CHAPTER 1

Introduction and Literature Survey

1.1 Introduction

The sustainable use of herbs in pharmacological treatment of diseases has been in practice since long ago. In developing countries, a large population still depends on use of these traditional medicines to meet their health care needs (Newman et al., 2000). Moreover studies on traditional plant resources has gained interest in the present, due to its rich source of bioactive compounds being isolated and used in the treatment of diseased conditions such as analgesic (Senthilraj et al., 2010), as anti-oxidant (Krishnaiah et al., 2011; Nimse et al., 2015), anti-inflammatory (Azab et al., 2016; Ghasemian et al., 2016; Shah et al., 2011),diuretic (Wright et al., 2007) anti-cancer (Shukla and Metha, 2015) and many other like neurodegenarative diseases, antidepressants and many other (Cechinel-Filho, 2012). Ayurveda being the most ancient of all traditional medicines that has began 5000 years ago. The ayurvedic system of medicine deals with the disease prevention and health promotion in a holistic way taking in to concern of the entire body, mind and spirit in the treatment of diseases (Satyapal et al., 2014).

According to the recent report 70% of the new chemical entities (NCE) approved for clinical trials by the Food and Drug Administration (FDA) were mainly naturally occurring or naturally derived. The data compiled also states 77 % of the anti-cancer agents, 78 % of anti-parasitic agents, 80% of the anti-viral agents identified during the last 30 years were naturally derived (Carg et al., 1997; Newman and Carg 2007; 2012). The invaluable significance of the natural products in medicinal field is substantiated from the obtained data. Though 250, 000 species of plants exists, only a few species have been studied for their pharmacological properties. Thus the World health Organization (WHO) in order to promote research on natural products formed the " Protection and Promotion of Traditional Medicine" report (WHO, 2014). The discovery of compounds from natural source would be advantages in content with the safety,

efficacy and low cost efficient providing scientific evidences to the traditional claims. Based on the traditional uses and efficacy, two plants *Curcuma zedoaria* and *T. involucrata* have been selected for the present study.

1.2 *Curcuma zedoaria*

1.2.1 Occurrence

C. zedoaria is a tuberous perennial herb of the Zingiberaceae family. *C. zedoaria* is commonly known as " Kichilikizhangu or Kachuur". The rhizomatous species is an indigenous plant of the Indian and Indonesian tropical and sub tropical regions. The images of the plant and rhizome was downloaded from turn-it-trpoical.co.uk and trade india.com. The plant is characterized by its purple, yellow flowers and the fragrant tuberous (Fig 1.1) rhizomes (Nair, 2000).

Fig. 1.1 Photograph of the Plant – A) *Curcuma zedoaria* and its B) Rhizome.

1.2.2 Taxonomic Classification of ***C. zedoaria***

Kingdom: Plantae
Phylum: Tracheophyta
Class: Liliopsida
Order: Zingiberales
Family: Zingiberaceae
Genus: *Curcuma*
Species: *C. zedoaria*

1.2.3 Botanical Description

- *C. zedoaria* is an herb that contains broadly ovoid, camphor-smelling rhizomes underground the stem (Nair, 2000).
- The stem is of 7 X 8 cm in size and deep yellow inside. Sessile are palmately branched tubers and branched fleshy roots. The sessile tubers appear pearl-white inside with a size up to 4.5 X 1.5 cm. The tubers are fusiform and fleshy.
- The leaves are 4-6 in numbers and appear simple with petiolates of (30-55 X 12-18 cm) arranged alternately in two opposite vertical rows. The leaves are flat with uneven adjacent sides and have a narrow oval shape tapering to a point at each end with purple colour patches on the upper side along the midrib.
- The *C. zedoaria* contains yellow coloured flowers which are15-20 cm long. They are stout with lateral spikes wrapped by obtuse sheaths.
- It has 4-6 numbers of coma bracts of dark pink colour fused at the base.
- Fertile bracts of 20-25 numbers with green pink margins were found to ovate with recurved tip.
- The flower also contains a small white bracteoles and greenish white calyx. Funnel shaped white or pinkish corolla of 3 cm long were found which are unequally lobed with the dorsal lobe.
- The middle lobe of *C. zedoaria* plant emarginate and produces anthers up to 0.5 cm long. This stimulates the formation of lateral staminodes which appears pale yellow, oblong and grows up to 1.2 cm long.

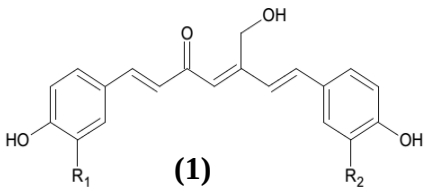
- The pistil contains trilocular ovary and many ovuled on axile placenta. The capsules are trigonous, smooth and the seeds are ellipsoid with white lacerate aril.

1.2.4 Traditional Uses

The rhizome of *C. zedoaria* is used in spice, vegetable and as salads (Ochse and Van den brink, 1980; Facciola, 1990). Traditionally the rhizomes are used as tonic, carminative, expectorant, rubefacient and as a diuretic (Wilson et al., 2005). The rhizomes are also used in combination with astringent, in conditions of dyspepsia, menstrual disorder and as a blood purifier. The dry rhizome powders are also used for the treatment of infections of the skin, skin eruption and to improve one's complexion.

1.2.5 Literature Survey – *C. zedoaria*

Syu et al., (1998) reported the isolation of curcuminoids **(1)**, demethoxycurcumin, curcumin, bisdemethoxycurcumin, 3, 7-dimethylindan-5-carboxylic acid **(2)**, curcolonol **(3)** and guaidiol **(4).**



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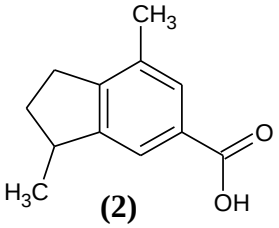
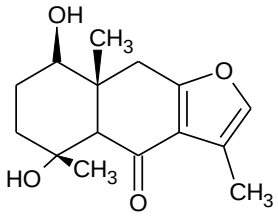


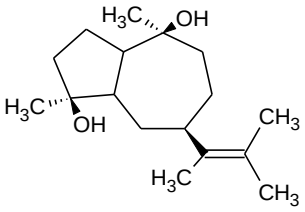
(2)

Curcumin (R_1=R_2=OCH_3),
Demethoxycurcumin (R_1=H, R_2= OCH_3),
Bisdemethoxycurcumin (R_1=R_2=H),

3, 7-dimethylindan-5-carboxylic acid **(2)**



Curcolonol **(3)**

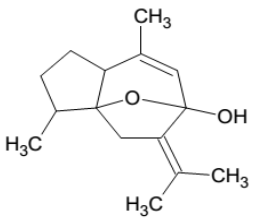


Guaidiol **(4)**

Fig. 1.2 Structures of Molecules Isolated from Ethanolic Extract of *C. zedoaria*

The molecules were obtained through bioassay-directed fractionation of ethanolic extract of *C. zedoaria*. The ethanolic extract of rhizome of *C. zedoaria* was reported to be cytotoxic against human ovarian cancer OVCAR-3 cells.

De Fátima Navarro et al., (2002) reported the isolation of curcumenol **(5)** from the rhizomes of *C. zedoaria*. The isolated compound curcumenol and the dichloromethane fraction of the hydroalcoholic extract of rhizome of *C. zedoaria* showed potent analgesic property in a dose dependant manner.

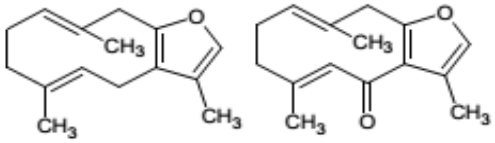


(5)

Fig. 1.3 Structure of Curcumenol

Jeng-Leun Mau et al., (2003) reported the antioxidant activity of the essential oils obtained by hydro-distillation method from the rhizomes of *C. zedoaria*. The essential oil extract contained 17 terpenes, 13 alcohols and 6 ketones.

Makabe et al., (2006) reported the potent anti-inflammatory property of sesquiterpenes furanodiene and furanodienone isolated from the methanolic extract of *C. zedoaria*.

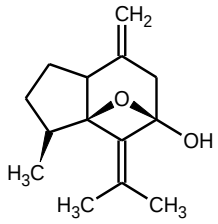


(6) **(7)**

Fig. 1.4 Structure of Furanodiene and Furanodienone

This molecules furanodiene **(6)** and furanodienone **(7)** inhibited the 12-O-tetradecanoylphorbol-13-acetate TPA-induced inflammation of the ears of mouse which was comparable to the standard indomethacin.

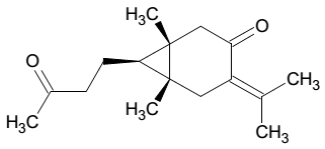
Lakshmi et al., (2011) reported the isolation of isocurcumenol **(8)** and its tumor inhibitory effect on DLA-challenged mice murine cancer cells in a dose dependent manner with no toxicity to the normal cells.



(8)

Fig. 1.5 Structure of Isocurcumenol

Kimura et al., (2013) reported the protective role of *C. zedoaria* extract and curcumenone **(9)** isolated on alcohol-induced drunkenness in mice and decrease in the elevation of blood alcohol concentration through increased liver alcohol dehydrogenase activity.



(9)

Fig. 1.6 Structure of Curcumenone

Xiu-fei Gao et al., (2014) reported the cancer cell proliferation inhibitory activity of *C. zedoaria* extracts in Human Breast cancer cell MDA-MB-231.

Si Lay Khaing et al., (2017) reported the identification of the active extracts and the phytocomponents essential of the cytotoxic acitity on Metastatic ovarian cancer cells and human umbilical vein endothelial cells in rhizomes of *C. zedoaria* collected from Myanmar.

Desmiaty et al., (2018) reported the potent antioxidant and antielastase activity of *C. zedoaria* extracts.

1.3 *Tragia involucrata*

1.3.1 Occurrence

T. involucrata is a perennial evergreen twiner and belongs to the Euphorbiaceae family. The plant is native of the Indian subcontinent distributed widely from Punjab and lower Himalayas ascending up to Assam and Meghalaya with an altitude of 750 m and southwards to Kerala. *T. involucrata* is commonly known as "Chenthatti' in Tamil. The plant has the characteristics stinging bristles and grows faster in the dry land.

1.3.2 Taxonomic Classification of *T. involucrata*

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Malpighiales
Family: Euphorbiaceae
Genus: Tragia
Species: *T. involucrata*

1.3.3 Botanical Description

- *T. involucrata* is a perennial evergreen herb with hispid stinging hairs. The image of the leaf (Fig: 1.7) was downloaded from te.wikipedia.org.
- The leaves are arranged alternatively and are 2.5-10 cm long. The leaves are oblong-lanceolate, ovate or elliptic in shape.
- Flowers are small of 2.5-5 cm long without petals. Spike axillary monoecious male flowers are found in the upper part and a few number of 1- 2 female flowers are found at the base.

- The male flowers are 1.5 mm across and have spathulate (broad at the apex and thinner at base) bracts with three sepals and stamens. The anthers of the flower are subsessile (has a small stalk).
- Whereas the female flowers are 3 mm across and are bracteate; It has six tepals (a part in a flower that has similar feature of petals and sepals) of 1 mm long. The ovate lanceolate are found thinning out towards the fruits.
- The seeds are three lobed of 0.6 X 1 cm, 8 mm diameter and are globose in nature (Patil et al., 2015).

Fig. 1.7 Photograph of the Plant – *Tragia involucrata*

1.3.4 Traditional Uses

The leaves of *T. involucrata* have been traditionally used for the treatment of skin infections, burns, scabies and burns. The leaf decoction of the *T. involucrata* along with *Cipadessa baccifera and Aristolochia talaga* are being used for curing insect and snake bites (Ayyanar and Ignacimuthu, 2005). The dried leaves of *T. involucrata* in combination with oil of *Brassica campestris* seeds have been traditionally used for the treatment of paralysis. The plant decoction is also predominantly used to treat asthma and

they are also a major constituent of the anti-diabetic formulations available in the market (Kar et al., 2003, Savithramma et al., 2007).

The roots of the *T. involucrata* are traditionally used as diuretic, treat itchy skin eruptions venereal diseases, haeorroids, gastropathy, dipsia and as a blood purifier (Samy et al., 1998; Sarada et al., 2002). The infusion of the roots is also given as diaphoretic in case of fever and the roots are also used externally in the treatment of leprosy. Decoction of the root is used for relieving bronchitis and fever (Kirtikar and Basu, 1977). The fresh extract of the roots of *T. involucrata* are traditionally used ot cure elephantiasis and tumor (Rahmatullah et al., 2010). The fresh roots of *T. involucrata* are also used externally to treat migraine (Udayan et al., 2008) and internally to treat piles (Silja et al., 2008). The fruits of this *Tragia* species are used in the form of tonic for the treatment of baldness.

1.3.5 Literature Survey of *T. involucrata*

Dhara et al., (2000) reported the *in vivo* analgesic and anti-inflammatory activity of various methanolic fractions of the roots of *T. involucrata* on Charles-Foster rats and Swiss albino mice respectively. The study reported that the anti-inflammatory and analgesic activities of the fractions of extract might be due to its lipoxygenase or cycloxygenase inhibitory mechanisms.

Samy et al., (2006) reported the separation of vinyl hexylether, shellsol, 2-methylnonane, 2, 4-dimethyl hexane, 2, 6-dimethyl heptane from *T. involucrata* species. The compounds were also tested for its antimicrobial and wound healing property in rat models.

Samy et al., (2006) reported the wound healing potential of extract of *T. involucrata* in *Staphylococcus aureus* induced excision wound in rats.

Panda et al., (2012) reported the isolation of the phytoconstituent **(10-14)** and antimicrobial activity of various extracts and isolated compounds obtained from the roots of *T. involucrata*.

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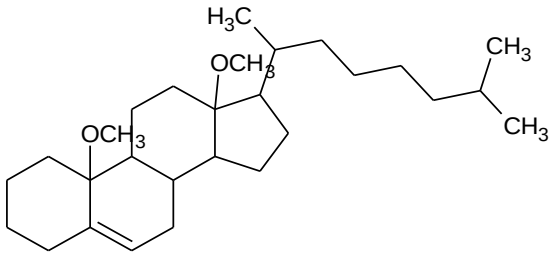

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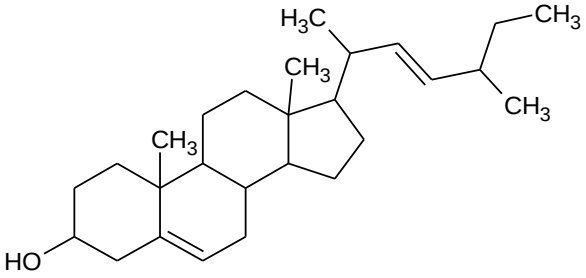

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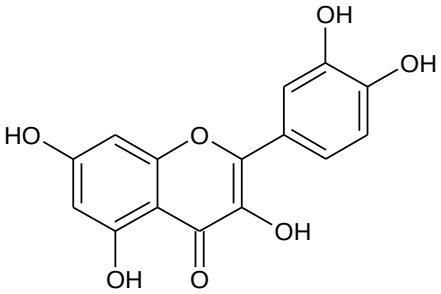

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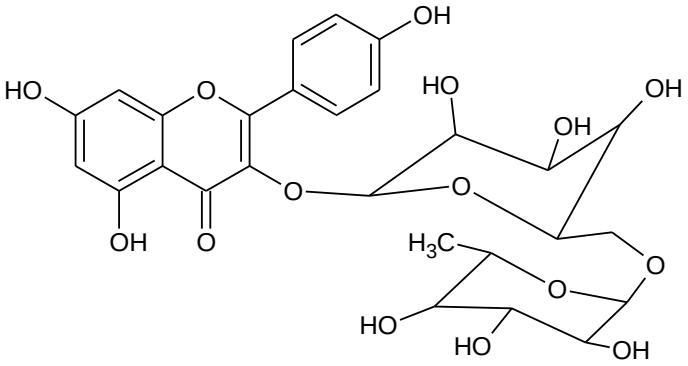

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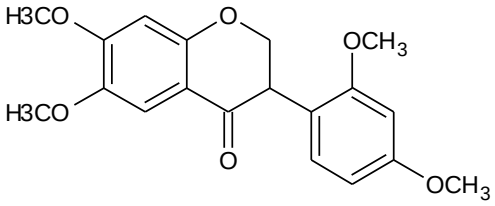


Fig. 1.8 Structures of Phytoconstituents Isolated from Roots of *T. involucrata*

Varma et al., (2014) reported the *in vivo* antiepileptic activity of extracts of *T. involucrata* in rat models. The convulsion was induced by different methods like use of electroshock and drugs such as pentylenetetrazole and picrotoxin. On treatment with various extracts of *T. involucrata* the biochemical parameter analysis revealed a decrease in duration of convulsion and also a decrease in MDA level in lipid peroxidation profile. The brain cells also revealed increase in brain glutathione levels in mice. These results suggested the dose dependant antiepileptic activity of *T. involucrata*.

Sangilimuthu et al., (2015) reported the fractionating of the molecules from the leaf extracts of *T. involucrata* and their antihistamine properties.

Bonam Srinivasa et al., (2017) reported the phytochemical, pharmacological and biological profiles of *Tragia* species.

The extensive literature survey on the plants *C. Zedoaria* and *T. involucrata* suggested the importance of the plant in the treatment of various diseased conditions. Though many traditional claims have been made on its importance in treatment of diseases studies there is a need to analyse the phytoconstituent contribution the pharmacological action and isolate the phytoconstituent possessing the medicinal property. Hence efforts have been made to identify and isolate the phytoconstituent which are expected to possess potent pharmacological activities.

1.4 Silver Nanoparticles (AgNPs)

In recent years, nanotechnology has evolved as a promising field for its potential application in the field of medicine (Aswathi et al., 2016; Madan et al., 2014 & 2016). The colloidal silver nanoparticles have gained its importance in the field of science and technology because of its application as optical receptors, bio-labeling material, anti-microbial agents, as intercalating agents and as sensors.

The metallic silver nanoparticles (AgNPs) are synthesized by both chemical as well as biological methods. The biological synthesis of silver nanoparticles by plants has gained importance because of the eco-friendly and non-toxic procedure being involved

(Singh et al., 2016). The silver nanoparticles (AgNPs) obtained were found to have high chemical stability, catalytic activity, conductivity and antimicrobial properties (Swathy, 2014). The size of the nanomaterial also plays a major role, as with decrease in the size, the surface area of the particle increases resulting in increased surface reactivity, dissolution rates, and bioavailability and altered toxicity profile (Nel 2006; Gorajana et al., 2015). Recent studies have reported the green synthesis of AgNPs using the extracts of *Lantana camara* (Kumari et al., 2015), *Hibiscus rosa-sinensis* (Nayak et al., 2015) and *Azadirachta indica* (Ahmed et al., 2016). The method involves the role of plant secondary metabolites as surface active molecules which are potent reducing and capping agents involved in the formation of stable nanoparticles.

1.5 Pharmacological Activities

The pharmacological studies are carried out to explore the efficacy, biocompatibility and the toxicity of the drug molecules.

1.5.1 Antimicrobial Activity

The early 1900's saw an increase in the number of researches performed on microorganisms. The studies on discovery of antimicrobial agents focus on two common reasons-The first is to find new molecules for the microbes developing resistance and the second reason is aimed at identifying new molecules that can inhibit microorganism which were not targeted by the drug currently in use. The organisms that have developed resistance include *Staphylococcus aureus, Enterococcus, Pseudomonas, klebsiella pneumoniae* and *Acinetobacter* strains (Hososaka et al., 2007; Abbo et al., 2005). The increasing resistance have limited the use of antimicrobials used to treat infections caused by specific organisms. With the discovery of new infectious diseases and development of resistance by the organisms it is necessary to find new anti-microbial effective to treat infections. The treatment of microbial infections involves use of antimicrobials obtained from natural sources and its analogs like penicillins, cephalosporins, and vancomycin (Walsh and Wencewicz, 2014). Approximately one quarter of the currently used anti-infective drugs were all derived from natural products (Alvi, 2000; Cutler and Cutler,

1999). Hence, necessity of identifying new antimicrobial drugs for the treatment of infections needs to be continued.

1.5.2 Antioxidant Activity

The oxidation processes are intrinsic, which involve energy management of the cell and thereby controlling the cellular functioning of the living organisms (Halliwell and Gutterridge, 2007). However, excessive free radical production disturbs the cellular mechanisms and results in the onset of new diseases and also accelerates the process of oxidative damage. The nitrogen intermediates, peroxyl, superoxide and the hydroxyl ($NO^{\cdot}$, $ROO^{\cdot}$, $O_2^{\cdot}$, $HO^{\cdot}$) free radicals, arise either from mitochondrial electron-transport chain or excessive stimulation of NAD(P)H electrons.

These free radicals with enhanced production results in oxidative stress and cause cellular damage even at the DNA level (Valko, 2007). There is increasing evidence to suggest that with increased oxidative stress, the free radicals generated can lead to degenerative disorders such as alzheimer's, cancer, arthritis, diabetics and cardiovascular diseases (Dalle Donne, 2006; Dhalla, 2000; Jenner, 2003; Sayre, 2001). The plants being a good source of antioxidants and the plant secondary metabolites like phenols and flavonoids are well reported for their antioxidant activities. The secondary metabolites prevent the oxidative damage caused by the free radicals and oxidizing agents (Matkowski 2006, Sarikurkcu et al., 2009, Antolovich et al., 2000) and prevent the occurrence of many diseases. In the present study *in vitro* antioxidant activity was performed with the dried rhizomes of *C. zedoaria* and leaves of *T. involucrata*.

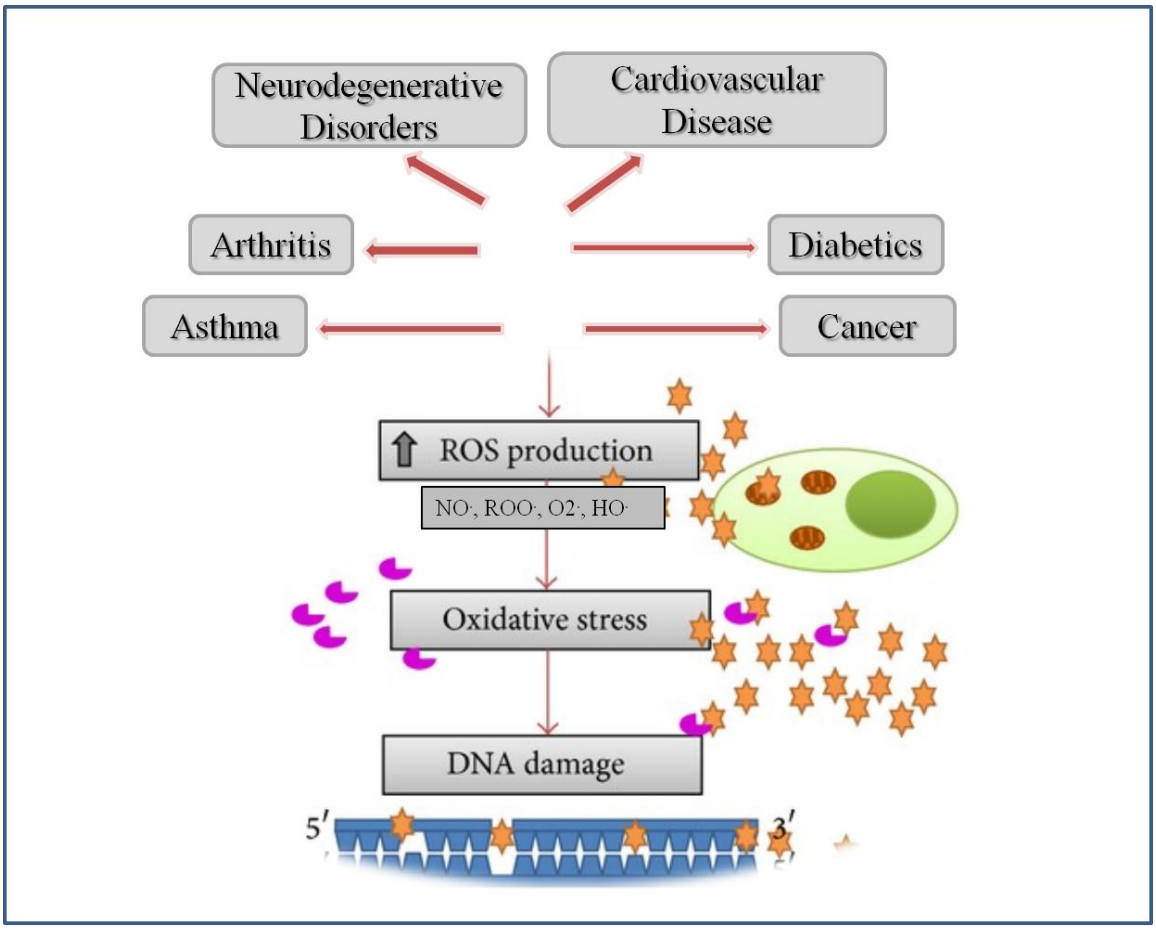
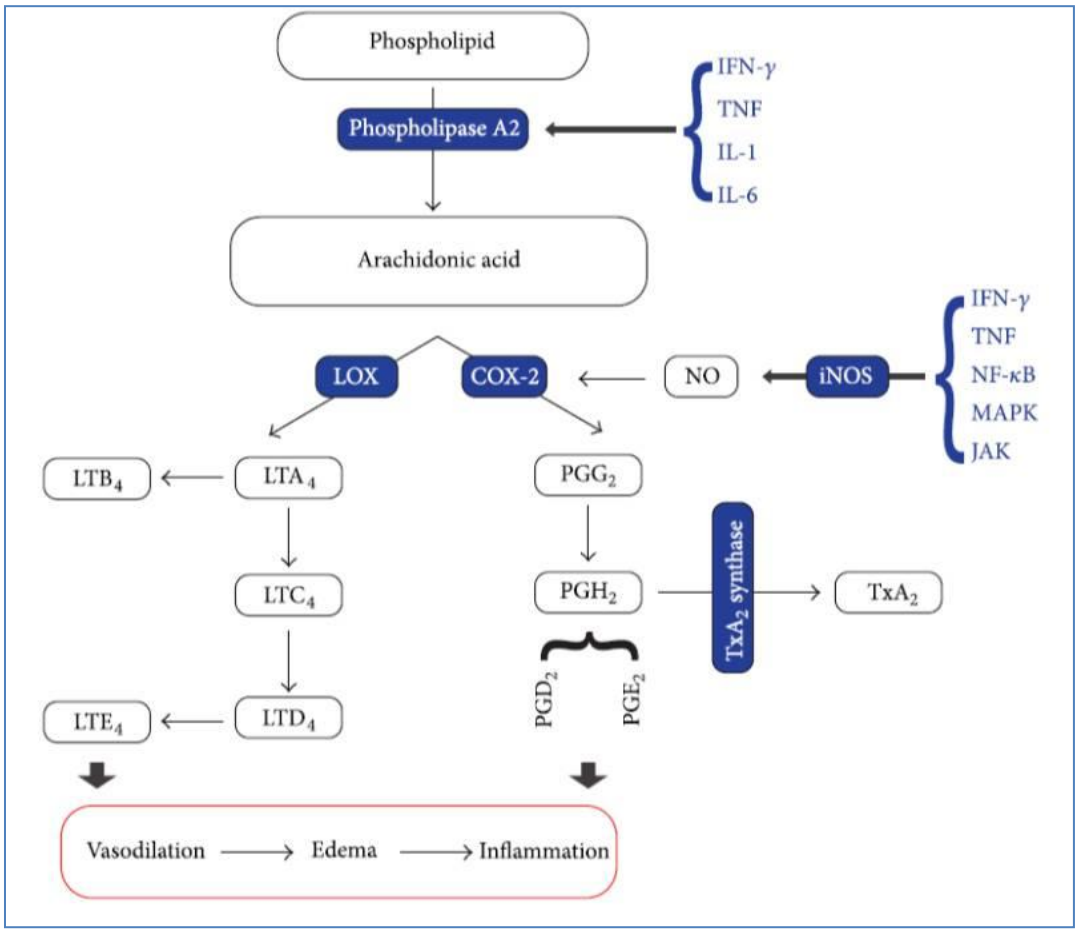


Fig. 1.9 Free Radicals a Cause for Various Diseases

1.5.3 Anti-inflammatory Activity

Inflammation of the cells are characterised by the proliferation of fibroblasts, vascular endothelium of joints, as well as macrophages and lymphocytes (Matsumoto et al., 2007). Reports state that the release of mediators like cytokines, chemokine, apoptosis intermediates-lipoxins and leukotrienes from various signal transduction pathways, which is also dependent on the free radical signalling molecules, aggravates this pathological process and contributes to diverse chronic degenerative diseases (Gilroy, 2004). The drugs currently being used in the treatment of inflammation, act either by inhibiting the Cyclo-oxygenase (COX) or Lipoxygenase (LOX) pathways (Eling, 2006; Iguchi, 2009; De Gaetano, 2003; Geronikaki, 2008) of the cell. A long term administration of these drugs affects gastrointestinal, cardiovascular and renal functioning. As a result there is a need to search for better alternatives with less or no side effects.



Note: COX-Cycloxygenase; LOX-Lipoxygenase; PG-Prostaglandin; LT-Leukotriene; TX-Thromboxane; NO-Nitric Oxide; iNOS-Inducible NO Synthase; IFN-Interferon; TNF-Tumor Necrosis Factor; MAPK-Mitogen Activated Protein Kinase; JAK-Janus Kinase; IL-Interleukin.

Fig.1.10. Inflammatory Pathway

1.5.4 Anti-diabetic Activity

Diabetes is an emerging endocrine disorder, caused by progressive insulin resistance, and impaired glucose utilization in adipose tissues and impairment of functioning beta cells (Cnop et al., 2005; Withers et al., 1998). The drugs currently in use for the treatment of diabetes focus on preventing the increase of glycemic index and also help in preventing complications like diabetic neuropathy and nephropathy (Maria et al., 2014) etc., occurring in hyperglycemic condition. The prolonged use of those drugs leads to various side effects like nausea, diarrhea, bloating and hypoglycemia, decreased body weight and

can sometimes affect the functioning of the liver (Bastaki, 2005). The α-amylase and β-glucosidase are enzymes that catalyzes the hydrolysis of α (1-4) and 1, 6-glycosidic linkage of polysaccharides and leads to post-prandial hyperglycemia. The inhibitors of these enzymes will help in regulating the blood glucose level (Adejumo et al., 2013).

Diabetics are of two type one is type I and type II. Type I diabetic's accounts for only about 5-10 % of the cases. Type I diabetics occur due to the beta cell destruction in the pancreas which is caused by the autoimmune response leading to insulin deficiency (Daneman, 2006; WHO, 1999). Type I diabetic patients require exogenous insulin to prevent serious complications like ketoacidosis, diabetic nephropathy, neuropathy and increased risk of heart disease and stroke (Wyatt and Ferrance, 2006; Cade, 2008). Type II diabetic is the most commonly found accounting for over 90% of all diabetics worldwide. Type II diabetics is caused by abnormality in insulin level or inability of the muscles to utilize insulin a resistance condition (Alberti et al., 1998; Zimmet 2001; Wild et al., 2004). Thus the glucose is not utilized by the cells for the conversion as energy in daily use which lead to increased glucose level in blood. Therefore diabetics and the complications associated with it, reduce the overall life expectancy of the people. The practice of traditional medicine for the treatment of diabetics has gained importance as they are reported to be less toxic and cheap (Cordell and Colvard, 2012). It is still noteworthy that there is a lack of knowledge about active plant compounds producing the desired effects. Thus the search of molecules with high therapeutic efficiency provides opportunities for identification of novel leads.

1.5.5 Anti-arthritic Activity

Rheumatoid arthritis is a progressive autoimmune disorder characterized by chronic inflammation and hypertrophy of synovial membranes, hyperplasia and deformity of the bones and cartilages (Lipsky et al., 1968). It progressive leads to destruction of the bones, joints and articular cartilages (Paval et al., 2009; Banji et al., 2011). The pathology of rheumatoid arthritis is very complex and the reason underlying the mechanism also remains unknown. Arthritis can affect everyday task of a person, leading to severe disability and may cause premature deaths (Murugananthan et al., 2013).

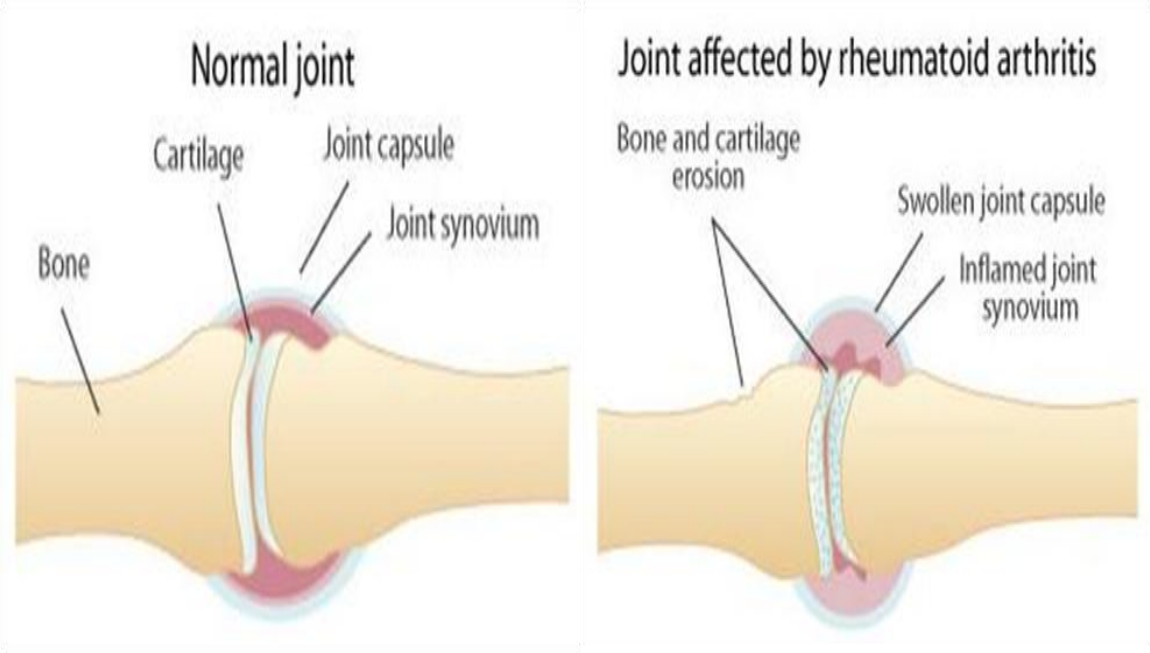


Fig. 1.11 Inflammation and Bone Deformity in Rheumatoid Arthritis Condition.

The conventional drugs used for the treatment of this disease such as non-steroidal anti-inflammatory agents, immunosuppressants and corticosteroids to newer biological molecules such as monoclonal antibodies and TNF-α failed to give long term effects with adverse side effects like ulcer, cardiovascular complications and nephro and hemato toxicity (Campbell, 1988; Nandi et al., 2008). Keeping in mind of the side effects associated with convention methods there is a need to find alternative method for treatment of this complex disorder. Hence, the complementary and alternative medicines traditionally claimed to be safe and effective and can meet the requirements of patients suffering from arthritis (Singh et al., 2011; Daniel, 2006).

1.5.6 Anti-urolithiatic Activity

Urolithiasis is a condition which involves the formation of stones in the kidney and bladder. The crystal aggregation or formation of calculi causes serious urethra obstruction with reported incidences of kidney failure and renal dysfunctions (Kok, 2002). There are increasing incidences of urolithiasis in humans and the clinical reports state that 80% of

these calculi are found to be composed of calcium oxalate and phosphates (Eknoyan, 2004).

The calcium oxalate stones are the most frequently observed stones in the clinical states and are claimed to have a greater affinity for renal cells. The pathogenesis of calcium oxalate stone formation involves a series of physicochemical events which includes the nucleation of the crystals, growth and aggregation of the crystals and finally the crystal aggregates retain on the epithelial cell linings of the renal tubules (Tsujihata, 2008). The formation of calcium oxalate stone is facilitated by the super-saturation of urine. The super saturation of urine occurs on increase in urinary pH, unbound ions such as calcium and oxalates. The super saturation of the urine also occurs due alteration in level of substances such as complexors and endogenous inhibitors which prevent the growth of the crystals (Basavaraj et al., 2007).

The struvite, an infectious stone, is the second common stone which occurs as crystallites in urine. Epidemiological reports state that the frequency of struvite stone formation is 38% of the total observed cases with urinary calculi (Chauhan and Joshi, 2013). The clinical data also revealed struvite stones to be a predominant constituent in 71.2% of clinically obtained stones in northern India. Statistical reports also state that 42.9% of Africans in the Saharan region and 20% of the US people were known to be infected with struvite stones (Daudon et al., 2004; Diamond et al., 1994).

The struvite stone formation is associated with infection of the urinary tract by infectious microorganisms like *Proteus*, *Pseudomonas, Klebsiella*, *Staphylococcus* and *Mycoplasma*. These microorganisms produce ammonium by hydrolysis of urea (Chauhan and Joshi 2008; Menon, 1998) and thereby increase the alkalinity of urine. As a result of which there is elevation in the ammonium and phsophate ions in the urine (Chauhan et al., 2009). The occurrence of the struvite stone is more frequent in women's and this stone with high degree of recurrence and rapid growth forms a staghorn calculi. The painful urological disorder also leads to obstructive or pyelonephritic episodes with evidences of renal dysfunction (Bichler et al., 2002).

Significant improvements have been attained in the management of urolithiasis like the use of modern techniques such as extracorporeal shock wave lithotripsy, percutaneous chemolysis and laparoscopic surgery for the removal of renal stones (Micali

et al., 2006). However, these techniques were found to be unconvincing because of the invasive methods and undesirable side effects such as increased hepatic hematoma, perinephric haematoma, tubular necrosis, ureteral obstruction and fibrosis. The treatment methods lead to injury of the kidney cell and the possibilities of recurrence of this renal stones are also consequent (McActeer and Evan et al., 2008).

Though there is a considerable progress in medical treatment of urolithiasis, the clinical use of pharmacological agents such as alkaline citrates, allopurinol, l-Methionine and anti-inflammatory drugs such as ibuprofen and naproxen for the treatment of urolithiasis was found to be unconvincing as these drugs doesn't target either the primary processes relating to crystal formation and aggregation nor prevents the complications arising due to it (McActeer and Evan et al., 2008). This leads to an utmost urge of exploring ideal inhibitors of stone formation and aggregation.

Traditional method of treatment of urolithiasis using various plant extracts and as food supplements are highly prevalent in India, China and other Asian countries. In the traditional medicine system like ayurveda, most of the preparations obtained from plants were proved to be effective in the treatment of urolithiatic conditions, although the rationale behind was not established through clinical evidences. Cystone is one of the ayurvedic polyherbal commercial medication currently (Himalaya Drug Company, India) prescribed for the treatment of urolithiasis (Rafiq et al., 2012). The extracts of plant source *Scoparia dulcis* (Reshma et al., 2014), *Boerhaavia diffusa* and *Tribulus terresteris* (Satish et al., 2010) were also used traditionally as preventive and curative medicine for urolithiasis.

It is of greater importance to identify lead molecules that might inhibit the crystal growth and prevent its recurrence so as non-surgerical management and prevention can be achieved. The traditional use of plant components or extracts in combination with other herbs provides wider opportunities in development of potent therapeutic drugs. Thus the present work was focused on exploring the anti-urolithiatic activity of *C. zedoaria* and *T. involucrata* plant.

1.6 Aim and Objective

- To perform extraction, phytochemical analysis, isolation and characterization of bioactive compounds from the rhizomes of *C. zedoaria* and *T. involucrata* leaf extracts.
- To carry out *in vitro* and *in vivo* biological activity studies of rhizome extracts of *C. zedoaria* and leaf extracts of *T. involucrata*.
- To biosynthesize and characterize silver nanoparticles from the leaf extracts of *T. involucrata*
- To compare the anti-urolithiasis activity of aqueous extract of *T. involucrata* and its silver nanoparticle.