Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Lawsozaheer, a new chromone produced by an endophytic fungus *Paecilomyces variotii* isolated from *Lawsonia Alba* Lam. inhibits the growth of *Staphylococcus aureus*

Zaheer Abbas, Bina Shaheen Siddiqui, Saleem Shahzad, Samia Sattar, Sabira Begum, Anum Batool & Mohammad Iqbal Choudhary

To cite this article: Zaheer Abbas, Bina Shaheen Siddiqui, Saleem Shahzad, Samia Sattar, Sabira Begum, Anum Batool & Mohammad Iqbal Choudhary (2020): Lawsozaheer, a new chromone produced by an endophytic fungus *Paecilomyces variotii* isolated from *Lawsonia Alba* Lam. inhibits the growth of *Staphylococcus aureus*, Natural Product Research, DOI: 10.1080/14786419.2020.1729148

To link to this article: https://doi.org/10.1080/14786419.2020.1729148

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1729148

Lawsozaheer, a new chromone produced by an endophytic fungus *Paecilomyces variotii* isolated from *Lawsonia Alba* Lam. inhibits the growth of *Staphylococcus aureus*

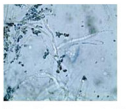
Zaheer Abbas[a], Bina Shaheen Siddiqui[a], Saleem Shahzad[b], Samia Sattar[b], Sabira Begum[c], Anum Batool[a] and Mohammad Iqbal Choudhary[a,c]

[a]HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan; [b]Department of Agriculture & Agribusiness Management, University of Karachi, Karachi, Pakistan; [c]Department of biochemistry, faculty of science, Icing Abdnlaziz university, Jeddah, Saudia Arabia

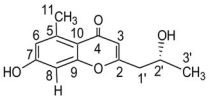
ABSTRACT

One new chromone, lawsozaheer (**1**), and five known compounds 4-(2-hydroxyethyl) phenol (**2**), viriditoxin (**3**), stigmasta-4,6,8(14),22-tetraen-3-one (**4**), β-sitosterol (**5**) and stigmasterol (**6**) were isolated from the fungal broth of *Paecilomyces variotii*. Their structures were elucidated using spectroscopic data. The configuration of **1** was determined by Horeau's method. The broth extract and compound **1** showed highly selective activity against *Staphylococcus aureus* (NCTC 6571) bacterium with 83.19 and 84.26% inhibition respectively at 150 μg/mL, comparing well with that of standard drug ofloxacin (87.013% inhibition at 100 μg/mL). Broth extract also showed 75, and 40% inhibition of *Candida albicans* and *Fusarium lini,* respectively.

Lawsonia inermis Lam



Paecilomyces variotii



Caused 84.26% inhibition of *Staphylococcus aureus*

ARTICLE HISTORY
Received 10 July 2019
Accepted 9 February 2020

KEYWORDS
Lawsonia alba Lam; *Paecilomyces variotii*; antimicrobial activity; *Staphylococcus aureus*; *Candida albicans*

1. Introduction

Lawsonia (family *Lythraceae*) is a monotypic genus characterized by *Lawsonia inermis* Linn. (syn. *L. alba* Lam.). It is known as henna in Arabic, mehndi in Hindi and Urdu. It is a native of North Africa and South-West Asia, and partially cultivated in West Africa (Sastri 1962; Zafar et al. 2006). The natural products isolated from this plant include lawsone (Cox 1938), aromatic compounds (Bhardwaj et al. 1976; Siddiqui et al. 2003; Uddin et al. 2011), saponins, triterpenoids (Khan et al. 1996; Siddiqui et al. 2005) and

CONTACT Bina Shaheen Siddiqui siddiqui_bina@yahoo.com

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1729148.

dioxin derivatives (Siddiqui et al. 2009). Previous investigations revealed that *L. alba* exhibits a wide range of biological activities, such as antibacterial (Habbal et al. 2011), antifungal (Begum et al. 2007), antiviral (Chaudhary et al. 2010), anticancer (Priya et al. 2011; Pradhan et al. 2012; Singh and Luqman 2014), hepatoprotective (Latha et al. 2005) and enzyme inhibition (Uddin et al. 2013) activity.

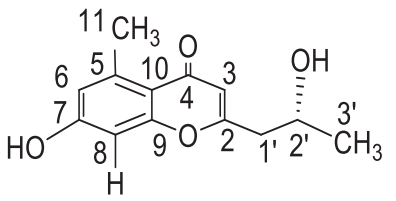
Endophytic microorganisms are fungi or bacteria which animate inside the plant tissues during their life cycle at any moment, without causing damage to their host. Endophytic fungi are significant source of a number of novel bioactive secondary metabolites, possibly valuable as human medicines, possessing antimicrobial, anticancer and several other activities (Amirita et al. 2012; Mousa and Raizada 2013) and could also serve as a prospective source of agrochemical and industrial products (Souza et al. 2011). Previously a few studies on *Lawsonia alba* endophytic secondary metabolites have been undertaken (Sarang et al. 2017) but there is no record on the chemical constituents of secondary metabolites of the fungus *Paecilomyces variotii* from *Lawsonia alba*. Therefore present studies were undertaken on the fungal broth extract from this source. Separation of broth extract using ethyl acetate extraction and various chromatographic techniques led to isolation of one new ((lawsozaheer, **1**) and five known compounds. The broth extract and compound **1** showed pronounced activity against Gram-positive bacterium *Staphylococcus aureus* (NCTC 6571; and moderate activity against two fungi *Candida albicans* and *Fusarium lini*.

2. Results and discussion

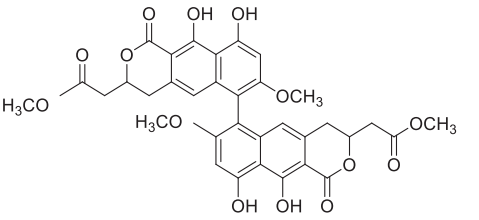
One new chromone, lawsozaheer (**1**) and five known compounds 4-(2-hydroxyethyl) phenol (**2**) (Kimura and Tamura 1973), viriditoxin (**3**) (Silva et al. 2013), stigmasta-4,6,8(14),22-tetraen-3-one (**4**) (Kobayashi et al. 1992), β-sitosterol (**5**) and stigmasterol (**6**) (Kamboj and Saluja 2011) were isolated in the present studies (Figure 1). The broth extract and compound **1** were evaluated for their antimicrobial activity. It may be noted that viriditoxin (**2**) has earlier been shown to possess broad spectrum antibacterial activity against clinically relevant Gram-positive pathogens including methicillin-resistant *S. aureus* (Silva et al. 2013). However this is a new source of its isolation.

2.1. Structure elucidation of lawsozaheer (1)

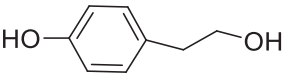
1 was isolated as white powder; $[\alpha]_D^{27}$: − 43.89 (***c,*** 0.09, MeOH). Its IR spectrum (KBr) showed characteristic bands at 3421, 3376 (OH) and 1614, 1662 (sh.), (C = O) cm^{-1} and UV spectrum (MeOH) showed λ_{max} at 243, 248 and 260 nm. Its EI-MS displayed molecular ion peak at *m/z* 234 ((Figure S1) and peak matching of the molecular ion gave exact mass *m/z* 234.0892 corresponding to the molecular formula $C_{13}H_{14}O_4$ (calc. 234.08913) displaying seven unsaturations. A significant peak at *m/z* 190 was due to loss of ethyl alcohol. A benzopyrone ring was deduced from analysis of the ^{1}H- and ^{13}C-NMR data (Supplementary material, Table S1; Figures S2-S7) which showed two *meta* coupled aromatic protons at δ 6.63 (1 H, d, $J = 2.4$, H-6) and 6.65 (1 H, d, $J = 2.4$, H-8) showing connectivity to aromatic carbons at δ_C 118.0 (C-6) and 101.7 (C-8) in the HSQC spectrum (Supplementary material, Figures S6); an olefinic proton singlet at δ



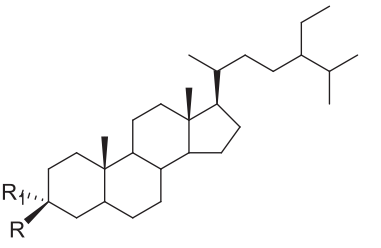
1



3



2



4 R, R^1 = O; $\Delta^{4, 6, 8 (14), 22}$
5 R= OH, R^1 =H; Δ^5
6 R= OH, R^1 =H; $\Delta^{5, 22}$

Figure 1. Structures of compounds **1–6**.

6.05 for H-3 connected to olefinic carbon at δ 112.4 (C-3); a carbonyl carbon at δ_C 182.0 (C-4), a quaternary carbon at δ 167.1 (C-2). The ^{13}C-NMR spectrum further showed four quaternary aromatic carbons at δ_C 163.2 (C-7), 143.6 (C-5), 161.5 (C-9) and 115.7 (C-10). The attached hydroxypropyl chain was recognized from proton signals at δ_H 4.19 (1 H, m, H-2′), 1.27 (3 H, d, $J = 6.0$, H-3′) and two diastereotropic protons at δ_H 2.64 (1 H, dd, $J = 14.4$, 7.8, H-1′a) and 2.70 (1 H, dd, $J = 14.4$, 6.8, H-1′b). Their respective carbons were identified at δ_C 66.3 (C-2′), 23.5 (C-3 ′) and 44.2 (C-1′) in the HSQC spectrum (Supplementary material, Figures S6). Signals for an aromatic CH_3 group were noted at δ_H 2.71 (3 H, s)/δ_C 23.2. The types of carbons were identified from broad band (Figure S3), DEPT 90 (Supplementary material, Figure S4**)** and DEPT 135 (Supplementary material, Figure S5) spectra and the protonated carbons were identified with the help of HSQC spectrum. The placement of various groups was confirmed from HMBC NMR spectrum (Supplementary material, Figure S7). Thus interactions were observed between H-3 (δ 6.05) and C-2 (δ 167.1), C-1′ (δ 44.2) and C-10 (δ 115.7); H-1′b to C-2, H-1′a (δ 2.64) and C-3 (δ 112.4), C-3′ (δ 23.5); H-3′ (δ 1.27) and C-1′ (δ 44.2), C-2′ (δ 66.3); H-11 (δ 2.71) and C-6 (δ 118.0), C-7 (δ 163.2) and C-10 (δ 115.7); H-8 (δ 6.65) and C-9 (δ 161.5) (Supplementary material, Figure S8). The stereochemistry at C-2′ was determined by Horeau's method (Horeau and Kagan 1964; *vide* Supplemental). **1** (4.5 mg) was acylated with racemic phenyl-α-butyric anhydride (20 mg) in dry pyridine. Ice cold water was added to the mixture to decompose excess anhydride and the mixture was extracted with ethyl acetate which was again extracted with 5% sodium bicarbonate. The alkaline layer was acidified with 10% hydrochloric acid and extracted with chloroform. The chloroform layer was worked up in the usual

manner to yield free phenyl-α-butyric acid $[\alpha]_D^{27} = +0.38$ (MeOH, $c = 3.5$) which led to determination of configuration at C-2′ as *R*. In the light of above data, the compound was identified as 2- (2′*R*-hydroxypropyl) −5-methyl-7-hydroxychromone (Figure 1). It is a new 2′*R* isomer of earlier reported 2- (2′*S*-hydroxypropyl) −5-methyl-7-hydroxychromone (Kashiwada et al. 1984).

Compounds **2-6** were identified through comparison of their spectral data with those reported in literature (*loc.cit*).

2.2. Antimicrobial activity

Initial antimicrobial activities were determined by Micro plate Alamar Blue Assay (MABA). Antimicrobial activity of the broth extract was determined against 15 Gram –positive bacteria including multi drug resistant strains *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus fecalis, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Micrococcus luteus, Corynebacterium xerosis, Corynebacterium hoffmanii, Streptococcus pneumonia, Staphylococcus aureus* clinical isolate, *Staphylococcus aureus* 13277, *Staphylococcus aureus* 13143, *Staphylococcus aureus* EMRSA-17, and *Staphylococcus aureus* VRSA 700699; twelve Gram-negative bacteria *Shigella boydii, Salmonella typhi, Salmonella typhi* para A, *Salmonella typhi* para B, *Shigella flexneri, Proteus mirabilus, Proteus vulgaris, Escherichia coli, Klebsiella pneumonia, Shigella dysenteriae, Enterobacter,* and *Pseudomonas aeruginosa* PA0286 and thirteen fungi *Trichopyton rubrum*, *Microsporum canis, Trichopyton mentegrophyte, Microsporum gypsium, Trichopyton tonsurans, Saccharomyces cerevisiae, Candida albicans, Helementho sporain, Aspergillus flavus, Aspergillus niger, Penicillium* sp, *Rhizopus* sp., and *Fusarium* sp.

The broth extract (LA-B-EA) and new compound (**1)** showed highly significant activity against *Staphylococcus aureus* (NCTC 6571) with 83.19 and 84.26% inhibition respectively at 150 μg/mL. The standard drug used was ofloxacin which showed 87.013% inhibition at the concentration of 100 μg/mL (Supplementary material, Table S2). Since compound **1** showed significant activity at 150 μg/mL it was evaluated at further increasing concentrations in order to determine the MIC_{90} *via* MABA which was found to be 225 μg/mL (Supplementary material, Table S3).

Compound **1** did not show any activity against other strains of *S. aureus* although the broth extract (LA-B-EA) showed moderate activity against *S. aureus* clinical isolate (39.76%) and *S. aureus* VRSA 700699 ((37.81%) (Supplementary material, Table S2).

The broth extract showed moderate activity against two fungi *Candida albicans* (75% inhibition) and *Fusarium lini* (40% inhibition) at 400 μg/mL (Supplementary material, Table S4). The activity of the extract and compound **1** against rest of the bacteria and fungi was marginal and not shown in the table.

3. Conclusions

The broth extract (LA-B-EA) and new compound (**1)** showed highly selective activity against *Staphylococcus aureus* (NCTC 6571) bacterium with 83.19 and 84.26% inhibition respectively at 150 μg/mL, comparing well with that of standard drug ofloxacin

(87.013% inhibition at 100 μg/mL). Broth extract also showed 75 and 40% inhibition of *Candida albicans* and *Fusarium lini,* respectively. The discovery provides a direction for further studies to obtain and develop potent antimicrobial agents from endophytic metabolites of *Lawsonia alba* Lam.

Disclosure statement

The authors confirm that this article content has no conflict of interest.

Funding

This work was supported by grants from the Pakistan Academy of Science by the grant # 5-9/PAS/52.

References

Amirita A, Sindhu P, Swetha J, Vasanthi N, Kannan K. 2012. Enumeration of endophytic fungi from medicinal plants and screening of extracellular enzymes. World J Sci Technol. 2:13–19.

Begum J, Yusuf M, Chowdhury JU, Khan S, Anwar MN. 2007. Antifungal activity of forty higher plants against phytopathogenic fungi. Bangla J Microbiol. 24(1):76–78.

Bhardwaj DK, Murari R, Seshadri TR, Singh R. 1976. Lacoumarin from Lawsonia inermis. Phytochemistry. 15(11):1789.

Chaudhary G, Goyal S, Poonia P. 2010. *Lawsonia inermis* Linnaeus: a phytopharmacological review. Int J Pharm Sci Drug Res. 2:91–98.

Cox H. 1938. Hair dyes. I. The chemistry and analysis of henna. Analyst. 63(747):397–404.

Habbal O, Hasson S, El-Hag A, Al-Mahrooqi Z, Al-Hashmi N, Al-Bimani Z, Al-Balushi M, Al-Jabri A. 2011. Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*. Asian Pac J Trop Biomed. 1(3):173–176.

Horeau A, Kagan H. B. 1964. [Determination of configurations by partial resolution. 3. Steroidal alcohols]. Tetrahedron. 20(11):2431–2441.

Kamboj A, Saluja AK. 2011. Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). Int J Pharm Sci. 3:94–96.

Kashiwada Y, Nonaka G.I, Nishioka I. 1984. Studies on rhubarb (*Rhei Rhizoma*). V. Isolation and characterization of chromone and chromanone derivatives. Chem Pharm Bull. 32 (9): 3493–3500.

Khan MAA, Jain D, Bhakuni R, Zaim M, Thakur R. 1991. Occurrence of some antiviral sterols in *Artemisia annua*. Plant Sci. 75:161–165.

Khan MAA, Singh N, Dhawan K. 1996. Occurrence and identification of a new antiviral saponin from *Lawsonia alba* Lam fruits. Natl Acad Sci Lett India. 19:145–148.

Kimura Y, Tamura S. 1973. Isolation of l-β-phenyllactic acid and tyrosol as plant growth regulators from *Gloeosporium laeticolor*. Agr Biol Chem. 37(12):2925–2925.

Kobayashi M, Krishna M.M, Ishida K, Anjaneyulu V. 1992. Marine sterols. XXII.2. Occurrence of 3-oxo-4, 6, 8(14)-triunsaturated steroids in the sponge *Dysidea herbacea*. Chem Pharm Bull. 40 (1):72–79.

Latha P. G, Suja S, R, Shyamal S, Rajasekharan S. 2005. Some hepatoprotective garden plants. Nat Prod Radiance. 4(4):278–279.

Mousa WK, Raizada MN. 2013. The diversity of anti-microbial secondary metabolites produced by fungal endophytes: an interdisciplinary perspective. Front Microbiol. 4:65.

Pradhan R, Dandawate P, Vyas A, Padhye S, Biersack B, Schobert R, Ahmad A, H Sarkar F. 2012. From body art to anticancer activities: perspectives on medicinal properties of henna. CDT. 13(14):1777–1798.

Priya R, Ilavenil S, Kaleeswaran B, Srigopalram S, Ravikumar S. 2011. Effect of *Lawsonia inermis* on tumor expression induced by Dalton's lymphoma ascites in Swiss albino mice. Saudi J Biol Sci. 18(4):353–359.

Sarang H, Rajani P, Vasanthakumari MM, Kumara PM, Siva R, Ravikanth G, Shaanker RU. 2017. An endophytic fungus, *Gibberella moniliformis* from *Lawsonia inermis* L. produces lawsone, an orange-red pigment. Antonie Van Leeuwenhoek. 110(7):853–862.

Sastri B. 1962. The Wealth of India, vol. 6. New Delhi: Council of Scientific and Industrial Research, p. 382–383.

Siddiqui BS, Kardar MN, Ali ST, Khan S. 2003. Two new and a known compound from Lawsonia inermis. HCA. 86(6):2164–2169.

Siddiqui BS, Kardar MN, Khan S. 2005. Two new triterpenoids from *Lawsonia alba*. Z Naturforsch B. 60(1):37–40.

Siddiqui BS, Uddin N, Begum S. 2009. Two new dioxin derivatives from the aerial parts of Lawsonia alba. Nat Prod Res. 23(18):1740–1745.

Silva MO, Kawai K, Hosoe T, Takaki GC, Gusmão NB, Fukushima K. 2013. Viriditoxin, an antibacterial substance produced by mangrove endophytic fungus Paecilomyces variotii. Microbial pathogens and strategies for combating them: Microscopy: science, technology, applications and education. [A Méndez-Vilas; J Díaz;] Publisher: Badajoz: Formatex Research Center, 2010, pp. 1406–1411.

Singh DK, Luqman S. 2014. *Lawsonia inermis* (L.): A perspective on anticancer potential of Mehndi/Henna. Biomed Res Ther. 1(4):112–120.

Souza JJd, Vieira IJC, Rodrigues-Filho E, Braz-Filho R. 2011. Terpenoids from endophytic fungi. Molecules. 16(12):10604–10618.

Uddin N, Siddiqui BS, Begum S, Ali MI, Marasini BP, Khan A, Choudhary MI. 2013. Bioassay-guided isolation of urease and α-chymotrypsin inhibitory constituents from the stems of *Lawsonia alba* Lam.(Henna). Fitoterapia. 84:202–207.

Uddin N, Siddiqui BS, Begum S, Bhatti HA, Khan A, Parveen S, Choudhary MI. 2011. Bioactive flavonoids from the leaves of *Lawsonia alba* (Henna). Phytochem Lett. 4(4):454–458.

Zafar S, Ahmad S, Jameel S. 2006. Lawsonia inermis Linn.a review. In Medicinal plants: traditional knowledge. New Delhi: IK International Publishing House Pvt Ltd, p. 34.