INTRODUCTION

Kohat lies in the Khyber Pakhtunkhwa Province. It is located at 71°26’29” to the east and 33°35’13” to the North. Kohat includes 3 ecological zones, with winters and summers both being moderate (Shinwari et al., 2011). Ethnobotanical survey of medicinally important plants against diarrheal diseases was conducted in district Kohat. Various studies were conducted regarding the survey of indigenous knowledge about medicinal plants (Shinwari & Gilani, 2003; Deeb et al., 2013), but focus now is on biological screening of the knowledge to prove its validity and to check toxicity of plants on other hand (Gilani et al., 2010).

Many native communities or tribes all over the world still are dependent on their own tribal medicinal practitioners (TMPs) for treatment of both human and livestock diseases (Shinwari et al., 2006; Nadeem et al., 2013). Diarrhea is the third leading cause of death in developing countries (Thapar & Sanderson, 2004). More than 1.8 million people (mostly children under the age of 5 years) died annually because of diarrhea (Anon., 2004). Diarrhea is caused by various bacterial species belonging to the Aeromonas, Cryptosporidium, Campylobacter, Salmonella, Shigella, Escherichia coli etc.

People with poor hygiene, children and adults are at high risk. Diarrhea leads to malnutrition, dehydration, and electrolyte imbalance and may even cause death if untreated (Anon., 1995). Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the therapy of diarrhea but have few side effects. Drugs with lesser side effects should be used (Hardman & Limberd, 1992). The active chemical constituents present in different plant parts proved to be helpful in curing the disease (Mitscher et al., 1980). Shah et al., (2011) have reported effect of plants on diarrhea and antispasmodic activity.

In tropical countries infectious diseases are the number one cause of death (Colegate & Molyneux, 2008). Pharmaceutical companies have made a number of new antibiotics in the past three decades (Nascimentom et al., 2000). Microorganisms have developed resistance to certain antibiotics due to excessive and improper usage. There is a need for a quick resolution of untreatable bacterial infections by finding new infection-fighting approaches (Sieradzki et al., 1999). Chemicals of plant origin are active against both plants and human pathogenic microbes (Mitscher et al., 1987).

To supplement efforts on recognizing importance of wild plants as a major source of healing various diseases and to bridge between humans and infectious diseases (Shinwari, 2010; Shinwari et al., 2012). The research focus on one hand is to correct identification of the resources (Shinwari et al., 2011a), and biological screening of the herbal plants/ medicine on other hand (Shinwari et al., 2009). Present research work aimed at determining the antimicrobial activities of some important traditional medicinal plants which are known to be effective against diarrheal diseases.

MATERIALS AND METHODS

An ethnobotanical survey was conducted in Kohat district to collect information of plants used to cure diarrhea by the local population. After completion of survey, 11 different plant species i.e. Acacia nilotica, Artemisia absinthim, Carum copticum, Cinnamomum zeylanicum, Curcuma longa, Fumaria officinalis, Mentha longifolia, Peganum harmala, Withania somnifera, Woodfordia fruticosa and Carum copticum were selected and collected from different areas of district Kohat and from herbal practitioner.

Preparation of extract: Each plant material in its powdered form was taken in the amount of 5gm and was dissolved in 50ml of solvent. The different solvents used were methanol, ethanol, n-hexane and acetone. The plants were soaked in each solvent for 7 days with stirring the
In vitro antibacterial assay: A total of 7 microbial cultures were examined during present study. Five gram-negative bacterial strains namely Escherichia coli, Shigellasonnei, Salmonella enterica, Klebsiella pneumonia and Yersinia enterocolitica along with 2 gram-positive bacterial strains namely Listeria monocytogenes and Staphylococcus aureus were used to check the antibacterial potential of the plant extracts. The identified microorganisms were obtained from Military Hospital (MH), Rawalpindi. E. coli, S. aureus, Y. enterocolitica, L. monocytogenes were cultured on Nutrient agar whereas S. sonnei, S. enterica, K. pneumonia were cultured on Muller Hinton agar. Above mentioned bacteria were grown on nutrient agar and Muller Hinton agar (MH) at 37ºC and stored in respective slants at 4ºC.

Antibiotic sensitivity test: The method of Gunasegaran et al., (2011) was followed for antibiotic sensitivity test. Muller Hinton medium was used. Three antibiotics i.e., ampicillin, erythromycin and gentamicin disc were used. The surfaces of the media were inoculated using sterile cotton swab. The antibiotics were then placed on culture plates and incubated at 37ºC for 24 hrs. The clear zones of inhibition formed around the zones of inhibition were measured. The sensitivity and resistance of the antibiotics towards isolates were determined.

Antibacterial assay: Antimicrobial assay was performed using agar well diffusion method modified by Parekh & Chanda, (2007). The 24 hrs old culture was used to prepare the inoculum. The inoculums were prepared in 0.9% saline solution. The turbidity of inoculum was adjusted to the McFarland 0.5 turbidity standard. The inoculums of about 100 μl were poured in one of the test tube of all sets. Those test tube sets were then serially two-fold diluted forming the concentrations of 50mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml for methanol, ethanol, acetone, n-hexane extract. Then inoculum was prepared in normal saline and the turbidity of inoculums was adjusted to the McFarland 0.5 turbidity standard. The inoculums of about 100μl was then inoculated into each tube. Then the tubes were incubated at 37ºC for 24 hrs in incubator. MIC was taken as the lowest concentration of the extract at which no growth of the microbes was observed.

Determination of the minimum bactericidal concentration (MBC): Minimum bactericidal concentration (MBC) of the plant extract was determined following method of Spencer & Spencer (2004). The tubes which have low MIC value were sub-cultured on fresh nutrient agar medium or Muller Hinton agar medium according to the type of bacterial strain used. The plates were then incubated at 37ºC for 24 hrs. The MBC was taken as the lowest concentration of the extract at which there is no growth on medium.

Results

The results of ethnobotanical survey, conducted in Kohat district that 11 plants are being used by the local population to cure diarrhea are given in Table 1.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Local name</th>
<th>Family</th>
<th>Part used</th>
<th>Ethnomedicinal uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia nilotica</td>
<td>Kikar</td>
<td>Mimosaceae</td>
<td>Pods</td>
<td>Pods of A. niloticae used in inflammatory condition of the respiratory, digestive and urinary tract, and is useful vomiting, diarrhea and dysentery</td>
</tr>
<tr>
<td>Artemisia absinthim</td>
<td>Afsanteen</td>
<td>Asteraceae</td>
<td>Leaves</td>
<td>Used to expel intestinal worms, indigestion, diarrhea, vomiting, tuberculosis and to stimulate appetite</td>
</tr>
<tr>
<td>Carum copticum</td>
<td>Spaerke</td>
<td>Umbelliferae</td>
<td>Seeds</td>
<td>Used in sore throat, diarrhea, dysentery, flatulence, vomiting and travel sickness</td>
</tr>
<tr>
<td>Cinnamonum zeylanicum</td>
<td>Dalchini</td>
<td>Lauraceae</td>
<td>Dried bark</td>
<td>Used in gastrointestinal disorder, vomiting, dysentery, diarrhea, flu</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>Haldi</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
<td>Used on burns, tonic for skin, arthritis, diarrhea</td>
</tr>
<tr>
<td>Fumaria indica</td>
<td>Shahtera</td>
<td>Fumariaceae</td>
<td>Whole herb</td>
<td>It is used in aches and pains, diarrhea, fever, influenza, vomiting</td>
</tr>
<tr>
<td>Mentha longifolia</td>
<td>Villanay</td>
<td>Lamiaceae</td>
<td>Whole herb</td>
<td>Used in gastrointestinal problems</td>
</tr>
<tr>
<td>Phyllanthus emblica</td>
<td>Amla</td>
<td>Phyllanthaceae</td>
<td>Fruit</td>
<td>As a tonic for hairs. Effective in diarrhea dysentery</td>
</tr>
<tr>
<td>Punica granatum</td>
<td>Anar</td>
<td>Lythraceae</td>
<td>Flowers</td>
<td>Used in diarrhea &amp; dysentery and for gum infection</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Khapyangae</td>
<td>Solanaceae</td>
<td>Leaves</td>
<td>Used in stomach problems &amp; in arthritis</td>
</tr>
<tr>
<td>Woodfordia fruticosa</td>
<td>Dhawai</td>
<td>Lythraceae</td>
<td>Flowers</td>
<td>It is used in skin diseases, anemia, diarrhea, dysentery, ulcers, UTI and jaundice</td>
</tr>
</tbody>
</table>

Table 1. Plants with their nomenclature arranged alphabetically by their plant name, local name, family and parts used for their Ethnomedicinal uses.
Antibiotic sensitivity test: Among the 7 bacterial strains tested for ampicillin, Gentamicin and Erythromycin. Two strains namely E. coli and K. pneumonia were resistant while all the remaining strains were found sensitive for ampicillin. Similarly therest results showed that E. coli, K. pneumonia and S. aureus were resistant to erythromycin. The other strains were more sensitive to erythromycin as compared to other 2 antibiotics. Gentamycin was found to be most effective against S. aureus and L. monocytogenes (Table 2).

<table>
<thead>
<tr>
<th>Antibiotic strain</th>
<th>Ampicillin</th>
<th>Gentamicin</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>23</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>9</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>30</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>30</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>16</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>18</td>
<td>22</td>
<td>24</td>
</tr>
</tbody>
</table>

Antibacterial activity of methanol extract: Methanol extract of above mentioned 11 medicinal plants showed various degrees of inhibition against the tested bacterial strains using the agar well diffusion method. Of the total medicinal plants, only C. coticum (10±0) and P. emblica (9.6±0.4) showed activity against E. coli at a concentration of 50mg/ml. Methanol extract of A. nilotica (15.33±1.3), A. absinthium (13.66±1.86), C. coticum (11±0), F. indica (11±0), P. emblica (17±0) P. granatum (20±4.47) and W. fruticosa (20.66±2.86) were effective against S. aureus. Results of methanol extract of eight medicinal plants active against S. sonnei were C. coticum (14±0), C. longa (12±0), F. indica (13±0), M. longifolia (10.6±0.51), P. emblica (15±0), P. granatum (26±3.22), W. somnifera (9±0), W. fruticosa (22±0). S. enteritidis was sensitive to A. nilotica (13±0), A. absinthium (10±0), C. coticum (11±0), P. emblica (16±0), P. granatum (10±0), W. somnifera (10±0), W. fruticosa (10.66±0.94). K. pneumonia which was sensitive to methanol extract of five plants i.e., A. nilotica (15±0), C. coticum (10±0), M. longifolia (10±0), P. emblica (12±2.16), W. fruticosa (11.66±0.47). Methanol extract of all selected medicinal plants was active against Y. enterocolitica and L. monocytogenes. There zones of inhibition were A. nilotica(8±0.894) and (14±0.89), A. absinthium (11±0.89) and (17.33±2.25), C. coticum (16±0) and (15±0), C. zeylamicum (17±0) and (13±0), C. longa (12.66±0.51) and (15±0), F. indica (11±0) and (10±0), M. longifolia (11±0) and (12.66±1.03), P. emblica (16±0) and (15±0), P. granatum (11±0.89) and (17.33±2.25), W. somnifera (11±0) and (20±0), W. fruticosa (17±2.94) and (17.33±1.24) respectively (Fig. 1).

Results of minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC): The minimum inhibitory concentrations of ethanol extract against all 7 strains were between 3.12-25mg/ml. The MBC of ethanol extract against all 7 strains lies between 6.25-50mg/ml. C. coticum was found to be most effective against strains of K. pneumoniae, Y. enterocolitica and L. monocytogenes. It also showed good activity against S. sonnei and S. enteritidis. Low effect was seen against E. coli and S. aureus. C. longa also showed good activity against S. sonnei (Fig. 4).

Antibacterial activity of acetone extract: Results of acetone extract of 11 medicinal plants tested against 7 bacterial strains revealed that E. coli was not affected by any of the medicinal plants tested except C. coticum that showed low activity against it (1.6±1.03). The gram positive bacterial strain S. aureus was greatly affected by 6 medicinal plants checked. Their zones of inhibition were A. nilotica (21±4.47), A. absinthium (11.33±0.51), C. coticum (11.6±1.03), F. indica (13.66±6.91), P. granatum (19±2.36), W. fruticosa (17±2.68). S. sonnei was highly sensitive to W. fruticosa (30±0) and P. granatum (25). It also moderately affected C. coticum (12±0), C. longa (15±0), P. emblica (13±0), W. somnifera (9±0.89). S. enteritidis was slightly affected by the ethanolic extract of A. absinthium (12.66±1.03), C. coticum (10±0), P. granatum (12.33±2.25), W. fruticosa (13±1.54). K. pneumonia those plants were A. absinthium (8±0), C. coticum (9±0), F. indica (10.66±5.39), W. somnifera (8.33±0.51). Both Y.enterocolitica and L. monocytogenes were sensitive to all of the medicinal plants (Fig. 3).
emblica (8.66±0.51). Y. enterocolitica and L. monocytogenes were sensitive to all of the medicinal plants except for M. longifolia. The zone of inhibition of other plants were A. nilotica (16.66±0.51) and (14.33±1.36), A. absinthium (13.33±0.51) and (16±0), C. copticum (11±0) and (14±0), C. zeylanicum (19±0) and (25±0), C. longa (13±0) and (13.66±0.51), F. indica (11.66±2.58) and (15.33±1.03), P. emblica (12±0.89) and (13±0.89), P. granatum (16.66±0.51) and (17±0), W. somnifera (11±0) and (10±0), W. fruticosa (13±0) and (15±0) (Fig. 5).

Results of minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC): The MIC of acetone extract against all 7 strains lies between 6.25-25mg/ml. The MBC of acetone extract against all 7 strains lies between 6.25-50mg/ml (Fig. 6).

Antibacterial activity of n-hexane extract: Antibacterial activity of n-hexane extract revealed that E. coli was sensitive to 2 plants out of the 11 selected plants, that were C. copticum (8±0), C. zeylanicum (10±0). Four plants were active against S. aureus. Their activity was arranged in ascending order P. emblica (9±0), A. absinthium (12±0), W. fruticosa (12.33±1.36), C. zeylanicum (13±0), F. indica (13.66±1.86). S. sonnei was sensitive to A. absinthium (9±0), M. longifolia (10.3±1.36), P. granatum (13±0). The zone of inhibition formed by C. copticum, F. indica, C. zeylanicum were (10±0), (10±0), (11±0) against S. enteritidis respectively. n-Hexane extract of A. absinthium (10±0), C. zeylanicum (10±0), F. indica (8.66±0.51), P. emblica (9±0), W. fruticosa (12±0.89) were active against K. pneumonia. Apart from C. longa n-hexane extract of all the selected medicinal plants were active against Y. enterocolitica i.e., A. nilotica (10±0), A. absinthium (14.33±0.51), C. copticum (12±0), C. zeylanicum (18±0), F. indica (11.66±1.36), M. longifolia (12±0.89), P. emblica (12±0.89), P. granatum (13.33±2.73), W. somnifera (10±0), W. fruticosa (18.33±1.86) respectively (Fig. 7).
BIOLOGICAL SCREENING OF PLANTS USED IN DIARRHEA TREATMENT

Fig. 3. Zones of inhibition of ethanol extract of medicinal plants against selected bacterial strains at concentration of 50 mg/ml.

Fig. 4. Minimum inhibitory concentrations (MIC) & Minimum bactericidal concentrations (MBC) of ethanol extract of medicinal plants. Legend: A. nilotica (A. n), A. absinthim (A. a), C. copticum (C. c), C. zeylanicum (C. z), C. longa (C. l), F. indica (F. i), M. longifolia (M. l), P. emblica (P. e), P. granatum (P. g), W. somnifera (W. s), W. fruticosa (W. f).

Results of minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC): The MIC of n-hexane extract against all 7 strains lies between 6.25-25mg/ml. The MBC of n-hexane extract against all 7 strains lies between 12.5-50mg/ml (Fig. 8).

Discussion

Current study was based on 44 different extracts from 11 medicinal plants. These plants are traditionally used against diarrhea. Aim of our research work was to determine antibacterial activities of these plants.

The antibiotic sensitivity test showed that E. coli was resistant to erythromycin and ampicillin, and was sensitive to gentamicin. The methanol extract of all the studied plants didn't inhibit E. coli except C. copticum and P. emblica. Selvamohan et al., (2012) reported similar activity of methanol extract of P. emblica against E. coli. S. aureus was found to be resistant against erythromycin and sensitive to ampicillin and gentamicin. The methanol and ethanol extract of A. nilotica showed very good inhibition. This result was in agreement with the result of Mahesh & Satish, (2008). They reported similar activity of methanol extract of leaves of A. nilotica against S. aureus. Among all plants, methanol extract of W. fruticosa exhibited the highest activity against S. aureus. Kumaraswamy et al., (2008) also found similar results. Raghu & Ravindra, (2010) separately reported similar activity of methanol extract of P. emblica. The lowest activity was exhibited by acetone extract of F. indica. The n-hexane extract of the plant that showed maximum inhibition against S. aureus was F. indica.
**Acetone Extracts**

**Fig. 5.** Zones of inhibition of acetone extract of medicinal plants against selected bacterial strains at 50 mg/ml.

**Fig. 6.** Minimum inhibitory concentrations (MIC) & Minimum bactericidal concentrations (MBC) of acetone extract of medicinal plants.


*S. sonnei* was found to be sensitive to all the tested antibiotics. Methanol extract of *P. granatum* showed maximum activity against *S. sonnei* followed by *W. fruticosa*. Ethanol extract of 7 plants were also active against *S. sonnei*. The highest zone was formed by *W. fruticosa*, followed by *P. granatum*. Acetone extract of 8 plants exhibited activity against *S. sonnei*. *S. enteritidis* was sensitive to erythromycin, ampicillin and gentamicin. The methanol extract of all plants checked were active against *S. enteritidis*. Methanol extract of *P. emblica* showed highest zone of inhibition against *S. enteritidis*. Ethanol extract of 5 plants were inhibiting growth of *S. enteritidis*. Highest zone of inhibition was found by *W. fruticosa*. The n-hexane extract of only three plants was active against *S. enteritidis*. The highest zone of inhibition in case of n-hexane extract was exhibited by *C. zeylanicum* followed by *C. copticum* and *F. indica*. *K. pneumoniae* was resistant to ampicillin and erythromycin but was found to be sensitive to gentamycin. Methanol extracts of 5 plants were active against *K. pneumoniae*. Maximum inhibition were done by *A. nilotica* followed by *P. emblica* and *W. fruticosa*. The n-hexane extract of *P. granatum* was inactive against *K. pneumoniae* and similar results were found by Malik *et al.*, (2010). *Y. enterocolitica* and *L. monocytogenes* were found to be sensitive to all three antibiotics i.e., erythromycin, gentamycin and ampicillin. Extracts of almost all plants in all solvents were active against *Y. enterocolitica* and *L. monocytogenes*. Therefore it is suggested that these two microorganisms can be treated by using these plants. Moreover results of present study suggests that these medicinal plants need further investigations as they could be a potential source of antimicrobial agents for drug formulation.
Conclusion

From present study it was concluded that the medicinal plants selected through ethnobotanical survey prove to be effective against the selected strains of diarrheal. Methanol extracts of W. fruticosa, P. granatum, P. emblica, A. nilotica, C. coticum showed good results as compare to other solvent used in the study. It proved that some plants in different crude extracts have ability to form higher zone of inhibition. So such plants could be helpful in discovery of antidiarrheal agents. From the comparative analysis of 4 solvents (methanol, ethanol, acetone, n-hexane) used for antibacterial activity. It can be concluded that there might be some compounds in the medicinal plants that were extracted by one or the other solvent hence showing different activities.

Acknowledgements

We are indebted to the Higher Education Commission for funding the project under Pak-US program.

References


(Received for publication 6 April 2012)