Antimicrobial activity of *Blumea balsamifera* (Lin.) DC. extracts and essential oil

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Abstract

Leaves from *Blumea balsamifera* (Lin.) DC. are used in traditional Thai and Chinese medicine for the treatment of septic wounds and other infections. In the present study, essential oil, hexane, dichloromethane, and methanol extracts of these leaves were evaluated for antibacterial and antifungal activity using the disc diffusion assay and agar microdilution method. The essential oil was the most potent, with an MIC of 150 µg/mL against *Bacillus cereus* and an MIC of 1.2 mg/mL against *Staphylococcus aureus* and *Candida albicans*. Activity was also detected from the hexane extract against *Enterobacter cloacae* and *S. aureus*. MBCs and MFCs were typically equal to or two-fold higher than the MICs for both extracts, indicating microbicidal activity. Data presented here shows that *B. balsamifera* extracts have activity against various infectious and toxin-producing microorganisms. This plant's active constituents could potentially be developed for use in the treatment and / or prevention of microbial disease.

Keywords: *Blumea balsamifera;* essential oil; antibacterial; antifungal; microbicidal

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1. Introduction

Antimicrobial resistance is a major global problem, with resistant strains of *Staphylococcus aureus* (Lee et al., 2009), *Salmonella enterica* serovar Typhi (WHO, 2009), *Candida albicans* (Cannon et al., 2007) and other microorganisms being responsible for much morbidity and mortality. Given that it takes between 12 and 15 years to develop new drugs (Watkins, 2002), urgent research and development is required to replenish our existing suite of anti-infective medicines before they are completely ineffective. In addition to the difficulties that antimicrobial resistance presents to the healthcare sector and pharmaceutical industry, recent reports of antibiotic resistance among foodborne bacteria such as *Bacillus cereus* (Park et al., 2009) mean that the food and drink industry is increasingly involved in the search for interventions to reduce pathogens in foods and protect consumers.

Humans have been using plants to treat infectious disease for thousands of years (Atta-ur-Rahman, 2008), and it was recently estimated by the World Health Organization (WHO) that 25% of modern medicines are derived from plants that were first used in traditional medicine (WHO, 2003). Potential applications for phytochemicals include use as antibacterial agents (Kumarasamy et al., 2005; Süzgeç et al. 2005), antifungal agents (Boonphong et al., 2007), and antibiotic resistance modulating agents (Fujita et al., 2005). In recent years, it has also been proposed that phytochemicals such as orange oil (Nannapaneni et al., 2009), oregano oil (Friedman et al., 2006) and green tea extracts (Friedman, 2007) could be used to enhance microbial food safety.

Blumea balsamifera (Lin.) DC. (Compositae) is a halfwoody, evergreen shrub that grows widely throughout East and South East Asia. This plant has numerous uses in traditional Thai and Chinese medicine including the treatment of septic

wounds (Ruangrungsi et al., 1985), respiratory infections and stomach pains (ICRAF, 2009). Preparation of the plant involves pounding the leaves for use in poultices, drying the leaves for smoking, or boiling the leaves so that the infusion may be used for bathing, inhalation or drinking (Ruangrungsi et al., 1985; ICRAF, 2009). The aim of the current study was to assess the potential for *B. balsamifera* components to be used in modern medicine and / or food safety by determining the spectrum, potency and nature of activity of various extracts of the plant.

2. Materials and methods

2.1 Plant material

B. balsamifera (Lin.) DC. was collected from Roi-Et Province in northeast Thailand at the end of the cool season (02-02-04). A voucher specimen (herbarium number WRBI 305) was deposited with the Walai Rukhavej Botanical Research Institute (Mahasarakham University, Thailand). Plant material was air-dried in the shade at room temperature for 10 days.

2.2 Extraction

Three aliquots of the dried leaves of *B. balsamifera* (3 x 300 g) were extracted with hexane, dichloromethane and methanol. Each of the different extractions was performed at room temperature a total of three times (3 x 800 mL), 48 hours each time. All extracts were evaporated *in vacuo* and stored in the dark at 4ºC until required. A fourth aliquot of the air-dried leaves of *B. balsamifera* (300 g) was subjected to hydrodistillation for 3 hours using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulphate and stored in a sealed vial in the dark at 4ºC until required.

2.3 Preparation of test solutions and discs

Test solutions were prepared containing 19.2 mg/mL extract in 5 % (v/v) aqueous dimethyl sulphoxide (DMSO). Whatman no. 1 sterile filter paper discs (6 mm) were impregnated with 20 µL of extract (corresponding to 384 µg extract) and allowed to dry at room temperature.

2.4 Microorganisms

Standard reference strains of bacteria used in the study were *B. cereus* ATCC 11778, *S. aureus* ATCC 25923, *Enterobacter cloacae* DMST 17206, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *S.* Typhi DMST 5784. The fungal strain *C. albicans* ATCC 10231 was also used. These were all obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. In addition, clinical isolates of *B. cereus, S. aureus*, *E. cloacae*, *E. coli*, *Klebsiella pneumoniae, P. aeruginosa*, and *S.* Typhi (strains MSH 001 to 007) were obtained from Mahasarakham Hospital, Mahasarakham Province, Thailand.

Prior to experiments, bacteria were cultured on nutrient agar for 24 hours at 37ºC, while *C. albicans* was cultured on potato dextrose agar for 48 hours at 25ºC. Inocula were prepared by suspending colonies of the above microorganisms in 0.9 % (w/v) sodium chloride solution, and adjusting these to achieve turbidity equivalent to a 0.5 McFarland standard.

2.5 Screening for antibacterial and antifungal activity

Antimicrobial screening was performed using the disc diffusion method. This involved preparing agar plates containing 10 mL nutrient agar (for antibacterial testing) or potato dextrose agar (for antifungal testing), inoculating the surface of

these with 0.1 mL of ~1 x 10^6 cfu/mL bacteria or *C. albicans*, allowing this to dry, and applying discs of the plant extracts. Discs containing penicillin, chloramphenicol, tetracycline and gentamycin were also tested for comparison. Agar plates of bacteria were then incubated at 37ºC for 24 hours, while agar plates of *C. albicans* were incubated at 25ºC for 48 hours. Inhibition zones were measured from the edge of the disc to the inner margin of the surrounding pathogens. Experiments were repeated to verify the reproducibility of results.

2.6 Determination of minimum inhibitory concentrations (MICs)

MICs were determined for the plant extracts using the method of Chandrasekaran and Venkatesalu (2004). This entailed preparing a dilution series of the different plant extracts in nutrient or potato dextrose agar and inoculating these with 50 µL of ~1 x 10^6 cfu/mL bacteria or fungi. Control tubes containing solvent but no plant extract were also tested. The final volume in each tube was 1 mL and the highest concentration of each plant extract tested was 9.6 mg/mL. Tubes inoculated with bacteria were incubated at 37ºC for 24 hours, while those inoculated with *C. albicans* were incubated at 25ºC for 48 hours. MICs were determined by identifying the lowest concentration of plant extract which completely inhibited microbial growth.

2.7 Determination of minimum bactericidal and minimum fungicidal concentrations (MBCs and MFCs)

MBC and MFC values were also determined according to the method described previously (Chandrasekaran and Venkatesalu, 2004). In brief, this involved identifying tubes from the MIC assay which did not show any growth, resuspending the microorganisms in nutrient or potato dextrose broth, and subculturing this onto the

surface of nutrient or potato dextrose agar. As before, plates inoculated with bacteria were incubated at 37ºC for 24 hours, while those inoculated with *C. albicans* were incubated at 25ºC for 48 hours. MBC and MFC values were determined by identifying the lowest concentration of extract that did not permit any visible growth.

3. Results and discussion

When different extracts of *B. balsamifera* were screened for activity using the disc diffusion method, it was the essential oil which was identified as having the most potent antimicrobial activity (Table 1). The essential oil was responsible for 19 mm and 12 mm zones of inhibition in agar plates inoculated with clinical isolates of the Gram positive bacteria *S. aureus* and *B. cereus*. This extract was also found to have activity against the pathogenic fungus *C. albicans*. Of the four plant extracts examined, it was the hexane extract which appeared to have the broadest spectrum of activity though (Table 1), inhibiting two strains of *S. aureus* and two strains of the Gram negative bacterium *E. cloacae*. Although this does not compare favourably with the spectrum of activity of the antibiotics tested (Table 1), it is worth noting that the bacteria against which the hexane extract is effective are very problematic species. Infections caused by *S. aureus*, in particular, are being seen with increasing prevalence in both the hospital (Rubinstein, 2008) and community (Moellering, 2006), and there is evidence to suggest that this species is becoming increasingly pathogenic (Otto, 2009) and decreasingly susceptible to the drug of last resort vancomycin (Gould, 2008). With regard to the dichloromethane extract, this was found to have a similar level of inhibitory activity against *E. cloacae* as the hexane extract, but no activity was detected against *S. aureus*. No activity whatsoever was detected from the methanol extract and work with this extract was discontinued.

Data from subsequent assays where the inhibitory and microbicidal activity of the extracts was measured (Table 2) correlates well with results from the screening work (Table 1). As before, the essential oil was found to be the most potent of the extracts, with an MIC of just 150 µg/mL against *B. cereus* and an MIC of 1.2 mg/mL against *S. aureus* and *C. albicans*. In addition to having inhibitory activity against Gram positive bacteria and the fungal pathogen *C. albicans*, results from the MBC and MFC assays suggest that *B. balsamifera* essential oil has microbicidal activity. Characterisation of plant extracts as microbiostatic or microbicidal has become a complicated matter in recent years (Cushnie et al., 2007). However, the method used to determine MBC and MFC values in the current study involved transferring all (as opposed to just a sample) of the original inoculum onto agar. This means that the observed results can be attributed to bacterial cell death, rather than cell aggregation. Antimicrobial agents with MBC and MFC values no higher than two to four times the MIC are considered microbicidal (Prescott et al., 1999) and this was the case for the essential oil.

As observed in the screening data, the hexane extract was found to have inhibitory activity against both Gram positive *S. aureus* and Gram negative *E. cloacae*, with MICs of 9.6 mg/mL and 4.8 mg/mL respectively (Table 2). Though these values are quite high, it should be borne in mind that the active constituent(s) of the extract may be present in small quantities. MBC data indicates that the hexane extract has bactericidal activity against *E. cloacae*. This finding is encouraging as Gram negative bacteria have notoriously low susceptibility to antibacterial agents due to the low permeability of their cell envelope (Delcour, 2009). Indeed, there is considerable concern among experts about the lack of drugs that are effective against Gram negative bacteria (Vergidis and Falagas, 2008). The dichloromethane extract of

B. balsamifera, in comparison to the hexane extract, was found to have quite weak inhibitory activity and no detectable bactericidal activity.

When data from Tables 1 and 2 are viewed together, it is clear that the hexane extract and essential oil of *B. balsamifera* are effective against different classes of microorganism. This indicates that different constituents are likely to be responsible for the activity of these two extracts. At the present time the identity of these constituents remains to be elucidated, but there has been a previous report of an antifungal compound, icthyothereol acetate, being isolated from *B. balsamifera* (Ragasa et al., 2005). Several flavonoids (Ali et al., 2005; Hasegawa et al., 2006) and sesquiterpenoids (Osaki et al., 2005) have also been isolated from *B. balsamifera*. Though none of these compounds have been tested for antibacterial or antifungal activity, certain flavonoids and sesquiterpenoids are known to be antimicrobial (Rabe and van Staden, 2000; Cushnie and Lamb, 2005), and it is conceivable that these constituents are contributing to the activity of this plant.

In summary, data presented here shows that *B. balsamifera* has antimicrobial activity, a finding which may explain the herb's use in traditional medicine. Of the various extracts of the plant examined, it was the essential oil and hexane extract which had the greatest activity in terms of potency and spectrum. Through future research, it may be possible to isolate and develop the compounds responsible for this activity. Data from the current study suggests that these agents could be useful against *S. aureus*, *E. cloacae* and *C. albicans* infections, especially if administered topically, where clinically effective doses would be more readily achievable. In addition, the compounds could potentially be developed as food additives for the prevention of foodborne diseases caused by *S. aureus* and *B. cereus*.

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References

Ali, D.M., Wong, K.C., & Lim, P.K. (2005). Flavonoids from *Blumea balsamifera. Fitoterapia, 76*, 128-130.

Atta-ur-Rahman (Ed.). (2008). Studies in natural products chemistry. Volume 35. Oxford: Elsevier Science (UK), p. 423.

Boonphong, S., Puangsombat, P., Baramee, A., Mahidol, C., Ruchirawat, S., & Kittakoop P. (2007). Bioactive compounds from *Bauhinia purpurea* possessing antimalarial, antimycobacterial, antifungal, anti-inflammatory, and cytotoxic activities. *Journal of Natural Products, 70*, 795-801.

Cannon, R.D., Lamping, E., Holmes, A.R., Niimi, K., Tanabe, K., Niimi, M., & Monk, B.C. (2007). *Candida albicans* drug resistance - another way to cope with stress. *Microbiology, 153*, 3211-3217.

Chandrasekaran, M., & Venkatesalu, V. (2004). Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *Journal of Ethnopharmacology, 91*, 105-108.

Cushnie, T.P.T., Hamilton, V.E.S., Chapman, D.G., Taylor, P.W., & Lamb, A.J. (2007). Aggregation of *Staphylococcus aureus* following treatment with the antibacterial flavonol galangin. *Journal of Applied Microbiology, 103*, 1562-1567.

Cushnie, T.P.T., & Lamb, A.J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*, 343-356.

Delcour, A.H. (2009). Outer membrane permeability and antibiotic resistance. *Biochimica et Biophysica Acta, 1794*, 808-816.

Friedman, M. (2007). Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Molecular Nutrition and Food Research, 51*, 116-134.

Friedman, M., Henika, P.R., Levin, C.E., & Mandrell, R.E. (2006). Antimicrobial wine formulations active against the foodborne pathogens *Escherichia coli* O157 : H7 and *Salmonella enterica*. *Journal of Food Science, 71*, M245-M251.

Fujita, M., Shiota, S., Kuroda, T., Hatano, T., Yoshida, T., Mizushima, T., & Tsuchiya, T. (2005). Remarkable synergies between baicalein and tetracycline, and baicalein and beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Microbiology and Immunology, 49*, 391-396.

Gould, I.M. (2008). Clinical relevance of increasing glycopeptide MICs against *Staphylococcus aureus*. *International Journal of Antimicrobial Agents, 31*, Suppl 2:1-9.

Hasegawa, H., Yamada, Y., Komiyama, K., Hayashi, M., Ishibashi, M., Yoshida, T., Sakai, T., Koyano, T., Kam, T.S., Murata, K., Sugahara, K., Tsuruda, K., Akamatsu, N., Tsukasaki, K., Masuda, M., Takasu, N., & Kamihira, S. (2006). Dihydroflavonol BB-1, an extract of natural plant *Blumea balsamifera*, abrogates TRAIL resistance in leukemia cells. *Blood, 107*, 679-688.

ICRAF (2009). *Blumea balsamifera* (http://www.worldagroforestrycentre.org/sea/Copyright .htm). Nairobi: International Center for Research in Agroforestry (Kenya).

Kumarasamy, Y., Nahar, L., Byres, M., Delazar, A., & Sarker, S.D. (2005). The assessment of biological activities associated with the major constituents of the methanol extract of 'wild carrot' (*Daucus carota* L) seeds. *Journal of Herbal Pharmacotherapy, 5*, 61-72.

Lee, C.Y., Chen, P.Y., Huang, F.L., & Lin, C.F. (2009). Microbiologic spectrum and susceptibility pattern of clinical isolates from the pediatric intensive care unit in a single medical center - 6 years' experience. *Journal of Microbiology, Immunology and Infection, 42,* 160-165.

Moellering, R.C., Jr. (2006). The growing menace of community-acquired methicillin-resistant *Staphylococcus aureus*. *Annals of Internal Medicine, 144,* 368-370.

Nannapaneni, R., Chalova, V.I., Crandall, P.G., Ricke, S.C., Johnson, M.G., & O'Bryan, C.A. (2009). *Campylobacter* and *Arcobacter* species sensitivity to commercial orange oil fractions. *International Journal of Food Microbiology, 129,* 43-49.

Osaki, N., Koyano, T., Kowithayakorn, T., Hayashi, M., Komiyama, K., & Ishibashi, M. (2005). Sesquiterpenoids and plasmin-inhibitory flavonoids from *Blumea balsamifera*. *Journal of Natural Products, 68,* 447-449.

Otto, M. (2009). Understanding the epidemic of community-associated MRSA and finding a cure: are we asking the right questions? *Expert Review of Anti-Infective Therapy, 7,* 141-143.

Park, Y.B., Kim, J.B., Shin, S.W., Kim, J.C., Cho, S.H., Lee, B.K., Ahn, J., Kim, J.M., & Oh, D.H. (2009). Prevalence, genetic diversity, and antibiotic susceptibility of *Bacillus cereus* strains isolated from rice and cereals collected in Korea. *Journal of Food Protection, 72,* 612-617.

Prescott, L.M., Harley, J.P., Klein, D.A. (1999). Microbiology. London: WCB / McGraw-Hill (UK).

Rabe, T., & van Staden, J. (2000). Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*. *Journal of Ethnopharmacology, 73,* 171-174.

Ragasa, C.Y., Co, A.L., & Rideout, J.A. (2005). Antifungal metabolites from *Blumea balsamifera*. *Natural Product Research, 19,* 231-237.

Ruangrungsi, N., Tantivatana, P., Tappayuthpijarn, P., Borris, R.P., & Cordell, G.A. (1985). Traditional medicinal plants of Thailand VI. Isolation of cryptomeridiol from *Blumea balsamifera* (Compositae). *ScienceAsia, 11,* 47-50.

Rubinstein, E. (2008). *Staphylococcus aureus* bacteraemia with known sources. *International Journal of Antimicrobial Agents, 32 Suppl 1,* S18-20.

Süzgeç, S., Meriçli, A.H., Houghton, P.J., & Çubukçu, B. (2005). Flavonoids of *Helichrysum compactum* and their antioxidant and antibacterial activity. *Fitoterapia, 76,* 269-272.

Vergidis, P.I., & Falagas, M.E. (2008). Multidrug-resistant Gram-negative bacterial infections: the emerging threat and potential novel treatment options. *Current Opinion in Investigational Drugs, 9,* 176-183.

Watkins, K.J. (2002). Fighting the clock - Pharmaceutical and biotechnology companies seek ways to reduce the time required to discover and develop medicines. *Chemical and Engineering News, 80,* 27-33.

WHO (2003). Traditional Medicine (Fact Sheet No. 134). Geneva: World Health Organization (Switzerland).

WHO (2009). Infectious diseases kill over 17 million people a year: WHO warns of global crisis (press release). Geneva: World Health Organization (Switzerland).

Table 1 Antimicrobial activity of *Blumea balsamifera* extracts and essential oil as determined by the disc diffusion assay.

Microorganism	Zones of inhibition (mm) for different extracts and antibacterial agents						
	Hexane 384 µg / disc	Dichloromethane 384 µg / disc	Essential oil 384 µg / disc	Penicillin 10 U / disc	Chloramphenicol 30 µg / disc	Tetracycline 30 µg / disc	Gentamycin 10 µg / disc
Gram positive bacteria							
B. cereus ATCC 11778	-	-	-	12	23	29	22
B. cereus MSH 001	-	-	12	12	24	32	22
S. aureus ATCC 25923	8	-	-	12	21	22	20
S. aureus MSH 002	6.5	-	19	11	22	28	21
Gram negative bacteria							
E. cloacae DMST 17206	7	8	-	14	7	7	10
E. cloacae MSH 003	6.5	7	-	12	11	7	7
E. coli ATCC 25922	-	-	-	13	26	23	15
E. coli MSH 004	-	-	-	12	24	21	18
K. pneumoniae MSH 005	-	-	-	13	22	21	14
P. aeruginosa ATCC 27853	-	-	-	-	23	24	24
P. aeruginosa MSH 006	-	-	-	-	26	15	20
S. Typhi DMST 5784	-	-	-	19	26	20	14
S. Typhi MSH 007	-	-	-	-	27	26	18
Fungi							
C. albicans ATCC 10231	-	-	9	-	9	8	8

Note: Zones of inhibition were measured after 24 hours incubation for bacteria and 48 hours for *C. albicans*; No inhibitory activity was detected from the methanol extract against any of the microbial spp.; -, no zone of inhibition

Table 2 Antimicrobial activity of *Blumea balsamifera* extracts and essential oil as determined by MIC, MBC and MFC assays.

Microorganism	Hexane		Dichloromethane		Essential oil	
	MIC (mg/mL)	MBC/MFC (mg/mL)	MIC (mg/mL)	MBC/MFC (mg/mL)	MIC (mg/mL)	MBC/MFC (mg/mL)
Gram positive bacteria						
B. cereus ATCC 11778	-	NT	-	NT	0.15	0.15
S. aureus ATCC 25923	9.6	-	-	NT	1.2	1.2
Gram negative bacteria						
E. cloacae DMST 17206	4.8	9.6	9.6	-	-	NT
Fungi						
C. albicans ATCC 10231	-	NT	-	NT	1.2	1.2

Note: Results were recorded after 24 hours incubation for bacteria and 48 hours for *C. albicans*; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; -, no activity detected; NT, not tested due to insufficient level of activity in MIC assay

List of table captions

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Table 2 Antimicrobial activity of *Blumea balsamifera* extracts and essential oil as determined by MIC, MBC and MFC assays.