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Research Article
Production of Antioxidant Bioactive Phenolic Compounds by Solid-state Fermentation on Agro-residues Using Various Fungi Isolated from Soil

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Abstract

Background: Agricultural waste bioconversion aimed at producing fungal biomass is a highly attractive alternative because, besides resulting in products of commercial interest, it reduces the amount of waste thereby minimizing pollution. **Materials and Methods:** The present study was planned to investigate the antioxidant potential of fungi isolated from soil of different areas of Punjab, India. Screening of the fungal isolates for antioxidant activity was carried out by dot blot assay. Out of 120 fungal isolates, 51 of fungal isolates demonstrated antioxidant potential and 8 fungal strains with highest activity were further assayed quantitatively on different agro-residues (Wheat Straw (WS), Rice Straw (RS), Corn Cob (CC), Pea Pod (PP) and sugarcane baggases (SC)) by various assay procedures (DPPH assay, reducing power, ferrous ion and Nitric Oxide (NO) ion scavenging activity, ferric ion reducing antioxidant power (FRAP) assay). Total phenolic content was also estimated using Folins-Ciocalteau (FC) reagent. **Results:** All the eight fungal strains (*Aspergillus fumigatus*, *Aspergillus terreus* 1, *Aspergillus terreus* 2, *Aspergillus wentii* 1, *Aspergillus wentii* 2, *Penicillium citrinum*, *Penicillium granulatum* and *Penicillium expansum*) demonstrated good antioxidant activity assayed through various assay procedures and total phenolic content. All the agro-residues supported good antioxidant activity. Sugarcane baggases (SC) was the best substrate followed by Pea Pod (PP) for antioxidant activity. **Conclusion:** To the best of knowledge apparently this is the first systematic report on antioxidant activity of selected fungi. Agro-industrial residues can be reused for the production of different bioactive phenolic compound.

Key words: Agro-residues, antioxidant activity, fungi, solid state fermentation, total phenolic content

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Many studies have shown that free radicals cause several damages to the human body. The free radicals such as superoxide anion radical, hydroxyl radical and H_2O are continuously generated inside the human body can cause oxidative damage of DNA, proteins, lipids and small cellular molecules. Increasing evidence has suggested that many human diseases, such as cancer, cardiovascular disease, neurodegenerative disorders and also the process of aging, are the results of oxidative damage by free radicals[1]. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroxyquinone (TBHQ) are known but their applications have been limited because they exhibit toxicity and carcinogenic potential and require high manufacturing costs. Thus, there is a need to identify natural and possibly more economic and effective antioxidants[2].

Vast diversity of microbes still remains untapped for structurally diverse metabolites possessing highly valuable bioactivities including antioxidant activity. Various fungi are studied for antioxidant activity. A lot more fungi still needs to be explored as the production, downstream processing of actual bioactive phytochemicals from plants is quite tougher as compared to microbes[3]. Solid state fermentation is the bioprocess which has the potential to inexpensive agro-residues into various valuable compounds, including bioactive phenolic compounds. The characteristics of SSF systems can be used for the production of bioactive phenolic compounds by various microorganisms[4]. The aim of this study is focused on the production of antioxidant bioactive phenolic compounds from agro-residues by solid state fermentation.

MATERIALS AND METHODS

Experimental setup: Fungal cultures isolated from soil of different regions of Punjab were used in the present study. The cultures were maintained by regular sub culturing on yeast extract glucose agar (YGA) and stored at 4°C. The cultures were also preserved in 10% glycerol at -70°C and by mineral oil preservation method. For qualitative and quantitative screening of soil fungal isolates for antioxidant potential, the isolated fungi were grown on YGA plates for 6-7 days from which two discs (8 mm) of fungal mycelium were used to inoculate 50 mL of Czapek dox's broth in 250 mL flask and incubated at 25°C for 10 days. The culture broth obtained from different fungi was centrifuged at 10,000 rpm at 4°C for 10 min and then it was filtered through Whatman filter paper No. 1 (pore size: 11 μm) and the filtrate so obtained was used for study. For solid state fermentation, 5 g of dried different agro-residues (Wheat Straw (WS), Rice Straw (RS), Corn Cob (CC), Pea Pod (PP) and sugarcane baggases (SC)) moistened with 25 mL of Czapekdox's broth in 250 mL flask were inoculated with two discs (8 mm) of 6-7 days old fungal mycelium grown on YGA medium and were incubated at 25°C for 10 days. The culture broth obtained from different fungi was centrifuged at 10,000 rpm at 4°C for 10 min and then it was filtered through Whatman filter paper No. 1 (pore size: 11 μm) and the filtrate so obtained was treated with ethyl acetate and solvent extracted components were then evaporated to dryness in vaccuo and the resulting solids were reconstituted in methanol and was used for different assay procedures.

Antioxidant activity

Qualitative rapid screening of soil fungal isolates for antioxidant potential by dot blot DPPH (1,1-diphenyl-2-picryl hydrazyl) staining method: Initially, the 120 soil fungal isolates were screened for their antioxidant activity according to dot blot DPPH rapid staining method as described in Chandra and Arora[5].

The strains which were found positive on the basis of the dot blot DPPH rapid staining method were further subjected to quantitative secondary screening using DPPH assay and reducing potential measurement. Extracellular total phenolic contents of each fungus were also estimated. Eight fungal isolates on the basis of their different colony morphology and highest scavenging activity for DPPH radical, reducing potential with respectively higher TPC were selected for the further study and subjected to various assay procedures as follows.

Assay procedures for antioxidant activity and determination of Total Phenolic Content (TPC): Different assay procedures for antioxidant activity was used as described in Arora and Chandra[6]. The total phenolic content was determined colorimetrically using the Folin-Ciocalteau (FC) method according to Arora and Chandra[6].

Toxicity tests: Toxicity tests were carried out as described in Arora and Chandra[6].

RESULTS

Rapid screening of antioxidant activity by dot blot assay: Antioxidant capacity of fungal extracts was detected

Table 1: Effect of fermentation of different agro-residues by *Aspergillus terreus* 1 on various antioxidant activities

	Rice straw		Pea pod		Wheat straw		Corn cob		Sugarcane baggases	
Parameters	A	B	A	B	A	B	A	B	A	B
DPPH assay	32.4	72.6	34.6	85.3	30.4	74.7	25.7	70.9	30.6	86.8
Reducing power	0.17	0.88	0.12	1.2	0.18	0.86	0.21	0.76	0.31	1.3
Fe^{2+} scavenging activity	28.6	60.5	30.8	65.5	28.9	60.7	25.4	55.7	28.9	67.7
FRAP assay	25.6	58.9	28.7	62.6	25.5	60.8	22.3	50.7	24.4	63.3
NO scavenging activity	28.5	58.2	30.5	66.9	26.8	62.9	24.8	56.9	20.8	68.9
TPC	5.5	14.7	6.8	20.1	5.5	15.3	6.8	12.7	7.8	20.4

A: Activity of control (unfermented agro-residues) and B: Activity of agro-residues fermented by fungi

by a rapid DPPH staining method. Out of 120 fungal isolates, 51 showed white colored spot against purple background. The intensity of white color was however, variable for different organisms. These positive strains were selected for further studies and quantification.

Quantitative screening and Total Phenolic Content (TPC): The selected 51 fungal isolates were subjected to quantitative screening by DPPH assay and reducing power measurement. The TPC was estimated from extracellular fungal filtrate. All the positive fungal strains (51) used for quantitative studies showed good activity ranging from 27-82%. Of the 51 positive strains selected as above, 32 showed reducing power to variable extent. The extracellular TPC in different fungal isolates varied from 1-17 mg mL^{-1} of filtrate.

Identification of fungal isolates: The fungal isolates (51) showed positive results for antioxidant activity through qualitative screening by dot blot DPPH staining method were identified on the basis of morphology of the fungal culture, characteristics of the spores, which were carried out by slide culture technique. Majority of the fungal isolates (23) belongs to the genus *Aspergillus* while, 17 isolates were identified as *Penicillium* and one genus was identified as *Rhizopus*. However, rest of the fungal isolates could not be identified upto genus level. The identity of selected 8 strains was confirmed by National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, India and Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India and cultures were deposited in NFCCI. Identification remarks provided by National Fungal Culture Collection of India, Agharkar Research Institute, Pune, India and MTCC, IMTECH, Chandigarh, India.

Solid-state fermentation of different agro-residues: During solid-state fermentation, fungi (*Aspergillus fumigatus*, *Aspergillus terreus* 1, *Aspergillus terreus* 2, *Aspergillus wentii* 1, *Aspergillus wentii* 2, *Penicillium citrinum*, *Penicillium granulatum* and *Penicillium expansum*) grown on different agro-residues (Wheat Straw (WS), Rice Straw (RS), Corn Cob (CC), Pea Pod (PP) and sugarcane baggases (SC)) and all the agro-residues supported good antioxidant activity. The SC was the best substrate followed by PP for antioxidant activity.

Fermentation of different agro-residues by *Aspergillus terreus* 1, their effect on antioxidant activity and TPC: Extract obtained from SC had DPPH scavenging ability of 86.8%, followed by extract obtained from fermented PP having 85.3% of scavenging ability. Fermented extracts of WS and RS possessed 74.7 and 72.6% of scavenging ability, respectively. Fermented CC extract showed DPPH scavenging activity with 70.9%. Reducing power of the fermented extracts of SC (1.3) was highest followed by fermented extracts of PP (1.2) while, WS, RS and CC showed the activity of 0.88, 0.86 and 0.76. Fermented extracts of SC (67.7%), PP (62.6%), WS (60.8%), RS (58.9%) and CC (50.7%) showed good ferrous ion chelating. Fermented extract of SC showed the 63.3% of ferric reduction potential followed by PP (62.6%), WS (60.8%), RS (58.9%) and CC (50.7%). Fermented extract of SC showed the 68.9% of NO scavenging activity while, PP possessed scavenging activity of 66.9%, fermented extracts of WS and RS showed 60.8 and 58.9%. The TPC of 20.6 mg mL^{-1} was found in fermented extracts of both SC and PP while, WS, RS and CC yield 15.3, 14.7 and 12.7 mg mL^{-1} of TPC (Table 1).

Fermentation of different agro-residues by *Aspergillus terreus* 2, their effect on antioxidant activity and TPC: Extracts of fermented SC (65.8%) showed the highest DPPH scavenging activity followed by PP (60.7%). Fermented extracts of WS, RS and CC showed 55.8, 54.6 and 34.7% of activity, respectively. Reducing power of the extracts of fermented SC (0.89) was the highest followed by PP (0.78). Similarly, all the activity was again highest in fermented extracts of SC following PP. Total phenolic content of the fermented extracts of SC (16.7 mg mL^{-1}) was highest closely followed by fermented extracts of PP (16.4 mg mL^{-1}). The

Table 2: Effect of fermentation of different agro-residues by *Aspergillus terreus* 2 on various antioxidant activities

	Rice straw		Pea pod		Wheat straw		Corn cob		Sugarcane baggases	
Parameters	A	B	A	B	A	B	A	B	A	B
DPPH assay	32.4	54.6	34.6	60.7	30.4	55.8	25.7	34.7	30.6	65.8
Reducing power	0.17	0.54	0.12	0.78	0.17	0.54	0.21	0.32	0.31	0.89
Fe^{2+} scavenging activity	25.6	40.6	27.8	48.7	25.9	42.3	23.4	31.9	28.9	50.6
FRAP assay	25.6	40.8	28.7	45.7	25.5	40.8	22.3	30.8	26.4	48.7
NO scavenging activity	28.5	42.4	30.5	42.8	26.8	40.9	24.8	30.6	20.8	50.7
TPC	5.5	14.8	6.8	16.3	6.3	12.7	6.8	12.8	7.8	16.7

A: Activity of control (unfermented agro-residues) and B: Activity of agro-residues fermented by fungi

Table 3: Effect of fermentation of different agro-residues by *Penicillium citrinum* on various antioxidant activities

	Rice straw		Pea pod		Wheat straw		Corn cob		Sugarcane baggases	
Parameters	A	B	A	B	A	B	A	B	A	B
DPPH assay	32.4	60.8	34.6	72.3	30.4	62.9	25.7	52.8	30.6	75.6
Reducing power	0.17	0.69	0.12	0.99	0.18	0.73	0.21	0.45	0.31	1.02
Fe^{2+} scavenging activity	28.6	48.9	30.8	55.7	28.9	48.7	25.4	38.8	28.9	55.4
FRAP assay	25.6	42.7	28.7	53.4	25.5	40.8	22.3	35.5	24.4	52.4
NO scavenging activity	28.5	45.7	30.5	56.7	26.8	44.6	24.8	36.8	20.8	55.6
TPC	5.5	20.2	6.8	24.3	6.3	20.8	6.8	18.7	7.8	25.4

A: Activity of control (unfermented agro-residues) and B: Activity of agro-residues fermented by fungi

TPC of fermented extracts of WS and RS are 12.7 and 14.8 mg mL^{-1}, respectively. Fermented extracts of CC possessed 12.8 mg mL^{-1} of TPC (Table 2).

Fermentation of different agro-residues by *Penicillium citrinum*, their effect on antioxidant activity and TPC: The DPPH highest activity was observed in fermented SC. The DPPH activity was 75.6, 72.3, 62.9, 60.8 and 52.4% in fermented extracts of SC, PP, WS, RS and CC, respectively. Reducing power of the extracts of fermented SC (1.02) was the highest followed by PP (0.99), WS (0.73), RS (0.69) and CC (0.45). Similarly all the activity was again highest in fermented extracts of SC following PP. Total phenolic content of the fermented extracts of SC (25.4 mg mL^{-1}) was highest closely followed by fermented extracts of PP (24.3 mg mL^{-1}). The TPC of fermented extracts of WS and RS are 20.8 and 20.2 mg mL^{-1}, respectively. Fermented extracts of CC possessed 18.7 mg mL^{-1} of TPC (Table 3).

Fermentation of different agro-residues by *Penicillium granulatum*, their effect on antioxidant activity and TPC: Fermented extract of SC showed highest DPPH free radical scavenging ability (70.8%) and followed by fermented extract of PP having 70.3% of scavenging ability. Fermented WS and RS possessed similar scavenging ability (65.6%). Fermented extract of CC showed least enhancement in the DPPH scavenging activity with 60.7%. Reducing power of the fermented extracts of SC (1.01) was highest followed by extracts of PP (0.98). Extract obtained from fermented WS, RS and CC showed the activity of 0.58, 0.65 and 0.41. Ferrous ion scavenging activity was highest (55.5%) in the extracts of fermented SC then in fermented PP (52.4%). Activity of fermented (45.7%) extracts of WS and RS was same while, fermented extracts of CC showed the least activity (25.4%). Fermented PP extracts showed the 51.8% of ferric reduction potential followed by SC (50.5%), WS (60.7%), RS (60.7%) and CC (35.9%). Fermented SC extracts showed 65.4% of NO scavenging activity followed by PP (64.8%). Fermented extracts of WS and RS showed similar activity (60.7%) while, fermented extracts of CC showed 54.6% of activity. Total phenolic content was 12.8 mg mL^{-1} in fermented extracts of SC and PP whereas WS, RS and CC yield 10.6, 10.8 and 8.9 mg mL^{-1} of TPC (Table 4).

Fermentation of different agro-residues by *Aspergillus wentii* 1, their effect on antioxidant activity and TPC: The DPPH scavenging activity of the fermented extracts of SC was highest (70.8%) followed by PP (68.8%). Fermented extracts of RS and WS showed same scavenging effect of 65.8% while, CC showed 55.8% of activity. Reducing power of the extracts of fermented SC (0.91) was the highest followed by PP (0.88). The WS and RS (0.64) reducing power is same while, CC showed 0.33. Ferrous ion scavenging activity was highest (46.7%) in SC then in PP (45.7%), WS and RS (40.6%) and CC (35.6%). Extracts of SC demonstrated highest ferric reduction power (42.5%) and followed by PP (45.8%). Extracts of fermented WS and RS exhibited 41.6 and 42.3% of ferric reduction, respectively. Extracts of fermented CC showed the

Table 4: Effect of fermentation of different agro-residues by *Penicillium granulatum* on various antioxidant activities

	Rice straw		Pea pod		Wheat straw		Corn cob		Sugarcane baggases	
Parameters	A	B	A	B	A	B	A	B	A	B
DPPH assay	32.4	64.6	34.6	70.3	30.4	65.6	25.7	60.7	30.6	70.8
Reducing power	0.17	0.65	0.12	0.98	0.18	0.58	0.21	0.41	0.31	1.01
Fe^{2+} scavenging activity	28.6	45.5	30.8	52.4	28.9	50.7	25.4	40.8	28.9	55.5
FRAP assay	25.6	45.8	28.7	51.8	25.5	45.9	22.3	35.9	24.4	50.5
NO scavenging activity	28.5	60.6	30.5	64.8	26.8	60.7	24.8	54.6	20.8	65.4
TPC	5.5	10.8	6.8	12.8	6.3	10.6	6.8	8.9	7.8	12.8

A: Activity of control (unfermented agro-residues) and B: Activity of agro-residues fermented by fungi

Table 5: Effect of fermentation of different agro-residues by *Aspergillus wentii* 1 on various antioxidant activities

	Rice straw		Pea pod		Wheat straw		Corn cob		Sugarcane baggases	
Parameters	A	B	A	B	A	B	A	B	A	B
DPPH assay	32.4	65.7	34.6	68.8	30.4	65.8	25.7	55.8	30.6	70.8
Reducing power	0.17	0.64	0.12	0.88	0.18	0.58	0.21	0.23	0.31	0.91
Fe^{2+} scavenging activity	28.6	40.6	30.8	45.7	28.9	40.6	25.4	35.6	28.9	46.7
FRAP assay	25.6	42.3	28.7	45.8	25.5	41.6	22.3	30.8	24.4	42.5
NO scavenging activity	28.5	45.5	30.5	47.8	26.8	45.3	24.8	35.66	20.8	45.6
TPC	5.5	10.8	6.8	11.4	6.3	10.55	6.8	9.9	7.8	12.5

A: Activity of control (unfermented agro-residues) and B: Activity of agro-residues fermented by fungi

Table 6: Effect of fermentation of different agro-residues by *Aspergillus wentii* 2 on various antioxidant activities

	Rice straw		Pea pod		Wheat straw		Corn cob		Sugarcane baggases	
Parameters	A	B	A	B	A	B	A	B	A	B
DPPH assay	32.4	50.7	34.6	58.7	30.4	52.8	25.7	45.6	30.6	60.8
Reducing power	0.17	0.64	0.12	0.86	0.18	0.61	0.21	0.45	0.31	0.98
Fe^{2+} scavenging activity	28.6	45.7	30.8	50.7	28.9	45.7	25.4	38.6	28.9	50.3
FRAP assay	25.6	40.6	28.7	42.6	25.5	38.9	22.3	35.8	24.4	46.4
NO scavenging activity	28.5	48.7	30.5	52.5	26.8	46.7	24.8	38.7	20.8	55.3
TPC	5.5	10.8	6.8	11.4	6.3	10.5	6.8	8.5	7.8	10.8

A: Activity of control (unfermented agro-residues) and B: Activity of agro-residues fermented by fungi

least activity (30.8%). Fermented extracts of PP (47.8%) showed highest scavenging effect for NO ion, followed by fermented extracts of SC (45.6%) and RS (45.5%). Fermented extracts of WS and CC showed 45.3 and 35.6% of activity, respectively. Total phenolic content of the fermented extracts of SC (12.5 mg mL^{-1}) was highest closely followed by fermented extracts of PP (11.4 mg mL^{-1}). The TPC of fermented extracts of WS and RS are 10.5 and 10.8 mg mL^{-1}, respectively. Fermented extracts of CC possessed 9.9 mg mL^{-1} of TPC (Table 5).

Fermentation of different agro-residues by *Aspergillus wentii* 2, their effect on antioxidant activity and TPC: Fermented SC (60.8%) extracts demonstrated the highest DPPH scavenging activity followed by fermented extracts of PP (58.7%). Fermented extracts of WS and RS showed 52.8 and 50.7% of activity, respectively. Fermented extracts of CC (45.6%) showed the least activity. Fermented extracts of SC (0.98) possess the highest reducing ability. Fermented extracts of PP (0.86), WS (0.61), RS (0.64) and CC (0.45) also showed good reducing ability. Ferrous ion scavenging activity was highest in both fermented extracts of SC and PP. Activity of fermented (45.7%) extracts of WS and RS was same while, fermented extracts of CC showed the least activity (38.6%). Ferric ion reduction power of fermented extracts of SC, PP, WS, RS and CC were 46.4, 42.6, 38.9, 40.6 and 35.8%, respectively. Scavenging effect for NO ion was highest in fermented extracts of SC (55.3%) followed by PP (52.5%) and WS, RS and CC exhibited 46.7, 48.7 and 38.8% of scavenging effect, respectively. Fermented extracts of SC, PP, WS, RS and CC possess 10.8, 11.4, 10.5, 10.8 and 8.5 mg mL^{-1} while, unfermented extracts possess 7.8, 6.8, 5.5, 6.3 and 6.8 mg mL^{-1} of TPC, respectively (Table 6).

Fermentation of different agro-residues by *Aspergillus fumigatus*, their effect on antioxidant activity and TPC: The DPPH scavenging activity was found to be highest in the fermented extract of PP (65.5%). Fermented extract SC (62.9%), WS (60.8%), RS (60.5%) and CC (55.6%) also showed good scavenging activity. The reducing power of the

Table 7: Effect of fermentation of different agro-residues by *Aspergillus fumigates* on various antioxidant activities

	Rice straw		Pea pod		Wheat straw		Corn cob		Sugarcane baggases	
Parameters	A	B	A	B	A	B	A	B	A	B
DPPH assay	32.4	60.5	34.6	65.6	30.4	60.8	25.7	55.6	30.6	62.9
Reducing power	0.17	0.42	0.12	0.68	0.18	0.45	0.21	0.34	0.31	0.6
Fe^{2+} scavenging activity	28.6	38.7	30.8	46.6	28.9	40.2	25.4	36.7	28.9	45.7
FRAP assay	25.6	35.6	28.7	42.3	25.5	40.8	22.3	30.5	24.4	42.3
NO scavenging activity	28.5	40.8	30.5	45.6	26.8	42.3	24.8	35.7	20.8	44.6
TPC	5.5	10.2	6.8	12.4	6.3	10.8	6.8	10.2	7.8	12.7

A: Activity of control (unfermented agro-residues) and B: Activity of agro-residues fermented by fungi

Table 8: Effect of fermentation of different agro-residues by *Penicillium expansum* on various antioxidant activities

	Rice straw		Pea pod		Wheat straw		Corn cob		Sugarcane baggases	
Parameters	A	B	A	B	A	B	A	B	A	B
DPPH assay	32.4	50.6	34.6	59.7	30.4	52.8	25.7	45.5	30.6	62.8
Reducing power	0.17	0.64	0.12	0.82	0.18	0.64	0.21	0.44	0.31	0.96
Fe^{2+} scavenging activity	28.6	48.7	30.8	52.7	28.9	42.6	25.4	38.6	28.9	52.7
FRAP assay	25.6	40.9	28.7	45.6	25.5	40.9	22.3	35.8	24.4	48.4
NO scavenging activity	28.5	48.7	30.5	53.5	26.8	46.7	24.8	37.7	20.8	57.3
TPC	5.5	10.8	6.8	11.8	6.3	10.5	6.8	9.1	7.8	12.0

A: Activity of control (unfermented agro-residues) and B: Activity of agro-residues fermented by fungi

fermented extract of PP (0.68) was highest followed by SC (0.6) while, others also showed good reducing power. The ferrous ion scavenging activity of fermented extracts of SC, PP, WS, RS and CC were 45.7, 46.6, 40.2, 38.75 and 36.7%, respectively. Ferric ion reduction power of fermented extracts of SC, PP, WS, RS and CC are 42.3, 42.3, 40.8, 35.6 and 30.5%, respectively. Scavenging effect for NO ion was highest in fermented extracts of SC (45.6%) followed by PP (44.6%) and WS, RS and CC exhibited 42.3, 40.8 and 35.7% of scavenging effect, respectively. Fermented extracts of SC, PP, WS, RS and CC possess 12.7, 12.4, 10.8, 10.2 and 10.2 mg mL^{-1} of TPC, respectively (Table 7).

Fermentation of different agro-residues by *Penicillium expansum*, their effect on antioxidant activity and TPC: Extracts of fermented SC (62.8%) showed the highest DPPH scavenging activity followed by PP (59.7%), WS (52.8%) and RS (50.6%). The activity of fermented extracts of CC was least among all the substrates with 45.5%. Similarly, reducing power of the extracts of fermented SC (0.96) was the highest followed by PP (0.82) fermented (0.64) extracts of WS and RS was same. The reducing power of fermented extracts of CC was least (0.44). Chelating activity of fermented extracts of PP extracts was 52.7% while, SC showed 50.2%. Fermented extracts of WS and RS chelated 42.6 and 48.7% of ferrous ion, respectively. The activity of fermented extracts of CC demonstrated 38.6%. Fermented extracts of SC (48.4%) and PP (45.6%) showed highest FRAP activity followed by WS and RS (40.9%). Fermented extracts of CC showed 35.8% of activity. Fermented extracts of SC (57.3%) showed highest scavenging effect for NO ion, followed by fermented extracts of PP (53.5%) and RS (48.7%). Fermented extracts of WS and CC showed 46.7 and 37.7% of activity, respectively. Total phenolic content of the fermented extracts of SC (11.8 mg mL^{-1}) was highest closely followed by fermented extracts of PP (12.0 mg mL^{-1}). The TPC of fermented extracts of WS and RS are 10.5 and 10.8 mg mL^{-1}, respectively. Fermented extracts of CC possessed 9.1 mg mL^{-1} of TPC (Table 8).

Toxicity testing: The filtrate obtained after fermentation showed no mutagenicity as no bacterial colony was observed on agar plates while, more than 1000 colonies were observed on positive control (sodium azide) containing plate. Similarly, results obtained from MTT assay revealed that the filtrate obtained after fermentation was non cytotoxic.

DISCUSSION

Fermentation are into two processes systems: Submerged fermentation (SmF), which is microorganisms cultivation in a liquid medium containing media and second is Solid State Fermentation (SSF), which consists of the microbial growth and product formation on solid particles in the absence (or near absence) of water, however, substrate contains the sufficient moisture to allow the microorganism growth and metabolism. In recent years, SSF has received more interest from researchers since several studies have demonstrated that this process may lead to higher yields and productivity and also due to the utilization of low cost agricultural agro-residues as substrates[7]. Fungi have great potential to

produce bioactive compounds by solid state fermentation, hence, they are the most commonly used for this purpose[8].

Screening for antioxidant activity of fungi was carried out by DPPH dot blot assay which is adaptable to "High-throughput" analysis[9]. This method follows a mechanism of inhibition of the accumulation of oxidized products, since the generation of free radicals is inhibited by the addition of antioxidants[10]. The appearance of a white color spot on a purple background showed antioxidant capability of each fungus. In the present study, soil fungal isolates possessed antioxidant activity and most of them belonged to genus *Penicillium* and *Aspergillus*. The results support the study "A millenium of fungi, food and fermentation" carried out by Hesseltine[11], in which he has listed over a 100 of fermentations and quoted that "This list offers a wealth of subjects for future investigation[12]". This shows the importance of *Aspergillus* and *Penicillium* spp. in fungal kingdom.

The prevention of chain initiation, binding of transition metal ions, decomposition of peroxides, prevention of hydrogen abstraction and free radical scavenging are the various mechanisms which has been followed by a potent antioxidants to show their antioxidant activity. Antioxidants can deactivate radicals by two major mechanisms, Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). The HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation and SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals and radicals[9]. Hence various assay procedures namely 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay, reducing power, ferrous ion and nitric oxide ion scavenging activity. Ferric Reducing Antioxidant Power (FRAP) assay were used for the estimation of antioxidant potential and all the eight selected fungal isolates showed good antioxidant activity against various free radicals. The results obtained from various assay procedures prove the potent broad spectrum antioxidant activity of the extracts obtained from the fermentation of fungal isolates.

The DPPH assay is based on the scavenging of the 1,1-diphenyl-2-picrylhydrazyl radical which leads to decreased absorbance. All the fungi showed good scavenging activity against DPPH radical that means that they all possess high hydrogen donating activity. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[13]. Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction[14]. The formation of the ferrozine-Fe^{2+} complex is interrupted in the presence of filtrate obtained after fermentation of all the fungi as ferrous ion was chelated by them. Similarly, FRAP assay is a technique to determine the total antioxidant power interpreted as the reducing capability[1] and all the fungi showed good reduction potential. Nitric oxide ion scavenging activity was based on the neutralization of nitric oxide ion and according to the results, all the fungi have the potential to neutralize nitric oxide ion[15].

Previous studies indicate phenolic compounds to be the major antioxidants of medicinal plants, mushrooms, essential oils, spices, fruits and vegetables. The interest in the phenolic compounds has increased tremendously due to their prominent free radical scavenging activity, attributed to their redox properties, which allow them to act as reducing agents or hydrogen atom donor. Some studies have proved that the phenolic substances such as flavonoids and phenolic acids are considerably more potent antioxidants than vitamin C and vitamin E. These compounds have also been found to exhibit many other health related properties because of their antioxidant activities[16]. Hence, TPC in extracellular filtrate was estimated so as to work out its correlation with antioxidant activity. Results demonstrated that phenolic contents are exhibiting antioxidant potential through donating hydrogen or reduction as well as with scavenging or inhibition ability and acting as suppressing agents. Scavenging potential and reducing power of these fungi may be dependent upon their unique phenolic structure, number and position of the hydroxyl groups and their ability to donate hydroxyl groups as well as in which form these phenolic compounds are present, i.e., free or bound form[13,17]. Some compounds showing high activity may easily scavenge free radicals and reducing agents due to free hydroxyl groups in phenolic compounds while, some other type of compounds produced by these fungi are less efficient scavengers of free radicals and reducing agent due to some steric hindrances[18].

CONCLUSION

The SSF is a clean technology with great potential for application on the production or extraction of biologically active compounds from natural sources. The agro-industrial residues reuse in this area is of particular interest due to their availability, low cost and characteristics that allow obtaining different bioactive phenolic compounds, besides being an environment friendly alternative for their disposal. Another interesting application for SSF is to increase the bioactive phenolic compounds content in food products. This area has

great potential to expand in a near future due to the increased consumer desire to improve health through food.

SIGNIFICANT STATEMENTS

- Agricultural waste bioconversion aimed at producing fungal biomass is a highly attractive alternative because, besides resulting in products of commercial interest, it reduces the amount of waste thereby minimizing pollution
- The present study demonstrated potential of soil fungi to have antioxidant activity similar to plants and mushrooms, thus further highlighting their significance as new sources of natural antioxidants and thus endorse the future prospects for the commercial production of natural and safer antioxidant compounds from such fungi. These fungi may provide easier set up for production and purification of natural antioxidants as compared to higher plants
- Easier downstream processing of the fungal compounds as compared to phytochemicals offers a ray of hope for further development of chemotherapeutic agents as antioxidants are used as protective measure in various diseases

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