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Studies on *In vitro* antibacterial efficacy of *Curculigo orchioides Gaertn*

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Abstract
The antibacterial activity of *Curculigo orchioides* root extracts were tested against ten bacterial species by disc diffusion method. Ethanol, ethyl acetate, acetone, chloroform and petroleum ether solvents were used for the extraction. Among the five solvents used ethyl acetate extract exhibited greater inhibitory effect against *Klebsiella oxytoca (29±1.7), Klebsiella pneumoniae (19.66±5.68), Staphylococcus lentus (16.66±2.5), Eschericia coli (14±1), Klebsiella oxytoca (13±2.6), Staphylococcus haemolyticus (13.33±5.50), Bacillus cereus (12.33±1.52), Staphylococcus aureus (11±1)* and *Enterobacter amnigenus (11.33±1.52)* followed by acetone, chloroform and ethanol extracts against *Staphylocoocus lentus*, *Bacillus cereus* and *Staphylococcus lentus* respectively. Petroleum ether extract of the plant root was not exhibited any inhibitory activity against all the tested organisms.

Keywords: antibacterial activity, *Curculigo orchioides*, disc diffusion assay, human pathogens, secondary metabolites

Introduction
The search for effective antibacterial drugs has switched to medicinal herbs in recent decades. The majority of them are highly successful in treatment of a variety of infectious disorders in the body, and the antibacterial efficiency value is given to particular plants is often astounding. (Parastoo Karimi Alavijeh *et al.*, 2012) [17]. According to conservative estimates, around 10% of all blooming plants in the world were being used by local groups at some point in the past, but just 1% have attained popularity recognition by modern scientists (Lopez *et al.*, 2001) [8]. Only 95 species of medicinal plants are used to make the 120 organic medications that are recommended around the universe. About 5000 of the approximately 2 million blooming higher plants already have their medicinal value examined. (Davis, 1994) [5]. Numerous reports have demonstrated high prevalence of microbial resistance towards many drugs in the treatment of serious diseases with antimicrobial drugs in medical advancements. Due to increased resistance of many microorganisms towards established antibiotics, investigation of the chemical compounds within traditional plants has become desirable (Shelef, 1983) [28]. Pharmacological therapy of infectious disorders continues to be a challenge in medical advancements, with numerous studies revealing a rise as in occurrence of bacterial resistance to a variety of antibiotic. (Evans *et al*, 2001) [7].

Curculigo orchioides is a sub-tropical Himalayan acaulescent plant species occurring from Kumaon to the West Bengal, Khasi Hills, Manipur, Bihar, Chota Nagpur, and the Western Ghats. (Sharma *et al*., 2002) [27]. In Siddha medicine, the root tubers is being used to cure pains, hyperglycemia, and leucorrhoea, as well as as an aphrodisiac; in Ayurveda it is used for treatment of diseases such as sprue, piles, disorders of blood, and also as an aphrodisiac and rejuvenator. These can also be used in association with flavourings and bitters for skin issues, as a demulcent, diuretic, tonic, diarrhoea, piles, jaundice, and asthmatic (Yoganarasimhan, 2000). Which is said to have anti-oxidant (Venukumar and Lathanm, 2002; Wu *et al.*, 2005) [29, 33], anti-inflammatory, and hepatoprotective (Rao and Mishra, 1996a, 1996b) [19] efficiency (Venukumar and Lathanm, 2002; Wu *et al*., 2005) [29, 33]. Antihepatotoxic effects have been found for curculigenin A and curculigol (Rao and Mishra, 1997) [21]. It has even been found being used as a Yang efficient and effective herb, with regulatory effects on glandular malfunction in rabbits with glandular ectomies (Min *et al*., 1998) [15]. In accordance with International Union for Conservation of Nature (IUCN) status (2012), Curculigo orchioides Gaertn. is indeed a near endangered herbaceous perennial belongs to the Hypoxidaceae family. It has anti-diabetic, anti-cancer, hepatoprotective and anti-neurodegenerative qualities, among other things (Wang *et al*., 2012; Chauhan and Dixit, 2007; Gulati *et al*., 2015) [32, 4, 10].

People of China, India, and a few other Asian countries have been utilized it all in traditional system of medicine procedures since ancient times. It is also employed in Ayurveda and Unani herbal pharmaceuticals. *C. orchioides* is being used in traditional medicine for a long time. It's pleasant, cold, mucilaginous, promotes Kapha and lowers Pitta daha (burning feeling), functions as a booster, and offers vigour, according to Raj Nighantu. Musali rhizome, when made into a paste with goat's milk or honey and applied topically to the face, enhances the skin. It is widely utilised by Ayurvedic practitioners in modern times, particularly as a component in aphrodisiac formulations. (Agrahari *et al.*, 2010) The rhizome has been used to treat piles, asthma, diarrhea, jaundice and on pimples (Wala and Jasrai, 2003) [31]. It is also used as anti-oxidant (Venukumar and Latha, 2002) [29], spermatogenic hepatoprotective (Venukumar and Latha, 2002) [30], immunostimulant (Bafna and Mishra, 2006) [3], anticancer (Raaman *et al*., 2009) [18], antibacterial (Nagesh and Shanthamma, 2009) [16],

antiosteopoprotic (Jioa *et al*., 2009) and hypoglycaemic (Jain *et al*., 2010) [11]. Phytochemical constituents seem to be in short supply due to their low adverse effects and compatibility with human physiology. (Sen et al. 2010) [26]. *Curculigo orchioides* Gaertn contain three steroids, sitosterol, stigmasterol (Rao *et al*., 1978) [22] and yuccageninCurculigo orchioides Gaertn (Garg et al., 1989) [9] has only one alkaloid, lycorine, which has been isolated and identified. Gas liquid chromatography was used to separate a variety of fatty acids using its root oil. Mehta *et al*. (1980) [14] identified them as oleic, palmitic, arachidic, linoleic and behenic acids. Numerous people all over the universe have investigated and tested on it both *in vivo* and *in vitro* due to its enormous value and utilisation. (Sahay *et al*., 2016) [24].

Therefore, the goal of this study was to assess the antibacterial activity of *C. orchioides*, a plant utilised in Ayurveda and traditional medicine to treat microorganism-caused symptoms. The potential action of *C. orchioides* extracts against bacterial infections was investigated in this study.

Materials and Methods

The plant material of *C. orchioides* was collected (Fig.1) and the healthy plant parts were selected and it is allowed to shade dried until they dried well and broken by hand easily in the laboratory at room temperature. After drying the plant parts were ground to a fine powder by using an electronic blender and stored in a closed container at room temperature for further uses. 50 gms of the plant leaf and stem powdered material was separately impregnated with 300 ml of each of the solvents *viz.*, ethanol, ethyl acetate, acetone, chloroform and petroleum ether and it was filtered through Whatman No.1 filter paper. The paste like extracts were stored in pre-weighed screw cap vials and the yield of extracts was calculated based on initial and final weight of the container. The extractions have been maintained in the screw cap vials in the refrigerator at 4 degree Celsius. Before to usage, each extraction being separately regenerated with a small amount of such extractant. The ten bacterial species were selected and the species were purchased from the Department of Microbiology, K.AP Viswanathan medical college, Tiruchirappalli, Tamil Nadu. The following gram-positive bacteria *viz.*, *Staphylococcus lentus*, *Staphylococcus haemolyticus*, *Staphylococcus aureus* and *Bacillus cereus and t*he gram-negative bacteria *viz.*, *Escherichia coli*, *Serratia marcescens*, *Enterobacter amnigenus*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Brevibacterium paucivorans* were used for testing. Whatman No.1 filter paper is the most convenient material for preparing the discs. The plant extract is soaked into uniformly sized filter paper discs, which are then sterilized in an autoclave. The standard procedure was used for the antibacterial test. The sterile Nutrient Agar medium was poured (10-15 ml) into each sterile petriplates. After solidification, 100 µl of suspension containing 10^8CFU/ml of each test bacteria were spread over Nutrient Agar plates. The sterile filter paper discs (6 mm in diameter) were impregnated with 10 µl of the 3 mg/ml extracts (30 µg/disc) placed on the inoculated agar. The same solvents used to dissolve the leaf extract, have been used to make negative control. Chloramphenicol (30 µg/disc) were used as positive reference control to determine the sensitivity of plant extract on each bacterial species. At 37° C the inoculated plates were incubated for 24 hrs. Antibacterial activity was evaluated by measuring the zones of inhibition against the test organisms. Each assay was conducted in triplicate.

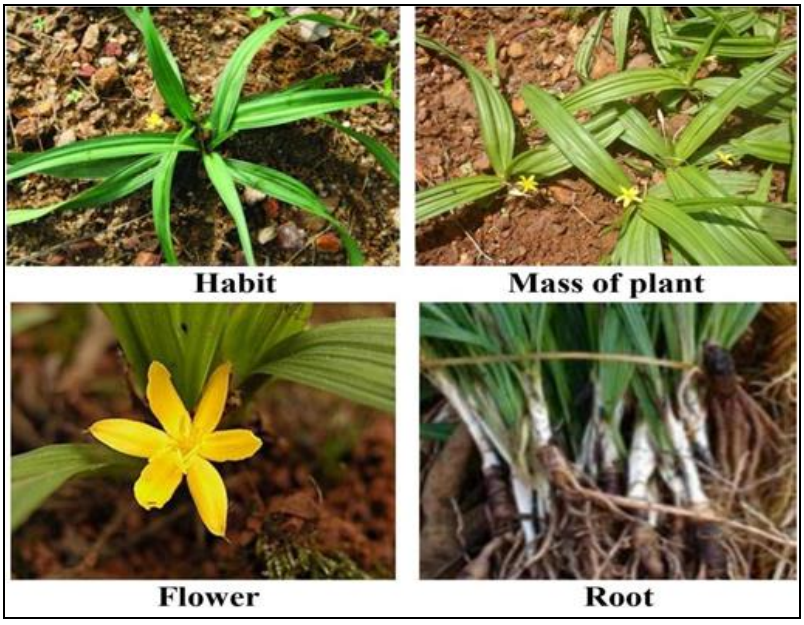

Fig 1: Habit of *Curculigo orchioides*

Results and Discussion

In vitro antibacterial activities of ethanol, ethyl acetate, acetone, chloroform and petroleum ether extracts of root was evaluated by disc diffusion assay against clinical pathogenic bacteria. The bacteria include both gram–positive and gram–negative. Comparing the five extracts and with standard antibiotic (Chloramphenicol), the ethanol, ethyl acetate, acetone and chloroform of the root extracts observed to have highest potential compared to petroleum ether extract (Fig.2).

The antibacterial efficacy of ethyl acetate root extract exhibited much greater inhibition against *Klebsiella oxytoca* (29±1.7), *Klebsiella pneumoniae* (19.66±5.68), *Staphylococcus lentus* (16.66±2.5), *Eschericia coli* (14±1),

Klebsiella oxytoca (13±2.6), *Staphylococcus haemolyticus* (13.33±5.50), *Bacillus cereus* (12.33±1.52), *Staphylococcus aureus* (11±1) and *Enterobacter amnigenus* (11.33±1.52) (Table-1). Moderate results were observed against *Serratia marcescens* (8.66±0.57) and *Salmonella typhi* (8.66±1.15) (Fig.2). Ethanol root extract showed a highest activity against *Enterobacter amnigenous* (20±5), *Staphylococcus haemolyticus* (17.33±1.52), *Staphylococcus lentus* (16±1), *Eschericia coli* (16.33±1.52), *Staphylococcus aureus* (15.33±2.08) and *Serratia marcescens* (15.66±1.15). Whereas, on gram-negative bacteria, tuber and leaf extracts of *C. latifolia* had a greater inhibitory zone than other extracts, particularly on Klebsiella spp. and P. aeruginosa. (Reza et al., 2016) [23].

Moderate inhibition was observed in the most tested bacteria on chloroform and acetone extracts. No results found in Petroleum ether extract against all tested bacteria (Table-1). Among the various organic solvents used the antibacterial activity of ethanol extracts was higher than that of aqueous extracts, which could be owing to the antibacterial principle being either polar or non-polar and only extractable via organic solvents. (Essawi and Srour, 2000) [6]. It's possible that this is due to the fact that different solvents possess varying levels of solubility for distinct phytocompounds. (Majorie, 1999) [13]. The effect of antibacterial in medicinal plants varies intensely depending on the phytochemical features of plant families and subfamilies and even the grown area (Al-Mariri and Safi, 2014; Sarac and Ugur, 2009) [2, 25]. However in this present study revealed that the ethyl acetate extract of *C. orchiodes* were exhibited maximum inhibition zone compared with other organic solvents.

Conclusion

The results of this study back up the claims that *Curculigo orchioides* (root) has been used in folk medicine to treat a variety of infectious diseases caused by bacteria and fungi. Further research is required, to determine the pure extracts' potential efficacy as antibacterial agents. The current findings will serve as a foundation for selecting medicinal flora for further research into the identification of new biologically active chemicals.

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Table 1: Antibacterial screening of root extracts of *Curculigo orchioides* Gaertn. On pathogenic bacteria (Disc diffusion method)

Inhibition zone diameter in mm (mean ± SD)											
Test bacteria	Ethyl acetate		Ethanol		Acetone		Chloroform		Petroleum ether		Positive Control * (30 mcg/disc)
	E (30 µg/disc)	N	E (30 µg/disc)	N	E (30 µg/disc)	N	E (30 µg/disc)	N	E (30 µg/disc)	N	
Staphylococcus haemolyticus	13.33 ± 5.50	-	17.33 ± 1.52	-	9.66 ± 2.08	-	8.66 ± 1.15	-	-	-	15.3 ± 0.57
Staphylococcus lentus	16.66 ± 2.51	-	16 ± 1	-	9.66 ± 2.88	-	9.66 ± 1.15	-	-	-	20.6 ± 1.55
Staphylococcus aureus	11 ± 1	-	15.33 ± 2.08	-	-	-	8 ± 0	-	-	-	10.6 ± 0.57
Bacillus cereus	12.33 ± 1.52	-	14.66 ± 5.77	-	9.66 ± 2.88	-	10.66 ± 0.57	-	-	-	9.0 ± 1.00
Salmonella typhi	8.66 ± 1.15	-	14.66 ± 2.51	-	8.33 ± 0.57	-	10.33 ± 1.52	-	-	-	13.3 ± 1.52
Eschericia coli	14 ± 1	-	16.33 ± 1.52	-	12.33 ± 2.08	-	9.66 ± 2.08	-	-	-	19.0 ± 1.00
Serratia marcescens	8.66 ± 0.57	-	15.66 ± 1.15	-	9.66 ± 0.57	-	8.33 ± 0.57	-	-	-	18.6 ± 1.51
Enterobacter amnigenous	11.33 ± 1.52	-	20 ± 5	-	10 ± 2.82	-	10.66 ± 2.08	-	-	-	19.6 ±0.57
Klebsiella pneumoniae	19.66 ± 5.68	-	13.33 ± 5.13	-	9 ± 1.41	-	9.66 ± 0.57	-	-	-	18.6 ± 1.51
Klebsiella oxytoca	29 ± 1.7	-	13.33 ± 5.50	-	-	-	9.66 ± 2.08	-	-	-	20.6 ± 1.15

Note: *'*' represents as Chloramphenicol, '–' represents as 'no inhibition, 'E' represents as 'Experimental' 'N' represents as 'Negative control'*

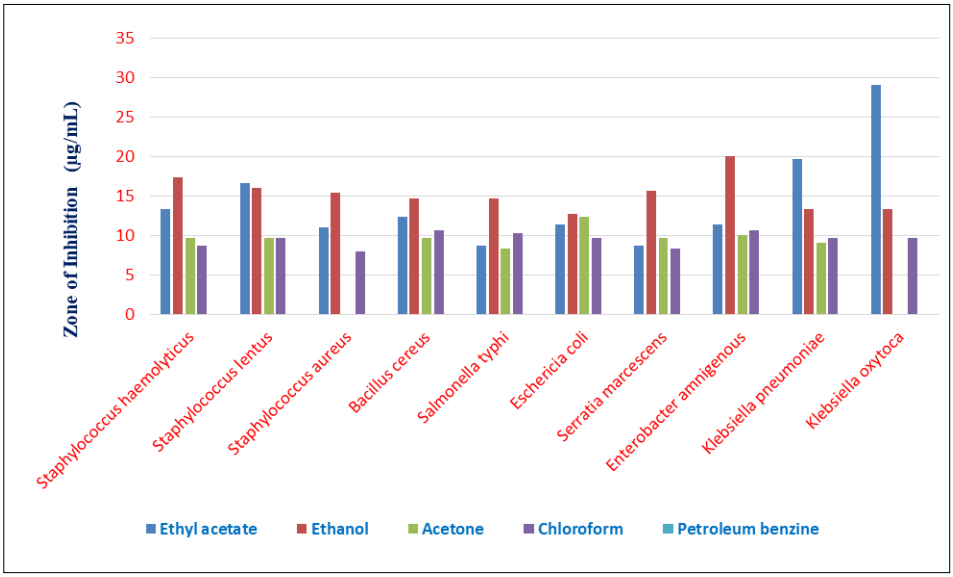

Fig 2: Antibacterial screening of root extracts of *Curculigo orchioides* Gaertn. on pathogenic bacteria (Disc diffusion method)

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