4.1 PLANT PROFILE

Plants belonging to the genus Sida have been used in traditional medicine for a variety of therapeutic purposes as astringent, cooling stomachic, analgesic, anti-inflammatory, hypoglycemic and nervous, urinary and cardiac diseases. This genus belongs to family Malvaceae and is a genus of herbs, shrubs and small trees which are distributed worldwide, mostly found in the tropics and subtropics, although some species extend into temperate regions. It contains 125 to 150 species, about a dozen species occur in India.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
<th>Subfamily</th>
<th>Malvoideae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order</td>
<td>Malvales</td>
<td>Tribe</td>
<td>Malveae</td>
</tr>
<tr>
<td>Family</td>
<td>Malvaceae</td>
<td>Genus</td>
<td>Sida</td>
</tr>
</tbody>
</table>

4.1.1 Sida cordifolia

**Botanical name**: *Sida cordifolia*

**Family**: Malvaceae

**Common Name**: Bala, Country mallow,

It is a perennial subshrub of the mallow family Malvaceae native to India. It has naturalized throughout the world, and is considered an invasive weed in Africa, Australia, the southern United States, Hawaiian Islands, New Guinea, and French Polynesia. The specific name, *cordifolia*, refers to the heart-shaped leaf.

**Botanical description**

*Sida cordifolia* grows well through the plains of India, especially, in damp climates. The shrub grows up to 0.75 – 1.5 meters in height. The root and the stem are stout and strong. The leaves are 2.5-7 cm long and 2.5-5 cm broad, with 7-9 veins. They are heart shaped, serrate and truncate. The flowers are small, yellow or white in colour, solitary and axillaries. The fruits are moong-sized, 6-8 mm in diameter. The seeds are called as Bijabanda in Ayurveda, are grayish black in colour and smooth. The plant flowers from August to December and fruiting occurs from October to January (Pole et al., 2006).

**Occurrence & Distribution**

Country Mallow of Malvaceae family is widely distributed along with other species are common throughout the tropical and sub tropical plains all over India and Srilanka up to an altitude of 1050 m., growing wild along the roadside.

**Part used**: Whole plant
Chemical constituents
Ephedrine, Pseudoephedrine, Sterculic, malvalic and coronaric acid. Fatty acids, Saponine, Betaphenethylamine, Hypaphorine, Ecdysterone, Indole alkaloids, Palmitic, stearic and β – sitosterol.

Medicinal use
S. cordifolia is used in Ayurvedic medicine, known as "malva branca", is a plant used in the folk medicine for the treatment of inflammation of the oral mucosa, asthmatic bronchitis and nasal congestion. It has been investigated as an anti-inflammatory (Franzotti et al., 2000), for treating cancer (Jenny et al., 2005), and for encouraging liver re-growth (Silva et al., 2006). Due to its ephedrine content, it possesses psychostimulant properties, affecting the central nervous system and also the heart (Adam and Steven, 2006). A 50% ethanolic extract of Sida cordifolia tested on rats showed potent antioxidant and anti-inflammatory activity, activity comparable with the standard drug deprenyl (Franzotti et al., 2000). The plant has demonstrated anti-pyretic and anti-ulcerogenic properties. The aqueous extract of Sida cordifolia stimulates liver regeneration in rats. No tannin or glycosides have been identified from the plant. The roots and stems contain the alkaloid ephedrine, normally observed in the different varieties of the gymnosperm genus Ephedra (Franzotti, 2000). Recent analyses have revealed that ephedrine and pseudoephedrine constitute the major alkaloids from the aerial parts of the plant, which also show traces of sitosterol and palmitic, stearic and hexacosanoic acids (Sutradhar et al., 2006). The flavones: 5,7-dihydroxy-3-isoprenyl flavone (1) and 5-hydroxy-3-isoprenyl flavone (2), β-sitosterol and stigmasterol have been isolated from the plant (Sutradhar et al., 2008). The analgesic alkaloid (5′-Hydroxymethyl-1′-(1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-1-yl)-heptan-1-one) has also been found. Sterculic, malvalic and coronaric acids have been isolated from the seed oil, along with other fatty acids (Joseph et al., 2011).
Figure 4.1: *Sida cordifolia* Linn.
### 4.1.2 Sida acuta Burm

<table>
<thead>
<tr>
<th><strong>Botanical Name</strong></th>
<th><em>Sida acuta</em> Burm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family</strong></td>
<td>Malvaceae</td>
</tr>
<tr>
<td><strong>Common Name</strong></td>
<td>Wireweed</td>
</tr>
</tbody>
</table>

**Biological Description**

*Sida acuta* an erect perennial under shrub (or) shrub, 1.5m high. The bark is smooth, greenish, the root is thin, long, cylindrical and very rough; leaves are lanceolate, the flowers are yellow, solitary or in pairs; seeds are smooth and black.

**Distribution**

*Sida acuta burm* is a shrub found as a major weed throughout the hotter parts of India and Sri Lanka.

**Part Used** : Whole plant

**Chemical Constituents**

Roots contain 3 types of alkaloids constituents viz., β- phenethylamines, quinazolines, carboxylated tryptamines; alkaloid cryptolepine, ephedrine, phytosterols, α- amyrin, starch, ecdysterone (Yoganarasimhan 2000).

**Medicinal Use**

In Indian traditional medicine, the root of *Sida acuta* is extensively used as a stomachic, diaphoretic and antipyretic. It is regarded as cooling, astringent, tonic and useful in treating nervous and urinary diseases and also disorders of the blood, bile and liver (Kirtikar and Basu, 1987; Khare et al., 2002). It is also used to treat gonorrhoea, elephantiasis and ulcers and is claimed to have aphrodisiac properties. The juice of the root is applied to wounds (Anonymous, 1972). The whole plant is used to treat snake bite and it lessened the haemorrhagic effect of *Bothrops atrox* venom (Anonymous, 2003). Decoction of the leaves and root is credited with emollient and tonic properties leaf juice is given for relief in chest pain and as an anthelmintic. This species is not only important as a medicine, but also yield a good fibre.
Figure 4.2: *Sida acuta Burm.*
4.1.3 *Sida retusa* Linn

**Botanical Name**: *Sida retusa* Linn  
**Family**: Malvaceae  
**Common Name**: Janglimethi

**Biological Description**  
An erect, minutely hairy, branched undershrub with a firm woody stem and intricate branches; leaves short petioled, obovate, truncate or more often retuse and serrate; flowers yellow, solitary and axillary; fruits enclosed within the persistent calyx, separating into one seeded cocci; seeds black, smooth (Warrier and Nambiar, 1995).

**Distribution**  
Throughout the warmer parts of India, as a weed of waste places

**Plant Used**: Whole plant

**Chemical constituents**  
Roots contain 3 types of alkaloidal constituents similar to *S. acuta*. Roots and leaves contain ephedrine.

**Medicinal Use**  
The roots and leaves are bitter, sweet, emollient, cooling, aphrodisiac, unctuous, strengthening and promote sexual vigour and vital factor. They are good for rheumatism, flatulence, colic, haemothermia, emaciation, vitiated conditions of *tridosa*, seminal weakness, arthritis and diarrhoea (Yoganarasimhan, 2000).
Antiviral activity

Figure 4.3: *Sida retusa* Linn
4.1.4 *Sida Spinosa Linn.*

**Botanical Name**: *Sida spinosa Linn*

**Family**: Malvaceae

**Common name**: Prickly sida, Gulsakari

**Biological Description**

A small, erect, grey, pubescent, branched undershrub with a slender erect stem, the young shoots being covered over with soft, grey stellate down; leaves with 2 or 3 small, stiff, minute spiny projections at the nodes adjacent to the place of insertion; flowers pale yellow to cream white, axillary and solitary on slender peduncles; fruits 5-6 or 3 chambered with one seed in each chamber, seeds brownish black, smooth.

**Distribution**

Throughout the hotter parts of India

**Chemical constituents**

Roots contains 3 types of alkaloidal constituents similar to *Sida acuta*

**Parts used**: Whole plant

**Medicinal Use**

The roots are diaphoretic, antiperiodic, aphrodisiac and tonic. They are administered in debility, fever, malarial fever, vitiated conditions of vata, haemorrhoids, swellings and in irritability of the bladder. The leaves are emollient and refrigerant and are useful in gonorrhoea, gleets, scalding urine and for the treatment of skin diseases and as oral snake bite treatment. The fruits are astringent and cooling (Warrier and Nambiar, 1995)
Figure 4.4: *Sida spinosa* Linn.
4.2 Cell culture
Cell culture is the complex process by which cells are grown under controlled conditions, generally outside of their natural environment. In practice, the term "cell culture" now refers to the culturing of cells derived from multi-cellular eukaryotes, especially animal cells. However, there are also cultures of plants, fungi and microbes, including viruses, bacteria and protists. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture. Animal cell culture became a common laboratory technique in the mid-1900s, but the concept of maintaining live cell lines separated from their original tissue source was discovered in the 19th century (Freshney, 2005; Carrel, 1912).

Cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. Growing viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines. The injectable polio vaccine developed by Jonas Salk was one of the first products mass-produced using cell culture techniques. This vaccine was made possible by the cell culture research of John Franklin Enders, Thomas Huckle Weller, and Frederick Chapman Robbins, who were awarded a Nobel Prize for their discovery of a method of growing the virus in monkey kidney cell cultures.

4.2.1 Vero
This cell line was first established in 1962 by Y.Yasumara & Y. Kawakita at Chiba University, Japan from a primary kidney culture of vervet monkey (Cercopithecus aethiops) otherwise known as African green monkey.

Vero cell is a hypoploid cell possessing a model number of 58 chromosomes instead of 60. In the past quarter of a century Vero cells has become one of the most valuable cell lines used in the entire fields of animal virology. The cell line has been useful for propagation and assay of a wide range of viruses such as measles, arbo virus, reo virus, rubella, simian adeno virus and poliovirus.

Polio and Rabies vaccine prepared from this cell line has been successfully used in several countries since many years. It’s also the only certified cell line used for the production of viral vaccines.
**Antiviral activity**

**Source**: *Cercopithecus aethiops* (African Green Monkey)

**Origin**: Normal, Kidney

**Morphology**: Fibroblast

**Susceptibility**: Adenovirus type 12, African swine fever virus, Arbovirus, Bluetongue virus, Bovine leucosis, Echovirus, Getah arbovirus, Herpes simplex virus, Influenza virus, Orbivirus, Orthomixovirus, Paramyxovirus, Poliovirus Type 3, Porcine Epidemic diarrhoea virus, Reovirus, Rubella virus.

**Use**: Virology; virus titration; virus replication; plaque assays; bacterial cytotoxicity.

The cell line was obtained from National Centre for Cell Sciences, Pune, India.

**Figure 4.5 Normal Vero cell culture**
4.2.2 HEp-2

The HEp-2 cell line has been described to originate from tumours which were produced in irradiated-cortisonised weanling rats after injection of epidermoid carcinoma tissue isolated from the larynx of a 56 year old male. STR (DNA)-profiling has revealed that the HEp-2 cell line is almost identical to the HeLa cell line.

**Source**: *Homosapiens*

**Species**: Human, Caucasian

**Origin**: Larynx

**Morphology**: Epithelial

**Susceptibility**: Adenovirus type 3, Arbovirus, Coxsackie virus, Echovirus, Herpes simplex virus, measles, Newcastle disease virus, Poliovirus Type 1, Respiratory syncytial virus, Vesicular stomatitis (Indiana)

**Use**: Carcinoma, squamous cell, tumorigenic in nude mice, virus production, cytotoxicity; virology; virus titration; bacterial adhesiveness; tumorigenicity

The cell line was obtained from National Centre for Cell Sciences, Pune, India.

*Figure 4.6 Normal HEp-2 cell culture*
4.2.3 A-549

A549 cells are adenocarcinomic human alveolar basal epithelial cells. The A549 cell line was first developed in 1972 by D. J. Giard, et al. through the removal and culturing of cancerous lung tissue in the explanted tumor of a 58-year-old caucasian male. In nature, these cells are squamous and responsible for the diffusion of some substances, such as water and electrolytes, across the alveoli of lungs. If A549 cells are cultured in vitro, they grow as monolayer cells, adherent or attaching to the culture flask. The human alveolar epithelial cell line A549 may be anchored or suspended in a solution in vitro (Giard et al., 1973).

Another characteristic of these cells are, they able to synthesize lecithin and contain high level of desaturated fatty acids, which are important to maintain the membrane phospholipids in cells.

Source: Homosapiens
Species: Human, Caucasian
Origin: Lung
Morphology: Epithelial
Characteristic: Synthesizes surfactant
Use: A549 cell line are widely used as an in vitro model for a type II pulmonary epithelial cell model for drug metabolism and as a transfection host.

The cell line was obtained from National Centre for Cell Sciences, Pune, India.

Figure 4.7 Normal A-549 cell culture
4.2.4 MDCK

Madin-Darby Canine Kidney cells are positive for keratin by immunoperoxidase staining. MDCK cells have been used to study processing of beta amyloid precursor protein and sorting of its proteolytic products.

**Source**
: Dog (*Canis familiaris*)

**Origin**
: Kidney

**Strain**
: cocker spaniel

**Morphology**
: Epithelia

**Characteristic**
: Domes, transport

**Use**
: This cell line is a suitable transfection host.

The cell line was obtained from National Centre for Cell Sciences, Pune, India.

**Figure 4.8 Normal MDCK cell culture**
4.3 Virus Profile

4.3.1 Herpes Simplex Virus Type-I (Jones, 2004; Liuzzi et al, 2004)

Herpes Simplex Virus (HSV) is a double stranded, enveloped, DNA virus of the family Herpesviridae. The structure of the herpes virus particle is very complex. The core consists of a toroidal shape with the large DNA genome wound around a proteinaceous core which is surrounded by complex capsid. Outside the capsid is the tegument, a protein-filled region which appears amorphous in electron micrographs. The Herpesviridae family is divided into three sub-families Alphaherpesvirinae which are neutrotropic and have a rapid replication cycle and usually a broad host and cell range, Betaherpesvirinae and Gammaherpesvirinae which differ in genome size and structure but both of which replicate more slowly and in a much more restricted range of cells of glandular and/or lymphatic origin. The same host can be infected with multiple distinct and unique types. Approximately 100 Herpes viruses have been isolated, at least one for most animal species and to date, there are eight known human Herpesviruses.

Herpes simplex viruses (HSV-1 and HSV-2) are important pathogens for humans, especially in the case of highly susceptible adults. After establishing latency, HSV can reactivate, causing frequent recurrent infections in some patients, which most people experience few recurrences. Among HSV-related pathologies, genital herpes is an important sexually transmitted disease (STD) commonly caused by HSV-2, with the exception of a minority of cases caused by HSV-1 (Johnson and Nahmias, 1989; Kalinyak et al., 1977). HSV-1 infections are very common and mostly affect adult people (Whitley and Kimberlin, 1998). HSV-1 infection is usually mild, especially when it affects the lips, face or genitals. However, in some cases type 1 can recur spontaneously in the eye, causing ocular herpes, a potentially serious infection which can lead to blindness. In very rare cases HSV-1 can spread spontaneously to the brain, causing herpes encephalitis, a dangerous infection that can lead to death. After primary infection, HSV persists in host for the lifetime, thus considered a lifelong infection. Nucleoside analogues such as acyclovir (ACV), pencyclovir etc., are the only approved drugs for the treatment of HSV infections.

Herpes simplex virus type I and II were procured from Christian Medical College, Vellore, Tamil Nadu, India and were grown in Vero (Normal African Green Monkey, Kidney) cell culture.
Figure 4.9 Morphology of Herpes infected Vero cell culture
4.3.2 Human Adeno Virus (Zock et al 1993; Sprengel et al., 1994)

Human adenovirus is a double stranded, non enveloped, DNA virus of the family Adenoviridae. To date, 51 human adenovirus serotypes have been described, grouped into six species (A-F) based on genome size, composition and organization, DNA homology, hemagglutinating properties and oncogenicity in rodents. This subdivision had some clinical relevance as distinct adenovirus species show a preference for specific organs: C, E and B species typically infect the respiratory tract, other B species the urinary tract; species A and F target the gastrointestinal tract and species D the eyes (Kojaoghlanian et al., 2003).

Adenovirus was first isolated in 1953 and these viruses are a frequent cause of acute upper respiratory tract (URT) infections and they also cause a number of other types of infections most of which are mild. The pathology is primarily from inflammation and loss of infected epithelial cells. Primary adenovirus infections usually occur in young children; approximately 5% of the acute respiratory illnesses in children up to 5 years are due to an adenovirus infection (Brandt et al., 1969) and enteric adenoviruses are a major cause of viral gastroenteritis in infants. In immunocompetent individuals, infections can manifest in diverse clinical syndromes, such as upper and lower respiratory tract disease, (Kerato) conjunctivitis, gastroenteritis and hemorrhagic cystitis. In rare cases, hepatitis, myocarditis, meningoencephalitis or nephritis are also encountered (Straussberg et al., 2001; Chuang et al., 2003). In individuals with an impaired immune response, life threatening adenovirus infections are common. Among these are patient hereditary immunodeficiency and patient with acquired immunodeficiency syndrome (AIDS) (Kojaoghlanian et al., 2003)

Due to the growing number of transplant recipient and AIDS patients, the impact of severe adenovirus infections, and concurrently, the need for effective antiviral therapy is increasing. Unfortunately, no formally approved drugs are yet available. Two antiviral compounds, i.e., cidofovir and ribavirin, have been used in clinical studies, with variable outcome (Lenaerts and Naesens, 2006).

Adenovirus strain was obtained from Christian Medical College, Vellore, Tamil Nadu, India and was grown in A-549 (Human small lung carcinoma) cells.
Figure 4.10 Morphology of Adeno infected A-549 cell culture
**4.3.3 Polio Virus** (Kitamura et al., 1980; Kitamura et al., 1981)

The human enteroviruses (EVs) comprise more than 60 and the rhinoviruses (RVs) more than 100 distinct serotypes within the family Picornaviridae (‘pico’ meaning small, ‘rna’ for ribonucleic acid) (Miller, 1997). The EVs are among the most common and most important viral pathogens of humans. The paralytic potential of the polioviruses, has been recognized as early as the 14\(^{th}\) century B.C in Egyptian art. Summer epidemics of paralytic poliomyelitis ravaged the United States through the 1950s. since the introduction of vaccines in the late 1950s and early 1960s, much of the developed world is now virtually free of poliovirus disease. In many developing countries, eradication programs have made dramatic progress (CDC, 1996, 1997 a, b).

Polio virus is single- stranded, positive sense, naked, icosahedral, RNA virus with three different serotypes 1, 2 and 3. It belongs to Picornaviridae family. Poliomyelitis (Greek: polios-gray and myelos- marrow or spinal cord). Poliovirus is the causative agent of poliomyelitis, acute and highly infectious disease involving invasion of the gastro-istttinal tract by one of the three serotypes. In a minority of patients it invades the nervous system, and can causes total paralysis in a matter of hours. The incubation period is from 3-21 days. Signs and symptoms include fever, fatigue, headache, vomiting, stiffness in the neck and pain in the limbs.

The first effective vaccine against polio was developed by Jonas Salk in 1955, which is based on formalin inactivated poliovirus. Later in 1961, Albert Sabin developed the oral vaccine, which is a live-attenuated vaccine produced by the passage of virus through non-human cells. Sabin vaccine is being used for modern mass vaccination to eradicate the poliomyelitis.

Poliovirus type I, attenuated strain was obtained from Christian Medical College, Vellore, Tamil Nadu, India, and was grown in Hep-2 (Human epithelial laryngeal cancer) cells.
Figure 11 Morphology of Polio infected Hep-2 cell culture
4.3.4 Influenza virus

Influenza A (H1N1) virus is single stranded enveloped RNA virus, the subtype of influenza A virus belongs to the family orthomyxoviridae, that was the most common cause of human influenza (flu) in 2009. Some strains of H1N1 are endemic in humans and cause a small fraction of all influenza-like illness and a small fraction of all seasonal influenza. H1N1 strains caused a small percentage of all human flu infections in 2004–2005 (Flu view 2004-05). Other strains of H1N1 are endemic in pigs (swine influenza) and in birds (avian influenza).

Swine influenza

Swine influenza (also called swine flu, or pig flu) is an infection by any one of several types of swine influenza virus. Swine influenza virus (SIV) is any strain of the influenza family of viruses that is endemic in pigs. As of 2009, the known SIV strains include influenza C and the subtypes of influenza A known as H1N1, H1N2, H3N1, H3N2, and H2N3. Swine influenza virus is common throughout pig populations worldwide. Transmission of the virus from pigs to humans is not common and does not always lead to human influenza, often resulting only in the production of antibodies in the blood. If transmission does cause human influenza, it is called zoonotic swine flu. People with regular exposure to pigs are at increased risk of swine flu infection. The meat of an infected animal poses no risk of infection when properly cooked.

Pigs experimentally infected with the strain of swine flu that is causing the current human pandemic showed clinical signs of flu within four days, and the virus spread to other uninfected pigs housed with the infected ones (Roos, 2010).

During the mid-20th century, identification of influenza subtypes became possible, allowing accurate diagnosis of transmission to humans. Since then, only 50 such transmissions have been confirmed. These strains of swine flu rarely pass from human to human. Symptoms of zoonotic swine flu in humans are similar to those of influenza and of influenza-like illness in general, namely chills, fever, sore throat, muscle pains, severe headache, coughing, weakness, and general discomfort. The recommended time of isolation is about five days.

Influenza A virus PR/8/34 (strain A/Puerto Rico/8/1934 H1N1) was obtained from Kyushu University of Health and Welfare, Nobeoka, Miyazaki, Japan, and grown in MDCK (Madin- Darby canine, kidney) cell culture.
Figure 4.12 Morphology of H1N1 infected MDCK cell culture