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PHYTOCHEMISTRY AND PHARMACOLOGY OF THREE *PIPER* SPECIES: AN UPDATE

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ABSTRACT: In this review, the current knowledge on the phytochemistry and pharmacology of *Piper betle* (betel), *Piper sarmentosum* (wild pepper) and *Piper caninum* (wild betel) is updated with some description of their botany and uses. Leaves of *P. betle* contain polyphenols, alkaloids, and essential oils, and display broad-spectrum antibacterial activity, substantial quorum sensing inhibition and tyrosinase enhancement activity. They also possess anti-malarial, anti-diabetic, anti-inflammatory, antinociceptive, hypoglycaemic, neuro-protective and hepatoprotective properties. Leaves of *P. sarmentosum* contain phenylpropanoids, phenylpropanoyl amides, dihydroxyflavone, and essential oils. Wild pepper displays a wide array of pharmacological properties including antioxidant, antibacterial, antifungal, anti-amoebic, anti-dengue, anti-tuberculosis, cytotoxic, antiplasmodial, neuromuscular-blocking, antinociceptive, anti-inflammatory, hypoglycaemic, anti-atherosclerosis and anti-osteoporosis activities. Leaves of *P. caninum* contain phenolic compounds, alkaloids, and essential oils. Pharma-cological properties of wild betel include antioxidant, antibacterial, antifungal, DNA-damaging, DNA strand-scission, and anticancer activities. All three Piper species reviewed possess pharmacological properties, which confer their traditional and contemporary uses as food and herbal medicine.

INTRODUCTION: In this review, the phytochemistry and pharmacology of three *Piper* species (*P. betle*, *P. sarmentosum*, and *P. caninum*) are updated with some description of their botany and uses. Of these species, *P. betle* is well reviewed while there is only one review for *P. sarmentosum* and none for *P. caninum* to date. This review is deemed appropriate and relevant in terms of content and timeliness.

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The genus *Piper* of the family Piperaceae has about 1000 species in the Neotropics with some 300 species found in Southeast Asia [1]. *Piper* species are rather uniform morphologically, with simple, alternate leaves and jointed stems with enlarged nodes.

Inflorescences are distinctive with many tiny flowers packed into upright or pendant spikes. Each flower matures into a tiny one-seeded drupelet, which together forms the multiple fruits. Species in the New World are bisexual while those in the Old World are dioecious. *Piper betle* L. or betel is a widely cultivated dioecious woody vine that can grow up to 20 m in length [2]. Stems are swollen at the nodes with adventitious roots for adhering in

climbing. Leaves are bright green, alternate, and heart-shaped, with 2-3 pairs of secondary veins and acuminate apex **Fig. 1**. They are aromatic and taste from sweet to pungent.

Recognized as one of the important plants in South Asia, *P. betle* has been ranked second to coffee and tea in terms of daily consumption [3]. The species has earned a reputation of being the "Green Gold of India" [4]. Its leaves are best known as a component of betel quid **Fig. 2**, consisting of slices of areca nut (*Areca catechu*) wrapped in *P. betle* leaves with a spread over of slake lime [5]. Often, other components such as tobacco or spices are added for flavoring. Betel chewing is common in countries of South and Southeast Asia, the Pacific Islands and the Middle East. Chewing the betel quid discolors the teeth, and stains saliva, mouth and lips red [2].

FIG. 1: BETEL LEAVES

It results in copious salivation, inducing frequent spitting. Chewing the quid produces a sense of well-being, alertness, warm sensation, and exhilarating feeling. Traditional uses of betel leaves include remedy for a headache, difficulty in urination, cough, sore throat, constipation, arthritis, wounds and boils [4].

Betel leaves can prevent bad breath (halitosis), improve vocalization, harden the gum, protect the teeth and reduce flatulence [6]. The leaves are believed to be effective in treating indigestion, bronchitis, constipation, congestion, cough, and asthma. They have been reported to possess anti-microbial, insecticidal, antioxidant, antinociceptive, antidiabetic and gastroprotective properties. The juice of *P. betle* leaves is given to children with a cough and indigestion.

FIG. 2: BETEL QUID

Piper sarmentosum Roxb. or wild pepper is a creeping stoloniferous herb with slender erect plantlets [7, 8]. Leaves are bright green, thin, papery and ovate to sub-orbicular with 5-7 distinct veins radiating from the base **Fig. 3**. Male and female flowers are white, and fruits are an obovoid berry.

The aromatic leaves with a pungent taste are consumed raw as ulam. In Southeast Asia, the plant has various uses in traditional medicine [9, 10]. In Malaysia and Indonesia, the leaves and roots are used for treating headache, toothache, coughs, asthma, fungal dermatitis and pleurisy. In Thailand, the roots are used as carminative and stomachic while the fruits and leaves are used as an expectorant.

FIG. 3: LEAVES OF *PIPER SARMENTOSUM*

Piper caninum Blume or wild betel (Piperaceae) is also a dioecious woody vine climber with stems having swollen nodes, which produce adventitious roots [7, 11]. Leaves of *P. caninum* are chartaceous, variable in shape with an acuminate apex and 2-3 pairs of secondary veins **Fig. 4**.

Photo by CSIRO

FIG. 4: LEAVES AND FRUITS OF *PIPER SARMENTOSUM*

The under the surface is glaucous, and the upper surface is green when fresh, darkening when dry. Inflorescences are terminal, erect with dense flowers. Fruits are globose with a persistent stigma and red when ripe. Leaves of *P. caninum* are chewed as a substitute for *P. betle* and for treating hoarseness [12]. Mothers would bathe with *P. caninum* leaves after childbirth. The Mah Meri aboriginal people in Peninsular Malaysia use the fruits as a food flavoring.

***P. betle* (Phytochemistry):** Polyphenols and alkaloids isolated from *P. betle* leaves were *β*-sitosterol, dotriacontanoic acid, tritriacontane, stearic acid, cepharadione and piperine [13]. Other phenolic compounds were hydroxychavicol, chavibetol, chavibetol acetate and eugenol [14].

Some 36 compounds representing 98% of the essential oil of *P. betle* leaves have been identified [15]. Eugenol (36%), chavibetol acetate (17%), 4-allyl phenyl acetate (9%) and 4-allylphenol (7%) were the main components. In the Philippines, major constituents of *P. betle* leaf oil were chavibetol (53%) and chavibetol acetate (16%) [16].

***P. betle* (Pharmacology):**

Antioxidant Properties: Among leaves of 10 ulam herbs studied, *P. betle* ranked fifth suggesting that its antioxidant properties were moderate [17].

A recent study on the antioxidant properties of the various components of betel quid showed that *P. betle* leaves had significantly lower phenolic content, ferric reducing power and free radical scavenging activity than areca nut and gambir [18].

Tyrosinase Enhancement Activity: Using the modified dopachrome method, negative tyrosinase inhibition (−20%) has been reported in leaves of *P. betle* [19]. The tyrosinase enhancement effect of *P. betle* leaves suggests their melanogenic or skin-darkening properties, unlike plant species with positive tyrosinase inhibition or skin-lightening properties [20].

Antibacterial and Anti-QS Properties: Betel leaves inhibited Gram-positive bacteria of *Brevibacillus brevis*, *Micrococcus luteus* and *Staphylococcus cohnii*, and Gram-negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* [19]. Minimum inhibitory dose ranged from 0.50−2.00 mg/disc. Similarly, the aqueous extract of *P. betle* leaves has been reported to inhibit *Streptococcus mitis*, *Streptococcus sanguinis*, and *Actinomyces viscosus*, which are early colonizers of dental plaque [21].

Allylpyrocatechol, the major active principle of *P. betle* leaf extract, showed promising activity against obligate oral anaerobes responsible for halitosis or mouth odor [22]. Betel leaves exhibited strong quorum sensing (QS) inhibition against the Gram-negative *Chromobacterium violaceum* bacteria [23]. Diameters of the inhibition zone of violacein production and *C. violaceum* growth were 20 mm and 16 mm, respectively. Mean inhibition doses were 0.01 and 0.25 mg/disc. Although the anti-QS activity of *P. betle* leaves has earlier been reported [24], no quantitative data were provided. Leaves of *P. betle* have been reported to possess antibacterial including anti-QS activity [23]. It is interesting to note that both these properties have also been reported in areca nut [25, 26]. Since. both *P. betle* leaves and areca nut are essential components of betel quid, it would imply that the traditional and customary practice of chewing betel quid do have the therapeutic effect of inhibiting growth and virulence of oral pathogens including QS bacteria.

Cytotoxic Activity: Ethyl acetate and hexane leaf extracts of *P. betle* had a dose-dependent inhibitory effect on the MCF-7 human breast cancer cells with IC_{50} values of 65 and 163 µg/ml, respectively [27]. Using the neutral red cytotoxicity assay, the aqueous *P. betle* leaf extract has been reported to display antiproliferative activity against KB cancer cells with an IC_{50} value of 30 µg/ml [28].

However, no activity was observed against HeLa cells. Methanol leaf extract of *P. betle* at 40 μg/ml showed strong activity toward Epstein-Barr virus (EBV) activation in Raji cells [29].

Anti-malarial Activity: The methanol leaf extract of *P. betle* has demonstrated significant in vivo anti-malarial properties against *Plasmodium berghei* during early and established infections [30]. Evaluations were based on three evaluation models of suppressive, curative and prophylactic anti-plasmodial activities at doses of 50, 100, 200 and 400 mg/kg. The acute oral toxicity limit test on mice showed a median lethal dose (LD_{50}) greater than 5000 mg/kg, suggesting that the extract is safe to use.

Platelet Inhibition Activity: Hydroxychavicol (HC), a component of *P. betle* leaves, was tested for its inhibition effect on platelet aggregation [31]. Results showed that HC could inhibit the cyclooxygenase activity of COX-1/COX-2, platelet calcium signaling, and thromboxane B_2 production and aggregation, and to scavenge reactive oxygen. The study concluded that HC could be a potential therapeutic agent for the prevention and treatment of atherosclerosis and other cardiovascular diseases through its anti-inflammatory and anti-platelet activities.

Anti-halitosis Activity: The ether betel leaf extract and fractionated allylpyrocatechol were found to have promising inhibitory activity against obligate oral anaerobic bacteria responsible for halitosis [22]. Due to its antimicrobial activity, the extract and compound have the potential to reduce methyl mercaptan and hydrogen sulphide responsible for oral malodor. Betel leaves could thus be used in the prevention of halitosis and the treatment of periodontal infection caused by oral anaerobes.

Anti-diabetic Activity: The anti-diabetic activity of *P. betle* leaves was tested using normoglycaemic and streptozotocin (STZ)-induced diabetic rats by oral administration of aqueous and ethanol extracts [32]. In normoglycaemic rats, both extracts significantly lowered the blood glucose level in a dose-dependent manner. The anti-diabetic activity of the aqueous extract was comparable to that of the ethanol extract. In the glucose tolerance test, both extracts markedly reduced external glucose load and blood glucose level. The ability to lower blood glucose levels of STZ-induced diabetic rats suggested that the extracts have insulinomimetic activity. Another study evaluated the feasibility of using *P. betle* leaves for treating diabetes mellitus [33]. Newly diagnosed Type-2 diabetic patients from either sex were selected (n = 50 per group). Betel leaves were given to the patients for 30 days in comparison with Triphala (an anti-diabetic herbal drug).

Results showed that the blood glucose levels of betel-treated patients were significantly reduced by 22% and 25% at the end of the second and fourth week, respectively. The blood glucose levels of triphala-treated patients were significantly reduced by 14% and 24% over the same period. There were no toxic effects in terms of hepatotoxicity, renotoxicity and hematological parameters in both groups. The study showed that *P. betle* leaves can be used as a potential pharmaceutical for Type 2 diabetic patients.

Anti-fertility Effect: The anti-fertility effect of the leaf stalk extract of *P. betle* in male albino mice was evaluated [34]. Initially, 500 mg of the extract were orally fed for 30 days and then 1000 mg for the next 30 days. The extract reduced fertility to 0% within 60 days. Suppression of sperm count and motility was observed.

Two months after extract administration, the altered parameters recovered including organ weight and fertility. Similarly, the extract had anti-fertility effects on female albino mice, which showed a decrease in reproductive organ weight, circulating level of estrogen, fertility, number of litters, serum glucose concentration and acid phosphatase activity [35]. One month after extract administration, these parameters were completely or partially restored.

Radioprotective Activity: The radioprotective activity of ethanol extract of *P. betle* leaves was evaluated using rat liver mitochondria and pBR 322 plasmid DNA as in vitro model systems [36]. The extract effectively prevented gamma ray-induced lipid peroxidation assessed by measuring thiobarbituric acid reactive substrates, lipid hydroperoxide and a conjugated diene. It also prevented radiation-induced DNA strand breaks in

a concentration-dependent manner. The radio-protective activity of the extract was attributed to its hydroxyl and superoxide radical scavenging ability along with its lymphoproliferative activity. Its radical scavenging capacity was probably due to its phenolic constituents comprising mainly of chevibetol and allylpyrocatechol.

Anti-ulcerogenic Activity: The ethanol *P. betle* leaf extract, orally administered to rats at a dose of 200 mg/kg for 10 days, was found to have a significant protective effect against gastric lesions induced by indomethacin [37]. The extract resulted in significant increase in superoxide dismutase and catalase activity, increase in mucus, hexosamine and total thiol group content, but marked reduction in oxidative protein and peroxidized lipid levels as compared to the control.

The protective and healing effects of ethanol *P. betle* leaf extract against the indomethacin-induced gastric ulceration in rats were also reported [38]. The superior anti-ulcerogenic activity of the extract was attributed to its high antioxidative content and its ability to augment the stomach mucin level.

Anti-hyperglycemic Activity: The anti-hyper-glycemic activity of methanol *P. betle* leaf extract was evaluated using the oral glucose tolerance test in glucose-loaded albino mice [39]. The extract showed dose-dependent effects with significant lowering of blood sugar in the mice.

At extract doses of 50-400 mg/kg, blood sugar levels declined by 31-47%. Glibenclamide, a standard anti-hyperglycaemic drug, when orally administered at a dose of 10 mg/kg lowered blood glucose levels by 46%. As such, the results strongly indicated that *P. betle* leaves possess potent anti-hyperglycaemic properties. In an earlier study, oral administration of two doses of *P. betle* leaf extract (75 and 150 mg/kg) to STZ-induced diabetic rats for 30 days resulted in significant reduction in blood glucose level [40].

Antinociceptive Activity: The antinociceptive activity of methanol *P. betle* leaf extract was demonstrated in albino mice with gastric pain induced by intraperitoneal administration of acetic acid [39]. At extract doses of 50-400 mg/kg, the reduction in the number of writhings was 47-71% as compared to the control. Aspirin, the standard antinociceptive drug, when administered at doses of 200 and 400 mg/kg, reduced the number of writhings by 51% and 67%, respectively.

The extract, therefore, appeared to be more potent than aspirin in the alleviation of pain. In another related study, the analgesic activity of methanol *P. betle* leaf extract in albino mice was evaluated by a hot plate, writhing and formalin tests [41]. At doses of 100 and 200 mg/kg, the extract resulted in a significant increase in the pain threshold using the hot plate method, and a significant reduction in the number of writhings caused by acetic acid and the number of licks induced by formalin.

Anti-inflammatory Activity: The anti-inflammatory activity of methanol *P. betle* leaf extract in albino mice was evaluated using carrageenan-induced hind paw edema method [41]. At doses of 100 and 200 mg/kg, the extract caused a significant inhibition of carrageenan-induced paw edema after 4 hours in a dose-dependent manner.

Neuroprotective Activity: The protective effect of aqueous leaf extract of *P. betle* has been demonstrated in the brain of ethanol-treated rats [42]. The brain of ethanol-treated rats exhibited increased levels of lipids, lipid peroxidation and disturbances in antioxidant defense. Subsequently, administration of 100, 200 and 300 mg/kg of extract for 30 days, resulted in a significant reduction of lipid levels and lipid peroxidation. Extract dose of 300 mg/kg was the most effective.

Hepatoprotective Activity: The hepatoprotective activity of aqueous leaf extract of *P. betle* against ethanol toxicity was evaluated using ethanol-treated rats [43]. Administration of ethanol (8 g/kg) for 60 days resulted in significant elevation of hepatic markers such as alkaline phosphatase, γ-glutamyl transferase, and bilirubin in serum compared to the control. Co-administration of *P. betle* leaf extract for 30 days at doses of 100, 200 and 300 mg/kg significantly decreased the level of hepatic and lipid peroxidation markers. The leaf extract at 300 mg/kg was the most effective. The results were comparable with silymarin, a known hepatoprotective drug.

***P. sarmentosum* (Phytochemistry):** Early phytochemical isolation reported one new and three known phenylpropanoids from the leaves of *P.*

sarmentosum [44], and six isobutylamides in homologous series and a new methyl butyl amide isolated from the hexane plant extract [45].

Subsequently, eight amides, two lignans, and four other compounds were isolated from the sequential hexane and methanol fruit extracts of *P. sarmentosum* [9]. From the roots of *P. sarmentosum*, chemical isolation yielded 16 compounds of which three were new (sarmentamide A, B, and C) [46]. Of the 13 known compounds, six were new to the species. Guided by the mitochondrial transmembrane potential assay, the four new C-benzylated dihydroxyflavone together with 13 known compounds have been isolated from the chloroform plant extract of *Piper sarmentosum* [47].

Recently, bioassay-guided fractionation of the sequential leaf extract of *P. sarmentosum* led to the isolation of three new phenylpropanoid amides of chaplupyrrolidones A and B containing a unique 5-oxygenated-Δ^3-2-pyrrolidone moiety and deacetyl-sarmentamide B [48]. A study of the chemical composition of essential oils from four *Piper* species from Vietnam reported that the leaf and stem oil of *P. sarmentosum* showed a chemical profile characterized mainly by aromatic compounds and devoid of monoterpene hydrocarbons [49].

The major constituents were benzyl benzoate (49%), benzyl alcohol (18%), 2-hydroxybenzoic acid phenylmethyl ester (10%) and 2-butenyl benzene (8%). Earlier, myristicin (65%), trans-caryophyllene (14%) have been reported to be the two predominant components of the leaf oil from *P. sarmentosum* [50].

***P. sarmentosum* (Pharmacology):**

Antioxidant Properties: Among leaves of 10 ulam herbs studied, *P. sarmentosum* ranked eighth suggesting that its antioxidant properties were weak [17]. Total phenolic content and free radical scavenging values of *P. betle*, which ranked fifth, were 1.8 and 3.4 times those of *P. sarmentosum*, respectively.

Earlier, the methanol leaf extract of *P. sarmentosum* at 250 μg/ml was reported to exhibit 88% superoxide scavenging compared to superoxide dismutase as standard [51]. Naringenin, identified in the HPLC chromatogram of the extract, had 76% superoxide scavenging activity. Oral supplementation of *P. sarmentosum* extract of 125 mg/kg for 28 days was able to significantly reduce lipid peroxidation and glutathione peroxidase due to oxidative stress induced by carbon tetrachloride in rats [52].

Antibacterial Activity: Out of four phenylpropanoids isolated from *P. sarmentosum* leaves, 1 - allyl - 2, 6-dimethoxy - 3, 4-methylenedioxybenzene showed antibacterial activity against *Escherichia coli* and *Bacillus subtilis* [44]. The methanol leaf extract of *P. sarmentosum* exhibited antibacterial activity against both Gram-positive *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), and Gram-negative *Pseudomonas aeruginosa* [53].

Antifungal Activity: Isolated from the roots of *P. sarmentosum*, brachyamide B and sarmentosine displayed antifungal activity against a clinical isolate of *Candida albicans* [46].

Anti-amoebic Effect: Orally administered crude methanol root extract of *P. sarmentosum* root at a daily dose of 1000 mg/kg for five days was reported to have anti-amoebic effects against *Entamoeba histolytica* infection in the caecum of mice [54].

Anti-dengue Activity: The ethanol plant extract of *P. sarmentosum* possessed larvicidal effect on larvae of dengue mosquitoes of *Aedes aegypti* (LC_{50} of 4.06 ppm) [55]. Concurrently, the extract was also found to exert adulticidal activity (LC_{50} of 0.14 μg) when tested against female *A. aegypti* mosquitoes [56].

Anti-tuberculosis Activity: Of 78 methanol plant extracts from 70 Malaysian plant species screened for anti-tuberculosis activity against *Mycobacterium tuberculosis* using a colorimetric microplate-based assay, positive activity was found in *P. sarmentosum* with minimum inhibition concentration (MIC) of 800 μg/ml [57].

The amides isolated from fruits of *P. sarmentosum* displayed anti-tuberculosis activities against *Mycobacterium tuberculosis* [9]. From the roots of *P. sarmentosum*, seven compounds exhibited anti-mycobacterial activity [46].

Cytotoxic Activity: The chloroform leaf extract of *P. sarmentosum* was evaluated for cytotoxic activity using the MTT cell viability assay [58]. Results showed that the extract inhibited HepG2 and HUVEC human cancer cells with IC_{50} values of 76 and 64 µg/ml, respectively. Phytochemical investigation of sequential petroleum ether and chloroform extract of *P. sarmentosum* yielded three amides and a sterol [59]. When the chemical constituents were tested for their cytotoxic activity using the sulforhodamine B (SRB) assay, none of the compounds was active as an anticancer agent.

Anti-plasmodial Activity: Leaf extracts of *P. sarmentosum* have been reported to possess anti-malarial properties [60]. Against *Plasmodium falciparum*, the chloroform extract at 0.05 mg/ml and the methanol extract at 0.80 mg/ml resulted in 100% inhibition after 48 h. Sarmentine and 1-piperettyl pyrrolidine isolated from the fruits exhibited anti-plasmodial activities [9], and sarmentine and sarmentosine isolated from the roots exhibited anti-plasmodial activity [46].

Neuromuscular-Blocking Activity: When tested on the phrenic nerve hemi-diaphragm of rats, the methanol leaf extract of *P. sarmentosum* has been reported to possess a marked neuromuscular-blocking activity at the neuromuscular junction [61]. The authors postulated that the inhibition of neurotransmitter (acetylcholine) release at the presynaptic terminal as a possible mechanism.

Antinociceptive and Anti-inflammatory Activities: When tested on mice, the aqueous leaf extract of *P. sarmentosum* at doses of 30, 100 and 300 mg/kg showed significant antinociceptive and anti-inflammatory activities [10]. Antinociceptive activity evaluated by abdominal constriction and hot-plate tests, and anti-inflammatory activity evaluated using carrageenan-induced paw oedema test showed that the extract exerted significant activities in a dose-dependent manner at all doses used. Concurrently, the ethanol root extract of *P. sarmentosum* has also been reported to possess and anti-inflammatory antinociceptive and antipyretic properties [62].

Hypoglycemic Effect: The hypoglycemic effect of the water aqueous plant extract of *P. sarmentosum* was examined in normal and streptozotocin-diabetic rats [63]. In the oral glucose tolerance test, a single oral administration of the extract at doses of 0.125 and 0.25 g/kg significantly lowered the plasma glucose level in the normal rats but not in the diabetic rats. However, repeated oral administration of the extract at 0.125 g/kg for seven days produced a significant hypoglycemic effect in the diabetic rats.

Following report that *P. sarmentosum* has anti-diabetic properties, a study was conducted to evaluate its effects on diabetic cardiovascular tissues [64]. Rats with the extract (0.125 g/kg orally administered daily for 28 days) showed increase in body weight, and decrease in fasting blood glucose and urine glucose level compared to the D group. Under transmission electron microscopy, they showed lesser ultra-structural degenerative changes in the cardiac tissues and proximal aorta, suggesting that *P. sarmentosum* can restore the ultra-structural integrity in diabetic cardiovascular tissues.

Anti-atherosclerosis Activity: Rabbits administered with 500 mg/kg aqueous extract of *P. sarmentosum* for 10 weeks displayed a significant reduction in the fatty streak (30%) compared to the high cholesterol group (86%) [65].

Anti-osteoporosis Activity: A study was conducted on radiological changes in fracture calluses in ovariectomized osteoporotic rats following the administration of an aqueous leaf extract of *P. sarmentosum* (125 mg/kg) for six weeks [66]. Results showed that the extract improved fracture healing, as assessed by the reduced callus volumes and reduced callus scores, suggesting that the extract is beneficial for fractures in osteoporotic rats.

Pharmacokinetics Study: This pioneering study was the first on the pharmacokinetics on an ethanol fruit extract of *P. sarmentosum* fruit in Sprague-Dawley rats at an oral dose of 500 mg/kg [67]. HPLC analysis with ultraviolet detection was employed to quantify pellitorine, sarmentine and sarmentosine in plasma, tissues, feces, and urine to calculate the pharmacokinetic parameters. Sarmentosine exhibited zero oral bioavailability because it was not detected in the plasma, tissues or urine. Pellitorine was found distributed in the intestinal wall, liver, lungs,

kidney, and heart, whereas sarmentine was found only in the intestinal wall and heart. The cumulative excretion of pellitorine, sarmentine, and sarmentosine in feces in 72 h was 0.08, 0.98 and 0.44 µg, respectively. This study shows that pellitorine and sarmentine have good oral bioavailability while sarmentosine is not absorbed in the gastrointestinal tract.

***P. caninum* (Phytochemistry):** Isolated from leaves of *P. caninum* were three flavonoids (5, 7 -dimethoxyflavone, 5, 7 -dimethoxyflavanone and 4', 5, 7-trimethoxyflavone), and two amides [68]. Safrole (17%), β-pinene (9%), linalool (7%) and β-caryophyllene (7%) were the main components of the leaf oil of *P. caninum* while safrole (26%), β-caryophyllene (10%) and germacrene D (8%) were the main components of the stem oil [69].

***P. caninum* (Pharmacology):**

Antioxidant Properties: Among 10 ulam herbs studied, *P. caninum* ranked fourth suggesting that its antioxidant properties were moderately high [17]. Total phenolic content and free radical scavenging values of *P. caninum* were 2.0 and 2.2 times higher than those of *P. betle*, which ranked fifth.

Antibacterial Properties: Methanol leaf extract of *P. caninum* has been reported to inhibit the growth of Gram-positive and Gram-negative bacteria *B. brevis*, *M. luteus*, *S. aureus*, *E. coli* and *S. enterica*, at mean inhibitory doses of 1.0-2.0 mg/disc [70]. Isolated from *P. caninum* leaves, 5, 7-dimethoxyflavone inhibited the growth *Bacillus subtilis* and *E. coli* while 4',5,7-trimethoxyflavone was effective against *B. subtilis* [68].

Leaf and stem oils of *P. caninum* have been reported to inhibit *S. aureus*, *Pseudomonas putida*, *E. coli*, *B. subtilis* and *P. aeruginosa* [69]. The antibacterial activity was attributed to safrole and β-caryophyllene. The chloroform bark extract of *P. caninum* has also been reported to exhibit antibacterial activity against *Bacillus cereus*, *Streptococcus pneumoniae* and *S. aureus* [71]. The antibacterial agents have been isolated and identified as (+)-bornyl *p*-coumarate and bornyl caffeate.

Antifungal Activity: Using the agar diffusion assay, methanol extract of *P. caninum* (20 µl per disc) inhibited pathogenic yeasts of *Candida albicans*, *Rhodotorula rubra* and *Torulopsis glabrata* but not *Cryptococcus neoformans* [72].

DNA-damaging Activity: Cepharadione A (a 4, 5-dioxoaporphine alkaloid) isolated from the dichloromethane-methanol (1:1) twig extract of *P. caninum* has been reported to possess DNA-damaging activity [73]. Using a yeast cytotoxicity assay, cepharadione an exhibited potent inhibitory activity against RS321NpRAD52 grown on glucose with IC_{50} of 50 nM. However, inhibition against the same strain of yeast grown on galactose was weak with IC_{50} of 293 nm.

DNA Strand-scission Activity: In a related study on the dichloromethane-methanol twig extract of *P. caninum*, isolated phenolic acid amides (N-cis-feruloyl tyramine, N-trans-feruloyl tyramine and 1-cinnamoyl pyrrolidine) displayed the ability to cleave DNA [74]. Using a DNA strand-scission assay, these three compounds was found to induce the relaxation of super-coiled pBR322 plasmid DNA in the presence of Cu^{++} and may represent a structurally new type of DNA strand-scission agent.

Anticancer Activity: Bornyl caffeate, found in the bark of *P. caninum*, has been reported to induce apoptosis in human breast cancer MCF-7 cells in a dose- and time-dependent manner via the ROS- and JNK-mediated pathways [75]. Bornyl caffeate increased Bax (pro-apoptotic protein) and decreased Bcl-xl (anti-apoptotic protein), resulting in the disruption of mitochondrial membrane potential (MMP) and subsequent activation of caspase-3.

CONCLUSION: *Piper* species of *P. betle*, *P. sarmentosum* and *P. caninum* reviewed possess pharmacological properties, which confer their traditional and contemporary uses as food and herbal medicine. Betel contains polyphenols, alkaloids and essential oils, and displays broad-spectrum antibacterial activity, substantial quorum sensing inhibition and tyrosinase enhancement activity.

They also possess anti-malarial, anti-diabetic, anti-inflammatory, antinociceptive, hypoglycaemic, neuroprotective and hepatoprotective properties. Wild pepper display a wide array of pharmacological properties including antioxidant,

antibacterial, antifungal, anti-amoebic, anti-dengue, anti-tuberculosis, cytotoxic, antiplasmodial, neuromuscular-blocking, antinociceptive, anti-inflammatory, hypoglycaemic, anti-atherosclerosis and anti-osteoporosis activities. Pharmacological properties of wild betel include antioxidant, antibacterial, antifungal, DNA-damaging, DNA strand-scission, and anticancer activities. Active research is being conducted on *P. sarmentosum* with studies on its pharmacokinetics initiated. There are good opportunities for further and new studies on the pharmacological properties of *P. caninum*. The prospects of all three *Piper* species for development into herbal and pharmaceutical products are promising.

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