Journal of Medicinal Plants Research Vol. 6(10), pp. 1853-1859, 16 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1138
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Pharmacological screening of *Morchella esculenta* (L.) Pers., *Calvatia gigantea* (Batsch ex Pers.) Lloyd and *Astraeus hygrometricus* Pers., mushroom collected from South Waziristan (FATA.)

Hussain Badshah[1], Rizwana Aleem Qureshi[1], Javed Khan[2], Farman Ullah[3], Shah Fahad[1], Faizan Ullah[1], Amir Muhammad Khan[4], Ishtiaq Hussain[1]* and Naeem Khan[1]

[1]Department of Plant Sciences, Faculty of Biological Sciences, Quaid-I-Azam University Islamabad, Pakistan.
[2]National Agricultural Research Centre, Islamabad, Pakistan.
[3]Department of Physiology, Kohat Institute of Medical Sciences, University of Science and Technology Kohat, Pakistan.
[4]Department of Plant Sciences, Kohat University of Science and Technology, Kohat KPK, Pakistan.

Accepted 11 October, 2011

The mushrooms were screened for their antimicrobial, and cytotoxicity, six strains of bacteria viz. *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Vibrio cholerae* (ATCC6643), *Escherichia coli* (ATCC15224), *Klebsiella pneumoniae* (MTCC618) and *Enterobacter aerogenes* (ATCC13048) were utilized as test organisms for determination of antibacterial activity. Maximum antibacterial activity was observed at 30 mg/ml concentration of extracts. Greater inhibitory activity against *S. aureus* was possessed by methanolic and ethanolic extracts of *Calvatia gigantean*. The methanolic extract of *Morchella esculenta* exhibited maximum inhibitory activity against *V. cholerae* and also the ethanolic extract showed higher bactericidal activity against *E. coli*. But the selected mushrooms exhibited no significant antifungal activity against *Aspergillus fumigatus* and *Aspergillus niger*.

Key words: Mushrooms, medicinal, South Waziristan Agency.

INTRODUCTION

Mushrooms have great potential to be used as a source of nutritionally functional food and a source of biologically active, physiologically beneficial and non-toxic medicines (Wasser, 1999). Many previous studies showed that mushrooms were attractive as a functional food and as a source for the development of medicines and drugs due to their pharmacological effects against pathogenic microbes (Rosa et al., 2003; Jonathan and Fasidi, 2003; Gbolagade et al., 2005; Gezer et al., 2006; Turkoglu et al., 2006; Demirhan et al., 2007; Turkoglu et al., 2007; Gbolagade et al., 2007). It is estimated that approximately 50% of the annual 5 million metric tons of cultivated edible mushrooms contain functional therapeutic properties.

Morchella esculenta, commonly known as morel, true morel, sponge morel, common morel, is considered as the most expensive product because of its nutritional value and having a distinctive taste (Prasad et al., 2002). *Calvatia gigantea,* also called Gaint puffball, are used not only as a source of food, but are also explored for their therapeutic value in traditional systmes of treatments (Peschel, 1998).

Similarly, *Astraeus hygrometricus*, known as hygroscopic earthstar, false earthstar or the barometer earthstar, is another kind of fungus having been used in the treatment of several ailments. Several steroid derivatives have been reported from this fungal species like 3-oxo-25*S*-lanost-8-eno-26 and 22-lactone and 3-*epi*-astrahygrol (Moradali et al*.,* 2007). In Pakistan, only

*Corresponding author. E-mail: hussain.badshahqau@gmail.com.

Table 1. Antibacterial activity for methanolic extract (30 mg/ml) of selected mushrooms (fungi) collected from South Waziristan. Antibiotic erythromycin was used as positive control while pure DMSO was used as negative control. The data is mean of three replica per treatment.

Mushrooms	Mean zone of inhibition (mm)					
	B. sub	***S. aur***	***E. coli***	***E. aer***	***V. cho***	***K. pnu***
M. esculenta	19.5±0.05	17.5±0.05	23.5±0.15	18 ±0.1	23.5 ±0.05	28.5 ±0.05
A. hygrometricus	19.5±0.05	21±0.1	22±0.3	24.5±0.15	21.5±0.15	20.5±0.15
C. gigantea	19±0.1	24±0.1	41±0.1	21±0.4	27.5 ±0.05	18.5 ±0.05
Erythromycin	29.5±0.5	33.5±0.5	40.5±3.5	33.5±3.5	47 .5±2.5	39±1
DMSO	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

± represent the value of standard error. *B. sub - Bacillus subtilis, S.aur - Staphylococcus aureus, E. coli - Escherichia coli, E. aer - Enterobacter aerogenes, V. cho - Vibrio cholera* and *K. pnu - Klebsiella pneumonia.*

M. esculenta is marketed, whereas; *C. gigantea* and *A. hygrometricus* have no trade in Pakistan. Therefore, the aim of the current investigation was to explore pharmacological potential of selected mushrooms growing in South Waziristan Agency (FATA), Pakistan.

MATERIALS AND METHODS

The selected mushrooms viz. *M. esculenta*, *A. hygrometricus* (Earth star) and *C. gigantea* (giant puffball) were collected during April-May from South Waziristan and identified at Pakistan Museum of Natural History, Islamabad.

Antibacterial assay

The dried material was grinded finely in electric grinder and stored at 4℃ for preparation of crude extracts in several solvents of different polarity 20 g of each mushroom powder was soaked in flask in 200 ml of the solvent (methanol, ethanol and chloroform) for 3 days with occasional shaking to facilitate extraction. Each mushroom extract was dissolved in pure dimethylsulphoxide (DMSO) to give stock solution of 100 mg per ml (Bbosaet al., 2007). As a result 30 and 15 mg/ml concentrations were prepared. Solutions of erythromycin (2 mg/ml) in DMSO were prepared for positive control whereas; pure DMSO was used as negative control. After sterilization, the Broth medium was allowed to cool under aseptic conditions. Then the refreshed test micro organism was added to the Broth culture in test tube with the help of sterile wire loop. This mixture in test tube was placed in shaker for 24 h so that the test organism grows well. The standard was prepared by adding 0.5 ml of 0.048 M $BaCl_2$ to 99.5 ml 0.36N $H2SO_4$. Six strains of bacteria were used; which were *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Vibrio cholerae*, (ATCC6643) *Escherichia coli* (ATCC15224), *Klebsiella pneumoniae* (MTCC618) and *E. aerogenes* (ATCC13048). The first two are Gram positive and the later four are Gram negative. Four different concentrations of each mushroom extract (30 and 15 mg/ml) were dispensed into the separate wells. The two 2 mg per ml solutions of the positive control (erythromycin) were also applied on each test organism in the same way. The plates were incubated at 37℃ for 24 h. Triplicate plates were prepared for each extract. Mean clear zones of these plates was calculated. All the materials used in these experiments were subsequently inactivated and autoclaved at 121℃ and 15 lb pressure per square inch for 30 min.

Antifungal activity

The agar tube dilution method was used for antifungal activity of methanolic extract at 12 mg/ml as reported by choudary et al. (1995). Just two strains were used (*A. nigar* and *A. fumigatus*). Percentage inhibition of fungal growth for crude methanolic extracts was determined by

$$\text{Percentage inhibition of fungal growth} = 100 - \frac{\text{Linear growth in test tube (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

RESULTS AND DISCUSSION

Antibacterial activity of methanolic extract chloroform extract and ethanolic extract

Three kinds of crude extracts viz. methanolic, chloroform and ethanolic were prepared from fruiting bodies of selected fungi and were screened for their antibacterial activity.

Methanolic extract

The results presented in Table 1 revealed that at 30 mg/ml concentration of methanolic extract, all the selected fungi exhibited similar antibacterial activity against *B. subtilis*. However, maximum (24 mm) inhibitory activity against *S. aureus* was possessed by methanolic extract of *C. gigantea* followed by *A. hygrometricus* (21 mm). *E. coli* showed maximum susceptibility to methanolic extract of *C. gigantea* at this concentration (30 mg/ml).

The ranking of selected fungi for their antibacterial activity against *E. coli* was as follow: *C. gigantean* > *M. esculenta* > *A. hygrometricus*. The results further revealed that maximum antibacterial activity against *Enterobacter* was exhibited by extracts prepared from fruiting body of *A. hygrometricus*. The inhibitory effects of *C. gigantea* against *Enterobacter* were more pronounced than *M. esculenta*. At the same 30 mg/ml concentration of methanolic extract, *C. gigantea* was highly effective in

Table 2. Antibacterial activity for methanolic extract (15 mg/ml) of selected mushrooms (fungi) collected from South Waziristan. Antibiotic erythromycin was used as positive control while pure DMSO was used as negative control. The data is mean of three replica per treatment.

Mushrooms	Mean zone of inhibition (mm)					
	B. sub	***S. aur***	***E. coli***	***E. aer***	***V. cho***	***K. pnu***
M. esculenta	17.5±0.15	17.5±0.25	20.5±0.05	17±0.1	20.5±0.05	22±0 .05
A.hygrometricus	1.6±0.25	16.5±0.05	18±0.1	21.5±0.15	13±0.1	19.5±0.05
C. gigantea	17.5±0.2	17±0.1	19.5±0.05	20.5±0.05	17±0.1	16.5±0.0 7
Erythromycin	29.5±0.5	33.5±0.5	40.5±3.5	33.5±3.5	47 .5±2.5	39±1
DMSO	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

± represent the value of standard error. *B. sub - Bacillus subtilis, S.aur - Staphylococcus aureus, E. coli - Escherichia coli, E. aer - Enterobacter aerogenes, V. cho - Vibrio cholera* and *K. pnu - Klebsiella pneumonia.*

Table 3. Antibacterial activity for chloroform extract (30 mg/ml) of selected mushrooms (fungi) collected from South Waziristan. Antibiotic erythromycin was used as positive control while pure DMSO was used as negative control. The data is mean of three replica per treatment.

Mushrooms	Mean zone of inhibition (mm)					
	B. sub	***S. aur***	***E. coli***	***E. aer***	***V. cho***	***K. pnu***
M. esculenta	18±0.1	15±0.1	17.5±0.25	17±0.3	22±0.2	23±0.2
A. hygrometricus	19±0.1	26±0.15	22±0.5	18±0.1	27.5±0.25	23.5±0.35
C. gigantea	21.5±0.05	21.5±0.2	24.5±0.15	25.5±0.05	28.5±0.05	22 ±0.2
Erythromycin	29.5±0.5	33.5±0.5	40.5±3.5	33.5±3.5	47 .5±2.5	39±1
DMSO	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

± represent the value of standard error. *B. sub - Bacillus subtilis, S.aur - Staphylococcus aureus, E. coli - Escherichia coli, E. aer - Enterobacter aerogenes, V. cho - Vibrio cholera* and *K. pnu - Klebsiella pneumonia.*

inhibiting the growth of *V. cholerae*. The sequence of selected fungi for antibacterial against *V. cholerae* was as: *C. gigantea>M. esculenta>A. hygrometricus*. The results showed that *K. pneumoniae* was highly susceptible to *M. esculenta* as compared to other fungi species. It was found that at 30 mg/ml, the methanolic extract of *A. hygrometricus* was more inhibitory against *K. pneumoniae* than *C. gigantea*.

The Table 2 showed that at 15 mg/ml concentration, the methanolic extracts prepared from fruiting bodies of *M. esculenta* and *C. gigantea* exhibited similar inhibitory effects (17 mm) against *B. subtilis* and *S. aureus*. The *A. hygrometricus* showed 16 mm zone of inhibition against both *B. subtilis* and *S. aureus*. Maximum (20.5 mm) antibacterial activity against *E. coli* was exhibited by *M. esculenta* followed by *C. gigantea* (19.5 mm) and *A. hygrometricus* (18 mm), respectively. *A. hygrometricus* possessed 21.5 mm zone of inhibition against *Enterobacter* which was 21 and 5% higher than *M. esculenta* and *C. gigantea*, respectively. *M. esculenta* showed 35 and 21% higher antibacterial activity against *V. cholerae* as compared to *A. hygrometricus* and *C. gigantea*, respectively. Similarly, the same concentration (15 mg/ml) of methanolic extract prepared from fruiting body of *M. esculenta* exhibited 22 mm zone of inhibition against *K. pneumoniae* which was 11 and 25% higher than *A. hygrometricus* and *C. gigantea*, respectively.

During present investigation, it was found that methanolic extract of *M. esculenta* exhibited higher inhibitory activity against *K. pneumoniae.* Previous studies showed that *M. esculenta* is used in health care in different parts of the world and it posses antimicrobial activities (Nautiyal et al., 2001). The methanolic and chloroform extracts of *C. gigantea* showed greater inhibitory activity against G-ve bacterial strains. A report indicates the traditional use of *C. gigantea* in cure of severe wounds by some tribes of North America (Peschel, 1998). Likewise, *C. gigantea*s are used to treat wounds in traditional Chinese medicine (Ying et al., 1987). Baker et al. (1987) has investigated the antibacterial activity of some Nigerian mushrooms.

Chloroform extract

Table 3 shows the antibacterial activity of chloroform extract (30 mg/ml) prepared from fruiting body of selected fungi. *M. esculenta* and *A. hygrometricus* showed 19.5 mm zone of inhibition against *B. subtilis* which was 3% higher than zone of inhibition produced by puff ball against same bacterial strain. However, the *S. aureus* exhibited greater susceptibility showing 24 mm zone of

Table 4. Antibacterial activity for chloroform extract (15 mg/ml) of selected mushrooms (fungi) collected from South Waziristan. Antibiotic erythromycin was used as positive control while pure DMSO was used as negative control. The data is mean of three replica per treatment.

Mushrooms	Mean zone of inhibition (mm)					
	B. sub	***S. aur***	***E. coli***	***E. aer***	***V. cho***	***K. pnu***
M.esculenta	13±0.3	15.5±0.25	18±0.1	18.5±0.05	19.5±0.05	21.5±0. 15
A. hygrometricus	20.5±0.15	27.5±0.15	18.5±0.05	30.5±0.15	12.5±0.05	24±0.1
C. gigantea	17.5±0.15	13.5±0.025	31.5±0.25	28.5±0.05	18±0.1	14± 0.1
Erythromycin	29.5±0.5	33.5±0.5	40.5±3.5	33.5±3.5	47 .5±2.5	39±1
DMSO	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

± represent the value of standard error. *B. sub - Bacillus subtilis, S.aur - Staphylococcus aureus, E. coli - Escherichia coli, E. aer - Enterobacter aerogenes, V. cho - Vibrio cholera* and *K. pnu - Klebsiella pneumonia.*

inhibition towards puff ball extract as compared to *A. hygrometricus* and *M. esculenta*, respectively. The chloroform extract of *A. hygrometricus* possessed 21 mm zone of inhibition against *S. aureus* which was 19% higher than zone of inhibition produced by *M. esculenta*. The chloroform extract at 30 mg/ml was highly effective against *E. coli*. The extract prepared from puff ball caused 24.5 mm of zone of inhibition against *E. coli* whereas, that of *A. hygrometricus* produced 22 mm zone of inhibition followed by *M. esculenta* (17 mm). The tested fungi also exhibited antibacterial activity against *E. aerogenes*. However, maximum inhibitory activity (25.5 mm) was shown by *C. gigantea*. The ranking of different fungi for antibacterial activity against *E. aerogenes* was as follow: *C. gigantea>A. hygrometricus>M. esculenta.* The *V. cholerae* was highly susceptible to chloroform extract of *C. gigantea* showing 28.5 mm of inhibition. *A. hygrometricus* exhibited 27.5 mm zone of inhibition against *V. cholerae* which was 20% higher than *M. esculenta*. Maximum growth inhibitory effects against *K. pneumoniae* were shown by *M. esculenta* producing 23.5 mm zone of inhibition. The selected fungi were ranked as follow according to their antibacterial activity against *K. pneumoniae*: *M. esculent a> A. hygrometricus > C. gigantea*.

The results presented in Table 4 showed that chloroform extracts prepared from fruiting body of selected fungi at 15 mg/ml also exhibited antibacterial activity against selected bacterial strains. Maximum (20 mm) inhibitory zone against *B. subtilis* was produced by *A. hygrometricus.* Whereas, *M. esculenta* showed 17.5 mm zone of inhibition against same bacterial strain. Like *B. subtilis*, the *S. aureus* also exhibited greater susceptibility to chloroform extract (15 mg/ml) of *C. gigantea* as compared to *A. hygrometricus* and *M. esculenta*, respectively. The chloroform extract (15 mg/ml) prepared from fruiting body of *C. gigantea* was highly effective in inhibiting the growth of *E. coli* as compared to *M. esculenta* and *C. gigantea*. It caused 31.5 mm zone of inhibition which was 41 and 43% higher than antibacterial activity of *A. hygrometricus* and *M. esculenta* against *E. coli*. At the foregoing described dose of chloroform extract, significantly higher antibacterial activity (30.5 mm) against *Enterobacter* was exhibited *A. hygrometricus*.

The inhibitory effects of *M. esculenta* and *C. gigantea* did not differ significantly. The results showed that *V. cholerae* was more susceptible to chloroform extract (15 mg/ml) of *M. esculenta* as compared to *A. hygrometricus* and *C. gigantea*. The same concentration (15 mg/ml) of chloroform extract prepared from fruiting body of *A. hygrometricus* produced 24 mm zone of inhibition against *K. pneumoniae* which was 12 and 43% higher than bacterialcidal activity of *M. esculenta* and *C. gigantea*, respectively. Similarly, the inhibitory activity of *M. esculenta* against *K. pneumoniae* was 16% higher than *C. gigantea*.

Ethanolic extract

Table 5 shows the antibacterial activity of ethanolic extract prepared from fruiting body of selected fungi. *A. hygrometricus* showed 20.5 mm zone of inhibitions against *B. subtilis*. The inhibitory activity of *M. esculenta* and *C. gigantea* against *B. subtilis* did not differ significantly as both showed 18.5 mm zone of inhibition. Maximum (25 mm) growth inhibitory activity against *S. aureus* was exhibited by *C. gigantea* as compared to *A. hygrometricus* and *M. esculenta*, respectively.

Similarly, the ethanolic extract (30 mg/ml) of *C. gigantea* was also highly effective against *E. coli* than other selected fungi. It showed 23 and 28% higher inhibitory activity against *E. coli* than *M. esculenta* and *A. hygrometricus*, respectively. Both *M. esculenta* and *A. hygrometricus* produced 22.5 mm zone of inhibition against *Enterobacter* which was 13% higher than antibacterial activity of *C. gigantea* against same bacterial strain. The results revealed that maximum inhibitory zone (21.5 and 19.5 mm) against *V. cholerae* and *K. pneumoniae* was produced by *A. hygrometricus*as compared to other two fungi types. The inhibitory activity of *M. esculenta* against *V. cholerae* was 8% higher than *C. gigantea.*

Table 5. Antibacterial activity for ethanolic extract (30 mg/ml) of selected mushrooms (fungi) collected from South Waziristan. Antibiotic erythromycin was used as positive control while pure DMSO was used as negative control. The data is mean of three replica per treatment.

Mushroom	Mean zone of inhibition (mm)					
	B. sub	***S. aur***	***E. coli***	***E. aer***	***V. cho***	***K. pnu***
M. esculenta	18.5±0.15	20±0.1	20.5±0.15	22.5±0.25	20±0.3	17.5±0. 35
A.hygrometricus	20.5±0.45	21.5±0.15	19±0.1	22.5±0.2	21.5±0.15	19.5±0.05
C.gigantea	18.5±0.45	25±0.00	26.5±0.05	19.5±0.05	18.5±0.15	15± 0.1
Erythromycin	29.5±0.5	33.5±0.5	40.5±3.5	33.5±3.5	47 .5±2.5	39±1
DMSO	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

± represent the value of standard error. *B. sub - Bacillus subtilis, S.aur - Staphylococcus aureus, E. coli - Escherichia coli, E. aer - Enterobacter aerogenes, V. cho - Vibrio cholera* and *K. pnu - Klebsiella pneumonia.*

Table 6. Antibacterial activity forethanolic extract (15 mg/ml) of selected mushrooms (fungi) collected from South Waziristan. Antibiotic erythromycin was used as positive control while pure DMSO was used as negative control. The data is mean of three replica per treatment.

Mushrooms	Mean zone of inhibition (mm)					
	B. sub	***S. aur***	***E. coli***	***E. aer***	***V. cho***	***K. pnu***
M. esculenta	16.5±0.35	19±0.1	20.5±0.05	18±0.1	14.5±0.45	17.5±0. 15
A.hygrometricus	16.5±0.05	18.5±0.05	11.5±0.1	17.5±0.25	25±0.1	19.5± 0.05
C.gigantea	14.5±0.05	14.5±0.45	15±0.15	19.5±0.05	18±0.2	16.5±0 .55
Erythromycin	29.5±0.5	33.5±0.5	40.5±3.5	33.5±3.5	47 .5±2.5	39±1
DMSO	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

± represent the value of standard error. *B. sub - Bacillus subtilis, S.aur - Staphylococcus aureus, E. coli - Escherichia coli, E. aer - Enterobacter aerogenes, V. cho - Vibrio cholera* and *K. pnu - Klebsiella pneumonia.*

The results presented in Table 6 revealed that ethanolic extracts prepared from fruiting body of selected fungi at 15 mg/ml showed antibacterial activity against tested bacterial strains. However, their inhibitory effect was lower than 30 mg/ml. The results showed that maximum bacterialcidal activity against *B. subtilis* and *S. aureus* was exhibited by *M. esculenta* and *A. hygrometricus.* At this concentration (15 mg/ml) of ethanolic extract, *M. esculenta* produced 20.5 mm zone of inhibition against *E. coli* which was significantly higher than inhibition zone produced by the other two fungal types against same bacterial strain.

The ethanolic extract of *C. gigantea* at same concentration of 15mg/ml produced 19.5 mm zone of inhibition which was 5 and 10% higher than antibacterial activity of *M. esculenta* and *A. hygrometricus*, respectively. The methanolic extract of *A. hygrometricus* at 15 mg/ml was highly effective in inhibiting the growth of *V. cholerae*. It showed 25 mm zone of inhibition against this bacterial strain. The ranking of selected fungi for their antibacterial activity against *V. cholerae* was as follow: *A. hygrometricus* > *C. gigantean* > *M. esculenta.*

The results further revealed that like *V. cholerae*, the *Klebsiella pneumoniae* was also highly susceptible to ethanolic extract (15 mg/ml) of *A. hygrometricus* showing 19.5 zone of inhibition. There were found variations among the antibacterial activities of different fractions prepared in different solvents. The crude extract prepared in methanol from fruiting body of *C. gigantea* exhibited higher antibacterial activity against *E. coli*. Similarly, the methanolic, extract of *M. esculenta* showed maximum inhibitory activity against *K. pneumoniae*. The methanolic extract of *A. hygrometricus* showed higher activity against *Enterobacter aerogenes*.

It was also found that antibacterial activity of methanolic extract of selected mushrooms was concentration dependant. The higher concentration of extracts that is, 30 mg/ml was more effective in inhibiting the bacterial growth than 15 mg/ml. *S. aureus* (G+ve) was found as more susceptible bacterial strain to chloroform extract prepared from fruiting bodies of selected fungi. Like methanolic extracts, the chloroform extract prepared from fruiting body of *C. gigantea* showed maximum inhibitory activity against *Escherichia coli* (24.5±0.15 mm), *E. aerogenes* (25.5±0.05 mm) and *V. cholerae* (28.5±0.05 mm), respectively. However, the chloroform extract prepared from fruiting bodies of *A. hygrometricus* and *M. esculenta* were equally effective against *K. pneumoniae* both showing 23±0.2 and 23.5±0.35 mm zones of inhibition, respectively.

B. subtilis was found as more susceptible bacterial strain showing 20.5±0.45 mm zone of inhibition to

Table 7 Antifungal activity for methanolic extracts (12 mg/ml) of selected mushrooms collected from South Waziristan. *Aspergillus fumigatus* and *Aspergillus niger* were used as test fungal strains. Antifungal activity was determined by agar tube dilution method.

Treatment	Linear inhibition of growth (%)	
	A. fumigatus	***A. niger***
M. esculenta	5±0.00	3.5±0.5
C. gigantea	1.5±0.5	50±10
A. hygrometricus	10±0.00	15±5
Turbenafine	100 ±0.00	100±0.00
DMSO	0.00	0.00

ethanolic extract of *A. hygrometricus* as compared to ethanolic extracts prepared from fruiting bodies of *M. esculenta* and *A. hygrometricus*, respectively. The ethanolic extract of *C. gigantea* exhibited greater zone of inhibition (25±0.00 and 26.5±0.05 mm) against *S. aureus* and *E. coli as* compared to other mushrooms. Whereas, the ethanolic extract prepared from fruiting body of *C. gigantea* was more effective in inhibiting the growth of *V. cholerae* and *K. pneumoniae*, respectively. These results are in agreement with previous findings of Black (2002) that mushrooms posses antibacterial activities. Mau et al*.* (2002) had reported in their studies that mushrooms posses medicinal properties. A research showed that the most significant antibiotics have been derived from fungi such as penicillin, streptomycin, chloramphenicol and vancomycin (Griffin, 1994) where humans can benefit from the natural defensive strategies of the fungi that produce antibiotics to fight infection from microorganisms. Moreover, the Chinese traditions indicate that certain *C. gigantea*s have anticancer properties (Ying et al., 1987). The best evidence supporting medicinal use of *C. gigantea*s comes from studies showing that extracts from certain species inhibit bacterial growth. For example, both *Calvatia craniformis* and *Calvatia lilacina* (*C. gigantea*s) produce an antibiotic called calvatic acid. The extracts containing calvatic acid have rather impressive antibacterial properties.

In laboratory tests, a fairly wide range of both Gram positive and Gram negative bacteria were sharply inhibited by calvatic acid (Imtiaj and Lee, 2007). Basidiomycete (*C. gigantea*s) mushrooms are receiving attention as potential sources of new classes of antibiotics with the development of new fermentation and purification technologies (Anke, 1989). Estrela et al*.* (2000) reported that the size of the microbial inhibition zone depends on the solubility and diffusibility of the test substance in the agar diffusion method and therefore may not express its full potential. This indication was evident in the present study that crude extracts prepared from fruiting bodies of selected mushrooms exhibited clear zones of inhibition against bacterial strains.

Antifungal activity

The results presented in Table 7 revealed that all the selected fungi did not show any significant antifungal activity against tested fungal strains as compared to fubgicideturbenafine except for *C. gigantea* that caused 50% inhibition of *Aspergillus niger*. The same mushroom showed 15% inhibition of *Aspergillus fumigatus*. The methanolic extract of *A. hygrometricus* caused 10% growth inhibition of *A. niger*. *M. esculenta* showed inhibitory activity (5 and 3.5%) against *A. fumigatus* and *A. niger*, respectively. Higher (10±0.00%) antifungal activity against *A. fumigatus* was exhibited by methanolic extract prepared from fruiting body of *A. hygrometricus*. The methanolic extracts of *M. esculenta* and *C. gigantea* showed 5±0.00 and 1.5±0.5% inhibition of *A. fumigatus* growth, Maximum antifungal activity against *A. niger* (10±0.00%) was possessed by methanolic extract of *A. hygrometricus*. Whereas, the methanolic extract prepared from fruiting bodies of *M. esculenta* and *C. gigantea* showed least inhibitory activity against *A. niger*. These results are an agreement with previous finding of Park et al. (2009) who isolated a protein from a mushroom called *Cordycepsmilitaris.* Previously, it has been reported that mushrooms show antimicrobial effects (Sheena et al., 2003; Hur et al*.,* 2004). Suayet al*.* (2000), in their study, described that Basidiomycetes have been reported to produce a large number of metabolites which show antibacterial and antifungal activity.

As for the antifungal activity, methanolic and water extracts of *Schizophyllum commune* were found to show no activity, whereas ethyl acetate and dichloromethane extracts were partially active against the fungal species tested (salahudin, 2008). Abraham (2001) stated that a compound from *M. conigenus* known as marasmic acid was shown to have antibacterial and antifungal properties. Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Hence, it is not surprising that antimicrobial compounds with more or less strong activities could be isolated from many mushrooms and that they could be of benefit for human (Lindequist et al*.,* 2005). The present results support the findings of Suayet al. (2000) that the antibacterial activity of polypores and gilled mushrooms was found to be more pronounced than antifungal activity (Suayet al*.,* 2000).

As in most cases, it appears that the fungal and yeast strains are more resistant to antimicrobial compounds than bacterial strains (Nishizawa et al*.,* 1990; Papadopoulou et al*.,* 2005). An extract of mushroom called *Lentinus adherens* were observed to be less effective against pathogenic fungi compared to pathogenic bacteria (Lauer et al., 1991). Another report by Jonathan and Fasidi (2003) also suggested that the antifungal activities of the mushrooms *Lycoperdon pusilum* and *Lycoperdon giganteum* extract against pathogenic tested were very low. Sokmen et al. (2004), in their study, described that there was no antifungal or

or anticandidal activity recorded from *Thymus spathulifolius* but the extract had antibacterial activity.

The effectiveness of the extracts, *S. commune* depends on the extraction solvent and higher antibacterial activity was exhibited compared to antifungal activity. Dichloromethane extract was the most effective, being able to significantly ($P<0.05$) inhibit the growth of most of the Gram-positive bacteria, Gram-negative bacteria and fungi.

Conclusions

The biological activities of natural compounds help to discover new antibiotic compounds that could serve as selective agents for controlling pathogenic diseases of both plants and animals. The methanolic and chloroform extracts of all selected fungi (mushrooms) exhibited greater bactericidal activity against *V. cholerae*. The *E. coli* was more susceptible towards the methanolic extract of *M. esculenta*. The results of present study established that mushrooms collected from South Waziristan Agency (FATA) Pakistan are useful for medicinal point of view and further works are needed to isolate and purify the bioactive compounds present.

REFERENCES

Abraham WR (2001). Bioactive sesquiterpenes produced by fungi: are they useful for human as well? Cur. Med. Chem., 8: 583-606

Anke T (1989). Basidiomycetes: A source for new bioactive secondary metabolites. In: Bioactive Metabolite from Microorganisms. Bushel ME, Grafe U, (eds), Amsterdam. Els. Sci. Pub., 51-65. J. Antibiot., 34(10): 1271-1277

Black JG (2002). Microbiology: Principles and Explorations (5th edition). New York: John Wiley and Sons, 11: 3–9.

Baker CC, Phelps WC, Howley PM (1987). Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. J. Virol., 61: 926-971.

Bbosa GS, Kyegambe OB, Ogwal J, Kan O, Ziraba RB, Odyed O, Waako SP (2007). Anti bacterial activity of *Mangifera indica* leaves. Afr. J. Ecol., 45 (Supp-1): 13-36.

Choudhary MI, Shahwar D, Parveen Z, Jabbar A, Ali I, Rahman A (1995). Antifungal steroidal lactones from *Withania coagulance.* Phytochemistry, 40(4): 1243-1246

Demirhan A, Yesil OF, Yildiz A, Gul K (2007). A research on antimicrobial activity of some macrofungi species. Sci . Eng. J. Firat Univ., 19: 425-433.

Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD (2000). Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal Braz. Dent. J., 11: 3-9.

Gbolagade JS, Fasidi IO (2005). Antimicrobial activities of someselected Nigerian mushrooms. Afr. J. Biol. Res., 8: 83-87.

Gbolagade J, Kigigha L, Ohimain E (2007) Antagonistic effect of extracts of some Nigerian higher fungi against selected pathogenic microorganism. Am-Eur. J. Agric. Environ. Sci., *2*: 364-368.

Gezer K, Duru ME, Kivrak I, Turkoglu A, Mercan N, Turkoglu H, Gulcan S (2006) Free-radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey. Afr. J. Biot., 5: 1924-1928.

Griffin DH (1996). Fungal Physiology, 2nd edition.Wiley-Liss, Inc.

Hur JM, Yang CH, Han SH, Lee SH, You YO, Park JC (2004). Antibacterial effect of *Phellinus linteus* against methicilin-resistant *Staphylococcus aureus.* Fitoterapia*,* 73: 603-605

Imtiaj A, Lee ST (2007). Screening of antibacterial and antifungal Activities from Korean wild mushrooms. World J. Agric*.* Sci., 3: 316–21

Jonathan SG, Fasidi OI (2003). Antibacterial Activities of Two Nigerian Edible Macro Fungi – Lycoperdon *Pusilum* (Bat.Ex) Lycoperdon Giganteum, Coperdon Giganteum (pers). Afr*.* J. Biomed*.* Res*.,* 6: 85-90

Lauer U, Anke T, Hansske F (1991). Antibiotics from basidiomycetes XXXYIII. 2-methoxy-5-methyl-1,4-benzoquinone, a thromboxane A2–receptor antagonist from *Lentinusadherens*. J. Antibiot., 44(1): 59-65.

Lindequist U, Niedermeyer JT, Jullich W (2005). The Pharmacological Potential of Mushrooms eCAM, 2(3): 285–299.

Maua lJ, Lin CH, Song FS (2002). Antioxidant properties of several specialty mushrooms. Food Res. Int*.,* 35, 519-526.

Nishizawa I, Nakata A, Kishida WA, Ayer LM, Browne LL (1990). Some biologically-active tannins of *Nuphar variegatum.* Phytochemistry, 29: 2491-2495.

Nautiyal B, Prakash V, Maithani UC, Chauhan RS, Purohit H, Nautiyal M.C (2003). "Germinability, productivity and economic viability of *Rheum emodi* Wall. ex Meissn. cultivated at lower altitude," Curr. Sci., 2: 143-148.

Park TB, Heum NK, Cha JE, Park HW, Hyung WJ, Kim H (2009). Antifungal and Anticancer Activities of a Protein from the Mushroom *Cordycepsmilitaris,* Korean J. Physiol. Pharmacol., 13: 495*.*

Papadopoulou C, Soulti K, Roussis IG (2005). Potential antimicrobial activity of red and white wine phenolic extracts against strains of *Staphylococcus aureus, Escherichia coli* and *Candida albicans.* Food Technol. Biotechol., 43 (1): 41-46

Prasad P, Chauhan K, Kandari LS, Maikhuri RK, Purohit A, Bhatt P, Rao KS (2002). *Morchella esculenta* (Guchhi). Need for scientific intervention for its cultivation in Central Himalaya. Curr. Sci., 82(9): 1098-100

Peschel K. (1998). Puhpohwee for the people: A Narrative Account of Some Uses of Fungi among the Ahnishinaabeg. DeKalb, IL, LEPS Press*,* p 70.

Rosa LH, Cota BB, Machado TK, Rosa AC, Zani LC (2003). Antifungal and other biological activities from *Oudemansiella canarii* (Basidiomycota). World J. Microbiol. Biotech*.,* 21: 983–98.

Sokmen A, Güllüce M, Akpulat HA, Daferera D, Tepe B, Polissiou M, Sokmen M, Sahin F (2004). The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control, 15: 627-634.

Suay I, Arenal F, Asensio FJ, Basilio A, Cabello MA, Díez MT, García JB, Val AG, Gorrochategui J, Hernández P, Peláez F, Vicente MF (2000). Screening of basidiomycetes from antimicrobial activities. Antonie van Leeuwenhoek, 78 (2): 129-139.

Turkoglu A, Kivrak I, Mercan N, Duru E, Gezer K, Turkoglu, H (2006). Antioxidant and antimicrobial activities of *Morchella conica* Pers. Afr. J. Biotechnol*.,* 5 (11); 1146-115

Wasser SP (1999). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. Crit. Rev. Immunol., 19 (1): 65-96.

Ying JX, Mao Q, Ma Y, Zong H (1987). Icons of MedicinalFungi from China. Bei. Sci. Press., 575.