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Natural Essence of Malay Poison from *Melaleuca cajuputi* as Potential Natural Herbicide and Microbes Inhibitor

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Abstract **- Poisoning is an art that Malays are well skilled at and recognized for. Numerous poisons are utilized differently; also, most characteristics relating to intoxication, poisoning, or therapeutic use are directly dependent on the origin of the substance such as animals or plants. *Melaleuca cajuputi* (gelam) belongs to the Myrtaceae family, and the Malay population has traditionally used it for several functions and reasons. Nevertheless, its natural essence has not been deeply researched; hence, this work aimed to assess the chemical characteristics of *M. cajuputi* and evaluate their impact on microbial processes and weed development. The observations indicated that three primary phenolic acids (Vanillic acid, Ferulic acid, and Caffeic acid), including one volatile substance, were identified, indicating a total phenolic content of 493.92±6.88 µg GAE/g DW. Extracts from *M. cajuputi* leaves inhibited aquatic weed activity against *Rotala rotundifolia* and *Glossostigma elatinoides*; treatment levels were between 10 and 50 g/L. Similarly, there were inhibitory observations against microbes like *Candida albicans, Escherichia coli, Methicillin-resistant Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Staphylococcus aureus* and *S. epidermis*. Hence, these observations are vital since it is the foundational study concerning the use of poisonous Malay plant species as antimicrobials and natural herbicides in Malaysia.**

Index Terms **- aquatic weed, Malay poison, *Melaleuca cajuputi*, natural herbicide, phenolic compound.**

INTRODUCTION

A Myrtaceae family aromatic species called *Melaleuca cajuputi* is found in Australia and other proximal nations like Malaysia, Indonesia, and Papua New Guinea [1]. Malaysia has a plant species called *M. cajuputi*, also called "gelam" [2]. It is extensively employed as a conventional medicinal plant, cosmetic ingredient, and cooking and freshening agent [3]. This species comprises rapidly developing trees that grow around riverbanks, coastal and sub-coastal areas, and inland regions. Alternately arranged plant leaves are dark green with a lanceolate shape [4]. Wetlands, mangroves, and heath forests are ecosystems with rich diversity, enabling socio-economic phenomena, including noteworthy contributions toward sustaining ecology [5], [6]. A pH range below 7 is best suited for *M. cajuputi* growth. Interestingly, Melaleuca is native to Australia and grows at a soil pH of 6 or less [7]. Flowers develop in groups across the stem; the fruit comprises several tiny seeds [8]. Budding *M. cajuputi* leaves are suitable for human consumption. Conventional medicine employs these leaves to treat stomach cramps, digestion issues, cough, influenza, and internal dysfunctions [4]. Though several plants demonstrate therapeutic potential, many are toxic. These plants provide pain relief for head, tooth, and earaches.

Moreover, they are used to treat convulsions, rheumatic conditions, colds, coughs, asthma, and other respiratory issues. It is also added to cosmetic or therapeutic fragrance products because it exhibits antifungal, antioxidant, antibacterial, anti-inflammatory, and preservative characteristics [2], [9]. Researchers [10] indicate that essential oils derived from *M. cajuputi* are effective in reducing SARS-CoV-2 likelihood. Society desperately requires vaccines that prevent or cure diseases, including the ability to prevent or halt the transmission of SARS-CoV-2. Further, extract derived from *M. cajuputi* stem is a promising insecticide that prevents carpenter ants from damaging wooden installations [11].

Natural products are vital for drugs and their development. Research suggests that one-third of all medicines approved by the FDA in the previous two decades are based on natural substances and other forms [12]. Unani, Ayurveda, and traditional Chinese medicine are no longer culture-restricted treatments; they are used by Western societies, too [13]. Tocopherols, flavonoids, phenolic acids, and nitrogen-containing products like chlorophyll derivatives, amino acids, alkaloids, and amines are phenolic compounds that exhibit antioxidant characteristics. Ascorbic acid and carotenoids are substances that provide antioxidant properties [14], [15]. In the micro-ecosystem of the *M. cajuputi* specimen origin, spatially distinct essential oils are present [5].

Agricultural crop output has been significantly enhanced due to herbicide use. Nevertheless, scavenging soil herbicide remains is a time-intensive process especially in the present agricultural pattern which may lead towards soil toxicity [16]. Synthetic pesticides are widely employed to reduce weed development at this time. Glyphosate is an extensively sprayed herbicide that generates amino acids like tryptophan and tyrosine that inhibit photosynthetic efficiency. High doses culminate in the aggregation of antioxidant enzymes [17].

In the context of human well-being, extensive synthetic pesticide exposure increases silicosis and pancreatic cancer risk. Continued exposure to herbicides and pesticides increases the likelihood of other health conditions like cognitive impairment, reproductive issues, liver problems, and immune disorders [18]. A substitute weed handing technique is vital to protect human well-being and food security. [15] assert that phenolic radicals have lesser electron reduction and reactive potential compared to oxygen radicals.

Hence, the objective of this study is to assess the chemical characteristics of *M. cajuputi* and evaluate the impact of weed development and microbial processes through HPLC and GCTOF-MS analysis to identify active *M. cajuputi* compounds. To evaluate the effect of microbial processes and aquatic weed development, the current study used *in-vitro* treatment and bioassay processes. Identifying active natural herbicidal substances from *M. cajuputi* allows for broader use of this study.

It can help understand new uses of pharmaceutical additives and antimicrobial characteristics.

MATERIALS AND METHODS

I. Plant Sample Preparation

Melaleuca cajuputi (Figure 1) leaves were gathered from the heath forests in Kampung Jambu Bongkok, Marang, Terengganu in Malaysia. Kampung Jambu Bongkok is situated on Peninsular Malaysia's East Coast; Kuala Lumpur is about 388 km from this place (Figure 1). The leaves were cleaned and freeze-dried before they were ground to a fine powder that was preserved at –20ºC for evaluation.

FIGURE 1 *Melaleuca cajuputi* (GELAM)

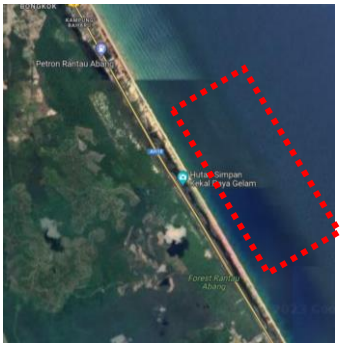

Figure 2 Location Of Heath Forest At Kampung Jambu Bongkok, Terengganu

II. Water Extraction

Every sample was processed using 10 g powdered freeze-dried substances added to 100 ml distilled water; an incubator shaker mixed the material for 30 minutes at 200 RPM and room temperature. Subsequently, the specimen was incubated for 30 minutes using an oven set at 60°C. It was then kept overnight in a dark environment at room temperature. Filtration was performed the next day, and just the clear supernatant liquid was available. The specimen was re-processed using several solvents like ethyl acetate, petroleum ether, and butanol, as recommended by [19], [20].

III. Identification of Volatile Compounds by Gas Chromatography Time-of-Flight Mass Spectrometry (GCTOF-MS)

Qualitative analysis of M. cajuputi leaf derivatives was performed using the Agilent 7890 GCTOF-MS system.

A 30 m x 0.25 mm, 0.25 µm capillary column was used as suggested by [21]; the system used in the present study differed mildly from the researchers' apparatus. A 1 µL sample was used based on split-less injection. Solvent delay and purge periods were set to four and one minutes, respectively. Helium flow at 1.0 mL min^{-1} comprised the carrier medium. The starting column temperature was set at 80°C. After 2 minutes, the initial temperature increase to 80°C was achieved at a speed of 5°C min^{-1}, followed by a 10°C min^{-1} increase to 250°C. Detector and inlet temperatures were 340°C and 220°C. The time-of-flight mass spectrometer commenced in the mass range of 50-1000 m/z, processing one spectrum every second. Peak identification needed >90% similarity index determined using mass spectra, as recommended by the National Institute of Standards and Technology library (NIST 14).

IV. Determination of Total Phenolic Content

This research used the Folin-Ciocalteau assay technique to evaluate the total phenolic presence [22]. A solution comprising 20% v/v deionised water and 90 µL Folin-Ciocalteau reagent was formulated. The specimen was split across wells on a 96-well flat-bottom microplate. Next, a 1.0 mg/g DW comprising distilled water (1000 µg/mL) diluted sample was incubated for five minutes at room temperature. Further, 7.5% w/v deionised water was employed to reduce the concentration of a 90 µL sodium carbonate solution that was incubated for two hours at room temperature. The TECAN microplate reader ascertained absorption characteristics and standards at λmax = 725 nm specific to a blank.

V. Determination of Phenolic Acids by High Performance Liquid Chromatography (HPLC)

The phenolic acid HPLC evaluation was conducted using the LC rapid resolution apparatus Agilent 1200 series (Agilent Technologies, Palo Alto, CA, USA). The apparatus comprised a binary pump equipped with an auto-injection mechanism, micro vacuum degassing areas, thermostat-regulated column area, and a diode array detector (DAD). Some modifications were made to the apparatus suggested by [23]. Zorbax SB-C_{18} Eclipse 100 × 2.1 mm, 1.8 µm column fitted with a diode-array-detector was employed for this research. The evaluation phase used linear gradient elution comprising two mobile solutions: Phase A comprising 90:10 v/v 1% formic acid in water/acetonitrile and Phase B comprising acetonitrile. The system used a flow rate of 0.5 ml/min, 25°C column temperature, and 10 µL injections. Sigma-Aldrich was selected for procuring phenolic acids standard such as Ferulic acid, Caffeic acid, Hydroxybenzoic acid, Vanillic acid and Coumaric acid.

VI. Determination of Antibacterial Assay

The antibacterial characteristics of *M. cajuputi* were evaluated using five gram-negative bacterial strains: *Escherichia coli*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. epidermidis*. The broth medium was mixed with Muller Hinton (MH) to create a nutrient-rich medium to prepare the inoculum supporting bacterial development. Agar well diffusion method were used by gently swipe the specimens on the MH agar medium [24]. Extracts exhibiting growth inhibition regions larger than 7 mm were identified, and the minimum inhibitory concentration (MIC) was ascertained. A 24-hour incubation was performed at 37°C for the identified zones, and MIC was re-evaluated. The most minor concentration blocking bacterial growth was considered the sample's MIC level.

VII. Determination of Antifungal Assay

The antifungal properties of *M. cajuputi* were evaluated using several pathogens: *Microsporum gypseum*, *Fusarium* sp., *Aspergillus niger*, *Candida albicans* and *Phanerochaete chrysosporium*. Swabbed pathogen samples were created using Potato Dextrose Agar (PDA) plates.

IX. Assessment of Inhibitory Effect Towards Aquatic Weeds

Pusat Penyelidikan Perikanan Air Tawar, FRI Glami Lemi, Jelebu, Negeri Sembilan, were contacted to purchase tissue cultures belonging to two aquatic species: *Rotala rotundifolia* (dwarf rotala) and *Glossosstigma elatinoides* (glosso). The suggestions by [25] was adapted and changed as required to process the culture. Water extraction concentrations were 100g/L; concentration levels varied (10, 20, 30, 40 and 50 g). Post-emergent observations were recorded. Germination rate, radicle and shoot lengths, radicle and shoot numbers, and plant height were recorded and evaluated with the control specimen (MS without of extract) after 4 weeks of incubation.

RESULTS AND DISCUSSION

I. Assessment of volatile compounds by Gas Chromatography Time-of-Flight Mass Spectrometry (GCTOF-MS)

GCTOF-MS assessment of *M. cajaputi* leaves exhibited 191 substances comprising distinct concentrations (peak area%), chemical groups, retention period, molecular weight (MW), chemical formula and structure, accurate mass, and MS fragments-ions, as suggested by library data obtained from the National Institute Standard and Technology (NIST). The outcomes were evaluated using a >8-% similarity compared to NIST.

Analysis indicated about 12% hydroxyl (-OH) compounds, which are active in *M. cajaputi*. The outcomes inferred from the chromatogram are listed in Table 1; they indicate three conspicuous peaks in the retention period range, i.e., from 16.36 to 16.45. However, only one compound comprised the tallest peak in the 0.90 % to 2.72 % range; this compound was 4h-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- having a 16.36 – 16.45.

Table 1
Phytochemical Screening Of Volatile Compounds Identified In Petroleum Ether Of *M. Cajaputi* Leaves Extraction

Volatile compound	Rt (min)	Area (%)	Molecular weight/ Formula	Exact mass	Chemical structure	MS Fragment-ions
4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl-	16.36	2.72	206 $C_{11}H_{10}O_4$	206.06		206, 191, 177, 148, 123, 95, 69

II. HPLC Analysis of Total and Individual Phenolic Content

M. cajaputi total phenolic content (TPC) exhibited a notable phenolic constitution (>300 mg GAE/g DW) of 493.93 mg GAE/g DW [26]. Phenolic acid was subjected to HPLC assessment; five organic solvent-based compounds were identified: 4-Hydroxybenzoic acid, *trans-p*-Coumaric acid, Ferulic acid, Vanillic acid, and Caffeic acid, as specified in Table 2. Plants are equipped with several mechanisms to handle abiotic or biotic environmental stresses. Biotic aspects like pathogens and predators comprise severe environmental stressors that inhibit growth and crop yield despite well-irrigated land anywhere in the world [26]. At the same time, the abiotic aspects might add to plant stress due to drought, flooding, water level reduction, changes in salinity and temperature, light (shade or radiation), CO_2, and nutrients will precipitate outcomes that affect plant survival, growth, development, and, seldom, resource distribution changes at the plant level [27]. [26] also indicated two plant defence systems:

- *direct defence* – plant aspect (physical properties, primary and secondary metabolites) that enhance plant fitness for certain conditions. This phenomenon is also called constitutional (ever ready) or induced (in response to a stressor) and;
- *indirect defence* - plant aspects that help create a symbiotic system comprising predators and herbivore parasitoids.

Responses to abiotic aspects require specific resources like reactive oxygen species (ROS) that act as vital regulatory entities necessary for plant cells and metabolism; these were devised by lesser activation of the "normal" metabolic route (a complex system of enzymes) to regulate plant stress [27], [28].

Drought stress affects plants immensely at their cellular level or the complete plant by increasing phytohormone abscisic acid (ABA) production. Moreover, the plant also exhibits numerous physiological and biochemical phenomena [29]. A plant could exhibit allelochemical phytotoxicity to build its community using inductive, constitutive, and regulatory techniques to act as physical defences against the external environment. In the research context, varying concentrations of phenolic substances were identified.

TABLE 2
COMPOSITION OF *M. cajaputi* PHENOLIC CONTENT (µg/g DW)

Solvent	Phenolic Acids						
	HBA	CA	VA	TCA	FA	3CA	2CA
Petroleum ether	ND	0.06±0.00	ND	ND	ND	ND	ND
Ethyl acetate	0.25± 0.00	0.05± 0.00	0.38± 0.00	0.06± 0.00	0.17± 0.00	ND	ND
Butanol	ND	0.20± 0.00	0.57± 0.00	0.08± 0.00	0.21± 0.00	ND	ND

*HBA:4-Hydroxybenzoic acid, CA: Caffeic acid, VA: Vanilic acid, TCA: *trans-p*-Coumaric acid, FA: Ferulic acid, 3CA:3-Coumaric acid, 2CA: 2-Coumaric acid and ND: not detected

III. Assessment of Antimicrobial Assay

Antimicrobial *M. cajuputi* leaf extract and antimicrobial inhibition phenomena in response to several solvents are listed in Table 3. This research classifies the outcomes into five sets, as suggested by [30]:

(i) zero antibacterial activity (inhibition zone < 1 mm);
(ii) slight (inhibition zone between 1-3 mm);
(iii) moderate (inhibition zone between 3-4 mm);
(iv) clear (inhibition zone between 4-10 mm) and
(v) strong (inhibition zone > 10 mm).

The present study did not provide evidence of strong antimicrobial inhibitory characteristics against fungi and bacteria. [31] highlighted that a phenolic substance derived from *M. cajaputi* and *Cocos nucifera* honey exhibits promising antimicrobial characteristics against *E. coli*, *S. aureus*, *and* MRSA. It contains substances like benzoic, gallic, and caffeic acids. Along the same lines, the research also identified that *M. cajaputi* demonstrated promising antibacterial characteristics for all evaluated solvents (ethyl acetate, petroleum ether, and butanol). Further, plant polyphenols are classified as antimicrobial substances, and researchers suggest they can be used for food preservation[32]. Gallic, ferulic, and chlorogenic acids are phenolic acids that exhibit pharmacological characteristics reducing the development of fungal pathogens responsible for plant infections [33]. In addition, mixtures comprising essential oils like aliphatic or aromatic compounds, including terpenoids, exhibited antimicrobial properties because they reduced bacterial growth, restricted exopolysaccharide formation, and reduced bacterial adherence [34].

TABLE 3
ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENTS SEPARATION EXTRACTS OF *M. cajuputi*

Antibacterial Activity					
Solvents	*S. aureus*	*S. epidermidis*	*E. coli*	*MRSA*	*P. aeruginosa*
Petroleum ether	++	~	~	++	+
Ethyl acetate	++	~	++	++	+
Butanol	++	~	++	++	+
Antifungal Activity					
	C. Albicans	*Fusarium sp.*	*M. Gypseum*	*P. Chrysosporium*	*A. Niger*
Petroleum ether	~	-	-	-	-
Ethyl acetate	~	-	-	-	-
Butanol	-	~	-	-	-

*Note:
A) - : no antimicrobial activity, B) ~ : slight antimicrobial activity,
C) + : moderate antimicrobial activity, D) ++ : clear antimicrobial activity,
E) +++ : strong antimicrobial activity

IV. Assessment of Inhibitory Effect Towards Aquatic Species

The *in-vitro* model aims to evaluate the post-emergent growth of two aquatic species: *Glososstigma elatinoides* and *Rotala rotundifolia*. A plant was assessed after the fourth week for treatment using an in-vitro mechanism cultured using the MS basal medium, as listed in Tables 4 and 5. Observations concerning the two aquatic species indicate that *M. cajuputi* extract exhibited progressive reduction concerning seedling development in the post-emergent phase of *G. elatinoides* and *R. rotundifolia* as higher concentrations were used. Seedling count for the two species had inhibited growth in the 10-50 g/L range compared to control. Hence, *M. cajuputi* affected *G. elatinoides* and *R. rotundifolia* in the post-emergent phase by reducing seedling count, plant height, and radicle and shoot lengths.

The previous study indicated a reduction in seed germination rate, leading to changes in metabolic enzyme activity (glycolysis) and oxidative pentose phosphate pathway (OPPP) [35]. Moreover, mitochondria were disturbed because of compromised respiration ability [36]. Similar outcomes were seen for *T. erecta* and *T. patula.* pre-emergence indicated a decrease in growth and germination indicators [37]. [38], [39] mentioned that phytotoxic phenomenon caused by phenolic substances could affect metabolic phenomena necessary for germination. [40] suggested that plants' ability to handle allelochemicals to regulate uptake and reduce toxicity at allelochemical targets varies. [36] reported that the precise steps comprising the inhibitory phenomena were precipitated by allelochemicals disrupting the biochemical and physiological systems of the target species.

TABLE 4
EFFECT OF *M. cajuputi* PHENOLIC CONTENT ON *G. elatinoides* GROWTH AT DIFFERENT CONCENTRATIONS.

Extract Concentration (g/L)	Length of shoot (mm)	Number of shoots	Length of radicle (mm)	Number of radicles	Height of plant (mm)	Number of seedlings
0 (control)	6.7 ± 0.3	10	5.7 ± 0.3	2	20.5 ± 0.6	25
10	3.9 ± 0.1	8	0.6 ± 0.1	1	7.9 ± 0.2	25
20	3.8 ± 0.1	6	0.8 ± 0.1	1	6.8 ± 0.2	21
30	3.0 ± 0.1	6	0.5 ± 0.1	1	5.2 ± 0.2	15
40	3.0 ± 0.1	4	0.7 ± 0.1	1	5.4 ± 0.3	19
50	2.8 ± 0.1	4	0.5 ± 0.1	1	4.9 ± 0.1	13

TABLE 5
EFFECT OF *M. cajuputi* PHENOLIC CONTENT ON *R. rotundifolia* GROWTH AT DIFFERENT CONCENTRATIONS.

Extract Concentration (g/L)	Length of shoot (mm)	Number of shoots	Length of radicle (mm)	Number of radicles	Height of plant (mm)	Number of seedlings
0 (control)	1.0 ± 0.0	6	3.6 ± 0.1	2	9.4 ± 0.2	25
10	0.7 ± 1.4	1	0.0 ± 0.0	0	10.0 ± 0.0	2
20	1.0 ± 0.0	3	0.0 ± 0.0	0	9.3 ± 0.1	6
30	1.0 ± 0.0	4	0.0 ± 0.0	0	8.5 ± 0.2	4
40	1.0 ± 0.0	2	1.0 ± 0.1	0	1.0 ± 0.0	4
50	1.0 ± 0.0	2	1.3 ± 0.1	0	1.0 ± 0.0	3

CONCLUSION

This research creates a new body of knowledge concerning using Malay poisonous plant species *Melaleuca cajuputi* as promising and eco-friendly natural herbicides. The outcomes suggest that phenolic substances and dominant phenolic acids comprised Caffeic, Vanillic, and Ferulic acids, including volatile substances affecting inhibitory phenomena associated with the development of *Glososstigma elatinoides* and *Rotala rotundifolia.* Further, *M. cajuputi* extracts exhibited antimicrobial properties against *Escherichia coli*, *Staphylococcus aureus*, *P. aeruginosa*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Candida albicans*, and *S. epidermis*. It is noteworthy that high phenolic concentrations (>300 mg GAE/g DW) were associated with *M. cajuputi* extract-based antimicrobial characteristics. Developing bio-herbicides using Malay poison creates a promising avenue to use natural substances to protect plants. Moreover, there are indications of coping against herbicide-resistant weeds. Naturally occurring compounds' structures might be responsible for such properties, allowing selective use as environmentally-friendly herbicides.

Hence, nature-based weed regulation highlights the importance of bio-herbicides that are less cost-intensive but safer.

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