Review of Literature

2.0 REVIEW OF LITERATURE

The review of literature pertaining to the study entitled '**A Comparative Study On The Antioxidant Properties Of Unripe And Ripe Fruits Of *Citrullus Colocynthis* (Linn.)**' is explained under the following heading.

2.1 INTRODUCTION

Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radicals. Besides, well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables, spices and some natural antioxidants are already exploited commercially either as additives or as nutritional supplements. More over, many other plant species have also been investigated in the search for novel antioxidants (Pourmorad *et al.*, 2006). Diets rich in fruits and vegetables contain a complex mixture of antioxidants. Clinical studies indicate that increased dietary intake of fruits and vegetables lowers oxidative stress and a high-fat diet is associated with increased oxidative stress (Prior, 2010).

2.2 ROS RADICALS AND REACTIVE OXYGEN SPECIES (ROS)

Plants are potential sources of natural antioxidants. They absorb the sun's radiation and generate high levels of oxygen as secondary metabolites of photosynthesis. On the other hand, oxygen is easily activated by ultra violet (UV) radiation and heat from the sunlight to produce toxic Reactive Oxygen Species (ROS). These ROS are highly reactive because they can interact with a number of cellular molecules and metabolites thereby leading to a number of destructive processes causing cellular damage (Hafidh *et al.,* 2009). Reactive oxygen species (ROS) include radicals as well as chemicals that can take part in radical type of reactions, but are not true radicals. They do not have unpaired electrons (Dowling and Simmons, 2009).

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atom or molecular orbital. This unpaired electron usually gives a considerable degree of reactivity to the free radicals. Radicals derived from oxygen represent the most important class of radical species generated in living system (Valko *et al.*, 2007).

The importance of free radicals and reactive oxygen species (ROS) has attracted increasing attention over the past decade. ROS, which include free radicals such as superoxide anion radicals ($O_2\bullet-$), hydroxyl radicals ($OH\cdot$) and non free-radical species such as H_2O_2 and singlet oxygen (O_2), are various forms of activated oxygen (Lee *et al.,* 2010).Free radicals are produced in the body during drug metabolism, exposure to ionizing radiation, UV light, pollution etc (Hermann, 2005). Free radicals are frequent products of biological redox reactions and invariably those involving one electron transfer processes (Cheel *et al.,* 2007).

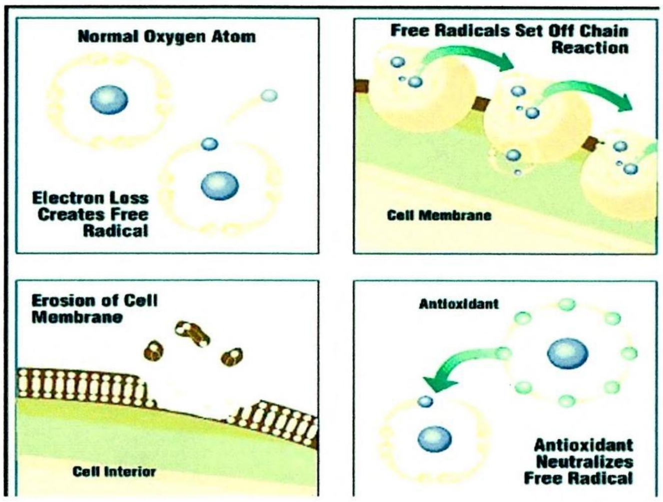
Generation of free radicals is an integral feature of normal cellular functions, in contrast excessive generation or inadequate removal of free radical results in destructive and irreversible damage to the cell (Shirwaikar *et al.*, 2006). The inducers of free radical reactions are many, but iron, particularly in the ferrous form, has been show to be very effective (Ebrahimzadeh *et al.*, 2010).Free radicals e.g. pro-oxidants are able to cause oxidative damage to lipids, protein and nucleic acids, causing deterioration of physiological function and aging (Costantini and Verhulst, 2009).

Oxidative damage is postulated to be the cause of many disparate disease processes including cancer, muscular degeneration of the retina, and coronary atherosclerosis. The oxidative hypothesis holds that aerobic metabolism produces free oxygen radicals (superoxide, singlet oxygen, hydrogen peroxide) which initiate lipid oxidation, possibly contributing to oxidative damage of DNA, proteins and carbohydrates within the cell (Gursoy and Tepe, 2009). Antioxidants are a diverse group of substances which have been shown to 'scavenge' free radicals *in vitro*, thereby slowing the oxidative process. The best studied antioxidants are vitamin C, vitamin E, and beta-carotene (McRae *et al.,* 2007)

2.3 FREE RADICALS

A free radical is an unbound compound having one or more unpaired electrons.

ANTIOXIDANTS & FREE RADICALS

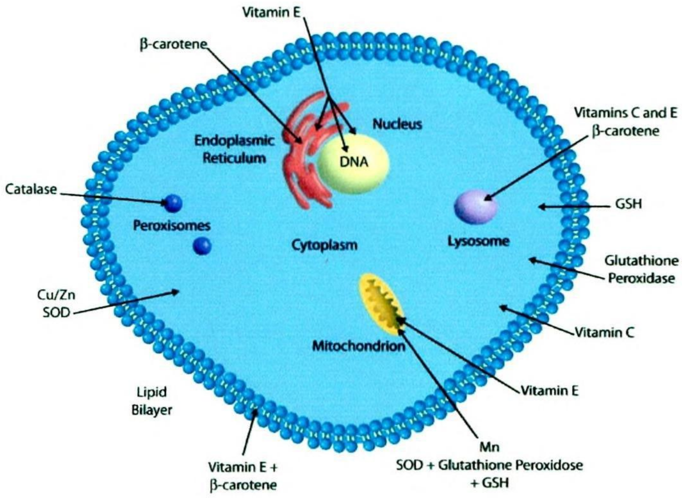


FREE RADICAL FORMATION

- Oxidation of substrates with high oxygen affinity (for example, fatty acids)
- Microbial lysis
- Environmental exposure (sunlight, radiation, high-oxygen levels)

2.4 ANTIOXIDANT SYSTEMS IN THE CELL

The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in cellular membrane or intracellular molecules (Gulcin *et al.*, 2007)



Life evolved from an intense free radical-rich primordial 'soup', and without the simultaneous evolution of powerful antioxidants, life would never have got past the first cell division. (Gutteridge and Halliwell, 2010). Several methods have been developed to measure the free radical scavenging capacity (RSC), regardless of the individual compounds which contribute towards the total capacity of a plant product in scavenging free radicals. (Choi *et al.*, 2002)

2.5 SOURCES OF REACTIVE OXYGEN SPECIES

Free radicals and reactive oxygen species including the hydroxyl radical ($^{\bullet}OH$), superoxide radical ($O_2^{\bullet-}$), peroxyl radical ($ROO^{\bullet}$), nitric oxide radical ($NO^{\bullet}$) and hydrogen peroxide (H_2O_2) are highly reactive, as the result of the presence of unpaired valance shell electrons (Laura *et al.*, 2010).

2.5.1 Superoxide anion radical ($O_2^{\bullet-}$)

Superoxide anion radicals increase under stress conditions such as heavy exercise, certain drugs, infection and various disease states (Chanda and Dave, 2009).

2.5.2 Hydrogen peroxide (H_2O_2)

A major antioxidant defence of human body is to prevent O_2^- and H_2O_2 from dangerous species such as hydroxyl ions, by binding transition metal ions in form that will not stimulate free radical reaction (Gupta and Sharma, 2006).

2.5.3 Singlet oxygen (O_2)

The appearance of large quantities of molecular oxygen (itself a free radical) some 2.2 billion years ago provoked the need to further adapt to an aerobic environment, with a requirement for specialised protection against reactive forms of oxygen (Gutteridge and Halliwell, 2010).

2.5.4 Nitric oxide radical ($NO^•$)

Nitric oxide radical is involved in several physiological processes like blood pressure control, neural signal transduction, platelet function and antimicrobial defence. Over production of the reactive nitrogen species is associated with several type of biological damage, such as lipid peroxidation, protein oxidation and nitration, enzyme inactivation and DNA damage (Ebrahimzadeh *et al.*, 2010).

2.6 OXIDATION AND REDUCTION

Oxidation is a gain of oxygen or a loss of electrons, whereas reduction is a loss of oxygen or a gain in electrons. So called 'pro-oxidants' promotes oxidative damage in laboratory model systems whereas antioxidants inhibit oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free-radical formation (Lekameera *et al.*, 2008).

2.7 OXIDATIVE STRESS

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems (Chanda and Dave, 2009).

2.8 PLANTS AND ANTIOXIDANTS

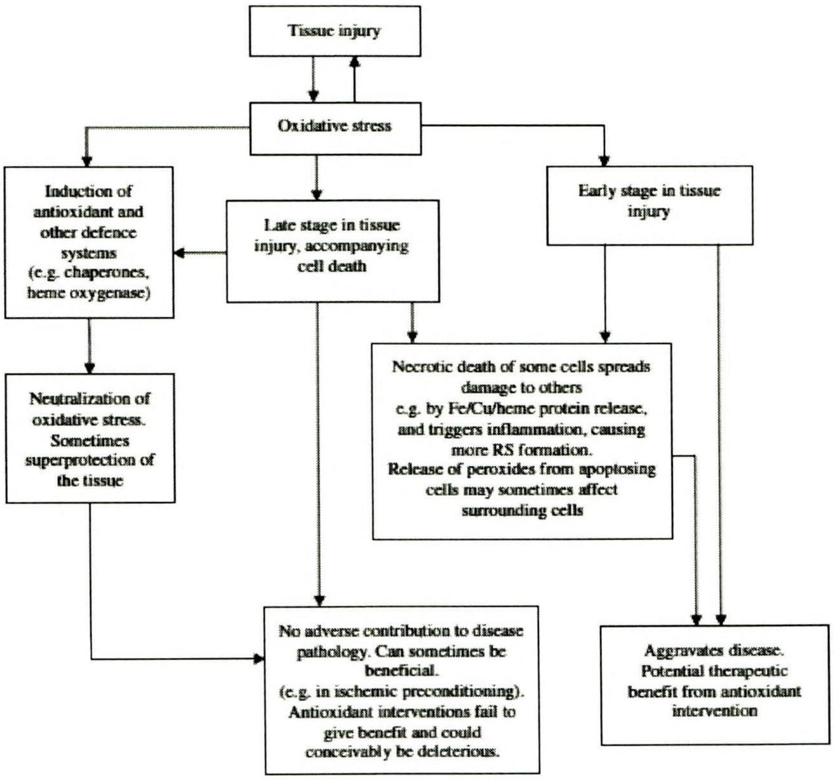
Many of the herbs contain potent antioxidant compounds that provide significant protection against chronic diseases. These compounds may protect LDL cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, inhibit lipid peroxidation, or have antiviral or antitumor activity. Research interest has focused on various herbs that possess hypolipidemic, antiplatelet, antitumor, or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer. Several herbs can help to reduce high blood cholesterol concentrations, provide some protection against cancer, and stimulate the immune system. Furthermore, a diet in which culinary herbs are used generously to flavour food provides a variety of active phytochemicals that promote health and protect against chronic diseases. Charlemagne was correct when he said 'a herb is a friend of physicians and the praise of cooks' (Craig, 2010).

Evidence suggests that compounds especially from natural sources are capable of providing protection against free radicals. This has attracted a great deal of research interest in natural antioxidants. It is necessary to Screen out medicinal plants for their antioxidant potential (Khalafet *et al.*, 2008).

2.9 OXIDATIVE DAMAGE TO BIOLOGICAL MOLECULES

The imbalance between reactive oxygen species and antioxidant defence system may increase the oxidative burden and lead to the damage of macromolecules such as DNA, carbohydrates or proteins (Leonardis *et al.*, 2008).

TISSUE DAMAGE DUE TO OXIDATIVE STRESS



2.9.1 Protein damage

Oxidative damage of proteins can result in several key events that alter cellular activity including changes in protein activity, proteosomal quality control, cellular redox balance and interference with the cell cycle. Protein carbonylation in particular is an event caused by direct attack of ROS and conjugation with highly reactive carbonyl compound produced as end products of lipid peroxidation. All ROS examined thus far including RNS, also give rise to protein carbonyls which, therefore have been regulated as broad biomarkers of oxidative stress (Ding *et al.*, 2007).

2.9.2 DNA damage

DNA is one of the molecules likely to suffer oxidative damage. Uracil, thymine glycols, 8-oxoadenosine, 8- oxoguanine and 5-formamide pyridine are products of DNA degradation after a ROS radicals attack. During DNA oxidation the formation of a basic sites, mutations and double helix breakage and rearrangement can be found (Jovanovic *et al.*, 2010).

2.9.3 Lipid peroxidation

The ROS formed under oxidative stress has the tendency to react with double bonds of polyunsaturated fatty acids. The oxidative degradation produces lipid hydroperoxides, leading to complex changes that eventually manifest themselves in the development of food rancidity and off flavours. ROS such as superoxide anions, hydroxyl radicals, hydrogen peroxide along with the metals $Fe3^{+}/Fe^{2+}$ are important mediators for the initiation of lipid peroxidation. Lipid peroxidation occurs mainly in bio membranes (Neli and Mermelstein, 2008).

2.10 DISEASES ASSOCIATED WITH OXIDATIVE STRESS

ROS have been implicated in more than 100 diseases; including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, cancer and gastric ulcer (Gulcin *et al.*, 2007).

2.10.1 Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory disease of joints that results in joint pain, swelling and destruction. It affects an estimated one percent of the adult population throughout the world. Progression of the diseases results in joint destruction deformity and significant disability. The importance of oxygen free radicals and related activated oxygen intermediates in the pathogenesis of rheumatoid arthritis has been identified with increasing incidence (Ponist *et al.*, 2010).

2.10.2 Diabetes mellitus

Diabetes mellitus is a chronic and major endocrine disorder caused by inherited or acquired deficiency in the production of insulin by the pancreas or by the ineffectiveness of the insulin produced. The formation of ROS is involved in the aetiology and pathogenesis of diabetes and the development of diabetic complications. Prolonged hyperglycemia causes oxidative stress and reduces the capacities of the endogenous antioxidant defence system via the production of several reducing sugars (Matsunami *et al.*, 2010).

2.10.3 Cataract

Formation of cataracts is believed to involve photolytic-free radical mediated damage to lens protein which causes the lens to lose its transparency. Lens tissue of the eyes maintains high concentrations of vitamin C, a natural oxygen and oxyradical scavenger (Panz *et al.*, 2008).

2.10.4 Aging

Aging is a universal but complex biological process. Free radicals produced during aerobic metabolism cause cumulative oxidative damage that eventually results in aging and death of the human and animals. Oxidative stress and oxidative damage in mitochondria have been proposed to play a critical role in the aging processes. Oxidized and cross linked proteins tend to accumulate in aging cells (Shin *et al.*, 2009).

2.10.5 Cancer

Reactive oxygen species play an important role in the initiation as well as the promotion phases of carcinogenesis. Tumours in the human body or in the experimental animals are known to affect many functions of the vital organs, especially the liver, even if the site of tumour does not interfere directly with that of organ's functions. Higher levels of free radicals and malondialdehyde, the end products of the lipid peroxidation, were reported in cancer tissue than in non- diseased organs (Naidu *et al.*, 2008).

2.10.6 Alzheimer's Disease

Multipotent antioxidant is of great interest for the treatment of complex diseases, for example Alzheimer's disease, in which diverse pathogenetic factors (other than free-radicals and metal ions) are implicated, and this group is, therefore, given special attention (Zhang *et al.*, 2001).

2.11 ANTIOXIDANTS

An antioxidant is any substance that, when present at low concentration significantly delays or prevents oxidation of cell content like proteins, lipids, carbohydrates and DNA. Antioxidants can be classified into three main types: first line defence antioxidants, second line defence antioxidants and third line defence antioxidants. (Sharma *et al.*, 2010).

Antioxidants can be complex molecules such as the superoxide dismutases, catalases and peroxiredoxins, or simpler ones such as uric acid and glutathione. The origin of antioxidants goes back to antiquity. In particular we are reminded of the remarkable technical knowledge of the ancient Egyptians in preserving dead bodies, in part due to the use of plant extracts rich in polyphenols (Reddy *et al.*, 2009).

The extraction method of the antioxidants affects the total phenolic contents and antioxidant capacities of the extracts. The antioxidant potential was highest in the ethyl acetate extract, followed by methanol extract, whilst the water extract was found to be the least effective antioxidant, which cannot be explained by the total phenolic content (Ozsoy *et al.*, 2009).

Antioxidant capacity is usually measured in food extracts obtained with chemical aqueous-organic solvents (methanol, ethanol, acetone, chloroform, etc.) (Jimenez *et al.,* 2008).

2.12 ENZYMIC ANTIOXIDANTS

Superoxide dismutases, catalase, glutathione reductase and some minerals like Se, Mn, Cu, Zn come under first line defence antioxidants (Poblete *et al.*, 2009).

2.12.1 Superoxide dismutase

Superoxide dismutase (SOD) is a metal enzyme whose active centre is occupied by copper and zinc, occasionally manganese or iron. SOD plays an important role in the protection of all aerobic life system against oxygen toxicity and the free radicals derived from oxygen. As an enzyme, SOD has particular value as an antioxidant that can help to protect against cell destruction. It has the clear ability to neutralize superoxide, one of the most damaging free radical substances in nature (Pereria *et al.*, 2009).

2.12.2 Catalase

Catalase is an important scavenging enzyme against ROS, as it removes hydrogen peroxide produced during metabolic processes. The enzyme is localized in the cytosol and peroxisomes of cells (Nasrabadi, 2008).

2.12.3 Glutathione peroxidase

Glutathione peroxidase, which uses GSH to remove hydrogen peroxide, lipid peroxides and hydroperoxides, is presently the only selenoenzyme clearly established as essential for humans. This is a selenium containing enzyme which catalyses the reduction of H_2O_2 and lipid hydroperoxide, generated during lipid peroxide, to water using reduced glutathione as substrate (Jacob, 1995).

2.12.4 Peroxidase

Peroxidases belong to the important enzymes removing active oxygen species in plants. Plant peroxidases have been studied for important role in lignification and suberization, active participation in the formation of diphenyl bridges, cross linking of hydroxyproline rich proteins in the cell wall matrix, control function of redox state in apoplast (Zahra *et al.,* 2009).

2.13 NON- ENZYMIC ANTIOXIDANTS

Glutathione (GSH), vitamine C, uric acid, albumin, bilirubin, vitamin E (mainly α- tocopherol), carotenoids, flavonoids, ect comes under second line defence antioxidants (Jaleel *et al*., 2009).

2.13.1 Tocopherol (Vit E) & Ascorbic Acid (Vit C)

The antioxidant vitamins C and E are localized, and presumably operate, in specific aqueous and lipid (membrane) compartments *In vivo,* yet there is considerable evidence of synergistic interaction between the vitamins. Results of most *in vivo* studies support the hypothesis derived fromin vitro studies that vitamin C can regenerate or "spare" vitamin E, a few studies report an antagonisti relationship between the two vitamins, or no interaction that is deemed to be significant. Many reports of vitamin C - vitamin E interaction studies continue to appear, and the synergism is mentioned frequently in the literature (Yogendrasinh *et al.,* 2010).

Vitamin E scavenges peroxyl radical intermediates in lipid peroxidation and is responsible for PUFA (poly unsaturated fatty acids) present in cell membrane and low density lipoprotein (LDL), against lipid peroxidation. (Shebly, 2009).

2.13.2 Polyphenols

Total phenolics are the major contributor to the antioxidant activity of fruits and vegetables (Karadenuz *et al.*, 2005). Plant polyphenols, a diverse group of phenolic compounds (flavanols, anthocyanins, phenolic acids, etc.) possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Heim *et al.*, 2002).

2.13.3 Carotenoids and Glutathione

Carotenoids in fruits and vegetables are believed to provide antioxidant protection to lipid rich tissues. Newer evidence suggests that p-carotene and tocopherol may act synergistically against lipid peroxidation, and that polyphenols, such as flavonoids provide antioxidant protection which is enhanced by vitamin C. Recent studies in humans have also shown interactions between GSH and AA. Plasma total glutathione (GSH + GSSG) and the ratio GSH/GSSG, an indicator of oxidant stress, were decreased in healthy men fed a low AA diet of 5 to 20 mg/d for 60 d in a metabolic unit (Niki, 2010).

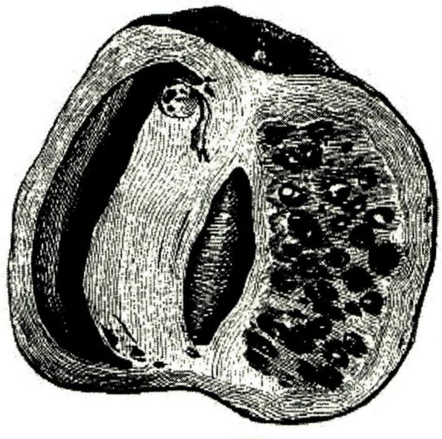
2.13.4 Phenols

Phenolic substances, which are known to possess high antioxidative activity, are actually common phytochemicals in fruits and leafy vegetables. Most of these phenolics are classified into two principal groups of phenol; carboxylic acids and flavonoids, the latter being the most significant. Phenolics in fruits, vegetables, herbs and spices possess potent antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activities (Jayaprakasha *et al.*, 2007).

2.14 FRUITS- A NATURAL SOURCE OF ANTIOXIDANT FROM MOTHER NATURE

Plant species still serve as a rich source of many novel biologically active compounds. However, very few plant species have been thoroughly investigated for their medicinal properties. Thus, there is renewing interest in phytomedicine during last decade and nowadays many medicinal plant species are being screened for pharmacological activities. The protective effects of diets high in fruits and vegetables have been attributed to the presence of these compounds (Cindy and Oliveri, 2003). *Citrullus colocynthis*(L.). Colocynthis includes wild in Western and Central India though Sahara and Sahel.

Transverse section of colocynth fruit.



Longitudinal section of colocynth fruit.

Citrullus colocynthis also known as bitter apple is a desert plant of the family cucurbitaceae naturally adapted to arid environments. It is a non-hardy, herbaceous perennial vine, branched from the base. Originally from tropical Asia and Africa, it is now widely distributed in the Saharo-Arabian phyto-geographic region in Africa and the Mediterranean region. Each plant produces 15-30 round fruits, about 3-4 inches in diameter, green with undulate yellow stripes, becoming yellow all over when dry. Seeds are small (1-4 inch or less in length) smooth and brownish when ripe. The fruits are widely used medicinally, especially for stomach pains the pulp, because of its content of glucosides such as colocynthin, it is an effective cathartic and laxative (Jayaraman *et al*., 2009).

2.15 FRUIT DESCRIPTION

Citrullus colocynthis belongs to the family Cucurbitaceae. Members of this family are generally dioecious herbs which may be prostate or climbing by means of tendrils. The fruit is fleshy and they are used as vegetable or as edible fruits. The colocynth plant is a native of arid soils. It has a large, fleshy perennial root, which sends out slender, tough, angular, scab rid vine like stems (Memon *et al,* 2003).

The melon vine bears a small, hard fruit with a bitter pulp. The fruit is globular, smooth, with a hard but thin rind, something like a gourd. It is filled with a soft, white pulp, in which are imbedded numerous seed. This pulp is used in medicine. The colocynth, or bitter-apple, of commerce, when deprived of its rind, as is mostly the case, presents a white, light and spongy pulp that readily breaks into three wedge-shaped pieces, each holding imbedded near its outer rounded surface a number of flat, ovate seeds. The proportion between pulp and seed varies according to different authors, from 23 to 33 per cent of pulp and 67 to 77 per cent of seed. The intensely bitter taste of colocynth resides in the pulp only, while the seeds at best contain only traces of it; The bitter taste and the powerful medicinal virtues of the pulp are due to the presence of a probably amorphous glucosid *colocynthin,* first identified and named by Meissner and by Vauquelin (1818), (Giwa *et al.,* 2010).

2.16 PHARMACOLOGICAL PROPERTIES

In moderate doses a drastic hydrogogue, cathartic and diuretic; in large doses emetic and gastro-intestinal irritant; in small doses it is expectorant and alterative. Physicians use this drug extensively as a drastic purgative in ascites and jaundice and in various uterine conditions, especially in amenhorroea. *C. colocynthis* in the form of the solid extract enters in to many of the purgative pills of modern pharmacy. It is useful in biliousness, fever, intestinal parasites, constipation, hepatic and abdominal, visceral and cerebral congestions, dropsy, etc. Juice of the fruit mixed with sugar is a house-hold remedy in dropsy (Giwa, *et al*., 2010).

Citrullus colocynthis parts of plants most often used for medicinal purposes are fruits and/or seeds, though other parts of the plants can be used, for example roots to treat urinary infection. With these analgesic and anti-inflammatory properties, *Citrullus colocynthis* can be considered an effective agent to treat inflammation diseases (Marzouk, 2010). Equal amount of seeds of the plant and castor are fried in mustard oil till the mixture turns black and used for Earache. The concoction is then filtered and used as ear drops. Small pieces of the stem of the plant and the bulb of onion are fried in mustard oil, and taken orally along with some salt for cough. Roots are chewed to get rid of constipation. Fruit powder is mixed with fenugreek, thymol and salt and a spoon of this mixture is taken orally for pile (SRISTI, 2009).

2.17 DETERMINING ANTIOXIDANT ACTIVITY: *IN VITRO* METHODS

The level of antioxidant nutrients present in a food is a nutrient content claim, and an antioxidant nutrient content claim can only be made for nutrients for which a Reference Daily Intake (RDI) has been established. The nutrient must have recognized antioxidant activity, i.e., there must be scientific evidence that after it is eaten and absorbed from the gastrointestinal tract the substance participates in physiological, biochemical, or cellular processes that inactivate free radicals or prevent free radical-initiated chemical reactions. The nutrient must also meet the requirements for nutrient content claims, such as high, good source and more (Erdemoglu *et al.*, 2006).

In vitro methods can be divided into two major groups: 1) Hydrogen atom transfer reactions like Oxygen Radical Absorbance Capacity (ORAC), Total radical trapping antioxidant potential (TRAP) and carotene bleaching; 2) Electron transfer reactions like trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP), diphenyl-_-picryl-hydrazyl radical scavenging assay (DPPH), Superoxide anion radical scavenging assay, Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay and Total phenol assay (Lavitschka *et al.*, 2007).

These methods are popular due to their high speed and sensitivity. However, it is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals (Bruijn *et al.*, 2009).

2.17.1 DPPH scavenging

Diphenyl-picryl-hydrazyl radical scavenging (DPPH) Assay. The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non radical form DPPH. This transformation results in a colour change from purple to yellow, which is measured spectrophotometrically. The DPPH free radical (DPPH) does not require any special preparation (Bhuiyan *et al.*, 2009).

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 colour. The colour turns from purple to yellow as the molar absorptive of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourization is stoichiometric with respect to number of electrons captured (Jimenez *et al.*, 2008).

2.17.2 Superoxide radical scavenging

Superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Qusti *et al.*, 2010). Numerous biological reactions generate superoxide anions which are highly toxic species. Superoxide indirectly inhibits lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical (Jung *et al.*, 2010).

2.17.3 Hydrogen peroxide radical scavenging

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as a bleaching agent in the textile, paper and pulp industries. Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapour or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage (Sun *et al.*, 2010).

2.17.4 Hydroxyl radical scavenging

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. The model used is ascorbic acid-iron- EDTA model of $HO^{\cdot}$generating system. This is a totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radicals. Hydroxyl radical eliminates hydrogen atoms from the membrane lipid which results in lipid peroxidation (Jung *et al.*, 2010).

2.17.5 ABTS scavenging

ABTS reacts quickly with ethanol/hydrogen donors to form colourless 2, 2'-azinobis (3-ethyl-benzothiazoline 6- sulfonate (ABTS). The reaction is pH independent. A decrease of the $ABTS^{+}$ concentration is linearly dependent on the antioxidant concentration. The ABTS radical cation ($ABTS^+$) must be generated by enzymes or chemical reactions, $ABTS^+$ can be dissolved in aqueous and organic media, in which the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples (Alothman *et al.*,2009).

With this background information, the experimental design of the work was planned as given in the next chapter.