Iranian Journal of Pharmaceutical Research (2005) 2: 87-91
Received: April 2004
Accepted: October 2004

Original Article

In Vitro Antibacterial Activity of *Rheum ribes* Extract Obtained from Various Plant Parts Against Clinical Isolates of Gram-Negative Pathogens

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Abstract

Rheum ribes is among the Polygonaceae family which is endemic in Iran and a few neighboring countries. In this investigation, antibacterial effects of root, stalk and leaves extracts of *Rheum ribes* on a few common clinical isolates of gram negative pathogens were studied, using the cup plate and paper disc methods. Gram negative microorganisms studied were *Escherichia coli, Klebsiella pneumoniae, Proteus* spp., *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*.

For examination of pathogenic microorganisms in the cup plate and disc diffusion method, methanolic extracts of different plant parts at concentrations of 250 and 500 micrograms per cup or disc, were used.

The root and leaves extracts have demonstrated significant antibacterial activities in both methods. However, stalk extracts showed a lower degree of antibacterial activity than the root and leaves extracts. Generally, the extracts showed a broad spectrum of activity, although they were more effective against *P. aeruginosa* and *Proteus* spp. in comparison with the positive control. The results suggested that extracts of *Rheum ribes* could be effectively used against clinical isolates.

Keywords: Antibacterial activity; Agar diffusion; Gram-negative pathogen; *Rheum ribes*.

Introduction

The treatment of infectious diseases with antimicrobial agents continues to present problems in modern day medicine with many studies showing a significant increase in the incidence of side effects and the resistance that pathogenic microorganisms build against several antibiotics (1-3). However, attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine to treat infections recently (4).

Rheum ribes L. (Polygonaceae) is a hardy perennial, cultivated in some temperate countries for its edible red leaf stalks. It grows in Iran and Turkey. Its Persian name is “Rivas” (5).

There are no previous studies on the antimicrobial activity of this plant, but there are some reports indicating the antimicrobial activities of Polygonaceae family (6, 7). However, antiviral effects of extracts from different parts of this plant have been reported previously (8). Also there are reports on the ethnomedical use of different parts of *R. ribes* in Turkey (9) and Iran (5).

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In the present work, the antimicrobial activities of root, leaves and stalk extracts of this plant against clinical isolates of gram-negative pathogens are described.

Experimental

Plant materials

Leaves and stalks of the plant were collected from Neishaboor (Khorasan Province, Iran) in June 1999, whereas roots were collected in September 1999. The plant was identified by the Herbarium of Ferdowsi University, Mashhad, Iran, and voucher specimens were deposited. Different parts of plant were cleaned from debris, air-dried and finally grounded to a coarse powder. Stalks were cleaned from debris, washed and then cut into small pieces before drying.

Preparation of extracts

Powdered plant materials (50 g) were extracted with methanol (300 ml) by the aid of a Soxhelet apparatus for 12 h. After filtration, the solvent was removed under reduced pressure using a Rotavapor - RE to give a concentrated extract and the residue was refrigerated until use.

Microorganisms

The hospital strains were from different specimens (vaginal, urine, scars, etc) of patients referring to Ghaem Hospital, Mashhad, Iran. These microorganisms were *Escherichia coli, Klebsiell penumoniae, Neisseria gonorrhoeae*, *Proteus vulgaris, Pr. mirabilis and Pseudomonas aeruginosa.* The numbers of strains for each microorganism were fifty, except for *N. gonorrhoeae,* which were thirty strains. To identify the microorganisms, specimens were cultured on different general, enrichment, selective and/ or differential media and underwent biochemical tests (10).

Microbiological assay

The antibacterial activities of extracts were determined by the agar diffusion method, using different reservoirs [cup (11) and disc (12)].

To inoculate the media for assay, 1 ml from 10^6 cfu/ml bacterial suspension, already harvested from surface of soybean casein digest agar (Merck), was added to 100 ml molten Muller-Hintone agar (Oxoid), (except for *N. gonorrhoeae,* in which the culture and assay media was supplemented with 5% sheep's blood). Thirty ml of this inoculated medium were poured into petri dishes and left until hardened. For the cup plate method 6 holes were punched within the medium in each plate and 100 µl of each extract solutions (250 and 500 mcg/cup) were pipetted in triplicate into holes. For the disc plate method, blank filter papers were placed on the surface of the medium and impregnated with 10 µl, containing either 250 or 500 mcg of various extracts.

Two antibiotic standard discs were used as the positive controls for both methods. Sensitivity was deduced by comparing the inhibition zone diameter produced by the gentamicin disc (for *E. coli, Proteus* spp. and *K. pneumoniae*) or tetracycline disc (for *P. aeruginosa* and *N. gonorrhoeae*) to those produced by extracts in both methods.

The petri dishes were incubated at 35ºC for 24 h, except for *N. gonorrhoeae* plates which were incubated 48 h in the presence of 5% CO_2. The inhibition zones were measured with a caliber and recorded as the mean diameter of 3 replications in mm.

Data analysis

Fisher's exact test was used to analyses data obtained from the zone of inhibition produced by different extracts and positive controls. A p-value less than 0.05 were taken as the critical criterion for statistical significance.

Results and Discussion

The results of the antibacterial activities of different parts of extracts on hospital strains are presented in Tables 1 and 2. Comparison of the effect of extracts and positive controls is shown in Figures 1-5 (cup plate and disc diffusion methods).

Figure 1 shows that different concentrations of root (250 and 500 mcg/cup or disc) and leaves extracts (500 mcg/cup or disc) have a similar effect on *E. coli,* which is comparable with the positive control (Gentamicin). However, a lower antibacterial effect was observed in comparison with the positive control for leaves (250 mcg/

Table 1. Antibacterial activities of methanolic extracts of different parts of *R. ribes* L.on hospital isolates of *E.coli, K. pneumoniae, Proteus* spp., *P. aeruginosa and N. gonorrhoeae* using cup plate method.

Extract		*E.coli* [a]				*K. pneumoniae* [a]				*Proteus* [a]				*P.aeruginosa* [a]				*N.gonorrhoeae* [b]			
Amount in cup (Mcg)		R	S	%S	R/S	R	S	%S	R/S	R	S	%S	R/S	R	S	%S	R/S	R	S	%S	R/S
Root	(250)	19	31	62	0.61	20	30	60	0.66	24	26	52	0.92	25	25	50	1	18	12	40	1.5
	(500)	12	38	76	0.31	17	33	66	0.51	21	29	58	0.72	23	27	54	0.85	16	14	46	1.14
Leaves	(250)	27	23	46	1.17	32	18	36	1.77	36	14	28	2.57	16	34	68	0.47	17	13	43	1.30
	(500)	19	31	62	0.61	29	21	42	1.38	31	19	38	1.63	13	37	74	0.35	13	17	56	0.76
Stalk	(250)	36	14	28	2.57	36	14	28	2.57	47	3	6	15.66	47	3	6	15.66	30	0	0	-
	(500)	33	17	34	1.94	35	15	30	2.33	43	7	14	6.14	47	3	6	15.66	29	1	3.33	29

a= 50 strains b= 30 strains S: No. of sensitive strains, R: No. of resistant strains, R/S: ratio of resistant to sensitive strains. %S= per cent of sensitive strains Sensitivity was deduced by comparing the inhibition zone diameter produced by Gentamicin disc (for *E.coli*, *K. pneumoniae*, *Proteus* spp.) or Tetracycline disc (for *P. aeruginosa* and *N. gonorrhoeae*) to the extract discs.

cup or disc) ($P<0.001$ and $P<0.05$ respectively) and stalk extracts (250 and 500 mcg/cup or disc) ($P<0.001$).

Figure 2 shows that a higher concentration of root extract (500 mcg/cup or disc) exhibits a significantly greater antibacterial effect, than the positive control, on *K. pneumoniae* ($P<0.05$). On the other hand a lower concentration (250 mcg/ cup or disc) showed no significant difference, compared with the positive control ($P>0.05$). There were no significant differences between the effect of different concentrations of leaves extracts (250 and 500 mcg/cup or disc) and the positive control ($P>0.05$). Different concentrations of stalk extracts (250 and 500 mcg/cup) have a lower degree of antibacterial activity than the positive control, and this difference was significant ($P<0.01$ and $P<0.05$ respectively). However, there is no significant difference between the effect of stalk extract and the positive control ($P>0.05$) in the disc method.

Figure 3 shows that there are significant differences between the effect of root (250, 500 mcg/cup) and leaves extracts (500, mcg/cup) with the positive control ($P<0.001$ and $P<0.001$, $P<0.05$ respectively) on *Proteus* spp. However, the effect of stalk extract (250 mcg/cup) is significantly lower than the positive control ($P<0.001$).

The antibacterial effect of root extract (500 mcg/disc) on *Proteus* spp. is significantly higher than the positive control ($P<0.05$). Root (250 mcg/disc) and leaves (250, 500 mcg/disc) extracts have a similar effect in comparison with the positive control. Stalk extract (250 and 500 mcg/disc) have lower effects in comparison with the positive control ($P< 0.001$, $P<0.05$ respectively).

Figure 4 shows the effects of extracts and positive control on *P. aeruginosa.* Root (250 and 500 mcg/cup or disc) and leaves (250 and 500 mcg/cup) extracts have a significantly higher effect than the positive control ($P<0.001$). The

Table 2. Antibacterial activities of methanolic extracts of different parts of *R. ribes* L. in terms % sensitivity of microorganisms on hospital strains of *E.coli,*[c] *K. pneumoniae,*[c] *Proteus* spp., [c] *P. aeruginosa* [c]*and N. gonorrhoeace* [d] in comparison with standard discs of antibiotics using disc diffusion method.

	E.coli [c]				*K. pneumoniae* [c]				*Proteus spp.*[c]				*P.aeruginosa* [c]				*N.gonorrhoeae* [d]			
Disc	R	S	%S	R/S	R	S	%S	R/S	R	S	%S	R/S	R	S	%S	R/S	R	S	%S	R/S
Amp	46	4	8.0	11.50	47	3	6.0	15.66	48	2	4.0	24.0	49	1	2.0	49.0	23	7	23.33	3.28
Amo	46	4	8.0	11.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ts	31	19	38.0	1.63	34	16	32.0	2.12	45	5	10.0	9.0	47	3	6.0	15.66	27	3	10.0	9.0
Gen	17	33	66.0	0.51	26	24	48.0	1.08	38	12	24.0	3.16	43	7	14.0	6.14	25	5	16.67	5.0
Te	-	-	-	-	-	-	-	-	-	-	-	-	41	9	18.0	4.55	14	16	53.3	0.87
Root [a]	22	28	56.0	0.78	22	28	56.0	0.78	33	17	34.0	1.94	21	29	58	0.72	22	8	26.67	2.75
Root [b]	16	34	68.0	0.47	17	33	66.0	0.51	30	20	40.0	1.50	18	32	64.0	0.56	19	11	36.67	1.72
Leaves (a)	26	24	480.	1.08	21	29	58.0	0.72	36	14	28.0	2.57	40	10	20.0	4.0	18	12	40.0	1.50
Leaves (b)	24	26	52.0	0.92	18	32	64.0	0.56	34	16	32.0	2.12	37	13	26.0	2.84	14	16	53.33	0.87
Stalk (a)	32	18	36.0	1.77	32	18	36.0	1.77	48	2	4.0	24.0	50	0	0	-	30	0	0	-
Stalk (b)	29	21	42.0	1.38	29	21	42.0	1.38	47	3	6.8	15.66	50	0	0	-	29	1	3.33	29.0

Amp: Ampicillin, Amo: Amoxicillin, Ts: Co-trimoxazole, Gen: Gentamycin, Te: Tetracycline a: 250 mcg/disc, b: 500 mcg/disc, c: 50 strains, d: 30 strains. Sensitivity was deduced by comparing the inhibition zone diameter produced by Gentamicin disc (for *E.coli*, *K. pneumoniae*, *Proteus* spp.) or Tetracycline disc (for *P. aeruginosa* and *N. gonorrhoeae*) and the extract discs.

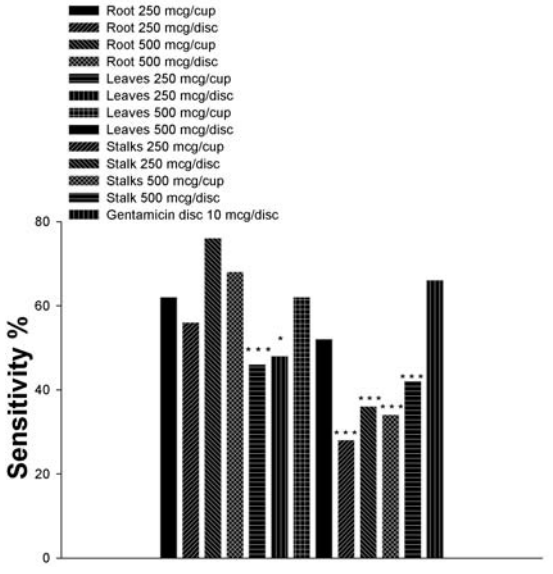

Figure 1. Antibacterial activity of methanol extracts of different parts of *R. ribes* L. on 50 hospital isolates of *E.coli*. Sensitivity was deduced by comparing the inhibition zone diameter produced by gentamicin disc and the extracts using cup plate and disc method; (*: P<0.05, ***: P<0.001, Fisher test).

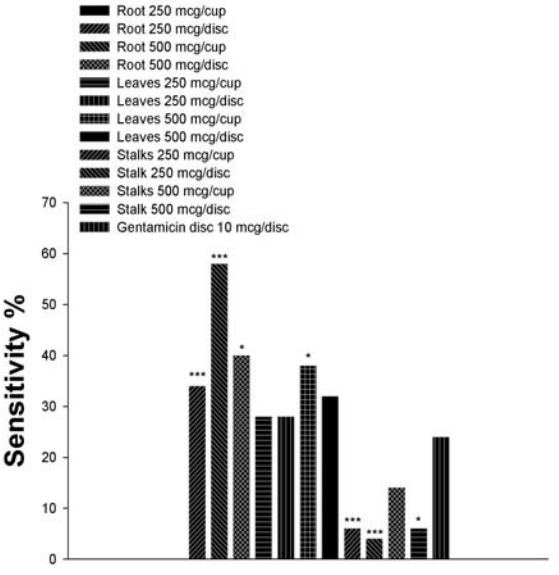

Figure 3. Antibacterial activity of methanol extracts of different parts of *R. ribes* L. on 30 hospital isolates of *Proteus* spp. Sensitivity was deduced by comparing the inhibition zone diameter produced by tetracycline disc and the extracts using cup plate and disc method; (*:P<0.05,***: P<0.001, Fisher test).

effect of leaves (250 and 500 mcg/disc) and stalk (250 and 500 mcg/cup) extracts is similar to the positive control. Stalk extracts in the disc method do not have any effect on *P. aeruginosa* (P<0.001).

Figures 5 shows nearly the same effect of root (250 and 500 mcg/cup) and leaves (250 and 500 mcg/cup) extracts on *N. gonorrhoeae*, comparable with the positive control. The antibacterial effect of root extracts (250 and 500 mcg/disc) is lower than the positive control (P<0.001 and P<0.05 respectively), while the stalk extracts had almost no effect on *N. gonorrhea* (P<0.001).

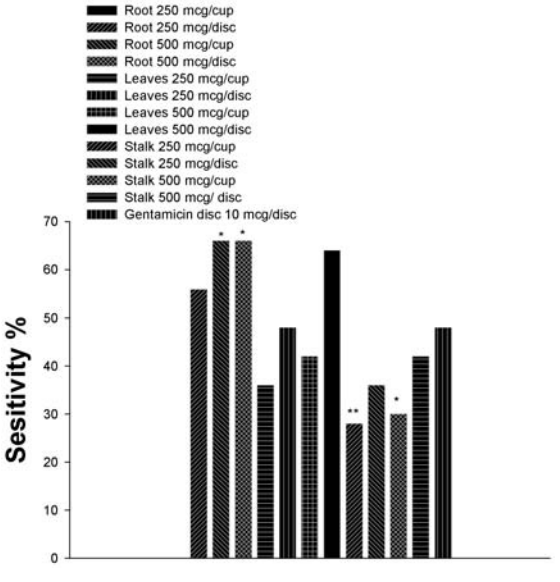

Figure 2. Antibacterial activity of methanol extracts of different parts of *R. ribes* L. on 50 hospital isolates of *K. pneumoniae*. Sensitivity was deduced by comparing the inhibition zone diameter produced by gentamicin disc and the extracts using up plate and disc method; (* : P<0.05, **: P<0.01, Fisher test).

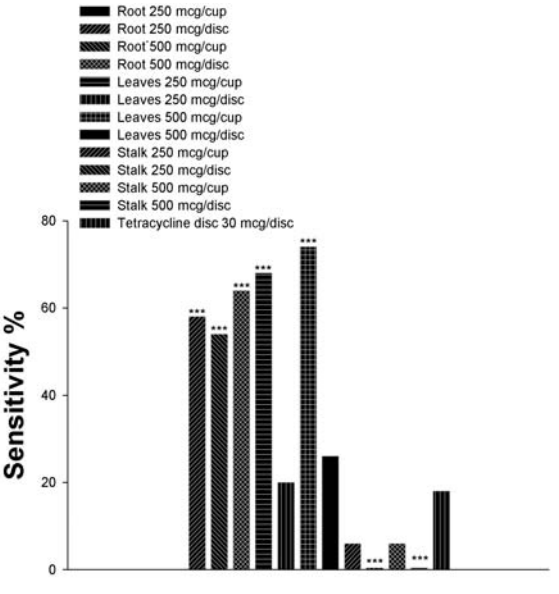

Figure 4. Antibacterial activities of methanol extracts of different parts of *R. ribes* L. on 50 hospital isolates of *P. aeruginosa*. Sensitivity was deduced by comparing the inhibition zone diameter produced by tetracycline disc and the extracts using cup plate and disc method; (***: P<0.001, Fisher test).

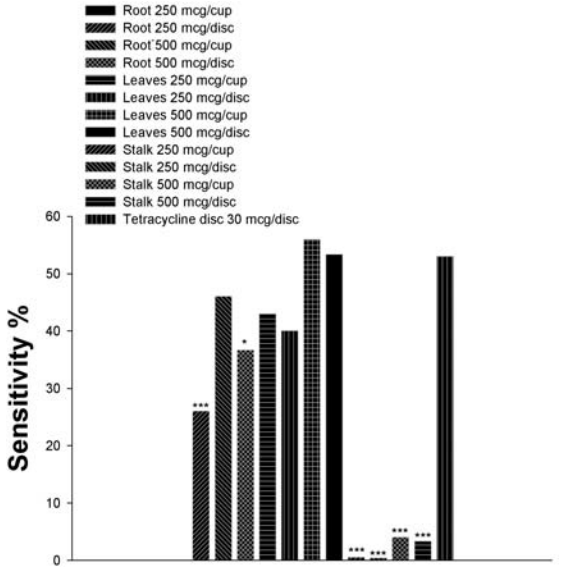

Figure 5. Antibacterial activity of methanol extracts of different parts of *R. ribes* L. on 30 hospital isolates of *N. gonorrhoeae*. Sensitivity was deduced by comparing the inhibition zone diameter produced by tetracycline disc and the extracts using cup plate and disc method; (*: P<0.05,***: P<0.001, Fisher test).

Comparing the two assay methods for antibacterial activity, there is not much difference between them.

Furthermore, the order of antibacterial activity of extracts on isolates was root >leaves> stalk, except for *N. gonorrhoea* which was leaves > root > stalk.

However, the antibacterial effect of stalk extract on isolates is negligible.

Extracts of different parts of *Rheum ribes*, especially root and leaves, have an antibacterial activity. This is very important, especially in the case of resistant microorganisms such as gentamicin or tetracycline resistant *P. aeruginosa.* The antibacterial effect of leaves extract on *N. gonorrheae* could be considered for its use in the Iranian folk medicine for the treatment of gonorrheae.

Finally, although the sensitivity of microorganisms towards the extract has been established from these two methods, never the less no conclusion on the antibacterial potency of the extracts and control antibiotics could be drawn (13).

Acknowledgement

The authors would like to thank Miss Z. Sabeti and Dr. T. Zeiaei for preparing the figures.

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