- Cold extraction and
- Hot extraction.

The extract obtained is then concentrated and constituents are separated by different methods such as chromatography. As a standard precaution against loss of material, concentrated extracts should be stored in the refrigerator.

### 4.1.1 Drying and Grinding

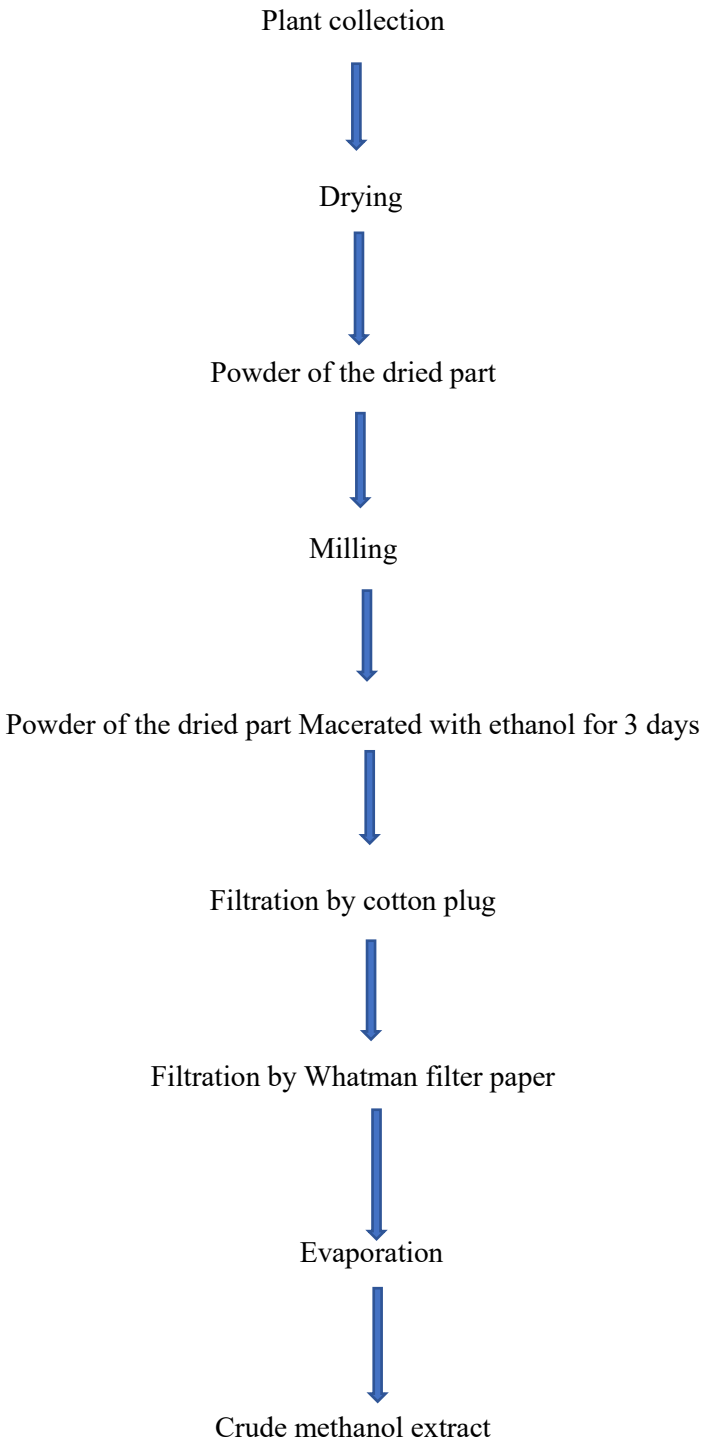
The collected plant parts were separated from undesirable materials or plants or plant parts and were washed with water. They were sun-dried for one week. The plant parts were grinded into coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

### 4.1.2 Cold Extraction (Methanolic extraction)

About 200 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 1000 mL ethanol. The container with its contents was sealed and kept for a period

of 8 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton. Then it was filtered through Whatman filter paper.

## Extraction at a glance



### 4.1.3 Partitioning with Methanol

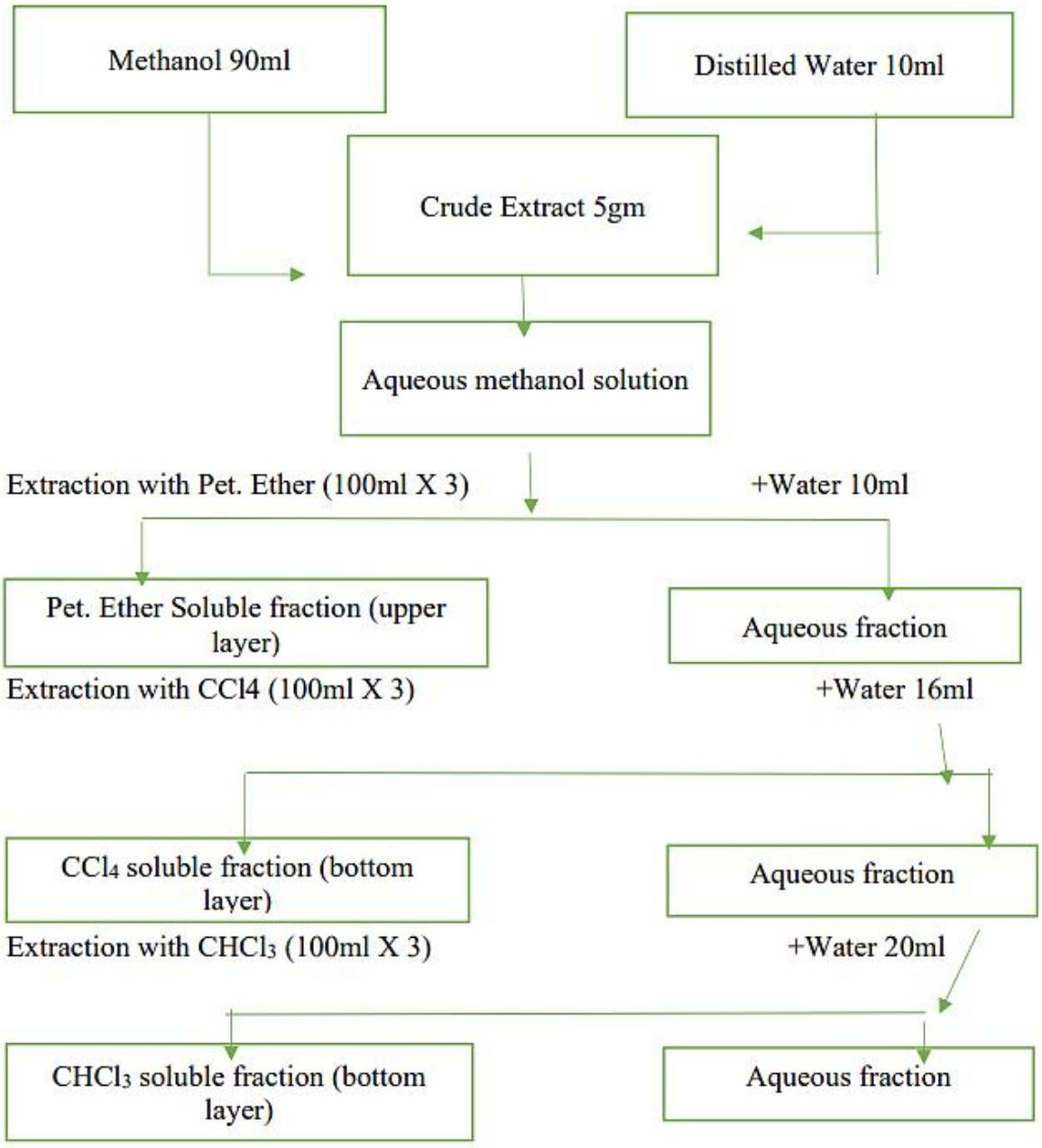



**Figure 4.1 :** Partitioning with Methanol

## 4.2 Phytochemical Screening of Pet Ether Extract

### 4.2.1 Introduction

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals. often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids. flavonoids, terpenoids, tannins, and many others., Nearly 50% of drugs used in medicine are of plant origin. and only a small fraction of plants with medicinal activity has been assayed. There is therefore much current research devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them. The phytochemicals isolated are then screened for different types of biological activity (Harborne et. al., 1998).

### 4.2.2 Test Material

Methanol extract of Pet Ether Extract from *Ampelocissus barbata* fruits.

### 4.2.3 Reagents of Chemical Group Tests

- Dragendroff's Reagent
- Fehling's Solution A
- Fehling's Solution B
- Methanol
- Sodium bicarbonate
- Sodium hydroxide
- Nitric acid
- Distilled water
- Libermann-Burchard Reagent
- Molish Reagent
- Benedict, Reagent

## 4.2.4 Preparation of Reagents (Trease & Evans 1989)

- Dragendroff's Reagent: 1.7 gm basic bismuth nitrate and 20 gm tartaric acid ware dissolved in 80 ml water. This solution was mixed with a solution containing 16 gm potassium iodide and 40 ml water.
- Fehling's Solution A: 34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.
- Fehling's Solution B: 176 gm of sodium potassium tartarate and 77 gm of sodium hydroxide were dissolved in sufficient water to produce 500 ml. Equal volume of above solution were mixed at the time of use.
- Benedict's reagent: 1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.
- Molish Reagent: 2.5gm of pure α-naphthol was dissolved in 25 ml of ethanol.
- Libermann-Burchard Reagent: 5 ml acetic anhydride was carefully mixed under cooling with 5ml concentrated sulfuric acid. This mixture was added cautiously to 50 ml absolute ethanol with cooling.

## 4.2.5 Methods of Phytochemical Tests

Testing of different chemical groups present in extract represents the preliminary phytochemical studies. In each test 5% (w/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test. The chemical group test, which are performed as follows- (Trease & Evans, 1989; Sofowara, 1982).

### 4.5.6 Tests for Reducing Sugar

- Benedict's test

0.5 ml of aqueous extract of the plant material was taken in a test tube. 5ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. A red color precipitate of cuprous oxide indicates the presence of a reducing sugar.

- Fehling's Test (Standard Test)

2ml of an aqueous extract of the plant material was added to 1ml of a mixture of equal volumes of Fehling's solutions A and B and boiled for few minutes. A red or brick red color precipitate formation indicates the presence of a reducing sugar.

- **Tests for combined Reducing Sugar**

1ml of aqueous extract of plant material was boiled with 2 ml of dilute hydrochloric acid for 5 minutes, then cooled and neutralized with sodium hydroxide solution and then Fehling's test was performed as described above. A red or brick red color precipitate formation indicates the presence of a combined reducing sugar.

- **Tests for Tannins**
- Ferric Chloride Test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 5%Ferric chloride solution was added. Greenish black precipitate indicates the presence of tannins.

- Potassium dichromate test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 10% Potassium dichromate solution was added. A yellow precipitate indicates the presence of tannins.

- **Test for Flavonoids (Agoha, 1981)**
- 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H2S04. A yellow coloration observed in each extract indicates the presence of flavonoids. The yellow color disappeares on standing.
- 0.2gm extract was dissolved in dilute sodium hydroxide and then neutralized with dilute hydrochloric acid. Formation of yellow color and disappearance of color indicate the presence of flavonoid.

- **Test for Saponins**

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A layer of foam above the solution indicates the presence of saponins.

- **Test for Carbohydrates / Gums**

5 ml solution of the extract was taken and then Molish's reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicates the presence of gums and carbohydrate.

- **Test for Alkaloids**

- Dragendroff's test:

2 ml solution of the extract and 5 ml of dilute hydrochloric acid (1%) were taken in a test tube. Then 1 ml of Dragendroff's reagent was added. Orange brown precipitate indicates as the presence of alkaloids.

- **Test for Glycosides**

A small amount of an alcoholic extract was taken in 1ml of water. A few drops of aqueous NaOH were added. A yellow color indicates the presence of glycosides.

- **Tests for Proteins-Xanthoprotein**

To 1 mL of extract, few drops of nitric acid were added by the sides of the test tube and observed for formation of yellow color. This indicates the presence of xanthoprotein.

## 4.3 Observations

## 4.4 Tests for Pet Ether Extract of *Ampelocissus barbata* fruits

The pet ether extract and its different fractions were subjected for chemical group tests and investigations of different group tests are given in table.

**Table 4.1 :** Different Chemical group tests for Pet Ether Extract from *Ampelocissus barbata* fruits Extract

| Test | Reagent | Observation | Inference |
|---|---|---|---|
| **Reducing sugar test** | Benedict's reagent | Brick red colored precipitate was not found | Absence of reducing sugar |
| | Fehling's Solution | Brick red colored precipitate was not found | Absence of reducing sugar |
| **Tests for combined reducing sugar** | Fehling's Solution | Brick red colored precipitate was not found | Absence of combined reducing sugar |
| **Tests for Tannins** | Ferric chloride solution | Greenish black precipitate was formed | Presence of tannins |
| | Potassium dichromate solution | A yellow precipitate was not formed | Absence of tannins |
| **Tests for Flavonoids** | Concentrated H2SO4 | A yellow coloration was not observed | Absence o flavonoids |
| | Dilute HCl<br>Dilute NaOH | A yellow coloration was not observed | Absence of flavonoids |
| **Tests for Saponins** | Distilled water | layer of foam was not produced | Absence of saponins |
| **Tests for Carbohydrates & gums** | Molisch's reagent | Red –violet ring was not produced at the junction of two liquids. | Absence of gums |
| **Test for Glycoside** | NaOH | Yellow color was not | Absence of glycoside |

| | | | |
|---|---|---|---|
| | | formed | |
| **Test for Alkaloids** | Dragendroff's test | Orange brown precipitate was not formed | Absence of alkaloids |
| **Tests for Xanthoprotein test** | Nitric acid | A yellow color was formed | Presence of xanthoprotein |

## 4.5 Determination of Total Tannin Content

### 4.5.1 Apparatus

- Volumetric flask
- Conical flask
- Pipette
- Beakers
- Test tubes
- Electronic balance
- UV spectrophotometer

### 4.5.2 Reagent

- Gallic acid
- Distilled water
- Sodium carbonate
- Folin-Ciocalteu (FC) reagent

### 4.5.3 Preparation of Sample

10 mg dried plant extract was weighed and mixed with 10 ml distilled water to make the concentration 1mg/1ml.

### 4.5.4 Preparation of Standard

0.5mg, 0.4mg, 0.3mg, 0.2mg and 0.1mg Gallic acid was dissolved in 5ml water to get the final 5 concentrations 100, 80, 60, 40 and 20 mg/L.

### 4.5.5 Preparation of Sodium Carbonate

3.5g sodium carbonate was dissolved in distilled water and the volume was adjusted to 10 ml with distilled water to make 35% sodium carbonate.

### 4.5.6 Working procedure

- First of all, 0.1 ml Gallic acid solution of each concentration (100, 80, 60, 40 and 20 mg/L) was taken into volumetric flask designed for each concentration.
- Then 7.5 ml of distill water was added and 0.5 ml of FC reagent was added to every volumetric flask with shaking and kept for 5 minutes.
- Then 1 ml 35% sodium carbonate solution (Na 2 CO 3 ) was added and diluted with 10ml distilled water.
- This preparation was then shaken well.
- Then they were kept for 30 minutes at room temperature.
- After 30 minutes the UV absorbance was measured at 725 nm against blank for Each concentration, the absorbance was taken for two times and mean was used for accuracy.
- Blank was prepared by following all the above steps except the addition of Gallic acid and sample.

## 4.6 Determination of Total Flavonoid Content

### 4.6.1 Apparatus

- Volumetric flask
- Conical flask
- Pipette
- Beakers
- Test tubes
- Electronic balance
- UV spectrophotometer.

### 4.6.2 Reagent

- Quercetin
- Distilled water
- Sodium nitrous
- Aluminum Chloride
- Sodium hydroxide.

### 4.6.3 Preparation of Sample

10 mg dried plant extract was weighed and mixed with 10 ml distilled water to make the concentration 1mg/1ml.

### 4.6.4 Preparation of Standard

0.1mg, 0.08mg, 0.06mg, 0.04mg and 0.02mg Quercetin was dissolved in 1mL water to get the final 5 concentrations 100, 80, 60, 40 and 20µg/mL.

### 4.6.5 Working Procedure

- First of all, 1 mL quercetin solution of each concentration (100, 80, 60, 40 and 20 µg/L) was taken into 10mL volumetric flask designed for each concentration.
- Then 4ml of distill water was added to every volumetric flask with shaking.
- Then 0.3 mL 5% sodium nitrous solution (NaNO2) was added and kept for 5 minutes.
- Then 0.3 mL of 10% Aluminum chloride was added.
- In 6th min, 2mL 1M sodium hydroxide (NaOH) was added.
- Immediately this preparation was shaken well and the final volume was adjusted up to 10 mL.
- After that, the UV absorbance was measured at 510 nm against blank for Each concentration, the absorbance was taken for two times and mean was used for accuracy.
- Blank was prepared by following all the above steps except the addition of quercetin and sample.

## 4.7 Determination of Total Phenolic Content

### 4.7.1 Apparatus

- Volumetric flask
- Beakers
- Test tubes
- Centrifuge apparatus
- Pipette
- UV spectrophotometer

- Electronic balance
- Cotton.

### 4.7.2 Reagents

- Folin-Ciocalteu (FC) reagent
- Gallic acid
- Methanol
- Distilled water.

### 4.7.3 Preparation of Sample

30 mg dried plant extract was weighed and mixed with 3 mL of 80% aqueous methanol. It was then sonicated for 20 min. Two ml of the extract was centrifuged for 15 min.

### 4.7.4 Preparation of Standard

12.5 mg Gallic acid was dissolved in methanol to make final volume 25 mL. Standard solution of 6 concentrations of the Gallic acid was prepared by serial dilution method. These concentrations were 500, 250, 125, 62.5, 31.25, and 15.62 mg/L.

### 4.7.5 Dilution of Folin-Ciocalteu (FC) reagent

1 mL FC reagent was mixed with 9 mL distilled water to make 10 times diluted.

### 4.7.6 Working procedure

- First of all, 1 mL solution of each concentration (500, 250, 125, 62.5, 31.25 and 15.62 mg/L) was taken into volumetric flask designed for each concentration.
- Then 9 mL of distill water was added and 1 mL of FC reagent (1/10)) was added to every volumetric flask with shaking and kept for 5 minutes.

- Then 10 mL solution of 7% Na2CO3 was added to it and volume was adjusted with distilled water to make final volume 25 mL.
- Then they were kept for 30 minutes at room temperature.
- After 30 minutes the UV absorbance was measured at 750 nm against blank for each concentration, the absorbance was taken for two times and mean was used for accuracy.
- Blank was prepared by following all the above steps except the addition of gallic acid and sample.

## 4.8 Determination of DPPH Scavenging Assay

### 4.8.1 Apparatus

- Test tubes
- Beakers
- Sonicator
- Pipette
- Thermometer
- UV spectrophotometer
- Electronic balance

### 4.8.2 Reagents

- Ethanol
- 0.004% DPPH ethanol solution
- Ascorbic acid (as positive control)

### 4.8.3 Method

- At first 10 test tubes were taken to make aliquots of 5 concentrations (1, 10, 20, 50, and 100 µg/ml) for sample (plant extract) and standard (ascorbic acid) each.

- Both the plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentrations by dilution technique. Here ascorbic acid was taken as positive control.
- The DPPH was weighed carefully and dissolved in ethanol to make 0.004% (w/v) solution. Sonicator was used to dissolve homogeneously.
- 1ml of different concentrations of plant extracts and ascorbic acid was taken in each test tube.
- After making the desired concentrations 3 ml of 0.004% DPPH solution was applied on each test tube by pipette.
- The room temperature was recorded and the test tubes are then kept in dark for 30 minutes to complete the reaction.
- DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank.
- After 30 minutes, absorbance of each test tube was determined by UV spectrophotometer at 517 nm.
- % of inhibition was calculated as-

% inhibition = [(Blank absorbance - Sample absorbance) / Blank absorbance] X 100

$IC_{50}$ was then determined from % inhibition vs. log conc. graph.

## 4.9 Determination of Hydrogen Peroxide Scavenging Assay

### 4.9.1 Apparatus

- Test tube
- Beaker
- Pipette
- UV spectrophotometer

- Electronic balance
- Tissue paper
- Measuring cylinder
- Spatula
- Vortex machine
- Sonicator

## 4.9.2 Reagents

- Ethanol
- Distilled water
- Phosphate buffer (40 mM $p^H$ 7.4)
- $H_2O_2$
- Ascorbic Acid (as positive control)

## 4.9.3 Method

## 4.9.4 Preparation of phosphate buffer

3.56 gram of $Na_2HPO_4.2H2O$ was dissolved in 100ml distilled water. Again 3.12 gram of $NaH_2PO_4.2H2O$ was dissolved in 100ml distilled water. Then 37.5 ml of prepared $Na_2HPO_4.2H2O$ solution and 62.5ml of prepared $NaH_2PO_4.2H2O$ solution were mixed together to get 100ml phosphate buffer. Then $p^H$ was checked by $P^H$ meter. Hcl or NaOH was added to adjust $p^H$ 7.4.

## 4.9.5 Preparation of $H_2O_2$

0.136gm dissolved in 100 ml phosphate buffer.

### 4.9.6 Preparation of Sample

Plant extracted was weighed and 8mg of it was mixed with 0.01L of ethanol. It was sonicated for 20 min. Sample solution 8 concentrations of the extract was prepared by serial dilution method. These concentrations were 800, 400, 200, 100, 50, 25, 12.5 and 6.25 mg/L.

### 4.9.7 Preparation of Standard

8 mg Ascorbic acid was dissolved in ethanol to make final volume 0.01L. Standard solution 8 concentrations of the ascorbic acid were prepared by serial dilution method. These concentrations were 800, 400, 200, 100, 50, 25, 12.5 and 6.25 mg/L.

### 4.9.8 Procedure

- 1ml of different concentrations of sample extract/ standard was transferred into the test tubes.
- 3ml of 40 mM phosphate buffer (pH 7.4) was added to each test tube to make a volume of 4ml.
- Then 6 ml hydrogen peroxide solution was added to each test tube.
- A blank solution containing phosphate buffer without extract and sample was prepared.
- Tubes were vortexed. After 10 minutes, absorbance of all the solutions was determined at 230nm using UV spectrophotometer.
- % Scavenged ($H_2O_2$) = [(Ao-A1)/Ao] x 100 where Ao is the absorbance of the control and $A_1$ is the absorbance in the presence of the sample of extract and standard.

## 4.10 Determination of Hydroxyl Radical Scavenging Assay

### 4.10.1 Apparatus

- Test tube
- Beaker
- Pipette

- UV spectrophotometer
- Electronic balance
- Tissue paper
- Measuring cylinder
- Spatula
- Vortex machine
- Sonicator.

## 4.10.3 Reagents

- Ethanol
- Distilled water
- $H_2O_2$ (1mM)
- Ascorbic Acid (as positive control)
- 2-deoxy 2-ribose (2.8mM)
- $Fecl_3$ (200 μM)
- EDTA (1.04 mM)
- Trichloroacetic acid, TCA (2.8%)
- Thio barbituric acid, TBA (1%)

## 4.10.4 Method

## 4.10.5 Preparation of Sample

Plant extracted was weighed and 8mg of it was mixed with 0.01L of ethanol. It was sonicated for 20 min. Sample solution 8 concentrations of the extract was prepared by serial dilution method. These concentrations were 800, 400, 200, 100, 50, 25, 12.5 and 6.25 mg/L.

## 4.10.6 Preparation of Standard

8 mg Ascorbic acid was dissolved in ethanol to make final volume 0.01L. A standard solution 8 concentration of the ascorbic acid was prepared by serial dilution method. These concentrations were 800, 400, 200, 100, 50, 25, 12.5 and 6.25 mg/L.

## 4.10.7 Procedure

- Hydroxyl radical scavenging activity of the examined compounds was measured based on the method of Halliwell (Halliwell et al.;1987) with a slight modification according to jiang (Jiang et al.;1993)
- 0.5 ml 2-deoxy 2- ribose solution (2.8 mM) was mixed with 12.5 µL of different concentrations (6.25,12.5,25,50,100,200,400 & 800 mg/L) of sample extracts or standard.
- Then 1 ml of 200 µM $FeCl_3$, 1mLof 1.04 mM EDTA, 0.5 mL of 1mM $H_2O_2$ and 0.5 ml of 1 Mm of ascorbic acid were added to prepare the reaction mixture.
- After an incubation time of 1 hour at $37^0$ C, 3.75 ml of 2.8% TCA and 3.75 ml of 1%
- TBA were added and kept at $100^0$ C for 20 minutes.
- The absorbance was measured at 530 nm. Blank was prepared simultaneously containing all the reagents except extract and standard.
- The percentage of hydroxyl radical scavenging activity by the extracts and standard compounds was calculated as follows: % Scavenged ($H_2O_2$) = [(Ao-A1)/Ao] x 100.

Where Ao is the absorbance of the control and $A_1$ is the absorbance in the presence of the sample of extract and standard.

## 4.11 Determination of Nitric Oxide (No) Scavenging Assay

## 4.11.2 Reagents used

- Sodium Nitroprusside
- Phosphate Buffer saline
- Griess Reagent

## 4.11.3 Preparation of Griess reagent:

- 2.3ml Phosphoric acid (85%)
- 1g Sulfanilamide
- 0.1g Naphtylethylenediamine
- 97.7ml water

## 4.11.4 Procedure

Nitric oxide scavenging activity was measured spectrophotometrically according to Govindarajan R *et al*., 2003. Sodium nitroprusside (5 mmol) in phosphate buffered saline was mixed with different concentrations of the extract (5-100 µg/ml) dissolved in suitable solvent system and incubated at 25$^{O}$C for 30 minutes. After 30 minutes of incubation 1.5 ml solution was taken and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthylethylene diamine dihydrochloride).The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm with a double beam Analykjena UV/Visible spectrophotometer. The process was done for ethanol extract *of Extract*. The nitric oxide (NO) radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I\ (\%) = [(A_{Blank} - A_{Sample}) / A_{Blank}] \times 100$$

Where $A_{Blank}$ is the absorbance of the control reaction (containing all reagents except the test compound), and $A_{Sample}$ is the absorbance of the experimental sample with all reagents. $IC_{50}$ value is the concentration of sample required to scavenge 50% nitric oxide free radical and was calculated from the plot of inhibition (%) against the sample concentration. All the determinations were carried out in triplicate and average of the absorptions was noted. Ascorbic acid was used as standard for this study.

# Chapter Five
# Results & Discussions

## 5.1 Result of Phytochemical group test

The Pet Ether Extract was subjected for chemical group tests and investigations of different group tests are given in below (Table 5.1).

**Table 5.1: Result of Phytochemical group test of Pet Ether Extract of *Ampelocissus barbata* fruits.**

| Phytochemical Group | Result |
|---|---|
| Reducing sugar (Benedict's) | - |
| Reducing sugar (Fehling's) | - |
| Combined reducing sugar | - |
| Tannins (Ferric Chloride) | + |
| Tannins (Potassium dichromate) | - |
| Flavonoids | - |
| Saponin | - |
| Carbohydrates & Gums | - |
| Alkaloids (Dragendroff's) | - |
| Glycoside | - |
| Proteins & Xanthoprotein | + |

+ indicates Presence and - indicates Absence

### 5.1.1 Discussion

The table indicates that the plant sample being tested contains Proteins & Xanthoprotein & Tannins (Ferric Chloride) as indicated by the plus sign in the result column. The presence of these Phytochemical groups on methanolic extract of Pet ether of *Ampelocissus barbata.*

## 5.2 Determination of DPPH Scavenging Assay

**Table 5.2.1 : DPPH scavenging assay of *Ampelocissus barbata***

| Concentration | Abs 1 | Abs 2 | log concentrat | Average | % inhibition |
|---|---|---|---|---|---|
| 0 | 0.6376 | 0.6373 | | 0.63745 | 0 |
| 1 | 0.6184 | 0.6176 | 0 | 0.618 | 3.051219704 |
| 10 | 0.6149 | 0.6145 | 1 | 0.6147 | 3.568907365 |
| 20 | 0.6121 | 0.6118 | 1.301029996 | 0.61195 | 4.00031375 |
| 50 | 0.6073 | 0.6067 | 1.698970004 | 0.607 | 4.776845243 |
| 100 | 0.587 | 0.5868 | 2 | 0.5869 | 7.930033728 |

**Table 5.2.2 : DPPH scavenging assay of Ascorbic Acid**

| Concentration | Abs 1 | Abs 2 | log concentration | Average | % inhibition |
|---|---|---|---|---|---|
| 0 | 0.3605 | 0.3604 | | 0.36045 | 0 |
| 1 | 0.3066 | 0.3062 | 0 | 0.3064 | 14.99514496 |
| 10 | 0.2851 | 0.2855 | 1 | 0.2853 | 20.84893883 |
| 20 | 0.1343 | 0.134 | 1.301029996 | 0.13415 | 62.78263282 |
| 50 | 0.1154 | 0.1152 | 1.698970004 | 0.1153 | 68.01220696 |
| 100 | 0.1035 | 0.1034 | 2 | 0.10345 | 71.29976418 |

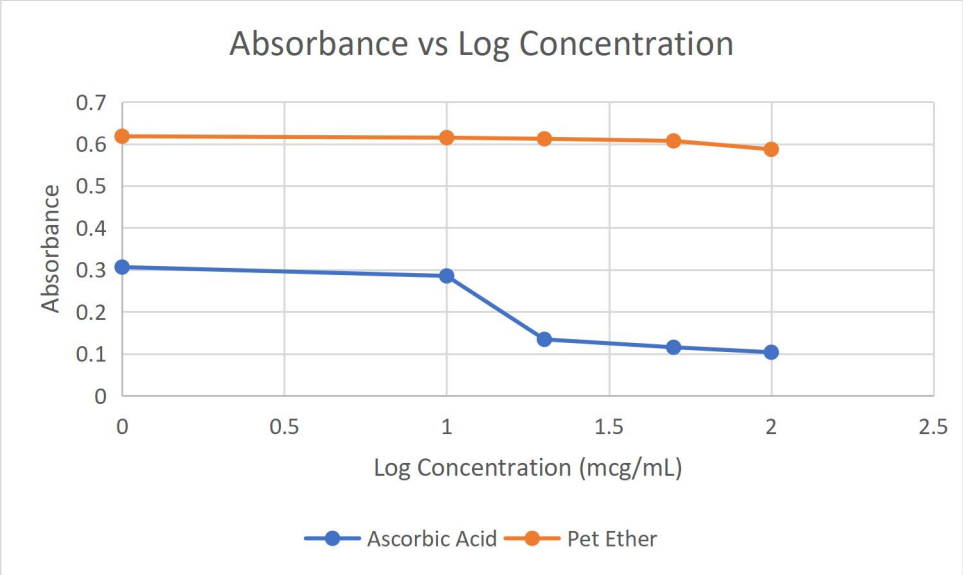



**Fig. 5.1 : Comparison of absorbance vs. log concentration graph for standard (ascorbic acid) vs. Pet Ether Extract of *Ampelocissus barbata.***

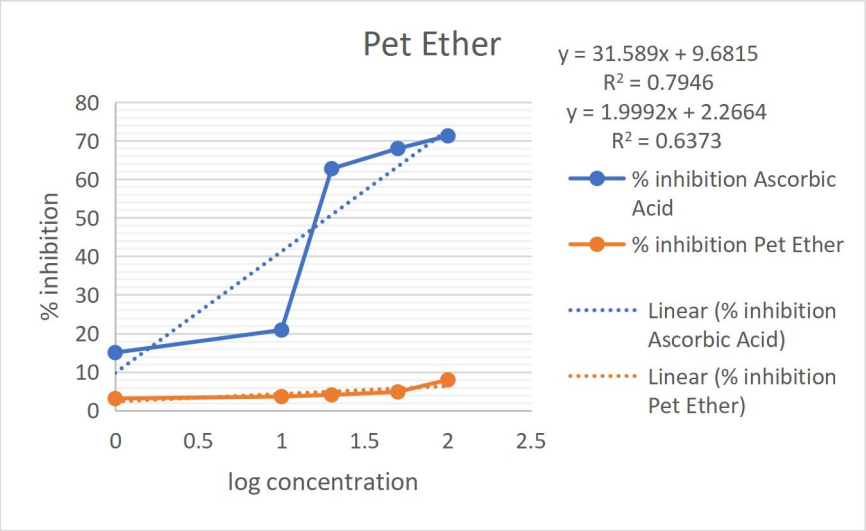



**Fig.5.2 : Comparison of % inhibition vs. log concentration graph for standard (ascorbic acid) vs. Pet Ether Extract of *Ampelocissus barbata.***

## 5.2.2 Results:

From the above Graph:

- $IC_{50}$ of Ascorbic acid (standard) : 1.27 µg/ml
- $IC_{50}$ of *Ampelocissis barbata* ( fruits extract of Pet ether) : 23.87 µg/ml

## 5.3 Determination of Total Tannin Content

**Table 5.3.1 : UV Absorbance of Gallic acid (standard) at 725 nm**

| Concentration (mg/L) | Average | Absorbance I | Absorbance II | Absorbance III |
|---|---|---|---|---|
| 20 | 0.077593333 | 0.0766 | 0.07878 | 0.0774 |
| 40 | 0.192633333 | 0.193 | 0.192 | 0.1929 |
| 60 | 0.346933333 | 0.3451 | 0.3473 | 0.3484 |
| 80 | 0.4409 | 0.4386 | 0.4427 | 0.4414 |
| 100 | 0.547266667 | 0.5464 | 0.5484 | 0.547 |

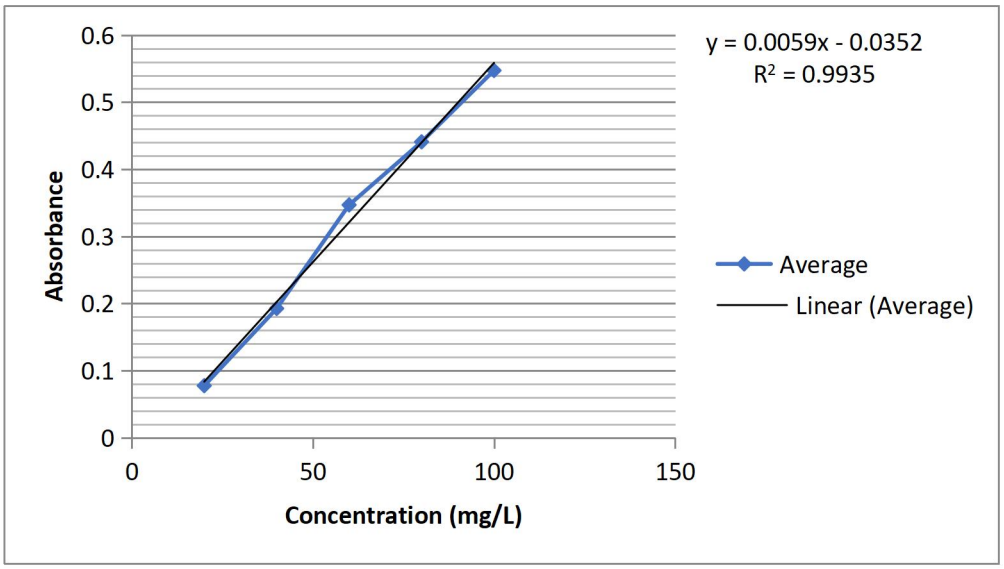



**Figure 5.3 :** UV absorbance of Gallic Acid (STD) at 725 nm.

| No | 1st Reading | 2nd Reading | Average | SEM | Grand Average |
|---|---|---|---|---|---|
| Pet Ether *Ampelocissus barbata* | 0.0916 | 0.0912 | 0.0914 | | |
| | 0.0899 | 0.0908 | 0.09035 | 0.000525 | 0.0908±0.0005 |
| | | | 0.090875 | | |

| x | y | m | c |
|---|---|---|---|
| 21.3559322 | 21.3559322 | 0.0059 | 00.0352 |

## 5.3.1 Results:

The Total Tannin Content of extract showed to be 21.3559322 mg GAE/g of dried plant extract of Pet ether from A.barbata fruits.

## 5.4 Determination of Total Flavonoid Content

**Table 5.4.1 : UV Absorbance of Quercetin (standard) at 510 nm**

| Concentration | Average | Abs 1 | Abs 2 | Abs 3 | Abs 4 |
|---|---|---|---|---|---|
| 20 | 0.3531 | 0.3521 | 0.3528 | 0.3535 | 0.354 |
| 40 | 0.441875 | 0.4453 | 0.4447 | 0.435 | 0.4425 |
| 60 | 0.578225 | 0.5789 | 0.5775 | 0.5773 | 0.5792 |
| 80 | 0.799275 | 0.7964 | 0.7991 | 0.8014 | 0.8002 |
| 100 | 0.8032 | 0.8021 | 0.8035 | 0.8027 | 0.8045 |

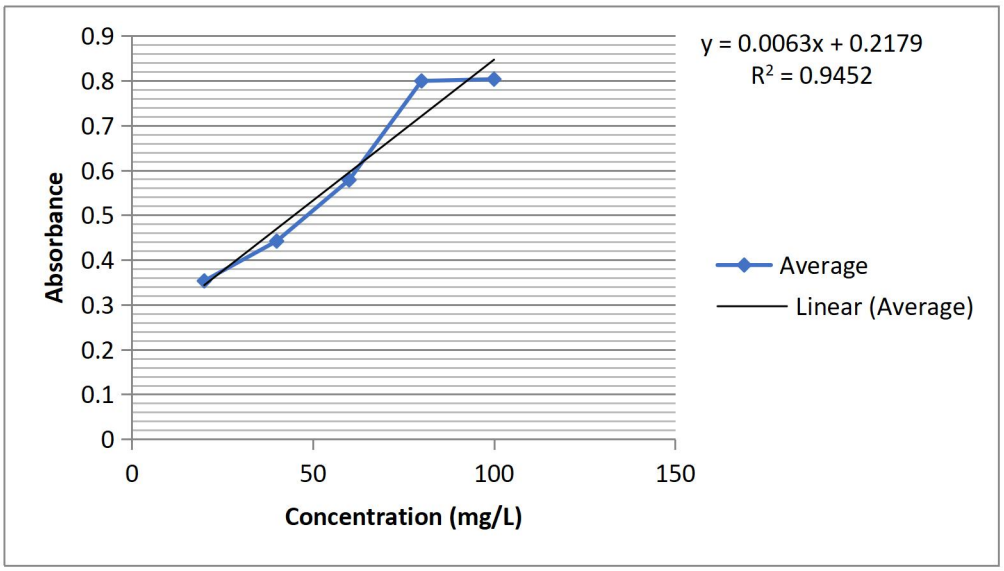



**Fig. 5.4 :** Total Flavonoid content determination of Pet ether extract of *Ampelocissus barbata* with the help of Quercetin standard calibration curve.

**Table 5.4.1 :** UV absorbance of quercetin (STD) at 510 nm.

| No | 1st Reading | 2nd Reading | Average | SEM | Grand Average |
|---|---|---|---|---|---|
| Pet Ether *Ampelocissus barbata* | 0.2377 | 0.2412 | 0.23945 | | |
| | 0.2426 | 0.2407 | 0.24165 | 0.0011 | 0.240±0.0011 |
| | | | 0.024055 | | |

| x | y | m | c |
|---|---|---|---|
| 3.507936508 | 0.24 | 0.0063 | 0.2179 |

Grand average = mean ± SEM (n=2)

## 5.4.1 Result:

The total flavonoid content of extract exposed to be 3.507936508 mg QE/g of dried plant Extract of Pet ether from A.barbata fruits.

## 5.5 Determination of Total Phenolic Content

**Table 5.5.1 : UV Absorbance of Gallic acid (standard) at 750 nm**

| Concentration | Average | Abs 1 | Abs 2 | Abs 3 |
|---|---|---|---|---|
| 15.62 | 0.0735 | 0.0755 | 0.0744 | 0.0706 |
| 31.25 | 0.2715 | 0.2649 | 0.2735 | 0.2761 |
| 62.5 | 0.2908 | 0.2839 | 0.293 | 0.2955 |
| 125 | 0.344066667 | 0.3401 | 0.3443 | 0.3478 |
| 250 | 0.355366667 | 0.3557 | 0.3544 | 0.356 |
| 500 | 0.403566667 | 0.403 | 0.4026 | 0.4051 |

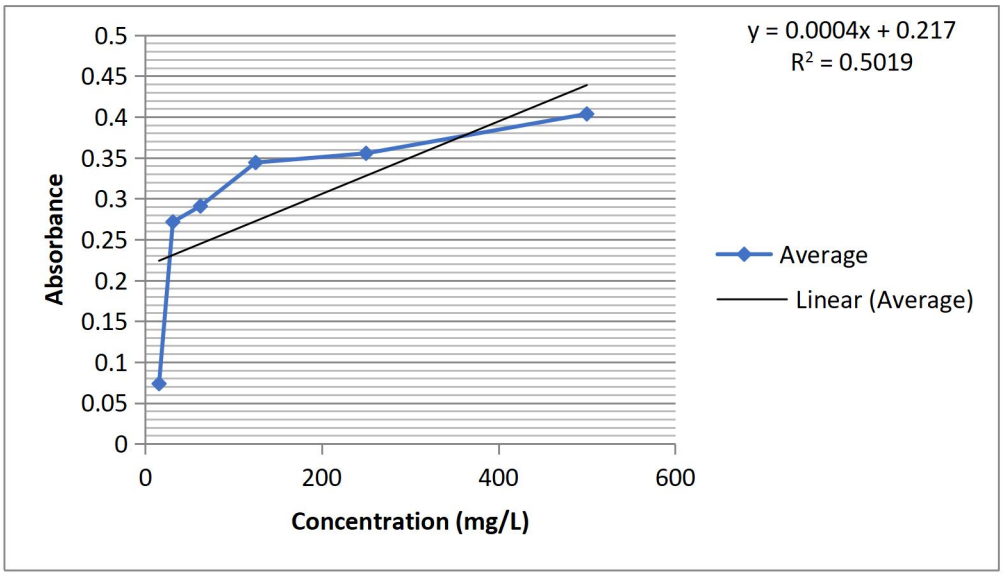



**Fig. 5.5 :** Total phenolic content determination of *Ampelocissus barbata* with the help of Gallic acid standard calibration curve.

**Table 5.5.2 : UV absorbance of Gallic Acid (STD) at 750 nm.**

| No | 1st Reading | 2nd reading | 3rd reading | Average | SEM | Grand Average |
|---|---|---|---|---|---|---|
| Pet Ether *Ampelocissus barbata* | 0.534 | 0.8127 | 1.496 | | | |
| | | | | 0.947 | 0.285 | 0.947±0.285 |
| | | | | | | |

| x | y | m | c |
|---|---|---|---|
| 1817.5 | 0.944 | 0.0004 | 0.217 |

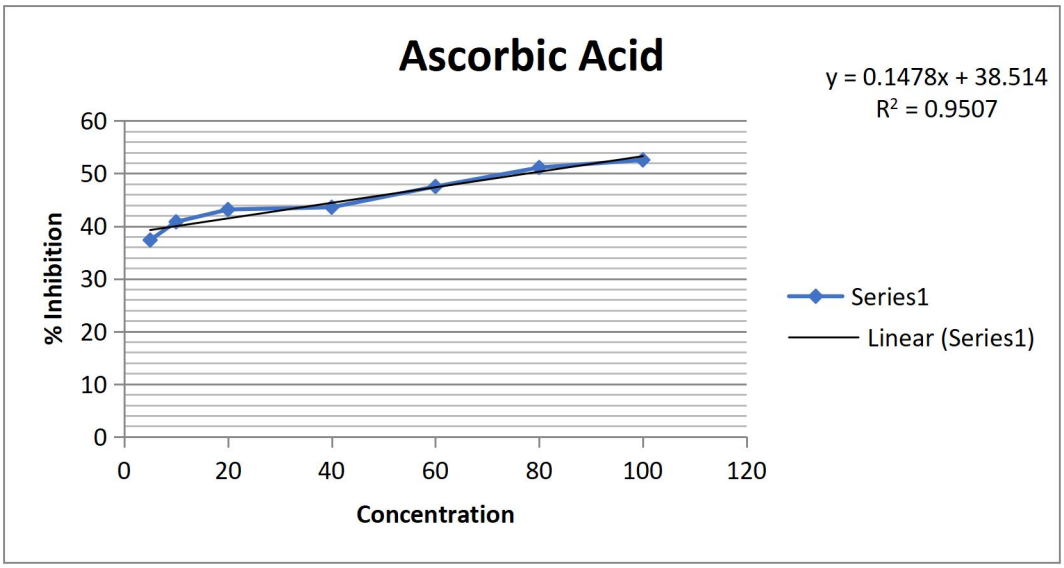
## 5.5.1 Result :

The Total Phenolic Content of Pet ether extract from *Ampelocissus barbata* fruits extract exposed to be 1817.5 mg GAE/g of dried plant extract.

## 5.6 Determination of Nitric Oxide (No) Scavenging Assay

**Table 5.6.1 : Nitric oxide (NO) scavenging assay of Ascorbic acid**

| | | Ascorbic Acid | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration | % Inhibition | Ab1 | Ab2 | Ab3 | Ab4 | Aver Ab | IC50 |
| Blank | | 0.7766 | 0.776 | 0.7786 | 0.7738 | 0.77625 | |
| 5 | 37.35587762 | 0.4858 | 0.4865 | 0.4863 | 0.4865 | 0.486275 | |
| 10 | 40.8115942 | 0.4581 | 0.4578 | 0.4628 | 0.4591 | 0.45945 | 77.71313 |
| 20 | 43.15942029 | 0.4407 | 0.4412 | 0.4413 | 0.4417 | 0.441225 | |
| 40 | 43.58454106 | 0.4429 | 0.4358 | 0.4364 | 0.4366 | 0.437925 | |
| 60 | 47.52657005 | 0.4045 | 0.4067 | 0.4084 | 0.4097 | 0.407325 | |
| 80 | 51.14009662 | 0.3715 | 0.3799 | 0.3822 | 0.3835 | 0.379275 | |
| 100 | 52.57971014 | 0.3667 | 0.3677 | 0.3687 | 0.3693 | 0.3681 | |



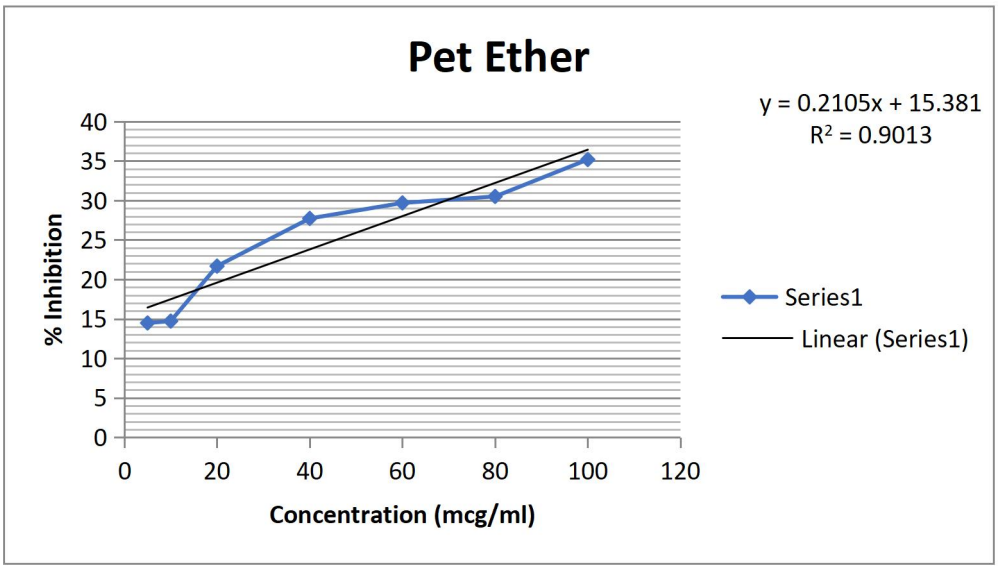

**$IC_{50}$ = 77.71313 µg/ml**

**Figure 5.6 :** $IC_{50}$ determination of Ascorbic acid

**Table 5.6.2 : Nitric oxide (NO) scavenging assay of Extract**

| | | Pet Ether | | | | | |
|---|---|---|---|---|---|---|---|
| Concentra | % Inhibiti | Ab1 | Ab2 | Ab3 | Ab4 | Aver Ab | IC50 |
| Blank | | 0.7766 | 0.776 | 0.7786 | 0.7738 | 0.77625 | |
| 5 | 14.47665 | 0.6637 | 0.6639 | 0.6637 | 0.6642 | 0.663875 | |
| 10 | 14.71498 | 0.6622 | 0.6617 | 0.662 | 0.6622 | 0.662025 | 24.46 |
| 20 | 21.68116 | 0.6086 | 0.6082 | 0.6086 | 0.6064 | 0.60795 | |
| 40 | 27.71981 | 0.5598 | 0.5609 | 0.5615 | 0.5621 | 0.561075 | |
| 60 | 29.68438 | 0.5458 | 0.546 | 0.5457 | 0.5458 | 0.545825 | |
| 80 | 30.50242 | 0.536 | 0.5392 | 0.5439 | 0.5388 | 0.539475 | |
| 100 | 35.18196 | 0.5123 | 0.5079 | 0.4987 | 0.4937 | 0.50315 | |

**Figure 5.7 :** $IC_{50}$ determination of Pet ether Extract




**Note:** $IC_{50}$ for both standard and sample was calculated from LDP Line software.

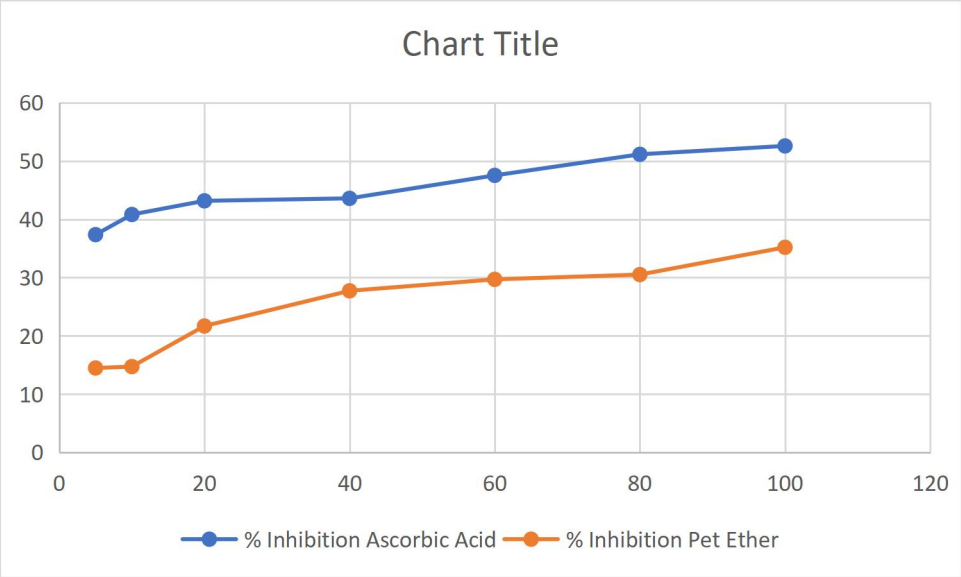



**Figure 5.7 :** Comparison of % Inhibition vs. Concentration graph for Ascorbic Acid and Extract of Pet ether.

## 5.6.2 Results

From the above Graph:

- $IC_{50}$ of Ascorbic acid (standard) : 77.71313 µg/ml
- $IC_{50}$ of *Ampelocissis barbata* (fruits extract of Pet ether) : 24.4608 µg/ml

## 5.6.2 Discussion

Direct tissue toxicity and vascular collapse associated with septic shock may result from a sustained production of the nitric oxide radical; moreover, chronic expression of the radical contributes in many carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Okuda T *et al.,* 1983). Extract showed significant NO. scavenging activity as compared to the standard.

## 5.7 Determination of Hydrogen Peroxide Scavenging Assay

**Table 5.7.1 : Hydrogen Peroxide Scavenging assay of Ascorbic Acid.**

| | | Ascorbic Acid | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration | % scavanged | Ab1 | Ab2 | Ab3 | Ab4 | Aver Ab | SC50 |
| Blank | | 3.6123 | 3.4362 | 3.6123 | 3.6123 | 3.568275 | |
| 6.25 | 34.40738172 | 2.2506 | 2.2504 | 2.601 | 2.2601 | 2.340525 | |
| 12.5 | 39.53871268 | 2.1575 | 2.1572 | 2.1576 | 2.1574 | 2.157425 | 96.04 |
| 25 | 55.8953556 | 1.5748 | 1.5748 | 1.5709 | 1.5746 | 1.573775 | |
| 50 | 59.37042408 | 1.448 | 1.4495 | 1.4492 | 1.4524 | 1.449775 | |
| 100 | 60.37300937 | 1.4122 | 1.4137 | 1.415 | 1.4151 | 1.414 | |
| 200 | 75.92113837 | 0.8575 | 0.8588 | 0.8599 | 0.8606 | 0.8592 | |
| 400 | 80.83808002 | 0.6836 | 0.684 | 0.6836 | 0.6838 | 0.68375 | |
| 800 | 83.86965691 | 0.5757 | 0.5754 | 0.5755 | 0.5757 | 0.575575 | |

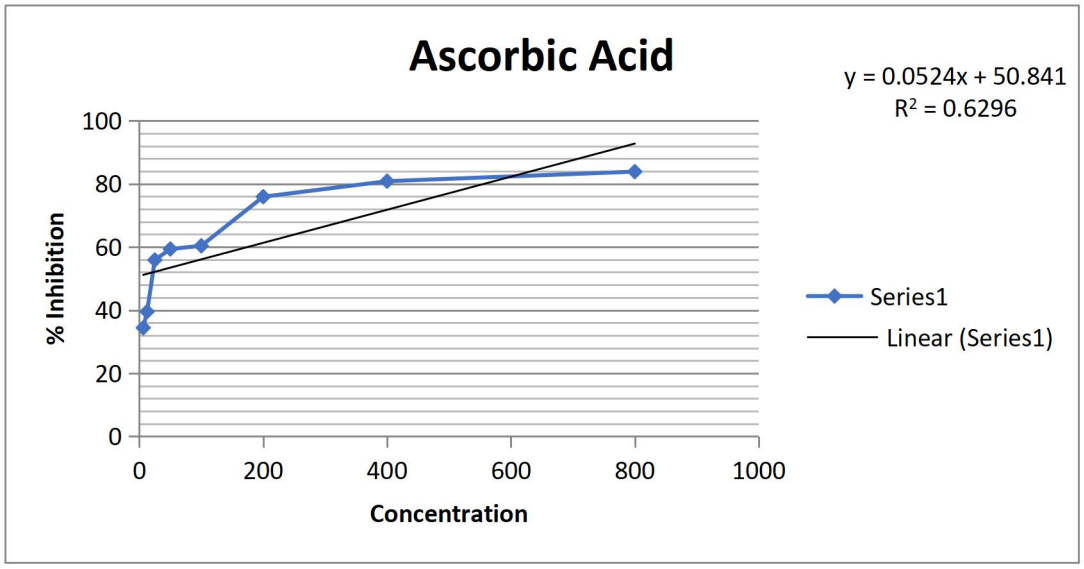



**Figure 5.8 :** $SC_{50}$ determination of Ascorbic acid

**Table 5.7.2 : Hydrogen Peroxide Scavenging assay of Extract**

| Concentra | % scavang | Ab1 | Ab2 | Ab3 | Ab4 | Aver Ab | SC50 |
|---|---|---|---|---|---|---|---|
| Blank | | 3.6123 | 3.4362 | 3.6123 | 3.6123 | 3.568275 | |
| 6.25 | 37.31565 | 2.2321 | 2.2506 | 2.2321 | 2.2322 | 2.23675 | |
| 12.5 | 75.10352 | 0.8888 | 0.8876 | 0.8893 | 0.8878 | 0.888375 | 29.2 |
| 25 | 75.27026 | 0.8827 | 0.8823 | 0.882 | 0.8827 | 0.882425 | |
| 50 | 75.59885 | 0.8711 | 0.8707 | 0.8706 | 0.8704 | 0.8707 | |
| 100 | 77.28244 | 0.8102 | 0.8105 | 0.8113 | 0.8105 | 0.810625 | |
| 200 | 80.65382 | 0.6906 | 0.6906 | 0.6903 | 0.6898 | 0.690325 | |
| 400 | 80.95438 | 0.6793 | 0.6798 | 0.6796 | 0.6797 | 0.6796 | |
| 800 | 84.75033 | 0.5439 | 0.5443 | 0.5441 | 0.5443 | 0.54415 | |

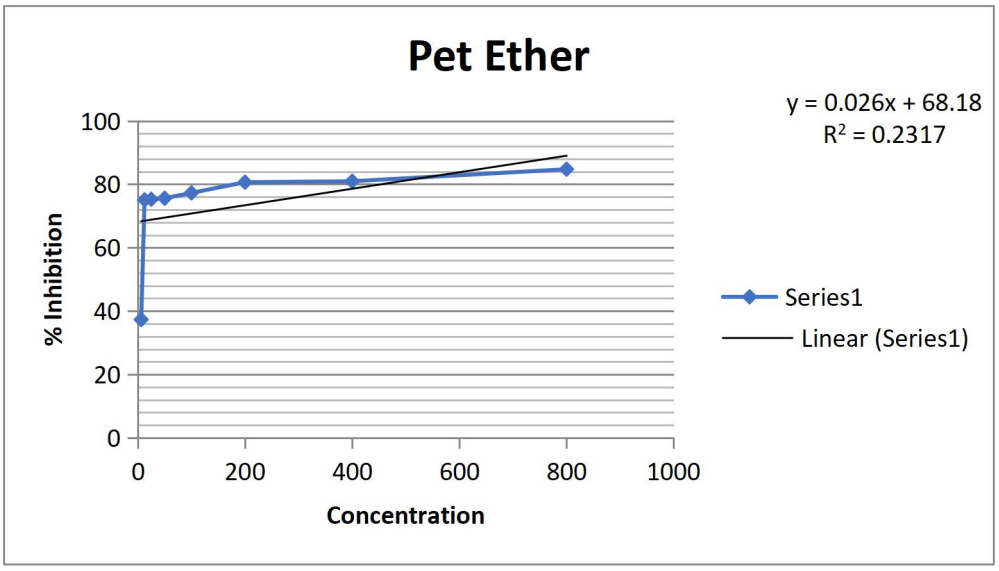



**Figure 5.9 :** $SC_{50}$ determination of Extract

$SC_{50}$ = 43.22 µg/ml

for Ascorbic acid

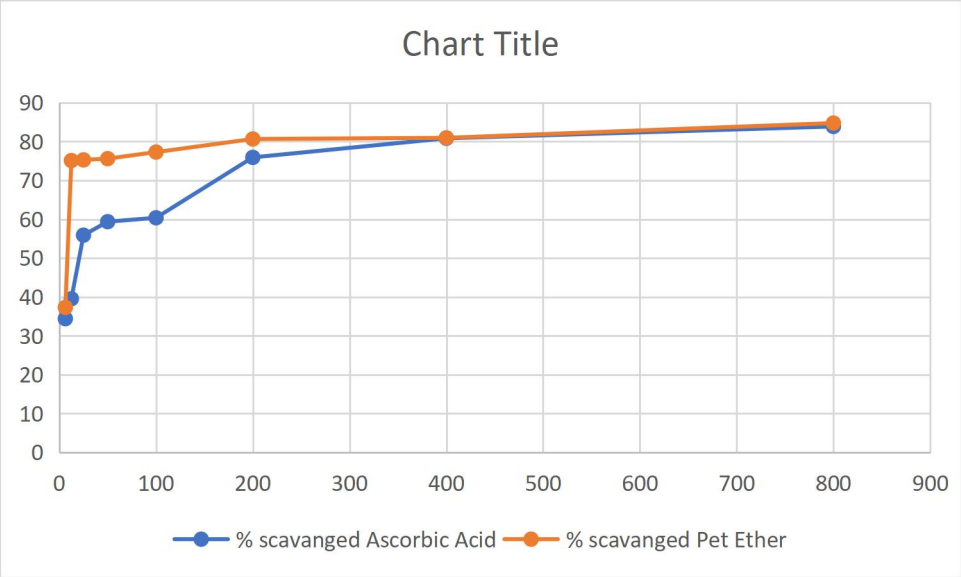



**Figure 5.10 :** Comparison of % Scavenged vs. Concentration graph for Ascorbic acid and Extract of Pet ether.

## 5.7.1 Results

From the above Graph:

- $SC_{50}$ of Ascorbic acid (standard) : 96.04 µg/ml
- $SC_{50}$ of *Ampelocissis barbata* (fruits extract of Pet ether) : 29.2 µg/ml

## 5.7.2 Discussion and Conclusion

- In the present study, Extract bulb extract showed a $SC_{50}$ value of 29.2 µg/ml which is comparable to the standard ($SC_{50}$ = 29.2 µg/ml) used. This scavenging activity may be due to the presence of various Phytochemicals including Phenolics and Flavonoids in methanolic extract of pet ether from *Ampelocissus barbata* fruits Extract.

## 5.8 Determination of Hydroxyl Radical Scavenging Assay

**Table 5.8.1 : Hydroxyl Radical Scavenging Assay of Ascorbic Acid.**

| | | Ascorbic Acid | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration | % scavanged | Ab1 | Ab2 | Ab3 | Ab4 | Aver Ab | SC50 |
| Blank | | 0.1531 | 0.1536 | 0.1538 | 0.1542 | 0.153675 | |
| 6.25 | 32.95916707 | 0.1028 | 0.1029 | 0.1028 | 0.1036 | 0.103025 | |
| 12.5 | 48.44639662 | 0.0789 | 0.0793 | 0.0792 | 0.0795 | 0.079225 | 138.8 |
| 25 | 50.07320644 | 0.076 | 0.0764 | 0.077 | 0.0775 | 0.076725 | |
| 50 | 51.16316903 | 0.0751 | 0.075 | 0.0751 | 0.075 | 0.07505 | |
| 100 | 54.28664389 | 0.0702 | 0.0702 | 0.0703 | 0.0703 | 0.07025 | |
| 200 | 56.0924028 | 0.0668 | 0.0673 | 0.0677 | 0.0681 | 0.067475 | |
| 400 | 57.14982918 | 0.0657 | 0.0658 | 0.0659 | 0.066 | 0.06585 | |
| 800 | 58.25605987 | 0.064 | 0.0642 | 0.0641 | 0.0643 | 0.06415 | |

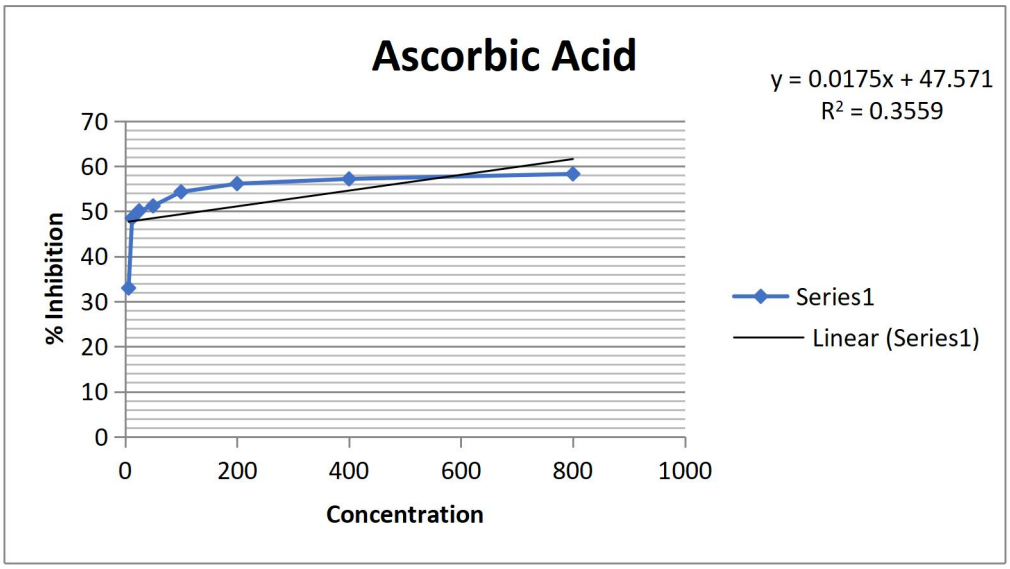



**Figure 5.11 :** $SC_{50}$ determination of Ascorbic acid

**Table 5.8.2 : Hydroxyl Radical Scavenging Assay of Extract.**

| Concentra | % scavang | Ab1 | Ab2 | Ab3 | Ab4 | Aver Ab | SC50 |
|---|---|---|---|---|---|---|---|
| Blank | | 0.1531 | 0.1536 | 0.1538 | 0.1542 | 0.153675 | |
| 6.25 | 1.854563 | 0.1506 | 0.151 | 0.1508 | 0.1509 | 0.150825 | |
| 12.5 | 11.90825 | 0.1348 | 0.1353 | 0.1355 | 0.1359 | 0.135375 | 39.17 |
| 25 | 15.30828 | 0.1299 | 0.1301 | 0.13 | 0.1306 | 0.13015 | |
| 50 | 21.24614 | 0.1208 | 0.121 | 0.1211 | 0.1212 | 0.121025 | |
| 100 | 38.08362 | 0.0946 | 0.0948 | 0.0953 | 0.0959 | 0.09515 | |
| 200 | 46.42915 | 0.0789 | 0.0798 | 0.0848 | 0.0858 | 0.082325 | |
| 400 | 50.10574 | 0.0763 | 0.0766 | 0.0768 | 0.077 | 0.076675 | |
| 800 | 53.45697 | 0.0717 | 0.0714 | 0.0715 | 0.0715 | 0.071525 | |

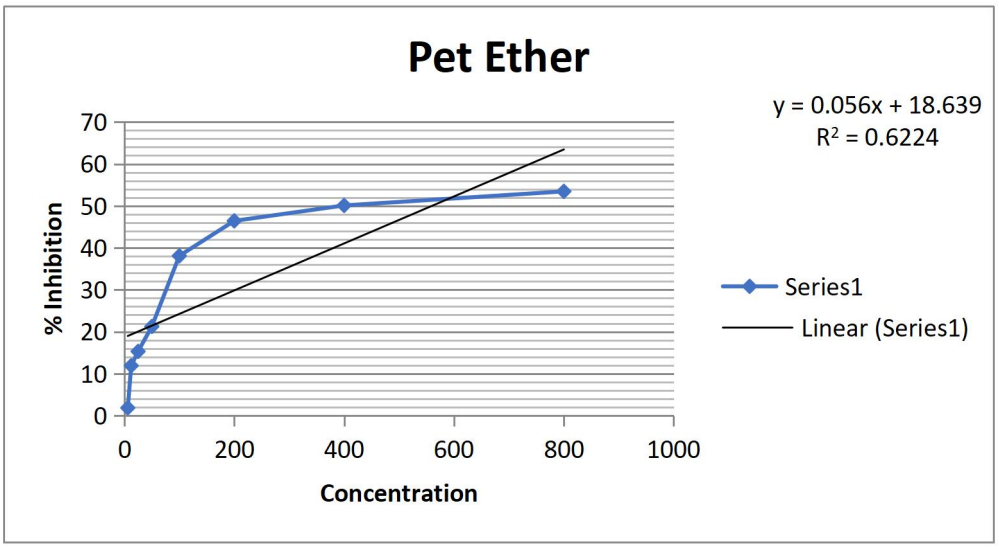



**Figure 5.12 :** $SC_{50}$ determination of Extract

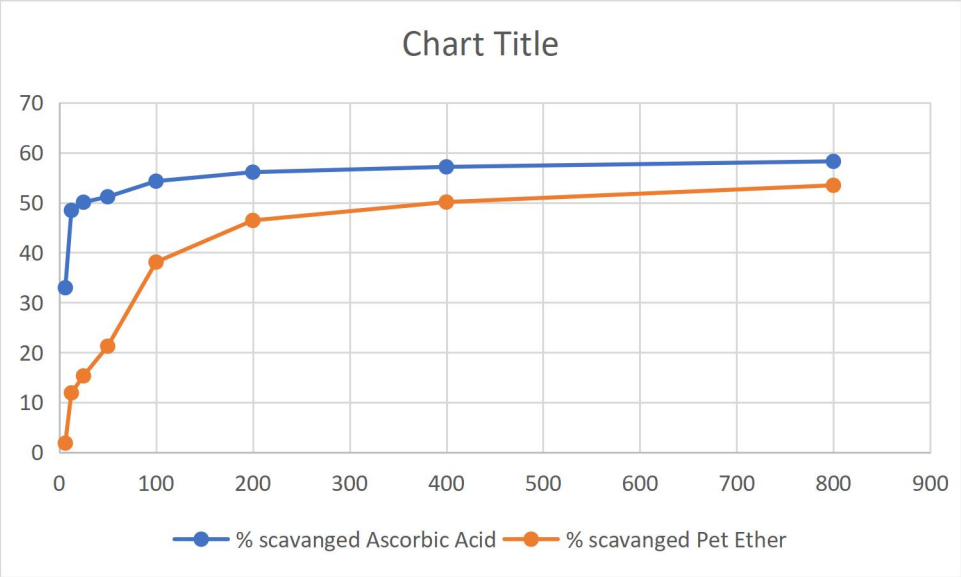



**Figure 5.13 :** Comparison of % Inhibition vs. Concentration graph for Ascorbic acid and Extract of Pet ether.

## 5.8.1 Results

From the above Graph:

- $SC_{50}$ of Ascorbic acid (standard) : 138.8 µg/ml
- $SC_{50}$ of *Ampelocissis barbata* ( fruits extract of Pet ether) : 39.17 µg/ml

## 5.8.1 Discussion and Conclusion

Hydroxyl Radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell (Battu, C.R. et al., 2011). In the present study, Extract bulb extract showed moderate hydroxyl radical scavenging activity which is comparable to the standard used. The hydroxyl activity may be due to the presence of various phytochemicals including Phenolics and Flavonoids in methanol extract of Extract.

# Chapter Six
# Conclusion

## 6.1 Conclusion

This study is an emerging field of research in evaluating many Antioxidant effects. If the proposed research can be performed successfully, a valuable resource for potent drug can be obtained. That may provide a safe, effective, economic and convenient source of potential drugs with desirable clinical properties. If we get opportunity we will carry on our research to evaluate other pharmacological properties.

Fruits of *Ampelocissus barbata* was macerated in 70% ethanol to produce extract which was tested for a number of in vitro biological experiments e.g. Antioxidant activity (Total Phenolic Content, Total Tannis Content and Total Flavonoid Content, Hydrogen Peroxide Scavenging Assay, Hydroxyl Radical Scavenging Assay, Nitric Oxide (No) Scavenging Assay, DPPH Scavenging Assay and Phytochemical Screening Test. Different concentration of this extract was statistically evaluated and significant activity was seen for the in vitro tests. Test groups were significantly different for more time points from control as the concentration increased. Only higher concentration of extracts was significantly exerting these activities.

From the above discussion, it can be concluded that the methanolic extract of the plant has DPPH radical scavenging, NO scavenging, hydrogen peroxide scavenging, hydroxyl radical scavenging and antioxidant activity as compared to standard. Different concentration of this extract was statistically evaluated and significant activity was seen. Therefore, fruits of *Ampelocissus barbata* can be used to prepare natural products which can be safely taken by all people.

This study provides valuable information about the plant extracts composition and biological activities and may serve as a basis for further research into the development of new antioxidant.

Furthermore, significant Phenolic compounds are present in plants, have antioxidant activity due to their redox properties, and therefore play a vital role in counterbalancing the free radicals.

## Conclusion

Further comprehensive pharmacological and Phytochemical study for the isolation and characterization of the specific compound is required to get a more potent agent with significant activity. Since the polyphenol compounds as well as other components with potent antioxidant activity are not known, thus advanced level of work should be performed for the isolation and identification of the antioxidant components in *Ampelocissus barbata* fruits. However, extensive and detailed Phytochemical investigations into the plant are required in future in order to identify and characterize potential bioactive components.

More in-depth studies should follow this investigation.

# Chapter Seven
# Reference

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