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International Journal of Current Research
Vol. 6, Issue, 10, pp.9104--9107, October, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *CURCULIGO ORCHIOIDES* GAERTN RHIZOME, AN ENDANGERED MEDICINAL HERB

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ARTICLE INFO

Article History:
Received 05th July, 2014
Received in revised form
17th August, 2014
Accepted 26th September, 2014
Published online 25th October, 2014

Key words:

Curculigo orchiodes Gaertn,
Ethanolic extract,
Aqueous extract,
Antimicrobial activity.

ABSTRACT

In the present study, aqueous and ethanolic extracts of *Curculigo orchioides* was investigated for antimicrobial activity. The microorganisms employed were *Erwinia amylovora, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Enterobacter cloacae.* The susceptibility of bacterial strains against the two extracts were determined using the disk diffusion method. In the aqueous extract of *C. orchioides* showed maximum antimicrobial activity against Klebsiella pneumonia (18.49 mm) than other organisms. Proteus mirabilis shows minimum (6.34 mm) inhibitory activity against aqueous extract while Enterobacter cloacae have no inhibitory activity. Likewise, ethanolic extracts of C.orchioides rhizome exhibited maximum inhibitory activity against the all microorganism but differ in zone of inhibition (Klebsiella pneumonia – 27.3 mm) than other organisms. Among the two extracts ethanolic extract has maximum antimicrobial activity against the six microorganisms.

INTRODUCTION

Approximately 80% of the world's population still relies on traditional plant medicines for the treatment of common illness (WHO, 2002; Zhang, 2004). The bacterial strains developed its genetic ability to various pharmacological antibiotics (Cohen, 1992). The synthesized drugs associated with adverse effects which lead to immunosuppression and allergic reactions (Lopez, Hudson and Towers, 2001). The formulation of appropriate and efficient antimicrobial drugs to the patient is ultimate goal in this decade. Plants are traditionally used in the treatment of bacterial and fungal infections for its wide range of bioactive molecules. Phytochemicals are applied as natural anti pathogenic which can be derived from leaves, stems, barks and flowers of plants (SajadYousuf *et al*., 2011). The traditional plant medicine is getting back with modern science all over the globe. The extracts from medicinal plants are used in the treatment of different diseases of humans, plants and animals (Nostro *et al*., 2000). *Curculigo orchiodes* Gaertn is a spreading endangered seasonal plant belongs to the family Hypoxidaceae. *C.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). The active compounds that have been reported are flavones, glycosides, steroids, saponins, triterpenoids and other secondary metabolites (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. In the present study, we investigated the potential of aqueous and ethanolic extracts of *C.orchiodes* in antimicrobial property against the six gram-negative bacteria.

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MATERIALS AND METHODS

Plant materials

Curculigo Orchioides Gaertnunderground bulbils were collected in August, 2009 from Madurai, identified by local experts and authenticated by Dr.P.Brintha, Associate Dean, Department of CARISM, SASTRA University, Thanjavur. Voucher specimen was deposited in the herbarium of the Department of CARISM, SASTRA University, Thanjavur, Tamil Nadu, India. Collected plant materials were cut and shade dried, powdered and used for extraction. Shade dried powder was used for the phytochemical screening.

Preparation of extract

Exactly 250g of the powdered bark of the plant was cold extracted using 70% (v/v) aqueous and likewise ethanol for 4

days. The mixture was later filtered and the filtrate was first concentrated in vacuum using rotary evaporator to remove the solvent. The aqueous residue left was then lyophilized to obtain the crude extract. The extract was brownish in colour and the yield obtained was 23.6% of the powdered bark.

Microorganisms

The test organisms were (*Erwinia amylovora, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Enterobacter cloacae*) obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune. The strains were kept at 4 °C on agar slant and sub cultured at 37 °C for 24 hr nutrient agar (Sigma–Aldrich, Germany) before any susceptibility test.

Phytochemical Analysis

Phytochemical tests for the screening and identification of bioactive chemical constituents in the C.orchioidies were carried out with the extracts using the standard procedure as described (Harborne 1973; Scalbert, 1991; Tanaka *et al*., 1992). For each test, 1mL of each solvent extract was used for analysis, in exception for the saponin test in which 3mL solvent extract was used.

Test for Saponins

Extract was placed in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Phenols and Tannins

Extract was mixed with 2mL of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Terpenoids (Salkowski's Test)

Extract was mixed with 2 mL of chloroform. Then 2mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration of the interphase was formed to show positive results for the presence of terpenoids.

Test for Flavonoids (Shinoda Test)

Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink,or purple coloration indicates the presence of flavonoids.

Test for Glycoside

Extract was mixed with 2mL of glacial acetic acid containing 2 drops of 2% $FeCl_3$. The mixture was poured into another tube containing 2mL of concentrated sulfuric acid. A brown ring at the interphase indicates the presence of glycosides.

Antimicrobial activity test

The antimicrobial activity of the crude extract was determined in accordance with the disc diffusion method described by Russell and furr (1977) and Irobi *et al*. (1994). The bacterial isolates were first grown in nutrient broth for 18h before use at 37^{o}C. One hundred microliter of the standardized bacterial suspension was evenly spread on Mueller-Hinton agar using a glass spreader. Commercially prepared sterilized disc was used to fill with the solution of the extract taking care not to allow spillage of the solution to the surface of the disc. The plates were allowed to stand on the laboratory bench for 1hr to allow proper diffusion of the extract into the media. The bacterial isolates were thereafter incubated at 37^{o}C for 24hr after which they were observed for zones of inhibition. The effects of the extract on the test bacterial isolates were compared with standard antibiotic Kanamycin.

Screening of antimicrobial activity

Disc diffusion method was carried out to evaluate the antibacterial activity by using Muller Hinton agar. Sterile filter paper disc whatmann (No:1, 6mm) was impregnated with concentration of 40 mg, plant extract. The disc was properly placed on already seeded Muller Hinton agar plates, sterile DMSO serve as a negative control. All the plates were incubated at 37^{o}C for 24hrs the antibacterial activity was interpreted by determining the diameter of zone of inhibition(in mm).

RESULTS

Phytochemical analysis of aqueous and ethanol extract of *C. orchioides* rhizome is shown in Table 1.

Table 1. Phytochemical analysis of aqueous and ethanol extract of *C. orchioides* rhizome

S.No	Test	Aqueous extract	Ethanol extract
1.	Saponin	+	–
2.	Tannin	+	+
3.	Terpenoids	–	–
4.	Sterol	–	+
5.	Flavones	–	+
6.	Coumarin	+	–
7.	Quinone	+	+
8.	Lignin	+	+
9.	Alkaloid	+	+
10.	Carbohydrates	+	+
11.	Starch	–	–
12.	Protein	+	+

Tannin, quinone, lignin, alkaloid, carbohydrates and protein were found in both extract of *C. orchioides* rhizome. Saponin and coumarin were found only in aqueous extract of *C. orchioides*rhizome. Sterol and flavones were found only in ethanolic extract of *C. orchioides* rhizome. The present study was aimed to evaluate the in vitro antimicrobial activity of aqueous and ethanolic extract of *C. orchioides* rhizome extract against six cultures namely *Erwinia amylovora, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Enterobacter cloacae* (Table 2). Both aqueous and ethanolic extracts showed antibacterial activity; however, the extracts differ in their activities against the microorganisms tested. In aqueous extract of *C. orchioides* showed maximum antimicrobial activity against *Klebsiella pneumonia* (18.49 mm) than other organisms. *Proteus mirabilis* shows minimum

(6.34 mm) inhibitory activity against aqueous extract while *Enterobacter cloacae* have no inhibitory activity. Likewise ethanolic extracts of rhizome exhibited maximum inhibitory activity against the all microorganism but differ in zone of inhibition (*Klebsiellapneumonia* – 27.3 mm). *Enterobacter cloacae* shows minimum (6.34 mm) inhibitory activity than other organisms. Among the two extracts, ethanolic extract has maximum antimicrobial activity against the six microorganisms.

Table 2. Zone of inhibition for aqueous and ethanol extracts of *C. orchioides* activity against Gram-negative bacteria

Microorganisms	Zone of Inhibition (mm)		
	Aqueous extract	Ethanol extract	Kanamycin
Erwiniaamylovora	13.39 ± 0.94	18.23 ± 0.92	22.51 ± 1.73
Klebsiellapneumoniae	18.49 ±1.02	27.3 ± 1.24	24.91 ± 1.96
Escherichia coli	10.72 ± 0.84	15.09 ± 0.95	26.37 ± 1.92
Proteus mirabilis	6.34 ± 0.42	9.28 ± 0.74	18.71 ±1.03
Pseudomonas aeruginosa	7.92 ± 0.58	12.72 ± 0.41	28.41 ±1.83
Enterobacter cloacae	–	6.82 ± 0.48	20.33 ± 1.51

Diameter of a sterile disc is 6mm. Concentration of each plant extract was 40 mg/disc. Values are mean inhibition zone (mm) ±S.D of three replicates; No inhibition (–).

DISCUSSION

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search of new antimicrobial drugs of plant origin (Ahmad and Beg, 2001). Bioactive compounds from plants serve as an novel source for infectious disease management as an alternate to synthetic drugs and several phytochemicals have been derived from the plant materials like bark, stem, leaves, roots, fruits, seeds, fruit rind, flowers and whole plants (Chanda *et al*., 2010). Thousands of diverse natural products are produced by plants and many of these are involved in plant defense mechanism. The phytochemical diversity of antimicrobial compounds include terpenoids, saponins, phenolics and phenyl propanoids, pterocarpans, stilbenes, alkaloids, glucosinolates, hydrogen cyanide, indole and also elemental sulphur, the sole inorganic compound (Cooper *et al*., 1996). Both the extracts of *C. orchioides* rhizome showed the presence of saponin, tannin, terpenoids, sterol, flavones, coumarin, quinone, lignin, alkaloid, carbohydrates, starch and protein, is in agreement with previous phytochemical studies of herbal plants.

The analysis of the plant extracts revealed the presence of phytochemicals which are known to exhibit medical and physiological activities. For example, tannins are polyphenolic compounds that bind to proline rich protein that interferes with protein synthesis (Sanches *et al*., 2005; Tsuchiya *et al*, 1996) and has shown to have antibacterial activity (Akiyama *et al*., 2001). Flavonoids are hydroxylated polyphenolic compounds known to be produced by plants in response to microbial infections to which this aspect has been extensively studied and found to have antimicrobial activity against an array of microorganisms in vitro (Cowan, 1999). Their ability has been attributed to form complexes with extracellular soluble proteins and bacterial cell walls (Trease and Evans, 1989). Terpenoids although mainly used for their aromatic qualities have also been found to be potential agents against inhibiting bacteria (Tsuchiya *et al*, 1996). Saponins which are glycosides have been found to have inhibitory effects on gram-positive organism, *S. aureus*. Therefore, the phytochemical analysis revealed that the aqueous and ethanolic extract have chemical compounds that have been found to possess antibacterial activity, which could contribute to the results obtained from antibacterial analysis.

Antibacterial activity obtained in this study varied with solvents (aqueous and ethanol) used for extraction. In the present study, Ethanol extract of *C. orchioides* displayed effective antimicrobial activity against selected pathogens with the inhibition zone in the range of 7–27 mm. These results are in parallel to the finding of previously reported study by AnbuJeba *et al*. (2009). The antimicrobial activity of the rhizome may be due to presence of phenolic active compounds in *C. orchioides* (Xu *et al*., 1992). Vinoth *et al*. (2012) reported predominant antibacterial activity in the organic solvent as compared to water, which indicates that the active compounds responsible for the bactericidal activity are more soluble in the organic solvents. Similar to this, in the present study, ethanol extract had the maximum inhibitory activity when compared to water extracts. Thus, the present study establishes that the organic solvents are having more powerful antibacterial activity than the water extracts, as opined by Maji *et al*. (2010).

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *Klebsiella pneumoniae* is the most important member of the Klebsiella genus of Enterobacteriaceae and it is emerging as an important cause of neonatal nosocomial infection (Gupta *et al*., 1993). *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitated and immune deficient patients. Infection caused by Salmonella typhimurium is a serious public health problem in developing countries and represents a constant concern for the food industry (Mastroeni, 2002). The demonstration of antimicrobial activity against Gram-negative 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.

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