Piper betle Linn. a maligned Pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery

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***Piper betle* L. is one of the important plants in the Asiatic region which ranks second to coffee and tea in terms of daily consumption. Though the plant is known for abuse, in recent years several reports have been published on the effects of the plant extract and chemical constituents on different biological activities *in vitro* and *in vivo*. The leaf extract, fractions and purified compounds are found to play a role in oral hygiene, anti-diabetic, cardiovascular, anti-inflammatory/ immunomodulatory, anti-ulcer, hepato-protective and anti-infective, etc. Patents were also awarded for some of the biological activities like anti-inflammatory, anti-cancer and immunomodulatory associated with leaf extracts and purified compounds. The active compounds isolated from leaf and other parts are hydroxychavicol, hydroxylchavicol acetate, allypyrocatechol, chavibetol, piperbetol, methylpiperbetol, piperol A and piperol B. Phenol-rich leaves of *P. betle* show high antioxidant activities. A number of biologically active compounds from *P. betle* have potential for use as medicines, neutraceuticals and industrial compounds. Since the traditional use of *P. betle* involves chewing, it offers possibilities of use in drug delivery through buccal mucosa bypassing the gastric route.**

Keywords: Chewing, drug discovery, *Piper betle*, pharmacological activity.

HUMAN dependence on plants as a source of medicine dates back to prehistoric times. Even now, more than three-fourths of the world's population relies mainly on plants and plant extracts for healthcare. Several prescription drugs in the developed countries contain plant components and more than 120 important prescription drugs are derived from plants[1]. Recent years have witnessed a resurgence of interest in plants as a source of medicine, especially those of antiquity and ethno-medicine. Betel vine (*Piper betle* L.; PB), a shade-loving, perennial evergreen climber of tropical origin, generally known as 'paan' in the Indian subcontinent and by different names in the Asiatic region is a plant of antiquity. According to estimates, it is consumed daily by nearly 600 million people and the custom of betel chewing encompasses a vast area of the world (Figure 1), extending 11,000 km west to east and 6000 km north to south, an area stretching from east Africa to Polynesia[2].

P. betle is a plant with known ethnomedicinal properties and its use in India, Indonesia and other countries of the Indo-China region – Malaysia, Vietnam, Laos, Kampuchea, Thailand, Myanmar, Singapore and the Far East is well known. Use of PB leaf (PBL) was known for centuries for its curative properties such as: to reduce/prevent body odour and bad breath, throat and lung problems, cough prevention and healing, to reduce unwanted vaginal secretion and bad odour and to prevent itching caused by fungus and internal/external bacteria[3]. In Chinese folk medicine betel leaves are used for the treatment of various disorders and claimed to have detoxication, antioxidation and antimutation properties. It may be mentioned that the traditional health systems recognized the value of PB and discovered many uses. Several tribes in India still use it as cure and protection from different ailments and several of the claimed PB uses have been validated over a period of time. Some of the work done earlier did show the useful effects of PB, including a sense of well-being[4]. PB use involves chewing, ingestion and topical applications.

In spite of the above-mentioned uses and research findings, PB is primarily known for abuse, which played a major role in the decline of its use in younger generation and perhaps also deterred earlier researchers to investigate the effects of this plant. A majority of the publications related to this plant in the past (primarily originating from Europe and other parts of the industrial world) projected it as one of the major causes of oral cancer in betel-chewing regions of the world. This was primarily due to abuse and addiction and not due to PB as such. Studies during the last two decades, primarily by the scientists from the regions where betel chewing is common, have brought to focus its beneficial properties and validated several uses which were known to the Asiatic communities. This review presents an overview

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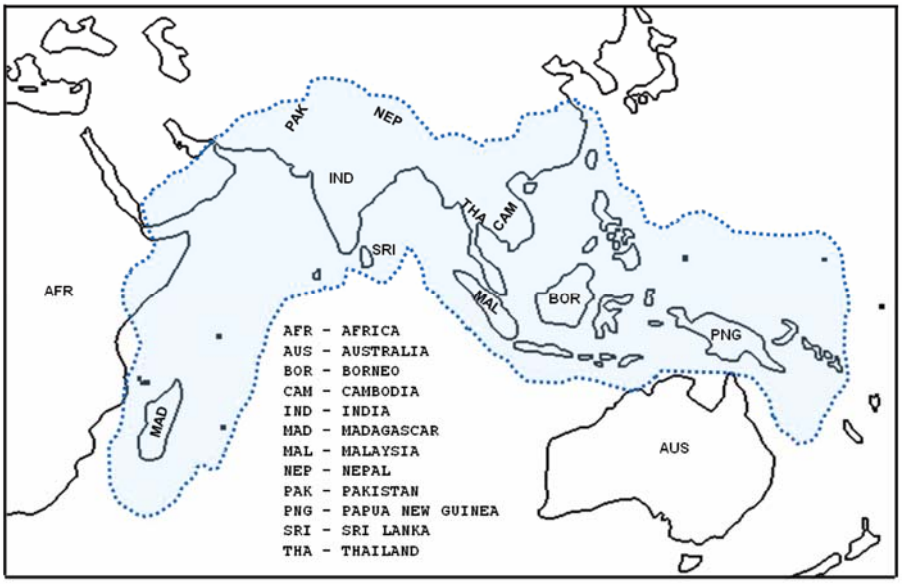


Figure 1. Regions within the dotted lines show major areas of *Piper betle* consumption. Barring the areas where the climatic conditions (high or low temperature accompanied by very low humidity) do not support its cultivation, the areas of cultivation and consumption overlap (redrawn after Rooney[2]).

on the diverse medicinal properties of PB and attempts to highlight the need for communities to have their own perspective and not be overwhelmed by findings which are fragmented views of the whole and hence opinions.

Therapeutic uses of PB and its chemical constituents

Though PB as a part of quid has been implicated in oral cancer, many scientists did not agree with these observations. The first indication of it being noncarcinogenic emerged from the work of Bhide and his group[5], when they showed non-mutagenic properties in betel leaves and the presence of hydroxychavicol (HC), a phenol in PBL with anti-mutagenic properties. This proved to be the turning point in PB research, when it was established that PBL per se do not contribute to oral cancer. This provided opportunities to explore the properties of PB. Since then, many biological activities have been demonstrated in betel leaf. Several medicinal properties have been attributed to PB, which include antioxidant, anti-infective, analgesic, anticancer, antidiabetic, hepatoprotective, immunomodulatory, cardiovascular, etc. (Table 1). Some of the activities have been patented (Table 2).

Since the primary use of PB is 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 PB have been described.

Effects of PB chewing on oral hygiene

Oral hygiene is maintained by the saliva which contains peroxidase, lysozyme and secretory antibodies to combat bacterial growth in the oral cavity, which is otherwise highly conducive to microbial growth. Any natural substance which is part of daily use and also chewed is the best candidate for oral hygiene, as it would also contribute to increased salivation and by default increase the levels of antimicrobials. If the candidate plant also happens to possess antimicrobial properties then it is the best option. With all these properties, PB is the best choice for oral hygiene because while chewing active phytochemicals are released into the oral cavity.

The bioenhancer effect of PB on salivary peroxidase activity was shown in *in vitro* experiments using human saliva and crude leaf extract[6]. Later studies showed that phenolic antibacterials from PBL cause suppression of bacterial activity in the oral cavity and prevent halitosis. Activity-directed purification led to the identification of allylpyrocatechol (APC)[7] possessing antimicrobial activity against oral bacteria, *Staphylococcus aureus*. Crude aqueous extract of PB was found effective against another oral microbe, *Streptococcus mutans* and caused changes in the ultrastructure and its acid-producing properties[8]. Suppression of overall oral microbial activity by PB was demonstrated by Bissa *et al.*[9]. The effectiveness of PB extract, essential oil and hydroxyl-chavicol (HC)[10] was also demonstrated on oral pathogenic fungi, *S. faecalis* and *Candida albicans*[11,12].

Aqueous extracts of PB (PBAq) affected the adhesion of early plaque settlers (*Streptococcus mitis*, *Streptococcus sanguis* and *Actinomyces viscosus*) on saliva-coated

Table 1. Biological activities in *Piper betle* extracts and its identified chemical constituents

Biological activity	Extract/chemical constituent	Reference
Oral hygiene/antibacterial/antifungal *Staphylococcus aureus* *Staphylococcus mutans* *Staphylococcus faecalis* *Candida albicans* *Vibrio cholerae ogawa* *Diplococcus pneumoniae* and *Klebsiella aerogenes*	Leaf extract (aqueous), ethyl acetate and ethanol extracts, allylpyrocatechol (APC), hydroxychavicol (HC)	6–13, 55
Cardiovascular effects	Leaf/inflorescence extract (water, ethanol, acetone) HC, piperbetol, methylpiperbetol, piperol A and piperol B	10–22
Digestion stimulant hepatoprotective/ glucose metabolism	Leaf powder, leaf extract	23–29
Anti-inflammatory/pain reliever/ antiallergic	Leaf extract (ethanol), APC, HC	10, 28–30
Antioxidant	Leaf extract, polyphenols – APC, chavibetol (CHV), HC	10, 18–26, 32, 33, 38–40
Cholinomimetic activity	Leaf extract	15, 41
Immunomodulatory	Leaf extract (ethanol/methanol), *n*-hexane and chloroform fractions, HC	40–46
Antiulcer/wound healing	Leaf paste, leaf extract, APC	3, 28, 34, 37, 47, 48
Chemopreventive/anticancer activity	Leaf extract, HC	5, 49–54
Anti-infective Antileishmanial Antifilarial	 Leaf extract (methanol) Crude extract, *n*-hexane and chloroform extracts, essential oil	 56, 57, 59 42, 59
Abiotic stress protective	Phenols – APC and CHV	34, 58, 60

glass surfaces[13]. Application of HC also decreased adhering properties of oral microbes and its antibacterial effect persisted for 7–8 h after application[10]. These findings validate the beneficial role of PB in oral hygiene and the potential of PB as antiplaque agent.

Effect on cardiovascular responses

Ayurveda uses certain thumb rules like shape of the plant part and its relationship to body organ for the purpose of treatment. The heart shape of PBL makes it a suitable candidate for heart-related curative properties/medicine. PB is considered to provide strength to the heart (cardio tonic) and regulates irregular heart beat and blood pressure. Cardiovascular response of PB acquires great significance by the fact that it is consumed globally, making it a feasible substitute for *Digitalis purpurea*[14]. The effect of PB chewing can be observed within minutes[15], which includes cardio-acceleration, sweating and salivation. It induces catecholamine secretion from the adrenal cortex contributing to increase in stamina, heart rate, blood pressure, blood glucose levels and sympathetic neural activity[16].

The effect of PBL on vasorelaxation has been studied on isolated perfused mesenteric artery rings, wherein it was observed that the vasorelaxant effect of PB was mainly endothelium-dependent and nitric oxide (NO)-mediated, as the effect was prevented by pretreatment with *N*(omega)-nitro-*L*-arginine (NOLA), a nitric oxide synthase (NOS) inhibitor, or by removal of endothelium[17].

Platelet hyperactivity is important in the pathogenesis of cardiovascular diseases due to intravascular thrombosis. Piperbetol, methylpiperbetol, piperol A and piperol B, isolated from PB, selectively inhibited platelet aggregation induced by platelet activating factor (PAF) in a concentration-dependent manner. Thus, these constituents are effective PAF receptor antagonists *in vitro*. These phenols had no effect on the cAMP contents in resting rabbit platelets[18].

Aqueous extract of inflorescence PB (PBI Aq) was also shown to be a scavenger of H_2O_2, superoxide radical and hydroxyl radical[19]. PBI extract also inhibited arachidonic acid (AA), collagen, and thrombin-induced thromboxane B_2 (TXB_2) production by more than 90%. However, PBI extract showed little effect on thrombin-induced aggregation. These results indicate that aqueous components of PBI have reactive oxygen species (ROS) scavenging property and prevent platelet aggregation possibly via scavenging ROS or inhibition of TXB_2 production[19]. HC, a purified compound from PB, was tested for platelet aggregation, TXB_2 and ROS production, cyclooxygenase (COX) activity, *ex vivo* platelet aggregation, mouse-tail bleeding time and platelet plug formation *in vivo*. HC was a potent COX-1/COX-2 inhibitor, ROS scavenger and inhibited platelet calcium signalling, TXB_2 production and platelet aggregation. Therefore, HC could be a potential therapeutic agent for the prevention of intravascular thrombosis due to anti-inflammatory and antiplatelet effects, without affecting the haemostatic functions[20]. PBI and betel quid (BQ) were evaluated with other indigenous

Table 2. Patents filed and awarded for various activities of *Piper betle*

Patent filed for activity	Patent title and abstract	Place of filing	Filing country/year	Grant date/patent no
Antileishmanial	Antileishmanial activity of betel-leaf extract. (This invention relates to the method of treating VL/kala-azar by administering an effective amount of betel-leaf extract or lyophilized extract together with or associated with an additive and a composition comprising betel-leaf extract with a pharmaceutically acceptable additive.)	India (CSIR/ IICB, Kolkata)	World/2000; USA/2001, 2002; China/2003; India/2003	WO/20002/045731 USA/2003/6610332
Anticancer	Antimonocytic activity of extracts of PBL. (This invention relates to anti-monocytic activity of betel-leaf extracts and suggest its use in the treatment of myeloid leukaemia in animals and human beings.)	India (CSIR/ IICB, Kolkata)	World/2000; USA/2001; Australia/2003; GB/2003; Japan/2003; China/2003; India/2003; Denmark/2003	GB/2004
Immunomodulatory	Use of betel-leaf extract to induce IFN-gamma production from human peripheral blood T-cells and as a Th1-type immunomodulator	India (CSIR/IICB, Kolkata)	World/2000; USA/2002, 2005; India/2003; China/2003; Japan/2003 Denmark/2003	WO/2002/049655 USA/2003/6531166
Anti-5 lipoxygenase	Herbal formulation of a combination of PB and *Murrya koenigii* extracts for blocking 5-lipoxygenase activity leading to the inhibition of leukotriene synthesis, suppression of interleukin-4 production, and enhancement of gamma interferon release with implications in arthritis and asthma.	India (CSIR/IICB, Kolkata)	USA/2001; World/2002; Australia/2004; Europe/2004; Japan/2004; China/2004	USA/2004/6773728 Europe/2005
Anticancer	A herbal composition for treating $CD33^{+}$ acute and chronic myeloid leukaemia and a method thereof.	India (CSIR/IICB, Kolkata)	USA/2004; USA/2002, 2003	USA/2007/7306817 USA/2005/6852344 2005/6967034
Antiwart	Method for instantaneous removal of warts and moles.	USA (Deer-field, IL)	USA/2001	USA/2001/6312735
Dye	Herbal dye and process of preparation thereof. (The present invention provides a herbal black dye from natural materials comprising *Juglans regia*, *Indigofera tinctoria*, *Terminalia chebula*, *Acacia accocina*, *Lawsonia inermis*, *Trigonella foenum-graecum*, *Sapindus mukorossi*, *Eclipta alba*, *Embelica officinalis*, *Acacia catechu* and PB. The dye derived is safe, non-toxic, antiallergic, antidandruff and free from toxic symptoms like itching.)	India (CSIR/NBRI, Lucknow)	USA/2004	USA/2007/7186279
Bronchial disorders	Herbal composition of a blend of active components prepared. (This invention relates to a herbal composition for the treatment and as a remedy for bronchial respiratory difficulties. More particularly, this invention describes the process of separation, physico-chemical characterization and biological response evaluation of active components obtained from extracts of any plant parts, including leaves, barks, roots and seeds of *M. koenigii* and PB plants in order to establish their role in the treatment and as a remedy for bronchial respiratory troubles.)	India (CSIR/IICB, Kolkata)	USA/2001	USA/2004/6773728
Anti-inflammatory	Analgesic and refreshing herbal composition and a process for preparing the same. (This invention provides an analgesic and refreshing herbal composition useful as dentrifrices; composition comprising 50–60% wt of betle extract; from PBL.)	India (CSIR/CIMAP, Lucknow)	USA/2001	USA/2003/6531115

plants for their ability to scavenge the 1,1-diphenyl-2-picryl-hydrazyle (DPPH) radical, to protect human low-density lipoprotein (LDL) from Cu^{2+}-catalysed oxidation and to protect cultured bovine aortal endothelial cells (BAEC) from oxidized LDL (oxLDL)-induced cytotoxicity. Polyphenol-rich extracts of PBI and BQ were potent DPPH scavengers, having similar activity to quercetin and were able to protect LDL from oxidation, but were pro-oxidants at lower concentrations, suggesting the possibility of protective effect against atherosclerosis[21]. HC from PBL was shown to be a potent inhibitor of xanthine oxidase implicated in ROS-generated ischaemic damage of the heart[22]. These experimental evidences indicate the potential of PB in cardiovascular activities.

Effect on digestive system

PB is a customary post-prandial offering in Indian subcontinent as it is considered to help in digestion. The first experimental evidence[23] in this respect was obtained on rats by oral administration of leaf extract of two PB landraces, the pungent Mysore and non-pungent Ambadi, and evaluating its effect on the digestive enzymes of the pancreas and intestinal mucosa as well as on bile secretion. The results indicated that although there was no effect on bile secretion and composition, there was a significant stimulatory influence on pancreatic lipase activity. Besides, the Ambadi showed a positive stimulatory influence on intestinal digestive enzymes, especially lipase, amylase and disaccharidases, thus promoting digestion. Later studies have also shown the beneficial effect of PB on digestion[24,25].

Hepatoprotective activity

Liver is one of the most important organs having a wide range of functions including detoxification, protein synthesis and production of biochemicals necessary for digestion. PB has a significant effect on various metabolic activities of the liver. The antihepatotoxic effect of PB extract was evaluated on ethanol[26] and carbon tetrachloride (CCl_4)-induced liver injury in a rat model[27]. Fibrosis and hepatic damage, as revealed by histology and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were induced in rats by CCl_4. PB extract significantly inhibited the elevated activities of AST and ALT and also attenuated total glutathione *S*-transferase (GST) and GST alpha isoform activity. PB also led to a rise in antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). The histological examination showed that the PB extract protected liver from the damage induced by CCl_4 by decreasing alpha-smooth muscle actin (alpha-sma) expression, inducing active matrix metalloproteinase-2 (MMP2) expression through the Ras/Erk pathway, and inhibiting TIMP2 level that consequently attenuated the fibrosis of liver. These findings support a chemopreventive potential of PBL against liver fibrosis[27].

The *in vivo* antioxidant potential of PBL extract was evaluated against oxidative stress induced by D-galactosamine intoxication in male albino Wistar rats[24]. Toxicity was induced by an intraperitoneal injection of D-galactosamine, 400 mg/kg body wt (BW) for 21 days. Rats were treated with extract (200 mg/kg BW) via intragastric intubations. The activities of liver marker enzymes (AST, ALT, alkaline phosphatase, gamma glutamyl transpeptidase) and levels of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides, SOD, CAT, glutathione peroxidase, vitamin C, vitamin E and reduced glutathione were monitored. The extract significantly improved the status of antioxidants and decreased TBARS, hydroperoxides and liver marker enzymes when compared with the D-galactosamine-treated group, demonstrating its hepatoprotective properties.

Effect on glucose metabolism

Evaluation of the effect of PB on glucose metabolism is of significance as it is recommended for consumption after meals. Oral administration of hot water extract (PBHWE) and cold ethanol extract (PBCEE) in normoglycaemic rats showed that both PBHWE and PBCEE significantly lowered the blood glucose level in a dose-dependent manner and were found to be non-toxic and well tolerated following chronic oral administration; no overt signs of toxicity, hepatotoxicity or renotoxicity were observed[28]. In glucose tolerance test, both extracts markedly reduced the external glucose load. Use of leaf suspension of PB[29] led to significant reduction in blood glucose level, glycosylated haemoglobin, and decreased activities of liver glucose-6-phosphatase and fructose-1,6-bisphosphatase, whereas liver hexokinase increased ($P < 0.05$) in STZ diabetic rats compared with untreated diabetic rats.

Antioxidant activity

Due to the oxidative atmosphere all the substances tend to get oxidized in nature. Compared to humans, plants have a highly developed antioxidant systems due to their sessile nature and are a good source of antioxidants for therapeutic uses. The PB extract and HC also showed significant *in vitro*/*in vivo* free-radical scavenging activity[10,18–22,24–26,30–37] and the overall activity of PB was superior to tea[35]. Polyphenol-rich extracts were potent DPPH scavengers[32,34–36] offering overall protection against various stresses. PB showed activity similar to quercetin and protected LDL from oxidation in a dose-dependent manner at concentrations higher than 10 μg/ml, but was pro-oxidant at lower concentrations[20]. Recently,

in vivo antioxidative effect was also shown by different workers[24,25]. PB showed promising antioxidant activity against erythrocytes from patients with HbE-beta thalassemia[36]. Significant differences in the antioxidant activity were also observed in PB landraces[37]. Considering the involvement of oxidative stress in an array of biological events, PB could possibly be a natural source of antioxidants for therapeutic uses.

Anti-inflammatory and antiallergic response

PBL have long been in use in the Indian traditional system of medicine for pain relief. Its application on inflammed parts to subside inflammation has also been well documented.

At non-toxic concentrations of 5–25 μg/ml PB, extracellular production of NO in murine peritoneal macrophages decreased in a dose-dependent manner. Down-regulation of transcription of inducible NOs in macrophages, and concomitant dose-dependent decrease in the expression of interleukin-12 p40 indicated the ability of PB to down-regulate T-helper 1 pro-inflammatory responses. Thus, the anti-inflammatory and anti-arthritic activities are attributable to the down-regulation of generation of ROS[38,39]. Significant suppression of tumour necrosis factor alpha (TNF-alpha) expression in human neutrophils by HC shows its role as an anti-inflammatory agent[10].

The effects of PB ethanol extract (PBE) on the production of histamine and granulocyte macrophage colony-stimulating factor (GM-CSF) by murine bone marrow mast cells (BMMCs) and on the secretion of eotaxin and IL-8 by the human lung epithelial cell line, BEAS-2B, were studied *in vitro*[40]. PBE significantly decreased histamine and GM-CSF produced by an IgE-mediated hypersensitive reaction, and inhibited eotaxin and IL-8 secretion in a TNF-alpha and IL-4-induced allergic reaction. Thus PB may offer a new therapeutic approach for the control of allergic diseases through inhibition of production of allergic mediators.

Cholinomimetic effect

Use of PB in cough and cold is well known among the tribes of North East India, like the Khasis, who still use it for this purpose. Leaf extract mixed with honey is a common remedy for cough and cold, which is given to infants and children during winter months. Studies have shown that use of PB quid leads to rise in body temperature due to cholinergic responses[15]. Aqueous (PBAq) and ethyl acetate (PBEtAc) extracts of PB were evaluated for their cholinergic responses using isolated guinea-pig ileum. It was observed that the spasmogenic activity was more in PBAq than PBEtAc. In isolated rabbit jejunum K^+-induced contraction was inhibited by PBAq as well as PBEtAc, suggesting blockade in calcium channel. Thus, PB contains cholinomimetic and possible calcium channel antagonist constituents which may provide the basis for several activities shown by this plant[41].

Immunomodulatory activity

The cytokine-mimetic properties of PB were tested by its ability to stimulate proliferation of mouse spleen cells and bone marrow cells[42,43]. The extract strongly stimulated proliferation of both bone marrow cells and splenocytes, and significant increase in cell concentrations. The cytokine-mimetic protein in PB was 26 kDa, which stimulated proliferative response in a dose-dependent manner. They also showed the effect of methanol extract in immunomodulatory activity *in vitro* and *in vivo*.

Singh *et al.*[44] demonstrated immunomodulatory efficacy of the crude methanolic extract and the various fractions of PB landrace, Bangla Mahoba, at different dose levels in BALB/c. The crude methanol extract and *n*-hexane fraction were found to potentiate significant enhancement of both humoral (plaque-forming cells, haemagglutination titre) as well as cell-mediated (lymphoproliferation, macrophage activation, delayed type hypersensitivity) immune responses in mice. Enhanced populations of T-cells ($CD4^+$, $CD8^+$) and B-cells ($CD19^+$) were observed. The *n*-hexane fraction was found to induce biased type-2 cytokine response as revealed by increased IL-4^+ and decreased IFN-γ^+ T-cell populations, whereas the chloroform fraction induced a predominant type-1 cytokine. Crude methanol extract demonstrated a mixed type 1 and type 2 cytokine response, suggesting a significant immunomodulatory property in this plant.

The effect of HC on pro- and anti-inflammatory cytokine levels in arthritic paw tissue homogenate supernatant was studied. HC showed significant lowering of pro-inflammatory (Th1) cytokine levels, viz. IL-2, IFN-gamma and TNF-alpha, with maximum inhibition at higher dose levels of 2 and 4 mg/kg per os and enhanced the production of anti-inflammatory (Th2) cytokines IL-4 and IL-5, as estimated by cytometric bead array immunoassay[45]. HC at graded doses also significantly decreased the expression of IL-1beta, PGE (2), LTB(4) and NO levels, showing significant inhibition of these parameters. Elevated level of CD4(+) T-cell-specific IFN-gamma in splenocytes of arthritic animals was also inhibited in treated animals. Immunomodulatory activity in HC was shown by Min *et al.*[46]. Patent on immunomodulatory activity of PB has been awarded (Table 2).

Antiulcer and wound-healing property

Use of PB in wound dressing and wound healing was known to Indian communities and is still being practised

as application of crushed leaf (paste) on cuts and wounds[3]. This property was studied in rats and found to be effective in non-steroidal anti-inflammatory drug (NSAID)-induced peptic ulcer. Significant healing effect of PBE was observed on induced peptic ulcer in albino rats[32]. It was inferred that free-radical scavenging activity of the plant extract may be responsible for its healing action. One of the phenol constituents, APC, in PBL imparts significant protection against indomethacin-induced ulcers in Sprague–Dawley rats. Treatment with APC and misoprostol could effectively heal the stomach ulceration, as revealed by the ulcer index and histopathological studies[35]. The protection against ulcer was correlated with antioxidative and mucin-protecting properties. Down-regulation of generation of reactive nitrogen species accompanied by T-helper 1 pro-inflammatory response by PB helps in wound repair[38]. Management of stoma[47] by application of PBL in patients has been demonstrated.

The role of ariginine metabolism in the healing action of APC and omeprazole against indomethacin-induced stomach ulceration in mouse was evaluated[48]. Indomethacin (18 mg/kg) was found to induce maximum stomach ulceration in Swiss albino mice on the third day of its administration, which was associated with reduced arginase activity, endothelial nitric oxide synthase (eNOS) expression, and IL-4 and TGF-beta levels, along with increased inducible nitric oxide synthase (iNOS) expression, nitrite, IL-1beta and IL-6 generation. The healing effect of APC was comparable to omeprazole, wherein the iNOS/NO axis shifted to the arginase/polyamine axis as revealed by the increased arginase activity, eNOS expression and reduced iNOS expression and nitrite level. The authors attributed these to a favourable anti-/pro-inflammatory cytokines ratio generated by APC.

Chemopreventive and anticancer activity

The first evidence of possible involvement of BQ components in suppressing the overall effect of mutagenicity of BQ came from two independent studies[49,50]. Betel leaf phenolic compounds, eugenol and HC, were tested in various strains of *Salmonella typhimurium* with or without metabolic activation, which showed dose-dependent suppression of dimethylbenzanthracene-induced mutagenesis in *S. typhimurium* strain TA98 with metabolic activation. HC was more potent than eugenol in this respect[5]. Betel-leaf extracts were nonmutagenic and suppressed mutagenicity of a number of known mutagens like benzo (a) pyrene and dimethylbenzanthracene; two tobacco-specific *N*-nitrosamines (TSNA), *N*′-nitrosonornicotine (NNN) and 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)[51,52]. It was further shown that betel leaf is also anticarcinogenic[53,54]. HC also inhibits the growth, adhesion and cell-cycle progression of oral KB carcinoma cells, whereas the induction of KB cell apoptosis (HC > 0.1 mM) was accompanied by cellular redox changes[31]. In recent years two patents on anticancer activities have been awarded (Table 2).

Anti-infective activity

Antibacterial

PBL extract, essential oil and its phenolic constituents, APC and HC, have been demonstrated to have antimicrobial properties against a number of oral bacteria[7–11,13]. Its activity against pathogenic, *Vibrio cholerae ogawa, Diplococcus pneumoniae* and *Klebsiella aerogenes* has also been shown[55].

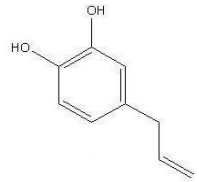
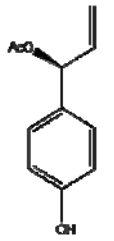
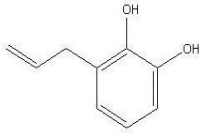
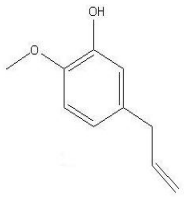
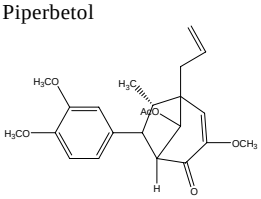
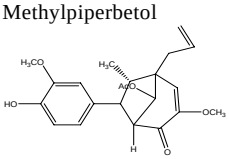
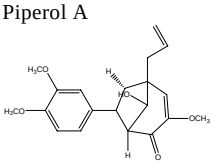
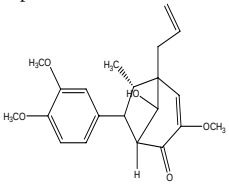
Antileishmanial activity

PBL has long been used in the Indian indigenous system of medicine for skin infections; however, its antileishmanial potential was not explored. Of late, the decrease in response to antimonial compounds has highlighted the need to develop new antileishmanial agents. Accordingly, methanol extract of PB was tested for its antileishmanial activity and found to be active against both promastigotes and amastigotes[56]. This leishmanicidal activity of PB was mediated via apoptosis as evidenced by morphological changes, loss of mitochondrial membrane potential, *in situ* labelling of DNA fragments by terminal deoxyribonucleotidyltransferase-mediated deoxyuridine triphosphate nick end-labelling, and cell-cycle arrest at the sub-G0/G1 phase[57,58]. A patent (Table 2) on antileishmanial activity has also been awarded.

Antifilarial activity

Antifilarial activity *in vitro* in the leaf extract of PB was demonstrated by Tripathi *et al*.[59]. Attempts were also made to observe antifilarial activity of the active extracts and correlate it with the antigen-specific immune responses in a rodent model, *Mastomys coucha* experimentally infected with human lymphatic filarial parasite, *Brugia malayi*. A significantly high level of antifilarial IgG antibody was observed throughout the observation period and *n*-hexane fraction elicited highest IgG titre followed by the crude methanol extract and chloroform fraction respectively. High degree of *in vitro* cellular proliferation of splenocytes of *B. malayi*-infected animals was observed in the presence of T-cell mitogen ConA as well as filarial antigen after *in vivo* administration of crude methanol and *n*-hexane fraction, in contrast to the usual suppressed cellular-responsiveness in infected untreated animals. Treatment with the extracts also led to increased NO production[42]. The induction of differential T-helper cell immune response appears ideal to overcome

Table 3. Specific biological activities and their mode of action of *Piper betle* extracts

Chemical constituent	Specific biological activity	Mode of action	Reference
Hydroxychavicol (HC)/ Hydroxychavicol acetate (HCA) 	Hyperuricemia (antidiabetic)	Acts via xanthine oxidase inhibition	22
	Cytokine production in Th cells (increased IL-2 production and attenuates IFN-gamma expression in Th cells) (immunomodulatory)	Suppressed T-bet expression, which is responsible for IL-2 suppression and IFN-gamma induction in Th cells and inhibited T-bet-mediated Th1 cell differentiation	46
	Oral hygeine	Probably works through the disruption of the permeability barrier of microbial membrane structures	10, 12
	Inhibits platelet aggregation	A potent COX-1/COX-2 inhibitor, ROS scavenger and inhibits platelet calcium signalling, TXB(2) production and aggregation	20
	Chemopreventive against the tobacco-specific carcinogens	Suppressed the mutagenic effects of tobacco-specific *N*′-nitrosonornicotine and 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone	5, 52
	Oral KB carcinoma cells	Inhibits the growth, adhesion and cell cycle progression of KB cells, whereas induction of KB cell apoptosis (HC > 0.1 mM) was accompanied by cellular redox changes	31
Allylpyrocatechol (APC) 	Gastric ulcer-healing action	Mediated by modulation of arginase metabolism and shift of cytokine balance	48
	Protection against ulceration	Protects indomethacin-induced gastric ulceration due to its antioxidative and mucin-protecting properties	37
	Anti-inflammatory effect	Targets the inflammatory response of macrophages via inhibition of iNOS, COX-2 and IL-12 p40 through down-regulation of the NF-kappaB pathway, indicating that APC may have therapeutic potential in inflammation associated disorders	29
Chavibetol (CHV) 	Photoprotective/radioprotective	Protects photosensitization-mediated lipid peroxidation of rat liver mitochondria; prevents γ-ray induced lipid peroxidation as assessed by measuring TBARS	34, 60, 61
Piperbetol 	Platelet hyperactivity/cardiovascular diseases due to intravascular thrombosis	Selectively inhibited platelet aggregation induced by platelet activating factor (PAF) in a concentration-dependent manner. These constituents are effective PAF receptor antagonists *in vitro*. These phenols had no effects on the cAMP contents in resting rabbit platelets.	18
Methylpiperbetol 			
Piperol A 			
Piperol B 			

immunosuppression as observed in the case of lymphatic filarial infection of *B. malayi*, which may be extended to other parasitic infections as well.

Protection against abiotic stress (photo/ionizing radiation)

The PB phenolics, APC and chavibetol (CHV), were found to protect photosensitization-mediated lipid peroxidation (LPO) of rat liver mitochondria. APC was significantly more potent than CHV on LPO. Better activity of APC compared to CHV could be attributed to its higher reactivity with $O_2^{\bullet-}$ as revealed by the rate constant values of $O_2^{\bullet-}$ quenching by the respective phenolics. APC also prevented the detrimental effects of the type II photosensitization-induced toxicity to mouse fibroblast L929 cells. Thus, APC may play an important role in protecting biological systems against photodamage, by eliminating reactive oxygen generated from certain endogenous photosensitizers[60]. PBE was shown to possess radio-protective activity. The extract effectively prevented γ-induced LPO as assessed by measuring TBARS, lipid hydroperoxide and conjugated diene in rat liver mitochondria. Likewise, it prevented radiation-induced DNA strand breaks in a concentration-dependent manner in plasmid DNA. The radical scavenging capacity of PBE was primarily due to its constituent phenolics, CHV and APC[34,61].

Studies with purified compounds/constituents of PB

Most of the studies on PB used crude extracts or fractions; only a few studies have been carried out using purified compounds (Table 3). Generally, the activities were related to phenolic constituents such as HC, hydroxylchavicol acetate, APC, CHV, piperbetol, methylpiperbetol, piperol A and piperol B isolated from leaf. An array of activities has been attributed to these compounds (Table 3). Briefly, the activities include: hyperuricemia (antidiabetic), cytokine production in Th cells (increases IL-2 production and attenuates IFN-gamma expression in Th cells), immunomodulatory, oral hygiene, inhibits platelet aggregation, against the tobacco-specific carcinogens, oral KB carcinoma cells, gastric ulcer-healing action, indomethacin-induced stomach ulceration and anti-inflammatory effect were some of the responses studied. HC was found to be a more potent xanthine oxidase inhibitor than allopurinol, which is clinically used for the treatment of hyperuricemia.

To conclude, PB is used in chewing, ingestion and topical application. PB chewing causes a warm feeling and increased blood circulation within a short time. This is primarily due to rapid entry and transport of molecules in the blood stream through buccal mucosa and freshening of breath by suppression of halitosis and rapid destruction of oral bacteria. Other effects are due to ingestion and they are observed over a period of time, such as digestive stimulant, carminative antiworm, hepatoprotective, etc. Topical application of PBL on wounds as a dressing and use of petiole as suppository in infants are still being practised in the Indian subcontinent.

Future perspectives

PB – a plant of antiquity with its global spread in terms of distribution, its acceptance by diverse cultural groups and known for ethnomedicinal properties – is bestowed with a unique position in the list of medicinal plants. Due to the higher phenol content in the leaf, the plant possesses high antioxidant activity and other pharmacological activities. A number of pharmacological activities such as antidiabetic, immunomodulatory, cardiovascular and anticancer were demonstrated in the last two decades. Some patents were also awarded on the biological activities in the last ten years.

PB also offers a possibility for use in drug delivery through buccal mucosa bypassing the gastric route, where the drug has to endure gastric juices and acidic pH. Importance of buccal drug delivery has been underlined[61]. Recently, the potential of transgenic plants as vaccine has been highlighted[62]. Due to its therapeutic properties and obligate vegetative propagation, PB offers an excellent system for making transgenics. Better understanding of the biological effects and chemical constituents of PB landraces with respect to dioecy will help in developing drugs by adopting out-of-the-box approaches.

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