

Research Article

Comparison of Anti-oxidant Activity of Five Elected Wild Commercial Mushrooms in Four Different Solvents as an Alternative Oxidative Stress Reliever

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Received: 3 Oktober 2019; Revised: 3 November 2019; Accepted: 13 November 2019; Published: 6 December 2019

Abstract: Mushroom also known as a lit source of nutritional and therapeutical component so in order to evaluate the usefulness of the Pakistani wild mushroom strains as the possible nutraceuticals, a detailed study entailing several tests for the determination of anti-oxidant potential was carried out on the elected mushrooms in four different solvents. Strains of *Lentinus edodes*, *Pleurotus ostreatus*, *Volvariella volvacea*, *Pleurotus eryngii* and *Ganoderma lucidum* were extracted for this particular purpose in the water, n-butanol, n-hexane and chloroform solvents. Test models including Total Phenolic Content assay, Total Flavonoid Content assay, 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) Scavenging Activity assay and Reducing Power assay were utilized for carrying out the comparative analysis among the selected mushrooms. The acquired results indicated that the extract of *Volvariella volvacea* and *Pleurotus eryngii* possess copacetic anti-oxidant potential and can be utilized as oxidative stress reliever in humans. Furthermore, extraction studies also indicated that water is the most suitable solvent for anti-oxidants extraction for these mushrooms. Finding of the anti-oxidant potential for elected wild mushrooms contra indicate that these mushrooms can be safely exploited as a amended natural anti-oxidant in comparison to its synthetic counterpart

Keywords: anti-oxidant; mushrooms; nutraceutical; oxidative stress; pakistan

1. INTRODUCTION

Generation of the reactive oxidative groups as by products owing to the metabolic activities (normal/pathological) of human beings could be regarded as the phenomenon of natural occurrence. But the excess production of these reactive species has been lately found out to be associated with some serious health issues such as cardiovascular ailments, immune system weakening, neurological diseases, onset of cancer and various other degenerative illnesses [1]. Almost every living organism is furnished with some sort of defense methodologies in order to handle with these potential oxidative stresses. Chemical substances like carotenoids, ascorbic acid, tocopherol or polyphenol compounds and oxidative enzymes like catalases or superoxide dismutase are few examples of natural anti-oxidant materials which are used for dealing oxidative species [2]. Similarly, synthetic antioxidants including Butylatedhydroxyanisole (BHA) and Butylatedhydroxy toluene (BHT) are also employed as food additives for stopping such oxidative deterioration. However, the controversial safety problems regarding the usage of these compounds have left nutritionists

somewhat concerned and now-a-days, focus has already been shifted towards figuring out natural anti-oxidants and nutraceuticals for enhancing the nutritional quality of the consumers [3].

The antioxidant ability or the potential nutraceutical use of any natural resource is entirely dependent upon its composition particularly the flavonoids or phenolic content. In Asian region, the fruiting bodies/caps of wild mushrooms is not only favored as the traditional cuisine component (owing to its explicit taste and rich aromatic qualities) but also is considered one of the highly regarded therapeutic and nutritional supplement employed excessively in human diet for dealing with illnesses of hypertension, cancer and hypercholesterolemia [4]. This is attributed to the amassing of various secondary metabolites like steroids, poly-ketides, terpenes and phenolic compounds in the tissue of the mushrooms [5]. These compositional attributes of mushrooms either commercial or wild are regional specific and are responsible for the enhanced usage of this particular fungi group as an anti-oxidant dietary supplements [6].

Positive correlations between the phenolic amount and anti-oxidant ability of commercial mushrooms have already been reported in numerous studies [7-9]. However, very little material is available on the reducing potential of the wild mushrooms of Pakistan. To the best of our information, the region elected for studies (i.e. city of Faisalabad) and the species under investigation (*Lentinusedodes*, *Pleurotusostreatus*, *Volvariellavolvacea*, *Pleurotuseryngii* and *Ganodermalucidum*) both are novel and are not studied before for their potential use as a nutraceutical. The categorization of mushrooms done in this research in accordance to their anti-oxidant prospective will significantly aid the nutritionists to gauge out health benefits from these species. Furthermore, this region selective study will also be beneficial for exploiting the nutritious and medical welfares specified to the commercial mushrooms present in this characteristic region of Pakistan.

2. MATERIALS AND METHODS

2.1. Materials

Fresh, refined and shade-dried strains of *Lentinusedodes*, *Pleurotusostreatus*, *Volvariellavolvacea*, *Pleurotuseryngii* and *Ganodermalucidum* were personally acquired from Medicinal Mushroom Lab Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Domestic blender was employed for pulverization of specimens into fine powder. The labeled samples were preserved at 4°C in plastic bags. Solvents, like hot water, chloroform, *n*-hexane and *n*-butanol; that were used for the preparation of mushroom extracts were all obtained from the Merck Company (Germany) and were used as such. All the utilized reagents were of analytical grade.

2.2. Extract preparation

Four types of solvents (i.e. hot water, chloroform, *n*-hexane and *n*-butanol) were used for the extract preparation. 20g of mushroom powder of each strain was extracted separately with 100% solution (200mL) of each respective solvent by using an Orbital Shaker at room temperature for 8 hours. Concentrated residue was yielded by the use of filtration and evaporation techniques. The storage temperature of 4°C was maintained throughout the span of the experiment for the engineered concentrated extracts [10].

2.3. Investigation of anti-oxidant potential of extracts

Following assays were used for the evaluation of anti-oxidant activity of the selected wild mushrooms.

2.3.1. Total phenolic content (TPC) determination

The Total Phenolic Content (TPC) of the mushroom samples were determined by using the previously reported Folin-Ciocalteu method with slight modifications [11]. The wavelength of 765 nm was used for measurement of absorbance and the phenolic concentration of the extracts was determined by using the acquired standard calibration curve of Gallic acid. The obtained results regarding TPC were recorded in the form of Gallic Acid Equivalents (GAE) of the dry weight.

2.3.2. Total flavonoids contents (TFC) determination

A well-documented spectrophotometric method reported by Benedec and co-workers was used for the determination of Total Flavonoid Content (TFC) in the mushroom strains [12]. Absorbance of the reaction mixture was measured after the incubation period of 15minutes and at the wavelength of 510nm. TFC of the extracts were expressed as Catechin Equivalents (CE) acquired from the analysis of linear regression curve of Catechin.

2.3.3. DPPH scavenging activity assay (DPPH)

The ability of extracts to scavenge the stable radical of 2, 2-diphenyl-1, 1-picrylhydrazyl is another commonly used indicator used for accessing the anti-oxidant activity of the substances. The DPPH assay was performed according to the method prescribed by [13]. The 50 μ L aliquots of respective samples in various concentrations were introduced into the 0.004% methanolic solution of DPPH and incubation was done at room temperature for 30minutes. The absorbance was recorded against a blank at the wavelength of 517nm and following formula was used for measuring DPPH inhibition.

$$DPPH \text{ inhibition (\%)} = \left[1 - \frac{A_1}{A_0} \right] \times 100$$

Here, A_1 represents the absorbing capacity of the sample while A_0 shows the absorbing capacity of the control.

2.3.4 Reducing power assay

The opted methodology for the determination of the reducing power of extracts was quite similar to the reported method of Pereira and his co-workers used with minor simplifications [14]. For absorbance detection, the wavelength of 700nm and Microplate reader (BioTek, USA) were utilized. The apparent increase in the absorbing capacity of the reaction media was indicative of its enhanced reducing power.

3. RESULTS AND DISCUSSION

3.1. Evaluation of Anti-oxidant activity

Table 1 and figure 1 represents the acquired results for all the protocols followed for the evaluation of antioxidant potential of mushroom extracts in various solvents. Assays are discussed below in separate sub-headings.

Table 1. Anti-oxidant activity of elected mushrooms extract

Solvents	TPC (mg/g)	TFC (mg/g)	DPPH (mg/g)	Reducing Power (Λ_{\max} 700 nm)
<i>Ganodermalucidum</i>				
Water	5.48±0.82	1.71±0.51	18.70±0.60	1.79±0.8
<i>n</i> -butanol	3.37±0.24	2.31±0.3	22.21±0.31	2.61±0.8
<i>n</i> -hexane	0.82±0.26	0.47±0.30	70.40±0.6	1.43±0.13
Chloroform	1.25±0.21	1.60±0.17	40.6±0.40	1.69±0.12
<i>Volvariellavolvacea</i>				
Water	6.60±1.12	2.58±0.8	5.80±0.39	2.90±0.31
<i>n</i> -butanol	6.12±0.60	1.40±0.8	8.90±0.22	2.65±0.68
<i>n</i> -hexane	1.52±0.40	0.69±0.17	26.70±0.38	1.89±0.59
Chloroform	3.89±0.8	1.66±0.29	20.30±0.19	1.59±0.29
<i>Pleurotuseryngii</i>				
Water	9.49±1.09	2.67±0.71	8.89±0.20	2.79±0.49
<i>n</i> -butanol	6.72±0.9	3.70±0.9	12.70±0.49	2.77±0.40
<i>n</i> -hexane	0.82±0.18 ^a	0.62±0.20 ^a	26.40±0.28	1.89±0.3
Chloroform	3.59±0.49	2.79±0.38	18.49±0.78	2.13±0.26
<i>Pleurotustreatus</i>				
Water	6.29±2.1	3.15±0.19	16.19±1.2	2.90±1.17
<i>n</i> -butanol	5.60±0.6	2.60±0.08	26.69±2.0	2.60±0.81
<i>n</i> -hexane	1.26±0.70	1.03±0.15	49.60±1.39	1.69±0.7
Chloroform	2.89±0.40	2.90±0.30	30.70±1.59	1.59±0.6
<i>Lentinusedodes</i>				
Water	3.11±0.6	2.21±0.26	13.40±0.08	2.90±0.09
<i>n</i> -butanol	2.57±0.5	3.44±0.22	22.1±1.19	2.89±0.05
<i>n</i> -hexane	0.76 ±0.2	1.103±0.18	39.10±0.18	1.60±0.06
Chloroform	1.69±1.02	1.75±1.2	30.1±0.49	1.79±0.11

*Values are mean ± SD of carefully conducted triplicate experiments

*Mean carrying different superscripted alphabets vary ($p < 0.05$) with 95% confidence.

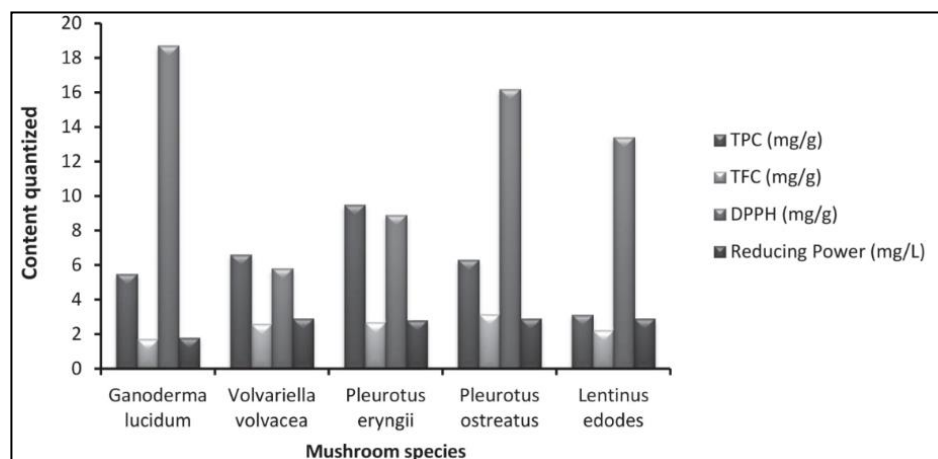


Figure 1. Comparative graph showing the extraction results for five elected mushrooms (Solvent: water).

3.1.2. Total flavonoids contents (TFC) determination

Flavonoids are very important group of secondary metabolites that contain substantial chelating as well as anti-oxidant potential but their extraction is considered somewhat difficult with the above tested assay [18]. Hence, separate TFC method was employed for the accurate determination of flavonoid content. Acquired results were found to be significantly similar to that assimilated from the TPC assay consolidating its validity. The maximum flavonoid content of $3.70 \pm 0.9 \text{ mg/g}$ of dry weight was detected in the *n*-butanol extract of *P.eryngii* while the lowest amount ($0.47 \pm 0.30 \text{ mg/g}$) was documented in the *n*-hexane extract of *G.lucidum*. Order with respect to the TFC protocol could be designated as *P.eryngii* > *P. ostreatus* > *L.edodes* > *V.volvacea* > *G.lucidum*. In terms of reagents, both hot water and hexane was found to be compatible for the flavonoid extraction. Results correspond with the finding of Sarikurkcu and co-workers [19].

3.1.3. DPPH scavenging activity assay (DPPH)

Scavenging of reactive free radicals that could be easily scrutinized through any sort of visual color/chemical change is one of the most important methods which are generally utilized for evaluating the anti-oxidant potential of any substance. DPPH method, OH quenching assay and metal chelating are the example of few protocols that falls into this respective category [20]. In the context of this work, the free-radical scavenging ability of the mushrooms extract was tested using a stable methanolic solution of DPPH. Fresh solution of DPPH shows characteristic absorption band at the wavelength of 517nm and exhibits deep violet color. Presence of the anti-oxidant specie in the reaction media results in the fading of the original color of DPPH solution [21]. In the present study mushroom extracts the water extract was showed the highest radical scavenging activity in the *V.volvacea* species and also other species *P.ostreatus*, *G.lucidum*, *L.edodes*. The present study mushroom species results resemble with the previous study mushroom species [22].

3.1.4. Reducing power assay

Reducing power determination is one of the major parameters to determine the anti-oxidant potential of any substance in its solution form. In this assay, the reduction power is measured by observing extend of reduction caused by this particular substance for converting the oxidized form of iron i.e. Fe (III) ion/ferricyanide into its reduced form i.e. Fe (II) ions/ferrous cyanide. This change can also be visually observed by the change in the color of the employed solution. The more apparent

the change in the intensity of the color the more will be the antioxidant potential of that substance (Fernandes et al., 2016). The recorded reducing power of all the specie was found to be phenomenal with the least value of 1.4 and maximum value of 2.90 at 50mg/mL in every solvent. Visually, a clear change in the test samples, depending upon the type of solvent and the specie intrinsic anti-oxidant power, from the yellow color to the numerous shades of green or blue was observed. Water extract was again found to be the most suitable solvating medium for the anti-oxidant extraction while the n-hexane showed the least capability in this accord. The acquired results collaborate with the findings of following researchers [23,24]. Hence, results evidenced that these understudy wild mushrooms exhibited excellent anti-oxidant potential and can be employed as an alternative nutraceutical in food industry.

4. CONCLUSION

The study for determination of the anti-oxidant potential for some wild mushrooms (*Lentinus edodes*, *Pleurotus ostreatus*, *Volvariella volvacea*, *Pleurotus eryngii* and *Ganoderma lucidum*) indicated that these mushrooms can be safely employed as a better natural anti-oxidant in comparison to its synthetic counterpart. The aqueous medium was found to be the most suitable extraction solvent for these species which also enhances its economic/momentary value in terms of commercialization. Acquired results indicated that out of the elected mushroom species, species of *Volvariella volvacea* and *Pleurotus eryngii* showed the best results in terms of anti-oxidant potential indicating that these species hold ample potential for exploitation as the natural nutraceutical.

Acknowledgements : Authors would like to acknowledge the supervisory assistance provided by Dr. Muhammad Shahid for the completion of this article.

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