CHAPTER 01

1.0 INTRODUCTION

1.1 SCOPE AND IMPORTANCE OF THE TOPIC:

Nature is an infinite source of discovery of new drugs and its further development. Nature has a wide and complete repository of remedies to cure almost all ailments in human beings. Nature, since its creation is a first rate drug store comprising of a wide flora and infinite range of living entities. Innumerable materials derived directly or indirectly from plants and animals are reported to possess anti oxidant and anti microbial properties. The aim of this study is centred on the utilisation of such precious and holy animal derived material: cow urine, which has these properties. (Jerald E et al., 2008) Cow, with a scientific name ‘Bos indicus’, is considered in the Vedas to be a holy and precious animal and is adored as the ‘mother of mankind’. A composition of cow excretion, urine, dung, with milk, curd, and ghee in appropriate proportions is known as “Panchgavya”. This is administered to women during delivery. Panchgavya is the prime constituent of almost all Ayurvedic preparations (Pathak M et al., 2003). Cow urine, which is one of the main ingredients in Panchgavya has an important therapeutic value. Cow urine is beneficial; cow has developed a symbiotic relationship with human beings (Mishra P et al., 2005).

1.2 SIGNIFICANCE OF THE SUBJECT MATTER

1.2.1 The Cow as a Provider:

Cow is perhaps the only harmless animal in the entire creation possessing an inherent donor nature. It means that its demands for survival are quite negligible compared to what it gives back to the creation. The diet of man starts from tea, which consists of milk, the major product of this holy animal. It is followed by many other dairy products made out of milk like Butter, Ghee, and Curd. Hindu rituals are incomplete without Ghee and curd is considered as a lucky charm by many; it being essentially consumed when people embark on an important mission or a long voyage. Cow dung is the major source of natural fertilizer that stimulates land fertility, thereby enhancing the farm output. Bio-gas is another resource obtained from cow dung, which is quite cheap, and finds a basic use as an environmental friendly fuel and also in generation of electricity for lighting purposes in the remote villages of India where grid supply has
yet not reached. In the past when agricultural automation was unknown, for centuries, the bull was extremely important as a power house to till the land for the ultimate production of food grains.

**Religious Significance of the Cow:**
Lord Krishna, one of the important Gods of the Hindus has been considered to signify the holiness of cow. According to Hindu scriptures, Lord Krishna spent his childhood in Gokul near Mathura. He is also popularly known by the name: Govinda or Gopala, which means ‘protector and friend of cows’. Both cow and the bull are highly esteemed animals in the Hindu Mythology. Cow and bull are the mother and the father of mankind. ‘Kamadhenu’ cow has a very high religious significance, being considered miraculous, fulfilling all the desires of her devotees. She is considered to be the mother of all cows. The followers of the three main religions in India: Hindus, Jains and the Zoroastrians consider the cow and the bull as holy animals. Important ceremonies in all these religions involve the use of cow urine in specified forms as per the tenents of each religion.

**Ayurvedic Significance of the Cow:**
From time immemorial, Ayurveda has understood the value of a cow. It is a great producer of milk and the dairy products, having many ‘sattvic’ properties. Yogurt, Butter Milk, Organic Milk, ghee and other milk bi-products have a high nutritional value by providing Calcium and Protein to the human body tissues and cells. Ghee and cow dung fed in fire ceremonies popularly known as Yagna, has been found to help in purification of air. Ayurveda has a firm belief with proper facts to prove that some diseases cannot be totally cured by medicines alone; and here ‘Panchamrit’ has been propagated; a drink that is meant to heal. It is considered to be the ‘nectar of gods’ composed of five chief ingredients – yogurt, ghee, honey, sugar and milk. It is distributed in the congregation at the end of religious ceremonies. ‘Panchamrit’ has been believed to fill the in-taker with divine energy as it exhibits healing action from within.

**Importance of conservation of the Cow:**
As discussed earlier, the cow plays a vital role in the lives of human beings, but still man has failed poorly to acknowledge the significance and importance of this precious
animal on earth. Actually, the sad fact is that since the cow is a harmless animal having no demands for its upkeep and is very gentle by nature, people have failed in the proper care to be given to the cow. But the time is ripe for man to come out of the cocoons and start giving attention to their actual care-takers. It is imperative to save and provide them due care, or the time is not far when they will be extinct, to be seen by future generations in books or museums. Time is ripe for us not to undermine their importance any more. Just imagine that if our days when our children are deprived of the nutrition provided by the cow and the condition of agriculture industry in the absence of cow dung! Cow service is a very sanctifying act, helping our body and soul to find attainment. People working in the cow shed (Gau-shala) or giving land for cow ranging and cow rehabilitation are the real benefactors of this animal in particular and the future human generations to come in general (Shri Swami Rajendra Das Ji Maharaj; Srila Prabhupada instructs about cows).

Now coming to another main product obtained from the cow, being its body discharge in the form of urine. Panchgavya prepared from cow urine is great elixir, proper diet, pleasing to heart and giver of mental and physical strength enhancing longevity. It has capacity to balance bile, mucous and airs. It has been observed to prevent heart diseases and lessen the effect of poison.

Cow urine after process is available in two forms:
1) Go Ark (Cow urine distillate), and
2) Ganavati (Cow urine tablet).

Three U.S. patents have been registered on Gomutra Ark (researched and authorized by U.S. government).
U.S. patent No. 1 = 6410059, dated June 25, 2002
U.S. patent No. 2 = 6896907, dated May 24, 2005.

In India, cow urine based products are available in different forms like:
1) Medicines prepared from cow urine as a main ingredient.
2) Cool drinks prescribed as an anticancer potion.
3) Fertilizers using cow urine and cow dung.
4) Insecticides for farming activities.
Figure: 01

Products for Domestic and Out-door use made from cow urine

Cow urine based shampoo

Cow urine – fermented; used as insecticide

Cow urine based after-shave lotion

Cow urine based cool drink

Medicines prepared from cow urine

People drinking cow urine in Indore

(Above six images; Courtesy IRC – SNR, India.)
5) Personal use products like after shave lotion; shampoos and hair oil for hair-care.
6) Disinfectants for home use.

Different types of medicines are prepared from cow urine like general tonics for better health, special tonics for mensurating women, tonics for growing children, tablets to improve digestion and functioning of the liver, oils for joint pain relief. Cool drinks are specially made to curb the growth of cancer.

Farming without the use of synthetic fertilisers is known by the term ‘Organic farming’. It involves the use of the mixture of both cow urine and dung in different proportions as per the requirement of the particular soil and the type of crop to be grown. It avoids total use of any synthetic fertilizers and pesticides, thereby avoiding deadly chemical pollution in the soil and environment, and reduces the expenditure involved in farming.

The five products of the cow: Milk, Ghee, Curd, Urine and Dung are mixed together in appropriate proportions and stored in a big vessel for 28 - 32 days. The mixture is stirred well and diluted with water to be finally used as an effective fertiliser.

Another type of organic fertilizer is ‘Amudhaa-ras’. It is prepared by mixing cow urine and dung with jaggery and banana. All the four constituents are put in a vessel and churned intermittently for 25 days. Finally it is diluted with water and is sprayed on to the fledging crop to get an increased growth and quality.

Stored cow urine works very efficiently as a pesticide. It curbs the growth of many pathogens singularly or mixing with different plant products like leaf and roots (IRC – SNR, India).

Conclusions derived in the Medical Science Scriptures of India states that diseases are mainly caused by the disturbance of three factors: Air (Vata), Bile (Pitta), and Mucous (Kapha). The findings show that cow urine maintains a balance between these three factors referred popularly in Sanskrit as “Samya dosharogata”. It implies that when a balance is maintained between these three factors in the human body, ailments are eliminated (Mandavgane S et al., 2005). Rural population in India use cow urine as a remedy to get rid of various diseases. Ayurveda has highlighted its antifungal,
Figure: 02

Cow urine based products used as medicine

General Tonic

Oil for joint pain relief

Tablets for better digestion

Cream for skin diseases

Tonic for menstruating women

Eye drops

(Above six images; Courtesy IRC – SNR, India.)
antibacterial, antiviral, anti allergic properties. Hence, it is referred as “Sanjivani” or “Amrita” in the Ayurveda.

Cow urine is a liquid discharge consisting of nontoxic waste material from the cow body. The main constituents of cow urine are Water: 95%, followed by Urea: 2.5%, and the rest 2.5% is a mixture of different minerals, salts, hormones, and enzymes. Cow urine exhibits the property of “Rasayana tattwa”, responsible for modulating various body functions, providing immunity. It augments B and T lymphocyte blastogenesis and Ig G, Ig A, and Ig M antibody titers in the human body. It also increases the secretion of Interleukin 1 and Interleukin 2. Phagocytic activity of macrophages, which is thus helpful in the control and prevention of infections (Randhawa G, 2010). Antimicrobial and germicidal properties of cow urine are due to the presence of urea, creatinine, aurum hydroxide, carbolic acid, phenols, and salts of calcium, and manganese. Its anticarcinogenic effect is due to uric acid, which has antioxidant property. Aurum hydroxide improves immunity, and allantoin promotes wound healing. Hence, administration of a dosage of cow urine helps in regulating an appropriate balance of the above mentioned substances, thus curing incurable diseases. Cow is hence considered a live dispensary; a store house of medicines (Jain N et al., 2010).

Today many AIDS patients are being administered cow urine therapy. People who were suffering with migraine and headache for the past 15 years have recovered within six months of undergoing this therapy. Many prominent natural health care centres in India like the Cow Urine Treatment and Research Centre, Indore have provided vital and cheap free treatment to thousands of populace. Most of the people suffering from common ailments like constipation had a quick and great relief with improved bowel movement, thus enjoying good health; thus proving the age old proverb: ‘if the stomach is clean half of the diseases get cured automatically’ to be true.

Cow urine also helps in boosting proper liver functioning. This leads to more production of healthy pure blood; finally imparting higher disease resistance power to the human body. Our body requires many salts in extremely minute amount to be present in order for the upkeep of our health. They are known as micro nutrients, and they are flushed out of the body through passing of urine. This leads to signs of early aging in a human body. On the other hand cow urine consists of all these micro
nutrients; hence its consumption in any form will fill the gap of the deficiency of these particular elements in the human body. Thus the process of ageing is deferred; hence cow urine is referred to as ‘Elixir of life’.

Cow urine has not only proved to protect the human physique from ulterior effects, but on the contrary, imparts strength to the human heart and brain thus avoiding mental tension disorders, hence curtails many nervous system problems.

Cow urine also possesses many salts in extremely minute amounts like that of gold and copper. Consumption of cow urine compensates this salt deficiency in our body. The presence of these salts in a human body is good for imparting resistance power against diseases. Moreover, it has been found that the rays of electric currents, generally known as electro-magnetic radiations, present in the environment prove to have a beneficial effect on the human body when such currents are absorbed by the body. Copper has the basic property of higher conductivity of electric current. Hence the presence of copper absorbed from cow urine by the human body, helps in the easy passage of these extremely small currents through our body, ultimately leading to better health.

Common synthetic antibiotics and other drugs always leave undesirable residues in our body which have other side effects. These poisonous residues are nullified by the consumption of cow urine; hence the overall health improves tremendously.

As cow urine imparts disease resistance power, people are freed from undue and false superstitions of any ghostly effects, which is actually not true. Many diseases in our body originate from the disturbed digestive power of the stomach. Cow urine therapy improves digestion, destroys poisonous substances, generally referred to as toxins present in our body. Thus it is a very good antitoxin for our body.

The chemical contents of cow urine with their power to control and cure diseases can be observed in brief from the following sequence in which the chemicals found in cow urine are shown against the positive effects observed on the human health regulatory system.
### Table: 01

**Constituents of cow urine and their effects**

<table>
<thead>
<tr>
<th>Substance present in Cow Urine</th>
<th>Positive effects on the human body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia; NH₃</td>
<td>Helps in stabilising the three properties: bile, mucous and air of body. Also improves blood formation</td>
</tr>
<tr>
<td>Aurum Hydroxide; AuOH</td>
<td>It has a germicidal nature; AuOH is also antibiotic and anti-toxic, thus increases immunity power of the body</td>
</tr>
<tr>
<td>Calcium; Ca</td>
<td>Imparts basic strength to bones, and has also germicidal power with blood purification improvement,</td>
</tr>
<tr>
<td>Carbolic acid; HCOOH</td>
<td>It has a germicidal nature, preventing the growth of germs and is also able to prevent gangrene.</td>
</tr>
<tr>
<td>Copper; Cu</td>
<td>Improves the absorption power of magnetic rays and also controls build up of excessive fats</td>
</tr>
<tr>
<td>Creatinin; C₄HgN₂O₂</td>
<td>Improves action against germs.</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Improves digestion and increases immunity</td>
</tr>
<tr>
<td>Hipuric acid; CgNgNox</td>
<td>Helps in the removal of toxins through urine</td>
</tr>
<tr>
<td>Iron; Fe</td>
<td>Maintains balance and helps in production of red blood cells &amp; haemoglobin.</td>
</tr>
<tr>
<td>Lactose; C₆H₁₂O₆</td>
<td>Helps in strengthening the heart; decreases nervousness.</td>
</tr>
<tr>
<td>Manganese; Mn</td>
<td>It imparts better germicidal power. Avoids decay leading to gangrene as it stops growth of germs.</td>
</tr>
<tr>
<td>Nitrogen; N₂,NH₂</td>
<td>Prevents abnormalities in blood and prevents the effect of toxins, its diuretic nature makes it to be a natural stimulant of urinary track, and activates the kidneys.</td>
</tr>
<tr>
<td>Phosphate; P</td>
<td>Helps in preventing urinary tract stone formation.</td>
</tr>
<tr>
<td>Potassium; K</td>
<td>Cures hereditary rheumatism. Increases appetite. Removes muscular weakness and laziness.</td>
</tr>
<tr>
<td>Sodium; Na</td>
<td>It helps in purification of blood as NaCl decreases the acidic content of blood; also has an antacid nature.</td>
</tr>
<tr>
<td>Sulphur; S</td>
<td>Improves bowel action of the intestines. Cleanses blood.</td>
</tr>
<tr>
<td>Substance</td>
<td>Description</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Urea; CO(NH$_2$)$_2$</td>
<td>Improves urine formation and exhalation action. Also has a germicidal action.</td>
</tr>
<tr>
<td>Uric Acid; C$_5$H$_4$N$_4$O$_3$</td>
<td>Prevents the swelling of heart inflammation. It is diuretic therefore destroys toxins</td>
</tr>
<tr>
<td>Vitamins A,B,C,D,E</td>
<td>Vitamin B is active ingredient for energetic life and prevents the effects of nervousness and thirst. It also helps in boosting the bone strength and reproductive power of the individual.</td>
</tr>
<tr>
<td>Water; H$_2$O</td>
<td>It maintains fluidity of blood, and helps in controlling the body temperature.</td>
</tr>
<tr>
<td>Other Minerals</td>
<td>Help in boosting immunity power of the body.</td>
</tr>
</tbody>
</table>
The overall benefits of consumption of potions consisting of cow urine can be in short summarised and enumerated as follows:

Ayurveda describes cow urine as a therapeutic agent. It is used as a cure for various diseases such as anaemia, jaundice, leprosy, coronary problems, piles; to name a few.

Cow urine has been found to be a good digestive, laxative and a toxin-neutralizing agent. It is a sure remedy against the Ayurvedic concept of ‘tridosha’, and is said to be a controller of kapha to be able to limit vata and pitta disorders.

Diseases of the mouth, and common ailments like common cold and hoarseness of voice are also brought under control by the administration of this therapy.

Cow urine therapy plays a major role in the cure of Anaemia. Anaemia is a pathological deficiency in which the number of red cells or their oxygen carrying capacity is insufficient to meet the physiological needs of the body. Depletion of the iron reserves in the blood is one of the major causes of anaemia. Vitamin A and B12 deficiencies, chronic inflammation and infections due to parasites are the other factors for anaemic condition of the body. Presence of erythropoietin hormone, iron and vitamins in cow urine may be one of the reasons why it is useful in the treatment of anaemia.

Many skin irritations and disorders are cured when cow urine is administered along with Hareetaki.

Panchagavya Gritha, an Ayurvedic formulation, is highly useful in the treatment of psychiatric disorders such as Mania, schizofrenia and depression. Even in case of orthopaedic problems like Osteomyelits, Panchagavya gritha has exhibited positive results.

For pain relief, Sciatica, castor oil are prescribed along with cow urine for time duration of one month.

The healing properties of cow urine have found reference in the ancient texts of Ayurveda, India’s natural medicine encyclopaedia.
Hence, from all these citations it is proved that cow urine alone can be used as the main treatment; or as anupan; meaning, a base for combination with other plant derived drugs or minerals such as Tulsi, Shatavari, Ashwagandha and Gaduchi for better synergistic effects. Here it is important to know that fermented or stored cow urine is traditionally used in African countries as a means of protecting cattle from malaria; cattle, although having babesiosis, an infection of red cells, do not suffer from malaria.

In Sushrita Samhita, cow urine has been described as an extremely effective substance or material secretion of animal origin having a wide and innumerable therapeutic value. In India, oral consumption of cow urine has been prescribed and practiced since ages. Effect of cow urine along with a combination of medicinal plants, has been found to enhance its effectiveness in the cure of diabetes, cancer, and AIDS. This type of an alternative and combo treatment is referred to as “Cowpathy”.

Indian Ayurvedic doctors regularly use cow urine as a natural medicine to treat many common disorders. Very few attempts have been made to correlate scientifically the nature of cow urine. In order to correlate ancient and traditional knowledge of use of cow urine with the scientific methodologies and parameters of modern times, this study has been done using cow urine in different forms as immunomodulatory and antioxidant agent (Gosavi D et al., 2011).

Antibiotics are very commonly and widely used for the treatment of many microbial infections and diseases. Usage of such enormous quantity of antibiotics should have created a situation today in which there would be no infectious diseases in the world. But the scenario is totally opposite. The actual fact is that, the problem of infectious diseases is ever increasing daily. Some of the main obstacles noticed are that the bacteria acquire a genetic ability of resistance towards these antibiotic drugs, and these drugs also have an adverse effect on the host. Hence, many natural products have been explored to combat such problems (Shah CP et al., 2011). As discussed earlier, cow urine has natural disinfectant and antiseptic properties. Traditional medicines have recommended direct consumption of cow urine as an effective medicine which is easily available and very easily affordable. It contains twenty four types of salts, phenol, potash, iron, calcium, phosphorus and lactose. Phenol, being the major
material constituent of cow urine exhibits antiseptic activity. It is a mixture of phenol and cresol.

The objective of the present study is to make a deep search into the bio enhancing property and the impact of the naturally available products, so that they can be used to combat many common diseases. Bio enhancing is one of the many properties of cow urine. It promotes and increases the effectiveness of antimicrobial, antifungal, and anticarcinogenic drugs. It also leads to an increase in the activity of gonadotropin releasing hormone conjugate with bovine serum albumin and zinc (Ganaie J et al., 2010).

Cow urine also has bio enhancing activity for Rifampicin, the main antibacterial drug used against tuberculosis, increasing its action up to seven times against *Escherichia coli* and up to ten times against Gram positive bacteria. Cow urine distillate enhances the movement or transport of antibiotics such as Rifampicin, Tetra cycline, and Ampicillin across the gut wall, as well as across artificial membranes. Such transport enhancement varies from a minimum of two to seven times (Patent storm.us/6896907 2010).

Cow urine has also been observed to lead to the increase in potency of “Taxol” (paclitaxel) against MCF -7, a human breast cancer cell line “invitro assays” (U.S. patent No. 6,410,059). These significant achievements highlights the potential role of cow urine in the treatment of various bacterial infections and also cancer, thus proving that cow urine plays a prominent role in enhancing the efficacy and potency of other drugs. Holy Scriptures like Amritsagar, Atharvaveda, Bhavprakash, Charak Samhita, Rajni Ghuntu, Sushrut Samhita and Vridhabhagabhatt, contains a vivid and detailed description regarding this matter. Centres at different locations where cow urine treatment and research are conducted since last few years have commonly concluded that cow urine is able to cure diseases like acidity, asthma, arthritis, AIDS, blockage in arteries, cancer, cardiac failures, constipation, diabetes, eczema, E.N.T. problems, fits, gynaecological problems and abortion, high or low blood pressure, migraine, piles, prostrate, psoriasis, thyroid, ulcer, and several other diseases (Jain NK et al., 2012).

The qualitative study of cow urine has shown to contain calcium salts, carbolic acid, chlorine, copper, creatinine, enzymes, hormones, gold acids, iron, lactose, magnesium,
manganese, minerals, nitrogen, phosphate, silicon, sodium, sulphur, acids like citric, maleic, tartaric, succinic, and Vitamins: A, B, C, D, E. Most of the illness in human beings are primarily due to either a lack or excessiveness of the above substances in the body. As cow urine consists of all these chemicals in appropriate proportions required by the human body, intake of cow urine maintains a proper balance of these chemicals, thus eliminating many diseases (Pathak ML et al., 2003).

Cow urine has an amazingly powerful germicidal power to destroy a variety of germs, thus controlling and curing almost all germ generated diseases. Cow urine balances the three ailments, hence diseases are cured. Cow urine improves the functioning of the liver, thus leading to better production of pure and healthy blood. It imparts immunity to the body against diseases. As cow urine consists of many different chemical substances and nutrients, thereby helping in maintaining a balance and avoiding their deficiency in the human body for a healthy disease free life. Cow urine contains various minerals, mainly copper, which helps in compensating the mineral deficiency in the human body, thus shielding it from diseases. Cow urine itself is non toxic, and also is able to neutralise the toxins as it is antitoxin by nature. Many different compositions of cow urine which are sold on commercial basis in the market have specific and high medical applications.

1.2.2 Medicinal Plants

Importance of plants for medicinal purposes:

Plants have been traditionally used for healing human ailments for centuries in the past. Actually, plants were the only source of remedy until the advent of synthetic pharmaceutical products since last few years. (Bassam Abdul Rassol Hasan (2012); Evita Ochel (2010); Francine Raymond (2014); Introduction to medicinal plants, Canada).

As of today, it is found that almost 38 - 42 % of common drugs and 65 % of anticarcinogenic drugs available to us are of plant origin. It can be very safely predicted that almost 75 - 80 % of the world’s population still consume medicines having plant origin.
Plants are natural chemical factories providing an in-exhaustive supply without any external demands like energy and skilled work force; which is a must in case of synthetic drugs. Plants from marginal desert zones, polar areas and the depths of the oceans are being explored for their medicinal potential. Gardens are a medicine cabinet full of useful everyday remedies that will treat a wide range of common ailments in a gentle but effective way.

Here it is utmost necessary to quote the words of expert herb grower Jekka McVicar: “Grow herbs in your garden and you grow history”. According to him, Herbs have been used since man has existed on earth primarily as a food and then simultaneously as a medicine. Just a simple cup of herb tea can ease tension, soothe a sore throat and lift the spirits.

There are a huge number of medicinal plants on the crest of earth. In U.S.A. alone, more than 1700 plants are commercially available. The medicinal qualities of plants are directly dependent on to the chemicals present in them. Plants synthesize many compounds called primary metabolites that are extremely necessary for their existence in the first place. These substances consist of proteins, fats, and carbohydrates that serve different purposes indispensable for their own substance and reproduction, and also for herbivores that feed on them.

Plants have their inbuilt capacity to synthesize an exceptional range of additional components, called secondary metabolites. Many secondary metabolites, due to their antibiotic nature, helps in imparting protection to the plants against fungi, bacteria, animals, and even other plants. These secondary metabolites are being explored by man for their antibiotic properties to combat various human ailments. This is due to the safe interpretation that every plant species contains chemicals that can affect some animals or micro-organisms negatively. Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defences against predation or infection.

The traditional medicinal usage of herbs by people in the past can be considered to be imperfect and unscientific by the standards of today. They were put to use on trial and error basis, and after a series of such tests on the patient, the potion was modified to secure the optimum natural therapeutic usage. Some herbal products are extremely effective but are equally dangerous, so their administration should only be in the hands
of skilled medical professionals only. Others, however, are sufficiently safe that they can be used by ordinary people to help prevent or alleviate minor health problems.

Man has accepted the fact that a diet for optimal health should be plant based. This actually proves true the olden saying of Hippocrates: “Let thy food be medicine and thy medicine be food”. Healthy eating in itself is preventive medicine. Plants serve the primary purpose as our food. Plants also have numerable healing qualities which directly benefits the human health; so they play a significant role in the upkeep of human health. Hence a diet of various plants is recommended for optimum health. Numerous plant food sources can also be used to heal, should we find ourselves in such a situation.

For centuries the native people of various cultures have used plants in medicine and for a varied range of treatment purposes. For centuries, the primary base of Chinese medicine was plants, which they still continue as of today. Actually it is the Western world which had lagged behind in this aspect. But the tables have turned today and now the full focus is on towards the exploration of natural resources.

Nowadays, the term “Alternative medicine” is widely used. It is concentrated on the use of plants for medicinal purposes. This is exactly contrary to our common, but wrong belief that medicines come in only pills, capsules which are to be swallowed orally, or as intravenous administrable liquids. Their usage finds even in simpler medicinal products like laxatives to complex medicinal aids such as blood thinners, antibiotics and anti malarial drugs.

Medicinal plants have a promising future because there are about half million plants around the world, and in most of them their medicinal activities requires deep investigations as of today. Their medical activities on proper investigation could be of great help in the treatment of current and future generations.

**Characteristics of Medicinal Plants:**
The three major characteristics of medicinal plants can be described as follows:

- **Synergic medicine:** The ingredients of plants all interact simultaneously, so their use may be either helpful or on the contrary will lead to further damage to the host,
or neutralize their possible effect due to the positive – negative reactions between the ingredients.

- **Support to official medicine:** In the treatment of complex illnesses like cancer diseases the components of the plants proved to be very effective.
- **Preventive medicine:** Active components of the plants are also characterized by their ability to prevent the occurrence of some diseases. This directly leads to the decreased usage of synthetic drugs which are used when the disease is already present. Hence plants helps in the reduction of the side effects associated with synthetic treatment.

Over the last few decades the popularity of Ayurveda has increased several folds in India and abroad as well. More than a thousand institutions throughout the world are now offering courses in Ayurveda. World Health Organization (WHO) has also accepted Ayurveda of possessing remedies based on the doctrines, skills and on the theories, knowledge, and indigenous applications, which are proprietary of different sects, incorporated for the overall management of the public health aspect, showing important routes for the prevention of epidemics and diseases, and also in diagnosis and treatment in the case of a wide range of illness in humans and also in animals.

Chinese Medicine and Ayurvedic herbs have provided valuable leads for further discovery of natural medicines. A major parcel of ‘Materia Medica’ is centred on the medicinal plant assets. Materia Medica is a branch of medicinal science devoted to the properties and action of various substances that are used by humans for the prevention or cure from sicknesses. It consists of medical benefits of the different types of preparations and their dosage to be taken; over and above the constitution of the products. It also consists of the properties, benefits and the right way of usage of different mineral waters, warm, cold and vapour baths at different parts of the world, especially Europe (Thomas J Graham, 2006).

Latest investigations and surveys of different pharmacological literary works reason that no less than one hundred twenty distinctive items recovered from medicinal plants have life saving properties. Here it is important to mention that the present information is acquired from only six percent of the sum accessible plant species that have been examined. Further researches are in progress at different locations on the investigation of new medicinal herbs for their positive healing actions against some common
centuries old ailments like diabetes and Alzheimer’s disease and some newer ones like cancer affecting different body parts and also AIDS. Ayurveda is a systematically studied and well organized medical science. It offers classic contents to the medical fraternity, especially in the fields of new nutraceutical and cosmeceutical product development. India is a major agro climatic zone of the planet having a large reserve of medicinal plants out of which quite a number of species are on the verge of extinction. The application of newer tissue culture and DNA finger printing methods, several of the endangered species are being artificially cultivated for their revival through proper selection of the germ plasm.

Since previous many years, natural characteristic items were, still going on today, and will continue in future to be an essential and important source of New Chemical Entities (N.C.E.) in the disclosure, examination and advancement of remedial executors against many infective and non-infective disorders. The bio atoms from these common sources are observed to be clinically more stable, more particularly action oriented at specific sites, and accessible from sources which are never exhausted by the adoption of proper conservation and growing techniques (Dhawan BN, 2005). Medicinal plants available in India have provided innumerable newer leads in the past (Mukherjee PK et al., 2007) and will still continue to yield more N.C.E. in the coming future.

The research based on the natural products centred towards anti microbial chemotherapy has been comparatively more modest. Several factors contribute to the current situation. Many bacterial and viral ailments like common cold and gastroenteritis are self restricted and require just symptomatic medication.

India lags behind in research and investigation of plants available on our soil compared to other foreign countries like Japan, U.S.A. and South Korea who are well ahead in this activity on plants of Indian origin. The major and primary reason for this limited input is the non availability of strict containment facility at most of the institutions in India required for such works (Dhawan BN, 2012).

Substances derived from medicinal flora and fauna have gained greater importance in recent years in the prevention and cure of human diseases as they are fully bio-friendly. According to a general estimate, around six thousand Indian medicinal plants
have gained use since centuries as medicines from that is mentioned in old folk cultures. It has been observed that these medicinal herbs cater in meeting approximately seventy five percent medicinal requirements of the third world countries (Veerachi U et al., 2011).

Many irresistible infections are cured and regulated utilizing plant inferred or obtained regular cures since ages. Today also these products available from agriculture sources continue to play a vital role in essential health awareness in a number of developed and under developed countries. Studies conducted on medicinal plants in many parts of the globe is of importance to both the agriculture and medicinal fields in the focus and work on newer leads towards development of alternative medicinal varieties that finally offer better economic and social benefits. The applications of plant preparations have been documented in many literatures. The control over bacterial infections has been positively and effectively achieved by the discovery of synthetic antibacterial drugs. But, quite a number of pathogens rapidly gain resistance towards the initially discovered synthetic antimicrobial drugs. Such an issue of medication safety, and the growing undesirable reactions of certain synthetic antimicrobials, has expedited the exploration of new antibacterial operators especially from characteristic assets from medicinal plants, whose supply dependably accessible in plenitude, likewise is renewable and is contamination free. Various research reports regarding antibacterial properties of plant concentrates from medicinal plants have been published and distributed in diverse literary works (Segni L et al., 2011). Infectious afflictions are one of the main reasons of human mortality on the planet. Resistance acquired by the organisms towards the synthetic antibiotics is also a global problem. The consistent rise of new multi drug resistant pathogens has undermined the clinical viability of numerous prevailing antitoxins. Herbal preparations are known to be utilized for the medication of numerous irresistible sicknesses all through the history of humankind. This is carried out by the utilization of commonly accessible items; either as unadulterated mixes or as in institutionalized plant concentrated form, furnishing expansive chances for new drug findings due to their nonstop accessibility and wide chemical diversity. The essential and primary need of today is for the enduring finding of new antimicrobial compounds with chemical structures that are broad in applications and have latest and new modes of action against recently rising and redeveloping irresistible infections. Henceforth, specialists are progressively focussing
their exploration and experimentation on common people, and searching for the advancement and synthesis of better drugs to combat microbial infections.

India is a vast and wide storehouse of medicinal flora and fauna. It is known to be very rich amongst other countries in the world possessing these wide medicinal plants resources. Indian geographical situation has a wide range in topography and climate, which has a direct influence on its vegetation, flora and fauna. Moreover, the agro climatic conditions are always conducive for introduction, culture and domestication of newer exotic plant varieties which are new to our soil or have disappeared in the past due to factors not favourable during those times. But the current trend since the last one or two decades has been towards the extensive investigation of secondary plant metabolites (phytochemicals) previously with unknown pharmacological activities as a source of newer medicines. Thus the aim of this research is towards the discovery and segregation of phytochemicals who exhibit strong anti microbial properties, which can be used for treatment of a range of microbe generated diseases.

Nano particles have gained major attention in the discovery of new drugs. The nano particle size of Ayurvedic powders or ‘bhasamas’ has been the subject of extreme examinations, as the nano particles reach the target area through fast spread in the cells due to their extremely small size. Ensuing activities the DNA / RNA atoms and protein synthesis inside the unit are hypothesised further as could reasonably be expected components for the quick onset of restorative movements of these Ayurvedic powders also known as Bhasamas as therapeutic agents. This most recent headway in exploration of natural drugs has opened another window by the utility of Ayurvedic bhasamas in herbo-mineral antidotes (Khare CP et al., 2012).

Discussion on medicinal plants from countries other than India:

In the rural areas of Libya, medicinal plants are a traditional source of remedies to fight many ailments. The main five of them: Thapsia garganica, Hammada scoparia, Euphorbia serrata, Hyoscyamus albus and Retama rateam have scientifically investigated for their antimicrobial activities. The extracts of the plants were evaluated for their gross phenolic and flavonoid content on which their antimicrobial potential is directly dependent. Research showed that the ratio between their anti oxidant activity and the gross phenolic content to be 1: 0.77 and to the flavonoid content to be 1: 0.98
The crude, aqueous and methanol extracts of these plants were tested against Gram positive and Gram negative bacteria. Here it was deduced that the extracts of *Hammada scoparia*, *Euphorbia serrata* gave good results against *Escherichia coli* and the extracts of *Hammada scoparia* and *Hyoscyamus albus* exhibited an exceptionally good antifungal and antimicrobial effect due to their potently high alkaloid content. Hence their antioxidant nature made them favourable for use since ages by the local natives (Rabia A *et al.*, 2012).

1.3 COW USED FOR THE STUDY:

The cow urine selected for this study was of a pure breed Gir cow (Junagadh, Gujarat). Its prominent physical characteristics are its reddish brown colour, with a bulging fore head and drooping ears. The cow is fondly known and addressed by the name ‘Godavri’ by the care taker of goshala at Gurukul Supa. It is seven years old, free of diseases, being vaccinated regularly as per veterinary schedule from time to time. The vaccine to counter worms and mumps is administered every three months interval, and vaccination for foot and mouth diseases is given twice in a year. Hence this cow was found medically fit and suitable for this study. The general and regular diet of the cow mainly consists of Green grass, Jowar, Maize, Green tea. Over and above this, a daily diet consisting of 5 Kg cotton oil cakes and maize oil cakes, 4 to 5 Kg Rice husk, and 40 Grams of mineral powder Vetcomin Forte Chelated is also given.

Vetcomin Forte Chelated is an animal feed supplement for proper maintenance of the animal, better health, better milk yield, and better reproductive performance. It is manufactured by Meteoric Life Sciences, 501, Samedh building, near Associate petrol pump, CG road, Ahmedabad 380006 and marketed by Vetco Pharma, b/h telephone exchange, Veraval 360024.

Each Kg of Vetcomin Forte Chelated consists of

- Macro minerals
  - Calcium.....................250.0 gm
  - Phosphorus..................100.0 gm
• Sulphur.........................8.0 gm
• Magnesium......................6.0 gm
• Iron..............................1.0 gm
• Iodine..........................0.4 gm
• Selenium.........................0.008 gm
• Chromium....................0.008 gm

  o Micro minerals:
    • Zinc..........................6.0 gm
    • Copper........................1.2 gm
    • Manganese..................1.2 gm
    • Cobalt.........................0.15 gm

  o Vitamins:
    • Vitamin A.....................600000 IU
    • Vitamin D3..................120000 IU
    • Vitamin E.......................0.3 gm
    • Niacin..........................1.0 gm
    • Protected Chlorine.........8.0 gm, especially for better fat metabolism.
Figure: 03

GIR COW
1.4 MICROORGANISMS USED IN THE STUDY:

BACTERIA:

Bacteria are extremely minute microscopic organisms possessing a quite simple and primitive form of cellular structure, and are commonly known as ‘prokaryotic’. Denish physician Christian Grams discovered in 1884, the differential staining technique familiarly known as Gram staining, which has grouped all bacteria as either Gram Positive or Gram negative bacteria. The Gram Positive bacteria appear deep violet in colour as they retain the crystal violet colour and also resist decolourization with acetone or alcohol. Contrary to it, the Gram negative bacteria loose the crystal violet colour in the presence of acetone or alcohol, and are counter stained by saffranin leading them to appear red in colour. Bacteria are broadly classified according to their morphological characteristics as lower and higher bacteria. The lower bacteria are generally unicellular structures, never found in the form of mycelium or sheathed filaments, e.g. cocci, bacilli, etc. Whereas, the higher bacteria are filamentous organisms, few being sheathed and also having special cells for reproduction. Those microorganisms capable of imparting any disease in humans or animals are known as ‘Pathogenic microorganisms’ or simply as ‘Pathogens’ (Prescott, 2008).
Figure: 04

GRAM POSITIVE BACTERIA

*Bacillus subtilis*

*Staphylococcus aureus*
Staphylococcus aureus:

Genus: Staphylococcus

In 1878, Koch observed some micrococcus type organisms in pus. Louis Pasteur, in 1880, cultivated these cocci in liquid media. They are found to be gram positive cocci, having a ovoid or spherical shape. They are non motile, and are arranged in a group of clusters. They grow on nutrient agar producing a colony which is observed to be of golden yellow, white, or lemon yellow colour. Pathogenic strains lead to coagulation, ferment glucose, lactose and mannitol with production of acid, which liquefies the gelation, and finally produces pus in the lesion. Staphylococcus aureus produces a golden yellow pigment.

Staphylococcus differs from micrococcus and other genus of the same family because it anaerobically utilizes glucose, manitol, and pyruvate. Staphylococci, are generally present on the skin or mucous membrane of the animal body.

The individual cells are observed to be 0.8 to 0.9 μ in diameter. They are spherical or ovoid in shape, non motile, non capsulated, non-sporulating. They are stained with aniline dyes or triphenyl methane dyes, and is gram positive. They are typically arranged in groups or irregular clumps resembling bunch of grapes, and are seen single or in pairs. They grow easily at an optimum temperature of 35°C on nutrient agar. They are notorious for causing supurative (pyogenic or pus forming) conditions in the host body (Frobisher M. 2002).

Bacillus subtilis:

Genus: Bacillus

Its members are large gram positive rods, occurring in chains, growing aerobically, and also form heat resistant spores. Most of these organisms exist as saprophytes in soil, water, air, and on vegetation, e.g. Bacillus cereus and Bacillus subtilis.

The species are differentiated by a number of criteria, of which one main is the size of the bacillus. There are classified into two groups, one being of the large cell, e.g. Bacillus megaterium, Bacillus cereus; and the other of the small cell, e.g. Bacillus subtilis, Bacillus stereothermophilus.
These species are of special interest either with reference to antibiotics, or in tests determining the effectiveness of sterilization procedures. Some *Bacillus subtilis* strains are observed to produce an extracellular penicillinase. The enzyme concerned is an adaptive one, being formed in appreciable amounts only when the organism is grown in the presence of penicillin. *Bacillus subtilis* is generally used as a test organism in determination of the efficiency of ethylene oxide sterilization. The spores of *Bacillus pumilis* are routinely used to test the efficiency of the ionizing radiation methods of sterilization (Frobisher M. 2002).
Figure: 05

GRAM NEGATIVE BACTERIA

Salmonella typhi

Escherichia coli

Proteus vulgaris
**Salmonella typhi:**

Genus: *Salmonella*

The genus *Salmonella* comprises of more than 1000 serotypes, and the pathogens causing enteric fever belong to it. Unlike other *Salmonella*, which are primarily the parasites of animals other than man, *Salmonella typhi* and its cousins, the three paratyphoid bacilli, are essentially observed as the parasites of man.

Species *Salmonella typhi* are present in large numbers in the blood, infected organs, ulcers, intestinal contents, and in faeces of humans. They are observed to be gram negative, motile, and non capsulated. They are pale coloured and show similarity to the other common pathogenic genus, *Shigella* (Prescott, 2008).

**Escherichia coli:**

Genus: *Escherichia*

They are gram negative rods, motile with peritrichous flagella. They do not form spores, and are always present in the intestinal tract of man or lower animals. This genus comprises of *Escherichia coli* and several of its variants.

Theoder Escherichi, in 1885 discovered *Escherichia coli*, routinely and commonly found in the human intestine, in sewage, water and or soil contaminated by faecal waste matters. *Escherichia coli* are gram negative rods, of 2 to 4 µ diameter, commonly observed in coccobacillary form. They are non spore forming, and have 4 to 8 peritrichous flagella. They are sluggishly motile, facultative anaerobes, and can be easily grown in laboratory media. *Escherichia coli* are generally non pathogenic, but in certain instances acts as opportunistic pathogens, as sometimes some strains of *Escherichia coli* are held responsible in producing septicaemia, inflammation of liver or gall bladder, appendix, and other infections. This species is an important pathogen in the veterinary field (Prescott, 2008).
**Proteus vulgaris:**

Genus: *Proteus*

*Proteus vulgaris* is a bar shape, Gram negative bacterium. They are basically detected in the intestinal tracts of people and other creatures. It is likewise discovered in soil, water and faecal matter. It is incorporated in the *Enterobacteriaceae* family, and is for the most part an entrepreneurial (opportunistic) pathogen which causes urinary tract problems, nosocomial infections and wound infections. *Proteus mirabilis* is the causative executor of almost eighty five percent of problems caused by *Proteus*. During clinical investigations of patients at medical care facilities and hospitals, and from patients having compromised immune systems, presence of *Proteus vulgaris* and *Proteus penneri* are commonly observed. *Proteus* and its other sister organisms like *Enterobacter, Enterococci, Klebsiella, Pseudomonas, Staphylococci* are commonly answerable for problems in patients with structural abnormalities of the urinary tract and catheterization or the patients acquiring nosocomial infections (Prescott, 2008).
YEASTS AND MOLDS

Aspergillus fumigatus

Candida albicans
YEASTS AND MOLDS:

*Aspergillus fumigatus*:

Genus: *Aspergillus*

*Aspergillus fumigatus* is a fungus commonly causing disease in patients with an immunodeficiency. It is a saprophytic fungi spread all over in nature, and has been mostly observed to be present in moist earth and other natural matter left in rotting conditions such as manure (compost) piles. *Aspergillus fumigatus* has been found to play an important part in the recycling of carbon and nitrogen. Settlements of this growth have been observed to be produced from a large number of conidiophores of very small greyish green coloured conidia of size 2–3 μm. They promptly become airborne. The fungus is capable of growth at the normal human body temperature of 37 °C or 99 °F, and their survival and growth is observed to be prominent in temperature as high as 51 °C. These conidia are able to survive even up to 71 °C, which is a general temperature of rotting manure due to self warming. Their spores are wide spread in the atmosphere, and it has been estimated that a person on an average inhales several hundred spores daily but they are rapidly destroyed by the immunity power of a normal healthy human body. On contrary to it, in patients with weak immune power like those who have acquired AIDS or leukaemia, or who have undergone major organ transplant surgery, *Aspergillus fumigatus* easily becomes pathogenic, by overcoming the weak defences of the host, and thus cause diseases generally termed as *Aspergillosis*.

*Aspergillus fumigatus* has been noted to be the most frequent cause of invasive fungal infection in immuno deficient patients, this may also include patients who receive immunosuppressive therapy for autoimmune or neoplastic disease, patients who have undergone vital organ transplants, and those affected by AIDS. *Aspergillus fumigatus* has been observed to primarily cause morbidity and mortality in patients who have acquired an invasive infection in the lung. Even in immuno competent hosts *Aspergillus fumigatus* has been able to surpass the immune power and is found to be responsible for causing chronic pulmonary infections, allergic broncho pulmonary aspergillosis, or allergic diseases. (Prescott, 2008)
**Candida albicans:**

Genus: Candida

*Candida albicans* is a dimorphic fungus that grows as yeast and filamentous cells. It causes opportunistic oral and genital infections in humans. RNA-binding protein Slr1 is responsible for the virulence power in *Candida albicans*. Systemic fungal infections commonly known as ‘fungemias’ includes those caused by *Candida albicans* are the main cause of morbidity and mortality in immuno deficient AIDS patients, patients receiving cancer chemotherapy treatment, or those who have undergone organ or bone marrow transplantation treatments. *Candida albicans* is known to form bio films on the surface of implantable medical devices like screws, rods, plates used in orthopaedic procedures; stents in cardiac surgery. In addition, *Candida albicans* is also responsible for causing nosocomial infections, and is a major health concern world-wide.

*Candida albicans* is a commensal found in the normal gut flora comprising of microorganisms that inhabit the human mouth and gastrointestinal tract. *Candida albicans* is generally present in 75 to 85% of the human populace with no detrimental effect. An excessive growth of *Candida albicans* leads to a problem known by the clinical term: ‘candidiasis’ or ‘candidosis’. It is often observed in immuno deficient patients such as those afflicted by HIV. Candidacies restricted to the mucosal membranes in mouth or vagina is known as thrush, which is very easily cured in those patients who are not immune compromised. Higher prevalence of *Candida albicans* has been observed in young individuals with tongue piercing, activity. The generally found unicellular yeast-like form of *Candida albicans* has been observed to infect the host tissue by adapting to the environmental conditions and converting itself to an invasive, multicellular filamentous form. This phenomenon has been termed dimorphism (Prescott, 2008).
MEDICINAL PLANTS USED IN THE STUDY

*Asparagus racemosus*

*Ocimum sanctum*
Tinospora cordifolia (Gaduchi)

Withania sominifera (Ashwagandha)
1.5 MEDICINAL PLANTS USED IN THE STUDY:

Asparagus racemosus:

*Asparagus racemosus* traditionally known as *Shatawari*, meaning ‘the curer of hundred diseases’ (Shat: hundred, and vari: cure). It is an important medicinal plant found in the tropical and sub tropical regions of India, Sri Lanka, and the Himalayas. The medicinal based literatures of the yore like *Ayurveda* (Hindu), *Unani* (Muslim) and *Siddha* and also other Indian and British Pharmacopoeias have made expositive expressions of its medicinal importance as a medicinal plant. It has gained usage as an adaptogen, to boost the non particular resistance of the creatures against various stresses. The plant is also used in the treatment of diarrhoea and dysentery. It exhibits prominent antioxidant, immune stimulant, anti-dyspepsia and anti tussive effects. The plant preparation is generally recommended as a galactogogue (breast milk output improver), as a tonic for controlling uterine disorders, in control of indigestion also known as ‘acid dyspepsia’ or simply as ‘hyperacidity’. It is also consumed as a common health tonic. The presence of saponins, also known as Shatavarins have been attributed to the adaptogenic effects of Shatavari (Goyal R et al., 2003).

Research in pharmacology suggests that *Asparagus racemosus* is well known for its anti nuclear, anti secretory and anti bacterial, anti depressant activity; anti-inflammatory, anti-cancer, anti oxidant, and anti tussive effects. It plays an important role in enhancing memory and protects against amnesia.

It also exhibits different effects like being, Aphrodisiac, Diuretic, Hepatoprotective, Immunomodulatory (extract), Immunoadjuvant, Neuroprotective, Oestrogenic, and Teratogenic effects. It has the capacity to prevent hepatocarcinogenesis, works in relieving stress and is also used in the treatment of diabetes reducing blood glucose levels.

Chemical constituents:

It is found in the form of a polycyclic alkaloid, Asparagamine A. It demonstrates antitumor activity against numerous cell lines. New steroidal saponins: Shatavaroside, Shatavaroside B, Shatavarins VI to X, Filiasparoside C and Saponins: Shatavarin I (Asparoside B), Shatavarin IV (Asparinin B), Shatavarin V, immunoside
and schidigerasaponin D5 (Asparagus A) have been successfully segregated by analysts from the roots of *Asparagus racemosus*. Isoflavone, 8-methoxy-5,6,4'-trihydroxyisoflavone 7-O-beta-D-glucopyranoside has also been obtained during the segregation.

**Ocimum sanctum:**

*Ocimum sanctum* is an aromatic plant found in the eastern world tropics across the whole of South Asia, India, and Nepal. Its common name is *Tulsi*, meaning a ‘holy herb’. *Tulsi* is cultivated and grown in front of people’s residence for sustaining religious customs and for medicinal purposes also as it contains essential oil. It has gained wide acceptability across India and Hinduism followers in Asia as a medicinal plant. An Ayurvedic preparation also known as herbal tea is commonly prescribed in the treatment many body disorders. The holy basil plants or leaves are worshipped particularly in mornings by the Vaishnavite followers of Hinduism. *Tulsi* has secured its usage value since centuries in Ayurveda for its wide healing properties. An ancient Ayurvedic text, *Charaka Samhita*, considers *Tulsi* as an adaptogen, it balances different and a peculiar astringent taste. Ayurveda has accepted to be the "elixir of life", promoting longevity. Many ayurvedic preparations use *Tulsi* extracts as medicines to encounter a numerous minor and major ailments. *Tulsi* is commercially available for use in different forms such as herbal tea, or in dry powder form or as dried leaves and even fresh leaves, which are consumed by people for health benefits blending the same with pure ghee. The key oils also extracted from Karpoora *tulsi* are of huge medicinal criticalness and is also widely used in the manufacturing of herbal cosmetics and skin preparations (Kothari *et al.*, 2008).

The fundamental substance constituents of *Tulsi* are: carvacrol, eugenol, linalool, oleanolic acid, Rosmarinic acid, Ursolic acid, beta-caryophyllene (7.9-8.1%), beta-elemene (10.9-11.1%), and germacrene D (1.9-2.1%).

Some potential pharmacological properties of *Ocimum sanctum* and its extracts have been indicated on in *vitro* studies and animal studies. Recent studies have concluded *tulsi* to be having a high concentration of eugenol necessarily found in modern day pain killers; hence it functions better as a COX-2 inhibitor. The combination of *Tulsi* with other hypoglycemic drugs has been found to play a vital
role in the reduction of blood glucose levels in type II diabetics. Similarly, it also helps in reduction of total cholesterol levels in the human body. The inherent antioxidant property of Tulsi renders its consumption directly or in purified concentrate form in imparting relief and giving beneficial effects on blood glucose levels because of its innate cell reinforcement properties. It offers a half way shield from radiation harming and is of great relief in cataracts. Anti-oxidant properties of Tulsi are of assistance in the repair of body cells damaged by exposure to radio activity. Experimental trials of the oil derived from Tulsi on the effects in rats sustained with a high fat diet have shown better antihyperlipidemic and cardioprotective impacts. Alcoholic extract of tulsi regulates resistance, accordingly improving and boosting the function of immune system in the human body. Investigation is carried out to evaluate the potential of β-Element for its anticaner properties, and its effectiveness is still to be confirmed by human clinical trials. Ocimum sanctum extracts shows antibacterial activity against Echerichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Proteus vulgaris.

Chemical constituents:

Main chemical constituents of tulsi are: carvacrol, eugenol, germacrene D (about 2%), linalool, oleanolic acid, rosmarinic acid, ursolic acid, beta-caryophyllene (7.9-8.1%), and beta-element (10.8 - 11.2%).

**Tinospora cordifolia:**

Tinospora cordifolia, also commonly known as Guduchi, belongs to the family Menispermaceae, found indigenously in the tropical areas of India, Myanmar and Sri Lanka.

Both the Ayurvedic and Jamu herbal practitioners regularly prescribe the concoctions of Tinospora cordifolia, and its other nearly related species: Tinospora crispa and Tinospora rumphii in direct or modified forms. The current research has concluded that a consolidated mixture of Tinospora cordifolia extract and turmeric extract proves to be very effective in diminishing the effects of hepatotoxicity, which is incited by the blend of ethambutol, isoniazid, pyrazinamide, and rifampicin; which is why it is also suitable as a medicine for tuberculosis. Consumption of the alcoholic concentrate of the stem
imparts antibacterial effect against *Escherichia coli*. Gout is treated with the decoction of the leaves of *Tinospora cordifolia*. Its fruit is used in the treatment of rheumatism and infectious hepatitis.

Chemical constituents:

*Tinospora cordifolia* contains active adaptogenic constituents like diterpene compounds, polyphenols, and polysaccharides, including arabinogalactan polysaccharide (TSP) (Guo et al., 2007)

*Withania somnifera*:

*Withania somnifera*, is customarily known by the name Ashwagandha, Indian ginseng, poison gooseberry or winter cherry. It is a plant belonging to the Solanaceae family. This plant is widely used in Ayurvedic preparations since hundreds of years. Meaning of *Ashwagandha* in Sanskrit is "resembling a stallion" (ashwa: horse; gandha: smell). The odour is from the root of the plant and is similar to that of a horse. The species name *somnifera* implies "slumber actuating" in Latin. *Withania somnifera* is grown mostly in the drier Indian states like Gujarat, some areas of Madhya Pradesh, Punjab, Rajasthan and also outside India in Sindh region of Pakistan and in Nepal. Ayurvedic treatment recommends the external application of the berries and leaves of *Withania somnifera* directly or in dried powder form on to tumours and ulcers.

Chemical constituents:

Leaves, root and stem of *Withania somnifera* primarily contains chemicals like alkaloids, steroidal lactones, tropine, and cuscohygrine withanolides; generally withaferin A, which was the first withanolide to be segregated from *Withania somnifera* (Pandit S et al., 2013).
1.6 SYNERGISTIC EFFECT OF COW URINE AND MEDICINAL PLANT EXTRACTS AND ITS IMPORTANCE:

Synergy extensively means that the result of consolidated activity of different constituents is more excellent than that which might have materialized from singular constituent commitment. As this action leads to better and beneficial results from the combined action of different constituents which is found to be better and greater than that which would have realised from individual constituent contributions, thus the age old proverb: “United we stand; divided we fall” proves to be true. In pharmacology synergy has a specific definition, but the term is often wrongly applied to describe any kind of interaction between the constituents of a single extract, as well as the components of a mixture of herbs. These interactions may involve a sum addition or potentiation of therapeutic effects, or an attenuation of toxicity, or side effects within the preparation. Whether any effect is truly synergistic or is just merely additive is difficult to define, hence rarely established, and evidence to define its conclusivity is sparse. Medical botanists after detailed research have come to the conclusion that effective results are achieved using the complete plant concentrates rather than using individual compounds. The institutionalization of these phytomedicines is more critical if true synergistic reaction has been observed, because the proportion between synergistic operators will play a discriminating role and any modest progressions can make the effects flighty, meaning not predictable.

If the blended impacts are simply an addition, then the progressions in the proportion will be less pivotal. It might along these lines be convenient to demarcate the cooperative energy decisively, and rather, routinely utilize the expression “polyvalent action” when the nature of action is vague or unclear (Trease and Evans, 2007).

1.6.1 ANTI MICROBIAL ACTIVITY:

Microbiology is the science dealing with the study of microbes, which are the microscopic organisms present in the plant and animal kingdom. The possibility of the microbiostatic compounds inhibiting the reproduction of pathogenic microbes and
enable the leucocytes and other defence mechanism of the host to cope up with few static invaders has been overlooked by the microbiologists and clinical personnel.

Paul Ehrlich, the discoverer of chemotherapy, used chemotherapeutic agents such as antibacterial, antifungal, antimicrobial, antineoplastic, antiprotozoal, antitubercular and antiviral agents for the cure of infectious diseases without any injury to the host’s tissue. Antimicrobial substances and its preparations are classified as antiseptics, disinfectants and chemotherapeutic agents. The term ‘disinfectants’ is used for the products which are utilised to eliminate or destroy infections, and should also be capable of destroying a wide range of microbes without any harm to the microbial spores. An antiseptic is a product possessing antimicrobial properties similar to those of the disinfectants, is used to control or eliminate microbial infections. Whereas, a chemotherapeutic agent is an antimicrobial substance, whose main function is to prevent the multiplication of the infective microorganisms, is administered systematically for the treatment of the infection.

**Antimicrobial agents:**

These are the chemotherapeutic agents generally used against microbial diseases and are divided into two types according to their action on microbes, namely microbistatic and microbicidal agents. A microbistatic agent inhibits multiplication or further growth of microbes, whereas a microbicidal agent controls infection by killing the bacteria. Antimicrobial agents are the chemotherapeutic substances that inhibit or destroy the growth of microorganisms in the living tissue. Antibiotics are substances produced by the living organisms, sufficiently non toxic to be used as antimicrobial agents. Natural substances like plant extracts, cowdung, cow urine etc have antimicrobial effect.

**1.6.2 PHYTOCHEMICAL ANALYSIS:**

Traditional remedies invariably involve crude plant extracts containing multiple chemical constituents, which vary in potency from highly active to very weak potency. A number of compounds having therapeutical properties that are present in medicinal plants and which are used for therapeutic purposes can be easily and perfectly identified by phytochemical analysis. Such chemicals extracted are also known as
phytochemicals. Such phytochemicals extracted from medicinal plants include alkaloids, flavonoids, steroids, tannins, and other different products. Out of the above products, alkaloids, flavonoids, and tannins are the main products that possess antibacterial properties. These products essentially help to identify newer structurally novel natural products with new modes of action and exhibiting antimicrobial activity. Plant metabolites of secondary stage with unknown pharmacological activities are under investigation for them to work as medicinal agents. It is anticipated that phytochemicals with adequate antibacterial efficacy can be used for the treatment of bacterial infections. HPTLC analysis can be carried out to detect the presence of these secondary metabolites.

HPTLC is a sophisticated and automated form of TLC (Planner chromatography). HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. It allows efficient and cost effective analysis of a broad number of compounds. Many samples can be analysed there by dramatically reducing analytical time. With HPTLC, the analysis can be viewed using different wavelengths of light thereby providing a more complete picture of the plant than is typically observed with more specific types of analysis. (Snyder LR, 1978; Chatwal GR, et al., 2002)

1.6.3 ANTIMUTAGENIC ACTIVITY:

Earth is made up of a number of entities either in living or non living form. Humans and animals exist surrounded by living but immobile substances called vegetation and non living substances which are commonly addressed by the term “chemical”. These chemicals are divided between naturally occurring and those synthetically produced by man. Some of these chemicals have an ability to cause mutation. These substances exist in all three forms: solids, liquid and gas. They are present in our food, or enter our body through the air we inhale, or still others gain entry into the body through absorption in our skin also. Such chemicals that enter our body and cause mutation are carcinogenous as they change the nucleic acid sequence of DNA.

Hence a proper, rapid and inexpensive method for testing the chemicals that are suspected as carcinogenic is the need of the day because a large number of new synthetic chemicals are being produced year after year. At the same time it is also extremely necessary to segregate naturally occurring and synthetically produced
substances that act as antimutagenic agents for safety aspect. These mutation inducing chemicals are fit to harm the germ line, that likewise prompts fertility problems mostly in females and additionally lead to unanticipated bad mutations in post generations. Many mutagenicity testing programmes have gone hay-wire due to the fact that the mutagenic products have the ability to initiate the growth of cancer.

The change in the development necessities of cells in microscopic organisms or other cell frame work might be measured by gene transformation. In the mammalian cells, breaks or re arrangements of chromosomes in the cells indicates gene mutation. The *Salmonella typhimurium* test is a transient bacterial technique generally acknowledged for distinguishing those substances that have the ability to generate hereditary damage, which finally results in gene changes.

In the *Salmonella typhimurium* assay test, usage of many *Salmonella typhimurium* strains having pre-existing mutation allowing the bacteria which are unable to synthesize the required amino acids and histidine is done, whereby their growth is stunted and in its absence form colonies. Synthesization of histidine by the cells or the restoration of the gene’s function is possible by new mutation near-by the site of these previously existing mutation or nearby in the genes. It has been observed that those cells which are newly mutated, able to from colonies and are also able to grow further in the absence of histidine. Hence this procedure is generally known as “reversion assay”.

The Salmonella strains that are utilized within the test have transformations that are distinctive in different genes in the histidine operon. Each of these transformations is intended to be receptive to mutagens that enactment as per distinctive mechanisms. The strains were made more sensitive to numerous substances by bringing extra changes into these strains. The outline of Salmonella Mutagenicity test has been explicitly done to catch the artificially affected mutagenesis. The importance of this test has gained importance since last few years by the government based scientific testing centres in the world, which test and access the mutagenic potential of new chemicals and drugs.

The *Salmonella* test that has been propagated by Bruce N. Ames and his associates has gained world-wide acceptance as an initial test for identification of chemicals that are able to cause mutagenic activity. Potential animal mutagens are those substances that
produce a positive mutagenic effect and are also carcinogens. Chemicals that are not mutagenic in *Salmonella* cannot be considered begnins or carcinogenic. Certain chemical classes like chlorinated hydrocarbons contain a large number of carcinogens but still are not mutagenic to *Salmonella*.

Mutagens act in different ways and all of them have a common ability to alter the DNA base sequence like point mutation and frameshift mutations within the genome. Researcher oncologists who have conducted many clinical trials have commonly agreed to the fact that most of the mutagens have the capacity to be carcinogenic and they play a vital role in the induction of neoplastic cell growth at many cancer sites.

The Ames test is a biological assay to screen and help in identification of the chemicals affecting the structure of DNA, thus also assessing the mutagenic potential of a chemical compound. Since the chemical nature of DNA is the same in bacteria and mammalian cells, a chemical that is shown to be a mutagen for bacteria is likely a to be a mutagen for mammalian cells. Also, rapid cell division and concurrent rapid DNA replication of most bacteria allow the detection of an expressed mutation in a very short period of time.

The assay procedure for a simple, quick and cheap screening of mainly commercial products acting as mutagens in bacteria was formulated by Bruce N. Ames *et.al* (1960), at the University of California, Berkeley. The Ames test identifies all mutagens irrespective of their carcinogenic or non carcinogenic nature due to the fact that the chemical nature of DNA is same in bacteria and mammalian cells. Still the procedure provides useful preliminary information and opens the doors for further investigations in sophisticated animal model systems to finally determine the actual carcinogenicity of a substance that was positive in the Ames test.

Recessive mutations can be detected readily due to the fact that bacteria have only one chromosome. Several modifications have been done to the original test, thus allowing detection of a wide range of mutations. For further increasing the relevancy of this test to human risk, a liver extract has been used to compare and access the biochemical modifications of chemicals that occur in the human liver.

The assay employs five strains of *Salmonella typhimurium* histidine auxotroph mutants (TA98, TA100, TA102, TA1535 and TA1537) deficient in the synthesis of
histidine, an amino acid essential for bacterial growth. All strains have gained recommendation from the Organization of Economic Corporation and Development (OECD) for Ames testing. The mutant strains can grow only in medium containing histidine. The goal of the assay is to determine whether compounds induce reverse mutations that restore the microorganism’s ability to synthesize histidine.

Only histidine prototroph (his+ revertants) will be able to grow and form visible colonies on an agar media containing a small quantity of histidine. If a mutagenic compound is introduced into the bacterial population, it will positively result in a higher number of revertants, this being compared against an untreated control culture. By using bacterial strains that carry different mutations, it is possible to determine the type of genetic mutation (e.g. deletion, substitution) induced by a mutagenic compound.

Characterization of *Salmonella* strain in the DNA repair mutation (*uvrB*) eliminates excision repair. The presence of the *uvrB* mutation makes the strains more sensitive to the test articles that induce damage. The *uvrB* change is part of an erasure transformation enlarging into a gene for biotin union; along these lines, the biotin prerequisite is a consequence of the cancellation of this area. The *uvrb* change is demonstrated by sensitivity to UV light. Notwithstanding the Histidine change these *Salmonella typhimurium* strains holds other transformation that extraordinarily expand their possibility to trace mutagens.

The rfa transformation can modify the qualities of the bacterial cell divider and cause incomplete loss of the lipopolysaccharide (LPS) hindrance by expanding porousness of cells to certain sorts of chemicals. The rfa change is seen by sensitivity test to crystal violet.

The R-component plasmid (*pKM101*) makes the strains more receptive to a mixture of mutagens. The plasmid conveys an ampicillin resistance gene; consequently ampicillin resistance demonstrates that the strain holds the plasmid.

Certain chemicals which are pro-mutagenic agents however are observed to be activated and become true mutagens in humans. Such activation due to a chemical modification generally is seen to start from the liver due to the activity of the normal liver on new substances. Enzymes required to activate the pro mutagens are not
produced in bacteria such as *Salmonella typhimurium*. These results in the non
detection of pro-mutagens by Ames test until they were first activated. Mixing the test
compound with catalysts from liver that change over pro mutagens into active
mutagens is an essential step included in the Ames test. Bruce Ames has effectively
illustrated that mutagenic executors are equipped for initiating updates in the unusual
gene that encodes the faulty his-compounds, accordingly bringing about to return once
again to the typical shape encoding the active protein. A transformation that returns a
trademark capacity to a mutant is known to be reverse mutation.

Those spontaneous changes happening by chance without any chemical treatment will
show up as colonies on the negative control petri plate. The amounts of mutant
colonies showing up on the positive control plates are additionally tallied which has
actuated change for the test to be recognized substantial. Failure from the positive
control compound to induce transformation is the main reason of tossing the
experiment

The Ames test has numerous noticeable focal points for the recognizable proof of the
hemicals bringing about gene changes. These focal points are recorded in short as:

1. **Quick results**: The test makes conceivable replicates and acquiring more reliable
   results in vicinity of only about 48 hours which is a very short period.

2. **Economic viability**: The Ames test empowers studying a large number of test
   materials at manageable cost offering budgetary profits.

3. **Different tester strains with numerous gene transformations**: The test
   empowers a synchronous research of the molecular impact mechanism of different
   test materials.

4. **Additional transformations and gene changes**: The test also permits additional
   sensitivity for distinctive chemicals.

5. **Mesophile character of Salmonella strains**: the test permits the investigation of
   mutagenic potential of the chemicals at 37 °C.

Apart from all the aforementioned focal points, the Ames/salmonella test framework is
exceptionally flexible in provision. Various alterations have been adequately done to
confirm mutagenic possibilities of different materials, for example natural chemicals
and its mixtures, body fluids, food, medications and numerous other physical operators.

The most common changes in the Ames test method are:

1. **The Spot test**: It is an essential technique for the determination of chemical mutagens.

2. **The Standard plate incorporation technique**: It is a more extensive strategy giving results compared to the spot test.

3. **The Pre-incubation technique**: This modification is done for procuring more successful studies with small amounts of test materials; the desiccator assay modifications are developed to study volatile materials and gases.

4. **The modified Salmonella microsuspension assay (Kado)**: It is a very delicate technique for testing the substances that are accessible only in minor amounts.

Diseases have been successfully treated since the advent of mankind by the utilization of plant extracts after trials and experiments on traditional medicine. In the days of yore there was no system for the exhaustive toxicological evaluations of the traditional medicinal plants, which the modern pharmaceutical compounds are subjected to today. Use for very long periods of time and getting positive results made them to be assumed to be safe. Research during the last few years has shown that a number of plants which are used for nutritional purpose or as therapeutics have *in vitro* mutagenic and antimutagenic properties. This proves that investigations centred on medicinal plants used since ages is valuable and important for the discovery of potential chemotherapeutic drugs that have no side effects like host resistance and are available from natural and renewable sources. The last few decades have been a witness to detailed study which has been centred on popular medicine available from medicinal plants with the aim of identifying, isolating and establishing natural products with powerful therapeutic properties.

At the same time cow urine also act as an antimutagenic agent. It is reported to contain no less than twenty four types of salts. Cow urine distillate (Go-Ark) is able to decrease the toxic effect of carcinogenic chemicals. According to our ancestors cow urine is considered to be the most effective medicine in the world which during their time was the main elixir of many incurable diseases. Though there are numerous examples stating the efficiency of cow urine, scientific reports are few. Thus, to reveal
the genoprotective potential of cow urine it has been used in the present investigation. In vitro genoprotective role of cow urine has been reported (Krishnamurthi K et al., 2004) but its efficiency against in vivo genotoxicity and its combined effect along with the various antioxidants is yet under investigation. Hence, this present study has been undertaken to fill the lacuna in this regard as far as possible to a major extent.