INTRODUCTION

*Every species of plant is a law unto itself.*

— Henry A. Gleason

Although the ages humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavors, fragrances and medicines (Crag and Newman, 2005). Primitive man noticed and acknowledged the great diversity of plants available to him. Eversince, the birth of mankind there has been an affinity between life, disease and plants. Primitive men started studying diseases and treatments (Lyons and Pertrucelli, 1987). There is no record that people in prehistoric times used synthetic medicines for their ailments but they tried to make use of the things they could easily procure. The most common thing they could find was their environment i.e., the plants and animals (Singh and Abarar, 1990).

Plants have formed the basis for sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties asserated to plants have proven to be specious, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use. The interest in nature as a source of promising chemotherapeutic agents holds on. Modern allopathic medicine has its crux in ancient medicine, and it is hopeful that many important new remedies will be discovered and commercialized in future, as it has been till now, by following the leads provided by traditional knowledge and experiences. The use of medicinal herbs for curing disease has been vouched in history of all civilizations. The plant itself is a biosynthetic laboratory,
not only for chemical compounds, but also a plentitude of compounds like glycosides, alkaloids etc. These exert physiological and therapeutic effect. Herbs are staging a comeback and herbal ‘renaissance’ is transpiring all over the globe. The herbal drug industry is growing at an astounding way all over the world. Herbal drug remedies are now available not only in drug stores, but also in food stores and supermarkets. Therefore, the efficacy and safety of herbal drugs is very critical. The herbal products today connote safety.

The word drug alone comes from the Dutch word “droog” which means 'dried plant'. Long before the advent of modern medicine, herbs were the main stream remedies for nearly all ailments. People commonly diagnosed their own illnesses, prepared and prescribed their own herbal medicines, or bought them from the local apothecaries (Tyler, 2000).

Substances deduced from the plants dwell the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems, example of the use of a herbal preparation in modern medicine is the foxglove plant. This herb had been in use since 1775. At present, the powdered leaf of this plant is known as the cardiac stimulant digitalis to the millions of heart patients it keeps alive worldwide.

Herbal medicines are being used increasingly as dietary supplements too to fight or prevent some common maladies like cancer, heart attacks and depression (Eisenberg et al., 1998). Based on ancient and experimental evidence, these culturally accepted herbal practices and beliefs are becoming quite common.
India, being one amongst the 12 Mega-Bio-diverse countries of the world, is a mansion of a wide variety of medicinal plants. It is evident that the Indian people have colossal passion for medicinal plants and they use them for wide range of health related applications. The demand for medicinal plants is increasing in both developing and developed countries and the bulk of their material trade is still from wild harvested plants (Pareek and Trivedi, 2011). Folk medicines, chiefly based on plants, enjoy an untainted position today, especially in the developing countries, where modern health service is limited. Safe, effective and real buy indigenous remedies are gaining popularity among the people of both urban and rural areas, especially in India and China. Information from ethnic groups or indigenous traditional medicine has played a vital role in the discovery of novel products from plants as chemotherapeutic agents (Katewa, 2009). The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants.

Recently, there has been an increase and use of herbal medicine for the treatment of various ailments. Traditional medicine has also been firmly gaining interest and consent even amongst the practitioners of modern medicine. With this, the vend potential of herbs and herbal products has being increased eloquently. India stays as one of the richest countries in the world as regards resource of medicinal plants. It constitutes 11% of the total known flora though its total land mass occupies only 2% of the globe. The Indian systems of the medicine have identified 1,500 medicinal plants of which 500
species are commonly used in the preparation of herbal drugs (Goyal, Samsher and Suresh Chandra, 2008). The use of plants as medicines is as senile as human civilization. History of Indian lore medicine has its origin in the Vedic period (2000 B.C to 800 B.C) for plants used in the art of healing. Synthetic medicines are being used to cure diseases. These medicines are highly potential in their action, but show many side effects in addition to being expensive. As a result, all the world countries again got renewed interest in traditional herbal medicines, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects.

Herbal medicine has gained momentum and it is evident from the fact that certain herbal remedies are more effective as compare to synthetic drugs. Medicinal herbs are designed to bring into perspective the massive knowledge acquired by man to retain his health by using the plants around him. Man examined all aspects of his environment by trial and error and classified material into remedial, harmful and nourishment. The use of herbal drugs in oriental or traditional medicine, being practiced in India, Pakistan, Bangladesh, Sri Lanka and China for centuries, is well established. However, the discovery and use of sulphonamides, antibiotics and synthetic drugs led to a dramatic decline in the popularity of herbal products used in therapy. Nevertheless, the realization of harmful toxic effects of profuse number of synthetic drugs led to for alternative sources, which would be safe and effective in sundry ailments. Though myriad of plants were known for their remarkable medicinal use in the vedic literature, they have not been brought into lime light in view of the present technological developments as much as the allopathic medicines.
In many developing countries, a large measure of the population leans on Traditional practitioners and their armamentarium of medicinal plants in order to meet up with healthcare needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often conserved their fame for historical and cultural reasons. Such products have become more widely attainable commercially, especially in developed countries. It is scaled that approximately one quarter of prescribed drugs comprise plant extracts or active ingredients bagged from or modeled on plant substances (Leena Tripathi and Jaiendra Nath Tripathi, 2003). Aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine and vinblastine are a few strategic examples of what medicinal plants have given us in the past. Most of these plants derived drugs were originally unmasked through the study of traditional cures and folk knowledge of indigenous people.

Herbal drugs fill out a major share of all the officially appreciated systems of health in India (Vaidya and Devasagayam, 2007). Herbalism has an elevated tradition of use outside of conventional medicine. The promise of preserving the natural qualities that promote a healthier method of healing different ailments contributed to the popularity of these medicines.

In India, very recently the weight of Ayurvedic medicine was appreciated and centralization of research in Ayurveda took place in 1965. The Indian Council of Medical Research (ICMR) to some extent tried on empirical grounds and has brought Ayurvedic principles of plants known in lore medicine into limelight. The Regional
Research Institute (RRI) at Poojappura in Trivandram undergone particularized phytochemical investigation for 40 plants and 55 Ayurvedic formulations were standardized. The institute scored commendable success in treating some skin, nervous and rheumatic diseases. Currently institutions like BSI, CSIR and IARI initiated exploration of medicinal flora. Nature is yet mankind’s greatest chemist and many compounds that remain discovered in plants are beyond the imagination of even our best scientists. Plant sources for new drugs may seem amaranthine. Though some plants have been proved their medicinal value in curing number of major and minor diseases, the potentialities of many plants are yet to be explored against pathogens causing dreadful diseases.

Rural people essentially depend on locally available traditional plants for health care. This method of health care is culturally relevant and also, the plants are generally easily available and affordable to the people. But, this kind of health care is hardly encouraged. Recently, research has increased significantly for a variety of reasons including an inability of many rural people and some governments to afford western-based pharmaceutical care, renewed interest in native resources and “traditional” health systems along with a greater appreciation for local and indigenous knowledge, international concerns for the conservation of biodiversity and their income-generating potential, (IDRC and Medicinal Plants, 1997).

For thousands of years products from natural sources have been used in caring for human health. Most of the drugs given even today are directly or indirectly from natural sources (Wang et al., 2007). These medicines which are safe, free from side effects and eco-friendly are derived from a wide variety of plants and are in use in every part of the
world (Chowdhury et al., 2005). Limitations of synthesized compounds in the treatment of chronic diseases and the potential of plant-based medicine as more effective and cheaper alternative was probably responsible for the fast growing industry of herbal medicine (Rojas et al., 1992). India is gifted with a rich wealth of medicinal plants. The Charak Samhita (1000 B.C.) gives details of about 340 medicinal plants of which only 85 are accepted by the Indian Pharmacopoeia (1966). Although more than 7500 medicinal plant species are reported to occur in India (Rao and Murugan, 2003) and 1000 medicinal plants in our state, many plants are yet to be analyzed for their medicinal properties. This implies or shows the lack of importance being given to the pharmaceutical potential of medicinal plants despite its being documented very long back by our ancestors.

The research carried out initially on medicinal plants gave encouraging results which prompted scientists to go on with an exploration of other new, more beneficial plant-derived drugs (Kumar et al., 2007). A multitude of compounds produced in a plant are responsible for its medicinal properties (Nair and Chanda, 2007; Nair et al., 2005; Kumar et al., 2007). The research on plants for the sake of drugs has evolved from small plants to, more recently, higher order ones. However, modernization of traditional medicine should not be simply Westernization. For herbal medicines, the purpose of a study is not only to screen out bioactive compounds from herbal extracts for new drug development, but also to standardize and control the quality of raw herbal materials and their products to ensure the safety and efficacy; and more importantly, to reveal their preventative and therapeutic mechanisms. So far, only a relatively small number of herbal medicines have been capably studied from all these aspects (Willow, 2011).
At present, there is an urgent need to pay pensive attention towards our traditional plants for our own health needs owing mainly to the emergence of resistance to antibiotics. Resistance to antibiotics has led to morbidity and death from treatment failures and augmented health care costs. This emergent antibiotic resistance is a serious global problem that can be tackled by traditional research along with other important new strategies including research for bioactive compounds.

Antibiotic research is aimed at the discovery and growth of novel bioactive metabolites. Natural products from plants are of significance in the discovery of biologically active compounds. Newly, higher plants are being screened at length for bioactive compounds.

The theme of improving human life and the challenge of increasing drug resistance has motivated a renewed interest in the hunt for new bioactive agents. Past research have evidences of antimicrobial and biological activity being exhibited by a number of plants belonging to different families.

Medicinal plants are often used in traditional medicine to treat different ailments in different areas of the world. This indigenous knowledge, passed down from generation to generation in various parts of the world, has significantly contributed to the advancement of different traditional systems of medicine (Jachak and Saklani, 2007) as well as helped in exploration of different medicinal plants to find the scientific basis of their traditional uses. This exploration of biologically active natural products have played an important role in finding New Chemical Entities (NCEs) for example, approximately 28% of NCEs between 1981 and 2002 were natural products or natural
product-derived (Newman et al., 2003). The pharmaceutical interest in plants as a source of medicines is less likely to raise issues of concern about sustainability of harvesting, as relatively small amounts of plant material are needed to conduct the screening for bioactivity which is the basis of many contemporary drug development strategies (Gerard Bodeker, 1995).

Plant drugs are generally less toxic and free from side effects than the synthetic ones (Momin, 1987). A resurgence of interest in the study and use of medicinal plant has been taking place during the last two decades. Also an ample growth has occurred in popular, official and commercial interest in the use of natural products.

The need of the hour is to screen a number of plants for promising biological activity. Considering the aforesaid, locally available, highly consumed leaves of *Piper betel* Linn, a local cultivar were selected and screened for their antimicrobial, biological activities and phytochemical studies.

**Origin and Distribution**

Betel plant is indigenous throughout the Indian Malaya region and also cultivated in Madagascar, bourbon and the West Indies, due to its various traditional and medicinal properties it has been propagated all over the world. According to estimates, it is used up daily by about 600 million people and the custom of betel chewing encompasses an enormous area of the world (Fig. 1), extending 11,000 km west to east and 6,000 km north to south, an area stretching from east Africa to Polynesia. Myanmar has an actual long tradition of cultivating betel leaves for green tea and chewing purpose. In India and Pakistan, it has been a well-known cultural tradition during marriages and also in holy
prayers. In Cambodia, there is a habit of Areca nut palm and betel leaves after the meal. In Vietnam, the leaves and juices are used ceremonially in Vietnamese wedding.

Fig. 1. Regions within the dotted lines show major areas of *Piper betel* consumption. Barring the areas where the climatic conditions (high or low temperature accompanied by very low humidity) do not support its cultivation, the areas of cultivation and consumption overlap (Rooney, 1996).
Different Names of *Piper betel*:

**Binomial Name:**  
*Piper betel* L.

**Vernacular Names:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Language</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paan</td>
<td>Urdu/Hindi</td>
</tr>
<tr>
<td>Nagavalli</td>
<td>Sanskrit</td>
</tr>
<tr>
<td>Veelaya / Veelaya / Vilya</td>
<td>Kannada</td>
</tr>
<tr>
<td>Taambuul</td>
<td>Sanskrit</td>
</tr>
<tr>
<td>Tamalapaku</td>
<td>Telugu</td>
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<td>Vidyachepan</td>
<td>Marathi</td>
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<td>Vettila</td>
<td>Malayalam</td>
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<td>Plu</td>
<td>Mangolia</td>
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<tr>
<td>Malus</td>
<td>Tetum</td>
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<tr>
<td>Maluu</td>
<td>Khmer</td>
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<tr>
<td>Plue</td>
<td>Thailand</td>
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<tr>
<td>Malu</td>
<td>Tokodede</td>
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<tr>
<td>Bulath</td>
<td>Sinhalese</td>
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<tr>
<td>Bileiy</td>
<td>Diveh</td>
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<td>Bulung Saamat</td>
<td>Kopampangan Language</td>
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<td>Daun Sirih</td>
<td>Malay Language</td>
</tr>
<tr>
<td>Papulu</td>
<td>Chamorro</td>
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<tr>
<td>Trau</td>
<td>Vietnamese</td>
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### Systematic Classification

<table>
<thead>
<tr>
<th>Taxonomic Rank</th>
<th>Classification</th>
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<tbody>
<tr>
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<td>Eulcarupota</td>
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<tr>
<td>Kingdom</td>
<td>Plentae</td>
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<tr>
<td>Sub-kingdom</td>
<td>Viridaeplantae</td>
</tr>
<tr>
<td>Phylum</td>
<td>Tracheophuyta</td>
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<tr>
<td>Sub-phylum</td>
<td>Euphyllophytina</td>
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<tr>
<td>Infra phylum</td>
<td>Radiotopses</td>
</tr>
<tr>
<td>Class</td>
<td>Mangoliopside</td>
</tr>
<tr>
<td>Sub-class</td>
<td>Mangolidae</td>
</tr>
<tr>
<td>Super Order</td>
<td>Piperaeae</td>
</tr>
<tr>
<td>Order</td>
<td>Piperales</td>
</tr>
<tr>
<td>Family</td>
<td>Piperaceae</td>
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<tr>
<td>Sub-family</td>
<td>Coliandinae</td>
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<tr>
<td>Tribe</td>
<td>Diapensieae</td>
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<tr>
<td>Genus</td>
<td>Piper</td>
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<tr>
<td>Species</td>
<td>Betle</td>
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<tr>
<td>Botanical Name</td>
<td><em>Piper betel</em></td>
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</tbody>
</table>
Plant Description

The betel vine is a perennial dioecious creeper belonging to the family Piperaceae i.e., the Black Pepper family (Gunther, 1952). *Piper betel* is a climber with broad heart shaped dark green, shiny juicy leaves. The betel plant is a branching vine, climbing as high as 10-15 feet, although it often grows as an under story ground lover. As a commercial crop, betel is planted in a large garden, trained on small tress like *Avathi Sesbania* grand flora (or) *Kalyana murungai erythrinavariiegata* (or) on wooden supports. It develops roots at the nodes and climbs by fixing them to the supports. Climbers, usually are aromatic in nature, it has scattered vascular bundles in Transverse Section (T.S.) like monocots. Shrubs, herbs, climbers are glabrous or pubescent, leaves - alternate, pubescent, pinnately veined, spikes, opposite arranged leaves, ascending arching, densely flowed distally dropping.

**Leaves:** Alternate, opposite, palmately veined.

**Flowers:** Polygamous, sessile, petiolate, carpascular.

**Stames:** 1-10 filamentous, anthers 2 locular, longitudinally dehiscent.

**Gynoeclium:** 2-5 carpellate, superior ovary - Single locular ovale, 1-5 orthotropous, stigmetous, short styles.

**Fruit:** Drupe or nutlet, pericarp fleshy.

**Seeds:** Copious starchy perisperus and minute embryo embedded in small perisperm.

**Flower:** Sesile on surface of rachis, floral bracts with whitish hairs

**Cultivation:** The betel vine is cultivated in most Southeast Asia, India, Burma, Srilanka, Pakistan, Vietnam, Cambodia, Myanmar, Philippines, Bangladesh and Manila. It is cultivated for Areca nut, Muckhirca, betel oil and betel acid.
Types of Cultivation

- Natural Conditions
- Partially controlled conditions
- Controlled conditions - mainly in sub-tropics (green house technology / environment chamber method and bareja technique)

Natural conditions

In states of Assam, Manipur; parts of Nagaland in north-east and Kerala; parts of Karnataka down, offer conditions to grow. Here it is grown along with areca nut (*Areca catechu*) and coconut (*Cocos nucifera*). Such plantation last for decades (30-40 years). Plant is a climber and the vine can be as tall as supporting tree, generally 10-15 mts height with profuse branching at top and lots of foliage.

Partially Controlled Conditions

- High humidity
- Low sunshine conditions
- Do not prevail round the year

So, plants are to be projected from excess sunlight and dry air. Vines are trained to live support on plants say, *Sesbania, Graniflora, S.seban (jayanthe), Serythrive indica (pavgava), Moringa oleifere* (drumstick) that provides a shade and contribute to increase in humidity. Close planting of vines helps in moisture retention, creation of microclimate conductive to growth, unlikely in north-east.

Vines are allowed to attain height only up to 1-2 mts height. This is brought about by suppressing linear growth and profuse branching.
**Controlled conditions**

Sub-tropical regions - Relative humidity low,

Temperature - Above 40°C in summer, below 10°C in winter.

Such weather conditions with adequate sunshine hypothetically active radiations 1200-1800 (I more m ~ 2s) one hardly conductive to good growth of shade loving native plant of tropics.

**Green House Technology**

Environmental chamber with materials available in native “BAREJA” older than 600-400 B.C.

**Process of Cultivation in Asia**

The betel leaf is cultivated in most of Southern Asia (Fig. 2). Different types of cultivars are cultivated throughout India (Table 1). Being a creeper, it needs a compatible free or a long pole for support. This cultivation is a special type of agricultural practice. High land and special fertile soils are best for betel. Water logged, saline and alkali soils are unsuitable for cultivation. Farmers who are called barvi prepare gardens for this. Paan gardens are called Barouj. Barouj is fenced with bamboo sticks and coconut leaves and on top it is also covered by paddy leaves. The land is dug out into furrows of 10-15 mts length, 75 cms width and 75 cms depth. Oil cakes, cow dung, cotton farm yard manure and leaves are thoroughly incorporated with the topsoil of the furrows. The creeper cuttings are planted after necessary dressing in the months of May and June, at the beginning of the monsoon season. Plants are mainly arranged in parallel rows about two feet apart and the saplings are twined around upright sticks of split bamboo and
reeds. Proper shade and irrigation are important for the successful cultivation of this crop. The plants are punctually watered in the hot months. The leaves of the plant become ready at maturity for plucking, after one year of planting and the production of the Barouj lasts for several years from date of planting. Betel needs a constant moisturized soil without excessive moisture. Hence, frequent light irrigation is given. The quantity of irrigation water should be such that the standing water should not remain for more than half an hour in bed. Best time for irrigation is mornings and evenings. In about 3-6 months time, vines grow to a height 150-180 cm. At this stage, branching is noticed in the vines. Leaves are removed along with the petiole with the right thumb. Once harvesting is commenced, it is continued almost every day or week. The interval of harvesting varies from 15 days to about a month. After each harvest, manuring has to be done.

![Piper betel L. Crop](image)

**Fig. 2.** *Piper betel* L. Crop
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Cv. and Origin</th>
<th>Colour &amp; Texture of Leaf</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Nature of leaf up</th>
<th>No. of secondary veins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>P. betel</em> L. Cv. Calcutta. Madhya Pradesh</td>
<td>Yellowish green. Glossey upper surface and coriacious</td>
<td>8</td>
<td>7.5</td>
<td>Acute</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td><em>P. betel</em> L. Cv. Despiran Uttar Pradesh</td>
<td>Dark Green, Glabrous</td>
<td>7.5</td>
<td>5</td>
<td>Curved. Acuminat e leaf tip</td>
<td>5 Intra marginal veins Present, asymmetric</td>
</tr>
<tr>
<td>3</td>
<td><em>P. betel</em> L. Cv. Desawari Uttar Pradesh</td>
<td>Greenish, Yellow, Glabrous</td>
<td>8.5</td>
<td>7</td>
<td>Curved Auminate leaf tip</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td><em>P. betel</em> L. Cv. Ghazipur West Bengal</td>
<td>Dark Green, Glossy Upper Surface and Coriacious</td>
<td>14</td>
<td>12</td>
<td>Acute</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td><em>P. betel</em> L. Cv. Bangladeshi West Bengal</td>
<td>Dark Green Coriacious</td>
<td>13</td>
<td>11</td>
<td>Acute</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td><em>P. betel</em> L. Cv. Benarasi Uttar Pradesh</td>
<td>Dark green, Glossy Upper Surface &amp; Coriacious</td>
<td>13</td>
<td>11.5</td>
<td>Acute</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td><em>P. betel</em> L. Cv. Hakeswar Madhya Pradesh</td>
<td>Yellowish Green, Glossy Upper surface &amp; Coriacious</td>
<td>15</td>
<td>14</td>
<td>Acute</td>
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<tr>
<td>8</td>
<td><em>P. betel</em> L. Cv. Vishnupuri Pan. Madhya Pradesh</td>
<td>Dark Green, Coriacious</td>
<td>15</td>
<td>14</td>
<td>Acute</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td><em>P. betel</em> L. Cv. (Kapuri) Tuni Andhra Pradesh</td>
<td>Light Green glabrous</td>
<td>12.5</td>
<td>7.5</td>
<td>Acute</td>
<td>7</td>
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<tr>
<td>10</td>
<td><em>P. betel</em> L. Cv. Saunfia Pan Madhya Pradesh</td>
<td>Dark Green Coriacious</td>
<td>9.5</td>
<td>7</td>
<td>Acumiant e</td>
<td>7</td>
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</table>

**Source:** Comparative Morphoanatomy of *Piper betel* L. cultivars in India, Seetha Lakshmi and Naidu, 2010)
Agro-economics of Betel in India

Betel vines are cultivated throughout south-east Asia. The leaves grow on betel vines, and the average size of vine plots range from 0.5 to 50 decimals (1 decimal = 0.01 acre). In India, a 2006 research reported betel vines being cultivated on about 55,000 hectares of farm land, with an annual production worth of about IN Rs.9,000 million ($200 million total, averaging $1,455 per acre). The betel farming industry, as per the surveyed report, supports about 400,000 - 500,000 agricultural families. A report in March 2011 claims that betel farming is on a decline in India. While in ideal conditions, some farms may report gross annual incomes after expenses of over IN Rs.26,000 per 10 decimal farm ($5,780 per acre), a betel farm income is highly erratic from year to year, because of rainfall patterns, temperature, and spoilage rates of 35% to 70% during transport over poor infrastructure. Simultaneously, the demand for betel leaves has been dropping in India because of contagious acceptance of gutkha (chewing tobacco) by consumers over betel leaf-based “paan” preparation; the report cites betel leaf trading has dropped by 65% from 2000 to 2010, and created an oversupply. As a result, the report claims Indian farmers do not find betel farming lucrative anymore.

Importance of Betel Leaves

A paste of betel leaves mixed with salt and hot water can be administered for filariasis. For treating obesity, one betel leaf mixed with *Piper nigrum* is prescribed for two months; Juice with honey or a liquid extract is useful in coughs, dyspnoea, deranged phlegm and indigestion in children. The application of leaves smeared with oil in solid promote secretion of milk when applied on the breasts of lactating women. A local
application is recommended for inflammatory swelling such as orchitis, arthritis and mastitis. In pulmonary infections of children and old age, leaves soaked in mustard oil are warmed and applied to the chest in order to relieve cough and dyspnoea; if mustard oil is lightly spread on the leaf, heated and placed on knees, it reduces pain. A traditional way to get relief from headache is to apply chunam (CaCO₃) to the tip of the leaf and paste it on the forehead. While applying medicines irritates the skin, the area is covered with betel leaves to reduce the irritation, betel leaf is eaten to increase digestion and the juice of the leaf wards off bad breath. The leaves are stimulant, antiseptic and sialogogue, the oil is an active local stimulant used in the treatment of respiratory catarrhs, as a local application of gargle, also as inhalant in diphtheria. Leaves are also used as counter–irritant to suppress the secretion of milk in mammary abscesses. The juice of the leaves is equivalent in power to one drop of the oil; Roots and stem base are used to make beverage. Taste is earthy and peppery – relaxing effect, used in large quantities for intoxicating effect, also used as Anti Anxiety and Anti Depressant. Chewing areca nut and betel leaf is used to cast out (cure) worms, according to traditional ayurvedic medicines chewing areca nut and betel leaf is a good remedy against bad breath (Halitosis). They are also said to have aphrodisia properties, which treat headaches, arthritis and joint pains. In Philippines, Thailand, Indonesia and China these are used to relive toothache, it is used as stimulant. In Indonesia, these are drunk as an infusion and as an antibiotic infusion to cure indigestion and as a topical cure for constipation as a decongestant and aid for lactation. Betel leaf is an aromatic antibacterial, stimulant herb, with a species clove like flavor. Chewing the leaves of herb increase the flow of saliva and also protect against intestinal parasites, betel leaf is a good
source of calcium, carotene and also helps in digestion. In India, the leaves are used as a masticatory, it is also used as condiment, roots, fruits and leaves are used in treatment of malaria, sores, bruises, ulcers, boil, ulceration in nose, as an antiseptic.

**Chief Constituents in Betel Leaf**

Chief constituents of the leaves are volatile oil, varying in the leaves from different countries and known as betel oil, which is primarily a class of allyl benzene compounds, several terpenes and terpenoids. Betel leaves are very nutritive and contain substantial amount of vitamins and minerals (Table 2) and therefore, six leaves with a little bit of slaked lime is said to be comparable to about 300 ml of cow milk particularly for the vitamin and mineral nutrition. The leaves also constitute the enzymes like diastase and catalase besides a remarkable amount of all the essential amino acids except lysine, histidine and arginine, which are found only in traces (CSIR, 1969; Gopalan, 1984; Guha and Jain, 1997).

**Use in modern medicine**

*Piper betel* L. leaves includes vitamin B, C; stimulates brain, lungs and heart and useful in *vata, kapha*; posses Anti Helminthic, stomachic action; used to treat Asthma, Leporsy, Alcoholism, Poisoning; improves Appetite; Juice of leaves is used as medicine for pain in eye and night blindness; powered fresh leaves are used in preparation of lotion and baths for patients suffering from fever, small pox, lymphangitis; also used as a pediatric medicine for indigestion, diarrhea, pulmonary catarrh and laryngeal problems in children.
Table 2. Nutritional composition of Betel leaf

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Constituents</th>
<th>Approximate composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>85-90%</td>
</tr>
<tr>
<td>2</td>
<td>Protein</td>
<td>3 – 3.5%</td>
</tr>
<tr>
<td>3</td>
<td>Fat</td>
<td>0.4 – 1.0%</td>
</tr>
<tr>
<td>4</td>
<td>Minerals</td>
<td>2.3 – 3.3%</td>
</tr>
<tr>
<td>5</td>
<td>Fibre</td>
<td>2.3%</td>
</tr>
<tr>
<td>6</td>
<td>Chlorophyll</td>
<td>0.01 – 0.25%</td>
</tr>
<tr>
<td>7</td>
<td>Carbohydrate</td>
<td>0.5 – 6.10%</td>
</tr>
<tr>
<td>8</td>
<td>Nicotinic acid</td>
<td>0.63 – 0.89 mg / 100 g</td>
</tr>
<tr>
<td>9</td>
<td>Vitamin-C</td>
<td>0.005 - 0.01%</td>
</tr>
<tr>
<td>10</td>
<td>Vitamin-A</td>
<td>1.9 – 2.9 mg/100 g</td>
</tr>
<tr>
<td>11</td>
<td>Thiamine</td>
<td>10 - 70 μg/100 g</td>
</tr>
<tr>
<td>12</td>
<td>Riboflavin</td>
<td>1.9 – 30 μg/100 g</td>
</tr>
<tr>
<td>13</td>
<td>Tannin</td>
<td>0.1 – 1.3%</td>
</tr>
<tr>
<td>14</td>
<td>Nitrogen</td>
<td>2.0 – 7.0%</td>
</tr>
<tr>
<td>15</td>
<td>Phosphorus</td>
<td>0.05 – 0.6%</td>
</tr>
<tr>
<td>16</td>
<td>Potassium</td>
<td>1.1 – 4.6%</td>
</tr>
<tr>
<td>17</td>
<td>Calcium</td>
<td>0.2 – 0.5%</td>
</tr>
<tr>
<td>18</td>
<td>Iron</td>
<td>0.005 – 0.007%</td>
</tr>
<tr>
<td>19</td>
<td>Iodine</td>
<td>3.4 μg/100 g</td>
</tr>
<tr>
<td>20</td>
<td>Essential oil</td>
<td>0.08 – 0.2%</td>
</tr>
<tr>
<td>21</td>
<td>Energy</td>
<td>44 kcal/100 g</td>
</tr>
</tbody>
</table>

**Source**: Betel leaf: The negelected Green Gold, Guha, 2006.

**Antibacterial activity**

If the world fails to mount a more serious effort to fight infectious diseases, antimicrobial resistance will increasingly threaten to send the world back to a pre-antibiotic age.

-- Gro Harlem Brundtland
Etymon and History

The term “antibacterial” derives from Greek ἀντί (anti), “against” (Ἀντί, et al.) + βακτήριον (baktērion), diminutive of βακτηρία (baktēria), “staff, cane” (Ἀντί βακτηρία, et al.), because the first ones to be discovered were rod-shaped, and the term “antibiotic” derives from ἀντι + βιωτικός (biōtikos), “fil for life, lively”, (Henry George Liddell, Robert Scott) which comes from βίωσις (biōsis), “way of life”, (Henry et al., βίωσις) and that from βίος (bios), “life”. (βίος Henry George Liddell, Robert Scott).

The history of antimicrobials has begun with the note of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. Bacteria were first identified in the 1670s by van Leeuwenhoek, soon after his invention of the microscope. However, it was not until the nineteenth century that their link with disease was appreciated. Then the liability that the microorganisms might be susceptible for disease began to take hold.

The antibiotic era enter on with the pneumatic application of nitroglycerine drugs, followed by a “golden” period of discovery from about 1945 to 1970, when a number of structurally contradictory, highly effective agents were discovered and developed. The first sulfonamide and first commercially available antibacterial antibiotic, Prontosil, was developed by a research team led by Gerhard Domagk in 1932 at the Bayer Laboratories of the IG Farben conglomerate in Germany. Domagk received the 1939 Nobel Prize for Medicine for his efforts. Research was frenzied apace by its fruition. The discovery and development of this sulfonamide drug embarked the era of antibacterial antibiotics.
Antibacterial antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. There are mainly two classes of antimicrobial drugs

1. Those obtained from natural sources
2. Synthetic agents

Whatever the source, the Antibacterials are used in the treatment and in the prevention of Infections

Mechanisms of action

Antibacterials are classified depending on the site of action (Fig. 3).

Inhibition of cell wall synthesis

Inhibition of protein synthesis

Inhibition of nucleic acid synthesis (DNA or RNA)

Antimetabolites

Fig. 3. Diagrammatic representations – target sites of action
Screening Methods

Determination of antimicrobial action against pathogens is essential for drug discovery. Screening is first the foremost step for determination of antimicrobial action. Screening assays for antibiotic susceptibility fall under two heads, viz.

Dilution Susceptibility tests

Agar diffusion tests

These are the two most popular techniques adopted to test microbial sensitivity to an antibiotic or a presaging extract for the presence of antibacterials.

Dilution Susceptibility tests

Dilution susceptibility tests can be used to determine the smallest amount of drug required to inhibit the growth of organism in vitro. That smallest amount is referred to as the Minimum Inhibitory Concentration (MIC).

Increasing amounts of the test drug sample under examination are placed in a series of culture tubes containing a suitable broth medium inoculated with the test microorganism. A generally recommended initial cell concentration is $5 \times 10^5$ CFU/tube. After incubation, the concentration of drug sample required to inhibit the growth of the organism used is determined by observing the absence of growth/turbidity (Fig. 4).
Fig. 4. Antibiotic susceptibility test-MIC Test

**Agar Diffusion tests**

The agar diffusion test has probably been the most widely used throughout history. The most widely used screening methods to measure the antimicrobial efficacy of medicinal plants, spice, their essential oil, and their constituents have been agar diffusion method (Kivanc and Anguel, 1986; Safak et al., 2003; Vilijoen et al., 2003; Thiem and Goslinska, 2004).

**Diffusion tests can be performed by two methods:**

Disc Diffusion method

Well Diffusion method
Disc Diffusion method

For routine tests, usage of disc diffusion method is more common. The paper disc plate method is the most commonly used method for determining susceptibility of microorganisms to specific drugs or herbal extracts. Small paper discs impregnated with known amounts of drug sample are placed upon the surface of an inoculated plate. After incubation, the plates are observed for any zones of inhibition surrounding the discs. A clear zone of inhibition around the discs corresponds to the degree of susceptibility of organism to the drug, which diffused into the agar from the disc. Kirby-Bauer disc diffusion assay is a rapid screening method for evaluating the antimicrobial properties of a specific antimicrobial agent (Fig. 5).

Well diffusion method

The well diffusion method is right suitable for direct aqueous extracts. After the medium gets solidified, a well was made in the plates with sterile borer (5 mm). The extract compound (50 μl) was fed into the well and plates were incubated at 37°C for 72 hrs. All samples were tested in triplicates. Microbial growth was determined by quantifying the diameter of zone of inhibition. A control with standard antibiotic was kept for all test strains and the control activity was deducted from the test and the results were recorded.
With both the agar and broth dilution assays, the objective is to generate a single statistic to describe the inhibition of a microorganism at a specific endpoint in time. The measurement of inhibition at a specific time is termed the minimum inhibitory concentration (MIC). The MIC may be defined as the lowest concentration at which no growth occurs in a nutrient medium. However, the definition of the MIC differs between publications. In some cases, the Minimum Bactericidal Concentration (MBC) or the bacteriostatic concentration is stated, both terms agreeing closely with the MIC.

There have been reports on antibacterial activities of many Piperaceae members, but so far the report on antibacterial activity of *Piper betel* L. Cv. Kapoori, a local cultivar is lacking. Hence, this variety is taken up for the antibacterial studies. The
antibacterial activity was seen against the organisms - *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Corynebacterium diphtheria* (ATCC 75415), *Xanthomonas citrovorum* (ATCC 8082), *Proteus vulgaris* (ATCC 638) and *Staphylococcus aureus* (ATCC 25923).

**Antifungal activity:**

**History**

Just before two to three decades, relatively few drugs were available for the treatment of fungal infections. The nature of fungal diseases is the only reason for the slow development of broad spectrum agents. The first antifungal agent, Nystatin was not discovered until 1949 and the first oral antibiotic being Griseofulvin, used for dermatophytosis management. Later, broad spectrum antifugals were developed. The first of its kind is iodinated trichlorophenol haloprogin 1969 heralded the discovery of azole antifungal agents. Then came the development of triazole antifungal agents that were less likely to cause heptotoxicity possibly. With the launch of newer broader spectrum agents - trichlorophenols, imidazoles, the stature of antifungal therapy transfigured greatly in the late 1960s.

**Mechanism of action**

Antifungals are classified by structure or mechanism and not by site of action (Fig.6)

They are:

- **Polyene antifungal agents:** Cell membrane disruption.

- **Azole and Triazole antifungal agents:** Ergosterol biosynthesis inhibitors.
• **Antifungal agents targeting squalene epoxidase:** Ergosterol biosynthesis inhibitors.

• Other antifungals affecting cell membrane stability

![Diagram of fungal cell and target sites](image)

**Fig. 6.** Target site of action for fungal agents

**Screening Methods**

Screening methods are the same as such for Bacteria. As the traditional use of *Piper betel* leaf extracts supports its antifungal activity, *Piper betel* L. Cv. Kapoori - a local cultivar is screened for its antifungal activity and the organisms used are *Aspergillus niger* (NCIM 596), *A. fumigatus* (NCIM 291) and *Candida albicans* (NCIM 670).

**Antioxidant Activity**

**History**

The up-growth of angiosperm plants between 50 and 200 million years ago resulted in the development of profuse antioxidant pigments – particularly during the Jurassic period – as chemical defenses against reactive oxygen species that are
byproducts of photosynthesis (Benzie.I, 2003). Originally, the term antioxidant specifically referred to a chemical that prevented the consumption of oxygen. In the late 19th and early 20th centuries, lengthy study concentrated on the use of antioxidants in important industrial processes. Early research on the role of antioxidants in biology centralized on their use in averting the oxidation of unsaturated fats, which is the cause of rancidity. However, it was the identification of vitamins A, C and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Covas et al., 2006 and Knight, 1998). The probable mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized (Moureu Charles et al., 1922).

**Description**

Free radicals are the atomic or molecular species with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed, these highly reactive radicals can begin a chain reaction, their chief danger comes from the damage they can do when they react with essential cellular components such as DNA or cell membrane. Cells may function poorly or die if it occurs. To prevent free radical damage, the body has a defence system of antioxidants.

Antioxidants are the body’s scavengers. These are the molecules, which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta –
carotene and vitamin C. Additionally selenium, a trace element that is required for proper function of one of the body’s antioxidant enzyme systems, is sometimes included in this category. The body cannot synthesize these micronutrients so they must be provided in the diet. When our body repair system cannot heal the damage caused by free radicals produced when exposed to UV, pollution, smoking then we need to take additional antioxidants.

Oxidation reactions are chemical reactions that involve the transfer of electrons from one substance to an oxidizing agent. Antioxidants can slow these reactions either by reacting with intermediates and halting the oxidation reaction directly or by reacting with the oxidizing agent and preventing the oxidation reaction from occurring. Antioxidants are chiefly used as ingredients in dietary supplements used for health purposes such as attempting to prevent cancer and heart disease.

**Role of oxygen free radicals in disease**

Oxygen, the most critical element for life, and is highly lethal to strict anaerobic systems is the main source of free radicals. Oxygen free radicals are highly reactive fragments capable of independent existence that contain one or more unpaired electrons.

**Five basic types of damage caused by free radicals**

**Lipid peroxidation:** Free radicals initiate damage to fat compounds in the body, causing them to turn rancid and release more free radicals.

**Cross linking:** Free radical reactions cause proteins / DNA molecules to fuse together.
Membrane damage: Free radical reactions disrupt the integrity of the cell membrane, which in turn interferes with the cell’s ability to take in nutrients and expel wastes.

Lysosome damage: Free radicals rupture lysosome cell (digestive particle) membranes. These then spill into the cell and digest critical cell compounds.

Accumulation of age pigment (lipofusion): Lipofuscin may interfere with cell chemistry.

Every time you breathe, oxygen uptake causes free radical production. Environmental factors, such as pollutants, smoke and certain chemicals also contribute to their formation. If left unchecked, they can wreak havoc on physique and cause a multitude of ailments including arthritis, cardiovascular disease, dementia and cancer.

Oxidative damage in the human body plays an important causative role in disease initiation and progression (Jacob and Burri, 1996). Damage from reactive oxygen species (ROS) including free radicals has been linked to some neurodegenerative disorders (Alzheimer’s disease and Parkinson’s and cancers).

Applications in Medicine and Nutrition

Researchers have found a high correlation between oxidative damage and occurrence of disease. For example, Low Density Lipoprotein (LDL) oxidation is associated with cardiovascular disease. The process leading to atherogenesis, atherosclerosis and cardiovascular disease is complex, involving multiple chemical pathways and networks, but the precursor is LDL oxidation by free radicals, resulting in inflammation and formation of plaques.

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Research suggests that consumption of antioxidants rich foods reduces damage to cells and biochemicals from the free radicals. This may slow down, prevent, or even reverse certain diseases that result from cellular damage and perhaps even slow down the natural aging process. This is the basis for the free radical theory of aging.

Few reactions in the body that yield free radicals involve metal ions. Several antioxidants, such as the tannins in walnuts and tea, chelate metal ions. This not only curtails the formation of ion-dependent free radicals, but also inhibits the metal ions from oxidizing cells and biochemicals directly. Some studies suggest that by abolishing free radicals and reducing cellular damage, antioxidants in the diet can have positive health effects. Such as preventing macular degeneration, keep up the immune system potentially preventing neurodegeneration due to oxidative stress (Wang et al., 2006), preventing DNA damage (Hillestrom, 2006) and lowering the risk of cardiovascular disease. Any particular antioxidant may perform only a small fraction of these functions.

Oxygen Radical Absorbance Capacity (ORAC) turned out to be the current industry standard for assessing antioxidant strength of whole foods, juices and food additives. Other measurement tests include reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating.

**Food sources**

Antioxidants are found naturally in varying amounts in vegetables, fruits, grain cereals, legumes, nuts, etc. Antioxidant sources includes Fruits (Berries and peppers, cider, wine); Vegetables (Spinach, Tea); Fungi (Mushrooms); Whole grain cereals (Hops,
Examples of antioxidants

Beta-carotene, Vitamin C (Ascorbic Acid), Vitamin A, Vitamin E (Alpha-tocopherol), Lutein, Lignan, Lycopene, Selenium (a mineral component of antioxidant enzymes), Flavonoids / Polyphenols; Vitamin-like Antioxidants include Coenzyme Q10 (CoQ10), Glutathione; Antioxidant enzymes made by the body are superoxide dismutase (SOD), catalase and glutathione peroxidase.

Much attention has been focused on the antioxidative compounds present in plants because of safety concerns associated with synthetic antioxidants.

Antihelminthic activity

Definition and History

An anthelmintic is a substance that expels or destroys gastro-intestinal worms. The more common name is dewormer or “wormer”. Anthelmintics are otherwise called parasiticides, endectocides, nematocides, parasitics, antiparasitics and drenches.

As with many areas of human parasitology, the development of anthelmintics has been slow, with only 11 compounds coming into general use in the past 50 years. The vast majority of anthelmintics do not lend themselves to community-based treatment because their dose regimens are complex or multi-day, or they are poorly tolerated.
Although knowledge of intestinal parasites predates Hippocrates, the Hippocratic Corpus furnishes the first scientific observations concerning the clinical perception and treatment of helminthic diseases. Before 1940, the only compounds used to deal with parasitism were natural substances that had some effect on parasites, but also risked toxicity to the host. The modern age of deworming began with the introduction of phenothiazine, which was administered to sheep as a drench and/or included in salt mixtures. It was sometimes combined with lead arsenate to control tapeworms. In the 1960s and 1970s, organophosphate anthelmintics were introduced. Although, nowadays anthelmintics are separated into classes on the basis of similar chemical structure and mode of action. Though anthelmintics are sold under several brand names, there are only three chemical classes of dewormers. All drugs in a chemical class kill worms in the similar manner, though the effectiveness within chemical families varies.

The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on the anthelmintic drug is sparse. According to the WHO, only a few drugs are used in treatment of helminthes in humans. Anthelmintics from natural sources could play a key role in the treatment of these parasite infections.

**Diseases caused by helminthes**

Lympathic filariasis (Elephantiasis), Schistosomiasis (Bilharzia), Ascariasis, Trichuriasis, Hookworm, Angiostrongylosis, Toxocariosis, Echinococcosis,
Cysticercosis (Taeniasis), Trichuriasis, Biliary Parasitic Diseases (Flukes), Strongyloidiasis and Schistosomiasis.

Helminthiasis is a highly prevalent disease worldwide that is caused by species of *Platyhelminthes* (flatworms) and *Nematoda* (roundworms). The disease may be transmitted by the fecal-oral route, active penetration of the skin by larvae from the soil, or vector arthropods. Helminthiasis is endemic in developing countries and places where sanitation is poor, but it also occurs in nonendemic areas because of immigration and travel. These infections are responsible for high levels of morbidity and mortality, including iron-deficiency anemia, seizures, portal hypertension, and chronic diarrhea (1–3). Children show high infection rates because they usually play in close contact with the soil and may put their fingers in their mouths. Children also are vulnerable to severe complications of helminthic infection, such as malnutrition, anemia, bowel obstruction, and learning disabilities.

**Drugs**

Pyrantel, palmoate, Albendazole, Mebendazole, Ivermectin, Praziquantel, Triclabendazole, Niclosamide, Closantel, Benzimidazoles, Oxamnaquine, Metrifonate, Antimonials.

As antimicrobials of plant origin have enormous therapeutic potential and are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antibacterials, potent anthelmintics are available today, and treatment is frequently done by using different types of drugs. However, the high costs of modern anthelmintics have limited effective
control of the parasites. In some cases, wide spread use of low quality anthelmintics (Barar, 2000). Only few plants are being used traditionally as anthelmintics e.g. *Aloe barberi*, *Trachyspermum ammi*, *Annona senegalensis* (Ibrahim *et al.*, 1983). So there is a need to develop anthelmintics drug from herbal source. In future, it is to be hoped that new anthelmintics from herbals come over.

**Antinutrient activity**

**Introduction**

An anti-nutrient is something that hinder a nutrient from being used from the body. The terms anti-nutrient and antinutrient are likely to be used inter-changeably in books and articles about substances that adversely affect the uptake and use of nutrients by the human body. These Anti-nutrients adversely affect the overall nutritional value of all foodstuffs consumed (including both food and drinks) by interfering with body processes such as (Hotz *et al.*, 2007), absorption of nutrients such as vitamin and minerals, the body's metabolic rate, Toxic effects, but not all antinutrients affect the body in all of these means. For example, a distinct antinutrient might only adversely affect the absorption of one particular vitamin or mineral.

**Anti-nutritional factors**

*Protease inhibitors* inhibit the activity of trypsin, chemotropism and other proteases. They are seen in legumes such as beans and peas, but also in cereals, potatoes, and other products. Their presence results in impaired growth and poor food utilization.
**Amylase inhibitors** have a similar activity against amylases. Amylases are important in breaking down the structure of carbohydrates, they hydrolyze sugar and starches. Ultimately, improving digestion of carbohydrates.

**Lectins** or humagglutinins are glycoprotein mainly found in legumes: beans, peas, lentils. Their presence results in poor food utilization and impaired growth.

**Glucosinolates** are found in cabbage and related species. Effects upon the thyroid function have been demonstrated.

**Saponins** are found in soybeans, peanuts, sugar beets and others. Toxic effect has been shown.

**Gossypol** is particularly important in cottonseed. Several toxic effects have been demonstrated.

**Phytic acid** occurs in several vegetable products. Its presence may affect bioavailability of minerals.

There are many anti-nutrients. Their individual effects on the body differ because different antinutrients affect the absorption, metabolism, utilization and/or excretion of different nutrients /including micro-nutrients. Depending on which nutrients are affected, the intensity of the effect, and intake of the nutrient(s) involved, various nutritional deficiencies and their effects may result.

**Examples of sources of anti-nutrients:** Coffee (caffeine); Tea (caffeine and tannin), but not all fruit/herbal teas; Carbonated Soft Drinks (acids and caffeine); Alcoholic Drinks (alcohols); Antibiotics – Few antibiotics kill even “friendly bacteria” in the gut;
Tranquilizers - reduce metabolic rate and depress appetite; Contraceptive Pills - affect uptake and activity of some nutrients; Medication - a huge category that includes many different types of drug.

**Examples of effects of anti-nutrients** (in general)

Nutrients, mainly vitamins and minerals that can be affected by anti-nutrients in general include **Vitamins** - vitamin A, vitamin B\(_1\), vitamin B\(_2\), vitamin B\(_3\), vitamin B\(_5\), vitamin B\(_6\), vitamin B\(_7\), vitamin B\(_8\), vitamin B\(_9\), vitamin B\(_{12}\), vitamin C, vitamin D, vitamin E, vitamin H (B\(_7\)) and vitamin K. **Minerals** - calcium (Ca), chlorine (Cl), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nitrogen (N), phosphorus (P), potassium (K), selenium (Se), sodium (Na) and zinc (Zn). **Amino Acids** - leucine, carnitine, cysteine and methionine. **Others** include beneficial gut bacteria, carotene and coenzyme Q10.

While several new types of antibiotic medicaments have been discovered over the eons, the hunt for discovering latest and further effectual drugs endure. As plant drugs are generally less toxic and free from side effects, rebound of interest in the study and use of medicinal plant has been taking place during the last two decades. As a part of this, a local variety of *Piper betel* plant was attentioned for its antinutrient screening.

**Bioassay Methods**

**Brine shrimp Assay and Cytotoxicity Assay**

Plants consumed as eatables should be free of toxicity or other adverse effects on their consumer. Conformable indicators of insecure plants include their toxic, cytotoxic and mutagenic potential. These harmful properties of plants are produced by the
chemicals that are found in certain plants as defense mechanisms. Although indigenous plants offer extensive medicinal value, one needs to caution their use, unless they have been tested in animals and cell culture for their toxicity, cytotoxicity, mutagenicity. Cytotoxicity is the scaling of a chemical’s ability to damage or kill cells whilst toxicity is the term used to describe the capacity to cause injury to a living organism or cause any diverse effects of a chemical on a living organism. The asperity of toxicity produced by any chemical is directly equivalent to the concentration and time of exposure. The relationship also rely on the developmental stage of an organism and its physiological status. It may be said that in natural foods of our everyday diet there are thousands of toxic substances, which leads to the distinction between toxicity and hazard. The toxicity of a substance is its intrinsic capacity to produce injury when tested by itself. The hazard of a substance is its capacity to produce injury under circumstances of exposure. Thus many substances have a high intrinsic toxicity but no hazard, when associated with its natural presence in foods. In spite of the multitude of toxic substances consumed daily in a normal diet by normal healthy individuals there is yet little evident hazard involved. There are three reasons for this. Firstly, the low concentrations of the toxicant present, secondly, because the effect of the many toxic substances present is not cumulative and thirdly because of the antagonistic effect of one toxicant upon another. However, the situation might be different if ‘toxic’ food is regularly eaten in excessive amounts (Fox and Norwood Young, 1982). The toxicity of the plants may be measured using the brine shrimp assay and the cytotoxicity may be measured using tests such as, MTT (3-[5, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide), flow cytometry and luciferase ATP. It can also be tested using stains such as neutral red and trypan blue.
A herb called berberine and berberine containing plants, although non-toxic at recommended doses has been found to be harmful when used during pregnancy and higher dosages may interfere with Vitamin B metabolism. When tested in rats, the LD$_{50}$ was 1.000 mg/kg body weight, which indicates that the toxicity is very low (Murray, 1995). Another example is the Siberian ginseng, which helps with chronic fatigue syndrome, athlerosclerosis and impaired kidney function at recommended levels. However, it can be toxic if taken at higher doses (4.5-6 ml 3 times daily) and the side effects include symptoms such as headache, irritability (Murray, 1995).

The identification of novel antitumor agents that evince efficacy is necessary to improve medical treatment based on new drugs. The pharmaceutical industries have been striving together in this area for many years trying to find novel compounds with good antitumor activity. Brine shrimp (*Artemia salina*) larvae have been reported as the laboratory organism that can detect bioactive compounds. Since there is a good enough correlation between the toxicity on *Artemia salina* and antitumor (McLaughlin, 1991; De Rosa, 1994) activity, this test is a good prescreening method for identifying new potential antitumor agents. The cytotoxicity may be evaluated using tests such as, MTT (3-[5, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) (Reddy, 2005).

**Mutagenicity Assay**

Mutagenicity is the capability of a chemical or physical agent to bring permanent alteration of the genetic material within the cells (Prescott *et al.*, 1999) and an agent that brings about these permanent alterations within the cell other than the normal growth is called a mutagen. There are many types of mutations. The first type is conditional
mutations that are expressed under certain environmental conditions, such as lethal mutations at high temperature. The second kind is biochemical mutations, which cause a change in the biochemistry of a cell. Since these mutations often inactivate the biosynthetic pathway, they frequently make a microorganism helpless to grow on a medium lacking an adequate supply of the pathway end (Prescott et al., 1999). An example of this is a strain of Salmonella typhimurium which is used in Ames mutagenicity testing (Ames et al., 1973) that carries a mutant gene making, it unable to synthesize amino acid histidine from the ingredients in its culture medium. However, few types of mutations (including this one) can be reversed and is then called a back mutation, with the gene regaining its function. These revertants are able to grow on a medium deficient of histidine (Ferguson et al., 2003).

Phytochemistry

**History and Use**

“Phyto” is a Greek word meaning plant and Phytochemistry is the study of Phytochemicals which are derived from the plants. Phytochemicals exist as long as plants exist. In a narrow sense phytochemistry is often used to describe the large number of secondary metabolic compounds found in plants. These are the non-nutritive plant chemicals, constituting a heterogeneous group of substances. In general, these plant chemicals are said to protect the plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack. Recently, it was clearly evident that they have roles in the protection of human health, when their dietary intake is significant. These compounds are known to possess the biological properties such as
antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer properties that protect humans against diseases. There are more than a thousand known and many unknown phytochemicals. Because of these properties, many researchers have been performed to reveal the beneficial health effects of phytochemicals.

**Classification of Phytochemicals** *(www.sigmaaldrich.com)*

As per the Chemical classification the Phytochemicals includes Aliphatic Compounds-(13); Alkaloid-(68); Anthocyanins and Anthocyanidins-(9); Aromatic Compounds-(14); Bioflavonoids-(23); Carotenoid-(18); Flavonoids, Flavonols, and Flavanols-(52); Glycosides, Including Glucosinolates-(11); Isoflavones-(6); Oils and Resin-derived-(9); Other Biochemical-(75); Phenols-(24); Plant Extracts-(24); Saponins-(11); Steroid-(19); Tannins-(12); Terpenes and Terpenoids (Isoprenoids)- (33).

**Mechanism of action of phytochemicals**

*Pronounced “fight-o-chemicals,” phytochemicals fight to protect your health*

Different mechanisms of action of phytochemicals have been suggested as there are many phytochemicals. Each phytochemical works differently.

Some of the general possible actions are Antioxidant, Hormonal action, Interference with DNA replication, Anti bacterial effect and Physical action. Altogether, they have complementary and overlapping mechanisms of action in the body.
Phytochemical Screening

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Phytochemical Screening

Phytochemical screening is the process of separation and isolation of biologically active principle from the plant sources. Screening is helpful to get lead discovery of new therapeutic agents, to find new sources for economic material, to help expand chemotaxonomy and to produce semi-synthetic derivatives.

Since the plant extracts constitute a number of chemical categories, no single method or attempt is used to elicit all the phytochemicals. Each phytochemical is to be screened individually following the basic prescribed series of tests.

Importance of Phytochemicals

Many of the phytochemicals possess antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer, help to reduce menopausal symptoms and osteoporosis, reduce the risk for breast cancer. Interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells have anti-bacterial properties. Some phytochemicals bind physically to cell walls preventing the adhesion of pathogens to human cell walls.
Only a few years ago, the term “phytochemical” was barely known. As phytochemicals may either be used as chemotherapeutic or chemo preventive agents (D’Incalci et al., 2005; Sarkar and Li, 2006), there is a need for the discovery of hidden phytochemicals.

Gas Chromatography–Mass Spectrometry (GC-MS)

Gas Chromatography–Mass Spectrometry (GC-MS) is a new technique that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances present within a test sample. The usage of a mass spectrometer as the detector in gas chromatography was developed during the 1950s after being originated by James and Martin in 1952. These sensitive devices were originally limited to laboratory settings. In early 1968, the first prototype quadrupole GC/MS instruments were developed. By the 2000s computerized GC/MS instruments employing quadrupole technology had become both essential to chemical research and one of the foremost instruments used for organic analysis. Today computerized GC/MS instruments are vigorously used in environmental monitoring of water, air, and soil; in the regulation of agriculture and food safety; and in the discovery and production of medicine.

GC-MS is used to perform a specific test that identifies the actual presence of a particular substance in a given sample, positively. For this reason, it has been widely heralded as a “gold standard” for forensic substance identification. Each peak of a chromatogram becomes a “fingerprint” of the compound. The fingerprints are compared with a library to identify the compounds.
History

Mass spectrometer as detector in gas chromatography was used in the 1950s by Roland Gohlke and Fred McLafferty (Gohlke, 1959; McLafferty Fred, 1993). The development of a computer controlled quadruple mass spectrometer was initiated in 1964 by the Electronic Associates, Inc. (EAI), a leading U.S. supplier of analog computers, under the direction of Robert E. Finnigan. The first prototype quadrupole GC/MS instruments were delivered to Stanford and Purdue University by the Finnigan Instrument Corporation, in 1967. FIC was eventually renamed Finnigan Corporation and went on to setup itself as the worldwide leader in GC/MS systems (Fig. 7).

Instrumentation

The GC/MS instrument is made up of two parts.

**Gas Chromatography (GC) part** - This separates the chemical mixture into pulses of pure chemicals based on their volatility, or ease with which they evaporate into a gas.

**Mass Spectrometer (MS) part** - This identifies and quantifies the chemicals based on their structure.

The sub-parts of each component are given below.

**Gas Chromatography (GC)**

**Injection port** – One microliter (1 µl, or 0.000001 L) of solvent containing the mixture of molecules is injected into the GC and the sample is carried by an inert (non-reactive) gas through the instrument, usually helium. The inject port is heated to 300°C to cause the chemicals to become gases.
**Fig. 7. GC-MS-QP 2010 [Shimadzu]**

**Oven** – The outer part of the GC is a specialized oven. The column is heated to bring the movement of molecules through the column. Typical oven temperatures range from 40° C to 320° C.

**Column** – Interior of the oven is the column which is a 30 meter thin tube with a special polymer coating on the inside. Chemical mixtures are separated depending on their volatility and are carried through the column by helium. Chemicals with high volatility travel through the column more quickly than chemicals with low volatility.

**Mass Spectrometer (MS)**

**Ion Source** – After moving through the GC, the chemical pulses continue to the MS. The molecules are blasted with electrons, which cause them to breakup into pieces and turn into positively charged particles called ions. This is important for the reason that the particles must be charged to pass through the filter.
**Filter** – As the ions continue to move through the MS, they travel through an electromagnetic field that filters the ions based on mass. The range of masses to be allowed through the filter is chosen. The filter constantly scans through the range of masses as the stream of ions come from the ion source.

**Detector** – A detector counts the number of ions with a specific mass. This information is sent to a computer and a mass spectrum is created. The mass spectrum is a graph of the number of ions with different masses that traveled through the filter.

**Computer**

The data from the mass spectrometer is then sent to a computer and plotted on a graph called a mass spectrum.

**Data Analysis**

The mass spectrum of an unknown compound can be compared to a library of mass spectra of known compounds.

**Applications of GC-MS**

Today computerized GC-MS instruments are more widely used in environmental monitoring of water, air and soil; in the regulation of agriculture and food safety; and in the discovery and production of medicine.

Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation and identification of unknown samples. GC-MS can also be used in airport security for detecting the substances in luggage or on human
beings. In addition, it can also identify trace elements in materials that were previously thought to have disintegrated beyond identification.

**Environmental monitoring and cleanup** - for tracking organic pollutants in the environment.

**Criminal forensics** – to analyze the particles from a human body in order to help link a criminal to a crime and also analysis of fire debris.

**Law enforcement** - for detection of illegal narcotics, commonly used in forensic toxicology, to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.

**Security** - as explosive detection systems.

**Food, beverage and perfume Analysis** - extensively used for the analysis of aromatic compounds which include esters, fatty acids, alcohols, aldehydes, terpenes, etc., in foods and beverages. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful.

**Astro-chemistry** - for analyzing the atmosphere of other planets and satellites.

**Medicine** - for detecting the in-born errors of metabolism.

In the Ayurvedic and the traditional medicinal systems of India, almost all the parts of *Piper betel* L. are used for the treatment of various diseases. Hence, on recognising the need of hour, an attempt was made to study the antimicrobial and biological activities of varied leaves of a local cultivar of *Piper betel* L. under both *in vitro* and *in vivo* conditions.
A local cultivar named *Piper betel* L. Cv. Kapoori was chosen and the selection of plant was made according to the survey method. It is cultivated locally and being used as masticatory and also in traditional medicine for curing number of disorders. Antimicrobial (MIC, MBC and MFC) screening of solvent extracts of different leaves (Mokkathotapapada, Mokkathotakalli and Kodithotakalli) of the plant were tested against the bacteria and fungi. Biological activities (antioxidant, antihelmenthic, antinutrient, cytotoxicity and mutagenicity) of solvent extracts of different leaves (Mokkathotapapada, Mokkathotakalli and Kodithotakalli) of the plant were carried out by different authenticated methods. Phytochemical studies of solvent extracts of different leaves of *Piper betel* L. were done by qualitative means using preliminary phytochemical analysis and GC-MS studies.

Ultimately the goal in surveying the different leaves (Mokkathotapapada, Mokkathotakalli and Kodithotakalli) of *Piper betel* L. Cv. Kapoori, a local cultivar for biologically active or medicinally useful compounds is to isolate the one or more constituents responsible for a particular activity. If successful, the drug would also fetch substantial amount of foreign exchange to the country and highlight the significance of betel leaf to a further extent.

**OBJECTIVES OF STUDY**

The research aimed to investigate solvent extracts of different leaves of *Piper betel* L. Cv. Kapoori for antimicrobial and biological activities such as antioxidant, antihelmenthic, antinutrient content, and their potential toxicity, cytotoxicity, mutagenicity and phytochemical studies using chemical methods and GC-MS analysis.
In order to reach the above said objectives, the below given experimental work was done:

- Collection and identification of different leaves of the *Piper betel* L. Cv. Kapoori, a local cultivar
- Obtaining aqueous and organic crude extracts from different types of betel leaves.
- Determination of the antimicrobial activity
  - Determination of the minimum inhibitory concentrations (MIC)
  - Determination of the antimicrobial activity by studying zone of inhibition.
- Determination of antihelmintic activity
- Determination of antioxidant activity
  - DPPH free radical scavenging assay
  - Reducing power methods
- Analysing the plant leaf materials for the following antinutrients:
  - Phytic acid using spectrophotometry;
  - Alkaloids using precipitation;
  - Trypsin inhibitors using enzyme substrate reaction;
  - Oxalic acid using high performance liquid chromatography (HPLC) analysis;
  - Cyanogens using picrate paper and spectrophotometry
  - Saponins using blood agar plates.
- Determination of toxicity of the plant extracts using the brine shrimp assay.
- Determination of cytotoxicity of the plant extracts using the 3-[4, 5-Dimethylthiazol- 2yl]-2, 5-diphenyl tetrazolium bromide (MTT) assay.
- Determination of mutagenicity of the plant extracts using the Ames test.

- Alkaloids, Carboxylic acids, Coumarins, Fixed oils, Flavonoids, Phenols, Quinones, Resins, Saponins, Steroids, Tannins, Xanthoproteins and Glycosides.
- GC-MS Studies of the best solvent extracts of the tested plant material exhibited effective biological activities.

**NEED FOR STUDY**

Need for study has become inevitable because in recent years, various plants are used as a subject to medical experiments. In particular, the *plant material* is recognized as one of the fascinating subjects, the extracts of which, can be used in healthcare settings and a range of other purposes in the form of diverse product lines. Synthetic chemicals used as food preservatives have toxic effects on prolonged use. So, the use of naturally occurring compounds as preservatives is a promising alternative. Natural substances having antimicrobial activity are potential preservatives and have the advantage of reduced/nil side effects over synthetic/chemical preservatives, even after prolonged use. Hence, the present research was undertaken.

Though literature is replete with examples of the various properties of *Piper spp.*

- The efficacy of the various parts of the plant as anti-ulcerogenic, anti-helminthic, analgesic and wound-healers has been widely documented.
We, however, are not aware of any published report on the anti-microbial properties, biological activities and phytochemical studies-qualitative tests and GC-MS analysis of different leaves (Mokkathotapapada, Mokkathotakalli and Kodithotakalli) of locally available *Piper betel* L. Cv. Kapoori Cultivar.

In the light of this, the present study was set up to investigate the anti-microbial activities, biological properties and phytochemical studies of different extracts of the given leaf varieties of *Piper betel* L. Cv. Kapoori, a local cultivar in order to unravel its potential use in various areas.

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