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Comparative Study of Antibacterial Activity and Toxicity of Certain Plants used in Unani Medicine

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ABSTRACT
In the present work we evaluated the antibacterial activity of Indian plants used in the Unani system of medicine against the pathogenic bacteria. The aqueous extracts of Tinospora cordifolia (stem), Sisymbrion irio (seeds), Viola odorata (flowers), Glycerrhiza glabra (roots and stolon) and Peristrophe bicalculata (whole plant) showed strong antibacterial activity against Salmonella typhi and Escherichia coli which is comparable to standard antibiotic Tetracycline. The aqueous extracts of these plants were also tested for their toxicity against human erythrocytes. These plant extracts were found to manifest the cellular toxicity to erythrocytes comparable to Tetracycline. The results reveal that the use of aqueous extracts of these plants may be effective in providing an alternative and effective method to eliminate the pathogens without producing much toxicity compared to the present antibiotics.
Key words: *Antimicrobial activity, Erythrocytes, Alternative Medicines*

INTRODUCTION

Antibiotics in modern therapeutic system have tremendous effect in controlling the infectious diseases [1]. However, the advent of escape mechanism (cf. drug resistance) adapted by most of the pathogens certainly needs a suitable replacement of the presently available antibiotics [2,3] . Moreover, many antibacterial and antifungal agents are known to exhibit serious untoward effects on host tissues leading to the system toxicity [4,5,6].

In the present study, five widely claimed crude drugs of Unani system of medicine (Table 1), for those symptoms that mostly appears due to the bacterial infections [7], have been screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Salmonella typhimurium*. The crude drugs were the stems of *Tinospora cordifolia*, seeds of *Sisymbriom irio*, flowers of *Viola odorata*, roots and stolon of *Glycerrhiza glabra* and whole plant *Peristrophe bicalyculata*. The antibacterial activities of these plant extracts were compared with standard antibacterial drug Tetracycline. To evaluate their toxicity the haemolysis assay [8] was performed to study the cellular toxicity of these drugs.

Table 1: Reported Therapeutic Uses of the Medicinal Plants Tested for Present Study

Source	Vernacular Name(Unani)	Claimed Therapeutic Uses
Sisymbrium irio	Khaksi	Antipyretic, Febrifuge and Expectorant
Tinospora cordifolia	Gilo	Antipyretic, Diuretic, Expectorant and Blood purifier
Viola odorata	Gul-e- Banafsha	Antipyretic, Astringent, Demulcent, Febrifuge and diuretic
Peristrophe bicalicutata	Chaksini	Analgesic, Anti-inflammatory, Antidote for Snake poison
Glycyrrhiza glabra	Aslesoos	Analgesic, Diuretic, Laxative, expectorant

MATERIALS AND METHODS

The media used for antibacterial test was Nutrient Broth (0.5% peptone, 0.5% sodium chloride, 0.15% beef extract and 0.15% yeast extract, pH 7.4) from Hi Media Pvt. Ltd. Mumbai, India. Bacteria (*B. subtilis* ATCC 6051, *S. typhimurium* ATCC 23564, and *E.coli* k-12 and *S. aureus* ATCC 9144), obtained from Institute

of Microbial Technology (IMTECH) Chandigarh, were incubated at 37ºC for overnight and diluted to get approximately 10^5 CFU/ml as described earlier [9].

The drugs were procured from the local suppliers and from Dawakhana Tibbiya College, AMU, Aligarh and their botanical identification was confirmed by Dr Shehbaz Ali, Department of Pharmacognosy, AMU, Aligarh. The classified reference vouchers were deposited at the Department of Ilmul Advia, AMU, Aligarh, India.

Preparation of extracts from plants

Plant materials were dried in the shade at room temperature, powdered and extracted by the method mentioned by Alade and Irobi[10]. In the Unani system of medicine, the use of alcoholic or other organic solvents is avoided thus most preferable way of preparing decoction remains the excessive boiling of the drugs with water. This will help in selective release of water-soluble active components in the decoction which makes it less toxic. Moreover, such aqueous extracts can be ingested by oral route and avoid the development of the formulations that become mandatory for the compounds isolated with the use of organic solvents to make them palatable by converting them into water soluble salts.

Determination of antibacterial activity

The antibacterial activity of the aqueous extract of different plants was determined using agar well diffusion method[11]. Wells (8 mm diameter) were punched in the agar. Aqueous solution of different extracts were dispensed in different wells (20 mg/ml) and incubated at 37 ºC for 24 hours. The control wells were loaded with saline (negative control) or Tetracycline (100 µg/ml) was considered as positive control. Blanks containing sterile water (negative control) viz. Tetracycline (100 µg/ml) were run parallel in the same plate. The antibacterial activity was assessed by measuring the zone of inhibition for respective drugs. The relative antibacterial activity of the given extracts was calculated by comparing its zone of inhibition with the standard drugs as shown earlier[9].

Table 2 shows antibacterial activity of various plants extracts compared to Tetracycline.

Determination of the toxicity of extracts to human erythrocytes

The cellular toxicity of the extracts from different plants was determined using published procedure with slight modification [8]. Erythrocytes were isolated from human blood by removing plasma and buffy coat and suspended in phosphate buffer saline (10 mM Phosphate, 150 mM Sodium Chloride) and dispended in sugar tubes (10^{10} cells/500 µl/tube). The serial dilutions of plant extract were made and mixed with erythrocytes keeping final volume 1 ml. The cells were incubated for 1 hr at 37 ºC and finally centrifuged at 1500 rpm for 10 minutes [7]. The lysis of the cells was observed by determining absorbance at 540 nm using colorimeter. The respective dilutions of the plant extracts (without erythrocytes) were completely lysed by treatment with 1% Triton-X100 and determining the absorbance of the released hemoglobin were taken as 100% lysis[7].

RESULTS AND DISCUSSION

The aqueous extract of the selected plants showed antibacterial activity (Table 2). Among them *T. cordifolia* emerged as the most potent antibacterial agent and is the only plant that was successful in inhibition of *S. typhi* and *E. coli*. On comparing with some standard antibiotics, its activity was found to be almost 70% to that of Tetracycline. The seeds of *S. irio* were also active against *E. coli*, *S. aureus* and *B. subtilis*. Surprisingly, it is not effective against *S. typhi*, although, it is one of the most preferable drugs in Unani system of medicine, and widely used for its antipyretic action in typhoid and influenza [7]. Other plants such as *V. odorata* (flower), *G. glabra* (roots) and *P. bicaliculata* (whole plant) were also found to be effective against *E. coli*, *B. subtilis* and *S. aureus*. However, like *S. irio*, they failed to inhibit *S. typhi* even at dose as high as high as 10 mg/ml. Although, *S. irio* was not effective against S. typhi but it showed strong inhibition against *S. aureus*. This certainly favours their indiscriminate use [8,12,13] in the treatment of such bacterial infections where it is usually accompanied by high fever. The effect of *P. bicalyculata* can easily be demonstrated due to its reported effect in inflammation in albino rats[14].

Table 2: Antibacterial Activity of some plants widely used as Antipyretic Agent in indigenous System of Medicine.

Name of the plant	*Percent inhibition*			
	E. coli	*B. subtilis*	*S. aureus*	*S. typhi*
Sysymbriom irio **(Khaksi)**	**66.0±2.4**	**46.0±2.2**	**56.0±1.8**	**00.0**
Tinospora cordifolia **(Gilo)**	**77.0±4.0**	**26.0±2.0**	**36.0±2.6**	**70.0±1.8**
Viola odorata **(Gul-e-Banafsha)**	**44.4±4.2**	**30.0±1.6**	**41.8±2.4**	**00.0**
Peristrophe bicalyculata **(Chaksini)**	**33.8±1.8**	**28.8±1.0**	**40.0±0.8**	**00.0**
Glycerrhiza glabra **(Aslusoos)**	**33.8±0.7**	**30.0±1.0**	**36.8±0.8**	**00.0**

The data represent mean of three different experiments ± S.D

The results show that most of these plant extracts are causing less haemolysis of the erythrocytes (Fig.1). The cellular toxicity results ensure the safe use of these herbal drugs in the treatment of various infectious diseases. *P. bicalyculata* has been found to show the anti-venom property [11, 15] presumably this may be due to protective effect of the drug against the toxicity of venom.

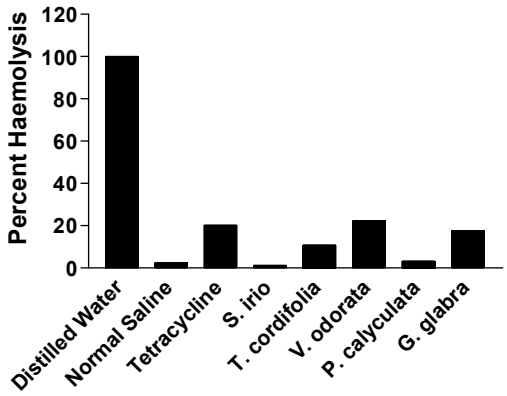

Fig. 1. Cellular toxicity against human erythrocytes

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REFERENCES

1. Finland M;(1978). The New Development in Antibiotics, *Ann. Intern. Med.* 89, 849-853.
2. Threlfall E. J., Frost JA., Ward L.R., and Rowe B.; (1996). Increasing spectrum of Resistance in Multi-resistant *Salmonella typhimurium. Lancet*, 347, 1053-1054.
3. Conly J., Rennie R., Johnson J., Farah S. and Hellman L; 1992Disseminated Candidiasis due to Amphotericin B Resistant *Candida albicans*. J. Infect. Dis., 165, 761-764.
4. Caldenwood S.B., Moellering R.C. (1980).Common adverse Effect of Antibacterial Agent on Major Organ System, *Surg. Clin. N. Am*., 60, 65-81.
5. Marr D.D. Drug induced hepatoxicity. (1982).*Am. J. Nurs.*, 81, 124-126.
6. Bolard J. (1986).How do the polyene Macrolide Antibiotics Affects the Cellular Membrane Properties? *Biochim. Biophys. Acta.,* 864, 257-304.
7. Safiuddin S. (1999).Unani Advia Mufrida, 8th edition, National Council for Urdu, Ministry of Human Resources and Development, Govt. of India, New Delhi, , 37, 82, 142, 243.

8. He, X.G., Mocek, U., Floss, H.G., Caceres, A. Giron, L., Buckley, H., Cooney, G., Manns, J., Wilson, B.W. (1994).Anti-fungal Compounds from *Solanum nigerescens*, *J. Ethnopharmacology*, 43, 173-177.
9. Owais, M., Sharad, K.S., Shehbaz, A., Saleemuddin, M. (2005). Antibacterial efficacy of Withania somnifera (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. *Phytomedicine*, , 12, 229-235.
10. Alade P.I., Irobi O.N. (1993).Antimicrobial Activity of Crude Leaf Extract of Acalypha wilkensiana, *J. Ethnopharmacology* 39, 171-174.
11. Perez C., Pauli M., Bazerques P. (1990). An Antibiotic Assay by Agar Well Diffusion Method, *Acta Biologiae et Medicine Experimentalis*., 15, 113-115.
12. Chopra R.N., Nayer S. L., Chopra I.C.; (1992).Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, pp., 126, 189, 228, 244, 255.
13. D'Souza M.T.; (1993).Tribal Medicine, 1st Society for Promotion of Wasteland Development, Delhi, , 21-7.
14. Sadrul Hoda; Afaq S.H and Tajuddin, (2000).Pharmacological studies of 'Chaksini' *Peristrophe bicalyculata* for Analgesic and Anti-inflammatory Effect on Albino Rat, *Hamdard Medicus,* 33, 4, 40-46.
15. Masker K.S. and Calus J.F.K. (1931). Indian Plant Remedies Used in Snakebite. *Indian Med. Res*. Memo, , 19, 60.