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***Piper betle*: a review on its bioactive compounds, pharmacological properties, and extraction process**

N I Azahar¹, N M Mokhtar² and M A Arifin¹

¹ Faculty of Chemical & Process Engineering Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

² Faculty of Civil Engineering Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

Corresponding author: mazmir@ump.edu.my

Abstract. *Piper betle* is a well-known medicinal plant that cultivated primarily in Southeast Asia. This plant is made up of a large number of bioactive compounds such as tannins, flavonoids (quercetin), eugenol, hydroxychavicol and chavibetol that represent the major components of the plant. This plant has been extensively studied for its pharmacological properties such as antimicrobial, anticancer, antioxidant, antidiabetic and anticancer. Many techniques have been used in *Piper betle* extraction such as soxhlet extraction, sonication extraction, maceration, ultrasound assisted extraction (UAE), supercritical fluid extraction (SFE) and microwave assisted extraction (MAE). Various benefits of *Piper betle* extract have been well utilized by the production of numerous types of plant-based products and to date, research on new products based on *Piper betle* is still being done. Application of *Piper betle* extract resulting in wide possibilities of usage in future product development. The quality and safety of *Piper betle* studies provide by toxicity test shows the *Piper betle* extract exhibit little to none toxicity level at respective concentration. This article aims to present a review of previous studies and research works conducted on *Piper betle* to serve as a source of additional information for future research related to the *Piper betle*.

1. Introduction

Piper betle (Family: Piperaceae) is a well-known plant mainly found in East Asian countries like Malaysia, Indonesia, Philippines, India and Sri Lanka. In these countries, there are large plantation farms of *Piper betle* to meet the industrial demand for the production of products such as toothpaste, shampoo, personal care products, herbal supplements and herbal drinks [3][4][5][6]. Previously, *Piper betle* with combination of lime betle, hard fruit, areca nuts and gambier used as mouth refresher [6]. The chewing goods that contains *Piper betle* has disguised benefit of helping to strengthen the teeth and refreshing the mouth [6][7]. Aside from oral usage, *Piper betle* paste is also frequently used to wash hair and intimate areas. Furthermore, the use of *Piper betle* leaves is also found in religious or ritual performances of Malay and Hindu cultures.

The medicinal properties possessed by *Piper betle* is utilized to treat wounds and cuts by crushing and spreading *Piper betle* leaves paste on the injured area [6][7]. The ability of this plant to cure disease is due to presence of many bioconstituents or bioactive compounds throughout the plant [7][8]. Bioconstituents in *Piper betle* is made up of various active ingredients such as tannins, flavonoids (quercetin), eugenol, hydroxychavicol and chavibetol [7][8][9][10]. *Piper betle* reported to possess



various pharmacological properties such as antimicrobial, antifungal, antioxidant, antidiabetic and anticancer [1][5][11][12].

Various parts of *Piper betle* plant are subjected to a series of procedures from the extraction process until compound isolation to retrieve the desired compounds. The extraction process can be executed in any preferred method such soxhlet extraction, sonication extraction, maceration extraction, ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE) [13][14][15].

To our knowledge, many researchers reviewed previous work focused on a few topics and there are limited papers that provide meticulous information and knowledge on different perspectives of *Piper betle*. Thus, the present paper aims to highlight various aspects of *Piper betle*; its bioactive compounds, pharmacological properties, extraction process, applications, quality and safety related to the plant.

2. Taxonomy and cultivation

Piper betle (Figure 1) belongs to Piperaceae family with genus Piper [16][17]. The genus Piper has more than 1000 species across the globe and about 300 species can be found in Southeast Asia [3][11][18]. Among the many species, *Piper betle*, *Piper sarmentosum* and *Piper caninum* are the three (3) most common species in Malaysia [18]. Table 1 shows the taxonomic hierarchy for *Piper betle* [10][19][20][21].



Figure 1. *Piper betle* leaves

Table 1. Taxonomic hierarchy of *Piper betle*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnolipsida
Order	Piperales
Family	Piperaceae
Genus	Piper
Species	betle
Binomial name	<i>Piper betle</i>

The creeper plant has heart-shaped leaves with pointed apex [5][11][22]. *Piper betle* leaves length can extend approximately to 18 cm and 12 cm in width [12]. The leaves give off a sharp and pungent aromatic smell [4][18][22]. The stalk is smooth and long [12][23]. The plant usually cultivated from 150 cm to 180 cm in height in plantation [1]. The height may grow up to 20 metres when left grow

wildly without trimming and cutting [18]. *Piper betle* preferred neutral soil between pH 7 to 7.5 as bed soil [22].

3. Bioactive composition

Piper betle ability to act as a medium for any possible treatment practice in traditional medication is due to its many bioactive components. The bioactive components can be obtained from the extraction process performed on different parts of *Piper betle* such as leaves, vines, branch or roots. *Piper betle* major components (Figure 2) consist of tannins, flavonoids (quercetin), eugenol, hydroxychavicol and chavibetol [7][8][9][10]. Chemical compounds extracted out after series of extraction can be divided into few types depending on the types of solvent immersed and solubilized in the extraction process such as ethanol, methanol, butanol, acetone and aqueous extricate same or different compound respectively such as (1) phenolic compounds, (2) ethanolic compounds, (3) methanolic compounds, (4) butanolic compounds, (5) acetone compounds, and (6) aqueous compounds [10]. Table 2 shows the chemical compounds and bioactive constituents of *Piper betle*.

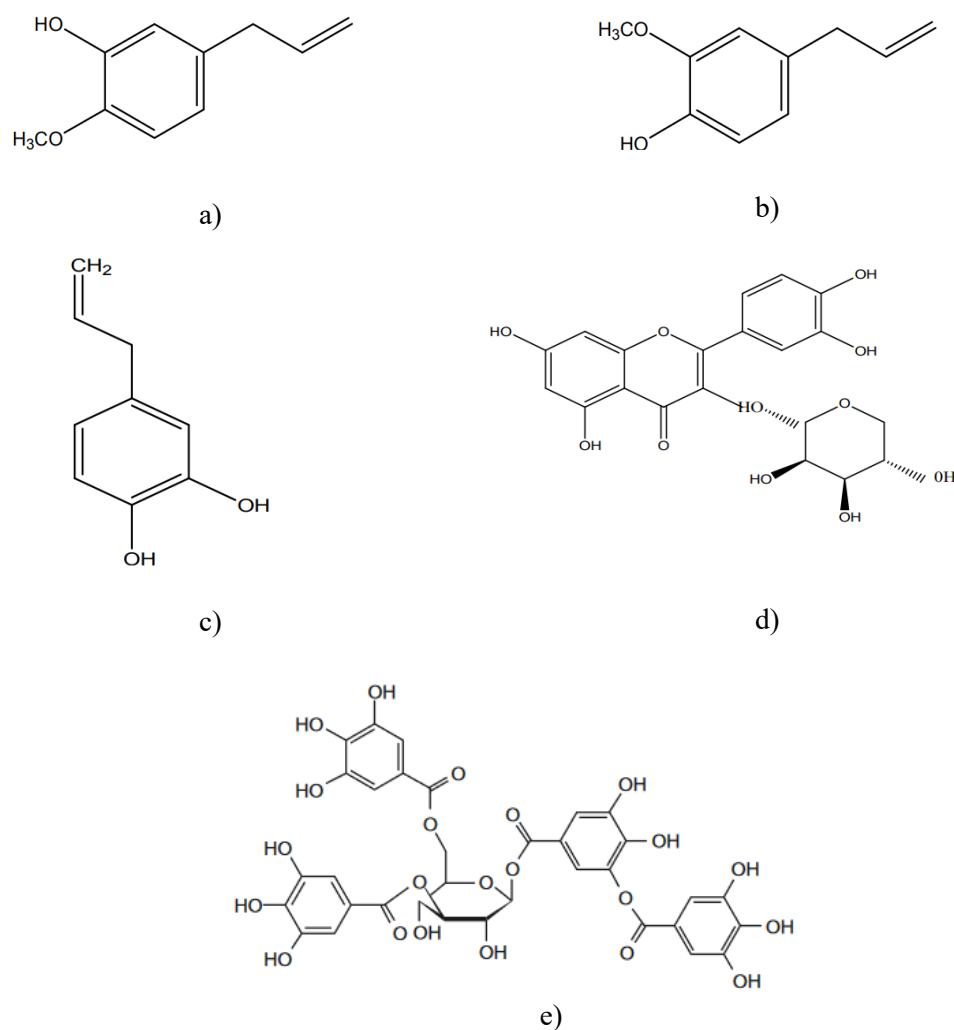


Figure 2. Molecular structure of major bioactive constituents of *Piper betle* a) Chavibetol, b) Eugenol, c) Hydroxychavicol, d) Quercetin (Flavonoids), e) Tannin acid. (Source: Dwivedi & Tripathi, 2014; Pradhan et al., 2013)

Table 2. Chemical compounds and bioactive constituents of *Piper betle*

Chemical Compounds	Bioactive Constituents	References
Phenolic	Chavicol, Hydroxychavicol, Chavibetol, Chavibetol Acetate and Eugenol	[7][8][10][24]
Ethanollic	Steroids, Diterpenes, Tannin, Cardial Glycosides, Flavonoids, Saponin, Phenols, Coumarin and Alkaloids	[7][10][24]
Methanollic	Steroids, Diterpenes, Tannin, and Saponin	[7][10][24]
Butanollic	Steroids, Diterpenes, Tannin, Flavonoids, Emodins and Alkaloids	[7][10][24]
Acetone	Steroids, Diterpenes, Tannin, Flavonoids, Saponin and Coumarin	[7][10][24]
Aqueous	Steroids, Diterpenes, Tannin, Cardial Glycosides, Flavonoids, Saponin, Phenols, Coumarin and Alkaloids	[7][10][24]

4. Pharmacological properties

4.1. Antibacterial

The antibacterial treatment using herbal or plant extracts served as an alternative option for treating bacterial infection. Studies show that *Piper betle* have significant antimicrobial properties that capable in reducing the formation of biofilm induced by the infectious bacteria [10][17][25][26][27]. The antibacterial properties believed to derive from multiple combinations of phytoconstituents such as phenols, sterols, flavonoids, polyphenols, tannins and hydroxychavicol [7][9][10][19][28][29][30].

Antibacterial efficiency has been tested with effective procedures such as disk-diffusion test and agar well diffusion method [31][32]. Based on many experiments, *Piper betle* extracts have proven to be effective in treating gram-positive and gram-negative bacteria by the presence of inhibition zone on nutrient agar [33][34][35]. Areas of inhibition for gram-negative bacteria were smaller compared to gram-positive bacteria due to the presence of cell wall made from lipopolysaccharides (LPS) in gram-positive bacteria. This cell wall is responsible for reducing the penetration of *Piper betle* extract into the bacterial membrane thus lowering the effectiveness of *Piper betle* extract to counter-kill the bacteria [31][36][37][38][39][40].

Hoque et al. performed extraction of *Piper betle* using ethanol as the main solvent for producing extract [6]. The extract was used against *E. coli* ATCC 25922, *E. coli* O157:H7 NCTC 12049, *Vibrio cholerae* ATCC 6395, *S. aureus* ATCC 25923 and *S. dysenteriae-1* MJ-84. Antibacterial properties were displayed with the presence of area of inhibition having diameter ranging from 12.33 mm to 14.67 mm. Budiman et al. treated two (2) different bacterial strains *P. acne* and *S. aureus* with *Piper betle* extract and found that the extracts are capable to reduce the bacterial growth [28]. *Piper betle* contained fatty acid compounds, hydroxy fatty acid ester and hydroxychavicol [7][8]. Anionic surfactants present in fatty acids have an antibacterial and antifungal activities at low pH especially on the gram-positive bacteria cell wall and membrane [41]. Furthermore, hydroxychavicol have positive feedback against bacterial strains [42].

Datta et al. have investigated the effect of *Piper betle* extract against four (4) strains of bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus vulgaris* by using disc diffusion method and found that the minimum inhibition concentration (MIC) for both *Klebsiella pneumonia* and *Proteus vulgaris* are 25 µg followed by *Pseudomonas aeruginosa* 35 µg and *Staphylococcus aureus* 40 µg [32]. Bangash et al. had extracted *Piper betle* with three (3) different solvents namely ethanol, chloroform and petroleum ether and tested against eleven (11) bacterial strains namely *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Pseudomonas putida*, *Klebsiella pneumonia*, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus*

mirabilis and *Vibrio cholera* [33]. The experiment revealed that ethanolic *Piper betle* extracts produced bigger area of zone of inhibition compared to the other two (2) solvents, possibly due to presence of phenolic compounds.

4.2. Antifungal

Fungal infections often cause the infected area to experience various discomfort such as itching, redness and irritation. In addition to bacterial infections, *Piper betle* extract is also often used to treat fungal infections. The use of *Piper betle* extract was proven in accordance with experiments conducted in the laboratory using disk-diffusion test and agar well diffusion method.

Ali et al. has experimented with hydroxychavicol, a compound isolated from *Piper betle* extract against two (2) species of fungus namely *Candida* and *Aspergillus* [17]. The study was focused on finding the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of hydroxychavicol. The clinical result of MIC for *Candida* species were determined to be ranging from 15.62 µg/ml to 500 µg/ml and 125 µg/ml to 500 µg/ml for *Aspergillus* species. As for MFC, the test showed similar or a double fold result compared to MIC. This study also confirmed the important reaction of hydroxychavicol to *Candida* species where hydroxychavicol was found to be a bioconstituent responsible for minimizing biofilm formation. The formation of biofilms is measured by the escalated uptake of propidium iodide by *Candida albicans* proving the membrane was indeed disrupted by hydroxychavicol.

Whereas Sarma et al. investigated the capability of *Piper betle* leaves extract against both bacteria and fungi [22]. *Piper betle* extracted with four (4) different solvents; water, hexane, acetone and ethanol, to determine the most suitable solvent for extracting *Piper betle*. The research proceeded by testing two (2) fungi strains *Aspergillus niger* and *Candida albicans* with *Piper betle* extract. All extracted indeed showed an inhibition area against both bacteria and fungi. The study revealed hexane, acetone and aqueous extracts provided larger area of inhibition compared to ethanol. Kaypetch & Thaweboon used the disk diffusion method to study three (3) different strains of *Candida* species namely *C. albicans*, *C. krusei* and *C. tropicalis* [43]. Results exposed that *Piper betle* extracts contained antifungal properties against all tested fungi species with the presence of zone of inhibition with diameter ranging from 32 mm to 35 mm.

Sivareddy et al. had extracted *Piper betle* mature leaves using ethyl ethanol [44]. The extract was used against *Candida albicans* resulting in the maximum inhibitory zone of 26 mm, higher when compared to Tulsi leaves extract that yielded maximum inhibitory zone of only 13 mm. Grag & Jain examined multiple fungi strains against *Piper betle* neat oil [45]. The study showed that *Piper betle* neat oil produced inhibition zone for all tested fungi strains such as *Ctenomyces serratus* (69 mm), *Arthroderma benhamiae* (65 mm), *Microsporium gypseum* (62 mm), *A. fumigatus* (62 mm), *Asper niger* (60 mm), *Trichophyton mentagrophytes* (60 mm), *A. ochraceous* (51 mm), *Aspergillus candidus* (40 mm) and *A. flavous* (35 mm).

4.3. Antioxidant

Antioxidant act as an agent that slows down the damage faced by cell towards free radical and chemical reactions performed by our body system against environmental stresses and other possible causes. An antioxidant is known as free-radical scavenger derived from either synthetic, natural or combination of both. The antioxidant level in certain extract may be tested using DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenger test [22]. DPPH scavenging activities occurs due to hydrogen-donating properties that may be present in the tested compound [22]. The ability to donate electrons and reducing power are due to the presence of reductones [22][46][47][48]. The antioxidant reacted with certain precursors of peroxides and hindering peroxide formation by breaking free radical chains [46].

Sarma et al. determined free radical scavenging activity of *Piper betle* extract by using DPPH [22]. The antioxidant properties owned by *Piper betle* extract are believed due to the phenol and flavonoid contents of the leaves. The higher the composition of both phenolic and flavonoid content, the higher the antioxidant and radical scavenging activity. The free radical scavenging activity was found

maximum in the ethanolic extract (89.46% inhibition) and lowest (62.03% inhibition) in the distilled water extract. Shinjini & Pramathadhip extracted *Piper betle* by using methanol as the solvent [49]. Methanolic extract of *Piper betle* presented the highest antioxidant properties by strongly inhibiting 50% of DPPH radicals at low concentration of 4.38 µg/ml. Jaiswal et al. prepared an experiment of determining antioxidant properties of *Piper betle* leaves [50]. The study shows that *Piper betle* that extracted with 80% ethyl acetate exhibit better solvent for antioxidant compound extraction. Sundang et al. compared antioxidant properties of *Piper betle* and *Leucosyke capitella* extract using DPPH assay [51]. Varying concentration (of 0, 5, 10, 20, 30, 40, 50 and 100 ppm) of *Piper betle* extracts were mixed with 0.5 ml of 1 mM of DPPH. The study conveyed that *Piper betle* possess higher antioxidant activity compared to *Leucosyke capitella* due to higher phenolic, flavonoid and tannin contents in *Piper betle* extract.

Venugopalan et al. also conducted a comparative study on antioxidant activities *Piper betle* leaves extract using DPPH assay [52]. Extracts were obtained using organic and aqueous solvents, and from observations it was determined that acetone water-acetic acid (AWA) mixed with *Piper betle* extract, exhibited maximum antioxidant properties whereas hexane showed the lowest in regard to inhibition. Furthermore, Abraham et al. also discovered that the highest DPPH radical scavenging activity was achieved when *Piper betle* was extracted using ethyl acetate, followed by hexane, methanol and finally aqueous extracts [53]. The ethyl acetate extract had an IC₅₀ value of 40 µg/ml, slightly higher compared to quercetin (IC₅₀=30 µg/ml) and rutin (IC₅₀=33.7 µg/ml). Whereas for extracts obtained using hexane and methanol, they required more than 100 µg/ml concentration. As for aqueous extracts, inhibition failed to reach at even 50%, indirectly proving the weakness of that solvent in extracting antioxidant compounds from *Piper betle*.

4.4. Antidiabetic

Diabetes mellitus (Type 1) is caused from the incapability of β-cell in the pancreas to produce an adequate amount of insulin to convert glucose to glycogen which consequently increase glucose levels in blood [54][55]. Current medication for treating diabetes have been reported to cause many side effects to the patients [54]. Therefore, studies to find alternative remedies using natural sources or herbs for diabetes are always ongoing.

Hossain analysed the antidiabetic activity of *Piper betle* extract using model rats [54]. The model rats were divided into three (3) groups and fed with different concentrations of *Piper betle* extract starting from 50 mg/kgbw for group A, 100 mg/kgbw for group B and 200 mg/kgbw for group C. The study found that the plasma glucose levels of the administered rats reduced significantly; 36.1% for group A, 16.6 % for group B followed by 23.76% for group C. Arambewela et al. induced rats with diabetes by destroying beta cells using Streptozotocin which later were sustained with *Piper betle* extract [56]. Based on the overall investigations, both *Piper betle* extract (hot water extract and cold ethanolic extract) possess marked hypoglycaemic activity (tested in fasted normoglycaemic rats) and antihyperglycaemic activity (by the improvement of glucose tolerance test and lowering blood sugar level in Streptozotocin-induced diabetic rats). Thus, the findings suggested the ability of *Piper betle* in doing insulinomimetic activity. Regardless of the beneficial claim, researchers faced some difficulties, in which they need to repetitively nourished the tested rats with *Piper betle* extract for the extract to keep on working.

Apart from that, Santhakumari et al. conducted a study on Streptozotocin-induced animals specifically the Wistar rats for testing the antihyperglycemic activity of *Piper betle* extract [57]. The STZ-induced rats showed decreasing body weight due to the destruction of proteins caused by diabetes. Whereas, the Streptozotocin-induced rat that had been treated with *Piper betle* showed normal to lowest weight loss among the rats due to *Piper betle* extracts possessing hyperglycemia activity. Malik et al. used *Piper betle* extract that was extracted using ethyl acetate and tested against α-glucosidase enzyme at a varying concentration from 50 ppm to 1000 ppm [58]. *Piper betle* extract exhibited a strong capacity to inhibit α-glucosidase enzyme especially at increased dosage. Kavitha & Perumal performed both in-vitro and in-vivo tests for antidiabetic properties of *Piper betle* extract [59]. The in-vitro test measures

the α -amylase activity using different concentration of *Piper betle* extract (3.125, 6.25, 12.5, 25, 50 and 100 $\mu\text{g/ml}$) with 0.25 ml of α -amylase solution. The researcher found that the ethanolic extract of *Piper betle* had better percentage (%) of inhibition compared to control compound, acarbose (anti-diabetic drug). For the in-vivo test, *Piper betle* extract was administered to catfishes (*C. gariepinus*). Catfishes were then cut open and few samples were taken from the desired organs such as blood, liver, tissue and epaxial muscle for glucose level determination. Catfish samples were divided into four (4) groups; group 1 represented the control, group 2: fish injected with glucose (diabetic control), group 3: fish injected with glucose and metformin hydrochloride and group 4: fish injected with glucose and *Piper betle* extract. The experiment showed that glycogenesis increased and comparable to other antidiabetic existed modules. This is probably due to the ability of *Piper betle* in inducing insulin activity which stimulates glycogen production, indirectly proving *Piper betle* of having equal or even better antidiabetic properties.

Subhani et al. performed an in-vivo experiment in which rats were induced by alloxan monohydrate to make the rats diabetic with a single intraperitoneal injection [60]. The rats were group into 5 groups; Group 1: control and did not receive any treatment, Group 2: diabetic control, received alloxan monohydrate and vehicle, Group 3: Alloxan + Glibenclamide (10 mg/kg, p.o.) and act as standard. Group 4: Alloxan monohydrate + *Piper betle* (250 mg/kg, p.o.), Group 5: Alloxan monohydrate + *Piper betle* (500 mg/kg, p.o.). The rat's plasma glucose level was observed periodically for a long duration of time. On 14th day, rats treated with 250 mg/kg of *Piper betle* shows slight decrease in blood glucose level whereas rats treated with with 500 mg/kg of *Piper betle* provided prominent result in lowering blood sugar level. On the 22nd day, the antihyperglycemic properties started to show up significantly and increased across the study. The antidiabetic effects shown in this study are believed to have a connection with the presence of glycosides, tannins and saponins where those compounds are commonly associated with antidiabetic activity. Furthermore, the rats administrated with *Piper betle* extract also displayed stable body weight with no significant weight loss, probably due to the increase of glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein by glycemic control.

4.5. Anticancer

Cancer treatments commonly employed on cancer patients include chemotherapy, radiation therapy and surgery [53][61]. Treatments that are associated with drug toxicity and drug resistance frequently results in side effects on cancer patients [62]. Thus, alternative medicine treatments that have a lower toxicity level is highly desirable [63]. The research on natural sources such as herbal and plant as chemotherapeutic agent increases over the year [53]. Studies show that various plant possesses concoction of bioactive compounds to act as anticarcinogenic and anti-proliferative towards cancer cells for anticancer drug development [64][65][66]. Antioxidant activities in plant also contributed to the positive correlation to the inhibition of cancer cell growth [67].

Abraham et al. used MCF-7 human breast cancer cell for anti-proliferation study [53]. Cells were cultured in 96-well culture plates and left for 24 hours to let the cells attached on plates prior to the treatment with *Piper betle* extracts that were obtained using four (4) different solvents; ethyl acetate, hexane, methanol and water. Based on the evaluation, ethyl acetate possess the highest antioxidant and anti-proliferative effects followed by hexane extract that showed dose-dependent inhibitory effects on MCF-7 cells with IC_{50} values $56.00 \pm 0.00 \mu\text{g/ml}$ and $163.30 \pm 2.89 \mu\text{g/ml}$ respectively. Boontha et al. assessed the cytotoxicity and cell migration effects of *Piper betle* extract against human breast cancer cells MCF-7 by incorporating the plant extract in transdermal patches [63]. Based on the experiment, the researcher found that *Piper betle* extract exerts both cytotoxicity and anti-migratory properties. Cytotoxicity activity of MCF-7 manifest that increasing the dosage of *Piper betle* extract will reduce the viability of MCF-7 cells. Whereas, cell migration of MCF-7 was suppressed with a remarkable result at a dose of 25 $\mu\text{g/ml}$ in which cell migration was repressed by 30 % higher than the control group.

Paranjpe et al. investigated the properties on *Piper betle* leaf extract against prostate cancer by identifying the active principle of the *Piper betle* extract [67]. In vivo research was done on 6-week-old male BALB/c nude mice. PC-3-luc cells (1×10^6) were subcutaneously injected to induce the tumour

growth on the mice. As the tumour perceptible, the mice were divided into four (4) groups randomly: (1) control group received vehicle (phosphate-buffered saline, PBS with Tween-80 and pH 7.4); (2) 200 mg/kg of *Piper betle* extract dissolved in Tween-80 with pH 7.4; (3) 400 mg/kg of *Piper betle* extract dissolved in Tween-80 with pH 7.4; (4) 650 mg/kg of *Piper betle* extract dissolved in Tween-80 with pH 7.4 by oral administration daily. The *Piper betle* leaf extract appears to possess antiproliferative, antimicrobial and immunomodulatory properties. At the end of week 6, the resect tumour were weighed post-euthanasia and approximately 59% reduction in tumour weight tumour were observed in mice that have been administered with 400 mg/kg *Piper betle* extract.

Ng et al. determined that *Piper betle* leaf extract is able to increase the cytotoxic of 5-fluorouracil capability in restraining the growth of HT29 and HCT116 of colon cancer cells [68]. The experiment classes four (4) groups of cell line: (1) a control group that consists of cancer cells without treatment; (2) cancer cell treated with 5-FU; (3) cancer cells treated with *Piper betle* extracts in range of 100 to 500 µg/ml; and (4) cancer cells treated with 5-FU and *Piper betle* extract. Aside from that, HT29 cells were also treated with (1) 4-allylpyrocatechol (synthetic HC) (62.5 to 1000.0 µmol/L) and (2) with 5-FU in combination with 4-allylpyrocatechol. The result showed that the combination of 4-allylpyrocatechol and *Piper betle* extract notably alleviated 5-FU effects and amplified the cytotoxic level of the drug that aim to diminish colon cancer cells. Alam et al. studied *Piper betle*'s antitumor activity by augmenting antioxidant potential [69]. Tumour transplantation was performed by introducing EAC cells in Swiss albino mice by intraperitoneal transplantation using (2×10⁶) cells per mouse every 10 days. In the study, *Piper betle* extract decreased the intraperitoneal tumour burden by directly reducing the tumour volume, tumour weight and viable tumour cell count and indirectly lengthen the life span of tumour-bearing mice.

5. Extraction Process

5.1. Maceration or Infusion Extraction

Maceration is a very simple extraction process that has been practised for ages. Table 3 provides an overview of the extraction for *Piper betle*. Every part of plants or herbs can be extracted by maceration. Maceration or also known as infusion commonly requires two (2) main components namely the subjected plants and also a solvent system. Maceration process requires the plants to be soaked in the solvent over a particular duration of time [3][27][70]. Time is an important parameter in this process because the desired compound may be extracted partially or completely within the allotted time. [71]. The solvent used is depended on the polarity of the solvent and also phytochemicals or bioactive compounds that are needed to be extracted out from the selected plants [27]. The preferred solvents system used in maceration are water, ethanol, methanol, hexane, ethyl acetate, dichloromethane, acetone, chloroform and etc. [1][3][22][27][28][31]. The volume of solvent used is depended on the volume or weight of the plant sample [27]. The higher the volume of the solvent used, the greater the contact surface area with the plant sample which may lead to higher extraction of the desired compounds. Maceration process also sometimes accompanied with support from either continuous agitation or induced heat during the maceration process [27][71]. Continuous agitation may be provided by using homogenizer, incubator shaker or magnetic stirrer [22][27]. The agitation can increase surface contact area between plant sample and solvent which may indirectly increases the extraction process. In addition, elevated temperature may also contribute to higher rate of extraction because more compounds are increasingly soluble at higher temperatures, thus allowing then to be extracted more efficiently.

Strength: Simple procedure compared to other conventional methods, cost-efficient [14]

Limitation: Large volume of solvent [14]

Table 3. An overview of maceration extraction for *Piper betle*

Solvent	Ratio <i>Piper betle</i> extract to solvent	Duration of Maceration	Temperature	Agitation	References
85% methanol	1:3	4 Hours	Room Temperature	Yes	[1]
95% ethanol		24 Hours	Room Temperature	No	[3]
95% ethanol	1:4	Overnight	Room Temperature	No	[6]
Distilled water, hexane, acetone, ethanol	1:10	Overnight	28 °C	Yes	[22]
Ethanol, ethyl acetate, acetone, dichloromethane	1:5	24 hours	Room Temperature	No	[27]
70% ethanol			Room Temperature	No	[28]
Absolute ethanol, Absolute methanol	3:10	7 days	Room Temperature	Yes	[31]
Water		2 Hours	27 to 60 °C	Yes	[71]
50% ethyl alcohol	4:5	A week	Room Temperature	No	[72]
Ethyl acetate	1:5	24 Hours	Room Temperature	No	[73]

5.2. Soxhlet Extraction

Soxhlet extraction process require the movement of steam or vapour generated by a boiling solvent towards the plant's sample contained inside a thimble of the soxhlet apparatus setup. This process requires the assemble of several glass apparatus such as condenser, extraction chamber, thimble, siphon arm, and a boiling flask which usually located on top of a heating system [14]. The heat applied is depended on the user preference but most commonly at the boiling temperature of solvent used [14][33]. The preferred solvents used in soxhlet extraction process are hexane, chloroform, methanol, ethanol, water, petroleum ether and etc [11][32][33][73]. Usually the plant sample containing desired compounds is placed inside a thimble, which is then inserted into the extraction chamber. The chamber is placed onto a boiling flask containing the extraction solvents. The chamber is then equipped with a condenser. The solvent later is heated to reflux. The solvent vapor moves up the distillation arm, and floods into the chamber that houses the thimble. The condenser ensures that the solvent vapor cools, and drips back into the chamber that houses the thimble. The thimble containing the plant sample will slowly be filled up with warm solvent. Plant compounds will then dissolve in the warm solvent. After the soxhlet chamber is near full, it will be automatically emptied by the siphon arm, with the solvent goes back down to the boiling flask [14][32]. The process is then continued for more cycles until a concentrated extract is obtained [13][32]. Table 4 shows an overview of the soxhlet extraction for *Piper betle*.

Strength: Using small amount of solvent compare to maceration [74]

Limitation: Exposure to dangerous organic solvent [75]

Table 4. An overview of soxhlet extraction for *Piper betle*

Solvent	Ratio <i>Piper betle</i> extract to solvent	Duration of Soxhlet	Temperature	References
Ultra-pure water	1:10	2 Hour	60 °C	[11]
Ethanol	1:6	16 Hours		[32]
Chloroform, Petroleum ether, Ethanol	1:50	4 Hour	100 °C	[33]
Hexane, Ethanol, Methanol,	1:10	1 Hour		[73]

5.3. Ultrasound-assisted extraction (UAE) or Sonication

Ultrasound-assisted extraction (UAE) or sonication use electromagnetic waves produced by a sonicator probe or a sonicator water bath [76][77]. For sonication using water bath, distilled water is first added into the water bath and the temperature is maintained at ± 2 °C of the desired temperature [76]. The high frequency waves produced causes vibration and water ripples. By adding the sample and solvent inside a container that is later placed in the sonicator allows waves to be transmitted to the mixture inside the container [77]. The waves or water ripples produce tiny bubbles. The extraction will occur when high frequency waves cause the bubble to moves at incredible high speed resulting in collisions between the bubbles. As bubbles burst at the plant cell wall, the bursting increases the penetration of the solvent towards the sample [14][78][79]. Thus, the extraction process will be increased rapidly. Table 5 provides an overview of ultrasound assisted extraction (UAE) for *Piper betle*. In some cases, heat is also introduced during sonication process to speed up the extraction process. The heat applied usually is in the lower range of 50°C to 60°C for plants or herbs due to sensitivity of plant sample that easily ruptured or denatured at higher temperature [78][80]. The temperature of the water bath can always be monitored at the control panel [76]. Sonication is capable in extracting phenolic compound at relatively low temperature and at shorter period of time compared to other conventional methods [77][78]. The extraction yield usually is higher compared to other conventional methods due to ultrasonic waves that can efficiently improve the mass transfer rate during solvent penetration stage [77][78][80].

Strength: High extraction yield, short extraction time, low solvent, low energy consumption [13][14]

Limitation: High frequency may affect the quality of phytoconstituents due to release of free radical [14][74][81]

Table 5. An overview of ultrasound assisted extraction (UAE) for *Piper betle*

Solvent	Ratio <i>Piper betle</i> extract to solvent	Duration of Sonication	Temperature	Frequency	Power	References
Distilled Water	1:4	15 min	Room Temperature	20 kHz		[76]
90% ethanol	1:30	5, 10, 20, 30, 40, 60 and 80 min	50°C, 60°C, 70°C	37 kHz	400 W	[78]
70%, 80%, 90% Ethanol	1:10, 1:20, 1:30	30 min	50°C, 60°C, 70°C	37 kHz	400 W	[80]
99.7% acetone	1:50	1 Hours	Room Temperature	40 kHz	135 W	[82]

5.4. Super Critical Fluid (SFE)

Super critical fluid (SFE) extraction require fluid that exists in super critical phase that lies above the critical points such as carbon dioxide (CO₂) which can be compressed and behave as a dense fluid [14][15]. Carbon dioxide is used as the main solvent whereas other solvents that are allowed to be the co-solvents include methanol and ethanol [14][31][77]. The process begins after the sample is placed inside the extractor. Filling up of carbon dioxide inside the extractor is assisted by a pump [15]. The mixture of sample and carbon dioxide gas breaks apart in a separation chamber [15]. Carbon dioxide which has a lower density compared to the sample will be sucked back into the system and will be recovered for use in the next cycle of extraction [14][15]. Whereas the extract will fall into a collection tank. The fluid strength can be varied by altering the temperature and pressure of the system [15][31][77][82]. Super critical fluid (SFE) is considered to be safe and free from toxics due to the absent of toxic solvent that might contaminate the extract [82]. Super critical fluid (SFE) may produce low yield of extraction due to the non-polar solvent and low solubility of carbon dioxide to react with the sample [77]. Table 6 shows an overview of super critical fluid (SFE) extraction for *Piper betle*.

Strength: Environmental save, faster diffusion, no solvent remained [15]

Limitation: Required high pressure, carbon dioxide is limited solvent (expensive) [14]

Table 6. An overview of super critical fluid (SFE) extraction for *Piper betle*

Solvent	Weight of <i>Piper betle</i>	Temperature	Flow Rate	Extraction Pressure	Duration of SFE	Co-solvent	Reference
Carbon Dioxide	100 g	35 °C - 60 °C	0.5 m ³ hr ⁻¹	15 - 20 MPa			[31][83]
Carbon Dioxide	5 g	70 °C	5 ml/min	30 MPa	120 min		[77]
99.5% w/w pure Carbon Dioxide	10 kg	60 °C	0.3 Lhr ⁻¹	6 - 8 MPa	3 hours + 1 hour	Ethanol	[82]

5.5. Microwave Assisted Extraction (MAE)

Microwave assisted extraction (MAE) principle based on direct effect of microwaves on molecules by ionic conduction and dipole rotation [84][85]. MAE divided into two (2) different methods: (1) with solvent (non-volatile compounds) and (2) without solvent (volatile compounds) [13][86][87]. In general, polar solvents have a permanent dipole moment, ionic solution heavily absorb microwave energy and nonpolar solvent temperature remain stable in microwave radiation [88][89]. MAE increases solubility for compounds of interest by rapid heating effect on solvent [90]. Selection of solvent depending on microwave-absorbing properties of solvent, selectivity towards the analyte and the interaction of solvent with matrix [91]. Typically, organic solvents are used in extraction of most bioactive compounds [91]. MAE that operated without solvent mainly used to recover essential oils [92]. The heat emitted from the generated electromagnetic waves will penetrate into the sample [93]. MAE used the principle of direct heating of the cell inside or in-situ heating of water in the cell [15][94]. The process results in water inside the plant cell to explode and consequently allows bioconstituents or phytochemical to be released out of the cell [15]. Table 7 illustrated an overview of microwave assisted extraction (MAE) for *Piper betle*.

Strength: Avoid degradation of the bioactive compound, high quantity with quality extract, reduced extraction time and solvent [94]

Limitation: Limited phenolic compound extracted as most phytoconstituents unstable in microwave condition [14]

Table 7. An overview of microwave assisted extraction (MAE) for *Piper betle*

Solvent	Weight of <i>Piper betle</i> extract	Ratio <i>Piper betle</i> extract to solvent	Duration of time (minute)	Temperature of evaporation (°C)	Reference
80% ethanol	100 g	1:5	1.0, 1.5, 2.0, 2.5, 3.0	40, 50	[95]

6. Applications

The use of *Piper betle* has been around since ancient times in various ways. The common applications for *Piper betle* can be classified into two categories: (1) traditional usage and (2) modern usage. Traditional usage is divided into several groups: (1) culture, (2) religion, (3) marriage. and (4) medicinal. Culture usage is pictured by the munching or chewing of *Piper betle* leaves with multiple constituents such as lime betle, hard fruit, areca nuts and gambier during conversations [6]. People in Malaysia, Thailand and India used *Piper betle* leaves in different occasions of religious practices, pray ceremonies and god's offering [96][97][98][99]. *Piper betle* is decorated as wedding offerings for the partner to symbolize strong bond and prosperity [100]. Medicinal usage focuses on physical or surface treatment specifically for skin or topical treatment. *Piper betle* is mixed with honey for the treatment of sore throat [7]. *Piper betle* is also prepared as a wound healing paste by crushing the leaves with mortar with the addition of few drops of water and then apply on the wound or swollen areas [6][7][20].

Modern society uses *Piper betle* extract in a variety of products especially natural care products such as shampoo, women's cleansers, toothpaste, tonic and food additives [3][4][5][6]. Natural based shampoo attracts consumers lately due to magnificent wonders such as strengthen, smoothing, and treating hair with added value of lowering chemical components in shampoo ingredients. *Piper betle* incorporated shampoo used to treat anti-dandruff, help in promoting hair growth and decreases hair fall [101][102]. Older generation used herbs such as *Quercus infectoria* and *Piper betle* to clean female intimate area [103]. The hassle preparation and availability of herbs in modern society causes people to neglect the practice. Due to that reason, company that produced feminine wash introducing natural herbs into their formulation. Feminine care focusing solely on female used to cleanser to maintain stable pH, treating germs infection and self-hygiene [103]. Toothpaste induced with *Piper betle* helps in oral health specifically as effective antimicrobial properties in oral cavity [104]. Tonic used to improved health in general and good to boost immune system [8][10][105]. The synthetic food additives in form of preservatives, coloring agents and antioxidant increases each year. Thus, researcher came with multiple test on natural based food additives such as from *Piper betle* to substitute existing food additives [22].

7. Quality and safety (toxicology and side effects)

Many toxicity tests have been performed on the extracts of *Piper betle* and its products to ensure it is safe for general human use. Valle et al. performed test of ethanolic *Piper betle* extracts against normal human dermal fibroblast (HDFn) at different concentrations using MTT assay and results revealed that the plant sample yielded very low cytotoxicity index, less than IC₅₀, even after the administration of the highest concentration of 100 µg/ml [31][106]. Arawwawala et al. used *Piper betle* extracts as rats' gastroprotective agents demonstrated no explicit indication of toxicity, hepatotoxicity, renotoxicity and any adverse side effects after administration of a high potent dosage of *Piper betle* (1500 mg/kg/bw) [107]. Furthermore, *Piper betle* extract do not exerts any physical sign of toxicity, stress or aversive behaviours on rats [108].

Sagupta et al. evaluated pre-clinical toxicity evaluation of leaf stalk extractive of *Piper betle* linn in rodents [109]. Multiple doses of *Piper betle* extract were administered to rodents for toxicity studies for

acute toxicity study (single dose) and chronic toxicity study (daily dose). In acute toxicity study, even after oral dosage of *Piper betle* extract up to 3200 mg/kg, the study failed to exhibit mortality after 24 hours. Whereas in chronic toxicity study, *Piper betle* extract had no general metabolic toxicity during 60 days of drug treatments. Choudhary et al. tested *Piper betle* extract toxicity level by inducing *Piper betle* extract to mice's liver showed non-toxic effect that supported by non-potential of lipid peroxidation [99]. Albeit the study prolongs for 21 days, the *Piper betle* extracts did not display any toxic effect in tissues such as liver and kidney. *Piper betle* extracts and its derivatives are believed to have minimal side effects due to derivation from food categorize materials identify as "generally recognize as safe" (GRAS) [110]. However, precautions must be taken for both pregnant and breastfeeding mothers [111].

8. Future and potential uses of *Piper betle*

Piper betle potential uses can be developed and diverse near future. *Piper betle* extract studies proved that *Piper betle* capable becoming an antimicrobial medium. Voon et al. had carried out research on the probability of using *Piper betle* extract as a substitute preservative in chilli paste or known as chilli bo [3]. The outcome showed that *Piper betle* had lowered the growth of aerobic bacteria by 6% and also had reduced the formation of mould and fungi by 7.41%. The observation opens the possibility for *Piper betle* advanced into natural food preservative production.

Ataguba et al. conducted in-vivo and in-vitro tests to investigate the antimicrobial abilities of *Piper betle* extract against viral strains isolated from fish and shrimp diseases [112]. In-vitro tests showed positive effects on pathogenic strains. While in-vivo tests successfully minimized *S. galalactiae* infection in *Nile tilapia*. Thus, *Piper betle* extract feasibility as a food supplement in feeding livestock could be employed as an economical option.

Hoppy et al. coordinated research on the practicality of *Piper betle* as an alternative tooth enamel for fluoride topical gel [113]. The main task was to maintain the pH of tooth enamel as the composition may change in colour scheme if pH falls out from the neutral range. The outcome reported that the composition of *Piper betle* in tooth enamel by 15%, 25% and 35% showed no color change.

Piper betle is also occasionally tested as an active ingredient in wound healing formulations. Research performed by Lien et al. on the proliferation of fibroblast NIH3T3 cells demonstrates that *Piper betle* extract was able to promote wound healing on Swiss mice without any psychological effects [73]. Further study shows that *Piper betle* extract induces wound healing by reducing the malondialdehyde (MDA) levels in the liver and legs soft tissues of the tested mice.

9. Conclusions

Piper betle possess adequate therapeutic potential based on the evidence provide from available literature research on the *Piper betle*. Thus, strengthen the possibility of *Piper betle* to be used extensively in commercial production. In conclusion, *Piper betle* can be accredited as a potential compound in alternative treatment to prevent or cure certain diseases due to the presence of various bioactive compounds such as tannins, flavonoids (quercetin), eugenol, hydroxychavicol and chavibetol inside the plant. Numerous researches conducted have proven *Piper betle* extract to possess antibacterial, antifungal, antioxidant, antidiabetic and anticancer abilities. However, the safety, side effects and toxicity level need to be addressed using scientific reports instead of only classified *Piper betle* as safe to consume due to "generally recognize as safe" (GRAS) as *Piper betle* classified as food category [112]. Further critical research works need to be developed on *Piper betle* extract to determine the possibility to introduce *Piper betle* into medicine production.

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