doi.org/10.36721/PJPS.2022.35.5.REG.1371-1377.1

Estimation of some trace metals, bioactive compounds, curative antimicrobial and antioxidant agents from *Russula foetens* and *Russula cf. foetentoides*

Abdul Rehman Niazi[1*], Muniba Shafique[1], Muhammad Imran[2] and Shoomaila Latif[3]
[1]Institute of Botany, University of the Punjab, Lahore, Pakistan
[2]Centre for Inorganic Chemistry, School of Chemistry, University of the Punjab, Lahore, Pakistan
[3]School of Physical Sciences, University of the Punjab, Lahore, Pakistan

Abstract: The current study is an attempt to estimate minerals, antioxidant and antimicrobial activities of two medicinal mushrooms (*Russula foetens* and *Russula cf. foetentoides*) along with their mycochemicals. Among the metal analysis, iron was most abundantly (7.428mg/g) present in *R. cf. foetentoides*. The mycochemical screening revealed the presence of active secondary metabolites and phenolic composition by HPLC showed five stable compounds with maximum concentration of gallic acid (22.31%). The highest antibacterial activity (27.8±0.152mm) was displayed in ethanol macerate of *R. foetens* in contradiction of *Staphylococcus aureus*. Similarly, highest antifungal potential was exhibited by *R. foetens* (ethanolic macerate) against *Fusarium equiseti* with zone of inhibition 23±0.572mm. The antioxidant potential was assessed by five assays and the *R. foetens* exhibited maximum % inhibition (43.045±0.037%) by DPPH radical protocol. Maximum % inhibition by ABTS protocol was exhibited by *R. cf. foetentoides* i.e., 88.057±0.021. The highest reducing potential was revealed in *R. foetens* (0.684±0.001mmol/L of $FeSO_4$). The highest flavonoids and total phenolic contents (TPC) were shown by *R. cf. foetentoides* i.e., 0.115±0.002mg/100g of Catechin and 0.064±0.001mg/100g of GAE (gallic acid equivalents), respectively. All the obtained results were analyzed statistically by applying the ANOVA to determine the significant and non-significant ranges.

Keywords: Mycochemical screening, antimicrobial, antioxidant, medicinal mushrooms, *Russula cf. foetentoides*, *Russula foetens.*

INTRODUCTION

Russula foetens and *Russula cf. foetentoides* are commonly found in deciduous and coniferous forest. The members of genus *Russula* make symbiotic mycorrhiza associations with higher plants. These mutualistic relationships help different verities of plants to develop more. . The genus is the largest in Agaricales and globally distributed with more than 100 *Russula* species reported from China, where mixed forests are characteristic features of habitat (Varo *et al*., 1980).

Phenolics are considered non-essential dietary components which inhibit many diseases like atherosclerosis and cancer. The role may be associated with their capability to chelate compound metals, prevent lipoxygenase and scavenging activity against the free radicals (Mallavadhani *et al*., 2006; Huie and Di, 2004; Niazi & Ijaz, 2021). There are several diseases such as bleeding, high blood pressure, gastrointestinal disorder and bacterial infections that have been cured by the use of mushrooms (Barros *et al*., 2007; Oso, 1977; Oso, 1981; Chapela, 1993). The fruiting bodies of mushrooms have potential to bio accumulate the minerals from growth medium (Rajarathnam *et al*., 1998; Kalač, 2010). Due to ecological and some genetic aspects, the fruiting bodies of advanced macrofungi exhibited maximum number of mineral components (Schmitt *et al*., 1977; Varo *et al*., 1980; Vetter, 1990; Gebreyohannes *et al.,* 2019).

Various antimicrobial drugs have been found to be for beneficial against many infectious diseases with reports of several problems of drug-resistant bacterial strains (Klein *et al*., 2007; Steinkraus *et al*., 2007; Sikandar *et al.,* 2020; Asemoloye *et al.,* 2022). These problems may lead the scientists to explore new antimicrobial substances that may be effective against virulent microorganisms. The production of free radicals occurs due to the chain reactions of oxidation which may cause the destruction of the cells of organisms (Cheung and Cheung, 2005). Almost all organisms are well protected against free radical damage. The disturbance in the antioxidant defense mechanism may lead towards the declining of physiological functions. So, there is the interest leading towards the usage of natural dietary supplements comprising of antioxidants. Now, the most frequently used synthetic antioxidants are butylhydroxytoluene (BHT), propyl gallate and butylated hydroxyanisole (BHA) (Mau *et al*., 2002).

Mushrooms could be used as potential natural therapeutic agents for human against numerous diseases. In this direction, we have observed that up till now, a very little research has been done on qualitative and quantitative

**Corresponding author:* e-mail: drarniazi.botany@pu.edu.pk

analysis of mushrooms from Khanspur, northern area in Pakistan. Hence, in the present manuscript evaluation of trace metals, antimicrobial and antioxidant activities of two wild medicinal mushrooms is presented that could be a potential source of curative antioxidant and antimicrobial compounds for making effective therapeutics.

MATERIALS AND METHODS

Samples

Russula foetens and *Russula cf. foetentoides*, the selected species of wild mushrooms, were collected from Khanspur-Ayubia during monsoon season of August, 2020. These mushrooms were photographed, vouchered, dried and packed in polyethene bags. Then, theses samples were transported to the FBSR Laboratory, University of the Punjab, Lahore and well-preserved at <4 °C within 24 h. The specimens were authenticated by Dr. Abdul Rehman Khan Niazi and the vouchered specimens were submitted in the LAH Herbarium.

Preparation of crude extracts in non-polar and polar solvents

By using maceration method, crude extracts were formed in solvents at room temperature using reported protocol (Ćujić *et al*., 2016; Niazi & Ijaz, 2021; Niazi *et al.,* 2021). Both of the mushrooms (5 g) were extracted in petroleum ether (50mL) for one week. Then, the extract of each mushroom sample was obtained by the filtration process using Whatman filter paper No. 1 and the procedure was done thrice. Then, the residual sample was extracted with chloroform (50mL), ethanol (50mL) and distilled water (50mL), respectively. These extracts were filtered and concentrated using a rotatory evaporator.

Estimation of trace metals

Standard procedures were adopted for the detection of metals from mushrooms (AOAC, 1990). Briefly, each sample was dried up to 1.0 g and thrice digested by wet digestion method using HNO_3. The flame photometer (JANWAY PEF 7) was used for the detection of sodium (Na) and potassium (K), while the other metals i.e., Ni, Co, Cu, Cr, Mn, Zn, Fe and Ca were detected by the Atomic Absorption (AA) spectrophotometer (PerkinElmer AAnalyst100).

Mycochemical analysis

Mushrooms were subjected for the evaluation of some mycochemicals i.e., alkaloids, anthraquinones, flavonoids, reducing sugars, saponins, tannins, cardiac glycosides and terpenoids (Ayoola *et al*., 2008).

Test organisms

Two gram -ve bacteria (*Escherichia coli* & *Pseudomonas stutzeri*), two gram +ve bacteria (*Bacillus subtilis* & *Staphylococcus aureus*) and two fungal strains (*Pennicillium vancouvernse* & *Fusarium equiseti*) were used as test organisms. Bacterial cultures were acquired from Institute of Microbiology and Molecular Genetics (MMG) and fungal strains from FBSR Laboratory, Institute of Botany, University of the Punjab, Lahore, Pakistan.

Agar well diffusion method

Antifungal and antibacterial potential was determined by following Jorgensen and Turnidge (2015). Briefly, the overnight cultures of bacterial and fungal strains were mixed with freshly prepared LB medium and MEA medium by swabbing technique in petri plates. Then, the media plates were left for solidification. After that, a well was made by a sterilized cork borer no. 4 and agar plug was removed. Almost 1000-2000ul of each mushroom extract was employed in each prepared well and placed in incubator at 25°C and 37°C for 48h and 25h, for antifungal and antibacterial, respectively. After incubation, the antimicrobial activity was calculated by measuring zone of inhibition in millimeter (mm). Antibiotics (Amikacin, Cephalaxin, Erythromycin) and antimycotic (Terbinafine) were used as standards (Niazi *et al.,* 2021).

DPPH free radical scavenging assay

Different concentrations (300mg/L, 600mg/L, 900mg/L) of standards (BHT, BHA) and both extracts were prepared in this assay. The prepared concentration (0.1mL) and then in each test tube, 3.9mL of DPPH (0.06mM) solution were added and noted the absorbance at 517nm against the blank i.e. ethanol (Brand-Williams *et al*., 1995; Niazi *et al.,* 2021).

$ABTS^+$ Assay

This assay was carried out by following Re *et al*. (1999). Same ratio of Potassium persulphate- $K_2S_2O_8$ (2.45mM) and ABTS (7mM) were added. Then, for 4 to 16 hours obtained mixture was left-hand till the changing of colour from blue green to yellow or light green and dilute it with ethanol. Thereafter, 0.1mL of each sample was taken in tube and added with 0.9mL of the prepared ABTS solution in each tube and noted the absorbance at 734nm (Niazi *et al.,* 2021).

Ferric reducing antioxidant power assay

About 2.5mL TPTZ (mmol/L in 40mmol/L HCL) was mixed with 25mL of acetate buffer (300mmol/L). Then, about 2.5mL $FeCl_3.6H_2O$ (20mmol/L) was poured for preparing FRAP reagent. Then, in each 0.01mL of macerates 0.03mL water is added with 0.3 mL of FRAP reagent. Ultimately, $FeSO_4.7H_2O$ (0.2mM-1mM) was used as standard and taken the absorbance at 593nm beside blank (Benzie and Strain, 1996).

Quantification of total phenolics and flavonoids

The phenolic contents were assessed by standard procedures as Folin-Ciocalteu (FC) reagent. First of all,

fresh FC reagent was prepared according to reported procedure. 0.2mL of different crude extracts were mixed this fresh FC reagent (0.8mL). Then added 2.0mL of 7.5% Na_2CO_3 and 7.0mL of dist. H_2O after 5min. This mixture was retained in the dark for 2 hours and then, absorbance was taken at 765nm (Iqbal *et al*., 2008). Almost, 1.0mL of the each extracts was mixed with dd H_2O. Then 0.3mL of $NaNO_2$ (5%) was mixed and after about 5 min, 10% aluminum chloride ($AlCl_3$) was added to it. After 6min, 2.0mL of 1M NaOH was added followed by addition of 2.4mL of dd H_2O. In this method catechin was selected as standard and absorbance was recorded at 510nm (Ardekani *et al*., 2011).

HPLC Analysis

For phenolic compound extractions, 5.0grams of each mushroom sample were pitted and macerate was prepared. To avoid the degradation of phenolic compounds, this maceration was carried out in the dark. Then, each macerate was moved to a volumetric flask and 50mL of ethanol was added. The Chromatographic separation was performed using C18 HPLC column (150mm x 4.6mm, 5µm) and heated at 30°C. For elution, the mobile phase was made of TFA 0.1% (A) and acetonitrile (B) in gradient mode following a standard procedure using an Alliance 2695 HPLC system (Waters Corp., Milford, MA) (González-Gómez *et al*., 2010). All compounds were identified by comparing the retention time and UV absorption spectrum with the standards (Lone *et al*., 2015)

STATISTICAL ANALYSIS

The results were expressed as means ±standard error (SE) and evaluated statistically using Analysis of Variance (ANOVA) and Duncan's multiple range tests using co-stat software (Version 3.03) to find out the significant differences ($P<0.05$).

RESULTS

Estimation of essential and non-essential trace metals

The two mushroom species, *Russula foetens* and *Russula cf. foetentoides* were subjected for the estimation of some essential and non-essential metals (minerals) i.e., Potassium (K), Calcium (Ca), Sodium (Na), Cobalt (Co), Copper (Cu), Chromium (Cr), Iron (Fe), Manganese (Mn), Zinc (Zn) and Nickel (Ni) using flame emission and atomic absorption spectroscopy. The mineral distribution in these species showed that magnesium, calcium, sodium, iron, manganese, zinc and potassium were present in substantial amount, while Cu, Cr and Co were in very low amount. Nickel was absent in both mushroom species (table 1). Among all these elements, Fe is found to be maximum in both species i.e., 5.715 and 7.428mg/g.

Mycochemical screening

The qualitative mycochemical screening of *Russula foetens* and *Russula cf. foetentoides*, revealed the presence of a variety of secondary metabolites like reducing sugars, alkaloids, anthraquinones, terpenoids, tanins, saponins and cardiac glycosides. Ethanol extracts showed more

Table 1: Estimation of essential and non-essential metals in *Russula foetens* and *Russula cf. foetentoides*.

Mushrooms	Essential and non-essential metals (mg/g)									
	K	Na	Cu	Zn	Co	Mn	Cr	Fe	Ca	Ni
Russula foetens	0.285	0.675	0.915	0.690	0.494	1.8	0.214	5.715	0.48	ND
Russula cf. foetentoides	0.212	0.03	0.666	1.51	0.345	3.6	0.301	7.428	0.288	ND

ND: Not detected

Table 2: Zone of inhibition (mm) exhibited by crude extracts of *Russula foetens, Russula cf. foetentoides* and antibiotics against bacterial strains.

Mushroom	Extraction solvent	Zone of inhibition (mm)			
		Escherichia coli	*Pseudomonas stutzeri*	*Staphylococcus aureus*	*Bacillus Subtilis*
Russula foetens	Petroleum Ether	15.5±0.288f	0±0d	0±0k	14.16±0.6g
	Chloroform	11.8±0.152hi	0±0d	19.63±0.137h	9.13±0.633h
	Ethanol	14.76±0.145fg	12.16±0.166bd	27.8±0.152b	19.33±0.881de
	Distilled water	16.6±0.208e	9.73±0.145cd	14.86±0.088h	15.83±0.166f
Russula cf. foetentoides	Petroleum Ether	9.33±0.333r	0±0d	25±0.577c	0±0m
	Chloroform	0±0p	0±0d	18.66±0.88de	9.3±0.7h
	Ethanol	14.6±0.208g	10.5±0.288bd	21.4±0.208d	24.83±0.166h
	Dist.H_2O	17.7±0.175d	14.7±0.153bc	14.76±0.121h	18.66±0.440e
LSD		0.923	1.041	2.143	1.20
Escherichia coli		Amikacin		19±0.776 mm	
Pseudomonas stutzeri		Cephalaxin		25±0.075 mm	
Staphylococcus aureus		Erythromycin		30±0.99 mm	
Bacillus subtilis		Amikacin		23±0.57 mm	

Table 3: Zone of inhibition (mm) exhibited by crude extracts of *Russula foetens, Russula cf. foetentoides* and antimycotics against fungal strains

Mushroom Samples and antimycotics	Extraction solvents	Zone of inhibition (mm)	
		Pennicillium vancouvernse	*Fusarium equiseti*
Russula foetens	Petroleum Ether	9.0±0.576hi	17±0.577fg
	Chloroform	18±0.578c	14±0.531jk
	Ethanol	23±0.571a	23±0.499bcd
	Dist.H_2O	11±0.498fgh	21±0.579e
Russula cf. foetentoides	Petroleum Ether	0±0k	17.3±0.321fgh
	Chloroform	9.0±0.320i	14.3±0.333jk
	Ethanol	21±0.323b	21±0.299e
	Dist.H_2O	11±0.333fgh	19±0.355f
LSD		1.234	1.631
Pennicillium vancouvernse		Terbinafine	14±0.987 mm
Fusarium equiseti		Terbinafine	30±0.322 mm

Table 4: Total phenolic contents, Total flavonoid contents of *Russula foetens* and *Russula cf. foetentoides*.

Mushrooms	Total Phenolics contents[a]	Total Flavonoids contents [b]	FRAP Assay [c]
Russula foetens	0.061±0.004c	0.111±0.002ab	0.684±0.001b
Russula cf. foetentoides	0.064±0.002bc	0.115±0.001a	0.468±0.001e

[a] (mg GAE/g extract); [b] (mg CE/g extract); [c] (mmol/L of $FeSO_4$)

Table 5: DPPH Free Radical Scavenging assay and ABTS assay of crude extracts.

Samples	Concentration of extract (mg/ml)	Concentration of extract (ppm)	Percentage inhibition of DPPH	Percentage inhibition of ABTS
Russula foetens	300	0.3	11.353±0.012t	23.947±0.021w
	600	0.6	30.367±0.015n	61.542±0.022m
	900	0.9	43.045±0.037b	83.849±0.012f
Russula cf. foetentoides	300	0.3	15.941±0.020p	22.476±0.016x
	600	0.6	36.953±0.008i	63.762±0.016l
	900	0.9	40.077±0.011e	88.057±0.021b

Note: In tables 2, 3, 4 and 5,
*The results were based on experiments carried out in triplicates and given as Mean± Standard error
*LSD refers to the least significant difference
*Alphabets show significant difference ($p<0.05$) between the mean, according to Duncan's new multiple range test; while ± sign designates standard error

number of mycoconstituents followed by aqueous, petroleum ether and chloroform extracts.

HPLC Analysis

For clarification of the phenolic composition of extracts, mushrooms extracted by ethanol were evaluated by HPLC. Overall, five the phenolic compounds were stable in these macerates and the same were selected as the representative compounds during this study, their detail is as follow. *Russula foetens* exhibited phenolic contents i.e., gallic acid (R_t 2.774min), catechin (R_t 3.369min), vanillic acid (R_t 7.29min), sinapic acid (R_t 12.13min), querctin (R_t 25.125min). The most abundant phenolic compound is gallic acid i.e. 22.31% of total identified compounds. In the macerate of *Russula cf. foetentoides* major compunds were gallic acid (R_t 2.730min), catechin (R_t 3.189min), p-coumeric acid (R_t 5.421min), vanillic acid (R_t 8.017min), sinapic acid (R_t 13.213min), ferulic acid (R_t 13.167min) has been identified. The most abundant phenolic compound is gallic acid that is 3.59%.

Antimicrobial activity of mushroom extracts

All mushrooms extracts showed a various degree of antagonizing effects against the tested microbial strains. This antibacterial activity of crude extracts ranged from 9.13±0.633 to 27.8±0.152mm (table 2). In case of all antibiotics, the maximum zone was shown by *Bacillus subtilis* (23±0.59mm), whereas minimum zone was exhibited by *E. coli* (19±0.776mm). The antifungal activity ranged from 9.0±0.577 to 23.5±0.572 mm with the highest potential reported by ethanolic extract and the lowest efficacy was put forward by petroleum ether extract against *Pennicillium vancouvernse* of *Russula foetens* (table 3). In case of antimycotics, zones formed by

terbinafine against *Pennicillium vancouvernse* and *Fusarium equiseti* were 14±0.987mm and 30±0.322mm, respectively.

DPPH Free Radical Scavenging Assay

The DPPH is a stable nitrogen containing free radical. Its color changes from the violet to yellow on the reduction due to hydrogen or electron donation. The % inhibition for DPPH radical scavenging activity is given in table 5. In general, highest level of flavonoid and phenolic contents may lead to better DPPH scavenging activity. But in case of *Russula cf. foetentoides* extract with the lowest amount of phenols and flavonoids leads towards the highest DPPH scavenging in the present study

ABTS Assay

The phenothiazine drug i.e., ABTS that reacts with potassium persulfate and makes the green blue radical, as 2, 2 - azinobis- (3 - ethylbenzothiazoline - 6 - sulfonate) ($ABTS^+$) by oxidation. This cation actually appraises the antioxidant potential of the sample. Both mushrooms showed great inhibition against ABTS (table 5).

Reducing power

FRAP assay is carried generally carried out to determine the reduction of Fe^{3+} to Fe^{2+} from the sample. Both the mushrooms showed great reducing power of Fe^{3+}, therefore exhibiting the antioxidant potential. There is a significant difference ($P<0.05$) among the extracts. *Russula foetens* having lower amount of phenolics and flavonoids disclosed the highest reducing power (table 4).

Total phenolic and flavonoid contents

The phenolic and flavonoid contents of *Russula foetens* and *Russula cf. foetentoides* are presented in the table 4. Total phenolic contents were reported as gallic acid; whereas, total flavonoid contents were reported as quercetin (equivalent to grams). *Russula cf. foetentoides* showed highest amount of phenolics and flavonoids.

DISCUSSION

Both mushrooms possess significant amount of metals. It is established in literature that the uptake of any mineral is under the influence of other that may trigger it or antagonize it (Latiff *et al*., 1996). The minerals characterize the ash that was left behind after the complete ignition of dry mushroom. Hence, these two species could be considered nutritional source of this element. Adequate amount of iron in a diet is very important as it reduces the incidence of anemia. Copper contents (0.666 & 0.915mg/g) can be a significant dietary component for vegetarians. The low concentration of sodium particularly in *Russula cf. foetentoides* makes it an anti-hypertensive diet. Similarly, presence of potassium can reduce blood pressure. Overall, the results indicate that the concentrations of elements are in agreement with the results of (Niazi *et al.,* 2021) and both could be used as good source of dietary supplements. The results of mycochemical screening were quite similar to the findings of Jonathan & Fasidi (2003) & Fujita *et al*. (2005). Chloroform was found to be poor solvent as compared to the other one. This is the point with research consequences that some mushroom constituents are more soluble in polar solvents than non-polar (Kawagishi *et al*., 1988). The results of HPLC analysis are quite similar to the findings of Prabu *et al*. (2019). Generally, presence of phenolics in both species is good evidence regarding their antioxidant potential, an important phenomenon for human health. The findings of antimicrobial activity seem in line with the results of studies of Brain *et al*. (1951); Hirasawa *et al*. (1999) & Waithaka *et al.* (2017). However, all the crude extracts of mushrooms in this study showed inhibition against some virulent strains. These findings proposed that these mushrooms are potential sources of new antimicrobial agents. There is work in progress for the screening of mycochemicals, which are responsible for the antimicrobial activities. Although, the antimicrobial drugs have been practiced for many healing purposes; but there are many drug resistant bacterial strains that may create serious problems. These circumstances required the search of new antimicrobial materials that may be effective against the virulent bacterial strains. Natural resources such as mycoconstituents in the considered wild medicinal mushrooms could be a substitute of drugs that are resistant to the bacterial and fungal strains (Kuete, 2010; Sikandar *et al.,* 2020). The constituents that have capability to do such reactions are named as antioxidants (Nabavi *et al*., 2009). These results of our study were in covenant with the results reported by Ebrahimzadeh *et al*. (2009). The mushroom sample having antioxidant activity scavenged the radical cation of $ABTS^+$ and changes the green-blue color to yellow or light green (Mau *et al*., 2002). Moreover it has been observed that by increasing concentration of each extract, inhibition also increased with maximum value @ 900mg/mL of each extract. Our findings were also in agreement with the studies of Dehpour *et al.* (2009) and Niazi & Ijaz, (2021). The amount of the Fe^{2+} complex can be examined by determining the development of blue colored complex at 700nm (Nabavi *et al*., 2008). These phenolic and flavonoid compounds are extensively present in the food products derived from natural sources and have displayed the significant antioxidant activities (Ebrahimzadeh *et al*., 2008; Cateni *et al.,* 2021). These findings displayed that these mushrooms have rich antioxidants and may be used in pharmaceutical industries for making novel therapeutics in future.

CONCLUSION

On the basis of obtained findings, it was concluded that the both mushrooms could be used as potential antimicrobial and antioxidant agents. *Russula foetens*

exhibited higher results as compared to *R. cf. foetentoides. R. foetens* should be names as medicinal healing mushroom due to having better antimicrobial and antioxidant agents along with bioactive compounds.

REFERENCES

AOAC. 1990. Official Methods of analysis (15th Ed.). Washington, DC: of the Association of Official Analytical Chemists. Virginia (USA), pp.1-5.

Ardekani MRS, Hajimahmoodi M, Oveisi MR, Sadeghi N, Jannat B, Ranjbar AM, Gholam N and Moridi T (2011). Comparative antioxidant activity and total flavonoid content of Persian pomegranate (*Punica granatum* L.) cultivars. *IJPR*, **10**(3): 519.

Asemoloye MD, Sunmola N, Jonathan G and Chikwem J (2022). Mycochemical screening reveals exopolysaccharide secretion, antioxidant and larvicidal activities of three oyster mushrooms. *J. Sci. Food Agric*., **102**(5): 2120-2126.

Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC and Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Trop. J. Pharm. Res.*, **7**(3): 1019-1024.

Barros L, Calhelha RC, Vaz JA, Ferreira IC, Baptista P and Estevinho LM (2007). Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *Eur. Food Res. Technol.*, **225**(2): 151-156.

Benzie IF and Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal. Biochem*.,**239**(1):70-76.

Brand-Williams W, Cuvelier ME and Berset CLWT (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.*, **28**(1): 25-30.

Brian PW (1951). Antibiotics produced by fungi. *The Bot. Rev.*, **17**(6): 357-430.

Cateni F, Gargano ML, Procida G, Venturella G, Cirlincione F and Ferraro V (2021). Mycochemicals in wild and cultivated mushrooms: nutrition and health. *Phytochem Rev*., **21**(2022): 1-45.

Chapela IH and Lizon P (1993). Fungi in the stone age: Letter to the mycologist. *Mycologist*, **7**(3): 121.

Cheung LM and Cheung PC (2005). Mushroom extracts with antioxidant activity against lipid peroxidation. *Food Chem.*, **89**(3): 403-409.

Ćujić N, Šavikin K, Janković T, Pljevljakušić D, Zdunić G and Ibrić S (2016). Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chem.*, **194**(2016):135-142.

Dehpour AA, Ebrahimzadeh MA, Fazel NS and Mohammad NS (2009). Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasasy aceites*, **60**(4): 405-412.

Ebrahimzadeh MA, Pourmorad F, Hafezi S (2008). Antioxidant activities of Iranian corn silk. *Turk. J. Biol*., **32**(1): 43-49.

Ebrahimzadeh MA, Nabavi SF and Nabavi SM (2009). Essential oil composition and antioxidant activity of *Pterocarya fraxinifolia*. *Pak. J. Biol. Sci.*, **12**(13): 957.

Fujita R, Liu J, Shimizu K, Konishi F, Noda K, Kumamoto S, Ueda C, Tajiri H, Kaneko S, Suimi Y and Kondo R (2005). Anti-androgenic activities of *Ganoderma lucidum*. *J. Ethnopharmacol.*, **102**(1): 107-112.

Gebreyohannes G, Nyerere A, Bii C and Berhe Sbhatu D (2019). Determination of antimicrobial activity of extracts of indigenous wild mushrooms against pathogenic organisms. *Evid. Based Complement. Altern. Med*., **2019**: 1-7.

González-Gómez D, Lozano M, Fernández-León MF, Bernalte MJ, Ayuso MC and Rodríguez AB (2010). Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). *J. Food Compost. Anal*., **23**(6): 533-539.

Hirasawa M, Shouji N, Neta T, Fukushima K and Takada K (1999). Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (Shiitake, an edible mushroom). *Int.J. Antimicrob. Agents.*, **11**(2): 151-157.

Huie CW and Di X (2004). Chromatographic and electrophoretic methods for Lingzhi pharmacologically active components. *J. Chromatogr. B*., **812**(1-2): 241-257.

Iqbal S, Haleem S, Akhtar M, Zia-ul-Haq M and Akbar J (2008). Efficiency of pomegranate peel extracts in stabilization of sunflower oil under accelerated conditions. *Int. Food Res. J.*, **41**(2): 194-200.

Jonathan SG and Fasidi IO (2003). Antimicrobial activities of two Nigerian edible macro-fungi-*Lycoperdon pusilum* (Bat. Ex) and Lycoperdon giganteum (Pers.). *Afr. J. Biomed. Res*., **6**(2): 85-90.

Jorgensen JH and Turnidge JD (2015). Susceptibility test methods: dilution and disk diffusion methods. *J. Clin. Microbiol.*, 1253-1273.

Kalač P (2010). Trace element contents in European species of wild growing edible mushrooms: a review for the period 2000-2009. *Food Chem*., **122**(1): 2-15.

Kawagishi H, Nomura A, Yumen T, Mizuno T, Hagiwara T and Nakamura T (1988). Isolation and properties of a lectin from the fruiting bodies of *Agaricus blazei*. *Carbohydr. Res.*, **183**(1): 150-154.

Klein E, Smith DL and Laxminarayan R (2007). Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999-2005. *Emerg. Infect. Dis*., **13**(12): 1840.

Kuete V (2010). Potential of Cameroonian plants and derived products against microbial infections: A review. *Planta Medica*, **76**(14): 1479-1491.

Latiff LA, Daran ABM and Mohamed AB (1996). Relative distribution of minerals in the pileus and stalk

of some selected edible mushrooms. *Food Chem.*, **56**(2): 115-121.

Lone SH, Bhat KA and Khuroo MA (2015). Phytochemical Screening and HPLC Analysis of Artemisia amygdalina. *In*: Chemical and Pharmacological Perspective of Artemisia amygdalina. Springer, pp.7-13

Mallavadhani UV, Sudhakar AV, Satyanarayana KVS, Mahapatra A and Li W (2006). Chemical and analytical screening of some edible mushrooms. *Food Chem.*, **95**(1): 58-64.

Mau JL, Lin HC and Chen CC (2002). Antioxidant properties of several medicinal mushrooms. *J. Agric. Food Chem.,* **50**(21): 6072-6077.

Mau JL, Lin HC and Song SF (2002). Antioxidant properties of several specialty mushrooms. *Food Res. Int.*, **35**(6): 519-526.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M and Eslami B (2009). *In vitro* antioxidant and free radical scavenging activity of Diospyros lotus and *Pyrus boissieriana* growing in Iran. *Pharmacogn. Mag.,* **5**(18): 122.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A and Bekhradnia AR (2008). Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline*, **2**(9): 560-567.

Niazi AR and Ijaz H (2021). Proximate Analysis and *In Vitro* Biological Activities of Cauliflower Mushroom, *Sparassis crispa* (Agaricomycetes), from Pakistan. *Int. J. Med. Mushrooms*, **23**(2): 79-81

Niazi AR, Naz H, Shafique M and Imran M (2021). Mycochemical Screening and Study of Medicinal Properties of Two *Amanita* Species (Agaricomycetes) from Pakistan. *Int. J. Med. Mushrooms*, **23**(5): 33-39.

Oso BA (1981). Fungi and Mankind. University of Ibadan (Nigeria) Inaugural Lecture, pp. 40.

Oso BA (1977). Mushrooms in Yoruba mythology and medicinal practices. *Econ. Bot.*, **31**(3): 367-371.

Prabu K, Rajasekaran A, Bharathi D and Ramalakshmi S (2019). Anti-oxidant activity, phytochemical screening and HPLC profile of rare endemic *Cordia diffusa*. *J. King Saud Univ. Sci.*, **31**(4): 724-727.

Rajarathnam S, Shashirekha MNJ and Bano Z (1998). Biodegradative and biosynthetic capacities of mushrooms: Present and future strategies. *Crit. Rev. Biotechnol.*, **18**(2-3): 91-236.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, **26**(9-10): 1231-1237.

Schmitt JA, Meisch HU and Reinle W (1977). Heavy metals in higher fungi, II, manganese and iron. *Z. Naturforsch. C*, **32**(9-10): 712-723.

Sikandar A, Zhang M, Wang Y, Zhu X, Liu X, Fan H and Duan Y (2020). Mycochemical screening and analysis, antioxidant activity and biochemical composition of fermentation strain Snef1216 (*Penicillium chrysogenum*). *J. Anal. Chem.*, pp.1-8.

Steinkraus G, White R and Friedrich L (2007). Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001-05. *J. Antimicrob. Chemother.*, **60**(4): 788-794.

Varo P, Lähelmä O, Nuurtamo M, Saari E and Koivistoinen P (1980). Mineral element composition of Finnish foods. VII. Potato, vegetables, fruits, berries, nuts and mushrooms. *Acta Agric. Scand.*, **22**(Suppl.): 89-113.

Vetter J (1990). Mineral element content of edible and poisonous macrofungi. *Acta Aliment.*, **19**(1): 27-40.

Waithaka PN, Gathuru EM, Githaiga BM and Onkoba K M (2017). Antimicrobial activity of mushroom (*Agaricus bisporus*) and fungal (*Trametes gibbosa*) extracts from mushrooms and fungi of Egerton main campus, Njoro Kenya. *J. Biomed. Sci.*, **6**(3): 1-6.