IJBPAS, October, Special Issue, 2021, 10(10): 322-335

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIMICROBIAL SCREENING AND CHARACTERIZATION OF SOIL FUNGI AT KALABURAGI REGION OF KARANATAKA, INDIA

SANGEETA M. KAMRADGI AND VIDYASAGAR GUNAGAMBHIRE M*

Department of PG Studies and Research in Botany, Gulbarga University Kalaburagi-585106, Karnataka, India

***Corresponding Author: Vidyasagar Gunagambhire M; E Mail: gmvidyasagar@gmail.com**

Received 25th April 2021; Revised 24th June 2021; Accepted 25th Sept. 2021; Available online 1st Oct. 2021

https://doi.org/10.31032/IJBPAS/2021/10.10.1031

ABSTRACT

Fifty one fungi were isolated from eight soil samples collected from distinct habitats of Kalaburagi region and screened for their antimicrobial activity against nine *Candida* spp. and five bacterial strains. Twenty isolates were picked up by primary screening using perpendicular streak plate method and subjected to secondary screening by agar well diffusion assay. Among the fungal isolates, two with code VSGF1 and VSGF2 have shown significant zone of inhibition against all tested pathogenic microbes. The crude ethyl acetate extract of VSGF1 displayed significant zone of inhibition 16.67±0.33mm and 15.00±0.57mm against *Candida glabrata* (MTCC3981), *C. albicans* (MTCC1637) and *C. tropicalis* (MTCC1406), respectively. However, the VSGF2 exhibited highest of 15.00±0.57mm inhibition zone against *Escherichia coli* and 14.33±0.33mm against *Bacillus subtilis* and *Staphylococcus aureus*. Mycochemical screening of both fungal isolates showed the presence of alkaloids, flavonoids, phenols, saponins and tannins, with highest contents of flavonoids followed by alkaloids and phenols. The molecular identification of VSGF1 and VSGF2 was done and confirmed their identity as *Talaromyces islandicus* and *Aspergillus terreus*, respectively. The efficient antimicrobial activity of both the fungi shall be considered while using the fungi as potential source for new antimicrobial drug.

Keywords: Antimicrobial activity, *Candida* spp., Mycochemical screening, Molecular identification, Soil fungi

INTRODUCTION

Fungi are eukaryotic microorganism occur as unicellular (yeast) and multicellular filamentous form **[1]**. Out of approximately 2.2 to 3.8 million fungal species exist on the planet, about 75,000 species occurring in soil **[2]**. These fungi play a major role in food, pharmaceutical and textile industries, agriculture, natural recycling, bioremediation and so on **[3]**. In addition, most of the fungal species cause skin infections, mucosal infections, serious allergies and even life threatening invasive infections (IFI). Among the fungi, *Candida* spp. and *Aspergillus* spp. are the most common fungal pathogens **[4]**. The recent scientific studies reported that, *Candida* spp. are spreading severe infections in human beings, particularly in immune compromised patients and gaining resistance against most of the available antifungal drugs. Fungi are a highly diverse group of organism, but narrowly less explored for the production of pharmaceutical products **[5]**. Most of the fungal species are known for the production of antimicrobial compounds **[6]**. Out of 10,700 antimicrobial compounds reported from the whole living world, 1600 are from fungi mainly from the soil fungi. Soils are highly complex with numerous constituents performing diverse functions due to the activity of soil microorganism **[7]**. Fungi from rarely exploited environment may lead to the discovery of novel strains with high potential and a wide spectrum of biologically unexploited products. Kalaburagi district with hot and dry environmental conditions proved to have fascinating and distinct microbial communities. Hence, the present studies carried out to identify the potential soil fungi for the production of antimicrobial agent from the Kalaburagi region of Karnataka, India.

MATERIALS AND METHODS

Study area

The Kalaburagi region is located in the northern part of the Karnataka state, lies between 17^0 10' and 17^0 45' N (Latitude) and 76^0 10' and 77^0 45' E (Longitude). It is a biggest district covering 8.4% (16174 Sq. Kms) area with 5.9% population of the state. The vast stretch of fertile black cotton soil of the district is known for the cultivation of pigeon pea (*Cajanus cajan*) and Jawar (*Sorghum bicolar*) crops. Being located in the center of the Deccan plateau, most part of the district is covered by the fertile black soil. The average rainfall is 777mm and the region is a drought prone area. The temperature ranges between 42 to 46 ^oC in summer and 26 ^oC in winter. The period from December

to May is the driest part of the year. Hence, the district is well known as "Sun city" of the state **[8]**.

Collection of soil samples

About 5 to10 cm deep soil samples were collected in a sterile polythene bag from unique and different habitats such as agricultural fields, gardens, composts, sand, river bank and rocky habitats of Kalaburagi region. The samples were brought to the laboratory, sieved to obtain fine particles and dispensed in the sterile polythene bag for further studies.

Isolation of fungal isolates The isolation of fungi was carried out by using standard soil dilution method **[9]**. The typical colonies of fungi were picked and subcultured on fresh slants of potato dextrose agar (PDA) media and maintained at 4^{o}C for further use.

Antimicrobial activity of fungal isolates

Test organisms

The antimicrobial activity was performed against pathogenic *Candida* spp. such as *C. albicans* MTCC183, *C. albicans* MTCC1637, *C. albicans* MTCC3017, *C. glabrata* MTCC3019, *C. glabrata* MTCC3814, *C. glabrata* MTCC3981, *C. tropicalis* MTCC230, *C. tropicalis* MTCC1406, *C. haemulonii* MTCC2766 (Microbial Type Culture Collection and Gene Bank, Chandigarh-India) and bacterial strains of *Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (The bacterial test organisms were obtained from Department of Microbiology, Gulbarga University, Kalaburagi).

Perpendicular streak plate method

Primary screening of fungal isolates has been carried out by the perpendicular streak plate method against *Candida* spp. using yeast peptone dextrose agar (YPDA) medium. A briefly single streak of fungal culture was inoculated at the centre of the plate and incubated at 28 ^{o}C for 5 days. After incubation at 90 ^{o} angles, the *Candida* spp. was inoculated by single streak. The antagonistic activity was measured by Zone of inhibition (ZOI). Isolates showing maximum inhibition zone were selected for secondary screening **[10]**.

Agar well diffusion assay

Preparation of fungal crude extract

5 mm of freshly subcultured mature fungal colony was inoculated in 100 ml of potato dextrose broth (PDB) (Potato-200g, dextrose-20g/liter of distilled water without agar) in 250 ml of an Erlenmeyer flask and incubated at 28 ^{o}C on rotary shaker with 120rpm for 7days. After incubation, the fungal biomass was separated by centrifugation at 10,000rpm for 10min. The supernatant was separated

and treated with an equal volume of ethyl acetate in separating funnel. The solvent layer was collected and evaporated to dryness. The dried residue thus obtained was used as crude extract. About 10 mg/ml of crude extract dissolved in dimethyl sulfoxide (DMSO) was used for antimicrobial activity against test organisms **[11]**.

Method

The secondary screening of fungal isolates was performed by agar well diffusion assay **[12]**. Sterilized YPDA (Yeast extract-10g, peptone-20g, dextrose-20g, agar-15g/liter of distilled water) and NA (Beef extract-3g peptone-5g, sodium chloride-5g, and agar-15 g/liter of distilled water) media were used to perform the anticandidal and antibacterial activity, respectively. The 24h old test organisms of *Candida* spp. and bacterial strains were streaked on respective medium (10^6 - 10^7 colony forming unit) poured into the petriplates. 5mm diameters of 3 wells were made with sterile cork borer. The wells were loaded with 50µl of the fungal crude extract and other two wells were filled with positive and negative controls. The standard drugs fluconazole and streptomycin were used as positive controls for *Candida* spp. and bacterial strains, respectively, whereas dimethyl sulfoxide was used as negative control. The plates were incubated at 28 ^{o}C (*Candida* spp.) and 37 ^{o}C (bacteria) for 24h. The sensitivity of test organisms was determined based on the clear zone of microbial growth inhibition and measured by using Antibiotic Zone scale-C PW297 (HiMedia Laboratories).

Mycochemical screening for secondary metabolites

The crude ethyl acetate fungal extracts were sequentially separated by dissolving in chloroform, petroleum ether and methanol. The solvent extracts were analyzed for qualitative screening for the presence of alkaloids, flavonoids, phenols, saponins and tannins **[13, 14]**. The quantitative estimation of these secondary metabolites was performed by using conventional methods **[15-17]**.

Morphological and molecular characterization of fungal isolates

The potent organisms were observed under Carl Zeiss microscope (AXIO Imager 2, Germany) and recorded the morphological features and submitted the cultures to National Fungal Culture Collection of India (NFCCI–ARI), Agharkar Research Institute, Pune. Molecular characterization of both the potent isolates was carried out at the Department of Microbiology and Biotechnology, Bangalore University, Bangalore. The obtained sequence was

analyzed with the help of Basic Local Alignment Search Tool (BLAST) and submitted to the National Center for Biotechnology Information (NCBI) and Genebank database. A phylogenetic tree was constructed with MEGA-X software by the neighbor-joining method **[18]**.

RESULTS AND DISCUSSION

Isolation of fungi

Eight soil samples were collected from different habitats of Kalaburagi region. A total of fifty one fungal species belonging to thirteen genera were isolated. Maximum of 9 isolates were obtained from vermicompost soil, followed by the soils of botanical garden (8 isolates), sorghum field (7 isolates), barren land (7 isolates), termites heap (6 isolates), rock weathering (5 isolates), river bank (5 isolates) and rock covering (4 isolates). Among the isolates, the species of *Aspergillus* and *Penicillium* were dominant in most of the soil samples and supported the results of earlier studies **[19]**.

Antimicrobial activity of fungal isolates

Among the fungal isolates, twenty isolates showing antagonistic activity were subjected to secondary screening by agar well diffusion assay and found VSGF1 and VSGF2 effective against all tested *Candida* spp., and bacterial strains. The ethyl acetate crude extract of VSGF1 showed 16.67±0.33 mm ZOI against *Candida glabrata* (MTCC3981), 15±0.57mm against *C. albicans* (MTCC1637) and 15±0.57mm against *C. tropicalis* (MTCC1406), as compared to 16±0.57mm against fluconazole (Standard). VSGF2 displayed highest of 15.33±0.33mm activity against *C. glabrata* MTCC3814 and *C. tropicalis* MTCC1406 as compared to 16.67±0.33mm and 16.00±0.57mm, activity respectively with standard drug **(Table 1)**. Similarly, the antibacterial activity of VSGF2 was highest (15.00±0.577mm) against *E. coli* and followed by against *B. subtillus* (14.33±0.33mm) and *S. aureus* (14.33±0.33mm). VSGF1 showed 11.33±0.33mm ZOI against *P. aeruginosa* and *B. subtillus* (**Table 2**). VSGF2 isolate was found better than the standard drug streptomycin.

Both the fungal extracts showed significant activity as compared to existing reports **[7]**. The anticandidal activity of fungi isolated from Saudi Arabia soil exhibited 12mm, 19mm and 20mm ZOI against *C. albicans*, *C. glabrata* and *C. tropicalis* **[1]**. Whereas, antibacterial activity of fungi isolated from Sultanate Oman soil showed moderate activity against *Pseudomanas aeruginosa* and *streptococcus* spp. **[20]**. Several fungal isolates have been screened for their antimicrobial activities against pathogenic

Candida species and bacterial strains **[21, 22]**. The current situation is more complicated due to poor response in clinical isolates of pathogenic microbes and emerging resistance to existing antimicrobial drugs **[23]**. The genus *Candida* is considered as most pathogenic group of microorganism. Among the species, *C. albicans* is a well known species causing infections in a large number of human beings, but the recent studies have documented a shift towards non albicans *Candida* (NAC) spp. **[24]**. In India, *C. tropicalis* alone or in association with *C. parapsilosis* are the second most prevalent *Candida* species after *C. albicans* **[25]**. Among NAC spp., fluconazole resistance was higher in *C. krusei* (97.3%), followed by *C. glabrata* (49.5%), *C. tropicalis* (34.3%) and *C. rugosa* (33.3%) **[26]**. Another most important public health issue is the rapid emergence of drug-resistance in bacteria. Back in 2004, the rate of resistant was estimated and about 70% of pathogenic bacteria became resistant to least one of the available antibiotics. The drug resistance in *E. coli* and *P. aeruginosa* was reported against penicillin (32.99%) and tobramycin (34-37%) antibiotics, respectively **[27, 28]**. Hence, to overcome these difficulties, one has to significantly improve or employ other successful strategies to control the spread of drug resistant microbes through appropriate drugs.

Mycochemical screening of fungal isolate

The mycochemical screening of VSGF1 and VSGF2 was performed to determine the presence of secondary metabolites in the fungal crude extract. Both fungal extracts showed the presence of alkaloids, flavonoids, phenols, saponins and tannins (**Table 3**). Highest amount of secondary metabolites were recorded in chloroform extracts, followed by methanol and petroleum ether. Maximum amount of flavonoids (28.8%) and phenols (260mg/g) were reported from VSGF1, whereas in VSGF2, alkaloids (8%) and tannins (260mg/100g) were highest. The saponins content was minimum in both VSGF1 (0.1%) and VSGF2 (0.07%) **(Table 4).** The presence of the highest amount of flavanoid is an indicative of its potential use as an anticancer, antibacterial, cardioprotective, anti-inflammation, antioxidative property and widely distributed in nature for its biological and therapeutic properties including antifungal potential **[29, 30]**. A new diphenylketones and xanthone derivatives are isolated from *Talaromyces islandicus* EN-501 exhibited antioxidant activity and active against several pathogenic bacteria **[31]**. More than 38 percent of biological active metabolites were extracted

from fungal isolates **[32]**. Thus the presence of alkaloids, phenols, flavonoids, saponins and tannins in VSGF1, and VSGF2 supports for their antimicrobial property.

Morphological and molecular identification of fungal isolate

The colony colour of VSGF1appeared initially dull green and turned orange colour on maturation and densely grown towards periphery with regular and circular margins. Colony appeared smooth and velvety and produceed red soluble pigment after 4 to 5 days of incubation (**Fig. 1a**). Conidiophores were small to medium and branched; primary branches of conidiophores were paired and held in whorls of two phialides. Phialides were symmetrical biverticillate and were typically flask shaped, swollen in the middle with pointed tip slightly narrowed base produced green to orange colour long chains of single celled and smooth surface conidia (**Fig. 1c**). Isolate VSGF2 appeared yellowish to cinnamon in colour and turned dark on maturation (**Fig. 1b**). The conidial heads were compact, biseriate, and densely columnar. Conidiophores were smooth and hyaline. The conidia were small, globose-shaped, smooth-walled, and vary from light yellow to hyaline and produced yellow soluble pigment (**Fig. 1d**). Molecular characterization of isolates VSGF1 and VSGF2 were carried out with ITS (internal transcribed spacer) gene sequences and submitted to Gen Bank (Accession number MN818685.1 and MN18690.1). BLAST search result indicated 96% sequence similarity of VSGF1 with the sequence of *Talaromyces islandicus* MDL_18167 (Accession no. MK601841.1) and 100% sequence similarity of VSGF2 with the sequence of *Aspergillus terreus* DTO 403-C9 (Accession no. MT316343.1) **(Fig. 2a & 2b)**. Phylogenetic tree constructed showing relationships within species among the isolates by choosing the maximum likelihood method with 1000 bootstrap replications. Based on cultural characteristics, microscopic observations and molecular characteristics, the fungal isolates VSGF1 and VSGF2 were identified as *Talaromyces islandicus* and *Aspergillus terreus*, respectively.

The recent investigations revealed that the genus *Talaromyces* is capable of producing large number of pharmaceutical products and antimicrobial substances **[33, 34]**. Significant antimicrobial activity has been reported in *Talaromyces convolutes*, *Talaromyces derxii* and *Talaromyces flavus* against *Candida albicans* and *Bacillus subtilis* **[35, 36]**. *Aspergillus terreus, A. niger and A. fumigatiaffinis* have also shown effective

antibacterial activity against a wide spectrum of bacterial species **[23]**. The present study reveals the antimicrobial activity of *Talaromyces islandicus* VSGF1 and *Aspergillus terreus* VSGF2 against pathogenic fungi and bacteria and suspects their significant role in the production of novel antimicrobial agents.

Table 1: Anticandidal activity of fungal isolates VSGF1 and VSGF2 against *Candida* spp.

	ZOI in mm		
MTCC *Candida* spp.	**Ethyl acetate extract of VSGF1**	**Ethyl acetate extract of VSGF2**	**Fluconazole (Standard)**
***C. albicans* 183**	**6.33±0.33**	**6.667±0.33**	**14.33±0.66**
***C. albicans* 1637**	**15.00±0.57**	**6.667±1.85**	**16.00±0.57**
***C. albicans* 3017**	**6.66±1.45**	**13.0±0.57**	**15.33±0.33**
***C. glabrata* 3019**	**6.33±0.66**	**8.0±0.577**	**16.33±0.33**
***C. glabrata* 3814**	**14.33±1.20**	**15.33±0.33**	**16.67±0.33**
***C. glabrata* 3981**	**16.67±0.33**	**6.00±0.57**	**16.00±0.57**
***C. tropicalis* 230**	**9.33±0.33**	**7.667±0.33**	**13.00±0.57**
***C. tropicalis* 1406**	**15.00±0.57**	**15.33±0.33**	**16.00±0.57**
***C. haemulonii* 2766**	**6.66±0.33**	**5.33±0.33**	**15.33±0.33**

Note: Data are represented as mean± SD of three independent experiments and each experiment was conducted in triplicate

Table 2: Antibacterial activity of fungal isolates VSGF1 and VSGF2 against bacterial strains

	ZOI in mm		
Bacterial strains	**Ethyl acetate extract of VSGF1**	**Ethyl acetate extract of VSGF2**	**Streptomycine**
Gram positive			
Bacillus subtilis	**6.67±0.33**	**14.33±0.33**	**11.67±0.33**
Enterococcus faecalis	**7.67±0.66**	**----**	**15.33±0.33**
Staphylococcus aureus	**8.667±0.33**	**14.33±0.33**	**12.33±0.33**
Gram negative			
Pseudomonas aeruginosa	**8.±1.000**	**11.67±0.33**	**11.67±0.33**
Escherichia coli	**10.00±0.57**	**15.00±0.57**	**10.33±0.33**

Table 3: Qualitative screening of secondary metabolites of VSGF1 and VSGF2

Secondary metabolites	**Mycochemical Tests**	**VSGF1**			**VSGF2**		
		C	**M**	**P**	**C**	**M**	**P**
Test for Alkaloids	**Dradendroff's test**	+	+	-	+	+	-
	Mayer's test	-	-	+	-	-	+
	Wagner's test	+	-	-	+	+	-
Test for Phenols	**$FeCl_3$ test**	+	-	-	+	-	-
	Elagic acid test	-	+	-	-	+	-
Test for Flavanoids	**Shinoda test**	+	-	+	-	-	-
	$FeCl_3$ test	+	-	-	+	+	-
	Zn/HCl test	-	+	-	-	-	-
	NaOH test	+	-	-	+	-	+
	Lead acetate test	+	-	+	+	-	-
Test for Saponins	**Foam test**	-	+	-	+	+	-
Test for Tannins	**$FeCl_3$ test**	-	+	-	+	-	-

(Note: C-Chloroform, M-Methanol, P-Petroleum ether)

Table 4: Quantitative estimation of secondary metabolites of VSGF1 and VSGF2

Secondary metabolites	Quantity	
	VSGF1	VSGF2
Alkaloid	6%	8%
Flavonoid	28.8%	26.6%
Phenol	260mg/100g	176mg/100g
Saponin	0.11%	0.07%
Tannin	180mg/100g	260mg/100g

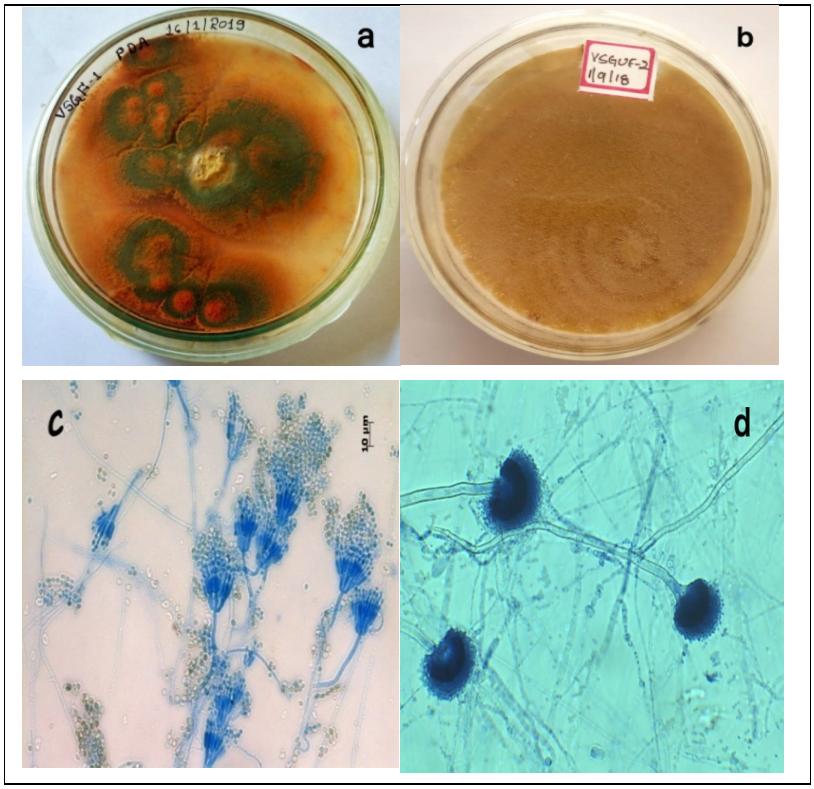

Fig. 1: Fungal culture colony plates on PDA medium a) VSGF1, b) VSGF2, c) Microscopic images and morphological identification of c) VSGF1 d) VSGF2

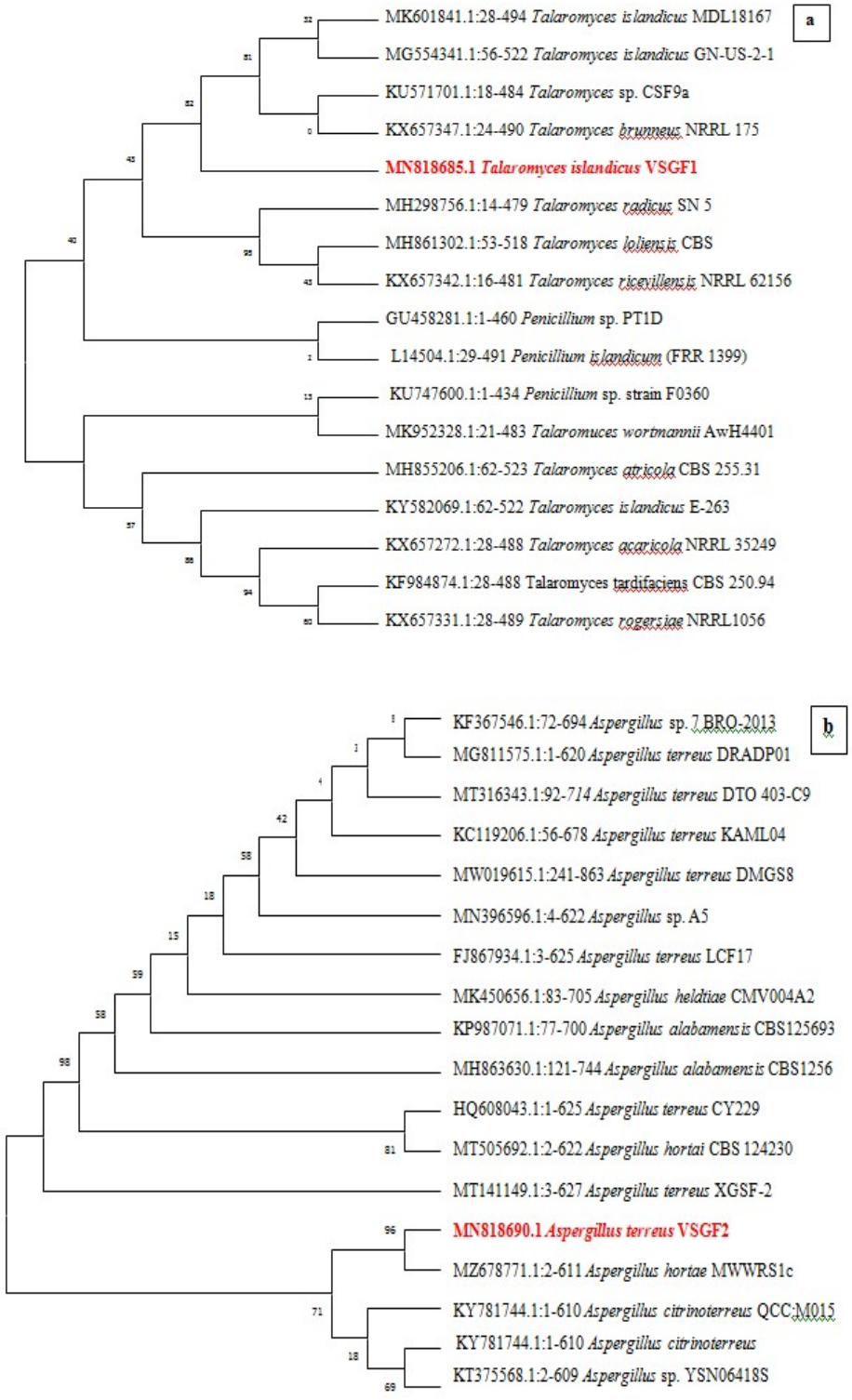

Fig. 2: Phylogenetic tree constructed and showing relationships within species among the isolates by maximum likelihood method by using Mega-X with 1000 bootstrap replications (a) *Talaromyces islandicus* VSGF1 (b) *Aspergillus terreus* VSGF2

CONCLUSION

The present study reveals the production of secondary metabolites in fungi isolated from the soil samples of Kalaburagi, India. Two fungal strains *Talaromyces islandicus* VSGF1 and *Aspergillus terreus* VSGF2 isolated from the region were found to be efficient in inhibiting the growth of pathogenic *C. albicans*, *C. glabrata* and *C. tropicalis* on par with the standard drug fluconazole. The results were also effective against *E. coli*, *B. subtilis* and *P. aeruginosa.* Further studies such as, characterization of the active compound and mechanism of antifungal action are required.

ACKNOWLEDGEMENT

The authors are grateful to Late Dr. M. K. Sateesh (Asst. Prof.), Department of Microbiology and Biotechnology, Bangalore University, Bangalore, Karnataka-India, for their kind support for Molecular identification of fungal isolates.

REFERENCES

[1] Nouf MA, Amani SA, Monerah RAO, Nour KAA, Saleh IA. Isolation, identification and anti-candidal activity of filamentous fungi from Saudi Arabia soil, *Saudi Pharm. J.* 26(2), 2018, 253-257.

[2] Hawksworth DL and Lucking R. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol. Spectr.* 5(4), 2017.

[3] Dick R. Lecture on Soil Bacteria in Soil Microbiology, Personal Collection of R. Dick, Ohio State University School of Environment & Natural Resources, Columbus, OH, 2009.

[4] Gow NAR, Netea MG. Medical mycology and fungal immunology: new research perspectives addressing a major world health challenge. *Philos. Trans. R. Soc. Biol, Sci*. 371(1709), 2016, 20150462. Doi: 10.1098/rstb.2015.0462.

[5] James BM, Brian OB, Mahmood P, Steve T, Anne-Marie A, Emmanuel Z, *et al*. Microbial genomics as a guide to drug discovery, structural elucidation: ECO02301, an antifungal agent, as an example. *J. Nat. Prod.* 68(4), 2005, 493-496. Doi: 10.1021/np0401664.

[6] Dobashi K, Matsuda N, Hamada M, Naganawa H, Takita T, Takeuchi T. Novel antifungal antibiotics A and B; taxonomy, fermentation and isolation, physicochemical properties and biological activities. *J. Antibiot.* 41(11), 1998, 1525-1532. Doi: 10.7164/antibiotics.41.1525.

[7] Bramki A, Ghorri S, Jaouani A, Dehimat L, Kacem CN. Antibacterial activity of

Aspergillus isolated from different Algerian ecosystems. *Afr. J. Biotechnol.* 16(32), 2017, 1699-1704. Doi.org/10.5897/AJB2017.16086.

[8] Kalaburagi at a glance from 2016-17. https://kalaburagi.nic.in/en/document-category/district-profile.

[9] Williams ST and Davies FL. Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. *J. General Microbiol.* 38, 1965, 251-261. Doi: 10.1099/00221287-38-2-251.

[10] Chakraborty RD, Chakraborty K and Thilakan B. Isolation and characterization of antagonistic Streptomyces spp. from marine sediments along the southwest coast of India. *Indian J. Geo-Marine Sci.* 44(1), 2015, 1742-1749.

[11] Chioma N, Njoku EN and Pharamat T. Antimicrobial activity of secondary metabolites of fungi isolated from leaves of bush mango. *J. Next Gener. Seq. appl.* 3(3), 2016, 135. Doi: 10.4172/2469-9853.1000135.

[12] Magaldi S, Mata-Essayag S, Hartung de CC, Perez C, Colella MT, Olaizola C, *et al*. Well diffusion for antifungal susceptibility testing. *Int. J. Infect. Dis.* 8(1), 2004, 39-45. Doi.org/10.1016/j.ijid.2003.03.002

[13] Tiwari P, Kumar B, Kuar M, Kaur G. Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia*, 1(1), 2011, 98-106.

[14] William CE and George ET. Trease and Evans' pharmacognosy, 13th ed. London Philadelphia: Baillière Tindall, 1989.

[15] Harborne JB. Phytochemical methods. Chapman and Hall Ltd. London, 1973; 49-188.

[16] Jia Z, Tang M and Wu J. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64(4), 1999, 555-599. Doi.org/10.1016/S0308-8146(98)00102-2.

[17] Bray HG and Thorpe WV. Analysis of phenolic compounds of interest in metabolism. *Methods Biochem. Anal.* 1, 1954, 27-52. Doi:10.1002/9780470110171.ch2.

[18] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Mol. Biol. Evol*. 28(10), 2011, 2731-2739. Doi.org/10.1093/molbev/msr121.

[19] Saravanakumar K, Krishnan N and Venkatesan K. Seasonal distribution and

abundance of soil fungi from forest soils of wet evergreen forest of Tamil Nadu, India. *J. modern Biotechnol.* 1, 2012, 8-14.

[20] Manal MA, Amani SA, Hind AA, Alothman MR, Alqasoumi SI, Zain SM. Evaluation of antimicrobial activity of secondary metabolites of fungi isolated from Sultanate Oman soil. *Saudi Pharm. J.* 27(3), 2019, 401-405. Doi.org/10.1016/j/jsps.2018.12.12.009.

[21] Ullah I, Khan NA, Jadoon MA, Hameed UR, Khan H, Mujaddad UR, *et al*. Isolation and identification of different rhizospheres fungi of Mansehra region. *Pakistan. J. Entomol. Zool. Stud.* 5(2), 2017, 437-442.

[22] Awaad AS, Al-Mudhayyif HA, Al-Othman MR, Zain ME, El-Meligy RM. Amhezole, a novel fungal secondary metabolites from *Aspergillus terreus* for the treatment of microbial mouth infection. *Phytother. Res*. 31(3), 2017, 395-402. Doi: 10.1002/ptr.5760.

[23] Pfaller MA. 2012. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. *Am. J. Med.* 125(1), 2012, 3–13. Doi: 10.1016/j.amjmed.2011.11.001.

[24] Rajendra JK, Kura MM, Arvind GV, Panthaki MH. *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole. *J. Med. Microbiol.* 59, 2010, 873-880. Doi: 10.1099/jmm.0.013227-0.

[25] Sardi JCO, Scorzoni L, Bernardi V, Fusco-Almeida MA, Mendes GMJS. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J. Med. Microbiol.* 62, 2013, 10-24. Doi:10.1099/jmm.0.045054-0.

[26] Deorukhkar SC and Roushani S. Fluconazole resistance in *Candida* Species: ten years' experience at a rural tertiary care teaching hospital in India. *J. Infect. Dis. Pathog.* 1(1), 2017, 1-8.

[27] Stephen TO and Kennedy KA. Prevalence of multidrug-resistant *Escherichia coli* isolated from drinking water sources. *Int. J. Microbiol.* 7, 2018. Doi: 10.1155/2018/7204013.

[28] Jung R, Fish DN, Obritsch MD, Maclaren R. Surveillance of multidrug resistant *Pseudomonas aeroginosa* in an urban tertiary-care teaching hospital. *J. Hosp. Infect.* 57(2), 2004, 105-111. Doi:10.1016/j.jhin.2004.03.001.

[29] Silva CR, Andrade-Neto JB, Campos RS, Figueiredo NS, Sampaio LS, Magalhaes HIF, *et al*. Synergistic effect of the flavonoid catechin, quercetin, or

epigallocatechin gallate with fluconazole induces apoptosis in *Candida tropicalis* resistant to fluconazole. *Antimicrob. Agents Chemother.* 58(3), 2014, 1468–1478.

[30] Andriy S, Jutarnart M, Roman B, Anna M, Tomas R, Juhee A, *et al*. Antimicrobial activity of crud extracts prepared from fungal mycelia. *Asian Pac. J. Trop. Biomed.* 7(3), 2017, 257-261. Doi: 10.1016/j.apjtb.2016.12.011.

[31] Hong LL, Xiao-Ming L, Hui L, Ling-Hong M, Bin-Gui W. Two New Diphenylketones and a New Xanthone from *Talaromyces islandicus* EN-501, an Endophytic Fungus Derived from the Marine Red Alga *Laurencia okamurai*. *Mar. drugs*. 14(12), 2016, 1-8. Doi: 10.3390/md14120223.

[32] Berdy J. Bioactive microbial metabolites-a personal view. *J. Antibiot.* 58(1), 2005, 1-26. Doi.org/10.1038/ja.2005.1.

[33] Suzuki K, Nozawa K, Nakajima S, Udagawa S, Kawai K. Isolation and structure of antibacterial binaphtho-a-pyrones, Talaroderxines A and B, from *Talaromyces derxii. Chem. Pharm. Bull.* 40(5), 1992, 1116-1119. Doi:10.1248/cpb.40.1116.

[34] Marino T, Nishimoto M, Masuda A, Fujita S, Nishikior T, Saito S. NK374200, a Novel insecticidal agent from *Talaromyces*, found by physic-chemical screening. *J. Antibiot.* 48(12), 1995, 1509-1510. Doi: 10.7164/1ntibiotics.48.1509.

[35] Aria M, Tomoda H, Okuda T, Wang H, Tabate N, Masuma R, *et al*. Funicone related compounds, potentiators of antifungal miconazole activity produced by *Talaromyces flavus* FKI-0076. *J. antibiot.* 55(2), 2002, 172-180. Doi: 10.7164/antibiotics.55.172.

[36] Suzuki S, Hosoe T, Nozawa K, Kawai K, Yaguchi T, Udagawa S. Yaguchi and Udagawa S. Antifungal substances against pathogenic fungi, Talaroconvolutins, from *Talaromyces convolutes*. *J. Nat. Prod.* 63(6), 2000, 768-772. Doi: 10.1021/np990371x.