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Alpinia Conchigera Griff. AS NATURAL ANTIMICROBIAL RESOURCES: A REVIEW

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Abstract: Natural products based on alternative medicines are getting popularity among consumers nowadays. This review aimed to explore the diverse use and effects of *Alpinia conchigera* Griff. as an antibacterial and antifungal. Keywords such as *Alpinia conchigera*, antimicrobial, antifungal and biology activity were used to search related studies from available databases. The review revealed that *Alpinia conchigera* exhibits antibacterial and antifungal activities to inhibit and kill pathogens because its essential oil that contains numerous phytochemical compounds such as chavicol acetate, β -sitosterol, alkaloids, steroids, saponins and other functions. Although studies have shown its general health medical value, there are still limited in-vitro and in-vivo studies mainly on the effectiveness of *Alpinia conchigera* Griff. on general health and oral health. Thus, more research is warranted in this area to encourage using natural resources for health.

Keywords: Antibacterial, antifungal, antimicrobial, biology activity, Alpinia conchigera.

1.0. Introduction

Alternative medicines which use natural products from plant sources as their main ingredients have started to draw people's attention, mainly because of their abundant availability and thus cost advantages. It has also been claimed that natural-based medicines are safer for long-term use, with less toxicity and side effects (George, 2011). The pharmaceuticals-based medications have caused more than 100,000 fatalities yearly, with non-fatal adverse drug reactions of about 2.2 million cases which was higher when compared to botanical-based products. The botanical-based medicines were reported to be low, ranging from 12 to 24 yearly. A study by Giménez et al. (2023)compares the safety of natural based medicines, biological disease-modifying anti-rheumatic drugs (bDMARDs)with conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs), andfound that the adverse effect was lower in systemic juvenile idiopathic arthritis (sJIA) treated with nDMARDs compared to those treated with csDMARDS. Most natural-based medicines do not contain synthetic chemicals and drugs and are fast becoming popular in developing and developed countries (Bachtel & Israni-Winger, 2020). Thus, this review aimed to explore the diverse methods used to extract and identify the constituents, as well as the effects of *Alpinia conchigera* Griff. as a natural antibacterial and antifungal agent.

A search was performed using several databases such as *PubMed*, and *Science Direct* for related articles published from 2007 until 2022. *Google Scholar* was also used in the search. Keywords, such as *Alpinia conchigera* Griff., antibacterial activity, antifungal activity, and biological activity were used, and only related papers were selected for further evaluation. Related information was extracted such as the place and parts where the samples were collected, methods used to extract the plant, methods applied to identify the constituents and bioactive compounds, and antimicrobial activities of *Alpinia conchigera* Griff.

The selection criteria set for this study were divided into two categories, inclusion, and exclusion. As for the former, the journal articles to be referred to must be in English, the studies conducted must be *in vitro* setting and they must include various parts of *Alpinia conchigera* Griff. such as the leaves, pseudostems, and rhizomes. Moreover, the studies must have used crude extract or bioactive compounds, which had been first identified such as through phytochemical

analysis to be tested, against the various types of microorganisms. As for the latter studies which used other species of *Alpinia* sp., such as *Alpinia galanga*, *Alpinia ligulata*, and *Alpinia nieuwenhuizii* were excluded (Phitak et al., 2009; Yusoff et al., 2011). Also excluded were studies which used the combined extract of *Alpinia conchigera* Griff. with other plants to observe their synergistic effect.

As a result, from the search a total of 465 related papers were found and only ten of them were shortlisted for further reviews and evaluation before the number was further reduced to seven to be included in the final analysis. Five of the studies were analysed for antimicrobial effects. Relevant information, such as area of sample collection, method of extraction, method to determine the bioactive compound, bioactive compounds present in *Alpinia conchigera* Griff., and antibacterial and antifungal activity of *Alpinia conchigera* Griff. was extracted and tabled.

2.0. Alpinia conchigera Griff

Malaysia is one of the countries with abundant natural resources, especially herbs. When modern medicine was not yet widely available, people utilized home remedies made of natural herbs and ointments to cure a variety of ailments like pain, injuries, and inflammation (Sam, 2019). Even now, in this modern technology era, natural herbs are still being used in many countries as an alternative to modern treatments (Bhardwaj et al., 2018; Gupta et al., 2018). In Malaysia, the local people continue using herbs extensively, as a substitute of modern medicine (Mainasara et al., 2018; Mohamad et al., 2019). In fact, because of the alleged benefits to treat a variety of ailments, researchers are becoming increasingly interested in the use of traditional medicinal herbs incorporated into modern treatment (Kim et al., 2018; Yong Lu, 2019).

Alpinia conchigera Griff is one of the many varieties of plants used in Malay traditional medicines (Qamaruz Zaman et al., 2022), which belongs to the family of *Zingiberaceae*, also known as *Languas conchigera* Burkill. Locally, this plant is known as "lengkuas ranting", "lengkuas kecil", "lengkuas padang", "lengkuas geting" and "chengkenam" which is native to the Peninsular Malaysia (Ibrahim et al., 2000). *Alpinia conchigera* is a flowering seed, perennial and herbaceous plant with creeping rhizomes, and it can grow between 0.6 metre to 1.5 metres tall (Burkill, 1966). This herbal plant grows well in tropical and sub-tropical climate zone countries, such as countries in South East Asia, Bangladesh, India and China. Most of its habitats are terrestrial, including open ground, secondary rainforest, freshwater swamp forest, and agricultural land. Additionally, it can thrive in wet, open areas like the boundaries of rice fields and behind the cover of rubber and oil palm trees.

A. conchigera collected from the wild, has been widely used by local people for generations, because of its medicinal uses and edibility. It has been reported that this plant is useful in medicine and food preparation, as spice, condiment, dye, and flavoring. The Malays used rhizome extract of *A. conchigera*, for the treatment of skin fungal infections and rashes (Ibrahim et al., 2009). In Chinese medicine, dyspepsia and insect bites are treated with the pounded rhizome of *A. conchigera*. The rhizome is considered diaphoretic and stimulating, and it is also commonly used in the treatment of bronchitis, jaundice, headache, ringworm, indigestion, abscess, and vertigo. Meanwhile, rheumatism has been treated with a poultice made from the cooked leaves or a mixture of both; the leaves and rhizomes (Yuandani et al., 2023). The pounded leaves have been used as one of the confinement treatments in Malay culture (Ibrahim et al. 2009).

The rhizome is often used in the preparation of rice alcohol, for its distinctive flavoring such as pungent hot and spicy taste. Because of its pungency, such countries as China and Thailand also use it as food condiments. Due to the presence of diverse phytochemicals, each component of the *A. conchigera* plant has distinct chemical properties that it uses to cure issues. Additionally, several significant bioactive substances, including chavicol, chavicol acetate, 1-acetoxyxhavicol acetate, eugenol, terpenoids, essential oils, 1,8-cineole, -caryophyllene, and cardamomin, are present in the rhizome and leaf essential oil (Qamaruz Zaman et al., 2022).

3.0 Samples Collection and Method of Extraction

The samples collected from the studies were from different places and countries, namely Malaysia (Aziz et al., 2013; Ibrahim et al., 2009; Taib et al., 2020), Bangladesh (Ibrahim et al., 2012; Saha & Paul, 2014), and Vietnam (Giang et al., 2007; Mohammad et al., 2010). Fresh rhizome samples of *A. conchigera* were collected from the Military Institute of Pharmaceutical Control and Research in Thai Nguyen, Vietnam, Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratory, Chittagong (Mohammad et al., 2010) and Jeli, Kelantan (Aziz et al., 2013; Ibrahim et al., 2009; Taib et al., 2020).

The fresh rhizome samples of *A. conchigera* were heated in the oven to dry to be in the powder form and extracted using methanol (MeOH), n-hexane, ethyl acetate (EtOAc), and n-butanol (*n*-BuOH) (Giang et al., 2007). In the study by Mohammad et al. (2010),some of the samples were homogenized using a hydro distillation process, turning them into slurry, to isolate the essential oils. Anhydrous sulfate was used to dry the essential oils, before being stored at 0°C inside an airtight container. Meanwhile a study by Taib et al. (2020), had the samples transferred to another state to be cultivated, dried, and ground before being extracted by a maceration technique using *n*-hexane, dichloromethane (DCM) and MeOH.

A rotary evaporator was used to dry the extracts. The presence of their bioactive components was subsequently examined in the n-hexane and DCM crude extracts.

Besides rhizomes, other parts of *A. conchigera* were also sampled such as the leaves and pseudostems. These samples collected from Jeli, Kelantan, Malaysia, were extracted by steam distillation and the essential oils were dried and stored at 4 to 6°C before being analyzed (Ibrahim et al., 2009). In the study by Ibrahim et al. (2012), the whole plant of *A. conchigera* Griff. collected from Naramuk, Bangladesh was first dried in the oven before it was grounded into powdered form and extracted using methanol for 18 hours through Soxhlet extraction. The whole plant was extracted to maximize the effectiveness of the extracts when tested.

Wild A. conchigera Griff. pseudostems and rhizomes were also collected from Jeli Kelantan, Malaysia, and dried and powdered prior to Soxhlet extraction using n-hexane, DCM, and MeOH. (Aziz et al., 2013). The crude extracts were dried in a vacuum after extraction. The chemical components and antibacterial activity of the n-hexane and DCM extracts were further analyzed (Aziz et al., 2013). *A. conchigera* Griff. leaves and stems were gathered from Naramuk, Bangladesh, and pre-cleared to remove unwanted components before being thoroughly dried and milled into coarse powder. This powder was then stored in an airtight container in a cool, dark, and dry environment. The powder was soaked in MeOH for 10 days, continuously shake. The products were filtered using white cotton and filter paper (Saha & Paul, 2014).

Based on the studies, the same methods were used to extract different parts of *A. conchigera* Griff. Soxhlet extraction or rotary evaporator were commonly used for this purpose, but with different types of solvents such as *n*-hexane, DCM, *n*-hexane-ethyl acetate (EtOAc) and MeOH to extract the compounds based on the polarity of the compounds.

4.0 Methods to Analyze Bioactive Compounds

Phytochemical screening is conducted to reveal the constituents of the plant extracts and to search for bioactive agents that can be used in the synthesis of valuable drugs. Therefore, the crude extracts are further analyzed to identify the presence of bioactive compounds. In several studies, it was found that the bioactive compounds extracted were more effective when compared to the crude extracts (Aziz et al., 2013).

Pseudostem and rhizome extracts in *n*-hexane and DCM were chromatographed on silica gel utilizing a stepwise gradient method with EtOAc as the solvent (Aziz et al., 2013). *A. conchigera* crude extracts of *n*-hexane and DCM were characterized, and the bioactive compounds were isolated using gas chromatography-mass spectrometry (GCMS) and column chromatography (CC) respectively (Taib et al., 2020). In another study *A. conchigera* crude extracts of *n*-hexane and ethyl acetate (EtOAc) were further fractionated to isolate the bioactive compounds using column chromatography (CC) and flash chromatography (FC) repeatedly (Giang et al., 2007). Ibrahim et al. (2009) used gas chromatography-mass spectrometry (GCMS) to analyze the presence of the essential oils compound of the leaf, pseudostem, and rhizome. The same method was used by Mohammad et al. (2010) but only to analyze the compounds present in the rhizome essential oils.

The rhizomes' essential oils of *A. conchigera* were also identified through preliminary phytochemical screenings using Fehling's solution test to screen for reducing and non-reducing compounds(Ibrahim et al., 2012). In the same study, several other tests were also performed such as Salkowski test to identify the presence of terpenoids, Liebermann-Burchard test to detect unsaturated steroid and cholesterol, Potassium dichromate, Hager's reagent to find out the total content of alkaloids and Shaking test for foaming and to detect present of saponins.

5.0 Compounds Present in Alpinia Conchigera Griff.

The percentage and proportion of the compounds present in the rhizome, pseudostem, and leave oils are different. The volume of essential oils yielded from the rhizomes was found to be higher than that the leaves and pseudostems (Ibrahim et al., 2012). In a study reported by Ibrahim et al. (2009), several compounds have been identified, which included monoterpenes, sesquiterpenes, esters, aldehydes, hydrocarbon, and phenol. It was found that β -bisabolene was the main compound present in the leave and pseudostem oils, while 1,8-cineole was a major compound found in rhizome oil. There were several essential oils common compounds that can be isolated from the leaves, pseudostems, and rhizomes namely β -bisabolene, β -sesquiphellandrene, and β -elemene. Mohammad et al. (2010) found that the essential oil obtained from the extraction process was only 1.10%. Eucalyptol, which made up 25.85% of the rhizome essential oil, was the predominant component of the rhizome. The other chemicals included chavicol, β -pinene, caryophyllene, and 4-terpineol.

The extraction of different parts of the plant such as rhizomes, leaves, and pseudostems produced different volumes of extracts. It was found that these compounds were a mixture of stigmasterol and β -sitosterol, chavicol acetate, *p*hydroxy cinnamaldehyde, 1'S-1'-acetoxychavicol acetate, trans-*p*-coumaryl diacetate, 1'S-1'- acetoxyeugenol acetate, 1'-hydroxychavicol acetate, *p*-hydroxycinnamyl acetate and 4-hydroxybenzaldehyde (Aziz et al., 2013). Taib et al. (2020) reported that the compounds found in the rhizomes of *A. conchigera* Griff. include 1'S-1-acetoxychavicol acetate, trans-*p*-coumaryl diacetate, *p*-hydroxycinnamyl acetate, 1'S-1'-hydroxychavicol acetate, *p*-hydroxybenzaldehyde, stigmasterol, and β -sitosterol. While Giang et al. (2007) reported four major compounds present in the rhizomes of *A*. *conchigera* crude extracts, namely cardamomin in the form of yellow needles, chalconaringenin 2'-O-methyl ether isolated as orange cylindrical crystals, alpinetin, and naringenin 5-O-methyl ether, both present as colorless needles.

Previous studies showed that compounds existing in the rhizome, leaves and pseudostems essential oils were different. The β -bisabolene and β -sesquiphellandrene compounds were reported at higher concentration from all parts of the tree. Table 1 below shows the bioactive compounds present after the isolation process from the crude extract of *Alpinia conchigera* Griff. from various parts of the plant such as the leaves, pseudostems, and rhizomes.

Study by	Country	Area/District	Samples	Method to Isolate or Analyze Compound	Compounds Presence
Giang et al. (2007)	Vietnam	Military Institute of Pharmaceutical Control and Research	Rhizomes	Column Chromatography (CC) Flash Chromatography (FC)	-Cardamomin -Chalconaringenin 2'- <i>O</i> -methyl ether -Alpinetin -Naringenin 5- <i>O</i> -methyl ether
Ibrahim et al. (2009)	Malaysia	Jeli, Kelantan	Leaves Pseudostems Rhizomes	Gas Chromatography- Mass Spectrometry (GC-MS)	-β-bisabolene -β-sesquiphellandrene -β-elemene -1,8-cineole
Mohammad et al. (2010)	Vietnam	Campus of BCSIR Laboratory, Chittagong	Rhizomes	Gas Chromatography Mass Spectrometry (GC-MS)	-Eucalyptol -Chavicol -β-pinene -Caryophyllene -4-terpineol
Ibrahim et al. (2012)	Bangladesh	Naramuk, Rajasthali of Rangamati district	Whole plant	Biochemical Tests: -Fehling's solution test -Benedict's test -Salkowski test -Dragendorff's reagent -Wagner's reagent -Hager's reagent -Tannic acid -Shaking test	-Reducing sugar -Steroids -Tannins -Alkaloids -Saponins
Aziz et al. (2013)	Malaysia	Jeli, Kelantan	Pseudostems Rhizomes	Column Chromatography (CC)	-Stigmasterol -β-sitosterol -Chavicol acetate -p-hydroxy cinnamaldehyde -1'S-1'-acetoxychavicol acetate - <i>trans-p</i> -coumaryl diacetate -1'S-1'-acetoxyeugenol acetate -1'-hydroxychavicol acetate -p-hydroxycinnamyl acetate -4-hydroxybenzaldehyde
Saha & Paul (2014)	Bangladesh	Naramuk, Rajsthali of Rangamati district	Leaves Stems	-	-
Taib et al. (2020)	Malaysia	Jeli, Kelantan	Rhizomes	Gas Chromatography Mass Spectrometry (GC-MS) Column Chromatography (CC)	 -1'S-1-acetoxychavicol acetate -trans-p-coumaryl diacetate -p-hydroxycinnamyl acetate -1'S-1'-hydroxychavicol acetate -p-hydroxybenzaldehyde -Stigmasterol -β-sitosterol

Table 1: Details of the five studies on Alpinia conchigera Griff.

6.0 Antibacterial Activity of Alpinia Conchigera Griff.

The antibacterial properties of A. conchigera were reported to be caused by crude extracts of the plant, phytochemicals, and bioactive compounds. The five studies have been conducted to test the efficacy of A. conchigera as an antimicrobial agent against several species of bacteria (Ibrahim et al., 2009; Ibrahim et al., 2012; Saha & Paul 2014; Taib et al., 2020; Giang et al., 2007). Three studies used American Type Culture Collection (ATCC) such as that by Taib et al. (2020) which used Methicillin Resistant Staphylococcus aureus ATCC 43300. Meanwhile the study conducted by Saha & Paul (2014), gram-positive and gram-negative bacteria were isolated from clinical samples of the Faculty of Microbiology, Chittagong University, Chittagong, Bangladesh. Ibrahim et al. (2012) also used pure culture from the Faculty of Biology of the same University. However, Giang et al. (2007) did not mention the source of the bacteria used in their study. Giang et al. (2007), also tested on gram-positive and gram-negative bacteria namely Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The tests were performed against Alpinia conchigera Griff. extracts and their bioactive compounds. Broth microdilution method was conducted to evaluate the minimum inhibitory concentration (MIC). The crude extract of *n*-hexane showed inhibitory action against *E. coli* (200 μ g/ml), *P. aeruginosa* (100 μ g/ml), and B. subtilis (100 µg/ml). Meanwhile, the crude extract from ethyl acetate inhibited the growing of E. coli (100 µg/ml) and S. aureus (50 µg/ml), and butanol crude extracts of the rhizomes of A. conchigera only showed antibacterial activity against S. aureus (100 µg/ml). From the characterized compounds it was found that three out of four compounds, cardamomin, chalconaringenin, and alpenetin showed good properties as antibacterial agents compared to the crude extracts of the plant against E. coli, B. subtilis, and S. aureus with the MIC in the range of 25 to 50 µg/ml to inhibit the bacteria strains. Another compound, naringenin only showed good inhibitory activity against E. coli and S. aureus $(50 \mu g/ml)$.

Ibrahim et al. (2009) applied the leaf, rhizome, and pseudostem essential oils to four strains of bacteria which were *Pseudomonas aeruginosa* UI 60690, *Pseudomonas cepacia, Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 1228. MIC was selected to determine the antimicrobial activity of the rhizome essential oil of *A. conchigera* by using the microdilution method. The effectiveness of the essential oil extracted from the leaves, rhizomes, and pseudostems of *A. conchigera* showed weak inhibitory activities. A high concentration of the extract was needed to inhibit the growth of four strains of bacteria ranging from 25.9 μ g/ μ l to 33.3 μ g/ μ l, in contrast the antibiotic concentration required to inhibit the bacteria in the range of 0.0036 μ g/ μ l to 0.014 μ g/ μ l. From the findings, the essential oils extracted from different sections of *A. conchigera* showed weak antibacterial activities indicating that antimicrobial activity of this plant could be due to the presence of bioactive compounds from the plant.

Ibrahim et al. (2012) evaluated the antibacterial activity of *A. conchigera* crude extract against four gram-positive bacteria namely *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *and Staphylococcus aureus* and seven gram-negative bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphii*, *Salmonella typhi*, *Shigella dysenteriae*, *Shigella sonnei*, *and Vibrio cholerae*. The methods selected for the antimicrobial screening were disc diffusion assay (DDA) and broth microdilution. The positive controls used in this experiment were ciprofloxacin and fluconazole. In DDA, the inhibitory properties were indicated by the zone of inhibition diameter. Based on the preliminary screening of antimicrobial activity results, it showed that the inhibition zone of *A. conchigera* Griff. was in the range of 19.0 to 23.0 mm. This indicates *A. conchigera* Griff. had a wide spectrum to inhibit gram-positive and gram-negative bacteria. A low concentration of *A. conchigera* extract (31.25 µg/ml) was used to inhibit the growth of *B. cereus*, *S. typhi, S. sonnei*, and *V. cholerae*, indicating the presence of strong antimicrobial bioactive compounds which can act as an antibacterial agent.

A preliminary screening of antibacterial activity of the essential oils extracted from the leaves and stems was conducted against eleven types of bacteria which include gram-positive and gram-negative bacteria namely, *B. subtilis, B. megaterium, B. cereus, S. aureus, P. aeruginosa, E. coli, S. dysentariae, S. sonnei, S. typhi, V. cholerae,* and *S. paratyphi* using disc diffusion technique. In this procedure, the positive and negative controls selected were standard disc of ciprofloxacin (500 μ g/disc) and a blank disc respectively and there were two different concentrations of crude extract used 250 μ g/disc and 500 μ g/disc. Based on the results obtained, the disc impregnated with 250 μ l of extract showed an inhibition range from 8.0 to 13.0 mm while that with 500 μ l had its diameter of inhibition zone in the range of 20.0 to 24.0 mm which reflected that the extract had antimicrobial properties. MIC was conducted to determine the lowest dosage that can be used to impede the growth of gram-positive and gram-negative bacteria. From the result, this plant extract with the concentration of 62.5 μ g/mL showed inhibition towards *S. aureus, S. typhi*, and *V. cholerae* (Saha & Paul, 2014).

A study conducted by Taib et al. (2020) determined the antimicrobial activity of bioactive compounds selected using broth microdilution technique against Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300. Dimethyl sulfoxide (DMSO) was used to dissolve the bioactive components that were isolated from the crude extract to a final concentration of 31.25 g/mL. The positive control used in this experiment was tetracycline. The bioactive compounds selected include 1'S'-1'-acetoxychavicol acetate, *trans-p*-coumaryl diacetate, and *p*-hydroxybenzaldehyde. The result of this study showed that 1'S'-1'-acetoxychavicol acetate exhibits antimicrobial effect with a MIC value of 0.5 mg/mL compared to the tetracycline used as a as positive control, which a much lower MIC value of 0.0005 mg/mL.

7.0 Antifungal Activity of Alpinia Conchigera Griff.

Besides bacteria, *A. conchigera* Griff. and the bioactive compounds isolated from this plant were also claimed to be antifungal agents. Three studies reported on antifungal activities of *A. conchigera* Griff. A study by Ibrahim et al. (2009) used *Microsporum canis* ATCC 36299 and *Trichophyton rubrum* ATCC 28188 isolated from lesion and *Trichophyton mentagrophytes* ATCC 18748 isolated from mild dermatophytosis. *Microsporum canis* (ATCC 36299), *Trichophyton mentagrophytes* (ATCC 18748), and *Trichophyton rubrum* (ATCC 28188) were tested with the essential oils collected from the leaf, rhizome, and pseudostem of *A. conchigera* Griff. The effectiveness of these essential oils was compared with the positive control which was cycloheximide with a MIC value of 2.27 $\mu g/\mu l$. Results obtained from the microdilution procedure conducted found that the MIC for essential oils from the leaf, rhizome, and pseudostem were 28.6 $\mu g/\mu l$, > 23.1 $\mu g/\mu l$, and > 31.0 $\mu g/\mu l$ respectively for all three dermatophytes. The essential oils were found not effective in repressing the dermatophytic fungi, thus further highlighting that the antifungal effect of *A. conchigera* could be due to other phytochemicals.

According to Giang et al. (2007), the *n*-hexane, ethyl acetate, and butanol crude extracts and four compounds isolated from this plant were tested against *Aspergillus niger, Fusarium oxysporum, Candida albicans,* and *Saccharomyces cerevisiae* through broth microdilution. It was reported that 100 μ g/ml butanol crude extract of *A. conchigera* could inhibit *F. oxysporum* and *S. cerevisiae* while 50 μ g/ml was needed to inhibit the growth of *C. albicans.* The lowest concentration of ethyl acetate crude extract needed to inhibit both *C. albicans* and *S. cerevisiae* which were 50 μ g/ml and 100 μ g/ml respectively. However, all four bioactive compounds did not show any inhibitory activity against the selected fungi and yeast.

In a study by Ibrahim et al. (2012), MeOH extract of *A. conchigera* was tested for its antifungal properties against seven types of fungi namely *A. niger, B. dermatitidis, C. albicans, C. neoformans, Microsporum* spp., *P. ovale*, and *Trichophyton* spp. using two different methods which were DDA and MIC. For DDA, the zone of inhibition diameter was found to be in the range of 15.0 mm to 26.0 mm compared to that of the positive control in this study, fluconazole (21.0 to 29.0 mm). After the preliminary test (DDA) was completed, three selected fungi namely *A. niger, B. dermatitidis,* and *C. albicans* were further tested through the broth microdilution technique to determine the lowest concentration of the extract needed to inhibit the fungi. It is found that, all three types of fungi were inhibited at 31.25 µg/ml of MeOH extract of *A. conchigera*. Table 2 below shows the inhibitory activity of *Alpinia conchigera* Griff. against gram-positive, gram-negative bacteria, and fungi.

Author	Microorganisms Used	Positive Control	Methods of Testing	Findings
Giang et al. (2007)	Gram-Positive: S. aureus, B. subtilis Gram-Negative: E. coli, P. aeruginosa Fungi: A. niger, F. oxysporum, C. albicans, S. cerevisiae	Not stated	Minimum Inhibitory Concentration (MIC)	-Crude extracts range from 50 to 200 μg/ml -Bioactive compounds range from 25 to 50 μg/ml -Bioactive compounds did not inhibit any type of fungi
Ibrahim et al. (2009)	Gram-Positive: S. aureus, S. epidermidis Gram-Negative: P. aeruginosa, P.cepacia Fungi: Microsporum canis (ATCC 36299), Trichophyton mentagrophytes (ATCC 18748), Trichophyton rubrum (ATCC 28188)	Streptomycin sulphate (0.0036 to 0.014 µg/ml) Cycloheximide 2.27 µg/ml	Minimum Inhibitory Concentration (MIC)	-Leaf oil: 25.9 µg/µl (bacteria) and 28.6 µg/µl (fungi) -Rhizome oil: > 33.3µg/µl (bacteria) and > 23.1µg/µl (fungi) -Pseudostem oil: > 28.6 µg/µl (bacteria) and > 31.0 µg/µl (fungi)
Ibrahim et al. (2012)	Gram-Positive: B. cereus, B. megaterium, B. subtilis, S. aureus Gram- Negative: E. coli, P. aeruginosa, S. paratyphi, S. typhi, S. dysenteriae, S. sonnei, V. cholerae Fungi: A. niger, B. dermatitidis, C. albicans, P. ovale, C. neoformans, Microsporum spp., Trichophyton spp.	Ciprofloxacin 50 µg/disc Fluconazole 50 µg/disc	Disc Diffusion Assay (DDA) Minimum Inhibitory Concentration (MIC)	DDA (diameter of inhibition zone) Bacteria: range from 19.33 mm to 23.67mm Fungi: 15.0 mm to 26.0 mm MIC 31.25 µg/ml: <i>B. cereus, S. typhi, Sh.</i> <i>dysenteriae, V. cholerae, A. niger,</i> and <i>B. dermatitidis</i> 62.50 µg/ml: <i>E. coli</i> and <i>S. aureus,</i> and <i>C. albicans</i>
Saha & Paul (2014)	Gram-Positive: B. subtilis, B. megaterium, B. cereus, S. aureus Gram-Negative: P. aeruginosa, E. coli, S. dysentariae, S. sonnei, S. typhi, S. paratyphi, V. cholerae	Ciprofloxacin 500 µg/disc	Disc Diffusion Assay (DDA) Minimum Inhibitory Concentration (MIC)	DDA (diameter of inhibition zone) Range from 8 mm to 24.5 mm MIC 62.50 µg/ml: <i>S. aureus, S. typhi</i> and <i>V. cholerae</i>
Taib et al. (2020)	Gram-Positive: Methicillin Resistant <i>S. aureus</i> (MRSA) ATCC 43300	Tetracycline (0.0005 mg/ml)	Minimum Inhibitory Concentration (MIC)	0.5 mg/ml: 1'S'-1'- acetoxychavicol acetate

Table 2: Inhibitory activity of Alpinia conchigera Griff. Against Gram-Positive, Gram-Negative Bacteria and Fungi

Based on the review, *Alpinia conchigera* Griff. has shown promising antibacterial and antifungal activities against various microorganisms. It has also been reported to have anti-inflammatory, and anti-cancer properties against prostate cancer cells, breast cancer cells, urinary bladder cancer cells, oral squamous cancer cells, and liver cancer cells. *A. conchigera* may be used as antimicrobial agents to replace commercial antibiotics and cancer treatment to treat infection, inflammation, and cancer problems. However, several challenges and issues can be foreseen when dealing with biocontrol agents, especially when they are used to substitute the functional properties of commercialized antibiotics and cancer treatments. Firstly, the safety issue and toxicity levels of antimicrobial agents which need to be determined before use. Besides, the bioactive compounds isolated from this plant differ, in relation to its different places of origin and its different parts namely leaves, pseudostems, and rhizomes. Different chemotypes rather than geographic or ecological differences

account for the cultivar's variance (Mohammad et al., 2010). Besides, thorough experiments are needed to ensure that the usage of the crude extract or the bioactive compounds is safe, as it may cause an allergy reaction in some individuals.

In addition, the consistency of the major compounds isolated from the essential oils needs to be monitored for every study conducted. The results obtained from the GCMS procedure need to be compared with those available in the National Institute of Standards & Technology (NIST) library data of peaks. This is due to the fact that likelihood scores are frequently assigned to results based on how closely the mass spectral data of the investigated molecule resembles those of its closest hit in the library. When attempting to clearly identify substances in complicated matrices, it is also crucial to use the NIST library (Valdez et al., 2018).

In the present review, most of the studies reported are *in vitro* and used ATCC samples. Nevertheless, these studies demonstrated the potential of *A. conchigera* Griff. as an effective antimicrobial agent to treat different health conditions which warrant further studies, especially with regards to the applicability of *A. conchigera* Griff. against various types of microorganisms. This may require some biotechnological tools and applications to be introduced to study a specific mechanism of inhibitory and cytotoxic activity. Besides, the stability of the crude extract of *A. conchigera* when combined with other ingredients to produce medication has not yet been ascertained. For example, the extract may be sensitive to other elements which may degrade its functions and efficacy properties. Moreover, the faster and easier technique to optimize the yield of the extract should also be investigated for time and cost efficiency.

Finally, the research regarding the use of natural resources especially, herbs as biocontrol agents against various species of microorganisms such as oral opportunistic pathogens potentially causing infection in the lower respiratory tract have not been comprehensively conducted. Therefore, further research on the antimicrobial action of the isolated crude extract and bioactive compounds isolated needs to be done actively, so that *A. conchigera* Griff. can be commercialized as reliable biocontrol agents in the pharmaceutical industry.

8.0. Conclusion

Alpinia conchigera Griff. is a herb that is abundant and easily accessible in our country, Malaysia. In addition to being utilised in traditional recipes, this plant has also been used as a post-partum remedy and used in traditional medicines to treat fungal infections. Its exhibited potential as good antibacterial and antifungal agents have been proven in the studies, where its crude extract and its bioactive compounds proved to efficiently inhibit and kill various types of microorganisms.

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