STUDIES ON MACROMOLECULES, PHOTOCHEMICAL IN VARIOUS ALOE VERA EXTRACTS AND ITS ANTIOXIDANT

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Technology degree in

Chemical Engineering

by

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DEPARTMENT OF CHEMICAL ENGINEERING

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafidework of **D.DURGA DEVI (38070002)**who carried out the projectentitled **"**Studies on macromolecules, phytochemicals in various aloe vera extracts and its antioxidant and antibacterial activity**"** under our supervision from October 2020 to March 2021.

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DECLARATION

I D.DURGA DEVI **(38070002)** hereby declare that the Project Report entitled **"STUDIES ON MACROMOLECULES, PHOTOCHEMICAL IN VARIOUS ALOEVERA EXTRACTS AND ITS ANTIOXIDANT ACTIVITY"** done by me under the guidance of **Dr.R.THYAGARAJEN** at Sathyabama institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biotechnology.

DATE:

PLACE: **SIGNATURE OF THE CANDIDATE**

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ABSTRACT

Aloe vera is the oldest known medicinal plant and the most commonly used medicinal plant on the planet. Aloe vera extract is a well-known skin healer. Burning, skin irritations, bruises, and insect bites are all soothed by aloe vera, and its bactericidal properties alleviate itching and skin swellings.Aloe vera is a strong detoxifier, antiseptic, and nervous system tonic. Vitamin B12, Vitamin A, B-Group vitamins, Vitamin C, Vitamin E, and folic acid are among the vitamins present in aloe vera gel. Aloe vera gel contains important ingredients such as 19 of the 20 amino acids required by the human body, as well as seven of the eight essential amino acids that cannot be produced by the body. In the present study was performed to investigate the bioactive constituents of Aloevera.

Extracted by using ethanol and water. Qualitative analysis of phytochemicals from the extracts showed the presence of carbohydrates, glycosides, phenols, terpenoids, saponins and proteins. The amount of carbohydrates, phenols and proteins was found to 310ug/g and 210ug/g, 490u/g and 280ug/g and 190ug/g and 390ug/g in aqueous and ethanol extracts. The DPPH radical scavenging activity ranged from 11.5% to 51.6% for ethanol extract and from 11.0% to 50.10% for aqueous extract.Hydrogen peroxide scavenging activity range from 12.5% to 51.7% for ethanol extract and from 11.5% to 50.15% for aqueous extract.Reducing power assay range from 12.5% to 56.7.7% for ethanol extract and from 11.0% to 49.10% for aqueous extract. Nitric oxide assay range from 13.5% to 52.67% for ethanol extract and from 11.5% to 51.10% for aqueous extract.

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LIST OF ABBREVIATIONS AND SYMBOLS

gms	-	Grams
ml	-	Millilitre
min	-	Minute
w / v	-	Weight by volume
nm	-	Nanometres
mg	-	Milligrams
mg/ml	-	Milligram per millilitre
mM	-	Millimolar
ug/ml	-	Microgram per millilitre
°C	-	Degree celcius
cm	-	Centimetre
%	-	Percentage
HCl	-	Hydrochloric acid
H_2SO_4	-	Sulphuric acid
GAE	-	Gallic acid equivalents
PBS	-	Phosphate buffer solution
Na_2SO_3	-	Sodium sulfite
NaOH	-	Sodium hydroxide
UV	-	Ultra Violet

CHAPTER 1

INTRODUCTION

Aloe vera (Aloe barbadensis), a plant with incredible medicinal properties, is commonly used in Ayurvedic, Homoeopathic, and Allopathic medicine. This plant is one of the most abundant natural health sources for mammals, including humans. The existence of more than 200 different biologically active substances has been discovered in the plant's chemistry, including antimicrobial, antibacterial, antifungal, and antiviral activities of the nonvolatile constituents of the leaf gel. Aloe species can be found around the African and Eastern European continents, as well as almost everywhere else on the globe. While the genus Aloe includes over 400 species, only a few, such as Aloe vera, Aloe ferox, and Aloe arborescens, are commonly traded. The inner gel of the leaves contributes to many biological properties associated with Aloe plants. Anti-diabetic, anti-inflammatory, peptic ulcers, antitumor, anticancer properties, activity impact on the immune system, allergic reactions, laxative effects, wound healing, antiseptic, vitamins, minerals, enzymes, amino acids, tension, sugars(GajendraMahor et al 2016).

1.1 CHEMICAL CONSTITUENTS OF THE PLANT

Aloe vera contains over 100 active biologic constituents (Ronald M. S. et al.,2007). The plant contains a number of natural, health-promoting compounds, including:

Polysaccharides :

Aloe gel contains 99 percent water and has a pH of 4.5, making it a common ingredient in over-the-counter skin salves. Glucomannan, an emollient polysaccharide, is present in the gel. It's a strong moisturizer that's used in a lot of cosmetics. From the Aloe vera inner leaf gel section, other polysaccharides such as arabinan, arabinorhamnogalactan, glactan, galactogalacturan, glucogalactomannan, and glucuronic acid containing polysaccharides are isolated (Choi S et al 2003).

Anthraquinones /Anthrones

Other polysaccharides containing arabinan, arabinorhamnogalactan, glactan, galactogalacturan, glucogalactomannan, and glucuronic acid are extracted from the Aloe vera inner leaf gel portion (Choi S et al 2003).

Vitamins/ Minerals: Vitamins C, A, E, B, B Carotene, Zinc, Calcium, Copper, Magnesium, Manganese, and Phosphates are all contained in it.

Enzymes : Alliase, alkaline phosphatase, amylase, catalase, lipase peroxidase, and corboxy peptidase are among the enzymes present in it.

Amino acids : It contains 20 of the 22 amino acids needed by humans, as well as seven of the eight essential amino acids. Campestrol, cholesterol, bsitosterol, and lupeol are four plant sterols.

1.2 THERAPEUTIC USES OF ALOE VERA

Aloe vera exudates are a clear, slick mucilage or gel produced by thin-walled tubular cells in the leaf's inner central zone (parenchyma). This mucilage was used to treat inflamed skin, and it was also used to treat radiation burns in the twentieth century. The bioactive compounds are used as astringents, homeostasis, antidiabetic, antiseptics, antibacterial, antioxidants, and anti-tumor agents, and are also useful in the treatment of stomach problems, gastrointestinal problems, constipation, radiation injury, wound healing, burns, dysentery, diarrhea, and skin diseases. Shelton R M et al.,1991) say that the plant is now commonly used in skin care.

Vitamins, enzymes, minerals, starch, lignin, saponin, salicylic acid, and amino acids are among the 75 potentially active constituents in aloe vera.Wound healing: The use of Aloe Vera for the healing of first and second degree burns is supported by a growing body of evidence (Maenthaisong R.et al 2007). Mannose-6 phosphate7 has been linked to the wound-healing properties of Aloe Vera gel. When applied topically to the skin, Brady kinase enzymes aid in the reduction of excessive inflammation.

Anti-oxidant activity Vitamins A, B, Carotene, C, and E are all present. Antioxidants are what they are. It contains minerals like calcium, magnesium, potassium, sodium, and zinc, which are essential for the proper functioning of antioxidant enzymes.

Anti-ulcer activity: Aloe vera gel contains a variety of glycoproteins that have anti-tumor and anti-ulcer properties (Sai Krishna Borra et al 2011).

Anti-septic and analgesic properties: Cholesterol, campesterol, B-sisosterol, and lupesol are some of the fatty acids present in aloe vera gel. They all have anti-inflammatory effects, with lupesol having antiseptic and analgesic properties (Barcroft et al 2003).

Anti-bacterial and anti-viral activity: Aloe vera gel contains 12 anthraquinones, which are phenolic compounds that have been used as laxatives in the past. Aloin and Emodin have anti-inflammatory, antibacterial, and antiviral effects (Lawrence R et al 2009).

Other medicinal uses: Traditionally, Aloe vera gel has been used topically (to treat wounds, burns, and skin irritations) and internally (Tanaka, M.et al., 2009) to treat constipation, ulcers, diabetes, headache, arthritis, and immune system deficiency. Polysaccharides found in aloe vera raise insulin levels and have hypoglycemic properties (Eshun, K. et al 2004).Cosmetics and skin protection application: For pimples, aloin and its gel are used as a skin tonic. In harsh and dry weather, aloe vera is often used to soothe the skin and keep it moist to help prevent flaky scalp and skin (West D.P et al 2003). Because of the presence of anthraquinone, aloe vera has a laxative effect. It is also said to have moisturizing and anti-aging properties, as well as anti-septic and anti-diabetic properties.

Helps digestion: Aloe vera juice helps the body to naturally cleanse the digestive system. If an individual is constipated, it allows the bowels to travel and aids in elimination. It will also assist in the slowing down of diarrhea.

Increases energy levels : Many substances in our diets can lead to fatigue and exhaustion. Aloe vera juice, when eaten on a daily basis, encourages a sense

of well-being, raises energy levels, and assists in the maintenance of a healthy body weight.

Builds immunity : Since the polysaccharides in Aloe vera juice activate macrophages, the white blood cells that battle viruses, it is particularly helpful for those with chronic immune disorders like polysaccharides or fibromyalgia.

Detoxifies : Aloe vera juice is an outstanding natural detoxifier. We also need to cleanse our systems from time to time because of our stressful lives, the pollution around us, and the junk foods we consume. Drinking Aloe vera juice contains a fantastically rich cocktail of vitamins, minerals, and trace elements to assist our bodies in coping with these stresses and strains on a regular basis.

Reduces inflammation: It increases joint stability and aids in body cell regeneration. It strengthens joint muscles, which decreases pain and inflammation in joints that are weak or old. While it is unclear when aloe vera's medicinal properties were first discovered, it has a long history with herbal medicine. If the aloin has been extracted by processing, aloe vera is non-toxic and has no reported side effect. Excessive ingestion of Aloe vera containing aloin has been linked to a number of side effects. However, the species is commonly used in Chinese, Japanese, Russian, South African, American, Jamaican, and Indian traditional herbal medicine. Aloe vera is thought to be helpful in wound healing.

Some studies, for example, indicate that Aloe vera gel facilitates wound healing, while others show that wounds treated with Aloe vera gel heal much slower than wounds treated with traditional medical preparations.

This study is focused on efficiency of aloevera extract (ethanol and aqueous) identified by quantification of photochemical, estimation of carbohydrates, proteins and phenols from aloe vera (ethanol and aqueous),Antioxidant activity(Antioxidant activity by DPPH assay, Hydrogen peroxide scavenging assay, Reducing power assay, Nitric oxide scavenging activity) and Antibacterial activity by well diffusion method.

CHAPTER 2

REVIEW OF LITERATURE

Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20% of the plants are found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources (Mothana R.A et.al,2005). The genus Aloe belonging to family Alliaceae is a succulent herb of 80 - 100 cm in height which matures in 4 - 6 years and survive for nearly 50 years under favorable conditions. Aloe vera (L.)Burm. f. syn. Aloe barbadensis Miller, is most biologically active among 400 species(Joshi S.P et.al,1997, West S.P et.al ZhuY.F et.al,2003 , Yagi.A , Kabash.A , Mizuno.K , Moustafa S.M , , YaKhalifa T.I , Tsuji.H et.al , 2003).

According to World Health Organisation, medicinal plants would be the best source for obtaining a variety of drugs (Santos.P.R.V,Oliveria.A.C.X , Tomassini.T.C.B et.al,1995). The plant is native to southern and eastern Africa along the upper Nile in the Sudan, and it subsequently introduced into northern Africa and naturalized in the Mediterranean region and other countries across the globe. The plant is commercially cultivated in Aruba, Bonaire, Haiti, India, South Africa, the United States of America, and Venezuela (Yeh.G.Y,Eisenberg.D.M, Kaptchuk.T.J ,Philips.R.S et.al, 2003).while the finest quality of Aloe is grown in desert of Southern California. The plant can survive in hot temperatures of 104˚F and with stand in below freezing temperatures until root is not damaged.

2.1 OUTER PROTECTIVE LAYERS OF LEAF

The bitter yellow latex of pericyclic tubules in the outer layer of the leaves contain derivatives of hydroxyanthracene, anthraquinone and glycosides aloin A and B from 15% - 40% in different investigations (Saccu.D , Bogoni.P , Procida.G et.al,2001 Bradley.P.R et.al, 1992 Bruneton.J et.al, 1995). The other active principles of Aloe include hydroxyanthrone, aloeemodin-anthrone 10-C-glucoside and chrones.

2.2 MIDDLE LAYER OF LEAF

The bitter yellow latex containing anthraquinones and glycosides has been reported from the middle layers ofThe juice that is originated from cells of the pericycle and adjacent leaf parenchyma, flowing spontaneously from the cut leaf get dried with or without the aid of heat and get solidified should not be confused with Aloe vera gel which is also the colourless mucilaginous gel that is obtained from the parenchymatous leaf cells (Bruneton.J et.al,1995). The parenchymatous tissue or pulp shown to contain proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds and small organic compounds in addition to the different carbohydrates. There is some evidence of chemotaxonomic variation in the polysaccharide composition [(Ni.Y ,Tizard .I.R et.al, 2004, Reynolds.T , Dweck.A.C et.al,1999) 16-different polysaccharides and 12 major polypeptides (molwt 15 - 77 kD), and various glycoproteins (29 kD in leaf gel).

2.3 INNER LAYERS OF LEAF

The innermost layer of leaf gel contains water upto 99%, with glucomannans, amino acids, lipids, sterols and vitamins (Brown.J.P et.al,1980 , Reynolds.T , Dweck.A.C et.al, 1999). Structure of Anthraquinone and structure of glycosides The other potentially active ingredients include vitamins, enzymes, minerals, sugars, lignin, saponins, saliOpen Access PP Therapeutic and Medicinal Use of Aloe vera: A Review Open Access PP 3 cylic acids, and amino acids (Vogler.B.K ,Ernst.E et.al, 1999 , Townsend.J et.al, 1998 , Antherton.P et.al, 1998 , Shelton.M.S et.al, 1991). It has numerous monosaccharide's and polysaccharides; vitamins B1, B2, B6, and C; niacinamide and choline, several inorganic ingredients, enzymes (acid and alkaline phosphatase, amylase, lactate dehydrogenase, lipase) and organic compounds (aloin, barbaloin, and emodin) as described by (Hayes.S.M et.al, 1999).

The main functional component of Aloe vera is a long chain of acetylated mannose (Femenia.A ,Sanchez.E.S, Simal.SRosello.C , et.al, 1999. Djeraba.A, Quere. P ,et.al, 2000, Lee.J.K, Lee. M. K ,Yun.Y. P, Kim.Y ,Kim.J.S ,Kim.Y.S, Kim.k,Han.S.S , Lee.C.K et.al,2001). Aloe gel is often commercialized as powdered concentrate. The therapeutically, it is used to prevent progressive

dermal ischemia due to burns, frostbite, electrical injury and intra arterial drug abuse. In vivo analysis of these injuries demonstrates that this gel acts as an inhibitor of thromboxane A2, a mediator of progressive tissue damage (Antherton.P et.al, 1998).

The Aloe vera gel play chief role in stimulation of the complement linked to polysaccharides, hydration, insulation, and protection. Application of fresh gel to normal human cells in vitro promoted cell growth and attachment, whereas a stabilized gel preparation was cytotoxic to both normal and tumour cells. This cytotoxicity was attributed to additional substances added to gel during processing (Davis.R.H,DiDonato.J.J, Hartman.G.M , Hass.R.C , et.al, 1994). The wound healing powers were due to a high molecular weighted polypeptide in healing of rat's excision wounds (Heggers.J.P , et.al,1996).

This glycoprotein promotes cell proliferation, so gel improves wound healing by increasing blood supply and increased oxygenation (Yagi.A, Kabash.A, Mizuno.K, Moustafa S.M,YaKhalifa.T.I, Tsuji.H et.al, 2003 Davis .R.H, Leitner.M.G,Russo.J.M ,Byrne.M.E,et.al,1989.). Growth of new blood capillaries (angiogenesis) and tissue regeneration in the burn tissue for a guinea pig has been reported, however, no specific constituents were identified (Heggers.J.P , et.al,1996). Further, a low molecular weight compound from freeze-dried gel stimulated angiogenesis in chick chorioallantoic membrane, and a methanol-soluble fraction of the gel stimulated proliferation of arteries in endothelial cells and induced them to invade a collage substrate (Lee.M.J, Yoon.S.H, Lee.s.k, Chung.M.H ,Park.Y.I, Sung.C.K , Choi.J.S , Lim.K.W,1995.)

2.4 WOUND HEALING

Wound healing is a dynamic process, occurring in 3 phases. The first phase is inflammation, hyperaemia and leukocyte infiltration. The second phase consists of removal of dead tissue. The third phase of proliferation consisting of epithelial regeneration and formation of fibrous tissue (Reddy uma .C.H, Reddy .S.K, Reddy .J et.al, 2011). A more recent review concludes that the cumulative evidence supports the use of Aloe vera for the healing of first to second degree burns (Maenthaisong.R, Chaiyakunapruk.N, Niruntraporn et.al, 2007).

The wound healing property of Aloe vera gel has been attributed to Mannose-6-phosphate (Davis .R.H, Leitner.M.G,Russo.J.M ,Byrne.M.E,et.al,1989). Actually, glucomannan and plant growth harmone gibberellins interacts with growth factor receptors of fibrobroblast and stimulate its activity and proliferation for increases collagen synthesis in topical and oral administration of Aloe according to Hayes (Hayes.S.M et.al, 1999). The Aloe administration influence collagen composition (more type III) and increased collagen cross linking for wound contraction and improving breaking strength (Reynolds.T ,Dweck.A.C, et.al,). It also increases synthesis of hyaluronic acid and dermatan sulfate in the granulation tissue of a healing wound (Chithra.P ,Sajithal.B , Chandrakasan.G et.al, 1998).

Acemannan is considered the main functional component of Aloe vera, is composed of a long chain of acetylated mannose ((Femenia.A ,Sanchez.E.S, Simal.SRosello.C , et.al, 1999. Djeraba.A ,Quere. P , et.al, 2000, Lee.J.K, Lee. M. K ,Yun.Y. P, Kim.Y ,Kim.J.S ,Kim.Y.S, Kim.k,Han.S.S , Lee.C.K et.al,2001). This complex carbohydrate accelerates wound healing and reduces radiation induced skin reactions(Castleman.M et.al,1991, de witte.P,et.al 1993). Macrophage-activating potential acermannan may stimulate the release of fibrogenic cytokines (de witte.P,et.al 1993,Ishii.Y,Tanizawa.H,Takino.Y, et.al,1994).

Direct binding of acemannan to growth factors and their stabilization may lead to promotion of prolong stimulation of granulation tissue (Castleman.M et.al,1991). The Aloe gel has been used for the treatment of radiation burns and radiation ulcers (Syed.T.A, Afzal.M ,Ashfaq.A.S. et.al, 1997) and complete healing has been observed in two radiation burns patients (Yeh.G.Y,Eisenberg.D.M, Kaptchuk.T.J ,Philips.R.S et.al, 2003). The fresh gel was more effective than the cream (Yeh.G.Y,Eisenberg.D.M,Kaptchuk.T.J ,Philips.R.S et.al, 2003, Visuthikosol.V,Sukwanarat.YChowchuen.B, et.al, 1995) as Aloe gel-treated lesions healed faster (11.8 days) compared to burns treated with petroleum jelly gauze (18.2 days) by Fulton (Fulton.J.E et.al, 1990). The 27 patients with partial thickness burns have been treated with Aloe gel in a placebo-controlled study (Montaner.J.S ,Gill.j , Singer.J ,et.al, 1996).

Anti-Inflammatory Action The anti-inflammatory activity of Aloe vera gel has been revealed by a number of in vitro and in vivo studies through bradykinase activity (Tyler.V.Eet.al , 1994 Che.Q.M , Akao.T , Hattori.M , Kobashi.K , Namba.T et.al, 1991). The peptidase bradykinase was isolated from aloe and shown to break down the bradykinin, an inflammatory substance that induces pain (Teradaira.S.Ito .R ,Beppu.HObata.M , Nagastu.T, Fujita.K et.al, 1993). A novel anti-inflammatory compound, C-glucosylchromone, was isolated from gel extracts (Haller.J.S et.al, 1990). Aloe vera inhibits the cyclo-oxygenase pathway and reduces prostaglandin E2 production from arachidonic acid. Fresh Aloe vera gel significantly reduced acute inflammation in rats (carrageenin-induced paw oedema), but not in chronic inflammation (Che.Q.M ,Akao.T , Hattori.M , Kobashi.K , Namba.T et.al, 1991).

In croton oil-induced oedema in mice, three Aloe vera gel sterols were able to reduce inflammation by up to 37%. Lupeol, the most active antiinflamatory sterol, reduced inflammation in a dose dependent manner. The data suggest that specific plant sterols may also contribute to the anti-inflammatory activity of gel (Haller.J.S et.al, 1990). The aloe sterol includes campesterol, β-sitosterol, lupeol, and cholesterol which are anti-inflammatory in nature, helps in reducing the inflammation pain and act as a natural analgesic. Other aspirin-like compound present in Aloe is responsible for anti-inflammatory and antimicrobial properties [44]. Even, Aloe vera extract (5.0% leaf homogenate) decreased inflammation by 48% in a rat adjuvant-induced arthritic inflammatory model (Davis.R.H ,Parker.L , Samson.R.T , Murdoch.D.P. et.al, 1991. Hanley.D.C , Solomon .W.A , Saffran.B , Davis. R.H et.al, 1982).

Effects on the Immune System

Alprogen inhibit calcium influx into mast cells, thereby inhibiting the antigen-antibody-mediated release of histamine and leukotriene from mast cells (Hansel.R ,Keller.K , Rimpler.H , Schneider.G et.al, 1994). In a study on mice that had previously been implanted with murine sarcoma cells, acemannan stimulates the synthesis and release of interleukin-1 (IL-1) and tumor necrosis factor from

macrophages in mice, which in turn initiated an immune attack that resulted in necrosis and regression of the cancerous cells (Peng.S.Y ,Norman.J , Curtin.G , Corrier.D , McDaniel.H.R , Busbee.D et.al, 1991). Several low-molecular-weight compounds are also capable of inhibiting the release of reactive oxygen free radicals from activated human neutrophils(Hart.L.A , Nibbering.P.H , Van den Barselaar.M.T , Van Dijk.H , Van den Burg,A.J , Labadie.R.P , et.al,1990).

Moisturizing and Anti-Aging Agent

Muco-polysaccharides help in binding moisture into the skin. The amino acids also soften hardened skin cells and zinc acts as an astringent to tighten pores. Its moisturiz- ing effects have also been studied in treatment of dry skin associated with occupational exposure where Aloe vera gel gloves improved the skin integrity, decrease appearance of acne wrinkle and decrease erythema (West.D.P ,Zhu.Y.F , et.al, 2003).The Aloe gel gives cooling effect and also acts as a moisturizing agent. It also has role in gerontology and rejuvenation of aging skin. This property of Aloe is be- cause it's biogenic material. Aloe vera is used as skin tonic in cosmetic industry.

2.5 ANTITUMOR ACTIVITY

A number of glycoproteins present in Aloe vera gel have been reported to have antitumor and antiulcer effects and to increase proliferation of normal human dermal cells (Choi.S.W ,Son.B.W , Son.Y.S , Park.Y.I , Lee.S.K , Chung.M.H et.al, 2001 cYagi.A , Kabash.A, Mizuno.K , Moustafa.S.M , Khalifa.T.I , Tsuji.H et.al, 2003). However, statistically significant clinical studies on the efficacy of Aloe vera gel on human health are very limited and often inconclusive (Eshun.K, He.Q,et.al, 2004). In recent studies, a polysaccharide fraction has shown to inhibit the binding of benzopyrene to primary rat hepatocytes, thereby pre- venting the formation of potentially cancer-initiating benzopyrene-DNA adducts. An induction of glutathione S-transferase and an inhibition of the tumor-promoting effects of phorbolmyristic acetate has also been reported which suggest a possible benefit of using aloe gel in cancer chemoprevention (Kim.H.S ,Lee.B.M , et.al, 2004 Kim.H.S , Kacew.S , Lee.B.M , et.al,1999).

2.6 LAXATIVE EFFECTS

Anthraquinones present in latex are a potent laxative; it's stimulating mucus secretion, increase intestinal water content and intestinal peristalsis (Ishii.Y ,Taniwaza.H , Takino.Y , et.al, 1994). The Aloe are due primarily to the 1, 8-dihydroxyanthracene glycosides, aloin A and B (formerly designated barbaloin) (Tyler.V.E et.al,19940, Tyler.V.E, Bradley.L.R, Robbers.J.E, et.al, 1988). After oral administration aloin A and B, which are not absorbed in the upper intestine, are hydrolysed in the colon by intestinal bacteria and then reduced to the active metabolites (the main active metabolite is aloe-emodin- 9-anthrone) (Che.Q.M ,Akao.T , Hattori.M , Kobashi.K , Namba.T et.al, 1991, 58), which like senna acts as a stimulant and irritant to the gastrointestinal tract (Reynolds.J.E. et.al, 1993). Aloe latex is known for its laxative properties. The laxative effect of Aloe is not generally observed before 6 hours after oral administration, and sometimes not until 24 or more hours after.

CHAPTER 3

AIM AND SCOPE

3.1 AIM

Studies on macromolecules, photochemical in various aloe vera extracts and its antioxidant and antibacterial activity

3.2 OBJECTIVES

1. To evaluate the phytochemical compounds.
2. To quantify the amounts of proteins, carbohydrates and phenols.
3. To estimate the antioxidant activity by dpph assay.
4. Hydrogen peroxide scavenging assay.
5. Reducing power assay
6. Nitric oxide scavenging activity

CHAPER 4

MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 *Equipments used*

1. Conical flask
2. Test tubes
3. Weighing balance
4. Shaker incubator

5. Millipore filter
6. Centrifuge
7. Eppendorf
8. Petri plates
9. Measuring cylinder
10. UV-spectrophotometer

4.2 METHODOLOGY

4.2.1 Sample Collection

The sample was collected from the forest regions of Southern India and they were thoroughly washed with tap water and then shade dried.

4.2.1 Procedures Used for the Extraction

The process of(Rajasekaran et al., 2005) was used to make aloe vera gel, with minor modifications. New and healthy leaves were gathered and washed under running water before being washed twice with distilled water. The upper leaf layer, which is the upper epidermis of the leaves, was removed, and the gel was collected and homogenized. It was centrifuged and then blended with 95 percent ethanol before being left for 5 days with intermittent shaking. After 5 days, the ethanol and aqueous

portions were separated by incubation at 360°C in an incubator. In a rotary evaporator, the filtrate was collected and evaporated to dryness under reduced pressure. Following that, a qualitative phytochemical study of individual ethanolic extracts and Aqueous was performed.

4.2.2 *Procedure For Phytochemical Test*

Alkaloids, hormones, triterpenoids, glycosides, sugars, flavonoids, tannins, phlobatannins, antiquinones, and saponins were all screened for phytochemically (Sofowora, 1993)

4.2.2.1 Tests for Glycosides

(a) The evaluation of Liebermann 2 mL extract was dissolved in 2 mL chloroform, then 2 mL acetic acid was carefully added. The presence of a steroidal nucleus is shown by a color shift from violet to blue to green (i.e. a glycone portion of glycoside).

(b) Salkowski's experiment Two milliliters of each extract is dissolved in two milliliters of chloroform. 2 mL sulphuric acid was carefully applied and gently shaken. The existence of a steroidal ring is indicated by a reddish brown color (i.e., glycoside) (Harborne, 1973)

4.2.2.2 Tests for Steroids

1) When 2 ml of the extract is dissolved in 2 ml of chloroform and 2 ml concentrate sulphuric acid is applied to the test tube, a red color is created in the lower chloroform layer, indicating the presence of steroids.

2) When extract(2ml) was dissolved in chloroform(2ml) and treated with sulphuric and acetic acids, a greenish color developed, indicating the presence of steroids. (Ogbuewu, 2008).

4.2.2.3 Tests for Anthraquinones

BORNTRAGER'S TEST : After shaking 3 ml of ethanolic extract with 3 ml of benzene, the mixture was filtered and 5 ml of 10% ammonia solution was applied to the filtrate. The ammonical (lower) phase was shaken, and the appearance of a pink, red, or violet color indicated the presence of free anthraquinone. (Sofowora, 1993)

4.2.2.4 Test for Tannins

2 mL ethanolic extract, 2 mL distilled water, and a few drops of FeCl3 solution (5 percent w/v) were applied to the mixture. The presence of tannins was suggested by the formation of a green precipitate. (Harborne, 1973)

4.2.2.5 Test of Saponins

In a test tube, 5 ml of ethanolic extract was shaken vigorously with 5 ml of distilled water before being warmed. The existence of saponins was determined by the formation of stable foam.(Ogbuewu,2008).

4.2.2.6 Test for Phlobatannins

2 ml of ethanolic extract was added to 2 ml of 1% HCl and the mixture was boiled. The presence of phlobatannins was suggested by the formation of a red precipitate. (Sofowora, 1993)

4.2.2.7 Test for Flavonoids

1 mL of ethanolic extract was mixed with 1 mL of 10% lead acetate solution. The presence of flavonoids was revealed by the formation of a yellow precipitate. (Harborne, 1973).

2) Three milliliters of ethanolic extract were combined with three milliliters of aqueous sulphuric acid and filtered while still wet. Three milliliters of benzene were applied to the filtered and shaken mixture. After separating the benzene layer, 3 mL of 10% NH3
was applied. The presence of anthraquinone derivatives in the ammonical (lower) process is indicated by a pink, red, or violet coloration.

4.2.2.8 Test for Alkaloids

On a steam bath, 3 ml of ethanolic extract is mixed with 3 ml of 1% HCl. The reagents of Mayer and Wagner were then added to the mixture. The existence of alkaloids was determined by the turbidity of the resulting precipitate.(Ogbuewu,2008).

4.2.2.9 Test for Terpenoids :

2 mL extract was dissolved in 2 mL chloroform and evaporated until dry. After that, 2 mL of concentrated sulphuric acid was added and heated for 2 minutes. The existence of terpenoids is indicated by a greyish color. (Sofowora, 1993)

4.2.2.10 Tests for Carbohydrates

Molisch‟s test: 3 ml of the ethanolic extract was added to 2 ml of Molisch‟s reagent and the resulting mixture shaken properly, then 2 ml of concentrated H2SO4 was added carefully to the test tube. The existence of carbohydrate is suggested by a violet ring around the interphone.(Harborne, 1973).

4.3.1 QUANTIFICATION OF PROTEINS BY LOWREY'S METHOD

The aromatic amino acids tyrosine and tryptophan in the ethanolic crude extract and aqueous of Aloe vera produce a blue color complex when they react with folin's phenol reagent. At 620nm, the color intensity was colorimetrically determined. Pipette 0.5, 1.0, 1.5, 2.0, and 2.5ml of the protein working standard solution into a clean dry test tube labeled S1 to S5, respectively.The crude extract was measured in milliliters (mL) and poured into the T1 test tube. With distilled water, the final volume is increased to 3.0 ml. As a blank, 1.0 mL of distilled water is used. All of the tubes receive 4.5ml of Alkaline copper reagent. The contents are thoroughly combined and kept at room temperature for 30 minutes. At 620 nm, the strength of color produced is measured calorimetrically.The obtained values are used to build a standard graph. The amount of protein present in the given unknown solution is determined using the graph. (Kresge, N.; Simoni, R. D.; Hill, R. L. et.al2005)

4.3.2 QUANTIFICATION OF CARBOHYDRATES

Carbohydrates are essential components of plant storage and structural materials. They are polysaccharides and free sugars. The carbohydrate content can be determined by acid hydrolyzing polysaccharides into simple sugars and calculating the monosaccharide that results.Dilute hydrochloric acid is used to hydrolyze carbohydrates into simple sugars. Glucose is dehydrated to hydroxyl

methyl furfural in a hot acidic medium. This compound produces anthrone, a green-colored substance with a maximum absorption wavelength of 630 nm. 200 mg anthrone dissolved in 100 mL ice-cold 95 percent H2SO4 1mg/ml is the standard glucose concentration. (F.Mahomoodally,et.al 2014)

4.3.3 QUANTIFICATION OF PHENOLS :

The Folin-Ciocalteu assay was used to calculate the total phenolic content. In a 25 ml volumetric flask containing 9 ml distilled water, an aliquot (1 ml) of extracts or a regular solution of Gallic acid (100, 200, 300, 400, and 500g/ml) was added. A reagent blank was made with distilled water. The mixture was shaken after adding 1 ml of Folin-Ciocalteu phenol reagent.After 5 minutes, the mixture received 10 mL of a 7% Na2CO3 solution. The volume was then increased to the required level. An UV-Visible spectrophotometer was used to measure the absorbance against the reagent blank after 90 minutes of incubation at room temperature. The amount of total phenolics was measured in milligrams of gallic acid equivalents (GAE). (Arunachalam,.K.et.al)

4.3.4 *ESTIMATION OF ANTIOXIDANT ACTIVITY BY DPPH METHOD*

The ethanol extract's free radical scavenging activity was tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) with some modifications.Briefly, a 0.4mM DPPH solution in methanol was prepared, and 2ml of this solution was applied to various concentrations of methanol sample (20, 40, 60, 80, 100ug/ml) and allowed to stand at room temperature for 30 minutes before being measured against a blank sample at 517nm.Higher free radical scavenging activity was demonstrated by the reaction mixture's lower absorbance. The following equation is used to measure the percentage of DPPH radical scavenging activity:

Radical scavenging activity (%) = (Absorbancecontrol - Absorbance sample/Absorbance control) x 100 (Bondet, V., Brand-Williams, W., & Berset, C. (1997).

4.3.5 HYDROGEN PEROXIDE SCAVENGING ASSAY

In nature, the hydrogen peroxide molecule is not poisonous. It can transform to toxic substances including hydroxyl radicals after undergoing many enzymatic activities. In this test, antioxidant components in plant extract inhibit free radical formation, and absorption was measured at 230nm using a UV spectrometer. In a 1:1 ratio, hydrogen peroxide (20mM) in phosphate saline buffer was applied to plant extracts and incubated for 30 minutes at room temperature. It was vortexes, and the absorbance was measured at 230nm using a UV spectrometer.

% of inhibition= Absorbance (control) – Absorbance (sample(aloe vera)) / Absorbance (control) × 100 (Jayanthy et al).

4.3.6 REDUCING POWER ASSAY

The components in the plant extract that have a reduction potential react with potassium ferric cyanide [Fe3+] to form potassium ferrocyanide [Fe2+], which then reacts with ferric chloride to form ferric ferrous complex, whose absorbance was calculated at 700nm using a UV spectrometer. Increased observance suggests the presence of more reducing components in plant extracts (Jayanthy et al.). Plant extracts were treated with potassium ferric cyanide. It was incubated for 20 minutes in a water bath at 500°C. Then 10 percent trichloroacetic acid was added, and the mixture was centrifuged for 10 minutes at 3000rpm. The supernatant was extracted, and distilled water was added in an equal amount. Finally, ferric chloride was applied, and the UV spectrometer was used to test absorbance at 700nm.

% of inhibition= Absorbance (control) – Absorbance (sample(aloe vera)) / Absorbance (control) ×100

4.3.7 *NITRIC OXIDE SCAVENGING ACTIVITY*

Endothelial cells produce nitric oxide, which is an essential chemical intermediate. Nitric oxide is an unstable molecule that, under aerobic conditions, reacts with oxygen to form the stable compounds nitrate and nitrite (Rozinaparole., et al.,), which act as free radicals that can disrupt cell physiological processes. The antioxidant in plant extract prevents the formation of nitrite ions. Griess reagent was used to calculate the amount of nitrite ion. In phosphate buffer saline, sodium nitroprusside (10mM) was mixed with different concentrations of P. Zeylanica extract and incubated at 300 C for 2 hours 30 minutes. Finally, Griess reagent was applied, and the chromophore formed was measured at 590nm using a UV spectrometer.

% of inhibition= Absorbance (control) – Absorbance (sample(aloe vera)) / Absorbance (control) × 100

CHAPTER 5

RESULTS AND DISCUSSION

5.1 PHYTOCHEMICAL STUDY

Alkaloids, saponins, reducing sugars, tannins, flavonoids, phenols, terpenoids, and Anthraquionones were screened for the existence or absence of phytochemicals on extract. The existence of alkaloids was verified using Mayer's reagents. The presence of alkaloids was detected by a pale precipitate, indicating that the test was positive (Table 5.1). The presence of saponins was determined using a froth test; a stable, permanent froth was produced, suggesting the

presence of saponins, and the saponins test was thus positive. The presence of tannins in the extract was confirmed by a lead acetate examination, which yielded yellow precipitate. The presence of resins was determined by the lack of turbidity in the lead water examination. Ferric chloride was used to screen for the existence of phenols. The presence of phenols caused a bluish color to appear. The Salkowaski test was used to determine the existence of terpenoids. The presence of terpenoids was detected by a reddish pink hue, indicating that the test was positive. The appearance of yellow colors indicated that the flavonoids test was positive.The presence of reducing sugars was determined using the Fehling test, and the formations of brick red precipitate revealed the presence of reducing sugars. The presence of Anthraquionones was calculated using Bontrager's test; no pink or violet color was observed in the ammonia process, suggesting the absence of Anthraquionones

Table 5.1: Phytochemical Study Result of Aloe Vera

S.NO	TEST NAME	RESULT	
1.		AQUEOUS	ETHANOL
2.	GLYCOSIDES	++	+
3.	STEROIDS	++	++
4.	ANTHRAQUINONES	+	++
5.	TANNINS	++	+
6.	SAPONINS	++	++
7.	PHLOBATANNINS	+	++
8.	FLAVONOIDS	++	++
9.	ALKALOIDS	+	+
10.	TERPENOIDS	++	++
11.	CARBOHYDRATES	+	+

+++^{ve}Faster reaction

+^{ve}Slower reaction

5.2 ESTIMATION OF CARBOHYDRATES, PROTEINS AND PHENOLS FROM ALOE VERA (ETHANOL AND AQUEOUS)

Aloe vera, ethanol and aqueous extract was evaluated and results were shown in table (table-5.2,graph-5.2). Total carbohydrates, total crude protein and total phenol content from ethanol and aqueous extracts. Carbohydrates is function of cell energy and cell structural components (Chavan and Patil, 2015). It is major component of lichen species viz. P. tinctorum (72.13%) R.conduplicans(79.80%), Ramalinahossei (59.9%) and Parmotremap seudotinctorum (53.2%) as reported by Kambar et al (2014). But on analysis by UV-vis spectrophotometry in the present study, which is a specific method, the carbohydrate content was found to be little high in aqueous extracts is310 µg/g and in ethanol extract is 280 µg/g .

Similarly,estimation of proteins content evaluated from both extracts. Protein is essential for cell growth and metabolic functions. In this study, protein content was present in aqueous extracts is 490 µg/g and 390 µg/g ethanol. These studies reported that aqueous extracted protein content was higher than ethanol extracts. It was found that the aqueous extract had more amount of protein content than the ethanol extract. According to Bahadur et al (2017) [33] certain lichen

species recorded highcrude protein, content and thus have good food value viz.*Dermatocarponmoulnsii* (20%), *Lobariaisidiosa* (20%),*Roccellamontagnei* (14%) and *Parmotrematinctorum* (14%).

Among the natural antioxidants, phenolic compounds have been studied as promising non-toxic antioxidants in various biological systems. Phenolic compounds, such as flavonoids, are commonly found in plants and have increasingly value to their antioxidant effects and to their potential role in preventing and treating diseases related to the oxidative stress. In this present study the highest level of total phenolic contents were observed in aqueous extract is 210u/g where as the ethanol extract is 190µ/g. Similar study was carried out by Aoussar et al (2017)with acetone extract of P. furfuracea with respectively 237.66 ± 25.18 µg of GAE/mg of dry extract and 19.2 ± 1.15 µg CE/mg of dry extract. Whereas the methanol extract of E. prunastri showed the low content of TPC (94.33 ± 24.82µg of GAE/mg dry extract) and of TFC (13.53 ± 1.07 µg CE/mg of dry extract).Earlier study was confirmed by Parizadeh et al (2018) that 2.96 mg/g was estimated in Leptogium sp.

Table 5.2: QUANTITATIVE ESTIMATION OF CARBOHYDRATES, PROTEINS AND PHENOLS FROM ALOE VERA

S.no	Phytochemical	Aqueous (µg/g)	ethanol (µg/g)
1	Carbohydrates	310	280
2	Proteins	490	390
3	Phenols	210	190

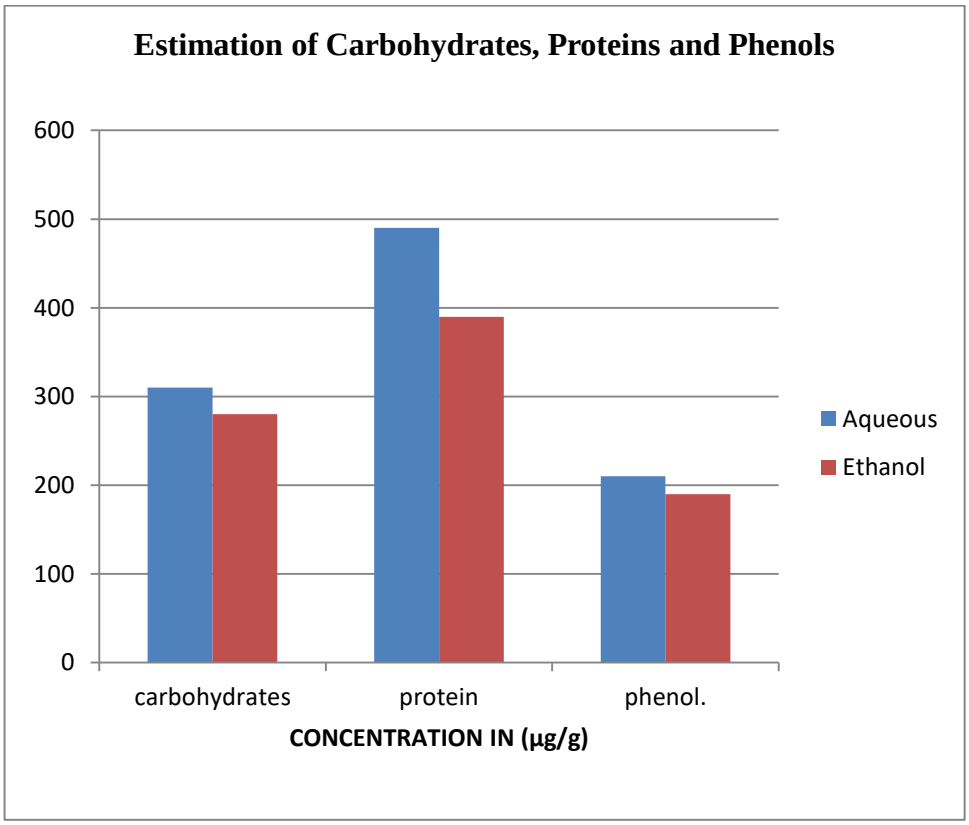

Figure 5.1 : Estimation of Carbohydrates Proteins and Phenols

5.3 ESTIMATION OF ANTIOXIDANT ACTIVITY BY DPPH ASSAY

The extracts were checked for their antioxidant activity. Both the extracts (ETHANOLl and AQUEOUS) were subjected to DPPH assay. Both extracts ethanol and aqueous extracts were found to have moderate antioxidant activity. The percentage inhibition of ETHANOL extract at 100 µ/g concentration was 51.6%, whereas the percentage inhibition of AQUEOUS extract at 100µ/g

concentration was 50.10%. The percentage inhibition of ascorbic acid at 100u/g concentration was 52.93%.Parizadeh et al (2018) reported that *Leptogium*sp19.86±0.00 µ/g scavenging antioxidants activity. (Sharma *et.al*., 2012) had quantified the antioxidant capacity of methanolic extracts of *Parmotremareticulatum* as 1.58µg Vitamin E equivalent / mg, and that of *Usnea* sp. was 0.690 µg Vitamin E equivalent /mg respectively(TABLE-5.3,GRAPH-5.3).

Table 5.3 : DPPH ASSAY OF ALOEVERA (AQUEOUS AND ETHANOL EXTRACT)

S.NO	CONCENTRATION (µg)	% INHIBITION OF DPPH ASSAY			
		CONTROL	STANDARD (VITAMIN –C)	AQUEOUS EXTRACT	ETHANOL EXTRACT
1	20µL	0.925	12.51	11.0	11.5
2	40 µL	0.925	24.5	22.2	23.1
3	60 µL	0.925	39.92	30.44	32.5
4	80 µL	0.925	45.26	42.11	43.25
5	100 µL	0.925	52.93	50.10	51.6

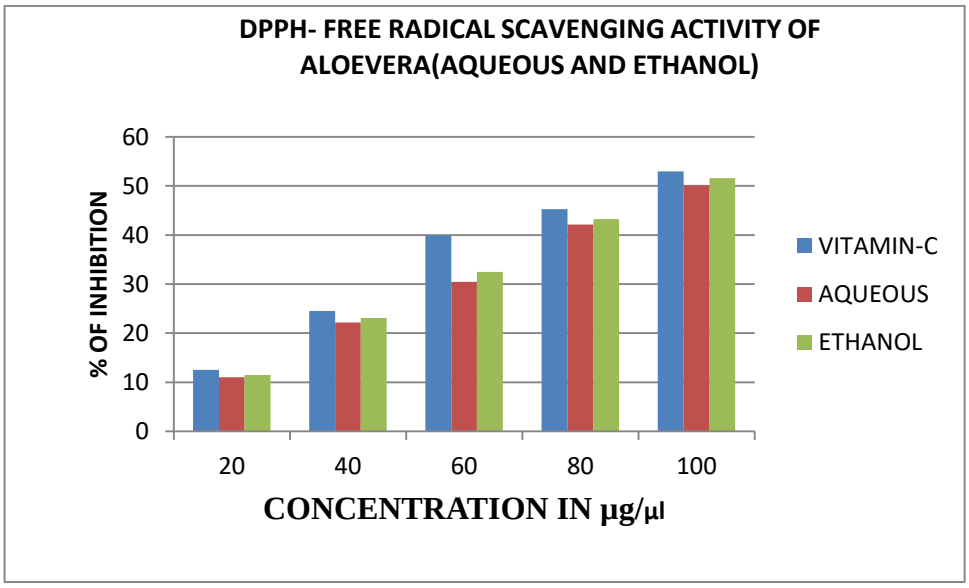

Figure 5.2 : DPPH- Free Radical Scavenging Activity of aloevera (Aqueous and ethanol)

5.4 HYDROGEN PEROXIDE SCAVENGING ASSAY

Hydrogen peroxide is a weak oxidizing agent that can deactivate a variety of enzymes by oxidizing thiol (-SH) classes.. Hydrogen peroxide may cross over the cell membrane and in the cell, hydrogen peroxide may immediately react with Fe2+, and probably Cu2+ to produce hydroxyl radical, which may initiate some toxic effects inside the cell (Kumaran et al., 2007). The percentage inhibition of ETHANOL extract at 100 µ/g concentration was 51.7%, whereas the percentage inhibition of AQUEOUS extract at 100µ/g concentration was 50.15%. The percentage inhibition of Vitamin-C at 100u/g concentration was 52.93% for ethanol, Aqueous and Vitamin-C, respectively. Ethanol extract showed high Hydrogen peroxide scavenging activity than Aqueous extract. (Graph-5.4 & Table-5.4).

Table 5.4 : HYDROGEN PEROXIDE OF ALOEVERA (AQUEOUS AND ETHANOL EXTRACT)

S.NO	CONCENTRATION (µg)	% INHIBITION OF DPPH ASSAY			
		CONTROL	STANDARD (VITAMIN –C)	AQUEOUS EXTRACT	ETHANOL EXTRACT
1	20µL	0.925	12.3	11.5	12.5
2	40 µL	0.925	24.5	22.5	24.1
3	60 µL	0.925	39.92	30.45	33.5
4	80 µL	0.925	45.26	43.11	44.25
5	100 µL	0.925	52.93	50.15	51.7

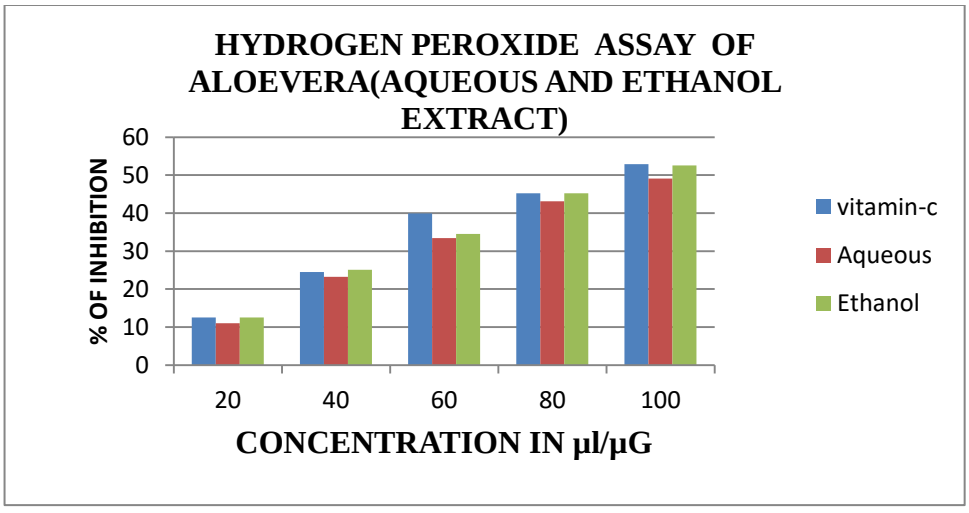

Figure 5.3 : HYDROGEN PEROXIDE ASSAY OF ALOEVERA (AQUEOUS AND ETHANOL EXTRACT)

5.5 REDUCING POWER ASSAY

By donating an electron to free radicals, antioxidants neutralize them. In the reduction of Fe3+ (CN-) 6-Fe2+ (CN-) 6, the direct electron donor was calculated using a reducing power assay (Yen et al., 1995).The neutralized radical color was blue and it was measured at 700nm. Ethyl acetate extract showed high antioxidant potential. The percentage inhibition of ETHANOL extract at 100 µ/g concentration was 52.6%, whereas the percentage inhibition of AQUEOUS extract at 100µ/g concentration was 49.10%. The percentage inhibition of Vitamin-C at 100u/g concentration was 52.93% for ethanol, Aqueous and Vitamin-C, respectively. Ethanol extract showed high scavenging activity than Aqueous extract. (Table-5.5 and Graph-5.5).

Table 5.5 : REDUCING POWER ASSAY OF ALOEVERA (AQUEOUS AND ETHANOL EXTRACT)

S.NO	CONCENTRATION (µg)	% INHIBITION OF DPPH ASSAY			
		CONTROL	STANDARD (VITAMIN –C)	AQUEOUS EXTRACT	ETHANOL EXTRACT
1	20µL	0.925	12.51	11.0	12.5
2	40 µL	0.925	24.5	23.2	25.1
3	60 µL	0.925	39.92	33.44	34.5
4	80 µL	0.925	45.26	43.11	45.25
5	100 µL	0.925	52.93	49.10	52.6

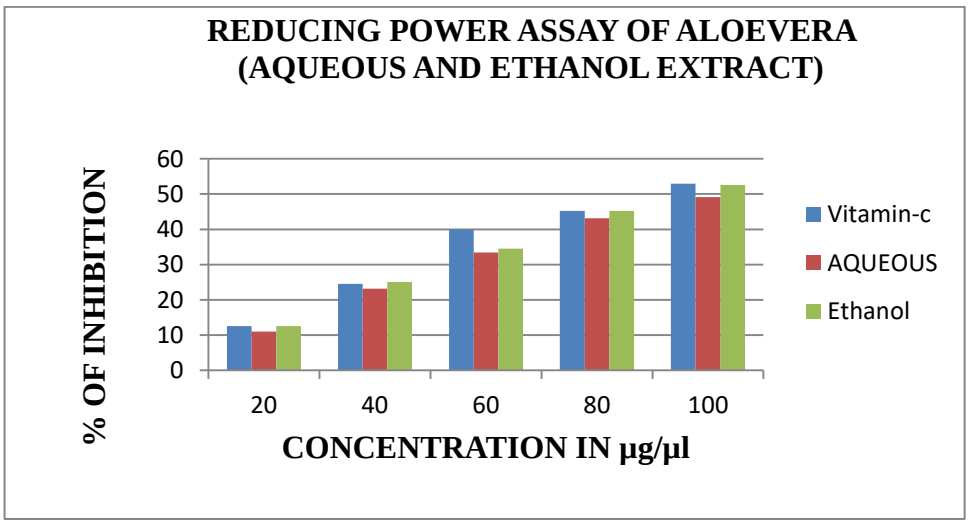

Figure 5.4 : REDUCING POWER ASSAY OF ALOEVERA(AQUEOUS AND ETHANOL EXTRACT

5.6 NITRIC OXIDE SCAVENGING ACTIVITY

Nitric oxide scavenger activity is a dominant various type inhibitor of the physiological process of cell, such as neuronal signaling, smooth muscle relaxation, effect of platelet aggregation and induce the toxicity of the cell. Nitric oxide is a well known free radical, which commence effector molecule in diverse biological system carrying neuronal messenger vasodilation, antitumor activities and antimicrobial. (Hagerman, et al., 1998).

Nitric oxide scavenging activity result wasgiven in Table-6 & Graph-6 and The percentage inhibition of ETHANOL extract at 100 µ/g concentration was 52.67%, whereas the percentage inhibition of AQUEOUS extract at 100µ/g concentration was 51.10%. The percentage inhibition of Vitamin-C at 100u/g concentration was 52.93% for ethanol, Aqueous and Vitamin-C, respectively. Ethanol extract showed high scavenging activity than Aqueous extract. (Table-5.7 and Graph-5.7).

Table 5.6 : NITRIC OXIDE ASSAY OF ALOEVERA (AQUEOUS AND ETHANOL EXTRACT)

S.NO	CONCENTRATION (µg)	% INHIBITION OF DPPH ASSAY			
		CONTROL	STANDARD (VITAMIN –C)	AQUEOUS EXTRACT	ETHANOL EXTRACT
1	20µL	0.925	12.51	11.5	13.5
2	40 µL	0.925	24.5	24.2	26.1
3	60 µL	0.925	39.92	31.44	34.5
4	80 µL	0.925	45.26	41.11	44.25
5	100 µL	0.925	52.93	51.10	52.67

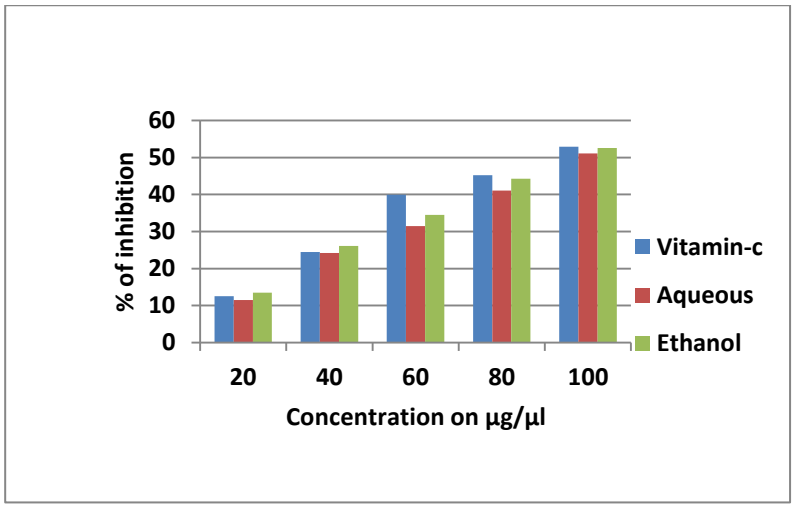

Figure 5.5 :

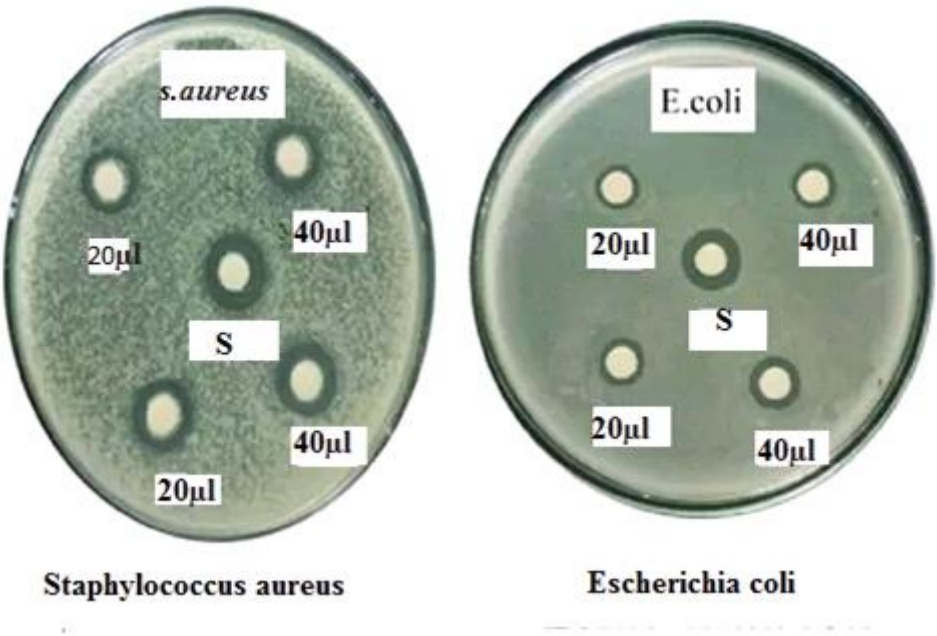

Figure 5.6 :

SUMMARY AND CONCLUSION

The existence of phytochemical constituents such as alkaloids, flavonoids, steroids, carbohydrates, and saponins in the Aloe vera gel extract (Ethanol and Aqueous) was confirmed by the above result and this present study confirmed that antioxidant. Even though the two Aloe vera extract ((Ethanol and Aqueous) performed efficiently, the Ethanol extract had highly performed than Aqueous extract.The medicinal functions of this plant can be linked to such bioactive compounds since it has been used in the treatment of various diseases such as diabetes, skin burns, cardiomyopathy, and so on. As a result, we should approach this plant for various medicinal purposes based on their bioactive compounds in order to fully utilize them.

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