International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 12, Issue 2, 2020

Original Article

ASSESSING COSMECEUTICALS PROPERTIES OF SOME MACROFUNGI FOR IMPROVED SKINCARE

AKASH SARASWAT[1], PURVI MATHUR[1], DOYELI SANYAL[1*]

[1]TERI-Deakin Nano Biotechnology Centre, Sustainable Agriculture Division, The Energy and Resources Institute, New Delhi 110003, India
Email: **doyeli.sanyal@teri.res.in**

Received: 14 Oct 2019, Revised and Accepted: 10 Dec 2019

ABSTRACT

Objective: This study has investigated cosmeceutical properties namely, antibacterial, sun protection factor and total phenolics contents of some selected macrofungi. The studies were conducted on five reference cultures collected from Indian type culture collection center (IMTEC, Chandigarh, India) and five isolates collected from TERI-Deakin Nano Biotechnology Centre facility at TERI Gram, Gurugram, India.

Methods: The cosmeceutical properties of the crude extracts from selected macrofungi were analyzed using standard bioassay techniques. Antibacterial activity was analyzed against *Staphylococcus epidermidis, Escherichia coli*, *Micrococcus luteus*, *Bacillus megaterium, Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Acinetobacter baumannii* using Agar well diffusion method. The sun protection factor was estimated and calculated using the Mansur equation. Free radical scavenging activity using DPPH was performed to assess the antioxidant activity of the extracts.

Results: Ethyl acetate extracts of the broth from *P. florida* and TERI-G1 cultures showed a broad-spectrum antibacterial activity against *S. epidermidis*, *E. coli*, *M. luteus* and *B. megaterium*. Ethyl acetate extracts of the broth from TERI-G3 showed the highest SPF activity of 34.02 at 200 µg. ml^{-1} concentration. Ethyl acetate extract of the broth from *F. velutipes*, *P. florida*, *P. ostreatus,* and TERI-G1 showed comparable antioxidant activity of 66.86%, 79.51%, 82.02%, and 69.58% respectively when compared to ascorbic acid (85.83%) and quercetin (83.09%) taken as positive control in the study and their total phenolic contents were found to be 6.93, 43.68, 20.88 and 13.77 Gallic acid equivalent (GAE) per gram. The minimal inhibitory concentration of F. *velutipes, P. florida, P. ostreatus, and* TERI-G1 was found to be 3552.89 µg. ml^{-1}, 1250 µg. ml^{-1}, 2418.9 µg. ml^{-1} and 3219 µg. ml^{-1} respectively.

Conclusion: The work is in progress to identify and characterize TERI-G1and TERI G3 cultures. Further studies on the anti-inflammatory, anti-tyrosinase, elastase inhibition properties of the cultures will be assessed to identify potential cosmeceutical active ingredients with promising applications in cosmeceutical products.

Keywords: Chemical analysis, Microbiology, Spectroscopy, Macrofungi, bioassay studies

DOI: http://dx.doi.org/10.22159/ijpps.2020v12i2.36074. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps

INTRODUCTION

Presently, there is increasing consumer demand for cosmetics that incorporate organic and natural ingredients as a result of which the cosmeceutical division of the personal care industry is rapidly thriving [1]. Cosmeceuticals are products that comprise creams, lotions, and ointments and have both cosmetic and therapeutic effects due to the presence of bioactive ingredients which enhances skin health [2]. Recently macrofungi have attracted a lot of attention as an interesting source of unique bioactive. These contain substances like carotenoids, resveratrol, omega fatty acids, ceramides, etc [3] which makes them a potent candidate for use in the cosmetic industry. They are easy to grow and maintain and their extracted secondary metabolites can be used as enhanced analogs with minimum side-effects. These macrofungi have been well documented as to the source of polyphenolic, phenolics, polysaccharides, terpenoids, selenium, vitamins and volatile organic compounds. Many of these compounds have been associated with excellent antioxidant, anti-inflammatory, anti-bacterial, anti-viral, anti-aging, anti-wrinkle, skin renewal, skin whitening and moisturizing effects [4-6]. Macrofungi like Reishi (*Ganoderma lucidum*), Shiitake (*Lentinula edodes*), Maitake (*Grifola frondosa*), cauliflower mushroom (*Sparassis latifolia*), Fu Ling (*Wolfiporia extensa*), etc are popularly used in facial creams and serums in East Asian countries including Korea, Japan and China [7-9]. Tinder fungus (*Fomes fomentarius*), oyster mushroom (*Pleurotus ostreatus*), elm oyster mushroom (*Hypsizygus ulmarius*), Portobello mushroom (*Agaricus bisporus*) prevail more in the Western region [10-12].

These macrofungi also show interesting anti-microbial activities. *Lentinula edodes* is very efficient against both gram-positive and gram-negative bacteria. Ganoderma, Lepista and Boletus species also show similar properties [13]. These macrofungi contain phenolic compounds that safeguard against viruses, bacteria, insects, and UV light [14].

Many well-known cosmetic brands nowadays use extracts of macrofungi in their products. For instance, the popular product range Aveeno Positively Ageless by Johnson and Johnson contains extract of *Ganoderma lucidum* and *Lentinula edodes*. Another brand, Vitamega cosmetics uses the extract of *Agaricus subrufescens* in their shampoo and moisturizing cream range. Earthherbs L. L. C. uses the extract of Siberian Chaga mushroom (*Inonotus obliquus*) in their antioxidant rejuvenating cream [15].

The present work is focused on exploring cosmeceutical properties namely; antibacterial, SPF, antioxidant activities and phenolics content of few selected identified (Pleurotus species, Flammulina species), and uncharacterized mushrooms collected from the TDNBC (TERI-Deakin Nano Biotechnology Centre) facility at TERI Gram, Gurugram, India.

MATERIALS AND METHODS

Reagents

All the reagents and chemicals used in the experiments were of analytical grade. Culture media (Nutrient Agar, Nutrient Broth, Potato Dextrose broth, Potato Dextrose Agar, Mueller Hinton agar no. 2) were collected from HiMedia Laboratories. Solvents (methanol, ethyl acetate, ethanol) and chemicals (Ascorbic acid, 1,1--Diphenyl-2-picrylhydrazyl (DPPH) Quercetin, Folin-Ciocalteu reagent, gallic acid) were purchased from Sigma Chemical Co., USA. Milli-Q water used in the studies has been obtained from Milli-Q® Integral by Merck.

Apparatus

1000 ml Erlenmeyer flask, Buchner funnel assembly, rotary vacuum evaporator, UV visible spectrophotometer (SHIMADZU, UV 2450) were used in the study.

Microbial culture

Mushroom cultures namely, *Flammulina velutipes* (MTCC-543), *Pleurotus florida* (MTCC-9194), *Pleurotus ostreatus* (MTCC-1801, 1802) and *Pleurotus sajor caju* (MTCC-141) and bacterial cultures for antimicrobial studies namely, *Staphylococcus epidermidis* (MTCC-3615), *Escherichia coli* (MTCC-443), *Micrococcus luteus* (MTCC-106), *Bacillus megaterium* (MTCC-453), *Pseudomonas aeruginosa* (MTCC-424), *Staphylococcus aureus* (MTCC-1144) and *Acinetobacter baumannii* (MTCC-1425) were collected from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The fungal cultures were maintained in Potato Dextrose Agar plates at 28 °C. The bacterial cultures were maintained in Nutrient Agar plates at 28 °C except for E. coli which was maintained at 37 °C.

Preparation of extracts

Three 6 mm discs of the mushroom cultures from their respective agar plates were used to inoculate 1000 ml Erlenmeyer flask containing 300 ml of Potato Dextrose broth in triplicate and incubated under shaking at 28 °C at 180 rpm for 21 d. After the completion of the incubation period, the mycelia were separated from the broth aseptically using a sieve (BSS 500 micron) and air-dried. The mycelia were extracted with methanol (50 ml) in a shaker for 24 h at 200 rpm. The extracts were filtered through Buckner funnel assembly and were washed twice with 25 ml methanol each. The extracts were collected and concentrated using a rotary vacuum evaporator at 45 °C. The broth samples were extracted with 100 ml ethyl acetate using liquid-liquid partitioning in a 2 liter separating funnel. The samples were further extracted two more times with 50 ml of ethyl acetate each. All the extracts were collected and concentrated using a rotary vacuum evaporator set at 45 °C. The concentrated crude extracts were then used for bioassay studies.

Table 1: Macrofungi and corresponding codes for the extracts

Microorganism	MTCC Number	Solvent	Extract	Fungal Code
Flammulina velutipes (FV)	543	Ethyl acetate	Broth	V1B
Flammulina velutipes (FV)		Methanol	Mycelia	V1M
Pleurotus florida (PF)	9194	Ethyl acetate	Broth	F1B
Pleurotus florida (PF)		Methanol	Mycelia	F1M
Pleurotus ostreatus (PO-S1)	1801	Ethyl acetate	Broth	O1B
Pleurotus ostreatus (PO-S1)		Methanol	Mycelia	O1M
Pleurotus ostreatus (PO-S2)	1802	Ethyl acetate	Broth	O2B
Pleurotus ostreatus (PO-S2)		Methanol	Mycelia	O2M
Pleurotus sajor caju (PSC)	141	Ethyl acetate	Broth	C1B
Pleurotus sajor caju (PSC)		Methanol	Mycelia	C1M
TERI-G1 (TG-1)		Ethyl acetate	Broth	T1B
TERI-G1 (TG-1)		Methanol	Mycelia	T1M
TERI-G2 (TG-2)		Ethyl acetate	Broth	T2B
TERI-G2 (TG-2)		Methanol	Mycelia	T2M
TERI-G3 (TG-3)		Ethyl acetate	Broth	T3B
TERI-G3 (TG-3)		Methanol	Mycelia	T3M
TERI-G4 (TG-4)		Ethyl acetate	Broth	T4B
TERI-G4 (TG-4)		Methanol	Mycelia	T4M
TERI-G5 (TG-5)		Ethyl acetate	Broth	T5B

***In vitro* antimicrobial study**

Agar well-diffusion method described by Perez *et al.* [16] was used to determine the antimicrobial activity. Mueller Hinton agar no. 2 was prepared and cooled to 40-45 °C and the bacterial inoculum (1.5×10^8 CFU. ml^{-1}, 0.5 McFarland) prepared above was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells (6 mm diameter) were made in each of these plates using a sterile cork borer. The wells were filled with 50 µl of test extract (5 µg. ml^{-1}). The plates were first incubated at 4 °C for 15 min and then at 37 °C for 18 h under aerobic conditions. The antimicrobial activity of the extract was determined by calculating the zone of inhibition. The diameters of zones of inhibition (ZOI) produced by the test were recorded and compared with those produced by positive control (Tetracycline at 5 µg. ml^{-1}, Sigma-Aldrich). For negative control, pure solvent (autoclaved Milli-Q water) was used in place of the extract. The experiment was performed two times in triplicates to minimize error and to check the reproducibility of results. The average values were recorded. The % antimicrobial activity was calculated with equation 1.

$$\% \text{ Inhibition} = 100-\{\frac{[(\text{ZOI control}-\text{ZOI sample})]}{(\text{ZOI control})}*100\} \text{Equation 1}$$

Sun protection factor

SPF determination was performed as described in Mansur *et al.* 1986 [17]. Absorbance readings of the crude extracts (200 µg. ml^{-1} concentration) dissolved in ethanol were taken in the wavelength range of 290 to 320 nm at 5 nm interval. A dilution to 100 µg. ml^{-1} was done with crude extracts showing exceeding spectroscopic readings. Mansur equation as mentioned in equation 2 was used to determine the SPF values of the crude extracts.

$$\text{SPF}= \text{CF}\times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda) \ldots \text{Equation 2}$$

where CF is 10 (correction factor), EE (λ) is erythemogenic effect of radiation at wavelength λ, I (λ) is the intensity of solar light at wavelength λ, and abs (λ) is the absorbance of wavelength λ by a solution of the preparation. The obtained absorbance values were multiplied by the EE (λ) values; their summation was taken and multiplied by the correction factor 10.

Antioxidant assay

Antioxidant activity of the crude extracts was determined as described by Clarke *et al.* 2013 [18] with slight modification. 1,1--Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was assessed with 20 ul of crude extract of 5000 µg. ml^{-1} concentration dissolved in methanol, mixed with 0.1 mmol of DPPH followed by incubation in the dark for 30 min. Absorption was taken at 517 nm with the help of a UV-Visible Spectrophotometer. Appropriate blank (methanol) and positive control (Ascorbic acid and Quercetin) were used. The inhibition of the DPPH free radical in percentage (I %) was calculated following equation 3.

$$\% \text{ Inhibition} = \{\frac{[(\text{A control} - \text{A sample})]}{(\text{A control})} * 100\} \ldots \text{Equation 3}$$

Where $A_{control}$ is the absorbance of the control reaction (full reaction, without the tested extract or Quercetin) and A_{sample} was the absorbance

in the presence of the sample. A fresh solution of DPPH was prepared before each experiment. Decreased absorbance of the reaction mixture indicates stronger DPPH free radical-scavenging activity. The minimum inhibitory concentration (MIC) values of the extracts showing good antioxidant properties were evaluated using the two-fold serial dilution method [19]. The samples were dissolved in methanol to obtain 5 mg. ml^{-1} stock solutions. With the help of serial dilution concentrations 500 µg. ml^{-1}, 250 µg. ml^{-1} and 125 µg. ml^{-1} were achieved and readings were taken at 517 nm wavelength.

Total phenolics content

The total phenolic content (TPC) was determined calorimetrically using a Folin-Ciocalteu 96-well microplate assay as described by Zhang et al. (2006) [20]. The total phenolic content was calculated from the linear equation of a standard calibration curve prepared with gallic acid and expressed as Gallic Acid Equivalent (GAE) per gram of extract.

Statistical analysis

All data are presented as mean±standard error of the means (SEM). Data for antimicrobial studies were analyzed using a 2way analysis of variance (ANOVA) using GraphPad Prism version 8.0.2. Differences were accepted as statistically significant when p-value<0.05.

RESULTS AND DISCUSSION

Antimicrobial studies

Continued search for antibacterial activity assessment of new potential active ingredients is crucial as gram-positive (*S. epidermidis, M. luteus, B. megaterium, S. aureus*) and gram-negative (*E. coli, P. aeruginosa, A. baumannii*) bacteria considered for this study are associated with systemic infections namely, urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and has been reported to have developed resistance against several commercial antibiotics [21]. As enumerated in table 2, extracts displayed different levels of antibacterial activities against the tested pathogenic bacteria. Broth culture of P. *florida* and TERI-G1 showed a broad-spectrum antibacterial activity against *S. epidermidis, E. coli, M. luteus, B. megaterium* cultures, with *P. florida* showing specific activity against *P. aeruginosa* and TERI-G1 against *A. baumannii* and *S. aureus*. Antibacterial activity as observed in remaining extracts was negligible or less than 50% relative to tetracycline, the positive control taken in the study. The antimicrobial activity of fungal extracts against different bacteria was found to be significant during statistical analysis (p-value<0.05).

Table 2: Antibacterial activities of the crude fungal extracts

Fungal code	Antibacterial activity (%)*						
	S. epidermidis	*E. coli*	*M. luteus*	*B. megaterium*	*P. aeruginosa*	*A. baumannii*	*S. aureus*
V1B	0.00±0.00	0.00±0.00	28.41±1.14	40.00±3.64	41.18±1.96	0.00±0.00	0.00±0.00
V1M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	39.22±3.92	0.00±0.00	0.00±0.00
F1B	21.51±0.00	26.67±0.00	22.73±0.0	45.45±1.82	43.14±3.92	0.00±0.00	0.00±0.00
F1M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	43.14±3.92	0.00±0.00	0.00±0.00
O1B	0.00±0.00	0.00±0.00	0.00±0.00	34.55±1.82	43.14±3.92	0.00±0.00	0.00±0.00
O1M	0.00±0.00	0.00±0.00	0.00±0.00	60.00±5.45	39.22±0.00	0.00±0.00	0.00±0.00
O2B	0.00±0.00	0.00±0.00	22.73±0.00	36.36±0.00	35.29±3.92	0.00±0.00	0.00±0.00
O2M	0.00±0.00	0.00±0.00	0.00±0.00	32.73±3.64	41.18±1.96	0.00±0.00	0.00±0.00
C1B	0.00±0.00	0.00±0.00	0.00±0.00	49.09±5.45	47.06±3.92	0.00±0.00	0.00±0.00
C1M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	39.22±0.00	0.00±0.00	0.00±0.00
T1B	50±0.00	51.28±0.00	44.44±0.00	56.86±9.8	0.00±0.00	33.85±3.08	64.79±5.63
T1M	21.43±0.00	0.00±0.00	25.56±1.11	56.86±9.8	0.00±0.00	0.00±0.00	50.7±0.00
T2B	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T2M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T3B	0.00±0.00	51.28±0.00	27.78±1.11	35.29±3.92	0.00±0.00	0.00±0.00	0.00±0.00
T3M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T4B	0.00±0.00	69.23±7.69	28.89±2.22	35.29±3.92	0.00±0.00	0.00±0.00	26.76±1.41
T4M	0.00±0.00	51.28±0.00	27.78±1.11	35.29±3.92	0.00±0.00	0.00±0.00	0.00±0.00
T5B	0.00±0.00	61.54±0.00	35.56±2.22	35.29±3.92	0.00±0.00	0.00±0.00	25.35±2.82

The % antimicrobial activity was calculated using equation 1 and expressed as a mean±standard deviation to the mean. (n=3) *(Data was found to be significant, p<0.05).

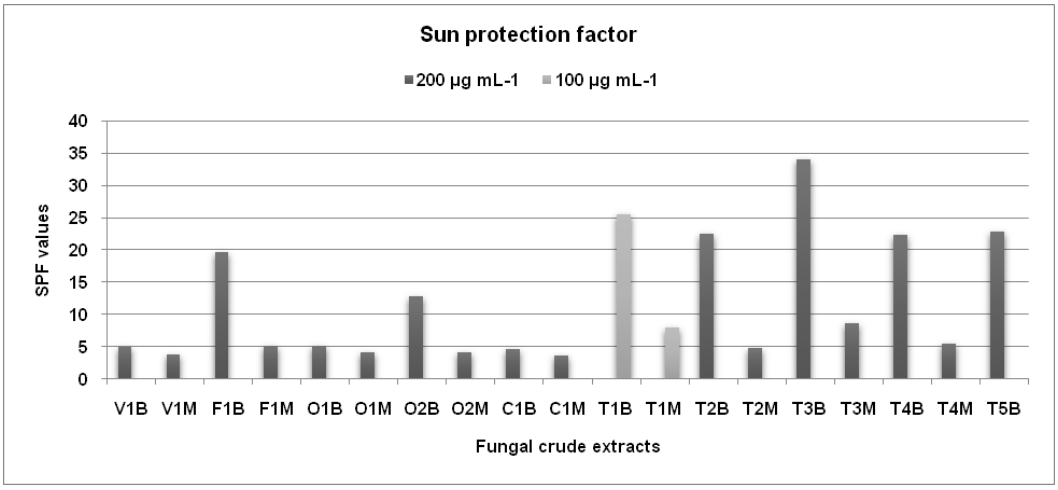

Fig. 1: Sun protection factor of the fungal extracts, SPF values were calculated using Mansur equation (equation 2) and expressed in fig. 1. (n=1)

Sun protection factor

Calculated values of SPF of the crude extracts isolated from the selected macrofungi were found to be in the range of 3.67-34.02. Dutra *et al.* (2004) [22] analyzed several commercial products containing a combination of active ingredients viz., benzophenone-3, octyl methoxycinnamate and octyl salicylate (in varying proportions) for their SPF values and compared them with the declared values in their product label and found to be in the range of 15-20. In the present study, crude extracts of TERI-G3 (T3B) showed promising SPF activity of 34.02 at 200 µg. ml^{-1}. *P. florida* (F1B), TERI-G1 (T1B), TERI-G2 (T2B), TERI-G4 (T4B) and TERI-G5 (T5B) also showed comparable SPF of 19.63, 25.50, 22.44, 22.31 and 22.87 respectively.

Antioxidant activity

Ascorbic acid and Quercetin were used as a positive control in this study which showed as 85.83% and 83.09% DPPH free radical scavenging activity respectively. Broth of *P. florida*, *F. velutipes*, *P. ostreatus*, TERI-G1 showed comparable scavenging activity as positive control with values of 79.51, 66.8, 82.02 and 69.58% respectively. Broth of *P. florida*, *F. velutipes*, *P. ostreatus* and TERI-G1-B showed MIC at 1250, 3552.89, 2418.9 and 3219 µg. ml^{-1}. Mycelia of *P. florida* showed MIC at 2903.76 µg. ml^{-1}.

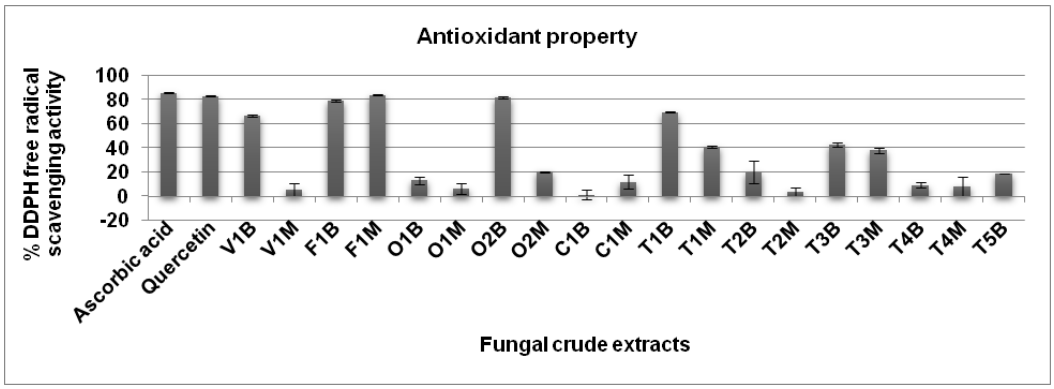

Fig. 2: DPPH free radical scavenging activity of the fungal extracts, the % antioxidant activity was calculated using equation 3 and expressed as mean±standard error to the mean (n=2)

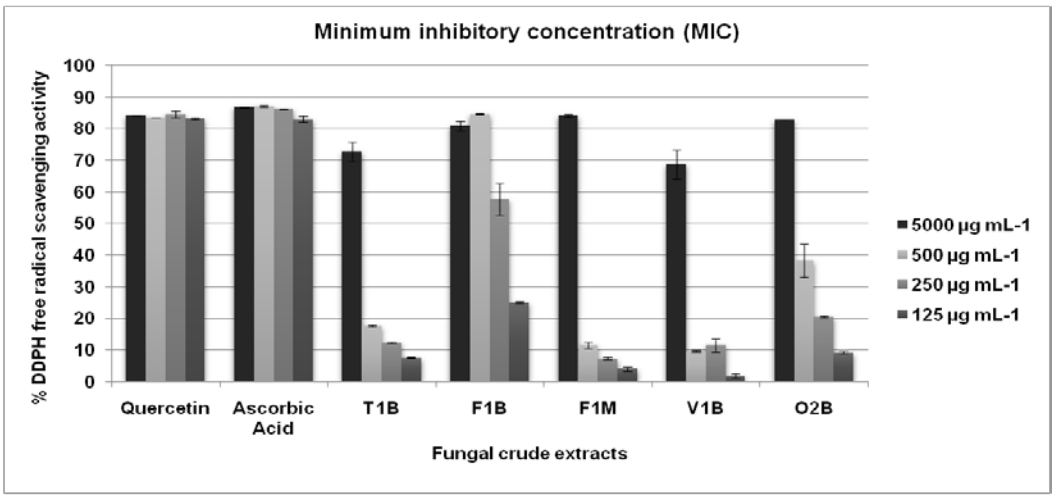

Fig. 3: Minimum inhibitory concentration of the selected fungal extracts, the MIC values are expressed as mean±standard error to the mean (n=2)

Total phenolic content

Phenolic compounds have been well documented for their contribution to antioxidant activity. These compounds are good electron donors, which directly contribute to antioxidant action [23]. Some phenolic compounds also stimulate the synthesis of endogenous antioxidant molecules in the cell [24]. Therefore, knowledge of total phenolic content is necessary for our study. Broth of *P. florida, F. velutipes, P. ostreatus* and TERI-G1-B showed total phenolics content of 43.68, 6.93, 20.88 and 13.77 gallic acid equivalent (GAE) per gram. Mycelia of *P. florida* showed a total phenolics content of 5.96 gallic acid equivalent (GAE) per gram.

Table 3: Total phenolic contents of the selected crude fungal extracts

Extract code	Total phenolics content (Gallic acid equivalent (GAE) per gram)
T1B	13.77±
F1B	43.68±
F1M	05.96±
V1B	06.93±
O2B	20.88±

Total phenolic content was plotted from a standard curve of Gallic acid equivalent. (n=2)

CONCLUSION

The most promising activity was shown by TERI G1, TERI G3, and *P. florida* extracts. The work is in progress to identify and characterize TERI-G1and TERI G3 cultures. Further studies on the anti-inflammatory, anti-tyrosinase, elastase inhibition properties of the cultures will be assessed to identify potential cosmeceutical active ingredients with promising applications in cosmeceutical products.

ACKNOWLEDGMENT

We thank The Director, Sustainable Agriculture Division, TD-NBC, TERI, India for providing necessary facilities to carry out research work.

AUTHORS CONTRIBUTIONS

All authors contributed extensively to the work presented in this paper.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

REFERENCES

1. Brandt FS, Cazzaniga A, Hann M. Cosmeceuticals: current trends and market analysis. Semin Cutaneous Med Surg 2011;30:141–3.
2. Mukta S, Adam F. Cosmeceuticals in day-to-day clinical practice. J Drugs Dermatol 2010;9:s62–6.
3. Camassola M. Mushrooms-the incredible factory for enzymes and metabolites productions. Ferment Technol 2013;2:1.
4. Wu Y, Choi MH, Li J, Yang H, Shin HJ. Mushroom cosmetics: the present and future. Cosmetics 2016;3:22.
5. Jandaik S, Anjana S. Oyster mushroom: an answer to human ailments: a review. Asian J Pharm Clin Res 2017;10:24-7.
6. Chandran PK, Ulagan MP. Antimicrobial and anticancer activity of silver nanoparticles from edible mushroom: a review. Asian J Pharm Clin Res 2017;10:37-40.
7. Jikai L. Biologically active substances from mushrooms in Yunnan, China. Heterocycles 2002;57:157-67.
8. Zhang Y, Mills GL, Nair MG. Cyclooxygenase inhibitory and antioxidant compounds from the mycelia of the edible mushroom *Grifola frondosa*. J Agric Food Chem 2002; 50:7581-5.
9. Choi MH, Han HK, Lee YJ, Jo HG, Shin HJ. *In vitro* anticancer activity of hydrophobic fractions of *Sparassis latifolia* extract using AGS, A529, and HepG2 cell lines. J Mushroom 2014;12:304-10.
10. Van Griensven LJLD. Culinary-medicinal mushrooms: must action be taken? Int J Med Mushrooms 2009;11:281.
11. Bilal AW, Bodha RH, Wani AH. Nutritional and medicinal importance of mushrooms. J Med Plants Res 2010;4:2598-604.
12. Wasser SP. Medicinal mushroom science: history, current status, future trends, and unsolved problems. Int J Med Mushrooms 2010;12:1.
13. Alves MJ, Ferreira ICFR, Dias J, Teixeira V, Martins A, Pintado M. A review on antimicrobial activity of mushroom (basidiomycetes) extracts and isolated compounds. Planta Med 2012;78:1707–18.
14. Heleno SA, Martins A, Queiroz MJRP, Ferreira ICFR. Bioactivity of phenolic acids: metabolites versus parent compounds: a review. Food Chem 2015;173:501–13.
15. Hyde KD, Bahkali AH, Moslem MA. Fungi—an unusual source for cosmetics. Fungal Diversity 2010;43:1-9.
16. Perez C, Paul M, Bazerque P. Antibiotic assay by agar well diffusion method. Acta Biol Med Exp 1990;15:113-5.
17. Mansur JS, Breder MNR, Mansur MCA, Azulay RD. Determination of sun protection factor by spectrophotometry. An Bras Dermatol 1986;61:121-4.
18. Clarke G, Ting KN, Wiart C, Fry J. High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential, and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the malaysian rainforest. Antioxidants 2013;2:1–10.
19. Siddiqi R, Naz S, Ahmad S, Sayeed SA. Antimicrobial activity of the polyphenolic fractions derived from *Grewia asiatica*, *Eugenia jambolana* and *Carissa carandas*. Int J Food Sci Technol 2011;46:250-6.
20. Zhang Q, Zhang J, Shen J, Silva A, Dennis DA, Barrow CJ. A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. J Appl Phycol 2006;18:445-50.
21. Gandra S, Mojica N, Klein EY, Ashok A, Nerurkar V, Kumari M, *et al.* Trends in antibiotic resistance among major bacterial pathogens isolated from blood cultures tested at a large private laboratory network in India, 2008–2014. Int J Infect Dis 2016;50:75-82.
22. Dutra EA, da Costa Oliveira DAG, Kedor Hackmann ERM, Maria Ines Rocha. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Rev Bras Cienc Farm 2004;40:381-5.
23. Bendary E, Francis RR, Ali HMG, Sarwat MI, El Hady S. Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. Ann Agric Sci 2013;58:173-81.
24. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. Plants 2019;8:96.