African Journal of Microbiology Research Vol 3(1) pp. 005-009, January, 2009
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0809 ©2009 Academic Journals

Full Length Research Paper

Antibacterial activity of *Curculigo orchioides* rhizome extract on pathogenic bacteria

K. S. Nagesh* and C. Shanthamma

Medicinal Plant Tissue Culture Lab, Department of Studies in Botany, University of Mysore, Manasagangothri, Mysore – 570 006, Karnataka, India.

Accepted 27 June, 2008

Curculigo orchioides **rhizome extracts were evaluated for antibacterial activity against pathogenic strains of Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) bacteria. Because the steam distilled preparation was found to be the most active amongst the different extracts, its antibacterial activity against the aforementioned strains was compared to the standard antibiotics gentamycin, ampicillin, doxycycline and erythromycin. Only the clinical isolate of *S. aureus* showed more sensitivity towards water extracts than the standard strain. Also, the steam distilled fraction was more effective against Gram-positive strains than Gram-negative strains. Therefore, the steam distilled extract from *C. orchioides* has a potential application as an antiseptic for the prevention and treatment of antibacterial infections, and the present findings support its traditional local uses.**

Key words: *Curuligo orchioides,* steam distilled extract, gram-positive and gram-negative strains.

INTRODUCTION

Curculigo orchioides Gaertn (Hypoxidiaceae) is popularly known as black musali in India. The rhizome, as well as the tuberous roots of the plant has been extensively used in indigenous systems of medicine in India, Pakistan and China for the treatment of various diseases, including cancer, jaundice, asthma and diarthrosis wound healing (Dhar et al., 1968). The juice extracted from the rhizome has also been used as a tonic to overcome impotency (Chopra, 1956). *C. orchioides* is a small geophilous, perennial herb with long cylindrical rhizomes. The plant is found from near sea level to 2300 m, especially in moist laterite soil. The active compounds that have been reported are flavones, glycosides, steroids, saponins, triterpenoids and other secondary metabolites (Misra et al., 1984; Misra et al., 1990; Xu et al., 1992). Therefore, the plants have long since been deemed a valuable source of natural products for maintaining human health. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance to therapeutic treatments. Therefore, such plants should be investigated to better understand their properties, safety profiles and levels of efficiency against pathogenic microbes.

*Corresponding author email: nageshinvitro@yahoo.co.in.

Gram-positive bacteria, such as *Staphylococcus aureus,* are mainly responsible for postoperative wound infection, toxic shock syndrome and food poisoning (Halcon and Milkus, 2004). Gram-negative bacteria, such as *Escherichia coli,* are present in the human intestine and cause lower urinary tract infections, and septicemia (Ehrlich et al., 2005). To the best of our knowledge, there are no reports on antimicrobial activity of *C. orchioides.* Hence, we report here the effect of various extracts of *C. orchioides* on pathogenic strains of Gram-positive bacteria (*S. aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) by zone inhibition assays. Their effects were compared to various antibiotics, namely gentamycin, ampicillin, doxycycline and erythromycin, which have different mechanisms of action on bacteria.

MATERIALS AND METHODS

Plant extract

Fresh rhizomes of *C. orchioides* were collected from B.R. Hills, Karnataka. The specimens were identified in the Department of Botany Manasagangotri, University of Mysore, Mysore. The rhizomes (200 g) were ground finely in a mortar and pestle with addition of water, and were subjected to steam distillation (Paech and Tracy,

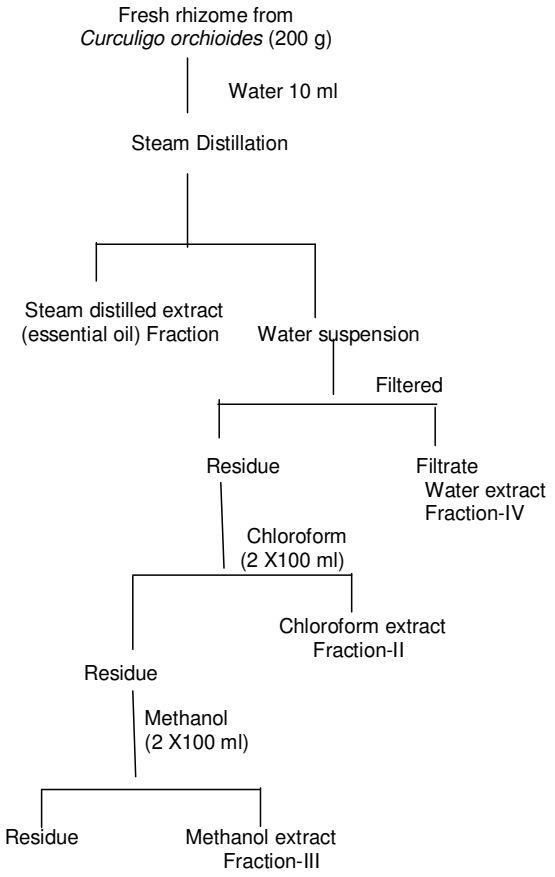

Figure 1. Preparation of extracts

1995; Mandal et al., 2007). The steam distilled fraction (Fraction-I, 0.763 g) was collected and the residue was resuspended in water and filtered. The filtrate was evaporated under a vacuum to produce the water extract (Fraction-IV, 2.0 g). The residue was air-dried and incubated overnight in chloroform (200 ml), filtered and re-extracted twice with chloroform (2 x 100 ml). All chloroform extracts were then combined and the solvent was evaporated to produce the chloroform extract (Fraction-II, 1.0 g). The residue which remained after chloroform extraction was then extracted with methanol to produce the methanol extract (Fraction-III, 3.8 g) (Figure 1).

Bacterial strains

The Gram-positive (*S. aureus* SR ATCC 6571 and clinical isolate, and *S. epidermidis* WHO-6) and Gram-negative (*E. coli* SR ATCC 10418 and clinical isolate, *P. aeruginosa* SR ATCC 10662 and clinical isolate, and *S. typhimurium* laboratory strain) bacteria used as test organisms were obtained from the Department of Microbiology, University of Mysore. Bacteria were cultured in Nutrient broth (Hi Media, Mumbai) at 37°C for 12 – 14 h and were maintained on Nutrient agar slants (Hi Media, Mumbai) at 4°C.

Antibacterial assay

The extracts were dissolved in ethylene glycol, filter-sterilized (0.47 µm diameter filters) and tested for antibacterial activity using the disc diffusion method. 4 mm.+ discs were impregnated with 2 mg of the sterile test material and placed onto the Nutrient agar surface, spread with 0.1 ml of bacterial culture (*ca.* 3 x 10^8 cells/ml using McFarland's 1 as a standard). The plates were incubated at 37°C for 12–14 h. Control discs contained ethylene glycol only. For comparison, the standard antibiotics gentamycin and ampicillin, which inhibit bacterial cell wall biosynthesis, and doxycycline and erythromycin, which inhibit bacterial protein synthesis, were included in the assay. The experiments were carried out in triplicate. The results (mean value $n = 3$) were recorded by measuring the zone of growth inhibition around the discs. Statistical analysis was carried out using Student's *t* test. (Chopade et al., 2008).

RESULTS

The antibacterial spectra showing the zones of inhibition in millimeters and as percentages (calculated using gentamicin as a positive control with 100% inhibition) for Gram-positive and Gram-negative bacteria are shown in Tables 1 and 2, respectively. Various extracts (Figure 1), such as the steam distilled (Fraction-I, essential oil), chloroform (Fraction-II), methanol (Fraction-III) and water (Fraction-IV) fractions, were tested for antibacterial activity in *in vitro* systems. All of the fractions were tested against the Gram-positive strains. For the *S. aureus* reference and clinical strains, both Fractions I and IV were active although to different extents. In the case of *S. epidermidis*, all four fractions were active, albeit to differing extents. In the *S. aureus* clinical isolate, Fraction-IV was also active, although Fraction-I showed more activity than standard antibiotics (Table 1). Among Gram-negative bacteria, Fraction-I displayed moderate activity against *E. coli* (CI) and *S. typhimurium* (laboratory strain), while all other extracts were inactive. Therefore, the minimum inhibitory concentration (MIC) was studied for Fraction-I only, and the results were compared with standard antibiotics (Tables 3 and 4). It was observed that dilution gradually altered the activity of Fraction-I in Gram - positive bacteria to 86 and 69% against *S. aureus* (SR) at 1/10 and 1/100 dilutions, respectively; however, *S. aureus* (CI) did not show a significant change (92%) at 1/10 dilution, although activity decreased to 73% at 1/100 dilution. In *S. epidermidis*, activity decreased with the 1/10 and 1/100 dilutions to 87 and 84%, respectively (Table 3). The Gram-negative bacteria *E. coli* (SR), *P. aeruginosa* (SR) and *S. typhimurium* (laboratory strain) showed decreases in activity with dilution. *S. typhimurium* showed no activity below the concentration of 200 µg/disc (1/10 dilution). The activities of Fraction-I against *E. coli* (SR) and *P. aeruginosa* (CI) were not affected at the 1/10 dilution (Table 4). Furthermore, the steam distilled fraction (Fraction-I) was more effective against Gram-positive compared to Gram-negative strains. *S. aureus* (CI) and *S. epidermidis* showed 175 and 86% inhibition, respectively, compared to Gram-negative strains of *E. coli* (CI), *P. aeruginosa* (CI) and *S. typhimurium* which showed inhibitions of 40, 29 and 55%, respectively.

However, no significant activity ($P > 0.1$) was shown in

Table 1. Zone of inhibition for various extracts from *C. orchioides* compared to reference drugs: activity against Gram-positive bacteria.

Microorgainsm	*Staphylococcus aureus* SR		*Staphylococcus aureus* Cl		*Staphylococcus epidermidis*	
Name of drug	Zone of inhibition		Zone of inhibition		Zone of inhibition	
	In mm Mean	As percentage $	In mm Mean	As percentage	In mm Mean	As percentage
Gentamycin 30mcg	28.33±1.17	100	12.12±0.21	100	20.66±0.87	100
Amplicillin 10 mcg	26.14±0.67	91	4.00±0.00	00	8.13±0.12	21
Doxycycline 30mcg	27.00±0.71	95	8.16±1.81	50	18.71±0.17	88
Erythromycin 0mcg	15.00±1.41	45	8.21±0.11	50	4.00±0.00	00
Fraction-1	13.66±0.77	37	18.66±0.12	175	18.33±0.87	86
Fraction-II	4.00±0.00	00	4.00±0.00	00	8.66±0.32	34
Fraction-III	4.00±0.00	00	4.00±0.00	00	9.71±0.16	24
Fraction-IV	7.41±0.33	12	10.33±0.66	75	8.60±0.16	27
Ethylene glycol	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00

SR: Standard reference, CI: Clinical isolates.
Mean: Mean value of the diameter of inhibition zone with standard error. The diameter of paper disc used was 4 mm.

Table 2. Zone of inhibition for various extracts from *C. orchioides* compared to reference drugs: activity against Gram-negative bacteria.

Name of drug	Microorgainsm									
	E. coil SR		*E. coil* C1		*S. typhimurium*		*P. aeruginosa* SR		*P. aeruginosa* C1	
	Zone of inhibition		Zone of inhibition		Zone of inhibition		Zone of inhibition		Zone of inhibition	
	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %
Gentamycin 30mcg	25±0.17	100	14.00±0.57	100	26.66±0.17	100	27.33±0.18	100	22.31±0.17	100
Amplicillin 10 mcg	17.00±0.67	61	4.00±0.00	00	4.71±0.12	03	7.33± 0.12	14	4.00±0.00	00
Doxycycline 30mcg	21.33±0.67	80	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00
Erythromycin 10mcg	16.66±0.87	57	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00
Fraction-1	9.66±0.82	26	9.66±0.00	50	13.66±0.87	40	10.33± 0.1	25	9.66± 0.32	29
Fraction-II	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00
Fraction-III	4.00±0.00	00	4.00±0.00	00	4.00±0.00	00	4.00± 0.00	00	4.00±0.00	00
Fraction-IV	9.00±0.67	23	7.33±0.66	30	5.60±0.16	04	8.00±067	17	8.17± 0.33	24
Ethylene glycol	4.00±0.00	00	0.00±0.00	00	0.00±0.00	00	0.00± 0.00	00	0.00± 00	00

SR: Standard reference, CI: Clinical isolates.
Mean: Mean value of the diameter of inhibition zone with standard error. The diameter of paper disc used was 4 mm.

standard reference strains of both Gram-positive and Gram-negative bacteria. Fraction-I (essential oil), as noticed by Dorman and Dean (2000), showed comparable activity to the standard antibiotics. It showed highest inhibition in Gram-positive *S. aureus* (CI, 246%) and comparable activity in *S. epidermidis* (83%) compared to the standard antibiotics ampicillin, doxycycline, erythromycin and gentamicin (Table 1). In Gram-negative bacteria, all antibiotics were active and depending on the antibiotic ranged between 57 - 80%, while Fraction-I showed significant ($P < 0.05$) activity against *E. coli* (CI, 38%) and *S. typhimurium* (laboratory strain, 35%) as compared to gentamycin (Table 2). The present study suggests that the essential oil fraction from *Curculigo orchioides* possesses significant ($P < 0.001$) antibacterial activity at very low concentrations (20 µg/disc) against the pathogenic Gram-positive bacterium *S. aureus* (CI).

DISCUSSION

The use of plants to treat diseases, including infectious ones, has been extensively applied by people. Plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds, as well as tannins. The components with phenolic structures were highly active against the microorganisms. Members of this class are known to be either bactericidal or bacteriostatic agents depending upon the concentration used (Rasooli et al.,

Table 3. Minimum inhibitory concentration of fraction 1 (essential oil) on Gram-positive bacteria with generation as standard reference.

Microorgainsm	*S. aureus* SR		*S. aureus* Cl		*S. epidermidis*	
	Zone of inhibition		Zone of inhibition		Zone of inhibition	
Name of drug	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %
Genetamycin 30 mcg	27.33± 0.74	100	10.67± 0.22	100	20.33 ± 0.66	100
Fraction	14.13± 0.12	43	17.13± 0.23	196	19-00± 0.48	91
1/10 dilution of fraction-1	12.66± 0.13	37	16.17± 0.23	192	17.13± 0. 48	80
1/100 dilution of fraction-I	11.13± 0.13	30	13.66± 0.13	144	16.67± 0.33	77
Ethylene glycol	4.00± 0.00	00	4.00± 0.00	00	4. 00± 0.00	00

SR: Standard reference, CI: Clinical isolates. Mean: Mean value of the diameter of inhibition zone with standard error. The diameter of paper disc used was 4 mm.

Table 4. Minimum inhibitory concentration of fraction 1 (essential oil) on Gram-negative bacteria with generation as standard reference.

Name of drug	Microorgainsm									
	E. coil SR		*E. coil* C1		*S. typhimurium*		*P. aeruginosa* SR		*P. aeruginosa* C1	
	Zone of inhibition		Zone of inhibition		Zone of inhibition		Zone of inhibition		Zone of inhibition	
	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %
Gentamycin 30 mcg	25.33±0.32	100	14.33 ± 0.12	100	25.10± 0.19	100	25.66± 0.17	100	22.33±0.66	100
Fraction -1	12.33±0.40	38	10.13± 0.32	59	12.67± 0.14	41	11.00± 0.58	32	9.0±0.57	27
1/10 dilution of fraction-1	9.71± 0.13	23	9.00 ± 0.13	48	10.59 ± 0.03	31	9.66± 0.33	26	10.33±0.32	35
1/100 dilution of fraction-1	8.00 ± 0.51	19	8.66± 0.34	40	4.00± 0.00	00	8.00± 0.00	18	8.33± 0.32	22
Ethylene glycol	4.00± 0.00	00	4.00± 0.00	00	4.00± 0.00	00	4.00±0.00	00	4.00± 0.00	00

2002). The antimicrobial activity of the rhizome may be due to presence of phenolic active compounds in *C. orchioides* (Xu et al., 1992). Furthermore, the steam-distilled fraction (Fraction-I) was more effective against Gram-positive and Gram-negative strains than other fractions. The reason may be that different solvents have varying degrees of solubility for different phytoconstituents (Majorie, 1999). Nevertheless, the present study suggests that the essential oil fraction from *Curculigo orchioides* possesses significant ($P < 0.001$) antibacterial activity at very low concentrations (20 µg/disc) against the pathogenic Gram-positive *S. aureus* (CI) bacteria. The results obtained might be considered for further studies aimed at isolating and identifying single active compounds within this fraction.

The demonstration of antimicrobial activity against both Gram-negative and Gram-positive bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. The results of the study also support the traditional application of the plant and suggest the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.

ACKNOWLEDGEMETS

The senior authors are grateful to the University of Mysore for providing financial assistance and research facilities.

REFERENCES

Chopade VV, Tankar AN, Ganjiwale RO, Yeole PGA (2008). Antimicrobial activity of *Capparis zeylanica* Linn . roots. Intl. J. Green Pharm. 2: 28-30.

Chopra RN, Nayar SL, Chopra IC, (1956). Glossary of medicinal plants CSIR New Delhi p. 84.

Dorman HJD, Dean SG (2000). Antimicrobial agents from plants; antimicrobial activity of plant volatile oils J. Appl. Microbiol. 88: 308-316

Dhar ML, Dhar BN, Dhawan DN, Mehrota C, Ray (1968). Screening of Indian plants for biological activity part 1. Indian J. Expt. Biol. 6(6): 232-249.

Ehrlich G, Hu F, Shen K, Stoodley P, Post J (2005). Bacterial plurality as a general mechanism driving persistence in chronic infections. Clin Orthop. Relat. Res. 20-24.

Halcon L, Milkus K (2004). *Staphylococcus aureus* and wounds: A review of tea tree oil as a promising antimicrobial. Amr. J. infect.

Cont. 32(7): 402-408
Majorie MC (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4): 564-582
Mandal SM, Mondal KC, Dey S, Pati BR (2007). Antimicrobial activity of the leaf extracts of *Hyptis suaveolens* (L.) poit. Indian J. Pharm. Sci. 69: 568-570
Misra TN, Singh RS, Tripathi, Sharma SC (1990). Curculigo a cycloartane, triterpine alcohol from *Curculigo orchiodies.* Phytochemistry 29: 929-931.
Misra TN, Singh RS, Upadhya J, Tripathi DNN (1984). Aliphatic compounds from *Curculigo orchiodies* Phytochemistry 23:1643-1645.
Paech K, Tracey MV (1995). Modern Methods of Plant Analysis Springer Verlag, Berlin. 3: 626-654.
Rasooli Seyed A, Mirmostafa (2002). Antimicrobial activity of Thymus Pubescens and T. Seyphyllum essential oils. Fitotherpia. pp.244-250.
Xu JP, Xu RS, Yuli X (1992) Glycosides of cycloartene sapogenine from *Curculigo orchiodies* Phytochemistry 31: 223-236.