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Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth

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ABSTRACT

The hydrodistilled essential leaves and stems oils of *Orthosiphon stamineus* Benth were analysed by GC–MS/MS. Sixty nine compounds representing 97.6 and 97.4% of the total leaves and stems oils, respectively were identified, of which β-caryophyllene (24.0 and 35.1%), α-humulene (14.2 and 18.4%), β-elemene (11.1 and 8.5%), 1-octen-3-ol (8.2 and 7.0%), β-bourbonene (3.4 and 3.0%), β-pinene (2.1 and 1.7%), caryophyllene oxide (1.6 and 2.2%), camphene (1.6 and 1.3%) and limonene (1.2 and 1.1%) were the major compounds. Thus, the monoterpenes and sesquiterpenes were the predominant portions of the oils. Essential oils and methanol extract of *O. stamineus* and the derived fractions of hexane, chloroform, and ethyl acetate were tested for anti-fungal activity, which was determined by disc diffusion and minimum inhibitory concentration (MIC) determination methods. The oils, methanol extract and derived fractions of methanol extract displayed great potential of anti-fungal activity as a mycelial growth inhibitor against the tested phytopathogenic fungi such as *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium solani*, *Colletotricum capsici* and *Phytophthora capsici*, in the range of 49.3–70.3% and minimum inhibitory concentration ranging from 500 to 1000 μg/ml.

1. Introduction

Fungi have long been recognized as causal agents of plant diseases. Rice sheath blight (*Rhizoctonia solani*), grey mold rot (*Botrytis cinerea*), fruit rot (*Fusarium solani*), vascular wilt (*F. oxysporum*), water soaked spot (*Sclerotinia sclerotiorum*) and fruit rot (*Phytophthora capsici*) are important plant diseases (Saini and Sharma, 1978; Purdy, 1979; Lee and Rush, 1983; Agrios, 1988; Mullins et al., 1992; Rojo et al., 2007). Chemical fungicides are known to be highly effective to control the postharvest diseases in various vegetables and fruits. However, they are not considered as long-term solutions due to the concerns associated with exposure risks, health and environmental hazards, residue persistence, and development of tolerance (Lingk, 1991; Radja Commare et al., 2002). The increasing recognition and importance of fungal infections and the difficulties encountered in their treatment have stimulated the search for synthetic chemical fungicide alternatives. Essential oils

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are made up of many different volatile compounds and have been shown to possess antimicrobial and fungicidal properties (Karmen et al., 2003; Ahmet et al., 2005). Essential oils and plant extracts are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional uses (Sawamura, 2000; Ormancey et al., 2001). So, essential oils and plant extracts are one of the most promising groups of natural compounds for the development of safer anti-fungal agents.

Orthosiphon stamineus Benth is used as a medicinal plant because of its diuretic, anti-fungal and bacteriostatic properties of leaves (Olah et al., 2003). Most of the papers dealing with this subject refer these effects to the content of potassium, inositol and lipophilic flavones in *Orthosiphon* leaves (Schneider and Tan, 1973; Schut and Zwaving, 1993). In addition to the mentioned components, saponins, sterols, polyphenols, rosmarinic acid and ursolic acid and essential oil have been also detected (Stecher, 1976; Tezuka et al., 2000; Hossain et al., 2006). The essential oil, in some cases, is the reason for diuretic effects of plant drugs, has not yet been described in detail. Based on preliminary analyses, Awale et al. (2003) have reported that it mainly consists of oxygenated sesquiterpenes. However, there is no report available in the literature on the detailed analyses of essential oil of *O. stamineus* and its anti-fungal property.

Therefore, the aim of the present study is (a) to examine the chemical composition of the essential oils isolated from the leaves and stems of *O. stamineus* by GC–MS/MS; (b) to evaluate the anti-fungal activity of essential oils and methanolic extract of *O. stamineus* and its derived fractions of hexane, chloroform and ethyl acetate against certain important phytopathogens causing severe diseases in the plants.

2. Materials and methods

2.1. Plant material

The leaves and stems of *O. stamineus* were collected from the hilly area at Penang in Malaysia, in July 2003 and initially identified by morphological features and data base present in the library, School of Biology, University Sains Malaysia, Malaysia and a voucher specimen has been deposited at the Forest Research Institute, Malaysia (FRIM) with voucher number ZAS 1113.

2.2. Isolation of the essential oils

The air-dried leaves and stems (250 g for each) of *O. stamineus* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oils were dried over anhydrous sodium sulphate and preserved in a sealed vial at 4 °C until further analysis.

2.3. Preparation of crude extracts

The air-dried leaves and stems of *O. stamineus* were pulverized into powdered form. The dried powder (50 g) was extracted three times with 70% methanol (3×200 ml) at room temperature and the solvents from the combined extracts were evaporated by a vacuum rotary evaporator (EYELA N-1000, Japan). The methanol extract was (5.3 g) suspended in water and extracted successively with hexane, chloroform and ethyl acetate to give hexane (1.97 g), chloroform (0.93 g) and ethyl acetate (0.78 g) and residual methanol fractions (0.58 g), respectively. Solvents (analytical grade) for extraction were obtained from commercial sources.

2.4. GC–MS/MS analysis

The GC–MS/MS analysis of the essential oils was performed using a Waters GC–MS/MS (Model 800) equipped with a ZB-1 MS-fused silica capillary column (30 m × 0.25m i.d., film thickness 0.25 μm). For GC–MS/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. Injector and mass transfer line temperature were set at 220 and 290 °C, respectively. The oven temperature was programmed from 50 to 150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100 (v/v), in methanol) of 1 μl was manually injected in the splitless mode. The relative percentage of the oil constituents was expressed as percentage by peak area normalization.

The identity of the components of the essential oils was assigned by comparison of their retention indices, relative to a series *n*-alkane indices on the ZB-1 capillary column and GC–MS spectra from the Wiley 6.0 MS data and literature data and whenever possible, by co-injection with authentic compounds (Joulain and Konig, 1998; Adam, 2001).

2.5. Microorganisms

The fungal cultures were obtained from the Herbal Laboratory, University Sains Malaysia, Malaysia (HL). Cultures of each fungal species were maintained on potato-dextrose-agar (PDA) slants and stored at less than 4 °C. The fungal species used in the experiment were *R. solani* HL 325, *B. cinerea* HL 206, *F. solani* HL 115, *Colletotricum capsici* HL 410 and *P. capsici* HL 97.

2.6. Preparation of spore suspension and test samples

The spore suspension of *B. cinerea*, *R. solani*, *F. solani*, *P. capsici* and *C. capsici* were obtained from their respective 10-daycold cultures, mixed with sterile distilled water to obtain a homogenous spore suspension of 1×10^7 spore/ml. Essential oils, methanol extract of *O. stamineus* and its derived fractions of hexane, ethyl acetate and chloroform were dissolved in methanol separately to prepare the stock solution with their respective known weights, which were further diluted to prepare test samples.

2.6.1. Determination of anti-fungal activity of essential oils and crude extracts

Petri dishes (9 cm diameter) containing 20 ml of PDA medium were used for anti-fungal activity assay, performed in solid media by disc diffusion method (Duru et al., 2003). Sterile Whatman paper discs of 6 mm diameter were pierced in the