Isolation and characterization of a bioactive compound with pharmacological potential from *Salacia oblonga* endophytome

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Abstract

The current work emphasizes the isolation and characterization of the bioactive compound from an endophytic fungus isolated from medicinal plant Salacia oblonga. Antimicrobial, antioxidant and antidiabetic activities of the active fraction were confirmed using standard methods. The extracts showed significant antibacterial activity against some pathogenic bacteria and were also positive to in vitro antidiabetic assays. The IC_{50} values obtained in the antioxidant assays (34.56 µg for SOEF fraction and 19.65 µg for ascorbic acid in the case of DPPH assay and 38.34 µg for SOEF fraction and 19.65 µg for quercetin in the case of ABTS assay) were positive and comparable to the standards used.

The purified active fraction was identified and characterized using LC-MS, NMR, FTIR, Homonuclear correlation spectroscopy sequence (COSY) and Heteronuclear Multiple Bond Coherence (HMBC). From the studies, the compound obtained in the active fraction was found to be 3-(4-hydroxyphenyl) propionic acid, a potent antioxidant compound that has several applications in food and pharma industries.

Keywords: Antimicrobial activity, Antioxidant effect, Endophytome, Characterization, *In vitro* antidiabetic assay

Introduction

Natural products have been an incredible source of compounds for drug discovery, which formed the basis of most of the early medicines followed by subsequent clinical, pharmacological and chemical studies[4]. Plants have been used as healers and health revitalizers since decades[8,24]. Their properties have been used as a primary source of medicines for early drug discovery[6]. Natural products have always played a major role in human therapy, they represent a huge reservoir of bioactive chemical diversity and help to understand the cellular pathways that are essential component of drug discovery process[21].

Recent advances in genomics and metabolomics during the past decades paint a clearer picture of the diversity of proteins targeted by natural-product molecules[10]. Endophytome refers to the microorganisms (mostly fungi and bacteria) colonizing the intercellular and intracellular regions of healthy plant tissues symbiotically. These associations are asymptomatic and potential sources of novel natural products for exploitation in the medicine and agriculture industry[22,23]. Many bioactive compounds and secondary metabolites have been extracted, isolated and characterized from endophytomes[11,16,19,23,25].

The discovery of novel antimicrobial metabolites from endophytome is an important alternative to overcome the increasing levels of drug resistance by plant and human pathogens, the insufficient number of effective antibiotics against diverse bacterial species and few new antimicrobial agents in development, probably due to relatively unfavorable returns on investment. There is some evidence that bioactive compounds produced by endophytes could be alternative approaches for the discovery of several novel drugs[5,7,9,13,15].

The current study is on the extraction and characterization of bioactive compounds from fungal endophytome from the medicinal plant *Salacia oblonga var. oblonga*. Our previous studies have reported on the fungal endophytic diversity from *Salacia* species in the Western Ghats of Karnataka, India and metabolite extraction followed through various bioassays[17,18]. The isolate which has shown the highest anti-diabetic and anti-bacterial activities, has been selected for the current study to isolate and characterize the active fraction using advanced methodologies and standard assay protocols.

Material and Methods

Extraction of metabolite: The endophytic fungi from *S. oblonga* were isolated and processed according to Roopa et al[17]. Fermentation of the isolated culture was carried out by providing alternate cycles of light (12 h) and dark (12 h) condition at 25 °C for 45 days for the secondary metabolite production. The metabolite was extracted from broth using ethyl acetate followed by evaporation at 45 °C, named as crude ethyl acetate extract of *S. oblonga* endophytic fungi (SOEF).

Purification of the metabolite: The SOEF extract was fractionated by silica gel column chromatography (60-120 mesh), eluted with step-wise gradient elution and collected fractions were observed under UV. From each group, a representative TLC (ALUGRAM® SIL G/UV254,

Macherey-Nagel, Germany) was developed with the solvent system (petroleum ether and ethyl acetate in the ratio 1:1) followed by visualization under low and high UV at 254 and 365 nm. Each fraction was collected separately (231 tubes) and numbered, consecutively differentiated into separate groups according to thin-layer chromatography monitoring.

Detection of antimicrobial metabolite using TLC Bioautography assay: The fractions obtained were tested for antimicrobial activity employing TLC bioautography assay. The SOEF fractions 1-79 and 80-101 were transferred to sterile Petri plates and overlaid with Mueller Hinton soft agar (for *Escherichia coli* and *Salmonella typhi*), Brain heart infusion (BHI) (for *Staphylococcus aureus*, *Bacillus subtilis*) medium incorporated with 2,3,5-triphenyltetrazolium chloride (TTC) and inoculated with 1% standardized microbial inoculum. After 10 h of diffusion at 8 ºC, the plates were incubated for 24 h at 37 ºC. After incubation, the upper agar was flooded with 10ml of microbiological agar 1% (w/v) containing 0.05% (w/v) of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml), which was converted into a formazan dye by the active dehydrogenase enzyme of the test microbe[2,14]. Zone of inhibitions were observed as clear spots against a red background on the chromatogram corresponding to metabolites having antimicrobial activity.

α-Amylase Inhibitor Activity Assay: α-Amylase inhibitory activity of pooled SOEF fractions was carried out according to the standard method with minor modification[1]. The reaction mixture containing phosphate buffer (100 mM, pH 6.8), α–Amylase (2 U/ml) and the SOEF fractions at concentrations ranging from 20 to 200 µg followed by the addition of substrate (soluble starch) was incubated at 37 °C for 30 min. DNS reagent was added and placed in a boiling water bath for 10 min. The absorbance of the resulting mixture was measured at 540 nm using a UV spectrophotometer (Bio-Rad, USA). Acarbose was used as a standard. Each experiment was performed in triplicates. The results were expressed as percentage inhibition which was calculated using the formula:

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

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

α-Glucosidase Inhibitory Activity Assay: α-Glucosidase inhibitory activity was evaluated according to Li et al[12]. α-glucosidase solution (0.6 U/ml) was pre-incubated with various concentrations of test samples ranging from 20 to 200 µg and the control sample for 5 min. The reaction was started by adding 200 µl of sucrose (37 mM) and it was terminated after 15 min incubation at 37 °C by heating in a water bath. The liberated glucose was determined. The enzyme activity is directly proportional to the liberated glucose and the liberated glucose is measured at 546 nm using a UV spectrophotometer. The assay procedures were done in triplicate. The activity of the extract was expressed as the minimum inhibitory concentration (IC_{50}) in µg.

Well Diffusion Assay: Antibacterial activity was tested by agar well diffusion assay[26] with some modifications. *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus epidermidis*, *Escherichia coli* and *Salmonella typhi* were tested for antibacterial activity using gentamycin as positive control and ethyl acetate as negative control. The diameter of the inhibition zone around the well is measured in millimeters (mm) and the average of three repeated agar discs was taken to assess the strength of antibacterial activity.

Minimal Inhibitory Concentration (MIC): MIC was determined by micro dilution method according to the standard protocol. Briefly, serial dilutions were performed to have the two-fold concentrations such as 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.2, 0.1 and 0.05 µg/ml, in 100 µl of sterile cation adjusted Mueller Hinton broth and brain heart infusion broth for Gram positive and Gram negative bacterial test pathogens respectively. 20 µl of standardized bacterial inocula were added to all the wells except the control well. Gentamicin (1 µg/mL) was used as standard positive control. Minimal inhibitory concentrations (MICs) were obtained by spectroscopy by measuring cell growth at OD 600 after incubation with compounds for 24 h at 37 °C. The above spectroscopic MIC was confirmed by the colorimetric method by adding 20 µl of 0.5 % resazurin aqueous solution[20].

Antioxidant Property Evaluation: The free radical scavenging capacity of extracts was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method[3] with minor modifications. The antioxidant activity was also analyzed by 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) method. Ascorbic acid and quercetin were used as positive controls. The results were expressed as total antioxidant capacity and a dose-dependent curve was plotted to calculate the IC_{50} value.

Physico-chemical Characterization: 10 µl of the sample was injected into HPLC having a PDA/UV detector (200-350 nm). LC-MS analysis of an active fraction of SOEF extract was performed in negative mode using Thermo LCQ Deca XP MAX with software Xcalibur using standard LCMS operating conditions. The column ACQUITY UPLC BEH C18 (250 mm length, ID of 4.6 mm and particle size 5 µm) was used. Water:Acetonitrile:Tri-fluoro-acetic acid (30:70:0.05) mixture was used as mobile phase for 0-16 min with a flow rate of 1 ml/min at a pressure of 15,000 psi at ambient temperature.

MS experimental conditions: probe/source voltage of 4.5 kV, sheath gas flow of 40.00 and auxiliary/sweep gas flow of 26.00. The source type: electrospray ionization (ESI) with a capillary temperature of 27 °C and capillary voltage of 16

V. The mobilization gas flow was helium at 1 ml/min, TOF of 6.15e-7 and the helium in the mass analyzer cavity was maintained at 0.1 Pa (10-3).

FTIR spectra were recorded on Perkin Elmer in diffuse reflectance (DRS) mode at a resolution of 2 cm^{-1}. A mass spectrum of active fraction was recorded using Agilent – NMR SC/AD/10-017 LC-MS instrument with the following chromatographic conditions: Column: Atlantis dC18, (50×4.6) mm, 5 µm; flow rate: 1.0 ml/min; mobile phase: 0.1% HCOOH in water with gradient elution; injection volume: 5 µl in methanol; Acq. Time: 0.319 sec; Width: 6410.3 Hz. ^{1}H, ^{13}C, ^{1}H-1H COSY and HMBC NMR spectra were recorded on a Bruker DPX 500 at 400 MHz instrument. The compound was dissolved in $CDCl_3$. Multiplicities of ^{13}C signals were assigned. This solution was constantly introduced by direct infusion using the equipment syringe pump at 5 ml/min.

Results and Discussion

Detection of Anti-microbial metabolite using TLC Bioautographic assay: The bioactive fractions 1-79 and 80-101 were pooled and analyzed for the antibacterial activity using bioautography. While fractions 1-79 showed no activity when tested against the 80-101 fractions, the latter were re-evaluated to confirm their antibacterial properties. The crude extract, analyzed on a TLC plate, displayed two distinct bands, with one corresponding to an Rf value of 0.52. This band, associated with the 80-101 fractions (Rf: 0.52), was confirmed to exhibit antibacterial activity through a bioautographic assay (Fig. 1). An undisclosed region corresponding to the Rf 0.52 stain was transferred from the chromatographic plate to a Petri dish and processed according to the protocol given. The inhibition zones were observed as clear areas in the Petri dish, corresponding to the activity of antimicrobial compounds present in the 80-101 fraction of SOEF extract (Fig. 1).

Determination of α-Amylase Inhibitory and α-glucosidase Inhibitory Activities: The potency of the inhibition was determined experimentally and the obtained data were expressed in IC_{50} values. The α-amylase inhibitory activity varied from 5.52 to 76.04 % for the concentration from 20 to 200 µg respectively (Table 1). The active fraction (80-101) of SOEF extract has shown a dose-dependent inhibition of α-amylase enzyme and IC_{50} value of the extract was found to be 134.73 µg. Similarly, the α-glucosidase inhibitory activity of the active fraction of SOEF extract varied from 13.95 to 67.81 % for 20 to 200 µg respectively with an IC_{50} value of 124.59 µg (Table 1).

Antibacterial Activity: The antimicrobial activity of the active fraction of SOEF extract against representative Gram-positive and Gram-negative bacteria was carried out by well diffusion Assay. The SOEF (50 µg) extract has shown effective antibacterial activity at varied levels (Table 2A). It was found that the highest inhibition zone was seen against *Staphylococcus aureus* of (12 mm and 22 mm for 50 µg and 100 µg respectively) and lowest with *Bacillus subtilis* (8 mm and 12 mm for 50 and 100 µg respectively). The minimum inhibitory concentrations (MICs) of isolated active fractions of SOEF extract were tested against various pathogenic bacteria (Table 2B). Among the tested bacteria, the active fraction of SOEF extract was more effective against *Bacillus subtilis* and *Staphylococcus epidermidis*, (MIC 3.13 µg) and the remaining were above ~6 µg. The gentamicin showed the lowest MIC activity ranging from 0.20 to 0.39 µg (Table 2B).

Antioxidant Assay: The dose-dependent progressive increase in the antioxidant activity of both the active fraction of SOEF extract and the standard samples (Ascorbic acid and Quercetin for DPPH and ABTS respectively) with an increase in their concentration was clearly evident (Table 3A and 3B). *In vitro* DPPH and ABTS assays exhibited good antioxidant activity comparable with standards (IC_{50} values 19.65 and 17.63 µg for standards and 34.56 and 38.34 µg for the active fraction of SOEF extract respectively) (Table 3A and 3B). Good radical scavenging activities of the samples were observed comparing with that of standards

Table 1
Inhibition of α-amylase and α-glucosidase enzyme acitivity by active fraction of SOEF extract.

Active SOEF fraction extract (µg)	α-amylase enzyme activity inhibition (%)	α-glucosidase enzyme activity inhibition (%)
20	5.52	13.95
40	10.59	28.10
60	17.49	35.36
80	24.59	41.44
100	34.18	43.73
120	42.65	48.14
140	53.18	55.67
160	63.18	61.32
180	69.47	64.24
200	76.04	67.81
Median inhibitory concentration of enzyme activity (IC_{50}) of active SOEF fraction were found to be 134.73 mg and 124.6 for α-amylase and α-glucosidase respectively.		

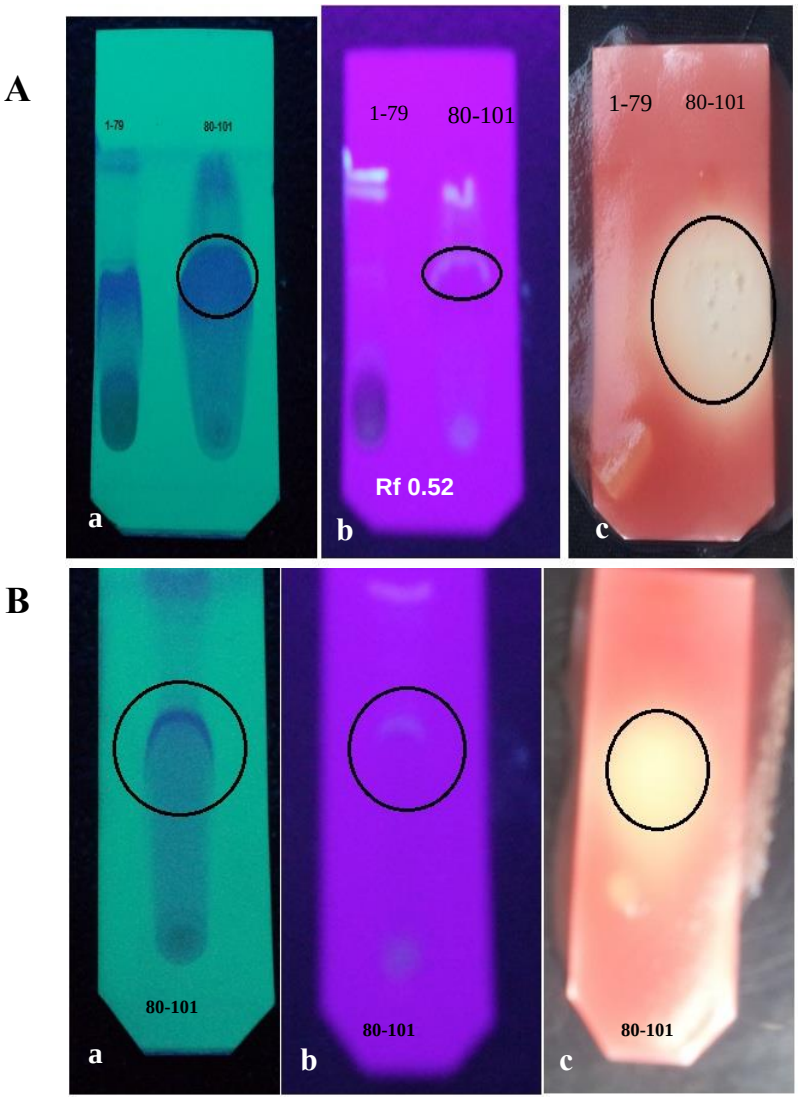

Figure 1: A. Chromatoplates of fractions 1-79 and 80-101 from column chromatography of SOEF extract; a) Under UV at 254 nm, (b) Under UV at 365 nm and (c) Bioactive spot (clear zone) against red background; B. TLC bioautoghraphy Chromatoplates of SOEF (80-101) fraction a) under UV at 254 nm, (b) under UV at 365 nm and (c) Bioactive spot (clear zone) against red background

Physico-chemical Characterization: Chromatogram of HPLC and LC-MS analysis of the extract in negative mode was shown in fig. 2A and fig. 2B. It showed molecular ion peak at m/z=166 [M-H], which is corresponding to 3-(4-hydroxyphenyl) propionic acid with molecular formula $C_9H_{10}O_3$, total ion chromatogram summing up intensities of all mass spectral peaks belonging to the same scan (Fig. 2B).

The FTIR spectra displayed vibrational stretches (spectral range of 400–4000 cm^{-1}) that were predicted based on their earlier reports. Accordingly, at 1672.31 cm^{-1} which corresponds to alkenyl C=O stretching of the carboxylic acid, 1553.74 cm^{-1} corresponds to aromatic C=C double bonding, 1453.44 cm^{-1} corresponds to O-H bending of COOH group, C-H bending, 1268.99 cm^{-1} O-H bending of phenol, C-O stretching of carboxylic acid, 1172.54 cm^{-1} corresponds to amine stretch, 896.97 cm^{-1} hydroxyl stretch and 722.98 cm^{-1} corresponds to C-H bending of 1,3 substitutions (Fig. 3). FT-IR and NMR are spectrometric methods that generate distinct and complementary information. IR and ^1H NMR (Fig. 4A) data provide prominent signals for the important functional groups and protons which are necessary for the formation of organic compounds.

In the ^1H NMR spectrum, signals were observed at d X and d Y, corresponding to aromatic hydrogens. The signals corresponding to the protons of n-CH_2 adjacent to C=O can be seen at δ1.267, δ1.248 and δ1.236. The up fielded signals at δ0.862, δ0.870 and δ 0.887 and δ 1.012, δ1.031 and δ1.048 belong to the protons of the n-CH_2-group attached to the hydroxy phenyl ring and are characteristic chemical shifts observed in fatty acid chains. The protons belonging to the aromatic ring have shifts at δ4.621, δ4.636 and δ 4.651. The ^{13}C NMR shifts observed at 29.65, 30.76 and 69.3 ppm indicated -CH_2, -C-H-O and C=O groups. The presence of -triplet signal 1:1:1 at 76.67, 76.98 and 77.3 ppm might be because of the CD_3Cl solvent.

Table 2A
Growth inhibition zone of pathogenic bacteria induced by active fraction of SOEF extract observed through well diffusion assay

Bacteria	Growth inhibition zone (mm)		
	Active SOEF fraction extract		Gentamicin (10 µg)
	50 µg	100 µg	
Klebsiella pneumonia	08	14	16
Bacillus subtilis	08	12	21
Listeria monocytogenes	08	15	20
Staphylococcus epidermidis	10	18	20
Staphylococcus aureus	12	22	22
Escherichia coli	-	20	22
Salmonella typhi	-	19	22

Table 2B
Minimum inhibitory concentration determined for active fraction of SOEF extract, against pathogenic bacteria

Bacteria	Minimum inhibitory concentration (µg)	
	Active SOEF fraction	Gentamicin
Klebsiella pneumonia	6.25	0.20
Bacillus subtilis	3.13	0.10
Listeria monocytogenes	12.5	0.20
Staphylococcus epidermidis	3.13	0.10
Staphylococcus aureus	6.25	0.20
Escherichia coli	6.25	0.39
Salmonella typhi	6.25	0.20

Table 3A
Total antioxidant capacity (% inhibition) determined for active fraction of SOEF, through DPPH assay.

Active SOEF fraction (µg)	Total antioxidant capacity (% inhibition)	
	Active SOEF fraction	Ascorbic acid
0.097	4.56	8.06
0.195	8.89	16.21
0.39	11.95	30.41
0.78	18.03	51.06
1.56	22.67	56.18
3.12	32.38	60.33
6.25	36.28	64.19
12.5	38.61	70.52
25	42.25	75.14
50	51.33	82.61
100	66.29	93.53
Median inhibitory concentration (IC_{50}): active SOEF fraction = 34.56 µg and ascorbic acid = 19.65 µg.		

Table 3B
Total antioxidant capacity (% inhibition) determined for active fraction of SOEF, through ABTS assay.

Active SOEF fraction (µg)	Total antioxidant capacity (% inhibition)	
	Active SOEF fraction	Quercetin
10	20.20	32.12
20	32.29	49.62
30	38.50	64.76
40	44.43	74.76
50	56.98	89.25
60	62.88	91.20
70	64.92	92.50
80	73.81	92.86
90	74.24	94.42
100	75.26	94.50
Median inhibitory concentration (IC_{50}): active SOEF fraction =38.34 µg and Quercetin = 17.63 µg.		

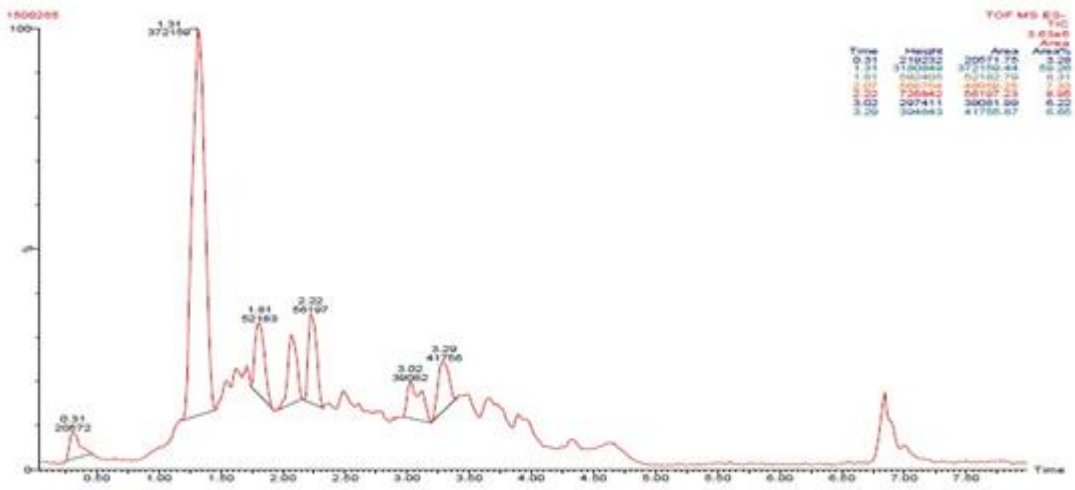

Figure 2A: HPLC Chromatogram profile of active fraction of SOEF.

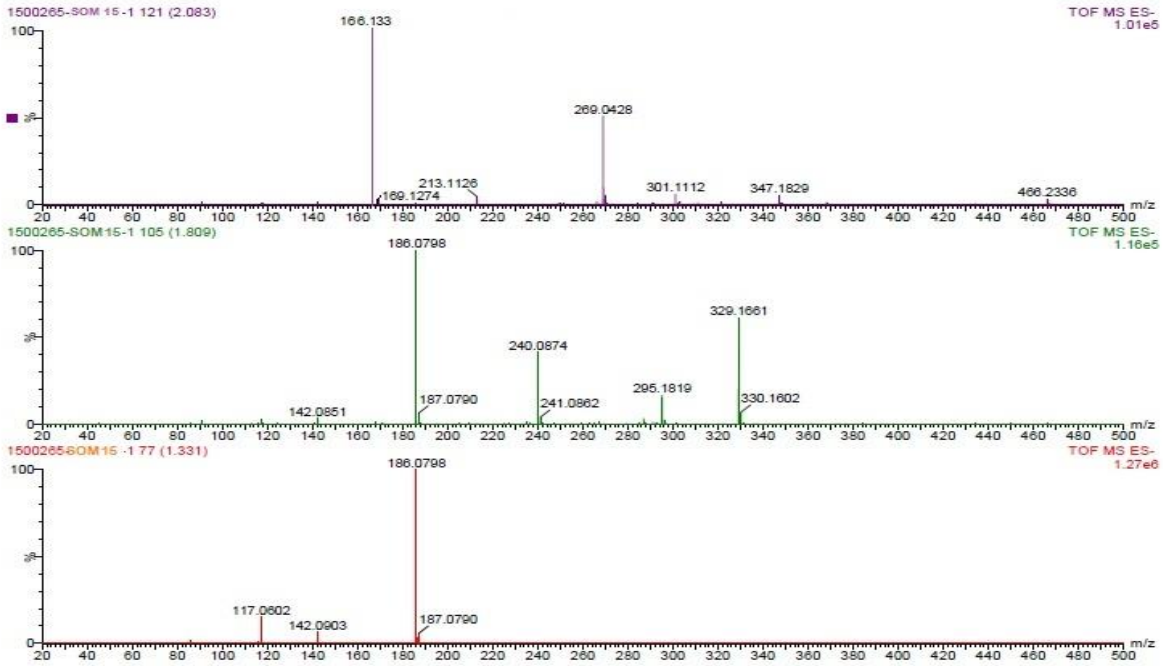

Figure 2B: LC-MS spectra of 3-(4-hydroxyphenyl) propionic acid isolated by active fraction of SOEF.

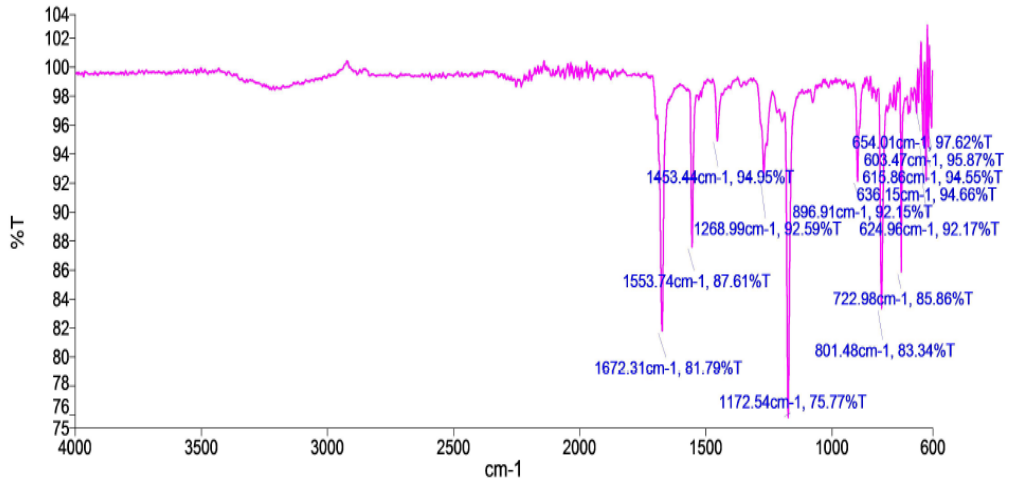

Figure 3: FTIR spectra of 3-(4-hydroxyphenyl) propionic acid from SOEF.

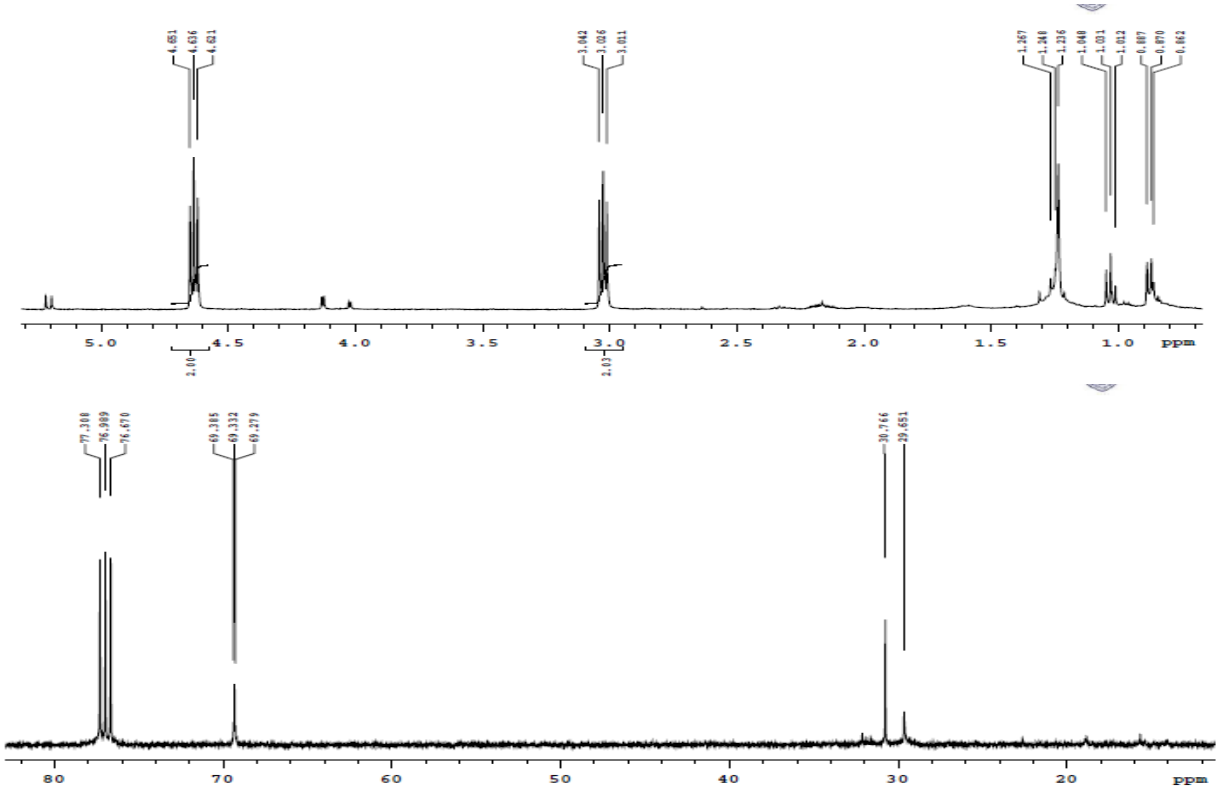

Figure 4A: ^{1}H and ^{13}C NMR spectra of 3-(4-hydroxyphenyl) propionic acid from active fraction of SOEF.

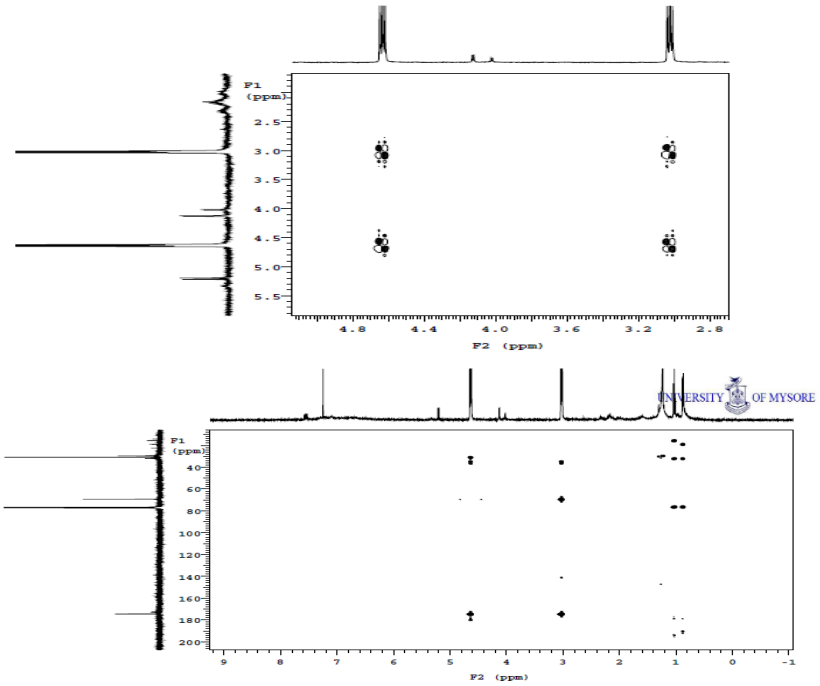

Figure 4B: COSY and HMBC NMR spectra of 3-(4-hydroxyphenyl) propionic acid from active fraction of SOEF.

The homonuclear correlation spectroscopy (COSY) sequence was used to identify spins coupled to each other at 4.6 ppm and 3.0 ppm (Fig. 4B). Heteronuclear Multiple Bond Coherence (HMBC) showed a 2-dimensional inverse of H, C correlation, which allowed and justified the determination of carbon to hydrogen connectivity (Fig. 4B). Through the spectral data it was possible to characterize the solid as 3-(4-hydroxyphenyl) propionic acid. It is commonly called 'Phloretic acid' which is a hydroxy monocarboxylic acid, having propionic acid comprising a 4-hydroxyphenyl group at the 3-position.

Conclusion

The obtained results confirm the potential of antioxidant, antidiabetic and antimicrobial properties of the active fraction of SOEF extract, comparable with the standards.

Further characterization studies of the active fraction have confirmed the lead molecule as 3-(4-hydroxyphenyl) propionic acid. It is an important therapeutic agent known for its antimicrobial, antioxidant and anti-inflammatory potential.

References

1. Ademiluyi A.O. and Oboh G., Soybean phenolic rich extracts inhibit key enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) *in vitro*, *Exp. Toxicol. Pathol.*, **65**, 305-309 **(2013)**

2. Bicalho B., Goncalves R.A., Zibordi A.P.M., Manfio G.P. and Marsaioli A.J., Antimicrobial compounds of fungi vectored by *Clusia* SPP. (Clusiaceae) pollinating bees, *Z Naturforschung C.*, **58**, 746-751 **(2003)**

3. Brand-Williams W., Cuvelier M.E. and Berset C., Use of a free radical method to evaluate antioxidant activity, *LWT*, **26**, 25-30 **(1995)**

4. Butler M.S., The role of natural product chemistry in drug discovery, *J. Nat. Prod*., **67**, 2141-2153 **(2004)**

5. Chandra S., Endophytic fungi: novel sources of anticancer lead molecules, *Appl. Microbiol. Biotechnol*., **95**, 47-59 **(2012)**

6. Dias D.A., Urban S. and Roessner U., A historical overview of natural products in drug discovery, *Metabolites*, **2(2)**, 303-336 **(2012)**

7. Fináková S., Šturdíková M. and Múčková M., Bioactive secondary metabolites produced by microorganisms associated with plants, *Biol*., **62**, 251-257 **(2007)**

8. Gopal N.M., Tejaswini J., Mantry S. and Kumar S.A., International standards of medicinal plants, *Int. J. Innov. Pharm. Sci. Res*., **2**, 2498-2532 **(2014)**

9. Guo L., Wu J.Z., Han T., Cao T., Rahman K. and Qin L.P., Chemical composition, antifungal and antitumor properties of ether extracts of *Scapania verrucosa* Heeg and its endophytic fungus *Chaetomium fusiforme*, *Molecules*, **13**, 2114-2125 **(2008)**

10. Harvey A.L., Edrada-Ebel R. and Quinn R.J., The re-emergence of natural products for drug discovery in the genomics era, *Nat. Rev. Drug Discov*., **14(2)**, 111-129 **(2015)**

11. Kumar D.S.S. and Hyde K.D, Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*, *Fungal Divers*, **17**, 69-90 **(2004)**

12. Li Y., Peng G., Li Q., Wen S. and Huang T.H., *Salacia oblonga* improves cardiac fibrosis and inhibits post-prandial hyperglycemia in obese Zucker rats, *Life Sci.*, **75(14)**, 1735-1746 **(2004)**

13. Madhusudhan M.C., Bharathi T.R. and Prakash H.S., A Review on Isolation and Purification of Bioactive Metabolites from Fungal Endophytes, *Curr. Biochem. Eng.*, **2(2)**, 111-117 **(2015)**

14. Mattana C.M., Satorres S.E., Sosa A., Fusco M. and Alcaraz LE., Antibacterial activity of extracts of *Acacia aroma* against methicillin-resistant and methicillin-sensitive *Staphylococcus*, *Braz. J. Microbiol*., **41(3)**, 581-587 **(2010)**

15. Newman D.J. and Cragg G.M., Natural products as source of new drugs over the 30 yrs from 1981 to 2010, *J. Nat. Prod*., **75(3)**, 311-335 **(2012)**

16. Reddy Gopa Sudheer Kumar, Brahman Pradeep Kumar and Usenireddy Mallu, Synthesis, Identification and Charcaterization of Potential impurities of Pomalidomide, *Res. J. Chem. Environ*., **27(3)**, 86-92 **(2023)**

17. Roopa G., Madhusudhan M.C., Nischita R. and Geetha N., *In vitro* studies on anti-diabetic, anti-bacterial and phytochemical activities of endophytic fungal extracts from Salacia species, *J. Med. Plant. Stud*., **10(4)**, 31-37 **(2022)**

18. Roopa G., Madhusudhan M.C., Prakash H.S. and Geetha N., Diversity of fungal endophytes from *Salacia* species in Western Ghats of Karnataka, India, *Res. J. Pharm. Biol. Chem. Sci*., **8(2)**, 449-455 **(2017)**

19. Saikkonen K., Forest structure and fungal endophytes, *Fungal Biol. Rev*., **21(2)**, 67-74 **(2007)**

20. Sarker S.D., Nahar L. and Kumarasamy Y., Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth and its application in the *in-vitro* antibacterial screening of phytochemicals, *Methods*, **42(4)**, 321-324 **(2007)**

21. Siddiqui A.A., Iram F., Siddiqui S. and Sahu K., Role of natural products in drug discovery process, *Int. J. Drug Dev. Res*., **6(2)**, 172–204 **(2014)**

22. Stone J.K., Bacon C.W. and White J.F., An overview of endophytic microbes: endophytism defined, In Bacon C.W. and White J.F., Eds., Microbial Endophytes, Marcel Dekker, New York, **3**, 29-33 **(2000)**

23. Strobel G., Daisy B., Castillo U. and Harper J., Natural products from endophytic microorganisms, *J. Nat. Prod*., **67(2)**, 257-268 **(2004)**

24. Surekha C., Srikanth R., Thupurani M.K., Neelapu N.R.R. and Peddireddy V., Antimicrobial Activities of *Salacia oblonga* Wall Leaf and Root Extracts Against Different Bacterial Strains and Fungal Isolates, *Curr. Microbiol*., **79(7)**, 204-213 **(2022)**

25. Tan R.X. and Zou W.X., Endophytes: a rich source of functional metabolites, *Nat. Prod. Rep*., **18(4)**, 448-459 **(2001)**

26. Zhang Y., Mu J., Feng Y., Kang Y., Zhang J., Gu P.J. and Zhu Y.H., Broad-spectrum anti-microbial epiphytic and endophytic fungi from marine organisms: isolation, bioassay and taxonomy, *Mar. Drugs*, **7(2)**, 97-112 **(2009)**.

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