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BIOLOGICAL SCREENING OF INDIGENOUS KNOWLEDGE BASED PLANTS USED IN DIARRHEALTREATMENT

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Abstract

A survey was conducted in Kohat district of Khyber Pakhtoonkhwa of Pakistan to document the plants which are used to treat diarrhea. Based on the survey, 11 medicinal plants were selected (*Acacia nilotica, Artemisia absinthim, Carumcopticum, Cinnamomumzeylanicum, Curcuma longa, Fumariaindica, Menthalongifolia, Phyllanthsemblica, Punicagranatum, Withaniasomnifera, Woodfordiafruticosa*). Their antibacterial activity against 7 pathogenic bacterial strains *(Escherichia coli, Staphylococcus aureus, Shigellasonnei, Klebsiellapneumoniae, Salmonella enteritidis*, *Yersinia enterocolitica, Listeria monocytogenes*) causing diarrhea was checked. Forty four crude extracts at concentration of 50 mg/ml were used for i*n vitro* antibacterial activity by agar well diffusion method. All of the crude extracts were found to inhibit one or the other bacterial strain examined. Minimum inhibitory concentration (MIC) was determined against susceptible bacterial strains. The (MIC) of the extracts against all tested bacterial strains ranged from 3.12 to 25 mg/ml. Minimum bactericidal concentration (MBC) was then determined for extracts with positive results for MIC that ranged between 6.25 to 50 mg/ml. *Woodfordiafruticosa, Punicagranatumand Carumcopticum* were found to be potential candidates for development of drugs for diarrhea.

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 include 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). The 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, Carumcopticum, Cinnamomumzeylanicum, Curcuma longa, Fumariaindica, Menthalongifolia, Phyllanthsemblica, Punicagranatum, Withaniasomnifera, Woodfordiafruticosa*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

plant material twice a day. Then the plant extract was filtered using Wattman No. 1 filter paper. The filtrate was then rotary evaporated, to separate the solvent and finally got the thick plant extract. Then the extract was then left for a few days to let all the solvent evaporated. The dry extract was dissolved in DMSO to get a concentration of 50 mg/ml, stored in capped vials at 4ºC.

***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 with 0.5 McFarland turbidity standards (1.5 x 108 cfu/ml).The Mueller Hinton agar plates were inoculated using the method of swabbing. The sterilized cotton swab was dipped in prepared inoculum. The cotton swab was streaked evenly. Then 6 mm wells were punched on a petri plate using sterile cork borer. 80µl of each plant extract was poured in their respective wells. The plates were incubated at 37ºC for 24 hrs. Then the zone of inhibition in mm was measured.

Determination of minimum inhibitory concentration (MIC): The method of Akinpelu & Kolawale (2004) was followed for the evaluation of minimum inhibitory concentration (MIC). For the determination of MIC, 2 ml TSA was taken in sixteen sterilized test tubes. These test tubes were then divided into four sets each containing four test tubes. Plant extract (50 mg/ml) was 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 37°C 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 1. Plants with their nomenclature arranged alphabetically by their plant name, local name, family and parts used for their Ethnomedicinal uses.

Plant name	Local name	Family	Part used	Ethnomedicinal uses
Acacia nilotica	Kikar	Mimosaceae	Pods	Pods of *A. nilotica*are used in inflammatory condition of the respiratory, digestive and urinary tract, and is useful vomiting, diarrhea and dysentery
Artemisia absinthim	Afsanteen	Asteraceae	Leaves	Used to expel intestinal worms, indigestion, diarrhea, vomiting, tuberculosis and to stimulate appetite
Carumcopticum	Spaerkae	Umbelliferae	Seeds	Used in sore throat, diarrhea, dysentery, flatulence, vomiting and travel sickness
Cinnamomumzeylanicum	Dalchini	Lauraceae	Dried bark	Used in gastrointestinal disorder, vomiting, dysentery, diarrhea, flu
Curcuma longa	Haldi	Zingiberaceae	Rhizome	Used on burns, tonic for skin, arthritis, diarrhea
Fumaria indica	Shahtera	Fumaricaceae	Whole herb	It is used in aches and pains, diarrhea, fever, influenza, vomiting
Menthalongifolia	Villanay	Lamiaceae	Whole herb	Used in gastrointestinal problems
Phyllanthsemblica	Amla	Phyllanthaceae	Fruit	As a tonic for hairs. Effective in diarrhea dysentery
Punicagranatum	Anar	Lythraceae	Flowers	Used in diarrhea& dysentery and for gum infection
Withaniasomnifera	Khapyangaea	Solanaceae	Leaves	Used in stomach problems & in arthritis
Woodfordiafruticosa	Dhawai	Lythraceae	Flowers	It is used in skin diseases, anemia, diarrhea, dysentery, ulcers, UTI and jaundice

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 theresults 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 2. Antibiotic sensitivity of tested isolates (mg/ml).

Antibiotic strain	*Ampicillin*	*Gentamicin*	*Erythromycin*
	Zone of inhibition (mm)		
Escherichia coli	0	10	0
Staphylococcus aureus	23	24	0
Shigellasonnei	9	18	12
Salmonella enteritidis	30	20	10
Klebsiella pneumonia	30	9	0
Yersinia enterocolitica	16	20	24
Listeria monocytogenes	18	22	24

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. copticum* (10±0) and *P. emblica* (9.66±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. copticum* (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. copticum* (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. copticum* (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. copticum* (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. entercolitica* 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. copticum (*16±0) and (15±0), *C. zeylanicum* (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 medicinal plants that were susceptible to bacterial strains and were analyzed for MIC. The MIC of methanol extract against all 7 strains lies between 3.12-25 mg/ml. *C. copticum* was found to be most effective against strains of *K. pneumonae.* It also showed medium results against *S. sonnei, Y. enterocolitica* and *L. monocytogens.* And low effect was seen against *E. coli, S. aureus* and *S. enteritidis.* The MBC of methanol extract against all 7 strains lies between 12.5-50 mg/ml (Fig. 2).

Antibacterial activity of ethanol extract: The ethanol extract of 11 medicinal plants against 7 bacterial strains revealed that *E. coli*was not affected by any of the medicinal plants tested except *C. copticum* 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. copticum* (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. copticum* (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. copticum*(10±0), *P. granatum* (12.33±2.25), *W. fruticosa* (13±1.54). *K. pneumonia* those plants were *A. absinthium* (8±0), *C. copticum* (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).

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. copticum* was found to be most effective against strains of *K. pneumonae, Y. enterocolitica* and *L. monocytogens.* 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 resistant to all medicinal plants tested. *S. aureus* was sensitive to 4 plants. The maximum zone formed against *S. aureus* by *P. emblica* (18±1.78), followed by *A. nilotica* (17.66±1.36), then *P. granatum* (14.66±1.03) and *F. indica* (8.66±1.36). *A. absinthium* (14±0), *C. copticum* (11±0), *C. longa* (12±0), *F. indica* (10±0), *M. longifolia* (10.3±1.36), *P. emblica* (16±3.22), *P. granatum* (26.6±2.58), *W. fruticosa* (12±0) were active against *S. sonnie*. The gram negative bacteria *S. enteritidis* was sensitive to four plants, its zone of inhibition with respective medicinal plants arranged in descending order: *P. granatum* (16.66±1.36), *P. emblica* (13.66±0.51), *C. copticum* (10±0), *W. fruticosa* (10±0). *K. pneumoniae* was sensitive to ethanolic extract of *F. indica* (11.66±2.58), *P.*

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. pnuemonia*. 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).

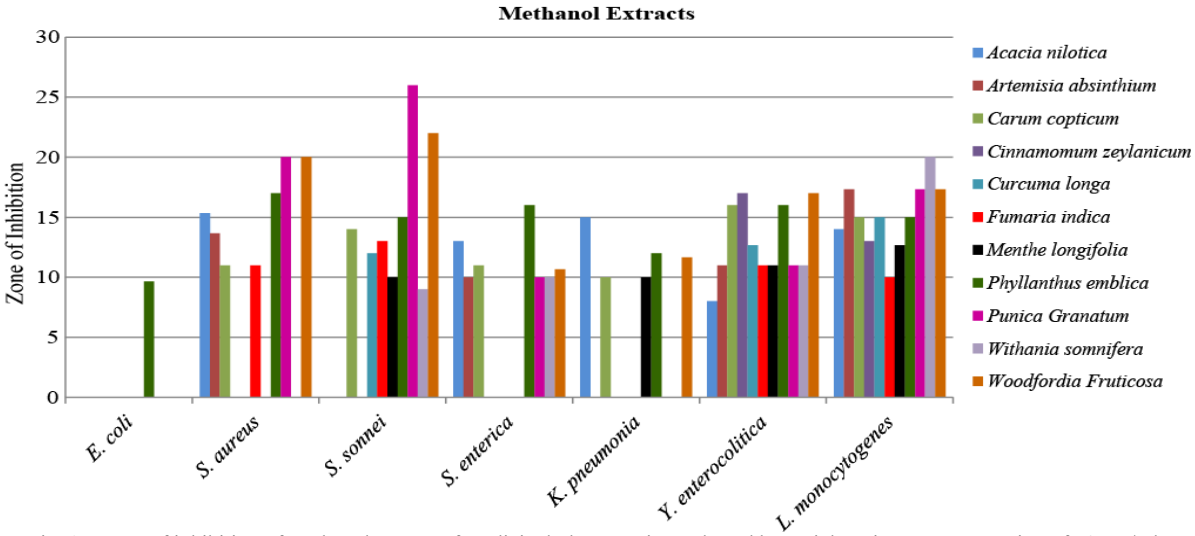

Fig. 1. Zones of inhibition of methanol extract of medicinal plants against selected bacterial strains at concentration of 50 mg/ml.

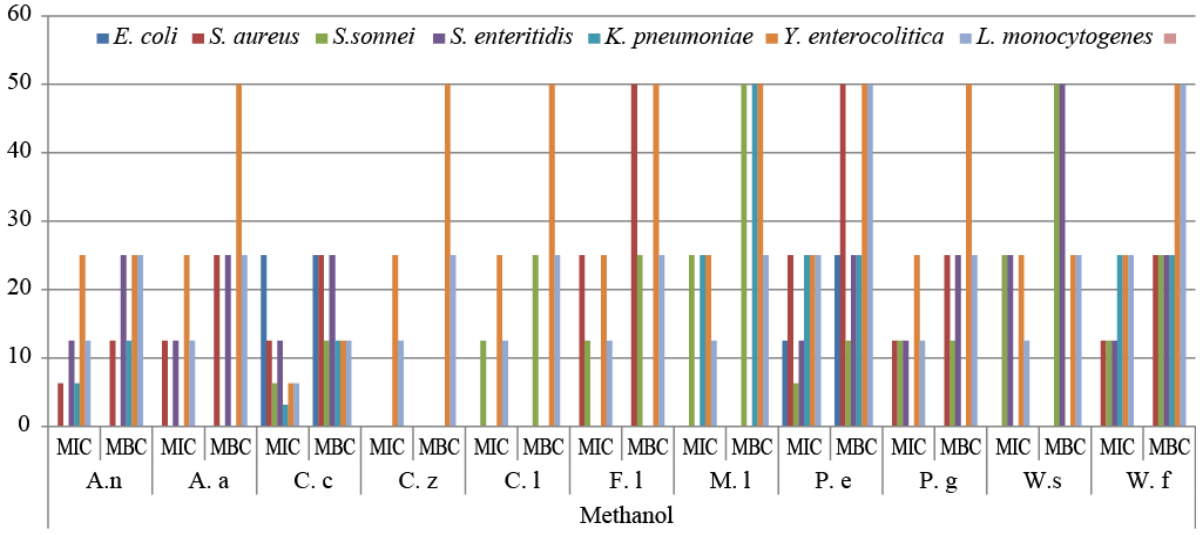

Fig. 2. Minimum inhibitory concentrations (MIC) & Minimum bactericidal concentrations (MBC) of methanol extract of medicinal plants.
Legend: *A. nilotica (A. n), A. absinthim (A. a), C. copticum (C. c), C. zeylanicum (C. z), C. longa (C. l), F. indica (F. i), M. longifolia (M. l), P. emblica (P. e), P. granatum (P. g), W. somnifera (W. s), W. fruticosa (W. f).*

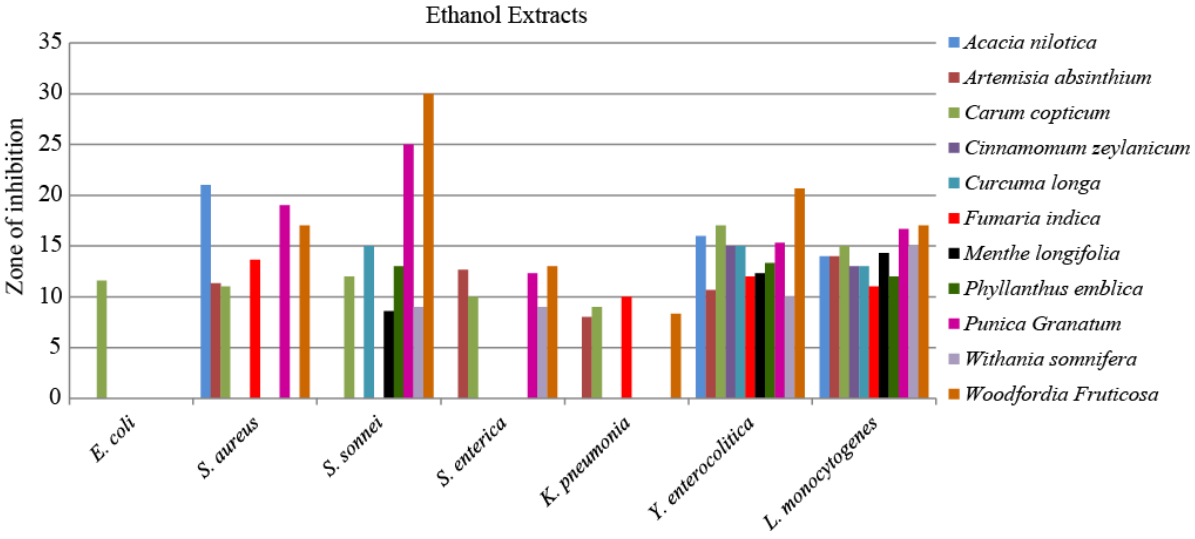

Fig. 3. Zones of inhibition of ethanol extract of medicinal plants against selected bacterial strains at concentration of 50 mg/ml.

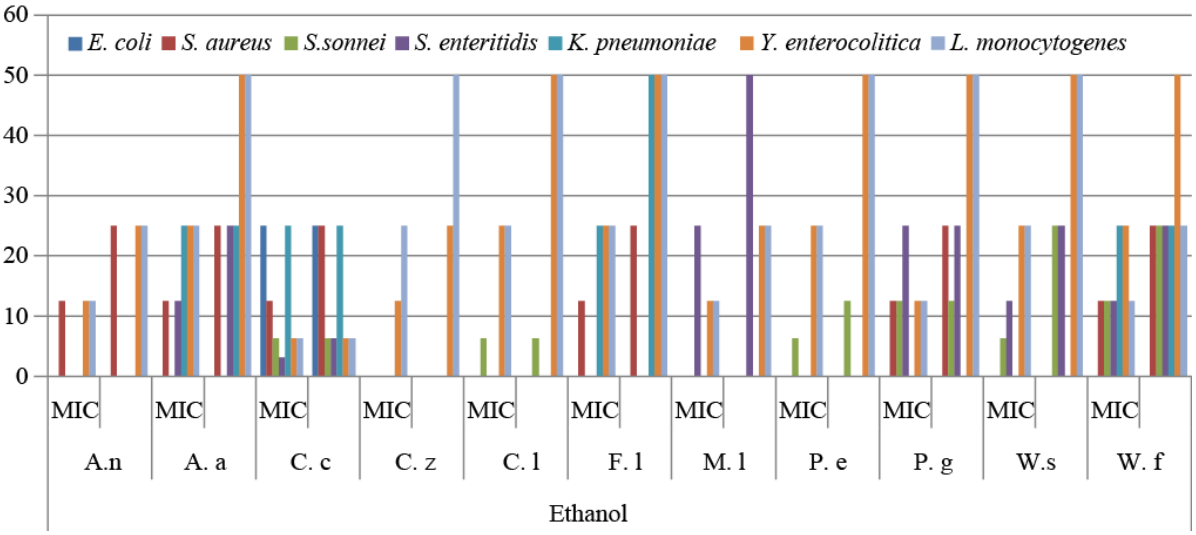

Fig. 4. Minimum inhibitory concentrations (MIC) & Minimum bactericidal concentrations (MBC) of ethanol extract of medicinal plants. Legend: *A. nilotica (A. n), A. absinthim (A. a), C. copticum (C. c), C. zeylanicum (C. z), C. longa (C. l), F. indica (F. i), M. longifolia (M. l), P. emblica (P. e), P.granatum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

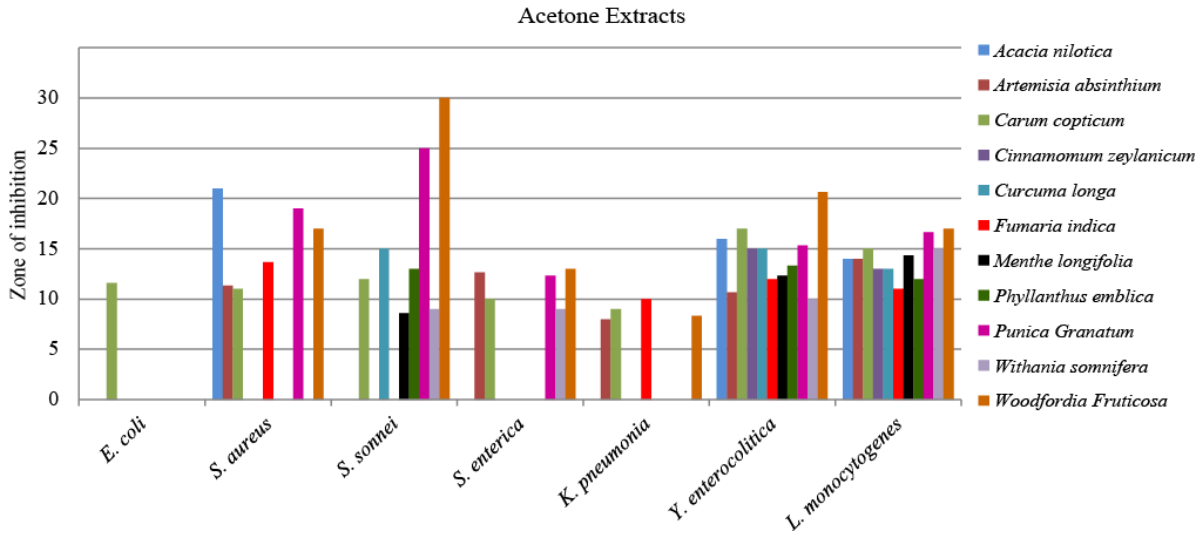

Fig . 5. Zones of inhibition of acetone extract of medicinal plants against selected bacterial strains at 50 mg/ml.

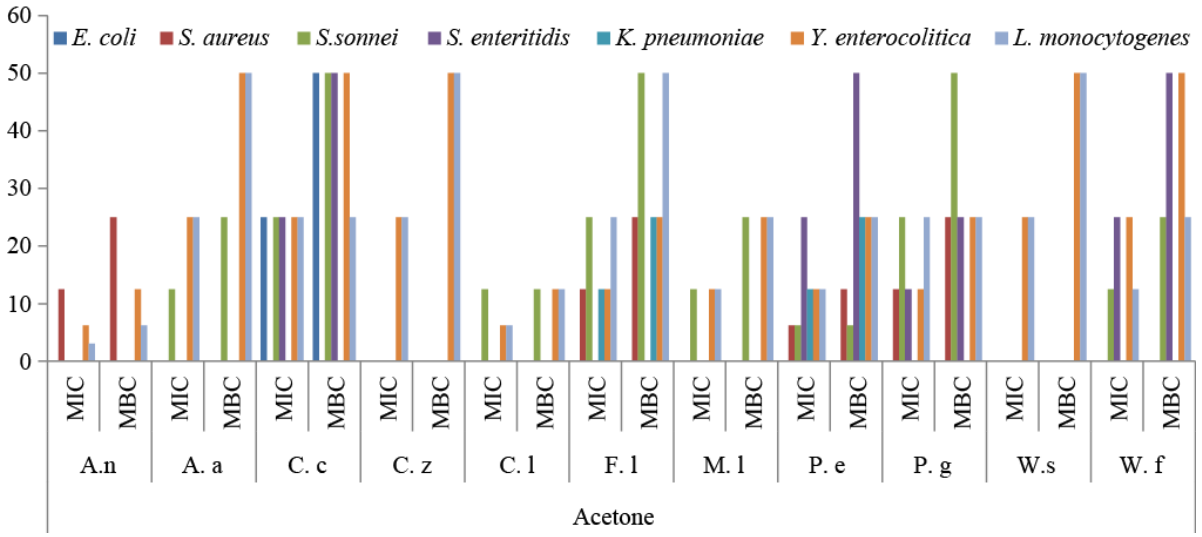

Fig. 6. Minimum inhibitory concentrations (MIC) & Minimum bactericidal concentrations (MBC) of acetone extract of medicinal plants. Legend: *A. nilotica (A. n), A. absinthim (A. a), C. copticum (C. c), C. zeylanicum (C. z), C. longa (C. l), F. indica (F. i), M. longifolia (M. l), P. emblica (P. e), P.granatum (P. g), W. somnifera (W. s), W. fruticosa (W. f).*

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. granitum.* 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. pneumonia* 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.

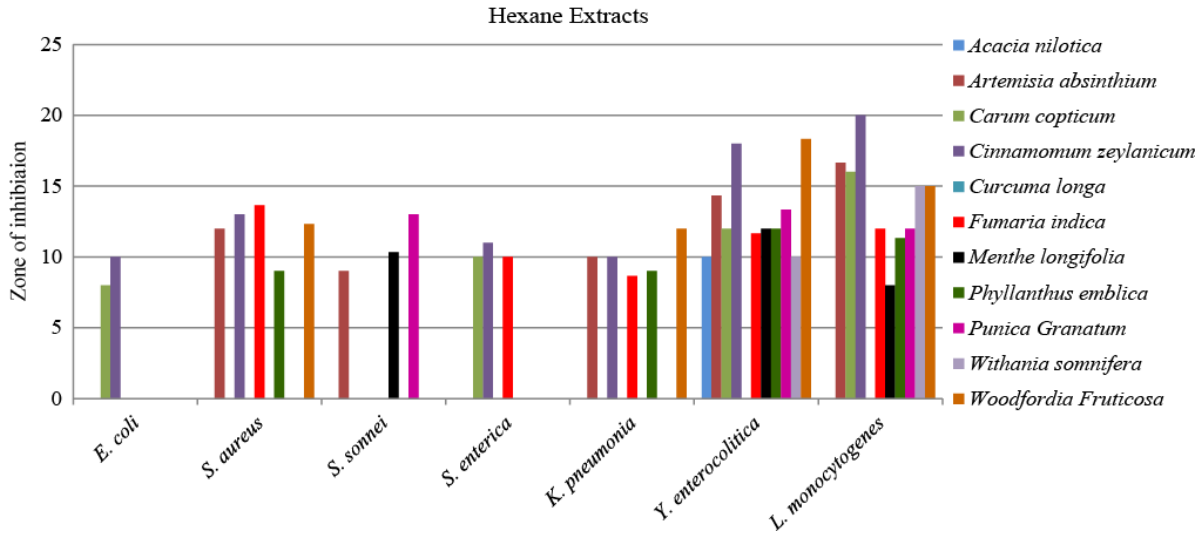

Fig. 7. Zones of inhibition of n-hexane extract of medicinal plants against selected bacterial strains at 50 mg/ml.

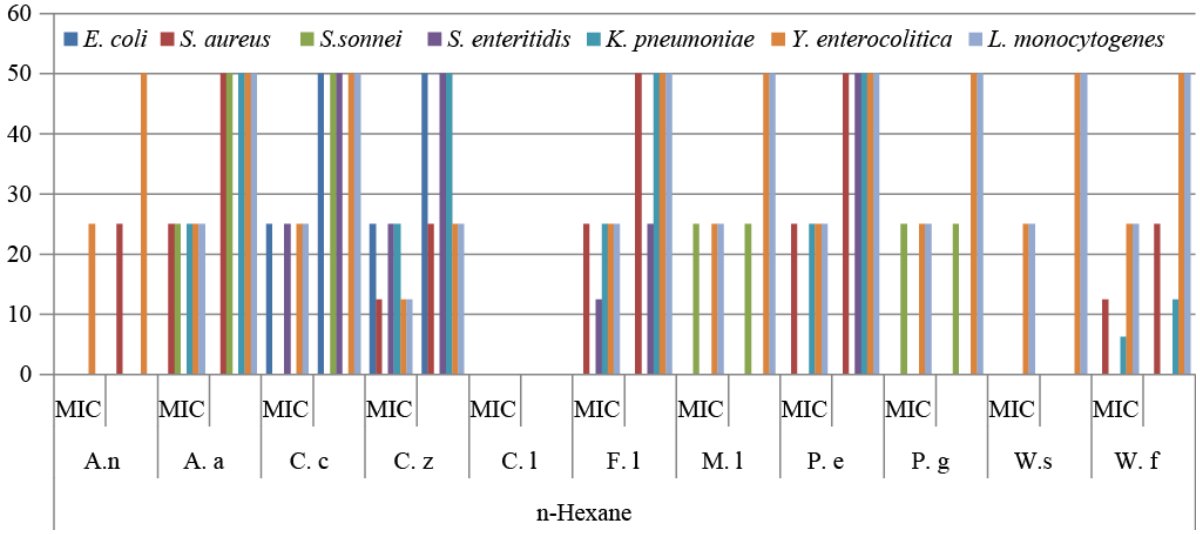

Fig. 8. Minimum inhibitory concentrations (MIC) & Minimum bactericidal concentrations (MBC) of n-hexane extract of medicinal plants. Legend: *A. nilotica (A. n), A. absinthim (A. a), C. copticum (C. c), C. zeylanicum (C. z), C. longa (C. l), F. indica (F. i), M. longifolia (M. l), P. emblica (P. e), P.granatum (P. g), W. somnifera (W. s), W. fruticosa (W. f).*

Conclusion

From present study it was concluded that the medicinal plants selected through ethnobotanical survey prove to be effective against the selected strains of diarrhea. Methanol extracts of *W. fruticosa, P. granitum, P. emblica, A. nilotica, C. copticum* 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.

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