2. REVIEW OF LITERATURE

Review of literature pertaining to the study comprises,

- Earlier work on *Pisonia grandis* R.Br.
- Bio-pharma potential of Pinitol
- Bio-pharma potential Allantoin
- Earlier work on *Andrographis stenophylla* C.B Clarke
- Bio-pharma potential of Andrographolide

2.1 Earlier Work on *Pisonia Grandis* R.Br.

The plant *Pisonia grandis* has been documented in many databases. The Ethno pharmacopoeia of Rotuman registered the use of leaves of the plant in Rotuman culture to control dysentery (McClatchey, 1996 and Buenz et al. 2005) identified *Pisonia grandis* R.Br. from the 17th century historic herbal text as a medicinal plant used by Rumphius and published those collections as Ambonese Herbal: Volume I. The ethnomedical and pharmacological uses of this plant has been documented in NAPRALERTM. Duke’s phytochemical database documents it as a diuretic and purgative agent. It’s use in the treatment of filariasis, inflammations, boils and edema has also been documented. (Dr. Dukes Ethnobotany database 2014)

The plant has been extensively investigated for its pharmacological potential. However not much work on isolation of active principles/secondary metabolites has been done. Earlier reports of scientific investigations on this plant have been reviewed for the period from 1990 to till date.

2.1.1 Pharmacognostical Studies

Microscopical studies and physio-chemical analysis of leaves of *Pisonia grandis* have been carried by (Jayakumari et al., 2011) that could serve in the identification and preparation of a monograph on this medicinal plant to maintain the quality of the plant.

2.1.2 Metabolites from *Pisonia grandis* R.Br.

There are only few reports on the chemical investigation of the leaves of the plant. As early as 1990, Natarajan et al. reported the presence of bio-active metabolites **β-sitosterol, α-spinosterol, dulcitol, β-sitosterol glucoside, octacosanol and quercetin** from the leaves of this plant.
UV fluorescence microscopy of petiole of *Pisonia grandis* collected from botanical garden of Oxford University confirmed its similarity to the *Gramineae* and revealed the presence of Ferulic acid *(Hartley et al., 1981)*.

The first report of chemical examination of *Pisonia grandis* revealed the presence of octacosanol (1), β-sitosterol (2), α-spinosterol (3), β-sitosterol glucoside (4), dulcitol (5) and quercetin (6) *(Natarajan et al., 1990)*.

Five new C-methylated flavonoids (7,2'-Dihydroxy-5,6-dimethoxy-8-methylisoflavone (7); 6,2'-Dihydroxy-5,7-dimethoxy-8-methylisoflavone (8), 3-Hydroxy-5,7,2'-trimethoxy-6,8-dimethylflavone (9), 3,5,2'-Trihydroxy-7,3'-dimethoxy-6,8-dimethylflavone (10), 5,7,2'-Trihydroxy-,3'-methoxy-6,8-dimethylflavanone (11), together with seven known compounds (β -sitosterol (12), 6,8-dimethyl-sogenistein
(13), leptorumol (14), 5,7,2'-trihydroxy-6-methoxy-8-methylisoflavone, pisonia-none (15), irilin A (16), 5,7,2'-trihydroxy-6,8-dimethylflavanone, 2'-hydroxydemethoxymatteucinol (17) and 3-methoxy-4-hydroxybenzoic acid (18) have been isolated after chromatographic separation of the hexane and CH$_2$Cl$_2$ extracts of the roots of *Pisonia grandis* (Sutthivaiyakit *et al.*, 2013).
2.1.3 Pharmacological Studies on *Pisonia grandis*

The plant *Pisonia grandis* is bestowed with immense pharmacological potential. Chewing two leaves of the plant has been found to reduce sugar levels in the body and the roots are considered to be purgative (Anonymous, 1969).

**Analgesic Activity:** The first scientific report on the medicinal potential of *Pisonia grandis* was published in 2002 which documented the analgesic potential of the leaves of *Pisonia grandis*. The methanol extract of leaves of *Pisonia grandis* showed significant analgesic activity in acetic acid induced writhing response method and in tail licks method (Anbalagan et al., 2002).

**Antipyretic Activity:** Antipyretic effect of a poly-herbal formulation using *Pisonia grandis* was evaluated by Elumalai et al. (2012). The poly-herbal formulation containing *Pisonia grandis* showed significant reduction of yeast induced pyrexia in rats with respect to the control group.

**Diuretic Activity:** The chloroform and methanol extracts of the leaves of *Pisonia grandis* were analysed for analgesic activity in dose response manner. The methanol extract possessed significant diuretic activity whereas the chloroform extract was completely devoid (Anbalagan et al., 2002).

**Anti-Inflammatory Activity:** The plant *Pisonia grandis* is well known for its anti-inflammatory activity. It has been extensively used in Indian Traditional Medicine to treat inflammatory. Fresh leaves, moistened with Eau-de-Cologne, find use in reducing inflammation of a filarioid nature in the leg and other parts of the body (Anonymous, 1969). The first scientific report on anti-inflammatory activity of leaves of *Pisonia grandis* was published by Anbalagan et al. (2002) indicated that chloroform and methanol extracts of leaves of *Pisonia grandis* were exhibited graded dose response in both acute and chronic model.

Methanolic extract and flavonoid rich fraction separated from the leaves of *Pisonia grandis* were evaluated for anti-inflammatory activity by Carrageenan induced paw edema model. The flavonoid rich ethyl acetate fraction was showed maximum inhibition due to the presence of quercetin in the leaves which strongly suggested that the defective effect inflammatory response by flavonoids (Jayakumari et al., 2012). Ethanolic extracts of roots of *Pisonia grandis* showed significant reduction in paw edema compared to standard drug indomethacin (Majumdar et al., 2012).

**Anti-Arthritic Activity:** Anti-arthritic activity of ethanol extract of *Pisonia grandis* was evaluated by Elumalai et al. (2012). The observed anti-arthritic potential of the plant
is due to the presences of phytoconstituents such as alkaloids, phenols and flavonoids.

**Wound Healing Activity:** Prabu et al. (2008) evaluated the wound-healing potential of methanolic extract of leaves of *Pisonia grandis* by incision and excision wound model. Ointments with 1% and 2% w/w extracts were capable of producing significant ($p<0.05$) wound healing activity in both models.

**Anti-Diabetic Activity:** The ethanolic extract of leaves of *Pisonia alba* Span was selected for administration in alloxan-induced diabetic rats. The extract reduced blood glucose levels of alloxan diabetic rats by elevating peripheral glucose utilization. Treatment with the ethanol extract of the plant restored the normal histological architecture of the liver, kidney and pancreas of alloxan-induced diabetic rats (Sunil et al., 2009a). Yet another report on the α-glucosidase inhibitory action and antidiabetic activity of *Pisonia grandis* revealed that the ethanolic extract of arial parts of *Pisonia grandis* showed intestinal α-glucosidase inhibitory activity. It also protects significantly from other metabolic aberrations caused by alloxan suggesting that ethanolic extract of aerial parts appears to be an attractive material for further studies leading to possible drug development for diabetes (Sunil et al., 2009b).

**Free Radical Scavenging Activity:** *Pisonia grandis* was found to contain very good anti-oxidant property. Methanolic extract of leaves of *Pisonia grandis* inhibited free radicals generated by DPPH, ABTS, lipid peroxides. Subhasree et al., (2009) found that the methanolic extract of leaves of *Pisonia grandis* has high potent in neutralizing ABTS cation radicals than the other radicals. The ethanolic extract of leaves of *Pisonia alba* Span showed dose dependent DPPH radical scavenging activity due to its ability for donating hydrogen molecule. The extract also possess antilipid peroxidant potential (Sunil et al., 2009a). The antioxidant and radical scavenging activity of methanolic extract of leaves of *Pisonia grandis* was established by Jagadeesan et al., (2011). Methanolic extract and it’s fractionates (ethyl acetate and ethanol fractions) of leaves of *Pisonia grandis* were investigated by nitric oxide radical scavenging assay method and DPPH method. The ethanol fraction showed maximum scavenging of nitric oxide and DPPH radicals (Jayakumari et al., 2012).

**Anti-microbial Activity:** The methanol extract of leaves of *Pisonia grandis* inhibited the growth of the gram-positive and gram-negative bacteria that aids in wound healing. The extract of 300 μg concentration showed inhibition equal to commercial antibiotics of 10 μg concentration (Prabu et al., 2008). Yet another evidence for antimicrobial activity of *Pisonia grandis* was reported by Nivedhitha and Rani (2011) against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus*
auresus, Aspergillus niger, Sacchraromyces cerevisiae. Recently the aqueous extract of leaves of Pisonia grandis and its ethylacetate fractions were screened against S.aureus, E.coli, K.pneumoniae and C.albicans by agar disc diffusion and agar well diffusion methods. The ethyl acetate fraction depicted significant antimicrobial activity with 1.2 mg/ml as minimum inhibitory concentration (Jayakumari et al.,2014).

**Hepatoprotective Activity:** Hepatoprotective activity of 95% ethanolic extract of roots of Pisonia grandis R.Br. was studied against paracetamol induced hepatic injury in Wister rats. Histopathological observations revealed that pretreatment with the extract protected the animals from paracetamol induced liver damage (Majumdar et al., 2012). The ethanolic and aqueous extracts of leaves of Pisonia grandis screened for its hepatoprotective potential against liver injury induced by carbon tetrachloride, paracetamol or thioacetamide and chronic liver damage induced by carbon tetrachloride in rats. Pretreatment of animals with the extract reduced inflammation and degenerative changes. Histological examination of liver tissues supported the hepatoprotection by both the extracts and thus the ethanolic and aqueous extracts showed significant hepatoprotective activity in carbon tetrachloride induced acute and chronic liver damage (Thenmozhi et al.,2013).

**Anxiolytic Activity:** The ethanolic extract of leaves of Pisonia grandis R.Br. possessed significant anxiolytic activity (Rahman et al., 2011). This investigation was carried out to find alternate plant derived medications with anxiolytic effect.

**In-vitro Antiplasmodial Activity:** Antiplasmodial activity of leaf and bark extracts of Pisonia grandis was well established by Sundaram et al., (2012).

**Toxicity Study:** The ethanol extract of leaves of Pisonia alba Span did not show any sign of drowsiness, restlessness, convulsions, piloerection and morality up to a dose of 4000 mg/kg (Sunil et al.,2009a). Non-toxic nature of the ethanol extract of leaves of Pisonia grandis was well established up to the dosage level of 2000 mg/kg body weights in rats (Elumalai et al., 2012). Hence the extracts of Pisonia grandis was consider being safe and non-toxic nature.

“The literature review during the period from 1990 to till date revealed that the plant pisonia grandis possessed analgesic, antipyretic, diuretic, wound healing, anti-diabetic, free radical scavenging, anti-inflammatory, anti-arthritis, antimicrobial, hepatoprotective, anxiolytic activity and in-vitro antiplasmodial activity”
2.2 BIO-PHARMA POTENTIAL OF PINITOL

Pinitol is the 3-O-methyl ether of D-chiroinositol, with both enantiomers occurring in various plant sources. The name pinitol derives from “pine”, as it was first isolated from pine tree. As the demand for pinitol as a food supplement and as a pharmaceutical increased, any attempt to isolate it from natural sources including plants is considered highly worthy. Isolation of D-Pinitol from plants has been reviewed recently (Poongothai et al., 2013). Number of scientific reports on the isolation of pinitol from plant families is illustrated in Chart 1.

Chart 1 Number of Scientific Reports on isolation of D-Pinitol

Pinitol has immense pharmacological significance. It is bestowed with antidiabetic (Ajuah et al., 2000), anti-inflammatory (Singhet al., 2001), antioxidant (Orthen et al., 1994) and immunosuppressive potential (Chauhan et al., 2011) and is used in the treatment of hypertension, rheumatism, cardiovascular diseases, AIDS and neurological disorders (Ostlund et al., 1996 and Kim et al., 2005). There is a growing interest in the use of D-pinitol as a food supplement because of its reported efficacy in lowering blood glucose levels with no side effects and nil toxicity (Bateset al., 2000) and thus D-pinitol has now become one of the better studied insulin mimickers in the food supplement industry. Report on pharmacological significance of pinitol is depicted in table 1.

2.2.1 Pharmacological Significance of D-Pinitol

Pinitol has immense pharmacological significance. As early as 1987, Narayanan et al., investigated the antihyperglycemic activity of pinitol from the leaves of Bougainvillea spectabilis which opened the door to the use pinitol as a food supplement in diabetes therapy. There are numerous reports on its pharmacological
potential. It is proven to possess anti-hyperglycemic, anti-inflammatory, anti-obesity, anti-oxidant anti-hypertension activities and immunosuppressive potential. It also finds use in curing asthma, bone metabolic disorders and hepatotoxicity.

A review of recent reports on the medicinal value of pinitol is presented.

**Anti-diabetic Potential of Pinitol**

D-pinitol is found to increase neural protection and memory ability in Wistar rat model of streptozotocin-induced diabetes by suppressing blood glucose and elevating insulin sensitivity (Lee et al., 2014). Oral ingestion of different doses of pinitol supplementation influences glucose tolerance, insulin sensitivity and plasma pinitol concentrations. Consumption of a nutritive beverage (Fruit Up) containing 2.5, 4.0 or 6.0 g of pinitol by thirty healthy subjects in two one-day trials revealed reduced serum glucose and insulin at 45 and 60 minutes at a dose of 6.0 g (Mijares et al., 2013). A low dose of pinitol isolated from the stem bark of *Piliostigma thonningii* showed significant anti-diabetic activity compared to that of the therapeutic dose of the anti-diabetic drug glibenclamide (Issac et al., 2013).

The pancreatic tissue protective nature of pinitol was analysed by its oral administration to streptozotocin-induced diabetic rats. Pinitol was found to enhance free radical-mediated alterations to near normalcy (Sivakumar and Subramanian, 2009a). The oral administration of D-pinitol to diabetic rats also showed alterations in the activities of key metabolic enzymes involved in carbohydrate metabolism (Sivakumar and Subramanian, 2009b).

Prolonged treatment of pinitol in Korean patients with type 2 *Diabetes mellitus* showed insulin-sensitizing effect without altering body weight and waist circumference of patients. This study reveals that pinitol can be effective as an oral agent in the treatment of type 2 diabetes and in the prevention of cardiovascular complications (Kim et al., 2005). Antihyperglycemic assay guided fractionation of roots of *Rhizophora apiculata* and its isolated constituent compounds revealed that the most active compound isolated from aqueous fraction was pinitol (Lakshmi et al., 2006). A study on the effect of D-pinitol, on the postprandial blood glucose response in type 2 diabetes patients revealed that ingestion of 1.2 g of pinitol one hour prior to consumption of rice controlled postprandial capillary blood glucose most effectively (Kang et al., 2006).
Hypoglycemic effect of pinitol isolated from soybeans has been reviewed (Shin et al., 2002). D-pinitol exerts insulin-like effect through post-receptor pathway of insulin action affecting glucose uptake in hypoinsulinaemic STZ-diabetic mice (Bates et al., 2000). A dose of 15 mg/kg promoted 21% decrease in plasma glucose in streptozotocin-treated rats (Fonteles, 2000). Pinitol or combinations of pinitol with insulin in a synergistic amount has been proved to be effective for controlling insulin-dependent diabetes (Weeks and Charles, 2000). Oral administration of pinitol isolated from soybean is reported to improve insulin sensitivity (Ajuah et al., 2000).

Anti-inflammatory Activity of Pinitol

Pinitol isolated from Abies pindrow leaves showed a significant anti-inflammatory effect in carrageenin-induced paw oedema in rats and it did not produce any behavioural change or mortality (Singh et al., 2001). When a combination of pinitol and glucosamine were administered, a synergistic anti-inflammatory effect against sub-acute inflammation was observed (Kim et al., 2005). The specific amount of glucosamine and pinitol needed to cure inflammatory diseases was determined by Yun et al., 2007. Pinitol suppresses inflammatory cellular response and inhibits cytokine secretion in LPS induced neutrophils (Gautam et al., 2008; Bhat et al., 2009).

Immunosuppressant and Neurological disorder suppressant Potential of Pinitol

The methods of using D-pinitol to promote a healthy nervous system and as a food supplement is well documented (US Patent 20130123370 A1). The immunosuppressant potential of the extracts of Argyrolobium roseum and its active constituent pinitol is well established (Chauhan et al., 2011). D-Pinitol is suggested as a potent immunosuppressor based on the results of well-established experimental models (Chauhan et al., 2011). The immunopharmacological functions of D-pinitol is documented (Lee et al., 2007).

Biosynthesis of Pinitol

Pinitol biosynthesis in the angiosperm family proceeds via the intermediate formation of ononitol (1D-4-O-methyl myo-inositol) whereas in gymnosperms, it proceeds via the intermediate formation of sequoyitol (1D-5-O-methyl myo-inosito). Since plants of the family Nyctaginaceae comprise of angiosperms, biosynthesis of pinitol may occur by the ononitol based pathway.
Pinitol biosynthesis pathway starts with the formation of myo-inositol-1-phosphate (myo-inositol 1-P) from glucose-6-phosphate (glucose 6-P) by inositol 1-P synthetase (INPSI). Then myo-inositol-1-phosphate dephosphorylated to myo-inositol by inositol 1-phosphatase (IMPI). Methylation of myo-inositol by inositol O-methyltransferase (IMTI) produces D-ononitol which is finally epimerased to d-pinitol by ononitol epimerase (OEPI). Epimerization of ononitol to pinitol is likely to proceed via a keto intermediate (www.pubchem.ncbi.nlm.nih.gov). Outline of biosynthetic pathway of pinitol was shown in Figure 19.

2.3 BIO-PHARMA POTENTIAL OF ALLANTOIN

Allantoin is 2,5-dioxo-4-imidazolidinyl-urea and is known as a keratolytic molecule that removes warts, corns and horny layer (hard layer) of the skin. It is a white, odourless, crystalline powder considered to be non-toxic, non-irritating and
non-allergenic ([www.balmtech.com](http://www.balmtech.com)). The U.S. Food and Drug Administration (FDA) confirmed that allantoin is a safe and effective skin protectant in the recommended dosage range of 0.5 to 2.0% ([Federal Register](https://www.federalregister.gov)). Allantoin is a compound that occurs naturally in wheat sprouts, tobacco seed, comfrey, and sugar beets. It is a 5-ureidohydantoin plays an essential role in the assimilation, metabolism, transport, and storage of nitrogen in numerous higher plants ([Wang et al., 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4913632/)). Allantoin has been isolated from the plants of the family **Apiaceae**, **Theaceae**, **Rubiaceae**, **Dioscoreaceae**, **Fabaceae**, **Poaceae**, **Leguminosae**, **Lamiaceae**, **Boraginaceae**, **Nyctaginaceae**, **Myristicaceae**, **Bignoniaceae**, **Bryaceae**, **Amaranthaceae**, **Selaginellaceae** and **Solanaceae**.

Allantoin is bestowed with lots of pharmacological significance. It is reported to be a free radical scavenger ([Guskov et al., 2002](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3551002/)) and wound healer ([Ranson 1984., Araújo et al., 2010](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3230511/)). It reduces plasma glucose in streptozotocin-induced diabetic rats ([Shan Niu et al., 2010](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3160868/)). Anti-inflammatory formulations ([Koho, 1984](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1822564/)), antimicrobial dressings ([Ying Ko Sai, 1983](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1822564/)), medicines that are used for treating gastroduodenal ulcer and chronic gastritis ([Dobrescu, 1998](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1822564/)) and ointments for treating plaque and psoriasis ([Pinheiro, 1997](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1588739/)) contain allantoin as one of the foremost ingredients.

Allantoin is used in a variety of skin care products ([Jewitt et al., 2013](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3990857/)), lip-care products([www.ipr.net](http://www.ipr.net)), hair care products([Wu Ke, 2013](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3990857/)), moisturizing cream ([Wang et al., 2013](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3990857/)), sunburn lotions, diaper rash ointments and mouthwashes. Report on pharmacological significance of allantoin is presented in table2.

### 2.3.1 Pharmacological activities of Allantoin

**Anti-diabetic potential of Allantoin**

The effect of allantoin on the plasma glucose of streptozotocin-induced diabetic rats (STZ-diabetic rats) exposed decrease in blood glucose level in the experimental animals in a dose-dependent manner ([Shan Niu et al., 2010](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3160868/)). Allantoin is suggested to have increased the GLUT4 gene expression in muscle by increasing β-endorphin secretion from the adrenal gland in STZ-diabetic rats.

The study conducted by [Chen et al., (2012)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3990857/) on allantoin suggest that it can activate the imidazoline receptor I(2B)R to increase glucose uptake into cells I(2B)R is being proposed as a new target for diabetic therapy.

**Anti-inflammatory effect and dermatological properties of Allantoin**

In the study on correlation between anti-inflammatory activity and SPF of sunscreen lotions *in vivo*, significant correlation is reported. Phorbol myristate acetate
on mice have been tested by Celine et al. (2012) anti-inflammatory effect of allantoin and glycyrrhetinic acid-based emulsions as well as commercial sun product containing any one of these ingredients revealed significant results suggesting use of these chemicals in sunscreen products.

The composition comprising a combination of methotrexate, bisabolol and allantoin is used to treat plaque psoriasis, atopic dermatitis and chronic eczema (Alario et al., 2013). A botanical basis soap of composition glycerol, 2,4,4′-trichloro-2′-hydroxyl diphenether, Hamamelis mollis ext., allantoin, 1-methyl-3-Ph propylamine, lanoline, anhydrous ethanol, and deionized water is capable of lubricating skin and softening hair, to shorten skin preservation (Wang et al., 2013).

The composition including propylene glycol, butanediol, betaine, sodium chloride, magnesium sulfate, methyl paraben, Propyl paraben, EDTA disodium salt, cetyl PEG/PPG-10/1 polydimethylsiloxane, simethicone, octanoic acid, triglycerides, shea butter oil, microcryst. wax, beeswax, polydimethylsiloxane, grape seed oil, sodium hyaluronate, essence, arbutin, adenosine, allantoin, iron oxide yellow, iron oxide red, black iron oxide, and water, in refreshing cream can effectively isolate harmful substances in skin cream for makeup and in the air (Jingyao, 2013).

The combination of Allantoin along with other chemicals are used to treat acarid-caused acne, and has effect of inhibiting seborrhea of oily skin, relieving discomfort, whitening, removing wrinkles, antiaging and brightening skin, and also can be used for caring skin, moisturizing, and reducing formation of age pigment. The red nose caused by acarid, can be cured by using allantoin along with other chemicals by inhibiting excessive lipid secretion, repairing hair follicle and rugged tissue, reducing pigmentation and indentations, and preventing acne formation (Wu Ke, 2013).

Endotoxin removal is efficiently achieved by the use of allantoin as a solid phase adsorbent which is effective than anion exchange, polymixin affinity and biological affinity methods for endotoxin clearance (Vincent et al., 2013). A skin-caring cream comprising allantoin significantly improved skin laxity, aging lines, dull skin and increased skin elasticity (Zhang et al., 2013).

Anti-bacterial Activity of Allantoin

The anti-bacterial, anti-viral, cytotoxicity and anti-microbial activities of allantoin along with the combination of some compounds are documented (Berrin et al., 2011).

Anti-ulcer Activity of Allantoin

Sixty-one alkaloids tested for anti-ulcer activity revealed fifty-five compounds to show significant antiulcer activity in ulcer induced animals (Heloina et al., 2008).
Anti-hypertensive Activity of Allantoin

Allantoin is recommended as an effective therapeutic agent for hypertension in the future. Administration of allantoin in rats decreased the blood pressure and in anesthetized rats inhibited cardiac contractility and heart rate. It is suggested for memory enhancement mediated by the PI3K–Akt-GSK-3β signal pathway and treating cognitive dysfunctions (Chen et al., 2014). Allantoin is also advocated for the cognitive dysfunctions observed in Alzheimer’s disease (Ahn et al., 2014).

Wound healing Activity of Allantoin

Comparison of the burn wound healing in rats by the application of extra ctumceae, heparin and allantoin gel (CTBX) and silver sulfadiazine (SSD) cream revealed significant positive effect on wound healing by the application of CTX (Durmus et al., 2012). Allantoin induces wound healing through the regulation of inflammatory response and stimulus to fibroblastic proliferation and extra cellular matrix synthesis. It is able to improve and fasten the reestablishment of the normal skin (Lorena et al., 2010).

Biosynthesis of Allantoin

Allantoin is nitrogen-rich compound derived from purine catabolism. A first step of purine catabolism occurs in the cytoplasm of infected nodule cell and leads to the production of oxopurines, such as hypoxanthine, xanthine and uric acid. Uric acid is then transferred to uninfected cells into the peroxisome and further metabolized to allantoin. The conversion of uric acid into allantoin is a single step process catalysed by urate oxidase. The urate oxidase reaction was determined by the conversion of urate to 5-hydroxyisourate (HIU), an unstable compound that decomposes spontaneously to 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU). Non-enzymatically decay of OHCU yield allantoin. The non-enzymatic decomposition of HIU generates a racemic mixture of allantoin (Rossi, 2007). Outline of biosynthetic pathway of allantoin was shown in Figure 20.
Fig. 6 Biosynthetic Pathway of Allantoin
2.4 Earlier Work on *Andrographis stenophylla* C.B Clarke

*Andrographis stenophylla* is a medicinal plant belonging to the family *Acanthaceae*. It is an erect shrub with very narrow leaves and stems from a stout rootstock; the corolla is pale with dark red stripes. In folk medicine the leaves are used for the treatment of diabetes and snake venom poisoning.

2.4.1 Pharmacognostical Studies

The physio-chemical and pharmacognostical properties of *Andrographis stenophylla* screened using light and confocal microscopy suggests use of physio-chemical, morphological and histological parameters as parameters to establish the authenticity of *Andrographis stenophylla* and that can possibly help to differentiate the drug from its other species (Vijaya Bharathi *et al*., 2007).

**Chemical constituents of the plants of the genus *Andrographis***

The genus *Andrographis* is rich in secondary metabolites particularly flavonoids. The following is a brief review of the scientific reports on isolation of flavonoidal compounds from the different species of *Andrographis*.

**Andrographis paniculata**

- Four flavonoids namely 7-O-methylwogonin, apigenin, onysilin and 3,4-dicaffeoylquinicwere isolated from *A.paniculata* (Wen-Wan Chao *et al*., 2010)
- Two new flavones, andropaniculosin and andropaniculoside and also 30 known compounds have been isolated from the plant *Andrographis paniculata* (Damu *et al*., 2008)
- Twelve flavonoids were isolated from the ethanol extract of *Andrographis paniculata*. Their structures were characterised by spectral analyses and chemical evidences (Chen *et al*., 2006)
- The roots and aerial parts of *Andrographis paniculata* yielded new flavones named 5-hydroxy-7,2′,6′-trimethoxyflavone and an unusual 23-carbon terpenoid, 14-deoxy-15-isopropylidene-11,12-didehydroandrographolide together with five known flavonoids and four known diterpenoids (Rao *et al*., 2004)
- Rao *et al*.,(2004) reported flavonoids and andrographolide diterpenoids, and other polyphenols from the whole plant of *Andrographis paniculata*
**Andrographis lineata**

- Three flavonoids, 5,7,2',3',4'-pentamethoxyflavone, 2'-hydroxy-2',4',6'-tri methoxychalcone and dihydrovoskullcap flavone together with 17,19,2'-tri hydroxy-5, 8 H, 9 H,1' -labd-13-en-16,15-olactone, a known diterpenoid and six known flavonoids, 5-hydroxy-7,8-dimethoxyflavanone, 5-hydroxy-7,8,2',3',4'-pentamethoxyflavone, 5,2'-dihydroxy-7-methoxyflavanone, 5,2'-dihydroxy-7,8-dimethoxyflavone, 5,2'-dihydroxy-7-methoxyflavone, 5,2'-dihydroxy-7-methoxyflavone 2'-dglucopyranoside were isolated from the whole plant of *Andrographis lineata*. The structures of these compounds were elucidated on the basis of spectral and chemical studies (**Hari Kishore et al., 2003**).

**Andrographis affinis**

- Three new 2'-oxygenated flavonoids, -5,7,2',3',4'-pentamethoxyflavanone, 5-hydroxy-7,8,2',5'-tetramethoxyflavone and echiioidinin 2'-O-beta-glucopyranoside, together with four known flavonoids, 7-O-methylidihydrowogonin, 7-O-methylwogonin, skullcapflavone 2'-methyl ether, and skullcapflavone and two diterpenoids, andrograpanin and 14-deoxy-11,12-didehydroandrographolide, were isolated from the whole plant of *Andrographis affinis* (**Reddy et al., 2003**).

**Andrographis alata**

- Das et al. (2006), isolated, five acylated 5,7,2',6'-oxygenated flavone glycosides along with the known 5,2',6'-trihydroxy-7-methoxyflavone-2'-glucopyranoside from the whole plant of *Andrographis alata*. The structures of the compounds were established from 1D and 2D NMR spectral studies and chemical studies.

- Damu et al. (1998), isolated a new flavone glycoside, echiioidinin 5-glucoside along with its known aglycone, echiioidinin from the whole plant of *Andrographis alata*. Based on the spectral and chemical studies, the compounds structure was established as 5,2'-dihydroxy-7-methoxy favone 5-glucopyranoside.

**Andrographis echiioides**

- Phytochemical investigation of the whole plants of *Andrographis echiioides* afforded two new 2'-oxygenated flavonoids two new phenyl glycosides along with 37 known compounds. The structure of new compounds was elucidated by spectral analysis and chemical transformation studies (**Shen et al., 2013**).
Jayaprakasam et al. (1999), found a new flavanone, dihydroechioidinin together with four known flavones, echiidinin, echioin, skullcap favone 2'-O-methyl ether and skullcap flavone 2'-O-glucoside. The structure of dihydroechioidinin was established as (2S)-5,2'-dihydroxy-7-methoxy flavanone on the basis of spectral and chemical evidence.

Govindachari et al. (1965), isolated a new flavone glucoside echiodin from Andrographis echioides and its structure was found to be 5-hydroxy-2'-β-d-glucosidoxy-7-methoxyflavone (echioidinin-2'-β-d-glucoside).

Andrographis viscosula

Phytochemical investigation of the whole plant of Andrographis viscosula led to the isolation of three new 2'-oxygenated flavonoids, (2R)-5-hydroxy-7,2',3'-trimethoxyflavanone, 7,2',5'-trimethoxyflavone, 5,7,2',3'-tetramethoxyflavone and eight known flavones (Rao et al., 2003).

Rao et al., (2002) isolated two new 2'-oxygenated flavones, 5,7,2'-trimethoxyflavone and 5,7,2',4',6'-pentamethoxyflavone from the whole plant of Andrographis viscosula.

Chemical constituents of Andrographis stenophylla

The phytochemical screening of the dichloroethane extract of the Andrographis stenophylla leaves showed the presence of a terpene and diterpenoid (Parasuraman et al., 2010). Methanol extract of leaves of Andrographis stenophylla was found to contain 2% of total phenolic compounds (Neelaveni et al., 2010). Activity guided isolation of extracts of Andrographis stenophylla yielded secondary metabolites acacetine (21), isosakuranetine (22) and andrographolide (23) (Neelaveni et al., 2006).
Pharmacological Studies on *Andrographis stenophylla*

The dichloroethane extract of leaves of *Andrographis stenophylla* significantly reduced hypoglycaemia compared with the glucose treated group (Parasuraman *et al.*, 2010). Various extracts of *Andrographis stenophylla* tested for its antioxidant potency, free radical scavenging activity and reductive ability revealed methanol extract to exhibit maximum antioxidant activity (Neelaveni *et al.*, 2010).

2.5 Bio-Pharma Potential of Andrographolide


Andrographolide exhibits immense biological activities. A literature review during the period from 1990 to till date revealed that the diterpenoid compound andrographolide is bestowed with anti-inflammatory, anti-diabetic, anti-oxidant anti-
tumour, anti-ulcer and anti-arthritic activities. A report on neuroprotective effects on Parkinson disease by andrographolide is well established (Zaijun et al., 2014). It is a non-steroidal anti-inflammatory agent.

Andrographolide inhibits viral penetrations, synthesis of viral proteins and inhibits viral DNA polymerase. There are more than 5000 consumer bands of andrographolide is currently available in the market which depicts the significance of the molecule (www.pubchem.ncbi.nih.gov). Andrographolide, has traditionally been used for the treatment of colds, fever, laryngitis, and other infections with no or minimal side effects. Andrographolide treatment of mdx mice, an animal model for Duchenne muscular dystrophy (DMD), exhibited less severe muscular dystrophy than untreated dystrophic mice and was found to improve grafting efficiency upon intramuscular injection of dystrophin-positive satellite cells. These results suggest the prospects of andrographolide in improving the quality of life in individuals with DMD (Cabrera, 2014).

The protective effects of andrographolide on the development of autoimmune diabetes (NOD) was tested with mice. Oral supplementation of andrographolide significantly inhibited insulitis, delayed the onset, and suppressed the development of diabetes in 30-week-old NOD mice in a dose dependent manner (Zhang, 2013).

The anti-bacterial and anti-oxidant activity of andrographolide and echiodinin of Andrographis paniculata by broth micro-dilution method and DPPH assay, respectively, revealed andrographolide to be more effective against most of the strains tested including Mycobacterium smegmatis, showing broad spectrum of growth inhibition activity. Moderate anti-oxidant activity was noticed in the study and the results have been suggested to provide scientific rationale for the use of this plant in folkloric medicine (Mohmmed, 2013).

Paracetamol overdose is often fatal due to progressive and irreversible hepatic necrosis. Engineered nanoparticles loaded with Andrographolide provides fast protection in Paracetamol induced acute liver failure due to the rapid regeneration of antioxidant capacity and hepatic GSH store (Roy et al., 2013).

The study of effect of dietary andrographolide on growth, non-specific immune parameters and disease resistance against Aeromonas hydrophila infections in Indian major carp, Labeo rohita fingerlings revealed the fishes fed with formulated diet containing andrographolide to show significant stimulatory effect on non-specific immune parameters along with improved growth performance and increased disease resistance against A. Hydrophila infection in L. Rohita fingerlings (Basha, 2013).
Andrographolide and its derivatives display anti-HIV activity in vitro. 3-nitrobenzylidene showed higher in vitro anti-HIV activity, whereas 2',6'-dichloronicotinoyl ester showed higher therapeutic index (Uttekar et al., 2012). A water-soluble polysaccharide (APP) isolated from Andrographis paniculata in synergism with andrographolide improved the metabolic abnormalities of diabetic mice and delayed the progression of diabetic renal complications suggesting its usefulness as a therapeutic agent for inhibiting the progression of diabetic nephropathy tested on renal complication in streptozotocin (STZ) induced diabetic mice. APP plus andrographolide increased the body weight and creatinine clearance rate (Ccr), and decreased the levels of serum creatinine, serum urea nitrogen, urinary albumin excretion (UAE), serum urea and blood glucose in diabetic rats, as well as the relative kidney weight (Jie Xu, 2012).

Andrographolide extracted from the herb Andrographis paniculata exhibited concentration-dependent inhibition of human monocytic matrix metalloproteinases activation, induced by either tumor necrosis factor or lipopolysaccharide (LPS), in THP-1 cells suggesting new opportunities for the development of new anti-inflammatory and leukemic therapies (Lee et al., 2012). Andrographolide induces apoptosis of SiHa cells via suppression of HPV16 transcription activity, leading to decreased E6 oncoprotein and restored p53. These findings imply that the andrographolide may be an effective agent for cervical cancer prevention and treatment (Fangkham, 2012). A series of andrographolide derivatives synthesized and evaluated for their anti-HIV activity in a cell-free virus infectivity assay using TZM-bl cell revealed andrographolide, 3-nitrobenzylidene derivative to show higher in vitro anti-HIV activity and 0-dichloro-nicotinoyl ester derivative to show higher Therapeutic Index suggesting andrographolide derivatives as promising candidates for prevention of HIV infection (Uttekar, 2012).

Acylated andrographolides synthesized through enzymatic acylation reactions using immobilized Candida antarctica lipase B (Novozym 435) as a biocatalyst revealed antibacterial activity against representative Gram-positive and Gram-negative bacteria with minimal inhibitory concentrations (MICs) as low as 4 microgram/mL (Chen, 2011).

Inflammation and endothelial cell dysfunction are important initiating events in atherosclerosis. Tumor necrosis factor-R induces the expression of cell adhesion molecules and results in monocyte adherence and atheromatous plaque formation. Andrographolide a major bioactive diterpene
lactone in Andrographis paniculata has anti-inflammatory activity. Heme oxygenase 1 plays a role in the inhibition of Tumor necrosis factor R (TNF-R) induced ICAM-1 expression by Andrographolide. The effect of Andrographolide on the IKK/NF-kB signaling pathway revealed Andrographolide to inhibit TNF-R-induced ICAM-1 mRNA and protein levels, its expression on the cell surface, and subsequent adhesion of HL-60 cells to EA.hy926 cells suggesting Andrographolide to be a potent cardiovascular-protective agent (Chao et al., 2011).

Andrographolide exhibits apoptosis of cancer cells at different concentrations portraying anticancer potential (Jayakumar et al., 2010). Andrographolides shows potent immunomodulatory and anti-angiogenic activities in tumorous tissues (Varma et al., 2011). Andrographolide, neoandrographolide, isoandrographolide, andrograpanin, 14-deoxy-11,12-didehydroandrographolide, 7-O-methylwogonin and skullcapflavone-I isolated from Andrographis paniculata exhibit anti-inflammatory/anti-allergic effects by modulating different inflammatory/allergic mediators and is suggested to provide useful phytomedical treatment against variety of inflammatory and allergic disorders (Chandrasekaran, 2011). Andrographolide analogues, have been tested for their pharmacological potential. 14-deoxy-11,12-didehydroandrographolide (21) is immunostimulatory, anti-infective and anti-atherosclerotic; neoandrographolide is anti-inflammatory, anti-infective and anti-hepatotoxic; 14-deoxyandrographolide is immunomodulatory and anti-atherosclerotic (Chao et al., 2010). Oral administration of andrographolide at dosage of 50 mg/kg body weight of male mice was found to affect vascular reactivity and serum testosterone level in experimental animals in week4 suggesting the potential of andrographolide in enhancing sexual properties (Sattayasai, 2010).

Andrographolide enhances 5-fluorouracil-induced apoptosis via caspase-8-dependent mitochondrial pathway involving p53 participation in hepatocellular carcinoma (SMMC-7721) cells suggesting its heady prospects in treating human carcinoma (Yang, 2009).

Andrographolide possesses strong anti-inflammatory activity. The ability of andrographolide to inhibit the release of inflammatory cytokines in in vitro non-specific inflammation model reveals it to be anti-inflammatory drug that is active in vitro and in vivo, and affects both non-specific as well as antigen/antibody-dependent lung inflammation (Abu-Ghefreh, 2009).

Invitro in vivo studies of the ethanolic extract of Andrographispaniculata and andrographolide reveal the potential inhibition of α-glucosidase and α-amylase enzymes (Subramanian et al., 2008). The synthesis of andrographolide derivatives,
3,19-isopropylideneandrographolide, 14-acetyl-3,19-isopropylideneandrographolide and 14-acetylandrographolide, and their in vitro antitumour activity against breast cancer cell lines and colon cancer cell lines is reported (Jada et al., 2007). The antiangiogenic activity of Andrographis paniculata extract (APE) and its major component andrographolide (ANDLE) studied both in vitro and in vivo models revealed significantly inhibition of B16F-10 melanoma cell line induced capillary formation in C57BL/6 mice demonstrating the potent inhibition of tumour specific angiogenesis by APE and ANDLE (Sheeja, 2007).

A series of analogues of andrographolide were found to be potent α-glucosidase inhibitors. Among them 23, 15-p-methoxybenzylidene 14-deoxy-11,12-didehydroandrographolide showed comparatively higher activity with IC50 value was 16 μM (Dai et al., 2006). Urea adducts of andrographolide were isolated from human urine administrated with andrographolide (Liang Cuia et al., 2008). Larvicidal activity of andrographolide was reported by Lingampally et al., (2012).

**Review of literature pertaining to quantification of Andrographolide**

A simple quantitative HPTLC method for determination of andrographolide in Andrographis paniculata at different stages of life cycle of crop from 30 days of plantation up to maturity of the crop was studied. Retention time for andrographolide was found to be 0.31. The average andrographolide content varied from 0.42% to 2.02% in the sample studied (Sharma and Sharma, 2013). High performance liquid chromatographic (HPLC) and high performance thin layer chromatographic (HPTLC) methods are established for quantitative determination of andrographolide (Vijaykumar et al., 2007; Kumora and Hasan 2007; Senthil Kumaran et al., 2003). The maximum andrographolide production by tissue culture was found to be 1.53 mg/g dry cell weight at the end of stationary phase during the growth curve. The accumulation of andrographolide, was stimulated by the presence of yeast (Gandi et al., 2012).

Herbal powder and polyherbal formulation containing Andrographis paniculata were standardized and validated by high performance thin layer chromatographic method. Andrographolide in herbal powder and polyherbal formulations was identified and amount was estimated densitometrically by HPTLC (jadhao, 2010).
Biosynthesis of Andrographolide

Andrographolide, a diterpene lactone richly isolated from the species *Andrographis paniculata* was biosynthesised via mevalonate and non-mevalonate pathway. But the major biosynthetic pathway to this diterpenoid operates through non-mevalonate pathway which is known as deoxyxylulose (DXP) pathway (Srivastava, 2010).