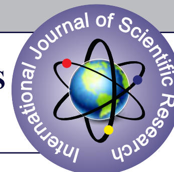
ORIGINAL RESEARCH PAPER

Volume - 10 | Issue - 04 | April - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH



ANTIOXIDANT, ANTIMICROBIAL AND ANTIVIRAL PROPERTIES OF INDIGENOUS EDIBLE MUSHROOM AGARICUS HETEROCYSTIS (AGH)

Microbiology

Jagadish Loganathan*	Center for Advanced Studies in Botany, University of Madras, India; PhD, Associate Professor, Department of Microbiology, PRIST Deemed University, Puducherry, India. *Corresponding Author
Suriya Kumaresan	Department of Biotechnology, PRIST Deemed University, India
Shenbhagaraman Ramalingam	Center for Advanced Studies in Botany, University of Madras, India
M.K. Premalatha	Center for Advanced Studies in Botany, University of Madras, India
Venkatakrishnan Venkatasubbu	Department of Biotechnology, Anna University, India
Kaviyarasan Venkatesan	Center for Advanced Studies in Botany, University of Madras, India

ABSTRACT

Antioxidant, antimicrobial and antiviral properties of Agaricus heterocystis (AGH) ethanolic extract were evaluated. The total antioxidant activity of the extract was assessed against ABTS and DPPH stable free radicals. Antioxidant components like phenol, flavonoid, β-carotene and lycopene were quantified. Antimicrobial and antiviral properties were analyzed by agar well-diffusion and plaque reduction methods respectively. The total antioxidant activity of the AGH extract were found to be strong and their EC_{50} values were 0.40±0.57 and 3.2±0.81 mg/ml against ABTS and DPPH radicals respectively. The AGH ethanolic extract showed strong antibacterial activity against gram positive bacteria than gram negative bacteria tested. The virucide action of the mushroom extract against human viruses tested exhibited prominent antiviral activity, the effective concentration inhibiting 50% of the viral activity were found to be of 116.3±1.97 and 110.2±1.88 µg/ml against HSV type 1 virus and Influenza viruses A and B respectively. In conclusion, it needs further study to identify the bioactive compounds responsible for antioxidant and antimicrobial properties and the role of antioxidant supplement under microbial stress.

KEYWORDS

Agaricus heterocystis (AGH); Antioxidant; Antimicrobial; Antiviral; Phenol; Flavonoid

INTRODUCTION

Mushrooms have been part of the human diet for thousands of years, involving a large number of edible species for their organoleptic, flavor, economic, ecological and medicinal properties. Nevertheless, an increase in the consumption of edible mushrooms has been observed in many Asian countries as food and medicine[1,2]. Recently, they have turned to become an attractive functional food that exhibit a large number of biological activities, making basidiomycetes an alternative target for natural drug research.

Free radicals or reactive oxygen species (ROS) produced in normal and pathological cells were essential to most living organisms to produce energy to enhance biological processes. Originally ROS were recognized as being instrumental for mammalian host defense as important intermediates in natural process that involved in cytotoxicity, control of vascular tone and neurotransmission by signaling the molecules in animals and plant host defense. But on the other hand, they lead to cause cellular damage produced uncontrollably. The oxygen derived free radicals produced involves in the onset of many diseases such as, cancer rheumatoid arthritis and atherosclerosis as well as degenerative processes associated with aging [3,4].

Almost all organisms are well protected against the damages caused by these free radicals, by having oxidative enzymes such as superoxide dismutase and catalase or chemical compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione [5-7]. However, these enzymes and compounds are frequently insufficient to totally prevent the damage and resulting in diseases, accompanied with accelerated ageing. Epidemiological studies have demonstrated that there is an inverse relationship exists between the intake of fruits and vegetables that are rich in antioxidants and their effect against free radical related diseases, set a milestone to screen the natural products like fruits, vegetables, herbs, mushrooms cereals, sprouts and seeds for their antioxidant properties[8,9].The Phytochemicals, especially of polyphenols present in fruits and vegetables are suggested to be the major bioactive health benefit compound, found to be associated with the inhibition of atherosclerosis and cancer [10,11].

The promising and positive effect of natural products against many human diseases had engrossed in design and discovery of new drugs. The appearance of resistant strains and the toxicity presented by some drugs have stimulated the research for the development of new substances like synthetic or natural antimicrobial agents without any side-effects [12-14]. The drugs derived from natural source is well established in many medicinal plants, but besides plants, fungi are an important source for anti-microbial and immunosuppressive compounds, e.g. penicillin and cyclosporine A. The secondary metabolites from higher fungi or mushrooms, exhibit many biological activities that includes variety of phenolic compounds, polyketides, terpenes and steroids[15,16].The antioxidant compounds present in mushrooms are of greater attention might support the human body to reduces oxidative stress induced damage without any interference [3]. Recently, some mushrooms such as *Fomes fomentarius*, *Lentinus edodes*, *Hypsizigus marmoreus*, *Ganoderma pfeifferi*, *Kuehneromyces mutabilis*, *Grifola frondosa* and *Rozites caperata* have been found to contain some compounds varied in their chemical structure including enols, sterols, organic acids, peptides (proteins) and with unidentified elements have antiviral and antimicrobial property[17-19].

Around the world many species of mushrooms are documented especially of indigenous edible and medicinal mushrooms and some are available or sold around, with great economic interests, as a source of income through self-employed. Hence, the main objective of the present study was to determine the biological properties of *Agaricus heterocystis* indigenously collected wild edible mushroom identified and optimized to grow under laboratory condition. To the best of our knowledge, no reports were available on their medicinal properties like antioxidant, antimicrobial and antiviral properties of this selected mushroom.

MATERIALS AND METHODS

Mushroom

Agaricus heterocystis (AGH) mushroom was collected from **Guindy Campus of the University of Madras, Chennai.** Identification and classification were carried out and the specimens deposited at the laboratory (Figure 1), having the gene bank accession number

FJ222602 given by NCBI EU028347 was taken for the study. Dried mushroom sample (50g) was extracted by stirring with 500 ml of ethanol at 30 ºC at 150 rpm for 24 h and filtered through Whatman No. 4 filter paper and then rotary evaporated at 40ºC to dryness, re-dissolved in ethanol to a concentration of 10 mg/ml and stored at 4 ºC for further use.

Figure 1 *Agaricus heterocystis* (AGH) mushroom cultivated under laboratory conditions.

Chemicals

Gallic-acid, Catechin Vitamin E, 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 1,1-Diphenly-2-picrylhydrazyl (DPPH) and MTT (3-(4,5- Dimethylthizaol-2-2yl)-2,5-diphenyltetrazolium bromide were purchased from Sigma (Sigma, Aldrich GmbH, Sternheim, GERMANY), Tween-20, Folin-Ciocalteu reagent (FCR), Sodium carbonate and ethanol.

Cell lines and Viruses

HEp2 and MDCK cell lines were grown in DMEM medium, supplemented with 10% of bovine fetal serum (BFS), penicillin (100 IU/ml), 2.5µg/ml of fungizones and streptomycin (100µg/ml) (Darmstat, GERMANY). The HSV-1 and Influenza A and B viruses were obtained from, Sri Ramachandra medical University, Department of Microbiology, Chennai. Virus stocks were prepared and stored at -80ºC.

Microorganisms

The following strains of bacteria were used: *Pseudomonas aeruginosa* ATCC 2036, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 27736, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Candida albicans* were collected from National cell culture collection (NCCC) Pune, India.

Antioxidant assay

ABTS radical scavenging activity

Free radical scavenging effect of *Agaricus heterocystis* (AGH) ethanol extract against ABTS free radical was determined according to the method Arno *et al.,*[20]. The capability to scavenge the ABTS radical was calculated using the following equation:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \text{ X } 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC) was calculated from the graph, plotted in percentage against extract concentration. Tests were carried out in triplicate.

DPPH assay

The free radical scavenging activities of extracts were measured by using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). Samples ranging from 0.1–20 mg/mL in 4mL of water or ethanol was mixed with 1 mL of methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma) radicals of 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank [21]. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = $[(A_0\text{-}A_1/A_0) \times 100]$

where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of DPPH scavenging effect against extract concentration. BHT was used as standard.

Antioxidant components

Total phenols were determined, and the content of total phenols was calculated on the basis of the calibration curve of gallic acid [22]. Total flavonoid was determined according to Barros & Ferreira *et al.,*[23]. The content of flavonoid was calculated on the basis of the calibration curve of catechins, and the results were expressed as mg of catechin equivalents (CEs) per gram of extract.

The dried ethanol extract (100mg) was taken to evaluate β-carotene and lycopene. The sample taken was vigorously shaken with acetone-hexane mixture (4:6, 10ml) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at γ = 453, 505 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100ml) = - 0.0458 A663 + 0.372 A505 – 0.0806 A 454: β-carotene (mg/100ml) = 0.216 A663 – 0.304 A505 + 0.452 A453. The results were expressed as µg of carotenoid/g of extract.

Phenol compounds and their antioxidant by thin layer chromatography

Thin layer chromatography (TLC) was performed on plates of 10cm x 10cm silica gel. Ethanolic extracts (10µl) were spotted on silica gel plates and developed in a horizontal chamber saturated with ethyl acetate: water: formic acid (85:15:10). After drying, the plates were developed using two sprays. Spray A (ferric chloride-Potassium ferricyanide [$FECL_3$-K_2Fe $(CN)_6$] was used to locate phenolic compounds. This spray was prepared at moment of the use by mixing equal volumes of each salt solution at 1%. The reagent has an orange-brown color and the phenolics present in the plate are detected by the formation of blue spots [24]. Spray B (0.04% DPPH in methanol) was used to locate antioxidant compounds. The use of purple reagent was to detect the presence of antioxidant compounds in the plate by the formation of white spots.

Antimicrobial activity of *Agaricus heterocystis* (AGH)

Antimicrobial activity of ethanol extract of *Agaricus heterocystis* (AGH) was determined by the agar-well diffusion method. The mushroom extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20% and sterilized by filtration through a 0.22µm membrane filter[25].

Determination of the maximum non-toxic concentration

The maximum non-toxic concentrations (MNTC) of the AGH extract in HEP-2 and MDCK cell lines was determined by a method like that described by Walker et al.,[26].

Determination of the antiviral activity

The antiviral activity was assessed by determining the reduction of the virus titers by determining the tissue culture infectious dose infecting 50% of the cells ($TCID_{50}$) or the infectious titer of any virus which can cause cytopathic effect (CPE). The HEp-2 (Human Caucasian larynx carcinoma) cell monolayers and MDCK (Madin-Darby Canine Kidney) Cells cultivated in 24 well TC plates were treated with two-fold dilutions of the extracts containing 500 l of 100 $TCID_{50}$ of the viruses added individually to different concentration of the extract and incubated for one hour in 37°C beginning from the MNTC. The plates were incubated for seven days at 36°C in 5% CO_2 environment. Cell control, Solvent control, Virus controls were performed along the assay. Cell Viability was confirmed by Tetrazolium blue reduction test (MTT).

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant properties assayed were summarized in Table 1 and the results were normalized and expressed as EC_{50} values (effective concentration scavenging 50% of free radicals) for comparison. The ethanol extract against ABTS radical was found to be effective and their EC_{50} value was found to be 400µg/ml. And at 5mg/ml the scavenging ability of *Agaricus heterocystis* ethanol extract against DPPH radical was found to be 70.24%. The EC_{50} value of 3.2mg/mL in *Agaricus heterocystis* ethanol extract was lesser about the other mushrooms such as *Lactarius deliciosus* (8.52mg/ml) and *Tricholoma protentosum* (22.9mg/ml)[27]. Similarly, the antioxidant property and the EC_{50} value of *Agaricus heterocystis* was effective when compared with a previous report made by Barros *et al.,* [28] on antioxidant property of *Agaricus* sp. against DPPH radical and their EC_{50} values varied from 5.37 -15.85 mg/ml.

Antioxidant components

Discovering natural antioxidants, especially of plant origin has been increasing in recent years, among which phenolics are of considerable

interest as dietary supplements or food preservatives [29]. Phenolic compounds are known to be powerful chain-breaking antioxidants and they possess scavenging ability due to their hydroxyl groups. Naturally occurring antioxidant components including Phenol, flavonoid, β-carotene and lycopene were found in the ethanol extract from *A.heterocystis* were given in (Table 1). Total Phenols were the major antioxidant components found in the ethanol extract of *A.heterocystis* of about 13.48±0.74 mg/g, followed by flavonoid 11.58±0.15 mg/g. β-carotene and lycopene were only found in remnant amounts of 8.35±0.34 µg/g and 3.36±0.27 µg/g and this amounts relates with the previous report concerning with β- carotene and lycopene from *Agaricus* sp.[30]. Studies have shown that polyphenols found in the dietary and medicinal plants could inhibit oxidative stress by chelate metals, inhibit lipoxigenase and scavenge free radicals. Besides phenols Flavonoids also reported as free radical scavenger by terminating the chain reaction during the oxidation of triglycerides[31].
The phenolic compounds present in the ethanol extract was analyzed in thin layer chromatography (TLC), using two specific reagents (Ferric chloride and Potassium ferricyanide) showed the presence of at-least three spots and relate to that the region spots sprayed with DPPH was decolorized by this spot shown in (Figure 2).

Correlation between Total phenol and antioxidant assays
Total phenols may account for the antioxidant capacity found among fruit and vegetables. Previous studies have shown linear correlation between phenols and antioxidant capacity [32]. Notably negative correlations were established between the phenols and antioxidant activities (Figure 2C and 2D). The EC_{50} values of DPPH scavenging and total phenol were (determination coefficient R^2 was 0.959 for phenols). ABTS scavenging and total phenols were (determination coefficient R^2 was 0.999 for phenols). This negative correlation proves that the samples with highest phenol content shows higher antioxidant activity and lower EC_{50} values.

Table1: EC_{50} values and bioactive compounds (mean±SD) obtained from the ethanol extract of *Agaricus heterocystis* (AGH)

Sample *A.heterocystis*	EC (mg/ml)		Phenol (mg/g)	Flavonoid (mg/g)	β-carotene (µg/g)	Lycopene (µg/g)
	ABTS	DPPH				
	0.4±0.57	2± 0.81	13.48±0.74	1.58±0.15	8.35±0.34	3.36±0.27

Each value is expressed as mean ± SD value (n=3).

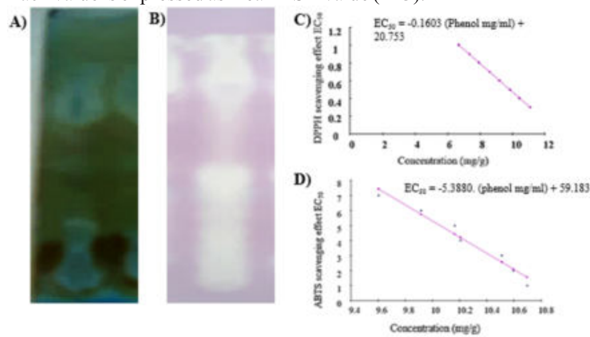


Figure 2 TLC of AGH ethanol extract and their correlation between DPPH, ABTS radical scavenging activity and total phenols. (A) revelation with $FeCl_2$ identifies the phenolic compound, (B) DPPH revelation to antioxidant activity. (C), correlation between DPPH radical scavenging and total phenol (D) correlation between ABTS radical scavenging and total phenol.

Antimicrobial activity of extract
The antimicrobial effect of ethanol extracts *Agaricus heterocystis* (AGH) tested against two species of Gram-positive bacteria, three species of Gram-negative bacteria and a yeast *Candia albicans* were given in Table 2. AGH ethanol extract had a narrow antibacterial spectrum against Gram-negative bacteria and strongly inhibited the growth of the Gram-positive bacteria tested, including *Bacillus subtilis*. The maximal zones of inhibition ranged from 10 to 21 mm. The most susceptible bacterium found to be was *Staphyllococcus aureus* (21±4 mm diameter) this was an important observation that *S.aureus* can produce several types of enterotoxins that cause gastroenteritis which created the major food borne disease in most countries and this species is exceptionally resistance to a number of phytochemicals [33]. Antibacterial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were absent at the concentration tested. Against *C.albicans* the ethanol extract exhibited anti-candidal activity. Similar observations were reported earlier in the ethanolic extract of a polyporus mushroom *Laetiporus sulphureus* (Bull). Murill mushroom[34].

Table 2 Antimicrobial activity of ethanol extracts *Agaricus heterocystis* (AGH) and antibiotic sensitivity of microorganisms (zone size, mm)

Test bacteria	A. heterocystis	N	A	P	G	O	T
Staphylococcus aureus **ATCC 25923**	21±0.5	NT	NT	31	16	NT	29
Bacillus subtilis **ATCC6633**	10±0.1	NT	NT	12	NT	NT	17
Escherichia coli **ATCC 35218**	14±0.5	NT	10	11	NT	NT	8
Klebsiella pneumonia **ATCC 27736**	-	NT	NT	18	NT	NT	5
Pseudomonas aeruginosa **ATCC 2036**	-	NT	NT	NT	16	NT	8
Candida albicans	16±0.5	19	NT	NT	NT	NT	NT

N, nystatin (100 U); A, ampicillin (10µg); P, penicillin (10 U); G, gentamycin (10µg); O, oxacillin (1µg); T, tetracycline (30 µg); (NT) not tested; (–) No inhibition.

Antiviral activity

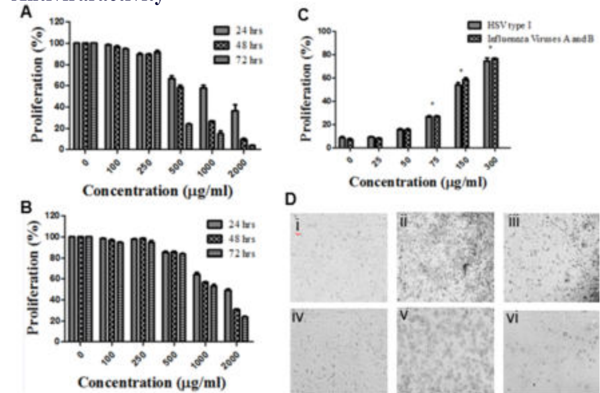


Figure 4 Antiproliferative effect of AGH extracts on HEP-2 and MDCK cell lines; cell proliferation assay using MTT dye. Antiviral property of AGH extract against HSV type 1 grown in HEp2 cell line (A) AGH extract on HEP2 cells, (B) AGH extract on MDCK cells (C) HEP2 cells and MDCK cells were treated with increasing doses of AGH extract and infected with HSV type 1 and Influenza virus A and B respectively D) HSV type 1 grown in HEp2 cell line i) Control ii) Viral infected plaque formed iii) drug treated, inhibited plaque formation, Influenza virus A and B grown in MDCK cell line iv) Control v) Viral infected plaque formed vi) drug treated inhibited plaque formation Results were expressed as percentage of inhibition with respect to untreated control. Solvent control had no effects on cells. Values are expressed in µg/ml as means ± SD of (n=6) experiments (One-way ANOVA, $^*P < 0.05$ control vs treatment)

The cell proliferation assay using MTT dye in HEP-2 and MDCK cell lines (host cell lines for viruses) treated with AGH extract demonstrated that AGH extract was not inhibiting the proliferation or not toxic to both the cell lines at the concentrations varying from 100-500µg/ml (Figure 3A and 3B). The nontoxic concentration ranged from 0 to 300µg/ml of AGH extract were treated on cells HEP-2 The mushroom extract exhibited a strong antiviral property against the viruses tested. The minimum concentration that inhibited 50% (EC_{50}) of the viral activation (Viral inactivation) were 116.3±1.97 and 110.2±1.88 against HSV type 1 and Influenza viruses A and B respectively (Figure 3C). The antiviral property of mushroom extract against HSV-1 and Influenza viruses A and B and the formation of CPE in cell lines with and without treatment of mushroom extract were shown in the Figure 3D respectively. The virucide activity of bio-active substances derived from fungi and plants have been demonstrated against herpesvirus [35]. Sorimachi *et al.,* [36] showed that

aqueous extract and fractions obtained by alcoholic extraction of *A. blazei* Murill were able to inhibit the cytopathic effect of Western Equine Encephalitis (WEE) virus, poliovirus and HSV in cultures of Vero cells and the authors demonstrated a greater activity of the alcoholic fractions in comparison to the aqueous extract. The observed results were in similar with the previous reports confirm that the ethanol extract of AGH observed to be having antiviral property effectively inhibiting HSV-1 and Influenza virus A and B infection *in vitro*.

CONCLUSION

In conclusion, edible mushrooms are regarded as a healthy food that constitute a good source of healthy compounds namely phenols, flavonoids, β-carotene and lycopene, suggesting that it could be useful in the prevention of diseases in which free radicals are implicated, because the role of oxidants in microbial or viral infections includes the regulation of both host metabolism and foreign substances. Antioxidants can therefore be expected to act at many different levels, calling for more investigations on the effect of antioxidant in microbial infections. And in case of herpetic infections, especially in resistant strains the mushroom extract may be used as a possible drug along with the existing drugs, needs further study.

ACKNOWLEDGMENTS

This research was supported by CAS Botany, University of Madras.

REFERENCES

1. Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L. Nutrients in edible mushrooms: an inter-species comparative study. Food Chemistry 1999;65:477-82.
2. Sanmee R, Dell B, Lumyong P, Izumori K, Lumyong S. Nutritive value of popular wild edible mushrooms from northern Thailand. Food Chemistry 2003;82:527-32.
3. Tan BL, Norhaizan ME, Liew W-P-P, Sulaiman Rahman H. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front Pharmacol 2018;9:1162-.
4. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry 2015;97:55-74.
5. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J 2016;15:71-.
6. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4:118-26.
7. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine 2018;54:287-93.
8. Chowdhury M, Kubra K, Ahmed S. Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh. Ann Clin Microbiol Antimicrob 2015;14:8-.
9. Radzki W, Slawinska A, Jablonska-Rys E, Gustaw W. Antioxidant capacity and polyphenolic content of dried wild edible mushrooms from Poland. International journal of medicinal mushrooms 2014;16:65-75.
10. Upadhyay S, Dixit M. Role of Polyphenols and Other Phytochemicals on Molecular Signaling. Oxid Med Cell Longev 2015;2015:504253-.
11. Fraga CG, Croft KD, Kennedy DO, Tomás-Barberán FA. The effects of polyphenols and other bioactives on human health. Food & Function 2019;10:514-28.
12. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74:417-33.
13. Li B, Webster TJ. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. J Orthop Res 2018;36:22-32.
14. Goyal S, Ramawat KG, Mérillon J-M. Different Shades of Fungal Metabolites: An Overview. In: Mérillon J-M, Ramawat KG, eds. Fungal Metabolites. Cham: Springer International Publishing; 2017:1-29.
15. Chen H-P, Liu J-K. Secondary Metabolites from Higher Fungi. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi Ji, eds. Progress in the Chemistry of Organic Natural Products 106. Cham: Springer International Publishing; 2017:1-201.
16. Baby S, Johnson AJ, Govindan B. Secondary metabolites from Ganoderma. Phytochemistry 2015;114:66-101.
17. Wu Y, Choi M-H, Li J, Yang H, Shin H-J. Mushroom Cosmetics: The Present and Future. Cosmetics 2016;3.
18. Ellan K, Thayan R, Raman J, Hidari KIPJ, Ismail N, Sabaratnam V. Anti-viral activity of culinary and medicinal mushroom extracts against dengue virus serotype 2: an in-vitro study. BMC Complementary and Alternative Medicine 2019;19:260.
19. Ganeshpurkar A, Rai G, Jain AP. Medicinal mushrooms: Towards a new horizon. Pharmacogn Rev 2010;4:127-35.
20. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 2001;73:239-44.
21. Garcia EJ, Oldoni TLC, Alencar SMd, Reis A, Loguercio AD, Grande RHM. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Brazilian Dental Journal 2012;23:22-7.
22. Safari P, Rezaei M, Shaviklo AR. The optimum conditions for the extraction of antioxidant compounds from the Persian gulf green algae (Chaetomorpha sp.) using response surface methodology. J Food Sci Technol 2015;52:2974-81.
23. Barros L, Baptista P, Ferreira ICFR. Effect of Lactarius piperatus fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food and Chemical Toxicology 2007;45:1731-7.
24. Barton GM, Evans RS, Gardner JAF. Paper Chromatography of Phenolic Substances. Nature 1952;170:249-50.
25. Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K. Antioxidant and antimicrobial activities of Laetiporus sulphureus (Bull.) Murrill. Food Chemistry 2007;101:267-73.
26. Walker WE, Waisbren BA, Martins RR, Batayias GE. A Method for Determining Sensitivities of Antiviral Drugs in Vitro for Possible Use as Clinical Consultation. American Journal of Clinical Pathology 1971;56:687-92.
27. Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chemistry 2007;100:1511-6.
28. Barros L. Antioxidant activity of Agaricus sp. mushrooms by chemical, biochemical and electrochemical assays. Food Chemistry 2008;v. 111:pp. 61-6-2008 v.111 no.1.
29. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines (Basel) 2018;5:93.
30. Sharma SK, Gautam N. Chemical, Bioactive, and Antioxidant Potential of Twenty Wild Culinary Mushroom Species. Biomed Res Int 2015;2015:346508-.
31. Roedig-Penman A, Gordon MH. Antioxidant properties of myricetin and quercetin in oil and emulsions. Journal of the American Oil Chemists' Society 1998;75:169-80.
32. Piluzza G, Bullitta S. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. Pharmaceutical Biology 2011;49:240-7.
33. Halpin-Dohnalek MI, Marth EH. Growth and Production of Enterotoxin A by Staphylococcus aureus in Cream. Journal of Dairy Science 1989;72:2266-75.
34. Wasser SP, Weis AL. Medicinal Properties of Substances Occurring in Higher Basidiomycetes Mushrooms: Current Perspectives (Review). 1999;1:31-62.
35. Bruggemann R, Matsuo Orlandi J, Benati FJ, et al. Antiviral activity of Agaricus blazei Murrill ss. Heinem extract against human and bovine herpesviruses in cell culture. Brazilian Journal of Microbiology 2006;37:561-5.
36. Sorimachi K, Ikehara Y, Maezato G, et al. Inhibition by Agaricus blazei Murill Fractions of Cytopathic Effect Induced by Western Equine Encephalitis (WEE) Virus on VERO Cells in Vitro. Bioscience, Biotechnology, and Biochemistry 2001;65:1645-7.