Identification of antimicrobial constituents in essential oil from *Paulownia fortunei* flowers

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Abstract

The aim of this study was to analyze essential oil from flowers of *Paulownia fortunei* through GC-MS for identification of possible antimicrobial constituents. The essential oil from flowers of *P. fortunei* was extracted by microwave assisted technique. The oil was analyzed by GC-MS that showed the presence of six compounds. Nerolidol was the principal compound in the extract with 82.81% peak area. Other compounds included pentacosane (3.95%), octadecane (4.77%), 5,9-undecadien-2-one, 6,10-dimethyl-, (E)- (3.02%), heptacosane (2.81%), and (E,E,E)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene (2.68%). Literature survey showed that the principal compound nerolidol possesses potent antibacterial and antifungal properties.
Keywords: Antimicrobial, Essential oil, Flower, GC-MS analysis, *Paulownia fortunei*.

Introduction

Essential oils (EOs) are natural and volatile compounds extracted as secondary metabolites from various aromatic plants (Perczak *et al*., 2019). These are insoluble in water and soluble in ether and alcohol. These are generally liquid at room temperature and colorless, often have a distinctive taste as well as pleasant odor (Sarkic and Stappen, 2018). These are a complex mixture of low molecular weight phytoconstituents extracted by steam distillation, dry distillation, cold pressing or solvent extraction (Khan and Dwivedi, 2018). These may constitute 20–200 different plant secondary metabolites belonging to a variety of chemical classes including phenylpropanoids, sesquiterpenes, monoterpenes, terpenoids, aliphatic and few aromatic compounds (Aziz *et al*., 2018). Plant EOs possess various applications mainly in agriculture, food industries, health and cosmetics (Reddy, 2019; Sharmeen *et al.*, 2021). These can be obtained from different plant organs such as flowers, buds, twigs, leaves, stem, fruits, bark, roots and seeds (Ghaffari *et al*., 2019). Several researchers have globally screened EOs as a potential source of novel compounds, food preservatives and also to treat infectious diseases (Singh *et al*., 2021). These exhibit biological properties including antioxidant, anticancer, antimutagenic, antiviral, antifungal, antibacterial, immunomodulatory, anti-inflammatory and antiprotozoal activities (Tariq *et al.*, 2019; Valdivieso-Ugarte *et al*., 2019).

Dragon tree [*Paulownia fortunei* (Seem.) Hemsl.], native to China and Taiwan, is a fast-growing perennial tree of family Scrophulariaceae. Its wood is highly suitable for paper production (Kiaei, 2013), with a fiber length of 1.42 mm (Rai *et al.*, 2000). Epicarp of its fruits contain antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Cercós, 1982). *P. fortunei* also possesses various medicinal properties. Its seeds are useful to treat diabetes and leaves show marked antioxidant property (He *et al.*, 2016). *Paulownia* spp. were introduced in Pakistan from 1989–95 at 13 locations in Kashmir, Punjab and Khyber Pakhtunkhwa by Pakistan Forest Institute and the seeds were obtained from Chinese Academy of Forestry (Siddiqui and Khan, 1989; Bajwa and Gul, 2000). Different plant species are known to possess a variety of antimicrobial compounds (Banaras *et al.*, 2020; Khan and Javaid, 2020). However, there is not any such report from flowers of *P. fortunei* growing in Pakistan. Therefore, in the present study, GC-MS analysis of essential oil of flowers of *P. fortunei*, collected from Lahore, Pakistan, was carried out for identification of antimicrobial compounds.

Materials and Methods

Few years back, seedlings of *P. fortunei* were brought from China and were sown at Faculty of Agricultural Sciences, University of the Punjab Lahore Pakistan. The mature fresh flowers were collected during spring 2021 from these plants. The flowers were kept in paper boxes and shifted to the laboratory for the essential oil extraction process.

Microwave assisted procedure was used to extract essential oil from *P. fortunei* flowers at Institute of Chemistry, University of the Punjab Lahore Pakistan. A modified microwave oven was utilized in the experiment, along with glass bends and a condenser. An electric grinder was used to crush 305 g of *P. fortunei* flowers into a paste. The sample was moved to a one-liter round-bottom flask and stabilized within the oven with bends, one of which was connected with a condenser using plastic clamps. The condenser is connected to the collecting flask by another bend. The oven's power level was set to 60 °C for 60 minutes, and the extraction process was initiated. After every 10 minutes, the orientation of the sample-containing flask was manually turned to ensure uniform heating. The essential oil-containing hydrosol from the collecting flask was transferred to the separating funnel and allowed to cool to room temperature. Dimethyl sulphoxide (5 mL) was added to extract the essential oil from the hydrosol. Oil containing DMSO layer sank down to the bottom and collected in screw vial.

The GC-MS analysis was carried out on a Gas Chromatograph (GC) machine model 7890B and that of Mass Spectroscopy (MS) machine model 5977A branded by Agilent Technologies. The column used was DB 5 MS, (30 m × 0.25 µm × 0.25 µm). Injection volume was 1 µL and carrier gas was helium. Oven ramping; initial temperature was 80 ºC and then raised 10 ºC per minute up to 300 ºC. Inlet temperature was 280 ºC. MS conditions were as: the source temperature was 230 ºC and quadrupole temperature was 150 ºC. Chemical compounds were identified by comparison of their spectra with NIST 2017 library and arranged in the ascending order of their retention times. The relative abundance was reported by using their peak areas.

Results and Discussion

There were six compounds in the methanolic extract of *P. fortunei* as shown in Fig. 1 and Table 1. Nerolidol was the principal constituent in this showing 82.81% peak area. Other compounds present in the extract were pentacosane (3.95%), octadecane (4.77%), 5,9-undecadien-2-one, 6,10-dimethyl-, (E)- (3.02%), heptacosane (2.81%), and (E,E,E)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene (2.68%). Mass spectra and structures of these compounds are given in Fig. 2. Essential oil of *P. tomentosa* flower from Egypt contained geranyl geraniol (18.05%) as the predominant compound followed by hexatriacontane (11.61%) with antibacterial activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* (Ibrahim *et al.,* 2013).

Nerolidol, the major compound in the extract is known to have marked antimicrobial potential. It is a sesquiterpene alcohol and is found in essential oils of many plant species including *Citrus aurantium*, *Hedychium coccineum* and *Jasminum grandiflorum* (Gurib-Fakim *et al.*, 2002; Jirovetz *et al.,* 2007; Ammar *et al.,* 2012). This compound is a flavoring agent, and is also used in as a fragrance agent in detergents and cosmetics (Lapczynski *et al.*, 2008). Nerolidol and its various derivatives namely (-)-α-bisabolol, *cis*-nerolidol, *O*-ethyl-nerolidol, and *O*-methyl-nerolidolare known for their antibacterial and antifungal activities (Krist *et al.,* 2015). Nerolidol isolated from essential oil of *Chamaecyparis obtusa* showed antifungal activity against *Microsporum gypseun* (Lee *et al.,* 2007). In addition to its own antibacterial potential, this compound also enhances action of other antibiotic drug by increasing bacterial permeability (Brehm-Stecher and Johnson, 2003). It disrupts barrier function of bacterial membrane by leaking potassium ion from *Staphylococcus aureus* (Inoue *et al.,* 2004).

Octadecane, an alkane, was the second most abundant compound with 4.77% peak area. It has been identified as a highly abundant compound in bulb oil of *Aliumnigrum* (30.5%) (Rouis-Soussi *et al.,* 2014). It has also been identified in many other plant species including *Rhaponticum acaule* and *Trichosanthes dioica* (Boussaada *et al.,* 2008; Khatua *et al.*, 2016). Similarly, two other compounds identified in the present study namely pentacosane and heptacosane were also alkanes. Presence of alkanes as volatile compounds in many plants species has been reported in many publications (Adeleye *et al.*, 2011). Marrufo *et al.* (2013) reported that hydrocarbons showed 91.1% of oils of *Moringaoleifera.* The major compounds were pentacosane (13.3%), hexacosane (13.9%), andheptacosane (11.4%). The oil was found having antibacterial and antifungal activities mainly due to the presence of these hydrocarbons. He (2009) reported that some alkanes are known for having marked antimicrobial effect particularly against *Escherichia coli* and *Staphylococcus aureus*.

5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-, also known as geranylacetone, is a colorless oil. It is a flavor component of various plant species such as tomato, mango and rice (Pino *et al.,* 2005). It has also been found as a minor compounds in oil of an endophytic fungus *Gliomastix murorum* isolated from *Paris polyphylla* var. *yunnanensis* (Zhao *et al.*, 2009). It has trypanostatic activity and can protect animals from *Trypanosoma congolense*-induced anaemia by inhibiting sialidase (Saad *et al.,* 2019).

(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene, also known as α- springene, is a diterpene. Previously, it was identified in *Ligularia fischeri* var. *spiciformis* with strong cytotoxicity against HL-60 (Lee *et al.,* 2002). This compound was also found in essential oils of *Murraya exotica* and *Teucriummarum* as a major component (Raina *et al.,* 2006; Djabou *et al.,* 2013).

Conclusion

The main component in essential oil of *P. fortunei* was nerolidol that is known for its antibacterial and antifungal activities.

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Dr. Muhammad Ashfaq brought seedlings of *P. fortunei* from China and cultivated at Institute of Agricultural Sciences, Punjab University Lahore.

Table 1: List of compounds in methanolic flower extract of *Paulownia fortunei* identified by GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	$C_{13}H_{22}O$	194.31	9.109	3.02
2	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	$C_{20}H_{32}$	272.46	14.825	2.68
3	Nerolidol	$C_{15}H_{26}O$	222.37	15.609	82.81
4	Octadecane	$C_{18}H_{38}$	254.49	17.932	4.77
5	Pentacosane	$C_{25}H_{52}$	352.68	19.568	3.91
6	Heptacosane	$C_{27}H_{56}$	383.73	21.079	2.81

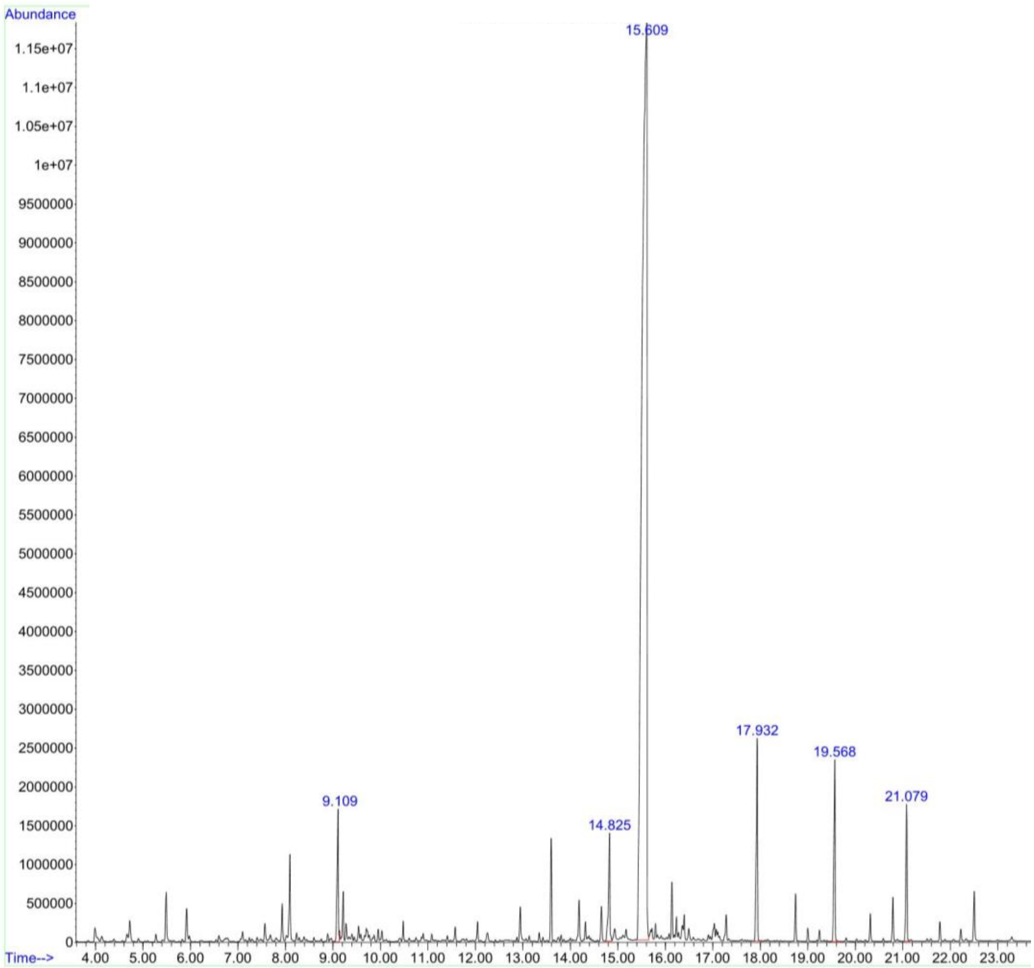
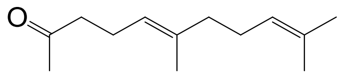
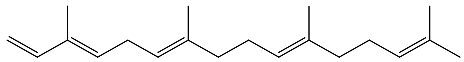


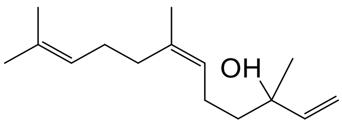
Fig. 1: GC-MS chromatogram of methanolic flower extract of *Paulownia fortunei.*



5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-



(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene



Nerolidol

Octadecane

Pentacosane

Heptacosane

Fig. 2: Structures of compounds identified in methanolic flower extract of *Paulownia fortunei.*

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