Indian Journal of Science and Technology Vol. 4 No.5 (May 2011) ISSN: 0974- 6846

Activity of commercially available herbal drugs against *Salmonella typhi*

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Abstract

The uses of herbal drugs are getting popularity in the market of India and Pakistan for the treatment of various diseases. In this study, the *Salmonella typhi* (ATCC 19430) susceptibility and sensitivity was evaluated against various herbal drugs. Seventeen herbal brands including liquid and solid dosage forms claimed to treat typhoid were purchased in this study. The serial dilutions of these samples were prepared and it was observed that herbal powder (AY1) sowed activity against *Salmonella typhi* while the rest of samples were without activity. The brands in liquid state were comparatively more active as compared to solid brands and in these samples taifax 120ml syrup (MZ1) made better zone of inhibition against *Salmonella typhi*while typhex plus 60ml (KF3) and temp out syrup (KF4) were less active. Minimum inhibitory concentration of AY1, MZ1 and KF4 brands were observed at 50µg/ml while the rest of the brands at this concentration were inactive. Levofloxacin antibiotic was taken as standard and its inhibitory concentration was 15.62µg/ml. The present study reveals that though herbal drugs are trendy in the market of India and Pakistan for the treatment of typhoid but it is misfortune to say that most of these drugs are not effective. Therefore appropriate measures must be taken against such biologically inactive drugs to secure the society.

Keywords: *Salmonella typhi,* typhoid, herbal drugs, marketed brands.

Introduction

Typhoid fever, a multisystemic disease with protean manifestations is an acute febrile illness caused by *Salmonella typhi,* a gram-negative *Bacillus* able to survive in hostile environments. Common worldwide, it is transmitted by the ingestion of food or water contaminated with feces from an infected person (Giannella RA, 1996). The pathogenic mechanisms of typhoid fever begin with *Bacilli* ingestion. The infecting dose of *Salmonella typhi* needs to be large to produce illness in healthy individuals, varying between 1000 and 1 million microorganisms (Gillespie SH, 2003).Typhoid fever is characterized by a sustained fever as high as 40 °C (104 °F), profuse sweating, gastroenteritis, and non-bloody diarrhea. Enteric fever (typhoid) is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate (WHO, 2003; John *et al*., 2003). The World Health Organization identifies typhoid as a serious public health problem and it has been reported that less commonly a rash of flat, rose-colored spots may appear with an estimated 16-33 million cases of annually resulting in 500,000 to 600,000 deaths in endemic areas, Its incidence is highest in children and young adults between 5 and 19 years old (http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index7.html).In developing countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics and a rapid rise in multidrug resistance. Resistance to the first line drugs chloramphenicol, ciprofloxacin and amoxycillin has been reported (Zulfigar*et al.,* 1994; Benoit *et al.,* 2003).

Herbal Medicine products are becoming increasingly popular in all over the world (Fisher & Ward, 1994; Brevoort, 1998; Eisenberg *et al.,* 1998). An estimated 80% of the world's population still depends on traditional herbal medicines for their health security (Carter, 2001). Herbal medicine is recognized as an important component of health care system, especially among rural dwellers (Esimone*et al*., 2002). Also, the ever increasing cost of orthodox health care services coupled with the side effects of certain synthetic drug therapies, has further caused a large proportion of patients in the developing countries to resort to alternative herbal health care which they feel is natural, safer, more accessible, more economical and takes into consideration the people's socio-cultural values (Nwaogu, 1997; Carter, 2001). The use of herbal drugs is very common for various ailments in India and Pakistan due to sense of safer therapy, easy availability and economical causes.

In order to promote Indian herbal drugs, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO guidelines (WHO, Geneva 2000). Ironically, not many Indian products are available in standardized form, which is the minimum requirement for introducing a product in the Western market (Dubey NK *et al.,* 2004). The aim of this study is to evaluate the biological activity of those commercially available herbal drugs that are claimed to be active against S*almonella typhi*, the causative agent of typhoid.

Material and methods

Samples collection and dilution preparations

Herbal drugs were purchased and collected from herbal stores. All of these samples are marketed as antityphoid drugs. A total of seventeen samples including solid and liquid dosage forms were used in this analysis (Table 1). The serial dilution of each sample was prepared by using dimethyl sulfoxide (DMSO) as solvent. The antityphoid and minimum inhibitory concentration of

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Table 1. Sources of various herbal brands used against typhoid

Serial #	Sample Code	Brand Name	Manufacturer [a]
1	MZ1	Taifax 120ml	KL Sukho District Rawalpindi
2	KF1	Doloex Tab	IR, Hazro, Attock
3	KF2	Malarian capsule	KL Sukho District Rawalpindi
4	KF3	Typhex Plus 60ml	QI (PVT) Ltd, Hattar
5	KF4	Temp Out syrup	IMP P.O. Box 9093 Lahore
6	KF5	Herbal Tablet	USL Hazro, Attock
7	KF6	Herbal Fine Powder (Whitish)	ABY BastiLalaRukhWahCantt
8	KF7	Marvaridi syrup 60ml	AUD Mianwalli
9	IS1	Aagura 130ml	BDK (Herbal) Faisalabad,
10	IS2	Bukharook Tab	AL (PVT) Ltd, Faisalabad
11	AY1	Herbal Powder (Reddish)	ABY BastiLalaRukhWahCantt
12	AY2	Herbal Grains (Dark orange state)	ABY BastiLalaRukhWahCantt
13	AY3	Herbal Grains (Whitish orange state)	ABY BastiLalaRukhWahCantt
14	AY4	Herbal Powder (White)	ABY BastiLalaRukhWahCantt
15	FA1	Bukharin	HL Pakistan
16	FA2	Feveroff	AL (PVT) Ltd, Faisalabad
17	FA3	Bukhareen	QI (PVT) Ltd, Hattar

[a] *KL, Kamal Labs; IR, IROP; QI, Qarshi Industries; IMP, Imran Pharma; USL, Usama Labs; ABY, Al-BadarYounaniDawaKhana; AUD, AltafUnaniDawakhana; BDK, BaraDawaKhana; AL, Ashraf Laboratory; HL, Hamdard Laboratories*

Table 2. Sensitivity of Salmonella typhi against various concentrations of antityphoid herbal drugs available in solid dosage form through agar well diffusion assay

Serial #	Sample Code	Zone of Inhibition (mm) against various concentration (mg/ml)					
		5	10	20	30	40	50
1	KF1	0	0	0	1	1	6
2	KF2	0	0	0	0	0	2
3	KF5	0	0	0	0	0	0
4	KF6	0	0	0	0	0	0
5	IS2	0	0	0	0	0	5
6	AY1	8	12	14	18	22	25
7	AY2	2	2	2	2	4	9
8	AY3	0	0	0	4	4	6
9	AY4	0	0	2	2	5	8
Standard	Levofloxacin	28	32	37	42	48	55

each herbal brand was evaluated. Levofloxacin was used as standard in this study.

Preparation of inoculum

Salmonella typhi (ATCC 19430) was grown to lag phase before inoculated in to nutrient broth medium for activation, the composition of which is as per the Indian Pharmacopoeia (1996) and incubated for 24 hours (Dykes *et al.,* 2003). For the test, 0.1 ml culture of this bacterium was inoculated in nutrient broth giving final cell load of 10^6-10^8 CFU/ml in nutrient broth media (Musumeci *et al.,* 2003; Sohn *et al.,* 2004).

Activity of herbal samples against Salmonella typhi (ATCC 19430)

The agar well diffusion assay was used (Perez C *et al.,* 1990) to test various concentrations of herbal drugs using Mueller Hinton Agar (MHA) media (Prescott *et al.,* 2005) against *Salmonella typhi* ATCC 19430. Mueller Hinton Agar (MHA) plates were seeded with *Salmonella typhi* (ATCC 19430) suspension (1.5×10^8 CFU/mL) using a sterile cotton swab. This tested bacterium suspension was adjusted previously using freshly prepared 0.5 McFarland turbidity standard. Wells were prepared by punching a stainless steel cylinder of 6mm diameter (Patel *et al.,* 2008) into the MHA plates to form wells. Samples of various dilutions were introduced into each well and allowed to stand for 30min at room temperature to diffuse before incubation at 37ºC for 24 hour. Levofloxacin solution was used as standard. The plates were incubated at 37˚C for 24 hours. After incubation, the antityphoid activity was evaluated by measuring the diameter of zone of inhibition for each concentration by using the digital vernier caliper (Model # GT04F037, China).

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of the antibiotic resulting in no growth after 16 to 20 hours of incubation (Prescott *et al.,* 2005) and it was determined using agar diffusion assay method as described by Mendoza, 1998. In this method, prepared inoculum of 0.1ml of *Salmonella typhi* strain was seeded in MHA plates against each sample concentration. A standard solution of Levofloxacin was run simultaneously. All plates were incubated at 37°C.

Table 3. Sensitivity of Salmonella typhi against various concentrations of antityphoid herbal drugs available in liquid dosage form through agar well diffusion assay

Serial #	Sample Code	Zone of Inhibition (mm) against various concentration (serial dilution in terms of %)				
		5%	10%	25%	50%	100%
1	MZ1	15	20	30	34	38
2	KF3	0	0	10	18	27
3	KF4	0	3	12	20	26
4	KF7	0	0	0	0	3
5	IS1	0	0	0	0	0

Results

Herbal drugs activity against Salmonella typhi

The antityphoid activities of herbal drugs through agar well diffusion assay were presented in Table: 2 & 3 by comparing with levofloxacin as standard. Out of 17 brands it was observed that sample AY1 made 25mm of inhibitory zone at 50mg/ml (Fig. 1a) that gradually decreases as the concentration is decreased. Herbal syrups including MZ1 (Fig. 1b), KF3 and KF4 showed best zones at their initial concentrations but there is reduction in the zone diameter as the sample was diluted. Inhibitory zone of 28mm was measured at 5mg/ml against

Indian Journal of Science and Technology Vol. 4 No.5 (May 2011) ISSN: 0974- 6846

Table 4. Minimum inhibitory concentration (MIC) of Herbal drugs against Salmonella typhi available in solid dosage form

Serial #	Sample Code	MIC against *Salmonella typhi* at various concentrations (µg/ml)				
		MIC_{250}	MIC_{125}	$MIC_{62.5}$	$MIC_{31.25}$	$MIC_{15.62}$
1	KF1	+	-	-	-	-
2	KF2	-	-	-	-	-
3	KF5	-	-	-	-	-
4	KF6	-	-	-	-	-
5	IS2	+	-	-	-	-
6	AY1	+++	++	+	-	-
7	AY2	++	+	-	-	-
8	AY3	+	-	-	-	-
9	AY4	++	+	-	-	-
10	Levofloxacin	+++++	+++++	+++++	++++	++++

Indications: - (strain is Resistant, no growth inhibition); + (Resistant, minute inhibition); ++ (Intermediate, slight inhibition); +++, ++++, +++++ (susceptible strain, complete inhibition)

Table 5: Minimum inhibitory concentration (MIC) of Herbal drugs against Salmonella typhi available in liquid dosage form

Serial #	Sample Code	MIC against *Salmonella typhi* at various concentrations (µl) (Serial dilution in terms of percentage)					
		MIC_{100}	MIC_{50}	MIC_{25}	$MIC_{12.5}$	MIC_{5}	MIC_{1}
1	MZ1	++++	+++	+++	+++	++	-
2	KF3	+++	-	-	-	-	-
3	KF4	+++	++	-	-	-	-
4	KF7	-	-	-	-	-	-
5	IS1	-	-	-	-	-	-

Indications: - (strain is Resistant, no growth inhibition); + (Resistant, minute inhibition); ++ (Intermediate, slight inhibition); +++, ++++ (susceptible strain, complete growth inhibition)

Salmonella typhi by using levofloxacin as standard (Fig. 1c). Similarly lesser zones were observed by AY2 (9mm), AY3 (6mm) and AY4 (8mm) respectively at 50mg/ml (Fig. 2a) while the remaining samples were biologically inactive (Fig. 2b).

Fig.1.Zone of inhibitions by (a) AY1, (b) MZ1and (c) Levofloxacin

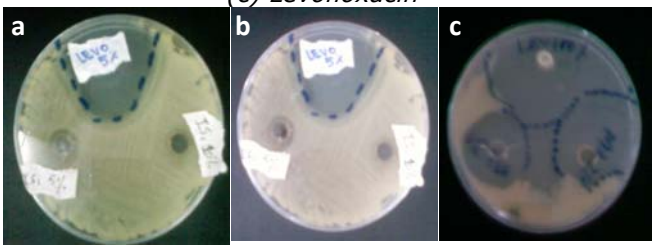
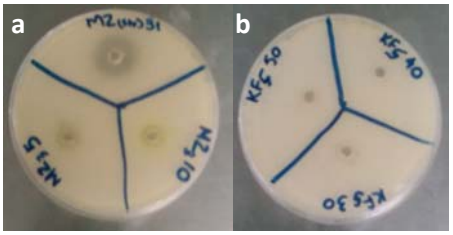


Fig. 2.Zone of inhibitions (a) Slight: AY4, KF4 (b) Nil



Minimum inhibitory concentration (MIC)

On the basis of usual MIC's criteria and finding of the result; *Salmonella typhi* strain (ATCC 19430) was resistant against 57% of the major portion among collected herbal samples (17 samples). Herbal samples including AY2, AY4 and KF3 showed lesser activity while only 21% of the brands showed remarkable activity including AY1 (250µg/ml), MZ1 (100µl) and KF4 (100µl). The MIC of levofloxacin, as standard was observed at 15.62µg/ml (Table 4; Table 5).

Discussion

Typhoid is more common (Malla*et al*., 2005). Therefore, number of drugs for the treatment of typhoid from synthetic to natural has been developed. Plants used in traditional Indian system of medicine have been found active against a wide variety of microorganisms (Khan *et al*., 1994). Many biochemical constituents of plants have been shown to possess excellent biological activities (Gupta *et al.,* 1993) against *Salmonella typhi.* However care must be taken during selection and prescription of antityphoid drugs. Since there are varieties of herbal drugs available in the market, therefore activity is the major concern for the treatment of problem. In this study, we have found that major portion of our drug samples (57%) were inactive against *Salmonella typhi* though they are used as antityphoid drugs. Herbal drugs are considered to be safe and effective due to number of reasons, however the composition of the ingredients, formulation and good manufacturing practices (cGMP) play a vital role that affects the activity of herbal drugs. Biological activities of these brands were measured through agar well diffusion assay and minimum inhibitory concentration (MIC) that showed desperate results against *Salmonella typhi.* The complete inhibition of this bacterium by AY1 (25mm), MZ1 (38mm), KF3 (27mm) and KF4 (27mm) was observed. Levofloxacin as standard was run simultaneously and its minimum inhibitory concentration was 15.62µg/ml while all herbal samples showed no activity at this concentration.

We conclude that such more evolutionary studies are needed to find out the activities of the drugs especially in the developing countries especially in India and Pakistan, so that good therapies can be ensured in time.

References

1. Benoit D, Renand L, Daniele M, Ame B, David B, Michael RM, Elisabeth C and Anel C (2003) Variant Salmonella genomic island 1antibiotic gene resistance cluster in *Salmonella enteric* Albany. *Emerg. Infect. Dis.* 9(5), 585-591.
2. Brevoort P (1998) The booming US Botanical Market. A new overview. *Herbalgram.* 44,33-36.

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3. Carter I (2001) Footsteps: A quarterly newsletter linking development workers around the world. *Tearfund, England,* No. 48, September 3.
4. Christie AB (1980) Typhoid and paratyphoid fevers. In: Infectious diseases: epidemiology and clinical practice.3rd ed. *Christie* AB (ed.). Churchill Livingstone, Edinburgh. pp: 47-102.
5. Corrado ML, DuPont HL, Cooperstock M, Fekety R and Murray DM (1992) Evaluation of new anti-infective drugs for the treatment of typhoid fever. Infectious Diseases Society of America and the Food and Drug Administration.*Clin Infect Dis*. Nov; 15 Suppl. 1. S236-40.
6. Dubey NK, Kumar R and Tripathi P (2004) Global promotion of herbal medicine, India's opportunity. *Cur. Sci*. 86 (1). 37-41
7. Dykes GA, Amarowicz R and Pegg RB (2003) Enhancement of nisin antibacterial activity by a bearberry (*Arctostaphylos uva-ursi*) leaf extract. *Food Microbiol.* 20, 211-216.
8. Eisenberg D, David RB, Ettner SL, Appel S, Van Rompay M and Kessler RC (1998) Trends in alternative medicine use in the United States, 1990-1997. *JAMA.* 280, 1569-1575.
9. Esimone CO, Chah KF and Ikejide SC (2002) Microbiological quality of herbal preparations marketed in Southeast Nigeria. *J. Nat. Remedies.* 2, 42-48.
10. Fisher P and Ward A (1994) Complementary Medicine in Europe. *Br. Med. J*. 309, 107-111.
11. Giannella RA (1996) Salmonella. In: Baron's Medical Microbiology. 4th edition. Baron S *et al.* (eds.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1. http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.section.1221.
12. Gillespie SH (2003) Salmonella infections. In: Manson's Tropical Diseases. 21st ed. Cook GC & Zumla A (eds.) WB Saunders/Elsevier, London. pp: 937-949.
13. Gupta A (1994) Multi-drug-resistant typhoid fever in children: epidemiology and therapeutic approach. *Pediatr. Infect. Dis. J*. 13, 134-140.
14. Gupta S, Yadava JNS and Tandon JS (1993) Antiscretory (antidiarrhoeal) activity of Indian medicinal plants against *Escherichia coli* enterotoxin-induced secretion in rabbit and guinea pig ileal loop models. *Intl. J. Pharmacol.* 31(3), 198-204.
15. Jayshree D Patel, Devang K Patel, AnshuShrivastava and Vipin Kumar (2008) Screening of plant extracts used in traditional antidiarrhoeal medicines against pathogenic *Escherichia coli. Scient. World*. 6 (6), 63-67.
16. John AC, Fouad GY, Stephen P, Maha T, Said AO and Frank JM (2003)Estimating the incidence of typhoid fever and other febrile illnesses indeveloping countries. *Emerg. Infect. Dis.* 9(5), 539-544.
17. Khan MA, Khan T and Ahmad Z (1994) Barks used as source of medicine in Madhya Pradesh, India. *Fitoterapia.* 65(5), 444-446.
18. Lin FY, Vo AH, Phan VB, Nguyen TT, Bryla D, Tran CT, Ha BK *et al* (2000) The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta region of Vietnam. *Am .J. Trop. Med*. Hyg. 62, 644-648.
19. Malla S, Kansakar P, Serichantalergs O, Rahman M and Basnet S (2005) Epidemiology of typhoid and paratyphoid fever in Kathmandu: Two years study and the trends of antimicrobial resistance. *J. Nepal Med. Assoc.* 44(157),18-22.
20. Mendoza MT (1998) What's new in antimicrobial susceptibility testing? *The Philippine J. Microbiol. & Infectious Diseases.* 27(3), 113-115.
21. Mirza SH, Beeching NJ and Hart CA (1996) Multi-drug resistant typhoid: a global problem. *J. Med. Microbiol*. 44, 317-319.
22. Musumeci R, Speciale A, Costanzo R, Annino A, *et al*. (2003) *Berberis aetnensis* C. Presl. extracts: antimicrobial properties and interaction with ciprofloxacin. *Intl. J. Antimicrobial Agents.* 22(1), 48-53.
23. Nwaogu MA (1997) Insight into herbal medicine: An approach to a healthier life. *Aba: Pan Aluminium Co.* Ltd. pp. 1-85.
24. Otegbayo JA, Daramola OO, Onyegbutulem HC, Balogun WF and Oguntoye OO (2003) Retrospective analysis of typhoid fever in atropical tertiary health facility. *Trop.Gastroenterol.* 23,9-12.
25. Perez C, Paul M and Bazerque P (1990) Antibiotic assay by agar-well diffusion method. *Acta Biologiae et Medicine Experimentalis*. 15, 13- 115.
26. Prescott LM, Harley JP and Klein DA (2005) *Microbiology* (Prescott Microbiology) (6th edition). McGrawHill Comp. ISBN-07-111216-2 (ISE). pp: 783-784.
27. Sohn HY, Son KH, Kwon CS, Kwon GS, et al. (2004) Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba* L., *Morus mongolica* Schneider, *Broussnetia papyrifera* (L.) Vent, *Sophora flavescens* Ait and *Echinosophora koreensis* Nakai. *Phytomedicine.* 11(7-8), 666-672.
28. Typhoid Fever. World Health Organization. http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index7.html.Retrieved 2007-08-28.
29. WHO (2003) Manual for the Laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. pp: 103-162.
30. WHO, Geneva (2000) General guidelines for methodologies on research and evaluation of traditional medicine, World Health Organization.
31. Zulfigar A, Tikki P, Bhutta B, Finly B and Altwegg M (1994) Typhoid fever and other salmonellosis, a continuing challenge. *Trends Microbiol*. 3 (7), 253-256.