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CHEMICAL COMPONENTS OF THE ESSENTIAL OILS FROM THREE SPECIES OF MALAYSIAN *PLUMERIA* L. AND THEIR EFFECTS ON THE GROWTH OF SELECTED MICROORGANISMS

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Abstrak: Komponen kimia minyak pati daripada bunga bagi tiga spesies *Plumeria* yang banyak terdapat di Malaysia, iaitu *P. rubra* L. (varieti bunga merah)*, P. acutifolia* Poir. (varieti bunga kuning) dan *P. obtusa* L. (varieti bunga putih) telah dianalisis menggunakan GC/MS dan 27 komponen telah dikenal pasti. Tujuh komponen telah disisihkan dan dikenal pasti daripada *P. rubra*, 14 komponen daripada *P. obtusa* dan 19 komponen daripada *P. acutifolia.* Kandungan utama minyak pati bagi ketiga-tiga spesies ialah asid 2-hidroksibenzoik fenilmetil ester. Ketiga-tiga spesies juga berkongsi dua hidrokarbon alkana, iaitu nanodekana and heneikosana. Kesan antimikrob bagi minyak pati tersebut (pada 2 μl bagi setiap disk) telah ditentukan menggunakan kaedah penyerapan agar. Lapan mikroorganisma telah digunakan untuk kajian ini, iaitu *Escherichia coli* (bakteria Gram negatif), *Staphylococcus aureus* dan *Bacillus cereus* (bakteria Gram positif), *Candida albicans* dan *C. humicola* (yis), dan *Trichophyton mentagrophytes*, *T. rubrum* dan *Microsporum canis* (kulat). Spektrum perencatan yang terluas ditunjukkan oleh minyak pati *P. obtusa*. Ekstrak tersebut merencat semua mikroorganisma yang diuji kecuali *E. coli*. Zon perencatan terbesar pula ditunjukkan oleh minyak pati *P. obtusa* terhadap *C. humicola*.

Kata kunci: Minyak Pati, Antimikrob, *Plumeria*

Abstract: The chemical components of the essential oils obtained from the flowers of three Malaysian species of *Plumeria*, namely *P. rubra* L. (red flower variety)*, P. acutifolia* Poir. (yellow flower variety) and *P. obtusa* L. (white flower variety) were analyzed by GC/MS and 27 components were identified. Seven components were separated and identified from *P. rubra*, 14 components from *P. obtusa* and 19 components from *P. acutifolia.* The major component that is found in all three species is 2-hydroxybenzoic acid phenylmethyl ester. Those three species also shared two alkane hydrocarbons, that is nanodecane and heneicosane. The antimicrobial properties of the essential oils (at 2 μl per disk) were determined using agar diffusion method. Eight different microorganisms were used in this study, that are *Escherichia coli* (Gram negative bacteria), *Staphylococcus aureus* and *Bacillus cereus* (Gram positive bacteria), *Candida albicans* and *C. humicola* (yeast), and *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum*

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canis (fungi). The broad spectrum of inhibition was exhibited by the essential oil of *P. obtusa*. The extract inhibited all tested microorganisms except for *E. coli*. The largest inhibition zone was shown by the *P. obtusa* essential oil against *C. humicola*.

Keywords: Essential Oils, Antimicrobial, *Plumeria*

INTRODUCTION

Genus *Plumeria* belongs to the Apocynaceae family and is native to the New World. The plants from this genus are widely cultivated in the tropical and subtropical regions throughout the world. They are recognized as excellent ornamental plants and often seen in the graveyards (Tung 1994). *Plumeria* plants are famous for their attractiveness and fragrant flowers. The essential oils from the flowers are used for perfumery and aromatherapy purposes. The decoction of the bark and roots of *P. rubra* is traditionally used to treat asthma, ease constipation, promote menstruation and reduce fever. The latex is used to soothe irritation (Wiart 2002).

Various scientific evaluations have been conducted to verify the traditional uses of this plant in the folk medicine. For instance, the extract obtained from the bark of *P. acutifolia* was found having antimutagenic properties (Guevara *et al.* 1996). The leaves extract of *P. rubra* exhibited antibacterial activity (Hamburger *et al*. 1991) while the barks extract showed cytotoxic effects against a number of *in vitro* human cancer cell lines (breast, colon, lung, fibrosarcoma and melanoma) (Kardono *et al.* 1990).

The essential oils obtained from the floral parts of three species of Malaysian *Plumeria* were the subjects of our studies. Our ultimate goals are to determine the composition of essential oils and their mutual synergistic effect against various microbes.

MATERIALS AND METHODS

Plant Material

Fresh flowers of *P. rubra, P. acutifolia* and *P. obtusa* were obtained from Universiti Sains Malaysia (USM) Campus. Their identities were checked by morphological examination and were compared with the herbarium specimens. Their voucher specimens were deposited at the Herbarium, School of Biological Sciences, USM.

Extract Preparation

Essential oils were extracted using the steam distillation apparatus.

GC/MS Analysis

The composition of essential oil was analyzed by gas chromatography-mass spectrometry (GC/MS). The identification of the compounds was performed by comparison of the obtained mass spectra with the mass spectra in the Wiley library.

A HP 5890 series II Plus (Hewlett-Packard) gas chromatography equipped with a HP 5971 mass spectrometer as a detector and SPB-5 (Supelco) capillary column (25 m x 0.32 mm i.d., 0.25 m film) was used with helium as a carrier gas (flow rate: 1 cm^3 min^{-1}). The injector, operated in splitless mode (1:50), was held at 230°C. The temperature program started at 50°C and after 2 min, ramped at 4°C min^{-1} to 220°C. The injection volume of essential oil used for each analysis was 2 μl.

Microorganisms

The microorganisms used in this study were obtained from the microbiology laboratory, USM. Eight microorganisms were used which are *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Candida albicans*, *C. humicola*, *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum canis*. All the bacteria were stored on nutrient agar slants, and all the fungi and yeast were stored in Sabouraud dextrose agar.

Culture Media

The nutrient and Sabouraud dextrose agar used for storage and antimicrobial susceptibility tests were obtained from Oxoid Ltd. England.

Preliminary Screening for the Antimicrobial Activities

Tests performed by agar diffusion method using 0.1 ml of inoculum containing 1.5 x 10^6 microbial cells per ml. Disks of 6 mm diameter were impregnated with 2 μl of extracts. The plates were incubated overnight at 37°C in the incubator. The diameter of the inhibition zones was measured. Each experiment was repeated at least three replicates.

RESULTS AND DISCUSSION

The GC/MS chromatograms of the essential oils obtained from the three species of *Plumeria* are shown in Figure 1. The list of identified constituents of the three essential oils is presented in Table 1.

It is noted that 27 compounds were detected and identified with typical library search match exceeding 90%. The most abundant component in the essential oils of the three species is 2-hydroxybenzoic acid phenylmethyl ester or also known as benzyl salicylate. Benzyl salicylate, with balsamic, sweet and floral odor is a major component of many essential oils obtained from fresh flowers. For examples, Ylang-ylang (*Cananga odorata*), Lilax (*Syringa vulgaris*) and Gardenia (*Gardenia jasminoides*). It is also an ingredient of many perfumes.

Only seven volatile constituents were detected in *P. rubra*. 2-methylbutan-1-ol, which was only found in this species could be considered as the chemical marker in characterizing its essential oil. The occurrence of this constituent together with β-phenylethyl alcohol, phenylacetaldehyde, nanodecane and heneicosane in the Malaysian varieties of *P. rubra* agrees with the finding of Omata *et al.* (1992) on the essential oil of Irma Bryan cultivar of *P. rubra* from Hawaii. However, the major constituent of the cultivar is β-phenylethyl alcohol,

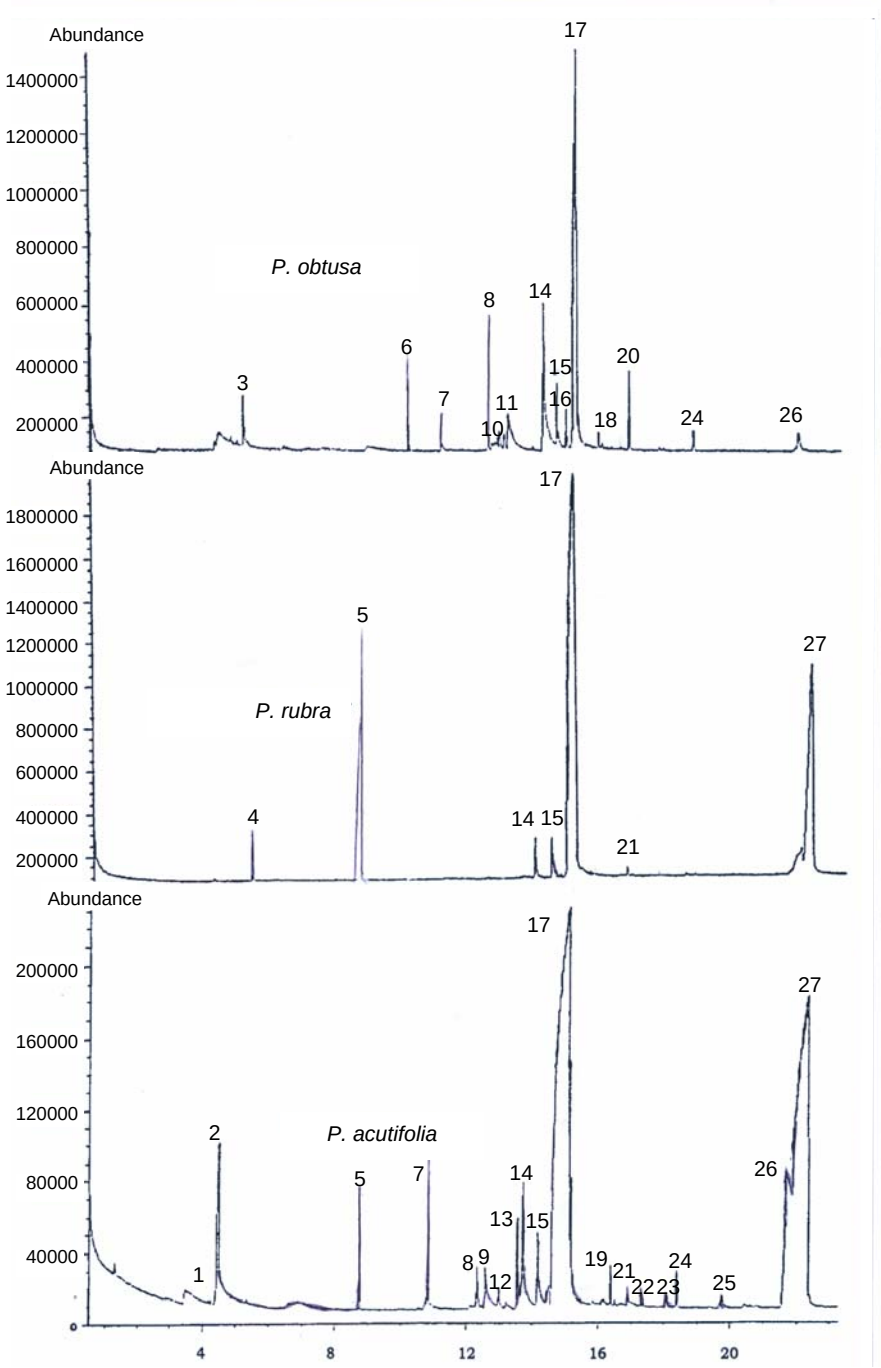

Figure 1: GC/MS chromatograms of the essential oils obtained from *P. obtusa, P. rubra* and *P. acutifolia.*

Table 1: List of the identified constituents in the essential oils of *P. obtusa, P. rubra* and *P. acutifolia.*

No.	Retention time (minutes)	Constituent	*P. obtusa*	*P. rubra*	*P. acutifolia*
1	4.32	α-terpineol	–	–	+
2	4.56	geraniol	–	–	+
3	4.96	linalool	+	–	–
4	5.22	2-methylbutan-1-ol	–	+	–
5	8.82	β-phenylethyl alcohol	–	+	+
6	10.12	farnesol	+	–	–
7	11.13	trans-farnesol	+	–	+
8	12.34	benzyl benzoate	+	–	+
9	12.50	neral	-	–	+
10	12.81	tetradecan-1-ol	+	–	–
11	12.91	2,3-dimethyl butane	+	–	–
12	13.00	geranial	–	–	+
13	13.52	dodecanoic acid	–	–	+
14	13.78	nanodecane	+	+	+
15	14.22	heneicosane	+	+	+
16	14.60	1 heptodecene	+	–	–
17	15.14	benzyl salicylate	+	+	+
18	15.87	9-eicosene-(E)	+	–	–
19	16.43	phenylethyl benzoate	–	–	+
20	16.79	nerolidol	+	–	–
21	16.97	tetradeconoic acid	–	+	+
22	17.20	tetracosane	–	–	+
23	18.03	octadecanoic acid	–	–	+
24	18.26	tricosane	+	–	+
25	19.82	docosane	–	–	+
26	21.78	eicosane	+	–	+
27	22.24	phenylacetaldehyde	–	+	+

Table 2: Diameter of inhibition zones of the tested microbes.

Tested microbial	Diameter of inhibition zone (mm)		
	P. obtusa	*P. rubra*	*P. acutifolia*
E. coli	–	–	–
B. cereus	11.5 ± 0.9	8.5 ± 1.8	7.0 ± 0
S. aureus	11.2 ± 0.3	–	–
C. albicans	10.7 ± 1.5	7.2 ± 0.3	–
C. humicola	15.7 ± 1.3	12.0 ± 0.9	13.3 ± 1.3
T. mentagrophytes	8.3 ± 0.3	–	–
T. rubrum	7.2 ± 0.3	6.5 ± 0	–
M. canis	7.7 ± 0.6	–	–

while the major constituent of the Malaysian variety of *P. rubra* used in this study is benzyl salicylate. β-phenylethyl alcohol and phenylacetaldehyde were also among the major constituents of *P. acutifolia*, while nanodecane and heneicosane were also detected in all the essential oils studied.

The antimicrobial activities of the essential oils were tested *in vitro* using disk diffusion method. The results are indicated in Table 2. The essential oil of *P. rubra* inhibited four microbes with the largest inhibition zones of 12.0 ± 0.9 mm against *C. humicola*. The essential oil of *P. obtusa* showed the best antimicrobial property compared to the other two species. It inhibited all microbes except for *E. coli*. Fourteen volatile components were detected in this essential oil. Besides the synergistic effects of the components, the presence of a monoterpene (linalool) and two sesquiterpenes (farnesol and nerolidol) only in this essential oil might be the contributing factor for its prominent antimicrobial property. Janssen *et al.* (1984) and Brehm-Stecher and Johnson (2003), respectively confirmed the antimicrobial properties of linalool, farnesol and nerolidol.

C. humicola appeared to be the most susceptible to the inhibitory effect of the essential oils. In contrast, the Gram negative bacterium, *E. coli* was resistant to all the three essential oils. Benzyl salicylate, the major constituent shared by all the three essential oils might play a major role in inhibiting *C. humicola.* Nikawa *et al.* (1995) discovered that the antifungal effect of benzyl salicylate was related to its ethyl alcohol content. The maximum inhibitory effect on *C. humicola* was indicated by the essential oil of *P. obtusa* with diameter inhibition zone of 15.7 ± 1.3 mm.

Previous study by Kamariah *et al.* (1999) on the essential oil of the fresh flower of *P. obtusa* collected in Brunei Darussalam also reported the highest percentage of benzyl salicylate in the species. They also reported the presence of benzyl benzoate, farnesol and nerolidol as major constituents of *P. obtusa*. As indicated in Table 2, benzyl benzoate and trans-farnesol are not only found in *P. obtusa* but also detected in *P. acutifolia*.

Despite having the highest number of volatile constituents (19), the essential oil of *P. acutifolia* showed the lowest antimicrobial activity. The essential oil only inhibited *B. cereus* and *C. humicola* with inhibition zones diameter of 7.0 ± 0 mm and 13.3 ± 1.3 mm, respectively. Four bioactive monoterpenes, that are α-terpineol, neral, geraniol and geranial were only detected in this essential oil. The failure to perform better antimicrobial activity might be due to the antagonism effect of the constituents in the essential oil. Some compounds might suppress the ability of another compounds to inhibit microbes.

CONCLUSION

The results obtained from this phytochemical and antimicrobial comparison have contributed in upgrading the scientific basis of these fragrant flowers and accomplished new scientific evidence by discovering the potential value of the essential oil of *P. obtusa* as an antimicrobial agent. The activity might be due to the synergistic effects of its constituents or the presence of bioactive compounds.

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