



Inhibitory effect of medicinal herbs on diarrheagenic bacteria

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INTRODUCTION

Diarrheic diseases represent a problem of great magnitude all over the world, and in particular in developing countries as those of Latin America, resulting in a considerable morbi-mortality.

In some cases of enteric infections caused by bacteria like *E. coli*, *Salmonella* and *Shigella*, antibiotics are prescribed, and it is alarming the strong resistance increase (Murray *et al.*, 2003; Fullá *et al.*, 2005).

The use of medicinal plants with therapeutic purposes is a practice that has been used from immemorial time, constitutes a challenge and it is offered like an alternative therapy. At present there is a great interest in the investigation of the antimicrobial properties. In Argentina presents high diffusion the use of plants in popular medicine, and it is very common the use of herbs for the treatment of diarrheas (Bartotto, 1964).

The aim of this work was to make a report of the popularly used medicinal herbs, and to demonstrate their antimicrobial effects against enteropathogenic bacteria.

METHODOLOGY

Inhibition studies by qualitative methods.

. Herb selection and preparation.

Decoction preparations were performed according to the herboristery indications of: agrimony (AG), cachiyuyo (CA), charrúa (CH), doradilla (DO), duraznillo (DU), holm-oak (HO), guava tree (GT), hamammelis (HA), fig tree (FT), rose petals (RP), oak (O), suico (ITS), blackberry (ZA) (25%) and pomegranate peel (4%).

. Bacterial strains.

S. typhimurium (strain 1), *S. enteritidis* (strain 2), *Sh. flexneri* (strain 3) and *Sh. sonnei* (strain 4), isolated from clinical samples.

. The assays were carried out in vitro by diffusion techniques in plates of Mueller Hinton agar (MH) of 4 mm thickness seeded with a suspension of 10^8

ufc/ml; (National Committee for Clinical Laboratory Standards, 2000) holes were punched and were filled with 50 microl of each of abovementioned preparations and were incubated at 37°C for 24 h. Then, inhibition halos were measured.

To study the inhibition of the different preparation forms, and the stability in the time of pomegranate peel against *Sh. flexneri*, 4 grams were weighed and 100 ml of water were measured and an infusion of 10 minutes was prepared, and decoctions of 1, 5, 10, 15, 20, 25 and 30 minutes, and the activity was assayed until two months of preparation.

Inhibition study by quantitative method.

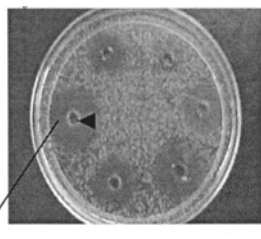
The sensibility of *Shigella flexneri* was determined quantitatively against the pomegranate peel tea by the dilution method in agar (MIC) according to NCCLS norms (National Committee for Clinical Laboratory Standards, 2002).

Although the decoction of 30 minutes was that which showed a major inhibition halo, as significant differences between decoctions from 10 to 30 minutes were not observed, the preparation of 10 minutes was selected for performing the quantitative studies.

A series of plates with MH agar were prepared, to which the tea in different concentrations was added. Each plate was inoculated with a suspension of 10^4 ufc/ml of *Sh. flexneri*. It was incubated at 37°C for 24 h, and the minimum tea concentration able to inhibit the strains of *Sh. flexneri* was determined (Table 3).

Only the decoctions of 10 min of pomegranate peel (4%), holm-oak, hamammelis, rose petal, oak (25%) showed inhibition halos against *Sh. flexneri*. None of the tested herbs showed inhibitory effects against the other strains.

The major inhibition in *Sh. flexneri* was observed with the tea of pomegranate peel.



Pomegranate

Figure 1. Inhibition halo of some teas tested on *Sh. flexneri*

RESULTS AND DISCUSSION

Table 1. Inhibitory effect of the different herbs on the strains under study

	Strain 1	Strain 2	Strain 3	Strain 4
AG				
CA				
CH				
DO				
DU				
HO			12 mm	
GR			20 mm	
GT			-	
HA			14 mm	
FT			-	
RP			16 mm	
O			16 mm	
SU			-	
ZA			-	

The inhibitory activity of the different times of preparations of pomegranate peel against *Sh. flexneri* is shown in Table 2.

Table 2

Teas	Inhibition halos (mm)						
	1	2	3	10	20	30	60
I. 10'	16	20	19	19	15	15	20
C. 1'	18	21	19	20	15	16	20
C. 5'	19	22	20	20	16	16	21
C. 10'	20	22	21	22	17	17	23
C. 15'	20	22	22	23	18	19	25
C. 20'	21	23	22	23	19	20	26
C. 25'	22	23	23	24	19	20	26
C. 30'	22	24	24	24	20	20	26

The decoction of pomegranate peel of 30 minutes was that of the major antimicrobial activity. This activity was kept until 2 months.

Table 3. MIC Determination in a solid medium.

Strains of <i>Sh. flexneri</i>	MIC in a solid medium
A	1/20
B	1/20
C	1/20
D	1/20
E	1/5
F	1/5
G	1/5

7 strains of *Sh. flexneri* isolated from clinical samples were assayed, of which 4 strains showed a MIC: 1/20, and 3 strains a MIC: 1/5.

CONCLUSIONS

From these results, it can be drawn the conclusion that the teas of holm-pak, pomegranate peel, hamammelis, rose petals and oak exercise inhibitory effect on *Shigella flexneri*. The pomegranate tea showed the highest inhibitory activity, with halos of 26 mm (diffusion) and a MIC ≤ 1/20. The studies of the antimicrobial active principles of these herbs were intensified, which could be used as a preventive alternative therapy and/or therapy. It is promising to continue with the investigations in order to be able to include them in a pharmaceutical product.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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IDECEFYN

vol 11 September-December 2006, 47-49

<http://www.idecefyn.com.ar>

ISSN 1666-888X

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