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ORIGINAL ARTICLE

ANTIMICROBIAL ACTIVITY OF *CURCULIGO ORCHIOIDES* GAERTN. CALLUS EXTRACT AGAINST SELECTED HUMAN PATHOGENS

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ABSTRACT

Antimicrobial efficiency of *Curculigo orchioides* callus extract was examined against *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans*. The zone of inhibitions are determined at 10 mg/ml concentration of methanol extracts of callus on agar well plate and MIC against tested microorganism. The zone of inhibition ranged between 8.7 ± 1.5 (*Pseudomonas aeruginosa*) and 16.0 ± 1.3 mm (*Staphylococcus aureus*). The finding's indicate that the callus extract of *Curculigo orchioides* show better inhibitory activity against only gram positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus* than gram negative bacteria such as like *Escherichia coli*, *Salmonella typhimurium* , *Pseudomonas aeruginosa* and *Proteus vulgaris*. However the methanol extract showed good zone of inhibition with fungi (*Candida albicans*). Similar results have been observed in the minimum inhibitory concentration assays. The results indicate that *Curculigo orchioides* callus extract has good antimicrobial activity against the tested organisms because of the presence of phytoconstituents. Further work is needed to isolate the active principle from the plant extracts and to carry out pharmacological studies.

Keywords: Antibacterial activity; Antifungal activity; *Curculigo orchioides*; callus Extract; Agar well diffusion method; Minimum inhibitory concentration.

1.INTRODUCTION

Plants are rich sources of effective and safe medicines that are often used in the treatment of various ailments. There are many published reports from different parts of the world on the antimicrobial properties of medicinal plants; plants are still recognized as the bedrock for modern medicine is treated infectious diseases (Tomoko *et al*., 2002). Systematic screening of them may result in the discovery of novel effective compounds (Wagh *et al*., 2007). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Balandrin and Klocke, 1988). Global researches have shown that different parts of the plants which include; stem, root, flower, barks leaves, etc. possess antimicrobial property (Samy and Ignacimuthu, 2000; Mahesh and Satish, 2008; Selvamaleeswaran *et al*., 2010).

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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, diarrhea and wound healing (Dhar *et al*., 1968). Active compounds such as flavones, glycosides, steroids, saponins, triterpenoids and other secondary metabolites have also been reported (Misra *et al*., 1990; Xu *et al*., 1992).

Plant tissue culture offers an effective method for enhancement of secondary metabolites, which are in high demand for their therapeutic value. It has been reported that callus and regenerated plants have shown enhancement of secondary metabolites, when compared to natural products (Bolda *et al*., 2011). These secondary metabolites may show antibacterial and antifungal activity. Hence, the aim of the present study was the antimicrobial activity of *Curculigo orchioides* callus extracts against six species of bacteria and a fungus.

2.MATERIALS AND METHODS

Callus Induction and Proliferation

The rhizome was cut into pieces of 2 cm size (proximal rhizome discs) and washed thoroughly with tap water for removing sand and microbes. Then the proximal discs were treated with fungicide (1 g/l of bavistin) and bactericide (100 mg/l of streptocycline) for 2 hrs. Finally the explants were washed with double distilled water for clearing the fungicide and bactericide on the explant. The treated proximal discs were sterilized with 0.1 % mercuric chloride for 6 min and finally washed with sterile doubled distilled water for five times (for clearing mercuric chloride from the surface of the explant). After surface sterilization, both ends of the explant were cut and trimmed to 1 cm size.

The explants were placed in a culture bottle individually on MS medium supplemented with the combination of various concentrations of auxin and cytokinin ranging from 0.5 -2.5 mg/l of 2.4-D and BAP for callus induction (Table – 1). All the culture bottles were incubated at 25 ± 1° C under a relative humidity of 50 to 60% (Ramawat, 2008). After forty five days, the replacement of MS-medium was supplemented with same concentration of hormones for the enrichment of callus development.

Extraction of Samples

The friable callus was removed from the culture bottle with the help of forceps, cut into pieces and shade dried. The organic constituents of callus materials were obtained by continuously extraction from shade dried callus (100 g) using a Soxhlet apparatus, with methanol as solvent. The extract was filtered through a filter paper (Whatman no.1), and then the solvent was removed under reduced pressure by using a rotary evaporator at 40ºC (Harborne, 1973). The final concentration of the crude extract was 2.5 %.

Test Organisms

The tests were performed on six species of selected bacteria *Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus* and one fungal species *Candida albicans*. All the bacterial species tested were maintained on Mueller-Hinton medium, Potato dextrose agar was used for fungus.

Antimicrobial Activity

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 microbial isolates were first grown in nutrient broth for 18h at 37°C. One hundred microliter of standardized bacterial suspension was evenly spread on Mueller-Hinton agar using a glass spreader and *Candida albicans* on PDA. 100 mg of crude extract was dissolved in 10 ml of DMSO (10 mg/ ml). 300 µl of crude extract was transferred in sterilized disc, taking care not to allow spillage of the extract from the disc surface. Then the extract treated discs were transferred on the surface of agar medium and time allowed for proper diffusion of the extract into the media (30 min). The petri plates were thereafter incubated at 37 °C for 24 hrs. (for bacteria) and 25 ºC for 72 hrs (for fungus), after which they were observed for zones of inhibition. The effects of the extract on the tested bacterial isolates were compared with the standard antibiotic - Ciprofloxacin (10 mg/ml). Negative controls were done using sterilized paper discs loaded with 300 µl of DMSO. The experiments were repeated in triplicate; and the zone of inhibitions observed.

Determination of MIC

The antibacterial activity of the callus extracts was determined using minimum inhibitory concentration (MIC) by the method described by Wiegand *et al*., 2008. The methanol extract (2.5 mg/ml) of *Curculigo orchioides* callus was taken and serial dilution of the extract with Luria broth for bacterial culture and potato dextrose for fungus. The broth medium was inoculated with the respective microbes. The micro plates were incubated for 24 hrs. at 37° C. The lowest concentration without visible growth was defined as MIC.

3.RESULTs

The maximum callus initiation and proliferation was observed in MS medium supplement with 1.5 & 2 mg/l concentration of 2,4-D. and BAP (Table–1). The antimicrobial activity of the methanol extract of *Curculigo orchioides* callus was examined, both qualitatively and quantitatively at 10 mg/ml (100µg) concentration by the presence or absence of microbial growth and zone of inhibition on agar well plates.

Table – 1. Concentration of Auxin and Cytokinin in MS medium for Callus Induction and Proliferation

Combination of Hormones supplemented in MS medium mg/l		Observation
2.4-D.	BAP	
0.5	0.5	Poor callus development
1	1	Minimum callus proliferation
1.5	1.5	Maximum callus proliferation
2	2	Maximum callus proliferation
2.5	2.5	Poor callus development and medium turned into block in colure

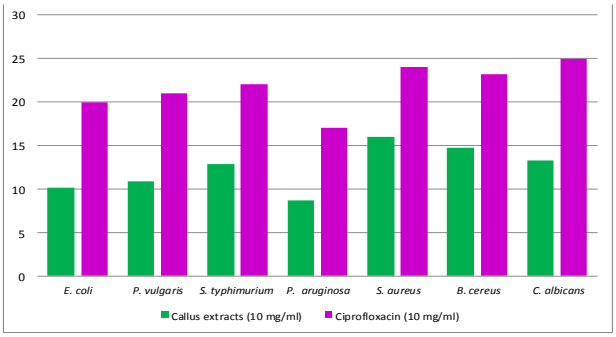
The antimicrobial effects of crude extracts of callus are summarized in Table – 2 & Graph -1. The callus extract of Curculigo orchioides showed good inhibitory activity against only gram positive organisms like *Staphylococcus aureus* (16.0 ± 1.3), *Bacillus cereus* (14.7± 2.5) and average antibacterial activity against gram negative organisms like *Escherichia* coli (10.1± 1.5), *Salmonella typhimurium* (12.9 ± 1.6), *Pseudomonas aeruginosa* (8.7 ± 1.5) and Proteus vulgaris *(10.9* ± 1.2). However the methanol extract showed good zone of inhibition in *Candida albicans* (13.3 ± 0.5). The zone of inhibition of Ciprofloxacin (10 mg/ml) against tested organisms shows the range between 17.0 ±

0.95 (*Pseudomonas aeruginosa*) and 25.0 ± 4.52 (*Candida albicans)*.

Table 2. Antimicrobial activity of methanol extract of *Curculigo orchioides* callus against human pathogenic organisms

Microorganisms	Diameter of Zone of inhibition (mm)	
	Callus extract (10 mg/ml)	Ciprofloxacin (10 mg/ml)
Escherichia coli	10.1 ± 1.5	20.0 ± 0.6
Proteus vulgaris	10.9 ± 1.2	21.0 ± 0.3
Salmonella typhimurium	12.9 ± 1.6	22.0 ± 0.41
*Pseudomonas **aeruginosa***	8.7 ± 1.5	17.0 ± 0.95
Staphylococcus aureus	16.0 ± 1.3	24.0 ± 0.9
Bacillus cereus	14.7 ± 2.5	23.2 ± 0.5
Candida albicans	13.3 ± 0.5	25.0 ± 4.52

Graph – 1: Antimicrobial activity of methanol extract of *Curculigo orchioides* callus against human pathogenic organisms

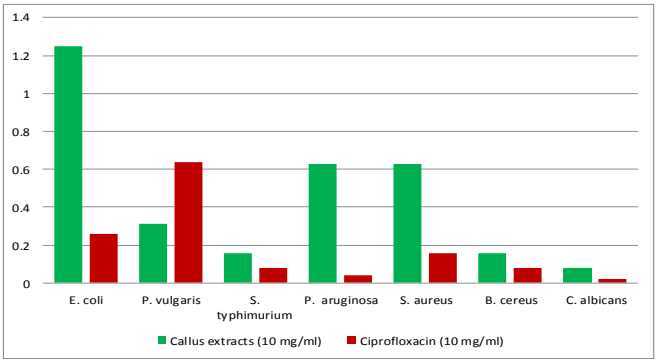


The minimum inhibitory concentration (MIC) of the extracts were revealed on 1.25mg/ml for *Escherichia coli*, 0.312 mg/ml for *Proteus vulgaris*, 0.156 mg/ml for *Salmonella typhimurium* and *Bacillus cereus*, 0.625 mg/ml for *Pseudomonas aeruginosa* and *Staphylococcus aureus*, 0.078 mg/ml *for Candida albicans* and (Table – 3 & Graph -2) the minimum inhibitory concentration (MIC) of the Ciprofloxacin against tested organisms shows the range between 0.019 (*Candida albicans*) and 0.625 *(Proteus vulgaris)*.

Table - 3. Minimum Inhibitory Concentration (MIC) of methanol extract of *Curculigo orchioides* callus against human pathogenic organisms

Microorganisms	Callus extract (mg/ml)	Ciprofloxacin (mg/ml)
Escherichia coli	1.25	0.156
Proteus vulgaris	0.312	0.625
Salmonella typhimurium	0.156	0.078
Pseudomonas aeruginosa	0.625	0.039
Staphylococcus aureus	0.625	0.156
Bacillus cereus	0.156	0.078
Candida albicans	0.078	0.019

Graph 2: Minimum Inhibitory Concentration (MIC) of methanol extract *Curculigo orchioides* callus against human pathogenic organisms



4.DISCUSSION

In ancient times, herbal medicines were the only cures various ailments in India. Now-a-days, in addition to other treatments available, plants are becoming active principle forms of medicine in developing countries. Several antibiotics used in the treatment of various infections have a limited antimicrobial spectrum, develop drug resistance in pathogens and lead to serious side effects. The efforts were directed to identify plant products, which have broad therapeutic use such plants, have been listed and described in ancient Indian treatises as having antimicrobial activity. The urge and need to find out a broad based inexpensive and alternate health care system have kindled interest and promoted studies on traditionally approved medicinal plants (Borris, 1996).

The present study is an attempt to evaluate the antimicrobial activities of the methanol extracts of *in vitro* propagated callus of *Curculigo orchioides*. The method used for testing of the antimicrobial activity was agar disc diffusion method (qualitative) and Minimum Inhibitory Concentration (Quantitative).

According to Bai (1990) the effectiveness of the plants was not due to one constituent, but to the combined action of other chemical compounds involved in it. Bioactive compounds like alkaloids, flavonoids show antimicrobial potential (Rojas, *et al*., 1992). In this study the gram positive organisms like *Staphylococcus aureus*, *Bacillus cereus* were more sensitive to methanol extracts of *in vitro* propagated callus of *Curculigo orchioides* than the gram negative organisms (Table 2 & 3) like *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* because the gram negative organisms have phospholipid membrane in addition to the inner peptidoglycan layer, which makes the cell more impermeable for exogenous molecules (Nikaido & Vaara, 1985) and also may be the presence of phenolic active compounds in callus extract is responsible for effective antimicrobial activity against Gram-positive than Gram-negative strains (Xu et al., 1992). The reasons for the variation of antimicrobial activity may be that different solvents have varying degrees of solubility in different phytoconstituents depending in the nature of bacterial resistant (Majorie, 1999). The present study suggests that the methanol extracts of *in vitro* propagated callus of *Curculigo orchioides* possesses significant antibacterial activity at very low concentrations against the *S. aureus*. The results of the study also support the traditional application of the plant and suggest that the plant extracts possess compounds with antibacterial properties that can be applied as antimicrobial agents.

The results indicate that the methanol extract of *in vitro* propagated callus of *Curculigo orchioides* possesses potential antibacterial and antifungal compounds. High level of activity was exhibited against both gram positive and gram negative organisms, qualitatively and quantitatively. Based on the above observations are may conclude that the callus extract contains significant antimicrobial phytoconstituents and that may be evaluate for their effect

on microorganisms causing infections like typhoid fever, urinary tract infections, septicemia, toxic shock syndrome, skin infection, nosocomial infection, arthritis and diarrhea.

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