2.0 REVIEW OF LITERATURE:

2.1 Heritage of India; Historical and Medicinal Significance of the Indian Medicinal Plants:

The Indian medicinal plants are the heritage of India from the great age due importance they have commanded from time immemorial. Ayurveda in itself is a deep study and experiments conducted over time to access the natural wealth of plants available on our land. This has resulted in to the development of drugs to curb varied ailments that affect man in particular and the animals in general. These drugs developed from natural resources which are mostly inexhaustible, cheap, abundantly available, and finally are fully eco-friendly with host resistance to them over the time period to be almost zero. Today even if the natural resources are on the exhaustion limits, then modern cell culture techniques help in cultivating those species that are on the verge of depletion.

Indian Ayurveda can be very safely expressed as a medical art since ancient times and the drugs so developed have been used in the days of antiquity as far back as history can take us. Medicine and treatment are two inter connected aspects which are directly responsible to combat the invasion and provide a cure to already invaded ailments. The earliest mention of Indian medicinal plants is in the ‘Rigveda’, one of the oldest repositories of knowledge, having been created approximately 4000 – 6500 years back from today. ‘Atharvaveda’ is the next repository in which the usage of medicinal plants for the preparation of drugs is more varied. Finally it is in the Ayurveda, or also known as ‘Úpaveda’, meaning supplementary, wherein detailed instructions regarding the properties of drugs and their dosage are available for the benefit of mankind. Hence Ayurveda in itself is now considered as the foundation stone of Indian medicinal treatment. Many western medicinal scientists assume Ayurveda to be as old back as 2500 – 3500 years.

Generally all Ayurvedic medicines consists of 15 or more drugs mixed together in a single potion; a poly herbal formulation. Some medicines which are meant to cure a
particular ailment like sore throat or common colds are equally effective against external skin disorders like dermatitis. The well-known Ayurvedic tonic: Dashmularishtha is a combination of 67 different herbal extracts. Similarly, Khadirradi Vati for sore throat contains 08 formulations and 05 to 37 herbal extracts. Allopathic healers will consider this to be absurd; doubting even the authenticity of certification from renowned Ayurvedic science institutes (Khare CP, 2012).

Surgery and therapeutics are dealt in detail in many chapters in the Ayurveda. Separate descriptions are available for Purgatives, Emetics; with the simpler medicines arranged in separate heads. The methods of administration of the drugs, their dosage and time interval which are described in detail, bears a very fine resemblance to the sanitary and antibiotic aspects taken care of today. From this point onwards, until the Muslim invasion on India, this art of medical science flourished. The other nations of the western world began to divert their attention towards the people of India, especially towards the Hindus of those days, to gain access to their knowledge of the healing art. Thus Indian medicine reached the far-off shores of Egypt, Rome and even Greece. The skill of the physicians of curing Greek soldiers from poisonous snake-bites and other ailments bears a testimony to this. There are ample reasons to believe that many Greek philosophers like Hippocrates, Paracelsus and Pythagoras had actually visited India in order to acquire this medicinal knowledge from their Indian counterpart. The work of the renowned physician Dioscoroides shows the extent the ancient Greeks were indebted to the Indians for their medicine.

The down-fall of Indian medicinal science started with the successive invasions by the Greeks, Scythians and Muslims. Original written and documented works were either destroyed in religious fervour of conversion to Christianity or Muslim; or they could not be taken proper care of during the circumstances prevailing in that period. The Muslims brought their own healing system: Unani; hence Ayurvedic medicine was thrown still further to the back seat.

With the advent of the British rule in India some 300 years back, western medicine began to gain importance. People began to accept the western medicine as its surgical aspects with prompt relief greatly appealed to the Indians. Western Materia-Medica intermingled with that of the Indians and it gave rise to the introduction of newer products and medicinal plants; some foreign to the Indian soil.
Hence, the combination of all these systems, mainly three: Ayurveda, Unani and the Western; led to the evolution of the commonly known Indian Indigenous Drugs (Chopra RN, Chopra IC; Indigenous Drugs of India, Academic Publishers, India).

Other factors which contributed to the downfall of Ayurveda were the total adamancy of the healers not to accept newer scientific innovations in their old knowledge dating a minimum of 600 years back; being reluctant to implement the minimum necessary changes to their classical tenets that were more than 2000 years old.

Ayurvedic literatures have a classical overtone, which has actually hidden its basic scientific principles, and hence it started to fall behind other modes of healing.

Most of the Ayurvedic healers possessed an unfriendly approach of not sharing their knowledge with their own brethren or counter parts practising other systems of treatment; and the lay man. Hence any drawbacks of a healer remained as it is and the basic sufferer was the patient. This led people to resort to alternate modes of cure.

But now the time has come for the Indians to come out of the coccons and embrace newer scientific procedures to update their original Ayurvedic courses. Prescriptions based on temperamental and humoral imbalances need to be changed. It is to be accepted that sharing of knowledge will lead to its increment. There should be no scope for any superstitions that sickness is the affliction of evil spirits. In circumstances of today, proper identification of main herbs, with their authentic nomenclature, the scientific evaluation of their chemical contents and their exact modes of action is the only way left to draw the proper benefits of the Ayurvedic system.

Actually Chinese medicine surpassed the Ayurvedic system by conquering the Western countries due to the nature of the Chinese people to dominate over rest of the population of the world; which was lacking in their Indian / Ayurvedic counter parts. In the US, there is a vast and detailed collection of Chinese medicinal literary works compared to that of the Ayurveda which have been confined to the archives. Even as of today, all hospitals in China have a ward dedicated to their traditional medicine which is conspicuously absent in India. Japan, known all over the world for having longest longevity of its people, is also favouring the Chinese system rather than the
Ayurveda for assistance in their conventional allopathic treatments. As of today a very few of Ayurvedic medicines are incorporated in prescriptions of allopathic practionners. This is due to a total incoordination between the experts of both the fields and hence only 12 – 15 herbal drugs are being prescribed by the allopathic doctors; a meagre figure of just 10 % of the total allopathic medicinal practionners that recommend herbal remedies (Khare CP, 2012).

India has a long cherished history of the prevalence of three systems: Ayurvedic, Siddha and Unani together; whose benefits could be reaped by the contribution and compiliation of the positive factors of each to get synergistic results. The Indian constitution also affords equal status to all these three systems of medicine with the prevailing allopathic one. Indian hospitals need to have additional wards / sections devoted to the tenents of these historical systems like that in the hospitals in China. All educational institutes imparting knowledge of medical science should have attached hospitals where the budding doctors will have an on hand experience of imparting treatment according to all the systems. The ministry of Health and Family Wellfare has a section named AYUSH, dedicated to simplifying and internally collaborating with all these systems.

The main aim of the AYUSH section is to:

1) Impart quality education and training of AYUSH doctors, teachers and scientists; thereby enhancing their knowledge by the covering of sylabbi based on paramedical, pharmacy and nursing education.
2) To construct modern facilities with centers of excellence in all aspects; whether inanimate or animate: materials and expert faculty in the field.
3) Cultivation and conservation of different medicinal plant species.
4) Promoting newer research and scientific approaches at all times; being the basic motto of AYUSH.

In India, Ayurvedic, Siddha and Unani products are regulated by the common Drugs and Cosmetics Act of 1945; wherein schedule T of the rules enforces Good Manufacturing Practices to be implemented strictly during the production of these classes of medicine. Schedule E1 contains a list of poisonous substances segregated from the Ayurvedic, Siddha and Unani systems. If any of the products contain any
intoxicating material, then it is to be sold and used under strict medical prescription and supervision; with the containers of such medicine displaying in bold the intoxicating nature of its contents. The Ayurvedic, Siddha and Unani medicines include all medicines to be administered externally and or internally; accept intravenous injections. AYUSH has also made it compulsory to conduct clinical trials on small scale before implementing in bulk for all proprietary products of all these three systems of medicine.

AYUSH has published *Ayurvedic Pharmacopoeia of India* (API) regarding the identification and quality aspects including dosage instructions of the medicines.

API Part 1 has 7 volumes with 540 monographs of individual Ayurvedic herbs.

API Part 2 has 3 volumes with 152 monographs of individual Ayurvedic preparations.

AYUSH controls the professional practicing of all these three Ayurvedic, Siddha and Unani systems of medicine and also Homeopathy under the jurisdiction of Central Council of Indian Medicine and the Central Council of Homeopathy (Khare CP, 2012).

Plants and trees exist on the surface of the earth right from the beginning of life on earth. Actually plants and trees, which are immobile living beings, are responsible for sustaining the life of all motile living beings as they convert carbon dioxide to oxygen by photosynthesis in presence of sunlight. There are 220000 to 250000 different species of plants and trees in some 300 genera and 10000 families, all having different characteristics. Most of them are useful to the motile living beings by providing shelter and food; but very few possess unpleasant or even poisonous chemicals that are unfit for use or consumption. Plants contain chemicals in natural form and this property of the plants has been exploited by man for medicinal purpose. Hence it is extremely necessary to study and assimilate different factors associated with their growth. Pharmacognosy is the answer to this important aspect.

**Pharmacognosy:**

Pharmacognosy is a unified approach of deciphering the minute details of individual medicinal plants. Many different aspects related right from the specific localised area
or site of growth of the plants like their chemistry, enzymology, genetics; procurement of the plants; their proper botanical codification; quality checks involved during their procurement; economic viability, are the major factors that are to be taken care of in the scientific evaluation of medicinal plants for their potential source of potent medicine for different human sickness. Hence pharmacognosy in itself is a phar-mo-commercial study of medicinal plants for their optimum usage. The term pharmacognosy was first used in the early part of nineteenth century for the above detailed knowledge regarding medicinal plants. It is a unified word developed by the combination of two Greek words: Pharmakon, meaning ‘a medicine’; and Giconosco, meaning ‘to get knowledge of’ (Trease and Evans, 15th edition; Ganzinger K (Dr), 1982).

Pharmacognosy is very closely associated with the botanical aspect and chemistry of the plants. It has greatly helped in the isolation of pure substances conforming to medical standards to be used as drugs. In the earlier days this aspect was a far off possibility and so the medicines used during the medieval ages were crude products prepared according to the individual knowledge of the traditional doctors of yore. Modern scientific methods for the extraction, purification and testing of the products from their crude sources is one of the main object of pharmacognosy which has helped the people on the universal scale. Newer products found their way in to the medicine cabinet of the working scientists. This led to another unexpected problem of depletion of the crude resources to an inordinate and sudden rise in its demand. This happened in the case of Catharanthus roseus, Coleus forskohlji, Arnica Montana, Taxus brevifolia and Leucojum aestiyum. Taxus brevifolia was found to be the source of drug Taxol, which found a positive application as a anti-cancer drug; and Leucojum aestiyum a source for drug Galanthamine to fight Alzheimer’s disease. Here, cell culture techniques play the role of a saviour for these crude resources which face extinction and The US National Cancer Institute has already embarked on this programme to avert a future crisis in supply of resources. Here the work of pharma-scientists possessing a varied and multi knowledge bank of the information regarding the terrain, climate, growth sustainability at the site, economic viability of production; will be of prime importance for the successful and continuous output of the crude raw material (Cragg GM et al., Journal of Natural Products and Plant Resources).
Initially Pharmacognosy was associated with the natural products used in allopathy, but in the days of Alternative medicine, which will be discussed on the coming pages, medicinal herbal products command greater importance in the subject. Pharmacognosy, though is mainly associated with plants, there are a few animal based products which are also associated in the classification. Plant based medicinal products are classified by different standard ways like:

1) Alphabetical arrangement according to their names,
2) Their taxonomy, their genera and species,
3) Their morphological characteristics, like seeds, stem, bark, leaves, flowers, fruits, roots, or the entire plant,
4) Their pharmacological or therapeutic use, and finally
5) The potent chemicals they contain.

Out of the 200000 to 250000 species of plants on the earth, till to date few have been studied for their medicinal content and an extremely large number of them remains to be explored. The screening of plants for their therapeutic potential is difficult. The easiest way is to first centre on those plants that are present in medicinal applications from the days of yore. The cultivation of medicinal plants in two areas having similar climatic conditions depends on the economic condition of the particular area. The production output changes with the economic viability. Cash crops of greater economic value supersede the cultivation of medicinal plants. With greater reliance on natural based medicinal products, developing countries like India, Sri Lanka and other Asian countries have increased the net cultivation of medicinal plants. Cultivation of one plant initially centred in a particular area a few decades back has been observed to shift in to more areas. The best example for this is the Cinnamon for centuries confined to Sri Lanka is found to grow in abundance in Seychelles.

India has the best climatic conditions which support the growth of various species of plants. China is also a major producer since last few years. Many countries still produce an abnormally low volume of medicinal plants due to their thrifty economic conditions and political instability. Individual government policies also play a major role in the output. Countries of the world began exploring for supplies of *Rouwolfia root*, an original produce of India towards Thailand due to the Indian government limiting the export to the best varieties only at a higher price.
Other factors that affect the cultivation of medicinal plants are the international restrictions imposed by the bodies having control over their procurement. The best example in this case are the restrictions placed on Aloe species (except Aloevera) by the Washington based Convention on International Trade in Endangered Species (CITES), caused problems in availability of all Aloe species in the world.

2.2 Steps involved in the discovery of new drugs:

A number of steps are involved in the scientific approach to the discovery, extraction, testing and finally incorporating as new drug are enumerated in brief as follows:

1) Scientists gather information from individuals and masses regarding their incorporating natural herbs in their daily activities.
2) Naturally occurring crude product being used in traditional medicine is separated for further examination according to their intensity of acceptance in the particular community.
3) Material is screened according to its scientific nomenclature like morphological characters and taxonomy.
4) A series of fractional extractions of the prominent active products is done; their chemical structures are understood for their medicinal importance.
5) Their activity is assessed on small scale initially on live models before final implementation in bulk.

The extract used for testing should be similar in almost all characteristics as their crude counterparts of the traditional healers. Simple extractions with hot water or with certain basic solvents like methanol are the preferable course of action. Fractionalization is done only in necessary cases to separate multiple compounds from a single source (Pharmacognosy, Trease and Evans, W.B.Saunders).

Today the world finds itself in the midst of a multiplicity of problems particularly in the area of health care. The situation in this sector is alarming because of the emergence of new diseases. Consequently the necessity of evolving new herbal remedies is on the ascendancy. In the present scenario, where 80% of the world population has no access to the benefits of western medicines due to financial constraints, it is quite necessary to emphasise the relevance of traditional remedies
which constitute an important part of the health care system in the developing countries, and are also entering the therapeutics in the developed countries.

New efforts in the above area have been initiated globally, and the new emerging scientific discipline of ethano-pharmacology forms part of it. The use and search of such drugs derived from Gomutra and Plants has accelerated in recent years. Almost 50% of the medicinal products available today in the commercial market are obtained from plants.

Modern medicine is also moving in this direction. Plants are a store house of a large number of secondary metabolites such as tannins, terpenoids, alkaloids, and flavanoids, which have demonstrated their antimicrobial properties invitro.

Antifungal activities of medicinal plant extracts against clinical isolates were determined against *Candida neoformans* and *Candida albicans* by using broth dilution method (Thirach S et al., 2003).

Volatile oils obtained from different plants like pepper, nutmeg, etc were accessed for antibacterial activity against twenty-five different types of organisms which included animal and plant pathogens, and the bacteria responsible for food spoilage and poisoning. The test results showed that the volatile oils exhibited considerable inhibitory effects against all organisms that were subjected to the test, while their other major components showed various degrees of growth inhibition (Dorman HJD et al., 2000).

### 2.3 Value of research on Indian medicinal plants:

Medicinal plant species found in India are mainly concentrated in the Himalayan ranges, the Western Ghats and the Andaman and Nicobar islands. Only 3000 species out of approximately 45000 have been scientifically taken note of; while almost 6000 of the species have been used by traditional healers. Many Indian plants have found usage in the treatment of various sicknesses. The smoke generated by burning the ‘Datura’ leaves is inhaled to cure Asthama. Similarly Croton is used as a purgative and ‘Nux vomica’ is good to secure relief from paralysis and dyspepsia.

Synthetic chemistry has made great strides in the recent history. Highly potent remedies like Arsenicals and anti malarial compounds have been synthesised by the
chemists for the treatment of protozoal illnesses and the sulphonamides for the treatment of bacterial attacks. These groups of antibiotics so developed acquired a masterly status in the medicinal world some 50 years back in the treatment of all bacterial and viral diseases. The picture was very rosy for a few years, but some new aspects like pollution created in the environment, high energy requirements which led to the depletion of natural energy resources, drastic undesirable changes in the climatic conditions, and finally developing of resistance by the host against these drugs; has led to the situation where naturally available medicinal plants need to be explored as the source of medicine for the ultimate benefit of mankind.

Coming to some lesser known Indian medicinal plants used as traditional medicine in local population, the first amongst them is the Chameleon plant, Botanical name: *Houttuynia cordatta*. The usually green coloured heart shaped leaves of Chameleon plant, a perennial ground cover plant found in the Manipur area of India are used by the local people in different ways. The dried and crushed leaves give out a peppery smell. The powder is mixed in the food items like salad, fried potato-and-flour biscuits for mainly flavouring purpose. The leaf extract is used as a medicine in case of dysentery, in the treatment of stomach ulcers and for relief from muscular pains (Medicinal Plants of India: Medicinal Flowers of India, 1).

Indian fumitory, botanical name: *Fumaria indica* is a herb with clusters of tiny pale white to pink flowers. They are found in the Himalayan areas up to a height of 2300 meters above the mid-sea level. It is commercially available under the name ‘Pitpapra’ in the Ayurvedic stores. The herb mixed with honey is ingested for the treatment of body aches, fever, to control diarrhoea, liver abnormalities and influenza. It also finds external application in the control of leucoderma and skin related infections (Medicinal Plants of India: Medicinal Flowers of India, 2).

Indian Elm, Jungle corked tree, South Indian Elm tree, commonly known in Hindi as Chilbil / Kanju / Papri; Botanical name: *Holoptelea integrifolia* is a 18 meter tall deciduous type of tree with a greyish and scaly bark. The crushed powder of this bark is used in the treatment of rheumatism. Paste prepared from the seeds and the bark is used to control ring-worm infection. The paste of its leaves along with the bark is used for the treatment of leprosy, skin diseases, intestinal problems and piles (Medicinal Plants of India: Medicinal Flowers of India, 3).
Arjun tree or simply known as Arjun, Botanical name: *Terminalia arjuna* is a large ever-green tree growing to a height of 100 feet. It is found in the Indian state of West Bengal, central India. It has a spreaded top and a smooth grey bark. As its mythological name, every part of this tree serves medicinal purposes. Ayurveda promotes the use of the crushed and powdered potions of this tree in the treatment of fractures, tumours, asthma, leucodermitis, ulcers, coronary ailments, excessive perspiration and urinary discharges; to name a few (Medicinal Plants of India: Medicinal Flowers of India, 4).

Indian tree of heaven, Botanical name: *Ailanthus excelsa* is another tall tree reaching a normal height of 60 to 80 feet. It grows with a straight trunk having a average circumference of 80 inches. It has a smooth greyish bark. The bark of this tree is of medicinal interest as it helps in the cure of body fever. The extracts of the leaves and the bark are prescribed as a general health booster for women undergoing labour and as recuperating tonic after pregnancy (Medicinal Plants of India: Medicinal Flowers of India, 5).

Pithraj tree, Botanical name: *Aphanamixis polystachya* is another deciduous tree found in the eastern state of Manipur in India reaching a height of 80 to 120 feet. The bark of this tree is used in the cure of liver and spleen abnormalities, and also in the control of tumours. The seeds are crushed and the seed-oil so obtained is useful in the treatment of rheumatism (Medicinal Plants of India: Medicinal Flowers of India, 5).

Indian butter tree, commonly known as Mahua /Mohwa in Hindi dialect, Botanical name: *Madhuca longifolia*, is a large tree with thick grey bark; found in forests of Central India. The flowers of this tree smell like musk and are edible, possessing good nutritional value. The flowers mature into fruits; berries, in few months. The berries are greenish, big and fleshy The wood of this tree is useful as timber, but is only used for this purpose when the tree dies. The bark of this tree is used in the cure of leprosy and the paste is applied on external injuries of the skin. The flowers of this tree are processed for medicines to get relief from acute cough, biliousness, and coronary problems. The berries consumed to get rid of blood irregularities (Medicinal Plants of India: Medicinal Flowers of India, 6).
Indian Borage, commonly known as *Chota kalp* in Hindi and *Undhanphuli* in Gujarati. Botanical name: *Trichodesma indicum*, is a herb reaching a height of 0.6 meters. It is found all over the grassy lands in India. The plant is bitter in taste. It is used as a medicine against a number of common ailments like: diarrhoea, dysentery, skin infections, for relief from acidity and constipation (Medicinal Plants of India: Medicinal Flowers of India, 7).

Ayurveda has recommended the benefits of vegetarian diet mainly comprising of green and leafy vegetables for their fibrous content. Greens are rich in fibre and contain antioxidants that help in combating many diseases like macular degeneration. After perfect washing with water, these leaves are cut into small pieces and eaten raw or are cooked by boiling them and then consumed. These greens help in improving the stomach and intestinal functioning and improve bowel movement giving immediate relief from constipation (Herbal Plants / Medicinal Plants of India, MeD India, Network for Health).

2.3.1 List of Main Medicinal Plants in India:

1) *Asparagus racemosus* (Shatavari)
2) *Ocimum sanctum* (Tulsi)
3) *Tinospora cordifolia* (Gaduchi)
4) *Withania somnifera* (Ashwagandha)
5) *Houttuynia cordata* (Chameleon plant)
6) *Fumaria indica* (Indian fumitory)
7) *Holoptelea integrifolia* (Chilbil / Kanju / Papri)
8) *Ailanthus excels* (Indian tree of heaven)
9) *Aloevera* (Ghritakumaari)
10) *Aphanamixis polystachya* (Pithraj tree)
11) *Bacopa monnieri* (Braahmi)
12) *Commiphora mukul* (Guggul)
13) *Commiphora wightii* (Guggulu)
14) *Convolvulus pluricaulis* (Shankhpushpi)
15) *Emblica officinalis* (Amla)
16) *Ferula foetida* (Hingu)
17) *Glycyrrhiza glabra* (Yashtimadhu)
18) *Peper longum* (Long pepper)
19) *Rauvolfia serpentine* (Sarpagandhhaa)
20) *Santalum album* (Chandana)
21) *Saraca asoca* (Ashoka)
22) *Syzygium aromaticum* (Lavanga)
23) *Syzygium cuminii* (Jambu)
24) *Terminalia arjuna* (Arjuna or Arjun tree)
25) *Tribulus terrestris* (Gokshura)

The anti-inflammatory, antioxidant nature, antimicrobial and analgesic actions of *Sansevieria roxburghiana* has been a subject of deep research (Olivia C, 2005; Aliero AA et al., 2008; Mimaki Y et al., 1996; Wasciky R et al., 1951; Da-silva AA et al., 2003; Mortan JF et al 1981; Sunilson J, et al., 2009; Onah JO et al., 1994).

Another serious and devastating problem encountered with medical treatment for microbial infections involving synthetic antibiotics is ‘Host Resistance’. Host Resistance is a phenomenon in which the person undergoing treatment with these synthetic products, over a passage of time finds these drugs to be ineffective in countering the same ailment which was effectively cured earlier. This has been due to the cropping up of many drug resistant organisms or simply called as pathogens. Hence scientists have diverted their attention towards developing new antimicrobial compounds with diverse chemical structures and newer modes of action based on plant or animal kingdom like medicinal plants and cow urine. Elaborating on this particular aspect further, research activities have been done to use cow urine with the extract of *Azadirachta indica*, a plant used by traditional healers as a mode of controlling microorganism growth. This experiment was done on the basis of folk method of using the mixture of bitter tasting and unpreferable odoured *Azadirachta indica* leaves soaked in pungent smelling cow urine to counter different sicknesses.

Both organic part of *Azadirachta indica* and its extract with cow urine were taken for testing against common microorganisms like *Escherichia coli* and *Klebsiella pneumonia*. The extract exhibited powerful antibacterial activity against the pathogens compare to that observed without cow urine. This was attributed to the presence of phenol in cow urine along with oxirane hepatadecyl, 1, 2-benzenedicarboxylic acid and dibutylphthalate, all acting as powerful antimicrobial agents. Also the extract of
Azadirachta indica in chloroform, without cow urine showed comparatively lower antimicrobial activity. This is attributed to the presence of natural oxiraine matter and other matters in minute proportions in cow urine that give and promote better synergistic effect with *Azadirachta indica* (Rajapandiyan *K* et al., 2011).

As discussed earlier, India has an extremely large geographical area of varying climatic conditions from one end in north to the other in south and in east to west. These variations in the climatic conditions; from extreme cold in the Himalayan regions to the hot desert conditions of Rajasthan, and the moist and salty climates of the seas in the western and southern regions to the comparatively drier regions in central and eastern India; support different types of plants and animals: flora and fauna. So a big library of plants is available, and research is being done to assess their therapeutic properties. But still as of today many of them have yet not been investigated as their sheer number is too large against the number of researchers engrossed in this field. Also facilities for containment / storage in hygienic conditions are far too short in India to promote the speed of research on these available resources. Purified grades of substances like andrographolide, curcumin and glycyrrhizic acid and the extracts of *Azadirachta indica* have shown powerful antimicrobial activity against a range of microbes. These products have shown better action against the viruses which spread chikungunya, bird-flu, measles and herpes in man. Similarly animals affected with fowl-pox, buffalo-pox have been cured by these potions. In case of chikungunya, the affected patient suffers from severe muscular pain, and the allopathic doctor prescribes a synthetic pain-killer for immediate relief to the patient. Here the pain killer generally plays a deadly role in case of patients with weak immunity or those who are old; by damaging the vital organs of their body like the kidneys, heart and or the liver. Thus rate of mortality from chikungunya being high is actually due to the faulty and uncontrolled prescription of heavy pain-killers without checking the medical history of the chikungunya affected patient. Hence it can be safely said that as chikungunya and herpes have no direct medication as of today, research using natural products available from plants, which still remains to be explored is the urgent need of the day to treat such ailments (Dhawan BN, 2012).
2.4 Value of research on cow urine:

Ayurveda also stresses on the importance of cow urine as a medicine. Pasture soil where bovines graze is always rich in nitrogen with the constant discharge of urine by the grazing animals. This nitrogen rich soil is exceptionally suitable for farming. Successful field trials have been organized by independent research institutes in India. Research workers of Mahatma Phule Krishi Vidyaeeth based at Rahuri conducted such trials to assess the increase in production of cash crops, pulses, on pasture land sprayed with cow urine. The soil on which the experiment was conducted was of clayey nature, poor in nitrogen content, phosphorous content was just average and potash content was on the higher side. Generally, the potassium content in most of the plantation soils in India is high, but its availability to the agricultural produce is comparatively less. Green Gram, a short time crop, was chosen for the experimental farming. Field trials were conducted in eight sections where different proportions of cowurine and fertilizer were employed to judge the exact effect. Cow urine was sprayed over the land in conjunction with other organic fertilizers in some sections. Trials showed that the plant height, dry matter content and the grain yield of the crop increased leading to an overall better quality on those areas where cow urine was sprayed along with the organic fertilizer compared to the sections where cow urine was sprayed less or not sprayed at all (Patil SM et al., 2011).

It is an accepted fact that cow urine increases the fertility of the pasturelands as nitrogen is returned back to the soil. However, sometimes excessive content of nitrogen released on the soil leads to its leaching and contaminating the under ground water sources used for drinking and other daily activities in the form of nitrates. These nitrate salts are detrimental for the health of both human and animals. Such a problem was encountered in Brittany, France. The problem was aggrevated due to the indiscriminate use of nitrogenous fertilizers in farming. The plants took up the nitrogen from the fertilizers and cow urine nitrogen leached in to the soil. Urea is initially deposited on the soil in form of urine in small patches of land. Within a period of 22 hours, urea is hydrolized and the freed nitrogen is taken up by the plants that grow on the surrounding soil or is retained unused in the soil for future uptake by the plants or evaporates in the environment in the form of ammonia gas. The evaporation into the atmosphere is dependent on the atmospheric conditions, mainly
temperature. Due to low temperature, ammonia evaporation will be less; hence, excess of the nitrogen will percolate into the soil and foul the ground water sources. Hence, it is utmost necessary to have better coordination between livestock grazing and the use of nitrogenous fertilizers in farming; as the lesser use of nitrogenous fertilizers will lead to lowering of agriculture costs and on the other hand will prevent ground water pollution (Philippe L, 2003).

A similar case has been noticed in New Zealand; the nation reputed for highest dairy products output in the world. New Zealand is a country blessed with large number of bovines and much of the land has been utilised by them for grazing. Studies conducted on the Nitrogen content of these lands showed the figure to be 1.0 Tonnes / Hectare. This high figure is due to the urination by the bovines and the use of artificial fertilisers by the farming community. This nitrogen content is too large for the budding agriculture produce to utilise it fully during their growth. This excess of nitrogen from the soil, sublimes in to the atmosphere in the form of either ammonia (NH$_3$) or Nitrous oxide (N$_2$O) gases; or seeps into the soil in form of Nitrates. This addition of nitrogen content in the atmosphere leads to acidic rain, increased acidic pH of the soil and water sources like streams and lakes, increased nitrate content of the land, abnormal increase in climatic temperature, and finally, it leads to the dangerous condition of damage to the Ozone layer in the atmospheric strata.

It has been observed that the bovine urine contains 80 – 90 % of the nitrogen it has taken up from with its food. This high nitrogen input on the land disturbs the natural cycle of Biological Nitrogen Fixation (BNF). Bovines take-up large amounts of nitrogen by eating from the land; and reintroduce it by urinating. In the normal course, approximately 07 – 30 % of the nitrogen in the land protein is converted to animal protein, and the rest from the animal returns back in the form of urine and dung. Nitrogen content in the urine and dung of the animal depends on the type of the animal and the dry food it has injested. Generally, bovine urine contains 70 % Nitrogen in the form of Urea, and the rest being other minerals and peptides (Haynes and Williams, 1993). The major amount of nitrogen in the dung is in organic form; about 22 % of the nitrogen present in dung is soluble in water, 17 – 18 % is in the form of undigested material, and the rest 55 - 60 % is in the bacterial cells (Oenema et al., 1997). New Zealand has an enormous population of more than 5.3 million milk-
cow and 4.5 million bovines reared for the table. The daily average excretion of all these animals is 3.0 $\text{LM}^3$ of dung and 175 million $\text{M}^3$ of urine. Their cow locations / houses give out 70 million $\text{M}^3$ of effluents; while the piggeries are at the second place expelling 3.8 million $\text{M}^3$ of effluents. Red meat processing units also contribute approximately 48 million $\text{M}^3$ annually (Saggar et al., 2004).

This enormous amount of nitrogen released from these animal facilities is somewhere in between 75 -125 million tonnes per year. This figure far exceeds the production of nitrogenous fertilizers of the whole world. In New Zealand the animals void approx 5 times more Nitrogen than the Nitrogen fertiliser input (Jagrati Singh, 2007).

Investigations are being focussed towards exploring the possibilities of using cow urine as a source of potent natural alkaloids and chemicals, which can cure many human and animal sicknesses.

The main product of the cow is milk, and from the milk ghee and curd are the secondary products prepared by man by modifying the constitution of milk. Urine and dung are the other two main product of the cow which is the waste discharge of the body. All these five products: milk, ghee, curd, urine and dung are used separately or in combination with each other for medicinal purposes related directly to human ailments.

Tobacco vice and exposure to ultra violet radiations are a major cause of human sickness. In the fast world of today, people have made tremendous leaps on the scientific front. With this people have adopted many vices. One of the major causes of human deaths in the world is the adoption of a vice: tobacco. Tobacco is taken by man either directly by chewing or by smoking. Free radical molecules are produced in the body due to exposure to the tobacco smoke and also by the exposure to harmful ultra violet radiations that reach the surface of the earth due to the ozone layer depletion. These free radicals play a dubious role of damaging the cells of the host body finally leading to sicknesses like coronary problems and carcinogenesis of the cells. Here, cow urine along with medicinal plants plays an important role of imparting protection against the detrimental effect of these free radicals as they possess antioxidants that protect the host body cells from the evil effects of these free radicals.
Obesity is a catastrophic problem encountered by people in this electronic era of automation and rapid transport, leading to many major health problems. Obesity can be defined as inordinate bulkiness or heaviness of the body of a person generally at the middle section and hips. A person having accumulation of fat leading to a 20% or above increase in body weight compared to the standard. It is due to the absence of minimum movement of parts of the body, termed as sluggishness. This happens due to in-take of above normal to heavy diet which exceeds the actual requirements of the body, leading to the accumulation of fat in the body, as all of the fat is not utilised in the metabolism function of the body. Obesity opens the doors for many problems like hypertension, ailments related to heart, impotency, infertility, stress, depression, diabetes. Obesity has affected about one-third of the population of the universe; a very alarming figure! It is one of the main causes, occupying a position within first ten, of human death: average mortality of 28000 to 32000 per year in Great Britain alone; while the figure in many countries of Europe goes in lakhs per year. India is also not lacking on this front: a very sorry picture; as we have adopted all the luxuries of the west without any considerations of their ulterior effects on our health. The proportion of death increases with obesity. A person who is 10% overweight have 13% increased risk of death and the same for 20% overweight is as high as 27 to 30%. Obesity may start from any stage in life, but generally children who are obese at a very young age carry on the abnormality to their adulthood also. It has also been observed that the fairer sex, women, are more prone to obesity than their counterparts. Urbanization, fast foods, breads and biscuits which are saturated with sugars increases the probability of obesity. The increased intake of calories with the corresponding lower usage of calories leads to obesity.

Ayurveda stresses on the importance and attention to be given to the food habits of a person. Food taken as medicine is better than taking medicine as food. Obesity is a condition fully and purely dependent on the unhealthy eating habits of a person. Early diagnosis of any disease helps in the start of its prompt treatment leading to better recovery. Obesity is a disorder which progresses slowly. In the starting phase it does not create any hindrances in the daily activities of a person. Obesity is a condition in which the progress of setting is slow but sure. Classification of obesity is dependent on factors like its onset conditions, its severity of affliction on to a person, the
distribution of excessive fat in the particular area of the body of the affected person and finally on the histopathology of the patient.

Treatment for obesity is based on a singular principle of reducing the acquirement of the calories by a person by consumption of food to the actual calories that are used by the person in their daily activities. High fat diet is to be avoided, paying extra attention to limiting foods that are low in nutrition but high in calories.

Proteins acquired from foods are necessary for the tissue repair activity of the body. About 1 gram / Kg body weight of protein intake is sufficient for a normal person. Ingestion of foods consisting of saturated fats should be avoided at all costs as these fats accumulate in the body. With prolonged restriction on fatty diet, there is a possibility of vitamin deficiency in the body as the intake of fat soluble vitamins A and D will also be less. Other minerals which are required for the daily activities of a body will also be depleted due to diet restrictions imposed on the person. Here cow urine therapy plays an important role in replenishing the above mentioned deficiency as stated earlier all these components are present in cow urine.

Obesity is measured by the term ‘Body-Mass-Index’ (BMI). BMI is measured by the formula:

\[ \text{BMI} = \frac{\text{Weight of the person in Kg}}{\text{Height of the person}}^2 \]

National Institute of Health has defined a Body Mass Index (BMI) of a person to be above 27 Kg / m² to be obese. Even a person having a BMI greater than 25 is considered to be obese.

The BMI values and the corresponding health of a person can be understood from the sequence below:

<table>
<thead>
<tr>
<th>BMI Value</th>
<th>Health Condition</th>
<th>Risk of Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 16 to 17</td>
<td>Very Thin</td>
<td>Very High</td>
</tr>
<tr>
<td>17.01 to 18.50</td>
<td>Thin</td>
<td>Moderate</td>
</tr>
<tr>
<td>18.51 to 25.00</td>
<td>Normal</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Studies on the effects of the administration of prescriptions of cow urine in powder form on the patients having BMI between 25 – 30 Kg / m², has shown positive results. The patients of both the sexes showed an equal better response by securing lower BMI within 90 days of the therapy. There were no side reactions in the patients undergoing the therapy and also no problems after the therapy was discontinued.

Similarly, cow urine has minerals, hormones, salts, and enzymes which exhibit antiseptic and antimicrobial properties. It increases the immunity of a person as it increases B cell and T cell blastogenesis, Serum Ig G, Ig M, Ig A level, and also enhances macrophage function level of Interleukin I and II, which finally enhances immunity (Ganguly S et al., 2011).

Many chemicals used as medicine to counter bacterial and fungal caused sickness are sourced from either plants or cow urine, or a combination of the two; or they may be artificially synthesised. As discussed earlier, those preparations procured from natural sources have been effective in providing relief against a number of sickness without any contrary side effects. The available literature in this respect has been reviewed in the following pages.

### 2.5 ANTIMICROBIAL ACTIVITY:

The antibacterial and antifungal properties exhibited by cow urine distillate were analyzed against the clinical pathogenic microorganisms. Antibacterial activity of cow urine distillate was analyzed against *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhi*. Maximum antibacterial activity was observed in *Pseudomonas aeruginosa* and *Salmonella typhi*. Antifungal activity of cow urine distillate was analyzed against *Aspergillus niger* and *Aspergillus flavus*. When the two fungal organisms were compared, maximum growth suppression was

<table>
<thead>
<tr>
<th>BMI Range</th>
<th>Obesity Type</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.01 to 30</td>
<td>Moderate Obesity</td>
<td>Moderate</td>
</tr>
<tr>
<td>30.01 to 40 and above</td>
<td>High to Very High Obesity</td>
<td>High</td>
</tr>
</tbody>
</table>
observed in *Aspergillus niger* than that observed in *Aspergillus flavus*. It was finally concluded that the inhibitory activity can be used in control of bacteria and fungi of various different origin (Sathasivan A *et al.*, 2010).

Evaluation of antimicrobial activity of Abhraka bhasma prepared with Gomutra was compared with standard drugs like Clotrimazole and Ciprofloxacin. The microorganisms used for evaluation were *Candida albicans*, *Candida tropicalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus albus* (Vijaykumar Babu Chavadi, 2011).

Binary combination of photo activated cow urine was carried out and these binary combinations were determined against seven different bacterial strains. Photo activated cow urine had shown a higher MIC value against *Staphlococcus aureus* & *Bacillus cereus*, *Lactobacillus acidophilus* and *Micrococcus luteus*. Whereas, the MIC value against *Escherichia coli* was comparatively less. Binary combinations of cow urine with Neem and Bavchi Oil had shown synergistic effect as the MIC value was observed to be more or less the same (Upadhyay *et al.*, 2010).

Analysis of the antioxidant and antimicrobial activities of cow urine was also carried out against different organisms. The antimicrobial cow urine and its distillate were tested by agar well method using microbes like *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The cow urine and its distillate that were tested for antimicrobial activities, exhibited the previously mentioned activities, and compared to it, effects in fresh cow urine were found to be better than its distillate (Jerald E *et al.*, 2008).

Cow urine and antibacterial plant extracts (Neem, Tulsi, Ritha & Pine oil) were used in combination as major components in the preparation of 100% natural disinfectants. The product so obtained has a pleasant aroma therapeutic vapour (Mandavgane SA *et al.*, 2005).

An invitro study has also been done for accessing the antibacterial capacity of cow urine against various pathogenic bacteria. The activities of the urine sample preparations under test were comparable with that of standard Streptomycin. According to the test results it was observed that the Gram positive bacteria were
more sensitive compared to the Gram negative bacteria. These results were most likely due to the differences in cell wall structure between Gram positive and Gram negative bacteria (Shah CP et al., 2011).

A study on the molluscicidal activity of different formulations of dried cow urine of different Indian breeds has also been done. These studies showed antibacterial as well as antifungal effect of different formulations of cow urine (Shivkumar et al., 2011).

The synergistic antimicrobial effect of cow urine and Azadirachta indica against bacterial, fungal and yeast strains was studied. Bacterial strains selected for the test were Escherichia coli, Pseudomonas aeruginosa, Streptomyces aureofaciens and Streptococcus mutans. Fungal strains used were Candida albicans and yeast like Candida parapsilosis, Candida tropicalis and Candida glabrata.

When equilibrated amount of cow urine is used with Azadirachta indica remarkable synergistic effects were observed in case of Candida tropicalis, Candida glabrata, Pseudomonas aeruginosa and Streptococcus aureofaciens (Vats S et al., 2011).

Cow urine is shown in the study as a bioenhancer. The distillate of cow urine is found to be more effective as a bioenhancer than the cow urine itself and it also increases the effectiveness of antimicrobial, antifungal and anticancer drugs. It increases the activity of gonadotropin releasing hormone conjugate with bovine serum albumin & zinc (Randhwa GK 2010).

Cow urine has bio enhancing activity for Rifampicin, the front line anti tubercular drug used against tuberculosis, increasing its action upto seven fold against Escherichia coli, and up to eleven fold against Gram positive bacteria. Cow urine distillate enhances the transport of antibiotics eg. Rifampicin, Tetracycline and Ampicillin, across the gut wall as well as across artificial membranes – transport enhancement varies from two fold to seven fold (Randhawa GK 2010).

Assessment of antibacterial and antifungal potentials of the methanol concentrates from the roots, stem and leaves of the plant Calendula officinalis was carried out against different bacterial strains and phytochemical analysis was performed. Their evaluated results showed that the medicinal plants play very important role in commercializing the highly valuable medicinal plants for its effective results and antimicrobial functions (Hussain I et al., 2012).
Both cow urine and its distillate have bio upgrading capacity in different proportions. The research was carried out to examine the extra impact on the bio upgrading capacity of Cow Urine Distillate (C.U.D) imparted on the antibacterial activity of methanolic extract of Capsicum frutescenes. This study of the antibacterial activity of methanol concentrate separately and its combination with C.U.D was assessed against different microorganisms by agar well diffusion method. The tests proved that the methanol extract showed more effect on microorganisms. While C.U.D on its own did not exhibit any inhibition of test bacteria, the synergistic antibacterial effect of the extract and C.U.D was higher than the inhibition caused by extract alone and this is a positive pointer towards the bio enhancing role of cow urine distillate (Rakshita M Vet al., 2010).

Since ages, Ayurveda has recommended the prescriptions of cow urine singularly or with its combination with medicinal plants to counter human health related aspects. Scientific approach in this field is as of today, still in the infancy needing many aspects to be deeply studied. The different positive effects of consuming cow urine in three ways: fresh urine, stored urine, or dried urine in powder form; and with their synergistic effect with medicinal plants is a subject of intense research which is the urgent requirement of today. Researchers on cow urine have come to some conclusions that cow urine in powder form promotes antioxidant and immunomodulatory activities. Their exact mechanism of working is still to be done (Gosavi DD et al., 2011).

Researchers have listed many important compounds in cow urine which successfully act to counter harmful microorganisms. Some of the major components are Urea, Uric acid and minerals. Urea is the final product after protein metabolism, and it functions as a powerful antiseptic with the capacity to curb carcinogenesis in conjunction with Uric acid. Many minerals contained in urine like Ammonia, Calcium, Copper, Iron, Manganese, Phosphate, Potassium, Sodium and Sulphur; are easily taken up by the human body from the potion of cow urine than that which could be done after digestion from normal diet. Also cow urine has all these elements together, whereas a varied diet is required to get an access to all of them. The other major elements present in cow urine include Allantoin, Carbolic acid, Enzymes, Lactose, Nitrogen and Vitamins A, B, C, D, E. All these elements play a common function of an
antiseptic and immunity booster for different parts of the human body preventing the advent of the dangerous pangs of cancer (Prakriti, Viman Nagar; Cow urine therapy - A research paper, 2010).

Cow urine is successful in non-surgical cure of Hemorrhoids, a condition of enlargement and displacement of the anal mucosa of the human rectal system. Oral administration of cow urine in Hemorrhoids patients has replaced the difficult, painful and costly surgical treatment. The symptoms of Hemorrhoids include severe pain during defecation and rectal bleeding. Out of the patients who underwent the treatment, 83 % of the patients were cured, 10 % of them had substantial improvement and the rest 7.33 % showed partial or nil (0.66 %) improvement (Talokar OW, 2013).

Cow urine is also found to work as an effective repellent for some species of mosquitoes and as a safe retardant of their growth right from the larval stage. Malaria causing mosquitoes, female Anopheles, are most affected by cow urine. To date, all mosquito repellents developed are synthetic chemical based; which over a period of time prove to be ineffective as the mosquito progeny develops a power to survive and sustain even in their presence, rendering them to be ineffective. Experiments of spraying 1 to 7 days old cow urine near the mosquito breeding sites gave positive results of hampering the growth of Anopheles larvae. This was safely attributed to the phenolic content of cow urine functioning as a safe antiseptic and growth retardant by affecting their breeding sites and rate of breeding. Still the results are not entirely positive, as according to their findings the growth of other culicine species was not affected (Eliningaya JK et al., 2011).

Rural and tribal residents in different pockets in the world have adopted cow urine therapy in different modes suitable to their life-style and the environment. Sudan is a country where illiteracy is very high and the per-capita income is extremely low in absence of proper income sources. Residents are unable to adopt any modes of modern health care; leaving aside the luxuries available to the common public of the world. Hence, they resort to herbs and other locally available products by incorporating it in their diet; and as medicine for any sickness according to the folk practice. The locale tribals have also used cow urine in different forms as medicine. Tribals use fresh cow urine for its mineral, carbohydrates and vitamin content; which
helps in replenishing the same in their bodies. Stored cow urine is also used as a medicine, because on long storage, the urine is free from bacteria; and it on the contrary helps to overcome many microbial sicknesses. Cow urine promotes an anti toxic action against cadmium chloride and works as an effective bio-enhancer for Zinc. Tribals of this area prefer a mixture of fresh cow urine and its fermented / stored version as a source of nutrition as it contains all the ingredients required for the body, and at the same time offers disease resistance power to the body (Hassan AA, 2013).

One of the main reasons of people not accepting cow urine therapy is the strongly repulsive pungent odour of the urine. The taste of cow urine is somewhat sweet and having pH in the range of 8.0 to 10.0. Also it was observed that some cows carry pathogenic bacteria due to the poor quality of food and garbage they graze on. These pathogenic bacteria are discharged from the body of the cow with the urine. Consumption of such urine for medicinal purpose has been found to be counter-beneficial. Trials have been done for the removal of the odour and the pathogenic bacteria from urine before it is prescribed for medicinal usage, and Arka-kalpana is a process regarding this subject matter (Sachin N, 2010).

Cow urine of the Indian pure breed cow is the most effective for therapeutic applications. It has got immunomodulatory effects in the host body against various infections. Research has been carried out on cow urine distillate and has found to have immunomodulation in mice. It has been found that it helps to enhance T-cell and B-cell growth, and also enhances the level of IgG. Cow urine distillate has synergistic properties with antibiotics, antifungal and anticancer drugs. Hence, CUD acts as a bio-enhancer. It has been found that CUD increases B and T lymphocyte blastogenesis. This study led to the investigation of effect of CUD on humoral and cell mediated immune response against NDV vaccination in broiler chickens when administered orally (Ganguly S, 2013).

Similar research has been carried out with cow urine to upregulate lymphoblastogenesis (Prabhakar K et al., 2005). The study was planned to investigate the blastogenic activity of lymphocytes and effect of in-vivo cow urine therapy on it in order to evaluate their potential to promote a protective immune response against disease. This therapy is not only boosting up the immune system of the living
creatures but it is also eco-friendly as there are no chances of pollution or discharge of harmful chemicals into the environment.

Cow urine therapy is concerned with utilization of the components of the urine for treating various human sicknesses. With reference to urological disorders, cow urine is very helpful because it provides important vitamins and minerals to the body. Vitamin C is one of the major constituent of cow urine. Besides that, it has traces of gold and copper. It acts as an effective medicine for In-Silico anti cancer activity caused by *Kappaphycus alvarezii* (Gowrishanker K et al., 2013).

Acquiring an infection and imparting immunity are two factors which share a close interaction with the host body. Microbes or bacteria or parasitic organisms are the causeative agents for an infection; whereas administration of certain drugs on a regular basis or once in a period of time or even once in a life-time of a person provides immunity to the body against these infections. Vaccines of Baccillus Calment Guerin, commonly known as B.C.G. vaccination are given to new-borns within seven days of birth to provide a life-long immunity against diseases like tuberculosis once in a lifetime.

Many synthetic antibiotics over an apart their facing the earlier discussed problem of host resistance, also have some immediate draw backs like them inducing vomiting, feeling of nausea and gastric abnormalities in the patient, and their leaving a metallic taste in the food pipe of the body; thus compelling the patient to reject the minimum required diet for their recuperation.

Many different methods are employed for the evaluation of antibiotic nature consisting of antimicrobial, antibacterial and antifungal activity of the available substance. These methods are as shown in the following heads:

1) Disc diffusion method.
2) Serial dilution method.
3) Solid dilution method.
4) Ditch plate technique.
5) Gradient plate technique.
6) Cup plate technique.
Disc diffusion method is the simplest of the available methods and is economically viable. Discs of size 0.8 cm prepared from Whatmann filter paper are impregnated with the products under test and applied on the walls of the agar plate previously containing the microbes under evaluation. The zone of inhibition is measured after incubation.

In the Serial dilution technique, graded doses of the product under test are poured into the tubes containing the test organisms. After incubation, the concentration at which the growth is stopped is the Minimum Inhibitory Concentration.

In the Solid dilution method, the products under test are diluted directly in the agar. This agar containing substance is poured into the plates and then incubated. The main benefit of this procedure is the evaluation of different test organisms at a single concentration of the antibiotic under test.

In the Ditch plate technique, one particular antibiotic product can be successfully and simultaneously evaluated against a large number of test organisms. The antibiotic is first mixed with the agar in the plate. The plate is then streaked with different test organisms at an angle of 90° to the ditch. Incubation is done and the zone of inhibition for each organism is measured.

In the Gradient plate technique, the concentration of the antibiotic under test is varied from zero to the maximum limit of its effect. The method consists of a dual layered agar plate. The nutrient agar containing the antibiotic under test is allowed to solidify in the first wedge of the plate. The second mount of the agar is done in the wedge. In this method a maximum of six test organisms can be evaluated simultaneously.

In the Cup plate technique, the test antibiotic is kept in a miniature cup and is sealed to the agar surface. After regular incubation, the zone of inhibition is measured.

Research has been diverted to the efficacy of Abharaka bhasama or Sahastraputi, the age-old Indian Ayurvedic product relied upon getting relief from gastric and nervous system abnormalities. Due to its extremely small size, it diffuses in the body very quickly and hence the damage to the cells is stopped and repairs initiated. Cow urine has been mixed these Abharaka bhasama in order to get an enhanced synergistic effect. Bacterial growth was retarded by this process against a number of test bacteria,
but the fungal attack was not affected as there was no effect on the fungi under test. So this mixture has definitely proved to be efficient in combating bacterial related sicknesses (Vijaykumar Babu Chavadi, 2011).

The positive action of Indian medicinal plants has been assessed on some bacterial strains like *Bacillus cereus*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Escherichia coli* and *Klebsiella pneumonia* by researchers in the near past. The aqueous and methanol extracts of the medicinal plants were taken for the study. The plants belonged to the arid regions of North-Gujarat where rain-fall is scanty. The plants under investigation were Galtoro, Dudhli, Sharu, Chanothi, Kagdonio, Durva, Gulmohar, Thor, Vad, Shivan, Shwet chandan and Ragat rohido. Different parts of the plants like leaf, stem, fruit, branches, pods or even the whole plant was used in preparation of the extracts. Their results showed that strongest antimicrobial effect was of Galtoro, which was followed by other two in decreasing order by Dudhli, Sharu. Here also it was observed that the methanol based extracts showed highest positive results against Gram negative species: *Klebsiella pneumonia*. Many different sicknesses affecting man like nervous disorders, diarrhoea, dysentery, fever, piles, general wounds, worms, bowel disorders, asthma were cured effectively by the first three plant species: Galtoro, Dudhli and Sharu. Greater emphasis was given to Galtoro as it exhibited its potential in curing many ailments. This type of investigations promotes the exploration of natural plant based resources to be incorporated as potent drugs to counter various microbial and viral sicknesses (Parekh J et al., 2006).

Four medicinal plants that are traditionally used in the Eastern Cape region for treating burn wound infections were collected for investigation and invitro antibacterial activity was carried out against eight different bacteria (Leizel W 2005).

The invitro antibacterial potential of the leaves of Annona muricata extracts in solvents like water and methanol was evaluated on bacterial strains like *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Staphylococcus aureus*, , *Streptococcus pyogenes* and *Salmonella typhimurium*. The invitro antibacterial activity was analysed by the application of agar cup method. The results showed that the Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* were most susceptible, and the Gram negative bacteria which
were observed to be most susceptible were *Klebsiella pneumoniae* and *Proteus vulgaris*. The antibacterial activity of the plant extracts was accessed using the standard antibiotic: Streptomycin (100 ppm). The results obtained in that study suggested that Annona muricata can be suitably used in treating diseases caused by the above mentioned organisms under test (Pathak P *et al.*, 2003).

Cattle urine is an important source of Nitrogen from pasture animals. It has been observed that the presence of Nitrogen in urine saturated soil is very high and this has a direct effect on the nett potential effect of the environment. This effect is substantial but also directly depends on the composition of the urine. (Monaghan RM *et al.*, 1992; Weilacher T *et al.*, 1996). Grazing cattle are unable to absorb all the nitrogen from their diet. This excess of nitrogen is excreted by the animal in the urine in form of urea. (Kebreab E *et al.*, 2001). Hence, understanding and evaluation and regulation of urea turnover in the soil of pastures are of great importance.

The study also deduced that with nitrogen, carbon transformation is extremely high in those pasture soil on which urine discharge is high. In these cases, the microbial community is found to be highly complex in the pasture soil. On analysis, if the recovery of carbon derived from urea was found to be low in PLFA’s, then it should not be implied that the intra cellular urea hydrolysis was a major reason for the low turnover of urinary urea. Certain information regarding the relation between microbial response and urine deposition is of great importance, which previously the overall PLFA dynamics had not revealed (Peterson SO, 2004).

Traditional Ayurvedic or Siddha medicines have relied since ages on cow urine as a main constituent of the naturally available medicinal products. A varied number of medicinal properties of cow urine have already been exemplified against different levels of infections. Cow urine works as a bio enhancer and increases the efficacy of antibiotics against the infectious agent with drugs like ampicillin, clotrimazole, isonized. Experiments using cow urine distillate have proved to decrease the toxic effect of Cadmium chloride in mice (Khan A *et al.*, 2002). Thus cow urine distillate has the power to cure disorders without inducing toxicity, and also has been observed to enhance the cellular and humeral immune system (Kumar A *et al.*, 2004). Research has also pointed to the fact that cow urine helps the lymphocytes to survive, and helps
in repairing the damaged DNA, thus proving to be effective in cancer therapy (Prabhakar K, 2004; Ambwani S, 2004).

The beneficial effects of cow urine on serum biochemical profiles which includes total serum protein, glucose, calcium, and cholesterol of birds have been proved (Chauhan RS et al., 2001). Experiments on rats have reported the usefulness of cow urine distillate as an antimicrobial agent, with a positive effect on weight gain, haematological profiles, immunomodulatory effect, and healing of surgical wounds (Gupta A et al., 2003).

2.6 PHYTOCHEMICAL ANALYSIS:
Pharmacological and phytochemical evaluation of plant Dhatura before and after Shodhana was carried out. It is a procedure by which both physical and chemical impurities are removed from the drug and thus enhancing the potency and efficacy of the drug. Many of the drugs contain various organic and inorganic impurities. Organic impurities include plant materials, animal materials and synthetic materials. Inorganic materials include stone, sand and unwanted minerals. This study is carried out to ascertain the changes in the chemical constituents of Dhatura beeja before and after purification procedures under different media viz. Gomutra and Goksheera and its toxicity effect on rats (Vasudev S, 2010).

Research scientists have observed that the phytochemicals extracted from medicinal plants have included alkaloids, flavonoids, with certain steroids, tannins, and a number of other chemicals. Chemicals possessing antibacterial properties are mainly alkaloids, flavonoids, and tannins. The studies regarding the general chemical and antibacterial activity of five South Algerian medicinal plants have been carried out. Here, the accessment of antibacterial activity was done on both Gram Positive and Gram Negative bacterial strains. The results so obtained showed the plants to have a M.I.C. ranging between 0.045 to 0.135 mg/ml depending on their species and also on the microbial strain under test (Ladjel S et al., 2011).

Qualitative tests were used for the near investigation of auxiliary metabolites. The procedures were conducted on leaf extracts of five Cassia species. Qualitative analysis was carried out for various phyto constituents. Solvents like methanol, ethanol and
ethyl acetate were taken to prepare the leaf extracts in order to distinguish the presence of the active components. The phytochemical screening demonstrated the vicinity of all the concoction constituents like alkaloids, flavonoids, steroids, tannins; and the conspicuous absence of tannins, anthraquinones, and phenolic flavonoids (Usha V et al., 2011).

A study dealing with the phytochemical analysis and different biological activities after the extraction and assessment of indole alkaloids from “Rauwolfia Serpentina” was carried out to observe their antimicrobial and antiproliferative activities. The detection or the deficiency of indole alkaloids was carried out using both T.L.C. and more reliable H.P.T.L.C. methods. The principle center of these routines was on the qualitative and quantitative determination of indole alkaloids. Four different alkaloid derivatives were detected. Spectrophotometry was done for the quantitative estimation of the alkaloids from Rauwolfia. A positive antimicrobial activity, M.I.C. and M.B.C were noted by using well diffusion assay technique. The study also reported that as the root extract performed better against Salmonella typhi, it has proved to be a better and suitable alternative for further drug development (Deshmukh SR et al., 2012).

Initial studies on alkaloids were carried out using medicinal plants from Lombok. They had collected one hundred plant species which were expected to have antimicrobial and especially anti malarial potentials. These plants represented forty nine families and eighty genera. Out of all the plants, 23% of them tested positive for alkaloids. Five positive tested plants were then selected for further investigation involving structure elucidation and antimicrobial testing on the extracted alkaloids (Surya H et al., 2011).

The leaf extract of Ancistrocladus heyneanus, a tropical liana plant had been taken up for study. The studies revealed it to be rich in Naphyl Isoquinolin alkaloids. Antimicrobial activity against one Gram positive and three Gram negative bacteria had been tested against crude aqueous and organic solvent extracts of Ancistrocladus heyneanus using agar well method. The alkaloids from crude organic solvent extract were isolated by H.P.T.L.C. Four distinct bands of the alkaloids were observed. Each one of them was tested for their antimicrobial activity. Fraction 1 showed positive action against only Gram positive bacteria but not against any Gram negative bacteria. Whereas, inhibition of growth of Gram positive bacteria Staphylococcus aureus were
obviously more when H2 and H4 fractions of alkaloids were tested. Both crude extracts as well as isolated alkaloid fractions showed considerable activity against Gram positive bacteria but there was no activity against Gram negative bacteria (Sudhakar M et al., 2012).

*Salmonella* has been detected to be the primary cause of food poisoning in the whole world, and many times such food poisoning out breaks are massive and affects a large world population. In USA alone, the Centre for Disease Control and Prevention has put up an estimate of a minimum of 14 lakhs cases of *Salmonella* infections per year. The European Union has reported on an average over 1.0 lakh cases annually. Certain pathogenic *Salmonella* serotypes like *Salmonella typhi* and *Salmonella paratyphi* are prevalent in man, usually leading to diseases such as enteric fever. A few pathogenic *Salmonella* serotypes such as *Salmonella enteritidis* and *Salmonella typhimurium* are able to infect humans and animals and so they are termed as ubiquitous. Synthetic antibiotics are mainly employed in the treatment employed and annihilation of these microbes. Antimicrobial operators are for the most part managed remedially and prophylactically to cure *Salmonellosis* in human and animal world-wide. However, a marked and increase resistance to these antibiotics is an inevitable side effect of these synthetic drugs, with the recent studies proving right the increased presence of *Salmonella* resistant to antibiotics in both humans and animals (Angulo et al., 2000; Gross et al., 1998; O’Brien, 2002).

Medicinal herbs have a long history of use as drugs in Asia either as crude or in extract form added in food or administered orally. These herbs possess bio active components, and this has brought about an improved comprehension of their numerous capable clinical, physiological, and therapeutic applications in the current medical fraternity (Merken et al., 2001; Zheng et al., 2001). A very large number of plants exceeding more than one thousand have been observed to exhibit antimicrobial effects (Nychas, 1995), which provide a safe and effective treatment against various diseases. A member of *Schizandraceae* family, *Schizandra chinensis*, bears a fruit known as *Schizandraceae Fructus*, is used in the treatment of dyspnea, irritability, palpitation, coronary illnesses, and likewise sleep deprivation in Asia (Lee et al., 2004). Elixirs of *Schizandraceae Fructus* have been discovered to show a positive hepatoprotective impact in both viral hepatitis and antimicrobial activities on *Staphylococcus aureus* (Sinclair, 1998).
In order to identify herbs with medicinal properties, twenty two medicinal herbs of Korean origin which were employed in the treatment of Gastro-intestinal infections were screened for their therapeutic potential in the treating three different serovar of Salmonella. The minimal inhibitory concentrations of the concentrates from Schizandrae Fructus were intensely studied by feeding the mice infected with Salmonella typhimurium with Schizandrae Fructus. Salmonella typhimurium affected mice which were not given a dose of Schizandrae Fructus demonstrated clinical side effects of lethargy, and damage in liver cells and tissues, kidney, intestine and spleen. In contrast, clinical abnormalities and tissue damage were less seen in Salmonella typhimurium affected mice administered with the concentrate of Schizandrae fructus. The aqueous and methanol concentrates of Schizandrae fructus demonstrated antmicrobial effect against all sixteen strains of eight different Salmonella serovars tested, wherein, the methanol concentrate showed to have a somewhat higher activity than the water extract. (Mi-Hyang Lee, 2006).

Schizandrae fructus had shown antibacterial activity against Helicobacter pylori and many other gram positive bacteria (Li et al., 2005; Sinclair, 1998). Its antimicrobial activity is due to the presence of some endotoxins and exotoxins or a wide range of antibiotics. It had been proved that, several secondary metabolites from plant origin such as alkaloids, tannins, saponins, and sterols have been associated with antimicrobial activity (Leven et al., 1979).

Even in the popular treatment of chemotherapy to control tuberculosis, Japanese researchers have identified Stephania cepharantha to be used in the treatment by the isolation of active alkaloids present in them (The Japanese Journal of Experimental Medicine, 1949, 20, 69). Similarly, Chinese researchers have been working on the usage of local plants to counter tuberculosis, have reported the activity of alkaloids in Coptis root (Chinese Medical Journal, 1950, 68, 169).

Sansevieria roxburghiana is another herb found in the geographical areas of Tamil nadu, Southern Andhra Pradesh of India and Sri Lanka. This herb had found its use as a medicine to counter common ailments through ages for the treatment of common human ailments like chest and stomach pains, ear-ache and diarrhoea. In case of ear-ache, the leaves are heated to boil and the warm potion is applied on the affected part to get immediate results. Also, the sap of the leaves is administered on to common
skin sores and graze injuries, and fungal skin infections like scabies. Microbial and phytochemical studies on the extracts of the leaves and rhizome of this herb showed the optimum presence of tannins, flavonoids, phenols; and also carbohydrates, saponin, anthocyanin, proteins, glycosides, phytosterols and β-cyanin. Prominent antimicrobial action was observed by subjecting the methanol and acetone extracts of the leaves to a number of common Gram positive bacteria, Gram negative bacteria and fungal strains. This action of the leave extracts was far greater than what that was observed from the ethyl acetate extract of the rhizome, which also promoted comparatively lesser anti microbial activity against a wide spectrum of microbes. The antimicrobial activity of both the leaves and the rhizome of *Sansevieria roxburghiana* against different pathogens have been investigated in detail (Deepa P et al., 2011).

The growth of the type of the organisms which can be retarded or controlled by the application of the extract of the leaves and rhizome of this plant can be broadly summarised as follows:

**Gram positive bacteria:** *Micrococcus luteus, Bacillus cereus, Enterococcus spp., Staphylococcus aureus.*

**Gram negative bacteria:** *Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescence, Salmonella typhi, Salmonella paratyphi, Klebsiella pneumonia, Shigella sonnei and E-coli.*

**Fungal strains:** *Candida albican* and *Cryptococcus* spp.

Researchers on natural plant based potent drugs have been trying to develop the extractions from the crude plants by newer techniques which are more efficient and rapid, using lesser quantities of solvent. Extractions of the alkaloids were successfully and rapidly accomplished by utilising non-fatty soap as the extracting agent. The soaps used were anionic and non-ionic in nature. In this process the precipitates of the alkaloids were dissolved at pH greater than 8.5, and finally extracted with chloroform. The quality of the alkaloids thus obtained had same characteristics as the older methods. This type of procedure can be made applicable to the extraction of secondary metabolites. A concentration of 0.1 to 0.2 % (m / v) of the soap yielded good results, but it was felt that if the concentration of alkaloids in the plant sample is
more; than a slightly higher concentration of the soap was required to get a better output (Abdelouaheb D et al., 2006).

2.7 ANTIMUTAGENIC ACTIVITY:

A drug for countering cancer developed from cow urine by an Indian organization: ‘Go Vigyan Anusandhan Kendra’ in collaboration with ‘National Environmental Engineer Research Institute’ has got a third U.S. patent for the anti genotoxicity properties. The research came to the conclusion that redistilled-cow-urine-distillate (RCUD) proved to be helpful in repairing the DNA from oxidative damage. RCUD works to control genotoxicity, a harmful action on the genetic material of the cell. Oxidative damage is the main reason for the early onset of ageing, cancer and various other diseases (Chakraborty T et al., 2010).

The anticarcinogenic activity of cow urine was evaluated using two stage in Swiss albino mice, first treated by a dosage of 7, 12-dimethylbenza(α) anthracene and one week later it was followed by repeated applications of croton oil three times in a week for sixteen weeks. The resultant effect of the treatment was compared against a control in which cow urine was not added as a drug. The results showed that the tumour incidence, tumour yield, tumor burden and the cumulative number of papillomas were higher in the control to that compared to the experimental mice which had been treated with cow urine. This effect and the difference in the value of the results of experimental groups so observed was statistically analysed and found to be greater in comparison to the control group. The study had demonstrated the chemo preventive potential of cow urine on DMBA induced skin tumorigenesis in Swiss albino mice (Vasim R et al., 2010).

Anticlastogenic effect of (RCUD) in human fringe lymphocytes was treated with manganese dioxide and hexavalent chromium. The study was carried out in vitro. Genotoxicants and Clastogenes caused the DNA strand break, chromosomal damage and micronucleus. Three different concentrations of redistilled cow urine distillate:
1.0 µL/ml, 50.0µL/ml and 100.0µL/ml were utilized in the experiments (Dutta D et al., 2006).

Manganese dioxide and hexavalent chromium were observed to cause prominent DNA strand break, chromosomal damage and development of micronucleus, yet, these were secured by the administration of redistilled cow’s urine distillate (Dutta D et al., 2006).

A clinical study of thirty patients fulfilling the criteria for inclusions were divided into two groups each consisting of 15 patients. In one group Gomutra arka administered in the dosage, whereas in other group, Placebo is being administered. All the patients were followed up for a period of 1 month for assessing the improvement. In the clinical study Group A (Gomutra arka), there were no samples which act complete remission, 27% sample showed marked improvement, 66% showed moderate improvement and 7% showed mild improvement (Sachin N, 2010).

A cow urine therapy camp for cancer patient was organised for 8 days in Mandsaur district. The main purpose was to access the efficiency of the application of cow urine on cancer patients who were from states all over India. It was deciphered that those patients who were being given cow urine therapy continuously since 2-3 months got major benefit (Jain N K et al., 2010).

Studies were undertaken for assessing the anti genotoxic antioxidant properties of cow urine distillate and re-distillate. Determinations of the antioxidant potential and volatile fatty acid levels were done. The products selected were: Actinomycine – D (0.1 µ mol/l)
Hydrogen peroxide (150 µ mol/l)

They were utilized for incorporating DNA strand break in conjugation with 0.1% DMSO as negative control. Dosage to check the antigenotoxic impact of cow urine was decided from the effects observed in studies that had been under taken earlier (Krisnamurthi K, 2004).

Further studies have also been done on Mitragyna speciosa Korth (Ketum), a medicinal plant that has found usage as a medicine since centuries for energy enhancement, and remedy for general illnesses like body fever and diarrhoea. A water concentrate of Mitragyna speciosa gained entrance for its energy of mutagenic and
antimutagenic action by the Salmonella/microsome mutagenicity test, famously reputed to be the Ames test. The test included the preincubation system against *Salmonella typhimurium* TA 98 and TA 100 bacterial strains, both in the presence and absence of metabolic activator S9 framework. With a specific end goal to verify the mutagenicity impacts, all the concentrates were assessed utilising double the worth of the amount of revertant colonies in negative control plate as cut-off focus. The outcomes showed nil mutagenic movement for frameshift transformation (TA98) and additionally base-pair substitution (TA100) in all the centralizations of *Mitragyna speciosa* in the presence and likewise without metabolic activator S9 framework. Antimutagenic movement of *Mitragyna speciosa* water extract with the synchronous expansion of mutagen was gained entrance to by utilisation of the Inhibition rate of revertant state. Three distinctive convergances of *Mitragyna speciosa* demonstrated unmistakable antimutagenic movement (p<0.001) as in spite of it mutagenicity was affected by 2-aminoanthracene for both TA 98 and TA 100 strains in the presence of metabolic activator S9 framework. Consequently it was inferred that, Mitragyana speciosa did not display or push any mutagenicity impacts in both analyzer strains in the presence and absence of metabolic activator S9 framework. *Mitragyna speciosa* displayed very effective antimutagenic impacts in both strains with the presence of metabolic activator S9 framework (Ghazali A R *et al.*, 2011).

Medicinal plants often represent the only therapeutic source for many communities and ethnic groups. The effectiveness of an aqueous extract of *Caryocar brasiliense* (Caryocaraceae) fruit pulp, popularly known in Brazil as Pequi, against clastogenicity induced by cyclophosphamide and bleomycin was evaluated using an in vivo mouse bone marrow cell micro nuclei test on in vitro Chinese hamster ovary cell (CHO-K1) chromosome aberration test and an in vitro antioxidant assay based on oxidative damage to 2-deoxy D ribose (2DR) induced by hydroxyl radicals generated by thr reactions between Ascorbic acid and Fe+++ EDTA. In the mouse bone marrow extract, all the plant extract showed protective effect against micronuclei induced by cyclophosphamide and bleomycin but did not interfere with polychromatic bone marrow erythrocyte proliferation, except when the mice had been previously treated with a highest dosage of cyclophosphamide. When CHO-K cells were pre-treated adding 0.01 ml, 0.05 ml, and 0.1 ml of concentrate per cc of cell culture medium 24 or 48 hours before bleomycin or cyclophosphamide, there was a noticed protective effect
against chromosome breaks, and also a major drop in the mitotic index of CHO-K cells. The extract also showed a protective effect against oxidative hydroxy radical damage to 2-DR (Juliana K et al., 2007).

Anticlastogenic effect was studied by using in vivo erythrocytes micro nuclease assay. Blood samples were collected and counted for reticulocytes with and without a micro nuclease using the fluorescent microscope. After comparing with the controls, we found that 25% of AP and IM leaves in diet have shown a significant decrease in micro nucleated peripheral reticulocytes (MNRETs) induced by MMC, CYP, and MMC, DMBA respectively (p<0.05). While IM fruit juice at 10 and 20ml/kg BW decreased MNRETs induced only by MMC in a very low dose, still the major decrease occurred only in high dose (p<0.05). The results showed that AP leaves and IM (leaves and fruits) were able to inhibit clastogenic activity of both direct and indirect acting clastogens in the mice, IM leaves showed the highest inhibitory effect (Piengchai, K et al., 2011).

Chemo treatment for cancer prevention is a new promising strategy by the infusion of either synthetic or naturally available chemicals to inhibit, reverse or retard tumorigenesis (Wattenburg, 1985; Greenwald et al., 1990; Hong et al., 1997). The work done on the above subject by Wattenberg, 1983; Steinmetz et al., 1991; Dragsted et al., 1993; Surh, 2003 showed that there are a large number of phytochemicals present in our diet which have shown antimutagenic and anti tumour inducing properties in various animal and cell culture systems. Also, the epidemiological studies have indicated that active agents found in the diet may reduce or in certain cases will increase the relative risk of cancer development (Weisburger, 1991). Chemopreventive agents function by different system directed at all locations and phases of cancer (Wattenberg, 1997). The anticancer causing potential of Thai bitter gourd fruits demonstrated that the rats fed with TBG diets at the concentration tested developed significantly higher incidence as well as multiplicity of colon tumours than the control group. These results showed that Thai bitter gourd fruits possess anticlastogenic potential against clastogen in the mice. It had no preventive potential against azoxymethane induced colon carcinogenesis in rat instead of increasing the occurrence of colon neoplasm when given during the primary stage. Thus the effect was no dose response relationship. The findings suggest that Thai bitter gourd fruits may have different chemo preventive potential on different organs,
because in the memory gland models it was able to decrease the multiplicity of tumour to a significant extent (Piengchai K et al., 2011).

Consumption of spices and herbal vegetables has been traditionally used in habitual diets in tropical regions. They contain nutrients along with phytochemicals such as polyphenols, terpenes, alkaloids, and phenolic acids. The crude or purified extracts of some medicinal plants play an important role in the prevention and treatment of some chronic diseases. Studies have reported that Thai vegetables, herbs and spices like Asiatic pennywort, Indian mulberry, Ivy gourd leaf, Lemon grass, Neem flowers and Sesbania flowers, possess antimutagenic, anticarcinogenic and anticlastogenic properties (Rojanapo et al., 1993; Kupradinun et al., 1997; 2008; 2011; Kusamran et al., 1998; Tepsuwaan et al., 1999; 2002).

*Eryngium foetidum* belonging to the family Apiaceae is widely used for the pungent aroma in habitual diets (Seaforth et al., 2005). It has also found a place as a home-made medicine for the treatment of cold, fits, fainting, head ache, and malaria (Mitchell et al., 2006; Roumy et al., 2007). The study of *Eryngium foetidum* was carried out in vivo in mouse peripheral blood erythrocyte. Micronuclease assay was carried out. The effects indicated that there were nil clastogenic effects of *Eryngium foetidum* in mice. Doses of *Eryngium foetidum* supplemented with the diets, diminished the amount of micro nucleated fringe reticulocytes in all the MMC groups that were treated in an manner which was fully concoction dependent. A visible and prominent reduction was observed at dosage concentrations of 1.6% and 3.2% *Eryngium foetidum* in the DMBA treated groups. This showed that *Eryngium foetidum* had no clastogenicity, but possessed anticlastogenicity against direct and indirect acting types of clastogens in mice.

Cancer is of the main reason of human death throughout the world, and that also mainly in the last 120 years. Rapid industrialisation has led to detrimental and irrevocable changes in the climate and the over-all environment in the world. This extreme growth of cancer in the human cells is most probably due to the changes occurring in the DNA sequence and its continuity, mutations taking place in the genes, genetic damage and changes in the chromosomes (Khosro Issazadeh et al., 2012; Shams A et al., 2012). This growth of cancer and the damage to the genes, is tried to be curbed by employing anti-carcinogens and antimutagens in the treatment
and also as preventive measures in daily life (Kim SY et al., 2000). Bruce Ames has deduced that the rate of mutation is slowed down by properties of some naturally occurring products which either interfere in mutagenesis or completely inactivate the mutagens (Maron DR et al., 1983). Such a retardation and control in the growth of cancer is due to the destruction of mutagens inside and outside the cells by the antimutagenic substances. The mutations taking place in the cells and the DNA damage is also stopped by these antimutagenic substances (Ruan C et al., 1989). This olive leaf extract has been able to control the growth and spread of many diseases over and above cancer like diabetes, high blood cholesterol, heart ailments, hypertension, microbial and fungal infections, common cold, herpes zoster, abnormal increase of body weight, and the early weakening of bones (Ritchason J, 2000). The antimutagenic potential of the olive leaf was accessed by the Ames test. Olive leaf extracts in hydro base were checked for their antimutagenic activity against sodium azide and 2-nitrofluorene in the presence of rat microsomal liver enzyme. All experiments were performed thrice simultaneously and the following equation was used to count the percentage inhibition:

\[
\text{Percentage of inhibition} = \left(1 - \frac{U}{N}\right) \times 100
\]

The results confirmed the anti-mutagenic potential of olive leaf extracts to curb the mutagenicity of sodium azide and 2-nitrofluorene, which rose prominently in the presence of rat microsomal liver enzyme.

Ames test gives the above results rapidly at a low cost. The incorporation of olive oil in our diet directly results into lessening the possibility of carcinogenesis in the body cells. The reduction in risk of colon cancer is attributed to the presence of mono saturated fatty acids, antioxidants and phenol groups in olive oil extract (Escrich E et al., 2007; Andreadou EK et al., 2006; Colomer R et al., 2006; Owen RW et al., 2004). Similarly, oleuropeinaglycone is a extremely powerful in controlling breast cancer (Menendez JA et al., 2007); the antioxidant properties of Aloe vera extracts also helps to negate cancerous growth in the host (Loots DT et al., 2007); consumption of herbal tea directly reduces the risk of colon cancer (Adhami V et al., 2003). Finally it can be said that olive leaf finds an important place in cancer treatment due to its year round abundant availability at negligible cost as it has great percentage of phenolic compounds which ultimately helps in fight against cancer.
Similarly, other researchers have accessed the antimutagenic potential of plants like *Peltastes peltatus* and *Maytenus ilicifolia*. As these plants found usage in traditional Brazilian medicine since ages, their functioning was attributed to the presence of flavonoids and tannins. Antimutagenic activity is generally attributed to the abundant presence of tannins and flavonoids which are phenolic products evenly spread in the plants. Flavonoids are generally ingested by people on consumption of beer, wine and even coffee; which are rich in them. Tannins are ingested by a person who drinks tea. These ingested products offer resistance to any mutagenic activity in the body. But, it is extremely necessary to mention here that freshly prepared tea is only good for health. Tannins are released when tea is heated to boil. If the heated tea is stored in a thermos-flask, then with the longer time duration, at temperature near to boil, the tannins begin to convert to tannic acid. This tannic acid is detrimental to the body; hence it is advisable to take only freshly prepared tea. Antimutagenecity of the above plants was assessed against standard mutagens, where both the plants promoted a high level of cytotoxicity. Test dosages containing 50% of the *Peltastes peltatus* and *Maytenus ilicifolia* extract gave better results when they were tested separately; but the mixture yielded complex results wherein their exact action was not conclusive. Their studies concluded that the aqueous extracts of *Peltastes peltatus* and *Maytenus ilicifolia* promoted a varied range of mutagenic inhibition dependent on the mutagen used and the concentration of the extract; or in some cases they actually promoted mutagenecity. These complex results were difficult to understand; and it was finally concluded that this action may be due to the presence of other reactive compounds in the plants whose chemical activity is unknown, which plays a role in such unpredictable results (Ruben CH *et al.*, 2003). Both flavonoids and tannins are slightly acidic and stable compounds that are generally unaffected by light, oxygen and heat in normal cases (MacGregor JT *et al.*, 1978). Studies have revealed that tannins promote antimutagenic effects even at low concentrations with the presence of S9 mix, even promoting mutagenic damage repair, This activity was absent at low concentrations in the absence of S9 mix (Imanishi *et al.*, 1991). Thus it can be very well concluded that certain plant products can help in the initial prevention, or decelerate the initiation and progression of cancerous growth in the human body cells. This is due to the fact that cancer is the result of the changes in the DNA structure, and these anti mutagenic substances helps in stopping the changes in DNA profile. The actions of these products are due to the balance maintained between the ingestion
of mutagenic and antimutagenic substances, which ultimately leads to the diminishing risk of cancer.

2.8 Role of medicinal plants and cow urine in agricultural and farming activities:

Medicinal plants also play an important role in increasing the agricultural production by controlling organisms that damage the crop and hampers the output and economy of the nation on a wider scale. Rice output of Asian countries including India was hampered on a massive scale due to infectious Bacterial Leaf Blight (BLB). Synthetic antibiotics like Carbendazim, Thiram, dithane M-45, Propiconazole, Sterpomycin sulphate; employed to control BLB left undesirable residues which were injurious to the surrounding fauna: animals and human health and disturbed the natural eco-cycle. It became necessary to resort to natural products for controlling BLB. Experiments were done using raw and stored cow urine singly and in conjunction with aqueous fractions of a sea weed red algae Kappaphycus alvarezii. Simple experiments were done by soaking Kappaphycus alvarezii overnight in cow urine and then spraying it on to the crop next day. Experiments were also done by collecting Kappaphycus alvarezii and cow urine in an earthen pot, and incubating it at atmospheric temperature under the soil for 480 hours. The clear extract was tested for its effectiveness to control the pathogens consisting of BLB on the infected paddy leaves. The results were quite encouraging as there were no synthetics employed; and economic viability, a major factor to be considered was also fulfilled as cow urine is abundantly available and there was no question of expensive equipments, energy requirements, their maintenance and skilled operative personnel (Venkatesh R, 2011).

Another pathogenic agent: Rhizoctonia solani, responsible for disease Sheath Blight of rice is the main cause for heavy losses encountered in rice production. In this case, initially synthetic antimicrobial agents were used. Their usage disturbed the ecosystem due to they being non biodegradable; and also adversely affected other insects and worms in the soil which actually help in the growth of paddy. Utilising natural products acting as bio-enhancer was the only recourse left. Seventeen medicinal plant extracts prepared in cow urine, cold water and hot water were evaluated for
controlling the growth of this pathogen. Extracts of the most of the plants in cow urine were more powerful in restraining the mycelia growth and finally the disease; than that observed in both cold and hot water. The cow urine extracts of the five medicinal plants that exhibited maximum powerful inhibitory action were *Psorelea corylifolia*, *Stereospermum suaveolens*, *Asparagus racemosus*, *Coleus forskohlii*, and *Canavalia gladiata*. Aqueous extracts showed less action compared to that in cow urine because they lacked in phenolic content required as an antiseptic. Extensive applications on a larger scale will give the exact effect of each plant extract in cow urine; thus, the few most effective plant extracts can be preferred over the others and necessary cultivation and / or procurement of these plants can be done for getting their regular supply (Tiwari RKS et al., 2011).

In a similar crop, wheat, which is main produce of the Northern states of Punjab and Haryana, and the next door country: Bangladesh. Their economy is directly affected due to any drop in the output of this crop. Bipolaris Leaf Blight (BiLB) affects the grains of wheat by markedly reducing their size, and resultant lowering down of both quality and output. Synthetic antibiotics employed to control BiLB left scars of extensive and irreparable damage to the surroundings. Aqueous and ethanol plant extracts in combination with cow urine and dung were tested to control this organism. The ethanolic plant extracts of *Adhatoda vasica* (leaves) and *Zingiber officinale* (rhizome) exhibited total inhibition of conidial germination of *Bipolaris sorokiniana*, a fungal strain of BiLB. *Oscimum sanctum* extract was capable of reducing the fungal growth by as high as 50 %. Aqueous plant extracts of 1 : 10 and with cow dung suspension of *Adhatoda vasica* and *Zingiber officinal; Piper betle, Vinca rosea and Azadirachta indica* extracts with cow urine in the ratio in the ratio 1 : 15 gave promising results (Nargis A, 2006).

Wheat crop is also damaged by different types of insects; right from germination stage to the adult crop being made ready after thrashing for the market, and during the storage. The insects generally bore through the grains thereby lowering the market value as the quality becomes inferior. *Rhyzopertha dominica* is one of this type of insect dubious for inflicting the above mentioned damage to the grains. This weight loss and damage caused by insect pests amounts to almost 5 % out of the average 10 % losses encountered on an average until the final produce reaches the
Initially these pests were controlled by fumigation or spraying using chemical agents like organophosphorous and pyrethroid based products. These products left their damaging effects in the atmosphere and the consumer. Hence attention was shifted towards various plant based products and cow urine and its dung to be helpful in controlling the proliferation of the borers. Different natural products have been used, namely: neem leaf powder, jatropha seed powder, mustard oil, dried cow dung powder, ashes of burnt cow dung and cow urine to control damage to the seeds, avoid damages during germination, growth and final storage by controlling the insect attacks. Treatment with the above mentioned natural insecticides offered immunity from damage on storage to a period of six months which is quite sufficient before the finished wheat reaches the kitchen. Use of mustard oil on the seeds is preferential as it has been observed to some times adversely affect the germination process; thereby decreasing the final yield of the crop; but the seeds treated with mustard oil give a crop which is stable from attacks at later stages. Neem leaf powder and cow-dung-ash powder are also effective in varied degrees in offering protection to the wheat crop. Experts who conducted different experiments in this direction concluded that neem leaf powder, cow dung ash powder, cow dung powder, jatropha seed powder, cow urine and mustard oil @ 2% were found highly effective in the management of Rhyzopertha dominica and as seed protectants. These studies clearly demonstrated the potential and possibilities of using locally available natural products in overall control of storage insect pests encountered in wheat growing and storage (Mamta Arya et al., 2013).

Other cereal crop affected by microorganisms is Sorghum, an important crop in the whole world. Sorghum is susceptible to damages caused by more than 100 fungi right from its germination stage to its full growth span; whereby its final output becomes as low as 25% of the actual. A study was conducted to impart better immunity to this crop by utilizing naturally available products like Panchgavya: the five products of cow: milk, ghee, curd, dung and urine; in conjunction with some medicinal plants like Adhatoda vesica and Pongamia glabra. The study showed promising results of the effect of Panchgavya with cow urine and medicinal plants like NSKE in the control of the microorganism; the results being comparable to those obtained using synthetic products; without any adverse effects on the surrounding environment or living beings (Shrinivas M et al., 2009).
Pathogens of species like *Fusarium, Alternaria, Pythium* inflict severe production losses of the harvest. Ginger production in the world suffers due to the rot of its rhizome due to microbial infections. Ginger being commonly consumed food material; synthetic antibiotics leave toxic residues on the surface and in the soil thus affecting humans: the consumer; and the soil, thus disturbing the eco-system. Hence, the rhizome rot disease management requires lesser involvement of chemical and biological modes. Cow urine along with plant extracts of *Artocarpus lakoocha* have proved to be very effective in curbing the negative effects of these species of organisms (Rakesh KN *et al*., 2013).

Betel, refered to as *Pan* in the local dialect is the major produce of the Indian state of Bangladesh crossing an annual average figure of 72000 tonnes. Since last few years the production is showing a downward trend due to factors like economic non-viability and the damage caused by insects and different diseases. Betel vine is very susceptible to harm from unfavourable environmental conditions like hot, dry and sunny climate. Though humid and shady conditions are required for the growth of the vine, it on the other hand also leads to diseases of root. The repetition of such diseases on regular basis annually, leads to total drop of produce within a few years. Foot and root decay caused by *Sclerotium rolfsii*, a pathogenic fungus found in soil; is the major reason for low output. This fungus is able to stay-put in any atmospheric conditions, and damages the crop year after year. Experiments have been conducted to retard the growth of *Sclerotium rolfsii* using cow urine alone; and in conjunction with some medicinal plant extract; and the results obtained were quite satisfactory. The plant extracts were of: neem leaves, rhizome of ginger and turmeric. The extracts were prepared by immersing the above items separately in water and cow urine for 24 – 36 hours. In-laboratory experiments clearly showed the retardance of growth of *Sclerotium rolfsii* in the presence of the above extracts in varying extents. Field trials to control this disease were necessary in order to get a binocular view of the findings, as the researchers who conducted the experiments were done on laboratory scale only (Amin R *et al*., 2013).

Cucumber is a major produce of Korea and many other nations of the world. Cucumber growth is hampered by the attack of fungus: *Sclerotinia sclerotiorum* which is responsible to cause decay in many other vegetables also. Humid climate
with cold facilitates the survival of this fungus for months together in soil. The fungal attack may either lead to the decay of the stem or the flowering cucumber bud or the complete plant leading to it withering out. Similar damage by the same fungus are observed in water-melon, lemon, pumpkin and other crops also. Laboratory trials involved separating the fungus: *Sclerotinia sclerotiorum* from the affected fruits and was dipped in cow urine for 22 – 25 hours and the effect on the fungi was noted by placing them on potato dextrose agar, checking the mycelia growth daily upto five days. Cow urine and dung were also kept surrounding the cucumber plants to check the mycelia growth. This had positive effects on the plants as the infections decreased and their growth was not stunted. The concentrations of the elements in cow urine and dung also had a similar proportionate bearing on their antiseptic properties. Direct application of cow dung on to the plants resulted into their death, but its application surrounding the plants gave good results. Results indicated the additional effectiveness of cow dung as it contained many digested as well as undigested matter so the poisonous attack of the fungus was easily stopped, compared to that observed with the application of cow urine singularly (Basak AB, 2002).

Honey is another natural product processed and made by honeybee insect. Honeybees collect the nectar from the flowers and plants and deposit the same in their colonies known as hives. The nectar mixed with the spitum of the bees in a complex natural mechanism, is converted into honey. It is sweet in taste, and is a refreshing drink in hot climate. It also acts as a powerful health tonic and has great therapeutic properties. Honey produce industry thrives on these minute insects; bees; being a source of lively hood to many. Wax is another bi-product of the honey industry. Honeybees are reared in special zones and when the comb is full, honey is extracted by centrifugal mechanical force without damaging the empty comb. Hence, honeybees are very important for this industry and their health is always a case of utmost importance. Also, honeybees play an important role in pollination in plants as they transfer pollen from one plant to the other in their quest for collecting nectar.

Honeybees are reared in the colder climates of the Indian states of Jammu and Kashmir, Himachal Pradesh, Punjab and also Bihar, Uttar Pradesh and Maharashtra. Honeybees in India have been adversely affected by Microbial Brood Disease since last few years in some areas in India. The disease affects the larvae and the insects
This has affected the industry due to high mortality rate of almost 30% of the insects. This disease is caused by the bacteria *Melissococcus plutonius*, a Gram positive, rod shaped. Synthetic antibiotics have been utilised to check this disease. Some of these antibiotics left poisonous residues in the honey and also in the pollen of plants. This hampered plant growth and the honey so polluted, was banned for human consumption. So once again, natural antibacterial agents were the only source of recourse to this devastating problem. Natural products like Neem, Turmeric, and cow urine were used to counter the attack of this bacterium. Spraying of cow urine in honeybee colonies curbed this bacterial invasion. The affected larvae were first removed from the combs, and new eggs and larvae were not affected as cow urine had started to function as a disinfectant. Reoccurrence of the disease was stalled by regular spraying at 12 – 14 days interval. In addition, cow urine had a positive effect in the attitude of worker bees as their stinging people who worked there was decreased. Wasp and Mite attacks on to the combs also stopped. The main concern of obnoxious odour of cow urine was not at all found in the honey. So once again, cow urine has proved to be useful in salvaging the honey bees in particular form mortality and the industry in general from financial losses (Aakash Chand *et al.*, 2012).

Similar work regarding the use of cow urine was carried out on other avian species, poultry; being a major contributer of white meat supply of the world. Cow urine has found its application as an antimicrobial agent to counter the attack of microbes in poultry birds. Better synergistic effects have been observed when CUD along with the extracts of *Aloe vera*, a cactus variety of plant; and wheat-grass, the young shoots of wheat were used together in the treatment of this avian species. *Aloe vera* is refered to as the gift of nature due to it being used since ages for medicinal applications for the benefit all living beings. The organisms that were tested for observing growth retardence were *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Listeria monocytogens*, *Klebsiella pneumoniae*, *Eschericha coli* and *Salmonella Typhimurium*. Bacterial growth was decreased by 30 to 40% of CUD, *Aloe vera* and wheat-grass when they were used separately. But their total synergistic concentration of 20% proved to be enough to retard the bacterial growth; thus giving a cheaper alternative against the costly synthetic antibiotics which had been employed earlier (Sharma RK, 2013).
Another breed of poultry, the White Leg horn chicken is also susceptible to microbial diseases resulting in lower egg laying and their death. In this case, *Aloe vera* extract with cow urine helped in stabilising the sero-biochemical aspects of this avian species. Cow urine *Ark* and *Aloe vera* were fed orally to the test birds for a period of three months. On serological testing it was noticed that the liver functioning of the birds was excellent as the readings of proteins and albumin were higher and that of liver marker enzymes AST, ALT and GGT were lower. Creatinine level was also on the lower side, but the blood-urea-nitrogen content showed no conspicuous variation. The main reason for *Aloe vera* being effective along with cow urine is its leaves and *Aloe gel* to be full of minerals like magnesium, calcium, chromium, copper and iron; which are also present in cow urine. The experiments proved that both cow urine *Ark* and *Aloe vera* exhibit enhanced hepatoprotective effect and lesser toxic effect as the values of AST, ALT and GGT are less; so they are a better substitute for liver protective drugs to be utilised on this breed of avian species (Panicker A et al., 2012).

Poultry rearing in India is a source of livelihood for the Indian farmers, who are under economic stress due to poor irrigation facilities in most of the parts of India. The conditions of draught have aggravated their problems to the extreme. The Indian government has also allotted the status of cottage and fast growing large commercial agriculture industry to the poultry rearing units. Vaccination programme are strictly followed by the farmers, but still infections suddenly affect the live stock leading to huge production and finally economical losses. These diseases are actually caused by the indiscriminate use of synthetic pesticides by the farmers, which lowers the immunosupressibility of the birds. Hence, natural immune stimulators are needed to overcome this problem. Herbal plant extracts offers a solution to this problem. *Tinospora cordifolia* (Guduchi) is one plant; which has given positive results as an immune booster. The meaning of Guduchi in local dialect is ‘to reactivate dead cells’. Powder of *Tinospora cordifolia* was fed to the avians along with their normal diet. Other medicinal plants that offered the same benefits were *Ocimum sanctum* (Tulsi), *Withania sominifera* (Ashwagandha) and *Asparagus racemoscus* (Satavari). Farmers have also incorporated the powders of *Withania sominifera* and *Asparagus racemoscus* in equal proportions in the poultry food to accelerate the immunomodulatory system of the birds. The extracts of all these plant species with
CUD has shown better immunomodulatory response in the birds (Ganguly S et al., 2010).

Some common human skin infections can be effectively cured using cow urine, citrus (lemon) fruit extract, boiled rice water and neem extracts. These products were effective in controlling the growth of fungus Malassezia; which is dimorphic, lipophillic yeast-like fungus on the human skin, the causative agent of dandruff. A mixture of cow urine with boiled rice water was far superior in retarding the growth of dandruff causing fungus. Effect with lemon was for short duration only. Neem also has antiinflammatory, antifungal effects and antimicrobial effects due to the presence of products like nimbene, nimbeneine, namdandial in the leaves. External injuries on the skin, ulcer growth on skin and lice in the hairs of the head can be effectively cured using Neem leaves extract. As lemon juice contains almost 5 % citric acid, it is also a weak antimicrobial agent. Experimental findings suggest cow urine to be the most powerful of all the above products due to it containing high levels of uric acid and urea. Whereas Neem leaves extract work better as a preventive against dandruff very successfully, and is also able to retard the growth before it is too severe (Saneeshkumar, 2012).

Experiments for assessing the antimicrobial activities of cow urine are being done on a wide scale. Generally, Agar well method is preferably used to judge the proportion of the antimicrobial activity of the sample under test. Some researchers have found fresh cow urine to be more effective as a disinfectant compared to CUD. Microbes like Staphylococcus aureus, Escherichia coli, Pseudomonas fragi, Bacillus subtilis, Streptococcus agalactiae and Proteus vulgaris are mainly problematic for healthy human life. One hindrance many times observed in cow urine therapy is the difference in concentration of the active elements present in urine to differ in the urine collected from a single cow during the different periods of a day. This is generally avoided by collecting urine as earlier mentioned before daybreak. This is due to the increase in concentration of the elements present in urine to be more due to a longer duration gap between two urinations as the animal generally sleeps during the night. The second major problem encountered in the variation in the components of urine is between two different animals; as their eating habits, living conditions, quality of diet and other physical and physiological factors play an important role in this case. Some times
fresh cow urine shows more antimicrobial activity compared to stored / incubated / photo activated cow urine as the pH of fresh cow urine is more acidic compared to the other versions (Anami A et al., 2012).

On the other hand, experiments conducted by another set of microbiologists using photo activated cow urine on human bacterial strains, secured good results of the antiseptic nature of the test product. This may be due to some unknown factors. Photo activated cow urine promoted anti bacterial activity in varying proportions against different Gram positive and Gram negative strains. Also, the concentration of mineral content in the urine played a role in deviating antimicrobial activity. The cow urine so collected was photo activated in sun light for exactly two days in a transparent and sterile glass container. It was then filtered by passing through a filter paper, and purified to remove any debris and insoluble material. pH of the solution before use was mildly alkaline in the range of 7.7 to 7.9. Unused product was stored at a constant temperature of 3.5 to 4.5 °C for future testing purpose. The antimicrobial activity was assessed against different bacterial strains by Agar well diffusion method, wherein different widths of zone of inhibition were obtained indicating the antimicrobial activity of the cow urine sample. The highest zone of inhibition was 22 mm for *Aeromonas hydrophila* and the lowest 13 mm was for *Enterobacter aerogenes*. Experiments also indicated that the results obtained by a 30 µL dose of photo activated cow urine were similar to that of the standard tetracycline. Researchers on this project have accepted that the antimicrobial activity of cow urine is due to it being rich in mineral content. Theoretically, the acidic pH of cow urine promotes antibacterial activity; but in this case antibacterial activity was observed even though the pH was mildly alkaline; which the researchers attributed to the formation of ketones, formaldehyde and sulfinoles on photoactivation (Sarsar V, 2013).

Research has been conducted to assess the lipase activity of cow urine. The disinfectant activity of cow urine depends on its chemical content. Various processes have been devised to get information of this chemical content along with its extent of lipase activity so that it can be incorporated as a potential drug in the fight against carcinogenesis of human body cells. The processes developed start from simpler Volumetric Analysis to the more intensive TLC, Spectrophotometry process and Tributyrin tests. Cow urine sample collected early just before sunrise contains the
maximum amounts of the present mineral ingredients, so these collected samples are preferable to others collected during day time. The antibacterial tests conducted from these samples showed that the activity increased with the increase in concentration of potent contents. This was measured by Well-plate and Pour-plate methods. Similar was the case with antifungal activity; which again was assessed by Pour-plate method. In the TLC procedure, the slides under investigation were sprayed with ninhydrin solution, which gave pink spots at the junction of the diffusion between the solvent and the urine sample; which indicated the presence of amino acids, which actually denotes the presence of enzymes. Volumetric analysis by titration proved the presence of lipase within the urine sample. This lipase presence was confirmed by spectrophotometry conducted under ultra violet light (Saneeshkumar, 2013).

Diabetes is a big silent killer to have afflicted a major populace of the world. In India alone, an average of 57500000 people has embraced this deadly disease; and people of rest of the nations of the world are not far behind in acquiring diabetes. Scientists are working to counter this disease since long and synthetic products have fallen short in offering a total cure for it. The next recourse is towards exploring natural products to offer solution to this burning problem. Cow urine has shown its ability to confine this affliction in its own shell. The effects of cow urine distillate (CUD) on diabetic mice have been investigated by researchers, getting promising results. Diabetes in the mice was introduced by injecting Streptozotocin in their bodies. The diabetic mice treated with CUD exhibited a higher increment of (High Density Lipo Protein) HDL levels with an increase in their body weight and decrease in blood cholesterol levels compared to the untreated samples; denoting improvements in their over all health. Glycogen synthesis in the mice liver and in the skeletal muscles was slowed down in the diabetic mice. As the level of insulin decreases in the diabetic body, the glycogen levels also showed a proportionate downward trend. But the specimens treated with CUD again showed higher glycogen level indicating a re-activation of the glycogen synthesis in their bodies. Scientists are yet unable to figure out the exact components of cow urine, and their mode of action; which is proving to be helpful in the fight against diabetes. The presence of antioxidants and free radical scavengers / destroyers could be one of the reasons for cow urine to be helpful in counterering diabetes. Hence cow urine therapy is recommended for people suffering from diabetes to ensure and safe guard people from other sicknesses like coronary problems, hypertension and
retinal damage which are the genuine followers of diabetes (Vijaya Bhaskara Reddy M, 2013).

The antimicrobial effect of cow urine on the growth of plants and on some common human pathogens has been under constant evaluation. The strains selected for evaluation were *Staphylococcus aureus* and *Escherichia coli*. Tests were conducted using cow urine distillate (CUD) in three different concentrations of: 5, 10 and 15 %. The test results at 15 % concentration of cow urine, indicated a powerful antimicrobial activity against both the organisms; suppressing their growth by almost 85 %. Also, suppression of *Escherichia coli* was greater than that of *Staphylococcus aureus*.

Cow urine also promoted the growth of cowpea plant. This was evident on checking the chlorophyll and protein content of the plant. In this case, *Vigna unguiculata* was grown under test conditions. Germination was done from the seeds of the plants in four separate earthen pots out of which one was maintained as a standard. Daily watering with varying proportion of cow urine was done twice in a day in the sequence given below. Rest of the conditions during rearing were maintained same for all the test samples.

<table>
<thead>
<tr>
<th>Pot Number</th>
<th>Cow Urine Content</th>
<th>Water Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 – Standard</td>
<td>0 %</td>
<td>100 %</td>
</tr>
<tr>
<td>02</td>
<td>2 – 10 %</td>
<td>98 – 90 %</td>
</tr>
<tr>
<td>03</td>
<td>50 %</td>
<td>50 %</td>
</tr>
<tr>
<td>04</td>
<td>100 %</td>
<td>0 %</td>
</tr>
</tbody>
</table>

The chlorophyll content of the leaves from each test pot was checked individually. Similar tests were conducted from other parts of the plants to assess the carbohydrate, protein and total nitrogen content in each plant. The test results indicated the chlorophyll content, carbohydrate content, protein content and total nitrogen content to be highest when cow urine administered was along with water in the ratio 1 : 1 (50
% cow urine + 50 % water). Hence, it can be summarised that cow urine, works as an effective antimicrobial agent against human pathogenic strains; and at the same time can be effective in promoting the growth of plants. Both these aspects need to be implemented on wide scale for the benefit of mankind (Vijayalakshmi R et al 2010).