2. REVIEW OF LITERATURE

It is hypothesized that plants possessing antimicrobial activity or diuretic effects may act towards direct remediation of ailments or relieving the diseased person of any secondary effects as the diuretic effects of *Hygrophiha spinosa* T. Anders. (Kumari & Iyer, 1967) likely actively contribute when the plant is used as a remedy for leucorrhea, where modern doctors also advocate drinking enough water to produce excessive urination.

Diuretic activity has also been reported for whole plant of *Eclipta alba* (L.) Hassk. (Rangineni *et al*., 2007) and for the flowers of *Spilanthes acmella* (L.) Murray (Ratnasooriya *et al*., 2004). It is interesting that these are precisely the parts used by the traditional healers as remedy for leucorrhea. Similarly, the roots of *C. ternatea*, used by local traditional healers for urinary tract infections reportedly demonstrated diuretic activity in dogs (Piala *et al*., 1962). The stem bark of *Holarrhena pubescens* Buch.-Ham. Wall. ex G. Don (used to treat leucorrhea by the traditional healers) reportedly contains antibacterial steroid alkaloids (Chakraborty & Brantner, 1999).

The antibiotic property of *A. conyzoides*, used by traditional healers as remedy for cloudy urination in women has also been reported (Durodola, 1977). *Emblica officinalis*, used by traditional healers as a remedy for leucorrhea is used in Ladakh against kidney and urinary disorders (Ballabh *et al*., 2008). The plant has also proven its efficacy in scientific studies against age-related renal dysfunction (Yokozawa *et al*., 2007). The plant reportedly also has antibacterial and antifungal properties (Ahmad *et al*., 1998, Dutta *et al*., 1998, Ghosh *et al*., 2008). The roots of *U. lobata* used by traditional healers of the Murong tribe for urinary problems and the plant *Hemidesmus indicus* (L.) R.Br. ex Schult. has also been shown to have antibacterial activity (Aqil & Ahmad 2007, Mazumder *et al*., 2001).

The stem bark of *Holarrhena pubescens* Buch.-Ham. Wall. ex G. Don (used to treat leucorrhea by the traditional healers) reportedly contains antibacterial steroid alkaloids (Chakraborty & Brantner, 1999). The antibiotic property of *A. conyzoides*, used by traditional healers as remedy for cloudy urination in women has also been reported (Durodola, 1977). Five Indian mangrove plants which are *Rhizophora apiculata*, *Rhizophora mucronata*, Bruguiera cylindrica, *Ceriops decandra*, Avicennia marina/parts
(hypocotyls, bark, collar and flower) were investigated to evaluate the antibacterial activity against UTIs bacterial pathogens. (Ravikumar et al., 2010)

Many plants have been used for the treatment of urinary tract infections in Indian system of medicine and in other ancient systems of the world, such as *Zingiber officinale, Punica grantum, Terminalia chebula, Ocimum sanctum, Cinnamomium Cassia, Azadirachta indica*. Out of these only a few have been evaluated as per modern system of medicine. From many such plants only extracts have been prepared and their usefulness evaluated. Since phytochemicals have fewer side effects, they have the potential as good antimicrobial drugs. They may also provide clues for the development of new and better drugs for urinary tract infections.

2.1 Phytochemicals as chemotherapeutic agents

Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases. The word ‘phyto-’ is derived from the Greek *phyto* which means - plant (Liu, 2004). The presence of these bioactive components are said to confer them with resistance against bacterial, fungal and pesticidal pathogens. These bioactive components are said to be responsible for the antimicrobial effects of plant extracts *in vitro* (Abo et al., 1991; Nweze et al., 2004). The interest in plants with antimicrobial properties has been revived due to current problems associated with the use of antibiotics with the increased prevalence of multidrug resistant (MDR) strains of a number of pathogenic bacteria such as methicillin resistant *Staphylococcus aureus, Helicobacter pylori*, and MDR *Klebsiela pneumonia*. On the other hand, infection with *Escherichia coli* involves the risk stimulation of verocytotoxin (VT) production (Yoh et al., 1997, 1999).

Herbal remedies are viewed as reemerging health aid in a number of countries (UNESCO, 1997). This can be traced to both the increasing cost of prescription drugs, for the maintenance of personal health and antibiotic-resistant strains in the case of infectious diseases (Levy, 1998; Van den Bogaard et al., 2000; Smolinski et al., 2003). In industrialized countries, the extraction and development of many drugs, and chemotherapeutics from medicinal plants have been increasing (UNESCO, 1998). Complications in the use of antibiotics in the treatment of hemolytic uremic syndrome
(HUS), and thrombocytopenic purpura (TTP) encouraged researchers to find effective medicinal plants as effective treatment for *E. coli* and related infections (Sandvig, 2001; Voravuthikunchai et al., 2005; Abong’o and Momba, 2009).

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, and that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. Since the levels of sanitation and hygiene are low for the majority of people in Africa compared to people in the First World countries, African people are therefore, to a large extent, exposed to a wider array of microbial pathogens, which increases their susceptibility to bacterial infections. However, because the African land is rich with medicinal herbs, they often resort to treating such infections with the local and indigenous plants, in situations where commercial drugs are not available or are too expensive (Fennel et al., 2004; McGaw et al., 2005; Yagoub, 2008; Lewu and Afolayan, 2009). For example, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich et al., 2004).

Different plant parts and components (roots, leaves, stem barks, flowers or their combinations, essential oils) have been employed in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin (Rojas et al., 2001; Ríos and Recio, 2005; Adekunle and Adekunle, 2009). Various chemical compounds (phytochemicals) with antibacterial activities exist in plants. Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources. These compounds are used by the plants as natural defences against bacteria, fungi and pests (Doughari and Obidah, 2008). In general, phenolics have been shown to be the predominant active chemical in plants, with gram positive bacteria being the most susceptible germs. Common methods used in the evaluation of the antibacterial and
antifungal activities of plant extracts and essential oils, include the agar diffusion method (paper disc and well), the dilution method (agar and liquid broth) and the turbidimetric and impedimetric monitoring of microbial growth (Ríos and Recio, 2005). These methods are simple to carry out under laboratory conditions, thus removing any barrier to the possible investigation of more plants for novel antibiotics.

2.1.1 Safety Concerns for Phytochemicals

Plants are natural reservoir of medicinal agents almost free from the side effects normally caused by synthetic chemicals (Fennel et al., 2004). The World Health Organization estimates that herbal medicine is still the main stay of about 75-80% of the world population, mainly in the developing countries for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side-effects (Kamboj, 2000; Yadav and Dixit, 2008). The over-use of synthetic drugs with impurities resulting in higher incidence of adverse drug reactions, has motivated mankind to go back to nature for safer remedies. Due to varied locations where these plants grow, coupled with the problem of different vanacular names, the World Health Organization published standards for herbal safety to minimize adulteration and abuse (WHO, 1999).

A number of modern drugs have been isolated from natural sources and many of these isolations were based on the uses of the agents in traditional medicine (Rizvi et al., 2009). Antimicrobial properties of crude extracts prepared from plants have been described and such reports had attracted the attention of scientists worldwide (Falodun et al., 2006; El- Mahmood and Amey, 2007; El- Mahmood, 2009). Herbs have been used for food and medicinal purposes for centuries and this knowledge have been passed on from generation to generation (Adedapo et al., 2005). This is particularly evident in the rural areas where infectious diseases are endemic and modern health care facilities are few and far; thus, compelling the people to nurse their ailments using local herbs.

Herbal treatments have been adjudged to be relatively safe (WHO, 1999). For instance, daily oral doses of epigallocatechin-3-gallate (EGCG) for 4 weeks at 800 mg/day in 40 volunteers only caused minor adverse effects (Phillipson, 2007). In a 90-day study of polyphenon E (a formulation of green tea extract with 53% Doughari et al. 841 EGCG), the oral no-effect level (NOEL) values are 90 mg/kg/day for rats and 600 mg/kg/day for dogs (Boocock et al., 2007). For curcumin, given to cancer patients at
3600 mg/day for 4 months or 800 mg/day for 3 months, only minor adverse effects are seen. For resveratrol, a single oral dose at 5 g in 10 volunteers only causes minor adverse effects (Boocock et al., 2007). Though herbs are relatively safe to use, their combined use with orthodox drugs should be done with extreme caution. Concomitant use of conventional and herbal medicines is reported to lead to clinically relevant herb–drug interactions (Liu et al., 2009).

The two may interact either pharmacokinetically or pharmacodynamically resulting into adverse herbal-drug interactions (Izzo, 2005). St John’s wort (Hypericum perforatum), used for the treatment of mild to moderate depression, interacts with digoxin, HIV inhibitors, theophylline and warfarin. Some medicinal herbs, when ingested, either affect cytochrome P450 isoenzymes by which drugs are metabolized, or, phosphoglycoprotein transporter systems that affect drug distribution and excretion. Concurrent use of some herbal medicines with other medicines may either lower blood plasma concentrations of medicinal drugs, possibly resulting in suboptimal therapeutic amounts, or lead to toxic concentrations in the blood, sometimes with fatal consequences (Phillipson, 2007). Despite this observation however, it has been reported that phytochemicals act in synergy with chemotherapeutic drugs in overcoming cancer cell drug resistance and that the application of specific phytochemicals may allow the use of lower concentrations of drugs in cancer treatment with an increased efficacy (Liu, 2004).

Another advantage with phytochemicals is that, among an estimated 10,000 secondary products (natural pesticides), it has been proposed that human ancestors evolved a generalized defense mechanism against low levels of phytochemicals to enable their consumption of many different plant species containing variable levels of natural pesticides (carcinogens) without subsequent ill health (Liu, 2004). Traces of phytochemicals found in fruits and vegetables may potentiate the immune system and help to protect against cancer (Trewavas and Stewart, 2003). Phytochemicals show biphasic dose responses on mammalian cells. Though at high concentrations they can be toxic; sub-toxic doses may induce adaptive stress response (Ames and Gold, 1991).

This includes the activation of signaling pathways that result in increased expression of genes encoding cytoprotective proteins. It is therefore suggested that hermetic mechanisms of action may underlie many of the health benefits of
phytochemicals including their action against cancer drug resistance (Mattson, 2008). Several phytoconstituents also act as antioxidants. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide anion, peroxyl radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage (Mattson and Cheng, 2006).

Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischemia, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing (Uddin et al., 2008; Jayasri et al., 2009). The antioxidants act by reacting with free oxygen radicals. The free radicals are metastable chemical species, which tend to trap electrons from the molecules in the immediate surroundings. These radicals, if not scavenged effectively in time, may damage crucial biomolecules like lipids, proteins (including those present in all membranes), and DNAs resulting in abnormalities leading to disease conditions (Uddin et al., 2008).

Thus, free radicals are implicated in a number of diseases including tumor inflammation (tumorigenesis), hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g. parkinsonism, Alzheimer’s diseases), AIDS and even early senescence (Chen et al., 2006; Uddin et al., 2008). Although in relatively insufficient amounts, the human body produces antioxidant metabolites (such as glutathione, melatonin, etc) and antioxidant enzymes (such as superoxide dismutase, catalases, etc) which are essential for preventing oxidative stress resulting from free radical accumulation in body tissues. Therefore, this insufficiency had to be compensated for by making use of natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, carotenoids and natural products in plants (Sen, 1995; Madsen and Bertelsen, 1995; Rice-Evans et al., 1997; Diplock et al., 1998).

Plants contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity (Madsen and Bertelsen, 1995; Cai and Sun, 2003). Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics which
possess the ability to scavenge the free radicals in human body. Significant antioxidant properties have been recorded in phytochemicals that are necessary for the reduction in the occurrence of many diseases (Hertog and Feskens, 1993; Anderson and Teuber, 2001). Many dietary polyphenolic constituents derived from plants are more effective antioxidants in vitro than vitamins E or C, and thus might contribute significantly to protective effects in vivo (Rice-Evans and Miller, 1997; Jayasri et al., 2009).

Studies to uncover other novel plant products especially those with potential activity against verocytotoxic bacteria has become very necessary. This is due to the emergence of bacteria producing these toxins and the abundance of predisposing factors ranging from fecal contamination of food and water sources to low level of hygiene and sanitation consciousness in the developing countries. The ready availability of these plants should be a motivating factor in embarking of such a research.

2.2 The genus Saccharum

The literature review on Saccharum emphasizes the traditional use and clinical potential of Saccharum species, especially S. munja. Additionally, it raises aquestion on traditional claims of these species, which have not been proven scientifically.

For review major databases such as Chemical Abstracts, Medicinal and Aromatic Plants Abstracts, Pub Med, King’s American Dispensatory, Raintree Nutrition Incorporation, Henriette’s Herbal Homepage, National Agricultural Library (AGRICOLA), Duke’s Phytochemical and Ethno botany database, UK Cropnet Ethnobotany database, Archives of American Folk Medicine and USPTO Patent Full Text and Image database has been referred.

Saccharum is a genus of 35-40 species that grow throughout the tropics and subtropics. Nine species can be found in the Flora region; five are native, two are grown as ornamentals, one is grown for agriculture, and one for research. Some species of Saccharum hybridize naturally with other, presumably closely related, genera such as Miscanthus, Imperata, and Sorghum. Species with awned lemmas are sometimes placed in a separate genus, Erianthus. The most familiar species of Saccharum is S. officinarum, sugar cane. (Hodkinson et al., 2002)

2.2.1. Saccharum officinarum
S. officinarum Linn having flowering region known as arrowing has soft-rinded, puffy nature. Antioxidant activity of inflorescence of S. officinarum L. could be linked to the presence of secondary plant products like flavonoids and phenols, which have the ability to scavenge hydroxyl radicals and lipid peroxy radicals. S. officinarum Linn is soft-rinded annual herb belongs to the Andropogonae tribe of the family Gramineae in order to Glumiflorae with a class Monocotyledoneae, subdivision Angiospermae and division Embryophita siphonogama. The subtribe is Sacharae and the genus, of course is Saccharum, derived from the Sanskrit "sarkara = white sugar". (Bachulkar, 2007; Bhore et al, 2012)

It is used as a folk medicine and also used as an antidote, antiseptic, antivenomous, bactericide, cardiotonic, demulcent, diuretic, intoxicant, laxative, pectoral, refrigerant, and stomachic. It is a folk remedy for arthritis, bedsores, boils, cancer, colds, cough, diarrhoea, dysentery, eyes, fever, hiccups, inflammation, laryngitis, opacity, penis, skin, sores, sore throat, spleen, tumors, and wounds. Powdered sugar is used as a drawing agent for granulations and proud flesh and in a 1:3 solution in water, for gonorrhea and vaginal discharges. A decoction of the root of the race of 'tebu lanjong' is used for whooping cough; and the cane juice is given for catarrh. It is used in elephant medicine; the juice is used to 'make an elephant sagacious', and in a poultice for sprains. In India, the plant as well as its juices is used for abdominal tumors. (Burkill, 1966; Duke and Atchley; Hartwell, 1967-71; Watt and Breyer-Brandwijk, 1962)

Sugarcane products are appreciated as food in social-economically disfavoured areas of Brazil and other countries. Results of the present work indicate that consumption of these products should be encouraged. Some of them, such as “rapadura”, are cheap sources of antioxidant substances, vitamins (A, C and D) and minerals (Fe, Ca, P, K, Mg) (Bernal, Guzman & Jimenez 2004). The extracts of Sugarcane inflorescence was carried out using petroleum ether, chloroform, Dichloromethane, Ethyl acetate, n-Butanol, Methanol and Distilled water as solvents. The Phytochemical investigation of extracts revealed the presence of alkaloids, tannins, anthraquinones, reducing sugars, saponins, flavonoids, polyphenols, steroids and terpenoids. Some of these fractions were screened for free radical scavenging activity (Antioxidant) using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). The better scavenging activity of Saccharum officinarum L. could be
linked to the presence of secondary plant products like flavonoids and phenols, which have the ability to scavenge hydroxyl radicals and lipid peroxyl radicals. (Bhore et al., 2012)

Tricin isolated from juice of *S. officinarum*. It is a flavone identified by spectroscopic methods as tricin-7-O-β-(6″-methoxycinnamic)-glucoside was isolated along with orientin. The tricin derivative was shown antioxidant activity higher than Trolox. It showed in vitro antiproliferative activity against several human cancer cell lines with higher selectivity towards cells of breast resistant NIC/ADR lines. (Duarte-Almeida et al., 2007).

![Tricin](image)

Tricin

Saccharum officinarum methanolic extract owing to its potent antiestrogenic nature alters the biochemical milieu of the reproductive tract which lead to change the normal status of the reproduction in female reproductive tract of rat and thus produce significant antifertility effect. (Balamurugan et al., 2009). Stem of *S. officinarum* reported to contain Abscisic acid, apigenin, glycoside, methyl lapigenin, arabinose, arunodin, benzoic acid, campesterol, coumarin, cylindrin, orientin, fructose, glucose, sucrose, invert sugar, ether, tricin and vicenin (Abbasi et al., 2009; Prajapati et al., 2006)

### 2.2.2 Saccharum spontaneum

*S. spontaneum* Linn. Synonyms, Ahlek, loa, wild cane, wild sugar cane, Family: Poaceae. The whole plant according to siddha the whole plant used to diseases of vatam
and pittam, vomiting, mental diseases, abdominal disorders, dyspnoea, anaemia, and obesity. The root according to ayurveda roots are sweet, astringent, emollient, refrigerant, diuretic, lithotriptic, purgative, tonic, aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles, sexual weakness, gynecological troubles, respiratory troubles etc. The stems (culm) are useful in vitiated conditions of pitta and vata burning sensation strongly, renal and vesicol calculi dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility. Leaves are employed for broom (cathartic and diuretics). It possess strong Allelochemicals and Allelopathic properties. Hence it may an absolute necessity to create a profile in regards to create a profile in regards to their identification and then Standardisation which may lead to further scientific investigations. (Anonymous, 1990; Harbone, 2005; Kumar, 2009; Suresh Kumar, 2010; Trease, 2002; Yognarashimhan, 2002)

2.3 Saccharum munja

_S. munja_, known as munja is a grass found in arid areas and along river banks in India. The grass is tall, panicles silky and greenish brown. The grass grows in excess and up to Seven feet in height. Leaf sheath shortly silky at extreme base, otherwise quite smooth, striate, pale straw colored, villous on margins at apex with long white hairs usually much longer than proper internode, uppermost sheath sometimes extending beyond the base of panicle. Its white flowers are of ornamental value.

The common name of the plant is _Kana_ or _sarkanda_ or _Moonja_ and is distributed from north and North West India to Pakistan and Afghanistan. There are 24 vernacular names of plant in Sanskrit language such as bahupraja, bana, bhadramunja, brahmanya, chakshuveshtana, darbhavhaya, dridhatrina, durmula, ikshukanda, maunji, munja, munjanaka, munjata, ranjana, sara, shakrabhanga, shara, shiri, sthuladarbha, sumekhala, tejana, tejanavhaya, trinakhaya, vaniraka. Flowering and fruiting are perennial and mostly from October to January. The plant is a large tufted grass and is of little account as fodder plant because cattle and buffaloes eat young leaves only during scarcity of food. The stem is used in making chiks and moorhas. The flowering scape is woven into winnowing trays (chhaj) and cover for grain heaps and carts, while the thick sheath next to scape is twisted into strings for weaving bed-streeds etc. (Chopra, 2005; The Ayurvedic pharmacopoeia of India, 2007; Kirtikar and Basu, 1935)
Figure 1: *Saccharum munja*

Table 2: Classification of *S.munja*

<table>
<thead>
<tr>
<th>Kingdom/Regno</th>
<th>Planate (Plants/Piante)</th>
</tr>
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<tbody>
<tr>
<td>Subkingdom/Sottoregno</td>
<td>Tracheobionta (Vascular plants/Piante Vascolari)</td>
</tr>
<tr>
<td>Superdivision/Superdivisione</td>
<td>Spermatophyta (Seed plants/Piante con Semi)</td>
</tr>
<tr>
<td>Division/Divisione</td>
<td>Magnoliophyta (Flowering plants/Piante Con fiori)</td>
</tr>
<tr>
<td>Class/Classe</td>
<td>Liliopsida(Monocotyledons/Monocotiledoni)</td>
</tr>
<tr>
<td>Subclass/Sottoclasse</td>
<td>Commelinidae</td>
</tr>
<tr>
<td>Order/Ordine</td>
<td>Cyperales</td>
</tr>
<tr>
<td>Family/Famiglia</td>
<td>Poaceae (Grass family)</td>
</tr>
<tr>
<td>Genus/Genere</td>
<td><em>Saccharum</em> L. (sugarcane)</td>
</tr>
<tr>
<td>Species/Specie</td>
<td><em>Munja</em>/Bengalense/Sara</td>
</tr>
</tbody>
</table>
Table 3: Synonyms of *S.munja*

<table>
<thead>
<tr>
<th>Language</th>
<th>Synonyms</th>
<th>Language</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanskrit</td>
<td>Bhadra, Munja</td>
<td>Orrisa</td>
<td>Sara</td>
</tr>
<tr>
<td>Bengali</td>
<td>Sara</td>
<td>Punjabi</td>
<td>Moonja, Sarkanda</td>
</tr>
<tr>
<td>Gujrati</td>
<td>Sarkat</td>
<td>Tamil</td>
<td>Munjipul, Munjapullu</td>
</tr>
<tr>
<td>Hindi</td>
<td>Sarkand, Munja</td>
<td>Telugu</td>
<td>Gendra, ponica</td>
</tr>
<tr>
<td>Kannad</td>
<td>Munji, Hullu, Hodake Hullu</td>
<td>Tibetan</td>
<td>Dambu</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Ama, Amaveru, Sara, Munjapullu</td>
<td>Urdu</td>
<td>Munja, Sarkanda</td>
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<tr>
<td>Marathi</td>
<td>Munja, Trikande, mole</td>
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Table 4: Species of genus *Saccharum.*

<table>
<thead>
<tr>
<th>Species/Specie</th>
<th>Species/Specie</th>
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</thead>
<tbody>
<tr>
<td><em>Saccharum alopecuroides</em></td>
<td><em>Saccharum japonicum</em></td>
</tr>
<tr>
<td><em>Saccharum alopecuroideum</em></td>
<td><em>Saccharum kanashiroi</em></td>
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<tr>
<td><em>Saccharum angustifolium</em></td>
<td><em>Saccharum koenigii</em></td>
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<tr>
<td><em>Saccharum arenicola</em></td>
<td><em>Saccharum longisetosum</em></td>
</tr>
<tr>
<td><em>Saccharum arundinaceum</em></td>
<td><em>Saccharum munja</em></td>
</tr>
<tr>
<td><em>Saccharum asperum</em></td>
<td><em>Saccharum munroanum</em></td>
</tr>
<tr>
<td><em>Saccharum balansae</em></td>
<td><em>Saccharum narenga</em></td>
</tr>
<tr>
<td><em>Saccharum barberi</em></td>
<td><em>Saccharum officinarum</em></td>
</tr>
<tr>
<td><em>Saccharum beccarii</em></td>
<td><em>Saccharum paniceum</em></td>
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<tr>
<td><em>Saccharum biflorum</em></td>
<td><em>Saccharum pappiferum</em></td>
</tr>
<tr>
<td><em>Saccharum brevibarbe</em></td>
<td><em>Saccharum perrieri</em></td>
</tr>
<tr>
<td><em>Saccharum ciliare</em></td>
<td><em>Saccharum procerum</em></td>
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</table>
2.3.1 Nutritive Elements in *S. munja*

Plants possessing their antibiotics properties have been well known since old civilization of Romans, Egyptians and Arabs. The plant barks, leaves, roots, juices, gums, fruits and seeds in heterogenous ground mixture or in solution form are used in the treatment of several diseases. Keeping in view the medicinal importance of Saccharum munja, inorganic nutrients were determined from its stem, flowers and adjacent soils. Table 5 shows that the sodium level in stem (5-10 ppm) is lower than the flower (10-40 ppm), while 10 ppm in their soils. Level of pottassium in stem (40.8-70.9) is higher compared to the level in flowers (0.4-10.4 ppm) of the plant and the soils (10-35 ppm). Sodium and Potassium cations perform various electrochemical functions in the human body such as emulsion stabilization, charge neutralization and supply of free energy during cell stimulation. During muscle contraction and nerve stimulation Na\(^+\) enters and Ka\(^+\) leaves the cell the potential difference across the cell membrane changes temporarily. The ATP (Adenosine triphosphate), the metabolic energy is then needed to pump Ka\(^+\) back and Na\(^+\) out for normal condition. Na\(^+\) and Ka\(^+\) stabilize the oil in water emulsion and maintain the solubility of proteins as globulin is insoluble in water but soluble in dilute salt solutions.

The plant stem (24-80 ppm) and flowers (24-120 ppm) has higher levels of calcium as compare to soil (0.14-6 ppm) in which saccharum munja is planted. Calcium plays an important role as factor IV in blood clotting mechanism. Calcium alongwith thromboplastin is required to convert prothrombin to thrombin, which activate fibrinogen to fibrin network. The level of magnesium in plant stem (43-430 ppm) and flowers (0.48-48 ppm) is higher than its level in soil (0.21-1.26 ppm). Magnesium plays an important role in the activation of enzymes which transfer phoshate from adenosine triphosphate to adenosine triphosphate which are fundamental and widespread and thus influence all basic life processes such as glycolysis. The physical stability of DNA, chromosomes and ribosomes are also depends on magnesium. Therefore a certain amount of magnesium is required for cell duplication. However there is no deficiency of Mg\(^{2+}\) in the body but during renal failure and alcoholism magnesium deficiency may be there which may cause muscular tremor, hallucination, depression and spasmophilia. Excess amount of magnesium may cause neuromuscular transmission blockage.
Table 5: Comparison of Nutrients in *S. munja*

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sample Type</th>
<th>pH</th>
<th>Moisture %</th>
<th>Organic Content</th>
<th>Soluble Salts %</th>
<th>Na⁺ PPM</th>
<th>k⁺ PPM</th>
<th>Ca²⁺ PPM</th>
<th>Mg²⁺ PPM</th>
<th>Fe³⁺ PPM</th>
<th>Cl⁻ PPM</th>
<th>HCO₃⁻ PPM</th>
<th>PO₄³⁻ PPM</th>
<th>SO₄²⁻ PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Soil</td>
<td>Soil</td>
<td>7.78</td>
<td>7.14</td>
<td>4.48</td>
<td>15.38</td>
<td>10.05</td>
<td>35</td>
<td>6.0</td>
<td>1.2</td>
<td>8600</td>
<td>17.8</td>
<td>410</td>
<td>0.13</td>
<td>100</td>
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<tr>
<td></td>
<td>Stem</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>40.8</td>
<td>40</td>
<td>430</td>
<td>285</td>
<td>178</td>
<td>490</td>
<td>1.04</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Flower</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>0.4</td>
<td>32</td>
<td>31.2</td>
<td>218</td>
<td>199</td>
<td>820</td>
<td>0.35</td>
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<tr>
<td>B Soil</td>
<td>Soil</td>
<td>7.60</td>
<td>3.45</td>
<td>7.40</td>
<td>10.29</td>
<td>10.20</td>
<td>10.15</td>
<td>0.27</td>
<td>0.21</td>
<td>229</td>
<td>78.4</td>
<td>106</td>
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<tr>
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<td>Stem</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>70.9</td>
<td>80</td>
<td>140</td>
<td>569</td>
<td>157</td>
<td>710</td>
<td>1.0</td>
<td>154</td>
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<tr>
<td></td>
<td>Flower</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>10.3</td>
<td>24</td>
<td>28.8</td>
<td>553</td>
<td>500</td>
<td>288</td>
<td>0.8</td>
<td>240</td>
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<tr>
<td>C Soil</td>
<td>Soil</td>
<td>7.30</td>
<td>13.63</td>
<td>3.93</td>
<td>14.50</td>
<td>10.00</td>
<td>10.00</td>
<td>0.14</td>
<td>1.26</td>
<td>172</td>
<td>71.2</td>
<td>600</td>
<td>0.25</td>
<td>250</td>
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<tr>
<td></td>
<td>Stem</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>60.1</td>
<td>24</td>
<td>43</td>
<td>704</td>
<td>203</td>
<td>300</td>
<td>0.7</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>Flower</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>10.4</td>
<td>120</td>
<td>48</td>
<td>540</td>
<td>311</td>
<td>600</td>
<td>0.35</td>
<td>48</td>
</tr>
</tbody>
</table>
(Average value, n = 4, Confidence interval at 95%)

High level of iron (172-860ppm) is present in soil as compared with plant stem (285-704) and plant flowers (218-553). The human body contain 4-6 gm of Fe out of which 1 gm is present in liver and spleen. Iron is present in body as a component of haemoglobin and myoglobin and responsible for oxygen transport and cell respiration. Iron is also present in intracellular cytochrome enzyme system, which is responsible for energy production. During pregnancy the high intake of iron is required due to iron deficiency. The daily requirement of iron is 10-20 mg if not maintain the deficiency will be there which may lead to hemolytic anemia, stomatitis, listlessness, palpitation, excretion and slow physical activity. The extra intake of iron (above 50 mg) accumulates in the liver in the form of haemosidrin and lead to haemachromatosis having symptoms of gray, skin pigmentation, hepatic enlargement, pancreatic infiltration with diabetes and myocardial diseases leads to heart failure.

Chlorides found in stem (157-203ppm) and flowers (199-500ppm) is higher than the soil (17.8-78ppm). Chloride is absorbed from the soil in the form of chloride ions (Cl\(^{-}\)) without becoming a structural unit and is essential for water balance, regulation in osmotic pressure and acid base equilibrium. It may be used in cell division in roots and leaves. Bicarbonates in stem (300-710ppm) and flowers (288-820ppm) as compared to soil (106-600ppm). Pancreatic secretion consists of an aqueous bicarbonate component from the duct cells and enzymatic component from the acinar cells and is alkaline in nature due to the high concentration of bicarbonate ions. This is useful in neutralizing the acidic gastric acid, allowing for effective enzyme action. Bicarbonate/chloride (HCO\(_3\)^{-}/Cl\(^{-}\)) exchangers regulate intracellular pH in the alkaline range.

Phosphate ions in stem (0.7-1.04ppm) and flowers (0.35-0.8ppm) is higher than soil (0.1-0.25ppm). Phosphate is used in transport of fatty acids within the cell. It is also used in storage and transfer of metabolic energy through phosphate bonds such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP). Sulphate ions lower in plant stem (86-154ppm) and plant flowers (20-240ppm) as compared to adjacent soil (100-250ppm). Sulphur is also responsible for high energy bond formation as in acetyl coenzyme-A and in derivative of lipoic acid (thioctic acid). The sulphahydryl group (-SH) binds with heavy metals by acting as chelating agent and is used in detoxification.
mechanism. The example of detoxication agent is “British antilewisite” which act by binding the arsenic atoms in chlorovinylarsine poison gas. Similarly B-penicillamine act as detoxification agent in lead poisoning. (Adrian, 1986; Gamick and Lamb, 1958; Hewitt and Smith, 1974; Kabata and Pendias, 1986; Tirmizi et al., 2005)

2.3.2 Organic Constituents

Stem yields 5.67% of furfural on dry basis. It also yields 19.5% of reducing sugars like glucose, xylose, galactose and rhamnose when digested with sulphuric acid. The hydrolysate contains about 34.5% of fermentable sugars as per Ayurvedic pharmacopoeia.

2.3.3 Therapeutic uses

*S. munja* is used as refrigerant, in burning sensation, thirst, herpes, dyspepsia, dyscaria, erysipelas, urinary complaints and diseases of eyes. Roots of saccharum munja used in dysuria, giddiness and vertigo. Saccharum munja are used to cure bleeding wounds. Roots of *S. munja* also used against fever and inflammation. *S. munja* grass as a type of gauze-pad to stop blood flow. The smoke of roots burnt near women after delivery and burns scalds. According to ayurveda *S. munja* has following properties: Aksiroga, Bastisula, Bhrama, Daha, Guna, Karma, Mutrakrcchra, Murcha, Rasa, Trsna, Virya, Vipaka, and Visarpa.

2.3.4 Important Formulations containing *S. munja*

There are number of Ayurvedic formulations containing *S. munja* manufactured and marketed by Ayurvedic companies such as Parvek and Aimil Pharmaceuticals (India) Ltd. Table 6 shows the different formulations containing *S. munja* as an active ingredient. These formulations are useful in many disorders such as burning and painful micturition, urinary stones and infections. (Rahar et al., 2010)

**Table 6:** Ayurvedic Formulations containing *S.munja*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation</th>
<th>Ingredients</th>
<th>Therapeutic Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ashmari Kalp</td>
<td><em>Saccharum munja</em></td>
<td>Burnings and painful micturition.</td>
</tr>
</tbody>
</table>
2. Neeri

Saccharum munja

Urinary stones and Infections

3. Tripancamula Kvatha Curna

Saccharum munja

Burning sensations, thirst and dysuria.

4. Brahman Rasayana

Saccharum munja

Used in painful micturations and urinary complaints.

5. Sukumara Ghra

Saccharum munja

Diuretic and painful micturations.

2.4 Standardization of plant drugs

India has 16 agro climatic zones, 45,000 different plant species and 15,000 medicinal plants (India Herbs, 2002). Indian systems of medicine have identified 1500 medicinal plants, of which 500 species are mostly used in the preparation of drugs. Three of the ten most widely selling herbal medicines in the developed countries, namely preparation of Allium sativum, Aloe barbadensis and Panax spp. are available in India (Dubey, Kumar and Tripathi, 2004). Despite a vast flora and fauna of medicinal plants, India’s share in global export of medicinal plants related trade is just 0.5%.

Global market of herbal medicines and herbal personal care products/herbal cosmetics was US $8 billion and US $22 billion respectively in year 1999 with annual growth rate of 20% (Herbal Sector, 2004; Wakdikar 2004). The European market of herbal medicines is US $6.8 billion (Calixto, 2000; Expo Europe, 2004). The German market corresponds to about 42% of European market. This market is followed by France 25%, Italy 9%, United Kingdom 8%, Poland 5%, Spain 3%, The Netherlands 3% and others 5%. The herbal medicine markets in Asia and Japan are US $2.3 and 2.1 billion
respectively. The domestic market of Indian systems of medicine and Homoeopathy was of the order of Rs. 4,000 crores in year 2000 (India Herbs, 2002).

During the last twenty years, herbal medicines have enjoyed renaissance among consumers throughout the developed world (Hylands, 2002). For centuries, preparations based on traditional folk uses have served as the main form of medical treatment. In most western countries, plant-based preparations or medicines were discarded during the early part of the twentieth century with the advent of modern medical science. However, in many parts of the world, especially, Asia, traditional herbal medicines are still the integral part of primary health care.

With the advent of twenty first century, plant based medicines and neutraceuticals have once again occupied the centre stage for treating many of today’s common illnesses and health problems as most of modern synthetic medicines are associated with severe adverse effects. While consumer acceptance of these products is high, there are concerns voiced mainly by regulatory agencies that current quality control and standardization procedures for botanical products are not sufficient to ensure their efficacy and safety. If plant-based medicines are to gain wider acceptance not only from the public, but from the regulatory authorities as well, then there must be new approaches to solve the problems of quality control and standardization.

Standardization of a drug guarantees its chemical consistency, therapeutic efficacy and reproducible biological activity (Hawkins, 2001). Standardization of plant derived drugs involves the collection of information and application of stringent quality control measures at every step of the process from the growing of a medicinal plant to the finished therapeutic substance (Bonati, 1991). It includes complete description of the starting drug, control and monitor on the factors viz., growing conditions, harvesting time, part of plant harvested, absence of toxic pesticides or other contaminants, drying methods, freshness and storage, extraction process, and analytical controls which are required for providing constancy of the quality of an extract. Nomenclature which includes the name of the drug, physical state, solvent for extraction and composition of the extract also form an important part of standardization.

Another aspect of standardization is that it guarantees the content of one or more active constituents or marker compounds. According to Willard “Standardization should
involve the compilation of complete data on a herb such as the season in which the herb is picked, the ripeness, the taste, smell, appearance, drying, storage, processing and fingerprinting which needs much larger spectrum of constituents, usually five or more active or marker constituents” (Willard, 1999).

Standardization is also considered a way to deal with the regulations, framed by regulatory authorities that require drug measurability, and active ingredients to be stated on product labels (Linda, 2002). Inconsistent and variable biological effects of plant-derived medicines are, perhaps, the main discouraging issues for researchers in the field of natural products. Reproducible efficacy and safety of the phyto-pharmaceuticals are based on reproducible quality. Therefore, a phyto-pharmaceutical could only be considered as a rational drug if it is standardized and its pharmaceutical quality is approved. Also, in pharmacological, toxicological, and clinical studies with herbal drugs, their composition needs to be well documented in order to obtain reproducible results (Bauer, 1998; Food and Drug Administration, 1998).

The World health Organization (WHO) has recognized this problem and has published guidelines to ensure the reliability and repeatability of research on herbal medicines (Bauer et al,1994). Besides research, this concept must be followed in the commercial production and therapeutic application of phyto-pharmaceuticals. Usually this undesirable biological and phytochemical variability of the plant material is due to different growth, harvest, drying and storage conditions. Therefore, cultivation of plants unde standardized conditions is desirable. The polarity of solvent, mode of extraction, instability of the constituents may influence the composition of extracts, and must, therefore, be kept constant (Bauer, 1998; Food and Drug Administration, 1998).

The WHO has issued the following guidelines for the assessment of herbal medicine.

1. **Quality assessment**: crude plant material, plant preparation and finished product.
2. **Stability**: shelf life.
3. **Safety assessment**: documentation of safety based on experience or toxicology studies.
4. **Assessment of efficacy**: documented evidence of traditional use and/or activity determination on animal or human models. (WHO, 1991)
The steps in standardization and quality control include:

1. Determining the identity of the plants from classical texts and equating it to scientific botanical identity.
2. Ensuring that the correctly identified raw material is supplied and used.
3. Ensuring that the correct formulation and the correct quantities are used.
4. Ensuring that the correct procedures of preparation, storage and packaging are adopted.

Standardized herbal extracts are of two main types: an active constituent extract where there is a known and accepted active biochemical principle, and a marker extract where the active biochemical principle is not known and a characteristic compound is used as a marker (Tierra, 1998). An active constituent extract, is like a drug and may have undesirable side effects which are normally absent in the herb. In the marker extract, a unique constituent is selected as a marker and as no single active constituent is known, so the entire extract is treated as active. Table 6 shows examples of active constituent extract and marker extract.

Standardization of Ayurvedic drugs is necessary seeing the growing popularity of medicine in western countries. The standardized medicinal herbs include:

1. Adhatoda vasica (0.5% vasicine)
2. Allium sativum (0.6% allicin)
3. Andrographis paniculata (10% andrographolide)
4. Asparagus racemosus (30% saponins)
5. Azadirachta indica (2% azadiractin)
6. Bacopa monnieri (20% bacoside)
7. Boswellia seratta (40 and 70% boswellic acid)
8. Capricum frutescens (0.62% capsaicinoids)
9. Commiphora mukal (5% guggal-sterones)
10. Embelia ribes (8% embelin)
11. Gymnema sylvestre (75% gymnemic acid)
12. Momordica charantia (3% bitters)
13. Ocimum sanctum (8% ursolic acid)
14. Phyllanthus niruri (2% bitters)
15. Picrorrhiza kurroa (10% kutkosides)
16. Pueraria tuberosa (7% diosgenin)
17. Saraca indica (8% tannins)
18. Terminalia arjuna (8% tannins)
19. T. belerica (40% tannins)
20. T. chebula (60% tannins)
21. Tribulus terrestris (20 and 40% saponins)
22. Trigonella foenum graecum (10% saponins)
23. Withania somnifera (1.5% withanolides)
24. Zingiber officinale (5% gingrols) (Singh A, 2004)

Bonati has described the standardized extracts as the ones:
1. Having consistent levels of specified compounds.
2. Having recognized active constituents as well as a variety of other plant constituents.

Standardized extracts have consistent activity which allows more accurate prescribing resulting in consistent clinical results. Further, extensive quality control ensures the quality and safety of standardized extracts. One of the major problems in standardizing plants is that they contain many complex and different chemical constituents (Hylands, 2002).

Oxford Natural Products (ONP) has developed an authority which has specified stringent criteria for standardizing plant based medicines. This helps manufacturers to standardize and validate plant products. Over the last two years, total quality profiling (TQP) has been developed which combines a number of technologies in a unique way to overcome problems associated with product definition and variability. Through the use of TQP manufacturing, one can achieve careful control on characterization of the plant materials and their preparation through an innovative combination of plant science, chemistry and biology applied in a uniquely complementary way. Phytotract – a proprietary software system contains the information about all key stages through which plant raw material passes from field to formulation such as, agronomic performance, soil
conditions and environmental elements including rainfall, chemistry and microbiology of the crop.

The quality and purity required in a drug are achieved by standards given in the official work of reference. To establish the identity and quality of the drug several methods may be considered (Handa, 1996; Evans, 1996; Anonymous (IP), 1996; Kokate, Purohit and Gokhale, 1999; Wallis, 1999).

These are enumerated below:

a. Morphology and Organoleptic evaluation. In case of whole drug, the macroscopic and sensory characters are usually sufficient to enable the drug to be identified. These include color, odor, taste, size, shape, fracture, etc.

b. Histology and Microscopy. These are valuable both for powders and ungrounded drugs. Type of epidermal parenchyma, stomata, trichomes, fibres, vessels, calcium oxalate crystals, etc., help in the identification of drug.

c. Quantitative Microscopy. Microscopical determinations such as vein-islet number, veinlet termination number, palisade ratio, stomatal number, stomatal index, determination of size of fibres, vessels, etc., help in the identification of the plant, and in differentiating closely allied species.

d. Solubilities, especially exceptional behaviour towards solvents, are useful for the examination of many oils, oleo-resins, etc.

e. Qualitative chemical tests. Most of these tests are color reactions which are specific for certain substances. These tests include the general tests for alkaloids, glycosides, tannins, flavonoids, etc.

f. Quantitative chemical tests. A number of quantitative chemical tests viz., acid value, iodine value, saponification value, ester value, unsaponifiable matter, acetyl value and volatile acidity are useful in the evaluation of fixed oils, resins, balsams, volatile oils and gums.

g. Physical constants such as specific gravity, optical rotation, viscosity and refractive index are especially valuable for the evaluation of oils and fats, oleo-resins, balsams, and similar substances.
h. Ash values. These are useful for detecting excess of sandy or earthy matters. The presence of ash is determined as total ash, acid insoluble ash, water soluble ash and sulphated ash.

i. Extractive values. The determination of extractable matter refers to the amount of constituents in a plant material extracted with specific solvents. It indicates the nature of constituents of the plant drug, and also helps in detecting low grade exhausted drugs.

j. Moisture content. Moisture content must be determined and controlled so as to prevent decomposition in the plant material. Methods which are commonly employed for the determination of moisture are loss on drying, toluene distillation, gas chromatographic method, Karl Fischer method and spectroscopic methods.

k. Volatile oil. Pharmaceutical significance of aromatic drugs is due to their odorous principles, i.e., volatile oils. These drugs are standardized on the basis of their volatile oil content.

l. Crude fibre. Determination of crude fibre is done to determine the presence of excessive woody material.

m. Microbial contamination. It is applied for crude drugs which are to be taken internally.

n. Toxic residues. These may arise due to pesticide application and fumigation. It can be reduced by use of infusions of the dried plant material.

o. Chemical fingerprinting. Various chromatographic procedures and spectroscopic methods are employed to develop the chemical fingerprint profile, thus allowing all components in the extract to be detected (Hylands, 2002).

p. Biological profiling. Biological profiling identifies biologically active plants allowing highly sophisticated standardization and quality control.

q. Assays. Crude drugs may be assayed for a particular group of constituents using chemical, spectrometric and radioimmunoassay.

2.4.1 Challenges in the standardization of plant drugs

1. Biological variations: Plants are rich source of chemicals and potential sources of effective medicines, but the chemical constituents of plants vary depending on the
species, variety and part of the plant, conditions of growth (soil, water and temperature) and age of the plant (Therapeutics Letter, 1998). These complexities and variations of chemical content make standardization essential.

2. **Selection of markers:** The major drawback associated with the process of standardization is selection of markers (Tierra, 1998). Most of standardized herbal extracts are not consistently standardized to one marker because it is not certain which of its constituents are responsible for its therapeutic actions. Nettle root is standardized by one company to 5% amino acids, by another to 8% sterols, and a third uses 35 ppm scopoline. Echinacea can be standardized to three different constituents, i.e., echinacosides, polysaccharides or polybutylides. Sometimes an active compound for any given herb may change in time such as the hyperforin of St. John’s wort recently understood to be more active than its previous marker, hypericin. Moreover, any medicinal herb does not exhibit single biological activity, but has long tradition of use in the treatment of various ailments and different constituents are responsible for different activities.

**Turmeric**

- **Analgesic**
  - Borneol, caffeic acid, curcumin, p-cymene, eugenol

- **Anti-dermatitic**
  - Guiacol

- **Anti-edemic**
  - Borneol, caffeic acid, caryophyllene, curcuminoids, eugenol

- **Anti-inflammatory**
  - Azulene, borneol, caffeic acid, caryophyllene, cinnamic acid, Curcumin, eugenol, protocatechuc acid, vanillic acid, b-sitosterol

- **Inhibit cyclooxygenase**
  - Curcumin, galangin

- **Inhibit lipooxygenase**
  - Borneol, caffeic acid, cinnamic acid

- **Inhibit 12-lipooxygenase**
  - Curcumin

- **Tumour necrosis fact.(inhibit)**
  - Curcumin

Turmeric is generally standardized on the basis of curcumin (95%) but this standardized extract can not be relied upon to be effective for skin conditions (antidermatitic).

3. **Role of Federal Regulatory Authorities (FRA):** Lack of proper control by FRA results in marked variation in strength of marketed herbal formulations. Consumer reports
found that 25% of ginseng products on health food store shelves did not contain any of the listed compounds, and the contents of products varied widely (Herbal Roulette, 1995). Another study commissioned by Los Angeles Times found that three of the ten different national brands of St. John’s wort over the counter remedies, contained less than half the active ingredients claimed on the label and seven products contained between 75 to 135% of the active ingredients as labeled hypericin (Barret and Gorski, 2002).

4. **Safety issues:** In the United States, botanical products are marketed as “dietary supplements”. Other countries treat the herbal preparations as drugs, and to be registered these products to be tested to prove their safety and chemical efficacy. There is general conception that herbs are ‘natural’ so these are completely safe. This is not true; the dangers of neutraceuticals are well documented (Holt, 1998; AAPS News Magazine, 2001). In 1994, use of Ephedra led to 39 deaths and 695 cases of serious illness, ranging from insomnia, nervousness and arrhythmia to hypertension, heart attack, seizures and stroke. Liquorice is reported to cause oedema and hypertension if used for a long time (Dubey, Kumar and Tripathi, 2004). Ginseng causes hypertension, gynaecomastia and vaginal bleeding.

Further, toxicities caused by herbal drugs have been shown in table 7 (AAPS News Magazine, 2001). Table 8 shows herbal drug interactions with modern drugs and their consequences (Saxena, 1985; Cupp, 1999; Meschino, 2003).
Table 7: Toxic effects of some herbal drugs.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Use</th>
<th>Possible toxin</th>
<th>Toxic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aristolochia spp.</td>
<td>Antidermatitis</td>
<td>Aristolonic acid</td>
<td>Dose dependent Carcinogenesis</td>
</tr>
<tr>
<td></td>
<td>Antirheumatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antigout</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>Adaptogen</td>
<td>Estrogen like</td>
<td>Gynaecomastia, Ginseng abuse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>substances</td>
<td>syndrome (excitation, arousal,</td>
</tr>
<tr>
<td>Pennyroyal oil</td>
<td>Abortifacient</td>
<td>Pulegone</td>
<td>Severe hepatotoxicity</td>
</tr>
<tr>
<td>(Mentha pulegium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Or Hedeoma pulgioides)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sassafras</td>
<td>Antirheumatic</td>
<td>Safrole</td>
<td>Hepatotoxic</td>
</tr>
<tr>
<td></td>
<td>Carminative</td>
<td></td>
<td>Hepatocarcinogenic</td>
</tr>
<tr>
<td></td>
<td>Flavoring agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senecio crotalaria</td>
<td>Stimulant</td>
<td>Pyrrolizidine</td>
<td>Hepatomegaly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>alkaloids</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Centrilobular necrosis</td>
</tr>
<tr>
<td>Symphytum officinale</td>
<td>Stimulant</td>
<td>Pyrrolizidine</td>
<td>Hepatocellular Damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>alkaloids</td>
<td></td>
</tr>
<tr>
<td>Viscum album</td>
<td>Diuretic</td>
<td>Alkaloids</td>
<td>Cytotoxicity</td>
</tr>
<tr>
<td></td>
<td>Antihypertensive</td>
<td></td>
<td>Haemagglutination</td>
</tr>
<tr>
<td></td>
<td>Anticancer</td>
<td></td>
<td>Mitogenic</td>
</tr>
<tr>
<td></td>
<td>Antispasmodic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8: Consequences of herbal drug interactions with modern drugs.

<table>
<thead>
<tr>
<th>Herbal drug</th>
<th>Modern drug</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aesculus hippocastanum</td>
<td>Coumarin</td>
<td>Synergism (potentiation of anticoagulant effect)</td>
</tr>
<tr>
<td>Convallaria majalis Nerium indicum</td>
<td>Digoxin</td>
<td>Synergism (potentiation of digitalis toxicity)</td>
</tr>
<tr>
<td>Echinacea</td>
<td>Immunosuppressive agents</td>
<td>Counteract effect of the drug</td>
</tr>
<tr>
<td>Ephedra sinica</td>
<td>Caffeine, Decongestant, Antidepressant</td>
<td>Synergism, Severe hypertension</td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>Aspirin, Warfarin, Clopidogrel, Dipyridamole</td>
<td>Synergism (increases bleeding, hemorrhages)</td>
</tr>
<tr>
<td>Glycyrrhiza glabra</td>
<td>Cortisone</td>
<td>Synergism</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>Antagonism</td>
<td></td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>Antidepressant</td>
<td>Synergism (dizziness, confusion, allergic reactions, fatigue and gastrointestinal symptoms)</td>
</tr>
<tr>
<td>Momordica charantia</td>
<td>Chlorpropamide</td>
<td>Synergism (upsets diabetic control)</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>Warfarin</td>
<td>Decreases warfarin effectiveness</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>Euphoria and CNS stimulation</td>
<td></td>
</tr>
<tr>
<td>Panax ginseng saponins</td>
<td>Antihypertensive</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Piper methysticum</td>
<td>Sedatives, Antipsychotics, Ethanol</td>
<td>Synergism (lethargic)</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Warfarin</td>
<td>Synergism (increases bleeding)</td>
</tr>
<tr>
<td>Allium cepa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanacetum parthenium</td>
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</tr>
</tbody>
</table>
2.4.2 Solutions for problems associated with plant drug standardization

1. Control and monitor on growing conditions, harvesting time, part of plant harvested, absence of toxic pesticides or other contaminants, drying methods, freshness and storage, processing, extract solvents, whole extraction process, and analytical controls.

2. Establishment of pharmacognostic standards as mentioned above.

3. Biological evaluation on the basis of traditional reports.

4. Toxicological studies (LD50 studies).

5. Isolation of biologically active constituent(s) using bioactivity-guided fractionation.

6. Estimation of biological markers using HPTLC/HPLC.

7. Preparation of standardized formulation.

8. Dosage schedule.

9. Control on formulation and manufacture of dosage form, packaging, storage and stability.

10. Control on batch to batch variation.

11. Control by FRA on finished products.

2.5 Experimental models for assessing antimicrobial activity

Various experimental models for evaluating antimicrobial activity are available which are discussed below.

2.5.1 Antibiotic sensitivity by disc method (Kirby-Baur Method)

The sensitivity of drug helps in selecting the appropriate line of treatment. The antibiotic sensitivity is quite significant due to development of resistance among various microorganisms. The effectiveness is based on size of inhibition zone. However, zone may vary due to diffusibility of drug size of inoculum, type of medium etc.

Plate the culture on the entire surface of the agar plate (Mueller Hinton agar plate) swabbed with organism to be tested. Prepare the solution of known concentration of an antibiotic in sterile distilled water and dip discs (0.5mm diameter) of whatman filter paper No.1. Place only 4-6 discs on one plate and incubate for 12-24 hours at 37°C. Following incubation the plates are examined for the presence of growth inhibition, which is indicated by clear zone surrounding each disc. Measure the diameter of the
clearing zones to the nearest millimeter. The faint growth or tiny colonies in the clearing zone may appear due to resistant nature of some bacteria. Avoid such growth.

2.5.2 Determination of minimum inhibitory concentration (MIC) of an Antibiotic

MIC is the lowest concentration of an antimicrobial agent that inhibits the growth of the test organism. There is different degree of inhibition in different bacteria with reference to a particular antibiotic. Usually the cell wall synthesis in bacteria occurs by synthesizing peptidoglycan layer. The synthesis of peptidoglycan occurs by lengthening long glycan polymers cross linked with amino acids chain to connect the long parallel glycan chains. Penicillin is effective against Gram-positive bacteria while streptomycin kills Gram-negative such as *E.coli*. Penicillin blocks the amino acid formation and such cells continue to enlarge with weak cell due to missing cross-link. Osmotic pressure exerts on the cell wall and cell breaks and lyses.

On the other hand, streptomycin used against Gram-negative bacteria binds the protein of 30S subunits of ribosome. The bacterial ribosome is inactivated by streptomycin, blocking protein synthesis in the cell. The cell stop dividing due to check of new protein synthesis and lose viability. Streptomycin is, therefore bactericidal. It enters the Gram-negative cells more easily in comparison to Gram-positive cells.

The procedure is stared by preparing the solution of standard antibiotic (4 units/ml of penicillin or 2mg/ml of streptomycin.) Mix 2 ml of antibiotic solution in 2 ml of nutrient broth in a test tube and shake well. Transfer 2 ml in tube 2 and subsequently transfer in rest of the tubes containing 2 ml of nutrient broth except the last one that does not contain antibiotic solution. It is possible to calculate the concentration of antibiotic in each tube. Inoculate each tube with one drop of culture and incubate them at 37° C for 48 hours. Measure the turbidity in terms of optical density (OD) by spectrophotometer and prepare a table or plot a graph between antibiotic concentration and turbidity. Low concentration of antibiotic will show maximum optical density due to less inhibitory effect whereas high concentration reveal minimum optical density as per Beer and Lambart’s Law of Spectrophotometry.(Dubey and Maheshwari, 2004; Cappuccin and Sherman, 2009)
2.6 Research Envisaged

Saccharum is a genus of 35-40 species that grow throughout the tropics and subtropics. Nine species can be found in the Flora region; five are native, two are grown as ornamentals, one is grown for agriculture, and one for research. Some species of Saccharum hybridize naturally with other, presumably closely related, genera such as Miscanthus, Imperata, and Sorghum. Species with awned lemmas are sometimes placed in a separate genus, Erianthus. The most familiar species of Saccharum is S. officinarum, sugar cane.

Study of literature revealed that only few species of Saccharum are known for their medicinal use. Among these S.munja is widely known for its medicinal properties. The species munja is useful in burning sensation, thirst, herpes, duypepsia, dyscaria, erysipelas, urinary complaints and diseases of eyes. Roots of saccharum munja used in dysuria, giddiness and vertigo. Saccharum munja are used to cure bleeding wounds. Roots of saccharum munja also used against fever and inflammation. S. munja grass as a type of gauze-pad to stop blood flow. The smoke of roots burnt near women after delivery and burns scalds. Despite of having very good medicinal properties no authenticate and systemic work has been carried out on S.munja. Not only a single pharmacological report available inspite of record of its medicinal value in Ayurvedic Pharmacopoiea and use of plant as an ingredient in Ayurvedic formulations. (Table 6).

Keeping in mind the traditional and complementary medicinal uses S.munja seems to hold a great potential for in depth investigation for various biological activities especially antibacterial (urinary tract infection). Therefore, it was considered worthwhile to undertake biological, phytochemical and standardization studies, and implement the following plan of work to fetch the goal.

1. S.munja subjecting to detailed pharmacognostic studies with a view to establish precise standards for its authentication.
2. Screening of antibacterial activity of S.munja.
3. Fractionation of S.munja to isolate bioactive constituent(s)/fraction.
4. Characterization of bioactive constituent(s)/fraction.
5. Evaluating the bioactive constituent(s) for antibacterial and antifungal activities.