In-Vitro Determination of Antioxidant Capacity for Methanolic extract of *Eichhornia crassipes, Lawsonia inermis L. and Cissus quadrangularis L.* by DPPH (1,1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Assay

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A thesis report, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled

"*In-Vitro* Determination of Antioxidant Capacity for Methanolic extract of *Eichhornia crassipes, Lawsonia inermis L. and Cissus quadrangularis L.* by DPPH (1,1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Assay" is an authentic and genuine research work carried out by me under the guidance of Mr. Amran Howlader, Senior lecturer, Department of Pharmacy, East West University, Dhaka.

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CERTIFICATE BY THE INVIGILATOR

This is to certify that the dissertation, entitled

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ENDORSEMENT BY THE CHIRPERSON

This is to certify that the dissertation, entitled

"Brine Shrimp Lethality Bioassay & Antimicrobial Investigation of the n-Hexane Extract of *Aphanamixis polystachya* Leaf"is a bonafide research work done by Md. Rakibul Islam, under the guidance of Mr. Apurba Sarker Apu, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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ACKNOWLEDGEMENT

I express my profound gratitude and deep respect to Mr. Amran Howlader, Senior Lecturer, Department of Pharmacy, East West University, for his expert and careful guidance, sincere help, constructive criticism, valuable time and honored suggestion without which I would not have been able to complete this work. He had been very enthusiastic and supportive in my research.

It is my great pleasure and privilege to acknowledge my deepest regards and gratitude to Dr. Sufia Islam, Chairperson and Associate Professor of the Department of Pharmacy, East West University, for her kind words during my troubling moments, and of course for her approval of the topic, constant inspiration and whole hearted cooperation.

I am also grateful to Mr. Abu Taiab Md. Jamaluddind and Mr. Apurba Sarker Apu, Senior Lecturer, Department of Pharmacy, East West University, for his professional helps which actually carved the path of our research.

I am thankful to the laboratory instructors Mr. Ajoy Roy, Mrs. Razia Sultana and Mrs. Tania for their kind support during the laboratory work.

I also like to thank my family members for their support and inspiration to complete this work.

Above all, I express my gratitude to Almighty Allah for giving me the strength, energy and patients to carry out this research work.

Dedicated to

The Strangers (Gurabah) who will

Revive the Nation

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ABSTRACT

Among the most used medicinal plants *Eichhornia crassipes*, *Cissus quagragualis L.*and *Lowsonia inermis L.* are very common. There were a goodmany work on these plant, but the antioxidant activity is not so satisfactory. To justify their antioxidant activity leaf of *Lowsonia inermis L.*, whole plant of *Cissus quagragualis L* and leaf of *Eichhornia crassipes* were taken. Their Methanolic extract was used and tested their antioxidant activity by DPPH Free radical scavenging assay (Quantitative analysis). Ascorbic acid was used as standard here. Activity of *Cissus quagragualis L* (IC_{50} = 11.3163 µg/ml) and *Lowsonia inermis L* (IC_{50} = 17.0689 µg/ml) are quite seticfactory. They have strong antioxidant activity. But, *Eichhornia crassipes* (IC_{50} =186.1196 µg/ml) gives almost no activity.

Key words: *In-vivo, in-vitro,* antioxidant, DPPH, IC_{50} etc.

Chapter One

INTRODUCTION

1 Introduction

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, “A medicinal plants is any plant which, in one on more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis.” When a plant is designated as ‘medicinal’, it is implied that the said plant is used as a drug or therapeutic agent or an active ingredient of a medicinal preparation.

“Medicinal plants may therefore be defined as a group of plants that possess some special properties that quality them as articles of drugs and therapeutic agents, and are used for medicinal purposes”. Nature is a best friend of our pharmacy field. Natural drugs are effective in action without side effects. (Ghani A 1998)

Natural products, including medicinal plants, have been the primary source for obtaining new drugs with therapeutic potential throughout history. It is estimated that approximately half of the drugs in use are derived from natural products. This means that around 4 billion people rely on natural products as a source of their primary medicinal needs. It is proved that half of the world's best selling drugs and many potential drugs under development are derived from plants. Some time ago the World Health Organization (WHO) reported that about 80% of the population in developing countries used some kind of traditional medicine in primary health care, emphasizing the use of herbal products (WHO 1993). According to Farnsworth (1985), up until 1985, of the 119 chemicals extracted from plants and used in medicine, 74% were first discovered through popular knowledge. Koehn & Carter reported that around 25% of prescribed drugs worldwide were obtained directly or indirectly from plants. Moreover, about 50% of drugs developed between 1981 and 2002 were obtained from natural products, semi-synthetic analogues or synthetic compounds based on natural products.

Medicinal plants represent an important health and economic component of biodiversity. Plants are the natural reservoir of many antimicrobial, anticancer agents, analgesics, anti-diarrheal as well as various therapeutic activities. Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for the treatment of various

diseases. Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders. About 25% of prescribed drugs in the world originate from plants and over 3000 species of plants have been reported to have anticancer properties. About 80% of the population in developing countries rely on traditional plant based medicines for their primary health care needs.In addition of possessing various other medicinal properties, 257 of these medicinal plants have been identified as efficacious remedies for diarrhoeal disease and 47 for diabetes. A large number of plants in different location around the world have been extracted and semi-purified to investigate individually their antimicrobial activity (Dranghon, 2004).

In Bangladesh, ninety percent of the medicinal plants are wild sourced. Out of approximately 5,000 species of indigenous and naturalized phanerogamic and pteridophytic plants growing in the country, more than a thousand of them, including many food, vegetable, beverage, spice and ornamental plants. Water plants are taxonomically different as there is generally a lack of adequate herbarium material and a paucity of critical studies in the development of various organs due to the high degree of adaptability in form and structure in relation to aquatic environment. Growing in the forests, jungles, wastelands, and along roadsides, the types of medicinal plants in Bangladesh are varied. Out of them more than a thousand have been claimed to posses medicinal or poisonous properties, of which 546 have recently been enumerated with their medicinal properties and therapeutic uses. Indigenous medicinal plants containing active and medicinal principles like glycerides, alkaloids, steroids, tannins etc. grow abundantly in Bangladesh. These indigenous medicinal plants are extremely used in both raw and semi-processed forms in the preparation of pharmaceutical, Homeopathic, Unani, and Ayurbedic medicines. Although our country is rich with this vast natural resource but due to lack of knowledge none processes these indigenous medicinal plants or its extracts locally. As a result every year Bangladesh imports a huge quantity of processed indigenous medicinal plants or its extracts from abroad at the cost of our foreign exchange to meet the country’s demand. So, efforts have been made to systematic processing and screening of indigenous medicinal plants as pharmaceutical raw materials.

In rural areas medicinal plants have been being used as remedy for disease for a long time. They not only cure the disease but also provide an important role in the economy. Medicinal plants are

cheap and easy to get to those people who knew it very well. Bioactive compounds are deposited in medicinal plants; it can serve as important raw materials for pharmaceutical manufacturing. They comprise a precious asset of a country and donate to its health care system. Well-judged and scientific investigation of this wealth can significantly contribute to the public health. Besides being available commodity of commerce, a country can also earn a good amount of foreign currency by exporting this natural wealth to other countries. More than 500 of such medicinal plants have so far been established as growing in Bangladesh. Almost all of these indigenous medicinal plants are extensively used in the preparation of unani, ayurvedic and homeopathic medicines in Bangladesh.

The goals of using plants as sources of therapeutic agents are (Fabricant DS and Farnsworth NR, 2001)

a) to isolate bioactive compounds for direct use as drugs, e.g. digoxin, digitoxin, morphine, reserpine, taxol, vinblastine, vincristine;
b) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g., metformin, nabilone, oxycodon (and other narcotic analgesics), taxotere, teniposide, verapamil, and miodarone, which are based, respectively, on galegine, Δ9-tetrahydrocannabinol, morphine, taxol, podophyllotoxin, and khellin;
c) to use agents as pharmacologic tools, e.g., lysergic acid diethylamide (LSD), mescaline, yohimbine; and
d) to use the whole plant or part of it as a herbal remedy, e.g., cranberry, echinacea, feverfew, garlic, etc.

1.1 *Cissus quadrangularis*

1.1.1 Plant Description

Cissus quadrangularis is a perennial plant of the grape family. It is commonly known as Veldt Grape or Devil's Backbone. *Cissus quadrangularis* reaches a height of 1.5 m and has quadrangular-sectioned branches with internodes 8 to 10 cm long and 1.2 to 1.5 cm wide. Along each angle is a leathery edge. Toothed tri lobe leaves 2 to 5 cm wide appear at the nodes. Each has a tendril emerging from the opposite side of the node. Racemes of small white, yellowish, or greenish flowers; globular berries are red when ripe. The flowers are also typical of the grape family are greenish white and come at the end of the summer. The stems are often leafless, when old. Few aerial roots arise from the joined nodes and grow towards the soil, The leaves are small, simple, heart shaped and toothed.

The flowers are small, greenish white, in short cymes. The fruits are globose or ovoid berries, red when ripe. The author of Nighantu Ratnakara mentions the there are two varieties of this climber, namely, of four – sided and three – sided stems. But only one variety of four – sided stem is reported; which at times may be seen shooting out branches having three sides. A two – sided horticultural variety is also grown in gardens. Precisely, it is also named as asthisandhani, which describes its peculiar quality of healing the bone fractures. Denoting the same property, is has few synonyms, like vafravati, asthisrnkhala, vajrangi, granthimala etc.

The plant has been mentioned in all ancient scriptures of Ayurveda. It is used in folklore medicine to heal bone fractures, throughout India. The Sanskrit names, mentioned above, also hint at the same use by ancient physicians. (Preciousproducts 2012)

1.1.2 History

Cissus quadrangularis is an ancient medicinal plant native to the hotter parts of Ceylon and India. It was prescribed in the ancient Ayurvedic texts as a general tonic and analgesic, with specific bone fracture and tendon healing abilities. Modern research has shed light on Cissus' ability to speed bone healing by showing it acts as a glucocorticoid antagonist. Since anabolic/androgenic compounds are well known to act as antagonists to the glucocorticoid receptor as well as promote bone and tendon growth and fracture healing, it has been postulated that Cissus possesses anabolic and/or androgenic properties. In addition to speeding the remodeling process of the healing bone, Cissus also leads to a much faster increase in bone tensile strength. In clinical trials Cissus has led to a fracture healing time on the order of 55 to 33 percent of that of controls. That cissus exerts antiglucocorticoid properties is suggested by a number of studies where bones were weakend by treatment with cortisol, and upon administration of Cissus extract the cortisol induced weakening was halted, and the healing process begun. A pure extract like CissusMax is suggested as to use all the active ingredients in the plants in a synergistic effect.

(Cissus Experts 2012)

1.1.3 Taxonomic Classification

Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Rosidae*

Order	*Rhamnales*
Family	*Vitaceae*
Genus	*Cissus* L.
Species	*Cissus quadrangularis* L.

(USDA 2012)

Figure 01: Some Identical Pictures of *Cissus quadrangularis L.*

1.1.4 Names

Synonyms

- *Cissus succulenta* (Galpin)
- *Cissus tetragona* Harv.
- *Vitis quadrangularis* (L.)
- *Vitis succulenta* Galpin (Cissus quadrangularis 2012)

Common Names

- English: edible stemmed vine (Dalziel).
- French: vigne de Bakel, cissus de Galam, raisin de Galam (Berhaut).
- West African: *BEDIK, DIOLA, FULA-PULAAR, TUKULOR*

1.1.5 Species Variation

It has a good many species, through out the world. Here a chart is given with their respective country.

Species	**Location**
C. araloides	Cameroon
C. assamica	China, India, Cambodia, Bhutan, Nepal, Thailand
C. debilis	Cameroon
C. hamaderohensis	Yemen
C. hypoglauca	Australia
C. ibuensis	Nigeria (Africa), Niger, Ghana
C. populnea	Nigeria (Africa)
C. quadrangularis	India, Sri Lanka (Asia)
C. rotundifolia	Africa, South America

C. rubiginosa	Congo
C. verticillata	Trinidad and Tobago (Carribean)
C. sicyoides	Brazil (South America)

(Fernandes & Banu 2012)

1.1.6 Macroscopic Characters

Stem: Stem is buff coloured with greenish tinge. Drug occurs as pieces of stem of varying lengths. Stem is slender dichotomously branched, sub-angular, glabrous, brown, fleshy, fibrous, smooth with 4-winged internodes Constricted at nodes . Internodes 4-5 cm long; and 1-2 cm thick; a tendril occasionally present at nodes aerial roots developing during the rainy season.

Leaf: Leafless when old, leaves are 5-15 cm long; cordate, broadly ovate or reniform, crenate-serrate, sometimes 3-7 lobed, glabrous 2.5-7.5 cm x 3 -9 cm; (Vasu, 2012)

Flowers: Flowers are small, greenish white, in short umbellate cymes.

Fruit: Berries are obovoid or globose in shape, succulent, very acrid, pea-sized 1-seeded.

1.1.7 Microscopic Characters

Mature stem shows squarish outline with prominent at each anular point. Epidermis is single layered, covered externally with thick cuticle; epidermis cells thin walled, rectangular and tangentially elongated, followed by 2-3 layers of cork and single layered cork cambium; cortex composed of 8-16 layers of thin-walled, circular to oval parenchymatous cells; four patches of collenchymatous cells present in all the four angular points embedded in cortical region like an umbrella arching over large vascular bundles; in the projected portion of angular region cortical cells filled with brown-red contents present; endodermis not distinct; stele consists of a large number of vascular bundles varying in size arranged in the form of a ring separated by rays of parenchyma; 3-4 vascular bundles larger in size, in each angular region,

below collenchymatous patch, while rest of bundles smaller in size; vascular bundles collateral and open type, capped by sclerenchymatous sheath which is well developed in larger bundles; cambium and interfascicular cambium quite distinct; central region occupied by a wide pith composed of thin-walled, circular to oval parenchymatous cells; idioblasts containing raphides and isolated acicular crystals of calcium oxalate present in the outer region of cortex also found in most of the cells in cortical region; starch grains present throughout the cortical and the pith region. (Vasu 2012)

Powder characteristics: Powder is Brown in colour; it shows fragments of vessels, fibers, parenchymatous cells and a few rosette crystals of calcium oxalate, starch grains and idioblast containing raphides and isolated acicular crystals of calcium oxalate. (Vasu 2012)

1.1.8 Distribution

It is probably native to India or Sri Lanka, but is also found in Africa, Arabia, and Southeast Asia. It has been imported to Brazil and the southern United States. It is very commonly known as asthisamharaka. As per Dr Jyotiraditya Agarwal (Chief Ayurvedic Consultant of Nidanam Clinic), Cissus has been used in various ayurvedic classical medicines to heal broken bones and injured ligaments and tendons. (Cissus quadrangularis 2012)

1.1.9 Use

Cissus quadrangularis stem is used medicinally in the indigenous systems of medicine both in the ayurvedic and unani systems. The stem is alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, irregular menstruation and asthma, fractures of bones

and in complaints of the back and spine .It is said to be very useful in piles, indigestion and anorexia, useful in anaemia, epitaxis, disorders of otorrhoea, given internally and applied topically for broken bones, beaten into a paste and administered in asthma. The stem juice is beneficial as alterative in scurvy and irregular menstruation, and in diseases of the ear and in nose bleeding. A paste of the stem is given in asthma, and may be useful for muscular pains, burns and wounds, bites of poisonous insects and for saddle sores of horses and camels. The powder of dry shoots is given in digestive troubles. A decoction of the shoots with dry ginger and black pepper is given for body-pains. The infusion of the plant is anthelmintic. The extract of the plant exhibits cardiotonic and androgenic property. The roots of Cissus quadrangularis are useful in treatment of fractured bones. Young shoots are used in treatment of dyspepsia and indigestion and considered as to be powerful alterative. It is also used in Malaria, Cancer, Upset stomach, Hemorrhoids, Stomach ulcers, Bodybuilding, Menstrual discomfort. (Vasu 2012)

1.2 *Lawsonia inermis L.*

1.2.1 Plant Description

The scientific name of the Henna Plant is Lawsonia inermis, the Genus being Lawsonia. It is from the family Lythraceae, also known as the loosestrife family. It has other names such as, Egyption Privet, Reseda, and Jamaica mignonette. Other names would be; El-Henna, Egyptian priest, and is sometimes known as Lawsonia Alba Lam. and Lawsonia Ruba. The Henna Plant is a tropical shrub or a small tree. A Henna plants flowers are sweet-scented, and are creamy or red (tattoo me, 2006). Each flower has 4 petals, 8 stamens, and 4 sepals. The Henna plant is a shrub, and at its highest it can reach up to 7 meters. The leaf of this plant is the shape of an almond. Its Bark is grayish-brown and the wood of the Henna plant is hard.

If temperatures never drop below 11C then the Henna plant can be grown outdoors. The best place to cultivate the Henna plant is in a place with the lowest moisture and highest heat, this creates the most amount of tannin. Tannin is what contributes to the rich colour of Henna. The shrub grows best in places without water, and dry soils.

It is a shrub that grows in North Africa, and Middle East. It is also native to Asia, and Australia. It is cultivated in India, Pakistan, Yemen, Morocco, Afghanistan, Sudan, Somalia, and Libya.

(Texiwala 2012)

Plant Class: Desert Shrub: glabrous, multibranched with spine tipped branchlets.

Leaves: opposite, entire, glabrous, sub-sessile, elliptical, and broadly lanceolate.

Fruit: Fruits are small, brownish capsules, 4–8 mm in diameter, with 32–49 seeds per fruit.

Flowers: Four sepals and a 2 mm calyx tube with 3 mm spread lobes. Petals are obvate, white or red stamens inserted in pairs on the rim of the calyx tube.

Figure 02: Some Pictures of *Lawsonia inermis L.*

1.2.2 Texonomical Classification

Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Myrtales*
Family	*Lythraceae*
Genus	*Lawsonia L.*
Species	*Lawsonia inermis L.* (USDA 2012)

1.2.3 History

Henna is a flowering plant and a native to tropical and subtropical regions of Africa, southern Asia and northern Australia. It is commonly used as hair dye and with the ascending trend of tattoos henna is used for body tattoos which is considered the most safe and painless alternative form of body ornamentation. It is been used for colouring hair and hands from thousands of years. Other than colouring hairs and hands henna has medicinal uses too.

(Lawsonia Inermis 2012)

1.2.4 Names

Trade Name: Henna

Most Popular Common Names:

Arabic: Henna, Henne, Hine, Hina, Hene, Heni

Pakistan, India & Bangladesh: Mendhi, Mehndi, Mehendi

Synonyms

Lawsonia spinosa L., *Lawsonia alba* Lamk.

Table 01: Some Lesser Known Common Names with Area.

Area	**Name**	**Area**	**Name**
Creole	Ene, Flè Jalouzi	Kashmir	Mohuz
Filipino	Cinamomo	Lao	Kaaw

Area	Name	Area	Name
English	Camphire, Egyptian Privet, Mignonette, Zanzibar Bark	Khmer	Krâpéén
French	Henné, Jalousie, Réséda de France	Malay	Inai, Pacar Kuku
Indonesian	Inai, Pacar Kuku	Sanskrit	Mendika, Ragangi, Raktgarbha
Javanese	Pacar Kuku	Somali	Erip
Spanish	Resedá	Swahili	Mheni, Mhina, Mkokoa, Mkokoa Muhina, Muina
Tamil	Marithondi, Maruthani	Thai	Thian Daeng, Thian Khaao, Thian King
Tigrigna	Elam	Vietnamese	Lâ Mòn, Nhuôm Móng Tay

(WEST63RD Internet, 2007)

1.2.5 Origin and Geographical Distribution

Lawsonia Inermis is native throughout the Middle East, North Africa and the Indian subcontinent. It flourishes in sunny positions in heavy soils that hold moisture well – though it will grow almost anywhere in these regions. These days it is also widely cultivated in China, The West Indies and Australia as well as in its native countries. (WEST63RD Internet 2007)

1.2.6 Uses

Leaves

They are treasured as a valuable source of natural dye. It is used cosmetically to decorate the skin; chiefly hands and feet of women and to decorate fabrics and other natural materials. Leaves also containing antiseptic, antibiotic and astringent properties, are used in infusions, decoctions, poultices and ointments in traditional medicines. To treat such ailments as; general headaches, epilepsy, tetanus, leprosy, jaundice, scurvy, beriberi,various skin and nail conditions (from athletes foot to herpes), open wounds, ulcers and lesions.

Flowers

Flowers are high in beta-ionone, a treasured olfactory ingredient. They are used in the manufacture of perfumes and in the manufacture of scented oils & incense. They are also used in traditional medicines to treat such ailments as; epilepsy and tetanus, general stomach pains and insomnia.

Fruit

It is a treasured olfactory ingredient. It is used in the manufacture of perfumes. and in the manufacture of scented oils & incense. It is also used in traditional medicines to treat such ailments as; excema and various skin conditions

Bark

Bark can be used in traditional medicines. To treat such ailments as; jaundice, excema and fungal infections it is used.

Roots

roots can be used in traditional medicines. To treat such ailments as; eye disease and skin various skin conditions it is used. It is also used as a diuretic and are also believed to have fertility enhancing properties. (WEST63RD Internet 2007)

1.3 *Eichhornia crassipes L.*

1.3.1 Plant Description

The plant is a perennial aquatic herb (*Eichhornia crassipes)* which belongs to the family Pontedericeae, closely related to the Liliaceae (lily family).Water hyacinth (*Eichhornia crassipes*) has been called the world's worst aquatic weed. It is a free floating water plant that is native to South America. It can vary in size from a few inches tall to over three feet. This plant has blue-green leaves, thick stalks and a showy purple or lavender flower. It thrives in tropical regions and in waters that are high in nutrients. Its beautiful, large purple and violet flowers have made it a popular ornamental, and the plant is now naturalized in most of the southern United States. It reproduces mostly by clonal propagation, but seeds also play a role in its survival and colonization. Massive weed colonies can grow when introduced into areas that are conducive for their proliferation. In addition, infestation can occur given a disruption in the natural ecological balance by human activities such as impounding of flowing waters by dams, channeling and allowing the buildup of eutrophication.

Water hyacinth prefer water temperatures of 65 degrees and higher, so they are considered tropical aquatic plants. They are very fast growing plants that can benefit from additional fertilizer to keep them lush and green. The water hyacinth is sometimes sold at aquarium shops, but more often available from pond plant dealers. Water hyacinth infestations increase most rapidly by the production of new daughter plants. During high water flows and flooding, infestations can break up and be moved to new locations. Most spread can be attributed to human activity such as the deliberate planting of water hyacinth in ornamental ponds or dams. Unwanted aquarium plants that are discarded into waterways are a major form of spread. Water

hyacinth can also be spread by contaminated boating equipment. Seeds are the main source of new infestations and are carried in water, mud (e.g. on machinery or boots) and by birds.

Water hyacinth grows in still or slow-flowing fresh water in tropical and temperate climates. Optimum growth occurs at temperatures of between 28°C and 30°C, and requires abundant nitrogen, phosphorus and potassium. Although this plant will tolerate a wide range of growth conditions and climatic extremes including frost, it is rapidly killed by sea strength salinity and will not grow in brackish water. Where water levels have receded, plants can survive on damp soil for several months.

1.3.2 Distribution

Water hyacinth is native to South America, where it forms vast mats on the water's surface and was introduced to the United States in the 1880s. It probably originated in the swamps associated with the great river systems of northern and central South America. It becomes naturalized in many warm areas of the world: Central America, North America (California and southern states), Africa, India, Asia, Australia, and New-Zealand (Gopal 1987).

Water hyacinth has not yet (1998) been found in the wild in Washington State, but has been sold as an ornamental in plant nurseries. Its use as an ornamental means that it could be introduced to our lakes and rivers, and this is expected to be its primary method of spread.

1.3.3 Broad Classification / Taxonomy

Kingdom	Plantae
Division	Magnoliophyta

Class	Liliopsida (monocotyledons or monocots, having one cotyledon, or embryonic leaf, in the seed).
Order	Liliales
Family	Pontederiaceae
Genus	*Eichhornia*
Species	*Eichhornia crassipes*

1.3.4 Names

Local name: Kochuripana

Synonyms

Eichhornia cordifolia Gand.

Eichhornia crassicaulis Schltdl.

Eichhornia speciosa Kunth

Heteranthera formosa Miq.

Piaropus crassipes Mart.

Common names: .

Area	**Names**
English	Nile lily, water hyacinth.
Fijian	Bekabekairaga, dabedabe ne ga, mbekambekairanga, ndambendambe ni nga.
French	Jacinthe d'eau, pensée d'eau.

Hindi	Jal khumbe.
Japanese	Hoteiaoi.
Maori (Cook Islands):	Riri vai.
Palauan	Bung el ralm.
Samoan	Aguapey, buchon, calamote, jacinto aquático, jacinto de agua, jacinto de rio.
Spanish	Aguapey, buchon, calamote, jacinto aquático, jacinto de agua, jacinto de rio, lechuguilla, liro de agua, tarulla.

1.3.5 Other species

Native Species

1. *Heteranthera dubia* (Jacq.) MacM.
2. *Heteranthera limosa* (Sw.) Willd.
3. *Heteranthera mexicana* Wats.
4. *Heteranthera multiflora* (Griseb.) Horn
5. *Heteranthera penduncularis* Benth.
6. *Heteranthera reniformis* Ruiz Lopez & Pavon
7. *Heteranthera rotundifolia* (Kunth) Griseb.
8. *Pontederia cordata*

Introduced Species

1. *Eichhornia azurea* (Sw.) Kunth
2. *Eichhornia crassipes* (Mart.) Solms.

3. *Eichhornia diversifolia* (Vahl) Urban
4. *Eichhornia paniculata* (Spreng.l) Solms
5. *Monochoria hastata* (L.) Solms
6. *Monochoria vaginalis* (Burm. f.) K. Presl

Figure 03: Some pictures of *Eichhornia crassipes L.*

1.3.6 Use

The primary attribute of water hyacinth is its ability to grow under a wide range of nutrient and environmental conditions. The plant is able to develop at an astounding rate, effectively out-competing other native aquatics. Its growth rate is among the highest of any plant known:

hyacinth populations can double in as little as 12 days. This rapid growth can cause an imposing amount of biomass. The level of biomass accumulation will determine its nuisance value and the impact on water quality. Excessive infestation by this weed can severely constrain human activities, affecting accesses to water, navigation, irrigation and fisheries. In other words, incredibly dense mats of free-floating vegetation block boat traffic and prevent swimming and fishing, and keep sunlight from reaching the water column and submerged plants (Gopal 1987).

Other Uses

Water hyacinths are potentially an excellent source of biomass. Through an anaerobic fermentation process, polluted hyacinths can be converted to the natural gas methane - a costly process that may become more economical as supplies of underground natural gas are depleted. Dried and cleansed plants can be used as fertilizer and plant mulch. Eventually, living aquatic plants might serve aboard long-distance manned spacecraft, absorbing wastes and converting carbon dioxide to oxygen, then being themselves converted into food. In recent years, the plant has been used to treat a variety of wastewaters and to produce high protein cattle food, pulp, paper, fiber, and more importantly, biogas as energy source (Agency for International Development, 1976; Bates and Hentges, 1976; Kojima, 1986). Since this biomass is a by-product of wastewater treatment, it has a positive environmental impact, and thus poses no threat as competitor to food, feed, or fiber-producing plants. Wilted water hyacinth, mixed with earth, cow dung, and wood ashes in the Chinese compost fashion, can yield useful compost in just two months. Although potential yields are incredible, so are the costs of removal or attempted eradication of this water weed. Standing crops have been estimated to produce 100-120 tonnes per hectare per year. Under ideal conditions, each plant can produce 248 offspring in 90 days. Water hyacinth roots naturally absorb pollutants, including such toxic chemicals as lead,

mercury, and strontium 90 (as well as some organic compounds believed to be carcinogenic) in concentrations 10,000 times that in the surrounding water. In Africa, fresh plants are used as cushions in canoes and to plug holes in charcoal sacks. The water hyacinths' flower is used as vegetable in Cambodia. Some scientists suspect that the water hyacinth contains a higher nitrogen content than most other invasive aquatic plants, but there is little evidence available to support this theory. The plant does store nitrogen within its root system for growth which can then be broken down into compost for the improvement of fertilizer.

Chapter Two

LITERATURE REVIEW

2.1 Literature Review of *Cissus quadrangularis L.*

Cissus quadrangularis L. is a succulent plant of family Vitaceae commonly found in tropical and subtropical xeric wood. It is a fleshy, cactus-like liana widely used as a common food item in India.

2.1.1 Review of Pharmacological Studies

The plant is prescribed in the ancient ayurvedic literature as a general tonic and analgesic, with specific bone fracture healing properties. The plant is believed to be useful in helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellings. Various folk claims for cure of various diseases, efforts have been made by researchers to verify the efficacy of the plant through scientific biological screening. The scrutiny of literature revealed some notable pharmacological activities of the plant such as antioxidant, free radical scavenging, anti microbial, anti bacterial, bone healing, antiulcer, analgesic, anti inflammatory and diuretic, presented in this review such that the potential use of the plant either in pharmaceutics or as an agriculture resource can be evaluated. The present review is an attempt to highlight phytochemicals, various traditional uses as well as pharmacological reports on *Cissus quadrangularis L.* (Shah U 2011)

Here a collection of plant extract and their pharmacological activity are given below:

Table 02: Summary of Pharmacological Activity of *Cissus quadrangularis L*

Plant Part	**Extracts**	**Pharmacological activity**	**References**
Whole Plant	Ethanolic	Analgesics activity	(Singh et al. 1984)
	Alcoholic	Antineoplastic activities	(Opoku et al., 2000)

Plant Part	Extracts	Pharmacological activity	References
	Aqueous	Pharmacological and Toxicological evaluation of an active principle obtained from the plant Vitis quadrangularis	(Venkata 1970)
	Alcoholic	Antioxidant Activity	(Oben 2007)
	Methanolic	Antioxidant Activity	(Shah 2011)
	Methanolic, Aqueous, Ethyl acetate, n-Hexane	Antioxidant Activity	(Murthy, Vanitha, Swamy & Ravishankar, 2003)
Stem	Aqueous, ethanolic	Anti bacterial activity	(Kashikar et al., 2006) (Jigna & Chandra, 2008) (Parekh & Chanda, 2008) (Parekh & Chanda, 2007)
	Methanolic	Accelerating healing process	(Deka et al 1994)
	Alcoholic, Ethyl Acetate	Anti-inflammatory	(Thisayakorn et al. 2007)
	Alcoholic	neutrophil mediated tissue protective	(Mallika et al. 2006)
	Alcoholic	obesity and obesity induced oxidative stress	(Oben et al. 2007)
	Alcoholic	Management of Weight loss	(Oben et al. 2008)
	Petroleum Ether	Stimulates the Growth of Fetal Bone	(Potu et al. 2008)
Stem	Alcoholic, Aqueous	Anti-inflammatory and Analgesic agents	(Singh et al. 2008)
	Methanolic	Gastroprotective effect	(Jainu et al. 2006)
	Methanolic	Antioxidant Activity	(Jainu & Devi 2005)

2.1.2 Review of Phytochemical Studies

The following types of compounds are found from *Cissus quadrangularis L.*

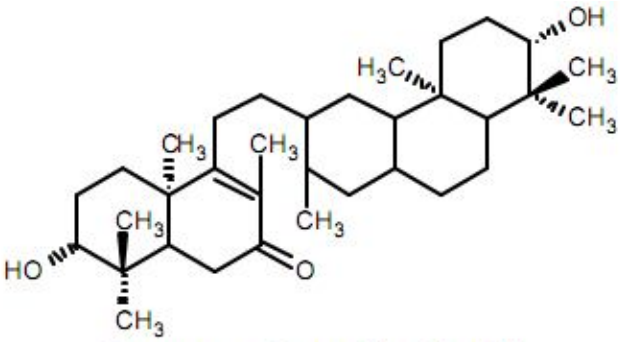
i. Phenolic compounds/Tannins
ii. Saponins
iii. Steroids
iv. Triterpenoids
v. Proteins
vi. Carbohydrates
vii. Glycosides etc.

Preliminary Phytochemical Screening of ethanolic extract *Cissus quadrangularis* gives the finding of the following class of compounds:

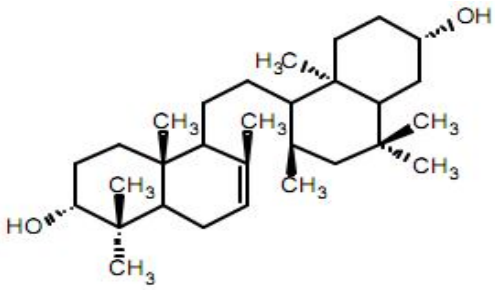
Table 03: Compounds Found from *Cissus quadrangularis*. (Mohanamba, 2012)

Plant part	**Chemical type**	**Compound**
Whole plant	1. Carotene	Chomolipid hydrocarbon
	2. Vitamin C	
	3. Triterpene	d-amyrin, d-amyrone
	4. Anabolic Steroids	ß-sitosterol, oxo-steroid, keto-steroid
Stem	1. Calcium Oxalate	
	2. Stilbene	Pallidol, Parthenocissin A, Quadrangularin A, Quadrangularin B, Quadrangularis C, Piceatannol
	3. Tetracyclic Triterpenoid or Saponin	Lupeol
	4. Flavonol	Kaempferol, Quercetin, Quercitrin
	5. Isoflavone	Geniszein, Daidtein
Aerial part	1. Alkene alkanol and alkenone	

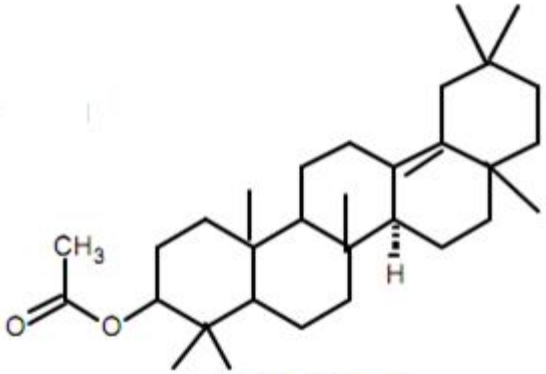
Some structures of the found compounds are given at next page:



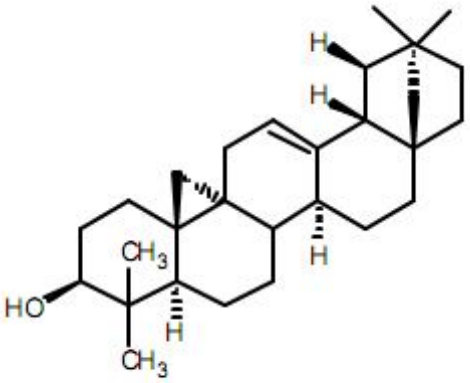
7-oxo-onocer -8-ene. 3-b , 21-a diol



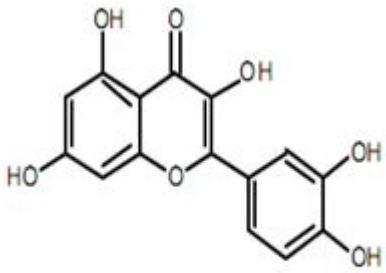
Onocer- 7- ene, 3-a, 21-b Diol



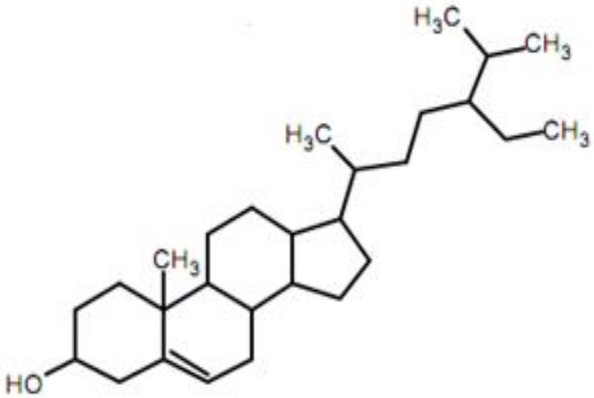
amyrin acetate



β–amyrin



Quercetin



β-Sitosterol

$CH_3(CH_2)_{17}CH_2COOCH_2(CH_2)_{18}CH_3$

Eicosyleicosanoate

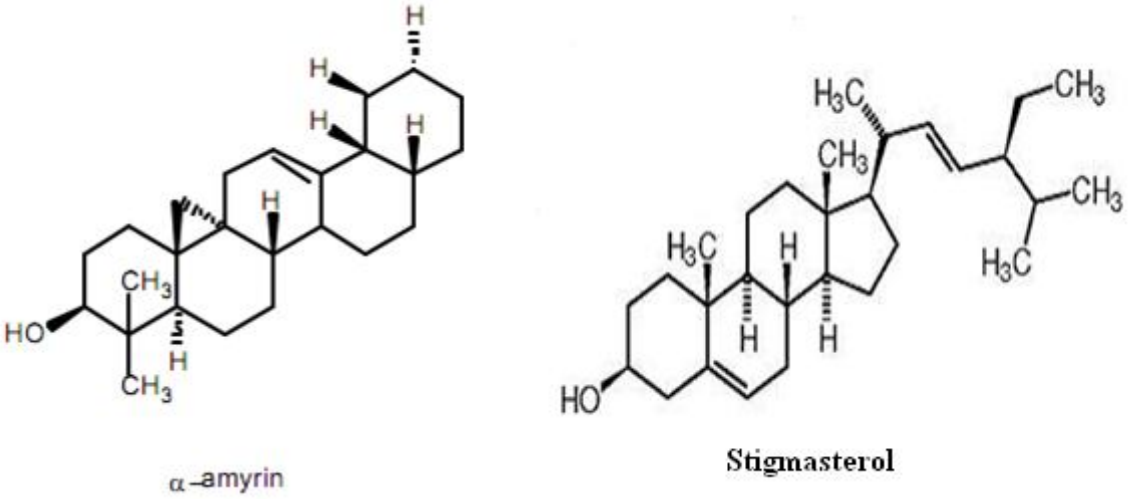


Figure 04: Compounds Found from *Cissus quadrangularis L.* (Shah 2011)

2.2 Literature Review of *Lawsonia inermis L.*

Lawsonia inermis L. is a much branched glabrous shrub or small tree (2-6 m in height), cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine.

2.2.1 Review of Pharmacological Studies

The plant has been reported to have analgesic, hypoglycemic, antimalarial, hepatoprotective, nootropic, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, anthelmintic, antifertility, tuberculostatic and anticancer properties. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.

Table 04: Summary of Pharmacological Activity of *Lawsonia inermis L.*

Plant Part	Work	Activity	Reference
Leaves	Ethanol (70%) extract showed significant Hypoglycaemic and hypolipidaemic activities in alloxan induced diabetic mice after oral administration.	Antidiabetic activity	(Syamsudin & Winarno 2008)
	Methanol (95%) extract showed significant in-vitro antihyperglycemic effect.	Antidiabetic activity	(Arayne et al. 2007)
	Methanol extract of 1 mg/ml concentration had displayed immunostimulant action as indicated by promotion of T-lymphocyte proliferative responses.	Immunomodulatory effect	(Arayne et al. 2007)
	Modulator effect of 80% ethanol extract on drug metabolising enzymes, antioxidant enzymes, lipid peroxidation in the liver of Swiss Albino mice.	Antioxidant activity	(Dasgupta, Rao&Yadava 2003)

Plant Part	Work	Activity	Reference
Leaves	Quinonic compounds from henna were studied in-vitro for antimicrobial properties.	Antibacterial activity	(Dama, Poul & Jadhav 1999)
	Chloroform extract of leaves of Lawsonia inemris had shown the highest activity (87.6%) followed by α tocopherol (62.5%) by using FTC method and based on TBA method significant activity (55.7 %) compared to α-tocopherol (44.4%).	Antioxidant effect	(Endrini, Rahmat , Ismail & Taufiq 2007)
	Methanolic extract	Antioxidant effect	(Mikhaeil, Badria, Maatooq & Ame 2004)
	Alcoholic extract	Antioxidant effect	(Khodaparast, Hosein & Zinab 2007)
	Alcoholic extract	Antioxidant effect	(Makhija 2012)
	Chloroform xtract	Antioxidant effect	(Endrini, Rahmat, Ismail & Taufiq 2002)
	The extract showed analgesic and antipyretic activity	Analgesic activity	(Mohsin et al. 1989)

Plant Part	Work	Activity	Reference
	The ethyl acetate extract of L. inermis L. was found to be the most active against all the bacteria in the test system.	Antibacterial activity	(Ali, Julich, Kusnick & Lindequist 2001)
	Aqueous extract of leaves of L. inermis showed the significant antibacterial effect against.	Antibacterial activity	(Baba-Moussa et al. 1997)
	Ethanol, methanol and aqueous extract of leaves of L. inermis are involved in defensive mechanism against spore germination of Drechslera oryzae.	Antifungal activity	(Natarajan & Lalithakumar 1987)
	Lawsone isolated from the leaves of L. inermis has shown significant antifungal antibiotic effect.	Antifungal activity	(Dixit, Srivastava & Tripathi 1980)
	Essential oil obtained by hydro-distillation from leaves of L. inermis growing in Iran were analysed by GC-MS and showed an antifungal activity.	Antifungal activity	(Aghel, Ameri & Ebrahimi 2005)
	Crude Methanolic extract of leaf of L. inermis showed in-vitro activity against Trypanosoma brucei at concentration of 8.3 mg/ml of blood in mice but not in-vivo.	Antitrypanosomal activity	(Wurochekke, Chechet & Nok 2004)
	The growth of Tuberclebacilli and of Mycobacterium tuberculosis H37Rv was inhibited by 6 µg/ml.	Tuberculostatic activity	(Sharma 1990)

Plant Part	Work	Activity	Reference
	The extract of leaves of henna showed significant analgesic as well as antipyretic activity.	Analgesic activity	(Mohsin et al. 1989)
	Ethanol extract	Anti-inflammatory	(Gupta, Saifi, Modi, & Mishra 1986)
	Chloroform extract of leaves of L. inermis displayed the cytotoxic effects against liver (HepG2) and Human breast (MCF-7) with IC50 values of 0.3 and 24.85µg/ml by microculture tetrazolium salt assay (MTT).	Cytotoxic activity	(Endrini, Rahmat, Ismail & Taufiq 2007)
	Aqueous extract of leaves of L. inermis was found to inhibit sickling and to increase the oxygen affinity of HbSS blood.	Antisickling activity	(Chang & Suzuka 1982)
	The ethanol extract of L. inermis L. leaves and lawsone tested for trypsin inhibitory activity showed an IC50 value of 64.87 and 48.6µg/ml, respectively.	Enzymes inhibitory activity	(Yogisha et al. 2002)
	Lawsone and its oxazine derivatives isolated from leaves of L. inermis had proven to be potential anticoagulant agent.	Anticoagulant effect	(Kumar, Kokate, Rambhau & Rao 1985)
	Chloroform and aqueous extracts inhibit the growth of microorganisms of burn wound infections.	healing effects	(Hamdi, et al. 1997)

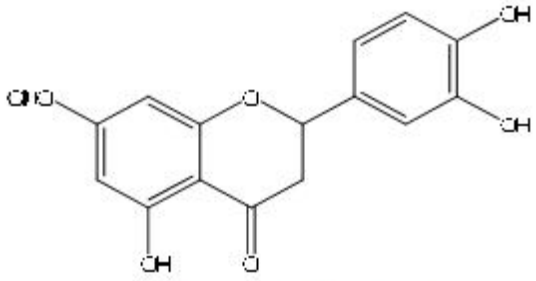
Plant Part	Work	Activity	Reference
Whole plant	with methanol and water	Antioxident	(Hosein & Zinab 2007)
	Aqueous and Methanolic Extracts	Antioxident	(Guha, Rajkumar, Kumar & Mathew 2011)
Seeds	Ethanol extract prepared from the powdered seeds of L. inermis L. failed to show any antifertility activity.	Antifertility activity	(Munshi, Shetye & Nair 1977)
	concluded that seed oil is devoid of behavioural and CNS effects and failed to produce any effect on isolated tissue though it possess significant analgesic activity.	analgesic activity.	(Bagi, et al. 1988)
	Alcoholic and Aqueous Extract	Antioxidant Activity	(Jacob, Madhumitha & Salar 2011)
	Chloroform extract	Antioxidant Activity	(Borade, Babasaheb, Kale & Shete 2011)

Plant Part	Work	Activity	Reference
Stem	Isoplumbagin and lawsaritol, isolated from stem bark of L. inermis L. showed anti-inflammatory activity against Carrageenan induced paw oedema in rats.	Anti-inflammatory activity	(Gupta, Ali, Pillai & Alam 1993)
bark	Alcoholic extract of the bark of L. inermis showed hepatoprotective effect against carbon tetrachloride-induced elevation in serum marker enzymes (GOT and GPT), serum bilirubin, liver lipid peroxidation.	Hepatoprotective activity	(Bhandarkar & Khan 2003)
Fruits	The ethanol extract shows potent activity against Sembiki forest virus (SFV) in swiss mice and chick embryo models exhibiting 100 to 65% activities	Antiviral activity	(Khan, et al. 1991)
Root	Isoplumbagin and lawsaritol, isolated from stem bark and root of L. inermis L. showed anti-inflammatory activity against Carrageenan induced paw oedema in rats.	anti-inflammatory activity	(Gupta, Ali, Pillai & Alam 1993)
	Methanol extract of roots of L. inermis was most effective in inducing abortion in mice, rats and guinea pig.	Abortifacient activity	(Aguwa 1987)

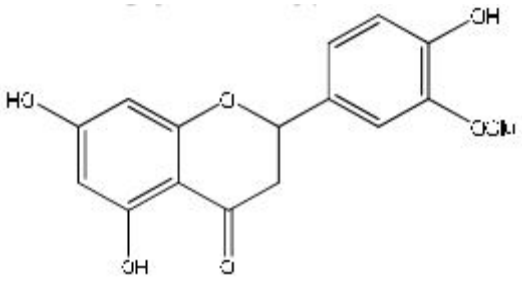
2.2.2 Review of Phytochemical Studies

The principal colouring matter of henna is lawsone, 2-hydroxy-1:4 napthaquinone ($C_{10}H_6O_3$) besides lawsone other constituents present are gallic acid, glucose, mannitol, fats, resin (2%), mucilage and traces of an alkaloid. Leaves yield hennatannic acid and an olive oil green resin, soluble in ether and alcohol. Flowers yield an essential oil (0.01-0.02%) with brown or dark

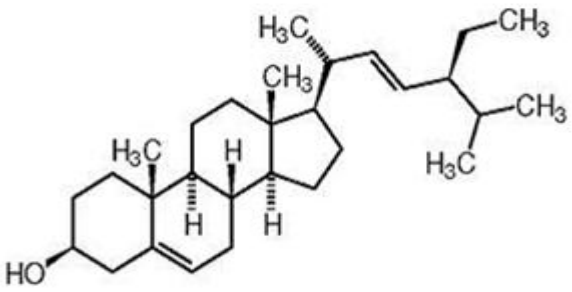
brown color, strong fragrance and consist mainly of α- and β-ionones; a nitrogenous compound and resin. Seeds contain proteins (5.0%), carbohydrates (33.62%), fibers (33.5%),fatty oils (10-11%)composed of behenic acid, arachidic acid, stearic acid, palmitic acid, oleic acid and linoleic acid. The unsaponified matter contains waxes and colouring matter. The root contains a red colouring matter. Phytochemicals reported in *L. inermis L.* are listed below with their structures.



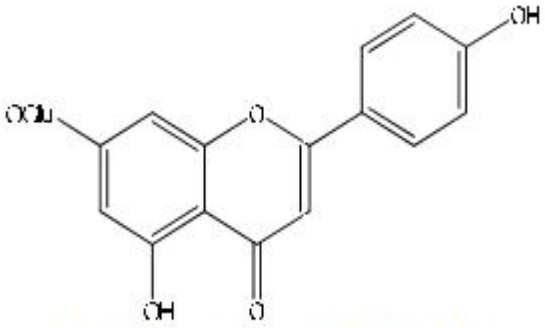
Luteolin-7-*o*-glycoside



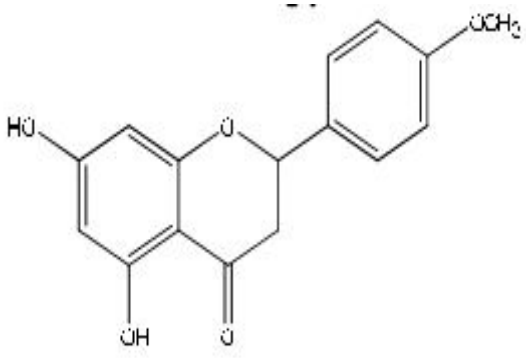
Luteolin-3'-*o*-glycoside



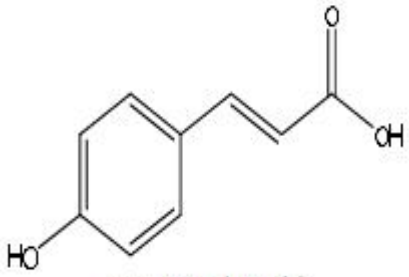
Stigmasterol



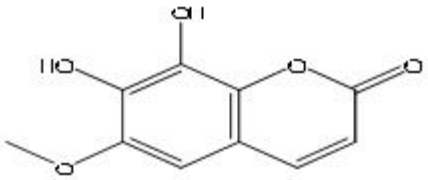
Cosmosiin (Acacetin-7-*o*-glucoside)



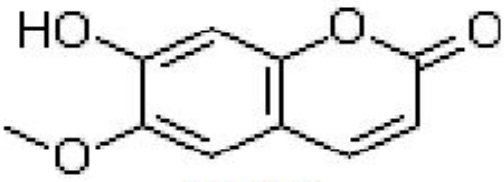
Acacetin



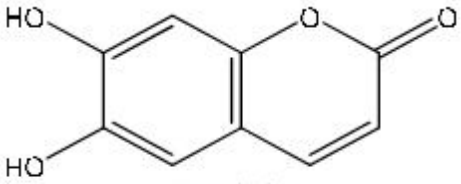
p-coumaric acid



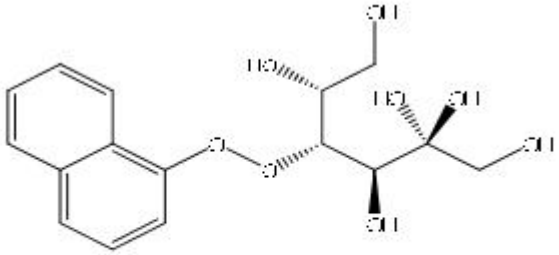
Fraxetin



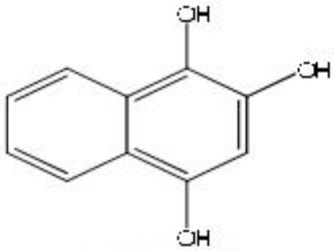
Scopoletin



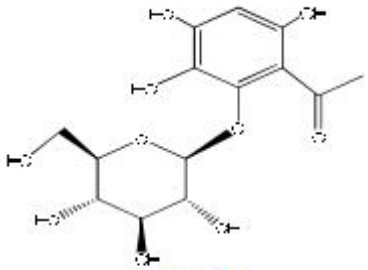
Esculetin



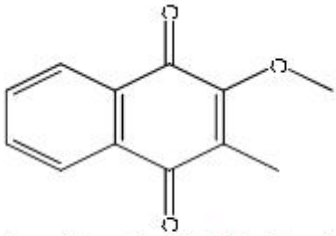
1,2-dihydroxy- 4-*o*-glucosyloxy Naphthalene



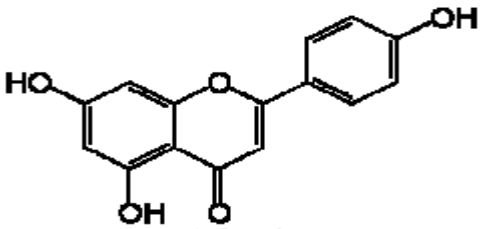
Lawsoniaside



Lalioside



2-methoxy-3-methyl-1,4-Napthoquinone



Apigenin

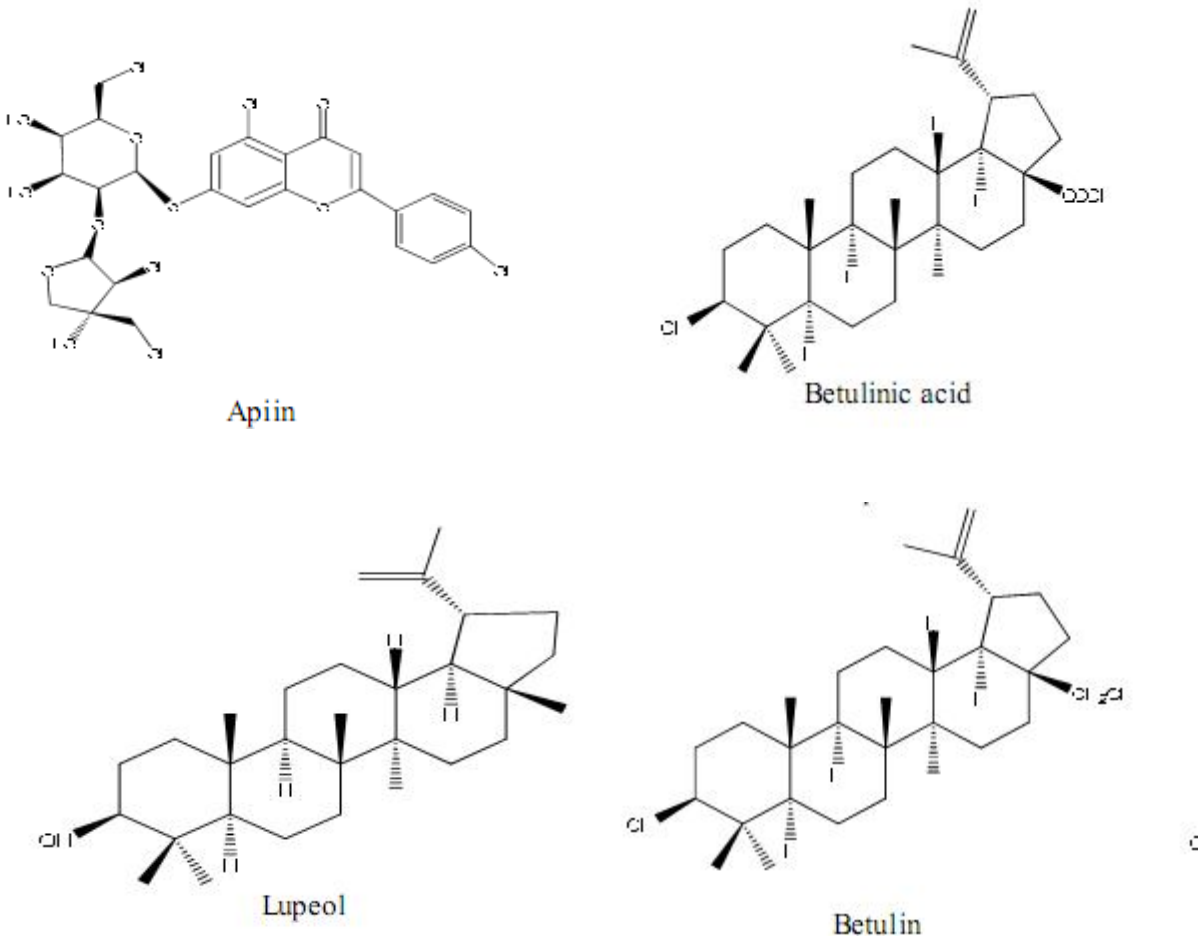
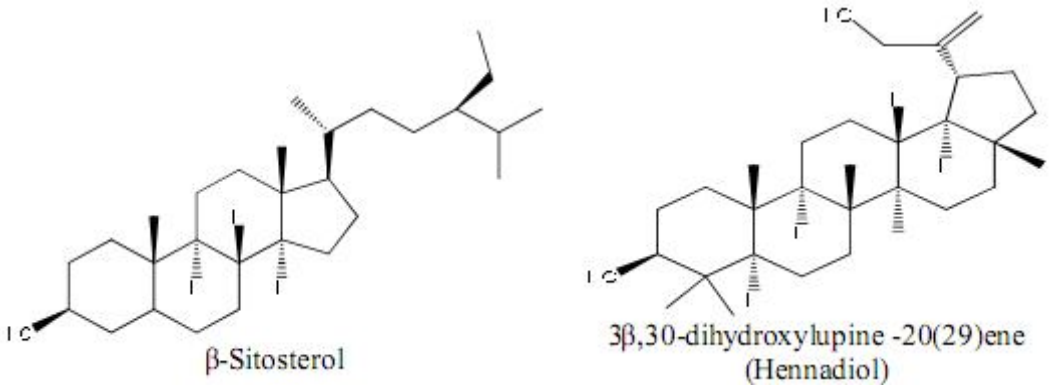


Figure 05: Compounds Found from Leaves. **[**(Hosein & Zinab 2007), (Pratibha & Korwar 1999), (Sastri 1962)]



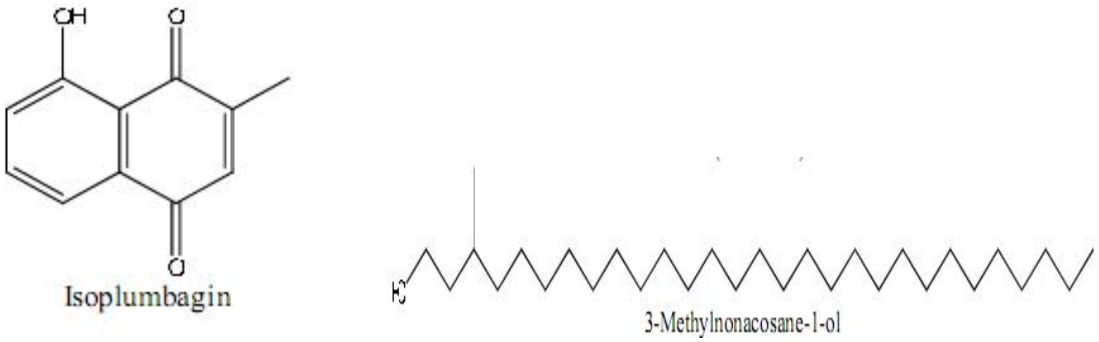


Figure 06: Compounds found from barks. (Gupta, et al. 1992)

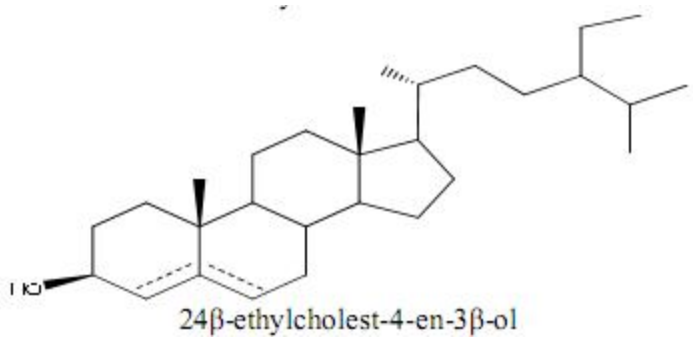


Figure 07: Compound Found from Roots. (Gupta et al. 1992)

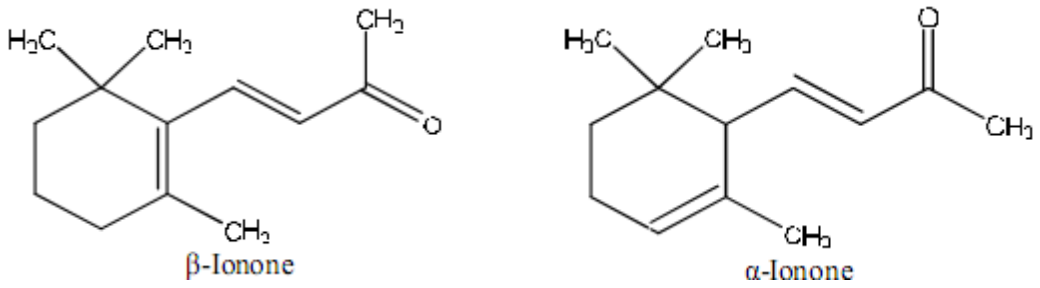


Figure 08: Compound Found from Flowers**.** (Anita & Kaushal 1950)

$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
Linoleic acid

$CH_3(CH2)_{18}COOH$
Arachidic acid

$CH_3(CH_2)_{20}COOH$
Behenic acid

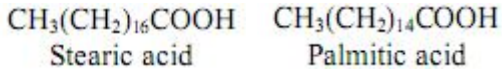


Figure 09: Compounds found from seeds. (Siddiqui, Kardar & Khan 2005)

2.3 Literature Review of *Eichhornia crassipes*

Till now a good many research works have been done. From these papers the pharmacological activity and the chemicals found are reviewed below.

2.3.1 Review of Pharmacological Studies

Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins. Alkaloids & flavinoids have been used as antiviral, antibacterial, antiamoebial & anticancer agents. Phenolic and polyphenolic are the other group of secondary metabolites. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance. Some works were done to prove some traditional use and some new use area. Here is a summery of the works done on this plant.

Table 05: Reported Pharmacological Activity of *Eichhornia crassipes.*

Work	**Result**	**Reference**
The aqueous, chloroform fractionates and the ethanol and ethyl acetate extracts were used for Micrococcus luteus and Rhodospirillum rubrum	Antibacterial activity	(Thamaraiselvi, Lalitha & Jayanthi 2012)
Ethanol and chloroform extracts showed significant activity against Monoscus ruber whereas	Anti fungal activity	(Thamaraiselvi, Lalitha & Jayanthi 2012)
aqueous and ethanol extracts showed significant activity against Aspergillus fumigates	Anti fungal activity	(Thamaraiselvi, Lalitha & Jayanthi 2012)
Chloroform extract	Antioxident activity	(Enein et al. 2011)

Work	**Result**	**Reference**
Aqueous and alcoholic extract	Antioxident activity	(Chantiratikul, Meechai & Nakbanpotec 2009)
Alcoholic extract	Antioxident activity	(Shanaba & Shalaby 2012)
Ethyl acetate extract was experimented.	Antioxident activity	Jayanthi & Lalitha 2011)
Extract of E. crassipes were tested electrochemically for the anticorrosion behavior.	Anticorrosive activity	(El-Mehalawy et al. 2008)
The cytotoxicity of crude methanolic extract and the fractionated compounds (5 fractions) of Eichhornia species were tested against HepG-2 and MCF-7 cells by sulforhodamine B (SRB) assay	Anticancer activity	(Freshney RI 2002)
Extract was experimented for arsenic accumulation reduction in liver of *Eichhornia crassipes*	Antiarsenic accumulator	(Shaheen *et al*. 2007).

2.3.2 Review of Phytochemical Studies

V. Kandukuri, J. G. Vinayasagar, A. Suryam, M. A. & Singara Charya reported the presence of alkaloid, phenol, steroid, tannin and saponin in methanol extract of dry Eichhornia crassipes where as reported the absence of flavonoids. J. A. Ndubuisi, E. O. Emeka, N. U. Luke revealed the presence of saponin, glycoside and anthraquinone but absence of alkaloid in chloroform extract of dry waterhyacinth. Presence of flavonoids in this plant was reported by Nyananyo. All

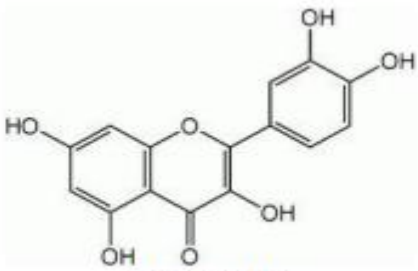
the above study was done in dry Eichhornia crassipes but the present study was carried out in fresh Eichhornia crassipes. This study was carried out to compare the potential of fresh plant with dry plant. (Thamaraiselvi, Lalitha & Jayanthi 2012)

Table 06: Metabolites Found from *Eichhornia crassipes.*

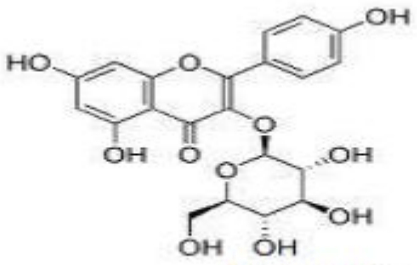
Plant type	**Extract**	**Metabolites**	**Researchers**
Dry	Acquous	alkaloid, flavonoids, steroid, tannins, phenolic contents, quinone and anthraquinone	N. Lata, &V. Dubey
Dry	Methanol	alkaloid, phenol, steroid, tannin and saponin	V. Kandukuri, J. G. Vinayasagar, A. Suryam, M. A. & Singara Charya
Dry	Chloroform	saponin, glycoside and anthraquinone	J. A. Ndubuisi, E. O. Emeka, N. U. Luke
Dry	Ethanol fractionate	alkaloids, flavonoids, sterols, terpenoids, anthroquinone, phenols and anthocyanins	P. Jayanthi, P. Lalitha
Fresh	Ethanol	alkaloids, flavonoids, sterols, terpenoids, anthroquinone, proteins, and phenols	Thamaraiselvi, P. Lalitha and P.Jayanthi
Fresh	Aqueous extract	phenol and tannin	S. A. Ahmed, M. Ito, K. Ueki

(Thamaraiselvi, Laqa1alitha & Jayanthi 2012)

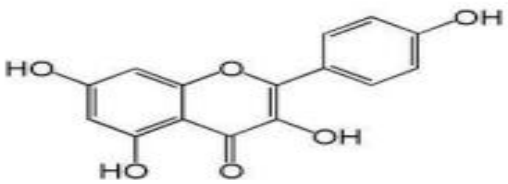
Among the reported compounds some structures are given at the next page.



Quercetin



Astragalin



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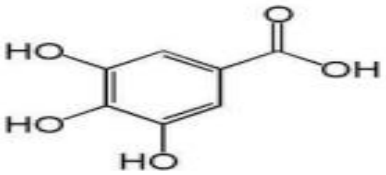
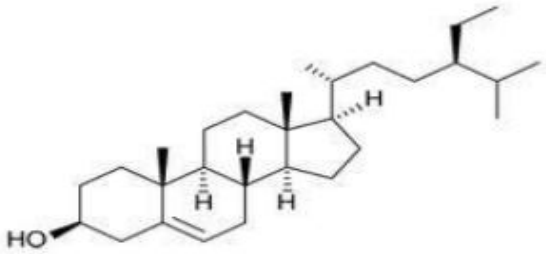
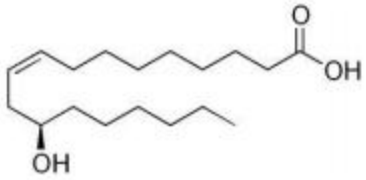


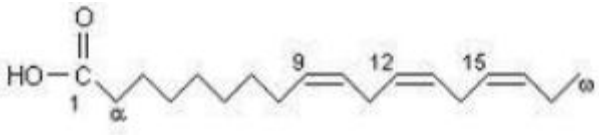
Gallic Acid



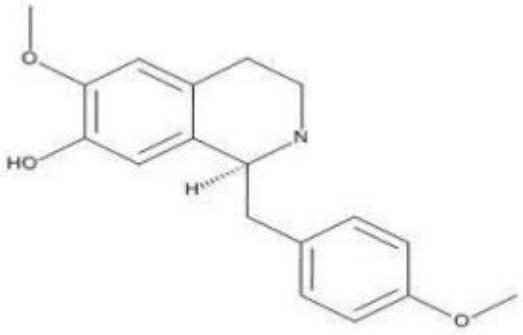
β-sitosterol



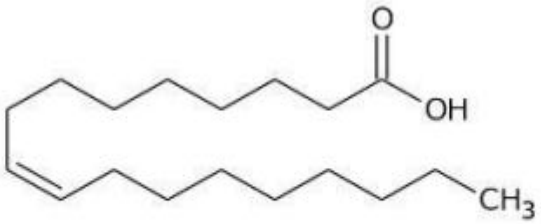
Ricinolic Acid



Linolenic Acid



Coclaurine



Oleic Acid

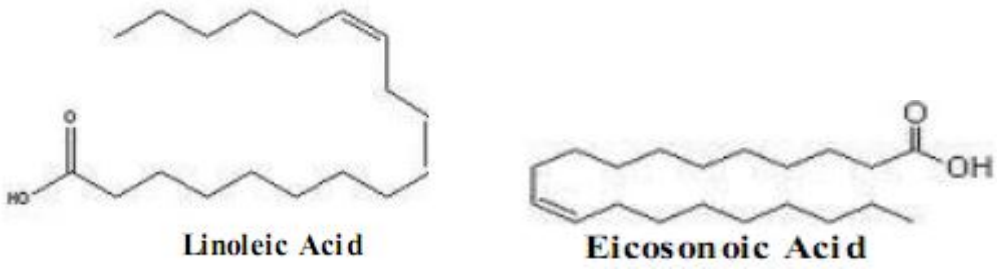


Figure 10: Some Compounds Found from *Eichhornia crassipes*.

Chapter Three

STATEMENT OF PURPOSE (SOP)

3 Statement of Purpose (SOP)

To meet the demand of the current age here I want to determine or measure the antioxidant property of the extract of *Cissus quadrangularis L, Lawsonia inermis L.* and *Eichhornia crassipes.* These three plants have a good many traditional or folk uses. Their scientific evidence of other activity is almost established. Though the evidence of antioxidant activity is not so strong, hence this study was undertaken.

Methanolic extract of plant was run with DPPH (1-diphenyl-2-picrylhydrazyl) test to determine the antioxidant activity of those plants.

Chapter Four

MATERIALS & METHODS

4 MATERIALS AND METHODS

For this experiment the following materials were used.

4.1 Equipments Required

Pipette (2ml)

Pipette (5ml)

Analytical balance

UV- visible spectrophotometer

Beaker (100 & 200ml)

Test tube

Aluminium foil

Spatula

Micro-pipette

Distilation machine

Test tube holder

4.2 Reagents Required

Distilled Methanol

Distilled water

1,1-diphenyl-2-picrylhydrazyl (DPPH)

4.3 Antioxidant Assay

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism.

Free radicals form when oxygen is metabolized or formed in the body and are chemical species that posses an unpaired electron in the outer (valance) shell of the molecule. This is the reason, why the free radicals are highly reactive and can react with proteins, lipids, carbohydrates and DNA. These free radicals attack the nearest stable molecules, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction, finally resulting in the description of a living cell. Free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived molecules are O^{-}_{2} [superoxide], -HO[hydroxyl] ,HO_2 [hydroperoxyl], ROO[peroxyl], RO[alkoxyl] as free radical and H_2O_2 oxygen as non-radical. Nitrogen derived oxidant species are mainly NO [nitric oxide], NO_2 [nitrogen dioxide] and N_2O_3[dinitrogen trioxide]. (Evans & Halliwall 1999)

In a normal cell, there are appropriate oxidant: antioxidant balance. However, this balance can be shifted, when production species is increased or when levels of antioxidants are diminished. This stage is called oxidative stress. Oxidative stress results in the damage of biopolymers including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates. Lipid peroxidation is oxidative deterioration of polyunsaturated lipids and it involves ROS and transition metal ions. It is a molecular mechanism of cell injury leading yield a wide range of cytotoxic products, most of which are aldehydes, like malondialdehyde (MDA), 4-hydroxynonrnal(HNE), Oxidative stress causes serious cell damage leading to a variety of human diseases 6 like Alzheimer's disease, Parkinson's disease, atheroscleorosis, cancer, arthritis, immunological incompetence and neurodegenerative disorders, etc. (Badarinath et al. 2010)

4.3.1 In-Vitro Determination of Antioxidant Capacity

Antioxidant capacity can be measured by both in-Vivo and in- Vitro method. Here only the in-Vitro methods are discussed. The various conventional and latest methods comes under invitro are listed in table below. It is very difficult to select a suitable antioxidant assay method. Antioxidants act by several mechanisms and no one assay can capture the different modes of action of antioxidant. (Badarinath et al. 2010)

Here are the in-Vitro methods by which the antioxidant tests can be done.

a. Hydrogen Atom Transfer methods (HAT)

i. Oxygen radical absorbance capacity (ORAC) method
ii. Lipid peroxidation inhibition capacity (LPIC) assay
iii. Total radical trapping antioxidant parameter (TRAP)
iv. Inhibited oxygen uptake (IOC)
v. Crocin bleaching Nitric oxide radical inhibition activity
vi. Hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline)
vii. Scavenging of H_2O_2 radicals
viii. ABTS radical scavenging method
ix. Scavenging of super oxide radical formation by alkaline (SASA)

b. Electron Transfer methods (ET)

i. Trolox equivalent antioxidant capacity (TEAC) decolourization
ii. Ferric reducing antioxidant power (FRAP)
iii. DPPH free radical scavenging assay

iv. Copper (II) reduction capacity

v. Total phenols by Folin-Ciocalteu

vi. N,N-dimethyl-p-Phenylenediamine (DMPD) assay

c. Other Assays

i. Total oxidant scavenging capacity (TOSC)

ii. Inhibition of Briggs – Rauscher oscillation reaction

iii. Chemiluminescence

iv. Electrochemiluminescence

v. Fluorometric Analysis

vi. Enhanced chemiluminescence (ECL)

vii. TLC bioautography

viii. Cellular antioxidant activity (CAA) assay

ix. Dye-substrate oxidation method (Badarinath et al. 2010)

Among these methods the most most widely methods are described below.

4.3.1.1 Ferric-Raducing Antioxidant Power (FRAP) assay

In order to assess the modifying effect of tea flavonoids on plasma antioxidant status, a variety of methods has been employed. Commonly used is the FRAP assay. This is a colorimetric assay that measures the ability of plasma to reduce the intense blue ferric tripyridyltriazine complex to its ferrous form, thereby changing its absorbance. (Benzie & Strain 1999)

It is simple, speedy, inexpensive, and robust does not required specialized equipment. It can be performed using automated, semiautomated, or manual methods. (Benzie & Strain 1999)

FRAP cannot detect species that act by radical quenching (H transfer), particularly SH group containing antioxidants like thiols, such as glutathione and proteins. (Huang, Ou & Prior 2005)

4.3.1.2 Total Radical Trapping Antioxidant Parameter (TRAP)

Another assay which has been applied in human plasma is the total radical trapping antioxidant parameter (TRAP). In this assay, the rate of peroxidation induced by AAPH(2'-azobis(2-amidinopropane) hydrochloride) is monitored through the loss of fluorescence of the protein R-phycoerythrin (R-PE). In the TRAP assay the lag-phase induced by plasma is compared with that induced by Trolox in the same plasma sample. (Huang, Ou & Prior 2005)

Used for measurements of in-vivo antioxidant capacity in serum or plasma because it measures nonenzymatic antioxidants such as glutathione, ascorbic acid. (Huang, Ou & Prior 2005)

Many different end points have been used, so comparisons between laboratories are difficult. It is relatively complex and time consuming. It also requires a high degree of expertise and experience.

4.3.1.3 Trolox Equivalent Antioxidant Capacity (TEAC)

This assay is based on the ability of molecules to scavenge the stable free radical of 2,2'-azinobis (3-ethylbenzothiozoline-6-sulfonic acid) in comparison with Trolox, a water soluble analogue of vitamin E. The activity of a compound is therefore expressed as TEAC. Of these assay, the ECL seems the least suitable to determine plasma antioxidant capacity because it relies on enzymatic activity. This technique has not been widely applied, which limits the possibility to compare results from different studies. All the other assays have been applied in plasma reproducibility.

4.3.1.4 ABTS {2,2'– azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)}

Miller et al (1993) described another technique for TAC measurement based on colorimetry. This assay is based on the principle that when 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) {ABTS} is incubated with a peroxidase (such as metmyoglobin and H2O2, a relatively stable radical cation, ABTS+ , is formed (see equation below).

The formation of ABTS+ on Interaction with Ferryl Myoglobin produces a relatively stable blue-green color, Measured at 600nm. Antioxidants in the fluid sample suppress this color production to a degree that is proportional to their concentrations. In this equation, HX-Fe III= metmyoglobin, X – [Fe IV = 0] = ferrylmyoglobin, ABTS = 2,2'– azino-di-[3-ethyl-bensthiazoline sulphonate]. (Miller & Rice-Evans 1993)

4.3.1.5 Oxygen Radical Absorbing Capacity (ORAC) Assay

Basically the same principle is applied as in the TRAP assay. The ORAC assay is another commonly applied antioxidant assay based on the ability of a test substance to inhibit the oxidation of B-phycoerythrin by reactive oxygen species, relative to Trolox. Proteins interfere with the analysis, partially protecting R-PE when all plasma antioxidants are exhausted. Determination of the lag-phase TRAP and ORAC assays can be performed with different radicals and thus different results will be obtained depending on the radical. For these reasons, results obtained with the TRAP or the ORAC assay in plasma have to be interpreted with care. (Caldwell 2001)

The advantage of the AUC approach is that it implies equally well for both antioxidants that exhibit distinct lag phase and those that have no lag phases. ORAC assay has been broadly

applied in academy and in the food and dietary supplement industries as a method of choice to quantify AOC. (Caldwell 2001)

ORAC is limited to measurement of hydrophilic chain but ignores lipophilic antioxidants. It requires fluorometers, which may not be routinely available in analytical laboratories. Temperature control decreases reproducibility.

4.3.1.6 DPPH Free Radical Scavenging Assay

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

Antioxidant compounds may be water-soluble lipid-soluble, insoluble, or bound to cell walls. Hence, extraction effciency is an important factor in quantifcation of antioxidant activity of foods. Trolox (as the reference standard) and the sample are reacted with DPPH solution in methanol/water for four hours at 35°C in a vessel mounted on a rotary shaker and the absorbance changes are measured at 517 nm. The quantity of sample necessary to react with one half of the DPPH is expressed in terms of the relative amount of Trolox reacted. Antioxidant activity of a sample is expressed in terms of micromole equivalents of Trolox (TE) per 100 grams of sample, or simply Trolox units per 100 gm or TE/100g. (Prakash, Rigelhof & Miller)

This technique is easy, effective, and rapid way to study plant extract profiles. No sample separation is needed. Potency of sample can be known.

4.3.1.7 Photochemiluminescence Method (PCL)

In the PCL assay (photochemiluminescence) the photo-chemical generation of free radicals is combined with the sensitive detection by using chemiluminescence. The PCL is based on the photo-induced autoxidation inhibition of luminol by antioxidants, mediated from the radical anion superoxide (Of) and is suitable to measure the radical scavenging properties of single antioxidants as well as more complex systems in the nanomolare range. Luminol works as photosensitiser as well as oxygen radical detection reagent. The PCL rnethod was carried out with the procedure described by Popov and Lewin (Popov& Lewin, 1999) and can be conducted by two difîerent protocols ACW and ACL that consent to measure the antioxidant capacity of the water- and lipid-soluble components respectively. In the water soluble fraction antioxidants such are ñavonoids, ascorbic acid, aminoacids, etc. are detected, while in the lipid soluble fraction tocopherols, tocotrienols, carotenoids, etc. are measured. The most widely used methods for measuring antioxidant activity involve the generation of radical species and the presence of antioxidants determining the dis-appearance of these radicals. Most of the assays determine the antioxidant activity in the micromolar range needing minutes or hours. The PCL assay, which is easy and rapid to perform, and although less reliable with lipohilic substrates, presents numerous advantages: it does not requires high temperatures to generate radicals and it is more sensitive to measure, in few minutes, the scavenging activity of antioxidants against the superoxide radical which is one of the most dangerous reactive oxygen species (ROS) also occurring in human body (Sch1esier, Harwat, Bohm, Bitsch 2002).

The methods presented can be divided into two groups depending on the oxidising reagent. Five methods use organic radical producers (TEAC I-III, TRAP, DPPH, DMPD, PCL) and one method works with metal ions for oxidation (FRAP). Another difference between these tests is the reaction procedure.

Table 07: Summary of Antioxidant Assays Methods.

Antioxidant assay	**Simplicity**	**Instrumentation**	**Biological relevance**	**Mechanism**	**Time required**
ORAC	++	+	+++	HAT	++
TRAP	— —	— —	+++	HAT	+++
FRAP	+++	+++	— —	SET	— —
TEAC	+	+	—	SET	—
F-C	+++	—	—	SET	+
TLC Autography	+++	+	— — —	SET,HAT	— — —
CAA Assay	—	—	+++	HAT	+++
Dye-substrate Oxidation	+	++	++	HAT	+
CUPRAC	+++	+++	— — —	HAT	+
Fluorometric	++	++	+	HAT	+
ECL	— — —	+++	+++	HAT	+++
ABTS	+	+	+	HAT	+

+, ++, +++ = Desirable To Highly Desirable Characteristic.

—, ——, ——— = less desirable to highly undesirable characteristic. (Badarinath et al. 2010)

Three assays use the delay in oxidation and determine the lag phase as parameter for the antioxidant activity (TEAC I, TRAP, PCL). They determine the delay of radical generation as well as the ability to scavenge the radical. In contrast, the assays TEAC II and III, DPPH, DMPD and FRAP analyse the ability to reduce the radical cation (TEAC II and III, DPPH, DMPD) or the ferric ion (FRAP). The three tests acting by radical reduction use preformed radicals and determine the decrease in absorbance while the FRAP assay measures the formed ferrous ions by

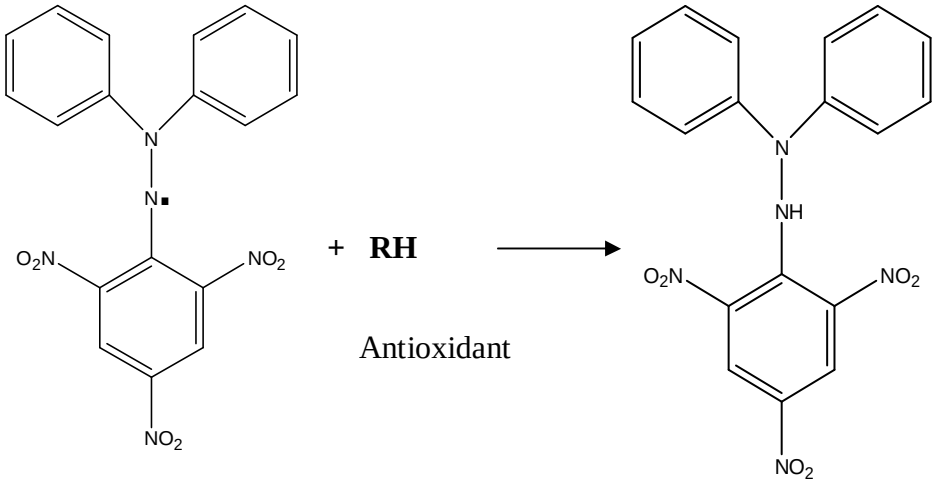
increased absorbance. Gallic acid was the strongest antioxidant in all tests with exception of the DMPD assay. In contrast, uric acid and ascorbic acid showed low activity in some assays. Most of the assays determine the antioxidant activity in the micromolar range needing minutes to hours. Only one assay (PCL) is able to analyse the antioxidant activity in the nanomolar range. (Schlesier, Harwat, Bohm & Bitsch 2002)

4.4 Method

4.4.1 Determination of DPPH Free radical scavenging assay (Quantitative analysis)

4.4.1.1 Principle

A rapid, simple and convenient method to measure free radical scavenging capacity of antioxidants involves the use of the free radical, 1,1-Diphenyl-2-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. DPPH is a stable nitrogen centered free radical with purple color and the odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm. When the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H, then the color turns from purple to yellow as the molar absorptivity of the DPPH radical reduces from 9660 to 1640 at 517 nm. Scavenging of DPPH free radicals by antioxidants decreases the absorbance. The lower the absorbance at 517 nm, the greater the free radical scavenging capacity of the crude extracts.



DPPH (oxidized form) DPPH (reduced form)

Diphenyl picrylhydrazyl

4.4.1.2 Procedure

- 2.0 ml of a methanol solution of the extract at different concentration (100, 50, 25, 12.5 etc. µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml).
- After 30 min reaction period at room temperature in dark place the absorbance was measured against at 517 nm against methanol as blank by using a UV- visible spetrophotometer.
- Inhibition free radical DPPH in percent (I%) was calculated as follows:

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

Where $A_{\mathbf{blank}}$ is the absorbance of the control reaction (containing all reagents except the test material).

- Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.
- Ascorbic acid was used as positive control.

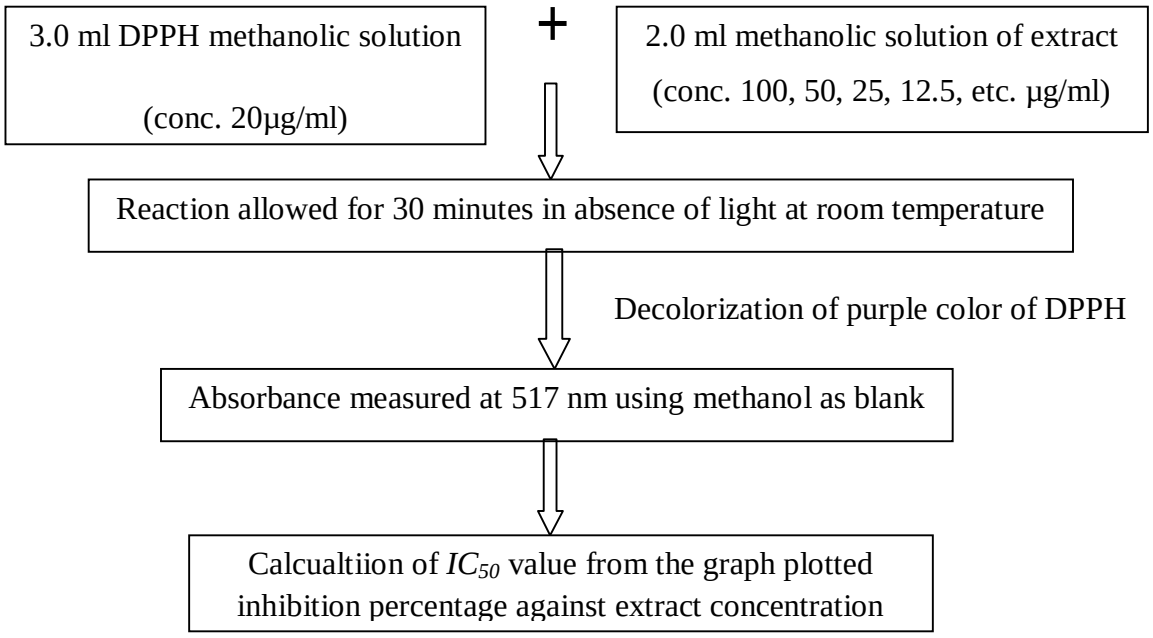


Figure 11: Representation of the Method of Assaying Free Radical Scavenging Activity.

Chapter Five

CALCULATIONS

5 CALCULATIONS

Table 08: Absorbance Data for Extracts.

Concentration (µg/mL)	**Aborbance**		
	Eichhornia crassipes	***Lawsonia inermis L.***	***Cissus quadrangularis L.***
400	0.196	0.198	0.154
200	0.128	0.137	0.141
100	0.212	0.133	0.133
50	0.269	0.12	0.115
25	0.375	0.103	0.129
12.5	0.394	0.154	0.154
6.25	0.37	0.278	0.222
3.125	0.379	0.337	0.278
1.562	0.415	0.371	0.329
0.7812	0.418	0.416	0.344

Absorbance of Blank (A_{Blank}) was measured: 0.419

5.1 Data Analysis for *Eichhornia crassipes*

Table 09: IC_{50} (µg/ml) Calculation for *Eichhornia crassipes.*

Serial No.	**A_{Blank}**	**Concentration (µg/ml)**	**A_{Sample}**	**% inhibition of free radical DPPH = $(1 - A_{Sample}/A_{Blank}) X 100\%$**	**IC_{50} µg/ml**
1		400	0.196	53.247	
2		200	0.128	69.47	
3		100	0.212	49.365	
4		50	0.269	35.697	
5	0.419	25	0.375	10.501	186.1196
6		12.5	0.394	6.025	
7		6.25	0.37	11.754	
8		3.125	0.379	9.658	
9		1.562	0.415	0.957	
10		0.7812	0.418	0.237	

Graphical representation of the data from where IC_{50} is calculated:

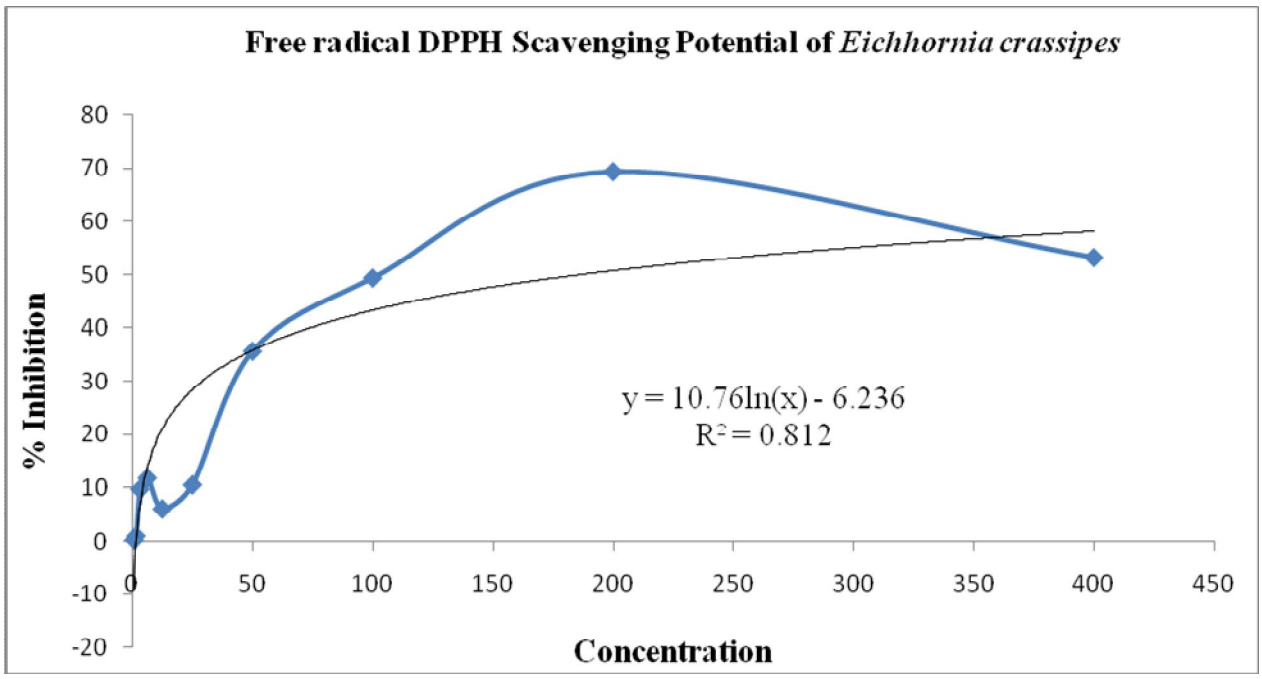


Figure 12: DPPH scavenging potential & IC_{50} value of mthanolic extract of *Eichhornia crassipes.*

5.2 Data Analysis for *Lawsonia inermis L.*

Table 10: IC_{50} (µg/ml) Calculation for *Lawsonia inermis L.*

Serial No.	**A_{Blank}**	**Concentration (µg/ml)**	**A_{Sample}**	**% inhibition of free radical DPPH = (1 – A_{Sample}/A_{Blank}) X 100%**	**IC_{50} (µg/ml)**
1		400	0.137	67.314	
2		200	0.133	68.365	
3		100	0.12	71.369	
4		50	0.103	75.327	

Serial No.	A_{Blank}	Concentration (µg/ml)	A_{Sample}	% inhibition of free radical DPPH = $(1 - A_{Sample}/A_{Blank}) \times 100\%$	IC_{50} (µg/ml)
5	0.419	25	0.154	63.128	17.0689
6		12.5	0.278	33.746	
7		6.25	0.337	19.456	
8		3.125	0.371	11.346	
9		1.562	0.416	0.743	

Graphical representation of the data from where IC_{50} is calculated:

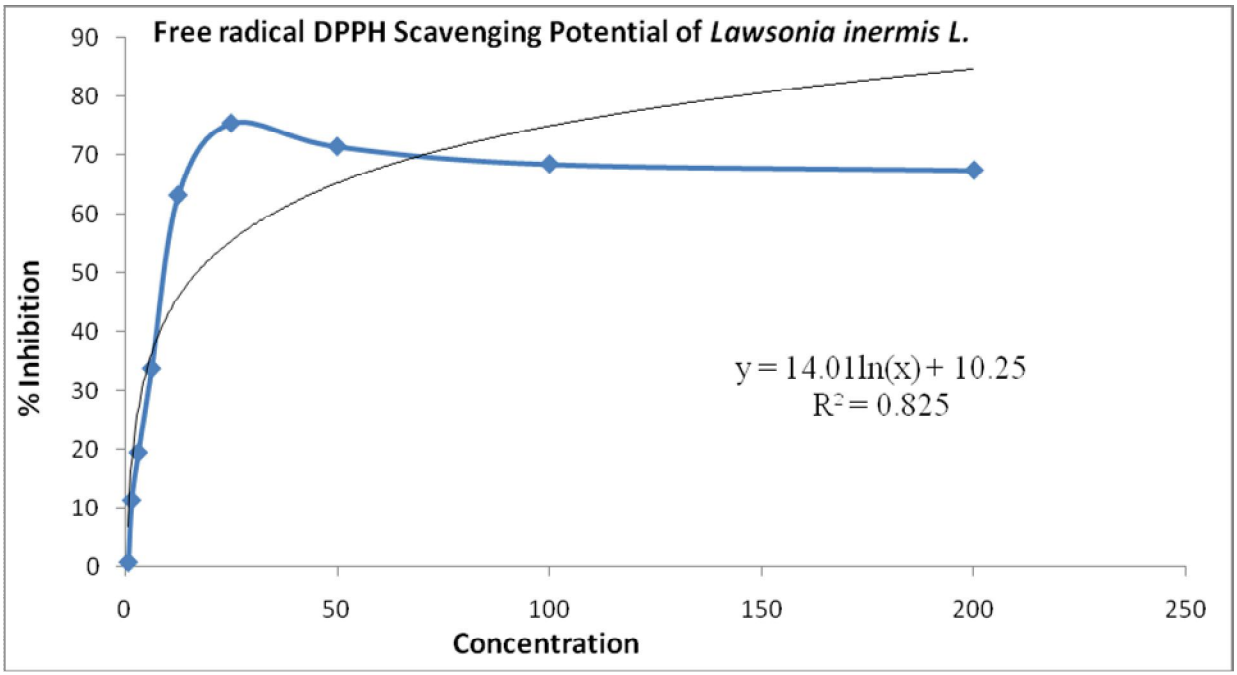


Figure 13: DPPH scavenging potential & IC_{50} value of crude methanolic extract of *Lowsonia inermis L.*

5.3 Data Analysis for *Cissus quadrangularis L.*

Table 11: IC_{50} (µg/ml) Calculation for *Cissus quadrangularis L.*

Serial No.	**A_{Blank}**	**Concentration (µg/ml)**	**A_{Sample}**	**% inhibition of free radical DPPH = $(1 – A_{Sample}/A_{Blank}) X 100$**	**IC_{50} (µg/ml)**
1		400	0.154	63.249	
2		200	0.141	66.369	
3		100	0.133	68.239	
4		50	0.115	72.554	
5	0.419	25	0.129	69.135	11.3163
6		12.5	0.154	63.246	
7		6.25	0.222	47.017	
8		3.125	0.278	33.652	
9		1.562	0.329	21.364	
10		0.7812	0.344	17.9	

Graphical representation of the data from where IC_{50} is calculated is in the following page.

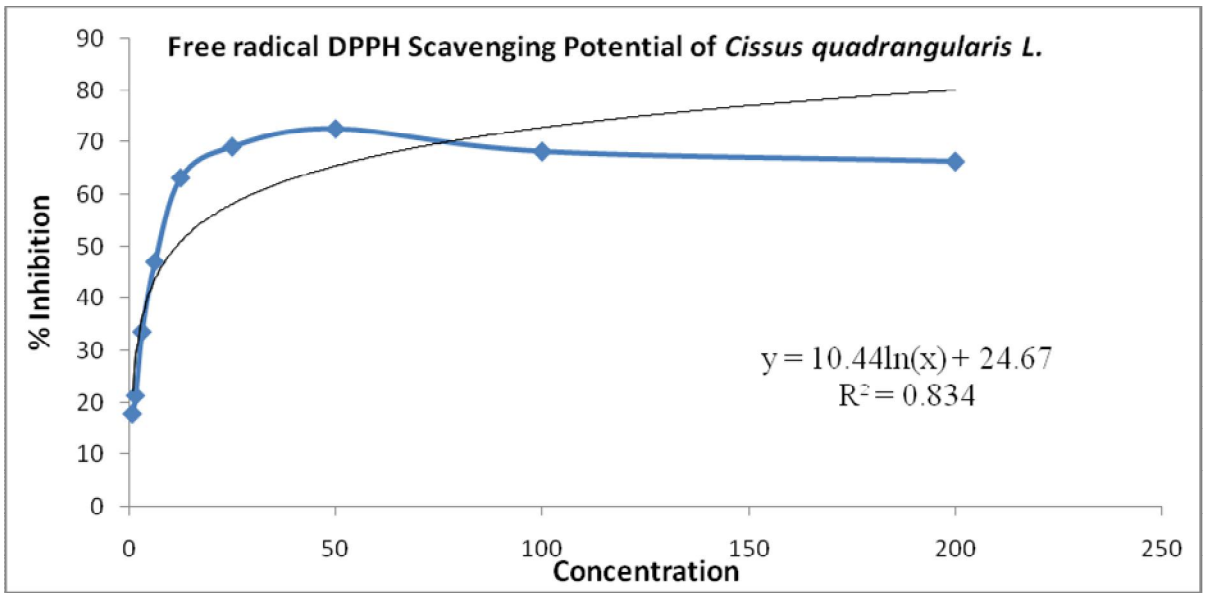


Figure 14: DPPH scavenging potential & IC_{50} value of crude methanolic extract of *Cissus quadragualis L.*

5.4 Data Analysis for Ascorbic Acid

Table 12: IC_{50} (µg/ml) Calculation for Ascorbic Acid.

Serial No.	**A_{Blank}**	**Concentration (µg/ml)**	**A_{Sample}**	**% inhibition of free radical DPPH = $(1 - A_{Sample}/A_{Blank}) \times 100$**	**IC_{50} (µg/ml)**
1		400	0.250	39.351	
2		200	0.259	38.249	
3	0.419	100	0.278	35.743	14.72
4		50	0.284	32.129	
5		25	0.331	20.982	
6		12.5	0.375	10.394	

Graphical representation of the data from where IC_{50} is calculated

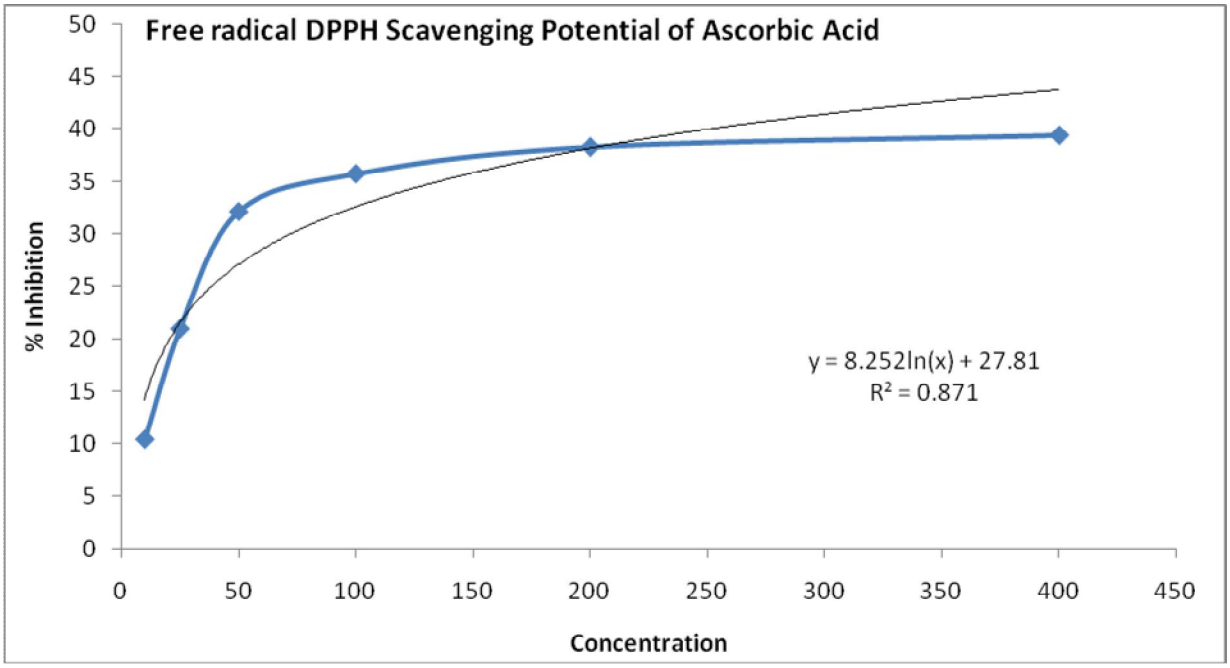


Figure 15: DPPH scavenging potential & IC_{50} value of pure Ascorbic Acid.

Chapter Six

RESULTS & DISCUSSIONS

6 RESULTS AND DISCUSSIONS

IC_{50} value of *Eichhornia crassipes* = 186.1196 µg/ml

IC_{50} value of *Cissus quadragualis L.* = 11.3163 µg/ml

IC_{50} value of *Lowsonia inermis L.* = 17.0689 µg/ml

Eichhornia crassipes shows activity with IC50 value of 186.12 µg/ml, which is actually not very significant. Over some years some number of people worked on this plant about same experiment. At 2009 Chantiratikul, P., Meechai, P. & Nakbanpotec, W. published a paper on the antioxidant activity of *Eichhornia crassipes.* Its procedure is quite similar to me and the result is also very close. Enein, A. M. A., Al-Abd, M. A., Shalaby, A. E., Ela, A. F., Nasr-Allah, A. A., Mahmoud, M. A. & El-Shemy, A. H. did the same research at 2011. They experimented DPPH scavenging activity to test the antioxidant properties of the crude extract of *Eichhornia crassipes* and its isolated fractions. Their IC_{50} ranged between 97.0 ± 5.4 and 97.4 ± 2.7 µg/ml, with a moderate activity. Variation of the result from their result can be due to that they used some specific isolated fractions from extract for DPPH test, but here the whole extract was used. At the same year Jayanthi, P & Lalitha, P. A. did the same experiment as mentioned in literature review part. They also got a moderate actioxidant activity. It varies slightly from this result. As they used ethyl acetate extract, while Methanolic extract was used in this study, which can be a considerable factor. At 2012, Shanaba, M. M. S. & Shalaby, A. E. made a paper on the same topic and find a moderate activity. They used some isolated compounds and they used ABTS method for antioxidant test where DPPH was used in this method. It can be said that the difference between the results are due to the methodological differences.

Cissus quadragualis L shows high antioxidant activity with a IC_{50} value of 11.3163 µg/ml. At 2003, K.N. Chidambara Murthy, A. Vanitha, M. Mahadeva Swamy, and G.A. Ravishankar found that the antioxidant activity of methanol extract and aqueous extract were comparatively less significant than that of ethyl acetate extract, and n-hexane extract showed the least activity. Here methanolic extract was used, but a good result. The mentioned researchers did both *in-vivo* (major) and in-vitro antioxidant test and they found a good result for ethyl acetate extract. At 2005, Jainu, M. & Devi, S. worked with Methanolic extract of stem of the plant and performed DPPH, OS and TBARS test and they claimed that the plant has a strong antioxidant activity. At 2007, Julius E Oben, Damaris M Enyegue, Gilles I Fomekong, Yves B Soukontoua and Gabriel A Agbor did the same work by ferric reducing antioxidant power (FRAP, antioxidant power) and 1,1-Diphenyl-2-picrilhydrazyl (DPPH, redical scavenging potential) and found same sort of activity. At 2011, Unnati Shah found strong antioxidant activity both *in-vivo* and *in-vitro* system for methanol extract and claimed that the activity is for the presence of β-carotene.

Lowsonia inermis L. having a IC_{50} value of 17.0689 µg/ml, has also strong antioxidant activity. At 2002, Endrini, S., Rahmat, A., Ismail, P. & Taufiq, Y. H tried to evaluate the antioxidant property of *Lowsonia inermis L.* and from their paper we know that the chloroform extract of henna had the highest activity (87.6 %), followed by α-tocopherol (62.5%) by using FTC method. Based on the TBA method, henna also had the significantly higher antioxidant activity (55.7 %) compared to α-tocopherol (44.4%). The comparison of both methods showed that henna had the significantly ($p<0.05$) higher total antioxidant activity in both methods. Antioxidant activity in henna was found to be the highest as compared to vitamin E or α -tocopherol. The strong cytotoxic properties of this extract could be due to its high antioxidant

activities. They used FTC method. At 2004, Mikhaeil, R. B., Badria, F., Maatooq, T. G., Ame, A. M. worked and found a very excellent activity, though they used whole plant part, methanol extract, ascorbic acid blank and ABTS method for experiment.

Mikhaeil, R. B., Badria, F., Maatooq, T. G., Ame, A. M worked with the plant leaves and found same result at 2007. At 2011, Jacob, P., Madhumitha, G. & Salar, M ; Guha, G., Rajkumar, V., Kumar, S. R. & Mathew, L. and Borade, S. A., Babasaheb, N., Kale, & Shete, V. R., three group of researchers worked separately and proved the antioxidant activity of henna plant by using different method. At 2012, Makhija, K. I., published a paper named, 'Lawsonia inermis - From traditional use to scientific assessment' and there he proved its antioxidant activity. These sorts of scientific works support this result; even they sometimes vary in methodology.

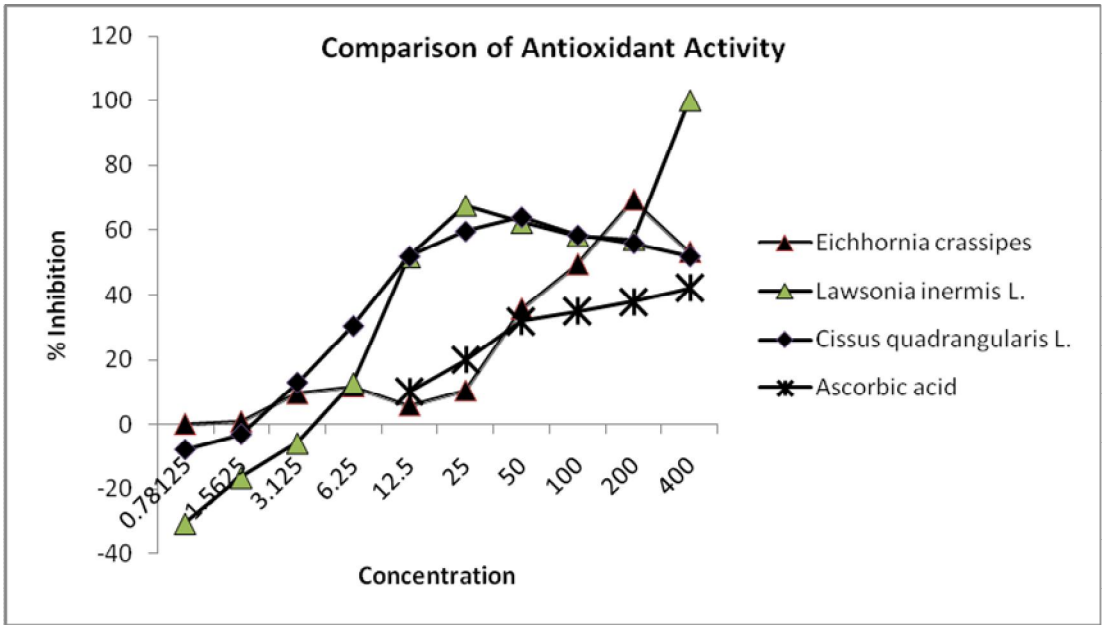


Figure 15: Comparison Between the Antioxidant Avtivity of the Plants.

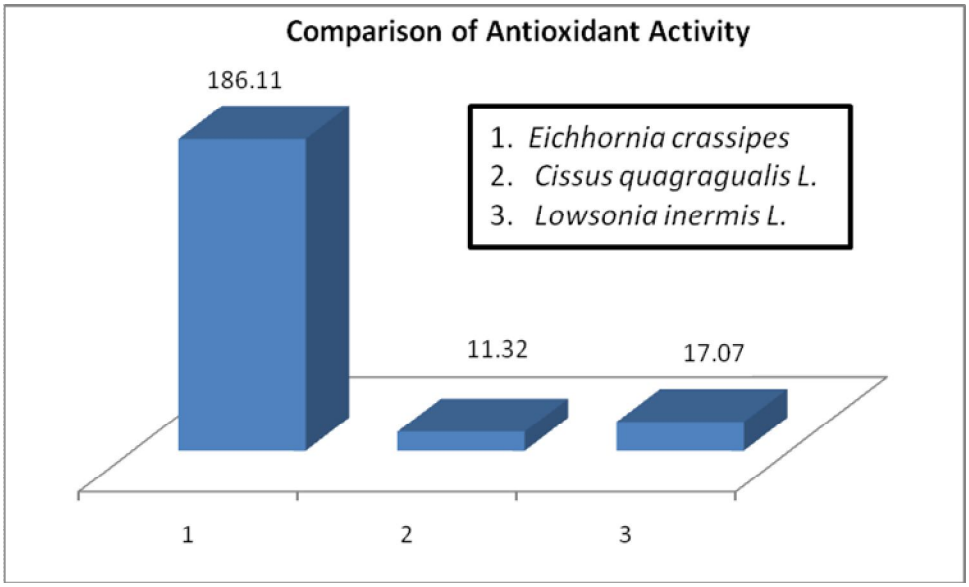


Figure 16: Compoarison of Antioxidant Activity.

These two figure proves that *Cissus quadragualis L.* is the strongest antioxidant among the three, as it has the lowest IC_{50} value.

CONCLUSION

In today life antioxidant is one type of indispensable for human and people are taking it very frequently. Unani and Ayourbedic medicines use these very commonly. They have also some preparations of antioxidants in market now. They are claiming their activity. This study has justified their claim with the quantitative value of results. Scientists can get pharmacophore from these plant. These plants can be used for further antioxidant experiment in different methods.

References

Aghel N, Ameri A, Ebrahimi P. 2005, Essential oil of Lawsonia inermis growing in iran: chemical composition and antifungal activity, first Seminar of Medicinal & Natural Products Chemistry Shiraz, Iran, 10-11 May.

Aguwa CN. 1987, 'Toxic Effects of the Methanolic Extract of Lawsonia inermis Roots', *International J Crude Drug*, Vol. 25, pp. 241-245.

Ali NAA, Julich WD, Kusnick C, Lindequist U 2001, ' Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities', *J Ethnopharmacol*, Vol. 74, no. 2, pp. 173-179.

Anita M. B. & Kaushal R. 1950, 'Essential oil from the flowers of camphire or henna plant', *Currnt Sci*, Vol. 19, pp. 284.

Arayne MS, Sultana N, Mirza AZ, Zuberi MH, Siddiqui FA 2007, ' In-vitro hypoglycemic activity of methanolicextract of some indigenous plants', *Pak J Pharm Sci,* Vol. 20, no. 4. pp 268-273.

Badarinath, V. A., Mallikarjuna, K., Chetty, S. M. C., Ramkanth, S., Rajan, S. V. T. & Gnanaprakash, K. 2010, 'A Review on In-vitro Antioxidant Methods: Comparisions, Correlations and Considerations', *International Journal of PharmTech Research*, Vol.2, No.2, April-June, pp 1276-1285.

Baba-Moussa F, Nacoulma O, Ouattara A, Nguyen HP, Akpagana K, Bouchet P 1997, 'Antibacterial activity of total aqueous extracts of Combretum micranthum, Lawsonia inermis and Waltheria indica, plants from west African pharmacopoeia', *Revue de Medecines et Pharmacopees Africaines*, Vol. 11, no. 12, pp. 197-203.

Bagi MK, Kakrani HK,Kalyani GA, Dennis TJ, Jagdale MH. 1988, 'Experimental evaluation of pharmacological activity of Lawsonia alba seed oil', *Fitoterapia*, Vol. 59, no. 1, pp. 39-42.

Bhandarkar M, Khan A. 2003, 'Protective effect of Lawsonia alba Lam.against carbontetrachloride induced hepatic damage in albino rats', *Indian J Exp Biol*, Vol. 41, no. 1, pp. 85-87.

Benzie, I. F. & Strain J. J., 1999, 'Ferric reducing antioxidant power assay; direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration methods enzymol', Vol. 299, pp. 15 – 27.

Borade, S. A., Babasaheb, N., Kale, & Shete, V. R., 2011, 'A phytopharmacological review on Lawsonia inermis (Linn.)', *INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES,* January, Vol. 2, no. 1.

Caldwell C. R. 2001, 'Oxygen radical absorbance capacity of the phenolic compounds in plant extracts fractionated by HPLC', *J Agric food chem*, Vol. 293, no. 2, pp. 232 – 238.

Chang H, Suzuka SE. 1982, 'Lawsone (2-OH-1, 4-napthoquinone) derived from the henna plant increases the oxygen affinity of sickle cellblood', *Bio-Chem. Biophys. Res. Commun,* Vol. 107, pp. 602-608.

Chantiratikul, P., Meechai, P. & Nakbanpotec, W., 2009, 'Antioxidant Activities and Phenolic Contents of Extracts from *Salvinia molesta* and *Eichornia crassipes'***,** *Research Journal of Biological Sciences,* September, Vol. 4, no. 10, pp. 1113-1117.

Cissus Experts, 2012, 'History of Cissus Quadrangularis' *Amazing New Discoveries of Cissus Quadrangularis* , Viewed at 30 June, 2012. < http://www.cissus.org/history-of-cissus-quadrangularis>

Cissus quadrangularis, 2012, Viewed at, 04 July, 2012, <www.en.wikipedia/ Cissus quadrangularis.org>

Dama LB, Poul BN, Jadhav BV 1999, ' Antimicrobial activity of Napthoquinonic compounds', *Journal of Ecotoxicology and EnvironmentalMonitoring*, Vol. 8, pp. 213-215.

Dasgupta T, Rao AR, Yadava PK 2003, 'Modulatory effect of Henna leaf (Lawsonia inermis) on drug metabolising phase I and phase II enzymes, antioxidant enzymes, lipid peroxidation and chemically induced skin and forestomach papillomagenesis in mice', *Molecular and Cellular Biochemistry*, Vol. 245, pp. 11-22.

Deka, D.K. 1994, 'Effect of Cissus quadrangularis in Accelerating Healing Process of Experimentally Fractured Radius-Ulna of Dog: A Preliminary Study by Using Radiological Examination', *Ind J. Pharmac*, VOl. 26, pp. 44-45.

Dixit SN, Srivastava HS, Tripathi RD 1980, 'Lawsone, the antifungal antibiotic from the leaves of Lawsonia inermis and some aspects of its mode of action', *Indian Phytopathol*, Vol. 31, pp. 131-133.

Dranghon, F.A. 2004. Use of botanicals as biopreservatives in foods. Food Technol.58:20-28.

El-Mehalawy A. A., Gebreel H. M., Rifaat H. M., El-Kholy I. M. & Humid A. A. 2008, 'Effect of antifungal compounds produced by certain bacteria on physiological activities of human and plant pathogenic fungi', *J. Appl. Sci*, Vol. 4, no. 4, pp. 425-432.

Endrini S, Rahmat A, Ismail P, Taufiq YH 2007, 'Comparing of the cytotoxicity properties and mechanism of Lawsonia inermis and Strobilanthes crispus extract against several cancer cell lines', *J Med Sci*, Vol. 7, no. 7, pp. 1098-1102.

Endrini, S., Rahmat, A., Ismail, P. & Taufiq, Y. H., 2002. 'Anticarcinogenic Properties and Antioxidant Activity of Henna (*Lawsonia inermis*). *Journal of Medical Sciences,*Vol. 2, pp. 194-197, viewed at. 06 July, 2012, <http://scialert.net/abstract/?doi=jms.2002.194.197 >

Enein, A. M. A., Al-Abd, M. A., Shalaby, A. E., Ela, A. F., Nasr-Allah, A. A., Mahmoud, M. A. & El-Shemy, A. H., 2011, '*Eichhornia crassipes* (Mart) solms', *Plant Signal Behav,* June, Vol. 6, no. 6, pp. 834-836.

Evans P. & Halliwall B. 1999, 'Free radicals and Hearing', *Ann N Y Acad Sci*, Vol. 19.

Fernandes, G. & Banu, J., 2012, 'Medicinal properties of plants from the genus Cissus: A review', *Journal of Medicinal Plants Research*, Vol. 6, no. 16, pp. 3080-3086, Viewed at, 28 June, 2012.< http://www.academicjournals.org/JMPR>

Freshney RI (2002). Cell line provenance. *Cytotechnology*, Vol. 39 pp.55-67.

Ghani, A. (1998). *Medicinal Plants of Bangladesh: Chemical Constituents and Uses*. Asiatic Society of Bangladesh, Dhaka.

Guha, G., Rajkumar, V., Kumar, S. R. & Mathew, L., 2011, 'Antioxidant Activity of Lawsonia inermis Extracts Inhibits Chromium(VI)-Induced Cellular and DNA Toxicity', *Evidence-Based Complementary and Alternative Medicin,* November, Vol. 2011.

Gupta S., Ali M., Alam M. S., Niwa M. & Sakai T. 1992, '24-beta ethylcholest-4-en-3-beta-ol from the Roots of Lawsonia inermis', *Phytochemistry*, Vol. 31, pp. 2558-2560.

Gupta S., Ali M., Alam M. S., Sakae T. & Niwa M. 1992, 'A new aliphatic hydrocarbon from Lawsonia inermis bark', *Indian Journal of Chemistry Section B Organic Chemistry Including Medicinal Chemistry*, Vol. 31, pp. 705-707.

Gupta S, Ali M, Pillai KK, Alam MS. 1993, 'Evaluation of anti-inflammatory activity of some constituents of Lawsoniainermis', *Fitoterapia*, Vol. 64, pp. 365-366.

Gupta, A., Saifi, A.Q., Modi, N. T. & Mishra, N. 1986, 'Anti-inflammatory activity of some active principles of Lawsonia inermis leaves', *Indian Journal of Pharmacology*, Vol. 18, no. 6, pp. 113-114.

Hamdi YP, Benazzouz M, Belkhiri H, Chari Z, Serakta M, Bensgni L. 1997, 'Healing effect of Lawsonia inermis L. (henna) as exemplified by the third degree burns', *Revue de Medecines et Pharmacopees Africaines*, Vol. 11, no. 12, pp. 151-156.

Hosein H. K. M. & Zinab D. 2007, 'Phenolic compounds and antioxidant activity ofhenna leaves extracts (Lawsonia Inermis)', *World Journal of Dairy & Food Sciences*, Vol. 2, no. 1, pp. 38-41.

Huang D., Ou B. & Prior R. L. ,2005, 'The chemistry behind antioxidants capacity assays', *J Agric food chem*., Vol. 53, no. 6, pp. 1841 – 1856.

Jacob, P., Madhumitha, G. & Salar, M., 2011, 'Free radical scavenging and reducing power of Lawsonia inermis L. seeds', *Asian Pac J Trop Med*, June, Vol. 4, no. 6, viewed at. 08 July, 2012, <http://www.ncbi.nlm.nih.gov/pubmed/21771698>

Jainu M. 2006, 'Gastroprotective effect of Cissus quadrangularis extract in rats with experimentally induced ulcer', *Indian J Med*, Vol. 123, pp.799-806.

Jainu, M. & Devi, S., 2005, 'In vitro and in vivo evaluation of free radical scavenging potential of Cissus quadrangularis', *Afr. J. Biomed*. Vol. 8, pp. 95-99.

Jayanthi, P. & Lalitha, P., 2011, 'Determination of the invitro reducing power of the aqueous extract of Eichhornia crassipes (Mart.) Solms', *Journal of Pharmacy Research,* 01 July, Vol. 4, no. 11, viewed at, 07 July, 2012, < www.jpronline.info>

Jayanthi, P. & Lalitha, P., 2011, 'REDUCING POWER OF THE SOLVENT EXTRACTS OF EICHHORNIA CRASSIPES (MART.) SOLMS', *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 3, no. 3.

Jigna, P. 2008, 'Antibacterial Activity of Aqueous and Alcoholic Extracts of 34 Indian Medicinal Plants against Some Staphylococcus Species', *Turk J Biol*, Vol. 32, pp. 63-71.

Kashikar, ND, George I. 2006, 'Antibacterial activity of Cissus quadrangularis Linn', *Indian J. Pharm Sci*, Vol. 68, pp.245-247.

Khan MM, Ali A, Jain DC, Bhakuni RS, Zaim M, Thakur RS. 1991, 'Occurrence of some antiviral sterols in Artemisia annua', *Plant Sci*, Vol. 75, no. 2, pp. 161-165.

Khodaparast, H., Hosein, M., Zinab, D., 2007, 'Phenolic compounds and antioxidant activity of Henna leaves extracts (Lawsonia inermis)', *World J. Dairy Food Sci*, Vol. 2, pp. 38-41.

Kumar RA, Kokate CK, Rambhau D, Rao YM. 1985, 'Studies in Lawsonia inermis lawsone and its oxazine derivatives as potential anticoagulant agents', *Indian Journal of Pharmaceutical Sciences*, Vol. 47.

Lawsonia Inermis, 2012, Species & Medicanal Herbs, viewed at, 28 June, 2012,< http://www.spicesmedicinalherbs.com/lawsonia-inermis.html>

Makhija, K. I., 2012, 'Lawsonia inermis - From traditional use to scientific assessment', *Pharmatutor,* ART-1022.

Mallika jainu, K. 2006, 'Protective effect of Cissus quadrangularis on neutrophill tissue injury induced by aspirin in rats', *journal of ethnopharmacology*, Vol. 104, pp. 302-305.

Miller N.J. & Rice-Evans, C., 1993, 'A novel method for measuring antioxidant capacity and its application tomonitoring the antioxidant status in premature neonates', *Clin Sci*, Vol. 84, pp. 407 – 412.

Mikhaeil, R. B., Badria, F., Maatooq, T. G., Ame, A. M., 2004, 'Antioxidant and Immunomodulatory Constituents of HennaLeaves', *Z.Naturforsch,*February, Vol, 59, pp. 468-476.

Mohanamba, E., Shobana, K., Sree, S. M., Kusuma, M. G., Satish, K. & Vijayakumar, B. 2012, 'Isolation of Alcoholic Extract of Cissus Quadrangularis and Evaluation of In-Vitro Anthelmintic Activity', *INTERNATIONL JOURNAL OF NOVEL TRENDS IN PHARMACEUTICAL SCICENCES*, Vol. 1, no. 1, viewed at, 16 June, 2012. < www.ijntps.com/ijnt/downloads/Volume%201/Issue%201/002.pdf>

Mohsin, A., Shah, A. H., Al-Yahya, M. A., Tariq, M., Tanira, M. O. M. & Ageel, A. A. 1989, 'Analgesic, antipyretic activity and phytochemical screening of some plants used in traditional Arab system of medicine', *Fitoterapia*, Vol. 60, no. 2, pp. 174-177.

Munshi SR, Shetye TA, Nair RK. 1977, ' Antifertility activity of three indigenous plantpreparations', *Planta Med*, Vol. 31, pp. 73-75.

Murthy, C. K. N., Vanitha, A., Swamy, M. M. & Ravishankar, A. G., 2003, 'Antioxidant and Antimicrobial Activity of *Cissus quadrangularis* L', *Journal of Medicinal Food*, July, Vol. 6, no. 2.

Natarajan MR, Lalithakumar D 1987, 'Leaf extracts of Lawsonia inermisas antifungal agent', *Curr Sci*, Vol. 56, pp. 21-22.

Oben, E. J., Enyegue, M. D., Fomekong, I. G., Soukontoua, B. Y. & Agbor, A. G., 2007, 'The effect of Cissus quadrangularis (CQR-300) and a Cissus formulation (CORE) on obesity and obesity-induced oxidative stress', *Lipids in Health and Disease, February, Vol. 6, no.4.*

Oben, E. J., 2007, 'The Effect (CQR-300) and Cissus formulation (CORE) on obesity and obesity induced oxidative stress', *Lipids in Health and Disease*, Vol. 6.

Oben, E. J., 2008, 'The Use of a Cissus quadrangularis/Irvingia gabonensis Combination in the Management of Weight loss: a double-blind Placebo-Controlled Study', *Lipids in Health and Disease*, Vol. 7.

Opoku, A. R. 2000, 'Preliminary Screening of Some Traditional Zulu Medicinal Plants for Antineoplastic activities versus the HepG2 Cell line', *PTR*, Vol. 14.

Parekh, J. Chanda, S.V. 2008, 'Antibacterial Activity of Aqueous and Alcoholic Extracts of 34 Indian Medicinal Plants against Some Staphylococcus Species', *Turk J Biol*, Vol. 32, pp. 63-71.

Parekh, J., Chanda, S. 2007, 'In vitro Screening of Antibacterial Activity of Aqueous and

Alcoholic Extracts of Various Indian Plant Species Against Selected Pathogens from Enterobacteriaceae', *African J. of microbiology research*, Vol. 1, pp. 92-99.

Popov, I. & Lewin, G. 1999, 'Oxidants and Antioxidants Part B -Antioxidative homeostasis: characterization by means of chemilumi-nescent technique', *Methods in Enzymology*, Vol. 300, pp. 437-456.

Potu, B.K. 2008, 'Petroleum Ether Extract of Cissus Quadrangularis L. Stimulates the Growth of Fetal Bone during Intra Uterine Developmental Period: A Morphometric Analysis', *Clinics*, Vol. 63.

Prakash, A. Rigelhof, F. & Miller, E. 'Antioxidant Activity', *Medallion Laboratories Analytical Progress*, viewed at, 29 June, 2012, < www.medallionlabs.com>

Pratibha G. & Korwar G. R. 1999, 'Estimation of lawsone in henna (Lawsonia inermis)', *Journal of Medicinal and Aromatic Plant Sciences*, Vol. 21, pp. 656-660.

Preciousproducts, 2012, Cissus quadrangularis (MUDU PAYALA NALLEDA) 3&4 FACES, Viewed at, 26 June, 2012.< http://preciousproducts.weebly.com/cissus-quadrangularis.html>

Sastri B. N. 1962, 'The Wealth of India: Raw Materials', *CSIR*, Vol. L-M, no. 6, pp. 47-50

Schlesier, K., Harwat, M., Bohm, V. & Bitsch, R. 2002, 'Assessment of Antioxidant Activity by Using Different In Vitro Methods', *Free Radical Research*, Vol. 36, No. 2 , Pages 177-187, viewed at 6 July, 2012, < http://informahealthcare.com/doi/abs/10.1080/10715760290006411>

Shah, U. 2011, 'CISSUS QUADRANGULARIS L.: PHYTOCHEMICALS, TRADITIONAL USES AND PHARMACOLOGICAL ACTIVITIES - A REVIEW', *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 3, no. 4, pp. 41-44, Viewed at, 06 June, 2012. <www.ijppsjournal.com/Vol3suppl4/2439.pdf>

Shanaba, M. M. S. & Shalaby, A. E., 2012, 'Biological activities and anticorrosion efficiency of water hyacinth(Eichhornia crassipes)', *Journal of Medicinal Plants Research*, June, Vol. 6, no. 23, viewed at. 30 June, 2012. < http://www.academicjournals.org/JMPR>

Sharma, V. K. 1990, 'Tuberculostatic activity of henna Lawsonia inermis Linn', *Tubercle*, Vol. 71, no. 4, pp. 293-296.

Siddiqui B.S., Kardar M. N. & Khan S. 2005, 'Two New Triterpenoids from Lawsonia inermis', *Helvetica Chimica Acta*, Vol. 86, no. 6, pp. 2164-2169.

Singh, A. 1984, 'An Experimental Study of Analgesic Activity of Cissus Quadrangularis', *Ind. J. Pharmac*, pp. 162-163.

Singh, A. 2008, 'Anti-inflammatory and Analgesic agents from Indian Medicinal Plants', *IJIB*, Vol. 3, pp. 58.

Syamsudin I. & Winarno H. 2008, ' The effects of Inai (Lawsonia inermis)leaveextract on blood sugar level: An Experimental Study', *Res J Pharmacol*, Vol. 2, no. 2, pp. 20-23.

Texiwala, G. 2012, Lawsonia inermis, viewed at 05 July, 2012. <http://dmt-sbi3u.wikispaces.com/Lawsonia+inermis>

Thamaraiselvi, Lalitha, P. and Jayanthi, P. 2012, 'Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.)Solms', *Asian Journal of Plant Science and Research*, Vol. 2, no. 2, pp. 115-122.

Thisayakorn, K 2007, 'Preliminary Anti inflammatory of Cissus quadrangularis Linn. in rats', *Journals of Ethnopharmacology*, Vol. 113, pp. 54-61.

USDA, 2012, *Cissus quadrangularis* L., Natural Resources Conversion Service, Viewed at, 07, June, 2012. <http://plants.usda.gov/java/profile?symbol=ciqu5>

USDA, 2012, Classification for Kingdom Plantae Down to Species *Lawsonia inermis* L, Natural Resourses Conversion Service, viewed at 29 June, 2012. <http://plants.usda.gov/java/ClassificationServlet?source=profile&symbol=LAIN5&display=31>

Vasu, 2012, 'About *Cissus quadrangularis(*Bonton Capsule)', *Published Report summary, India, Viewed at, 01 July, 2012,* <www.vasuhealthcare.com/pdf/aboutcissusquadrangularis.pdf>

Venkata subbu V. S. 1970, ' Pharmacological and Toxicological evaluation of an active principle obtained from the plant Vitis quadrangularis', *Isnd. J. Pharmac*, Vol. 2, no. 3, pp. 91-97.

WEST63RD Internet, 2007, Lawsonia Inermis L, viewed at, 30 June, 2012, <http://www.hennatattoos.com/lawsonia-inermis/>

WHO. 1993, Regional office for Western Pacific Research guidelines for evaluating safety and efficacy of herbal medicines, Manila.

Wurochekke, A. U., Chechet, G. & Nok, A.J. 2004, 'In-vitro and In-vivoantitrypanosomal brucei infection in mice', *J Med Sci*, Vol. 4, no. 3, pp. 236-239.

Yogisha S, Samiulla DS, Prashanth D, Padmaja R, Amit A. 2002, 'Trypsin inhibitory activity of Lawsonia inermis', *Fitoterapia*, Vol. 73, pp. 690-691.