ANTIBACTERIAL ACTIVITYOF ESSENTIAL OIL OF *PAULOWNIA FORTUNEI* (SEEM.) HEMSL. FLOWERS

MALIK F. H. FERDOSI[1], IQRA HAIDER KHAN[2], ARSHAD JAVAID[2*] AND TAYYABA SATTAR[3]

[1]*Department of Horticulture, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan*
[2]*Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan*
[3]*Department of Chemistry, Lahore College for Women University, Lahore, Pakistan*

[*]*Corresponding author's email: arshad.iags@pu.edu.pk, arshad.dpp@pu.edu.pk*

Abstract

This study was undertaken to assess the antibacterial activity of essential oil of *Paulownia fortunei* flowers. The flowers were collected from *P. fortunei* plants introduced from China few years back and cultivated in University of the Punjab Lahore, Pakistan. Essential oil was extracted using microwave assisted method. Flowers gave 0.020% oil yield. Activity of the essential oil was assessed against four pathogenic bacterial species viz. *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* There was a significant difference ($P \leq 0.01$) among the bacterial species with respect to their response to the oil. *K. pneumoniae* was the most sensitive bacterial species followed by *S. aureus* and *E. coli* with 12.5 mm, 11.5 mm and 10.5 mm zones of inhibition respectively. *P. aeruginosa* was the most resistant bacterial species showing 9 mm zone of inhibition. This study concludes that essential oil of *P. fortunei* flowers had variable antibacterial activity against different bacterial species.

Keywords: Dragon tree, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Pakistan.

Introduction

Essential oils (EOs) are complex mixtures of natural volatile organic compounds that are produced by aromatic plants in the form of secondary metabolites (Valdivieso-Ugarte *et al.*, 2019). They are liquid, limpid and soluble in organic solvents with lower densities than that of water (Perricone *et al.*, 2015). They can be synthesized by all plant organs such as flowers, buds, stems, twigs, leaves, fruits, seeds, bark and roots (Akthar *et al.*, 2014). They contain a number of organic compounds belonging to lactones, aldehydes, ketones, ethers, esters, alcohols, phenols, sesquiterpenes and terpenes (Aali *et al.*, 2017), which in pure form exhibit biological activities against various Gram negative and Gram positive bacterial pathogens (Sabo and Knezevic, 2019). Antibacterial efficacies of EOs and their components may vary accordingly with different bacteria as well as with oils (Artini *et al.*, 2018; Reyes-Jurado *et al.*, 2020). Many *in vitro* studies have reported the pronounced antibacterial efficacies of EOs against various food-spoilage and human pathogenic bacterial species (Naz *et al.*, 2014; Perricone *et al.*, 2015; Valerio *et al.*, 2021).

Paulownia (family Paulowniaceae) is a genus of nine fast-growing hardwood tree species (Bajwa and Gul, 2000). These are mostly present in China, Laos and Vietnam, and have

also been introduced in other countries of Asia, USA, Europe and Australia (Woods, 2008). In Japan, Korea and China, its wood is used in manufacturing of musical instruments. It is a part of agroforestry system in China due to its fast growth rate, light but strong wood, use of leaves as fodder, nectar rich flowers, and sparse canopy (Wu and Zhu, 1997). *Paulownia* was introduced in Pakistan from 1989-95 through a project financed by IDRC, Canada. In 1989, its seeds were obtained from Chinese Academy of Forestry and planted at 13 sites in Punjab, Kashmir and Khyber Pakhtunkhwa by Pakistan Forest Institute, Peshawar (Siddiqui and Khan, 1989; Bajwa and Gul, 2000). Most of the previous studies were carried out on chemical profile and antibacterial activities of essential oils from *Paulownia tomentosa* (Ibrahim *et al.*, 2013; Schneiderová and Šmejkal, 2015). Few years back, plants of *P. fortunei* were brought from China and cultivated at Faculty of Agricultural Sciences, University of the Punjab Lahore, Pakistan (Fig. 1). Recently, Ferdosi *et al.* (2020) studied chemical profile of essential oils of flowers of these plants and found nerolidol as the major compound in the oil. This compound is known to possess antibacterial activity (Inoue *et al.*, 2004; Krist *et al.*, 2015). However, Ferdosi *et al.* (2020) did not carry out antibacterial bioassays. The present study was, therefore, carried out to evaluate the antibacterial activity of essential oil of *P. fortunei* flowers.

A B

Fig. 1: *Paulownia fortunei* tree (A) and a fruiting branch (B).

Materials and Methods

Collection of *P. fortunei* flowers: Fresh flowers were collected from *P. fortunei* plants growing at Faculty of Agricultural Sciences, University of the Punjab Lahore Pakistan. Seedlings of these plants were brought by Dr. Muhammad Ashfaq from China and cultivated here five years ago. The flowers were kept in paper bags after collection and shifted to the laboratory for the extraction of essential oil.

Extraction of essential oil: For extraction of essential oil from the collected flowers, microwave assisted procedure was used following Ferdosi *et al.* (2020). Fresh *P. fortunei* flowers (350 g) were ground in an electric grinder to make a paste, and the sample was transferred to a 1 L round-bottom flask and stabilized within the oven with bends, one of which was connected with a condenser that was connected to a collecting flask. The oven power was set at 60 °C for 60 min. The orientation of the sample-containing flask was turned manually after every 10 min to maintain uniform heating. The essential oil-containing hydrosol was transferred to a separating funnel and cooled at room temperature. Five milliliters of dimethyl sulphoxide (DMSO) was added to separate the essential oil from the hydrosol mixture. The oil containing DMSO layer was settled down that was collected in screw vial.

Antibacterial activity: For antibacterial assays of essential oil of *P. fortunei* flowers, disk diffusion method was used. Four non-multi drug resistant bacterial strains namely *E. coli* 5964-LF, *K. pneumonia* 599-BLF, *S. aureus* 613-B and *P. aeruginosa* 5994-NLF were used in the present study. Growth medium LBA (Luria Bertani Agar) was autoclaved poured in the sterilized Petri plates and bacteria cultures were spread over it. A volume of 20 µL of the essential oil was poured on sterilized filter paper disk of 0.6 cm diameter using a micro pipette and placed over LBA media plate. A volume of 20 µL of DMSO was also used as a negative control. Plates were then incubated at 37 °C for 24 h and zones of inhibitions were recorded with the help of a scale.

Statistical analysis: All the data were analyzed by one-way ANOVA and the means were delineated by LSD test at $P \leq 0.05$.

Results & Discussion

Analysis of variance showed that there was a significant difference ($P \leq 0.01$) among the bacterial species with respect to their response to the applied essential oil of *P. fortunei* flowers (Table 1). *K. pneumoniae* was the most sensitive bacterial species where a highest zone of inhibition (12.5 mm) was recorded. *S. aureus* was the second most sensitive bacterial species followed by *E. coli* with 11.5 mm and 10.5 mm zones of inhibition. The most resistant bacterial species was *P. aeruginosa* showing 9 mm zone of inhibition. Recently, Ferdosi *et al.* (2020) studied the chemical profile of *P. fortunei* flowers and reported the presence of nerolidol in very high concentration (82.81%). This compound is known to possess antibacterial and antifungal activities (Inoue *et al.,* 2004; Krist *et al.,* 2015), and could be responsible for antibacterial activity of essential oil in the present study. *P. fortunei* flowers are a rich source of many flavonoids such as β-sitosterol, apigenin, daucosterol, luteolin, kaempferol, quercetin, hesperetin and thunberginol A (Zhang and Li, 2008; Li *et al.,* 2009). Among these, β-sitosterol isolated from roots of *Malva parviflora* (Ododo *et al.,* 2016), leaves of *Odontonema strictum* (Luhataab and Usuki, 2021), and stem bark of *Punica granatum* (Nweze *et al.,* 2019) exhibited potent antibacterial activity against a number of bacterial species including *S. aureus*, *E. coli* and *Salmonella typhi*. Apigenin (Cushnie

et al., 2003), quercetin (Jaisinghani, 2017), thunberginol A (Yoshikawa *et al.*, 1992), and luteolin (Guo *et al.*, 2020) are known to possess moderate to very high antibacterial activities.

Table 1: Analysis of variance (ANOVA) for the effect of essential oil of *Paulownia fortunei* flowers on bacterial growth.

Sources of variation	df	SS	MS	F values
Bacterial species	3	20.06	6.69	9.59*
Error	8	5.58	0.69	
Total	11	25.64		

*Significant at $P \leq 0.01$.

Essential oil of *P. fortunei* flowers showed variable antibacterial potential against the four tested bacterial species. *K. pneumoniae* was the most sensitive while *P. aeruginosa* was the least sensitive bacterial species to the applied essential oil. These results are in agreement with the findings of various earlier studies where extracts and essential oils of *P. fortunei* and other plant species showed variable antibacterial activity against different bacterial species. Epicarp extracts of *P. fortunei* and *P. tomentosa* were highly active against *S. aureus* and *Bacillus subtilis* while their activity was low against *Saccharomyces carlsbergensis* and *E. coli* (Cercós, 1982). Essential oil from aerial parts of *Baccharis dracunculifolia* showed better activity against *S. aureus*, *Bacillus cereus* and *P. aeruginosa* than against *Listeria monocytogenes*, *Micrococcus flavus*, *Enterobacter cloacae*, *E. coli* and *Salmonella enterica* (Cazella *et al.*, 2019). Similarly, Inouye *et al.* (2001) studied the antimicrobial activities of 14 essential oils and found and reported that *Haemophilus influenzae* was the most susceptible while *E. coli* was the least susceptible to most of the oils. Likewise, eucalyptus oil obtained from *Eucalyptus globulus* showed more pronounced activity against *S. aureus* than against *P. aeruginosa*, *K. pneumoniae* and *Acinetobacter baumannii* (Mulyaningsih *et al.*, 2011).

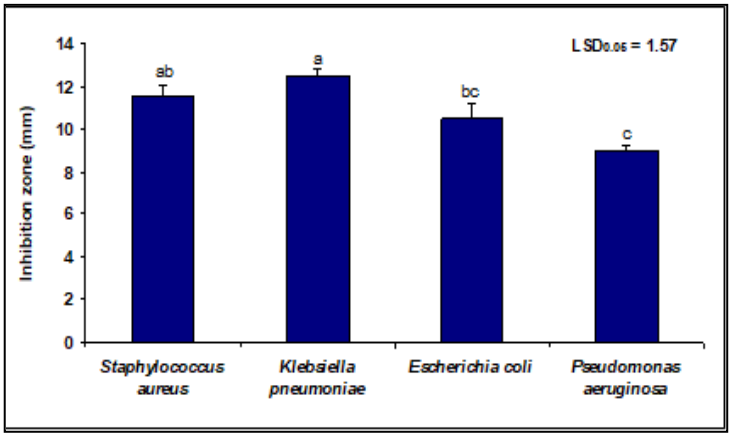

Fig. 2: Antibacterial activity of essential oil of *Paulownia fortunei* flowers. Vertical bars show standard errors of means of three replicates. Different letters show significant difference among the treatments at $P \leq 0.05$ as determined by LSD test.

Conclusion

Essential oil of flowers of *P. fortunei* showed a broad spectrum antibacterial activity. *K. pneumoniae* was found the most sensitive bacterial species followed by *S. aureus* and *E. coli.*

References

Aali, E., R. Mahmoudi, M. Kazeminia, R. Hazrati and F. Azarpey. 2017. Essential oils as natural medicinal substances. *Tehran Univ. Med. J.,* 75: 480-489.

Akthar, M.S., B. Degaga and T. Azam. 2014. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review. *Issues Biol. Sci. Pharm. Res.,* 2: 001-007.

Artini, M., A. Patsilinakos, R. Papa, M. Božović, M. Sabatino, S. Garzoli and L. Selan. 2018. Antimicrobial and antibiofilm activity and machine learning classification analysis of essential oils from different mediterranean plants against *Pseudomonas aeruginosa*. *Molecules*, 23: Article ID 482.

Bajwa, G.A. and H. Gul. 2000. Some observations on insect species of *Paulownia* species at Pakistan Forst Institute Campus, Peshawar. *Pak. J. For.,* 50: 71-80.

Cazella, L.N., J. Glamoclija, M. Soković, J.E. Gonçalves, G.A. Linde, N.B. Colauto and Z.C. Gazim. 2019. Antimicrobial activity of essential oil of *Baccharis dracunculifolia* DC (Asteraceae) aerial parts at flowering period. *Front. Plant Sci.,* 10: Article 27.

Cercós, A.P. 1982. Antimicrobial activity of the epicarp of the fruits of *Paulownia fortunei* and *Paulownia tomentosa*. *Rev Argent Microbiol.,* 14(2):111-114.

Cushnie, T.P.T., V.E.S. Hamilton and A.J. Lamb. 2003. Assessment of the antibacterial activity of selected flavonoids and consideration of discrepancies between previous reports. *Microbiol. Res.,* 158: 281-289.

Ferdosi, M.F.H., I.H. Khan, A. Javaid, T. Sattar and A. Munir. 2020. Identification of antimicrobial constituents in essential oil from *Paulownia fortunei* flowers *Mycopath,* 18(2): 53-57.

Guo, Y., Y. Liu, Z. Zhang, M. Chen, D. Zhang, C. Tian, M. Liu and G. Jiang. 2020. The antibacterial activity and mechanism of action of luteolin against *Trueperella pyogenes*. *Infect. Drug Resist.,* 13: 1697-1711.

Ibrahim, N.A., S.S. El-Hawary, M.M.D. Mohammed, M.A. Faraid, A.M. Nayera and E.S. Refaat. 2013. Chemical composition, antimicrobial activity of the essential oil of the flowers of *Paulownia tomentosa* (Thunb.) Steud. growing in Egypt. *J. Appl. Sci. Res*., 9: 3228-3232

Inoue, Y., A. Shiraishi, T. Hada, K. Hirose, H. Hamashima and J. Shimada. 2004. The antibacterial effects of terpene alcohols on *Staphylococcus aureus* and their mode of action. *FEMS Microbiol. Lett.,* 237: 325-331.

Inouye, S., T. Takizawa and H. Yamaguchi. 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *J. Antimicron. Chemother.,* 47: 565-573.

Jicrobiology, R.N. 2017. Antibacterial properties of quercetin. *Microbiol. Res.,* 8: 6877.

Krista, S., D. Banovaca , N. Tabancab , D.E. Wedgec , V.K. Gochevd , J. Wannere , E. Schmidta and L. Jirovetz. 2015. Antimicrobial activity

of nerolidol and its derivatives against airborne microbes and further biological activities. *Nat. Prod. Commun.*, 10: 143-148.

Li, X.Q., P.F. Zhang, W.D. Duan, D.L. Zhang and C. Li. 2009. Studies on the chemical constituents from flower of *Paulownia fortunei*. *Zhong Yao Cai*, 32: 1227-1229.

Luhataab, L.P. and T. Usuki, 2021. Antibacterial activity of β-sitosterol isolated from the leaves of *Odontonema strictum* (Acanthaceae). *Bioorg. Med. Chem. Lett.*, 48: Article 128248.

Mulyaningsih, S., F. Sporer, J. Reichling and M. Wink. 2011. Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. *Pharmaceut. Biol.*, 49: 893-899.

Naz S, A. Javaid, N. Ahmad, A. Shoaib. 2014. Antibacterial activity of essential oils of *Trachyspermum ammi* (L.) Sprague and *Ocimum basilicum* L. against *Acidovorax* sp. *Int. J. Biol. Biotechnol.*, 11(4): 671-675.

Nweze, C., H. Ibrahim and G.I. Ndukwe. 2019. Beta-sitosterol with antimicrobial property from the stem bark of pomegranate (*Punica granatum* Linn). *J. Appl. Sci. Environ. Manage.*, 23: 1045-1049.

Ododo, M.M., M.K. Choudhury and A.H. Dekebo. 2016. Structure elucidation of β-sitosterol with antibacterial activity from the root bark of *Malva parviflora*. *Springerplus*, 5(1):1210.

Perricone, M., E. Arace., M.R. Corbo., M. Sinigaglia and A. Bevilacqua. 2015. Bioactivity of essential oils: a review on their interaction with food components. *Front. Microbiol.*, 6: Article ID 76.

Reyes-Jurado, F., A.R. Navarro-Cruz, C.E. Ochoa-Velasco, E. Palou, A. López-Malo and R. Ávila-Sosa. 2020. Essential oils in vapor phase as alternative antimicrobials: A review. *Crit. Rev. Food Sci. Nutr.*, 60: 1641-1650.

Sabo, V.A. and P. Knezevic. 2019. Antimicrobial activity of *Eucalyptus camaldulensis* Dehn. plant extracts and essential oils: A review. *Ind. Crop Prod.*, 132: 413-429.

Schneiderová, K. and K. Šmejkal. 2015. Phytochemical profile of *Paulownia tomentosa* (Thunb). Steud. *Phytochem. Rev.*, 14: 799-833.

Siddiqui, K.M. and M. Khan. 1989. Introduction of *Paulownia* in Pakistan. *Pak. J. For.*, 39: 171-176.

Valdivieso-Ugarte, M., C. Gomez-Llorente., J. Plaza-Díaz and A. Gil. 2019. Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: A systematic review. *Nutrients*, 11: Article ID 2786.

Valerio, F., G.N. Mezzapesa., A. Ghannouchi., D. Mondelli., A.F. Logrieco and E.V. Perrino. 2021. Characterization and antimicrobial properties of essential oils from four wild taxa of Lamiaceae family growing in Apulia. *Agronomy*, 11: Article ID 1431.

Woods, V.B. 2008. *Paulownia* as a novel biomass crop for Northern Ireland. Publ. No 7, AFBI Hilsborough.

Wu, Y. and Z. Zhu. 1997. Temperate Agroforestry in China. In: Andrew M. Gordon and Steven M. Newman (ed.). Temperate Agroforestry Systems. Wallingford, Oxfordshire: CAB International. pp. 170-172.

Yoshikawa, M., E. Uchida, N. Chatani, N. Murakami and J. Yamahara. 1992. Thunberginols A, B, and F, new antiallergic and antimicrobial principles from hydrangeae dulcis folium. *Chem. Pharmaceut. Bull.*, 40: 3121-3123.

Zhang, P.F. and C. Li. 2008. Flavones from flowers of *Paulownia fortunei*. *Zhongguo Zhong Yao Za Zhi* 33: 2629-2632.