

## REVIEW ARTICLE

# A mini review on the methods for the extraction, isolation, and determination of *P. odorata*'s bioactive compounds

Nur Ain Syabila Kiraman, Hartini Yusof\*

Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA Cawangan Selangor Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia

## Abstract:

*Persicaria odorata* is a perennial herb of the *Persicaria* genus, which belongs to the *Polygonaceae* family. *P. odorata* is commonly known in Malaysia as *daun kesum* and is widely used in cuisine and has different medicinal benefits. The medicinal benefits from *P. odorata* are contributed by its various bioactive compounds and biological activities. Thus, phytochemical analysis has been researched extensively to maximize the benefits of the plant. For herbal studies, extraction plays a crucial step in extracting the preferred bioactive compounds hence several extraction techniques such as chromatography, electrophoresis, and spectroscopy have been developed to separate and identify bioactive compounds. In turn, these compounds play a big role on the biological activities of the plant. This review highlights the methods for extraction, separation, and identification of bioactive compounds and the biological activities of *P. odorata*.

## \*Corresponding Author

Hartini Yusof  
Email:  
tini01@uitm.edu.my

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## 1. INTRODUCTION

*P. odorata* (Lour.) Soja'k has been reclassified from *Polygonum odoratum* Lour. (Starkenmann et al., 2006). *P. odorata* is a perennial herb that owns to the family *Polygonaceae* and the genus *Persicaria* (Ridzuan et al., 2013). *P. odorata* is part of a fresh herb group called cilantro and imitates the 'cilantro' flavor. In Brunei, Singapore, and Malaysia, it is called *daun kesum* and is widely used in food preparations as a flavoring material. *P. odorata* is a tender perennial herb with a height of 30-35 cm and 6-15cm of pointed leaves with a conspicuous dark purple sign in the middle of the leaves, which are green, lanceolate, and reed stems. This herb has a pungent and spicy taste and an odour like coriander with a lemon scent hint. This unique strong smell is reported due to the presence of decanal, undecanal, dodecanal, (Z)-3-hexen-1-ol, and (Z)-3-hexanal (Starkenmann et al., 2006).

In plants and fruits, many antioxidants are found: phenolics, carotenoids, anthocyanins, and tocopherols. In pharmaceutical trials, 20% of recognized plants have been used, impacting the health care system in beneficial ways, such as treating cancer and other diseases (Altemimi et al., 2017). Plants that contain beneficial phytochemicals can act as natural antioxidants, supplements that the human body needs. There are several elements involved in phytochemical plant research: compound extraction from a plant, isolation of the substances to be analyzed, recognition and analysis of the substances extracted, inspection of the biosynthetic

mechanisms of a particular molecule, and quantitative evaluation.

Complementary and alternative medicine (CAM) has become more essential in treatment because it is secure, organic, and efficient in treating diseases. In general hospitals, CAM has been commonly used to resolve medical issues such as infections and complications and protect patients' health (Ridzuan et al., 2013). The leaves of *P. odorata* have been used as a traditional medicine to treat various ailments such as diarrhoea and inflammation. Aside from human ailments, *P. odorata* has the potential to cure zoonotic diseases like mastitis (Hashemi et al., 2008). They contain a wide range of bioactivities, including antioxidant, antifungal, antibacterial, and anti-inflammatory properties, owing to the presence of significant volatile compounds such as long-chain aldehydes and other secondary metabolites of plants (phenolic compounds and flavonoids) (Sim et al., 2019). Earlier research has found that *P. odorata* has a spectrum of therapeutic activities, and a few are highly useful, like antioxidant, anticancer, antibacterial, and anti-inflammatory. Using the herb in food is also a great idea as it may preserve or strengthen biological functions and decrease the incidence of illness. The association of plant extract with traditional antibiotics has not yet been recognized (Ridzuan et al., 2017).

Many extraction methods are used to obtain bioactive compounds from plant products, including distillation, solvent extraction, sublimation, and pressing. This review aims to demonstrate the process of extracting, isolating, and

identifying *P. odorata* for its bioactive compounds and biological activities.

## 2. DISCUSSION

### 2.1. Bioactive Compounds/Phytochemicals

Chemotherapeutic employments of the medicinal plant are simply the ancient proof of humankind (Alam et al., 2017). Initially, humans used herbs for their cooking only. Still, with the disclosure of therapeutic potential, this daily herb has become a valuable root of illness fixation and well-being in different human societies (Vinatoru, 2001). The ancient history of bioactive compounds is using medicinal plants in old age. In the past, individuals did not know about bioactive compounds. However, utilization of these molecules was adequately assorted in various possibilities.

Organic compounds produced by the plant can be categorized as primary or secondary metabolites. The primary metabolites include phyosterols, carbohydrates, proteins, organic acids, and lipids, are chemical elements essential for metabolic cellular respiration and sustaining life through photometric synthesis, enhancement, and development. At the same time, secondary metabolites have various chemical structures able to produce biological effects on human health (Alam et al., 2017). According to Martin & Demain (1978), specialized metabolites are those compounds that are typically developed in the post-growth process, do not have a growth function (even though it could have a reliability function role), make by taxonomic classes of microorganisms, have peculiar chemical composition, and sometimes produced as combinations of strongly linked natural compound members. Bioactive plant compounds are usually manufactured as secondary metabolites. Any of these compounds within the secondary metabolites affect biological processes considered to be bioactive. Hence, specialized metabolites that cause pharmacological or toxicological impacts in humans and wildlife are a simple definition of plant bioactive compounds (Bernhoft, 2010).

Six key groups of phytochemicals have been categorized based on their chemical compositions and characteristics: carbohydrates, alkaloids, lipids, terpenoids, phenolics, alkaloids, and other substances, including nitrogen. Under research, it is possible to distinguish phytochemicals into major groups, such as polyphenols and carotenoids, including flavonoids, stilbenes/lignans, and phenolic acids. Based on their similar chemical composition, flavonoids can be further classified, such as flavones, flavanols, isoflavones, flavanones, and anthocyanins (Huang et al., 2015).

### 2.2. Method Uses for Extraction, Isolation, and Identification of Bioactive Compounds

The beginning step for the study of herbal plants is extraction and one of the critical steps since it is mandatory to bring out the preferred composition for later isolation and detection. Primary extraction procedures like pre-washing,

drying of plant materials or freeze-drying and grinding maximize analytical extraction kinetics and improve the interface surface of the sample with the solvent (Sasidharan et al., 2011). In varying circumstances, multiple extraction techniques can be used to consider the selectivity of extraction from different natural sources. Various solvents with varying polarities must be applied to get numerous phenolic compounds from plants with high precision (Wong et al., 2006). Polar solvents like ethyl-acetate, methanol and ethanol are being used to extract the hydrophilic substances. Meanwhile, the solvents used for lipophilic compound extraction are dichloromethane or a 1:1 mixture of dichloromethane and methanol.

Digestion, percolation, infusion, maceration, and boiling under reflux are typical extraction methods. These methods are the easiest extraction method, but it takes a long time. Rapid solvent extraction, steam distillation, supercritical fluid extraction, sonication (ultrasound-assisted extraction), microwave distillation, and hydrodistillation were done to evade flaws in the traditional extraction technique, which is the modern extraction method (Zhang et al., 2018). The conventional extract techniques are maceration, soxhlet extraction, and hydrodistillation. While microwave-assisted extraction, pressurized liquid extraction, supercritical fluid extraction, enzyme-assisted extraction, pulsed electric field-assisted extraction, and ultrasound-assisted extraction is the non-conventional extraction technique.

The detection and isolation of plant bioactive compounds is a method that has been redeveloped in recent years (Altemimi et al., 2017). The objective of observing bioactive compounds is to search for a proper approach to classify biological activity, such as antibacterial, cytotoxicity, or antioxidant, combined with simplification, precision, and rate (Mulinacci et al., 2004). Plants extracts usually have a combination of various phytochemicals with different polarities. The steps of identification and characterization of the bioactive compound remain a big challenge. Dissimilar isolation methods, like High-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), sephadex chromatography, flash chromatography, and column chromatography must use as standard procedures in the isolation of these bioactive compounds to isolate pure bioactive compounds. Other than that, non-chromatographic methods like immunoassay using fourier-transform infrared spectroscopy (FTIR), phytochemical screening assay, and monoclonal antibodies (MAbs) can be utilized to achieve and promote the detection of bioactive compounds (Sasidharan et al., 2011).

### 2.3. Extraction, Isolation, and Identification Method of Bioactive Compounds from *P. odorata*

Extraction for GC profile, the *P. odorata* leaves been minced will cover with dichloromethane. Then the mixture stood overnight, dried with Na<sub>2</sub>SO<sub>4</sub> and inserted into a GC-MS on a polar and an apolar column. Using GC-MS-O, undecanal, (Z)-3-hexanol, (Z)-3-hexanal, dodecanal, and decanal which are the organic compound, were discovered.

Other than that, 3-sulfanyl-hexan-1-ol, 3-sulfanyl-hexanal, and aldehydes were uncovered (Starkenmann et al., 2006). Sasongko et al. (2011b) reported the volatile compound that was extracted by three solvents (petroleum ether, acetone, and ethanol) and the yield extract each solvent was 0.90, 6.33, and 4.49%. The result showed that the main volatile compound extracted by ethanol was ocimene (26.44%); acetone and petroleum ether extraction was dodecanal, with a relative peak area at 27.14% and 53.12%. Decanal, dodecanal, beta-caryophyllene, neophytadiene, and ethyl hexadecanoate are the other volatile compounds extracted by these three solvents (Sasongko et al., 2011b).

Ridzuan et al. (2014;2017) studies showed that the major volatile compounds in the n-hexane extract revealed by the GSMS analysis were  $\beta$ -citral, decanal,  $\alpha$ -citral, caryophyllene, drimenol, dodecanal, euparone, drimenol, Z-citral, 2,4-heptadiene,2,6 dimethyl, and alkene (Ridzuan et al., 2013; Ridzuan et al., 2017). Ahongshangbam et al. (2014) revealed that ferulic acid, apigenin, quercetin, gallic acid, p-coumaric acid, and ellagic acid were the bioactive compounds that have been identified thru high-performance liquid chromatography analysis. They extracted the dried *P. odorata* with 100ml of ethanol and then the whole extract was extracted three times with ethyl acetate (Ahongshangbam et al., 2014). Saad et al.

(2014) extract *P. odorata* using three different techniques, which are maceration, percolation and decoction, to determine which extract gives the most effective antimicrobial activity. The results show that the extract percolation and maceration techniques were more effective because the solubility of the active components is influenced by the type of solvent used in the extraction technique (Saad et al., 2014).

*P. odorata* were extracted with a methanol/water mixture (1:1, v/v) using an ultrasonic bath at room temperature thrice (3x15 min). High-performance liquid chromatography coupled with diode-array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-MSn) method was used to analyze the methanolic-aqueous extracts and kaempferol sulphate, quercetin 3-O- $\beta$ -D-glucuronide, methyl gallate, (+)-catechin, and tetrahydroxyflavonol derivative are the bioactive compounds that been identified (Pawłowska et al., 2020). Yanpirat & Vajroda (2015) reported the chemical constituent from the lipophilic extract. The results obtained from the TLC technique were terpenoids, steroids, and other unidentified organic compounds but not alkaloids (Zhang et al., 2018). Table 1 summarises the method used and the list of bioactive compounds identified from leaves extract of *P. odorata*.

**Table 1:** Method used and the list of bioactive compounds identified from leaves extract of *P. odorata*.

Method for extractions	Method for isolation and identification	Bioactive compounds	References
Leaves extracted with n-hexane (n-hex), dichloromethane (DCM), Methanol (MeOH) and water.	Gas Chromatography Mass Spectrometer (GCMS)	Major volatile compounds: Decanal, $\beta$ -citral, $\alpha$ -citral, dodecanal, caryophyllene, euparone, drimenol, Z-citral, 2,4-Heptadiene,2,6-Dimethyl, and alkene.	Ridzuan et al. (2017) Ridzuan et al. (2014)
Ethanol extract	High-performance liquid chromatography	Apigenin, gallic acid, quercetin, p-coumaric acid, ferulic acid, and ellagic acid	Ahongshangbam et al. (2014)
Hydrodistillation uses to extract the essential oil from the fresh and dry leaves of <i>Persicaria odorata</i> .	Gas Chromatography-Mass Spectrometry (GC-MS analysis)	Caryophyllene, dodecanal, decanal, drimenol, and alpha-caryophyllene	Sasongko et al. (2011a)
Hydrophilic extract and lipophilic extract with distilled water and chloroform.	Thin-layer chromatography (TLC)	Terpenoids, steroids, and other unidentified organic compounds but not alkaloids.	Yanpirat & Vajrodya (2015)
<i>P. odorata</i> was extracted with three types of solvents ethanol, 50% ethanol and aqueous extract.	Preparative HPLC	Quercitrin and scutellarein-7-glucoside.	Okonogi et al. (2016)
Essential oils obtained by hydrodistillation	Gas chromatography with flame ionization detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS)	N-dodecanal (37.1%), n-decanal (18.1%), 1-decanol (5.4%), 1-dodecanol (4.8%), $\alpha$ -humulene (4.5%), cis-caryophyllene (3.9%) and n-undecane (2.5%).	Rebíčková et al. (2020)

Extracted with 99.9% of methanol by ultrasonification	Phytochemical element test	Sulphate, Nitrate, Tanins & Phenols	Saad et al. (2015)
Methanolic-aqueous extracts	HPLC-DAD-MSn validated analytical method	Methanolic-aqueous extracts, kaempferol sulfate, Quercetin 3-O- $\beta$ -D-glucuronide, methyl gallate, (+)-catechin, and tetrahydroxyflavonol derivative	Pawłowska et al. (2020)
Macerated with methanol solvent then followed by Soxhlet extraction	Phytochemicals analysis is performed by using standard qualitative methods	Saponins, tannins, total phenols, flavonoids, and alkaloids	Sim et al. (2019)
<i>P. odorata</i> extract by three solvents, petroleum ether, acetone and ethanol	GC-MS analysis.	Caryophyllene, dodecanal, beta-pinene, ocimene, neophytadiene, and ethyl hexadecanoate	Sasongko et al. (2011b)

## 2.4. Biological Activities

### 2.4.1. Microbial activity

The ability for antibacterial, antioxidant, antifungal, anti-inflammatory, anti-diarrheal, anti-cytotoxic, anti-ulcer, and antigen-toxicity activities of *P. minor* has been identified (Qader et al., 2011; Uyub et al., 2010; Wasman et al., 2010). Few studies have stated that because of terpenes and aldehydes such as decanal, dodecanal, eremophilene, alpha-curcumene, and caryophyllene. *P. odorata* has antimicrobial properties as the significant essential oil compounds present in leaves (Abu Bakar et al., 2015; Sasongko et al., 2011a)

Abu Bakar et al. (2015) studied the antibacterial activity of *P. odorata* leaf material by using two types of solvent extractions which are 30% aqueous-ethanol and 100% aqueous solvents. *P. aeruginosa*, *S. aureus*, *E. coli*, and *E. faecalis* were utilized for the antibacterial assessment of *P. odorata* extracts. To get the active strains accordingly, each test organism's culture stock was sub-cultured at 37 °C for 24 hours on fresh nutrient agar plates. Kirby-Bauer disk diffusion was implemented for antibacterial activity screening of plant extracts, and the results indicate that both extracts of *P. odorata* show strong activity against certain pathogenic bacterial strains. The sensitivity of aqueous extracts and crude aqueous ethanol was tested using the disc diffusion method at four different concentrations, which are 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/mL DMSO. The result revealed that *S. aureus*, *E. faecalis*, and *E. coli* were susceptible to both extracts. Nevertheless, *E. faecalis* did not demonstrate any action at a concentration of 25 mg for aqueous-ethanol extract. *S. aureus* and *E. coli* were the most susceptible species to aqueous-ethanol extraction, followed by *E. faecalis*. For the aqueous extract, all bacteria were sensitive at all concentrations except at 25 mg all three bacteria did not show the inhibition zones. The inhibition zones for *E. faecalis*, *S. aureus*, and *E. coli* were highest when using the highest extract concentration. For aqueous-ethanol extract, the inhibition zone with a

diameter of 19.33mm, 19.50 mm and 18.00 mm, while for aqueous extract 15.70 mm, 16.60 mm, and 16.45 mm. But for the *P. aeruginosa*, it not susceptible to all concentrations of both extracts may because of gram-negative bacteria so it may be affected by the density of its membrane surface. The usage of low dosage or solvent during extraction also can be one of the reasons why *P. aeruginosa* does not sensitive to both extracts (Abu Bakar et al., 2015).

Various kinds of extractions have used to evaluate the antimicrobial activity of the most active plant extract (Ridzuan et al., 2013; Saad et al., 2014). To get the particular extract, *P. odorata* was extracted using the following extracts: n-hexane, dichloromethane, Methanol and water. Eight bacterial strains which included gram-positive bacteria (*S. epidermidis*, *S. aureus*, *S. pneumoniae*, and *S. pyogenes*) and gram-negative bacteria (*K. pneumoniae*, *P. aeruginosa*, *S. typhi*, and *E. coli*) were tested against all extracts with four different concentrations (400, 200, 100 and 50 mg/ml) (Ridzuan et al., 2013). While Saad et al. (2014) extract the *P. odorata* leaves by using maceration assisted by ultrasonication, percolation with soxhlet extractor, and decoction and extracts with three various concentrations (100mg/ml, 75mg/ml, and 50mg/ml) being tested against four bacterial strains which are *E. coli*, *S. aureus*, *B. subtilis*, and *Salmonella spp* (Saad et al., 2014). To determine and screen the antimicrobial activity disc diffusion method was used (Ridzuan et al., 2013; Saad et al., 2014). *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *S. pyogenes* were the bacterial strains that susceptible to n-hexane extract. The bacteria with the most susceptibility for DCM extraction were *S. epidermidis*, *S. aureus*, *S. typhi*, and *S. pyogenes*, but *S. typhi* was only vulnerable at 400 mg/ml concentration of DCM extract. Then, only two bacteria were efficient against the methanol extract, which is *S. epidermidis* and *S. aureus*. Lastly, for the aqueous extract, the inhibition zones were observed on *S. aureus* (10.00 $\pm$ 2.00mm), *S. pneumoniae* (16.00 $\pm$ 2.00mm), and *S. pyogenes* (14.33 $\pm$ 1.52mm) (Ridzuan

et al., 2013). Antimicrobial activity only shows on *E. coli* and *S. aureus* for the extract obtained from decoction with a zone of inhibition of 6mm at 100mg/ml concentration. Meanwhile, the extraction obtained from ultra-sonic maceration and percolation using a soxhlet extractor showed antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli*, and *Salmonella spp* with rising inhibition zone along with extract concentration. The antimicrobial test showed that extract from maceration and percolation technique are more successful than decoction technique because the solubility of bioactive compound of the leaves are affected by the type of solvent being used in extraction (Saad et al., 2014).

Antibacterial activity against Gram-negative bacteria such as *E. coli* and gram-positive bacteria such as *S. aureus* shows strong activity by using the essential oil extracted from the fresh and dry leaves of *P. odorata*. The average zone of inhibition (ZOI) produced by essential oil from fresh and dry leave against *S. aureus* were 21 and 26 mm, while the average against *E. coli* was 13 and 19mm. As essential oil performs better against gram-positive bacteria than against gram-negative bacteria, *E. coli* has a lower average inhibition zone than *S. aureus* (Sasongko et al., 2011a). Antimicrobial activity of *P. odorata* essential oil shows the various range, 1024  $\mu\text{g}\cdot\text{mL}^{-1}$ , 512–1024  $\mu\text{g}\cdot\text{mL}^{-1}$  in agar and 128–1024  $\mu\text{g}\cdot\text{mL}^{-1}$ , 512–1024  $\mu\text{g}\cdot\text{mL}^{-1}$  in broth. *P. odorata* showed the lowest Minimum inhibitory concentration against *E. faecalis*, *B. subtilis*, and *S. pyogenes* which is 512  $\mu\text{g}\cdot\text{mL}^{-1}$  in the liquid stage. While for the vapor stage, the lowest Minimum inhibitory concentration that being detected against *E. coli* is 512  $\mu\text{g}\cdot\text{mL}^{-1}$ . Essential oils are more sensitive to tested with gram-positive compared with gram-negative bacteria because essential oils have active compounds that can effectively separate the essential bonds in the cell wall of gram-positive bacteria (Řebíčková et al., 2020).

#### 2.4.2. Antifungal activity

The bioautography method, thin layer chromatography, was used to investigate the inhibitory activities of lipophilic extract from *P. odorata* against *Colletotrichum capsici* and *Colletotrichum gloeosporioides*. From the bioautography and microdilution bioassay, the results showed that the lipophilic extract of *P. odorata* showed more capable to inhibit spores of *C. gloeosporioides* compared to *C. capsici*. This is being shown by clear zones showing the inhibition of the fungus that can be detected for *C. gloeosporioides* but not for *C. capsici* (Yanpirat & Vajrodaya, 2015).

In a study carried out by Chan et al. (2018), extractions of the fresh leaves produced significant antifungal activities in two of its extracts, such as hexane and chloroform, which showed broad-spectrum fungicidal activity (Chan et al., 2018). The antifungal activities were done using hexane, dichloromethane, methanol, and water extracts against *Candida albicans* and the result proved that none of the extracts was active against *C. albicans* (Ridzuan et al., 2013). Other research used oven-dried leaves, so the bioactive

compounds in the plant may reduce or degrade during drying (Chan et al., 2018).

#### 2.4.3. Anti-inflammatory effect

In the ethanol extract of *P. odorata*, it was found that quercetin and scutellarein-7-glucoside are the main compounds for the anti-inflammatory effect. By decreasing IL-6-secretion, it shows that scutellarein-7-glucoside and quercitrin give about 50% of the anti-inflammatory effect of *P. odorata* (Okonogi et al., 2016). In the methanol extract of *P. odoratum*, two major compounds, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and E-15-heptadecenal, may be found associated with their anti-inflammatory effects. By hindering the production of nitric oxide in a concentration-dependent manner ( $\text{IC}_{50} = 53.75+0.72 \mu\text{g}/\text{mL}$ ), the DLE displayed a potent anti-inflammatory effect (Chansiw et al., 2019). The anti-inflammatory activity of *P. odorata* compounds can be used as an alternative or complementary treatment.

#### 2.4.4. Antioxidant activity and anticancer properties

*P. odorata* has antioxidant properties that can prevent or delay our body from suffering from oxidative damage, and the properties are a substance or nutrients in our food. Oxidation of other molecules is inhibited, which means that electrons or hydrogen are moved to an oxidizing agent from a material. Since free radicals are generated because of these oxidation reactions, in our body cells, these radicals can trigger chain responses that can cause cell damage or death (Khaki & Fathiazad, 2012). Natural antioxidants can be found mainly in plants with rich phenolic compounds. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay has been used to investigate the antioxidant activity of the *P. odorata* and the prominent  $\text{IC}_{50}$  value is  $190.19\pm 0.42 \mu\text{g}/\text{ml}$ .

The existence of polyphenols such as gallic acid, quercetin, ferulic acid, and apigenin are maybe the causes of the *P. odorata* prominent antioxidant effect. The ethanolic extract of the *P.odoratum* exhibited the ability to quench DPPH radical results showed that it provides a good antioxidant with free radical scavenging activity (Ahongshangbam et al., 2014).

*P. odorata* also has anti-cancer properties, and Mohamaed et al. (2006) has shown that homoisoflavone molecules from *P. odorata* generate apoptosis and detain G2/M cell cycles by modulating Bcl-2 protein in breast cancer cells. In several cell cancers, Bcl-2 is an anti-apoptotic enzyme that is over-expressed. Therefore, one of the methods to minimize cancer progression is the activation of Bcl-2 phosphorylation by the homoisoflavone enzyme (Rafi & Vastano, 2007).

### 3. CONCLUSION

Various techniques and solvents can extract the medicinal plants for their phytochemicals. Different solvents used in extractions will exhibit different types of bioactive compounds. Different extraction techniques also influence antimicrobial activity because the technique or solvent used

for extraction will affect the quality of the compounds from the leaves. From this review, the methods used for isolation and identification are High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GS-MS). As discussed above, bioactive compounds of *P. odorata* have biological activities antifungal activity, antimicrobial activity, anti-inflammatory effect, and antioxidant activity. It concludes that *P. odorata* is important to emerging as a potential green medicine and it is recommended for future research to use a more in-depth method to determine the mode of action of each bioactive compounds for further understanding.

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