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Research Article

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Fatty acid based antimicrobials from *Streptomyces* sp. SORS-24, an endophyte isolated from *Sonchus oleraceus*

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Abstract

Due to the rise in bacterial resistance towards various therapeutic agents, interest is now developing towards fatty acid based antimicrobials because of their non-specific mode of action. A strain SORS 24 isolated from *Sonchus oleraceus* (Sow thistle) showed significant activity against *Escherichia coli* ATCC 25922 (25 mm), *Chlorella vulgaris* (20 mm), *Bacillus subtilis* DSM 10 (ATCC 6051) and *Pseudomonas* sp. (15 mm). It displayed an LC_{50} value of 10 μg/ml against *Artemia salina* (Brine shrimp) nauplii and an EC_{50} value of 0.8 μg/ml in the (DPPH) diphenylpicrylhydrazyl antioxidant assay. The strain also displayed genotoxicity against a *PolA* deficient strain, *E. coli* K-12 AB 3027 (15 mm). Mass spectrometry (HPLC-MS) showed that the strain produced oleamide (9-Octadecenamide) and erucamide (13-Docosenamide). Both of the purified fatty acid amides showed prominent activity against *B. subtilis* DSM 10 (ATCC 6051) (20 mm) and *E. coli* ATCC 25922 (15 mm). Significant genotoxicity was observed against *E. coli* K-12 AB 3027 (15 mm). The 16S gene sequencing revealed that the strain belonged to species, *Streptomyces tanashiensis*. As far as our understanding, this is the first report of this species producing these fatty acid based antimicrobials.

Significance And Impact Of Study

With the issue of resistance in bacteria on the rise, interest is now developing towards fatty acid based antimicrobials. Because of their non-specific action, it is difficult for the bacteria to acquire resistance against them. They are also non-toxic, naturally abundant, and renewable compounds. Our study was aimed to screen the actinomycetes in medicinal plants of Pakistan for such fatty acid based antimicrobial compounds as potential therapeutic agents.

Keywords: antimicrobials, endophytes, fatty acid amides, *sonchus*, *streptomyces*

Introduction

Fatty acids consists of a methyl group and a reactive carboxylic acid connected through hydrocarbon chains (Fattahi et al. 2020). Their amide derivatives contain nitrogen and are formed when fatty acids react with heterocyclic groups (Yosief and Sarker 2021). Prior studies have observed that their bioactivity increases with an increase in the number of carbon atoms in the skeleton (Gopalakrishnan et al. 2016). Initial studies (Kabara et al. 1972) reported that the amide derivative imparts a stronger antimicrobial effect than free fatty acids (Chen et al. 2020). However, most recently the derivatives containing amide as well as an ester bond were observed to be more effective as compared to derivatives with only an amide bond (Fattahi et al. 2020).

The bioactivity of fatty acid amides have been known since a long time but recently more studies have demonstrated their immense pharmacological potential (Yosief and Sarker 2021). Due to their effect on cell proliferation of tumor cell lines, reports indicate their possible role in cancers (Jug et al. 2020). One of known amide derivative of oleic acid, oleamide is a bioactive signaling molecule in the mammalian brain (Çakir et al. 2020) and is reported to be biologically active with prominent inhibitory activity against pathogenic bacteria (Jug et al. 2020, Naumoska et al. 2020). The anti-inflammatory effect of oleamide has been proven as well and it was comparable to diclofenac, a non-steroidal anti-inflammatory drug (NSAID) (Naumoska et al. 2020).

Although the bioactivity of fatty acid amides has been known since long but not much interest has been put in developing them as potential pharmaceutical agents until now. Due to the increasing issue of antibiotic resistance, there is a renewed interest in them mainly because they are widely bioactive and have a mode of action that is non-specific. Hence, it is challenging for bacteria to acquire resistance against them. Their non-toxicity and abundance in nature as well as enhancement of activity through chemical modification makes them attractive candidates for new antimicrobials (Yosief and Sarker 2021).

The present study was carried out to explore the endophytic actinomycetes residing in the unexplored niche of medicinal plants of Pakistan. Since no earlier reports exist on the fatty acid amides from *Streptomyces tanashiensis* and their antimicrobial activity, therefore this study report new findings that may lead to novel therapeutic possibilities.

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Materials and methods

Collection of *Sonchus oleraceus* L. and strain isolation

Sonchus oleraceus L was observed to be growing in an empty flowerbed next to the gene lab building (31.49° N, 74.47° E) situated in the institute of microbiology and molecular genetics (iMMG), University of the Punjab, Lahore. The plant was dug carefully so its roots remained intact and it was brought to the lab in a labelled bag.

The plant was washed under tap water to remove any soil particles, cut into 0.5 cm segments, and surface sterilised (Qin et al. 2011). Briefly, the segments were immersed in 70% ethanol for 5 min and then in 0.9% NOCl for 20 min. It was followed by immersion in 10% $NaHCO_3$ for 10 min. In the last step, the segments were washed using autoclaved distilled water. The segments were placed on two media, actinomycetes isolation agar (Difco laboratories, USA) and glycerol casein KNO_3 agar (Küster and Williams 1964). They were incubated for 1–3 weeks at 28°C. A separate plate was incubated after dragging the cut segments on the surface of the media in order to check surface sterility. In cases of contamination, the sterilisation procedure was optimised (Qin et al. 2011) by changing the sterilisation time of each step until complete sterility was obtained. The growth resulting in rough, embedded colony was selected and sub-cultured on GYM (Glucose yeast extract malt extract) agar (Shirling and Gottlieb 1966).

Selection through preliminary screening

For determining the strains as biologically active, a preliminary antimicrobial activity testing was performed using agar plug method (Fatima et al. 2019). The results revealed a strain, SORS 24 to be bioactive against Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) test bacteria.

Morphological, biochemical, and physiological characterization

The morphological properties of SORS 24 were recorded after sub culturing it on GYM agar for up to 21 days. The slides of the strain were prepared (Kieser et al. 2000) and the aerial and substrate mycelium were studied under the microscope. The physiological properties of the strain was determined using different sugars as source of carbon and formation of melanin as described by the standard International *Streptomyces* Project (ISP). An additional nine biochemical tests were carried out (Shirling and Gottlieb 1966).

16S gene sequencing and GenBank submission

For the 16S rRNA sequencing, total genomic DNA was extracted (Tanvir et al. 2013) and sent to a commercial sequencing facility (Macrogen Inc., Seoul, Korea). To check the reliability of the sequence, a boot-strap test was performed (Efron and Tibshairani 1994) using 100 replicates. A phylogenetic tree was constructed through the neighbor joining method (NJ) (Saitou and Nei 1987) using MEGA 7 (Kumar et al. 2016) to compare all the sequences that displayed 99% homology with the sequence. The sequence of a closely related genus, *Streptacidiphilus jiangxiensis* was used as an out-group in the phylogenetic tree. The sequences were also compared for their average nucleotide identity (ANI) using the software EZBioCloud (Yoon et al. 2017) and a threshold limit of 98.65% for species demarcation was considered (Kim et al. 2014). The specie(s) giving >98.65% ANI value with the obtained sequence was considered and an accession number KC191697 was obtained through GenBank submission.

Small scale cultivation of SORS-24

The *Streptomyces* strain SORS-24 was grown in two flasks each containing 300 ml of GYM broth. The pH of the media was adjusted to 7.8 and it was autoclaved at 121°C for 20 min. The strain SORS 24 was pre-cultured in GYM broth and approximately 10% was used for the inoculation. The inoculated flasks were incubated at 180 rpm on a linear shaker at 28°C for 72–96 hours. Following incubation, the flasks were put in a sonicator bath for 10 min to disrupt the cells. Ethyl acetate (1:1) was added and the upper layer was separated using a separating funnel and evaporated under vaccum with a rotary evaporator (Heidolph 4000 efficient). The resulting extract was analysed further for the biological screening.

Determination of antimicrobial and cytotoxic activity of *streptomyces* sp. SORS-24

The disk diffusion method (Shan et al. 2010) was used to determine the antimicrobial activity of the crude extract. The set of pathogens that were used as test organisms included *Staphylococcus aureus* ATCC 25923, *B. subtilis* DSM 10 (ATCC 6051), *Klebsiella pneumoniae* ATCC 706003, methicillin resistant *Staph. aureus* (MRSA), *E. coli* ATCC 25922, *Pseudomonas* sp., *Enterobacter* sp., *Saccharomyces cerevisiae* ATCC 9080, *E. coli* K12 (W1130), *Chlorella vulgaris*, and *Candida tropicalis*. Antibiotic assay discs were prepared by cutting out 6 mm Whatmann filter paper discs and imbibed with 15 μl of the extract (5 mg/ml concentration) and placed on the Muller Hinton agar (Difco laboratories) plates seeded with the test organisms. The plates were incubated for 24 hour at 37°C and the resulting zone of inhibition were noted in mm(s).

For determining the cytotoxic activity, a microwell cytotoxicity assay was performed (Qin et al. 2011). The concentrations of the extracts used for the assay were 10, 50, 100, 150, 500, and 1 000 µg/ml and they were prepared after air drying the extract and re-dissolving it in 1% dimethyl sulfoxide (DMSO). The rate of mortality (M) was calculated (Tanvir et al. 2016). For the assay, actinomycin D was added as a positive control and 1% DMSO in seawater as a negative control.

In vitro screening for genotoxic activity

The genotoxicity testing was performed on *PolA* defected strain of *E. coli* (K-12 AB 3027) by the process of DNA damage and repair test (DDRT) (Zhang et al. 2013). For this test, the disk diffusion method was repeated with the *PolA* defected strain.

In vitro screening for antioxidant activity

The antioxidant assay was carried out (Qin et al. 2011) and for the assay, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was freshly prepared in absolute methanol (1 mM, O.D = 0.9 ± 0.2 at 517 nm). The concentrations of extract used for the assay were 100, 500, 1 000, 5 000, and 10 000 µg/ml. Ascorbic acid was taken as a positive control and 1% solution of DPPH was taken as a negative control.

Chemical screening through thin layer chromatography (TLC) and bioautography

Thin layer chromatography (TLC) of the extract was performed (Kirchner 1967). Briefly, a 20 × 20 cm silica gel plate (Merck, Germany) was cut and the extract was spotted with the help of a capillary tube, and superimposed. The plate was developed using chloroform ($CHCl_3$) methanol (MeOH) as a solvent system and observed under 254 nm and 366 nm wavelengths. The bands that displayed absorbance were marked. The developed plate was stained with anisaldehyde/H_2SO_4 solution and observed for different color patterns.

For bioautography (Marston 2011), the plates were developed by TLC as described previously (Kirchner 1967). A cell suspension of *E. coli* ATCC 25922 and *B. subtilis* DSM 10 (ATCC 6051) was prepared separately in 10 ml of 0.85% normal saline. Their turbidity was paralleled with 0.5 MCF (Mc Farland) turbidity standard (BD diagnostics, USA). Once the desired turbidity was achieved, 0.6% nutrient agar (N-agar) was seeded with 15 ml of these suspensions in the ratio 1:100 and it was used for overlaying the developed TLC plates. The plates were kept for 1–2 hour at 4°C and then incubated for 24 hour at 37°C. After incubation, they were sprayed with thiazolyl blue tetrazolium blue (MTT) solution (0.5 mg/ml) and the development of colours was observed after 10 min. The dead cells were indicated by the colour yellow whereas live cells were indicated with purple.

Scale up fermentation and extraction

A pre-culture was made by inoculating 200 ml GYM broth with the well-grown GYM agar blocks of the strain SORS-24 and incubating at 28°C for 3 days at 180 rpm on a linear shaker (Certomat BS1, Sartorius stedim biotech GmbH). Scale up fermentation was done in five 5 l EM baffled flasks, each flask containing 2 l GYM broth resulting in a total volume of 10 l. Each flask was inoculated with a pre-culture ratio of 10%. The fermentation was done at 28°C for 72–96 hours at 180 rpm on linear shakers (Certomat BS1, Sartorius stedim biotech GmbH). After fermentation, the culture broth was harvested and the mixture was filtered under vaccum (Knf lab laboport, Germany). The solid phase or the mycelium was mixed with celite and extracted with methanol. The liquid phase was separated and extracted with ethyl acetate. The methanol and ethyl acetate were evaporated under vaccum on rotary evaporators (Buchi Rotavapor R 200, R-215, Buchi labortechik Germany) to dryness.

Purification and mass determination by (UHR) HPLC-MS analysis

The extract from the liquid and solid phases was combined and fractionated on Amberlite XAD-16 N (Dows chemical company, Germany) resin using MeOH/H_2O. The fractions were purified using silica gel column (30 × 1.5 cm, silica gel 60H, Merck) with $CHCl_3$/MeOH and preparative TLC (PTLC) with $CHCl_3$/MeOH as mobile phase in a 9:1 gradient. The purified compounds were analysed using UHR-HPLC-MS. The obtained data were compared with reference compounds in databases such as dictionary of natural products (DNP), Scifinder, natural substance library (H.P Fiedler, Universität Tübingen, Germany) (Fiedler 1993) and other chemical abstracts. Compounds 1 and 2 were characterized further by (UHR) HPLC-MS and comparing it with the mass spectra of commercially available compounds. The (UHR) HPLC-MS analysis was carried out using Dionex RSLC 3000 HPLC system with Bruker Daltonics maXis 4 G. A 3 μm nucleosil C18-column (Maisch, Germany) of 100 x 2 mm length was used. The mobile phase was a gradient of MeOH/CH_2O_2 (999.9:0.1) from 0% to 100% in 20 min and 100% for 3 min. Scan started at 50 m/z and ended at 1000 m/z. The flow rate was adjusted at 300 μl/min and 3 μl of the sample was injected into the column. The UV–VIS detector was adjusted to monitor wavelengths at 210, 220, 254, and 272 nm. The electrospray ionization (ESI) was adjusted for positive and negative modes. The capillary voltage was at 3.5 kV and temperature was 350°C. Analysis was carried out through brucker daltonics data analysis software.

Statistical analysis

The results from each experiment were calculated from triplicate studies and expressed as mean values. The LC_{50} was calculated using probit regression analysis and EC_{50} was obtained using a dose response curve calculated through SPSS version 28.0 (SPSS, USA).

Results and discussion

Isolation and characterization of *Streptomyces* sp. SORS-24

Sonchus oleraceus L. was found growing near a damp field around the institute of microbiology and molecular genetics. Its stem, roots, and leaves were surface sterilized and plated on two selective media. After incubation, the actinomycetes strain was observed to be growing on actinomycetes isolation agar and it was further purified on GYM agar. The strain SORS-24 displayed the typical characteristics of *Streptomycetes*. It was a rough-embedded colony with olive-cream green aerial mycelium and cream substrate mycelium, and it displayed excellent sporulation. In gram staining, Gram positive cells were observed in long filamentous chains (Fig. 1). The strain utilized galactose, glucose, arabinose, sorbitol, sucrose, mannitol, raffinose, and lactose as a carbon source demonstrating the ability to grow on a diversity of sugars. It tested positive in biochemical tests like melanin production as well as hemolysis. Among several tests, the strain SORS-24 displayed melanin formation, utilization of organic acids, formation of organic acids, hemolysis of esculin, arbutin, and lecithovitellin reaction. The detailed physiological characterisation are described in Table 1. By comparing these characteristics with actinomycetes in the Bergey's manual of determinative bacteriology (Holt 1994), it was suspected that the strain was of the genus *Streptomyces*.

For confirmation, 16S rRNA gene sequencing was carried out and total nucleotides of 1066 bp were obtained for the strain SORS-24. The BLAST analysis was done and the obtained sequence gave 99% homology to a range of *Streptomyces* type strains. Therefore, a phylogenetic tree was constructed (Fig. 2). The values of the ANI was calculated of all the sequences that gave 99% homology with the obtained sequence. The analysis indicated that the strain was closely related to *S. tanashiensis* (ANI value 99.05%) (GenBank Accession no. KC191697).

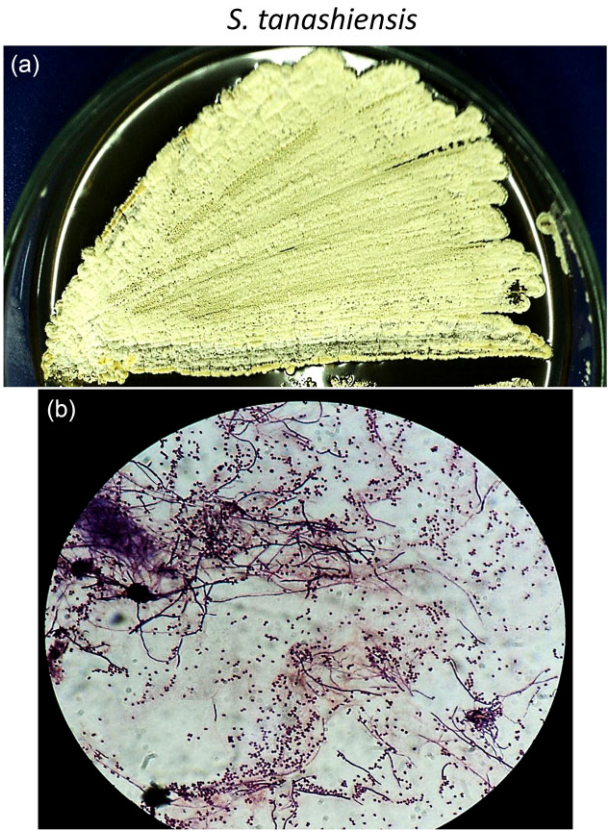

Figure 1. (a) Substrate and aerial mycelium of *Streptomyces* sp. SORS-24. (b) Gram staining under oil-immersion 100X magnification of *Streptomyces* sp.

Antimicrobial and cytotoxic activity of *Streptomyces* sp. SORS-24

The steps from preparation of sample to extraction and analysis by chromatography has a significant impact on the quality of the results obtained. Moreover, there is also a common trend to use faster and more effective methods that are environmental friendly (Krakowska-Sieprawska et al. 2022). In our study, we carried out a biological screening for *Streptomyces* sp. SORS-24 through various biological procedures in order to determine its full potential. Antimicrobial activity was determined using the disk diffusion method (Shan et al. 2010) against a variety of test organisms. The *Streptomyces* sp. SORS-24 showed the most activity against *E. coli* ATCC 25922 with a 25 mm zone of inhibition. The strain also showed noteworthy zones against *Ch. vulgaris* (20 mm), *E. coli* K-12 (15 mm), *Pseudomonas* sp. (15 mm) and *B. subtilis* ATCC 6051 (15 mm). No activity was observed against *Staph. aureus* ATCC 25923, MRSA, or *K. pneumoniae* ATCC 706003 (Table 2) which is interesting since a prior report (Singh et al. 2009) described prominent antimicrobial activity of endophytic *Streptomyces* sp. against both *Staph. aureus* and *K. pneumoniae*.

The *Streptomyces* sp. SORS-24 gave a very promising cytotoxicity profile. It produced compounds that gave 82.7% mortality at a concentration of 500 μg/ml and a maximum percentage mortality i.e. 100% at a concentration of 1 000 μg/ml in the crude extract. The LC_{50} was calculated as 10 μg/ml and upon comparing it with the reference values (Geran 1972) it was found to be toxic. Studies have proven that the brine shrimp cytotoxicity assay using *Artemia salina* nauplii can be correlated with cytotoxic activity on solid tumors. Although this test is able to identify anticancer activity of the compound under study but it is limited in terms of its ability to distinguish between strong, moderate and weak cytotoxicity. It is

Table 1. Morphological and physiological characteristics of *Streptomyces* sp. SORS-24.

Morphological characterization	**Size (mm)**		Pin Point
	Shape		Circular
	Margin		Filamentous
	Texture		Rough
	Consistency		Hard/Embedded Excellent
	Sporulation		
	Growth		Well
	Pattern		grown/ Partitioned
	Substrate mycelium		Cream
	Aerial mycelium		Dirty green
	Soluble Pigments		No
Physiological characterization using standard International *Streptomyces* Project (ISP) with sugar utilization as carbon source and melanin test	**Glu**	**7d**	+
		14d	+
	Gal	**7d**	+
		14d	+
	Ara	**7d**	+
		14d	+
	Man	**7d**	–
		14d	+
	Sor	**7d**	+
		14d	+
	Suc	**7d**	+
		14d	+
	Manni	**7d**	+
		14d	+
	Raf	**7d**	+
		14d	+
	Fru	**7d**	–
		14d	–
	Lac	**7d**	+
		14d	+
	Mel	**8d**	+
		14d	+
Physiological characterization using nine biochemical tests	**UA**	**6d**	**+**
		9d	**++**
		16d	**++**
	UO	**7d**	–
		11d	–
		17d	–
	FO	**5d**	–
		9d	+
	HEA	**5d**	++
		10d	++
	HUA	**5d**	–
		9d	–
	LV	**3d**	+
		5d	+
		8d	**+**

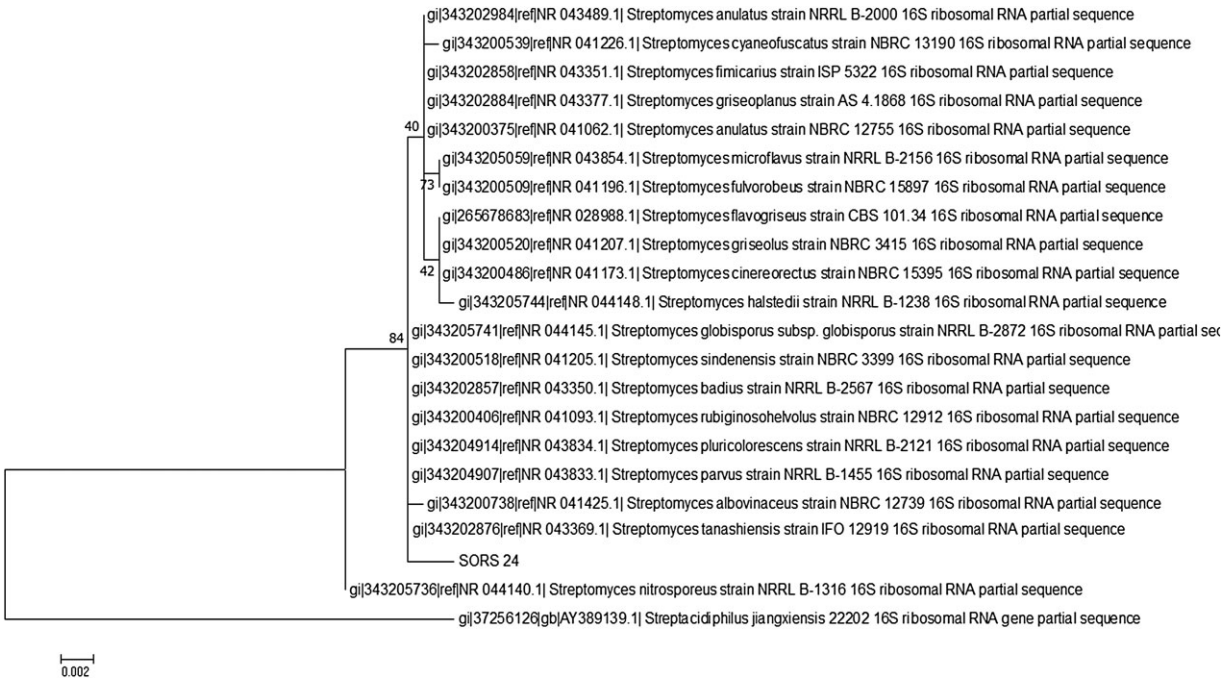

Figure 2. Phylogenetic tree of *Streptomyces* sp. SORS-24.

Table 2. Antimicrobial activity of *Streptomyces* sp. SORS-24.

Strain Code	*B. subtilis* ATCC 6051	*Ent. spp.*	*Pseudo. spp.*	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*K. pneumonia* ATCC 706003	*MRSA*	*E. coli K12*	*S. cerevisisae* ATCC 9080	*C. tropicalis*	*C. vulgaris*
SORS-24	15	14	15	–	25	–	–	15	–	–	20

B.subtilis Bacillus subtilis; S. aureus Staphlococcus aureus; E.coli Escherichia coli; K. pneumoniae Klebsiella pneumoniae; MRSA Methicillin Resistant Staphlococcus aureus; C. tropicalis Candida tropicalis, C.vulgaris Chlorella vulgaris; (–) no inhibition; zones of inhibition were measured in mm(s).

still a screening tool for testing potential cytotoxins (Niksic et al. 2021).

Genotoxic activity of *Streptomyces* sp. SORS-24

The testing for genotoxicity results in identifying chemicals that may result in genetic alterations. They may include tests for determining DNA damage. Such damage may not be permanent and may be reversible however; the accumulation of the damaged DNA in somatic cells signals the development of degenerative diseases and if it accumulated in the germ cells it may lead to abortions and infertility. Genotoxicity tests are performed *in vitro* by using bacterial cells or cultured mammalian cells. They are mostly used for screening for genotoxicity in somatic cells (Luan and Honma 2022). In this study, we applied the genotoxicity testing by using the DDRT that is mainly used to differentiate between substances with the ability to damage DNA. It is usually applied for antitumor pre-screening (Zhang et al. 2013).

In our study, the bacterial cells of *E. coli* K-12 AB 3027 were used. This is a SOS and PolA defective strain (Ljungquist et al. 1976) that is also a lexA mutant (Alam et al. 2009) as well as being defective in endonuclease activity. The extract of the *Streptomyces* sp. SORS-24 showed a prominent zone of inhibition (15 mm) against *E. coli* K-12 AB 3027. The inhibition indicated that the active metabolites in the extract caused damage to the DNA. This result gave us further confirmation of the toxic LC_{50} value obtained earlier.

Antioxidant activity of *Streptomyces* sp. SORS-24

Among the antioxidant assays, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is the most simple and widely used method (Kitima et al. 2018). DPPH is a stable free radical and the test is based on the scavenging of these radicals that result in a colour change from violet to pale yellow (Sharma and Bhat 2009). The assay only requires a freshly prepared DPPH solution and the absorbance is measured through a spectrophotometer (Anisha and Radhakrishnan 2017).

In our study, the antioxidant assay of 5 000 μg/ml concentration of the *Streptomyces* sp. SORS-24 extract gave a DPPH scavenging activity of 89.8 μg/ml and for the 10 000 μg/ml the scavenging activity increased to 92.5 μg/ml. The EC_{50} value was calculated as 0.8 μg/ml which was close to that of the ascorbate standard i.e. 0.3 μg/ml. With an increase in concentration of the extract, an increase in the activity was observed that indicated a dose dependent response. The bioactivities are described in detail in Table 3.

Table 3. Bioactivities of *Streptomyces* sp. SORS-24.

Bioactivity	Sample	Concentration studied (μg/ml^{-1})								LC_{50} (μg/ml^{-1})	EC_{50} (μg/ml^{-1})
		10	50	100	150	500	1000	5000	10000		
Brine shrimp cytotoxicity assay	Standard (DMSO 1% in seawater)	0	0	0	0	0	0	–	–	0	–
	SORS 24	54	59.6	65	67.8	82.7	100	–	–	10	–
DPPH antioxidant assay	Standard (Ascorbic acid)	–	–	100	–	100	100	100	100	–	0.338
	SORS 24	–	–	20.5	–	50	54.3	89.8	92.5	–	0.821

Score for LC_{50}: Highly toxic - <20(μg/ml^{-1}), toxic – up to 1000 μg/ml^{-1} and non-toxic - >1000 μg/ml^{-1}.

Antimicrobial, cytotoxic, and genotoxic activity of the purified compounds from the scale up fermentation

The antimicrobial and genotoxic activity of the purified compounds was determined by using disk diffusion method and through bacterial cells of *E. coli* K-12 AB 3027 respectively. Later the cytotoxic activity of the two purified compounds was determined through brine shrimp cytotoxicity assay. In the antimicrobial activity, compound 1 showed the most activity against *B. subtilis* ATCC 6051with a 20 mm inhibition zone. Compound 2 also showed the most noteworthy zone against *B. subtilis* ATCC 6051 (20 mm) and less against *E. coli* ATCC 25922 (15 mm). In the assay for cytotoxicity, compound 1 gave 77.7% mortality at a concentration of 500 μg/ml and a 97.7% at a concentration of 1 000 μg/ml. The LC_{50} was calculated as 490 μg/ml. Compound 2 gave 51.1% mortality at a concentration of 500 μg/ml and an 82.2% at a concentration of 1 000 μg/ml. The LC_{50} was calculated as 600 μg/ml. In the genotoxicity testing, compound 1 and compound 2 showed prominent zones of inhibition (17 and 15 mm, respectively, against *E. coli* K-12 AB 3027.

Purification and confirmation of the fatty acid based antimicrobials, oleamide, and erucamide by (UHR) HPLC-MS analysis

The 10 l shaking flasks with culture of *Streptomyces* sp. SORS-24 were extracted using Amberlite XAD-16 N resin and later purified using silica gel. Two compounds were separated through PTLC. Compound 1 was non-polar that gave a weak absorption at 254 nm, it was a white powder that weighed 9 mg. It was light violet after staining with anisaldehyde reagent. In the ESI + ve ion chromatogram, compound 1 was present at 20.0–20.3 min's retention time. The [M + H]+ signal was detected at *m/z* 282.2, the [2M + H]+ signal was observed at *m/z* 563.5 and the [M + Na]+ signal at *m/z* 304.2. The molecular weight was calculated to be 281.2 daltons with the molecular formula C_{18} H_{35} NO (Fig. 3a). It was confirmed to be 9-octadecenamide (Oleamide) after comparing it with the mass spectra of the commercially available oleamide reference compound CAS no. 301–02-0.

Studies have related the fatty acid and their amides derivatives to antifungal, antibacterial, nematicidal as well as antimalarial activities (Moon et al. 2018). The compound 1 identified in our study as 9-octadecenamide (Oleamide, C_{18} H_{35} NO) has been previously described to possess antimicrobial and anti-oxidant activity (Naumoska et al. 2020). A few studies have also reported its anti-inflammatory and bronchodilatory activity (Awakan et al. 2018, Moon et al. 2018). A study by Shao et al. 2016 showed that oleamide is also capable of inhibiting a lethal toxin producing cyanobacterial strain, *Microcystis aeruginosa*. Oleamide from the medicinal mushroom, *Ganderma lucidum* was reported to be the bioactive compound responsible for anti-inflammatory and analgesic effects (Jug et al. 2020). It was also reported from the green algae *Arctium lappa* and *Codium fragile* extracts and was responsible for its potent anti-inflammatory activity (Çakir et al. 2020).

Compound 2 was non-polar and displayed weak absorption at 254 nm wavelength. It was white in color and weighed approximately 10 mg. It turned light violet after staining with anisaldehyde reagent. In the ESI + ve ion chromatogram, compound 2 was present at 21.5–21.8 min's retention time. The [M + H]+ signal was present at *m/z* 338.3, a [2M + H]+ signal at *m/z* 675.6 and the [M + Na]+ signal at *m/z* 360.3. The molecular weight was calculated to be 337.3 daltons and the molecular formula was C_{22} H_{43} NO (Fig. 3b). It was confirmed to be 13- docosenamide (Erucamide) after comparing it with the mass spectra of the commercially available erucamide reference compound CAS no. 112–84-5.

Erucamide has been reported to be similar to an endocannabinoid analogue oleoylethanolamide (OEA). Evidence from a prior study by Li et al. 2017 have suggested that OEA may partake in the control of behaviors related to rewards. Therefore, increasing the OEA may be used as an approach to control depressive disorders. Hence, erucamide can be used as an anti-anxiety and anti-depressive agent. As far as we checked apart from the bioactivity mentioned above no other bioactivity of erucamide has been reported previously.

It should be noted that since fatty acids are natural and essential components of the cells therefore they can be used as a chemical conjugate to drug molecules to develop efficient prodrugs. A well-designed prodrug based on the chemical conjugation of fatty acid amides to the drug molecule may not only improve bioavailability and enhance delivery but may also result in better therapeutic efficacy (Fattahi et al. 2020).

Conclusions

In order to establish a collection of endophytes, particularly endophytic actinomycetes for new fatty acid based antimicrobials, our study focused on obtaining strains from an unexplored habitats. The plant environment has been explored for endophytes; however, there are few reports on the endophytes in the medicinal plants of Pakistan. Our aim was to make use of this environment and to obtain novelty in bioactive com-

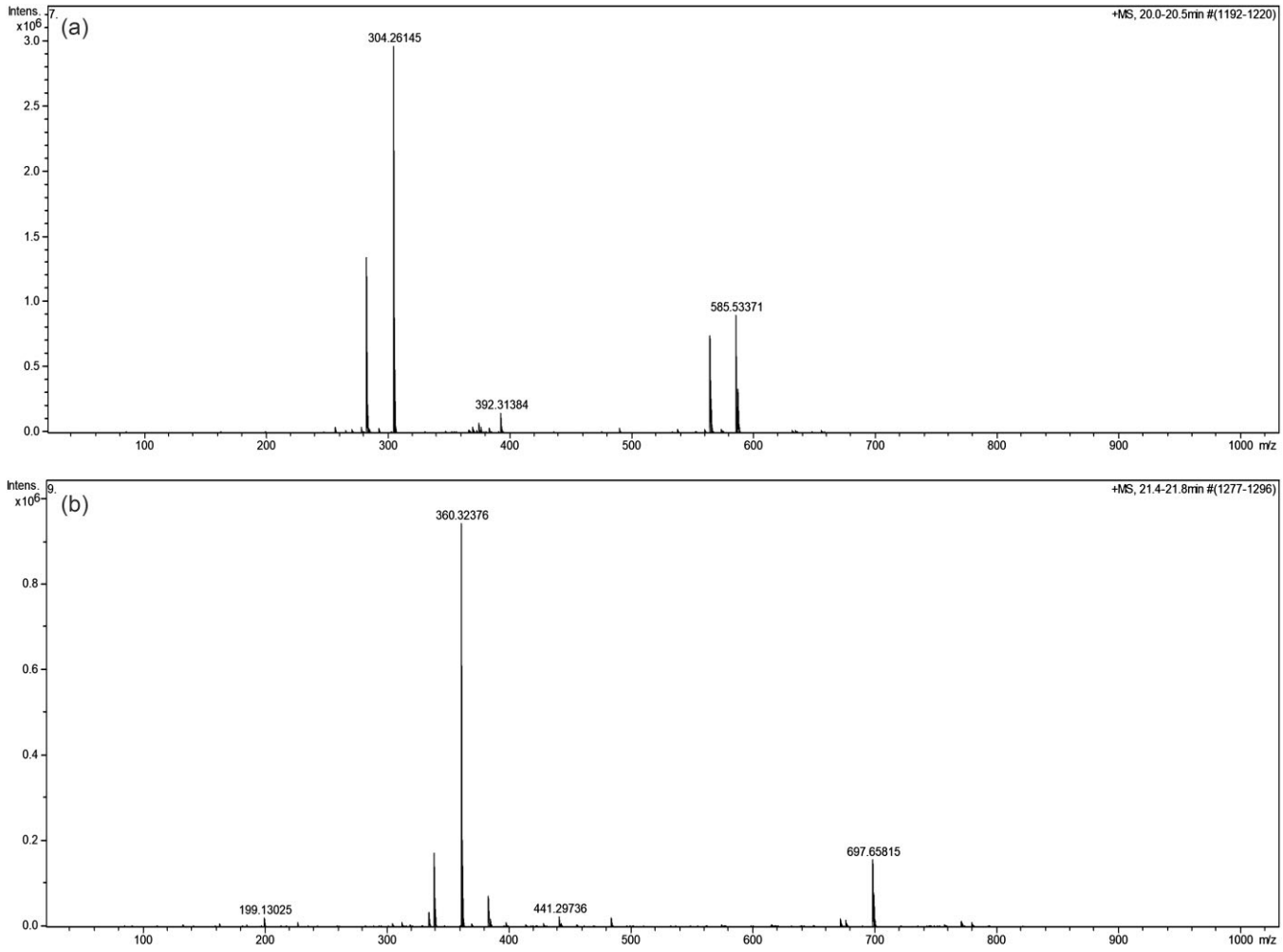

Figure 3. (a) Mass spectra for compound 1 (Oleamide C_{18} H_{35} NO). (b) Mass spectra for compound 2 (Erucamide, C_{22} H_{43} NO).

pounds. As a result, in our study, we successfully isolated two potent fatty acid based antimicrobials from *Streptomyces* sp. SORS-24. From the results, we can infer that this strain is a significant source of fatty acid based antimicrobial agents. In addition, the strain was also observed to be producing significant cytotoxic, genotoxic and antioxidant compounds. This study also reveals that medicinal plant environment of *S. oleraceus* is a significant ecological niche that is harboring a diversity of strains yet to be explored. The importance of fatty acid amides have already been established in industrial applications and in food but the pharmacological aspects of these bioactive compounds are now being explored. The outcome of the study encourages further exploration of such habitats for potential fatty acid based pharmaceutical agents. Further study on the activity of the isolated compounds from *Streptomyces* sp. SORS-24 can be conducted on common cancers prevailing in Pakistan, such as breast and liver cancers, since the compounds in the our study gave significant cytotoxic and genotoxic activities.

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Conflicts of interest

None declared.

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Author contributions

Rabia Tanvir (Data curation [lead], Investigation [lead], Writing – original draft [lead]), Imran Sajid (Conceptualization [equal], Resources [supporting], Supervision [supporting], Writing – review & editing [supporting]), Yasir Rehman (Data curation [supporting], Writing – review & editing [supporting]), and Shahida Hasnain (Conceptualization [lead], Re-

sources [lead], Supervision [lead], Writing – review & editing [supporting]).

Data availability statement

All data generated or analysed during this study is included in this published article.

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