Antibacterial Activity of Mother Tinctures of Cholistan Desert Plants in Pakistan

M. AHMAD, NAZIA GHAFOOR AND M. N. AAMIR[1]
Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur-63100, [1]Faculty of Pharmacy, University of Central Panjab, Lahore-54000, Pakistan

Ahmad, *et al*.: Antibacterial Activity of Cholistan Desert Plants

The mother tinctures of desert were screened for antibacterial activity against bacterial strains of Gram-positive and Gram-negative bacteria. Mother tinctures were prepared by maceration process and antibacterial activity of different plants was evaluated and compared by measuring their zones of inhibition. The results indicated that *Boerrhavia diffusa* mother tincture had excellent activity only against *Escherichia coli*. Mother tincture of *Chorozophora plicata* showed highly effective results against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* whereas *Echinops echinatus* mother tincture showed highly effectiveness only against *Salmonella typhi*. *Heliotropium europaeum* mother tincture exhibited highly effective results against *Bacillus subtilis* in all concentrations. *Tamrix aphylla* presented maximum activity only against *Bacillus subtilis* in all three concentrations. Among the selected species *Heliotropium europaeum*, *Chorozophora plicata* and *Tamrix aphylla* were more effective plants against many microorganisms. However, *Boerrhavia diffusa* and *Echinops echinatus* were less effective plants against tested pathogenic bacteria.

Key words: Antibacterial activity, Cholistan desert plants, homoeopathic mother tinctures

***Address for correspondence**
E-mail: mna19bwp@yahoo.com

Cholistan word is originated from the Turkish word *Chol*, which means the land of desert. Cholistan desert locally known as Rohi desert that surrounds near about 30 km from Bahawalpur, Punjab, Pakistan and covering an area of 26,000 km^2 which is full of resources of medicinal plants. Its length is 480 km and 32-192 km varying breadth[1]. Cholistan desert is uniquely located wild land of its own kind with scarcity of endemic flora, containing only 128 species belonging to 32 families. The specialized medicinal knowledge of plants is not common, but confined to the local specialist/herbalist called '*hakims*'. They offer human and animal disease treatments by providing herbal decoction, pellets, fresh, crushed or dried[2].

The purpose of this study was to determine the effectiveness of selected medicinal plants (mother tinctures) for the control of growth of selected pathogen as well as nonpathogen bacteria. This was an attempt to find a potential source of more active antibacterial agent and was great applications in homeopathic field. Most of the plants are used to treat various ailments associated with fever in Cholistan desert and adjoining area. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and antiinflammatory properties of plants[3-8] but the Cholistan desert have not been subjected to scientific investigation for their efficacy and safety. Therefore present study was designed: (1) To validate claim by traditional herbal practitioners about the effectiveness of local plants against various ailments, the study was designed to evaluate the antibacterial activity of five selected medicinal plants, (2) the general objective is to determine antibacterial property of homoeopathic mother tinctures against six common bacteria, (3) to determine which medicinal plant has maximum antibacterial activity against Gram-positive or Gram-negative bacteria, (4) to compare antibacterial property of selected plants to ciprofloxacin against Gram-positive and Gram-negative bacteria.

For the screening of antibacterial activity the arial parts of five medicinal plants, *Boerrhavia diffusa* (*Itsit*), *Chorozophora plicata* (*Neel Kanth*), *Echinops echinatus* (*Unt-kantalo*), *Heliotropium europium* (*Gidhar tambakoo*) and *Tamrix aphylla* (*Lao*), were collected from different areas of Cholistan Desert and they were identified at Cholistan Institute of Desert Studies.

The collected plant samples were crushed and dried in open air under shade for 15 days and milled to fine powder in an electric grinder and stored in a well closed container. To prepare homoeopathic mother tinctures 100 g of each powdered plant material was soaked in 900 ml of 70% ethanol[9,10]. After 15 days, the soaked material of each plant was filtered and mother tincture was preserved. For the essay of antibacterial activity, two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and four Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella sonnei*) pathogenic bacterial strains were selected. To avoid the strain to go to the death phase, the inoculated broth media was shifted to shaking water bath adjusted at 37° for about 24 h. A 0.5 ml inoculated broth media was poured into petri plates and then 15 ml of nutrient agar were poured in each of them[11,12]. After solidifying, sterile sharp end cork borer (8 mm diameter) was used to bore three wells in petri plates labelled with the name of bacterial strains along with a positive standard ciprofloxacin as control[13,14]. With the help of micropipette the wells were filled with three different doses of plants mother tinctures (200, 400 and 800 µg/ml) and a standard dose of ciprofloxacin and finally incubated for 24 h at 37°. After all dishes were observed for zones of inhibition and the diameters of these zones were measured. The experiments were done in triplicate and the values obtained were statistically analysed by using Statistical Package for the Social Sciences (SPSS, IBM Corporation, USA) software[15].

B. diffusa mother tincture obtained by maceration method showed moderate activity against *S. aureus*, *P. aeruginosa* and *S. sonnei* and less activity was observed against *E. coli*, whereas *B. subtilis* and *S. typhi* were found resistant at 200 µg/ml concentration. When dose was increased to 400 µg/ml, it was highly effective against *E. coli* and still showed moderate activity against *S. aureus*, *P. aeruginosa* and *S. sonnei* and was ineffective against *B. subtilis* and *S. typhi* bacterial strains. However, at 800 µg/ml concentration, mother tincture was highly effective against *E. coli*; moderate activity was shown against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *S. sonnei*; less activity was found in *S. typhi* microorganism (Table 1). The zone of inhibition in the range of 20-22 mm was considered moderate and more than 25 mm

was considered highly effective when compared to ciprofloxacin.

The data of antibacterial activity of *C. plicata* mother tincture are given in Table 1. Results showed its moderate activity against *S. aureus*, *P. aeruginosa* and *E. coil*; less activity was observed against *B. subtilis*, *S. typhi* and ineffective against *S. sonnei* bacterial strain at 200 µg/ml concentration. When dose of mother tincture was increased to 400 µg/ml, moderate activity was observed against *S. aureus*, *P. aeruginosa*, *S. typhi* and *E. coli*; less activity was found against *B. subtilis* and *S. sonnei* bacterial strains. However, at 800 µg/ml concentration, maximum antibacterial activity was observed against *S. aureus*, *P. aeruginosa* and *E. coli* and moderate activity was observed against *B. subtilis*, *S. typhi* and *S. sonnei* bacterial strains.

E. echinatus mother tincture was highly effective against *S. typhi*, moderate activity was observed in *E. coli and* less activity was found against *P. aeruginosa* microorganism; data revealed *that E. echinatus* mother tincture was ineffective against *B. subtilis*, *S. aureus* and *S. sonnei* at 200 µg/ml mother tincture concentration. When dose of mother tincture was icreased to 400 µg/ml, highly effectiveness against *S. typhi* was obsered and moderate activity was again noted against *E. coli* and less activity was found against *P. aeruginosa*. However, at 800 µg/ml concentration mother tincture was highly effective against *S. typhi*; moderate activity was shown against *P. aeruginosa* and *E. coli*; less activity was found against *B. subtilis* and *S. sonnei* compared to standard used.

Results of antibacterial activity of *H. europaeum* mother tincture are given in Table 1. The data showed that it was highly effective against *B. subtilis*, moderately active against *S. typhi* and less active against *S. aureus* and *S. sonnei* microorganism and was ineffective against *P. aeruginosa* and *E. coli* at 200 µg/ml concentration. At 400 µg/ml concentration, it showed high activity only against *B. subtilis;* moderate activity was observed against *S. typhi;* less activity was found against *S. aureus* and *S. sonnei* as well as least activity was recorded against *P. aeruginosa* and *E. coli* bacterial strain. However, at 800 µg/ml concentration, mother tincture was highly effective against two microorganism i.e. *B. subtilis* and *S. typhi*; moderate activity was found against *S. aureus*; less activity was found against *P. aeruginosa* and *S. sonnei* microorganism as well as negligible activity against *E. coli* compared to standard.

Zone of inhibition of *T. aphylla* mother tincture are reported in Table 1. Results did not show high or moderate activity against any bacterial strain but less activity was found against *S. aureus*, *S. typhi* and *E. coil*. however, *B. subtilis*, *P. aeruginosa* and *S. sonnei* were found strongly resistant to *T. aphylla* mother tincture at 200 µg/ml mother tincture concentration. At higher doses of 400 µg/ml, similar results were observed. However, at 800 µg/ml concentration, mother tincture was highly effective against *B. subtilis*

TABLE 1: ZONE OF INHIBITION OF MEDICINAL PLANTS AGAINST DIFFERENT PATHOGENIC BACTERIAL SPECIES

Plants	Dose (µl)	Zones of inhibition (mm)					
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Shigella sonnie*	*Escherichia coli*
Boerrhavia diffusa	200	12.67±6.36	20.00±0.88	20.00±1.15	10.67±5.81	20.00±1.15	18.00±2.00
	400	13.67±5.45	20.33±2.40	21.33±1.85	13.33±0.88	21.00±0.00	25.33±0.33
	800	21.67±1.20	21.00±2.88	22.67±0.66	16.00±0.00	22.33±1.15	25.67±2.30
Chorozophora plicata	200	16.33±0.66	23.67±0.57	23.67±1.85	19.33±2.18	12.67±0.66	21.00±0.00
	400	17.00±1.52	23.67±3.05	23.67±2.08	21.00±0.88	15.33±1.45	23.00±0.66
	800	21.00±0.57	26.33±1.85	26.00±4.58	21.67±057	20.00±2.88	26.33±2.18
Echinops echinatuss	200	Nil	10.67±4.00	18.67±2.84	26.00±0.57	13.00±1.52	23.00±5.85
	400	13.00±1.00	12.33±0.33	19.67±1.20	26.33±0.66	13.67±0.66	23.33±1.85
	800	14.67±2.66	12.67±0.33	20.67±6.00	27.33±2.02	17.33±0.33	24.00±2.08
Heliotropium europaeum	200	25.67±7.88	16.67±8.33	12.67±6.56	20.33±0.33	14.67±1.33	10.00±5.50
	400	26.67±1.33	18.00±2.51	13.00±0.57	20.67±7.88	15.67±1.66	11.33±5.36
	800	30.00±2.64	21.00±2.08	14.67±1.76	26.00±5.50	16.00±5.50	13.00±0.57
Tamarix aphylla	200	11.00±5.85	16.00±1.20	10.67±5.36	16.00±2.66	11.00±0.00	19.33±1.85
	400	15.67±1.33	16.67±2.00	11.33±0.33	16.67±2.30	12.33±0.33	19.67±2.66
	800	25.67±0.33	18.33±6.22	24.00±2.00	19.00±8.32	13.00±0.57	21.00±2.64
Ciprofloxacin (200 mg/100 ml)	200	33.67±0.88	31.33±0.66	34.00±1.52	36.33±0.88	33.33±1.85	34.67±0.88

and *P. aeruginosa*; moderate activity was observed against *E. coli* and least activity was found against *S. aureus* and *S. typhi*. *T. aphylla* mother tincture was found ineffective against *S. sonnei* at all concentrations when compared to standard ciprofloxacin.

These zone of inhibition showed that mother tincture of *B. diffusa* plant has moderate antibacterial activity. Tannins, favonoids, alkaloids and steroids found in number of medicinal plant might account for antibacterial activity[16]. The mother tincture of *B. diffusa* has bioactive agents present that are tannins and alkaloids[17]. The mother tincture of *C. plicata* plant has both highly effective as well as moderate activities. The presence of polyphenolic and favonoids in *C. plicata* might be responsible for effectiveness against pathogenic bacteria[18]. The mother tincture of *E. echinatus* plant exhibited both highly effective and moderate antibacterial activities. Aerial parts of *E. echinatus* plant contain alkaloids, echinopsine, echinopsidine and echinozolinone. Apigenin and its derivatives, echinacin and echinaticin are responsible for antimicrobial activity[19]. These results indicated that mother tincture of *H. europeaum* plant has moderate to highly effective and its antibacterial activities might be due to pyrrolizidine alkaloids. *T. aphylla* plant has moderate and high antibacterial activity against different species. Flavonoids and polyphenolic in arial parts of *T. aphylla* might be responsible for its antibacterial activity.

As compare to all medical plants results of *H. europaeum* showed highly effective activity against three bacterial strains *B. subtilis*, *P. aeruginosa* and *S. typhi*. *C. plicata* exhibited maximum zone of inhibition against three bacterial strains *S. aureus*, *P. aeruginosa* and *E. coli*. Tincture of *B. diffusa* was highly effective only against *E. coli*. Maximum activity of *E. echinatus* was against *S. typhi* bacterial strain. However, *T. aphylla* had shown activity against *B. subtilis* and *P. aeruginosa*. So it is concluded that *H. Europaeum*, *C. plicata* and *T. aphylla* are most effective plants against pathogenic microorganisms. However, *B. diffusa* and *E. echinatus* can also be utilised as antibacterial agents but their activity is comparatively lesser.

ACKNOWLEDGEMENTS

The author thank Dr. Muhammad Arshad, Senior Scientific Officer, Cholistan Institute of Desert Studies for plant authentication.

REFERENCES

1. Chouhan F, Memon FZ, Aziz-Ur-Rehman, Tufail M. Analytical investigation of inorganic nutritive elements grown in Cholistan desert. Nucleus 2000;39:195-9.
2. Arshad M, Akbar G, Rashid S. Wealth of medicinal plants of Cholistan desert, Pakistan. Hamdard Med 2003;4:25-34.
3. Stepanović S, Antić N, Dakić I, Svabić-Vlahović M. *In vitro* antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbiol Res 2003;158:353-7.
4. Bylka W, Szaufer-Hajdrych M, Matławska I, Goślińska O. Antimicrobial activity of isocytisoside and extracts of *Aquilegia vulgaris* L. Lett Appl Microbiol 2004;39:93-7.
5. Behera SK, Misra MK. Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India J Ethnopharmacol 2005;102:319-25.
6. Govindarajan R, Vijayakumar M, Singh M, Rao ChV, Shirwaikar A, Rawat AK, *et al*. Antiulcer and antimicrobial activity of *Anogeissus latifolia*. J Ethnopharmacol 2006;106:57-61.
7. Nagarsekar KS, Nagarsenker MS, Kulkarni SR. Evaluation of Composition and Antimicrobial Activity of Supercritical Fluid Extract of Leaves of *Vitex negundo*. Indian J Pharm Sci 2010;72:641-3.
8. Agnihotri S, Wakode S. Antimicrobial activity of essential oil and various extracts of fruits of greater cardamom. Indian J Pharm Sci 2010;72:657-9.
9. Nandi M. Alcohol concentration in the preparation of mother tinctures of vegetable origin. The example of *Holarrhena antidysenterica*. Homeopathy 2002;91:85-8.
10. Banerjee DD. Textbook of Homeopathic Pharmacy Including Pharmacological Principles for Homeopathic Practice. B. New Delhi, India: Jain Publishers (Pvt.) Ltd.; 2006.
11. Gopanraj G, Dan M, Shiburaj S, Sethuraman MG, George V. Chemical composition and antibacterial activity of the rhizome oil of *Hedychium larsenii*. Acta Pharm 2005;55:315-20.
12. Sayed HH, Abbas HA, Morsi EM, Amr Ael-G, Abdelwahad NA. Antimicrobial activity of some synthesized glucopyranosyl-pyrimidine carbonitrile and fused pyrimidine systems. Acta Pharm 2010;60:479-91.
13. Ghalem BR, Mohamed B. Antimicrobial activity evaluation of the oleoresin oil of *Pistacia vera* L. Afr J Pharm Pharmacol 2009;3:92-6.
14. Das K, Tiwari RK, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plant Res 2010;4:104-11.
15. Tanja M, Slavica S. Antimicrobial activity of the *Hypercum perforatum* plant. Bull Chem Technol Macedonia 2006;25:127-30.
16. Oyedeji OA, Adeniyi BA, Ajayi O, König WA. Essential oil composition of *Piper guineense* and its antimicrobial activity. Another chemotype from Nigeria. Phytother Res 2005;19:362-4.
17. Umamaheswari A, Nuni A, Shreevidya R. Evaluation of antibacterial activity of *Boerhaavia diffusa* L. leaves. Int J Green Pharm 2010;4:75-8.
18. Saha MR, Alam A, Akhter R, Jahangir R. *In vitro* free radical scavenging activity of *Ixora coccinea* L. Banglad J Pharmacol 2008;3:90-6.
19. Bhandari MM. Flora of Indian Desert. Jodhpur, India: MPS Repros Publisher; 1995.

Accepted 22 September 2012
Revised 15 September 2012
Received 30 September 2011
Indian J. Pharm. Sci., 2012, 74 (5): 465-468