



Bioactive compounds from mangrove derived rare actinobacterium *Saccharomonospora oceani* VJDS-3

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Abstract

A rare actinobacterium was isolated from Nizampatnam mangrove ecosystem of Andhra Pradesh, India, and was screened for its ability to produce bioactive compounds. The potential strain was identified as *Saccharomonospora oceani* VJDS-3 by polyphasic taxonomy. Purification of the biologically active compounds by column chromatography led to the isolation of three compounds, namely methoxy ethyl cinnamate (ethyl(E)-3-(4-methoxyphenyl)acrylate) (**R1**), 4-hydroxy methyl cinnamate (methyl(E)-3-(4-hydroxyphenyl)acrylate) (**R2**) and 4-methylbenzoic acid (**R3**). The structure of the compounds was elucidated on the basis of spectroscopic analysis including FTIR, EIMS, ¹HNMR and ¹³CNMR spectroscopies. The antimicrobial activity of the bioactive compounds produced by the strain was tested against a panel of bacteria and fungi, and expressed in terms of minimum inhibitory concentration. Compound (R1) exhibited higher antimicrobial potential (50 µg/ml) against *Staphylococcus aureus*, *Bacillus megaterium* and *Candida albicans* compared to R2 and R3. Antioxidant activity of compounds was determined by DPPH and ABTS radical scavenging activities. The results revealed that compound R3 effectively scavenged DPPH (73.08 ± 1.29) and ABTS (99.74 ± 0.00) radicals at a concentration of 25 and 50 µg/ml, respectively. Antidiabetic and anti-obesity activities were evaluated by inhibitory potential of compounds against alpha-glucosidase, alpha-amylase and pancreatic lipase by spectrophotometric assays. Compound R1 showed effective inhibition against alpha-glucosidase (66.8 ± 1.2) at 20 µg/ml while moderate to weak activities were found against alpha-amylase and pancreatic lipase. To the best of our knowledge, this is the first report on the isolation of supra said compounds from the genus *Saccharomonospora*.

Keywords *Saccharomonospora oceani* · Actinomycetes · Mangrove ecosystem · Antioxidant activity · Antidiabetic activity

Introduction

The frequent rediscovery of known antibiotics from the terrestrial actinobacteria has limited the development of novel and effective drugs, and raised concerns over the future treatment of infections (Manivasagan et al. 2014). At the same time, marine-derived compounds are more attractive and efficient in action against the pathogens resistant to the known

antibiotics (Donia and Humman 2003). In addition to drug-resistance problem, the frequent breakdown of the epidemic diseases and the magnitude of their dissemination among people, patient's sensitivity and inability to control certain infectious diseases have given necessity for continuous search of new antibiotics all over the world. The requirement for discovery and isolation of novel chemical structures from unique untapped niches is undisputed. This is achieved by investigating new microbial sources for production of antimicrobial agents from unexplored habitats (Xu et al. 2014). Microbial diversity of the mangrove ecosystem is one of the promising research areas which needs extensive exploration for understanding the biogeography, community assembly and ecological processes (Curits et al. 2002; Das et al. 2006).

Mangrove ecosystem is one of the most productive ecosystems of tropical and subtropical regions of the world. The environment of the mangrove ecosystem is saline and

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highly rich in organic matter because of its various microbial activities (Kizhekkedathu and Parukuttyamma 2005). The ecosystem existing between terrestrial and marine environment is an ideal habitat that supports rich and diverse group of microorganisms (Arifuzzaman et al. 2010). The secondary metabolites produced by mangrove plants are toxic to residing microbes, need to be degraded or detoxified for their survival. In this situation, microbes are under high pressure to sustain and eventually adapt to the existing conditions for their survival and evolve to produce novel bioactive secondary metabolites (Sengupta et al. 2015). There are evidences that compounds with unique structures and potential medicinal use have been isolated from mangrove actinobacteria in recent years which include alkaloids, benzene and cyclopentenone derivatives, dilactones, macrolides, 2-pyranones, sesquiterpenes, salinosporamides, xiamycins and novel indolocarbazoles (Xu et al. 2014).

Actinobacteria can be divided into two groups, namely *Streptomyces* or non-*Streptomyces* or rare actinobacteria (Azman et al. 2015). Even though species of *Streptomyces* are well known for their proven ability to produce antibiotics and other molecules of pharmaceutical interest, relatively rare actinobacteria are less scrutinized for bioactive metabolites. Rare actinobacteria have been considered as actinobacteria with lower isolation rates compared to *Streptomyces* strains using conventional isolation methods, due to the requirement of specific chemical and physical pre-treatment of the samples, enriched isolation media and fine-tuning of culture conditions (Khanna et al. 2011). The bioactive research of natural metabolites from the under explored habitats of mangrove rare actinobacteria have become popular. Compounds uncovered from the mangrove rare actinobacteria are uniquely structured and lead directly to the advancement of novel antibiotics that are active against antibiotic-resistant pathogens (Lam 2006). Many new compounds identified from the rare actinobacteria are proven effective in clinical and pharmaceutical industries (Azman et al. 2015).

Our research group have isolated diverse bioactive compounds from mangrove actinobacteria with effective antimicrobial and anticancer activities (Usha Kiranmayi et al. 2015, 2016a, b, c). A new semisynthetic compound from Nizampatnam mangrove ecosystem has also been reported (Usha Kiranmayi et al. 2016d). In the present study, a rare actinobacterium *Saccharomonospora oceani* was isolated from relatively unexplored regions of Nizampatnam mangrove ecosystem with the help of selective pre-treatment and enrichment procedures. The isolate was found to be active against a panel of pathogens including Gram positive and Gram negative bacteria as well as fungi. The optimum conditions were designed to achieve the highest quantities of secondary metabolites with functional properties retained (Manideepa et al. 2016b). The objectives of the existing study include fermentation, purification, chemical structure

elucidation and biological evaluation of purified compounds produced by *Saccharomonospora oceani* responsible for the antimicrobial potential.

Materials and methods

Isolation and identification

The actinobacterium, VJDS-3, was isolated from the Nizampatnam mangrove ecosystem by employing soil-dilution plate technique. The air-dried sediment samples were pre-treated with calcium carbonate (10:1 w/w) and plated on selective media such as humic acid vitamin agar supplemented with 3% NaCl. The strain was identified as *Saccharomonospora oceani* by morphological, cultural, physiological, biochemical and molecular (16 S rDNA analysis) approaches. The rDNA sequence was deposited in the NCBI GenBank with the accession number KP231373 (Manideepa et al. 2016a). Pure culture of actinobacterium was maintained on yeast-extract malt-extract dextrose (YMD) agar slants at 4 °C for further study.

Fermentation

To prepare the seed culture, a loopful of the strain VJDS-3 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of ISP-2 (International Streptomyces Project) medium composed of yeast extract 0.4%, malt extract 1%, dextrose 0.4% and CaCO₃ 0.2% with pH 7.0. The inoculated flasks were incubated on rotatory shaker (120 rpm) at 30 °C for 48 h. Seed culture at concentration of 10% was transferred into the optimized fermentation medium consisting of maltose (1%), tryptone (0.5%), K₂HPO₄ (0.05%) and NaCl (3%) with pH adjusted to 7.2. The bioactive metabolites from the fermented broth were harvested by filtration of biomass through Whatman filter paper no. 42 (Merck, Mumbai, India). The culture filtrate (50 l) obtained after cultivation of the strain for 8 days were extracted twice with an equal volume of ethyl acetate, pooled and the organic layer was concentrated by rotary evaporation and freeze dried to yield 4.2 g of deep brown semi-solid crude extract.

Purification and structure elucidation

The separation of crude extract was conducted via gradient elution system of hexane:ethyl acetate. The eluent was run over the column and small volumes of eluent collected in test tubes were analysed via thin-layer chromatography (TLC) using silica gel plates (Silica gel, Merck, Mumbai, India) with hexane:ethyl acetate solvent system. Compounds with identical retention factors (R_f) were combined and assayed for antimicrobial activity against Gram positive (*Bacillus*

megaterium), Gram negative (*Escherichia coli*) bacteria and yeast (*Candida albicans*) using agar well diffusion assay (Cappuccino and Sherman 2004).

Among the 20 main fractions eluted, 19 polar residues and one non-polar residue were obtained. Three polar fractions exhibited high antimicrobial activity while the non-polar fraction did not exhibit significant bioactivity. These three polar fractions designated as R1, R2 and R3 were collected at different eluent systems of hexane:ethyl acetate viz. 70:30 v/v; 60:40 v/v and 40:60 v/v, respectively. Fractions R1, R2 and R3 with slight impurity were rechromatographed to get pure compounds. The structures of active compounds were analysed employing Fourier transform infrared (FTIR), model: Thermo Nicolet Nexus 670 spectrophotometer with NaCl optics, electron ionization mass/electron spray ionization mass spectrophotometry (EIMS/ESIMS); model: Micromass VG-7070H, 70 eV spectrophotometer and Nuclear Magnetic Resonance (^1H NMR and ^{13}C NMR) model: Varian Gemini 200 and samples were made in CDCl_3 with trimethyl saline as standard.

Biological assays

Minimum inhibitory concentration (MIC) of bioactive compounds

The minimum inhibitory concentration (MIC) of the bioactive compounds produced by the strain was determined against Gram positive and Gram negative bacteria as well as fungi using agar well diffusion assay (Cappuccino and Sherman 2004).

Test microorganisms

The cultures of *S. aureus* (MTCC 3160), *B. megaterium* (NCIM 2187), *Bacillus subtilis* (ATCC 6633), *Xanthomonas campestris* (MTCC 2286), *Proteus vulgaris* (MTCC 7299), *Pseudomonas aeruginosa* (ATCC 9027), *E. coli* (ATCC 35218), *Enterococcus faecalis* (MTCC 439), *Streptococcus mutans* (MTCC 497), *Lactobacillus casei* (MTCC 1423) and *Lactobacillus acidophilus* (MTCC 495) were employed for antibacterial assay. *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 1015), *Aspergillus flavus* (ATCC 9643), *Fusarium oxysporum* (MTCC 3075), *Fusarium solani* (MTCC 4634) and *Penicillium citrinum* (MTCC 6489) were used for testing antifungal activity.

Testing the minimum inhibitory concentration of the metabolites by agar well diffusion assay

The minimum inhibitory concentration (MIC) of bioactive compounds produced by the strain was determined against Gram positive as well as Gram negative bacteria and fungi

using agar plate diffusion assay. Nutrient agar and Czapek-Dox agar media were used for culturing bacteria and fungi, respectively. Sterilized agar medium seeded with test bacterial suspension was transferred to Petri plates under aseptic conditions. After the solidification of agar medium, wells about 6 mm diameter were cut with a sterilized cork borer. In case of antifungal assay, test fungus (10^5 spores/ml) was plated on to the solidified agar medium. Metabolites dissolved in dimethyl sulfoxide (DMSO) at concentrations ranging from 0 to 1000 $\mu\text{g/ml}$ were added to the wells. After inoculation, the plates were incubated at 30 °C and examined after 24–48 h of incubation for bacteria and 48–72 h for yeast and filamentous fungi. The experiment was carried out in triplicates and the solvent (DMSO) alone was kept as negative control. tetracycline, griseofulvin and amphotericin-B were employed as positive controls for bacteria and fungi. The lowest concentration of bioactive compound exhibiting antimicrobial activity against the test microbes was taken as MIC of the compound.

Measuring antioxidant activity by DPPH

To identify the anti-oxidant activity of pure compounds of *S. oceani* VJDS-3, free-radical scavenging activity of each compound was assayed by DPPH (1,1-diphenyl-2-picrylhydrazyl) (Tiwari et al. 2011a). 25 μl concentration of each compound and standard ascorbic acid were mixed with 100 μl of 0.1 M Tris-HCl buffer (Ph 7.4) and 125 μl DPPH solution (0.5 mM in methanol) in separate tubes. The tubes were incubated in dark at room temperature for 30 min and the optical density was measured at 517 nm corresponding to blank using a UV-Vis spectrophotometer. All the experiments were made in triplicates and the results were represented in mean \pm SD. The percentage of free-radical scavenging activity is determined by the following equation.

$$\begin{aligned} \text{\% of free radical scavenging} \\ = (\text{Abs of control} - \text{Abs of test}) / \\ (\text{Abs of control}) \times 100. \end{aligned}$$

Abs = absorption.

IC_{50} values were calculated using linear regression analysis. The IC_{50} values signify the concentration of sample, which is appropriate in scavenging 50% of the DPPH free radicals.

ABTS radical scavenging activity

ABTS free-radical scavenging activity was performed as per the standard protocol (Tiwari et al. 2011a). 100 ml stock solution of ABTS (2,2'-azinobios diammonium salt) (0.5 mM) was prepared by addition of 1 ml potassium persulfate (6.8 mM PBS, pH 8.0) and stored in dark at room

temperature for 24 h. In a 96-well micro plate, 10 μ l of compound was mixed with 190 μ l of ABTS radical solution and incubated in dark for 15 min at room temperature. Decolorized ABTS absorbance was measured spectrophotometrically at 734 nm.

% of free radical scavenging

$$= (\text{Abs of control} - \text{Abs of test}) / (\text{Abs of control}) \times 100$$

Antidiabetic activity

Intestinal alpha-glucosidase inhibition assay

To investigate the inhibitory effect of the compounds from mangrove actinomycetes, an in vitro alpha-glucosidase inhibition test was performed. Inhibition of rat intestinal alpha-glucosidase enzyme was determined as per the standard protocols (Tiwari et al. 2011b). 20 μ l of compound (1 mg/ml DMSO) was incubated with 50 μ l of crude intestinal alpha-glucosidase for 5 min and then with 50 μ l of substrate 5 mM *p*-nitro phenyl-alpha-D-glucopyranoside. Acarbose was used as reference drug as alpha-glucosidase inhibitor. Absorbance was measured at 405 nm. Percent enzyme inhibition was calculated by the following equation.

$$\% \text{ of inhibition} = (\text{Abs of control} - \text{Abs of test}) / (\text{Abs of control}) \times 100$$

Pancreatic alpha-amylase inhibition assay

Inhibition of alpha-amylase enzyme was determined as per the standard protocols (Tiwari et al. 2011b). 40 μ l of compound was reconstituted in 100 μ l phosphate buffer (20 mM, pH 6.8) containing 6.7 mM sodium chloride in 2-ml Eppendorf tubes and incubated with 200 μ l porcine pancreatic alpha-amylase for 10 min. Then, 100 μ l of soluble potato starch solution (0.5%) was prepared and added to each of the tube and incubated again at 25 $^{\circ}$ C for 10 min. The ongoing reaction was stopped by addition of 400 μ l of DNS (Dinitro salicylic acid). Closed tubes were placed in water bath (85–90 $^{\circ}$ C) for 10 min to develop colour and then cooled at room temperature. The reaction mixture (50 μ l) was subsequently diluted with 175 μ l of distilled water in a 96-well micro plate and absorbance (540 nm) was read spectrophotometrically. Percentage of inhibition was calculated by the following formula:

$$\% \text{ of inhibition} = (\text{Abs of control} - \text{Abs of test}) / (\text{Abs of control}) \times 100$$

Pancreatic lipase inhibition assay

Inhibition of pancreatic lipase was used for evaluation of in vitro anti-obesity activities of the three compounds isolated from mangrove actinobacteria (Roh and Jung 2012). The standard drug taken for the present study is Orlistat. The test solution contains *p*-nitrophenyl butyrate as a

substrate using 0.1 M potassium phosphate buffer (pH 7.2). To 95 μ l of phosphate buffer, 25 μ l of porcine pancreatic lipase extract (1 mg/ml) was added to micro plate wells and then 30 μ l of compounds (50 μ g/ml) or 30 μ l of reference compound Orlistat was mixed and pre-incubated for 30 min at 37 $^{\circ}$ C. After pre-incubation, the reaction was initiated by adding 50 μ l of *p*-nitro phenyl butyrate (10 mM). The samples were incubated at 37 $^{\circ}$ C for 40 min and the amount of *p*-nitro phenol released in the reaction was measured using UV-Visible spectrophotometer at 415 nm. The results were compared with Orlistat. The percentage inhibition was calculated as:

% of inhibition = Absorbance of control

$$- [\text{Absorbance of sample with enzyme} \\ - \text{Absorbance of sample without enzyme}] / \\ \text{Absorbance of control} \times 100$$

Results and discussion

Culture filtrates pooled after 8 days of fermentation were extracted with ethyl acetate and concentrated to yield a deep brown semi-solid compound, which in turn was subjected to silica gel column chromatography using the solvent system of hexane/ethyl acetate. Among the 20 main fractions eluted, three fractions with slight impurities exhibited significant antimicrobial activity were rechromatographed on silica gel column yielding three pure compounds R1, R2 and R3.

Compound-R1 was obtained as white amorphous powder (5 mg). The IR spectrum displayed absorption bands at 1706.47, 1285.60, 1173.12 cm^{-1} (Supplementary Fig. A). The molecular formula $\text{C}_{12}\text{H}_{14}\text{O}_3$ established from its molecular ion peak at m/z 207 [$\text{M}^+ + \text{H}$] in the ESI-MS spectrum (Supplementary Fig. B). The ^1H NMR (CDCl_3 , 300 MHz) spectrum demonstrated signals at 7.65 (1H, d, $J = 16$ Hz), 7.45 (2H, d, $J = 8.8$ Hz), 6.90 (2H, d, $J = 8.8$ Hz), 6.30 (1H, d, $J = 16$ Hz), 4.25 (2H, t, $J = 6$ Hz), 3.80 (3H, -OCH₃), 1.32 (3H, CH₃) (Supplementary Fig. C). The ^{13}C NMR (CDCl_3 , 75 MHz) spectrum indicated peaks at δ 167.14, 161.01, 144.02, 129.30, 129.30, 129.68, 115.11, 113.89, 113.83, 59.97, 59.80 and 13.83 ppm (Supplementary Fig. D). The ^1H NMR spectrum displayed the two doublets at 7.65 (1H, d, $J = 16$ Hz), 6.30 (1H, d, $J = 16$ Hz) were assigned to olefinic protons. The higher coupling constants suggested the presence of *trans*-double bond. Further, triplets at 4.18 (2H, t, $J = 6.7$), and 0.88 (3H, t, $J = 6.9$ Hz) indicate the presence of a side-chain linkage in the compound. Two doublets at δ 6.90 (2H, d, $J = 8.8$ Hz), 7.45 (2H, d, $J = 8.8$ Hz) indicating the presence of 1,4-disubstituted aromatic ring pattern-type substitution. The ^{13}C NMR spectrum indicated the presence of 13 carbons. The peak at 1709 cm^{-1} (C-9) was assigned to ester carbonyl which was further correlated with the IR absorption band at 1675 cm^{-1} .

Further, the signals at 115.11 (C-2) and 129.30 (C-5) were assigned to the aromatic and double bond carbons. Thus, based on IR, mass, NMR data and in comparison with the reported literature, the structure of compound R1 was assigned as 4-methoxy ethyl cinnamate (ethyl(E)-3-(4-methoxyphenyl)acrylate) (Fig. 1).

Compound R2 was obtained as white amorphous powder (5 mg). The IR spectrum displayed absorption bands at 2936, 1685, 1597 cm^{-1} (Supplementary Fig. E). The molecular formula $\text{C}_{10}\text{H}_{10}\text{O}_3$ established from its molecular ion peak at m/z 179 [$\text{M}^+\text{+H}$] in the ESI-MS spectrum (Supplementary Fig. F). The ^1H NMR (CDCl_3 , 300 MHz) spectrum demonstrated signals at 7.70(1H, d, $J = 15.8$ Hz), 7.5 (2H, d, $J = 8.8$ Hz), 6.8(2H, d, $J = 8.8$ Hz), 6.3(1H, d, $J = 15.8$ Hz), 3.8 (3H, s) (Supplementary Fig. G). The ^{13}C NMR (CDCl_3 , 75 MHz) spectrum indicated peaks at δ 169.70, 161.294, 145.18, 129.68, 129.68, 126.95, 115.31, 114.18, 114.18 and 55.21 ppm (Supplementary Fig. H). The ^1H NMR spectrum which displayed the two doublets at 7.70 (1H, d, $J = 15.8$ Hz), 6.30 (1H, d, $J = 15.8$ Hz) were assigned to olefinic protons. The higher coupling constants suggested the presence of *trans*-double bond. Two doublets at δ 6.80 (2H, d, $J = 8.8$ Hz), 7.5 (2H, d, $J = 8.8$ Hz) indicate the presence of 1,4-disubstituted aromatic ring pattern-type substitution. The ^{13}C NMR spectrum indicated the presence of 10 carbons. The peak at 169.70 cm^{-1} (C-9) was assigned to ester carbonyl which was further correlated with the IR absorption band at 1685 cm^{-1} . Further, the signals at 115.31 (C-2) and 129.68 (C-5), were assigned to the aromatic and double-bond carbons. Thus, based on IR, mass, NMR data and in comparison with the reported literature, the structure of compound R2 was assigned as 4-hydroxy methyl cinnamate (methyl(E)-3-(4-hydroxyphenyl) acrylate) (Fig. 2).

Compound-R3 was obtained as a solid (15 mg). The IR spectrum displayed absorption bands at 2936, 1685, 1626, 1585, 1247 cm^{-1} (Supplementary Fig. I). The molecular formula $\text{C}_8\text{H}_8\text{O}_2$ established from its molecular ion peak at m/z 137 [$\text{M}^+\text{+H}$] in the ESI-MS spectrum (Supplementary Fig. J). The ^1H NMR (CDCl_3 , 300 MHz) spectrum demonstrated signals at 7.7 (2H, d, $J = 8.8$ Hz), 6.48 (2H, d, $J = 8.8$ Hz), 2.4 (3H, s, CH_3) (Supplementary Fig. K). The ^{13}C NMR (CDCl_3 , 75 MHz) spectrum indicated peaks at δ 172.90, 154.22, 154.22, 148.91, 143.10, 114.25, 114.25

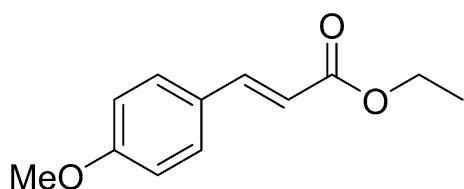


Fig. 1 4-Methoxy ethyl cinnamate (ethyl(E)-3-(4-methoxyphenyl) acrylate)

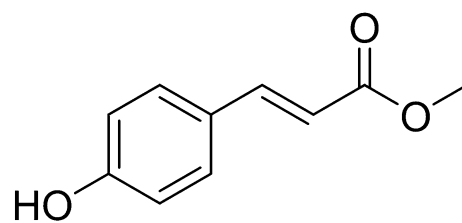


Fig. 2 4-Hydroxy methyl cinnamate (methyl(E)-3-(4-hydroxyphenyl) acrylate)

and 31.43 ppm (Supplementary Fig. L). Two doublets at δ 6.48 (2H, d, $J = 8.8$ Hz), 7.7 (2H, d, $J = 8.8$ Hz) indicate the presence of 1,4-disubstituted aromatic ring pattern-type substitution. The signal at 2.40 (s, 3H) was assigned to methyl group. The ^{13}C NMR spectrum indicated the presence of eight carbons. The peak at 172.90 (C-9) was assigned to acid carbonyl which was further correlated with the IR absorption band at 1685 cm^{-1} . Thus, based on IR, mass, NMR data and in comparison with the reported literature, the structure of compound R3 was assigned as 4-methyl benzoic acid (Fig. 3).

MIC of bioactive compounds produced by *Saccharomonospora oceani* VJDS-3

Antimicrobial activities of the bioactive compounds in terms of MIC are shown in Table 1. The bioactive compounds produced by *S. oceani* VJDS-3 exhibited significant antibacterial and antifungal activity. 4-methoxy ethyl cinnamate (R1) exhibited higher antimicrobial potential than that of 4-hydroxy methyl cinnamate (R2) and 4-methylbenzoic acid (R3). Compound R1 exhibited good activity against *S. aureus* and *B. megaterium* followed by *S. mutans*. Significant activity of R2 was recorded against *L. casei* and *L. acidophilus* followed by *S. aureus* and *B. megaterium* while compound 3 recorded good activity against *S. aureus*. Among the fungi tested, *C. albicans* exhibited high sensitivity to all the compounds.

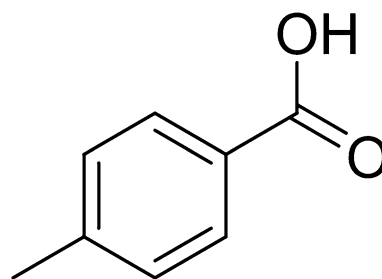


Fig. 3 4-Methyl benzoic acid

Table 1 MIC values of the bioactive compounds produced by *Saccharomonospora oceani* VJDS-3

Test organisms	MIC ($\mu\text{g/ml}$)			
	R1	R2	R3	#Antibiotic
Bacteria				
<i>Staphylococcus aureus</i>	50	70	70	25
<i>Bacillus megaterium</i>	50	70	175	30
<i>Bacillus subtilis</i>	75	90	200	40
<i>Bacillus cereus</i>	75	90	125	25
<i>Xanthomonas campestris</i>	75	100	100	25
<i>Proteus vulgaris</i>	100	120	250	40
<i>Pseudomonas aeruginosa</i>	90	100	225	30
<i>Escherichia coli</i>	90	100	100	30
<i>Enterococcus faecalis</i>	90	100	100	25
<i>Streptococcus mutans</i>	70	80	200	40
<i>Lactobacillus casei</i>	75	50	150	50
<i>Lactobacillus acidophilus</i>	75	50	150	25
Fungi				
<i>Candida albicans</i>	50	70	90	50
<i>Aspergillus niger</i>	100	120	200	5
<i>A. flavus</i>	150	175	150	10
<i>Fusarium solani</i>	175	190	250	10
<i>Fusarium oxysporum</i>	200	250	350	10

Data were statistically analysed by one-way analysis of variance (ANOVA) and found to be significant at 0.05% ($n = 3$)

MIC minimum inhibitory concentration, #Antibiotic tetracycline against bacteria, griseofulvin against yeast and amphotericin-B against fungi, R1 4-methoxy ethyl cinnamate, R2 4-hydroxy methyl cinnamate, R3 4-methylbenzoic acid

Table 2 DPPH and ABTS radical scavenging activities of isolated compounds

Name	DPPH % Inhibition at 25 $\mu\text{g/ml}$ (SC_{50} $\mu\text{g/ml}$)	ABTS % Inhibition at 20 $\mu\text{g/ml}$ (SC 50 $\mu\text{g/ml}$)
Compound-R1	ND	ND
Compound-R2	ND	ND
Compound-R3	$73.08 \pm 1.29(10.32)$	$99.74 \pm 0.00 (3.27)$
Ascorbic acid	$87.25 \pm 0.18(8.69)$	–
Trolox	–	$99.97 \pm 1.0 (2.35)$

Values are mean \pm SD ($n = 3$)

Data were statistically analysed by one-way analysis of variance (ANOVA) and found to be significant at 0.05% ($n = 3$)

DPPH assay

The DPPH radical scavenging activity of the three compounds isolated from *S. oceani* VJDS-3 in correlation with ascorbic acid as standard reference is shown in Table 2. Compounds R1 and R2 displayed no activity at 25 $\mu\text{g/ml}$ while R3 and standard ascorbic acid showed inhibition of

73.08 ± 1.29 and 87.25 ± 0.18 $\mu\text{g/ml}$, respectively, at the same concentration. Though the compound R3 was able to scavenge DPPH free radical and convert it into DPPHH, the scavenging effect of compound was less compared to standard.

ABTS radical scavenging activity

The antioxidant capacity of compounds was evaluated according to ABTS decolorization method. The compounds R1 and R2 exhibited no ABTS free-radical scavenging activity at the concentration of 20 $\mu\text{g/ml}$, whereas R3 displayed significant activity (99.74 ± 0.00) in comparison with reference compound Trolox (99.97 ± 1.0) at the same concentration (Table 2).

Oxidative stress mainly caused by free radicals leads to several diseases in human beings such as atherosclerosis, diabetes, cancer, inflammation, Alzheimer's and Parkinson's. Either increased free radicals or decreased antioxidants can lead to oxidative stress and associated complications. The free radicals (ROS) produced by biochemical reaction in human body cause structural and functional changes in human metabolism. Different types of secondary metabolites from plant and microbial origin that can give protection against oxidative stress induced disorders in humans (Tiwari 2004). Therefore, research is directed towards natural antioxidants from plants and microbes which serve as safe antioxidant agents. As part of our study, we tested for DPPH and ABTS free-radical scavenging activity of the constituents isolated from *S. oceani* VJDS-3, among which compound 3 displayed significant DPPH and ABTS free-radical scavenging activities.

Intestinal alpha-glucosidase inhibition assay

The inhibitory effect of three compounds on alpha-glucosidase was evaluated by in vitro method (Table 3). The compounds R1 and R2 (at a concentration of 20 $\mu\text{g/ml}$) exhibited 66.8 ± 1.2 and 3.8 ± 0.4 $\mu\text{g/ml}$ alpha-glucosidase inhibitory activities, respectively, whereas R3 displayed no activity at 20 $\mu\text{g/ml}$. Acarbose was taken as the standard reference drug that showed alpha-glucosidase inhibitory activity with an IC_{50} value of 93.2 ± 1.2 $\mu\text{g/ml}$.

Pancreatic alpha-amylase inhibition assay

The ability of the compounds to inhibit the alpha-amylase activity in vitro was executed and the results are presented in the Table 3. The compound R3 failed to inhibit alpha-amylase activity. However, R1 and R2 exhibited moderate to weak alpha-amylase inhibitory activity with an IC_{50} values 11.5 ± 0.5 and 3.1 ± 0.3 $\mu\text{g/ml}$, respectively, when compared with acarbose (86.6 ± 0.2 $\mu\text{g/ml}$) at the concentration of

Table 3 Effects of bioactive compounds on alpha-glucosidase, alpha-amylase and pancreatic lipase

Name	M-AGI % Inhibition at 20 µg/ml (IC ₅₀ µg/ml)	Alpha-amylase % inhibition at 40 µg/ml (IC ₅₀ µg/ml)	Lipase % Inhibition at 50 µg/ml (IC ₅₀ µg/ml)
Compound-R1	66.8 ± 1.2(13.54)	11.5 ± 0.5	28.8 ± 1.6
Compound-R2	3.8 ± 0.4	3.1 ± 0.3	18.8 ± 3.1
Compound-R3	ND	ND	ND
Acarbose	93.2 ± 1.2 (5.66)	86.6 ± 0.2 (7.02)	–
Orlistat	–	–	58.3 ± 2.1

Values are mean ± SD ($n = 3$)

Data were statistically analysed by one-way analysis of variance (ANOVA) and found to be significant at 0.05% ($n = 3$)

40 µg/ml. Alpha-amylase inhibitory activity was measured by the amount of starch in the presence of alpha-amylase enzyme. The increased intensity of blue colour indicates the presence of starch due to the presence of alpha-amylase inhibitory activity while the reduced intensity of blue colour in the reaction mixture indicates the hydrolysis of starch into mono-saccharides by alpha-amylase.

Pancreatic lipase inhibition assay

The percentage inhibition potential of pancreatic lipase of three compounds is documented in Table 3. The compounds R1 and R2 displayed moderate pancreatic lipase inhibition with an IC₅₀ values 28.8 ± 1.6 and 18.8 ± 3.1 µg/ml, respectively, at the concentration of 50 µg/ml, whereas compound R3 did not exhibit any inhibition.

Diabetes is becoming pandemic globally and it is widely accepted that most challenging goal in the management of patients of diabetes mellitus is to achieve blood glucose level as close to the normal as possible (Dalglish et al. 2002). In addition, postprandial hyperglycemia is independent risk factor for the development of macrovascular complications in diabetes mellitus (Paterson et al. 2003). Since alpha-glucosidase catalyses the final step in carbohydrate digestive process, it could retard the use of dietary carbohydrates to suppress postprandial hyperglycemia (Saklatvala et al. 2003). The enzymes alpha-glucosidase, alpha-amylase and pancreatic lipase are popular targets for the management of postprandial hyperglycemia and triglyceridemia which are components of metabolic syndrome, and specifically in the management of the diabetes and obesity. Pancreatic lipase inhibitory activity has been extensively used for the determination of potential efficacy of natural products as anti-obesity agents (Seyeden et al. 2015). In the course of our efforts in identifying anti-hyperglycaemics and anti-obesity agents from *S. oceani* VJDS-3, we have isolated three compounds from the crude ethyl acetate extract among which compounds R1 and R2 displayed significant antidiabetic and anti-obesity activities and can be used for further studies.

Saccharomonospora, a rare actinobacterium genus is much less exploited for bioactive metabolites than the other strains of the order *Actinomycetales*. Maloney et al. (2009) isolated an unprecedented alkaloid Lodopyridone A from culture broth of marine *Saccharomonospora* sp. active against HCT-116 human colon cancer cells with IC₅₀ of 3.6 µM. Yamanka et al. (2014) successfully expressed a 67-kb nonribosomal peptide synthetase biosynthetic gene cluster from the marine actinomycete *Saccharomonospora* sp. CNQ-490 and produced dichlorinated lipopeptide antibiotic taromycin A in the expression host *Streptomyces coelicolor*. In this way, by genome mining, the supra said strain has been shown to possess a full biosynthetic pathway to produce a new antibiotic taromycin A through direct cloning and refactoring methods.

Purification and structure elucidation of two novel natural products Lodopyridones B and C by HPLC–UV guided isolation of the culture broth of a marine actinobacterium *Saccharomonospora* sp. CNQ-490 were recorded (Le et al. 2017). Chemical structures of the active compounds were established from the interpretation of 2D NMR spectroscopic data and comparison of NMR data with Lodopyridone A. Lodopyridones B and C exhibited weak inhibitory activities on the β-site amyloid precursor protein-cleaving enzyme 1 (Le et al. 2017). Yim et al. (2017) purified three new α-pyrones, saccharomonopyrones A–C from the marine sediment-derived actinobacterium *Saccharomonospora* sp. CNQ-490. Saccharomonopyrone A is the first α-pyrone microbial natural product which possesses ethyl-butyl ether chain in the molecule, while saccharomonopyrone B and C have unusual 3-methyl and a 6-alkyl side chain within a 3,4,5,6-tetra substituted alpha-pyrone moiety. Saccharomonopyrone A exhibited weak antioxidant activity.

The natural, semisynthetic and synthetic cinnamic acid esters and their derivatives are proven to have diverse pharmacological activities such as anticancer, antibacterial, antifungal, antioxidative, antidiabetic, antiinflammatory, antitubercular, anti-HIV and antiparasitic activities. Especially cinnamic acid derivatives have significant antiparasitic and antifungal activities, and these are often used as

promising starting compounds for the development of novel and potential drugs (Zhou et al. 2017; Guzman 2014). In the present study, three bioactive molecules from *S. oceani*—VJDS-3 are reported. These bioactive compounds showed moderate to weak antimicrobial activity and notable antioxidant and alpha-glucosidase inhibition activities. However, this is the first report of purification and characterization of 4-methoxy ethyl cinnamate (ethyl(E)-3-(4-methoxyphenyl)acrylate), 4-hydroxy methyl cinnamate (methyl(E)-3-(4-hydroxyphenyl)acrylate) and 4-methylbenzoic acid from the genus *Saccharomonospora*.

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Compliance with ethical standards

Conflict of interest All the authors declare that there is no conflict of interest.

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