1.0 Introduction

1.1 Medical treatment

Modern medical treatments use the latest research to combat disease. Medicine is the applied science or practice of the diagnosis, treatment, and prevention of disease [1]. It encompasses a variety of health care practices evolved to maintain and restore health by the prevention and treatment of illness in human beings.

Contemporary medicine applies health science, biomedical research, and medical technology to diagnose, treat injury, and disease, typically through medication or surgery, and through therapies as diverse as psychotherapy, external splints, and traction, prostheses, biologics, ionizing radiation, and others. The word medicine is derived from the Latin airs medicine, meaning the art of healing [2, 3].

1.2 Classification of Medical Treatment

Medical treatment may be classified in two categories.

1.2.1 Allopathic medicine

German physician Samuel Hahnemann (1755-1843) invented the term “allopathy”. “Allopathy” is derived from the Greek words “allo,” (meaning “other”), and “pathos” (meaning “suffering”). A system of medical practice that aims to combat disease by use of remedies (as drugs or surgery) producing effects different from or incompatible with those produced by the disease being treated by homeopathy. Samuel Hahemann, the founder of homeopathy, coined the term “allopathy.” It is not a good way of treatment for the diseases because any disease cured by allopathic way of medication may also have some side effects of that cure or any other symptoms. Because allopathic medication does not remove the disease or does not kill the germs, it pushes them inside the body so patient feels that it is covered or removed from the body but in real it become stronger and attacks again after a time interval with high proportion. Antibiotics and different type of agents are used to cure the diseases. These antibiotics produce effects
different from the disease that is being treated. Allopathic medication killing people day by day because of different type of side effects, and it creating other diseases that do not exist yet. It is not a permanent way of medication to treat the diseases or to remove them from the body of suffering patient.

Though, the allopathic medicine is working in a wide range for the treatment of different kind of diseases because it is faster way of treatment than other alternative medicine but it has many side effects [4].

1.2.2 Alternative Medicine

The major schools of alternative medicine in India are Ayurveda, Unani, Naturopathy, Homeopathy, Yoga, and Meditation. Ayurveda traces its roots to 5,000 years back and is now fast catching up as the medical treatment of choice with urban people, who have not found satisfaction with allopathic treatments.

Naturopathy refers to the treatment of various mental and physical disorders using natural methods, like magnet therapy, mud therapy, osteopathy etc. Naturopathy stresses healthy dietary and living habits.

Yoga and meditation developed in India thousands of years ago. One of the most widely practiced of alternative medicine in India; Yoga has gained popularity in the West due to the extremely effective therapies and its offers to problems ranging from weight gain to cardiac disorders [5, 6].

Alternative medicine may be classified in two categories.

I. Homeopathy

II. Unani

III. Herbal

1.2.2.1 Homeopathy Treatment

Homeopathy, like Naturopathy, is another system of alternative medicine in India that has its roots in the West. The pioneer in the
field of homeopathy is Samuel Hahnemann, who postulated in 1796 that "like cures like", known as the principle of similar.

Like other systems of alternative medicine in India, homeopathy is a system of holistic medicine. Homeopathy treatments include a single medicine to treat all disorders of the body. Since, it is believed that entire body functions as a single entity, and a disorder in one system of the body causes a disturbance in the other system. Homeopathy practitioners believe that mere symptomatic treatment will not cure the ailment. Instead, the whole body is treated to bring back the harmony of the various systems.

The medicines used in homeopathy are sourced from natural sources like animals, plants, minerals etc. The substances are diluted and then various doses are prescribed based on the practitioner's diagnosis. Homeopathy is said to have cured diseases like asthma, measles, benign nodules, and cysts. India presently has many registered practitioners of homeopathy. The Central Council for Research in Homeopathy in New Delhi, India, and the Foundation for Homeopathic Research in Mumbai are some of the premier institutes offering homeopathy treatments in India [7].

1.2.2.2 Unani Treatment

The Unani system of medicine traces its origins to ancient Greece. However, it was the Muslims "Hakims" of India, who developed it further into an effective system of medicine. The Hakim Ibn Sina is considered the founder of Unani medicine.

One of the most well developed fields of alternative medicine in India; the Unani offers cures from cardiac problems to problems like hair loss, weight gain, and gum bleeding. India has over 100 hospitals specializing in the system of Unani medicine. The country also has 30,000 registered practitioners running private Unani clinics. The Tibbiya College at Aligarh and Ahmedabad are considered centres of excellence for Unani medicine.
The Unani therapies are based on the use of various herbs, and medicinal plants that have been used for centuries in traditional medicine in India. The basic principles of Unani medicine are similar to those of Ayurveda, and state that the well being of the body depends on the harmony between the Humors [8-10].

1.2.2.3 Herbal Treatment

Ayurveda: Theory for herbal medicine

Definition

Ayurveda originated in India several thousand years ago. The term “Ayurveda” combines the Sanskrit words “ayur” (life), and “veda” (science or knowledge). Thus, Ayurveda means “the science of life.” According to Charaka, ayurveda is knowledge, which seeks to weigh of life in the scales of wholesomeness and happiness against their opposites. Its main themes of health, disease, and recovery of health from disease take the stage against an inspiring background of intuitive philosophy, lofty idealism, and vivid compassion, which are the hallmarks of India’s cultural inheritance [11, 12].

Ayurveda is a type of healing craft practiced in India from the ancient era. Ayurvedic practitioners rely on plant extracts, both “pure” single-plant preparations, and mixed formulations. The preparations have lyrical names, such as Ashwagandha (Withania somnifera root), Cauvery 100 (a mixture), and Livo-vet. These preparations are used to treat animals as well as humans. In addition to their antimicrobial activities, they have been found to have ant diarrheal, immunomodulatory, anticancer, and psychotropic properties [13-18]. In-vivo studies of abana, an ayurvedic formulation, found a slight reduction in experimentally induced cardiac arrhythmias in dogs [19]. Two microorganisms against, which ayurvedic preparations have activity are aspergillus spp. [20], and propionibacterium acnes [21].

The toxicity of ayurvedic preparations has been the subject of some speculation since, some of them include metals. Prpic-Majic et al. [22]
identified high levels of lead in the blood of adult volunteers, who had self-medicated with ayurvedic medicines [23].

Propolis is a crude extract of the balsam of various trees; it is often called bee glue since, honeybees gather it from the trees. Its chemical composition is very complex; like the latexes described above, terpenoids are present as well as flavonoids, benzoic acids, esters, substituted phenolic acids, and their derivatives [24]. Synthetic cinnamic acids, identical to those from propolis, are found to inhibit hem agglutination activity of influenza virus [25].

1.2.2.3.1 History of medicine

All human societies have medical beliefs that provide explanations for birth, death, and disease. Throughout history, illness has been attributed to witchcraft, demons, astral influence or the will of the Gods. These ideas still retain some power with faith healing, and shrines still used in some places although the rise of scientific medicine over the past millennium has altered or replaced mysticism in most cases [26].

The ancient Egyptians had a system of medicine that was very advanced for its time, and influenced later medical traditions. The Egyptians and Babylonians both introduced the concepts of diagnosis, prognosis, and medical examination. The Hippocratic Oath, still taken by doctors today, was written in Greece in the 5th century BCE. In the medieval era, surgical practices inherited from the ancient masters were improved, and then systematized in Rogerius’s “the practice of surgery”. During the Renaissance, understanding of anatomy improved, and the invention of the microscope would later lead to the germ theory of disease. These advancements, along with developments in chemistry, genetics, and lab technology (such as the x-ray) led to modern medicine [27].
1.2.2.3.2 Introduction of herbal medicines

Herbal medicines are as old as mankind. Since, ancient time’s man has adopted remedies from nature. During 19th century a number of natural products were isolated, and subjected to detailed investigation of their structure, and pharmacological action. A review of ancient literature reveals that many drugs of the vegetable, mineral, and animal origin are being traditionally used for the treatment for the various diseases [28-32]. This involved the application of chemistry to isolate the active constituents in medicinal plants, resulting in next few centuries in the discovery of quinine, caffeine, morphine etc.

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants, and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology, and phytotherapy.

Herb plants produce, and contain a variety of chemical compounds that act upon the body, and are used to prevent or treat disease or promote health, and well being. A herbalist is a professional trained in herbalism, the use of herbs (also called botanical or crude medicine) to treat others. The first Chinese herbal book, the Shennong Bencao Jing, compiled during the Han Dynasty but dating back to a much earlier date, possibly 2700 B.C., lists 365 medicinal plants, and their uses-including Ma-Huang, the shrub that introduced the drug ephedrine to modern medicine. Sometimes, the scope of herbal medicine is extended to include fungi, and bee products as well as minerals, shells, and certain animal parts [33, 34]. People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times.

From the middle ages on, many practitioners have tried to classify herbal remedies by observation of their effects. This is closer to the modern scientific approach of gathering evidence. Eastern herbal
medicine still adheres to the mystical approach in its theories whilst Western herbalists tend to use herbs for the ingredients they contain; mixing, and matching them in the way that conventional medicine does with modern drugs.

The study of herbs dates back over 5,000 years to the Sumerians, who described well-established medicinal uses for such plants as laurel, caraway, and thyme. Ancient Egyptian medicine of 1000 B.C. are known to have used garlic, opium, castor oil, coriander, mint, indigo, and other herbs for medicine and the Old Testament also mentions herb use, and cultivation including mandrake, vetch, caraway, wheat, barley, and rye.

The Greek physician compiled the first European treatise on the properties, and uses of medicinal plants, De Materia Medica. In the first century AD, Dioscorides wrote a compendium of more than 500 plants that remained an authoritative reference into the 17th century. The ancient Greeks and Romans made medicinal use of plants. Greek and Roman medicinal practices, as preserved in the writings of Hippocrates. Similarly, important for herbalists and botanists of later centuries was the Greek book that founded the science of botany, Theophrastus’ Historian Planetarium written in the fourth century B.C.

In some cases, herbal medicines offer an inexpensive and safe alternative to pharmaceuticals. In the U.S., which has just 4% of the world’s population, 106,000 patients died from, and 2.2 million were seriously injured by adverse effects of pharmaceuticals in 1994, as reported in Journal of the American Medical Association.

Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization (WHO) estimates that 80 percent of the world’s population presently uses herbal medicine for some aspect of primary health care. However, most herbalists concede that pharmaceuticals are more effective in
emergency situations where time is of the essence \[35, 36\]. Within the past decade, Americans have consumed ever-increasing amounts of traditional Chinese herbs, and formulas. Some of these consumers are under the care, and guidance of practitioners, who have received specific training in the use of Chinese herbal preparations. However, many receive haphazard advice from both health practitioners, and lay people, who have no experience or training.

In the case of Chinese herbal knowledge, its use by people unfamiliar with its rules, and protocols invariably leads to mishaps: either the herbs or formulas fail to work as expected or worse side effects may result whenever herbs are used in contraindicated conditions. Whilst there is undoubtedly merit in testing plants for beneficial compounds they may contain, it is through a truly scientific approach that these benefits will be realized.

Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase \[37, 38\]. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic natural plant compounds \[39\]. Substances derived from the plants remain the basis for a large proportion of the commercial medications
used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems. For example, ephedra is herb used in Traditional Chinese Medicine for more than two thousand years to treat asthma and other respiratory problems. Ephedrine, the active ingredient in ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems. It helps the patient to breathe more easily. Another example of the use of herbal preparation in modern medicine is the foxglove plant. This herb had been in use since 1775. At present, the powdered leaf of this plant is known as the cardiac stimulant digitalis to the millions of heart patients it keeps alive worldwide.

Herbal Medicine can be broadly classified into various basic systems: Traditional Chinese Herbalism, which is part of Traditional Oriental Medicine, Ayurvedic Herbalism, which is derived from Ayurveda, and Western Herbalism, which is originally came from Greece and Rome to Europe, and then spread to North and South America.

Chinese and Ayurvedic Herbalism have developed into highly sophisticated systems of diagnosis, and treatment over the centuries. Western Herbalism is today primarily a system of folk medicine.

Interest in the United States had been growing in the recent years from the reported success stories from the use of herbs. For example, St. John’s Wort is widely used in the treatment of mild depression without the need for Prozac. St. John’s Wort does not have the side effects such as that of Prozac. There are some Ayurvedic herbs that are very useful for reducing cholesterol, diabetes etc. Similarly, the popularity of Ginseng and Ginkgo biloba (ginkgo) is rising due to its beneficial effects.

1.2.2.3.3 Timeline of Herbal Medicine

No one knows, for sure, when humans began using herbs for medicinal purposes. The first written record of herbal medicine use showed up in 2800 B.C. in China. Since, then the use of herbs has gained, and fallen out of, favor many times in the medical field. The
timeline that follows shows some of the key dates and major points in the history of herbal medicine.

2800 B.C.- The first written record of herbal medicine use showed up. (Titled the Pen Ts'ao by Shen Nung)

400 B.C.- The Greeks joined the herbal medicine game. Hippocrates stressed the ideas that diet, exercise and overall happiness formed the foundation of wellness.

50 A.D.- The Roman Empire spread herbal medicine around the Empire with the commerce of cultivating herbs.

200 A.D.- The first classification system that paired common illnesses with their herbal remedy appeared. This was prepared by the herbal practitioner Galen.

800 A.D.- Monks took over the herbal field with herbal gardens at most monasteries and infirmaries for the sick and injured.

1100 A.D.- The Arab world became a center of medicinal influence. Physician Avicenna wrote the Canon of Medicine, which gave mention to herbal medicines.

1200 A.D.- Black Death spread across Europe, and herbal medicines were used alongside “modern” methods such as bleeding, purging, arsenic, and mercury with equal or better results.

1500 A.D.- Herbal medicine, and herbalists were promoted, and supported by Henry VII, and the Parliament due to the large number of untrained apothecaries giving substandard care.

1600 A.D.- Herbs were used in treating the poor, while extracts of plant, minerals, and animals (the “drugs”), were used for the rich. The English Physician, an herbal explaining the practice of herbal medicine was written during this time.

1700 A.D.- Herbal medicine got another high profile endorsement from Preacher Charles Wesley. He advocated for sensible eating, good hygiene, and herbal treatments for healthy living.
Chapter 1

1800 A.D.- Pharmaceuticals began to hit the scene, and herbal treatments took a back seat. As side effects from the drugs began to be documented, herbal remedies came into favor again. The National Association of Medical Herbalists was formed, and later renamed the National Institute of Medical Herbalists (NIMH.)

1900 A.D.- Lack of availability of drugs during World War I increased the use of herbal medicines again. After the war pharmaceutical production increased and penicillin was discovered. Herbal practitioners had their rights to dispense their medications taken away, and then reinstated. The British Herbal Medicine Association was founded, and produced the British Herbal Pharmacopoeia. People began to express the concern over the large number of side effects and environmental impact of the drugs of the 1950s.

2000 A.D.- EU took action on regulation, and testing of herbal medicines similar to those used for pharmaceuticals. Herbal medicines have been documented for almost 4000 years. These medicines have survived real world testing, and thousands of years of human testing. Some medicines have been discontinued due to their toxicity, while others have been modified or combined with additional herbs to offset sideeffects. Many herbs have undergone changes in their uses. Studies conducted on the herbs and their effects keep changing their potential uses.

1.2.2.3.4 Plant derived drugs

Medicinal plants play an important role in the development of potent therapeutic agents [40]. In the USA deserpidine, reseinnamine, reserpine, vinblastine, and vincristine in all over the world, ectoposide, eguggulsterone, teniposide, nabilone, plaunotol, zguggulsterone, lectinan, artemisinin and ginkgolides, paciltaxel, toptecan, gomishin, and irinotecan are the drugs derived from higher plants. Plant based drugs provide outstanding contribution to modern therapeutics; for example; serpentine isolated from the root of Indian plant Rauwolfia serpentina in 1953, was a revolutionary event in the treatment of
hypertension, and lowering of blood pressure. *Vinblastine* isolated from the *Catharanthus roseus* is used for the treatment of hodgkins, choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular, and neck cancer [41]. *Vincristine* is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymphosarcoma, cervical, and breast cancer. *Phophyllotoxin* is a constituent of *Phodophyllum emodi* currently used against testicular, small cell lung cancer, and lymphomas. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension, and cancer.

In the Western world, as the people are becoming aware of the potency, and side effect of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Throughout the history of mankind, many infectious diseases have been treated with herbs. A number of scientific investigations have highlighted the importance, and the contribution of many plant families i.e., *Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceae* used as medicinal plants. The bioactive extract should be standardized on the basis of active compound. The bioactive extract should undergo safety studies. Almost, 70% modern medicines in India are derived from natural products [42, 43].

1.2.2.3.5 Herbal Medicine Today

Herbal medicines are still in use today. In some respects they have gained a new momentum in the medical field, as many people seek alternative treatments, and begin to check out traditional, and Eastern, medicine, herbs are becoming more popular. As physicians seek new treatments for many common illnesses they are beginning to revisit the traditional remedies, using herbal medicines. Pharmaceutical medications, with their potential for harmful side effects, and addiction are becoming less popular. People are seeking alternatives to the modern medical interventions. Improving, and
maintaining, health naturally is a very popular approach to overall wellness [44-52].

The herbs used today are generally cultivated for those purposes. Very few herbs are harvested from the wild with the exception of a few still found in the rainforests, and higher elevations. The cultivation of herbs for medicinal uses is a large field, and more people are beginning to plant their own herb gardens. Many monasteries continue to grow large herbal gardens within their walls.

Elderly people also metabolize medications differently, and generally are on more medications, and therefore, must also exercise caution when trying new herbal treatments. Underlying ailments that may affect the body’s ability to process or absorb medications are also an issue.

Herbal medicine has enjoyed a long and colorful history. From the early Chinese Empires to modern physicians’ offices, herbal medicines have continued to be a part of the medical field. Herbal treatments have matured throughout history, along with the methods of delivering them. In the beginning, the herbs were used in a hit or miss method and required major events to change their use. Research, and clinical trials have helped to shape the field of herbal medicine, and the future for herbal medicine looks bright [53-58].

Plants have been used in treating human diseases for thousands of years. Some 60,000 years ago, it appears that Neanderthal man valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found [59], which was used by Iranian.

Since, prehistoric time’s shamans or medicine men, and women of Eurasia, and Americas acquired a tremendous knowledge of medicinal plants. All of the native plant species discussed in detail elsewhere was used by native people in traditional medicine. The fact that hundreds of additional species were also used by First Nations Canadians [60], suggests that many of these also have important
pharmacological constituents that could be valuable in modern medicine.

Until the 18\textsuperscript{th} century, the professions of doctor, and botanist were closely linked. Indeed, the first modern botanic gardens, which were founded in 16\textsuperscript{th} century in Pisa, Padova, and Florence (Italy) were medicinal plant gardens attached to medical faculties or schools.

The use of medicinal plants is not just a custom of the distant past. Perhaps 90\% of the world’s population still relies completely on raw herbs, and unrefined extracts as medicines [61]. A 1997 survey showed that 23\% of Canadians have used herbal medicines. In addition, as much as 25\% of modern pharmaceutical drugs contain plant ingredients [62].

India is one of the 12 mega biodiversity centers having 45,000 plant species; its diversity is unmatched due to the 16 different agroclimatic zones, 10 vegetative zones, and 15 biotic provinces. The country has a rich floral diversity as represented in Table 1.1.

\textbf{Table 1.1 Floral diversity in India}

<table>
<thead>
<tr>
<th>Number</th>
<th>species</th>
</tr>
</thead>
<tbody>
<tr>
<td>15,000-18,000</td>
<td>Flowering plants</td>
</tr>
<tr>
<td>23,000</td>
<td>Fungi</td>
</tr>
<tr>
<td>25,000</td>
<td>Algae</td>
</tr>
<tr>
<td>1,600</td>
<td>Lichens</td>
</tr>
<tr>
<td>1,800</td>
<td>Bryophytes</td>
</tr>
<tr>
<td>30 millions</td>
<td>Microorganism</td>
</tr>
</tbody>
</table>

Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. Traditional preparation comprises medicinal plants, minerals, and organic matters etc. Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. The ancient record is evidencing their use by Indian,
Chinese, Egyptian, Greek, Roman, and Syrian dates back to about 5000 years. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments [63], which also forms a rich source of knowledge [64, 65]. The various indigenous systems such as Siddha, Ayurveda, Unani, and Allopathy use several plant species to treat [66]. In India around 20,000 medicinal plant species have been recorded recently [67]. More than 500 traditional communities use about 800 plant species for curing different diseases [68]. Currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects [69]. Plants are important sources of medicines, and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources [70].

1.3 Cassia genus and its importance

Cassia species: The genus Cassia is one of the most important genuses of sub family Caesalpinaceae (Family: Fabaceae), most distributed in tropics and sub tropics. It comprised of about 600 species, most of them are having medicinal values, and used to cure a broad range of diseases. The available literature on in vitro micropropagation of Cassia species described the differential response to cytokinin, and auxin treatment in the media for effective shoot regeneration, and plantlet propagation. It was also found that the explants type played a specific role in morphogenesis and multiplication.

General botanical description and properties of Cassia species

Cassia species belong to the family Caesalpiniaeae. Caesalpiniaceae is often treated as a sub family, Caesalpinioideae, of the large family Leguminosae. It is closely related to Mimosaceae, and Papilionaceae, but can be distinguished by few stamens, and five free petals.
Chapter 1

_Caesalpinioideae_ consist of trees, shrubs, and a few woody herbs found in the tropics. Economically, woody _Caesalpiniaceae_ is important for its timber. _Cassia_ and _Tamarindus_ species are used for medicinal purposes [71-74].


_Cassia_ species have been of keen interest in phytochemicals, and pharmacological research due to their excellent medicinal values. They are well known in folk medicine for their laxative, and purgative uses [75-77]. Besides, they have been found to exhibit anti-inflammatory [78], antioxidant [79, 80], hypoglycaemic [81, 82], antiplasmodial [83], larvicidal [84], antimutagenic [85, 86], and anticancer activities [87]. They are also widely used for the treatment of wounds [88], skin diseases such as ringworm, scabies and eczema, gastro-intestinal disorders like ulcers [89], and jaundice [90]. The phytochemical studies of the medicinal plants have provided some biochemical basis for their ethno pharmacological uses in the treatment, and prevention of various diseases, and disorders [91-93]. The medicinal properties of _Cassia_ species are due to their contents of hydroxyanthraquinone, alkaloids, flavonoids, phenolic acids, carbohydrates, glycosides, protein, amino acids, saponins, and triterpenoids derivatives. Looking to the importance two plants are selected for present study.
17

_Cassia siamea_ (Lam.) (syn: Senna siamea (Lam.) is a very widespread medicinal and food plant cultivated in Southeast Asia, and sub-Saharan Africa [94]. Its stem bark is traditionally used against constipation, malaria, and associated diseases such as fevers and jaundice [95-97]. A decoction of bark is given to diabetic patients, while a paste is used as dressing for ringworm, and chilblains. The roots are used as antipyretic and leaves for constipation, hypertension, insomnia and asthma. The ethnobotanical survey among the tradi practitioners of Brazzaville, and Dolisie (Congo) confirms these uses, with the addition of antinociceptive, and antiviral activities. Previous studies have confirmed some of these traditional uses: antiplasmodial activity [98, 99], antioxidant, and antihypertensive activity, laxative activity, sedative activity [100-101] have been validated by pharmacological studies. However, there are as yet no published studies concerning the analgesic activity of _Cassia siamea_, though it has been noted that the plant, thanks to barakol has anxiolytic and CNS inhibitory effects.

_Cassia javanica_ (Linn.) is a beautiful garden tree that belongs to family _Leguminosae_. It is cultivated throughout in India for beautiful pink blossoms. Previous literature provides meagre information about therapeutic uses of this plant. Bark of _C. javanica_ is used as one of the ingredients in antidiabetic ayurvedic formulation. Leaves are proved to be active against Herpes simplex infection [102]. Leaves are reported to contain variety of secondary metabolites, such as flavones, sterols, several hydrocarbons, anthraquinones, glycosides etc. Among these flavones, glycosides and sterols are considered to be antidiabetic compounds [103]. The presence of these antidiabetic phytochemicals of _C. javanica_ leaves may give desired pharmacological action. As there are no scientific data available regarding antidiabetic effects of leaves, it felt relevant to assess bioactivity of leaves of _C. javanica_ [104, 105].
1.4 Antimicrobial Survey

What are Antimicrobials?

A wide variety of active phytochemicals include the flavones, flavonoids, flavonols, phenolics, polyphenolics, quinones, tannins, coumarins, terpenoids, alkanoids, lectins, and polypeptides are believed to be present in plant parts, and possess antimicrobial properties. Some volatile essential oils of commonly used culinary herbs, spices, and herbal teas have also exhibited a high level of antimicrobial activity. From the literature and other surveys, we can tell that flavonoids are naturally occurring substances possessing several biological properties [106]. The antimicrobial effects of some flavonoids, and phenolics are studied by several researchers [107].

Antimicrobial chemotherapy has been an important medical treatment since, the first investigations of antibacterial dyes by Ehrlich in the beginning of the twentieth century. However, by the late 1940s bacteria resistant to antimicrobials were soon recognized as a serious problem in clinical environments such as hospitals, and care facilities [108]. Bacterial resistance forces the research community to develop methods of altering structures of antimicrobial compounds to avoid their inactivation yet structural modifications alone are not enough to avert bacterial resistance. The increasing use of household antibacterial products and agricultural antimicrobials fosters resistance to drugs specific for human therapy, and may have huge consequences particularly for children, and elderly persons [109, 110]. Antimicrobials contained in manure, and bio solids may enhance selection of resistant bacteria by entering the aquatic environment through pathways of diffuse pollution [111]. Surface water, and shallow groundwater are commonly used for drinking water, and antimicrobials are now found to pollute many aquatic sources [112, 113]. Antimicrobials are used worldwide in human medicine, food, agriculture, livestock and household products. In many cases, the use of antibiotics is unnecessary or questionable. An
overview of antimicrobial pathway used in human medicine is shown in Figure 1.1.

**Figure 1.1 Antimicrobials used in human cell and its biochemical pathway**

Consumption of antibiotics is linked to bacterial resistance. In hospitals, most common resistant bacteria include methicillin-resistant, *Staphylococcus aureus*, vancomycin-resistant enterococci, and gram-negative rods, including the *Enterobacteriaceae*, and *Pseudomonas aeruginosa* [114]. Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compounds with antimicrobial activity, with possibly new modes of action.

This expectation that some naturally occurring plant compounds can kill antibiotic-resistant strains of bacteria such as *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, and *Staphylococcus aureus* [115]. In the past few decades, the search for new anti infective agents has occupied many research groups in the field of ethnopharmacology. A pub med search for the antimicrobial activity of medicinal plants produced 115 articles from the period between 1966 and 1994. However, in the following decade between 1995 and 2004; this number increase more than doubled to 307. In these studies one finds a wide range of criteria related to the discovery of antimicrobial
compounds in plants. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes or naphthoquinones. After detection of antimicrobial activity in the plant extract, some of these compounds were isolated or obtained by bioassay-guided isolation. A second block of studies focuses on the random screening of natural flora of a specific region or country, and the third relevant group of papers is made up of in-depth studies of the activity of a plant or plant compound against a specific pathological microorganism [116].

Table 1.2 Some (semi)synthetic bioactive compounds derived from natural compounds, which demonstrate better activity and/or lower toxicity.

<table>
<thead>
<tr>
<th>(semi)synthetic compounds</th>
<th>natural compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>cocaine</td>
<td>morphine</td>
</tr>
<tr>
<td>metformin</td>
<td>galegine</td>
</tr>
<tr>
<td>nabilone</td>
<td>Δ9-tetrahydrocannabinol</td>
</tr>
<tr>
<td>oxycodon (and other narcotic analgesics)</td>
<td>morphine</td>
</tr>
<tr>
<td>taxotere</td>
<td>taxol</td>
</tr>
<tr>
<td>teniposide</td>
<td>podophyllotoxin</td>
</tr>
<tr>
<td>verapamil</td>
<td>khellin</td>
</tr>
<tr>
<td>amiodarone</td>
<td>khellin</td>
</tr>
</tbody>
</table>

The goals of using plants as sources of therapeutic agents are a) to isolate bioactive compounds for direct use as drug, e.g., atropine, scopolamine, digoxin, digitoxin, morphine, reserpine, taxol, vinblastine, and vincristine; (b) to produce bioactive compounds from novel or known structures, using them as lead compounds for (semi)synthesis of novel patentable entities with better activity, and/or lower toxicity (examples are shown in Table 1.2 ); (c) to use natural products as pharmacological tools, e.g., lysergic acid diethylamide,
mescaline, strychnine, yohimbine; and (d) to use the whole plant or part of it as a herbal remedy, e.g., Cranberry, Echinacea, Feverfew, garlic, Ginkgo biloba, St. John’s wort, and saw palmetto. The number of higher plant species (angiosperms and gymnosperms) is estimated between 215,000, and 500,000 species.

1.5 Antioxidants

1.5.1 Free radical generation: Role of oxidation process in body

To help our body, protect itself from the rigors of oxidation, Mother Nature provides thousands of different antioxidants in various amounts in fruits, vegetables, whole grains, cereals, millets, minor millets, nuts, and legumes. When our body need to put up its best defense, especially true in today’s environment, antioxidants are crucial to our health. As oxygen interacts with cells of any type an apple slice or in our body, the cells lining our lungs or in a cut on our skin oxidation occurs. This produces some type of change in those cells. They may die, such as with rotting fruit. In the case of cut skin, dead cells are replaced in time by fresh new cells, resulting in a healed cut. This birth and death of cells in the body goes on continuously, 24 hours a day. A process is necessary to keep the body healthy. Oxidation is a very natural process that happens during normal cellular functions. Yet, there is a downside, while the body metabolizes oxygen very efficiently, 1% or 2% of cells will get damaged in the process, and turn into free radicals.

"Free radicals" is a term often used to describe damaged cells that can be problematic. They are "free" because they are missing a critical molecule, which sends them on a rampage to pair with another molecule. These molecules will rob any molecule to quench that need.

1.5.2 Types of free radicals

Most free radicals are coming from oxygen atoms, and are called Reactive Oxygen Species (ROS), such as superoxide ion, hydroxyl radical, hydrogen peroxide and singlet oxygen.
Superoxide ion (or reactive oxygen species) is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria, deoxyribonucleic acid (DNA), and other biomolecules. Superoxide radical is produced in-vivo, and the superoxide dismutase enzymes are an important protective antioxidant defense [117,118].

Scheme 1.1 shows the free radical mechanism of oxygen. Dismutation occurs spontaneously at a rate that decreases with a rise in pH, but the rate can be accelerated enormously by addition of superoxide dismutase (SOD) enzymes, which are present in essentially all body tissues, and in most uncooked foods [119]. Fortunately, neither •O₂⁻ nor H₂O₂ is very reactive. H₂O₂ can cross membranes readily, which •O₂⁻ is unable to do; the charged natured of •O₂⁻ renders it membrane impermeable unless there is a trans-membrane channel through, which it can move, such as the anion channel in erythrocytes [120]. Much of the damage that can be done by •O₂⁻, and H₂O₂ in vivo is thought to be due to their conversion into more reactive species [121], probably the most important of which is hydroxyl radical (•OH). Hydroxyl radical is formed by the reduction of an oxygen molecule in the electron transport chain as shown in Figure 1.2.
Figure 1.2 Reduction of an oxygen molecule in the electron transport chain

It is a neutral (not charged) form of the hydroxide ion. Hydroxyl radicals are highly reactive and form an important part of radical biochemistry [122]. Unlike superoxide, the hydroxyl radical cannot be eliminated by an enzymatic reaction. It has a very short half-life, and will only react with molecules within its vicinity. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids, and proteins. Singlet oxygen is formed by immune system. Singlet oxygen causes oxidation of Low-density lipoprotein (LDL) cholesterol [123-125].

1.6 Flavonoids

The flavonoids, derivatives of 1, 3-diphenylpropane, are a large group of natural products, which are widespread in higher plants but also found in some lower plants, including algae. Most flavonoids are yellow compounds, and contribute to the yellow colour of the flowers, and fruits, where they are usually present as glycosides. Flavonoids are the largest group of phenols in the plant kingdom, and are metabolites with a benzopyran nucleus having an aromatic substituent at carbon number 2 (C-2) of the C ring [126, 127]. Figure 1.3 depicted here shows the basic structure of flavonoids ring.
Chapter 1

The most common types of flavonoids are flavanones, flavonols, flavones, and flavans. As shown in Figure 1.3, characteristic features of these flavonoids are a carbonyl at C-4 (flavanones), carbonyl at C-4, double bond between C-2, and C-3, and hydroxyl at C-3 (flavonols), carbonyl at C-4, and a double bond between C-2, and C-3 (flavones), and no carbonyl at C-4 but with a hydroxyl at C-3 for the flavans.

![Figure 1.3 Basic skeletons of flavonoid](image)

Among the types of flavonoids that exist in nature, flavans are the major group found in sorghum [128], and the major flavans are leucoanthocyanidins (hydroxyl at C-3 and C-4), (+)-catechin (hydroxyl at C-3), and anthocyanidin (hydroxyl at C-3, double bond between C-3 and C-4). Anthocyanidins such as luteolinidin, cyaniding, and apigeninidin have been reported in sorghum. Awika, Rooney, and Waniska identified apigeninidin and luteolinidin as the major 3-deoxyanthocyanidins (anthocyanidins that lack a hydroxyl group at the C-3 position) in black and brown sorghums [129]. Blessin, Van Etten, and Dimler found an anthocyanidin, fisetidin, in sorghum, and Yasumatsu, Nakayama, and Chichester reported pelargonidin. Strumeyer, and Malin however did not find any anthocyanidins in Leoti and Georgia 615 sorghums [130-132]. Some most common flavonoids are shown in Figure 1.4.
1.7 Phenolic acids

Phenolic compounds are substances that possess an aromatic ring bearing one or more hydroxyl (-OH) substituent [133]. As mentioned earlier, sorghum contains phenolic compounds that have antioxidant properties, and provide a protective role to the sorghum plant [134-138]. These phenolic compounds in grain may be classified very broadly, into phenolic acids, flavonoids, and condensed tannins.
<table>
<thead>
<tr>
<th>Name of acid</th>
<th>Functional group position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>H</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>H</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>H</td>
</tr>
<tr>
<td>p-hydroxy benzoic acid</td>
<td>H</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>-OH</td>
</tr>
</tbody>
</table>

Table 1.3 Position of functional groups with respect to R₁, R₂ and R₃ in benzoic acid

Basic skeleton of benzoic acid is depicted in the Figure 1.5 and basic skeleton of cinnamic acid is given in Figure 1.6. Tables 1.3 and 1.4 are given for the convenience to identify the derivatives of benzoic and cinnamic acid respectively, according to the position of functional group attached with basic skeleton. Phenolic acids are the simplest phenolic compounds, and are derivatives of benzoic or cinnamic acids. They contain hydroxyl or methoxyl groups substituted at various positions on the aromatic ring. Hahn et al., [139] separated and identified, by reverse phase high performance liquid chromatography (HPLC), eight main phenolic acids in sorghum grain extracts in both white and brown sorghum (gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, p-coumaric, ferulic and cinnamic acid). These phenolic acids are found in both the free and bound form, with brown sorghum containing the highest amount of free phenolic acids. Bound phenolic acids are those that are complexes or form conjugates with sugars, proteins or cell wall polysaccharides [140, 141].
Inflammation (Latin, inflamatio, to set on fire) is the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants [142]. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection, the two are not synonymous: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen.

In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, an inflammation that runs
unchecked can also lead to a host of diseases such as hay fever, atherosclerosis, and rheumatoid arthritis. It is for that reason that inflammation is normally closely regulated by the body.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma, and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells, which are present at the site of inflammation and is characterized by simultaneous destruction, and healing of the tissue from the inflammatory process.

1.8.2 Causes of Inflammation

Burns, Chemical irritants, Frostbite, and Toxins, Infection by pathogens, Physical injury (blunt or penetrating), Immune reactions due to hypersensitivity, Ionizing radiation, foreign bodies, including splinters, and dirt, are main cause of inflammation. Compression between acute and chronic inflammation is given in the Table 1.5.

**Table 1.5 Comparison between acute and chronic inflammation**

<table>
<thead>
<tr>
<th></th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causative agent</td>
<td>Pathogens, injured tissues</td>
<td>Persistent acute inflammation due to non–degradable pathogens, persistent foreign bodies, or autoimmune reactions</td>
</tr>
<tr>
<td>Major cells</td>
<td>Vasoactive amines,</td>
<td>Mononuclear cells (monocytes, macrophages,</td>
</tr>
<tr>
<td>involved</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Chapter 1

<table>
<thead>
<tr>
<th>eicosanoids</th>
<th>lymphocytes, plasma cells), fibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary mediators</td>
<td>Immediate</td>
</tr>
<tr>
<td>IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes</td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>Few days</td>
</tr>
</tbody>
</table>

#### 1.8.3 In vitro methods for anti-inflammatory activity [143]

An array of physiological substances, sometimes called autacoids, are involved in the process of inflammation and repair. These include histamine, serotonin, bradykinin, substance P, and the group of eicosanoids (prostaglandins, thromboxanes and leucotrienes), the platelet-activating factor (PAF) as well as cytokines and lymphokines. Their discovery makes the use of *in vitro* studies possible. The influence of non-steroidal anti-inflammatory agents (NSAID) on the eicosanoid pathway gave rise to numerous studies. These include 3H-Bradykinin receptor binding, Substance P and the tachykinin family, 3H-Substance P receptor binding, Neurokinin receptor binding. Assay of polymorphonuclear leukocyte chemotaxis, *in vitro*, Polymorphonuclear leukocytes aggregation induced by FMLP, Constitutive and inducible cellular arachidonic acid metabolism *in vitro*, Formation of leukotriene B4 in human white blood cells *in vitro*, Formation of lipoxygenase products from 14C-arachidonic acid in human polymorphonuclear neutrophils (PMN), *in vitro* formation of eicosanoids from 14C-arachidonic acid in human platelets, *in vitro*, stimulation of inducible prostaglandin pathway in human PMNL, COX-1 and COX-2 inhibition, Induced release of cytokines (Interleukin-1alpha, IL-1beta, IL-6, IL-8 and TNF-alpha) from human white blood cells. *In vitro* flow cytometric analysis of intracellular
cytokines, TNF-alpha antagonism, binding to interferon receptors, Screening for interleukin-1 antagonists, Inhibition of interleukin-1β converting enzyme (ICE) are main causes of inflammation.

1.8.4 In vivo methods for anti-inflammatory activity

Tissue injury or trauma initiates a cascade of reactions, which results in the proteolytic generation of Bradykinin and kallidin from high-molecular-weight precursors, kininogens found in blood, and tissue. The rapid enzymatic cleavage of kininogens is accomplished by the kallikreins, a group of proteolytic enzymes, which are present in most tissues and body fluids. Bradykinin produces pain by stimulating A and C fibers in the peripheral nerves, participates in the inflammatory reaction and lowers blood pressure by vasodilatation. Since, its breakdown occurs via the same enzyme responsible for converting angiotensin I into angiotensin II some of the effects of converting enzyme inhibitors may be due to presence of Bradykinin. The 3H-Bradykinin receptor binding is used to detect compounds that inhibit binding of 3H-Bradykinin in membrane preparations obtained from guinea-pig ileum. Two types of Bradykinin receptors (BK1 and BK2 receptors) are known. The existence of a pulmonary BK3 receptor has been proposed by Farmer et al. in 1989. Evidence was obtained for the existence of three subtypes of B2 receptors, B2a, B2b, and B2c

1.8.5 Methods for testing acute and sub acute inflammation

Carrageen induced Paw edema in rats, Ultraviolet erythema in guinea pigs, vascular permeability, Inhibition of leukocyte adhesion to rat mesenteric venules. In vivo Oxazolone-induced ear edema in mice, Croton-oil ear edema in rats, and mice, Pleurisy test, Granuloma pouch technique, Urate-induced synovitis, are some most common methods to induce inflammation in animal study.
1.8.5.1 Non-steroidal anti-inflammatory drugs (NSAIDS)

Non-steroidal anti-inflammatory drugs, usually abbreviated as NSAIDs or NAIDs, are drugs with analgesic, antipyretic (lowering an elevated body temperature and relieving pain without impairing consciousness), and in higher doses, with anti-inflammatory effects (reducing inflammation).

The term “non-steroidal” is used to distinguish these drugs from steroids, which (among a broad range of other effects) have a similar eicosanoid-depressing, anti-inflammatory action. As analgesics, NSAIDs are unusual in that they are non-narcotic. Figure 1.7 depicted here indicates mechanism of action for NSAID.

NSAIDs are sometimes also referred to as non-steroidal anti-inflammatory agents / analgesics (NSAIAs) or non-steroidal anti-inflammatory medicines (NSAIMs). The most prominent members of this group of drugs are aspirin, ibuprofen, and diclofenac because they are available over-the-counter in many areas. They are classified as under [144,145]:

![Figure 1.7 Mechanism(s) of Action. All of the non-steroidal anti-inflammatory drugs](image-url)
Nonselective COX inhibitors (conventional NSAIDs)

1. **Irreversible COX inhibitors** Aspirin

2. **Reversible COX inhibitors**
   a. Pyrazolone derivatives - Phenylbutazone, Oxyphenbutazone
   b. Indole derivatives - Indomethacin
   c. Propionic acid derivatives - Ibuprofen, Naproxen, Ketoprofen, Flurbiprofen
   d. Anthralinic acid derivative - Mephenamic acid
   e. Aryl-acetic acid derivatives - Diclofenac, Aceclofenac
   f. Oxicam derivatives - Piroxicam, Tenoxicam
   g. Pyrrolo-pyrrole derivative - Ketorolac

B. **Selective COX-2 inhibitors**

1. Preferential COX-2 inhibitors - Nimesulide, Meloxicam, Nabumetone, Etodolac
2. Highly selective COX-2 inhibitors - Celecoxib, Etoricoxib, Parecoxib, Lumiracoxib

C. Analgesics - antipyretics with poor anti-inflammatory action

1. **COX-3 inhibitors** - Paracetamol (Acetaminophen)

2. **Do not inhibit prostaglandin synthesis** – Nefopam

These drugs are not derived from opium. They do not interact with opioid receptors but act primarily on peripheral pain mechanisms. They may also act on CNS to raise the pain threshold. The Greek physicians Galen, and Hippocrates described the analgesic effects of willow bark from which aspirin was synthesized subsequently. They are considered as non narcotic analgesics [146]. They are also referred to as non-steroidal anti-inflammatory drugs (NSAIDs). These drugs are chemically diverse, but most are organic acids. During 1999 to 2000, per member per year costs for these drugs grew by 37.9%, the highest growth rate among the top 25 therapeutic categories, as measured by ‘Express Scripts Drug Trend Report’. Contributing to this growth was
the introduction of the newer, more expensive cyclo-oxygenase-2 (COX-2) specific inhibitors. The first COX-2 inhibitor approved in the United States was celecoxib in December 1998, and the second, rofecoxib, entered the market in May 1999. Valdecoxib was approved by the US-FDA in November 2001 (Cox et al., 2003).

Inflammation and oxidative stress plays an important role in various diseases. Inflammation is an immunological defense mechanism elicited in response to mechanical injuries, burns, microbial infections, allergens, and other noxious stimulus [147-149].

Use of anti-inflammatory agents may therefore, be helpful in the treatment of inflammatory disorders [150]. Non-steroidal or steroidal anti-inflammatory drugs are commonly used to treat different inflammatory diseases [151, 152]. The adverse effects of the currently available anti-inflammatory drugs, however, pose a major problem in their clinical use [153]. Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics [154, 155].

1.9 Aim and Objectives of present thesis

Looking to the importance of both plants of Cassia species, the present research programme is designed accordingly.

The main aim of this research programme is to find out therapeutic use of phytochemicals present in the aerial parts of two plants Cassia siamea and Cassia javanica.

The present work will be also focused on separation, isolation, characterization of various phytochemicals present in the aerial parts of two above-mentioned plants.

The study also emphasized possible applications of these phytochemicals in medicinal treatments of various diseases.

To achieve the aim of this research programme following points are considered.
i. It is assumed that phenolic acids and flavonoids are playing important role in various biological activities of plants and therefore, total phenolics and total flavonoids will be investigated.

ii. Thus, the present investigation is aimed to evaluate the antimicrobial activity of eight extracts of aerial parts of *Cassia siamea* and *Cassia javanica*.

iii. *In-vitro* antioxidant activity of three extracts of aerial parts of *Cassia siamea* and *Cassia javanica* will also be established using standard protocols.

iv. *In-vivo* anti-inflammatory activity of ethanolic extract of aerial parts of *Cassia siamea* and *Cassia javanica* will be evaluated using paw edema method on Swiss *Wistar* albino rats.
Chapter 1

References


Chapter 1


[21] H Hasegawa, S Matsumiya, M Uchiyama, T Kurokawa, Y
Inouye, R Kasai, S Ishibashi and K Yamasaki. Inhibitory effect
of some triterpenoid saponins on glucose transport in tumor
cells and its application to *in vitro* cytotoxic and antiviral

[22] A Chopra and V V Doiphode. Ayurvedic medicine: core concept,

effect of some propolis constituents and their analogues (esters
of substituted cinnamic acids), *J. Nat. Prod.*, 55(3) (1992) 294-
297.

Synergistic effect of flavones and flavonols against herpes
simplex virus type 1 in cell culture. Comparison with the
1740.

amine fraction of cucumber (Cucumis sativus) extract, *J. Appl.

1.

[27] S Dev. Ethnotherapeutic and modern drug development: The


[29] C Chang, C L Ashendel, Thomas C K Chan, R L Geahlen, J
McLaughlin and D J Waters. Oncogene Signal Transduction


Chapter 1


[63] S P Ambasta. The useful Plants of India, 1\textsuperscript{st} Ed. (CSIR, New Delhi, India) (1986) pp 65.


Chapter 1


Chapter 1


[99] W Thongsaard, S Chainakul, G Bennett and C Marsden. Determination of barakol extracted from *Cassia siamea* by


D T Longhi-Balbinot, D Lanznaster, C H Baggio, M D Silva, C H Cabrera, V A Facundo and A R S Santos. Anti inflammatory effect of triterpene 3b, 6b, 16b-trihydroxylup-20(29)-ene
obtained from *Combretum leprosum* Mart & Eichelmie, *J. Ethnopharmacol.*, 142 (2012) 59-64.