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Antifungal and Phytotoxic Activities of Ethyl Acetate Soluble Fraction and Purified Compound (Ethyl-3-hydroxy-5-methoxy-4-methylbenzoate) from *Lonicera quinquelocularis*

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Chromatographic separation and purification of ethyl acetate fractions of *Lonicera quinquelocularis* resulted in the isolation of a new compound namely ethyl-3-hydroxy-5-methoxy-4-methylbenzoate. The ethyl acetate fractions extract of *Lonicera quinquelocularis* and isolated compound ethyl-3-hydroxy-5-methoxy-4-methylbenzoate were screened for antifungal and phytotoxic activities. The ethyl acetate fraction was effective against *Aspergillus flavus* (55.5 %) and *Aspergillus niger* (61 %). Furthermore, the new purified compound and ethyl acetate soluble fraction showed phototoxic effect as well.

Keywords: *Lonicera quinquelocularis*, Antifungal, Phytotoxic.

INTRODUCTION

The genus *Lonicera* belongs to the family Caprifoliaceae comprises of 12 genera and 450 species, found in temperate region of Northern Hemisphere. Plants of this genus are used for the treatment of acute fever, headache, respiratory infections [1], antibacterial, antioxidant, cytoprotective, hepatoprotective, antiviral, antitumor and anti-inflamatory activities [2-6]. The literature of this genus showed the isolation of various phyto-constituents such as iridoids, bisiridoids, sulfur containing monoterpenoids, alkaloidal glycosides, triterpenoids, saponins, coumarin glycosides and flavone glycosides [7-10]. *Lonicera quinquelocularis* belongs to this genus mostly found in dry sunny places between 750-3000 m in many countries of Asia. Literature study found the isolation of triterpenoids, loganins, coumarins, iridoide glycosides, benzoates and alkaloids [11-15] from this plant. The diverse medicinal importance of this plant led us to find out some medicinally important phyto-chemicals.

EXPERIMENTAL

The plant was collected from Bara Galli, Hazara division, District Mansehra and was identified by Professor Dr. Manzoor Ahmad, Plant Taxonomist, Department of Botany, Government Degree College, Abbotabad, Pakistan.

Extraction and fractionation: The shade dried aerial part of *Lonicera quinquelocularis* (1000 g) was ground and extracted with methanol at room temperature. The extract was concentrated with rotary evaporator to obtain gummy crude. The methanolic crude was further partitioned with *n*-hexane (15 g), chloroform (20 g), ethyl acetate (13 g) and *n*-butanol (6 g), respectively.

Isolation of ethyl-3-hydroxy-5-methoxy-4-methylbenzoate: The ethyl acetate soluble fraction was further fractionated over silica gel column chromatography eluting with *n*-hexane (100 %), *n*-hexane: EtOAc (1: 99:1), EtOAc (100 %), EtOAc:MeOH (1:9-9:1), MeOH (100 %), in increasing order of polarity to obtain 13 fractions (A-M). The fraction E was re-chromatographed over silica gel column chromatography elutiong with *n*-hexane: EtOAc (4:1, 3:2, 1:1, 2:3 and 1:4). Elution of the fraction with 3:2 mixture of *n*-hexane:EtOAc afforded a pure compound ethyl-3-hydroxy-5-methoxy-4-methylbenzoate.

Antifungal assay: The antifungal activity of crude *Lonicera quinquelocularis* ethyl acetate fraction and purified compound ethyl-3-hydroxy-5-methoxy-4-methylbenzoate was determined by using the reported procedure [16]. 1 mg/mL stock solution of crude ethyl acetate fraction and ethyl-3-hydroxy-5-methoxy-4-methylbenzoate was prepared in DMSO. Furthermore, 1 mL solution of 200 µg/mL was prepared from the stock solution using DMSO as diluting solvent. In the same way, terbinofine stock solution of 1 mg/mL (positive control/antifungal agent) was prepared in DMSO. Moreover, 1 mL solution of the required concentration (200 µg/mL) was prepared from the stock solution in DMSO. Correspondingly,

1 mL DMSO was taken, used as a negative control from the bottle.

In order to grow fungus for inoculums preparation, sabouraud dextrose agar (MERCK) was used, composed of peptone complex 10 g/L, glucose 40 g/L and agar 15 g/L. 6.5 g SDA media was dissolved in 100 mL distilled/autoclaved water in flask for fungus growth and autoclaved at 121 °C for 15 min. 4 mL of this media was poured in all the autoclaved test tubes and marked up to 10 cm in the Laminar flow cabinet for two fungal strains. From the required concentration (200 µg/mL) of the solution, 67 µL of extract solution was put in all the 4 test tubes, which were specified and duplicate for the two fungal strains. In the same way, the terbinofine solution of 67 µL (for positive control) of the required concentration (200 µg/mL) was put in all the two test tubes (one for each) of the two fungal strains. Similarly, 67 µL DMSO (negative control) was poured in another set of two test tubes (one for each) of the two fungal strains. After the completion of this whole process, all the test tubes were place in the laminar flow in slanting position for solidifying the media in the test tubes at room temperature. After the solidification process, 15 spores from 7 days old culture were placed of each fungus strain in all the test tubes (extract + control) and were specified carefully for each strain. All the test tubes were packed air tightly and were placed in incubator at 36 °C for 7 days. Their growth was measured after 7 days and calculated their % inhibition.

Allelopathy/phytotoxic assay (petri plate study): Phytotoxic assays were performed using modified protocol [17]. 5 mg of the extract was taken on electronic digital balance and dissolved in 5 mL of methanol to prepare stock solution from the crud crude ethyl acetate fraction and compound ethyl-3-hydroxy-5-methoxy-4-methylbenzoate. The solutions of 100 µg/mL from the stock were prepared. Five plates were set for each of the two solution *i.e*. one plate in each of the five plates was control that contained distilled water and the remaining four contain the above solution of the extract. For 100 µg/mL solution 0.3 mL from the stock solution was taken and mix with 14.7 mL of methanol to prepare 15 mL volume of the 100 µg/mL solution. Each plate was set with filter paper and the solution was put in it with micropipette. Fresh seeds of rice which were already placed in distilled water for about 30 min were sowed in each plate. In each plate 8 seeds were sowed. In order to check the phytotoxic effect of the plant the radical and seedling were measured after 7 days and average means were taken.

RESULTS AND DISCUSSION

The chromatographic extraction and purification of the ethyl acetate soluble fraction resulted in isolation of new compound ethyl-3-hydroxy-5-methoxy-4-methylbenzoate (Fig. 1). The ethyl acetate soluble fraction and isolated compound

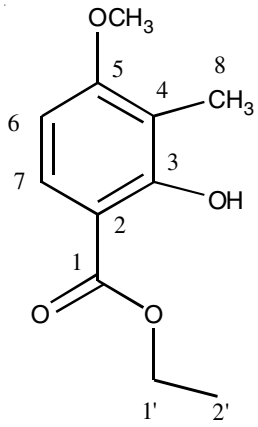

Fig. 1. Chemical structure of ethyl-2-hydroxy-4-methoxy-3-methylbenzoate isolated from ethyl acetate soluble fraction of *Lonicera quinquelocularis*

ethyl-2-hydroxy-4-methoxy-3-methylbenzoate decreased the fungal growth 55.5 and 61 % against *Aspergillus flavus* and *Aspergillus niger* respectively (Table-1). Furthermore, the ethyl acetate soluble fraction and isolated compound showed potent phytotoxic activity against rice seedling and radicals (Fig. 2).

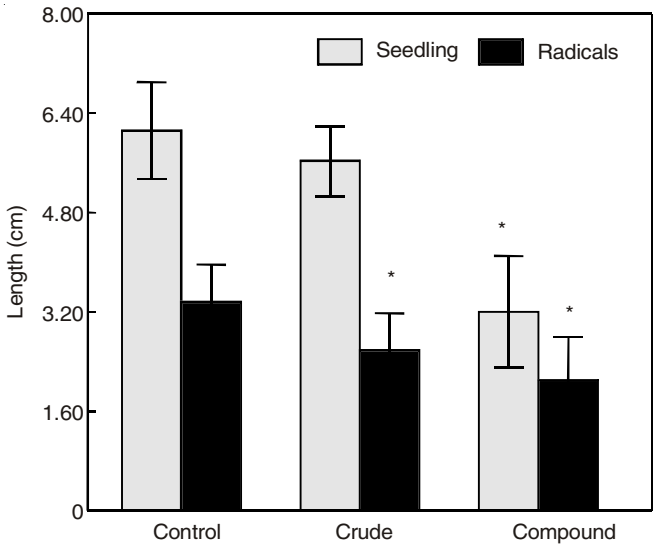

Fig. 2. Phytotoxic effect of *Lonicera quinquelocularis* ethyl acetate fraction and purified compound (ethyl-2-hydroxy-4-methoxy-3-methylbenzoate) against rice growth after 7 days treatment. The data represent the mean of three different experiments done in duplicate. The different letters represent statistically different from each other (*P < 0.05)

Ethyl -3-hydroxy-5-methoxy-4-methylbenzoate: Colourless needles (13 mg); UV (MeOH) λ_{max}, nm (log ε): 225 (4.02), 240 (4.05), 325 (4.35); IR (KBr, ν_{max}, cm^{-1}): 3420, 1720, 1650-1510, 1280, 1130, 1025; ^1H NMR (400 MHz, $CDCl_3$), δ 6.97 (1H, d, *J* = 7.6 Hz, H-6), 7.76 (1H, d, *J* = 7.6 Hz, H-7), 2.07

TABLE-1
ANTIFUNGAL ACTIVITY OF *Lonicera quinquelocularis* CRUD ETHYL ACETATE FRACTION AND PURIFIED COMPOUND AGAINST *Aspergillus flavus* AND *Aspergillus niger*

	Ethyl acetate fraction (cm)	DMSO (cm)	TER	Inhibition (%)	Purified compound (cm)	Inhibition (%)
Aspergillus flavus	6.9	9	–	22.5	4.0	55.5
Aspergillus niger	5.2	9	–	41.2	3.5	61.0

(3H, s, H-8), 4.15 (2H, q, J = 7.1 Hz, H-1'), 1.29 (3H, t, J = 7.2 Hz, H-2'), 3.99 (3H, s, OCH_3), 5.73 (1H, bs, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 171.2 (C-1), 163.7 (C-5), 158.1 (C-3), 127.9 (C-7), 117.4 (C-4), 108.57 (C-6), 106.8 (C-2), 60.4 (C-1'), 14.2 (C-2'), 11.4 (C-8). HR-EIMS *m/z* 210.2403 $[M+H]^+$ (calcd. for $C_{11}H_{14}O_4$, 210.2367).

On the basis of spectroscopic data the isolated compound was assigned as ethyl-3-hydroxy-5-methoxy-4-methylbenzoate (Fig. 1). It has been estimated that in developed countries such as United States, phytomedicines constitute as much as 25 % of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80 % [18]. The developing countries provide two third phytomedicines and the rural population largely depend on indigenous systems of medicine. In present work the ethyl acetate soluble fraction of *Lonicera quinquelocularis* and the isolated compound have been selected to screen for phytotoxic and antifungal properties.

Herbal remedies are used in the prevention, treatment and cure of disorders and diseases since ancient times [19]. It has been observed that during sowing, handling, storage and distribution, fungal attacks are common, which may be responsible for spoilage and production of mycotoxins, that are most dangerous [20,21]. Therefore, uses of antifungal components are highly recommended. The ethyl acetate soluble fraction of *Lonicera quinquelocularis* inhibited the growth of *Aspergillus flavus* (22.5 %) and *Aspergillus niger* (41.2 %), while the compound ethyl-3-hydroxy-5-methoxy-4-methylbenzoate decreased the growth of *Aspergillus flavus* and *Aspergillus niger* 55.5 and 61 % respectively (Table-1). Similarly, during germination of seed other herbs decreased the actual production. Medicinal plants and their secondary metabolites are important to overcome herbs. *Lonicera quinquelocularis* also act as pesticides that inhibit or decrease the growth of herbs (Fig. 2).

Conclusion

The ethyl acetate soluble fraction of *Lonicera quinquelocularis* and isolated compound ethyl-3-hydroxy-5-methoxy-4-methylbenzoate showed the best inhibition of *Aspergillus flavus Aspergillus niger* and a very good phototoxic activity.

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REFERENCES

1. P.J. Houghton, Z. Boxu and Z. Xisheng, *Phytother. Res.*, **7**, 384 (1993).
2. R. Puupponen-Pimia, L. Nohynek, C. Meier, M. Kahkonen, M. Heinonen, A. Hopia and K.M. Oksman-Caldentey, *J. Appl. Microbiol.*, **90**, 494 (2001).
3. M.P. Kahkonen, A.I. Hopia and M. Heinonen, *J. Agric. Food Chem.*, **49**, 4076 (2001).
4. J.Z. Shi, X.F. Chen and L. Wan, *China J. China Mater. Med.*, **24**, 363 (1999).
5. J. Wang, X.-Z. Zhao, Q. Qi, L. Tao, Q. Zhao, R. Mu, H.-Y. Gu, M. Wang, X. Feng and Q.-L. Guo, *Food Chem. Toxicol.*, **47**, 1716 (2009).
6. H.J. Yoo, H.J. Kang, Y.S. Song, E.H. Park and C.J. Lim, *J. Pharm. Pharmacol.*, **60**, 779 (2008).
7. K. Machida, J. Asano and M. Kikuchi, *Photochemistry*, **39**, 111 (1995).
8. F. Bailleul, A. Leveau and M. Durand, *J. Nat. Prod.*, **44**, 573 (1981).
9. I. Souzu and H. Mitsuhashi, *Tetrahedron Lett.*, **10**, 2725 (1969).
10. I. Souzu and H. Mitsuhashi, *Tetrahedron Lett.*, **11**, 191 (1970).
11. I. Ali, D. Khan, F. Ali, H. Bibi and A. Malik, *J. Chem. Soc. Pak.*, **35**, 139 (2013).
12. S. Kumar, O.P. Sati, V.D. Semwal, M. Nautiyal, S. Sati and Y. Takeda, *Phytochemistry*, **53**, 499 (2000).
13. D. Khan, M. Afzal, S. Woodward and S. Khan, *Rec. Nat. Prod*, **8**, 121 (2014).
14. D. Khan, H.U. Khan, F. Khan, S. Khan, S. Badshah, A.S. Khan and A. Samad, *PLoS ONE*, **9**, e94952 (2014).
15. D. Khan, S. Khan, S. Badshah, H. Ali, H. Ullah, Z. Muhammad and S. Woodward, *J. Nat. Prod.*, **30**, 74 (2016).
16. M.A. Gyamfi, M. Yonamine and Y. Aniya, *Gen. Pharmacol.*, **32**, 661 (1999).
17. J.L. McLaughlin, Proceedings of NIH Workshop, Bioassay for Discovery of Antitumoral, Antiviral Agents from Natural Sources, vol. 18, 22 (1988).
18. P.P. Joy, J. Thomas, S. Mathew and B.P. Skaria, Medicinal Plants, Conser-vation and Livelihoods; In: Tropical Horticulture, Naya Prokash, Calcutta, vol. 2, p. 449-632 (2001).
19. B. Tepe, D. Daferera, A. Sokmen, M. Sokmen and M. Polissiou, *Food Chem.*, **90**, 333 (2005).
20. E. Ernst, *Ann. Intern. Med.*, **136**, 42 (2002).
21. W. Tassaneeyakul, E. Razzazi-Fazeli, S. Porasuphatana and J. Bohm, *Mycopath*, **158**, 239 (2004).