Data Article

Data on the inhibitory effect of endophytic fungi of traditional medicinal plants against pancreatic lipase (PL)

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Abstract

This article describes isolation and pancreatic lipase (PL) inhibitory potential of 18 endophytic fungi isolated from the various parts of six indigenous medicinal plants. PL catalyzes absorption and hydrolysis of triglycerides into di-glycerides into mono-glycerides and free fatty acids. PL inhibitors are well-known for the disruption of pancreatic lipase activity. The quest for novel pancreatic lipase inhibitors is crucially important owing to their therapeutic potential in the treatment of obesity and related chronic diseases. The present dataset provides information about the presence of endophytic fungi in the internal tissues of selected plants and the PL inhibitory potential of their metabolites using bioassay based screening. Absence of the yellow zone surrounding the standard Orlistat and test extract indicated PL inhibition due to the cumulative effect of metabolites present in the extract. The data suggests that TLC bio-autographic method is simple, rapid and reproducible and therefore it could be effectively used for high throughput screening of PL inhibitors from natural sources.

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https://doi.org/10.1016/j.dib.2019.104797
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1. Data

The data summarized in Table 1 lists the percent inhibition of PL by inhibitors present in crude extract of endophytic fungi isolated from their host plant. A total of eighteen fungal endophytes were isolated from the different parts of the selected plants. Data shown in Fig. 1 represents inhibition of PL by extracts of endophytes using phenol red olive oil agar method. Complete absence of yellow halo surrounding standard, Orlistat well and extract of CLL-2 showed complete inhibition of pancreatic lipase by the metabolites present in the test extract. However, partial inhibition was seen in the well containing the extract CLL-1. Tube assay in Fig. 2 showed a dose dependent PL inhibition by the selected extract. Color intensity in the tube 2–6 in (Fig. 2) changed from pink to red as function of inhibition of PL. Data shown in Fig. 3 represents TLC bio-autography based screening of fungal extract for PL inhibition. In order to confirm the effectiveness of enzyme assay, extract of endophytes was screened for pancreatic lipase inhibition by modifying TLC bio-autographic method. This modified method is rapid and a large number of samples can be screened in a single TLC plate in a shorter duration [1,2]. Change in the colour of the plate from red to orange yellow indicated PL activity in the control while absence of yellow halo indicates PL inhibition (Fig. 3).

2. Experimental design, materials and methods

2.1. Microorganisms and preparation of extract

Endophytic fungi were isolated from various tissues of the selected plants as per standard isolation method [3]. After isolation, pure culture of each isolate was maintained on sterile potato dextrose agar (PDA) medium as a stock culture at 4 °C.

The isolated endophytic fungi were grown on agar plugs [4]. After visible growth, the agar plugs were crushed and metabolite extraction was carried out using ethyl acetate. All the crude extracts of each isolated fungi were screened out for qualitative inhibition of pancreatic lipase by various methods.

2.2. PL inhibition assay: chromogenic olive oil plate method

The ethyl acetate extract of the test endophytes was screened for pancreatic lipase inhibitory activity by chromogenic olive oil plate method. This method is based on change in the color of media due...
| Sr. No. | Host plant       | Tissue | Isolated endophytic fungi | Lipase inhibition | % inhibition |
|--------|------------------|--------|---------------------------|-------------------|--------------|
| 1      | *Citrus lemon*   | Leaves | CLL1                       | +++               | 100          |
| 2      | Leaves          | CLL2   | +                         | 50                |
| 3      | Leaves          | CLL3   | +                         | 50                |
| 4      | Bark            | CLB1   | +                         | 50                |
| 5      | Bark            | CLB2   | -                         | Nil               |
| 6      | *Nerium oleander* | Leaves  | NOL1                      | +                 | 40           |
| 7      | Bark            | NOB1   | -                         | Nil               |
| 8      | *Withania somnifera* | Leaves  | WSL1                      | -                 | Nil          |
| 9      | Leaves          | WSL2   | -                         | Nil               |
| 10     | Bark            | WSB1   | +++                       | 100               |
| 11     | Bark            | WSB2   | -                         | Nil               |
| 12     | *Aloe vera*     | Leaves | AVL1                      | +                 | 40           |
| 13     | Bark            | AVB1   | -                         | Nil               |
| 14     | Bark            | AVB2   | -                         | Nil               |
| 15     | *Punica granatum* | Leaves  | PGL1                      | -                 | Nil          |
| 16     | Bark            | PGB1   | -                         | Nil               |
| 17     | *Catharanthus roseus* | Leaves  | CRL1                      | -                 | Nil          |
| 18     | Bark            | CRB1   | ++                        | 60                |
| 19     | *Orlistat standard* | (120 mg/ml) |                         | +++               | 100          |

+++: Complete inhibition, ++: Moderate inhibition, +: Low inhibition, -: No inhibition. The values are average of triplicate samples (n = 3).
The isolates designated with bold font show 100% inhibition of the PL. It for the purpose of readers' attention only.

**Fig. 1.** Olive oil plate method for screening of endophytes, C: control; T1: test extract of CLL1; T2: test extract of CLL2; S: Standard Orlistat.

**Fig. 2.** Lipase assay in tube: 1: control, 2–6: crude extract of endophyte
to acid production as result of enzyme activity [5]. Plates of Olive oil agar were prepared and wells were punched. Then, 100 µL of preincubated master-mix of porcine pancreatic lipase (PPL, 50 µL) and extract (50 µL) of each endophyte was loaded in a well. The control well comprised of PPL preincubated with distilled water instead of culture extract. Orlistat (120 mg/mL) were preincubated with PPL and used as standard inhibitor of PPL. All plates were incubated at 37 °C for 24 hr.

2.3. Lipase inhibition assay using tube method

PPL enzyme (100 µL, 10 mg/mL) in phosphate buffer (pH 7.4) was preincubated with same volume of the test extract and the Orlistat standard (1–10 mg/mL) separately. Distilled water instead of test extract or Orlistat was used as control. All tubes were incubated at 37 °C for 20 min. After incubation, 100 µL of substrate prepared in distilled water (pH 7.4) was added in each tube and again incubated at 37 °C for 20 min. After incubation, phenol red indicator (10 µL) was added to each tube and the tubes were observed for the change in color. Assay was performed in triplicates.

2.4. TLC bio-autography

The aluminum coated TLC (silica gel F_{254} 25 × 25 cm) sheets were used for TLC bio-autography. The data was obtained by spotting test extracts and standard PL inhibitor, Orlistat (100, 50 and 10 µg/mL) onto a separate TLC plate (2.5 × 10 cm). Ethyl acetate was spotted on another TLC sheet and used as control plate. After complete drying of spots, Whatmann filter paper was impregnated with PPL solution (10 mg/mL) in phosphate buffer (pH 7.4) was overlaid on the all spotted plates. All the plates were incubated at 37 °C for 15 min for enzyme-inhibitor interactions. Substrate agar solution with few drops of phenol red indicator was poured on the all plates after the filter paper was peeled off [6,7]. Thereafter, all the plates were incubated at 37 °C for 20 min for enzyme substrate reactions.

2.5. Data analysis

Data reveals formation of yellow halo due to the enzyme (PL) activity; whereas absence of yellow halo surrounding the standard and test extract of endophytic fungus showed complete inhibition of PL by the metabolites present in the test extract. A dose dependent inhibition of PL was observed in tube assay. TLC bio-autographic data confirmed the presence of PL inhibitory metabolites in the test extract.
Acknowledgement

The financial assistance provided by Indian Council of Medical Research, New Delhi (File No.45/12/2018/TRM/BMS) in the form of RA fellowship to MPP is gratefully acknowledged. RHP is thankful to the management of RCPASC College, Shirpur for providing laboratory facilities.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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