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A REVIEW ON *Piper betle* EXTRACT AND HYPOCHLOROUS ACID ON INSECTICIDAL ACTIVITY

(Ulasan tentang Ekstrak *Piper betle* dan Asid Hipoklorus Terhadap Aktiviti Racun Serangga)

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Abstract

Piper betle L. is a well-known medicinal plant that originates from the Asian region. This crop was used daily by the older generation as natural medicine to cure certain types of diseases. A series of phytochemical screening procedures proved that the extract of *Piper betle* contains essential phytochemicals, such as tannin, saponin, flavonoid, and alkaloid, which possess the insecticidal activity potential towards a wide range of insects. Previous studies have led to the implementation of betel extract in the agriculture sector as a pesticide or a major constituent in the production of novel insecticides. The effectiveness of betel extract as a pesticide is quite promising and it can be used as a better alternative to replace the use of harmful, chemical pesticides. Similar to betel extract, hypochlorous acid shows antimicrobial and insecticidal effects, but its true potential has not been studied thoroughly because its current usage is mainly focused on the healthcare sector as disinfectants or to aid wound healing. Both betel extract and hypochlorous acid can be beneficial to the agriculture sector, especially in pest management procedures, if its true potential is studied thoroughly to validate its mechanism or mode of action towards insects.

Keywords: *Piper betle* L., phytochemical, insecticidal activity potential, hypochlorous acid

Abstrak

Piper betle L. ialah sejenis tumbuhan ubatan yang berasal dari Asia. Generasi terdahulu telah menggunakan tumbuhan ini secara harian sebagai ubatan semula jadi untuk merawat penyakit tertentu. Beberapa siri analisis fitokimia telah membuktikan bahawa ekstrak *Piper betle* mengandungi beberapa jenis fitokimia penting termasuk tannin, saponin, flavonoid, dan alkaloid, yang memiliki ciri anti-serangga terhadap pelbagai jenis serangga. Kajian yang telah dijalankan telah membawa kepada penggunaan ekstrak sireh sebagai racun perosak atau bahan utama dalam penghasilan racun serangga yang baharu. Keberkesanan ekstrak sireh sebagai racun perosak menunjukkan hasil yang meyakinkan dan boleh digunakan sebagai alternatif yang lebih baik untuk menggantikan penggunaan racun kimia yang berbahaya. Asid hipoklorus juga menunjukkan kesan antimikrob dan anti-serangga seperti ekstrak sireh, tetapi keupayaannya yang sebenar tidak dikaji secara mendalam kerana penggunannya lebih tertumpu dalam bidang kesihatan sebagai disinfeksi atau membantu dalam penyembuhan luka. Kedua-dua ekstrak sireh dan asid hipoklorus boleh menjadi bahan yang bermanfaat dalam sektor pertanian, terutamanya dalam kawalan makhluk perosak jika potensi sebenarnya dikaji dengan lebih mendalam bagi mengesahkan mekanisma atau cara tindakannya terhadap serangga.

Kata kunci: *Piper betle* L., fitokimia, ciri anti-serangga, asid hipoklorus

Introduction

Betel (*Piper betle* L.) from the Piperaceae family is a traditional medicinal plant that is widely used in Malaysia and Asia for various purposes [1]. It has its own common names in different regions worldwide, such as *Paan* in India and *Sireh* in Malaysia. It normally appears at the beginning originated from Malaysia, but there are also studies that have proven that this crop might have originated from Thailand since 5500 BC, Indonesia since 3000 BC, and Philippines in 2600 BC. It is estimated that around 100 varieties of betel exist worldwide and 40 of them can be found in India. Betel plants can be directly consumed through chewing or as additives to other types of plant to form a mixture. Aside from human health, betel is also used in the agricultural sector to aid in efficient plant growth.

Figure 1. Betel plant (*Piper betle* L.)

Pest management in agriculture will become more challenging due to the increasing pest resistance to chemical pesticides. The arising problems forced farmers to seek alternatives to controlling pests to avoid yield loss in the future. It is undeniable that the use of chemical pesticides was efficient initially, but it has led to undesirable side effects, such as environmental pollution. Researchers are moving forward in providing farmers with a better alternative that is more environment-friendly, effective at low cost, and able to cater to the main objective of pest control. Plants were proven to contain certain compounds that can largely ward off the insecticidal activity potential [2]. Certain regions in the country have started to use such crops in pest management.

Phytochemical screening of crops is carried out to identify the presence of a chemical or bioactive compound in a certain plant. This procedure is done in the laboratory because it involves the use of chemicals and requires specific conditions. The presence of a certain chemical compound in the plant can be clearly observed. Examples of tests conducted in the phytochemical screening are the froth test for saponin, the ferric chloride test for tannins, and test for anthraquinones. The plant extract is used as the main material for the screening procedure. Some tests require extract in powder form while others require extract in alcoholic form.

Botany of *Piper betle* L.

The Piperaceae family consists of approximately 1000 species of plants and shrubs, including *Piper betle L.* which is also known as betel vine parts. It is a well-known traditional plant due to its many medicinal benefits and can be used for many purposes. Its leaves are the most used plant parts. It has a pungent odour and requires another plant or support for the growth of its vine [3]. These plants can be classified and differentiated based on their leaves, sizes, and colour. Table 1 shows the taxonomy of *Piper betle* L. This type of medicinal plant can be widely found across the world, and most originate from Southeast Asian countries, such as Malaysia, Indonesia, Myanmar, Thailand, and the Philippines. Its name varies depending on the local community because the betel crops were named according to their local language and beliefs. India is one of the most significant countries that relate to the origin of betel. According to their beliefs, betel plant is known as Paan from the Sanskrit word “parn”, which means snake hood as the leaves resemble the shape of snakehead [3].

Table 1. Taxonomy of *Piper betle* L.

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Stretophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Magnolianae
Order	Piperales
Family	Piperaceae
Genus	*Piper* L.
Species	*Piper betle* L.

This crop can be planted in two types of cropping systems, i.e., open cropping system and the controlled cropping system. Under the open or natural cropping system, betels are grown attached to a supporting crop, such as *Cocos nucifera* and *Areca catechu*, which provides support to allow the betel crop to grow up to 15 meters in height.

Biological activity of *Piper betle* extract: Antibacterial Activity

Betel extracts show significant effects in terms of antibacterial properties towards various ranges of microbes that could be very useful not only in the pharmaceutical sector but also related to the agriculture sector. Generally, the antibacterial properties of a compound were studied to be implemented in the treatment of disease-causing bacteria. Some of the pathogens were also founded in food. Betel leaves have been employed as an antibacterial agent in India and other Southeast Asian countries since ancient times, and they are mentioned in various historical manuscripts dating back to the Vedic period (3,000 BC). The extracts of betel leaves, stalks, and stems, as well as the oil obtained from the plants, have all been investigated and proven to be effective against a variety of harmful bacteria [3].

The presence of several strong chemicals such as alkaloids, tannins, phenolic molecules, and glycosides might explain the considerable antibacterial action. Antibacterial properties were discovered in betel leaf extract with ethanol, ethyl acetate, and methanol as solvents, particularly in betel leaf extract [4]. Although generally the studies conducted on the antibacterial properties of betel extract were mainly focused on disease-causing bacteria in humans and animals, some of the bacterial strains were plant-associated. More research and studies should be conducted to study the antibacterial properties of betel extract against disease causing bacterial strains on plants instead of focusing on humans and animals. There are only a few studies reported that validates the effectiveness against plant pathogenic bacteria. According to Nagu [5], betel extract can be a great alternative to be treated against several phytopathogenic bacteria such as *Xanthomonas oryzae, Xanthomonas axonopodis pv. vesicatoria* and *Agrobacterium tumefaciens*. In the study, *Xanthomonas axonopodis pv. vesicatoria* was proven to be the most susceptible bacteria against all type of betel extract. The reported antibacterial properties against several bacterial species were summarized in Table 2 below.

Antilarval activity

The extract of *Piper betle* was studied and proven to contain antilarval activity against the larvae of several species. Among the studied insect species are *Chrysomya megacephala* and *Chrysomya bezziana* [3]. However, there is a significant difference in the effectiveness of different types of extract against the studied insects. Among the extracts, betel essential oil is ranked as the most effective as compared to aqueous extract and ethanol extract. In another study by [12], betel extract was also proven to have a similar effect when treated to the larvae of *Aedes aegypti*. These studies have demonstrated that betel extracts possess the antilarval activity potential that could be used as a major component in the anti-larval agent to curb the insect population.

Antifungal activity

Aside from antibacterial and antilarval properties, betel extract was also found to have antifungal properties, which were caused by several constituents in the extract, including chavicol, chavibetol, chavibetol acetate, and hydroxychavicol [3]. The tested fungi were *Aspergillus* *parasiticus*, *Trichophyton mentagrophyte*, and *Candida albicans*. Once again, the effectiveness of each type of extract varies with chloroform extract, showing the most promising result [13].

Table 2. Bacterial strains studied against betel extract

Bacterial strain	Disease Caused by Bacteria	Isolated	Ref.
Xanthomonas oryzae	Bacterial blight	*Oryza sativa* (rice)	[6]
Xanthomonas axonopodis pv. vesicatoria	Bacterial spot	*Solanum lycopersicum* (tomato) and *Capsicum anuum* Group (pepper)	[7]
Agrobacterium tumefaciens	Crown gall disease	Soil and crown gall	[8, 9]
Xanthomonas campestris p.v. campestris	Black rot	*Brassica oleracea var. capitata* (cabbage)	[10]
Erwinia carotovorum sub sp *carotovora*	Soft rot disease	*Solanum tuberosum* (potato)	[2]
Pseudomonas syringae	Bacterial canker	*Prunus spp.*	[11]

Extraction and phytochemical screening of *Piper betle*

Betel leaf extracts that are commonly studied are aqueous, methanol, ethanol and essential oil extracts. These extracts yield different results because certain active components are only present in a certain type of extract. The percentage of each compound depends mainly on the variety of the betel and environmental factor. Essential oil (EO) extracted from betel leaves contains more than 60 active compounds, each of which has unique characteristics, that are very beneficial. Phytochemical screening is done to determine the retain extract. It is a process to detect the presence of chemical components produced by plants. The detection of phytochemicals is done mainly to determine the presence of major chemicals, such as alkaloids and saponins, but further research has proven that it could also be used to detect the presence of tannins, saponins, essential oil, and other phytochemicals produced naturally by plants [14]. *Piper betle* L. was studied for its phytochemical and was found to contain flavonoids, saponins, isoeugenol, terpenoids, alkaloids, protein, and other naturally occurring beneficial chemicals [15]. There are several methods reported for the detection of phytochemicals in an extract, including for betel extract (Table 3).

According to Mazumder [18], betel crops were one of the most highly produced crops, averaging at Rs 9000 million worth of production yearly, but it is mostly wasted or sold at a very low price [19] introduced the oil extraction method. The first oil extraction method uses the clavenger apparatus, but was unsuccessful due to the rapid formation of an emulsion. Guha introduced a series of improvements that made the extraction process more efficient. The main consideration for the oil extraction process in India is in terms of technology availability and suitable chemicals that can be easily retrieved for the extraction process to be conducted on a large scale [19]. In general, different types of extracts will yield different types of phytochemicals in the extract. In a study conducted by Saini et al. [20], ethanol extract and aqueous extract of betel crops yield the highest quantity of phytochemical that can be traced by a series of phytochemical screening procedures. It recorded a total of six phytochemicals in the extract, which are proteins, saponins, carbohydrates, alkaloids, tannins, and phenol. In contrast, in a study by [21], chloroform extract of the same species of crop yields a high number of essential phytochemicals, including chavibetol acetate, chavicol, allylpyrocatechol, and chavibetol. It is very crucial to select a suitable extraction procedure to enable the presence of targeted phytochemicals to be studied. Phytochemicals are present in different parts, such as root, bark, stem, flower, and leaf, but their concentration varies depending on the crop variety. The Sri Lankan variety of *Piper betel* L. was studied and proven to contain safrole as the highest concentration of phytochemical with an average of 53%, followed by allylpyrocatechol acetate and eugenol with 15% and 6% concentration, respectively [22]. In contrast, the essential oil extracted from betel crops of the Malaysian variety contains a high amount of chavibetol, in approximately 70% concentration. These differences were proven to be caused by the geographical location as an environmental factor significantly affect the growth phase, as well as the physical and chemical properties of the variety.

Table 3. Series of tests conducted in phytochemical screening of betel extract

Test / Procedure	Observation (indicates presence of targeted phytochemical)	Ref.
Test for alkaloids		
Dragendraff's test	Orange-brown or reddish - brown precipitate	[16, 17]
Mayer's test	Cream precipitate	[17]
Test for glycosides		
Legal's test	Changes of color from pink to red	[17]
Keller-Killani test	Reddish-brown color	[17]
Test for saponins		
Haemolysis test	Changes of red blood cell structure observed under microscope	[17]
Foam test	Stable foam appeared	[17]
Shaking vigorously with distilled water	Lather formed	[16]
Test for flavonoids		
Shinoda test	Orange color formed	[17]
Test with concentrated sulphuric acid	Yellowish orange color formed	[16]
Test for carbohydrates		
Molisch's test	Violet color appeared	[17]
Fehling's test	Reddish-orange precipitate formed	[17]
Test for phytosterols		
Libermann-Buchard's test	Emerald green color appeared	[16, 17]
Salkowski's test	Brown color appeared	[16, 17]
Test for protein		
Biuret test	Violet color formed	[17]
Ninhydrin's test	Purple color formed	[17]
Test for phenolic compound		
Phenolic compound test	Blue color appeared	[17]
Test for tannins		
Addition of 1% gelatine and 10% sodium chloride	White precipitate formed	[17]
Test with Bramer's reagent	Brown precipitate formed	[17]
Ferric chloride test	Greenish black color appeared	[16]
Lead acetate test	White precipitate formed	[16]

Among the phytochemicals present in the betel leaf extract, the phenolic compound recorded the highest concentration compared to other groups of compounds [15]. Phenolic compounds, such as flavonoids, tannin, and carotenoids, are found in most plants. Phenolic compounds are the main compound that helps in UV protection, defence mechanism, and antioxidant properties [23]. The phenolic compounds in betel crops possess antioxidant properties. Researchers have proven and identified phenolic compounds as the most functional metabolite that can be extracted from *Piper betle* L. and it has potentials in many fields of study. These essential phytochemicals were also found in other plants that were studied for similar reasons, including antioxidant and antibacterial potential that provide better alternatives to replace harmful artificial chemicals used widely in the industry. Table 4 shows the phytochemicals present in different types of betel extracts.

Table 4. Essential phytochemicals in *Piper betle* L. extracts

Phytochemicals	Molecular Formula	Type of Extract	Ref.
Flavonoid	$C_{15}H_{10}O_5$	Ethanol extract	[20]
Alkaloids	$C_{21}H_{20}N_2O_3$	Chloroform, ethanol, and aqueous extract	[20]
Tannin	$C_{76}H_{52}O_{46}$	Aqueous and ethanol extract	[20]
Eugenol	$C_{10}H_{12}O_2$	Methanolic extract	[15]
Safrole	$C_{10}H_{10}O_2$	Methanolic extract	[15]
Chavibetol acetate	$C_{12}H_{14}O_3$	Methanolic extract	[15]
Phytosterols	$C_{29}H_{50}O$	Chloroform, ethanol, and aqueous extract	[20]
Oleic acid	$C_{18}H_{34}O_2$	Methanolic extract	[15]
Trymyristin	$C_{45}H_{86}O_6$	Methanolic extract	[15]

Figure 2 shows a molecular structure for several phytochemicals that are present in betel leaf extract. Although generally all cultivar of *Piper betle* contains similar phytochemicals, its concentration may vary due to several factors, mainly by the environmental factor at the cultivation area. Environmental factors, such as humidity, temperature, shading effect, light intensity, and nutrient availability, play a vital role in crops' growth leading to different chemical concentration for the same species that is collected from different regions and origins. Even though studies have been done to clarify the effect of aging towards the concentration of each phytochemical in the betel extract, it was concluded that the maturity stage of the crop does not largely affect the chemical constituent content of the extract.

Insecticidal activity potential of *Piper betle*

Table 5 shows the current usage of *Piper betle* extracts in pest management as an agent that significantly affects the treated pests. The studies validated the effect of the extract on different stages of insect pest. Some of the extracts acted on several stages and mechanisms, including the larvae and adult stages, affecting the egg-hatching process of insects and showing repellent properties.

Several species of the Piperaceae family, including *Piper betle* L., have been proven to contain insecticidal activity potential toward several species of pests [24]. All the *Piper* species studied were proven to contain similar essential phytochemicals that allow it to possess such potential. Traditionally, betel crops were used directly as a control agent for several insect species that could become vectors for harmful diseases. In modern agriculture, chemical components extracted from various parts of betel crops were studied and applied to produce novel insecticides [25]. Further research and advancement are in progress to produce more efficient insecticides that cause less environmental damage. The essential oil derived from plant species was proven to pose less harm to both humans and environment due to its biodegradable properties [26].

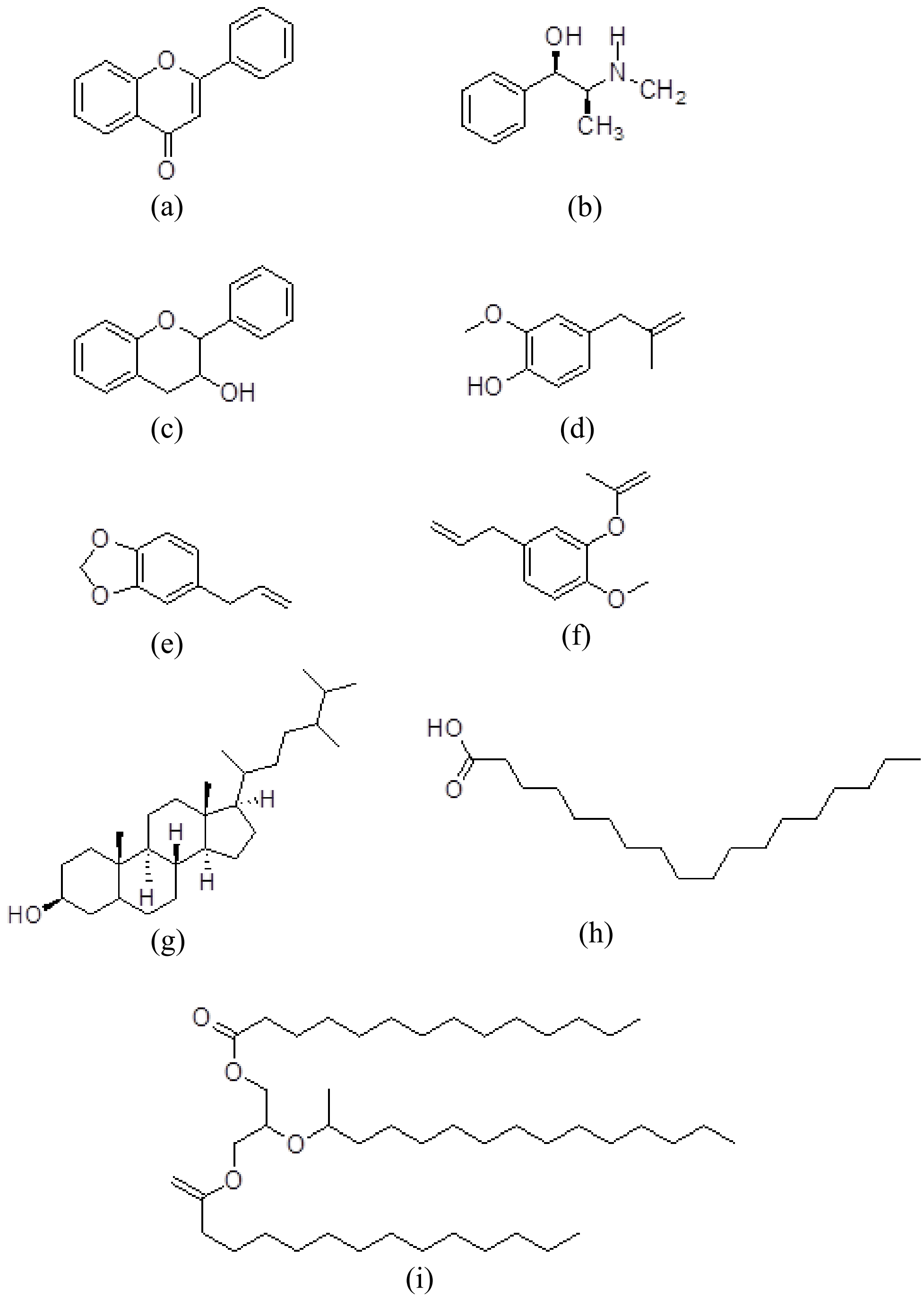

Figure 2. Molecular Structure of Some Phytochemicals Present in Betel Extract. (a) flavonoids (b) alkaloids (c) tannins (d) eugenol (e) safrole (f) chavibetol acetate (g) phytosterols (h) oleic acid (i) trymyristin

Table 5. Usage of *Piper betle* Extract as Pesticide

Insect	Test	Result / Observation	Ref.
Musca domestica	Fumigation toxicity	Provide moderate toxicity to treated insect	[27, 26, 28]
	Contact toxicity	Shows weak properties in topical application but promising result in diet exposure to larvae	
	Ovicidal effect	Shows toxicity effect but lower than eugenol	
	Oviposition repellence	Shows promising oviposition deterrence and increased with increasing concentration	
	Adulticidal bioassay	Betel essential oil with the concentration of 120 μg/cm^3 in ethanol causes 100% knock down effect	
Aedes aegypti	Larvicidal assay	Causes 100% mortality rate at the concentration of 500 ppm after 1 hour of treatment	[27, 12]
Chrysomya bezziana	Larvicidal bioassay	3% betel oil causes 100% mortality rate of 1st instar larvae after 2 hours and 74% mortality rate of 2nd instar larvae after 4 hours 4% betel oil causes 100% mortality rate of 1st instar larvae after 2 hours and 2nd instar larvae after 4 hours	[27]
Chrysomya megacephala	Larvicidal bioassay	2% betel oil causes 97% mortality rate of the larvae after 4 hours 3% and 4% betel oil causes 100% mortality rate of larvae after 3½ hours	[27]
Sitophilus oryzae	Adulticidal bioassay	1% betel oil causes 100% mortality rate of adult *Sitophilus oryzae* after 1½ hour	[27]
Cylas formicarius	Adulticidal assay	Almost all crude solvent extract provides significant effect to the adult *Cylas formicarius* with ethyl acetate recorded the highest mortality rate (100% after 48 hours), followed by crude acetone extract (70% after 48 hours) at the concentration of 20 ppm	[29]
Meloidogyne spp	Applied to tomato plant infested with *Meloidogyne spp*	The highest decrease of *Meloidogyne spp* population in the soil was recorded at 80.06% by 20% of concentration The highest decrease of *Meloidogyne spp* in the tomato root was recorded at 76.14% by 20% of concentration The highest decrease of eggs produced by *Meloidogyne spp* was recorded at 61.73% by 20% of concentration	[30, 31]
	Test on the survival of second stage juveniles (J_2s) *in vitro*	Causes higher mortality compared to control (untreated water)	
	Test on egg hatching – *in vitro*	Causes egg suppression at 100% by 80% of concentration	
Anopheles spp	Repellent test	80% of betel leaf extract shows the highest repellent percentage at 93.75% followed by 40% concentration (68.75%) and 20% concentration (50%)	[32]

Table 5 shows the insecticidal activity of betel extract for different stages of insects. Mohottalage et al. [22] conducted a study to compare the effectiveness of betel essential oil and *Ceylon citronella* oil extracted from *Cymbopogon nardus* towards adult houseflies (*Musa domestica L.*). It was found to be approximately three times more efficient than the *Ceylon citronella* oil. Adult houseflies were treated with both substances at 24 hours and 48 hours of exposure time. Another study conducted by Yushananta and Ahyanti [28] used different concentrations of betel essential oil to treat houseflies and compared the effect of concentration variation on the mortality rate. From the finding, a higher concentration of betel oil led to a higher mortality rate for houseflies. They proved that the toxicity of betel extract was mainly caused by the presence of eugenol and safrole in high concentrations. The toxicity potential of the active compound makes it more efficient than other naturally occurring chemicals. Commercially, safrole was applied in the production of insecticide as a synergist.

The essential oil of *Piper betle* acted on the larvae and adult stages of the targeted insects with different concentrations [12]. A recent study validated that the betel oil effectively controlled the larvae of *Aedes aegypti* at the concentration of 500 ppm, higher than the concentration of widely used insecticide, known as temephos, which requires only 0.1 mg/L dose in water to control the larvae efficiently. But there is a recent report that the larvae of *Aedes aegypti* tend to become resistant towards temephos in some countries. On the other hand, there is no report of resistance when betel oil is used to control the larvae [12]. A substance that affects different stages of insects has high potential to be developed into novel insecticides as it will help increase its effectiveness towards the targeted pests. Moreover, it is better to control the pests at the early stage to curb the population of infesting pests and simultaneously reduce the total loss done by the pests. As for small-scale or large- scale production, farmers will surely use this alternative to decrease their expenditures in controlling pests and diseases for the agricultural processes. Aside from affecting adults and larvae of pests, betel EO also shows potential in terms of oviposition deterrent activity. The total number of eggs produced in the oviposition assay at the concentration of 100 ppm by using *Piper betel* L. extract yields decreased quantity by five (5) times lower than the control condition [12].

Regarding the mode of action and mechanism, each phytochemical targets and acts differently to the treated insects or pests, but it still yields the same result, causing mortality to the organisms [26] studied the insecticidal activity of betel essential oil and other chemicals, including eugenol acetate, eugenol, and β-caryophyllene, by two modes of action or treatment to the *Musa domestica*, which are contact toxicity and through fumigation. All chemicals used can be traced in the betel essential oil, but the chemicals were tested individually as a comparison to the betel essential oil. Different concentrations of each chemical were prepared by diluting it in acetone and applied to filter paper placed inside a jar. Approximately 20 starved and an anesthetized uniformly sized houseflies were placed into the jar containing the filter paper and the mortality rate was recorded after 3 hours. Another mode of action by betel oil studied by Subaharan et al. [26] is through contact toxicity, which can further be divided into two categories, namely topical application and dietary exposure to larvae.

In conclusion, the studies show promising results for the individual constituent found in betel essential oil. Almost all results for the individual chemicals were superior to the betel essential oil. It can be concluded that the substances play an important role in insecticidal activity against houseflies. Another study conducted by Yushananta and Ahyanti [28] concluded that a higher concentration of betel oil and more prolonged exposure to the betel oil causes a higher mortality rate of *Musa domestica*. The highest concentration used was 25%, which yields about eight houseflies mortalities. The highest mortality was recorded for six houseflies at an exposure time of 20 minutes.

There are several causes of death for the treated insect, either by direct contact or through fumigation that causes the chemical to enter the insect through the respiratory system. Tannins are one of the phytochemicals that is responsible for the death of insects. Tannins' mechanism targets the cell wall and digestive system of pests [28]. The nature of certain pests, e.g., houseflies tend to lick anything in its surrounding, can provide a pathway for the tannins, also known as a contact poison, to enter the inner structure of the pests. After entering the body, it inhibits significant enzyme activity and causes digestive failure, leading to death. Another example of the phytochemical mechanism in insecticidal activity is the inhibition of the respiratory system by flavonoids. This chemical constituent pathway is mainly through the airways by entering the respiratory tracts. It causes paralysis and muscle depletion by acting on the central nervous system of the affected organisms. Chavicol and eugenol, another major component in betel oil, were mainly used as pesticide synergists due to their antiseptic properties.

Regarding the treatment at the larvae stage of certain insects, betel oil will first target the larvae's midgut epithelium, which then leads to malformed tubules that will then causes larvae mortality. Generally, different types of phytochemicals target different parts of the insect, but further research can be done to clarify which one of the phytochemicals acts, or if all phytochemicals mechanisms occur simultaneously as the mechanism was just stated individually.

Hypochlorous acid: A review

Hypochlorous acid (HOCl) is a substance that is naturally produced primarily by white blood cells, including neutrophils and eosinophils, of most mammals as a response to injury or infection [33]. This chemical is nontoxic and noncorrosive, but is effective and widely used in the pharmaceutical, health and a wide range of other sectors. For instance, this chemical can be easily found at home because it is used as an active compound in cleansers and bleaches [34]. These usages of HOCl have proven its potential, and in the future, more use of this beneficial chemical might be discovered, including in the agriculture sector, to aid the processes. There are also records showing that HOCl has already been used as one of the compounds to produce several types of pesticides. So, the implementation HOCl in the agriculture sector is not new and further development can be done to reduce the dependency on synthetic chemicals.

HOCl can be produced through several chemical processes. As stated earlier, HOCl is naturally produced by white blood cells, such as neutrophils of mammals. HOCl is produced due to oxidative burst, a condition whereby oxygen consumption rises remarkably [35]. During this process, also known as a respiratory burst, cells utilize oxygen (O_2) and, an enzyme called NADPHase, triggers the conversion of O_2 to hydrogen peroxide (H_2O_2). This enzyme is bounded to the membrane of mitochondria. The H_2O_2 then reacts with chlorine ion (Cl^-) from the cellular fluid, catalysed by a leukocyte-derived enzyme, known as myeloperoxidase, to yield HOCl. According to a study by [35], approximately 0.1 µM of HOCl can be produced from every 10^6 in vitro neutrophils that can kill *Escherichia coli* (*E. coli*) within 5 minutes. *E. coli* is a microorganism that can be found in the intestine; it is usually not harmful to health, but certain strains of *E. coli* can cause stomach pain and diarrhoea. Aside from natural production by neutrophils and eosinophils in mammals, HOCl can also be produced artificially or through the reactions of several chemicals.

There are several methods that can be used to produce HOCl. Among reactions that yield HOCl are hydrolysis of chlorine gas, electrolysis of salt solution and acidification of hypochlorite. In commercial industries, mild HOCl is produced by the acidifying of NaOCl with hydrochloric acid (HCl) [37]. Other methods to produce HOCl for industrial purposes depend on the size of the industry and available resources for the production procedures. The most widely known commercialized product containing HOCl in a low concentration as its active compound is disinfectants used in the healthcare sector. Disinfectants are used to neutralize wounds in humans due to their antimicrobial properties. The use of HOCl as the main ingredient for disinfectants is a success because it is effective against a wide range of microorganisms in a short period of time [35], and it does not interrupt the wound healing process [36]. These properties are the major factor that lead to the utilization of HOCl in the healthcare industry as suitable chemicals, especially in crucial medical processes. Each substance used was first proven to have no detrimental effects as it relates mainly to the safety of patients and the person in charge of handling the substance.

Two popular disinfectants sold in the market are Medilox® and Sterilox. The products were tested for their stability, which is a primary concern for products containing HOCl. The concentration of chlorine and the pH were proven to be in decreasing pattern over time [37]. In addition, its shelf life is short, estimated to be four days, when it is directly exposed to sunlight or UV radiation. Further research and development should improve their shelf life to produce better quality and long-lasting effects.

The potential of hypochlorous acid towards insecticidal activity

HOCl is widely known as an agent for wound healing treatment and disinfectants as it contains high potential for antimicrobial activity. This potential is also beneficial in sectors other than pharmaceutical. HOCl has the potential for an antimicrobial activity that helps to hinder and inhibit microorganism growth and population. This might lead to HOCI being used for pest control and result in a novel method to aid several processes of agricultural products for better food security. In general, not many studies have been done to verify the true potential of HOCl in controlling agricultural pests. Only a few studies have proven the benefit of using a compound containing chlorine to curb pest population, but there is evidence that household products, such as bleach, have been used as pesticides for a few varieties of pests. This method can be utilized by small-scale farmers who do not want to spend a large budget on controlling pests and diseases of their agricultural crops.

Table 6 shows the results of a study conducted by [38] to evaluate the effectiveness of commercial bleach towards the control of *Aedes aegypti*, which is the principal vector for the dengue virus. The eggs laid by the mosquitoes tend to stay dormant for a few months after it completes embryogenesis, producing an eggs bank for the mosquito species. This problem largely contributes to the rising cases of dengue fever. The mosquitoes have to be controlled as early as the egg stage. Ovicide has to be applied to kill potential eggs in an area. The main problem when using ovicide is the eggs' resistance to desiccation. The outer layer of the eggs can block the insecticide from penetrating the inner layer, and thus decreasing the effectiveness of insecticide at the larval stage of *Aedes aegypti*. In contrast, the active compound found in regular bleach was proven to be able to breakdown the outer layer, making a pathway for the insecticide to enter the eggs [39]. The active ingredient responsible for this action is sodium hypochlorite (NaOCl). The use of household bleach as an insecticide for *Aedes aegypti* is a cost-effective alternative because the bleach itself is cheap compared to insecticide, and the water applied with the bleach was proven safe for daily consumption if the concentration does not exceed four ppm.

Mackay et al. [38] conducted a study on the efficiency of commercial bleach against *Aedes aegypti.* Two types of solution were tested: bleach, and a mixture of bleach and smectite clay. The solution containing bleach and smectite clay was verified to be almost equally effective against the eggs of *Aedes aegypti* on plastic, rubber, and concrete, while the bleach solution alone was less effective against the eggs on concrete. Longer exposure time to sunlight yields fewer eggs at the end of the experiment, implying that exposure to sunlight plays a vital role in increasing the efficiency of bleach spray on eggs. In light of the discovery of the potential of bleach-containing HOCI for pest control, more studies should be conducted to uncover the true potential of HOCl in the agriculture sector. However, the use of bleach must be handled with care because household bleach is corrosive and, at a certain level of concentration, might harm the skin or cause damage to surfaces, such as plastics, some metals, paint, wood, and fabrics.

Potential pesticide formulation of hypochlorous acid and betel extract

HOCl solution and betel extract were proven to contain insecticidal activity potential towards many pests at different stages, including the egg, larval, and adult stages. Betel extract contains essential phytochemicals that provide its insecticidal activity potential, while HOCl contains antimicrobial and insecticidal activity. Improved formulations containing both HOCl and betel extract can be produced to increase their effectiveness. A pesticide formulation that can act on different mechanisms can be valuable to farmers because it can be a cost-effective alternative in pest management. The primary consideration in producing the formulation is the shelf-life of the pesticide and whether combining the compounds would produce adverse side effects. There are studies where the combination of HOCl and betel extract were tested for its effectiveness on fungi. These studies may lead to discoveries and further studies on the efficacy of formulation that combines the two essential compounds towards insecticidal activity.

Table 6. Insecticidal activity of HOCl

Insecticidal Activity Potential of HOCl	Targeted Insect Stage / Targeted Organism	Results	Ref.
Usage of HOCl in the control of Aedes aegypti	Egg stage of insect	Both solution containing bleach only and the formulation between bleach and smectite clay were proven to be effective against the egg of Aedes aegypti	[38]
Classified under the pesticide category	Adult stage of insect	Hypochlorous acid along with other disinfectants were classified under the pesticide categories by The Environmental Protection Agency (EPA)	[40]
Possess antimicrobial properties	Adult stage of insect	HOCl were proven to provide significant effect towards antimicrobial activity that was also possessed by betel extract that could lead to the production of novel insecticide	[41]; [35]
Used as germicide	Microorganism	A type of HOCl germicide known as warexin were proven to possess sporicidal and bactericidal activity potential against several bacterial strain and its effectiveness were higher compared to bleach.	[43]

Conclusion

Piper betle has considerable potential for insecticidal activity. The essential oil extracted from betel plants contains beneficial phytochemicals that can be used as a major component in novel insecticides and pesticides. Tannins, saponins, and flavonoids are examples of essential phytochemicals in betel crops. It is crucial to seek better alternatives for pest management that does not involve the use of chemicals that are harmful to the environment. Moreover, current artificial insecticides and pesticides tend to increase insect resistance up to a point that chemical insecticides are no longer effective. The formulation of betel extract and hypochlorous acid aimed to increase effectiveness as hypochlorous acid has antimicrobial activity potential. An investigation ought to be conducted for the use of betel extract against a broader range of pests. Betel extracts have been proven to be fatal against certain pest species. Future studies should focus on the extract's mechanism and the insects' taxonomy. A combination of hydroporous *Piper betle* extract and other possible solutions must be examined to increase their effectiveness. Currently, the primary use of HOCI is in the healthcare sector as disinfectant because of its antimicrobial and antifungal properties. These chemicals can be further studied to expand their usage to the agriculture sector. Household bleach that contains HOCl as its constituent has been proven to have the potential towards insecticidal activity that could largely contribute to the production of novel pesticides.

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