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

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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 gigantea*. 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; Guzer 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 Giant puffball, are used not only as a source of food, but are also explored for their therapeutic value in traditional systems 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-25S-lanost-8-eno-26 and 22-lactone and 3-epi-astrahydrog (Moradali et al., 2007). In Pakistan, only
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°C 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 dimethylsulfoxide (DMSO) to give stock solution of 100 mg per ml (Bbossa et 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 hygrometric potential of selected mushrooms growing in South Waziristan Agency (FATA), Pakistan.

RESULTS AND DISCUSSION

Antibacterial activity of 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. gigantea > 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>
<thead>
<tr>
<th>Mushrooms</th>
<th>B. sub</th>
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<th>V. cho</th>
<th>K. pnu</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. esculenta</td>
<td>19.5±0.05</td>
<td>17.5±0.05</td>
<td>23.5±0.15</td>
<td>18 ±0.1</td>
<td>23.5 ±0.05</td>
<td>28.5 ±0.05</td>
</tr>
<tr>
<td>A. hygrometricus</td>
<td>19.5±0.05</td>
<td>21±0.1</td>
<td>22±0.3</td>
<td>24.5±0.15</td>
<td>21.5±0.15</td>
<td>20.5±0.15</td>
</tr>
<tr>
<td>C. gigantea</td>
<td>19±0.1</td>
<td>24±0.1</td>
<td>41±0.1</td>
<td>21±0.4</td>
<td>27.5±0.05</td>
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<td>Erythromycin</td>
<td>29.5±0.5</td>
<td>33.5±0.5</td>
<td>40.5±3.5</td>
<td>33.5±3.5</td>
<td>47.5±2.5</td>
<td>39±1</td>
</tr>
<tr>
<td>DMSO</td>
<td>0±0.00</td>
<td>0±0.00</td>
<td>0±0.00</td>
<td>0±0.00</td>
<td>0±0.00</td>
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</tr>
</tbody>
</table>


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

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

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. gigantea > 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
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 possesses 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* 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

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**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.

<table>
<thead>
<tr>
<th>Mushrooms</th>
<th>B. sub</th>
<th>S. aur</th>
<th>E. coli</th>
<th>E. aer</th>
<th>V. cho</th>
<th>K. pnu</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. esculenta</em></td>
<td>17.5±0.15</td>
<td>17.5±0.25</td>
<td>20.5±0.05</td>
<td>17±0.1</td>
<td>20.5±0.05</td>
<td>22±0.05</td>
</tr>
<tr>
<td><em>A. hygrometricus</em></td>
<td>1.6±0.25</td>
<td>16.5±0.05</td>
<td>18±0.1</td>
<td>21.5±0.15</td>
<td>13±0.1</td>
<td>19.5±0.05</td>
</tr>
<tr>
<td><em>C. gigantea</em></td>
<td>17.5±0.2</td>
<td>17±0.1</td>
<td>19.5±0.05</td>
<td>20.5±0.05</td>
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<td>Erythromycin</td>
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<td>33.5±3.5</td>
<td>47.5±2.5</td>
<td>39±1</td>
</tr>
<tr>
<td>DMSO</td>
<td>0±0.00</td>
<td>0±0.00</td>
<td>0±0.00</td>
<td>0±0.00</td>
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</tr>
</tbody>
</table>

± 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.

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<th>E. coli</th>
<th>E. aer</th>
<th>V. cho</th>
<th>K. pnu</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. esculenta</em></td>
<td>18±0.1</td>
<td>15±0.1</td>
<td>17.5±0.25</td>
<td>17±0.3</td>
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<td>23±0.2</td>
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<tr>
<td><em>A. hygrometricus</em></td>
<td>19±0.1</td>
<td>26±0.15</td>
<td>22±0.5</td>
<td>18±0.1</td>
<td>27.5±0.25</td>
<td>23.5±0.35</td>
</tr>
<tr>
<td><em>C. gigantea</em></td>
<td>21.5±0.05</td>
<td>21.5±0.2</td>
<td>24.5±0.15</td>
<td>25.5±0.05</td>
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<td>Erythromycin</td>
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</tr>
<tr>
<td>DMSO</td>
<td>0±0.00</td>
<td>0±0.00</td>
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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. esculenta > 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>
<thead>
<tr>
<th>Mushrooms</th>
<th>Mean zone of inhibition (mm)</th>
<th>B. sub</th>
<th>S. aur</th>
<th>E. coli</th>
<th>E. aer</th>
<th>V. cho</th>
<th>K. pnu</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. esculenta</td>
<td>13±0.3</td>
<td>15.5±0.25</td>
<td>18±0.1</td>
<td>18.5±0.05</td>
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</tr>
<tr>
<td>A. hygrometricus</td>
<td>20.5±0.15</td>
<td>27.5±0.15</td>
<td>18.5±0.05</td>
<td>30.5±0.15</td>
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<tr>
<td>C. gigantea</td>
<td>17.5±0.15</td>
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<td>Erythromycin</td>
<td>29.5±0.5</td>
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<td>40.5±3.5</td>
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</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Mushroom</th>
<th>B. sub</th>
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<th>E. coli</th>
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<th>V. cho</th>
<th>K. pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. esculenta</td>
<td>18.5±0.15</td>
<td>20±0.1</td>
<td>20.5±0.15</td>
<td>22.5±0.25</td>
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</tr>
<tr>
<td>A. hygrometricus</td>
<td>20.5±0.45</td>
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<td>22.5±0.2</td>
<td>21.5±0.15</td>
<td>19.5±0.05</td>
</tr>
<tr>
<td>C. gigantea</td>
<td>18.5±0.45</td>
<td>25±0.00</td>
<td>26.5±0.05</td>
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<td>Erythromycin</td>
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</tr>
</tbody>
</table>

± 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. pneumonia.

Table 6. Antibacterial activity for ethanolic 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.

<table>
<thead>
<tr>
<th>Mushrooms</th>
<th>B. sub</th>
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<th>E. coli</th>
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<th>V. cho</th>
<th>K. pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. esculenta</td>
<td>16.5±0.35</td>
<td>19±0.1</td>
<td>20.5±0.05</td>
<td>18±0.1</td>
<td>14.5±0.45</td>
<td>17.5±0.15</td>
</tr>
<tr>
<td>A. hygrometricus</td>
<td>16.5±0.05</td>
<td>18.5±0.05</td>
<td>11.5±0.1</td>
<td>17.5±0.25</td>
<td>25±0.1</td>
<td>19.5±0.05</td>
</tr>
<tr>
<td>C. gigantea</td>
<td>14.5±0.05</td>
<td>14.5±0.45</td>
<td>15±0.15</td>
<td>19.5±0.05</td>
<td>18±0.2</td>
<td>16.5±0.55</td>
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<tr>
<td>Erythromycin</td>
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<td>33.5±0.5</td>
<td>40.5±3.5</td>
<td>33.5±3.5</td>
<td>47.5±2.5</td>
<td>39±1</td>
</tr>
<tr>
<td>DMSO</td>
<td>0±0.00</td>
<td>0±0.00</td>
<td>0±0.00</td>
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</tr>
</tbody>
</table>

± 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. 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 less than 30 mg/ml. The results showed that maximum bactericidal 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 15 mg/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. gigantea > 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±3.5 mm zones of inhibition, respectively.

B. subtilis was found as more susceptible bacterial strain showing 20.5±0.45 mm zone of inhibition to
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 possess antibacterial activities. Mau et al. (2002) had reported in their studies that mushrooms possess 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. giganteas* have anticancer properties (Ying et al., 1987). The best evidence supporting medicinal use of *C. giganteas* comes from studies showing that extracts from certain species inhibit bacterial growth. For example, both *Calvatia craniformis* and *Calvatia lilacina* (C. giganteas) 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. giganteas*) 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 furigicideturbenafine except for *C. giganteas* 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. giganteas* 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. giganteas* 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 *Cordyceps militaris*. 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 Suay et al. (2000) that the antibacterial activity of polyopes 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; Papadopoulos 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Linear inhibition of growth (%)</th>
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<tbody>
<tr>
<td></td>
<td><em>A. fumigatus</em></td>
</tr>
<tr>
<td><em>M. esculenta</em></td>
<td>5±0.00</td>
</tr>
<tr>
<td><em>C. gigantea</em></td>
<td>1.5±0.5</td>
</tr>
<tr>
<td><em>A. hygrometricus</em></td>
<td>10±0.00</td>
</tr>
<tr>
<td>Turbenafine</td>
<td>100±0.00</td>
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<td>DMSO</td>
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### 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.
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**


