Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

IN-VITRO ANTIBACTERIAL ACTIVITY OF LEAF AND ROOT EXTRACTS OF *HYPOCHAERIS RADICATA* L. (ASTERACEAE) – A MEDICINAL PLANT SPECIES INHABITING THE HIGH HILLS OF NILGIRIS, THE WESTERN GHATS

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Received: 14 Oct 2012, Revised and Accepted: 21 Nov 2012

ABSTRACT

The present study is focused onto investigate the antibacterial activity of different extracts (petroleum ether, chloroform, ethyl acetate, methanol and water) of leaf and root parts of the plant species, *Hypochaeris radicata* against certain both Gram-positive bacteria (*Streptococcus faecalis, S. pyogenes, Enterococcus faecalis, Bacillus subtilis, B. thuringiensis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Seratia marcescens, Klebsiella pneumoniae, Proteus vulgaris, P. mirabilis, Salmonella paratyphi, S. parathypi* A, *S. paratyphi* B, *Pseudomonas aeruginosa* and *E. coli.*) by following agar disc diffusion method. Methanolic extract displayed broad spectrum of activity against all the test organisms than that of the other extracts. Among the extracts of two parts attempted, the methanolic root extract showed highest antibacterial activity (17.1 ± 0.1). The antibacterial activity of the methanolic extracts of both parts was comparable to that of the standard drug, ampicillin. The minimum inhibitory concentration (MIC) of the methanolic leaf and root extracts was determined by the broth dilution method and was ranging between 200 and 400µg/mL, and 200 and 600µg/mL respectively. The results of this study support the species, *H. radicata* for its antibacterial agent. The leaf and root extracts can be used to treat various skin and gastrointestinal infections in humans and so has good potential use in the food and cosmetic industries as well.

Keywords: *Hypochaeris radicata*, Methanolic leaf and root extracts, Antibacterial activity, Disc diffusion method, Minimum inhibitory concentration.

INTRODUCTION

Plants have been an important source of medicine for thousands of years. The World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicines [1]. The varied phytochemical agents present in medicinal plants are the factors for their therapeutic value [2]. Plant based antimicrobials have been proved to be effective in the treatment of infectious diseases with lesser or no side effects, which are often associated with synthetic antibiotics [3]. The antimicrobial properties of plants have been investigated by a number of researchers world wide. In recent years many plants of traditional medicinal importance are being evaluated for their antibacterial activity [4,5].

Hypochaeris radicata L. (Asteraceae), native of Europe, seldomly distributed in high hills of Nilgiris of Western Ghats, India at around 2000m above msl in forest margins is used for traditional medical practice for its anticancer, anti-inflammatory, anti-diuretic, hepatoprotective activity and to treat kidney problems also. In the local area of Nilgiris, the traditional healers prescribed this species for wound healing and other skin diseases caused by pathogens. The leaves and root of this species are reported to have good antioxidant property [6]. The aim of the present study was to evaluate the antibacterial and minimum inhibitory concentration of *H. radicata* leaf and root extracts against certain pathogenic bacterial strains include both Gram-positive and Gram-negative types.

MATERIALS AND METHODS

Plant collection and identification

The leaves and roots of *H. radicata* were collected from Kattabettu, Nilgiris, the Western Ghats, India (2000m above msl). The authenticity of the plant was confirmed in Botanical Survey of India, TNAU Campus, Coimbatore by referring the deposited specimen. The voucher number is BSI/SRC/5/23/2010-11/Tech.153.

Preparation of plant extracts

The dust free leaves and roots of *H. radicata* were shade dried and powdered. About 50g of coarsely powdered plant materials (50g/250mL) were extracted in a soxhlet apparatus for 8 to 10 hours, sequentially with petroleum ether, chloroform, ethyl acetate, methanol and water separately in order to extract non-polar and polar compounds [7]. The extracts obtained were then concentrated and finally dried to a constant weight. Dried extracts were kept at 20°C until further test will be carried out.

Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient agar medium and were incubated without agitation for 24hrs at 37°C. The cultures were diluted with fresh nutrient agar broth to achieve optical densities corresponding to 2-10^6 colony forming units (CFU/mL) for bacteria.

Microbial strains used

In-vitro antibacterial activity was examined for the leaf and root extracts of the species, *H. radicata* against 15 bacterial strains which include the Gram-positive strains *viz., Streptococcus faecalis, S. pyogenes, Enterococcus faecalis, Bacillus subtilis, B. thuringiensis* and *Staphylococcus aureus* and Gram-negative strains *viz., Seratia marcescens, Klebsiella pneumoniae, Proteus vulgaris, P. mirabilis, Salmonella paratyphi, S. parathypi* A, *S. paratyphi* B, *Pseudomonas aeruginosa* and *E. coli.* All these bacteria were obtained from the Department of Biotechnology, Hindustan College of Arts and Science, Coimbatore.

Antibacterial assay

Plant extracts of *H. radicata* which was prepared with different solvents *viz.,* petroleum ether, chloroform, ethyl acetate, methanol and water were used to test their antibacterial activity by disc diffusion method [8]. Bacterial suspension is streaked on the Molten Mueller-Hinton agar medium containing the Petri plates. Discs of 5mm diameter were impregnated with the leaf and root extracts separately. Ampicillin was used as a positive control. The plates were incubated for overnight at 37°C and the results were obtained by measuring the zone of diameter in millimeter.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined through the broth dilution method [9]. Bacteria were grown in nutrient broth for 6 hrs. After this, 200µl of 10^6 cells/mL were inoculated in tubes with (1800µl) nutrient broth supplemented with eight different concentrations of 100-800µg/mL for both leaf and root extracts separately. For the determination of MIC, ampicillin

100µg/mL was used as positive control and the pure solvent (methanol) 100µl was used as negative control. All the tubes were incubated at 37ºC for 24hrs and they were examined for visible turbidity. The MIC values were taken as the lowest concentration that inhibited the visible growth of the tested organisms [10,11].

Statistical analysis

The antibacterial activity of *H. radicata* leaf and root extracts was indicated by clear zones of growth inhibition. All experiments were performed in triplicates and the results are presented as mean ± SD (Standard Deviation). The significancy in the difference of mean was determined according to New Duncan's Multiple Range Test [12].

RESULTS AND DISCUSSION

Tables 1 and 2 exhibit the data on antibacterial activity of leaf and root extracts of the study species, *H. radicata* respectively. The results of the study revealed that higher antibacterial activity was observed for methanol extract of both leaf and root parts than that of the other solvent extracts attempted *viz.,* chloroform, ethyl acetate, petroleum ether and water. Further it was observed that the inhibitory activity of the extracts both bacterial strain and solvent specific. Among the solvents tried, greater zone of inhibition was produced by methanolic leaf extract against the bacterium, *Enterococcus faecalis* (11.86 ± 0.15) while the petroleum ether extract showed lower inhibitory activity for all bacterial strains tested which was ranging between 5.8 ± 0.26 and 7.36 ± 0.4 (Table 1). The methanolic root extract showed higher zone of inhibition against the bacteria, *Bacillus subtilis* (17.1 ± 0.1) and *Staphylococcus aureus* (16.06 ± 0.92), while the petroleum ether and water extracts showed lower antibacterial activity *viz.,* 5.66 and 7.20, and 5.93 and 6.20 (Table 2). The bacteria *viz., Streptococcus faecalis, S. pyogenes, Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, P. mirabilis, Salmonella parathypi* A, *S. paratyphi* B and *Pseudomonas aeruginosa* were found to be most resistant to water extract showing no inhibition. It may be explained that the activity of antibiotics in plant extracts against bacterial growth may be due to their mechanism of action, chemical structure or spectrum of activity [13]. It was further observed that the inhibitory activity of methanolic leaf extract against the bacteria *viz., Bacillus subtilis, Klebsiella pneumoniae* and *Proteus mirabilis,* and inhibitory activity of root extract against the bacteria *viz., Bacillus subtilis, Staphylococcus aureus, Seratia marcescens, Proteus mirabilis* and *Salmonella paratyphi* B were significantly greater than that of the standard drug, ampicillin also.

Table 1: Antibacterial activity of various alcoholic and aqueous leaf extracts of *Hypochaeris radicata.*

Name of the bacteria	Diameter of the inhibition zone (mm)					
	Control*	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
Gram-Positive						
Streptococcus faecalis	19.50 ± 0.51^{a}	7.66 ± 0.57^{b}	8.33 ± 0.57^{b}	9.53 ± 0.56^{c}	10.2 ± 0.72^{c}	10.60 ± 0.57^{c}
S. pyogenes	19.50 ± 0.50^{a}	6.20 ± 0.20^{b}	7.26 ± 0.25^{b}	7.26 ± 0.37^{b}	10.63 ± 0.55^{c}	7.46 ± 0.41^{b}
Enterococcus faecalis	27.90 ± 0.36^{a}	5.80 ± 0.26^{b}	7.23 ± 0.25^{c}	7.13 ± 0.15^{c}	11.86 ± 0.15^{d}	9.90 ±0.17^{e}
Bacillus subtilis	8.26 ± 0.46^{a}	6.10 ± 0.10^{b}	7.86 ± 0.80^{a}	10.60 ± 0.35^{c}	9.20 ± 1.05^{c}	9.56 ±0.51^{c}
B. thuringiensis	14.70 ± 0.40^{a}	7.20 ± 0.20^{b}	9.50 ± 0.45^{c}	7.76 ± 0.25^{b}	8.10 ± 0.10^{b}	10.06 ± 0.11^{c}
Staphylococcus aureus	14.90 ± 0.10^{a}	6.26 ± 0.25^{b}	7.26 ± 0.25^{b}	8.53 ± 0.47^{c}	7.23 ± 0.25^{b}	7.40 ± 0.36^{b}
Gram-Negative						
Seratia marcescens	10.16 ± 0.28^{a}	6.03 ± 0.15^{b}	6.66 ± 0.57^{b}	7.66 ± 0.57^{c}	7.16 ± 0.76^{c}	8.50 ± 0.50^{d}
Klebsiella pneumoniae	9.00 ± 0.92^{a}	6.23 ± 0.20^{b}	7.33 ± 0.49^{bc}	8.03 ± 0.89^{c}	9.30 ± 0.35^{a}	6.86 ± 0.15^{b}
Proteus vulgaris	17.86 ± 0.50^{a}	6.60 ± 0.36^{b}	10.63 ± 0.60^{c}	7.63 ± 0.55^{b}	9.73 ± 0.25^{c}	7.66 ± 0.57^{c}
P. mirabilis	8.53 ± 0.47^{a}	7.36 ± 0.40^{b}	8.26 ± 0.25^{ac}	7.96 ± 0.25^{bc}	9.40 ± 0.36^{d}	8.36 ± 0.32^{ac}
Salmonella paratyphi	19.90 ± 0.17^{a}	6.30 ± 0.10^{b}	6.36 ± 0.15^{b}	6.46 ± 0.25^{b}	7.36 ± 0.37^{b}	9.26 ± 0.25^{c}
S. paratyphi – A	18.73 ± 0.30^{a}	6.20 ± 0.26^{b}	8.36 ± 0.35^{c}	8.23 ± 0.25^{c}	10.83 ± 0.15^{d}	8.93 ± 0.81^{c}
S. paratyphi – B	11.56 ± 0.51^{a}	7.03 ± 0.15^{b}	7.80 ± 0.26^{b}	7.00 ± 0.20^{b}	8.26 ± 0.25^{b}	7.36 ± 0.35^{b}
Pseudomonas aeruginosa	17.10 ± 0.10^{a}	5.86 ± 0.32^{b}	7.66 ± 0.35^{c}	7.73 ± 0.25^{c}	8.60 ± 0.36^{c}	8.60 ± 0.52^{c}
E. coli	14.90 ± 0.10^{a}	6.26 ± 0.15^{b}	7.30 ± 0.26^{c}	7.20 ± 0.20^{c}	8.60 ± 0.10^{d}	9.10 ± 0.10^{d}

*Ampicillin.

Values were performed in triplicates and represented as mean ± SD.

Mean values followed by different superscripts in a horizontal row are significantly different ($p<0.05$).

Table 2: Antibacterial activity of various alcoholic and aqueous root extracts of *Hypochaeris radicata.*

Name of the bacteria	Diameter of the inhibition zone (mm)					
	Control*	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
Gram-Positive						
Streptococcus faecalis	14.00 ± 0.20^{a}	6.40 ± 0.36^{b}	10.03 ± 0.05^{c}	8.23 ± 0.40^{d}	8.53 ± 0.47^{d}	-
S. pyogenes	19.56 ± 0.51^{a}	6.80 ± 0.26^{b}	8.56 ± 0.49^{c}	9.80 ± 0.26^{d}	10.13 ± 0.15^{d}	-
Enterococcus faecalis	27.50 ± 0.12^{a}	7.20 ± 0.10^{b}	7.15 ± 0.23^{b}	10.85 ± 0.12^{c}	9.80 ± 0.33^{c}	-
Bacillus subtilis	11.63 ± 0.55^{a}	5.90 ± 0.36^{b}	8.86 ± 0.15^{c}	7.30 ± 0.36^{d}	17.10 ± 0.10^{e}	6.76 ± 0.25^{bd}
B. thuringiensis	11.83 ± 0.15^{a}	5.66 ± 0.41^{bd}	8.30 ± 0.36^{c}	7.33 ± 0.41^{cd}	8.06 ± 0.11^{c}	6.80 ± 0.26^{d}
Staphylococcus aureus	14.83 ± 0.20^{a}	7.23 ± 0.20^{b}	11.40 ± 0.36^{c}	11.73 ± 0.25^{c}	16.06 ± 0.92^{a}	-
Gram-Negative						
Seratia marcescens	10.73 ± 0.25^{a}	6.43 ± 0.20^{b}	9.86 ± 0.15^{c}	9.01 ± 0.12^{c}	11.80 ± 0.75^{a}	6.03 ± 0.05^{b}
Klebsiella pneumoniae	10.76 ± 0.40^{a}	6.20 ± 0.20^{b}	9.73 ± 0.25^{a}	8.26 ± 0.25^{c}	10.16 ± 0.15^{a}	-
Proteus vulgaris	19.63 ±0.47^{a}	6.40 ± 0.40^{b}	12.26 ± 0.30^{c}	7.90 ± 0.17^{d}	10.13 ± 0.23^{c}	-
P. mirabilis	6.36 ± 0.35^{a}	6.40 ± 0.20^{a}	9.36 ± 0.35^{b}	10.36 ± 0.35^{b}	10.50 ± 0.45^{b}	-
Salmonella paratyphi	19.86 ± 0.15^{a}	6.46 ± 0.45^{b}	10.53 ± 0.47^{c}	9.66 ± 0.61^{c}	10.41 ± 0.45^{c}	6.76 ± 0.25^{b}
S. paratyphi – A	18.73 ± 0.25^{a}	6.13 ± 0.15^{a}	9.63 ± 0.56^{cd}	10.23 ± 0.25^{c}	8.30 ± 0.36^{d}	-
S. paratyphi – B	8.83 ± 0.15^{a}	6.13 ± 0.15^{b}	11.23 ± 0.20^{c}	10.16 ± 0.15^{c}	12.50 ± 0.50^{d}	-
Pseudomonas aeruginosa	14.03 ± 0.15^{a}	6.23 ± 0.25^{b}	9.83 ± 0.15^{c}	7.10 ± 0.10^{b}	9.90 ± 0.17^{c}	-
E. coli	13.63 ± 0.35^{a}	6.13 ± 0.15^{bd}	11.9 ± 0.10^{c}	9.76 ± 0.20^{d}	7.16 ± 0.28^{b}	5.93 ± 0.11^{d}

*Ampicillin, '-' indicates no activity.

Values were performed in triplicates and represented as mean ± SD.

Mean values followed by different superscripts in a horizontal row are significantly different ($p<0.05$).

From the overall performance of extracts, it is known that the methanolic leaf and root extracts of *H. radicata* showed broad spectrum of antibacterial activity in comparison to that of the other solvent extracts. Among the two parts attempted, the methanolic extract of root showed higher inhibition activity. This may be explained due to the presence of rich quantity and variety of bioactive compounds like flavonoids and phenolic acids, the more required bioactive compounds for antibacterial activity in the root part of *H. radicata* than that of the leaves [14]. The synergetic activity of that rich constituents may be the possible factor for the inhibition of the growth of the bacterial colonies [15]. Most prominent antibacterial activity for many other Asteraceae members has been well documented already [16-21].

As the methanolic extracts are more promising and effective, determination of minimum inhibitory concentration (MIC) was made only for this alcoholic extract of both leaf and root parts of *H. radicata* against 15 pathogenic bacteria of both Gram-positive and Gram-negative types (Tables 3 and 4). The MIC of the methanolic leaf and root extracts of *H. radicata* was ranging between 200 and 400µl, and 200 and 600µl respectively. For the methanolic leaf extract, the most susceptible bacteria identified were *Enterococcus faecalis, Bacillus subtilis, Proteus vulgaris, P. mirabilis, Salmonella paratyphi* B and *Pseudomonas aeruginosa.* The most resistant bacteria were *Streptococcus faecalis* and *Salmonella paratyphi.* For the methanolic root extract, the most susceptible and resistant bacteria were *Seratia marcescens* and *Bacillus thuringiensis* respectively.

Table 3: Minimum inhibitory concentration (MIC) of methanolic leaf extracts of *Hypochaeris radicata* on certain pathogenic bacteria.

Bacteria	Minimum inhibitory concentration (mg/mL)									
	Solvent[A]	Control[B]	100	200	300	400	500	600	700	800
Gram-Positive										
Streptococcus faecalis	+	+	+	+	+	+	-	-	-	-
S. pyogenes	+	+	+	+	+	-	-	-	-	-
Enterococcus faecalis	+	+	+	+	-	-	-	-	-	-
Bacillus subtilis	+	+	+	+	-	-	-	-	-	-
B. thuringiensis	+	+	+	+	+	-	-	-	-	-
Staphylococcus aureus	+	+	+	+	+	-	-	-	-	-
Gram-Negative										
Seratia marcescens	+	+	+	+	+	-	-	-	-	-
Klebsiella pneumoniae	+	+	+	+	+	-	-	-	-	-
Proteus vulgaris	+	+	+	+	-	-	-	-	-	-
P. mirabilis	+	+	+	+	-	-	-	-	-	-
Salmonella paratyphi	+	+	+	+	+	+	-	-	-	-
S. paratyphi – A	+	+	+	+	+	-	-	-	-	-
S. paratyphi – B	+	+	+	+	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	-	-	-	-	-	-
E. coli	+	+	+	+	+	-	-	-	-	-

A – Negative control (methanol); B – Positive control (Ampicillin).

(+) and (-) -indicate the presence and absence of bacterial growth respectively.

Table 4: Minimum inhibitory concentration (MIC) of methanolic root extracts of *Hypochaeris radicata* on certain pathogenic bacteria.

Bacteria	Minimum inhibitory concentration (mg/mL)									
	Solvent[A]	Control[B]	100	200	300	400	500	600	700	800
Gram-Positive										
Streptococcus faecalis	+	+	+	+	+	+	-	-	-	-
S. pyogenes	+	+	+	+	+	-	-	-	-	-
Enterococcus faecalis	+	+	+	+	+	+	-	-	-	-
Bacillus subtilis	+	+	+	+	+	+	+	-	-	-
B. thuringiensis	+	+	+	+	+	+	+	+	-	-
Staphylococcus aureus	+	+	+	+	+	-	-	-	-	-
Gram-Negative										
Seratia marcescens	+	+	+	+	-	-	-	-	-	-
Klebsiella pneumoniae	+	+	+	+	+	+	+	-	-	-
Proteus vulgaris	+	+	+	+	+	+	-	-	-	-
P. mirabilis	+	+	+	+	+	+	-	-	-	-
Salmonella paratyphi	+	+	+	+	+	-	-	-	-	-
S. paratyphi – A	+	+	+	+	+	+	+	-	-	-
S. paratyphi – B	+	+	+	+	+	+	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	+	+	-	-	-	-
E. coli	+	+	+	+	+	-	-	-	-	-

A – Negative control (methanol); B – Positive control (Ampicillin).

(+) and (-) -indicate the presence and absence of bacterial growth respectively.

CONCLUSION

The results of the study revealed that the methanolic root extract of the study species, *H. radicata* was more effective against both Gram-positive and Gram-negative bacteria to control the colonial growth than the leaf extract. This fact confirms the traditional knowledge of local healers on the wound healing property of this species. So the root part of this plant species can be used as a promising source for the development of new pharmaceuticals that address the therapeutic needs to cure infectious diseases.

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