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***In vitro* studies on anti-diabetic, anti-bacterial and phytochemical activities of endophytic fungal extracts from *Salacia* species**

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Abstract

In the current investigation, 474 endophytic fungi were isolated from 1200 plant parts of four species of Indian traditional medicinal plant *Salacia* collected in different seasons. Among them, *Fusarium oxysporum*, *Penicillium notatum*, *Pestalotiopsis* sps and *Phoma* sps were isolated as a dominant genus from *S. chinensis*, *S. oblonga*, *S. fruticosa* and *S. macrosperma* respectively. Most of the isolates have shown potential anti-diabetic activity through alpha-amylase inhibitor activity at different levels. Among them, metabolite extracted from endophytic fungi *Colletotrichum* species isolated from *Salacia macrosperma* resulted in significant inhibition exhibiting IC₅₀ values of 106.11 µg/ml and 124.62 µg/ml against α-amylase and α-glucosidase respectively. Out of 68 isolates, five isolates have shown anti-bacterial activity against all the tested organisms through dual culture method. Overall, this study strongly suggests that extracts of isolated endophytic fungi from *Salacia* can be developed as a potent drug molecule in the view of their pharmaceutical importance.

Keywords: *Salacia*, endophytes, hyperglycemia, pharmaceutical, anti-diabetic, anti-bacterial activity

1. Introduction

Endophytes are promising source of novel metabolites of various bioactivities, these microorganisms, which asymptotically reside in the tissues of higher plants, may produce several molecules of biotechnological interest. Further, medicinal plants are recognized as the major resources of endophytes, which can produce similar bioactives as medicinal plants. Endophytic fungi have a mutual relationship with the host, protecting the host against pathogen and in rare cases may be an opportunistic pathogen [1]. Fungal endophytes are known to protect their hosts from contagious agents and withstand at adverse conditions by discharging active metabolites [2]. Through the interaction with plants, endophytic microorganisms can produce several metabolites with pharmaceutical application, which is being used in the production of anti-microbials that inhibit the development of pathogens [3]. Several reports are demonstrated the anti-microbial potential of various endophytic extracts against pathogenic microorganisms [4-6].

Anti-microbial metabolites are natural low-molecular-weight organic molecules, which are produced by endophytes that are active at low concentrations against several pathogenic microorganisms [7]. Several investigations witnessed for the isolation of a large number of antimicrobial molecules from endophytes. These are mainly identified as terpenoids, alkaloids, steroids, peptides, phenols, isocoumarin derivatives, quinines and flavonoids with various pharmaceutical potentials [8-9].

Diabetes mellitus is a metabolic disorder resulting from the deficiency in insulin secretion, insulin action, or both promoting disturbance of carbohydrate, fat, and protein metabolism by α-amylase [10]. Diabetes mellitus has become epidemic in India due to societal influence along with changing lifestyles [11]. Many studies have confirmed the benefits of medicinal plants with hypoglycemic effects in the management of Diabetes mellitus. In addition, during the past few years, a few bioactive drugs isolated from medicinal plants and microorganisms showed anti-diabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy [10, 12-13]. The wide span of natural bioactive compounds derived especially from the plants associated microbes has been largely unexplored [14-15].

It was understood that medicinal plants and their fungal endophytic communities generate the same or analogous bioactive therapeutic products. These microbial communities have withdrawn an immense attention after the invention of anti-cancer drug producing fungi from *Taxus brevifolia*. Medicinal plants are known to harbor endophytic fungi that are thought to be associated with the production of several pharmaceutically important products [16]. Secondary metabolites produced by these endophytes have also received significant importance for a broad range of biological activities as that of plant metabolites [17-18].

The utilization of these fungal endophytes for the large scale production of the secondary metabolites has been still on rise and it will be the major interest of the pharmaceutical industry. Therefore, it is very necessary to explore the fungal endophytic microbes existing in the medicinal plants. *Salacia* belongs to the family Celastraceae, and it is widely used in ayurvedic medicine for its several known medicinal properties. It is mainly used to treat diabetes, rheumatism, gonorrhoea and skin diseases [13]. One of our previous work reports on fungal diversity of the endophytes isolated from *Salacia* species at different seasons [19]. The current study is the continuation of our previous work and it involves the investigation on biological properties of these endophytic extracts such as anti-diabetic and anti-bacterial activities and its phytochemical evaluations.

Materials and Methods

Isolation of endophytic fungi

The samples of *Salacia* species were washed thoroughly in running water before processing them for the experiment. Stem pieces were surface sterilized by sequential washes in 75% (v/v) ethanol (1 min), 4% (v/v) NaOCl (4 mins) and rinsed with sterile water to remove surface sterilizing agents and allowed to surface dry under sterile conditions [20]. One hundred stem segments of 0.5×1.0 cm from each plant collected at different seasons were dissected and placed on water agar (WA) (15 g/L) medium amended with Streptomycin (100 mg/L) contained in 9 cm diameter Petri dishes. Ten segments were placed on 20 ml WA medium in each Petri dish and incubated in a light chamber for 2 weeks at 12 h light/dark cycles at 23 °C [21]. After incubation for 15 days, individual fungal colonies were picked from the edge with a sterile fine tipped needle and transferred onto potato dextrose agar (PDA) without antibiotic [19].

Fermentation and Extraction

In order to obtain the crude extract of fungal endophytes isolated during all the above mentioned seasons were cultured in 100ml Erlenmeyer flask; a slightly modified version of the methodology described by Li *et al.* (2008) [22] was used. The endophytic fungi were incubated in PD (Potato Dextrose) medium at 28° C for 15 days. The fermentation medium was filtered using double layered sterile muslin cloth. The filtrate was transferred to a separating funnel to which was added the same volume of crude ethyl acetate. The funnel was strongly agitated and then the separation of the phases occurred by polarity difference. The process was repeated thrice. The obtained ethyl acetate extract was 98% concentrated using rotary evaporator (Heidolph) at 40° C and the material obtained was subjected to the biological activity.

Determination of α -amylase inhibitor activity

Alpha-amylase inhibitory activity of crude extract and fractions was carried out according to the standard method

with minor modification [23]. In a 96 well plate, reaction mixture containing 50 μ l phosphate buffer (100 mM, pH = 6.8), 10 μ l α -amylase (2 U/ml), and 20 μ l of extract was pre-incubated at 37°C for 20 min. Then, the 20 μ l of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min; 100 μ l of the DNS colour reagent was then added and kept on boiling water bath for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate Reader (Multiska thermo scientific, version 1.00.40). Acarbose (0.1-0.5 mg/ml) was used as a standard. Without test extract substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

Where,

A_s is the absorbance in the presence of test substance and A_c is the absorbance of control.

Anti-bacterial assay (dual-culture agar diffusion assay)

Test microorganisms

Test bacterial cultures were procured from Microbial Type Culture Collection (MTCC) of Institute of Microbial Technology, Chandigarh. Cultures of Gram positive bacteria *S. aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 121) and Gram-negative *E. coli* (MTCC 7410) and *Salmonella typhi* (MTCC 733) were grown on Mueller Hinton agar media used for anti-bacterial activity assay.

Test microbial inocula for anti-bacterial assay were prepared according to Clinical Laboratory Standards Institute (CLSI, 2005) [24] document M2-A8. For the growth method, a loop is used to touch the top of three to five colonies of the same morphological type from an agar plate culture. This is suspended in 10 ml of a sterile Mueller Hinton broth (MHB) aseptically and incubated at 37 °C. The turbidity of the actively growing cells were adjusted to the 0.5 McFarland standard (at 625 nm, 0.08-0.01 absorbance in UV-VIS Spectrophotometer) using sterile broth to produce a standardized microbial inocula of approximately $1-2 \times 10^8$ CFU/ml.

Isolates shown anti-diabetic inhibitory activity more than 25% were subjected to anti-bacterial activity. Anti-bacterial activity of selected endophytic isolates were tested for dual-culture agar diffusion assay with some modifications [16]. Petri dishes were prepared by pouring 20 ml of sterilized MHA media under aseptic condition and allowed to solidify. After solidification of the media, 100 μ l of standardized test microbial inocula of Gram positive bacteria *S. aureus*, *B. subtilis* and Gram-negative bacteria *E. coli* and *S. typhi*, were spread uniformly using sterile cotton swabs. Selected endophytic fungal isolates were grown at room temperature for 14 days on antibiotic free potato dextrose agar (PDA). 6 mm diameter of actively growing endophytic fungal agar blocks or discs were placed on the surface of the agar media seeded with test bacteria using sterile cork borer. Agar block or discs without endophytic fungal growth and antibiotic were used as negative control and positive control respectively. After keeping at 4 °C for 4 hours for the diffusion of antibacterial metabolites, thereafter plates were incubated at 37 °C for 24 h. The diameter of the inhibition zone around the discs is measured in millimeter (mm) and the average of three repeated agar discs were taken to assess the strength of

antibacterial activity.

Phytochemical analysis of the selected fungal extracts

The ethyl acetate extract of endophytic fungi exhibiting broad spectrum of anti-bacterial and anti-diabetic activities were subjected to chemical constituent analysis was conducted according to the standard methods. Using these methods, the presence of several phytochemicals like sterols, tannins, proteins, sugars, alkaloids, flavonoids, saponins, terpenoids and cardiac glycosides was evaluated.

Test of steroids (Salkowski's test)

0.2 g of the extracts was dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added from the sides of the test tube, to form a layer. A reddish brown colour at the inter phase indicated the presence of steroids.

Test for tannins (Ferric chloride reagent test)

0.2 g of each extract was taken separately in water, warmed and filtered. To a small volume of this filtrate, a few drops of 5% w/v solution of ferric chloride prepared in 90% alcohol were added. Appearance of a dark green or deep blue colour indicated the presence of tannins [20].

Test for sugars (Fehling's test for free reducing sugar)

About 0.5g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars [21].

Test for proteins

A little extract was taken with 2 mL of water and 0.5 mL of concentrated HNO₃ was added to it. Yellow colour is obtained if proteins are present [26].

Test for flavanoids (Ferric chloride test)

About 0.5g of each extract was boiled with 5ml of distilled water and then filtered. To 2ml of this filtrate, a few drops of 10% ferric chloride solution was added. A green-blue or violet colouration indicated the presence of Flavonoids [21].

Test for saponins (Foam test)

1g of each extract was boiled with 5ml of distilled water and filtered. To the filtrate, about 3ml of distilled water was further added and shaken vigorously for about 5min. frothing of which persisted on warming was taken as an evidence for the presence of saponins [21].

Test for terpenoids (Salkowski's test)

To 0.2g of each extract, 2ml of chloroform was added, followed by a further addition of 3ml of conc. H₂SO₄ to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids [21].

Test for cardiac glycosides (Keller-killiani test)

0.5g of the extract was dissolved in 5ml distilled water. 2ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycoside.

Results

Determination of alpha-amylase inhibitor activity

Isolates obtained from *Salacia chinensis* at all the three seasons

Total of 172 isolates from *Salacia chinensis* were subjected to anti-diabetic activity. Amongst them, 32 isolates have shown inhibition more than 25% in crude extracts are SCS5, SCS2, SCS6, SCS13, SCW9, SCS19, SCM15, SCM1, SCS36, SCS17, SCS27, SCM68, SCS14, SCW37, SCS4, SCS21, SCS20, SCS3, SCS26, SCM2, SCM55, SCM4, SCW7, SCS12, SCM33, SCM41, SCS37, SCS38, SCM8, SCM35, SCM51 and SCM26 as 39.71%, 36.12%, 35.39%, 34.46%, 33.60%, 33.07%, 32.14%, 31.94%, 31.74%, 30.21%, 30.01%, 29.88%, 29.75%, 29.28%, 27.89%, 26.89%, 26.83%, 26.69%, 26.63%, 26.63%, 26.63%, 26.56%, 26.56%, 26.43%, 26.43%, 26.43%, 26.36%, 25.96%, 25.86%, 25.63%, 25.63% and 25.10% respectively. The highest inhibition was shown by the isolate SCS5 were as the least inhibition was shown by SCM59 as 0.20% with the absorbance 0.1503 at 540nm which was equal to negative control.

Isolates obtained from *Salacia oblonga* in all the three seasons

A total of 124 isolates from *Salacia oblonga* were subjected to anti-diabetic activity, out of which 15 isolates have shown inhibition more than 25% in crude extracts are SOM15, SOW23, SOM32, SOW24, SOM1, SOW4, SOS25, SOS8, SOS15, SOS19, SOS27, SOS29, SOS30, SOS2 and SOS6 as 39.38%, 31.94%, 31.87%, 31.44%, 29.61%, 29.35%, 28.22%, 26.76%, 26.63%, 26.63%, 26.63%, 26.56%, 26.43%, 25.30% and 25.30% respectively. The highest inhibition was shown by the isolate SOM15 were as the least inhibition was shown by SOW30 as 1.0% with the absorbance 0.1491 at 540nm, which was nearer to negative control.

Isolates obtained from *Salacia fruticosa* in all the three seasons

A total of 128 isolates from *Salacia fruticosa* were subjected to anti-diabetic activity, out of which 15 isolates have shown inhibition more than 25% in crude extracts are SFM17, SFS33, SFS36, SFW1, SFM35, SFM34, SFM15, SFM31, SFS9, SFS13, SFS31, SFS39, SFS16, SFS20 and SFW15 as 33.60%, 31.94%, 31.94%, 31.94%, 31.87%, 31.27%, 29.88%, 29.35%, 26.83%, 26.63%, 26.63%, 26.43%, 25.63%, 25.30% and 25.30% respectively. The highest inhibition was shown by isolate SFM17 were as the least inhibition was shown by SFW23 as 0.13% with the absorbance of 0.1540 at 540nm which was nearer to negative control.

Isolates obtained from *Salacia macrosperma* in all the three seasons

A total of 50 isolates from *Salacia macrosperma* were subjected to anti-diabetic activity, out of which 06 isolates were shown inhibition more than 25% in crude extracts are SMW8, SMM1, SMW1, SMW3, SMS10 and SMS8 as 33.00%, 29.35%, 29.35%, 29.35%, 26.43% and 26.36% respectively. The highest inhibition was shown by isolate SMW8 were as the least inhibition was shown by SMS5 as 0.27% with the absorbance 0.1502 at 540nm which was nearer to negative control.

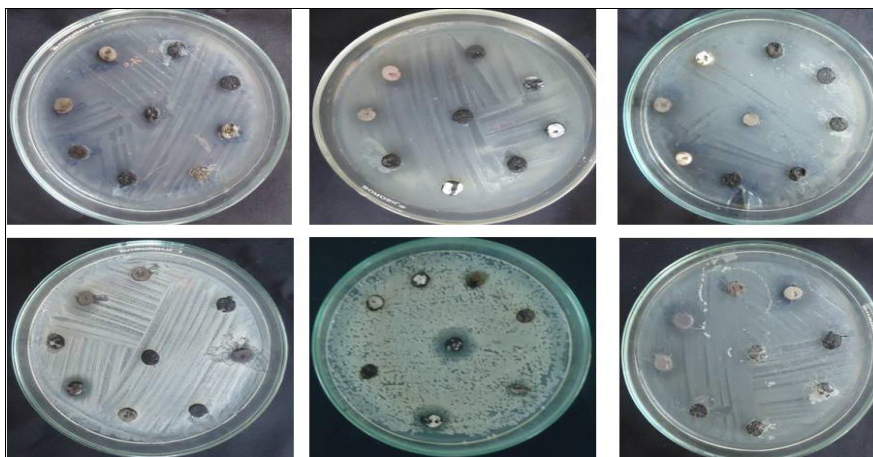


Fig 1: Anti-bacterial activity of ethyl acetate extracts of selected endophytic fungi against test pathogens by disc diffusion method

Primary screening of isolated endophytes for anti-bacterial activity from *Salacia* species

Anti-bacterial activity of selected endophytic isolates was tested for dual-culture agar diffusion assay (Fig 1). Out of 68 isolates 5 isolates have shown antibacterial activity against all the tested organisms, in which SOM15 was shown highest anti-diabetic inhibition and also anti-bacterial activity against all the tested pathogen (Table 1).

Phytochemical analysis

Phytochemical analysis was carried out on the isolated endophytic fungal extracts to determine the presence of chemical components as a prospective source for medicinal and industrial use. Their presence is an indicator that they can be exploited as precursors in the development and advancement of synthetic drugs. The active metabolites contain chemical groups such as steroids, tannins, sugars, proteins, flavanoids, saponins, terpenoids and glycosides. In the current study, phytochemical analysis of ethyl acetate extracts of 39 isolates out of 68 isolates showed the presence of steroids, 21 of tannins, 45 of sugars, 33 of proteins, 26 of flavonoids, 33 of saponins, 25 of terpenoids and 32 of glycosides (Table 2).

Discussion

Salacia has been used in the traditional Ayurvedic medicinal

system of India, which has been reported to possess hepatoprotective, anti-inflammatory, antitussive, anti-fungal, anti-bacterial, and wound healing properties [12, 22]. It is also found to be useful in treatment of diabetes, abdominal pain, constipation, skin diseases, fever, and leprosy. The various phytoconstituents/ secondary metabolites were reported in this plant recognized as alkaloids, flavonoids, and anthraquinone glycosides [27-29]. The plant endophytic fungi have been assumed to have symbiotic association and genetic exchange with host plants and reported to possess the special ability of producing the same or similar compounds as presented in their host plants [30-31]. A wide range of fungal endophytes has been isolated from many medicinal plants, which have a rich source of secondary metabolites [32]. In the current investigation, the endophytic fungi isolated from *Salacia* witnessed the presence of various phytochemicals, which is responsible for anti-diabetic, and anti-bacterial activity. The ethyl acetate extract of fungal culture was used to study of phytochemicals and confirmed the presence of phytochemicals such as phenols, flavonoids, alkaloids, terpenoids, and saponins at varied concentrations. The results revealed that the fungal extracts were found to have a significant amount of anti-diabetic and anti-bacterial activity. These results indicate that endophytes isolated from *Salacia* can be potential sources for anti-diabetic and anti-bacterial activity as *Salacia* plant extracts.

Table 1: Fungal endophyte isolates from *Salacia* and pathogenic bacteria tested

Sl no	Isolate No.	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>
1	SCM1	+	+	-	-
2	SCM2	-	+	+	+
3	SCM4	-	+	-	-
4	SCM8	+	-	+	+
5	SCM15	-	-	+	-
6	SCM26	+	-	-	+
7	SCM33	+	+	+	-
8	SCM35	+	+	+	+
9	SCM41	+	-	-	-
10	SCM51	-	+	+	+
11	SCM55	+	-	+	+
12	SCM68	+	-	-	+
13	SCS2	+	-	+	+
14	SCS3	-	-	-	-
15	SCS4	+	-	+	+
16	SCS5	+	-	-	+
17	SCS6	+	-	-	+
18	SCS12	+	-	-	-
19	SCS13	-	+	+	-
20	SCS14	+	+	+	-

21	SCS17	+	+	+	-
22	SCS19	+	+	-	-
23	SCS20	-	-	+	+
24	SCS21	+	+	+	+
25	SCS26	+	-	-	+
26	SCS27	+	+	+	+
27	SCS36	-	-	+	+
28	SCS37	+	+	-	-
29	SCS38	+	+	+	-
30	SCW7	+	+	+	-
31	SCW9	-	-	-	-
32	SCW37	+	-	+	-
33	SFM15	+	-	+	+
34	SFM17	+	-	-	+
35	SFM31	-	-	-	+
36	SFM34	+	+	+	+
37	SFM35	+	+	-	-
38	SFS9	-	+	-	+
39	SFS13	-	+	-	+
40	SFS16	-	-	-	+
41	SFS20	+	+	+	+
42	SFS31	-	-	+	-
43	SFS33	-	+	+	+
44	SFS36	+	+	+	+
45	SFS39	+	-	+	+
46	SFW1	+	+	-	+
47	SFW15	+	-	+	-
48	SMM1	+	-	+	+
49	SMS8	+	+	+	+
50	SMS10	-	-	-	-
51	SMW1	+	-	+	-
52	SMW3	+	-	+	+
53	SMW8	+	-	+	+
54	SOM1	+	-	-	+
55	SOM15	+	+	+	+
56	SOM32	-	-	+	-
57	SOS2	+	+	-	+
58	SOS6	+	+	-	+
59	SOS8	-	+	-	-
60	SOS15	+	+	-	+
61	SOS19	+	+	+	+
62	SOS25	+	-	+	-
63	SOS27	-	+	+	+
64	SOS29	+	+	+	-
65	SOS30	+	-	-	-
66	SOW4	+	+	+	+
67	SOW23	+	+	+	+
68	SOW24	+	-	-	+

Table 2: Phytochemical analysis of fungal endophyte isolates from *Salacia*.

Sl no	Isolate No	Steroids	Tannins	Sugars	proteins	Flavonoids	Saponins	Terpenoids	Glycosides
1	SCM1	+	-	-	+	+	+	-	+
2	SCM2	-	-	-	-	+	+	-	+
3	SCM4	-	-	-	-	-	-	-	-
4	SCM8	-	+	-	-	-	-	-	-
5	SCM15	-	+	-	-	-	-	-	-
6	SCM26	+	+	+	-	-	+	-	-
7	SCM33	+	+	+	-	-	+	-	-
8	SCM35	+	+	+	-	-	+	-	-
9	SCM41	+	+	+	-	-	+	-	+
10	SCM51	+	+	+	-	-	+	-	+
11	SCM55	+	+	+	-	-	+	-	+
12	SCM68	+	+	+	-	-	+	-	+
13	SCS2	+	+	+	-	-	+	-	+
14	SCS3	+	+	-	-	-	-	-	+
15	SCS4	-	+	-	-	-	-	-	+
16	SCS5	-	+	-	-	+	-	-	+
17	SCS6	+	+	-	-	+	-	-	+
18	SCS12	+	-	-	-	+	-	-	-

19	SCS13	+	-	-	-	+	-	-	-
20	SCS14	-	-	-	-	+	-	-	-
21	SCS17	-	-	-	-	+	-	-	-
22	SCS19	+	-	-	-	+	-	-	-
23	SCS20	+	-	-	-	+	-	-	-
24	SCS21	+	-	+	-	+	-	-	-
25	SCS26	+	-	+	-	+	-	-	-
26	SCS27	+	-	+	-	+	-	-	-
27	SCS36	+	-	+	-	+	-	-	-
28	SCS37	+	-	+	-	+	-	-	-
29	SCS38	+	-	+	-	+	+	-	-
30	SCW7	+	-	+	-	+	+	-	-
31	SCW9	+	-	+	-	-	+	-	-
32	SCW37	+	-	+	-	-	+	-	-
33	SFM15	+	-	+	-	-	+	-	-
34	SFM17	+	-	+	-	-	+	-	-
35	SFM31	+	-	+	-	-	+	-	-
36	SFM34	+	-	+	+	-	+	+	-
37	SFM35	-	-	+	+	-	+	+	-
38	SFS9	-	-	+	+	-	+	+	-
39	SFS13	-	-	+	+	-	+	+	-
40	SFS16	-	-	+	+	-	+	+	-
41	SFS20	-	-	+	+	-	+	+	+
42	SFS31	-	-	+	+	-	+	+	+
43	SFS33	-	-	+	+	-	+	+	+
44	SFS36	-	-	+	+	-	+	+	+
45	SFS39	-	-	+	+	-	-	+	+
46	SFW1	-	-	+	+	-	-	+	+
47	SFW15	+	-	+	+	-	-	+	+
48	SMM1	+	+	+	+	-	-	-	+
49	SMS8	+	+	+	+	-	-	-	+
50	SMS10	+	+	+	+	-	-	-	+
51	SMW1	+	+	+	+	+	-	-	+
52	SMW3	-	+	+	+	+	-	+	+
53	SMW8	-	+	+	+	+	-	-	+
54	SOM1	-	+	+	+	+	-	-	+
55	SOM15	-	-	+	+	+	+	-	+
56	SOM32	-	-	+	+	-	+	-	+
57	SOS2	-	-	+	+	-	+	+	-
58	SOS6	-	-	+	+	-	+	+	+
59	SOS8	-	-	+	+	-	+	+	-
60	SOS15	-	-	+	+	-	+	+	+
61	SOS19	-	-	-	+	-	+	+	+
62	SOS25	+	-	-	+	-	-	+	+
63	SOS27	+	-	-	+	-	-	+	+
64	SOS29	+	-	-	+	-	-	+	-
65	SOS30	+	-	-	+	+	-	+	-
66	SOW4	+	-	-	+	+	-	+	-
67	SOW23	+	-	-	+	+	-	+	-
68	SOW24	-	-	-	+	+	-	+	-

Conclusion

In the present investigation, the endophytes have been successfully isolated from *Salacia* plants. The endophytic fungi isolates have shown prominent anti-diabetic, and anti-bacterial activities. Phytochemical analysis witnessed the presence of secondary metabolites similar to *Salacia* plant extracts and comparable to the plant metabolites. As they are good microbial resource in producing these bioactive compounds from their host, they could be a satisfactory substitute for pharmacy, medical, agriculture and industries. Therefore, further detailed investigation is essential to exploit their potentiality as a novel anti-microbial and anti-diabetic agent.

Conflict of Interest

The authors declare no conflict of interest

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