REVIEW OF LITERATURE

Medicinal plants have got an indispensable role in the development of human culture; to cite with religions and different ceremonies are evident (eg. Dutura has long been associated with the worship of Shiva, the Indian God).

**Significances of medicinal plants to human being**

- Many of the neoteric medicines are provoked obliquely from medicinal plants. Example of such neoteric drug is Aspirin.
- Greater number of cultures round the world make use of plants directly, as medicine. **Example:** Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, of which the popular example being the Garlic.
- Medicinal plants are the reserve for novel drugs.
- Analysis of medicinal plants keeps us to understand the plant toxicity resulting the protection of humans and animals from natural poisons.
- Cultivation and preservation of medicinal plants preserve the biological diversity.

**Plant resources for newer medicines**

**Bryophytes** (non-vascular plants, e.g. Liverwort and moss) own about 15,350 species.

**Seedless vascular plants** (commonly called ferns) carry about 12, 157 species.

**Gymnosperms** hold 760 species approximately.

**Angiosperms** are estimated beyond 250,000 species.

The medicinal effects of plants are due to metabolites exceptionally, secondary compounds produced by plant species.
Plant metabolites include primary metabolites and secondary metabolites.

<table>
<thead>
<tr>
<th>Plant primary metabolites</th>
<th>Plant secondary metabolites (plant natural products)</th>
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<tr>
<td>1. Organic compounds produced in the plant kingdom</td>
<td>1. Organic compounds produced in plant kingdom.</td>
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<tr>
<td>2. Have metabolic functions essential for plant growth and development</td>
<td>2. Don’t have apparent functions involved in plant growth and development.</td>
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<tr>
<td>3. Produced in every plant</td>
<td>3. Produced in different plant families, in specific groups of plant families or in specific tissues, cells or developmental stages throughout plant development.</td>
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<tr>
<td>4. Include carbohydrates, amino acids, nucleotides, fatty acids, steroids and lipids.</td>
<td>4. Include terpenoids, special nitrogen metabolite (including, non-protein amino acids, amines, cyanogenic glycosides, glucosinolates, and alkaloids) and phenolics.</td>
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First pharmacopoeia was devised by the Greek physician Galen (AD 129-200) describing the appearance, properties and use of many plants of his time. Natural products chemistry actually began with the work of Sertuner, who first isolated morphine from Opium. It was obtained from *Papaver somniferum*. Quinine from *Cinchona* tree had its origin in the royal households of the South American Incas. Indigenous people derived medicines and poisons from thousands of plants. A review of some plants that originated from Central and South America indicates that most of them had potentially toxic characters (Steven King, 1992).

India has roughly 45,000 plant species and medicinal properties have been assigned to several thousands. Around 2,000 are found in literature and indigenous systems commonly employ almost 500-700 species (Jain, 1994).
Current estimates indicate that nearly 80% of people in developing countries still rely on traditional medicine based largely on various species of plants and animals for their primary health care. Four out of ten Americans utilized alternative medicine therapies in 1997, total visits to alternative medicine practitioners increased by almost 50% from 1990 that exceeded the visits number of all US primary care physicians (Grabley and Thiericke, 1999).

Cardiovascular disease, diabetes, rheumatism and AIDS require new effective drugs. Most developing countries relied and will continue to rely on traditional natural medicines due to the deterrence of high costs of modern allopathic medicines (Patwardhan et al. 2004).

It is a known fact that the diseases are mainly caused by certain pathogenic microorganisms. Bacteria are very common human pathogens. As they are present everywhere, even in the extreme conditions, they can attack human beings very easily by any natural means such as water, air and food. For being free from bacterial disorders number of synthetic drugs were discovered. Though they are effective in controlling bacterial disorders, they have some shortcomings like high cost, temporary cure and side effects. Reviewing these facts, attempts are on run in search of new herbal drugs towards disease control. Attempts have been made to control bacterial diseases by using herbal drugs. Some plants have been identified having antibacterial nature, and the antimicrobial potential of many plants is yet to be proved.

Farnsworth (1966) surveyed plants having antimicrobial properties. Dhar et al. (1968) tested many plants for biological activity. Bhakuni et al. (1969) screened Indian
plants for biological activity. Antimicrobial screening of compounds of plant origin has
been the source of innumerable therapeutic agents (Balandrin et al. 1985). India has a
great diversity of flora and a broad tradition in the use of medicinal plants for both
antibacterial and antifungal activities (Rastogi and Mehrotra, 1993).

Siddha, the South Indian traditional system has a compilation of antimicrobial
activity of more than 1500 plants (Siva Raman et al. 1995). Farooq and Pathak (1998)
evaluated the antimicrobial activity of indigenous medicinal plant preparations.

A range of industries like pharmaceutical, cosmetic, agricultural and food use
plants as a part of their production processes. History of many civilizations showed that
plants were used for treating diseases. Commencement of research in medicine concluded
that plants/herbs contain active principles that may be held responsible for their curative
action. This observation has a lot of significance in showing the potential of plants for
various applications especially in disease management in today’s synthetic era, as a
number of microorganisms are becoming resistant to existing drugs. Literature reveals
that only 20% of the plant vegetation on earth have been studied and 60% of man-made
medicines owe their origin to plants. There are around 2,50,000 species of plants in the
world and around 10% only have been tested for some type of biological activity or the
other (Verpoorte, 1998). Early knowledge and scientific values can combinely provide
potent remedies to eliminate the diseases.

The scientific name of betel vine is *Piper betel* L. It belongs to the family
Piperaceae, i.e., the black pepper family (Gunther, 1952). The vine is dioecious, shade
loving perennial root climber. There are around 100 varieties of betel vine in the world, of which about 40 are found in India and 30 in west Bengal (Guha, 1997; Maity, 1989; Samanta, 1994). In fact, this edible leaf has achieved an esteemed position in the human society right from the dawn of civilization, particularly in the countries like Bangladesh, Burma, China, India, Indonesia, Malaysia, Nepal, Pakistan, Philippines, South Africa, Sri Lanka, Thailand etc (Jana, 1996; Khoshoo, 1981; Samanta, 1994; Sharma et al. 1996).

**Economic potentiality of Betel Leaves**

The vast economic potentiality of the Betel crop can be adequately established by the fact that about 15-20 million people consume leaves in India on a regular basis (Jana, 1996) besides those in other countries of the world may include over 2 billion consumers (Jeng et al. 2002). This crop provides a national income to the tune of INR 6,000-7,000 million every year (Jana, 1995; Samanta, 1994). The leaves are of great demand in several other countries of the world like Bahrain, Canada, Great Britain, Hong Kong, Italy, Kuwait, Nepal, Pakistan, Saudi Arabia and many other European countries (Jana, 1996; Singh et al., 1990).

**Medicinal value of betel leaf**

Betel leaf is traditionally useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, hysteria, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, ringworm, swelling of gum, rheumatism, abrasion, cuts and injuries etc., as folk medicine. While the root is known for its female contraceptive effects (Chopra et al., 1956; Khanra, 1997). Further, the essential oil in the leaves possesses antibacterial, antiprotozoan and antifungal properties. Therefore, the oil
kills or inhibits growth of harmful bacteria causing typhoid, cholera, tuberculosis etc., that needs proper evaluation and exploitation (CSIR, 1969).

**Nutritive value of betel leaf**

The leaves are highly nutritive and contain substantial amount of vitamins and minerals 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. Besides, the leaves also contain several digestive enzymes along with significant amount of all the essential amino acids (CSIR, 1969; Gopalan, 1984; Guha and Jain, 1997). However, relevant data from a complete biochemical analysis is unavailable from any single source.

This being a lucid indication that the foreign exchange earning potentiality of the crop, medicinal applications and nutritive values are required to be strengthened by the interest of the nation. This may be achieved through proper research on various aspects that boost up the export, systematic utilization of betel leaves for various purposes.

**Morpho-anatomical studies of *Piper betel* L. cultivars**

Seetha Lakshmi and Naidu (2010) studied the comparative morpho-anatomical information of 10 common cultivars of *Piper betel* species available in India. The ten cultivars show some structural similarities. Four-layered upper and two layered lower epidermis was observed in all the varities of *P. betel* studied. Crystals and oil reserves were found in the epidermal cells. The Kapoori, Tuni variety has more stomatal and trichome frequency. Multi-cellular tector trichomes were seen on the abaxial face of midrib. Presence of parenchymatous bundle sheath was seen in all the varieties. The
developments of tracheoids idioblasts from the vessel elements have been noticed. These characteristics are typical xeromorphic anatomy of leaves which could preserve the aroma and shelf-life, longevity of betel leaves. In this study a clear distinction showed between Kapoori Tuni from other landraces.

**Antimicrobial and Biological activities of Piper betel L.**

Jesonbabu et al. (2012) conducted studies to investigate the antimicrobial activity of the *Piper betel* leaf extracts against the clinically isolated Extended Spectrum Betalactamases (ESBL) producing bacteria. Chloroform extract of *Piper betel* leaves was examined against human pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, *K. oxytoca*, *Proteus vulgaris*, *P. mirabilis*, *Citrobacter koseri*, *C. freundii*, ESBL producing, *E. coli*, *K. oxytoca*, *K. pneumonia* and found profound antimicrobial activity (>11 mm inhibition zone) MIC (400-200 μg/ml).

Niraj A. Ghanwate and Prashant Thakare (2012) studied the antimicrobial and synergistic activity of ingredients of betel quid against microbial population of oral cavity and four enteric pathogens namely *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Shigell flexneri*. The highest percentage of reduction in microbial population was shown by the combination of betel leaf, lime and kattha (Blk) followed by betel leaf, cardamom etc.

Pallavi et al. (2012) studied the anthelmintic activity of ethanolic and aqueous extracts of stems of *Piper betel* Linn. Indian adult earthworms were used for the assessment of anthelmintic activity. Albendazole (40 mg/ml) was used as standard and normal saline water was used as vehicle respectively. Observations were made for the
time taken to paralysis and death. Extract of stems of *Piper betel* Linn not only demonstrated anthelmintic property but also caused death of the worms when compared with marketed standard preparation i.e., Albendazole (40 mg/ml).

Tarun Agarwal *et al.* (2012) investigated the comparative study of antimicrobial properties of four varieties of *Piper betel*; namely Desawari, Desi, Bangladeshi and Jaleswar, cultivated in India. Cold Aqueous, Methanolic, Ethanolic, and Ethyl Acetate extracts of dried leaves of all the four varieties of *Piper betel* at a final concentration of 500 mg/ml were tested against pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* using agar well diffusion method.

Tarun Agarwal and Rachana Singh (2012) studied a comparative evaluation of antimicrobial properties of leaves of seven cultivars of *Piper betel* found in India namely; Desawari, Gahoba, Sofiya, Banarasi, Desi, Bangladeshi and Jaleswar, cultivated in India. The cold Aqueous, Methanolic, Ethanolic extracts of dried leaves of all the varieties of *Piper betel* were tested against pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.

Arani Datta *et al.* (2011) investigated the antimicrobial activity of ethanol extract of *Piper betel* leaves against human pathogenic bacteria (both gram-positive and gram-negative). The leaf powder was found to contain carbohydrate, protein, polyphenolic compounds, flavonoid, alkaloids and total antioxidant. The ethanol extract showed strong free radical scavenging activity. The extract confirmed significant antimicrobial activity against all bacterial strains tested. Crude ethanol extract of *Piper betel* showed strong antimicrobial activity against the tested pathogenic bacterial strains.
Arani Datta et al. (2011) investigated antioxidant and antimicrobial properties of the leaves of *Piper betel* L. against human pathogenic bacteria. The ethanol extract exhibited strong free radical scavenging activity as seen by DPPH model. The extract confirmed notable antimicrobial activity against all bacterial strains tested.

Balaji Kaveti et al. (2011) evaluated antibacterial activity of aqueous and ethanol extract of the leaves of *Piper betel* L. against three Gram positive, two Gram negative bacteria. The two extracts displayed different degrees of activity against the microorganisms investigated. The ethanol extract considerably was more effective than aqueous extract in inhibiting the investigated microbial strains.

Devjani Chakraborty and Barkha Shah (2011) studied Antimicrobial, Antioxidative and Antihemolytic Activity of *Piper betel* leaf extracts collected from Srilanka, and identified that leaf extracts exhibited good biological activities.

Himratul-Aznita et al. (2011) carried out experiments to screen the susceptibility of the aqueous extract of *Piper betel* towards seven species of oral Candida. It was found that *P. betel* extract exhibited high antifungal activity.

Jahir Alam Khan and Naveen Kumar (2011) studied the efficacy of ethanolic and methanolic extracts of leaves of *Piper betel* for antibacterial properties against pathogenic bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Both the extracts were effective against the used pathogens but methanolic extracts were found to be more effective in comparison to ethanolic extracts.
Mahfuzul Hoque et al. (2011) carried out screening of the ethanol extract of Betel leaf (*Piper betel* L.), an indigenous climber plant of Bangladesh for its antibacterial activity against some foodborne pathogens viz. *Vibrio cholera* (ATCC 6395), *E. coli* (ATCC 25922), *E. coli* (O157; H7 NCTC 12049), *Shigella dysenteriae*-1 (MJ-84) and *Staphylococcus aureus* (ATCC 25923). The ethanolic extract of betel leaf showed the highest MIC values for *E. coli* (ATCC 25922) (0.625 mm), *Vibrio cholera* (ATCC 6395) (0.625 mm), and *Staphylococcus aureus* (ATCC 25923) (0.625 mm).

Panagal Mani and Boominathan (2011) studied Antifungal activity of individual and combine solvent fractions of *Piper betel* against fungal pathogens isolated from infected patients. The methanolic mingled ethanol fraction of *Piper betel* (zone of inhibition was and 7.4 mm) showed pronounced inhibition.

Sharma et al. (2011) conducted studies to evaluate and establish the claim of antidermatophytic activities of *Piper betel* Linn and *Allamanda cathertia* Linn. *In vitro* and *in vivo* antifungal studies of chloroform and methanol extracts of these plants and their mixture were conducted against *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans* and *Microsporum gypseum* *in vitro*. Guinea Pigs were used in case of *in vivo* experiments. The MIC of the extracts were found ranging between 0.156-1.25 mg/ml. 5% extract ointments were prepared and applied against induced ringworm in Guinea Pigs, with subsequent removal of infections in less than 60 days.

Sugumaran et al. (2011) extracted the essential oil from Vellaikodi variety of *Piper betel* L leaves (*Piperaceae*) by using hydro-distillation method in a clevenger type apparatus. EO was analyzed by gas chromatography – mass spectroscopy. Sixty five
components were identified in the oil. The 5-(2-propenyl)-1,3-benzodioxole (25.67\%) was determined as the first chief constituent in the oil, the second was eugenol (18.27\%), and third 2-methoxy-4-(2-propenyl) acetate-phenol (8.00). The antimicrobial screening of the obtained or isolated essential oil was performed against dental pathogens such as *Staphylococcus aureus*, *S. mutans*, *Lactobacillus acidophilus*, *Candida albicans* and *Saccharomyces cerevisiae* and identified the pronounced antimicrobial potential of *Piper betel* oil against tested pathogens.

Sundeep Chaurasia *et al.* (2011) carried out experiments on *Piper betel* leaf extract from simple maceration process. Calibration curve of leaf extracts were prepared in phosphate buffer of pH 7.2 on three consecutive days at \(\lambda_{\text{max}} 280\) nm. The absorbance values (mean of three determinations) were taken with their standard deviations at different concentrations in the range of 20-100 \(\mu\)g/ml. Extract was found to obey Beer-Lambert’s law in the concentration range of 20-100 \(\mu\)g/ml with regression coefficient (r2) values 0.9998. The regression equations were calculated as \(y = 5.2766 + 324.9606X\) for phosphate buffer of pH 7.2. These studies can be concluded that the developed method of estimation of PBL extract using UV Spectrophotometric technique can be used for direct and rapid measurement of the extract.

Wahyu Widowati *et al.* (2011) conducted research to investigate anticancer activity of *P. betel* extracts on breast cancer cell line T47D, and antioxidant activity. *P. betel* extracts were able to inhibit T47D cell proliferation with IC\(_{50}\) 55.2 \(\mu\)g/ml, while DPPH scavenging activity (IC\(_{50}\)) of *P. betel*, was 5.49 \(\mu\)g/ml.
Ali et al. (2010) isolated Hydroxychavicol, from the chloroform extraction of the aqueous leaf extract of *Piper betel* L. (Piperaceae), investigated for its antifungal activity against 124 strains of selected fungi. Hydroxychavicol exhibited significant inhibitory effect on fungal species.

Arambewela et al. (2010) conducted clinical trial using the wound healing cream on dermatitis patients revealing that treatment was significantly effective on skin rashes.

Fathilah (2010) carried out experiments to study the efficacy of *Piper betel* and *Psidium guajava* extracts in dental plaque control. *Streptococcus sanguinis, S. mitis* and *Actinomyces* sp., the predominant bacteria present at this initial stage of plaque development were used as test organisms. Extracts suppressed the growth of these bacteria.

Intzar Ali et al. (2010) investigated antifungal activity of leaf extract of *Piper betel* L., (Piperaceae) against selected fungi. Hydroxychavicol extracted from the betle leaves exhibited inhibitory effect on fungal species of clinical significance, with the MICs ranging from 15.62 to 500 μg/ml - yeasts, 125 to 500 μg/ml - *Aspergillus* species, and 7.81 to 62.5 μg/ml for dermatophytes where as the MFCs were found to be similar or two fold greater than the MICs.

Kumar et al. (2010) tested the antibacterial efficacy of ethanol extracts of *Datura* and *Piper betel* against three standard microorganisms *E. coli* DH5 (MTCC 2804), *Bacillus amyloliquefaciens* (MTCC 4012) and *Pseudomonas aeruginosa* (MTCC 1265). *Piper betel* showed valuable antibacterial activities.
Nuraida (2010) studied the leaves of *Piper betel* Linn towards bacteria in the mouth i.e., *Streptococcus aureus*, *S. viridans* and *S. mutans*. Essential oils of the plant was found to possess phenolic compounds such as cavicol, cavibetol, carvacrol, eugenol and allyl pyrocatechol and are assumed to inhibit food borne pathogens as well as food spoilage microorganisms.

Pandita *et al.* (2010) studied the antimicrobial activity of the leaves of *Piper betel* L. (Piperaceae) relevant in oral care lead to the investigation of its anti-plaque activity. Two varieties (Banarasi and Calcutta) were tested for antimicrobial activity against *Streptococcus mutans*, *S. sobrinus*, and *Actinomyces viscosus*. Leaf extract of the two varieties were found significantly effective against the test organisms as compared to the standard.

Amin *et al.* (2009) carried out studies on the prevalence of natural gastrointestinal nematodes observed in cattle. The prevalence of gastrointestinal nematodes was 84.1% (rainy seasons-97%, summer-85.5% and winter seasons-69.8%). The prevalence of strongyles (*Haemonchus* sp., *Trichostrongylus* sp., *Oesophagostomum* sp. and *Mecistocirrus* sp.), *Bunostomum* sp., *Strongyloides* sp., *Trichuris* sp. and *Capillaria* sp. were 63.9%, 26.3%, 21.5%, 17.3% and 24.5%, respectively. Water extracts of betel leaf showed potential *in vitro* activities against adult parasites (100% efficacy against adult worms).

Fathilah *et al.* (2009) studied the bacteriostatic effect of *Piper betel* and *Psidium guajava* extracts on selected early dental plaque bacteria (*Streptococcus sanguinis*, *S. mitis* and *Actinomyces* sp.), investigated viewing the changes in the doubling time (g) and
specific growth rates (μ). It appears that *P. betel* and *P. guajava* extracts showed bacteriostatic effect on the plaque bacteria.

Rahul Shukla *et al.* (2009) studied antimicrobial activity of the successive extract of the fresh leaves of *Piper betel* Linn against Gram-positive and Gram-negative bacterial strains. The results revealed that all extracts exhibited effective inhibitory action against *S. aureas*.

Rahul Shukla *et al.* (2009) conducted experiments to investigate antimicrobial activity of Leaf extracts of *Piper betel* Linn against Gram-positive and Gram-negative bacterial strains. Leaf extracts showed effective inhibitory action against *S. aureas*. The aqueous, ethyl acetate and Petroleum Ether extracts showed very effective as compared to standard penicillin. Aqueous extract was also found significantly effective against *Bacillus* and *P. aureginosa* as compared to standard penicillin.

Nair and Chanda (2008) studied antibacterial activity. Aqueous and methanol extract of the leaves of *Piper betel* L. against ten Gram positive, twelve Gram negative bacteria and one fungal strain, *Candida tropicalis*. Piperacillin and gentamicin were used as standards for antibacterial assay, while fluconazole was used as standard for antifungal assay. The methanolic extract was considerably more effective than aqueous extract in inhibiting the investigated microbial strains.

Nair and Sumitra Chanda (2008) studied antimicrobial properties of Methanolic extracts of the leaves of *Terminalia catappa* L., *Manilkara zapota* L., *Piper betel* L. against 10 Gram +ve, 12 Gram –ve bacteria and 1 fungal strain. Among the three plants, the most active antimicrobial plant was *Piper betel* L.
Marina et al. (2007) studied the crude methanolic extract, some organic fractions and compounds isolated from *Piper solmsianum* C. DC. var. *solmsianum* (Piperaceae) for possible antimicrobial activity against Gram-positive and Gram-negative bacteria. The bioautographic assays disclosed three inhibition zones. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined showing excellent activity, particularly against the Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *S. saprophyticus* and *Streptococcus agalactiae*).

Sharad Bissa et al. (2007) carried out experiment to understand the effect of traditional *Piper betel* leaves on oral microorganisms that gave good results against the oral microbes.

Arambewela et al. (2006) carried out studies on the extracts obtained from the leaves of *P. betel* (Srilanka), had profound antioxidant activity. The scavenging effects of *P. betel* extracts on DPPH radicals increased.

Francis Parillon and Henie Edward (2006) studied five plants, namely *Psidium guajava* (guava), *Illicium verum* (star anise), *Annona squamosa* (sugar apple) and two cultivars (malaysian and caribean) of *Piper betel* Linn. (Betel leaf) were screened for antimicrobial activity against sixteen foodborne bacteria (both Gram-positive and Gram-negative pathogens) using the agar disc diffusion method. The methanolic extracts of green vein (gv) cultivars of *Piper betel* L., demonstrated greater antimicrobial activity.

Panuwat Suppakul et al. (2006) carried out studies on antimicrobial and antioxidant activities of Betel oil against ten pathogenic and spoilage bacteria and three strains of yeast. The Minimum Inhibitory Concentration (MIC) and Minimum Oxidative
Bleaching Inhibitory Concentration (MOBIC) of betel oil were determined. At the concentration of 50 mL mL-1, betel oil showed a zone of inhibition, ranging from 9.15 to 17.30 mm in diameter. The MICs of betel oil in a range of 12.5-100 mL mL-1 could inhibit the growth of all test microorganisms. The MOBIC of betel oil was 100 mL mL-1.

Rathee et al. (2006) conducted studies on 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay of ethanol extracts of three varieties (Bangla, sweet, and Mysore) of *Piper betel* (pan), revealed the Bangla variety to possess the best antioxidant activity that can be correlated with the total phenolic content and reducing powers of the respective extracts.

Arambewela et al. (2005) studied and reported that the EO from the *Piper betel* leaves showed activity against *Escherichia coli, Streptococcus pyogenes, S. aureus, Colletotrichum sp., Fusarium oxysporium sp., Corynospora cassicola, Rigidoporous sp.* and *Phytophthora sp.* The ethanol extract showed activity against test organisms.

Dasgupta and De (2004) investigated the antioxidant activities of three betel cultivars (Kauri, Ghanagete and Bagerhati) grown in India. The antioxidant activities of the 3 cultivars were in the order of Kauri > Ghanagete > Bagerhati.

Wiart et al. (2004) studied antibacterial activity of ethanol extract of leaves of *Piper porphyrophyllum* N.E. Br. The activity increased on fractionation (hexane, dichloromethane and aqueous), particularly in the aqueous fraction.

Nalina Thurairajah and Zubaidah Haji Abdul Rahim (2003) developed a fast and simplified thin layer chromatography technique in the separation of the active antibacterial compound from *Piper betel* by bioautography.
Ramji et al. (2002) carried out the biological studies with allylpyrocatechol (APC) extracted from *Piper betel* L. (Piperaceae) leaves, exhibited potential antimicrobial activity against obligate oral anaerobes responsible for halitosis.

Jenie et al. (2001) examined the effect of extraction methods on the antimicrobial activity of green and yellow varieties of *Piper betel* Linn towards some food borne (*Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium, Escherichia coli* and *Listeria monocytogenes*) and food spoilage microorganisms (*Pseudomonas aeruginosa, P. fluorescens, Lactobacillus plantarum, B. stearothermophilus, Aspergillus niger, Penicillium rubrum, Candida utilis* and *Saccharomyces cerevisiae*).

Shitut et al. (1999) studied *in vitro* antimicrobial activity of different varieties of *Piper betel* Linn, leaf stalk extracts against human pathogenic bacteria and phytopathogenic fungi. The ethyl acetate and ethanol extracts of the four varieties showed significant activity against bacteria *Vibrio cholerae ogawa, Staphylococcus aureus, Diplococcus pneumoniae* and *Klebsiella aerogenes*. In the same way, ethyl acetate and ethanol extracts of all the four varieties have showed moderate to significant activity against most of the fungi tested.

Shitut et al. (1999) studied the in vitro antimicrobial activity of different varieties of *Piper betel* Linn, leaf stalk extracts against human pathogenic bacteria and phytopathogenic fungi. The ethyl acetate and ethanol extracts have shown significant activity against bacteria *Vibrio cholerae ogawa, Staphylococcus aureus, Diplococcus pneumoniae* and *Klebsiella aerogenes*. The hexane and benzene extracts have shown moderate activity. In the same way, ethyl acetate and ethanol extracts have shown
moderate to significant activity against the tested fungi.

Garg and Jain (1992) conducted experiments for evaluation of *in vitro* anthelmintic activity of essential oils of *Piper betel* L. cultivar Sagar Bangla against hookworms (*Haemonchus contortus*) and tapeworms (*Ascaris lumbricoides*). The extracts showed effective activity on the test organisms.

Garg and Rajshree Jain (1992) studied biological activities of the essential oil from the leaves of *Piper betel* L. Sagar Bangla cultivar against the growth of four keratinophilic fungi, *Arthroderma benhamiae, Microsporum gypseum, Trichophyton mentagrophytes, Ctenomyces serratus* and five pathogenic *Aspergilli*. Bacterial organisms *Bacillus subtilis, B. pumilus, Staphylococcus aureus, Salmonella typhi* and *Vibrio cholera* were also found to be susceptible to the oil. The essential oil was also found to be more effective against tapeworms (*Taenia solium*) and hookworms (*Bunostomum trigonocephalum*), than the synthetic anthelmintics piperazine phosphate and hexyl resorcinol.

Prasad *et al.* (1992); Garg and Jian (1992); Baby *et al.* (1993) studied and found the biological activities of the crude extract of the *Piper betel* plant and its constituents.

Wang and Wu (1996) separated phenolics from leaves of *Piper betel* L. and studied the effect on the mutagenicity of arecoline. The extracts exhibited potent antioxidant activity.

Nagabhushan *et al.* (1989) isolated Hydroxychavicol and Eugenol from betel leaf (*Piper betel*) and conducted studies on the modulation of nitrosation of methylurea by sodium nitrite at pH 3.6 and 30°C. The accumulation of mutagenic N-
nitrosomethylurea was monitored by checking the mutagenicity of reaction mixture in *Salmonella typhimurium* strain TA100 and TA1535 without S9 mix.

Nik Haiha *et al.* (1987) conducted experiments to determine antimicrobial activity of Leaf extract of *Piper betel* for the alternative treatment against fish bacterial disease causing organisms such as *Vibrio alginolyticus*, *V. vulnificus*, *Photobacterium damsela* and *Pseudomonas sp*. Ethanolic extract with a maximum zone of inhibition of 24 mm and 25 mm for *V. vulnificus* and *P. damsela*, respectively. The MIC value *P. betel* was found to range from 3.25 -12.5 mg/ml. whilst, the MBC value of the *P. betel* ranged from 6.25 -25mg/ml.

Philip *et al.* (1984) carried out experiments and identified the presence of fungicidal and nematocidal components in different extracts of leaves of *Piper betel* L.

Nadkarni (1976) stated that the leaves of *Piper betel* L. are useful in treating bronchitis, difficulty in breathing and cough. The liquid extract of the plant has been in long use traditionally in curing inflammation and infections of the respiratory tract, cough, dyspnoea, indigestion, diphtheria, hysteria, as well as general and sexual debility.

Misra and Dixit (1979); Evans *et al.* (1984), investigated antifungal and nematocidal components from the leaves of *Piper betel* L.

Ali and Mehta (1970) conducted Adult Motility Assay (AMA) for assaying the anthelmintic activity of essential oils of *Piper betel* L. against the earthworms (*Pheritima posthuma*). The worms are exposed to varying concentrations of plant extracts and observed for their inhibited motility and/or mortality at different intervals. The extracts exhibited potent anthelmintic activity.
Chemical investigations of *Piper betel* L.

Hemamalini *et al.* (2012) screened the methanolic fraction of betel leaves using Gas Chromatography-Mass Spectrometry (GC-MS) and also tested antioxidant, ferric reducing ability and antibacterial activity of natural (NHC) and Synthetic Hydroxychavicol (SHC), a major phenolic compound in *Piper betel* leaf. The GC-MS analysis showed that betel leaf extract contained a variety of bioactive natural compounds.

Periyanayagam *et al.* (2012) studied the detailed micromorphology and physico-chemical analysis of the leaves of *Piper betel* L. var. Pachaikodi family Piperaceae. Phytochemical screening showed the presence of steroids, tannins, proteins, aminoacids, flavonoids, terpenoids, mucilage, volatile oils, saponins, carbohydrates and absence of alkaloids, fixed oils.

Arambewela *et al.* (2011) studied on *Piper betel* an economically important plant cultivated in Sri Lanka. Studies on the chemical constituents is indicative that safrole is the major constituent, followed by chavibitol acetate, in the essential oil of common betel leaves of Sri Lanka. Investigations on the bioactivities of *P. betel* revealed the presence of antimicrobial, insecticidal, antioxidant, antidiabetic and gastroprotective activities. In addition, *P. betel* was found safe in terms of hepatotoxicity, renotoxicity, hematotoxicity, gross morphology, weights of organs, stress or aversive behaviors in rats.

Chandra Vikash *et al.* (2011) conducted review on *Piper betel* L. and reported that the betel leaves contains large number of bioactive metabolites like polyphenols, alkaloids, steroids, saponin and tannin. It has light yellow aromatic essential oil with
sharp burning taste. The main constituents are Hydroxychavical/Hydroxychavicol acetate, Allylpyrocatechol, Chavibetol, Piperbetol etc. Other constituents are arecoline, carvacrol, caryophyllene, piperitol, eugenol, isoeugenol, allylpyrocatechol, chavicol, safrole, anethole, chavibetol, cadinene hydroxychavicol, beta-sitosterol, beta-sitosterol palmitate, dotriacontanoic acid, tritriacontane, steric acid, cephadione, piperine, piperlonguminine, chavibetol acetate, allylpyrocatechol monoacetate, allylacetoxyl benzene, estragole, methyl eugenol and hydroxycatechol, methylpiperbetol, piperol A and piperol B, carvacrol, eugenol acetate and allyl pyrocatechol diacetate. *Piper betel* leaf extracts possess various pharmacological activities.

Dwivedi and Mehta (2011) conducted experiments on Chemical investigation of aliphatic compounds of *Piper betel* (leaf stalk). The hexane extract of *Piper betel* (leaf stalk) yielded four aliphatic compounds in pure form i.e., Pentadecyl 6-hydroxytridecanoate (1), Pentatriacontanol (2), Methyl hexacos-7-enoate (3) and 6, 9-heptacosa diene (4).

Kushagra Nagori *et al.* (2011) reviewed on *Piper betel* L. and summarized about the information concerning the botany, ethnopharmacology, phytochemistry, biological activity and use of hyphenated analytical techniques like DART-MS (Direct Analysis in Real Time Mass Spectrometry) and other techniques for characterizing various compounds. This plant was known to possess antioxidant, antifungal, antiulcerogenic, antiplatelet, antidiabetic, immunomodulatory, antileishmanial, antiamoebic, anti-inflammatory, antifilarial and antimicrobial activity. A wide spectrum of chemical compounds including chavibetol, allyl pyrocatechol, eugenol, quercetin, caryophyllene,
safrole, hydroxychavicol, \( \text{-pinene, myrcene, chavicol, Germacrene-D, } \text{-terpineol, } \text{-pinene, camphene etc have been isolated.}

Patturajan Rajeshbabu et al., (2011) investigated the comparative study on the antibacterial activity and nutritive value of the different extracts of the leaves of *Piper betel* and Black betel cv. *Kammar*, against two human pathogenic bacteria namely *Staphylococcus aureus* and *Streptococcus pneumoniae*. Leaf extracts of *P. betel* and black betel showed significant activity against the tested organisms. Analytical study on the biochemical parameters indicated that carbohydrates, ash, alkalinity, sodium and potassium are higher in *P. betel* leaves and concluded that the traditional wisdom of chewing betel leaves would certain the usefulness in bacterial infections of mouth, throat and lung.

Periyanayagam et al. (2011) studied Phytochemical analysis, fluorescence analysis on the dried powdered leaves and gas chromatography-mass spectrometry (GC-MS) analysis on the essential oil from the fresh leaves of *Piper betel* L. var. *Sirugamani1* (SGM 1) (Piperaceae). Preliminary phytochemical screening of leaves revealed the presence of carbohydrate, phytosterols, saponins, tannins, proteins and free amino acids, mucilage, terpenoids and flavonoids. Considerable colour variations were observed in the fluorescence analysis, 0.8% wt/v of essential oil was obtained. GC/MS fingerprint showed the presence of 50 compounds in the oil. The major compounds were Germacrene-D (16.07%) followed by Lepidozene (14.99), \( \beta \)-caryophyllene (9.86%), 1,3,4–Eugenol (7.17%), \( \beta \)-Elemene, \( \beta \)-Murrolene (3.18%), \( \alpha \)-Selinenol (3.07), \( \beta \)-Cadinene (2.82%) and Cineole (2.80%).
Sugumaran et al. (2011) extracted essential oil from Vellaikodi variety of *Piper betel* L. leaves (Piperaceae) by hydro-distillation method in a clevenger type apparatus. The essential oil obtained was analyzed by gas chromatography – mass spectroscopy. Sixty five components were identified in the oil. The 5-(2-propenyl)-1,3-benzodioxole (25.67%) was found as the first major constituent in the oil. The second was eugenol (18.27%) and third 2-methoxy-4-(2-propenyl) acetate-phenol (8.00) were predominant components in this oil. *Piper betel* oil showed the pronounced antimicrobial potential against tested pathogens causing dental caries.

Sugumaran et al., (2011) extracted the essential oil from Sirugamani variety of *Piper betel* L leaves (Piperaceae) by hydro-distillation method in a clevenger type apparatus. The essential oil obtained was analyzed by gas chromatography - mass spectroscopy. Sixty seven components were identified in the oil. The major constituents of oil identified were 5(2-propenyl)-1,3-benzodioxole (32.7919%), eugenol (16.1787%), 2-methoxy-4-(2propenyl)-acetate-Phenol (8.0107%), t-Gurjunene (4.1482%) and Sabinene (3.4389%). The isolated essential oil showed good antimicrobial activity.

Vasuki et al. (2011) studied organoleptic, microscopic, fluroscence, physical constant investigations and preliminary phytochemical screening of *Piper betel* Linn. The phyto constituents detected in the betel leaf extracts were carbohydrates phytosterol saponins tannins proteins amino acids gums and mucilage flavonoids and terpenoids.

Adeltrudes et al. (2010) extracted the Betel oil from the leaves of *Piper betel* L through steam distillation and characterized for its organoleptic properties and physicochemical constants. The chemical components of the Betel oil identified via Gas
Chromatography-Mass Spectroscopy consist of 5-(2-propenyl)-1, 3-benzodioxole, eugenol isomer and caryophyllene. The oil was found to have significant antibacterial and antifungal activity.

Bajpai et al. (2010) conducted chemical profiling of leaves of different betel cultivars of India such as Saufia, Bangla, Desawari, J. Green, J. White, Kalkatiya, Mahoba, Deshi Paan using direct analysis in real time mass spectrometric technique (DART-MS). The analysis on the leaves of cultivars revealed the presence of Chavicol, allylpyrocatechol, chavibetol, Phenyl alanine, chavicol acetate, chavibetol acetate, allylpyrocatechol acetate and allylpyrocatechol diacetate.

Binit Kumar Dwivedi et al. (2010) investigated chemical profile of the oily fraction of the leaf stalk of Piper betel of Indian origin, led to the identification of number of ester compounds. The hexane eluates of n-hexane extract of P. betel yielded a waxy fraction, which was rechromatographed on silica gel column. Its hexane and 25% benzene eluates yielded waxy liquids, which were small in amount and was not separated by column chromatography. Hence, were separated by Gas Chromatography Mass Spectrometry (GC-MS) analysis, which revealed the presence of 19 compounds. The compounds were identified by comparing their retention time and covate indexes with that of literature and by interpretation of mass spectra. Many of them are used in industry for various applications like perfumes, flavors, deodorants, antiseptic and pharmaceuticals.

Pin et al. (2010) investigated the influence of solvents with different polarities on the antioxidant and anti-inflammatory properties of betel leaf extracts (Piper betel). The
solvents employed were water, ethanol, ethyl acetate and hexane. High Performance Liquid Chromatography (HPLC) was used in finding out the chemical profiles and concentrations of the active compounds, namely, hydroxychavicol (HC) and eugenol (EU). The HPLC results revealed that HC and EU were detected in all types of extracts and the concentrations were highest in the water extract. The highest extraction yield was obtained using water. All the extracts were highly active in both antioxidant assays with water extract showing the strongest inhibition. The extracts also exhibited significant inhibition in XOD and LOX assays. The results indicated that the bioactivity of the extracts was related to HC and EU.

Li-Ching Morgan Rowa and Jiau-Ching Hob (2009) assayed the essential oil and methanolic and aqueous extracts of *Piper betel* L. for various antimicrobial and biological activities. The chemical composition of its essential oil and its fractions were analyzed by GC/MS analysis. Eugenol (36.2%), chavibetol acetate (16.9%), 4-allylphenyl acetate (9.4%) and 4-allylphenol (7.2%) were the main components, comprising 69.7% of the oil. The fractionation of the essential oil gave two fractions. Fraction-I was rich in eugenol (71.3%) and fraction-II in eugenol (46.4%), chavibetol acetate (19.4%) and 4-allylphenyl acetate (11.8%). The essential oil exhibited significant antimicrobial and biological activities.

Yeap Soo Fong (2009) isolated and purified four chemical compounds such as chavibetol, 2-hydroxychavicol, β-sitosterol and 2-allyl-3, 4-dihydroxybenzaldehyde from the leaves extracts of *Piper betel*. 

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Mohottalage et al. (2007) conducted experiments on GC-MS analysis of leaves of Srilankan betel variety and found out the presence of Safrole, allyl pyrocatechol diacetate, eugenol and eugenol acetate.

Ghosh and Bhattacharya (2005) investigated chemical profile of the solvent extracts of *Piper betel* roots using column chromatography. The alcoholic extract consist of aristololactam A-II and a new phenyl propene, characterized as 4-allyl resorcinol, while the petroleum-ether extract yielded a diketosteroid, *viz.* stigmast-4-en-3, 6-dione. All the compounds were characterized by spectroscopic means.

Leopold et al. (1999) carried out analysis of the essential oil of *Piper betel* leaves from South-India using GC-MS. The analysis showed the presence of Chavibetol, eugenol, allyl pyro catechol derivatives.

Ramasarma and Sadhan Kumar Dutta (1995) extracted chemical using standard procedure and also investigated physico-chemical properties of Petioles of the plant *piper betel* - bengal variety, after extraction of chemicals they were subjected to evaluate diastase, biological and pharmacological activities.

Agnes Rimando et al. (1986) isolated and identified fourteen volatile components including eight allypyrocatechol analogues from the essential oil and ether soluble fraction of Philippine *Piper betel* leaves (Piperaceae). The major constituents of Philippine *Piper betel* oil were chavibetol and chavibetol acetate. Capillary GC analysis of the oil showed chavibetol (53.1%), chavibetol acetate (15.5%), caryophyllene (3.79%), allypyroacatechol diacetate (0.71%), campene (0.48%), chavibetol methyl ether (methyl eugenol (0.48%), eugenol (0.32%), α-pinene (0.21%), β-pinene (0.21%), α-limonene
(0.14%), safrole (0.11%), 1,8-cineol (0.04%) and allylpyrocatechol monoacetate. The major component of the ether soluble fraction was allylpyrocatechol (2.38% of the leaves).

Though *Piper betel* leaves as a part of quid has been implicated in oral cancer, many scientists did not agree with these observations. Amonkar *et al.* (1986) carried out experiments and found out *Piper betel* leaf extracts were non-carcinogenic, and also showed non-mutagenic properties in betel leaves and the presence of hydroxychavicol (HC), a phenol in *Piper betel* L with anti-mutagenic properties. This proved to be the turning point in *Piper betel* research, when it was established that *Piper betel* leaves do not contribute to oral cancer. This provided opportunities to explore the properties of *Piper betel* L. Since then, a variety of biological activities have been demonstrated in betel leaf. Many medicinal properties have also been attributed to *Piper betel*, which include antioxidant, anti-infective, analgesic, anticancer, antidiabetic, hepatoprotective, immunomodulatory, cardiovascular, etc. (Table 3). Since the primary use of *Piper betel* happens to be the chewing of leaves, its effect starts right from the buccal cavity (maintaining oral hygiene) through direct introduction in the blood stream via the buccal mucosa (cardiotonic) and continues till it is ingested and assimilated (effect on digestive system, other pharmacological activities) within the human body. In this review, the various pharmacobiological activities of *Piper betel* L. have been described.
Table 3. Biological activities in *Piper betel* extracts and its identified chemical constituents

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<th>Sl. No</th>
<th>Biological activity</th>
<th>Extract/chemical constituent</th>
<th>Reference</th>
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<tbody>
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<td>3.</td>
<td>Digestion stimulant hepatoprotective/ Glucose metabolism</td>
<td>Leaf powder, leaf extract</td>
<td>Prabhu, M.S. et al. (1995); Pushpavalli, G et al.. (2008); Pushpavalli, G et al. (2009); Saravanan, R et al. (2002); Young, S.C et al. (2007); Arambewela, L.S. et al. (2005) and Santhakumari, P et al. (2006).</td>
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<tr>
<td>Sl. No</td>
<td>Biological activity</td>
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The above literature reveals that noteworthy work has not been done on the selected leaves (Mokkathotapapada, Mokkathotakalli and Kodithotakalli) of *Piper betel* L. Cv. Kapoori, a local cultivar.

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