INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 5, p. 184-191, 2018

RESEARCH PAPER OPEN ACCESS

Biological Screening and Nutritional Assessment of Selected Mushrooms Collected from District Mansehra Khyber Pakhtunkhwa Pakistan

Hameed Shabab Alam[1], Muhammad fiaz[1], Nisar Ahmad[*2], Ijaz Ahmad[3], Hameed Ur Rehman[3], Jan Alam[4], Zain ul Wahab[5]

[1] Department of Botany, Hazara University, Mansehra, Pakistan

[2] Department of Botany, Kohat University of Science & Technology, Kohat, Pakistan

[3]Department of Chemistry, Kohat University of Science & Technology, Kohat, Pakistan

[4]Department of Botany, Hazara University, Mansehra, Pakistan

[5]Department of Conservation Studies Hazara University, Mansehra, Pakistan

Key words: Mushrooms, Biological activities, Proximate composition, Nutrition, Drug sighting

http://dx.doi.org/10.12692/ijb/12.5.184-191 Article published on May 30, 2018

Abstract

Our research summarized the results of biological activities and proximate composite of three culinary medicinal mushrooms species *Morchella esculenta, Boletus edulis* and *Calvatia cyathiformis*. The antibacterial results of the extract and its derived fractions from *Morchella esculenta* revealed that the *n*-hexane fraction showed outstanding inhibitions 82.35 % and 77. 20% against *Bacillus popilliae* and *Salmonella typhi* respectively followed by crude extract with 69.15% inhibition. The *n*-hexane fraction of *Morchella esculenta* was also found to be potential against *Vibrio cholerae* giving 76.50% inhibition while its crude extract showed 77.20% inhibition against *Salmonella typhi*. Antibacterial evaluation of extract/frations obtained from *Boletus* showed that *n*-hexane fraction showed 78.20% inhibition against *Bacillus popilliae* second by crude extract with 77.20% inhibition. The crude extract and *n*-hexane fraction of *Boletus* projected good inhibitions 72.20% and 70.50% respectively against *Vibrio cholerae*. In antibacterial screening of extract/fractions from *Calvatia cyathiformis* the *n*-hexane fraction is found to be most potential showing 72% to 78% inhibitions against all tested bacteria pathogens. The antifungal results revealed that all the extracts and its derived fractions from the three selected mushrooms showed moderate to good inhibition against the tested fungal pathogens. Crude extracts and its derived fractions from *Morchella esculenta*, *Boletus edulis* and *Calvatia cyathiformis* were investigated for their antioxidant capacity by using an assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical antioxidant activity. Between the extracts/fractions, the water extract from all three *tested mushrooms* showed the most potent radical scavenging activity followed by chloroform and ethyl acetate fractions respectively. Total phenolics in the water fraction were higher than that of the all fractions. Positive correlations were found between total phenolic content in the mushroom extracts and their antioxidant activities.

* **Corresponding Author:** Dr. Nisar Ahmad ✉ 03449002451h@gmail.com

Introduction

Medicinal fungi are those fungi which produce medically significant metabolites or can be incited to create such metabolites utilizing Biotechnology. Mushrooms could be an alternative source of new antimicrobial mixes, primarily auxiliary metabolites for example terpenes, steroids, anthraquinones, benzoic corrosive subsidiaries, quinolones, oxalic corrosive, peptides and proteins. Wild mushrooms are turning out to be more critical for their dietary, tangible and particularly pharmacological characteristics[1] (Ergonul *et al*., 2013). Mushrooms are the main source of the crucial cancer prevention agent's selenium and ergothioneine. It is well admitted fact that mushrooms always fall low in calories, fat free, cholesterol free and low in sodium but still they give a few supplements that are normally found in grains[2] (Chen and Seviour, 2007). Due to increased resistance by microbes and malnutrition the researchers started to scan for new antimicrobial agents from different sources to be utilized as novel antimicrobial chemotherapeutic specialists[3] (Turkoglu *et al*., 2006). Researchers while looking for new helpful options, has concentrated on numerous sorts of mushrooms and has discovered different remedial compounds against cancer and other human diseases[4] (Wong *et al*., 2009). The aim of the current research work was to find out the biological screening and nutritional assessment of selected Mushrooms collected from district Mansehra Khyber Pakhtunkhwa Pakistan.

Material and methods

Fresh selected mushrooms were collected randomly from Hazara Division Pakistan. The taxonomic identification of these mushrooms was determined by export and qualified mycologist *Dr. Muhammad Fiaz* Department of Botany, University of Hazara. Fresh mushrooms were washed under running tap water; air dried and afterward was homogenized to fine powder

A. Extraction and Fractionation

The homogenized powder of *Morchella esculanta* was soaked in methanol for 07 days. The powdered was extricated with 80% methanol three times and separated at room temperature. The filtrate was vanished in rotary evaporator to get greenish extract, which was further suspended in water and portioned progressively with n-hexane, chloroform, ethyl acetic and n-butanol solvent to get respective fraction[5] (Ahmad *et al.,* 2015). Same extraction and fractionation method was adopted for *Boletus edulis* and *Calvatia cyathiformis*.

B. Antimicrobial assay

Antibacterial and antifungal experiments were performed by using agar diffusion technique as per established protocols[6] (Shinwari *et al*., 2013). In this process, wells were made in petriplates and the extracts/fractions of mushrooms were poured in these wells and after incubation of 24hrs (for bacteria) and 48 hrs (for fungi), the inhibition zones made around these wells were measured and were compared with the inhibition zone made by the standard antibiotic used. Five bacterial strains and three fungi were used in antibacterial and antifungal activities. The stock solution was prepared by adding 150mg of the extract/fraction in 10ml of the dimethyl sulphoxide (DMSO) to get 15mg/ml which was then utilized for antibacterial activities

C. DPPH radical scavenging activity assay

The free radical searching movement of the portions was measured In vitro by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) test as indicated by the technique portrayed before[7] (Ahmad et al., 2008). The stock arrangement was set up by dissolving 24 mg DPPH with 100 ml methanol and put away at 20°C until required. The working arrangement was acquired by weakening DPPH arrangement with methanol to accomplish an absorbance of around 0.98±0.02 at 517 nm utilizing the spectrophotometer. A 3ml aliquot of this arrangement was blended with 100µl of the example at different fixations (10 - 500µg/ml).

The response blend was shaken well and brooded oblivious for 15 min at room temperature. At that point the absorbance was taken at 517nm. The control was set up as above with no example. The searching action was assessed in view of the rate of DPPH radical rummaged as the accompanying condition:

$$\text{Scavenging Effect \%} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

D. Proximate Analysis

Proximate investigation of a mushroom test decides the aggregate protein, fat, starch, cinder, and dampness reported as the rate structure of the item. Every one of the specimens was processed utilizing mortar and cylinder and powdered examples were utilized for proximate investigation. AOAC techniques were connected to carryout proximate examination of the specimens for dampness, fiery debris, unrefined filaments, rough fats, proteins and starches. The nitrogen was controlled by smaller scale Kjeldahl strategy depicted by Pearson (1976) and the nitrogen substance was changed over to protein by increasing by a component of 6.25. Starch was dictated by contrast. All the proximate qualities were accounted for in rate[9, 10] (AOAC, 2000; AOAC, 2003).

Result and discussions

In spite of the gigantic differences of antibacterial mixes, bacterial imperviousness to first-decision anti-microbials has been radically expanding. In addition, the relationship between multiresistant microorganisms and nosocomial diseases highlight the issue, and the pressing requirement for arrangements. Common assets have been abused in the most recent years and among them, mushrooms could be an option wellspring of new antimicrobials .Antimicrobial specialists are irrefutably a standout amongst the most vital helpful disclosures of the twentieth century. Be that as it may, with the anti-infection period scarcely five decades old, humankind is presently confronted with the worldwide issue of rising resistance in essentially all pathogens[11] (Peterson *et al.,* 2004). Plants are utilized as prescriptions because of the nearness of extensive variety of naturally dynamic particles. Restorative significance of these plants relies on upon the convergence of dynamic atoms making them a rich wellspring of various drugs which assume an over whelming part in the support of human wellbeing[12] (Edeoga *et al*., 2005). In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In perspective of this, the scans for new hostile to microbial operators from restorative plants are much more dire in the nations like India where irresistible illnesses of bacterial inception are uncontrolled, as well as the causative specialists are likewise building up an expanding resistance against large portions of the ordinarily utilized anti-infection agents[13] (Abebe *et al.,* 2003).

Nowadays, the normal items and therapeutic plants are the most elite wellspring of life sparing medications for most of the total populace are a subject of extraordinary worldwide enthusiasm for the revelation of new antimicrobial operators[14] (Sashikala *et al.,* 2009). This could be identified with the late disappointment of anti-microbials against the emotional rising of the multidrug safe pathogens notwithstanding the fast spread of the new diseases[15] (Abdullah, 2011). Accordingly, researchers are giving top most need looking for substitute antimicrobial specialists from therapeutic plants[16] (Islam *et al*., 2010). Three gm negative bacterial strains, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*, and one Gram-positive strain, *Staphylococcus aureus*, were chosen for this study. Bacterial diseases brought about by the class *Staphylococcus* are an extraordinary risk to both people and creatures[16] (Simor *et al*., 2001).

Typhoid fever, brought on by *Salmonella typhe*, is a worldwide contamination. *Pseudomonas aeruginosa* is a typical bacterium that can bring about maladies in people, most famous for creating lung diseases or pneumonia. On the off chance that *Pseudomonas aeruginosa* provinces happen in basic body organs, for example, the lungs, urinary tract and kidneys, the outcomes can be lethal[17] (Balcht and Raymond, 1994). *Escherichia coli* cause numerous diseases including contaminations, pneumonia, bone and joint diseases, and skin and delicate tissue contaminations[18] (Todar, 2007). As indicated by the World Health Organization (WHO), 80% of the total populace relies on upon conventional drug for their essential human service's needs. There are impressive monetary advantages in the advancement of indigenous medications and in the utilization of restorative plants for the treatment of different infections[19] (Azaizeh *et al*., 2003).

The antibacterial after effects of the concentrate and it's got parts from *Morchella esculenta* showed that the n-hexane division gives remarkable hindrances 82.35% and 77. 20% against *Bacillus popilliae* and *Salmonella Typhi* respectively followed by rough concentrate with 69.15% hindrance. The n-hexane division of *Morchella esculenta* is additionally observed to be potential against *Vibrio cholerae* giving 76.50% restraint while its unrefined concentrate indicated 77.20% hindrance against *Salmonella typhi*. Antibacterial assessment of concentrate/fractions got from *Boletus edulis* demonstrated that n-hexane part indicated 78.20% hindrance against *Bacilluspopilliae* second by rough concentrate with 77.20% inhibition. The unrefined concentrate and n-hexane division of *Boletus edulis* anticipated great hindrances 72.20% and 70.50% separately against *Vibrio cholerae*. In antibacterial screening of concentrate/divisions from *Calvatia cyathiformis* the n-hexane portion is observed to be most potential indicating 72% to 78% restraints against all tried microbes pathogens (Table 1, 2, 3). The present examination uncovered that the contemplated mushrooms are possibly a decent wellspring of antibacterial operators and accepts their customary restorative use, likewise a hotspot for regular antibacterial and a premise for further pharmacological assessment.

Organisms are pervasive in nature, and contamination because of contagious pathogens has turned out to be more normal[20] (Lopes and Martins, 2008). Several works have shown in research center trials that distinctive plant tissues, for example, roots, leaves, seeds and blossoms have inhibitory properties against parasites[21] (Davicino *et al.*, 2007). Keeping in perspective the above connections amid present study, the rough chloroform, ethyl acetic acid derivation, butanol and n-hexane portions of three chose mushrooms tried against three contagious strains. Antimicrobial properties of plant concentrates have been accounted for with expanding recurrence from various parts of the world[22] (Cowan, 1999). Medicinal plants have been a wellspring of wide assortment of naturally dynamic mixes for a long time and utilized broadly as unrefined material for treating different infection conditions[23] (Borris, 1996). On premise of these lines, an endeavor had been made to assess the antifungal movement of couple of therapeutic plants against *A.niger, A. flavus* and *F. oxysporum*.

The present study tried the antifungal action of rough concentrates of three restorative mushrooms. The antifungal results uncovered that every one of the concentrates and it's got portions from the three chose mushrooms demonstrated moderate to great restraint against the tried contagious pathogens (Table 1, 2, 3). Crude concentrates and it's got parts from *Morchella esculenta, Boletus edulis* and *Calvatia cyathiformis* were examined for their cancer prevention agent limit by utilizing a tests of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical rummaging movement.

Among the concentrates/portions, the water remove from all the three tried mushrooms showed the strongest radical rummaging action took after by chloroform and ethyl acetic acid derivation parts individually (Table 4). Complete phenolics in the water division were higher than that of the all parts. Positive relationships were found between aggregate phenolic content in the mushroom extricates and their cell reinforcement exercises. Consumable mushrooms may have potential as common cancer prevention agents. Raw contemplate from nature and mixes filtered from these ponder can serve as better medication sources as home grown drugs and have no or least reactions, bio friendly furthermore have advantage because of the blend of restorative fixings of minerals and also vitamins[24] (Saetung *et al.*, 2005).

Movement directed fractionation and disengagement of mixes is the beginning stage for medication disclosure. Bio says are useful and easiest devices for testing the action of plant concentrates and on the premise of these exercises concentrates are gone before for photochemical learning to seclude novel helpful specialists[6] (Shinwari *et al.,* 2013). Pharmaceutical exercises of plant concentrates /portions are because of the nearness of major phyto compounds, unsaturated fats[25] (Aqil *et al.*, 2006).

Proximate examination is utilized to evaluate the relative measures of protein, lipid, water, fiery debris and starch in a life form. Protein, lipid and starch each add to the aggregate vitality substance of a living being while water and fiery debris just contribute mass. Thus, the aggregate vitality substance of an example can be recreated from its proximate synthesis.

The aftereffects of the immediate examination of the mushroom species class with their standard deviation are condensed in Table 5. The dampness substance was observed to be most astounding in *Morchella esculenta* (91.78 ± 8.45) whereas *Calvatia cyathiformis* was observed to be the least (14.30 ± 0.95). As a consequence of this study, *Morchella esculenta* was found to contain the most astounding substance of the dampness. The diving request of the dampness substance was seen as *Morchella esculanta*>*Boletus edulis* > *Calvatia cyathiformis*. The most astounding fiery remains and protein quality were seen in *Morchella esculenta* during investigation. Fiery debris substance of *Morchella esculanta*, *Boletus edulis* and *Calvatia cyathiformis* were found to have 13.65 ± 0.90, 5.45 ± 0.75 and 2.50 ± 0.92 separately. The after effects of fiery debris content with standard deviation are introduced in Table 5.

Morchella esculenta was observed to be most astounding in its rough fat substance (2.85 ± 0. 52) However, boletus contains unrefined fat (2.38±0. 15) while *Calvatia ceyaphisormyes* were found to have 1. 90 ± 0.12 fats. Mushroom species were observed to be a moderate wellspring of fats especially *Morchella esculenta*. Rough fiber of *Morchella esculenta* was found to have estimation of 84.80 ± 0. 40, trailed by the *Calvatia cyathiformis* (82.50 ± 0.50) (Table 5). Protein substance of the mushroom species was computed on the premise of the accessible nitrogen utilizing Kjeldaahl technique and was seen in the scope of 0.44 to 10.22% with *Morchella esculenta* having the most elevated quality (98.70 ± 0.53) trailed by Boletus (41.50 ± 1.80) and *Calvatia cyathiformis* (10.45 ± 0. 70) (Table 5). The sugar substance of investigated tests uncovered that *Morchella esculenta* had most elevated measure of starches (81. 60 ± 0.86), trailed by the diminishing request of *Calvatia cyathiformis* (72. 60 ± 0. 78) and Boletus (49.50 ± 2.75) (Table 5). Taking into account the proximate piece examination they are low in fat and rich in protein, sugars and rough filaments. Generally, the data about the proximate arrangement and vitality are of awesome enthusiasm for mushrooms to be utilized as nourishments or sustenance enhancing materials or in the detailing of wellbeing sustenance.

The mycochemicals results revealed that alkaloids, flavonoids, proteins and carbohydrates are present in all three selected mushrooms while saponins and glycosides were found to be absent in all three samples (Table No. 6). The biological potential of these selected mushrooms can be directly correlated with the mycochemicals present in them. The antimicrobial and antioxidant activities of mushrooms may be due to the presence of alkaloids and flavonoids in mushrooms.

Table 1. Proximate Values of the Selected Medicinal Mushrooms with Standard Error (%).

Parameters	*Morchella*	*Boletus*	*Calvatia*
Moisture	91.78 ± 8.45	23.75 ± 0.80	14.30 ± 0.95
Ash	13.65 ± 0.90	5.45 ± 0.75	2.50 ± 0.92
Fats	2.85 ± 0. 52	2.38 ± 0. 15	1. 90 ± 0. 12
Fibers	84.80 ± 0. 40	80.35 ± 0.70	82.50 ± 0.50
Carbohydrate	81. 60 ± 0.86	49.50 ± 2.75	72. 60 ± 0. 78
Proteins	98.70 ± 0.53	41.50 ± 1.80	10.45 ± 0. 70

Table 2. Antioxidant activities of crude extracts.

Extracts/Fractions	DPPH IC_{50} ± SEM (mM)		
	Morchella	*Boletus*	*Calvatia*
Crude	16. 057 ± 0.02	33.053 ± 0.05	29.080 ± 0.02
n-hexane	-	-	-
Chloroform	2.02 ± 0.06	3.005 ± 0.03	2.090 ± 0.002

Extracts/Fractions	DPPH IC_{50} ± SEM (mM)		
	Morchella	*Boletus*	*Calvatia*
Ethyl acetate	3.015 ± 0.05	4.025 ± 0.02	3.055 ± 0.04
n-butanol	-	-	-
Aqueous	0.099 ± 0.15	1.035 ± 0.06	0.065 ± 0.02
3-t-butyl-4-hydroxyanisole (BHA)[g]	0.049 ± 0.03	0.049 ± 0.03	0.049 ± 0.03

BHA, Positive control used in DPPH assays

Table 3. Statistical analysis of Bacterial Inhibition (in Percentage) of Crude extract and its derived fractions of *Morchella esculenta*.

Pathogens	Crude extract	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Aqueous	Antibiotic drug
B. popilliae	69.15	86.35	55.1	52.2	40.45	27.2	91.5
M. lacunata	39.6	36.2	41.5	36.1	32.5	23.4	89.3
S. typhe	77.2	53.6	69.3	60.3	56.1	19.2	88.5
S. dysenteriae	67.4	57.5	49.2	52.2	33.1	23.3	91.5
V. cholera	67.2	76.5	48.5	48.3	66.2	40.2	92.2
Mean	64.11	62.03	52.72	49.82	45.67	26.66	90.6
SD	14.2994	19.746	10.44806	8.827627	14.90778	8.080718	1.603122

Note: Statistical analysis of fungal inhibition of *Morchella esculenta*

A. fumigates	69.4	57.1	61.5	66.3	56.4	38.4	84.5
P. chrysogenum	74.6	64.3	59.5	59.7	60.5	60.4	87.4
C. albicans	64.5	68.2	60.1	57.2	58.1	54.4	86.4

Table 4. Statistical analysis of Bacterial inhibition (in Percentage) of Crude extract and its derived fractions of *Boletus edulis*.

Pathogens	Crude extract	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Aqueous	Antibiotic drug
B. popilliae	77.2	78.2	41.4	37.3	27.5	26.5	93.3
M. lacunata	62.3	70.3	61.2	44.3	31.2	27.3	87.4
S. typhe	51.5	73.2	56.5	55.5	25.6	22.3	90.5
S. dysenteriae	61.2	69.3	59.3	58.5	31.5	19.5	88.2
V. cholera	70.5	72.2	55.4	52.5	25.3	26.3	91.5
Mean	64.54	72.64	54.76	49.62	28.22	24.38	90.18
SD	9.772564	3.465977	7.810442	8.686311	3.410767	3.348432	2.409772

Note: Statistical analysis of fungal inhibition of *Boletus edulis*

A. fumigatus	61.2	67.3	68.2	59.5	12.3	19.5	86.2
P. chrysogenum	64.3	67.5	63.3	60.2	17.5	11.5	86.5
C. albicans	61.2	59.5	64.5	60.5	20.1	14.2	83.2

Table 5. Statistical analysis of Bacterial Inhibition (in Percentage) of Crude extract and its derived fractions of *Calvatia cyathiformis*

Pathogens	Crude extract	*n*-hexane	Chloroform	Ethylacetate	*n*-butanol	Aqueous	Antibiotic drug
B. popilliae	55.4	72.3	61.2	55.2	38.2	21.3	90.5
M. lacunata	67.2	78.1	60.4	52.5	38.5	24.4	89.1
S. typhe	60.1	75.3	59.2	49.3	31.5	21.3	89.3
S. dysenteriae	60.4	72.3	57.1	60.1	36.2	21.2	90.5
V. cholera	64.2	78.4	60.2	65.3	35.4	20.5	90.2
Mean	61.46	75.28	59.62	56.48	35.96	21.74	89.92
SD	4.476382	2.976911	1.578607	6.323132	2.816558	1.524139	0.672309

Note: Statistical anaylsis of fungal inhibition of Calvatia cyathiformis

A. fumigatus	59.1	59.4	40.3	40.2	23.1	22.3	83.5
P. chrysogenum	54.2	54.3	43.1	41.5	10.3	10.1	89.1
C. albicans	56.3	57.2	42.5	40.3	22.2	17.4	90.3

Conclusion and recommendations

This study provides novel scientific information about our three selected mushrooms *Morchella esculenta, Boletus edulis* and *Calvatia cyathiformis* based on its biochemical potential andproximate analysis. The mushrooms under investigationshowedpromising antimicrobial and antioxidant activities. Thus the study authenticated the pharmacological uses and nutritional values of the tested mushrooms samples with special reference to most promising potential extracts/fractions. The biologically active extracts/fractions may be directly used to treat various infectious diseases. Furthermore all the three tested mushrooms are good source of nutrition and energy.

Abbreviations

DMSO, dimethyl sulphoxide; DPPH, 2,2′-diphenyl-1-picrylhydrazyl; AOAC, association of official analytical chemist;

References

Abdullah M, Ab Hamid MR, Mustafa Z, Mohd Suradi NR, Idris F, Liong CY, Ismail WR, Mohd SF. 2011. Value-Based Total Performance Excellence Measurement (VBTPEM): An Overview of Agenda and Transformation. J Qual. Measure. Anal **7(1),** 67-75.

Abebe D, Debella A, Urga K. (2003). Medicinal plants and other useful plants of Ethiopia.

Ahmad I, Chen S, Peng Y, Chen S, Xu L. 2008. Lipoxygenase inhibiting and antioxidant Iridoids from *Buddleja crispa*. J Enz Inhib Med Chem **23,** 140-143.

Ahmad N, Shinwari ZK, Hussain J Perveen R. 2015). Phytochemicals, antibacterial and antioxidative investigations of *Alhagi maurorum* Medik. Pak J Bot **47(1),** 121-124.

Aqil F, Ahmad I, Mehmood Z. 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish journal of Biology **30(3),** 177-183.

Azaizeh H, Fulder S, Khalil K, Said O. 2003. Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region. Fitoterapia **74(1),** 98-108.

Balcht A, Raymond S. 1994. *Pseudomonas aeruginosa*: Infections and treatment. Marcel Dekker, New York.

Bjordal JM, Lopes-Martins RA, Joensen J, Couppe C, Ljunggren AE, Stergioulas A, Johnson MI. 2008. A systematic review with procedural assessments and meta-analysis of low level laser therapy in lateral elbow tendinopathy (Tennis elbow). BMC Musculoskeletal Disorders **9(1),** 75.

Borris RP. 1996. Natural products research: perspectives from a major pharmaceutical company. Journal of ethnopharmacology **51(1-3),** 29-38.

Chan SI. 2009. A physical chemist's expedition to explore the world of membrane proteins. Annual review of biophysics **38,** 1-27.

Chen YQ, Wang N, Qu LH, Li TH, Zhang WM. 2001. Determination of the anamorph of *Cordyceps sinensis* inferred from the analysis of the ribosomal DNA internal transcribed spacers and 5.8 S rDNA. Biochemical systematics and ecology **29(6),** 597-607.

Cowan MM. 1999. Plant products as antimicrobial agents. Clinical microbiology reviews **12(4),** 564-582.

Crump JA, Luby SP, Mintz ED. 2004. The global burden of typhoid fever. Bulletin of the World Health Organization **82(5),** 346-353.

Davicino R, Mattar A, Casali Y, Porporatto C, Correa SG, Micalizzi B. 2007. In vivo immunomodulatory effects of aqueous extracts of Larrea divaricata Cav. Immunopharmacology and Immunotoxicology **29(3-4),** 351-366.

Edeoga HO, Okwu DE, Mbaebie BO. "Phytochemical constituents of some Nigerian medicinal plants".

Gunç Ergönül P, Akata I, Kalyoncu F, Ergönül B. 2013. Fatty acid compositions of six wild edible mushroom species. The Scientific World Journal 2013.

Islam MM, Hossain MA, Jannat R, Munemasa S, Nakamura Y, Mori IC, Murata Y. 2010. Cytosolic alkalization and cytosolic calcium oscillation in Arabidopsis guard cells response to ABA and MeJA. Plant and Cell Physiology **51(10),** 1721-1730.

Kim T, Oh PI, Simor AE. 2001. The economic impact of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals. Infection Control & Hospital Epidemiology **22(02),** 99-104.

Mahmood A, Mahmood A, Malik RN, Shinwari ZK. 2013. Indigenous knowledge of medicinal plants from Gujranwala district, Pakistan. Journal of Ethnopharmacology **148(2),** 714-723.

Saetung A, Itharat A, Dechsukum C, Wattanapiromsakul C, Keawpradub N, Ratanasuwan P. 2005. Cytotoxic activity of Thai medicinal plants for cancer treatment. Songklanakarin J Sci Technol **27(2),** 469-478.

Shinwari ZK, Ahmad N, Hussain J, Rehman NU. 2013). Antimicrobial evaluation and proximate profile of *Nepeta leavigata*, *Nepeta kurramensis* and Rhynchosia reniformis. Pak. J. Bot **45(1),** 253-259.

Todar K. 2007. Pathogenic E. coli. Online Textbook of Bacteriology. University of Wisconsin, Madison. Department of Bacteriology.

Turkoglu A, Kivrak I, Mercan N, Duru ME, Gezer K, Turkoglu H. 2006. Antioxidant and antimicrobial activities of *Morchella conica* Pers. African Journal of Biotechnology **5(11).**

Wong KH, Sabaratnam V, Abdullah N, Kuppusamy UR, Naidu M. 2009. Effects of cultivation techniques and processing on antimicrobial and antioxidant activities of *Hericium erinaceus* (Bull.: Fr.) Pers. extracts. Food Technology and Biotechnology **47(1),** 47-55.