2. REVIEW OF LITERATURE

2.1. Description of *Talinum Cuneifolium* Linn.\(^\text{42, 43}\):

**Botanical Name** - *Talinum Cuneifolium* Linn

**Family** - Portulaceae

**Regional Names**

- **English** - Ceylone bachali.
- **Telugu** - Palaku, Akukoora, Seema bachali.
- **Tamil** - Pasali.

![Talinum Cuneifolium](image)

**Figure: 2.1 Talinum Cuneifolium Linn.**
Geographical distribution:

This plant is weed in gardens, cultivated in S.V.U.Campus Tirupathi, Tirumala Gardens, Srilanka, Bangladesh, Pakistan, U.S.A., Puerto Rico, and Virgin Islands.

Description of plant:

Habit       -   A moderate Herb.
Stem        -   Erect
Leaves      -   Alternate, spathulate (or) oblanceolate, obscurly nerved
Inflorescence -   Terminal compound racame
Flowers     -   Small pink in terminal sub dichotomouslybarebed panicles
Perianth    -   Sepals 2, Petals 5
Androecium  -   Stamens numerous, decurved in fruit.
Gynoecium   -   Unilocular, Superior ovary, ovules numerous, free central.
Fruit       -   Decurved with Capsule globose, 3 valved.

Uses:

Leaves and roots are medicinally important parts. Powdered leaf is used in treatment of diabetes, hepatitis, mouth ulcers and aphrodisiac. The fresh leaves are used as stomachic. Roots possess tonic properties, used in treatment of cough, gastritis and pulmonary tuberculosis. They are also used to treat dehydrating diarrhea.
2.2. Description of *Flemingia wightiana* Graham ex Wt. & Arn.

**Botanical Name** - *Flemingia wightiana* Graham ex Wt. & Arn.

**Family** - Fabaceae

**Geographical distribution:**

This plant is widely distributed in Eastern and western Rocks of Southern India.

**Description of plant:**

- **Habit** - A moderate Shrub.
- **Stem** - Erect
- **Leaves** - The leaf is trifoliate, the leaflets are oblong. Abaxial side pubesecular
- **Flowers** - flowers are in axillary racemes
- **Fruits** - Fruits are legume with red glands
- **Seeds** - seeds are two in each pod, black in colour

*Figure: 2.2 – Leaves Flemingia wightiana Graham ex Wt. & Arn*
2.3. Review on Biological Studies:

Microbial agents will cause number of infections to the human beings, to treat these infections there are number of herbal plants or agents available to prevent or cure these infections. These agents are called as antimicrobial agents.

Antimicrobial activity of plants can be detected by observing the growth response of various micro-organisms to those plant tissues or plant extracts, which are placed in contact with them. Many methods are available to detect their antimicrobial activity. But all the methods are not equally sensitive or even based on the same principle. But in general the biological evaluation can be carried out much more efficiently on water soluble, fine crystalline substance than on mixtures like plant extracts\textsuperscript{44,45}. In order to detect antimicrobial activity in plant extracts, the following conditions must be fulfilled.
The plant extract must be brought into contact with the cell wall of the microorganisms that have been selected for the test.

Conditions must be adjusted so that the microorganisms are able to grow when no antimicrobial agents are present.

There must be some means of judging the amount of growth, if any, made by the test organism during the period of time chosen for the test.

**General methods for antimicrobial screening:**

There are number of methods available to evaluate the antimicrobial activity. They are,

1. Diffusion method.
2. Dilution Agar method.

**Diffusion method:**

In the diffusion technique a reservoir containing the plant extract to be tested is brought into contact with an inoculated medium (e.g. agar) and after incubation, the diameter of the zone around the reservoir (inhibition diameter) is measured. In order to lower the detection limit, the inoculated system is kept at a low temperature during several hours before incubation, which favors diffusion over microbial growth and thus increases the inhibition diameter. This method was originally designed to monitor the amounts of antibiotic substances in fermentation cultures and has also been used for obtaining biograms and for testing essential oils. Different types of reservoirs have
been employed, including filter paper discs, porcelain or stainless steel cylinders placed on the surface and holes punched in the medium. It is not necessary to sterilize the test samples since any bacteria present will be confined to the reservoirs and will not therefore be able to spread and ruin the place.

The hole-plate methods, however, is the only suitable diffusion technique for testing aqueous suspensions of plant extracts. In this method, the presence of suspended particulate matter in the sample being tested is much less likely to interfere with the diffusion of the antimicrobial substance into the agar than in the filter paper disc and the cylinder plate methods. Precipitation of water-insoluble substances in the cylinder or in the disc will indeed prevent any diffusion of antimicrobial substances into the agar. Nevertheless, in order to limit precipitation as much as possible in the whole plate methods, pre-incubation should be carried out at room temperature (25°C) rather than 4°C. Advantages of the diffusion methods are, the small size of the sample used in the screening and the possibility of testing five to six compounds per plate against a single microorganism.

**Dilution agar method:**

In the dilution methods, samples being tested are mixed with a suitable medium that has been previously inoculated with the test organism. After incubation, growth of the microorganism may be determined by direct visual or turbidimetric comparison of the test culture with a control culture which did
not receive the sample being tested, or by plating out both test and control cultures. Usually a series of dilutions of the original sample in the culture medium is made and then inoculated with the test organism. After incubation, the end point of the test is taken as the highest dilution which will just prevent perceptible growth of the test organism (MIC–Value). These methods are the best for assaying water-soluble or lipophilic pure compounds and to determine their MIC-values, which can be recorded using this method.

**Bioautographic methods:**

Bioautography, as a method to localize antibacterial activity on a chromatogram, has found widespread application in the search for new antibiotics from microorganisms. Most published procedures are based on the agar diffusion technique, whereby the antimicrobial agent is transferred from the thin layer or paper chromatogram to an inoculated agar plate through a diffusion process. Zones of inhibition are then visualized by appropriate vital stains. The problems due to the differential diffusion of compounds from the chromatogram to the agar plate are simplified by direct bioautographic detection on the chromatographic layer. This method, however, requires more complex microbiological equipment and is, in contrast to the contact bioautographic methodology, easily affected by possible contamination from airborne bacteria. It should be noted that there are some plants with antibacterial activity as shown in the Table: 2.1 and the polyherbal formulations as shown in Table: 2.2. Some of them have found their place,
especially in the folklore medicine (Allium sativum, Allium ursinum, Geranium macrorrhizum), but most herbs with antimicrobial activity have high toxicity (Veratrum lobelianum, Helleborus odorus, Artemisia vulgaris, Hedera helix etc.), which restrict their broader use. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with that of costly synthetic drugs that have adverse effects.

**Table: 2.1 Plants reported having antibacterial activity.**

<table>
<thead>
<tr>
<th>Plants</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aristolochia bracteate</em></td>
<td>49</td>
</tr>
<tr>
<td><em>Achillea clavennae</em></td>
<td>50</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>51</td>
</tr>
<tr>
<td><em>Barringtonia asiatica</em></td>
<td>52</td>
</tr>
<tr>
<td><em>Calotropis gigantea</em></td>
<td>53</td>
</tr>
<tr>
<td><em>Cichorium intybus</em></td>
<td>54</td>
</tr>
<tr>
<td><em>Cleome vicosa</em></td>
<td>55</td>
</tr>
<tr>
<td><em>Coscinium fenestratum</em></td>
<td>56</td>
</tr>
<tr>
<td><em>Curcuma longa</em></td>
<td>57</td>
</tr>
<tr>
<td><em>Daniellia oliveri</em></td>
<td>58</td>
</tr>
<tr>
<td><em>Gmelina asiatica</em></td>
<td>55</td>
</tr>
<tr>
<td><em>Gnaphalium oxyphyllum</em></td>
<td>59</td>
</tr>
<tr>
<td><em>Gnaphalium viscosum</em></td>
<td>59</td>
</tr>
<tr>
<td><em>Lithocarpus celebicus</em></td>
<td>60</td>
</tr>
<tr>
<td><em>Salacia beddomei</em></td>
<td>61</td>
</tr>
</tbody>
</table>
Table: 2.2 Some of the polyherbal formulations having antimicrobial activity:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV-07</td>
<td>64</td>
</tr>
<tr>
<td>Basant</td>
<td>65</td>
</tr>
<tr>
<td>Sudarshan churna</td>
<td>66</td>
</tr>
</tbody>
</table>

2.4. Review on Inflammation:

Inflammation is best defined as the reaction of vascularised living tissue to local injury\textsuperscript{67,68}. Repair starts along with inflammation and continues even after inflammation is cured. In repair the damaged cells are replaced by same type of cells or by formation of fibrous tissue resulting in scar formation.

Inflammation has different phases:

- The first phase is caused by an increase of vascular permeability resulting in exudation of fluid from the blood into the interstitial space.
The second phase by infiltration of leucocytes from the blood into the tissues.
The third phase by granuloma formation.

The classical symptoms of inflammation are rubar, calor, tumour, dolor and function laesa.

**Aetiopathogenesis:**

1. Physical or Mechanical: Abrasions, cuts, lacerations, etc.
2. Chemicals: Acids, Alkalis, etc.
3. Radiation: U.V radiation, Thermal trauma, etc.
4. Infections: Virus, Fungi, Bacteria, etc.
5. Immunological mechanisms: Rheumatoid arthritis etc.

Depending on the type of etiological factor and its duration inflammatory process can be studied under two broad headings.

- Acute inflammation.
- Chronic inflammation.

**Acute inflammation**:\(^{67,68,69}\)

It is of relatively short duration, lasting for minutes, several hours, (or) even few days and is a similar response by the vascular system to any type of
causative agent; characterized by vasodilation, changes in blood flow, exudation of plasma and emigration of leucocytes, predominantly neutrophils. The escape of fluid, proteins and blood cells from the vascular system into the interstitial tissues and body cavities is known as exudation. This inflammatory exudate is characterized by its high specific gravity above 1.020, high protein content and cellular debris; whereas transudate, an ultra filtrate of blood plasma, poor in protein content will be having specific gravity less than 1.012.

The main pathogenesis can be conveniently arranged under headings;

- Hemodynamic changes.
- Cellular events.

**Hemodynamic changes:**

Following an inconstant and transient vasoconstriction of arterioles lasting a few seconds, vasodilation occurs immediately. Vasodilatation is considered to be crucial event in the pathogenesis, which will be resulting increased blood flow, the reason for rubor and calor.

This local vasodilation and increased blood flow are due to secretion of chemical mediators and this view is supported by sir. Thomas Lewis “Triple response theory of redness, flare and wheal”. He postulated that neural mechanisms were responsible for flare but vascular changes are mediated by chemical means. At this stage increased blood volume in dilated vessels result
in increase in local hydrostatic pressure which in turn results in transudation of protein poor fluid. On the other hand, slowing of circulation occurs due to increased permeability of local microvasculature, known as “STASIS” resulting in increased viscosity of blood.

**Cellular events:**

It is followed by “migration” and event in which leucocytes, principally neutrophils, orient along the blood vessel walls. Later, leucocytes adhesion and transmigration occurs. It is the main cellular phase where in delivery of leucocytes to the site of injury occurs and they ingest offending agents, kill bacterial and other microbes, and degrade necrotic tissue and foreign antigens. During this chemotaxis and phagocytosis, activated leucocytes may release toxic metabolites causing tissue damage. Here the adhesion and transmigration of leucocytes are determined largely by binding of complement molecules on surface of leucocytes and endothelium.

The type of emigrating leucocytes varies with the age of inflammatory lesion and type of stimulus. In most forms of acute inflammation neutrophils predominate in the inflammatory infiltrate during first 6-24 hours and then replaced by monocytes in 24-48 hours. But exceptions, which are noteworthy are lymphocytes are 1st cells to arrive in viral infections, eosinophils in some hypersensitivity reactions, even neutrophils stay for 48-96 hours without being replaced by monocytes in pseudomonas infections.

**Chemical mediators of inflammation:**
Sources and classification of chemical mediators

I. Cell derived mediators:

i) Preformed:

   a. Vasoactive amines: Histamine, Serotonin.

   b. Lysosomal enzymes: Acid and Neutral proteases.

ii) Synthesized:

   a. Prostaglandins: \( \text{PGD}_2, \text{PGE}_2, \text{PGF}_{2\alpha}, \text{PGI}_2, \text{TXA}_2 \).

   b. Leucotrienes: \( \text{LTB}_4, \text{LTC}_4, \text{LTE}_4 \).

   c. Interleukin: IL-1 and 8 Families.

   d. Tumor necrosis factor: TNF \( \alpha \) and \( \beta \).

II. Plasma derived mediators:

   a. The complement system: C3a, C3b, C4a and C5a.

   b. The kinin system: Bradykinin, Kallikrein.

   c. Coagulation and fibrinolytic system: Fibrin Peptides, Fibrin degradation Products (FDP).

III. Other mediators:

   a. Nitric oxide.

   b. Oxygen derived free radicals.
c. Growth factors: Platelet derived growth factor (PDGF)

Transforming Growth factor $\alpha$ (TGF$\alpha$)

d. Neuropeptides.

**Outcome of acute inflammation:**

a. Complete healing, tissues retain normality.

b. Healing by fibrosis and scarring.

c. Worsening into abscess.

d. Progression to chronic inflammation.

**Chronic Inflammation:**

Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate pro-inflammatory agents. Chronic inflammations include a proliferation of fibroblasts and the infiltration of neutrophils and exudation. It is a prolonged process of inflammation, less uniform and characterized by the presence of

a. Lymphocytes.

b. Plasma cells.

c. Macrophages.

d. Proliferation of fibroblasts and small blood vessels.

e. Fibrosis.
f. Tissue destruction.

Clinically chronic inflammation:

a. An outcome of acute inflammation.

b. Repeated attacks of acute inflammation.

c. May begin insidiously and will not follow classic symptoms and signs of acute inflammation.

**Common mechanism of anti-inflammatory drugs**\(^{70,71}\):

By means of suppression of immune reaction, corticosteroids will be mitigating the inflammatory activity. Inhibition of biosynthesis of prostaglandin is one of the most common mechanisms of variety of anti-inflammatory drugs in vogue.

The anti-inflammatory action of NSAID’s is related to inhibition of COX-2 and COX-1, which in turn is responsible for adverse effects of NSAID’s, like peptic ulceration and nephrotoxicity.

Selective COX-2 inhibitors are in the limelight and pharmaceuticals boast that they are having spare gastric activity at therapeutic concentration. Even though they have shown amount of non-interference policy with gastro
protective prostaglandins, in total they don’t possess this activity on prolonged use. So all NSAID’s must be used cautiously until safer agents are developed72.

Some of the latest NSAID drugs

a. ETODOLAC – Selective COX-2 inhibitor.
b. ROFECOXIB – Novel COX-2 inhibitors.
c. CELECOXIB – Novel COX2 inhibitors.
d. VALDECOXIB – Novel COX2 inhibitors.
e. ZILIUTION – Lipo-oxygenase inhibitors.
f. NABUMETONE – Selectively inhibits PG synthetase.
g. SERRATIOPEPTIDASE – Proteolytic enzyme acts by
   hydrolyzing bardykinin, serotonin.

Other mechanisms suggested for anti-inflammatory activity of NSAIDS:

a. Stabilization of leucocytes-lysosomal membranes.
b. Interference with migration of leucocytes.
c. Inhibition of phagocytosis by leucocytes.
d. Interference with oxidative phosphorylation.
e. Inhibition of lipoperoxide generation.

**Screening methods of anti-inflammatory drugs73,74,75:**

The cardinal signs of inflammation *via*, redness, swelling, heat, pain and loss of function serve as targets (or) parameters for experimental investigation of anti-inflammatory activity. Since inflammation is a multi component dynamic forces (or) polyphasic tissue response, multiple or variety of screening
methods for individual anti-inflammatory compound are required; none of the available anti-inflammatory agents can suppress all the phases (or) even single phase of one component.

Clinically anti-inflammatory drugs are judged by their effects on pain stiffness, swelling. But the methods which use animals like mice, albino-rats, guinea pigs, rabbits, pigeons, swine and monkeys as model to evaluate the anti-inflammatory activity, can be worked out only on certain cut parameters like swelling, pain, exudates, granuloma etc.

The available screening methods are:

a. U.V. Erythema in guinea pigs.

b. Oxazolone induced ear edema in mice.

c. Croton Oil ear edema in rats and mice.

d. Paw edema in rats.

e. Pleurisy tests.

f. Granuloma pouch technique.

g. The proliferative phase is measured by methods for testing granuloma formation, such as:

1. Cotton wool granuloma

2. Glass rod granuloma.
3. PVC sponge granuloma.

2.5 Screening methods of analgesic drugs:

Analgesia is defined as the state of reduced awareness to pain and analgesics are the drugs which decrease pain sensation by increasing threshold to painful stimuli.

The methods for characterising potentially useful for the relief of pain are numerous. Despite the specific defenders and critics of the various methods, there is no single approach that gives complete parallelism between results in animals and clinical practice in man. A part of difficulty is directly related to the fact that clinical pain is a pathological condition and differs from experimental pain. The former tends to run a chronic course and usually more severe than experimental pain. In addition, clinical pain is not constant and is influenced by environmental conditions.

Some of the screening methods are

- Hot plate technique.
- Tail flick method.
- Flinch jump test.

Herbal drugs used in the treatment of inflammation and pain:

The greatest disadvantage of the presently available potent synthetic drugs lies in their toxic symptoms on gastro intestinal tract and kidney. Compared with synthetic drugs, drugs derived from plants are frequently
considered to be less toxic with fewer side effects. Therefore, the search for more effective and safer antiinflammatory agent has become an area of active research i.e. natural products. List of most commonly used plants in herbal formulations is shown in Table: 2.3. The polyherbal formulations is shown Table: 2.4

Table: 2.3 Plants reported having anti-inflammatory and analgesic activities.

<table>
<thead>
<tr>
<th>Plants</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhatoda vasica</td>
<td>76</td>
</tr>
<tr>
<td>Aegle marmelos</td>
<td>77</td>
</tr>
<tr>
<td>Alstonia macrophylla</td>
<td>78</td>
</tr>
<tr>
<td>Ammania baccifera</td>
<td>79</td>
</tr>
<tr>
<td>Anacardium occidentale</td>
<td>80</td>
</tr>
<tr>
<td>Arnebia euchroma</td>
<td>81</td>
</tr>
<tr>
<td>Atalantia monophylla</td>
<td>77</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>82</td>
</tr>
<tr>
<td>Boerhaavia diffusa</td>
<td>83</td>
</tr>
<tr>
<td>Bryonia laciniosa</td>
<td>84</td>
</tr>
<tr>
<td>Cassia fistula</td>
<td>85</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>86</td>
</tr>
<tr>
<td>Colchicum autumnale</td>
<td>87</td>
</tr>
<tr>
<td>Delonix elata</td>
<td>88</td>
</tr>
<tr>
<td>Eclipta alba</td>
<td>89</td>
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</tbody>
</table>
Table: 2.4 Some of the polyherbal formulations tested for anti-inflammatory and analgesic activities.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheriya rasnadi kashayam</td>
<td>98</td>
</tr>
<tr>
<td>Maharasandhi quath</td>
<td>99</td>
</tr>
<tr>
<td>Rasandiguggulu</td>
<td>100</td>
</tr>
<tr>
<td>Triphala guggul</td>
<td>101</td>
</tr>
<tr>
<td>Yogaraja guggul</td>
<td>101</td>
</tr>
</tbody>
</table>

2.6. Review on isolation and characterization of phytoconstituents

**Chromatography**

Russian botanist *Mikhail Tsvat* invented the first chromatography technique in 1901 during his research on chlorophyll. He used a liquid – adsorption column containing calcium carbonate to separate plant pigments. He first used the term *chromatography* in print in 1906 in his two papers about chlorophyll in the German botanical journal.
**Principle**

Chromatography is a powerful technique for separating mixtures. It involves passing the sample, a mixture which contains the analyte in the “mobile phase”, often in a stream of solvent, through the “stationary phase”. The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time. Each component has a characteristic time of passage through the system, called a “retention time”. Chromatographic separation is achieved when the retention time of the analyte differs from that of other components in the sample.

Various techniques for the separation of compounds rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary absorbing medium through which they pass; such as paper, gelatin, alumina or silica.

There are different types of chromatography such as paper, thin layer, or column chromatography each with its own advantages and disadvantages. Chromatography systems have a stationary phase (which can be solid or liquid) and a mobile phase (usually liquid or gas). In column chromatography both phases are placed in a column container.

*Analytical chromatography* is used to determine the identity and concentration of molecules in a mixture.
Preparative chromatography is used to purify larger quantities of molecular species.

Retention

The retention is a measure of the speed at which a substance moves in a chromatographic system. In continuous development systems like HPLC or GC, where the compounds are eluted with the eluent, the retention is usually measured as the retention time \( R_t \) or \( t_R \), the time between injection and detection. In interrupted developments systems like TLC the retention is measured as the retention factor \( R_f \), the distance moved by the compound divided by the distance moved by the eluent front.

\[
R_f = \frac{\text{Distance moved by compound}}{\text{Distance moved by the eluent}}
\]

The retention of a compound often differs considerably between experiments and laboratories due to variations of the eluent, the stationary phase, temperature, and the setup. It is therefore important to compare the retention of the test compound to that of one or more standard compounds under absolutely identical conditions.

Plate theory

The plate theory of chromatography was developed by Archer John Porter Martin and Richard Laurence Millington Synge. The plate theory describes the chromatography system, the mobile and stationary phases, as being in
equilibrium. The partition coefficient $K$ is based on this equilibrium, and is defined by the following equation.

$$K = \frac{\text{Concentration of solute in stationary phase}}{\text{Concentration of solute in mobile phase}}$$

$K$ is assumed to be independent of concentration, and can change if experimental conditions are changed, for example temperature is increased or decreased. As $K$ increases, it takes longer for solutes to separate. For a column of fixed length and flow, the retention time ($t_R$) and retention volume ($V_r$) can be measured and used to calculate $K$.

**Column chromatography**

Column chromatography consists of a vertical glass column filled with some form of solid support with the sample to be separated placed on top of this support. The rest of the column is filled with a solvent, which, under the influence of gravity, moves the sample through the column. Similar to other forms of chromatography, differences in rates of movement through the solid medium are translated to different outlet times from the bottom of the column for various compounds of the original sample.

In 1978, W. C. Stills introduced a modified version of column chromatography called flash column chromatography. The technique is very similar to the traditional column chromatography, but the solvent is driven through the column by applying pressure. When applying pressure on the top
of the column, the separations were performed within 20 minutes with improved separation$^{102}$. 