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Biotechnological intervention in betelvine (*Piper betle* L.): A review on recent advances and future prospects

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ABSTRACT

Betelvine (*Piper betle* L.) is cultivated for its deep green heart shaped leaf for (15–20) million Indian and 2 billion foreign consumers annually. The crop provides Rs (6 000–7 000) million of national income per year and at the same time leaves worth Rs (30–40) million is exported to other countries. The leaves are not only used directly for chewing purposes but also possesses antioxidant, anti-inflammatory, anti-apoptotic, anti-cancer and anti-microbial properties. Besides, the leaves also contain eugenol rich essential oil (1%–3%) which is the source for medicine, stimulant, antiseptic, tonic and other ayurvedic formulations. The essential oil also contains chavibetol, caryophyllene and methyl eugenol which are the potent source for preparation in ayurvedic medicine and herbal products. Cost of betelvine essential oil is 10$ per 5 mL. In spite of its great economical and medicinal importance betelvine is still neglected by the researchers for proper characterization and authentication for selection of elite landraces. Lack of awareness among people, use of same planting material for many generations, existing of many synonyms for a single landraces, no proper characterization of available landraces are some of the significant constraints for its commercialization. Our review endeavours a complete advance in the research on betelvine, existing lacunae for its proper characterization and commercial cultivation. It also attempts to provide a comprehensive account on biotechnological interventions made in betelvine aimed at complementing conventional programmes for improvement of this nutraceutically important cash crop.

1. Introduction

Betelvine is the most important and useful asexually propagated cash crop having various cultivars [1]. It belongs to Piperaceae family and is a shed loving plant. It has a perennial creeper and bears leaves that are 4–7 inch long and 2–4 inch broad. It bears both male and female flowers. It is originated from Malaysia but is distributed extensively in South and Southwest China. This crop is usually cultivated in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries [2]. *Piper betle* (*P. betle*) is called by different names in India *i.e.* Pan in Hindi, Tambula in Sanskrit, Villayadela in Kannada, Vettilakkoti in Malayalam, Vettilai in Tamil, Tamalapaku in Telugu, Videch-pan in Marathi, Nagarbel in Gujrati, Pan in Bangala also called Tanbol in Arabic and Burg-e-Tanbol in Persian [3]. This plant is economically, medicinally and traditionally important in the whole world [2]. The betel leaves are mainly used as mouth freshener and is also well known for curing many communicable and non-communicable diseases like cold, cough, bronchial asthma, rheumatism, stomachalgia and used to treat other diseases like bad breath, boils and abscesses, conjunctivitis, constipation, swelling of gums, cuts and injuries [4]. The essential oil of betel leaves possess anti-bacterial, anti-protozoan and anti-fungal properties. From the study it is known that the aqueous extract of betelvine reduces the adherence of early dental plaque bacteria [2]. It is also found that betel has significant anti proliferative activity *in vitro* and *in vivo* prostate cancer models [5]. Betel leaves consist of the most abundant phytochemicals *i.e.* hydroxychavicol which contributes to the antiproliferative efficacy of betel leaf extract [4]. This phenolic compound of betel leaf inhibits prostate cancer through ROS driven DNA damage and apoptosis [4]. Some of the important constituents of the leaf oil are α-Pinene, Safrole, Eugenol, Selinene, Hydroxychavicol,

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Cadinene, Caryophyllene *etc*. The list of the reported important constituents of betel leaf oil with their CAS number, molecular formula, molecular weight and uses are mentioned in (Table 1). The important constituents of *P. betle* have become a good source of income and mode of foreign exchange for the country. The size and colour of the betel leaf is the most important factor for the betel producers to categorise products in the market. On the basis of chemical constituents of leaf essential oils, five prominent groups of betelvine landraces, namely Bangla, Kapoori, Meetha, Sanchii and Desawari have been recognized [6]. The betelvine is called as 'green gold of india' as about 20 million people derive their livelihood directly or indirectly from production, processing, handling, transportation and marketing of betel leaves in India [7].

There are about 125–150 cultivars of betelvine available in India [8]. Cultivation of betel is done on about 55 000 ha with an annual production which worth about Rs 9 000 million. It is mainly cultivated in West Bengal, Assam, Andhra Pradesh, Bihar, Tamil Nadu, Karnataka, Kerala, Maharashtra, Odisha, Madhya Pradesh and Uttar Pradesh [9]. The annual production of betel leaves is about Rs 9 000 million and it was found from the survey that about 20 million people acquire their lives from maintenance, plantation, management, export and import of betel leaves in India [10]. The cultivation economy depends both in terms of benefit-to-cost ratio and net income. For the last 7 years an average productivity of 19.41 lakh leaves/ha has been observed [11]. It is also found to be one of the most important commercial crops which is able to attract adequate amount of foreign exchange to the country. Per year leaves worth about Rs (30–40) million are exported to other countries of the world like Baharin, Canada, Great Britain, Nepal, Pakistan and Saudi Arabia [10]. The annual turnover of betelvine is estimated to be Rs. 10 000 million. The betel leaves are also exported to UK, USA, Bangladesh, Malaysia, Singapore, Sri Lanka and other Arabian Countries from India which is able to earn about 198 lakh rupees per year through this foreign exchange.

2. Problems associated with betelvine

2.1. Cultivation practice and commercialization

Betelvine leaves are aromatic in nature, highly nutritional and are the economic product of *P. betle* [12]. The plant is raised by vegetative propagation method in shade areas for years, some in coconut fields while others in a particular field called Baroj which is like a small hut of 2 m height approximately. The

Table 1
Chemical compounds of *P. betle* and their uses.

Compound	CAS no.	Molecular formula	Molecular weight	Uses
α-Pinene	80-56-8	$C_{10}H_{16}$	136.23	Anti-inflammatory and antibiotic
Camphene	79-92-5	$C_{10}H_{16}$	136.24	Preparation of fragrances and food additive for flavouring
Sabinene	3387-41-5	$C_{10}H_{16}$	136.24	Antimicrobial activity
Myrcene	123-35-3	$C_{10}H_{16}$	136.24	Preparation of fragrances and food additive
A-Terpinene	99-86-5	$C_{10}H_{16}$	136.23	Cosmetics and food industries
B-Phellandrene	555-10-2	$C_{10}H_{16}$	136.23	Cosmetics and personal care industries, cleaning products
B-Ocimene	13877-91-3	$C_{10}H_{16}$	136.23	Perfumery
Terpinolene	586-62-9	$C_{10}H_{16}$	136.26	Perfumery and food additive
Cis-sabinene	15826-82-1	$C_{10}H_{16}$	136.23	Anti-Infective Agents
Terpineol-4	8000-41-7	$C_{10}H_{18}O$	154.25	Fragrance compositions in disinfectants, polishes and household products
Safrole	94-59-7	$C_3H_5C_6H_3O_2CH_2$	162.19	Beverages and candy preparation
Eugenol	97-53-0	$C_{10}H_{12}O_2$	164.22	Antiseptic and anaesthetic, in dentistry
Iso-safrole	120-58-1	$C_3H_5C_6H_3O_2CH_2$	162.19	Fragrance industry
B-Bourbonene	5208-59-3	$C_{15}H_{24}$	204.3511	Flavour and fragrance agents
B-Elemene	515-13-9	$C_{15}H_{24}$	204.35628000	Antiproliferative effect, used in chemotherapy for cancer treatment
Methyl Eugenol	93-15-2	$C_{11}H_{14}O_2$	178.22	Fragrance ingredient in perfumes, toiletries and detergents
Caryophyllene	87-44-5	$C_{15}H_{24}$	204.35	Antioxidant, anti-inflammatory, anti-cancerous and local anaesthetic.
Aromadendrene	489-39-4	$C_{15}H_{24}$	204.35	Antioxidants and anti ageing
B-Farnesene	18794-84-8	$C_{15}H_{24}$	204.35	natural insect repellent
A-humulene	6753-98-6	$C_{15}H_{24}$	204.39	Anti-inflammatory, effective in reducing platelet activating factor
Methyl isoeugenol	93-16-3	$C_{11}H_{14}O_2$	178.23	Flavour and fragrance agents
Germacerene-D	23986-74-5	$C_{15}H_{24}$	204.4	Analgesic and anti-inflammatory properties
B-Selinene	17066-67-0	$C_{15}H_{24}$	204.35628000	Antibacterial properties and used in aromatherapy
A-Selinene	473-13-2	$C_{15}H_{24}$	204.3511	Aroma active compound
A-Farnesene	502-61-4	$C_{15}H_{24}$	204.3511	Plant defence, biofuel precursor
Hydroxychavicol	1126-61-0	$C_9H_{10}O_2$	150.1745	Antimutagenic effect
Eugenyl acetate	93-28-7	$C_{12}H_{14}O_3$	206.24	Anti-virulence potential
A-Cadinene	24405-05-1	$C_{15}H_{24}$	204.36	Anticancer activity
Germacerene-B	15423-57-1	C_{15} H_{24}	204.356	Antimicrobial and insecticidal properties
E-Nerolidol	40716-66-3	$C_{15}H_{26}O$	222.37	Flavouring agent and in perfumery
Spathulenol	6750-60-3	$C_{15}H_{24}O$	220.4	Antibacterial activity
Globulol	489-41-8	$C_{15}H_{26}O$	222.37	Antimicrobial activity
Chavibetol	501-19-9	$C_{10}H_{12}O_2$	164.2	Aromatic compound with a spicy odour
Allylpyrocatechol Diacetate	13620-82-1	$C_{13}H_{14}O_4$	234.25	Antimicrobial activity against various obligate oral anaerobes
1,8-Cineol	470-82-6	$C_{10}H_{18}O$	154.28	Used in treatment of inflammatory diseases

crop is usually cultivated by small farmer's generation after generation following traditional methods due to lack of research and development of scientific methods for its proper cultivation. Market price of betelvine frequently varies due to agro-climatic factors, transport facilities, farm location and demand supply ratio which discourage the cultivation thereby disturbing the economic stability of the plant growers. Major problems in marketing are transport, too many middleman, absence of grading, fluctuating of practice and inadequacy of finance [11]. Regularity in commercialization of betelvine with proper marketing system is needed to enhance the export potential and revenue generation. Revenue generated through betelvine export can easily be exceeded if agronomic practices are scientifically explored [11].

2.2. Existence of synonym

Betelvine cultivation has been facing lot of problems due to the existence of its synonyms. In some places the same betel plant is being cultivated with different names and at some region different betel plants are cultivated with same names. Thus landraces with prefix 'Desi' in their names invariably refer to the landraces 'Bangla' in West Bengal, landrace 'Kapoori' in Maharashtra and landrace 'Desawari' in Madhya Pradesh [13]. Reports on proper identification or its characterisation are still scanty. As a result of which a lot of confusion is existing among the farmers. Cultivation is mainly through traditional farming system and is controlled by families or communities. The betelvine growers invariably named the betelvine according to the region where it is cultivated, taste and shape of the leaves. Though the geographical distribution of the betelvines under cultivation is vast but the genetic variation may not be so well distributed [14]. On the basis of morphological characteristics there are reports on hundred betelvine types into six cultivars like Bangla, Desawari, Kapoori, Khasi, Meetha and Sanchi. Chemical or molecular fingerprinting in betelvine has not yet been reported in most of the cultivars. Therefore it is always difficult to differentiate the cultivars which are invariable named by the local farmers.

2.3. Wastage of surplus betel leaves

The spoilage of betel leaves accounts for the post-harvest loss in the range of 35%–75% respectively [15,16]. Spoilage may be due to diseases, pest attacks, dehydration and discolouration of leaves. Besides, due to improper marketing, continuously changing price and unavailability of storage facility wastage of huge amount of betelvine leaves occur. So, it is very important to maintain the quality of betel leaves under different seasons by applying different advanced techniques for storage and transport of betel leaves. Such wastages can be reduced by extracting essential oils from its leaves. This essential oil has been reported as remarkably medicinal and aromatic which indicate a promising industrial future by development of its value added product like talc, perfumes, beverages and food additives [10,17]. The outcome of different experiments showed that the best season for longer storage of betel leaves in any of the form which may be petiolated or depetiolated is winter season i.e. December–January [16].

2.4. Betelvine and cancer: long lasting controversy

Betelvine leaves are important because of its medicinal, religious and ceremonial [18]. It also prevents halitosis, improves vocalization, and strengthens gum, treat indigestion, constipation, congestion, coughs and asthma. Despite of its medicinal uses, since long time betelvine has been a crucial point of argument that the consumption of betel leads to oral cancer. In many experiments, several scientists have proved that chewing of betel leaves along with areca nut, catechu, slaked lime and often tobacco induces cancer [19–25]. In contrary scientific studies have shown that betel leaf is itself devoid of mutagenic and carcinogenic effects. Subsequent studies of phytochemical constituents present in its leaf have shown prevention against chemical carcinogens causing oxidative stress and inflammation that has been tested in Swiss mice. Along with curing different diseases betel leaf is found to be useful in preventing different other types of cancer. The betel leaves are aromatic with varied taste *i.e.* sweet to pungent due to presence of its essential oils.

3. Biochemical and molecular profiling

3.1. Biochemical profiling

The essential oil produced from *P. betle* leaf is light yellow to dark yellow in colour with a pungent spicy fragrance. Percentage of leaf essential oil varies from 0.1% to 2.0%. The essential oil is hypersensitive, cardiac and respiratory depressant effects along with antibacterial, antifungal activity and many pharmacological activities due to the presence of eugenol in the oil [8,26,27]. Besides eugenol a phenolic compound hydroxychavicol has also been identified in betel leaves which possess anticarcinogenic activity [28]. Rawat *et al.* (1989) [6] studied five landraces of betelvine and characterized them on the basis of their volatile constituents present in its essential oil. Out of the 51 identified compounds eugenol was found to present in all landraces and in highest percentages. Similarly the essential oil and its constituents of most of the varieties were reported by many workers [29–32]. Constituents of essential oil vary due to several factors like soil quality, climate and agronomic practices. Besides the essential oil, betelvine leaf extracts possesses antioxidant activity [33].

3.2. Bioactivity studies

P. betle leaves have been used in preparation of medicine due to its antioxidant and antimicrobial properties. The ethanolic extract of leaves has antimicrobial activity against human pathogens including both gram-positive and gram-negative bacteria. By phytochemical screening it was found that carbohydrate, protein, polyphenolic compounds, flavonoid and alkaloids were present in its leaves. Strong free radical scavenging activity was observed in ethanol extracts by DPPH model assay in various betelvine landraces [34,35]. According to [36] Jagetia and Baliga (2004) hydroalcoholic extracts of betel leaf also possess nitrogen oxide scavenging effect. Pre clinical experiments have shown that betel leaf possess antidiabetic, antiulcer, antiplatelet aggregation, antiferitility, cardiotonic,

antitumour, antimutagenic, respiratory depressant and antihelminthic activities [37,38]. The oil of betel leaves consist of Eugenol which is found to have antifungal property. The leaves are known to consist of a good amount of nutritive value and also contain enzymes like diastase and catalase and traces of some amino acids namely lysine, histidine and arginine [39]. Multiple studies have shown that the betel leaf possesses anti mutagenic and anti clastogenic effects [40]. According to the studies of [41] Murakami *et al.* (2000) betel leaf extract contains anti tumour promoting agent.

3.3. Molecular marker based genetic diversity assessment

There are many landraces in India which are named according to their village names or cultivated localities. The genetic variations of the betelvines are not well studied due to its vast geographical distribution. Few studies on genetic variation were carried out by using RAPD method to distinguish between male and female betelvines as well as between different landraces [14,42]. In order to study the genetic diversity among different landraces, it is necessary to determine the relatedness of different cultivars of *P. betle*. There are reports on RAPD analysis using two related betelvines namely Kapoori and Bangla in which it was found that the Kapoori cultivars were more heterogenous but Bangla cultivars were mostly similar to each other. There are many cultivars of *P. betle* which are not properly distinguished due to similarities in their morphological characters and in certain region same cultivars are cultivated under different local name. Therefore RAPD and ISSR markers were used for analytical comparison to establish genetic identities and evaluate genetic diversity among fifteen cultivars of betelvine [1]. Maximum similarity was observed in the Balipana and Birkoli landraces and minimum between Banglamandesore Chitalpudi and Halisahar Sanchi landraces.

3.4. Application of SCAR marker for sex determination

Both the betelvine breeding and selection programmes are facilitated by the development of sex-specific markers. This new marker developed *i.e.* SCAR (Sequence Characterized Amplified Regions) marker will be useful in determining the sex type of betelvine plants and it will also help in designing economical breeding programmes [43]. Betelvine is a dioecious perennial crop where both male and female clones are cultivated based on local preference. The collection of new germplasm and its classification require knowledge of the sex of the plant which is a time consuming process. Different molecular tools were introduced for early sex determination and of developmental and evolutionary pathways of sexual dimorphism. A SCAR marker was developed for sex determination in betelvine plant is a PCR based marker which represents genomic DNA fragments that are identified by PCR amplification using sequence specific oligonucleotide primers [43]. The gender distinction and determination has not been well studied in betelvines so far. Studies have shown that gender preferences are correlated with levels of secondary metabolites where higher amounts of phenols and antioxidants are found in females as compared to that of males [44].

3.5. Chlorophyllase activity of P. betle

The role of chlorophyllase was studied in two contrasting *P. betle* landraces *i.e.* Kapoori Vellaikodi with light green leaves and Khasi Shillong with dark green leaves [45]. These landraces showed negative correlation between chlorophyll content and chlorophyllase activity in fresh as well as stored leaves. Seasonal variations in chlorophyll content and chlorophyllase activity were studied in Bangla and Mitha varieties of betelvine (*P. betle*) from January to December. Chlorophyll content of betel leaves is enhanced in the winter environmental conditions The experimental results indicates that the total chlorophyll is higher during winter and successively lower in summers in Bangla variety. In reverse activity of chlorophyllase enzyme was reduced in winter season in comparison to summer season. By studying the chlorophyllase activity of we can know the gene responsible for the change in leaf colour from green to yellow colour.

3.6. Disease management and cultivar development

The common serious problem in the cultivation of betelvine is its diseases like powdery mildew, foot rot, leaf rot caused by various pathogens. This leads to great loss in both national and international economy. Several diseases that cause serious damage to the betelvine crop are foot rot, stem rot, leaf rot, leaf spot, root rot and foliage that are mainly caused by *Phytopthora* species *i.e. Phytopthora parasitica* (*P. parasitica*), *Phytopthora nicotianae*, *Phytopthora palmivora*, *Phytopthora capsici* severally damage foot, stem, root and foliage [46]. The most common and widespread disease of betelvine is Powdery mildew disease. The disease should be detected at early stage to prevent damage caused to the whole plantation. The main source of powdery mildew is *Oidium piperis* [47]. The foot rot disease of betelvine is caused by *P. parasitica*. Leaf rot disease of betel leaf was influenced by high atmospheric humidity and rainfall from June to August annually. The incidence of leaf spot increased with less humidity gradient during the months of November to March. *Trichoderma harzianum* is used for management of foot rot disease in betelvine. The factors that influence the growth of betelvine inside 'baroj' are low temperature, high humidity and diffused light. The interrelationship between the climate and frequency of diseases should be studied before giving final recommendation to the farmers [46]. There are hemipteron insect pests that occur in betelvine ecosystem and harms the plant in its yield potential as reported in West Bengal varieties [48].

4. Conclusion and future prospects

Till date good progress has been made regarding the chemical profiling, bioactivity studies and disease management for betelvine which has been shown in (Table 2). Identification of constituents in different landraces using modern gas chromatographic techniques could be useful for future elite landraces selection and their improvement programmes. At the same time efforts should be made on characterization of most of the available landraces which could be useful for resolving synonym problem and their proper authentication. In consideration of the proven therapeutic value of *P. betle* proper

Table 2

Research works on *P. betle*.

Sl. No.	Landraces of *P. betle*	Research work	Year	References
1.	Chandrakala, Karpada local, Godibangla, Nahua, Balia, Desibangla, Dandabangla, Maghai	Chemical composition and antioxidant activity of some important betelvine landraces	2016	[49]
2.	Unknown *P. betle* cultivar	Phytochemical analysis of *P. betle* leaf extract	2015	[50]
3.	Unknown *P. betle* cultivar	Synthesis of silver nanoparticles using *P. betle* and its antibacterial activity	2014	[51]
4.	Unknown *P. betle* cultivar	Hypolipidemic activity of *P. betle* in high fat diet induced hyperlipidemic rat	2014	[52]
5.	Unknown *P. betle* cultivar	Cytotoxic effects of Betelvine leaf extracts using *Artemia salina* leach	2014	[53]
6.	Unknown *P. betle* cultivar	A study on Betelvine cultivation and market crisis in Karur district	2013	[11]
7.	Unknown *P. betle* cultivar	Hydroxychavicol and eugenol profiling of betel leaves from *P. betle* L. obtained by liquid–liquid extraction and supercritical fluid extraction	2013	[54]
8.	Unknown *P. betle* cultivar	Effect of ethanolic extract of leaf *of P. betle* L. as immunomodulatory agent: A unique role of phytochemicals	2013	[55]
9.	Unknown *P. betle* cultivar	Antibacterial effect of crude extract of *P. betle* L. against pathogenic bacteria	2013	[56]
10.	Unknown *P. betle* cultivar	Antioxidant and cytoprotective activities of *P. betle*, Areca catechu quid with or without calcium hydroxide	2013	[57]
11.	Unknown *P. betle* cultivar	Phytochemical screening and antioxidant potential of different extracts of *P. betle* leaves	2013	[58]
12.	Pachaikodi	Pharmacognostical and Phyto-physicochemical profile of the leaves of *P. betle* L. var Pachaikodi (Piperaceae) - Valuable assessment of its quality	2012	[59]
13.	Unknown *P. betle* cultivar	Characteristic differences in metabolic profile in male and female plants of dioecious *P. betle*	2012	[44]
14.	Unknown *P. betle* cultivar	Effects of ionic surfactants on the morphology of silver nanoparticles using paan (*P. betle*) leaf petiole extract	2012	[60]
15.	Unknown *P. betle* cultivar	The anti-biofouling effect of *P. betle* extract *Pseudomonas aeruginosa* and bacterial consortium	2012	[61]
16.	Simurali Deshi	Keeping quality of betel leaves (*P. betle* L.) as influenced by different methods and seasons	2012	[16]
17.	Bengaluru local, Madras type, Meetha paan	Antioxidant capacity, radical scavenging ability, total phenols and flavonoids in three types of betelvine (*P. betle* L.)	2012	[62]
18.	Unknown *P. betle* cultivar	Recognition of powdery mildew disease for betelvine plants using digital image processing	2012	[46]
19.	Kapoori, Bangla, Godibangla, Kali Bangla, Navbangla, Deshawari, Gach Pan, Kalipatti	Diversity analysis between male and female landraces through SPAR Profile	2011	[63]
20	Tellaku Ponnur, Kuljedu Cuddapah, Kapoori, Pachaikodi, Gangeri, Vellaikodi, Ramtek Bangla, Meetha Pan, Gach Pan	Identification of sex-specific DNA marker	2011	[64]
21.	Unknown *P. betle* cultivar	Evaluation of antihistaminic activity of *P. betle* leaf in guinea pig	2011	[65]
22.	Unknown *P. betle* cultivar	Evaluation of antibacterial properties of extracts of *P. betle* leaf	2011	[66]
23.	Unknown *P. betle* cultivar	Antibacterial activity of ethanol extract of Betel leaf (*P. betle* L.) against some food borne pathogens	2011	[67]
24.	Vellaikodi	Chemical composition and antimicrobial activity of vellaikodi variety of *P. betle* L. leaf oil against dental pathogens	2011	[32]
25.	Unknown *P. betle* cultivar	Determination of Antioxidant flavonoids and phenolic compounds in betel plant by high performance liquid chromatography	2011	[68]

26.	Unknown *P. betle* cultivar	Management of foot rot of betel vine *(P. betle* L.) caused by *Phytophthora parasitica* Dastur	2011	[9]
27.	Unknown *P. betle* cultivar	Antimicrobial, antioxidative and antihemolytic activity of *Piper betel* leaf Extracts	2011	[38]
28.	Calcutta, Desipan, Desawari, Ghazipur, Bangladeshi, Banarasi, Jaleswar, Vishnupuri, Kapoori Turi, Saunfia Pan	Comparative morphoanatomy of *P. betle* L. cultivars in India	2010	[8]
29.	Unknown *P. betle* cultivar	*In vitro* antifungal activity of hydroxichavicol isolated from *P. betle* L.	2010	[69]
30.	Unknown *P. betle* cultivar	Characterization and evaluation of antimicrobial activity of the essential oil from the leaves of *P. betle* L.	2010	[70]
31.	Unknown *P. betle* cultivar	Gas chromatography mass spectrometry (GC–MS) analysis of the hexane and benzene extracts of the *P. betle* (leaf stalk) (Family: Piperaceae) from India	2010	[71]
32.	Unknown *P. betle* cultivar	Changes in the antioxidant enzymes and lipid peroxidation in Betelvine subjected to water stress	2009	[72]
33.	Unknown *P. betle* cultivar	The n-hexane and chloroform fractions of *P. betle* L. trigger different arms of immune responses in BAL B/c mice and exhibit antifilarial activity against human lymphatic filarid Brugia malayi	2009	[73]
34.	Unknown *P. betle* cultivar	Pro-apoptotic effect of the Landrace Bangla Mahoba of *P. betle* on Leishmania donovani	2009	[74]
35.	Unknown *P. betle* cultivar	Antioxidant effect of ethanolic extract of *P. betle* L. (Paan) on erythrocytes from patients with HbE-beta thalassemia	2009	[75]
36.	Unknown *P. betle* cultivar	Antibacterial activity o f fresh leaves of *P. betle* L.	2009	[76]
37.	Unknown *P. betle* cultivar	Management of leaf spot disease complex of betelvine by bioagents and plant extracts	2008	[77]
38.	Unknown *P. betle* cultivar	Antimicrobial and antioxidant activities of betel oil	2006	[78]
39.	Unknown *P. betle* cultivar	Dose dependent effect of *P. betle* L. Leaf extract on erythrocytes of experimental mice	2006	[79]
40.	Unknown *P. betle* cultivar	Antioxidant activity of *Piper betel* leaf extract and its constituents	2006	[34]
41.	Unknown *P. betle* cultivar	Radioprotective property of the ethanolic extract of *Piper betel* leaf	2005	[40]
42.	Unknown *P. betle* cultivar	Antioxidant activity *of P. betle* L. leaf extract *in vitro*	2004	[33]
43.	Kapoori, Tellaku, Bangla, Meetha Bangla, Gach Pan, Kalipatti, Desawari, Awani pan	Genetic diversity amongst landraces of a dioecious vegetatively propagated plant, betelvine (*P. betle* L.)	2004	[42]
44.	Bangla and Mitha Pan	Seasonal variations in chlorophyll content and chlorophyllase activity in Bangla and Mitha varieties grown in different soil treatments	2003	[45]
45.	Bangla, Kapoori, Desavari	RAPD profile analysis among the betelvine cultivars	2002	[14]
46	Bangla, Desawari, Kapoori, Meetha, Sanchi	Essential oil components as markers for identification of *P. betle* L. cultivars	1989	[6]

characterization could be useful for long term research for drug development. Efforts are to be intensified towards obtaining promising landraces with high eugenol and chavibetol content. In addition to its bioactivity studies attention may also to be addressed towards proper authentication of a particular landrace and their conservation for continuous supply. As plant secondary metabolites are always affected by soil and environmental factors it is also imperative to study the effect of abiotic factors on production and quality of betelvine. Further standardization of factors for quality and quantity of betelvine essential oil and extracts could be the future line of research before clinical trial and large commercial cultivation. Recent biotechnological tools like chromatography, NMR other functional genomics techniques could be explored to find out new compounds with active potential from this unexplored plant species. Further study is needed in chlorophyllase activity for long term storage and improvement of export potential of betelvine leaf. Special attention is needed in pest and disease management in betelvine for development of new and improved varieties. Genetic diversity assessment using molecular markers should be intensified taking maximum number of landraces. In essence, biotechnological intervention has opened up new horizon for genetic improvement, proper authentication and identification of elite chemotypes of this medicinally and economically important cash crop.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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