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Enhancing Anti-*Candida* Potential: Optimizing Parameters for Increased Production of Bioactive Secondary Metabolites Extracted from Fungi Linked to *Saraca asoca*

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ABSTRACT

Ashoka (*Saraca asoca*) is a conventionally used medicinal plant in India, known to have curative properties. The present study elucidated the *in vitro* Anti-*Candida* potential of secondary metabolic compounds extracted from 21 fungal endophytes (SA-A to SA-T) obtained from *S. asoca*. Crude ethyl acetate extracts (25 mg/ml) were subjected to susceptibility test against *Candida albicans* (MTCC-227), *Candida tropicalis* (MTCC-3421), *Candida parapsilosis* (MTCC-998) and *Candida glabrata* (MTCC-3814). Ethyl acetate extract of SA-J (*Rhizopus* sp.) fungal strain was significantly effective against *C. albicans, C. tropicalis*, *C. parapsilosis* and *C. glabrata*, exhibiting a zone of inhibition of 24.33±0.44, 20.50±0.28, 19.66±0.44 and 22.83±0.60 mm, respectively. Secondary metabolites of SA-F (12±0.00 mm), SA-G (20±0.57 mm), SA-H (12.50±0.28 mm), SA-K (20.50±0.50 mm), SA-L (14.16±0.60 mm), SA-Q (16.66±0.88 mm), SA-R (24±0.76 mm) and SA-T (27.83±0.16 mm) expressed strong inhibition against *C. albicans*. Bioactive compounds of SA-F (21.66±0.33 mm), SA-G (18.16±0.44 mm) and SA-K (14±0.28 mm) were also active against *C. glabrata*. Growth of *C. tropicalis* was inhibited by extracts of SA-B (23.83±0.16 mm) and SA-C (22±0.28 mm). Metabolites of SA-D significantly suppressed the growth of *C. tropicalis* (22.16±0.44 mm) and *C. parapsilosis* (24.33±0.44 mm). MIC values ranging 0.048 to 0.097 mg/ml were determined for SA-J by Resazurin assay using 96-well microtiter plate. Physico-chemical parameters like growth medium (potato dextrose broth), temperature (30°C), pH (6), incubation period (8 days), carbon source (dextrose) and nitrogen source (yeast extract) were determined for the most promising *Rhizopus* sp. SA-J strain in terms of antimicrobial activity.

Key words: Anti-*Candida*, bioactive compounds, antimicrobial screening, *Saraca asoca*

INTRODUCTION

The evolution and widespread of the new contagious diseases is the most pressing challenge that humanity is fighting at this moment. Over the recent decades, the occurrence of drug resistance in pathogenic microbes has upstretched serious threats in terms of the well-being and health of the human population, while the emergence of novel and fatal viral infections has contributed to making the situation more threatening. Possibly the best illustration of this is the ongoing COVID-19 pandemic, which is the consequence of a novel corona virus epidemic that has killed over 6.5 million people worldwide (Caruso *et al.*, 2022). Excessive and frequent use of insecticides, herbicides, rodenticides and fungicides in agriculture and farming has also resulted in catastrophic environmental problems. As an unintended consequence these toxic chemical compounds inexorably accumulate in the ecosystem, resulting in increased resistance in microorganisms, loss of biological diversity and contamination of land and aquatic habitat (Badawy *et al.*, 2022). Overuse of antibiotics is leading to antimicrobial resistance in the microbial population. To cope up with some of these problems, a persistent requirement of discovering entirely novel and extremely potent biologically active compounds capable of alleviating some encumbrance auxiliary with utilizing injurious, synthesized and ineffectual chemical substances. Compounds extracted from natural sources are prominent, frequently exhibiting substantial and comprehensive biological activities, moreover possessing less hazardous and more environmentally-friendly characteristics (Stan

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et al., 2021). Conventionally natural products are derived from bacteria, fungi, plants and animals. In more recent years, technological advancements have shifted the focus for discovering novel compounds away from these natural sources and diverted to screening compound libraries composed of artificially synthesized molecules. Notwithstanding their apparent potential expectations, such automated, high-throughput technologies failed to demonstrate significant success. Consequently, there is once again a revival of interest in natural compounds as alternate reservoir of novel compounds having curative properties (Kumar *et al.,* 2023; Kaur *et al.,* 2024). Numerous plants, bacteria, fungi and animals have been investigated, providing a broad spectrum of novel therapeutic natural compounds, particularly those with anticancer and antimicrobial properties (Cui *et al.*, 2020). The use of naturally occurring antimicrobial compounds is recommended due to the formation of protein-protein linkages during the interaction resulting in infrequent development of microbial resistance (Nisa *et al.*, 2020). Recently, an unexplored class of microorganisms coined as endophytes has progressively emerged as the potential source of such naturally occurring compounds (Farooq *et al.*, 2020). Endophytic microorganisms thrive within the inner tissue structure of robust plant devoid of any pathogenic symptoms (Fouda *et al.*, 2015). They have been demonstrated to generate a broad spectrum of biologically active substances facilitating the host plant's inclusive healthy development while coexisting in a mutually beneficial arrangement. These substances were discovered to possess bioactivities with conceivable applications in sectors such as agriculture, medicine and industry (Strobel, 2018; Harrison and Griffin, 2020). Endophytic microorganisms associated with various plants are being explored and it is strongly believed that approximately three million existing plant species harbor at least one or more endophytes, nevertheless only a small fraction has been explored and assessed for microbial diversity associated with them (Grabka *et al.*, 2022). Nevertheless, the range of compounds associated with endophytes is astronomical, encompassing an enormous variety of structures and biological properties. Additionally, it has been anticipated that there could be approximately 10 lakh distinct species of endophytic fungi, the vast majority of which are still unidentified (Ahmed *et al.*, 2023). For the aforementioned reason, endophytic microbes are an extremely promising resource for exploring novel bioactive compounds.

Ashoka (*Saraca asoca*) commonly known as ashok briksh, is a conventionally used medicinal plant in India, possessing antibacterial, antifungal, anti-cancer, anti-menorrhagia, anti-oxidant and anti-inflammatory properties (Lahiry *et al.*, 2020). The plant has long been used in ayurvedic medicine considering almost every component of it has therapeutic value. Because of its curative properties and distinct geographic range, *S. asoca* is a perfect host for endophytic fungal isolation. The present study was intended to bio prospect the fungal diversity associated with *S. asoca* for anti-mycotic properties.

MATERIALS AND METHODS

Fresh and healthy plant specimen (leaf, bark and stem) free from any visible mechanical damage were obtained from medicinally valuable *Saraca asoca* (Ashoka) plant indigenous to district Kurukshetra (29° 52′ to 30° 12′ and 76° 26′ to 77° 04′) in Haryana province of India. Plant parts were collected and transported right away to the research facility in sterilized zip bags in order to minimize the risk of contamination and immediately processed (Mbilu *et al.*, 2018).

The particulate material on the outermost layer of healthy explant segments (root, stem and leaf) wiped out by 2-3 washings of normal tap water followed by distilled water. After being meticulously removed, plant samples were immersed in 70% ethanol for 60-70 seconds, soaked in 2% NaOCl solution for 120 seconds, and then again dipped in 70% ethanol for 60 seconds followed by 2-3 thorough washings of autoclaved distilled water. To ascertain the efficacy of sterilization process, 100 µl of distilled water obtained from the last washing was spread on Mueller Hinton Agar (MHA) at 28°C and 37°C ((Mikolajczyk *et al.*, 2021). Surface sterilized segments (5 × 5 mm) were aseptically transferred on Potato Dextrose Agar (PDA) plates and incubated for a period of 5-7 days at 28±2°C. For suppressing the growth of bacterial population PDA medium was

incorporated with broad spectrum antibiotic streptomycin (100 mg/l) (Boghsani *et al.*, 2020). The plates were frequently observed for fungal growth.

Selection of fungi was carried out on the basis of different morphological characteristics followed by purification by transferring them onto fresh potato dextrose agar medium plates. Macroscopic features including aerial mycelium, colony colour, pattern of mycelium and pigmentation were used for initial characterization. Further microscopic characteristic features including conidiophores, hyphae structure, conidia, etc. were studied using lacto phenol cotton blue staining (Samapti *et al.*, 2022). Pure cultures were preserved on PDA slants at 4°C and in 20% Glycerol cryo-vials at -20°C for further studies.

Freshly grown (4-5 days) funguses (6 mm diameter discs) were transferred in a 500 ml potato dextrose broth at 28±2°C for 10-14 days. After completion of incubation time period fermentation broth was separated from mycelium using Whatman filter papers. Bioactive secondary metabolites produced were extracted by mixing fermentation broth with ethyl acetate (1:1 ratio). This mixture was vigorously shaken for 30-40 min and rested overnight to form layers of ethyl acetate (top) and fermentation broth (bottom). Ethyl acetate was evaporated using vacuum evaporator at 30°C to produce crude metabolic extracts (Toppo *et al.*, 2024).

Four pure lyophilized test organisms were procured from MTCC-CSIR- Institute of Microbial Technology, Chandigarh, India; *Candida albicans* (MTCC-227), *Candida tropicalis* (MTCC-3421), *Candida parapsilosis* (MTCC-998) and *Candida glabrata* (MTCC-3814). Lyophilized cultures were revived by aseptically transferring into sterilized potato dextrose broth. Revived microbes were preserved on slants at 4°C and in 20% glycerol medium at -20°C. Freshly grown pure cultures of test microbes were transferred into 0.85% sterile saline solution and mixed uniformly using vortex shaker. Turbidity of microbial inoculums was adjusted according to 0.5 McFaland standards (Witasari *et al.*, 2022).

Agar Well Diffusion technique (Yanti *et al.*, 2021) was followed for assessing Anti-*Candida* properties of fungal metabolites extracted in ethyl acetate. Sterilized cotton swabs were used to spread 100 µl suspensions of standardized test microbes uniformly on MHA. With sterile cork borer, 6 mm-diameter wells were perforated in agar plates. Crude extract was mixed in DMSO (25 mg/ml) and 200 µl of this solution was filled in punctured wells. For diffusion of extracts, agar plates were left undisturbed for 2 h followed by incubation at 34±2°C for 24-48 h. The development of distinct zones of inhibition against the test organism surrounding the agar wells was measured with Hi Antibiotic Zone Scale in millimeter.

Freshly grown cultures of test microbe's were transferred into 10 ml PDB and incubated at 37°C overnight to achieve a turbidity equivalent to 0.5 McFarland standards. This standardized microbial inoculum was further diluted with PDB (0.5 ml into 9.5 ml broth) and mixed thoroughly using a vortex shaker to attain 0.5-2.5 × 10^5 CFU/ml. Autoclaved and UV-treated microtitre plate was filled with 100 µl PDB from the 1st - 12th well. Extracts were serially diluted in PDB filled first to tenth well. 100 µl extract (25 mg/ml) was transferred into 1st well and mixed thoroughly with pipette, 100 µl of this was transferred to the 2nd well using a fresh tip and again mixed as above. This procedure was repeated up to the 10th well and 100 µl from the 10th well was discarded to maintain the same volume of mixture in each well. The two-fold concentrations (12.50, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.097, 0.048 and 0.024 mg/ml) implemented in this study were finalized based upon the results of antifungal screening of ethyl acetate extracts. The 11th and 12th number wells, each comprising 100 µl of PDB, were utilized for testing growth and sterility, respectively. To achieve final population of 2.5 × 10^4 CFU/ml, 10 µl diluted yeast inoculum was transferred from 1st - 11th well, but 12th well was incorporated with 10 µl broth only. Microtitre plates were sealed with UV-treated parafilm followed by incubation at 37°C for 24 h. Subsequently, 0.01% Rezasurin dye solution (0.01 g in 100 ml autoclaved distilled water) was filled into the 1st - 12th well followed by incubation at 35±2°C for 120 min. The minimum inhibitory concentration of ethyl acetate extract was determined by colour change from blue/purple to pink/colourless (Kebede and Shibeshi, 2022).

Initially 100 ml of different growth medium including Muller-Hinton broth, Sabouraud dextrose broth, malt extract broth, potato

dextrose broth, Czapex dox broth and potato dextrose yeast broth were used to grow selected fungal strains SA-J. After an incubation of 10 days at 28±2°C, metabolic compounds were extracted from fermentation broth and weighed. Mycelium was dried at 80°C to remove moisture content and constant fungal biomass weight was also recorded. Similar process was implemented in case of detecting yield of metabolite and biomass during optimization of other parameters. Optimized medium was selected on the basis of maximum fungal biomass (mg/ml) and metabolite yield in µg/ml (Kalyani *et al.*, 2023). Similarly, other cultural growth parameters were optimized in selected growth medium by varying various physiological and chemical parameters comprising incubation period, temperature, pH, carbon source and nitrogen source.

While maintaining the remaining parameters constant, the SA-J strains were cultivated in 100 ml of potato dextrose medium for varying incubation period of 3-12 days at 28±2°C. Yield of bioactive metabolite and biomass produced was checked after an interval of 24 h (Zhang *et al.*, 2020).

Fungal strains SA-J were grown in 100 ml PDB at temperature ranging 15 to 50°C at 5°C inter valunder stationary condition. After completion of eight days incubation period weight of bioactive metabolite and biomass produced were recorded (Subedi *et al.*, 2021).

Keeping other parameters constant, selected fungal strains SA-J were subjected to pH range of 2-11 at an interval of one at 28±2°C for seven days. The biomass and bioactive metabolite produced at different pH were estimated (Deka and Jha, 2018).

Potato infusion was obtained by boiling 200 g thin potatoes slices into one litre distilled water for half an hour and filtered with cheese cloth. Each 250 ml flask containing 100 ml potato infusion and 2 g of different carbon source viz., galactose, starch, dextrose, mannose, sucrose, fructose, maltose, glycerol, D-mannitol and lactose. Bioactive metabolite and biomass production was analyzed in potato dextrose medium supplemented with various 0.5% nitrogen sources including ammonium sulphate, yeast extract, peptone $[(NH_4)_2S0_4]$, sodium nitrate $\{NaN0_3\}$ and beef extract. SA-J strains were inoculated in medium amended with various carbon and nitrogen sources followed by incubation at 28±2°C for seven days (Liu *et al.*, 2022).

RESULTS AND DISCUSSION

Twenty-one endophytic fungal strains (SA-A to SA-U) were obtained from leaf, stem and bark of *S. asoca* (Ashoka) plant collected from various locations. Isolated endophytic fungi were identified for the genus based on macroscopic (growth pattern, colony colour, aerial mycelium, pigmentation and surface texture) and microscopic (structure of hyphae, conidia, conidiophores and spore) examination. Fungal genus were identified as: *Aspergillus* sp. (28.57%), *Alternaria* sp. (14.28%), *Mucor* sp. (14.28%), *Penicillium* sp. (9.52%), *Fusarium* sp. (9.52%), *Rhizopus* sp. (9.52%), *Nigrospora* (4.76%), *Gliocladium* (4.76%) and Verticillium (4.76%; Table 1).

Extracts obtained from various fungal strains after extraction with ethyl acetate were tested for Anti-*Candida* activity against *C. albicans, C. tropicalis*, *C. parapsilosis* and *C. glabrata*. Ethyl acetate extracts were reconstituted in DMSO (25 mg/ml). Anti-*Candida* potential of bioactive metabolites mixture was confirmed by appearance of inhibition zone which was measured with the help of Hi Antibiotic Zone Scale in millimeter (Table 1).

As evident from the results, growth of *C. albicans* was significantly suppressed by extracts of fungal strains SA-G (20.00±0.57 mm), SA-J (24.33±0.44 mm), SA-K (20.50±0.50 mm), SA-R (24.00±0.76) and SA-T (27.83±0.16 mm). In case of *C. tropicalis*, SA-B (23.83±0.16 mm), SA-C (22.00±0.28 mm), SA-D (22.16±0.44 mm) and SA-J (20.50±0.28 mm) produced maximum growth inhibition. *C. parapsilosis* was found to be the most resistant test microbe as extracts of only three fungi SA-D (24.33±0.44 mm), SA-J (19.66±0.44 mm) and SA-U (17.16±0.60 mm) exhibited considerable growth inhibition. Bioactive metabolites of fungi SA-J (21.66±0.33 mm), SA-G (18.16±0.44 mm), SA-J (22.83±0.60 mm) and SA-K (14.00±0.26 mm) inhibited the growth of *C. glabrata*. Maximum diameter of inhibition zone was produced by extract of SA-T against *C. albicans* (27.83±0.16 mm).

From assessment of *Anti-Candida* potential of various fungal extracts, it was concluded that SA-J (*Rhizopus* sp.) effectively inhibited the growth of all four test microbes: *C. albicans*

Table 1. Isolation, identification of fungi and anti-*Candida* activity of ethyl acetate extracts

Fungal isolate	Plant part	Genus identified	Zone of inhibition (diameter in mm)			
			Opportunistic test pathogen			
			C. albicans	*C. tropicalis*	*C. parapsilosis*	*C. glabrata*
SA-A	B	*Mucor* sp.	-NO-	-NO-	-NO-	-NO-
SA-B	S	*Fusarium* sp.	-NO-	23.83±0.16	10.33±0.33	<10
SA-C	L	*Alternaria* sp.	-NO-	22.00±0.28	<10	-NO-
SA-D	L	*Rhizopus* sp.	-NO-	22.16±0.44	24.33±0.44	-NO-
SA-E	S	*Penicillium* sp.	-NO-	-NO-	-NO-	-NO-
SA-F	B	*Alternaria* sp.	12.00±0.00	-NO-	-NO-	21.66±0.33
SA-G	L	*Penicillium* sp.	20.00±0.57	-NO-	-NO-	18.16±0.44
SA-H	L	*Aspergillus* sp.	12.50±0.28	-NO-	-NO-	12.00±0.28
SA-I	S	*Nigrospora* sp.	10.00±0.28	-NO-	-NO-	-NO-
SA-J	B	*Rhizopus* sp.	24.33±0.44	20.50±0.28	19.66±0.44	22.83±0.60
SA-K	S	*Aspergillus* sp.	20.50±0.50	-NO-	-NO-	14.00±0.28
SA-L	L	*Aspergillus* sp.	14.16±0.60	-NO-	-NO-	-NO-
SA-M	L	*Aspergillus* sp.	-NO-	-NO-	-NO-	12±0.00
SA-N	B	*Mucor* sp.	-NO-	-NO-	-NO-	14±0.28
SA-O	B	*Gliocladium* sp.	11.16±0.16	13.00±0.57	<10	10.00±0.00
SA-P	L	*Fusarium* sp.	-NO-	-NO-	-NO-	12.66±0.44
SA-Q	S	*Aspergillus* sp.	16.66±0.88	-NO-	<10	<10
SA-R	L	*Verticillium* sp.	24.00±0.76	<10	-NO-	-NO-
SA-S	B	*Mucor* sp.	-NO-	-NO-	-NO-	-NO-
SA-T	S	*Alternaria* sp	27.83±0.16	12.66±0.44	<10	-NO-
SA-U	L	*Aspergillus* sp.	-ND-	-ND-	17.16±0.60	<10
+ve control (Itraconazole 5 mg/ml)			20.66±0.27	19.16±0.36	17.50±0.23	18.50±0.23
-ve control (DMSO) -NO-			-NO-	-NO-	-NO-	

Diameter of inhibition zone expressed as mean ± standard error; NO – No zone observed; L – leaf; S – stem; B – bark.

(24.33±0.44 mm), *C. tropicalis* (20.50±0.28 mm), *C. parapsilosis* (19.66±0.44 mm) and *C. glabrata* (22.83±0.28 mm) and as such was selected as the most promising fungal isolate. Minimum inhibitory concentration and growth parameters optimization for maximum metabolite and biomass were studied for selected isolate.

The lower most extract concentration, completely inhibiting the microbial growth was considered as MIC. The minimum inhibitory concentration of SA-J ethyl acetate extract was determined by Resazurin assay in 96-well microtiter plate. Blue/purple in Fig. 1 indicates no microbial growth in the well. Colour change from blue/purple to pink/ colourless indicated presence of viable *Candida* cells permanently reducing Resazurin (Blue) to fluorescent Resorufin (Pink; Blazic *et al.*, 2019). As shown in Fig.1, the values of MIC for ethyl acetate extract of SA-J (*Rhizopus* sp.) recorded from 0.048 mg/ml (*C. glabrata* and *C. albicans*) to 0.097 mg/ml (*C. parapsilosis* and *C. tropicalis*).

Comparison of statistical data produced by cultivating SA-J (*Aspergillus* sp.) in various growths medium revealed that maximum dry

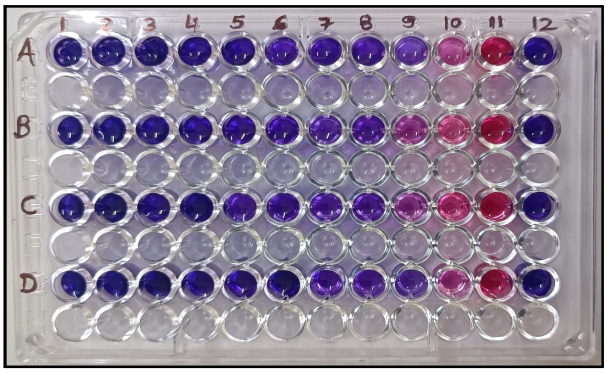

Fig. 1. MIC of ethyl acetate extracts obtained from fungal strain SA-J. A: *C. albicans*, B: *C. tropicalis*, C: *C. parapsilosis*, D: *C. glabrata*, +ve: Positive control (growth medium+test microbe) and -ve: Negative control (growth medium).

biomass (13.7 mg/ml) and metabolite (30 µg/ml) were recorded in PDB, followed by PDYB and SDB (Fig. 2a).

Henceforth, PDB was incorporated as basal medium for the optimization of other parameters. Uniform seven days incubation at 28±2°C was provided to fungal strain growing in different medium. In a similar study (Kalyani *et al.*, 2023) analyzed effect of different

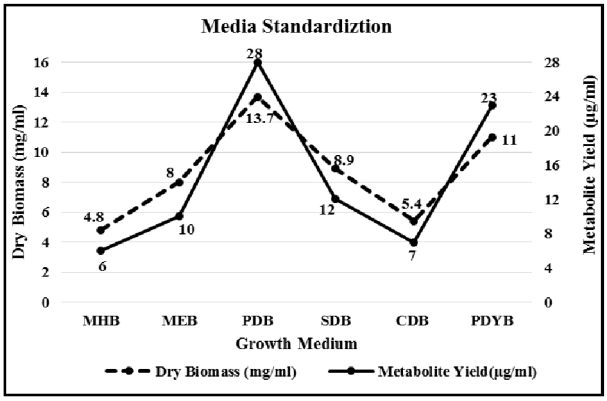

Fig. 2a. Media optimization for maximum dry biomass and metabolite.

growth medium for enhanced dry mycelium and metabolic compounds production by *Aspergillus fumigatus* (MF1). Growth (750.5 mg/ 50 ml) and metabolite production (22 mm against *S. paucimobilis*) in potato dextrose broth were highest as compared to other growth medium used.

The production of antifungal metabolites (29 µg/ml) and dry biomass (13.2 mg/ml) were determined to be optimal after an 8-day incubation period (Fig. 2b). Furthermore, after nine days of incubation, there was a relatively low reduction in both biomass (13 mg/ml) and metabolite yield (28 µg/ml). Maximum biomass and metabolite yield were recorded during the stationary phase and remained almost static up to 12 days of incubation. In a similar study, Liu *et al.* (2022) optimized medium composition and incubation period for *P. eryngii*-3 and results showed that maximum dry cell weight (9.3543 ± 0.2327 g/ l) was produced at 7th day of incubation.

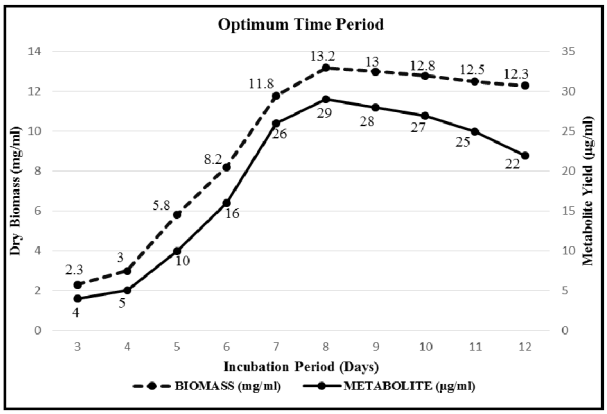

Fig. 2b. Incubation period optimization for maximum dry biomass and metabolite yield.

Temperature is the most significant physiological parameter responsible for the growth rate and yield of secondary metabolites. Fungal isolate SA-J (*Aspergillus* sp.) produced maximum biomass (14 mg/ml) and bioactive compound (32 µg/ml) after incubating at 28±2°C for seven days (Fig. 2c). Exponential growth pattern was observed from 15-30°C and a sharp decline in growth (10.5 mg/ml) along with metabolite produced (20 µg/ml) at 35°C. SA-J survived a temperature as high as 45°C and no fungal growth was observed at 50°C. Similar results were reported by Kalyani *et al.* (2023) during optimization of cultural conditions for *Aspergillus fumigates*. Fungus was grown at temperature ranging from 20-45°C and maximum dry cell weight (870.83±0.76 mg/50 ml) and metabolite (27±0.37 mm against *S. aureus*) production was observed at 30°C.

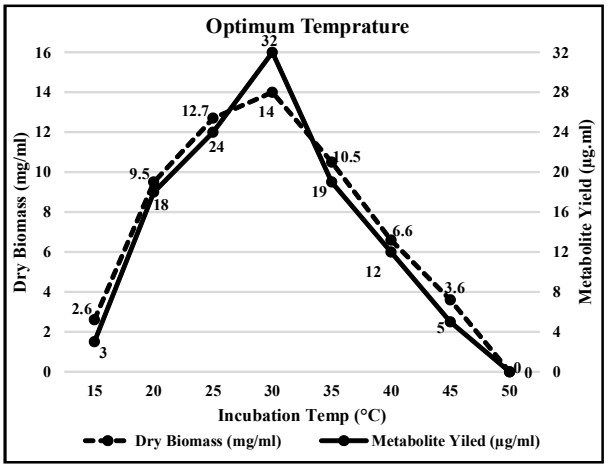

Fig. 2c. Incubation temperature optimization for maximum dry biomass and metabolite yield.

pH of growth medium exhibited strong effect on the microbial growth and secondary metabolites production. Permeability of microbial cell is dependent on the pH and effect the absorption of nutrients from the medium. Selected fungus SA-J was grown at a pH range 2-11 for the assessment of growth and metabolite production. Maximum value for dry biomass (14.1 mg/ml) and metabolic yield (37 µg/ml) were observed at pH 6 (28±2°C for seven days) in PDB followed by pH 5 and pH 7 (Fig. 2d). No growth was observed below pH 3 and above pH 10. Maximum dry mycelium weights (677.33±2.88 mg/250 ml) along with metabolic yield (258.66±3.51µg/250 ml) were observed by Farooq *et al.* (2023) at the pH of 4.

Fungal isolate SA-J was subjected to various carbon and nitrogen sources for the

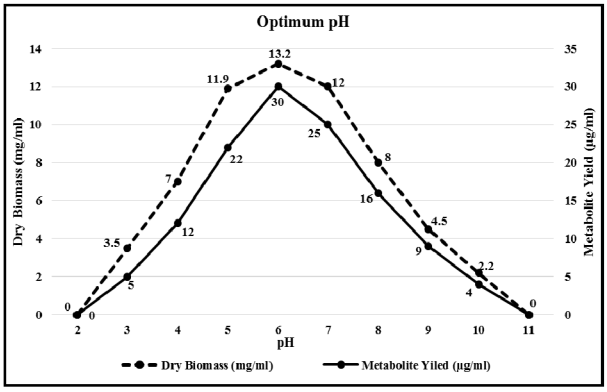

Fig. 2d. pH optimization for maximum dry biomass and metabolite production.

assessment of effect on the production of dry biomass and active metabolite yield. Potato infusion prepared was amended with dextrose, expressed highest growth of biomass (12.7 mg/ml) and metabolite production (24 µg/ml). Starch and sucrose triggered moderate production of fungal biomass and metabolite. Lowest amount of metabolite (8 µg/ml) and dry biomass (7.2 mg/ml) were produced in case of lactose (Fig. 2e).

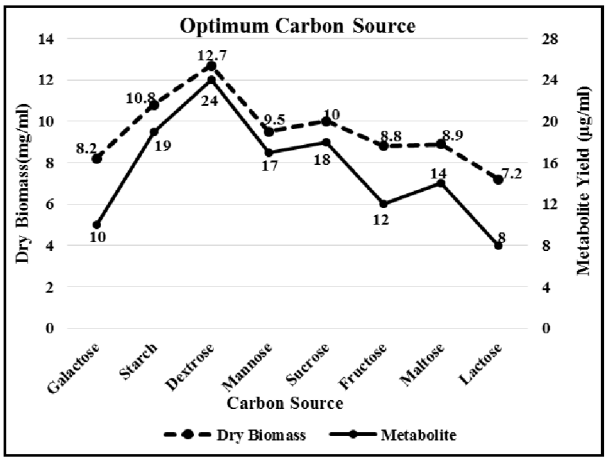

Fig. 2e. Carbon source optimization for maximum dry biomass and metabolite yield.

Nitrogen source can significantly affect fungal biomass and metabolite production by fungi. In case of SA-J strain yeast extract amended medium delivered maximum dry biomass weight (13.6 mg/ml) followed by peptone (11.9 mg/ml), beef extract (10.2 mg/ml), ammonium sulphate (9 mg/ml), malt extract (8.7 mg/ml) and sodium nitrate (8.2 mg/ml). Maximum amount of metabolite was recorded in yeast extract (25 µg/ml) thereafter moderate amount was yielded with peptone (21 µg/ml), beef extract (19 µg/ml) and ammonium sulphate (15 µg/ml). Lowest metabolic yield (µg/ml) was observed when growth medium was amended with sodium nitrate as nitrogen source (Fig. 3). El-Mahdy *et al.* (2023) studied effect of various carbon and nitrogen sources on production of exo polysaccharide by *F. nygamai*. Maximum EPSs production observed in sucrose and glucose was 0.32±0.02 and 0.30±0.02 g/ml and dry mycelium weight was 2.81±0.2 and 2.70±0.2 g/100 ml. Peptone proved to be the most effective nitrogen source for the production of EPS (0.39±0.02 g/100 ml) and mycelium (3.03±0.2 g/100 ml).

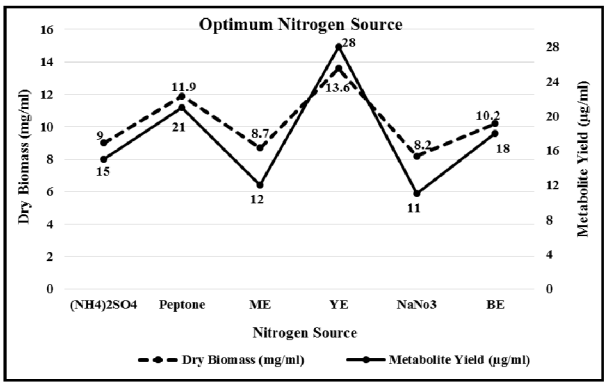

Fig. 3. Optimization of nitrogen source for production of maximum dry fungal biomass and metabolite.

CONCLUSION

The present study was focused on evaluating the anti-*Candida* efficacy of ethyl acetate extracts of fungal strains associated with medicinally valuable *Saraca asoca* (Ashoka) plant native to Krurkshetra, Haryana, India. As per the data of morphological and microscopic examination, 21 endophytic fungi were isolated belonging to nine genera. Results of agar well diffusion method revealed that crude ethyl acetate extracts of endophytic fungi were effective against the opportunistic test microbes used in the study. The study was also intended towards analyzing effect of physiological and chemical parameters on growth and metabolite production from *Apergillus* sp. SA-J. This study suggests that medicinal plants harbor endophytic microbial population, a valuable source for bio-prospecting of new bioactive compounds. Further bioactive compounds present in crude ethyl acetate extracts can be characterized using HPLC, GC-MS, NMR and FTIR.

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