Indian Journal of Experimental Biology
Vol. 60, October 2022, pp. 781-788
DOI: 10.56042/ijeb.v60i10.35574

Enhancement of mangiferin by fungal endophytes isolated from *Salacia chinensis* L. and *Salacia oblonga* Wall.

Jasmeet Kaur[1], Minakshi Verma[2], Bibhu Prasad Panda[1] & Vidhu Aeri[2]*

[1]Microbial and Pharmaceutical Biotechnology Laboratory, [2]Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi-110 062, India

Received 19 May 2020; *revised 05 September 2022*

Microbes residing in the internal tissues of a plant are called endophytes, and are known for producing phytochemicals such as taxol, podophyllotoxin, azadirachtin and vinca alkaloids. In this study, out of five isolates from *Salacia* species, two fungi *Penicillium capsulatum* and *Aspergillus fumigatus* have been evaluated and confirmed by polymerase chain reaction (PCR) amplification for their endophytic action to produce mangiferin. Mangiferin has been reported to possess protective properties, including antioxidant, antidiabetic and immunomodulatory. It has been reported that the content of mangiferin is 7-9% in *Mangifera indica*, and is also present in other plants like *Swertia chirata*, *Salacia chinensis*, and *Hypericum aucheri*. Therefore, an attempt was made to explore the biotechnological approach and regulation studies to increase the production of mangiferin in *S. chinensis* and *S. oblonga*. Endophytes were isolated, screened, and analyzed, to evaluate the mangiferin in fungal extracts in comparison with crude plant extracts. An HPLC analysis was used to determine the mangiferin content present in the fungal extract of *S. chinensis* stem (74.74 g/mL), followed by fungi extracts of *S. oblonga* root (33.75 g/mL) and *S. chinensis* root (30.50 g/mL), compared with the plant extracts. These results were confirmed by FTIR analyses.

Keywords: Antidiabetic, Antioxidant, *Aspergillus fumigatus*, *Chinese salacia*, Immunomodulatory, *Penicillium capsulatum*, Saptachakra

Endophytic fungi offer a prolific source of novel bioactive metabolites that have the potential to serve as a lead molecule for the pharmacological sector in the development of new drugs[1,2]. Examples of some well known phytochemicals produced by endophytes are taxol, podophyllotoxin, azadirachtin, vinca alkaloids, and many others. Mangiferin has gained more importance due to its consideration as a miracle herbal compound. It is a natural bioactive compound useful against lifestyle-related disorders.

Mangiferin is a natural polyphenolic ingredient present in different parts of mango such as leaves, bark, peels and kernel with beneficial health effects. The reported benefits of mangiferin range from antiaging, antioxidant, antiviral, anticancer, antioxidant, immunomodulatory, hepatoprotective, antidiabetic, antioxidant, and cardiovascular effects to enhanced cognitive brain function[3]. Mangiferin can reduce Ca^{2+} concentration, and thus protect T-cells against activation of induced apoptosis[4]. Surprisingly, Suya du *et al.*[5] demonstrated that mangiferin showed a significant reduction in biochemical and toxicological parameters, as well as improvement in hematological parameters observed in diabetic rats. Also, mangiferin showed a marked increase in insulin sensitivity and B cell function, further improving the metabolic parameters. Mangiferin has been reported to inhibit the expression of nitric oxide synthase and tumor necrosis factor. It has been reported to demonstrate a pro-hypoglycemic role by maintaining glucose metabolism and insulin resistance. Since mangiferin has been suggested to protect kidneys from diabetic nephropathy[6], the most common and serious diabetic complication, it can be widely used for the protection and treatment of chronic disorders. Mangiferin has been reported to play a key role in the treatment of diabetes by inhibiting enzyme sucrose, maltase, and isomaltase, which are responsible for the conversion of carbohydrates into simple sugars[7]. Antidiabetic activity of mangiferin has been shown to mitigate hyperglycemia and insulin resistance according to other experimental studies[8].

Salacia species (*Celastraceae*) is an important source of active phytochemicals viz., mangiferin,

*Correspondence:
Phone: +91 9873496942 (Mob.)
E-Mail: vaeri@jamiahamdard.ac.in (VA);
jasmeet.kaur94@hotmail.com (JK)

salacinol and kotalanol responsible for antidiabetic activity; and also phenols and flavonoids which are responsible for the management of free radicals that play an important role in the pathogenesis of disease[9]. The presence of several activities including antidiabetic, antimicrobial, antioxidant, and antihyperlipidemic in crude extracts of *Salacia* species has been reported earlier[10]. High levels of free radicals result in damaging the cellular proteins, membrane lipids, and nucleic acids and eventually lead to cell death. Thus, antioxidants with the ability to quench free radicals can help in subsidizing the cellular damage thereby eliminating the symptoms of the disease. Mangiferin has been considered a super antioxidant due to its iron-chelating properties, thus providing cellular protection by regulating polymer chain initiation and maintaining cellular oxidation-antioxidant balance. Duarte *et al.*[11] identified 20 different compounds, *viz*. triterpenes (3β-stearyloxy-oleanane, 3β-stearyloxy-ursane, seco-friedelane), xanthone, polyols, carboxylic acid, aromatic ester in *S. elliptica.* Similarly, Wang *et al.*[12] have reported the presence of triterpenes, including quinone methides, friedelanes, phenolics, polyol, and chromanone in *S. amplifolia.*

The species have been classified as endangered due to excessive and indiscriminate collection of supplements for global demand, being a natural potent antidiabetic drug. Also, the rare occurrence of active metabolites and difficulty in obtaining the plant led to exploring the biotechnological approach and regulation studies to increase the production of secondary metabolites. Keeping this in view, here, we isolated, screened and analyzed selected endophytes present in the two *Salacia* species (*Salacia chinensis* L. and *S. oblongata* Wall.) for investigating the mangiferin content in their fungal extracts in comparison to crude plant extracts. These isolates were subjected to characterization by ITS-PCR using DNA samples, and further subjected to sequencing for identification and determination of the nature of components responsible for the activity and phylogenetic linkage mapping.

Materials and Methods

Plant material and chemicals

Fresh roots of *Salacia oblonga* Wall. were procured from AIMIL Pharmaceuticals, New Delhi. The roots and stems of *Salacia chinensis* Linn. were collected in December from Cuddalore, Nagapattinam District, Tamil Nadu. The collected plant material was authenticated by Dr. Sunita Garg, at the Raw Materials, Herbarium & Museum, Delhi (RHMD), CSIR-National Institute of Science Communication and Policy Research (NIScPR), New Delhi. The standard mangiferin was procured from Sigma Aldrich (USA) having a percentage purity of ≥98%. HPLC grade acetonitrile and Orthophosphoric acid were obtained from Merck Ltd., Mumbai, India. All other chemicals used were of analytical grade.

Preparation of plant extract

The authenticated crude drug powder (30 g) was soaked in 500 mL of ethyl acetate (Merck Ltd., Mumbai, India) and extracted by continuous hot percolation method using Soxhlet apparatus at 60°C for 72 h. The extract was filtered and concentrated up to 25 mL using a Rota evaporator[13]. Since no waxy layer was found on the roots and stems of the plant material before grinding into a coarse powder, therefore, direct extraction was performed with ethyl acetate to extract both polar and non-polar components from the plant powder.

Isolation of endophytic fungi

The roots and stems were thoroughly washed with sterilized distilled water followed by surface sterilization in 70% ethanol for 2-3 min. They were then again rinsed with distilled water followed by 4% sodium hypochlorite for 3 min. The aseptically cut interior plant segments were placed on potato dextrose agar (PDA) supplemented with streptomycin. The plants were incubated at 27°C for 7 days or till colony formation occurs. Fungal mycelia originating from the plant were transferred to PDA media to obtain a pure culture of endophytic fungus. These were maintained on PDA slants for future experimental use[2].

Preparation of fungal extracts

The isolated fungus was cultured by inoculating in 10 Erlenmeyer flasks containing 50 mL of potato dextrose broth and incubated at 27°C for 10 days. After 10 days each fungal species was used for extraction. The mycelia were removed from PDB, dried, and to 1.0 g of cell mass, an approximate 10 mL volume of solvent (ethyl acetate) was added to extract intracellular secondary metabolites. For extraction of secondary metabolites from metabolic liquid, the solvent was added at the ratio of 2:1(v/v) to the liquid. Both the mixtures were subjected to agitation at 120 rpm for 2 h. Further, the organic layer was evaporated to dryness and the concentrated extract was used for further analysis[14].

Genomic DNA extraction

The genomic DNA was isolated using the CTAB method. The mycelia were grounded to a fine powder using liquid nitrogen. The lysis of cells was carried out using a CTAB lysis buffer (10% CTAB, 5 M NaCl, 5 mM EDTA, 1 M Tris- HCl, 0.3 gm PVP, 20 µL β-mercaptoethanol, 20 µL proteinase K). The solution was mixed properly and was kept in a shaker incubator at 65°C for 30 min. After incubation, the extraction was carried out by adding an equal volume of chloroform-isoamyl alcohol (24:1) and was subjected to centrifugation. The aqueous layer was collected and the genomic DNA was allowed to precipitate by the addition of chilled isopropanol followed by centrifugation. The DNA was further washed with 70% ethanol and stored for further analysis[14].

Identification of endophyte

The endophytic fungus showing maximum growth was identified based on macroscopic and microscopic characteristics such as shape, the appearance of colonies, and color. The molecular identity of all the isolates was confirmed by isolating the DNA from the cultures and DNA content was estimated by using NANODROP, 2000C spectrophotometer. The DNA was used in PCR to amplify the ITS region using ITS 1 and ITS 4 primers described in the literature[14]. The conditions used for PCR were as follows: initial denaturation was carried out at 94°C for 3 min followed by annealing of primers at 55°C and extension at 72°C. The reaction was carried out for 30 cycles. The sequencing reaction was carried out using forward and reverse primers. The 400-900 bp amplicon was gel eluted and the product was sequenced by Sanger's method of DNA sequencing. The sequencing results were assembled and compared with the NCBI data base[15].

Preparation of standard mangiferin solution

One mg/mL stock solution of the mangiferin powder using HPLC grade methanol (Merck Ltd., Mumbai, India) was made. Furthermore, dilution of stock solution was made to obtain 20, 40, 60, 80 and 100 µL concentration of standard.

HPLC analysis of plant and fungal extracts

To verify the mangiferin production, the extracts were subjected to TLC using pre-coated silica gel plates. TLC was used as the primary step to verify and confirm the elution of mangiferin from all the extracts before performing HPLC as it is simple and cost-effective. The standard mangiferin was dissolved in methanol. Toluene-ethyl acetate-formic acid-methanol (6:3:0.8:0.2 v/v/v/v) was used as elution system. The plates were developed in a saturated chamber.

All extracts were quantified using high-performance liquid chromatography[16]. HPLC system (Shimadzu LC 10AT) equipped with a PDA detector was used for the quantification of marker compounds in the extracts. The C-18 column 250 × 4.6 mm dimension was used for analysis. A mixture of acetonitrile (15%, HPLC grade) and 0.1% orthophosphoric acid (85%, HPLC grade) at a flow rate of 1.0 mL/min was used as the binary gradient mobile phase for the analysis. The separation was done at ambient temperature. Solvents were filtered through a 0.45 µm nylon membrane (Millipore, Billerica, MA, USA) before use.

A calibration graph of marker compounds was plotted by injecting 20,40,60,80 and 100 µL of standard methanol at a detector wavelength of 257 nm. The samples were delivered via a 20 µL injection loop using a 25 µL capacity Hamilton microlitre syringe. A run time of 10 min was given for each run. The HPLC was stabilized for about half an hour before the injection.

FTIR analysis

Further characterization of fungal extracts for mangiferin was done with FTIR spectroscopy using a KBr press. The pellets were prepared using pure KBr and analyzed by fixing them in a sample holder. The FTIR spectrum was recorded in the region of 4000-450 cm^{-1}.

Results

Isolation of fungal endophytes

A total of five endophytic fungal cultures were isolated from selected *Salacia* species, viz; two from *S. chinensis* stem, two from *S. oblonga* root, and one from *S. chinensis* root. Few cultures were identified on the morphological basis which belongs to the genera *Aspergillus.* The few cultures were found to be non-sporulating and could not be identified on a morphological basis. Proper growth was not observed in a few cultures while subculturing in PDB, hence these fungal cultures were excluded and other samples were processed.

Molecular characterization of fungal endophytes

Since macroscopical and microscopical identification was not sufficient to identify the cultures, therefore,

molecular characterization was carried out. The concentration of isolated DNA are reported in Table 1. Out of five isolates, the two endophytic fungi isolated from *S. chinensis* roots and stems were sequenced using Sanger's method, and the sequence was identified as *Aspergillus fumigatus* and *Penicillium capsulatum* by sequence analysis of the ITS region of the rDNA gene (Fig. 1). The amplification of the ITS region was carried out using universal eukaryotic primers of ITS1 and ITS4. A phylogenetic tree was constructed confirming both fungi (Fig. 2). All the isolates showed a homology of greater than or equal to 98%.

Table 1 — Quantification of DNA using NANODROP

Sample type	Nucleic acid conc.	Unit	A260	A280	260/280
DNA	4612.5	ng/µL	92.249	55.205	1.67
DNA	139.0	ng/µL	2.781	2.326	1.20
DNA	10465.3	ng/µL	209.307	109.820	1.91
DNA	719.0	ng/µL	14.391	11.428	1.26
DNA	2430.9	ng/µL	48.618	29.580	1.64

Estimation of mangiferin content by HPLC

The quantitative analysis of mangiferin using HPLC has shown the presence of the active molecule in all the samples. The amount of mangiferin in all the samples was calculated using a standard plot of mangiferin (Fig. 3). The mangiferin content in the *Salacia* species was influenced by fungal endophytes. Among the five isolates, endophytic fungi isolated from the *S. chinensis* stem reported the highest increment in mangiferin content. The concentration was determined by comparing the HPLC profile of

A

```
1 gcctgaaagc gcccgccgaa gcaacaagta catagacacg tggaggttga cccagagggc
61 cctcactcgg taatgtcata ggtgaactgc ggaaggatca ttaccgagtg agggccctct
121 gggtccaacc tcccacccgt gtctatcgta ccttgttgct tcggcgggcc cgccgtttcg
181 acggccgccg gggaggcctt gcgcccccgg gcccgcgccc gccgaagacc ccaacatgaa
241 cgctgttctg aaagtatgca gtctgagttg attatcgtaa tcagttaaaa ctttcaacaa
301 cggatctctt ggttccggca tcgatgaaga acgcagcgaa atgcgataag taatgtgaat
361 tgcagaattc agtgaatcat cgagtctttg aacgcacatt gcgccccctg gtattccggg
421 gggcatgcct gtccgagcgt cattgctgcc ctcaagcacg gcttgtgtgt tgggcccccg
481 tcccctctc ccgggggacg ggcccgaaag gcagcggcgg caccgcgtcc ggtcctcgag
541 cgtatggggc tttgtcacct gctctgtagg cccggccggc gccagccgac acccaacttt
601 atttttctaa ggttgacctc ggatcaggta gggatacccg ctgaacttaa gcatatcaat
661 aaagcggaag aacg
```

B

```
1 gcaacaagta catagacacg cggaggtgga cccggagctc cgcactcgtt aatgatatct
61 ctcaaggacg atgatttccg aaaggggtcc tcccggccca acctcccacc cgtctctatc
121 gtaccttgtt gcttcggcgg gcccgcccac cgtggccgcc ggggggcgtc cgccccgcgg
181 cccgtgcccg ccgaggaccc ctgggaactc gtgtgtgaag agtcacgtct gagcgagaag
241 cgaaatcagt caaaactttc aacaacggat ctcttggttc cggcatcgat gaagaacgca
301 gcgaaatgcg ataagtaatg tgaattgcag aattcagtga atcatcgagt ctttgaacgc
361 acattgcgcc ccctggcatt ccgggggggca tgcctgtccg agcgtcattg ctgccctcaa
421 ccggcttg tgtgttgggc cgccgtcccc ccgcctgccc ggggggacgg gcccgaaagg
481 gcggcggc accgcgtccg gtcctcgagc gtatggggg
```

Fig. 1 — Sequence of (A) *Aspergillus fumigatus*; and (B) *Penicillium capsulatum*

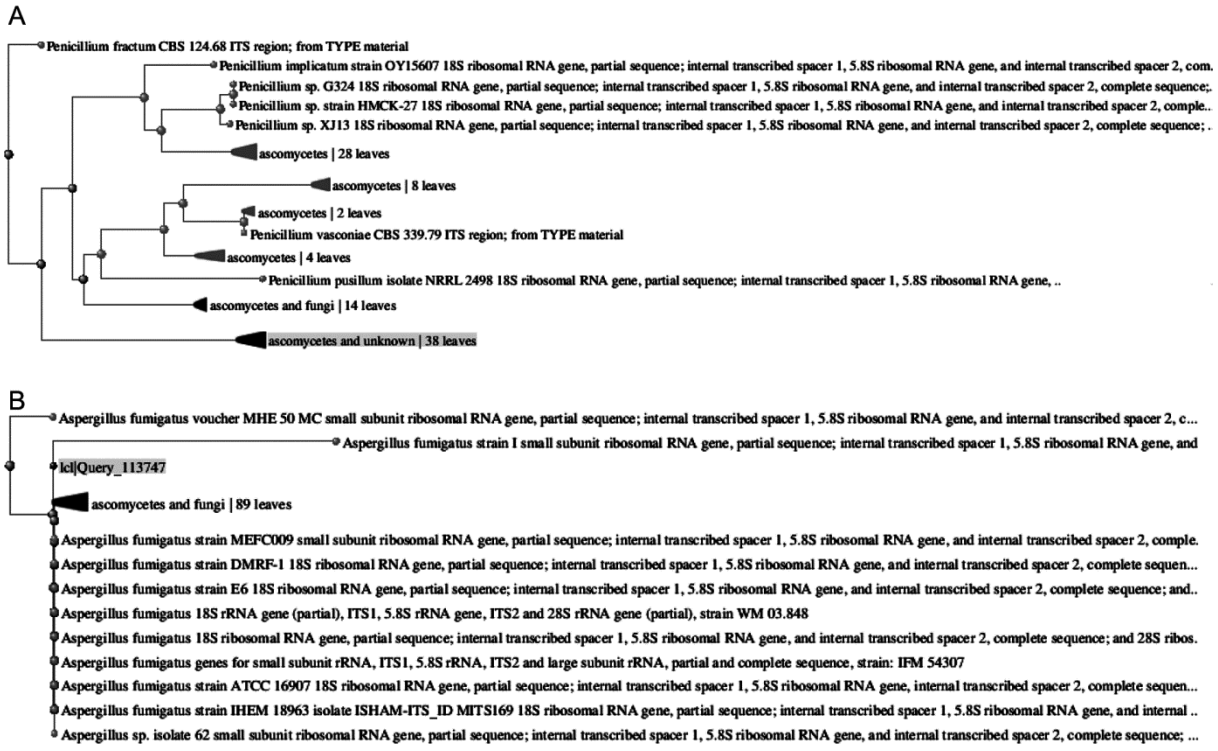

Fig. 2 — Phylogenetic relationship inferred by analysis of ITS sequence: (A) *Aspergillus fumigatus*; and (B) *Penicillium capsulatum*

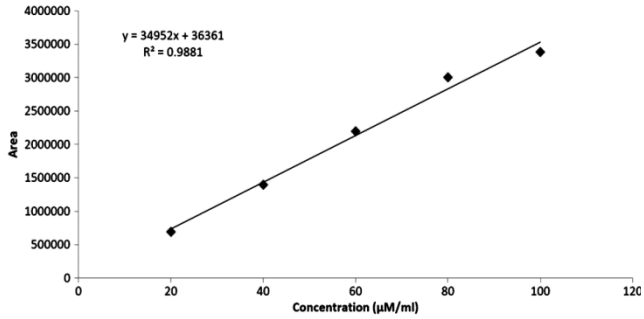

Fig. 3 — Standard plot of mangiferin

plant and fungal extracts. Each fungal and plant extract was analyzed for the presence of different phytochemicals. The solvent system comprising a mixture of acetonitrile (15%) and 0.1% ortho-phosphoric acid (85%) provided good resolution for the separation of all marker compounds from the conventional soxhlet and fungal extracts of *Salacia* species. The variation in the retention time of peaks in the chromatogram may be due to the presence of other chemical constituents (Fig. 4).

The plant extract of *Salacia chinensis* stem was found to contain the highest amount (26.89 µg/mL) of mangiferin followed by *S. chinensis* root (10.42 µg/mL) and *S. oblonga* root (4.809 µg/mL). The use of the biotechnological approach led to an increased amount of mangiferin in fungal extracts of the plant. The highest amount of mangiferin (74.74 µg/mL) was found in the fungal extract of *S. chinensis* stem followed by fungal extracts of *S. oblonga* root (33.75 µg/mL) and *S. chinensis* root (30.50 µg/mL), respectively (Table 2).

Mangiferin confirmation using FTIR

The UV spectrum and FTIR analysis (Fig. 5) were also similar to the standard mangiferin confirming the mangiferin production. The IR spectra of pure mangiferin were verified with the help of the literature and it was found that the absorption peaks of fungal extracts were similar to absorption peaks of standard mangiferin indicating the presence of mangiferin in the fungal extracts. The complex composition of fungal extracts results in differences in relative intensities and band positions.

Discussion

Mangiferin is a potential bioactive compound reported to treat many lifestyle associated disorders. Mangiferin being the active molecule responsible for the antidiabetic activity of *Salacia* members, the

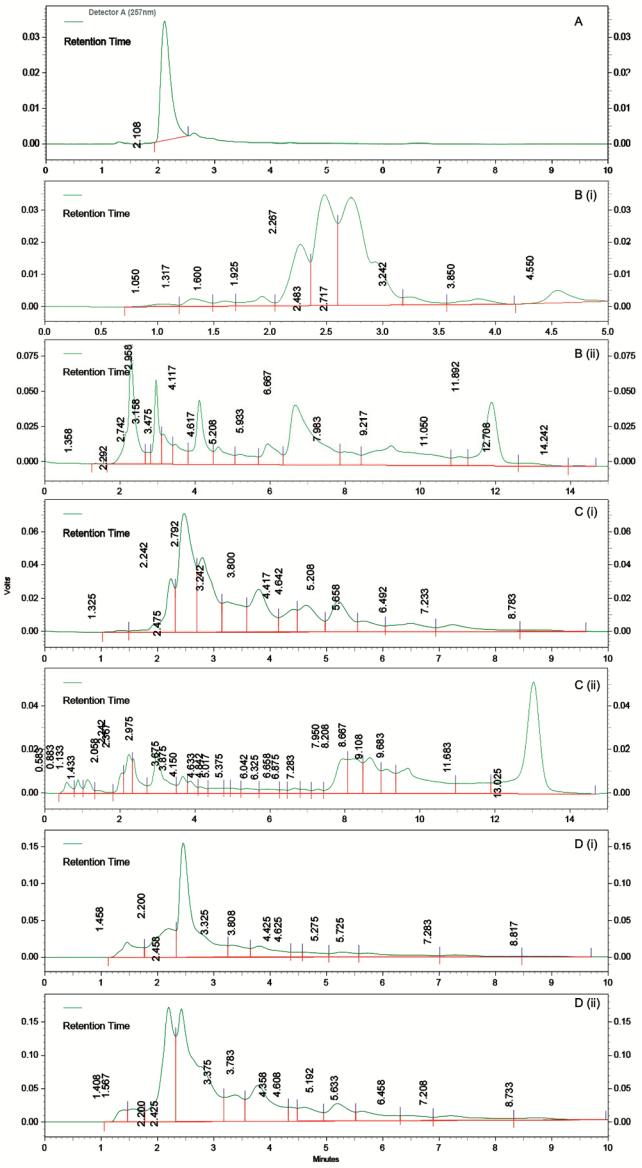

Fig. 4 — HPLC Chromatogram of (A) Standard Mangiferin; and (B-D) plant and fungal extract of *Salacia oblonga* root, *S. chinensis* root and *S. chinensis* stem.

Table 2 — Total mangiferin Content in extracts

Sample ID	Retention time	Mangiferin conc. (µg/mL)
Salacia oblonga root (plant extract)	2.267	4.809
S. oblonga root (fungal extract)	2.292	33.75
S. chinensis root (plant extract)	2.242	10.42
S. chinensis root (fungal extract)	2.275	30.50
S. chinensis stem (plant extract)	2.200	26.89
S. chinensis stem (fungal extract)	2.200	74.74

quantitative analysis of mangiferin provides an economical significance to the members of this genus[17-23]. A low yield of mangiferin from plant

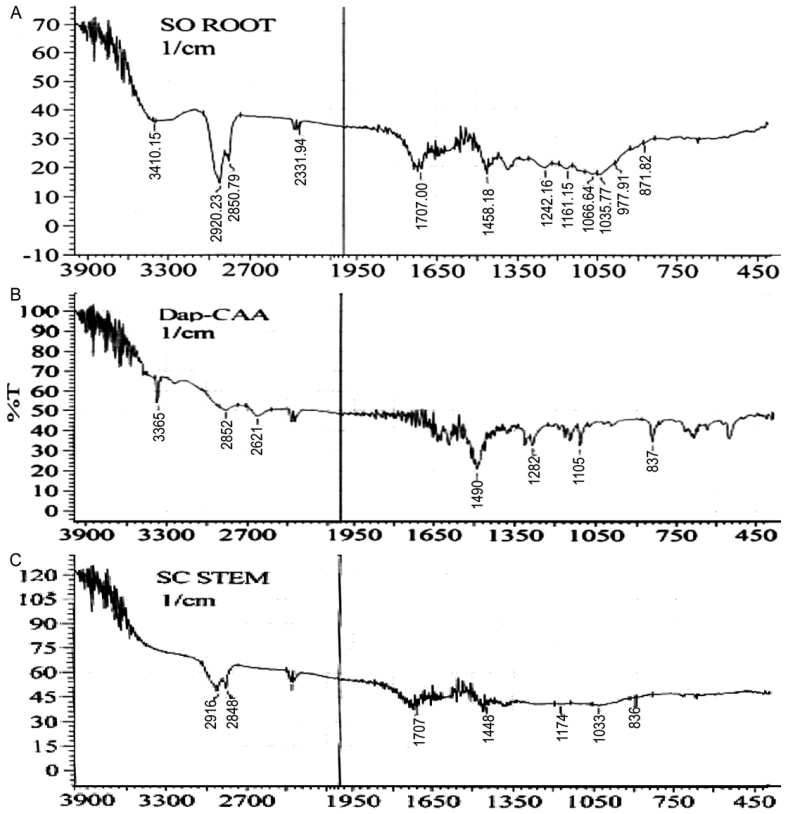

Fig. 5 — FTIR analyses of (A) *Salacia oblonga* root; (B) *Salacia chinensis* root; and (C) *Salacia chinensis* stem

extracts and difficulty in obtaining the plant led to exploring the biotechnological approach and regulation studies to increase the production of secondary metabolites. According to some researchers, the Nam Doc Mai methanolic extract contains 2.80 g of mangiferin per 100 g of extract, followed by the Keow Savoey variety at 2.40 g per 100 g, and the Gaew variety at 1.30 g per 100 g[24,25].

The quantitative analysis of mangiferin using the HPLC method has shown the presence of this molecule in all the samples. Among these, the fungal extracts of roots of *Salacia oblonga* and fungal extracts of *Salacia chinensis* (root and stems) showed the maximum mangiferin content as compared to the plant extracts of the selected *Salacia* species. This proves that endophytes are capable of producing active compounds that plant produces. Although mangiferin has usually been associated with *Mangifera indica*, this work reports the isolation of mangiferin from *Salacia* species.

It is often difficult to identify the culture based solely on macroscopic characters, therefore, nowadays molecular-based techniques are frequently used for identification, the most common being DNA identification[1]. Species identification and characterization revealed that the dominant species present in the samples were *Aspergillus fumigatus* and *Penicillium capsulatum*. These endophytic fungi isolated from *S. chinensis* root and stem were cultured in PDB media. Chromatographic separation of broth and mycelium led to the exploration of mangiferin in addition to other phytoconstituents.

Penicillium capsulatum was characterized as Fungi imperfecti, belonging to asexual stages of basidiomycetes and ascomycetes. The fungus was initially isolated from Panama. The genus *Penicillium* has been reported to be widely present in the soil. However, *A. fumigatus* belonging to the genus *Aspergillus* has been reported to be commonly present in the environment. The fungus is said to play an important role in the recycling of nitrogen and carbon present in the environment[26,27]. This indicates that these fungi may colonize in the host plant and are highly influenced by environmental factors. Although these fungi have been explored widely, their potential in natural product research needs much attention.

This study evaluated the potential of endophytes as a promising source of secondary metabolites that are produced by their host plant. Therefore, the study demonstrated that among the various methods for enhancement of secondary metabolite, utilizing the endophytic study can be widely accepted. Further study is needed to understand the mechanism of enhancement of mangiferin due to the interaction of plant and fungal endophytes.

Conclusion

The study reveals that both the species of *Salacia* viz., *Salacia chinensis* and *Salacia oblonga* are potential sources of pharmaceutically valuable molecule mangiferin. The fungal extracts reported a higher content of mangiferin as compared to the plant extracts, i.e., a three-fold higher content of mangiferin was produced by the application of a biotechnological approach. Hence, it is important to bioprospect endophytes for enhancing the content of therapeutically important phytoconstituents of respective plants.

Conflict of Interest

Authors declare no competing interests.

References

1 Verna CL, M Raja, RTPP, AK & Pratibha S, Opportunistic endophytism of *Trichoderma* species in rice Pusa Basmati-1 (PB1). *Indian J Exp Biol*, 56 (2018) 121.

2 Kaur J, Kaur R & Kaur A, Antidiabetic and antioxidant potential of endophytic fungi isolated from medicinal plants. *Int J Green Pharm*, 12 (2018) 6.

3 Dar A, Faizi S, Naqvi S, Roome T & Zikr-Ur-Rehman S, Analgesic and antioxidant activity of mangiferin and its derivatives: the structure activity relationship. *Biol Pharm Bull*, 28 (2005) 596.

4 Miura T, Ichiki H, Hashimoto I, Iwamoto N, Kato M, Kubo M, Ishihara E, Komatsu Y, Okada M, Ishida T & Tanigawa K, Antidiabetic activity of a xanthone compound, mangiferin. *Phytomedicine*, 8 (2001) 85.

5 Du S, Liu H, Lei T, Xie X, Wang H, He X, Tong R & Wang Y, Mangiferin: An effective therapeutic against several disorders. *Mol Med Rep*, 18 (2018) 4775.

6 Pal PB, Sinha K & Sil PC, Mangiferin attenuates diabetic nephropathy by inhibiting oxidative stress mediated signaling cascade, TNF alpha related and mitochondrial dependent apoptotic pathways in streptozotocin-induced diabetic rats. *PLoS ONE*, 9 (2014) e107220.

7 Muruganandan S, Srinivasan K, Gupta S, Gupta PK & Lal J, Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. *J Ethnopharmacol*, 97 (2005) 497.

8 Garrido G, Delgado R, Lemus Y, Rodríguez J, García D & Nunez-Selles AJ, Protection against septic shock and suppression of tumor necrosis factor alpha and nitric oxide production on macrophages and microglia by a standard aqueous extract of *Mangifera indica* L. (VIMANG). Role of mangiferin isolated from the extract. *Pharmacol Res*, 50 (2004) 165.

9 Chavan JJ, Ghadage DM, Kshirsagar PR & Kudale SS, Optimization of extraction techniques and RP-HPLC analysis of antidiabetic and anticancer drug Mangiferin from roots of Saptarangi. *J Liq Chromatogr Relat Technol*, 38 (2015) 963.

10 Matsuda H, Murakami T, Yashiro K, Yamahara J & Yoshikawa M, Antidiabetic principles of natural medicines. IV. Aldose reductase and alpha-glucosidase inhibitors from the roots of *Salacia oblonga* Wall. (Celastraceae): structure of a new friedelane-type triterpene, kotalagenin 16-acetate. *Chem Pharm Bull*, 47 (1999) 1725.

11 Duarte LP, Figueiredo RC, Sousa GFD, Soares DBDS, Rodrigues SBV, Silva FC, Silva GDDF & Filho SAV, Chemical constituents of *Salacia elliptica* (Celastraceae). *Quimica Nova*, 33 (2010) 900.

12 Wang Y, Chen WS, Wu ZJ, Xi ZX, Chen W, Zhao GJ, Li X & Sun LN, Chemical constituents from *Salacia amplifolia*. *Biochem Syst Ecol*, 39 (2011) 205.

13 Jayaraj R, Sasidharan N, Tom B & Muhammad AK, Comparative phytochemical profiling and quantification of Mangiferin content in species of *Salacia* from southern Western Ghats of India. *J Biol Act Prod Nat*, 6 (2016) 209.

14 Qadri M, Johri S, Shah BA, Khajuria A, Sidiq T, Lattoo SK, Abdin MZ & Hassan SR, Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. *SpringerPlus*, 2 (2013) 8.

15 Shah S, Shrestha R, Maharjan S, Selosse MA & Pant B. Isolation and Characterization of Plant Growth-Promoting Endophytic Fungi from the Roots of *Dendrobium moniliforme*. *Plants*, 8 (2019) 5.

16 Akaki J, Morikawa T, Miyake S, Ninomiya K, Okada M, Tanabe G, Pongpiriyadacha Y, Yoshikawa M & Muraoka O, Evaluation of *Salacia* species as anti-diabetic natural resources based on quantitative analysis of eight sulphonium constituents: a new class of α-glucosidase inhibitors. *Phytochem Anal*, 6 (2014) 544.

17 Ajila CM, Rao LJ & Rao UJ, Characterization of bioactive compounds from raw and ripe *Mangifera indica* L. peel extracts. *Food Chem Toxicol*, 12 (2010), 3406.

18 Musini A, Rao JP & Giri A, Phytochemicals of Salacia oblonga responsible for free radical scavenging and antiproliferative activity against breast cancer cell lines (MDA-MB-231). *Physiol Mol Biol Plants*, 21 (2015) 583.

19 Morikawa T, Akaki J, Ninomiya K, Kinouchi E, Tanabe G, Pongpiriyadacha Y, Yoshikawa M & Muraoka O, Salacinol and related analogs: new leads for type 2 diabetes therapeutic candidates from the Thai traditional natural medicine *Salacia chinensis*. *Nutrients*, 3 (2015) 1480.

20 Brownlee M & Cerami A, The biochemistry of the complications of diabetes mellitus. *Annu Rev Biochem*, 50 (1981) 385.

21 Chavan JJ, Jagtap UB, Gaikwad NB, Dixit GB & Bapat VA, Total phenolics, flavonoids and antioxidant activity of Saptarangi (*Salacia chinensis* L.) fruit pulp. *J Plant Biochem Biotechnol*, 22 (2013) 409.

22 Deepa MA & Bai VN, Antibacterial activity of *Salacia beddomei*. *Fitoterapia*, 75 (2004) 589.

23 Deokate UA & Khadabadi SS, Phytopharmacological aspects of *Salacia chinensis*. *J Pharmacogn Phytother*, 4 (2012) 1.

24 Imran M, Arshad MS, Butt MS, Kwon JH, Arshad MU & Sultan MT, Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. *Lipids Health Dis*, 16 (2017) 84.

25 Jangra A, Arora MK, Kisku A & Sharma S, The multifaceted role of mangiferin in health and diseases: a review. *ADV TRADIT* MED, 21 (2021) 619.

26 Abraham A, Philip S, Narayanan SP, Jacob KC, Sindhu R, Pandey A, Sang BI & Jayachandran K, Induction of systemic required resistance in *Hevea brasilienses* (Willd. Ex A.Juss.) Mull.Arg. by an endophytic bacterium antagonist to *Phytophthora meadii* McRae. *Indian J Exp Biol*, 57 (2019) 796.

27 Roopa G, Madhusudhan MC, Sunil KCR, Lisa N, Calvin R, Poornima R, Zeinab N, Kini KR, Prakash HS & Geetha N, Identification of Taxol producing endophytic fungi isolated from *Salacia oblonga* through genomic mining approach. *J Genet Eng Biotechnol*, 13 (2015) 119.

28 Huang TH, He L, Qin Q, Yang Q, Peng G, Harada M, Qi Y, Yamahara J, Roufagalis BD & Li Y, *Salacia oblonga* root decreases cardiac hypertrophy in Zucker diabetic fatty rats: inhibition of cardiac expression of angiotensin II type 1 receptor. *Diabetes Obes Metab*, 10 (2010) 574.

29 Ismail TS, Gopalakrishnan S, Begum VH & Elango V, Anti-inflammatory activity of *Salacia oblonga* Wall. and *Azima tetracantha* Lam. *J Ethnopharmacol*, 56 (1997) 145.

30 Jayawardena MHS, Alwis NMWD, Hettigoda V & Fernando DJS, A double blind randomised placebo controlled cross over study of a herbal preparation containing *Salacia reticulata* in the treatment of type 2 diabetes. *J Ethnopharmacol*, 97 (2005) 215.

31 Kajimoto T & Node M, Inhibitors against glycosidases as medicines. *Curr Top Med Chem*, 9 (2009) 13.

32 Karunanayake EH, Welihinda J, Sirimanne SR & Sinnadorai G, Oral hypoglycaemic activity of some medicinal plants of Sri Lanka. *J Ethnopharmacol*, 11 (1984) 223.

33 GM Vidyasagar, Naaz SQ, Ahmed MDL, Babanagre S, MK Sangeeta & V Ambika, *Penicillium citrinum Thom*: A potential antihyperglycemic endophyte from *Gymnema sylvestre*. *Indian J Nat Prod Resour*, 12 (2021) 557.

34 Aderibigbe AO, Emudianughe TS & Lawal BA, Antihyperglycaemic effect of *Mangifera indica* in rat. *Phytother Res*, 6 (1999) 504.

35 Kueh YC, Morris T & Ismail AA, The effect of diabetes knowledge and attitudes on self-management and quality of life among people with type 2 diabetes. *Psychol Health Med*, 2 (2017) 138.

36 Li Y, Huang TH & Yamahara J, *Salacia* root, a unique Ayurvedic medicine, meets multiple targets in diabetes and obesity. *Life Sci*, 21 (2008) 1045.

37 Stohs SJ & Ray S, Anti-diabetic and Anti-hyperlipidemic Effects and Safety of *Salacia reticulata* and Related Species. *Phytother Res*, 7 (2015) 986.

38 Morikawa T, Ninomiya K, Tanabe G, Matsuda H, Yoshikawa M & Muraoka O, A review of antidiabetic active thiosugar sulfoniums, salacinol and neokotalanol, from plants of the genus Salacia. *J Nat Med*, 75 (2021) 449.