# Analysis of Antidiabetic Herbal Drug by High Performance Thin Layer Chromatography

**By**

**Durga .B**

**(14PCH001)**

**Thesis submitted to**

**Avinashilingam Institute for Home Science and Higher Education for Women, University**

**(Estd.u/s of UGC Act 1956)**

**Coimbatore- 641043**

**Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry**

**April- 2016.**

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29/4/16

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**Durga.B**

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| TLC | Thin Layer Chromatography |
| HPLC | High Performance Liquid Chromatography |
| HPTLC | High Performance Thin Layer Chromatography |
| $R_f$ | Retention value |
| DPPH | 1,1-Diphenyl-2-Picryl-Hydrazyl |
| TBA | Tertiary Butyl Alcohol |
| OGTT | Oral Glucose Tolerance Test |
| BPA | Bisphenol A |
| IAA | Indole-3-Acetic acid |
| NMR | Nuclear Magnetic Resonance |
| LCMS | Liquid Chromatography Mass Spectrometry |
| PDB | Protein Data Bank |
| HRBC | Human Red Blood Cell |
| LDL | Low Density Lipoprotein |
| HPL | High Density Lipoprotein |

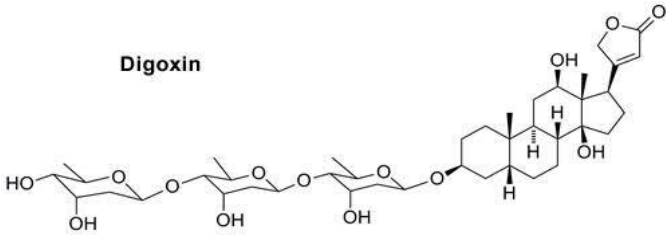
# 1. INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural product from plant, animal, and minerals have been the basis of the treatment of human disease. About 80% of people in developing countries still relays on traditional based largely on species of plants and animals for their primary health care since it has no side effects. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous system of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatment (***Chopra et.al.,1956***). Herbal medicines as the major remedy in traditional medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts world and have made a great contribution towards maintaining human health (***Sane et al.,2002***).

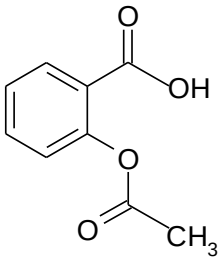
The varies indigenous systems such as siddha, ayurvedha, unani and allopathy use several plant species to treat different ailments (***Rabe and Staden 1997***).The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines. This lead to sudden increase in the number of herbal drug manufactures (***Agarwal et al.,2005***).In India around 20,000 medicinal plant species have been recorded recently but more than 500 traditional communities use about 800 plant species for curing different diseases.( ***Kamboj et al.,2000***).Plant are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the united states contain at least one-plant derived ingredient. In the last century, roughly 121

pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.

According to the world health organization (WHO) the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (***Evans et al.,1994***).Many conventional drugs originated from plant sources; a century ago, most of the few effective drug were plant based. Examples digoxin(1) (from foxglove), aspirin(2) (willow bark), morphine(3) (from the opium poppy), quinine(4) (from cinchona bark) ( ***Vickers and Zollman, 1999***).

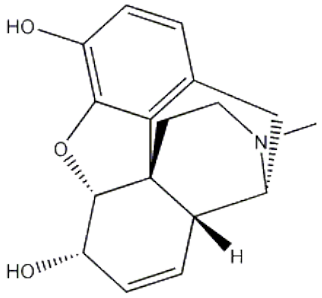



**1**



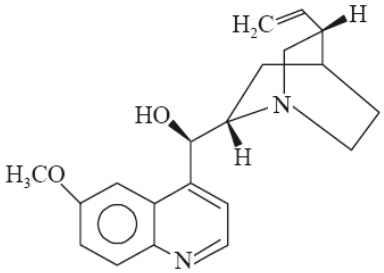

Aspirin

**2**



morphine

**3**



Quinine

**4**

Herbal products were discarded from conventional medicine use in the mild 20 th century, not necessarily because they were ineffective but because they were not as economically profitable as the newer synthetic drugs *( **Tyler et al., 1999**).*In rural areas, there are additional cultural factors that encourage the use of botanicals, such as the environment and culture, a " man earth relationship" people believe that where an area gives rise to a particular disease, it will also support plants that can be used to cure it ( ***Winslow and Kroll 1998***). In India vast sections of the population have no assess to morden medicine ( ***Mudur et al., 1998****).*

### 1.1. ADVANTAGE OF HERBAL MEDICINE

- Herbal medicine products are far cheaper than many pharmaceutical alternatives. They do not need to undergo long and expensive clinical testing and are subjected to healthy market forces due to this lack of regulation as well. All this adds up to more money in people's pocket in addition to increased health.
- Herbal medicine can be consumed without the aid of any kind of prescription. They can be found very easily from a local drug store.
- Herbal medicines are known to be more productive in comparison to other forms of medication in curing certain conditions unless mixed with chemical components, they are known to be all natural.
- One of the greatest benefit with herbal medicines is the nonexistence of side effects. Also, they tend to offer long lasting benefits in term of overall wellness.

- Obesity is a growing problem which is known to have hazardous issues on an individual's health. Herbal medicine can help one deal with the problem of obesity very effectively without consuming much time and efforts.

## 1.2. WHY STANDARDIZATION OF HERBAL MEDICINE

In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine are getting more apparent. It is the cardinal responsibility of the regulatory authorities to ensure that consumers get the medication, which guarantees purity, safety, potency and efficacy. The regulatory authorities rigidly follow various standards of quality prescribed for raw materials and finished products in pharmacopoeias, formularies and manufacturing operation through statutory imposed good manufacturing practices. These procedures logically would apply to all types of medication whether included in modern system of medicine or one of the traditional system.

Though herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control profile. The quality of herbal medicine that is, the profile of the constituents in the final product has implication in efficacy and safety. However, due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish quality control parameter though modern analytical technique are expected to help in circumventing this problem. Hence for herbal drugs and products, standardization should encompass the entire field of study from cultivation of medicinal plant to its clinical application. Plant materials and herbal remedies derived from them represent substantial portion of global market and in

this respect internationally recognized guidelines for their quality assessment and quality control are necessary (***kunle et al., 2012***).

### 1.3. HPTLC (High-Performance Thin Layer Chromatography)

High-performance thin layer chromatography based methods could be considered as a good alternative, as they are being explored as an important tool in drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risk and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters (***Najoshi and Khandelwal 2002***).

### 1.4. NMR (Nuclear Magnetic Resonance)

Drug in clinical use are mostly synthetic or natural products, NMR spectroscopy has been mainly used for the elucidation and conformation of structures. For the last decade, NMR methods have been introduced to quantitative analysis in order to determine the impurity profile of a drug, to characterise the composition of drug products, and to investigate metabolites of drug in body fluids. For pharmaceutical technologists, solid state measurements can provide information about polymorphism of drug powders, conformation of drugs in tablets etc. Micro-imaging can be used to study the dissolution of tablets, and whole-body imaging is a powerful tool in clinical diagnostics.

### 1.5. DIABETS

Diabetes is a chronic disorder in metabolism of carbohydrates, protein and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance (***Chand et al.,2000***). Also, it may be defined as a disease where the body either produces little insulin/ceases to produce insulin becomes progressively resistant to its action *(* ***Rajan et al.2002***).It has now

become an epidemic that the sadults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025 ( ***Torben et al.,2002***). There are more than 30 million people with diabetes mellitus in India and the incidence is increasing (***Shankar et al., 2001***).

Also there are many patients in the community with undiagnosed diabetes decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades.diabetes is being projected as the world's main diabler and killer in the net 25 year *(**Edwin et al.,2006**).*Patients with diabetes experience significant morbidity and mortality from micro vascular and macro vascular complications. The cost of treating diabetes and associated complications exceeds 100 billion per year. The complications are far less common and less severe in people who have well-controlled blood sugar level (***Andrew et al., 2000***)

### 1.5.1. CLASSIFICATION OF DIABETES

**TYPE I DIABETES ( Insulin-dependent diabetes)**

It is prevalent in 10% of diabetic patients, islet β-cell destruction usually leads to absolutes insulin deficiency. Patients are completely reliant upon exogenous insulin to prevent ketosis and there by preserve life *(**Lokesh et al.,2006***).

**TYPE II DIABETES (non-insulin dependent diabetes)**

This accounts for more than 85% of cases worldwide. It is a heterogeneous type, ranging from insulin resistance to insulin deficiency (***Williams and Wilkins et al.,2001***).Also type 2 diabetes is a multifactorial diseases with both a genetic component and an important non-genetic components (***Seshiah et al.,2000***).

## 1.6. ANTIDIABETIC PLANTS IN TRADITIONAL MEDICINES

The NAPRALERT database lists over 1200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with antidiabeticactivity.over half of these have been used ethopharmacologically in

traditional medicine as antidiabetic and some 50% of these traditional remedies have been studied experimentally.(***Marles et al.,1996***).

## 1.7. IN INDIAN TRADITIONAL MEDICINES

In India plants like,

- *Abromaaugusta(L.) L.f,*
- *Aerva lanata,*
- *BerberisaristataDC,*
- *Calamusrotang(L.),*
- *Cannabissativa(L.),*
- *Momordicachrantia(L.),*
- *GymnemasylvestreR. Br,*
- *Ocimim sanctum (L.)*
- *pterocarpusmarsupiumRoxb*,
- Tinosporacordifolia(Willd.) Hook.f. and Thomson, etc., are the mostcommonly used species in traditional medicine as antidiabetic agents (***Chhetri et al.,2005***)

## 1.8. PHYTOCONSTITUENTS WITH ANTIDIABETIC ACTIVITY

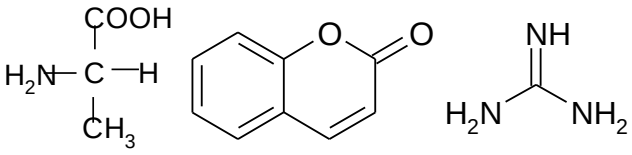
The constituents that comes under the category of,

- Coumarine(5),
- Amino acids(6),
- Guanidines(7),

- Alkaloids,
- Glucopeptides,
- Terterpenoids,
- Amino acids,
- Steroids,
- Xanthone,
- Flavonoids,
- Lipids,
- Phenolics,
- Alkyl disulphides,

polysacchraids are reported to have antidiabetic activity.

Few constituents with structure




alanine coumarin guanidine

**5** **6** **7**

(***Marles et al.,1995***) have reported the antidiabetic plants and their active constituents. Diabetics mellitus was a debilitating and often life-threatening disease with increasing incidence in rural populations throughout the world. There are more than 1200 species of plants which have been used to treat diabetes and or investigated for antidiabetic activity. The development of indigenous botanical resources as inexpensive sources for standardized crude or purified anti diabetic drugs and for the discovery of lead compounds for novel hyperglycemic drug development. (***Kanthi Abesundaraet al.,2004***) examined srilanka plant stuffs were examined regarding in vitro and in vivo α-glycosidase inhibitory actions. Water extracts and methanol extracts of dried fruits of Nelli ( *phylanthus embelica*) methanol extracts of dried flowers of Ranawara ( cassia

auriculata) and water extracts of latex of Gammalu ( pterocarpus marsupium) were found to have a potential α-glycosidase inhibitory activity in vitro.

## 1.9.OBJECTIVES

Diabetic mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from the defects in insulin secretion, insulin action or both. There are several classes of approved oral antidiabetic drugs, however, most of them exert undesirable side effects, drug interactions and the treatment is expensive. Apart from currently available therapy, herbal medicines recommended for treatment of diabetics throughout the world has increased, Herbal drugs are prescribed widely because of their effectiveness, less side effect and relatively low cost. Flavonoids are a group naturally occurring compounds widely distributed as secondary metabolites in the plants. They have been recognized for having clinical properties, such as anti-inflammatory, anti-allergic, anti-viral, anti-bacterial, and anti-diabetic activities. One of these flavonoid quercetin was found to have anti-hyperglycemic activity in both type 1 and type 2 diabetics.(***ketan Hatware 2014***).

In view of the above facts the present study aimed to analyse the antidiabetic herbal drug for,

- The presence of phytochemical constituents like terpenoid, flavonoid, and alkaloid by preliminary colour test.
- To quanitatively determine the amount of flavonoid present in the drug.
- To carry out the flavonoid and polyphenolic profile by HPTLC.

- To study the UV spectral pattern of the drug.
- To estimate the amount of quercetin present in the drug by UV method.

# 2. REVIEW OF LITRATURE

The review was done under following headings,

1). Analysis of herbal drug.

2). Analysis of *Aerva lanata*.

3). Analysis of *Pterocarpus Marsipium.*

## 2.1.ANALYSIS OF HERBAL DRUG

- **Chitravadivu *et al*.,(2009)** developed the qualitative analysis for identifying the compounds present in the medicinal plants. Four medicinal plants were collected and analyzed for phyto-constituents. The qualitative analysis revealed the presence of the biomolecules such as anthraquinone, alkaloids, catachol, flavonoids, phenolic compounds, steroids, tannins and triterpenoids respectively.
- Diabetes mellitus was one of the common metabolic disorders and 2.8% of the population suffers from this disease throughout the world and it may cross 5.4% by the year 2025. Oral hypoglycemic agents like sulphonylureas and biguanides are still the major players in the management of the disease but there was a growing interest in herbal remedies due to slide effects associated with the oral hypoglyamic agents. The medicinal plants, besides having natural therapeutic

that herbs are a growing part of high-tech medicine. The antidiabetic activity of medicinal plants was due to the presence of phenolic compounds, flavonoids, terpenoids, coumarins and other constituents which show reduction in blood glucose level **Upendra Rao *et al*., (2010).**

- **Akshada N.Kakade *et al*.,(2012)** developed the HPLC analysis of *β-sitosterol* in herbal medicine and vegetable oils. *Solal beta sitosterol* capsules was a herbal medicine and β-sitosterol is known to control cholesterol levels, reduce the activity of cancer cell, promote prostate gland health and enhance immunity in the human body. *β-sitosterol* can also be found in vegetable oil such as: wheat germ oil, cotton seed oil, etc,. The amount of *β-sitosterol* of the vegetable oils was detected by HPLC. The retention time was found to be 36.91 in wheat gram oil.
- **Chanida Palanvej *et al*.,(2014)** reported the usnic acid content in *usnea Siamensis* by TLC-densitometry and TLC image analysis. *Usnea Siamensis* was herbal drug that was used to treat diseases in folk medicine. The quantitative analyses of active compound(+)- usnic acid in *Usnea Siamensis* sample from 15 locations were done by TLC using chloroform and methanol (9:1). The usnic acid contents in *Usnea Siamensis* were found to be 2.32±0.29 and 2.26±0.25 g/ 100 g of dried crude drug respectively. It was found that usnic acid contents by two methods were not statistically different (p=0.0256)
- **Sudhir S. Kamal *et al*.,(2015)** reported the element analysis of herbal tablets by ICP-MS. Herbal medicines required standardization, with implementation and constant review of technical standards of production and effective quality control methods. Heavy metals are very toxic, they are soluble in water and may be readily absorbed into living organisms. After absorption, these metals can bind to vital cellular components such as structural proteins, enzymes, and nucleic acid and interfere with their functions. These metal cause physiological and health effects herbal medicines contains metals like Mn, Zn, Cr, Fe and Cu etc…these metals were determined quantitatively by using ICP-MS(inductively coupled plasma mass spectroscopy).

## 2.2. ANALYSIS OF*AERVA LANATA*

- *Aerva lanata* was a herbal medicine. The plant is known as pasahanahede/gorakshaganja in ayurvedic system of medicine. The roots are diuretic and used to cure urinary troubles including stones. Anti urolithic activity was carried out by simultaneous flow static method. The precipitate were determined by S.S.M and finally it was found one percent aqueous extract of *Aerva lanata* root was good at controlling calcium phosphate crystallization. Five percent of the aqueous extract was good on calcium oxalate crystallization, though it controls both type of crystal formation. **Sunil Kumar *et al*.,(2005).**
- **Tushar A. Deshmukh *et al*.,(2008)** have studied the *Aerva lanata*. The study demonstrated the anti-hyperglycemic activity of alcoholic extract of *Aerva lanata* leaves on serum glucose levels and on the oral glucose tolerance test (OGTT) in alloxan induced diabetic mice. *Aerva lanata* alcoholic ( 100,200 and 400mg/kg) and glyburide (10mg/kg) were administrated orally in alloxan induced diabetic mice. In the OGTT, *Aerva lanata* increased the glucose level. The *Aerva lanata* (400mg/kg) showed significantly more anti hyperglycemic activity than *Aerva lanata* (100 and 200mg/kg).
- **Deepak Kumar *et al*.,(2009)** have studied that the ethanolic extract of *Aerva lanata*. The plant exhibited the significant dose dependent antiasthmatic activity in-vitro and in-vivo.
- **Appia Krishnan *et al*.,( 2009)** have studied the phytochemical constituents and anti-diabetic effect of Arial parts of *Aerva lanata* in normal and alloxan induced

diabetic rats. Ethanolic extract of *Aerva lanata* was evaluated for hypoglycemic activity in diabetic rats at doses of 50,100 and 200 mg $kg^{-1}$ body weight. *Aerva lanata* extract given to diabetic animal's dose dependently reduced the blood glucose level, which was comparable to standard dose of motformin.

- **Anantha *et al*.,(2010)** have reported the antiparasitic activity of the seed and leaf extracts of *Aerva lanata* were tested against a tapeworm and an earthworm, particularly ethanolic extract showed to be better against tapeworms and earthworms than the standard albendazole, which were used in the treatment of helmentic parasite infections.
- **Yamunadevi*et al*.,(2011)** have elucidated the terpenoid profile of *Aerva lanata* using high performance thin layer chromatography. In that the preliminary phytochemical screening was done and HPTLC studies were carried out. The methanolic extract of stem, leaves-root, flower and seed of *Aerva lanata* showed the presence of 27 different types of terpenoids with 27 different $R_f$ values in the range 0.06 to 0.97.
- **Payal Chawla *et al*.,(2012)** have studied the biological activities of the genus *Aerva*. There were 28 species of *Aerva* genus. But only few species are medicinal. A number of flavonol glycosides has been reported from *Aerva* as major constituents. These plants were used as medicinal herb in traditional medicine system like diuretic, demulcent, purgative, emetic and tinder. *Aerva* plants were used to cure ulcer, lithiasis, dropsical affections, eye affections, toothache, headache, in disorders of abdomen and inflammation of internal organs. Roots and flowers were reported to possess hypoglycemic, antioxidant, anthelmintic, analgesic, antimalarial, antivenin activities and medicinal properties against rheumatism and kidney troubles.
- **Yamunadevi*et al*.,(2012)** have determined the saponins by HPTLC method. the medicinally important plant *Aerva lanata*, it was confirmed that the stem, root, leaves, flowers and seeds of *Aerva lanata* possessed the steroid, tepenoids and

saponins. *Aerva lanata* can be used to control insects, molluss etc. The biological and biochemical properties of saponins suggested that the *Aerva lanata* possess cardiac, antifungal, and hemolytic abilities to affect metabolism and biosynthesis.

- **Paramasivam Ragavendran *et al*.,(2012)** have studied the antioxidant potential of *Aerva* lanata. In this study of Phytochemical screening of aqueous, ethanol extract was selected. The plant exhibited the most potent radical scavenging activity at a maximum concentration 2.5mg/ml. natural antioxidants such as flavonoids, total phenol, tannins and lycopene were evaluated and also antioxidant activity against DPPH, super oxide anion, hydroxyl radical, nitric oxide radical, total antioxidant capacity assay and anti-lipid peroxidation activity were evaluated. Finally it was found that the *Aerva lanata* showed high anti lipid peroxidation against TBA. Strong antioxidant activity was observed in aqueous ethanol extract than water and ethanol extract.
- **Vijayalakshmi *et al*.,(2012)** have reported the phytochemical screening of root extracts of *Diospyrus Ferrea* and *Aerva lanata.* The phytochemical screening was one of the necessary step to find out the chemical constituents. *Diospurus Ferrea* was medicinally important and that the root has been used for the treatment of various ailment. *Aerva lanata*, the whole herb is medicinally important. Dry root powder was tested with different solvents such as hexane, chloroform, methanol, ethanol and water. And it was found that the tested root powder of *Aerva lanata* showed some of the chemical components such as flavonoids, saponins, tannins coumarines and terpenoids.
- **Rajendra Prasad Gujjeti *et al*.,(2013)** have studied the phytochemical screening and thin layer chromatography of *Aerva lanata* root extract. Phytochemical screening revealed the presence of phytochemical constituents. TLC studies of *Aerva lanata* root extract constituted different colored phytochemical compounds with different $R_f$ value. It was concluded that *Aerva lanata* root as a rich source of

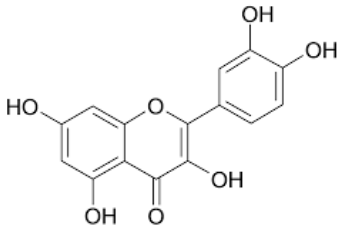
natural antioxidants, medicinally important bioactive compound and preventing oxidative stress related degenerative diseases and also provided lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of injections caused by the microorganisms.

- **Pandi Lakshmi *et al*.,(2013)** have reported the potential of two commonly used phytotherapeutic agents such as *scoparia Dulcis* and *Aerva lanata* in the management of calcium oxalate urolithiasis in male albino rats. *Scoparia Dulcis* and *Aerva lanata* whole plant and fruit parts with ethylene glycol induced lithiasis was used to reduce the levels of stone forming and prevent the formation of kidney stones. Fruit part of *Scoparia Dulcis* in combination with *Aerva lanata* reduces the risk of stone formation than others. The seed extract of the medicinal plants were moderate to good inhibitor of calcium oxalate, calcium carbonate and calcium phosphate.
- **Anita *et al*.,(2013)** have studied *Aerva lanata* which was a therapeutic agent for various diseases. The active components present in *Aerva lanata ,* such as flavonoids, glycosides, carbohydrates, alkaloids, tannins, saponins, terpenoids, phenols, and phytosterols were responsible for its pharmacological activities. The anti helminitic activity of *Aerva lanata* was due to carbohydrates, tannins and flavonoids. The antioxidant activity and antimicrobial activity were due to saponins, tannins, terpenoids and phenols present in *Aerva lanata.Aerva lanata* showed good antiastimatic activity and antimicrobial activity against various gram positive and gram negative bacteria. The diuretic activity of *Aerva lanata* was mainly due to flavonoids. The hydroalcoholic extract of *Aerva lanata* was proved to be a good herbal for liver ailments.
- **MannenDenni *et al*.,(2013)** have reported the comparison between white and yellow flowered variants of *Aerva lanata*. *Aerva lanata* were analyzed for various classes of compounds. Cold extraction of the plant material was performed using methanol to extract free phenolics while hot extraction in mild alkaline condition was done to extract bound phenolics, methoxy kaempferol has been identified for

the first time in the flowers of both varients. The yellow variant showed slightly higher flavonoid content than the white variant, especially when subjected to mild alkaline extraction using $Na_2Co_3$ solution. Chlorophyll content estimated to the yellow count part. HPLC fingerprint comparison of the methanolic extracts revealed that the phytochemical variation in the two sample.

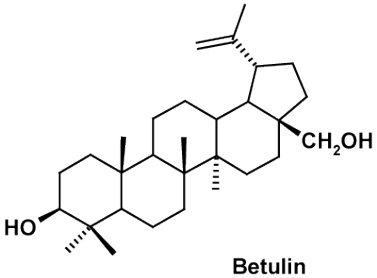
- **Manickam Murugan *et al*.,(2014)** have studied the phytochemical examination of therapeutic important of whole plant extract of *Aerva lanata* an important medicinal plant. Phytochemical analysis of methanol and ethanol extracts prepared from *Aerva lanata* revealed the presence of alkaloids, coumarins, flavonoids, quinines, saponins, steroids and xanthoproteins. The ethanol and methanol extract of whole of *Aerva lanata* were tested against *Klebsiella Pneumonia*, *ServatiaMarcescenes*. It was found that ethanol extract of *Aerva lanata* revealed the high degree of antibacterial activity against *Klebsiella pneumonia*. *Aerva lanata* had been displayed antibacterial activity against the plant to treat urinary tract infection, noscomial infection, pneumonia.
- **Nagaratna *et al*.,(2014)** have studied the *Aerva lanata* (Linn) a herbal drug widely used in urinary disorders. Phyto chemical investigations revealed the presence of steroids, tannins, flavonoids, terpenoids in different parts of the plant. Antiurolithic activity of the plant was also carried out. Besides, it also was proved that the plant possess pharmacological activities like anti-diarrhoeal, anti-hyperglycameic, anti-oxidant, anti-helmentic and analgesic.
- **Vinit Movaliya *et al*.,(2014)** have examined the *Aerva lanata* for nephroprotective activity. This plant was used to break the kidney stone. It has proved that the roots and flowers possess medicinal properties against rherematism and kidney troubles. The study indicated that the phytoconstituents of the various part of *Aerva lanata* was used to design novel drug against various diseases.
- **Basavaraj M. Dinnimath *et al*.,(2014)** have studied that the *Aerva lanata* a herbal medicine which is used as antiurolithiatic, astringent, diuretic, antimicrobial, anti inflamamatory and heptoprotective. The isolated compounds from two fractions n-butanol and ethyl acetate were characterized by modern

analytical technique such as IR, HPTLC, NMR and LCMS and was found to be as quercetin(8) and botulin (9). These two compounds were studied for antiurolithiatic activity by silico technique by docking with a protein 2 ETE of oxidase from PDB and the result indicated the region specificity with the enzyme.




quercetin

**8**




**Betulin**

**9**

- **Payal *et al*.,(2015)** have studied phytochemical and biological activities of *Aerva lanata*. *Aerva lanata* belongs to *Amaranthaceae* family, it contains flavonoids, alkaloids, phenol, tannin, proteins, amino acids, steroids, saponins and carbohydrates. It has anthelmintic, demulcent, anti-inflammatory, diuretic, hypatoprotective, hypoglycemic, anti-diabetic, anti-parasitic, and anti-microbial, anti-asthmatic, anti-fertility, hypolipidemic and nephroprotective property.
- **Nandhagopal *et al*.,(2015)** have studied the *Aerva lanata* which was an medicinally important plant known for its prolonged hypertensive and hypoglycemic activity. The study was focused on standardization of in vitro propagation and phytochemical studies of *Aerva lanata*. Direct organogenesis of shoot from nodal segment was achieved culturing on MS medium supplemented with different concentrations and combinations of growth regulators BPA,IAA and KIN. The maximum number of multiple shoots per explants was obtained in the combination of BPA and KIN. Full strength of MS medium with different concentration of auxins such as IAA and IBA were used for in vitro root formation. IAA with half strength MS media gave better response through morphogenesis investigation of *Aerva lanata*. The preliminary phyto chemical

screening of *Aerva lanata* plant extracts using different organic solvents showed positive results in *Aerva lanata* extracts.

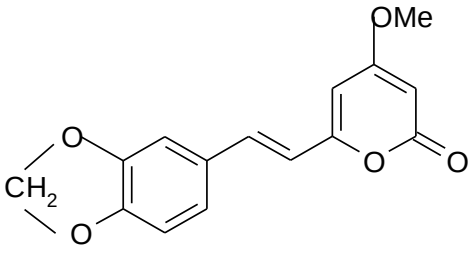
- **Vivek *et al*.,(2015)** have reported the invitro anti-inflammatory activity of *Aerva lanata*. The extract at different concentrations was incubated with HRBC and egg albumin in controlled experimental conditions and to absorbance were determined to assess the anti- inflammatory property. The effect of dichofenac sodium was found to be less when compared with the ethanol extract. From the study it was concluded that *Aerva lanata* possessed in vitro anti-inflammatory effects against the HRBC lysis and denaturation of protein. The effect was possibly due to the polyphenols and flavonoids content of *Aerva lanata.*

### 2.3. ANALYSIS OF *PTEROCARPUS MARSUPIUM*

- **Farboodniary Jahromi *et al.,(1993)*** have reported ethyl acetate extracts of *Pterocarpus Marsupium* heartwood and its flavonoid constituents, marsupin, pterosupin and liquitrigenin. The ethylacteate extract produced a significant reduction of serum triglyceride, total chlosterol and LDL-and VLDL- cholesterol levels without any significant effect on the level of HDL-cholestrol. Liquiritigenin in serum cholesterol, LDL- cholesterol and atherogenic index, pterosupin being additionally effective in lowering serum triglyceride.
- **Manickam *et al.,(*1997)** have reported the antihyperglycemic activity of phenolics from *Pterocarpus Marsupium*, Glucose level in rats with hyperglycemia induced by streptozotonic were determined after administration of marsupin, pterosupin and pterostillbene, three important phenolic constituents of the heartwood of lterocarpus marsupium. Marsupin and pterostillbene significantly lowered the blood glucose level compared to that of 1,1-dimethyl biguanide (metformin).
- **Anna Rita Bilia *et al*.,(2001)** have determined the efficiency of two-dimensional homonuclear ‘H-‘H correlated spectroscopy and two-dimensional reverse heteronuclear shift correlation spectroscopy in characterizing and evaluating the relative content of herbal extract constituents was demonstrated. These

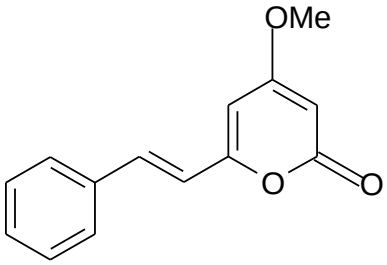
experiments are able to fully assign the proton and carbon resonances of all three classes of constituents present in dried commercial extract of St.John's wort, that is flavonols, phloroglucinols and naphthodianthrones, with particular regard to the very unstable phloroglucinols. In addition shikimic and chlorogenic acids, sucrose, lipids, polyphenols, and traces of solvents of the extractive process( methanol) were also identified. These experiments can be considered to be a very simple and fast analytical method for determining the quality and stability of the filled commercial extract. They represent a generally applicable technique for a rapid screening and a specific measurement of other commercial phytochemicals or, in selected cases, an alternative to the classical analytical technique such as high-performance thin-layer chromatography, high-performance chromatography, capillary gas chromatography, and electrophoresis.

- **Anna Rita Bilia *et al*.,(2002)** have studied the efficiency of one and two dimensional NMR experiments in characterizing the content of the constituents of herbal drugs preparations in demonstrated for kava-kava. These experiments directly detect active constituents presented in kava lactones in both finely powdered herbal drug and in a commercial extract. NMR spectroscopy detected all other compounds present in the extract. NMR characterization of the kava-kava herbal drug showed the presence of the following compounds.



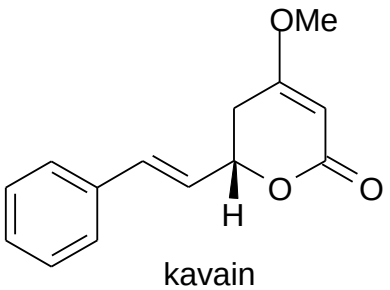

5,6-Dehydromethysticin

**10**



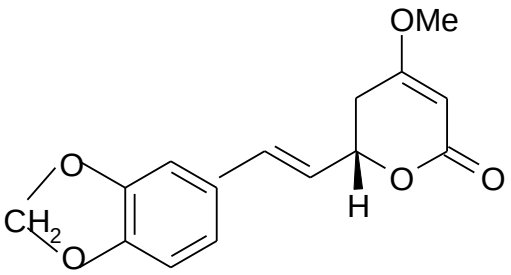

Demethoxyyangonin

**11**



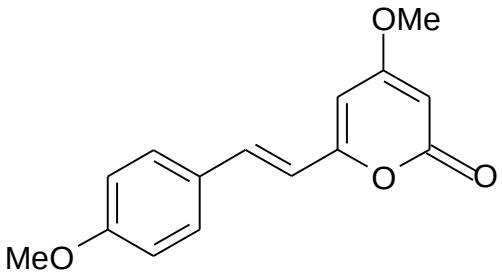

kavain

**12**



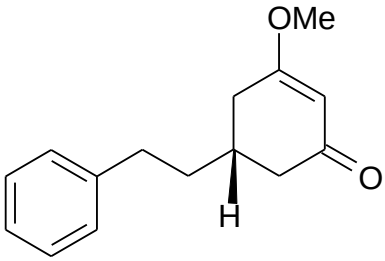

Methysticin

**13**



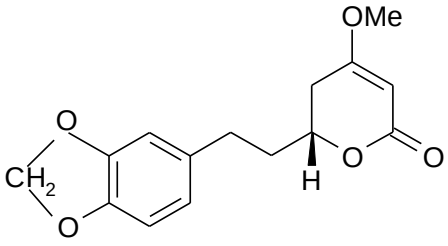

Yangonin

**14**




dihydrokavain

**15**




dihydromethysticin

**16**

- **Mankani *et al*.,( 2004)** have determined the hepato protective activity of *Pterocarpus Marsupium* stem and bark extracts against carbon tetrachloride induced hepato toxicity. Hepato toxicity was induced in male rats by intraperitoneal injection of $CCl_4$. Methanol and aqueous extracts of *Pterocarpus Marsupium* stem bark were administered to the experimental rats. The hepato protective effect of these extracts was evaluated by the assay of liver function

biochemical parameters and histopathological studies of the liver. It was found that the methanol extract- treated animals, the toxic effect of $CCl_4$ was controlled significantly by restoring the levels of serum bilirubin, protein and enzymes when compared to the normal and the standard drug silymarin-treated groups. Histology of the liver sections of the animals treated with extracts showed the presence of normal hepatic cords, absence of neurosis and fatty infiltration. The methanol extract of the stem and bark of *Pterocarpus Marsupium* possessed significant hepato protective activity.

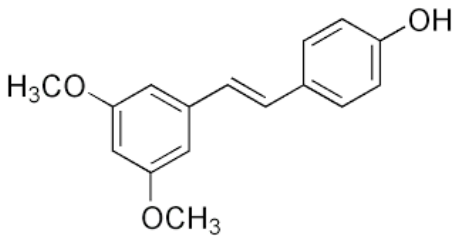
- **Blanca Rivero Cruz *et al*.,(2006)** have examined the composition of the spasmolytic essential oil of the medicinal species *Brickellia Veronicaefolia* was established by NMR spectroscopy. In addition to GC-MS analysis and HPLC studies. Seven major compounds, representing 86% of the oil, were identified as benzyl2,6-dimethoxybenzoate, 2-hydroxybenzyl 2'-methoxybenzoate , chamazulene , β-caryophyllene , germacrene D, bicyclogermacrene , and β-eudesmol . A sensitive and accurate analytical 'H NMR method has been developed for the quantification of the major compounds in the essential oil of *Brickellia Veronicaefolia.* The method was validated using benzyl 2,6-dimethoxybenzoate and β-caryophyllene, two of the active principles in the oil, and successfully applied to the determination of these pharmacologically active compounds in three different batches of the oil .
- *Pterocarpus Marsupium* belongs to Leguminoceal which is widely used in 'Ayurveda' as 'Rasayana' for the management of various metabolic disorders including hyperglycemia. Treatment of diabetic rats with *Pterocarpus Marsupium* methanol extracts for 7-14 days showed normalization of streptozotocin-distressed serum glucose. PMMtE exerted a protective effect by correcting glucosylated hemoglobin, serum protein, insulin, alkaline and acid phosphatase and albumin levels. Finally it suggested oral administration of *Pterocarpus Marsupium* have the ability to improve streptozotoncin-induced chronic diabetic stress. **Rajnish Gupta *et al*.,(2009).**

- The ethanol extracts of *Pterocarpus Marsupium* wood, bark and combined extracts were investigated for its anti-diabetic effects in wistro albino rats. Diabetes was induced in albino rats of alloxan monohydrate. The ethanol extracts of *Pterocarpus Marsupium* wood and bark at a dose of 150mg/kg of body weight and combined extracts at a dose os 150+150mg/kg of body weight were administered at a single dose. The effects of ethanol extracts of *Pterocarpus Marsupium* on blood glucose, plasma insulin, glycosylated haemoglobin, serum lipid profile, serum insulin, albumin, globulin, serum enzymes, antioxidant enzymes, lipoprotein peroxidation, reduced glutathione, glutathione peroxidase, erythrocytes in rats were determined. The ethanol extracts of *PterocarpusMarsupium* resulted significant reductions of blood glucose, lipid parameters. Except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant enzymes. It was concluded that the ethanol extracts of *Pterocarpus Marsupium* possessed significant antidiabetic, antihyperlipidaemic andantioxidant effects in alloxan induced diabetic rats. **Maruthupandian *et al*.,(2011)**
- **Okwuosa *et al*.,(2011)** have reported the triglyceride and glucose lowering potential of the leaf extracts of *Pterocarpus Santalinoides* in rats. It was found that the oral administration of the leaf extracts of *Pterocarpus Santalinoides* reduced glucose and triglyceride concentration in dexamethasone treated rats. These results were extra polated to humans, the *Pterocarpus Santalinoides* might prove useful in the treatment of NIDDM and or prevention of insulin resistance in non-diabetic states such as obesity and impaired glucose tolerance.
- **Kirana Halagappa *et al*.,(2011)** have studied the effect of aqueous extracts of *Pterocarpus Marsupium Roxb* on elevated inflammatory cytokine, tumor necrosis factor (TNF)-α in type 2 diabetic rats. It was concluded that the aqueous extract of *PterocarpusMarsupium* at both dose(i.e) 100 and 20 mg/kg, decreased the fasting and postprandial blood glucose in type 2 diabetic rats. The 200 mg/kg had more pronounced effects on postprandial hyperglycemia. The drug also improved the body weight of diabetic animals. Cytokine TNF-α was found to be an elevated in untreated diabetic rats due to chronic systemic inflammation. The

aqueous extracts at both doses significantly decreased the elevated TNF-α level in type 2 diabetic rat. The cytokine TNF-α by the rasayana drug *Pterocarpus Marsupium* is related with its potential anti-diabetic activity.

- **Udaysing Hari Patil *et al.,(*2011)** The different stem bark sample ( apical bark, middle bark and mature inner bark were analyzed for phytoconstituents, namely total reducing sugar, amylose, amylopectin, starch, crude fibers, crud protein, total polyphenols, water soluble tannins, total flavonoids, total alkaloids, nitrates, oxalate and total ash value. The concentration of constituents except oxalate and total ash was found higher in the apical stem bark then the middle and mature inner bark. The oxalate and total ash were higher in the mature inner bark than the apial stem bark and middle bark. Preliminary phytochemical analysis indicated the presence of pharamacologically active phytoconstituents phenols, tannins, flavonoids, flavones, alkaloids, terpenoids, saponins were found absent in all the three bark samples.
- **Abirami *et al*.,(2012)** have studied the antioxidant activity of aqueous, ethyl acetate and methanol extracts of the bark of *Pterocarpus Marsupium.* The total antioxidant potential has been assessed by FRAP assay. Methanol extract of *Pterocarpus Marsupium* was found to possess highest DPPH radical scavenging activity followed by aqueous and ethyl acetate extracts. The aqueous extract of *Pterocarpus Marsupium* exhibits potent ABTS scavenging activity. The nitric oxide scavenging activity of *Pterocarpus Marsupium* was found to possess higher superoxide scavenging activity at lower concentrations when compared to the other extracts.
- **Mohammed Rageeb *et al*.,(2012)** have determined the anti-inflammatory activity of *Pterocarpus Marsupium* stem bark extracts using carrageenan induced raw paw oedema method. The methanol and aqueous extracts had exhibited anti-inflammatory activity in carrageenan induced rat paw oedema method. It was reported flavonoids present in the stem and bark may be responsible for anti-inflammatory activity.

- **Manish Devgun *et al*.,(2012)** compared the extraction of phytochemical constituents using conventional and nonconventional methods such as ultrasonic and micro wave- assisted extraction methods. *Pterocarpus Marsupium* has been reported to contain bioactive phyto-chemicals, e,g., pterostlbene. The results showed that among the conventional extraction methods, percolation gave the highest yield. The non-conventional methods were optimized. The extraction yield was highest in the case of MAE. The phyto chemical screening of the extracted indicated similar group of compounds in all the extracts. The thin layer chromatography showed the presence of pterostilbene in the extracts by using percolation, MAE and UAE. The MAE method extracts significantly had higher amount of pterostilbene. The quantification of pterostilbene(17) was conducted by HPLC.

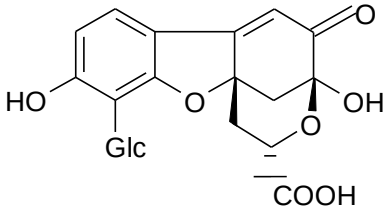



**pterostilbene**

**17**

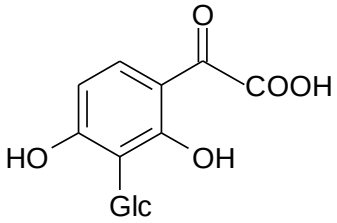
- **Kachhawa *et al.,*(2012)** have studied the antibacterial activity of *Pterocarpus Marsupium* Roxb stem with different concentration of methanol extract. Antimicrobial activity was tested against Gram-positive bacteria (i.e.) *Bacillus coagulans* and *Escherichia coil*, and Gram-negative bacteria. Evaluation were based on the inhibition zone using disc diffusion assay. The extraction showed significant activity against both bacteria.

- **Bhupendra Chauhan *et al*.,(2012)** have examined the memory enhancing activity of *Pterocrpus Marsupium Roxb*. The phytochemical tests of methanolic extract of *Pterocarpus Marsupium* showed the presence of various phyto constituents, such as carbohydrates, glycosides, saponins, tannins and flavonoids. It was known that saponins compound have neotropic activities. The methanolic extract of *Pterocarpus Marsupium* has potent neotropic activity.
- **Basudeb Achari *et al.,(*2012)** have studied the fluoresecene showed by extracts of the heartwood of *Pterocarpus Marsupium*. This was due to compound 1, whose structure was elaborated using spectroscopic/spectrometric studied. The plant material also contains the nonfluorescent compounds 2 and 3. The absolute configuration of 1 was determined by experimental and theoretically calculated electronic CD spectra, while that 3 was deduced from ECD comparison with reported results in the α-hydroxdihydrochalcone series. Along with that compound 2 and 3 were also isolated and characterized spectral techniques.




1

**18**

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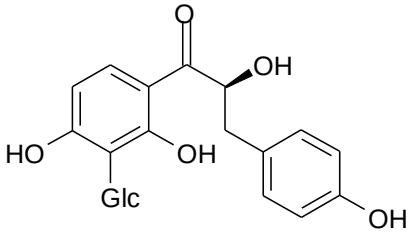
**19**

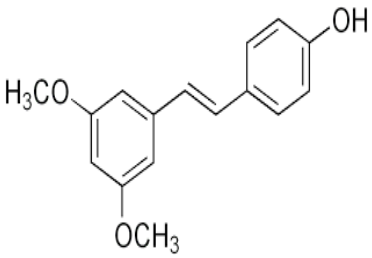



3

**20**

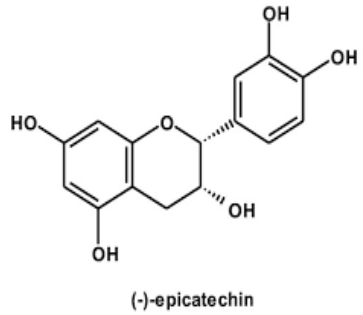
- **Kumaravel *et al*.,(2013)** have studied the in vitro antioxidant activity of ethyl acetate leaf extract of *Pterocarpus Marsupium* by using dipheny-picryl-hydrazyl assay, ferric reducing antioxidant potential FRAP assay, nitric oxide radical scavenging activity, hydroxyl radical scavenging activity, total peroxy radical trapping potential assay, hydrogen peroxide radical scavenging activity and reducing power assay. The percentage of scavenging activity at different concentration level were determind. The leaf extracts showed higher scavenging activity (i.e) 71% in ferric reducing antioxidant potential assay at a concentration of 100µg/ml. The ethyl acetate leaf extract of *Pterocarpus Marsupium* have the ability for free radical scavenging activity.
- **Arpita Sikdar *et al*.,(2013)** have studied the analgesic potential of *Pterocarpus Marsupium* commonly known as indian kino and it used for several medicinal purposes. The phytochemical studies revealed the presence of triterpenoids, steroids in the petroleum ether extracts, triterpenoids, steroids and poly phenolic compounds in the ethyl acetate extract, and triterpenoids, steroids, glycosides, polyphenolic compounds and carbohydrates in the methanol extracts of *Pterocarpus Marsupium* leaf. The three different solvent extracts from *Pterocarpus Marsupium* leaf have been studied for their analgesic potential by acetic acid induced writhing assay in albino mice. All the test extracts exhibited significant analgesic activity. The methanol extracts was found to be the most potent followed by the ethyl acetate and petroleum ether extracts respectively.
- *Pterocarpus Marsupium* is a shrub belonging to *fabaceal* family. It is commonly known as Indian kino tree or malbar kino. When injured, gum kino used in the treatment of polyuria, inordinate night sweat and phthisis pulmonalis. In traditional medicine, the plant bark is often assumed to treat diseases such kapha and pitta elephantiasis, erysipelas, urethrorrhea, rectalgia, opthalmopathy, hemorrhages, dysentry, cough, grayness of hair. *Pterocarpus Marsupium* has been used as anti-diabetic since time immemorial. The *Pterocarpus Marsupium* contain chemical constituents like pterostilbene, pterosupin, (-) epicatechin. *Pterocarpus Marsupium* can be used in variety of pharmacological disorders

such as anti-diabetic, anti-hyperglycemic/hypoglycaemic activity, anti-hyperinsulinaemic and anti-hypertriglyceridamic activity, cardiotonic activity, anti-cataract activities. **Dharashan *et al*.,(2014)**

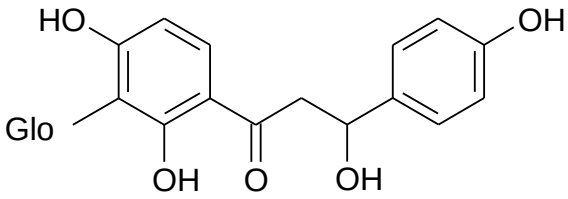



Pterostilbene

**21**




**22**




Pterosupin

**23**

- **Mithun Mukherjee *et al*.,(2015)** have performed the phytochemical screening of the bark extracts of *Pterocarpus Marsupium* which showed the presence of phytosterols, triterpenoids and saponins in the petroleum ether fraction and carbohydrates, flavonoids,tannins and phenols in the methanol fractions.
- Heartwood of the plant*Pterocarpus Marsupium* was used for various diseases like diabetes, angina, cancer, cardiotonic, brain tonic. Many researchers isolated several active principle like flavonoids, terpenoids and reported its pharmacological activity. Alcohol extracts of both leaf and heart wood of the plant were analyzed through HPTLC and result of leaf sample showed more or equal spot on HPTLC finger printing. This indicated that the leaf may have similar active potency like heart wood**. Mahajon Bidhan *et al.,(*2015)**

# 3. MATERIALS AND METHODS

The present work was aimed at the analysis of herbal medicine using HPTLC and UV methods.

**THE METHODOLOGY COMPRISES OF**

- Collection of the herbal drug.
- Identifying the phyto constituents by colour tests.
- Quantitative determination of total flavonoids by colorimetric and gravimetric method.
- HPTLC profile of the herbal drug.
- UV spectrum of the herbal drug.

## 3.1. COLLECTION OF HERBAL DRUG

The herbal drug powder was collected from a local doctor from gudalore.

## 3.2. PHYTOCHEMICAL COLOR TEST OF THE HERBAL DRUG

### 3.2.1.Test for Alkaloids

**A. Mayer's Test**

A fraction of the herbal drug was treated with few ml of Mayer's Test Reagent [1.36g of Mercuric chloride and 5g of potassium iodide in 100 ml of water] and observed for the formation of cream colored precipitate.

**B. Wagner's Test**

A fraction of the herbal drug was treated with few ml of Wagner's reagent [1.27g of Iodine and 2g of potassium iodide in 100ml water] and observed for the formation of reddish brown colored precipitate.

**C. Hager's test**

A few ml of the herbal drug was treated with Hager's reagent [Saturated aqueous solution of picric acid] and observed for the formation of prominent yellow precipitate.

**3.2.2.Test for Flavonoids**

**A. Test with Sodium hydroxide**

A small amount of the herbal drug was treated with aqueous sodium hydroxide and observed for the formation of yellow orange color.

**B. Test with concentrated Sulphuric acid**

A fraction of the herbal drug was treated with concentrated sulphuric acid and observed for the formation of orange color.

**C. Shinoda Test**

The herbal drug was dissolved in ethanol, warmed and filtered. Three pieces of Magnesium chips were then added to the filtrate followed by few drops of concentrated HCl. A pink, orange or red to purple coloration indicates the presence of flavonoids.

**3.2.3. Test for Tannins**

The herbal durg was dissolved in water and heated on a water bath for one hour. It was then treated with Ferric chloride and observer for the formation of dark green color.

### 3.2.4. Test for carbohydrates

#### A. Molisch's Test

Few drops of Molisch's reagent were added to each of the herbal drug dissolved in distilled water and 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of red or dull violet color at the interphase of the two layers was a positive test (Sofowora 1993)

#### B. Fehling's test

Herbal drug was dissolved in distilled water and filtered. The filtrate was heated with 2 ml of equal volume of Fehling's A and B. formation of red precipitate of cuprous oxide was an indication of the presence of red sugars. (Sofowora 1993)

### 3.2.5. Test for Quinone

A small amount of herbal drug was treated with concentrated Hydrochloric acid and observed for the formation of yellow colored precipitate.

### 3.2.6. Test for Terpenoids

#### A. Salkowski test

The herbal drug was taken in a test tube and few ml of chloroform was added. Then concentrated $H_2SO_4$ is added carefully along the sides of the test tube, Reddish Brown colored solution indicates the presence of terpenoids.

#### B. Libermann-Burchard test

The herbal drug was treated with $CHCl_3$, acetic anhydride and added few drops of con.$H_2SO_4$ and observed for the formation of Dark green color.

### 3.2.7. Test for Phenols

### A. FeCl3 Test

The herbal drug was treated with 5% $FeCl_3$ and observed for the formation of deep blue or black color.

### B. Lead acetate test

A small amount of the herbal drug was treated with lead acetate and observed for the formation of white precipitate.

### 3.3. TOTAL FLAVONOID DETERMINATION

**3.3.1.** Aluminum chloride colorimetric method was used for flavonoids determination (**Chang et al., 2002**). About 0.5 ml of the sample( 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% Aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8ml of distilled water. Kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm.The calibration curve was prepared by using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

**3.3.2.** Total flavonoid quantification was done using standard method(**Harborne et al.,1983**). About 10g of sample was mixed with 100ml of 70% methanol in a 250ml conical flask. Magnetic stirrer was used to mix the solution for 3 hrs and the mixture solution was filtrated using whatmann filter paper number1 ( 150mm). The filtrated sample was re-extracted once again with 70% methanol and filtrated in a similar way. Both the filtrate were transferred into a crucible and evaporated to dryness in a hot water bath at $60^{o}$ c and weighed.

### 3.4. HPTLC ANALYSIS OF THE SAMPLE

#### 3.4.1. Flavonoid profile

**Test solution preparation**

About 10 mg of the herbal drug was weighed accurately in an electronic balance (Afcoset), dissolved in 200µl of the respective solvent (Ethanol) and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

**Sample application**

2 µl of test solution and 2 µl of standard solution were loaded as 5mm band length in the 3 x 10 Silica gel 60$F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

**Spot development**

The herbal drug loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Flavonoid) and the plate was developed up to 90mm.

**Photo-documentation**

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 254nm and UV366nm.

**Derivatization**

The developed plate was sprayed with respective spray reagent (Flavonoid) and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

**Scanning**

After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 366 nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 versio

The mobile phase used for the analysis was ethyl acetate-butanone-formic acid-water (5:3:1:1) and the spray reagent used was 1% Ethanolic Aluminium chloride reagent. After derivatization from the chromatogram a yellow, yellowish blue coloured fluorescent zone at UV 366nm mode were observed in the tracks.

**3.4.2. Polyphenol profile**

**Test solution preparation**

The given herbal drug 10 mg was weighed accurately in an electronic balance (Afcoset), dissolved in 200µl of the respective solvent (Ethanol) and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

**Sample application**

2 µl of test solution and 2 µl of standard solution were loaded as 5mm band length in the 3 x 10 Silica gel 60$F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

**Spot development**

The herbal drug loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Polyphenol) and the plate was developed up to 90mm.

**Photo-documentation**

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 254nm and UV366nm.

**Derivatization**

The developed plate was sprayed with respective spray reagent (Polyphenol) and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

**Scanning**

Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366 nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 version.

The Mobile phase used for the analysis was Toluene-Acetone-Formic acid (4.5 : 4.5 : 1) and the Spray reagent used was Folin Cio-calteu reagent. After derivatization from the chromatogram a blue, bluish brown coloured zone at visible light mode were observed in the tracks.

**3.5.UV SPECTRUM**

The UV spectrum was recordedbetween 200-400nm range using UV Visible spectrophotometer model number AU 2701.The solvent used was water.

# 4.RESULTS AND DISCUSSION

In recent years, an extensive use of herbal drugs, herbal drug preparations, and herbal medicinal products in all countries of world has pointed out the need for overall appropriate methods of assay for a rapid screening and, in a few cases, for specific measurements of these commercial phytochemicals. The analysis of herbal drug constituents is generally achieved using UV spectroscopy and chromatographic techniques (TLC, HPTLC, GC). Hence in the present work analysis of an antidiabetic herbal drug was carried out and the results are presented below.

## 4.1.PHYTOCHEMICAL SCREENING

The phytoconstituents present in the herbal drug used for diabetes mellitus were identified by the colour test.The results revealed the presence of alkaloids, flavonoids, steroids, terpenoids, carbohydrates, tannins and phenols. The result of the preliminary studies were summarized in table-1.

**Table-1**

**Phyto-chemical screening of Herbal Powder**

| Phyto Constituents | Results |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Terpenoids | – |
| Carbohydrates | + |
| Phenols | + |
| Tannins | – |

## 4.2.QUANTITATIVE ESTIMATION OF FLAVONOID IN THE DRUG

Many herbs and plant products have been shown to have hypoglycemic action. Flavonoids are known to be bioactive antidiabetic principles. Flavonoid compounds such as Boswellic acid, Ellagic acid, Quercetin, and Rutin revealed maximum reduction in the blood glucose levels.( ***Puchchakayala 2012***).

The quantitative estimation of flavonoid was done by the method described by ***Change*** **and** ***Horborne***. The results showed 286.17µg/100mg of flavonoid in Change method and 0.348mg/1g in Horborne method.

## 4.3.HPTLC ANALYSI OF THE DRUG

HPTLC analysis were carried out to study the flavonoid and polyphenolc profile of the herbal drug using quercetin as standard. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolutions and reproducible peaks.The desires solvent system for flavonoid was achieved using ethylacetate-butanone-formic acid-water (5:3:1:1) and for the polyphenolic it was found to be toluene-acetone-formic acid (4.5:4.5:1). The ethanolic extract of the drug showed the presence of two different type of flavonoid with two different $R_f$ value in the range of 0.24 and 0.97 (Table 2). The ethanolic extract of the drug showed the presenceof five different type of polyphenol with 5 different $R_f$ value in the range of 0.07,0.50,0.78,0.83,0.96 and 0.75 respectively (Table 3). The results of the HPTLC profile for flavonoid and polyphenolics of the herbal drug are shown in figures 1, 2, 3, 4, 5.

**Table 2**

**HPTLC Flavonoid Profile of the Herbal drug**

| Track | Peak | Rf | Height | Area | Assigned substance |
|---|---|---|---|---|---|
| Sample D | 1 | 0.24 | 13 | 210.4 | Flavonoid 1 |
| Sample D | 2 | 0.97 | 98.6 | 2589.6 | Unknown |
| STD | 1 | 0.59 | 371.5 | 14657.2 | Flavonoid standard |

**Table 3**

**HPTLC Polyphenolic Profile of the Herbal drug**

| Track | Peak | Rf | Height | Area | Assigned substance |
|---|---|---|---|---|---|
| Sample D | 1 | 0.07 | 22.7 | 379.4 | Unknown |
| Sample D | 2 | 0.50 | 13.4 | 89.1 | Unknown |
| Sample D | 3 | 0.78 | 53.2 | 1690.2 | Polyphenol 1 |
| Sample D | 4 | 0.83 | 48.1 | 1541.3 | Unknown |
| Sample D | 5 | 0.96 | 69.5 | 2541.6 | Polyphenol 2 |
| STD | 1 | 0.75 | 813.7 | 23620.8 | Polyphenol standard |

## FLAVONOID CHROMATOGRAM OF THE HERBAL DRUG

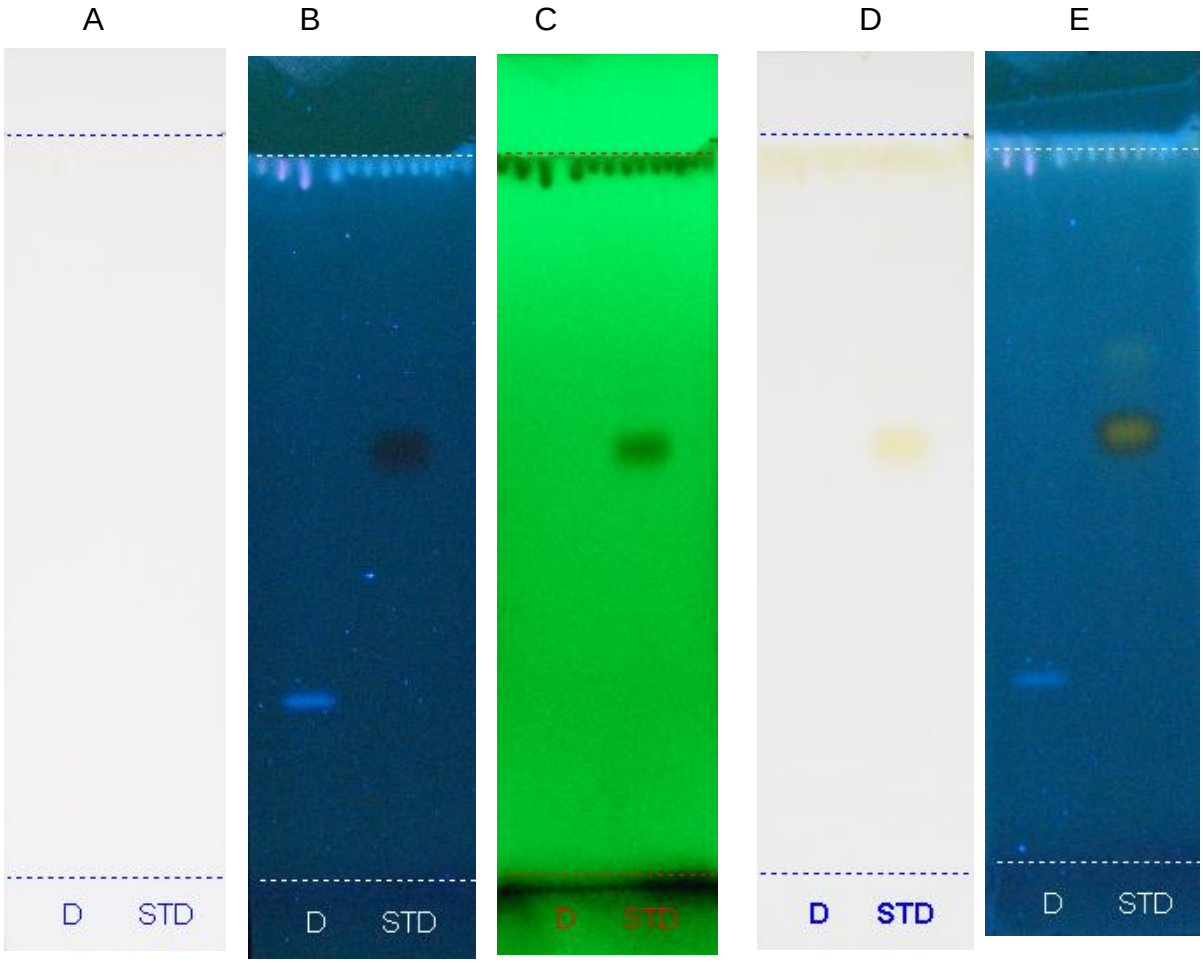



**Figure1**: HPTLC studies on the flavonoid of herbal drug.

**A**: HPTLC of the powder under daylight; **B:** HPTLC of the powder under UV 366nm; **C:** HPTLC of the powder under UV 254nm; **D:** HPTLC of the powder under daylight-after derivation; **E:** HPTLC of the powder under UV 366nm after derivation.

## POLYPHENOLIC CHROMATOGRAM OF THE HERBAL DRUG

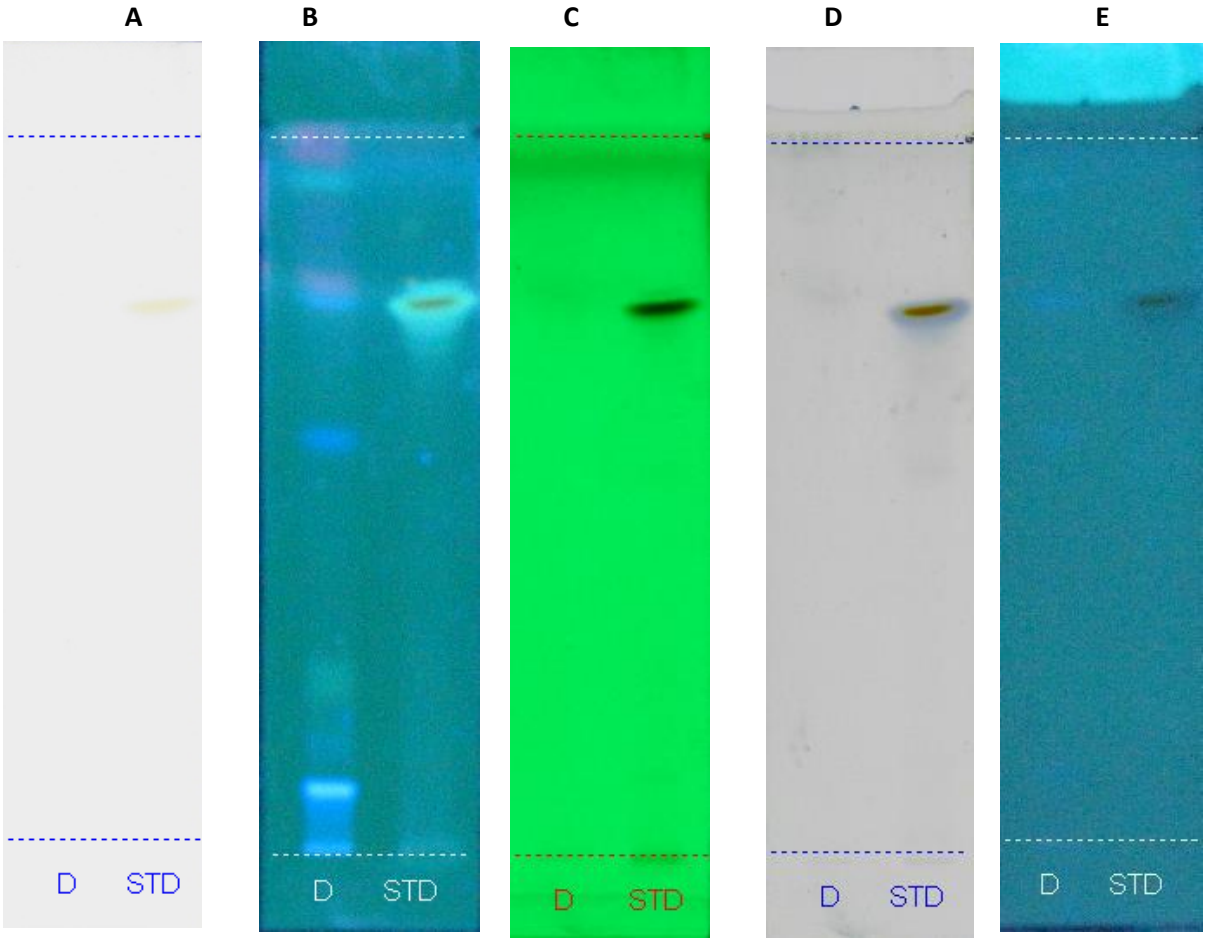
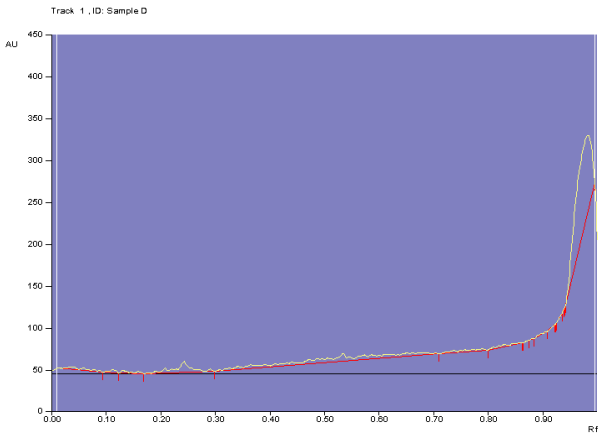
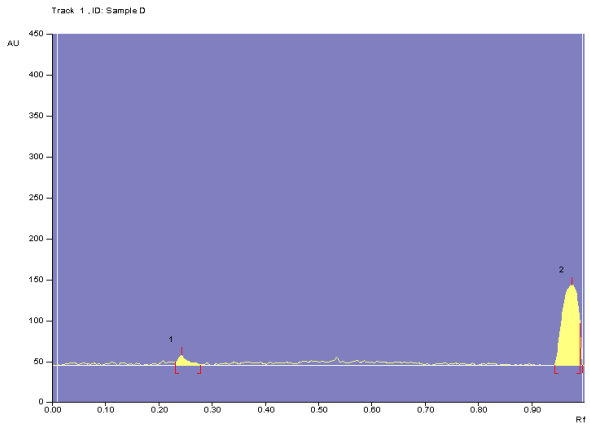



**Figure2**: HPTLC studies on the polyphenolic of herbal drug.

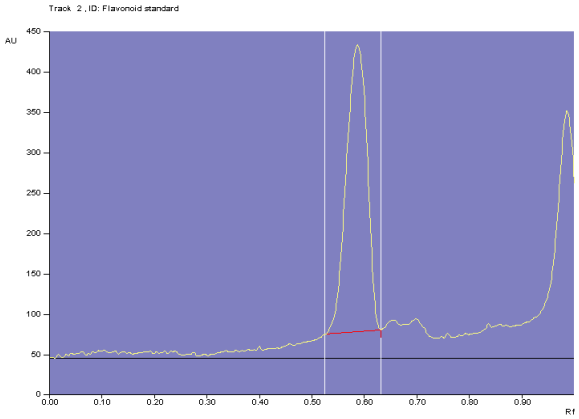
**A:** HPTLC of the powder under a daylight; **B:** HPTLC of the powder under UV 366nm; **C:** HPTLC of the powder under UV 254nm; **D:** HPTLC of the powder under daylight-after derivation; **E:** HPTLC of the powder under UV 366nm after derivation.



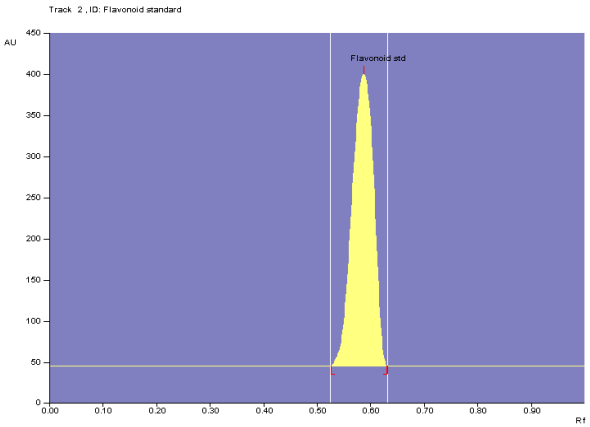

A



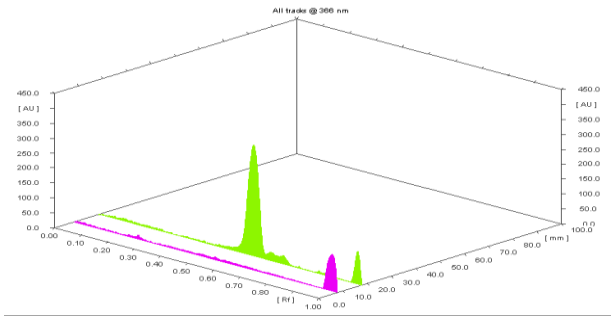

B




C




D




E

**Figure 3:**HPTLC flavonoid chromatogram of the herbal drug.

**A:** track1-sample D powder sample-Baseline display at 366nm; **B:** track1-sample D powder sample-peak densitogram display at 366nm; **C:** track STD-flavonoide standard baseline display at 366nm; **D:** track STD-flavonoide standard peak densitogram display at 366n; **E**: 3D display of all tracks.

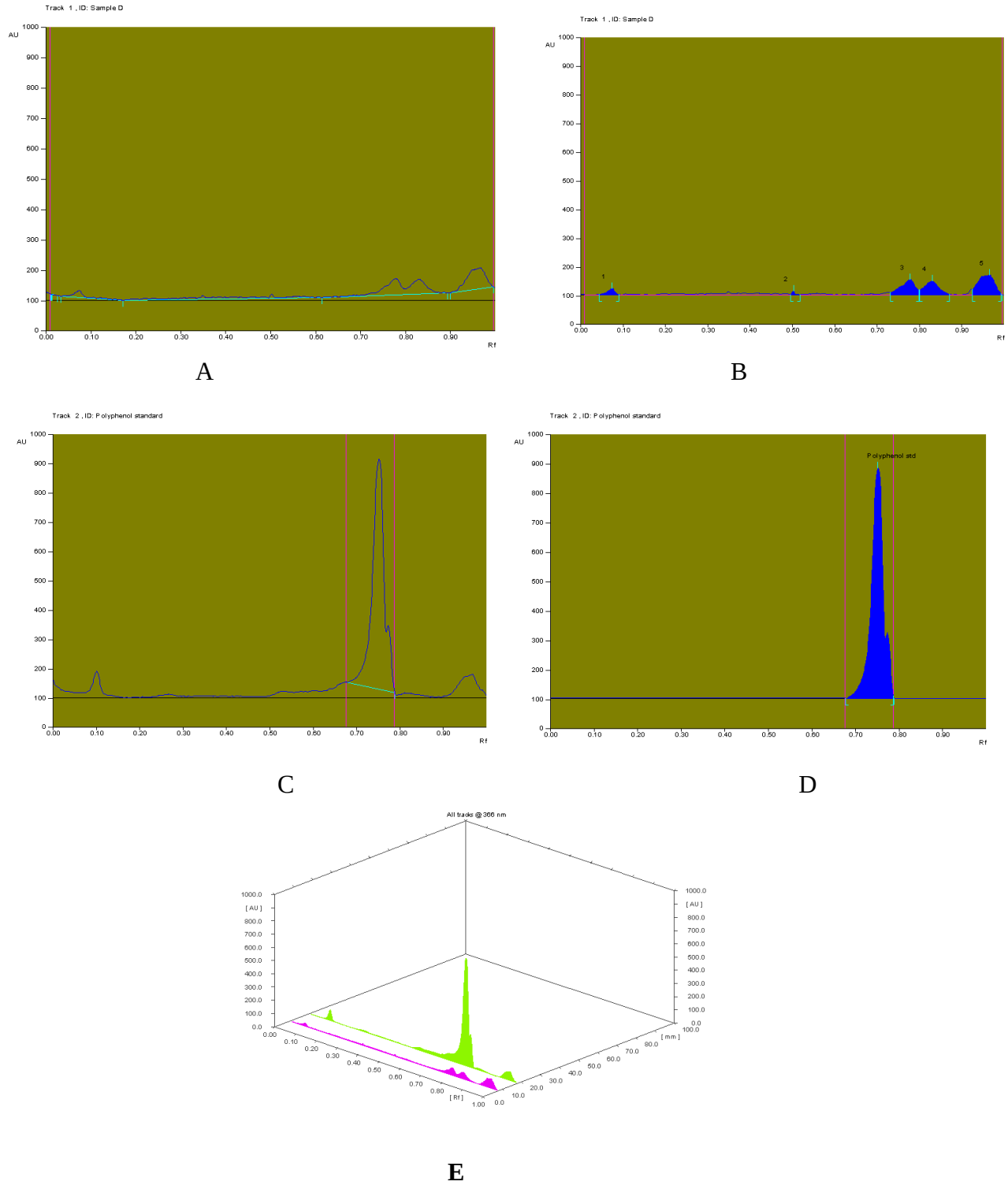



**Figure 4:** HPTLC polyphenol chromatogram of the powder.

**A:** track1-sample D powder sample-Baseline display at 366nm; **B:** track1-sample D powder sample-peak densitogram display at 366nm; **C:** track STD-polyphenol standard baseline display at 366nm; **D:** track STD-polyphenol standard peak densitogram display at 366n; **E**: 3D display of all tracks.

## 4.4.UV ANALYSIS OF THE HERBAL DRUG

The uv visible spectra of herbal showed two absorbance bands at 370 and 242nm. This may be due to the presence of flavonoids. Since the typical UV spectra of flavonoid include two absorbance bands. Band-A between 350 and 385nm and Band-B in the range of 250-290nm.(***Dimitrios Tsimogiannis 2007***). The UV spectrum of the herbal drug is given in figure-5.

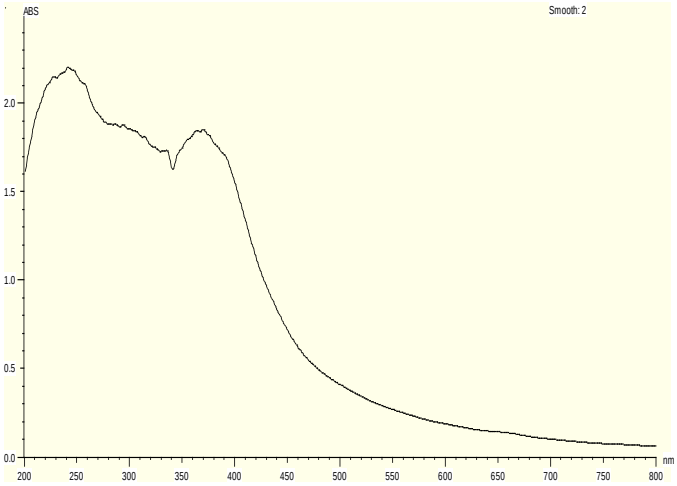



**Figure 5:** UV spectrum of the herbal drug

### 4.4.1.ESTIMATION OF QUERCETIN IN THE HERBAL DRUG

Quercetin has been found to be more effective antidiabetic compound among flavonoids. This may be due to the fact that quercetin may stimulate β-cells to release more insulin. Also the pronounced activity of the compound could be because of enhanced peripheral glucose utilization by skeletal muscle in addition to that of β-cell stimulation.( ***Ramulu Jadhav 2012***).

The quantitative estimation of herbal drug was carried out using UV visible spectra photo meter. Standard quercetin solutions were prepared in the ranfe from 300-1500ppm/l. In the herbal drug the amount of quercetin was found to be 1480ppm/. Since the quercetin level was found to be high in the drug, the antidiabetic activity of drug may be due to quercetin.

# 5.SUMMARY AND CONCLUSION

Natural products have been a successful source of bioactive molecule in medicine much before the advancement of modern drug system. Hence in the present study, analysis of a antidiabetic herbal drug has been carried out, and the summary of the results are given below.

- Preliminary phytochemical analysis of the herbal drug showed the presence of alkaloids, flavonoids, phenolic compounds.
- The amount of flavonoid in the herbal drug was found to 286.17µg/100mg by*Change* method and 0.348mg/1g by *Horborne* method.
- Flavonoid and polyphenolic profile of the herbal drug was carried out using HPTLC method.
- UV spectrum of the compound revealed the presence of two band set 370 and 242nm which may be due to the presence of flavonoid in the herbal drug.
- The amount of quercetin present in the herbal drug was also estimated and was found to be 1480/l.
- Hence quercetin may play a role in the antidiabetic activity of the drug.

# 6.BIBLIOGRAPHY


- Abirami B, Gayathri p and uma D(2012), **"In vitro antioxidant potential of *Pterocarpus Marsupium* bark"**, *International journal of chemical & pharmaceutical sciences*, 3(2), pp:17.
- Akshada N. Kakade and Magdum(**2012**), **"HPLC analysis of β- sitosterol in herbal medicine and vegetable oils"**, *International journal of pharmacy and life science,*3(5).
- Anantha D, Israiel Kumar T, Santhosh Kumar M, Manohar Reddy A, Mukharjee N.S.V, Lakshmanan Rao A(2010), **"Invitro anti-helmentic activity of aqueous and alcoholic extracts of *Aerva lanata* seeds and leaves",***J.Pharma.Sci. and Res*, 2(5), PP:317-321.
- Anita A and Malar Retna A(2013), "**Review on the medicinal plant-*Aerva lanata***", *Asian Journal of Biochemical and pharmaceutical Research*, 3(1),PP: 215.
- Anna Rita Bilia, Maria Camilla Bergonzi, Diamanto Lazari, and Franco Francesco vincieri (2002), **"Characterization of commercial kava-kava herbal drug & herbal drug preparations by means of NMR spectroscopy**", *J. Agric. Food chem*, 50,pp:5016-5025.
- Anna Rita Bilia, Maria Camilla Bergonzi, Giovanni Mazzi, Franco Francesco Vincieri(2001), **"Analysis of plant complex matrices by use of NMR spectroscopy St. John's wort extract**", *J. Agric. Food Chem*, 49,pp: 2115-2124.
- Appia Krishnan G, Rai V.K, Nandy B.C, Meena K.C, Dey S. Tyagi P.K, TyagiL.K (2009), **"Hypoglycemic and antihyperlipdamic effect of Ethanolic extract of aerial parts of *AervaLanta* Linn. In Normal And alloxan induced Diabetic Rats"**, *International Journal of pharmaceutical Sciences and Drug Research*,1(3), pp:191-194.

- Arpita Sikdar, Anirban Biswas, Sanjib Bhattacharya, Moulisha Biswas(2013), **"Assessment of analgesic activity of *Pterocarpus Marsupium* leaf extract in swiss albino mice",***journal of advanced pharmacy education and research, 3*(1), pp:42.
- Basavaraj M Dinnimath and Sunil S Jalalpura(2014), "**In silico antiurolithiatic screening of *Aerva lanata* (L.)isolated constituent"**, *International Journal of Pharmaceutical Education and Research*,49(2), PP:126.
- Basudeb Achari, Pradeep K. Dutta, Subodh K. Roy, Prarthana Chakraborty,Jhimli Sengupta, Durba Bandyopadhyay, Joy K. Maity, Ikhlas A. Khan, Yuanqing Ding and Daneel Ferreira (2012), **"Fluorescent pigment and phenol glucosides from the heartwood of *Pterocarpus Marsupium***", *Journal of natural products*, (75,) pp:655-660.
- Bhupendra Chauhan, Amrendra Kumar Chadhary (2012), **"Memory enhancing activity of methanolic extract of *Pterocarpus Marsupium* Roxb** *phytopharmacology*, 2(1), PP:72-80.
- Blanca Rivero- cruz, Isabel Rivero-cruz, Juan M. Rodriguez, Carlos M. cerda-Garcia-rojas and Rachel Mata((2006), **"Qualitative & Quantitative analysis of the active components of the essential oil from *Brickellia Veronicaefolia* by NMR ",***J.Nat.Prod*, 69,pp:1172-1776.
- Chayanon Chaowuttikul, Worthat Thitikornpong, Chanida Palanuvej and Nijsiri Ruangrungsi(2014),"**quantitive determination of usnic acid content in *usneasiamensis* by TLC- densitometry and TLC image analysis"**, *research journal of pharmaceutical, biological and chemical sciences*, 5(1), pp: 118.
- Deepak kumar, D.N. Prasad, Jyoti parkash, SP Bhatnagar, Dinesh kumar(2009), **"Antiasthamatic activity of Ethanolic extract of *Aervalanata*(linn)",***Pharmacology online*,2, pp:1075-1081.
- Dharshan S, Veerashekar T, Kuppast I.J, Raghu J.D(2014), **"A review on *Pterocarpus Marsupium Roxb*"**, *International journal of universal pharmacy & Bio sciences,* 3(6), pp:32.

- Dimitrios Tsimogiannis, Martina Samiotaki, George Panayotou and Vassiliki Orepoulou(2007), **characterization of Flavonoid subgroups and hydroxyl substitution by HPLC-MS/MS**,12,pp:593-606.
- Edwin Jarald, Siddaheswar Balakrishnan Joshi and Dharma Chandra Jain(2008), **" Diabetes and herbal medicines",***Iranian journal of pharmacology and therapeutics,* 7(1), pp:97-106.
- Farboodnlay Jahromi M.A, Anil B. Ray and Chansouria J.P.N (1993) "**Antihyperlipidemic effect of flavonoids from *Pterocarpus Marsupium*",***Journal of natural product*, 56(7), pp- 989-994.
- Gajalakshmi S, Vijayalakshmi S, Devi Rajeswari V(2012), "**pharmacological activities of *Aerva lanata*: A perspective review"**, *International Research Journal of Pharmacy*, 3 (1),PP:28.
- Kachhawa J.B.S, Sharman, Tyagi S, Gupta R.S, Sharma.K.K(2012), **"In vitro Evaluation of antibacterial activity of *Pterocarpus Marsupium* Roxb",***International journal of Pharmacy & Pharmaceutical science*, 4(1), 67-68.
- Ketan Hatware and Annapurna A(2014), **The effect of quercetin on blood glucose levels of normal and streptozotocin induced diabetic (type1 and type 2) rats**, *International journal of pharmaceutical, chemical and biological sciences*,4(3), pp:613-619.
- Kirana Halagappa, H.N. Girish, B.P Srinivasan(2011), **"The study of aqeous extract of *Pterocarpus Marsupium* Roxb on cytokine TNF-α in type 2 diabetic rats"**, *Indian Journal of Pharmocology* 42(6), pp:392-396.
- Kumaravel R.S, MaleekaBegam S.F, Parvathi H and SenthilKumar M(2013), **"Phytochemical screening & In vitro antioxidant activity of ethyl acetate leaf extracts of *Pterocarpus Marsupium* Roxb (Fabaceae)"**, *International Journal of curr.sci.* 9, pp:46-55.

- Kunle, Oluyemisi Folashade, Egharevba, Henry Omaoregie and Ahmadu, Peter Ochogu(2012), **" standardization of herbal medicines",** *International journal of biodiversity and conservation,* 4(3), pp:101-112.
- Mahajon Bidhan, A.B. Rema Shree, R. Remadevi (2015), **"HPTLC comparision of leaf & heart wood of *Pterocarpus Marsupium* Roxb- An endangered medicinal plant",***Journal of Scientific & Innovative Research* ,4(1), PP:27-32.
- Mammen Denni, Daniel Mammen(2013), **"Yellow and White flowered varients of *Aerva lanata*: A phytochemical variation study"**, *Indian Journal of Applied Research*, 3( 7), PP:94-96.
- Manickam M, Ramanathan M, Farboodniay Jahromi M.A, Chansouria J.P.N and Ray A.B(1997),**"AntihyperglycemicActivity of Phenolics from *Pterocarpus Marsupium"*,***J.Nat. Prod*, 60(6), pp:609-610.
- Manickam Murugan, Veerabahu Ramasamymohan (**2014**), **"phytochemical, FT-1R and antibacterial activity of whole plant extract of *Aervalanata(*L.) juss. Ex.Schult",***Journal of medicinal plants studies,* 2(3), PP:51-57.
- Manish Devgun, Arun Nanda and Shahid H. Ansari(2012), **"Comparison of Conventional & Non-conventional methods of extraction of heartwood *Pterocarpus Marsupium* Roxb"**, *Acta polonial pharmaceutical-drug research*, 69(3), pp-475-485.
- Mankani K.L, Krishna V, Manjunatha B.K, Vidhya S.M, Jagadeesh Singh S.D, Manohara, Anees Ur Raheman, Avinash K.R, **"Evaluation of Hepatoprotective activity of stem Bark of *Pterocarpus Marsupium* Roxb",***Indian J. Pharmacol*, 37(3), PP:165-168.
- Maruthupandian A and V.R. Mohan (2011), **"Antidiabetic, Antihyperlipidaemic and antioxidant activity of *Pterocarpus Marsupium* Roxb. inalloxan induced diabetic rats"**, *International journal of pharma Tech research*, (3)3, pp:1681-1687.

- Mithun Mukherjee and Sharmistha Gupta (2015**), "*Pterocarpus Marsupium* A phytopharmacognostic study"**, *International journal of current advanced research*, 4(10), pp:461-464.
- Mohammad Rageeb Mohammed Usman, Pathan Ekbal Kha H, Jain Bharath V. Pawar Sandeep R, (2012), **"In vitro anti-Inflammatory activity of *Pterocarpus Marsupium* Roxb. Stem bark on albino rats"**, *Journal of pharmaceutical and scientific innovation,* 1(2),pp: 21-25.
- Nagaratna A, Prakash L Hedge, Harini A (2014),**"A pharmacological rewiew on Gorakha ganja ( *Aerva lanata*(Linn.).Juss.Ex.Schult)**,*Journal of pharacognosy and phytochemistry*, 3(4), pp:253-257.
- Nandagopal S, Lalitha M, Abirami S, Saikrishna D and Priyan A(2015), "**Effect of phytohormones on micropropagation and phytochemical studies of *Aerva lanata*(Linn.)Juss.Ex.Schult-A seasonal and vulnerable plant"**, *Der pharmacia lettre*, 7(3), PP:291-298.
- Okwuosa C.N, Unekwe P.C, Achukwu P.U, Udeani T.K.C and Ogidi U.H (2011), **"Glucose &Triglyceride lowering activity of *Pterocarpus Santaniloides* leaf extracts against dexamethasone induced hyperlipidemia & insulin resistance in rats**", *African journal of Biotechnology* ,10(46), pp:9415-9420.
- Paramasivam Ragavendran, Dominic Sophia, Chinthamony Arul Raj, Thangarajan Starlin and Velliyur Kanniappan Gopalakrishnan(2012), "**phytochemical screening, anti oxidant activity of *Aerva lanata (L)*- An Invitro study"**, 5(2), PP:77-81.
- Payal Chawla, Amit Chawla, Neeru Vasadeva and Surendra Kumar Sharma(2012), **"A review of chemistry and biological activities of the genus *Aerva*-** *A Desert plant* , 69(2), pp:171-177.
- Pyal C, Gurlaganjeet K, Davinder K, Gagan S, Amit C and Dhawan R.K(2015), **A review on phytochemistry and biological Activities of *Aerva,*** pp:2-4.

- Raja Sundarajan, Akhil Bharampuram and Ravindranadh Koduru(2014), **"A review on phytoconstituents for nephroprotective activity"**, *pharmacophore an International Research Journal*, 5(1), PP:160-182.
- Rajendra Prasad Gujjeti, Estari Mamidala (2013), **"Phytochemical screening and thin layer chromatoglophic studies of *Aervalanata* root extract",** *International Journal of Innovative Research in Science, Engineering and Technology,* 2(10), pp:5725.
- Rajnish Gupta, Radhey Shyan Gupta (2009), **"Effect of *Pterocarpus Marsupium* in streptozotocin-included hyperglycemic state in rats**, **comparison with Glibenclamide***", research article*, pp:39.
- Ramulu Jadhav, Goverdhan Puchchakayala(2012), **"hypoglycaemic and antidiabetic activity of flavonoids: Boswellic acid, Ellagic acid, Quercetin, Rutin on streptozotocin-nicotinamide induced type 2 diabetic rats"**, *International journal of pharmacy and pharmaceutical sciences*,4(2).
- Rehana Banu .H,Nagarajan .N(2014), **" TLC and HPTLC fingerprinting of leaf extracts of Wedelia Chinensis (osbeck) Merrill,** *Journal of pharmacognosy and phytochemistry,* 2(6), pp:29-33.
- Sanjay Kumar Pal, Yogeshwer Shukla(2003), **" Herbal medicine; current status and the future",** *Asian pacific journal of cancer prevention,* 4,pp:281-288.
- Sheetal Verma and Singh s.p(2008), **"current and future status of herbal medicines",** *vertinary world,*1(11), pp:347-350.
- Sudhir S. Kamat and R.P Suryawanshi (2015), **"Elemental analysis of herbal tablets by ICP- MS"**, *journal of academic and industrial research (JAIR)*, 3(8), pp:362.
- Sunil Kumar K.N, Suchitra Narayan Prabhu, Ravishankan Sahana B, Yashovarma B, Sunil R. Dhaneshwari(2015), "**chemical analysis and in vitro evaluation of antiurolithiatic activity of *Aerva lanata* (Linn.) Juss. Ex Schult roots"**, *Journal of pharmacognosy and phytochemistry*, 3(3), PP:1.

- Tushar A. Deshmukh, Bapuso V. Yadav, Sachin .L badole, subhash L. bodhankar(2008), "**antihyperglycaemic activity of alcoholic extract of *Aerva lanata* (L.) A.L.Juss.Ex.J.A. Schultes leaves in alloxan induced diabetic mice"**, *Journal of Applied Biomedicine*, 6, PP:81-87.
- Udaysing Hari Patil, Dattatraya.K. Gaikwad(2011), **"Biochemical standardization of stem bark of *Pterocarpus Marsupium*(Roxb)"**, *International research journal*, 2(1), pp:65-71.
- Ulrike Holzgrabe, Bernd W.K, Diehl, Iwona Wawar(1998), **"NMR spectroscopy in pharmacy",** *Journal of pharmaceutical and biomedical analysis,* 1(7),PP:567-616.
- Upendra Rao M, Sreenivasulu M, Chengaiah B, Jaganmohan Reddy K, Madhusudhana Chetty C (2010), **"Herbal medicines for Diabetes mellitus",** 2(3), pp:1883-1892.
- Vijayalakshmi R and Ravindhran R (2012), "**preliminary comparative phytochemical screening of root extracts of *Diospyrus ferrea* (Wild.) Bakh and *Aerva lanata(L.)* Juss.Ex schultes"**, *Asian Journal of plant Science and Research*., 2(5), PP:581-587.
- Vinit Movaliya, MaitreyiZaveri (2014), **"A Review on the pashanbheda plant "*Aerva Javanica*",** *Int.J.Pharma. sci. Rev. Res*, 25(2), pp:268-275.
- Vivek D, Nimasha G Nair, Anju MP, Bijesh Vatakkeel, Siju EN, Aiswarya Lakshmi AG(2015), **"In vitro anti-inflammatory activity of *Aerva lanata*"**, *International Journal of Toxicological and Pharmacological Research*, 7(1), PP:57-59.
- Yamunadevi M, Wesely EG, Johnson M(2012), **"chromatographic finger print studies on saponins of *Aervalanata*(L.)Juss.Exschiltes by using HPTLC"**, *International journal of current pharmaceutical research*, 4(2), pp:52-57.
- Yamunadevi M, Wesely EG. Johnson M(2011), "**Phytochemical studies on the terpenoids of medicinally important plant *Aervalanata* L. using HPTLC"**, *Asian pacific journal of Tropical Biomedicine*, pp:220-225.