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Antibacterial activity of *Centella Asiatica* (Linn.,) leaves

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Abstract

Centella asiatica (Linn.) urban belonging to family Umbeliferae, it is a medicinal plant described by Charaka as an anti-aging plant. An experiment was carried out to study the antimicrobial activity of water extract of *Centella asiatica* plant by disc diffusion method. Zone of inhibition produced by water extract in dose of 62.5, 125, 250,500, 1000 ug/ml against various type of bacteria were analysed. *Centella asiatica* (500 ug/ml) have the potent antibacterial activity against *E.coli*.

1. Introduction

Humans have frequently used plants to treat common infectious diseases, and some of these traditional medicines are still part of the habitual treatment of various maladies. Due to their disease treatment and micro-organism elimination features, antimicrobials are very important chemicals. There are a great variety of antimicrobial agents currently available. Before choosing a particular antimicrobial agent to employ against a disease or a particular micro-organism, its selective toxicity must be taken into account, due to the fact that it is more important to eliminate the bacteria without harming the host organism. Antibiotics are important biochemical produced by micro-organisms and widely employed in current medical use for a long time in semi-synthetic forms. Unfortunately, uncontrolled use of antibiotics, caused from either patients or prescriptions made without cell cultures analyses, increased resistance of bacteria. Increment in resistance and some other problems caused an increasing interest in antimicrobial plant extracts (1). Each and every class of antimicrobial agents represents a unique mode of action against a particular microorganism. These actions are mostly dependent on the type of microorganism, which can be related to the cell structure. As an example membrane structures of gram negative and gram positive have essential differences which totally affect their antimicrobial resistance mechanisms (2). There is an increasing concern about safety and quality of foods, especially in meat products, which led numerous developments in meat preservation. Although, synthetic preservatives have usually been employed for this purpose but their use is limited due to their side effects. That is why, in order to overcome the microbial contamination in meat, the use of bioactive phytochemicals as natural preservatives are more preferred by both customers and food industry (3).

Many plants were found to contain compounds, which are used as natural medicines to treat common bacterial infections. Indian medicinal plants are regularly used in various system of medicine because of minimal side effect and cost effectiveness. The potential for developing antimicrobials from higher plants appears rewarding as it may lead to the development of phytomedicine against microbes. Hence the sensitivity study of bacterial strains to the plant *Centella asiatica* (CA) was evaluated. CA (Linn.) urban belonging to family Umbeliferae is very useful medicinal plant described by Charaka as an antiaging plant. It is creeping plants, but leaves are bigger and long petiolate (4). The leaves are entire, crenate, orbicular and reniform. Leaves are 1.5-6.5 cm in diameter, petioles 7.5-15 cm in length, stipules are short forming sheathing base. It bears an umbel inflorescence with 3-4 pink sessile flowers. The stems are red and show long internodes. This plant is found in marshy areas all over India, Sri Lanka, Madagascar and Africa up to an altitude of 650 m. Various chemical constituents are reported in CA like asiatic acid, asiaticoside, madecassoide, madecassic acid, glucose, rhamnose, terpenoids , sitosterol, stigmasterol, fatty oil consist of glycerides of palmitic acid, stearic acid, linoleic acid, linolenic acid, ascorbic acid, . It also contains calcium, iron, and phosphate (5). In Ayurveda, an Indian system of medicine, this is used in the management of central nervous system, skin and gastrointestinal disorder (6). CA has been shown to improve memory, general

mental ability of mentally retarded children (7). It was also shown to have wound healing property (8), anticancer property (9), antileprotic property (10), antistress activity (11), and is also shown to have increase antioxidant prominence in stress condition (12). Therefore, our study was concentrated on the role of CA in augmenting the antimicrobial properties.

2. Materials and Methods

2.1 Collection of plant materials

The fresh leaves of CA leaves were collected in Thanjavur, Tamilnadu, INDIA. The accuracy of plant selection was proved and authenticated by Department of Botany, Annamalai University, Tamilnadu, India.

2.2 Extraction of plant material

The fresh leaves of the plant were air-dried at 40^0C and ground to powder, which was then subjected to exhaustive extraction using water in a Soxhlet apparatus. The dark green liquid extract was concentrated under vacuum and the resulting dried extract was lyophilized and preserved in a refrigerator at 4^0C until use in the experiments.

2.3 Bacteria samples

Bacteria [*Klebsiella pneumonia* (Gram negative), *Escherichia coli* (gram negative), *Streptococcus aureus* (Gram positive), *Streptococcus pyogenes* (Gram positive)] were obtained from the Department of Microbiology, Annamalai University, Annamalai Nagar. Bacterial cultures were maintained on Muller Hinton Agar which was stored at 4°C.

2.4 Disc diffusion method

The paper disc diffusion method was used to determine antibacterial activity which is based on the method of Ali et al., (13). Muller-Hinton agar inoculated with bacteria (200 µl of bacteria suspension in 20 mL of medium) was poured into petridishes to give a solid plate. CA was dissolved in water at room temperature. Sterile paper disc (6mm) were loaded with different concentrations (62.5, 125, 250, 500 and 1000 µg/ml) of CA and then applied to the petridishes. The plates were incubated at 37°C for 24 hours. Incubation zone diameter around each of the discs were measured and recorded at the end of the incubation time (14). The activity of extract was compare with standard ciprofloxacin (10µg/ml). The minimum dimension of the zone of no microbial growth and minimum inhibitory concentrations (MIC) were determined**.**

3. Result and Discussion

Medicinal herbs used in ayurverdic are effective against wide range of diseases due to the presence of secondary metabolites such as phenolic compounds (15). Therefore, we also examined the antimicrobial activity of CA extracts against several pathogenic micro-organisms. Present research work deals with the evaluation of antimicrobial activity of CA. In this research work water extract was prepared and the antimicrobial activity was performed by using agar disc diffusion method. Our results indicate that the water extracts of CA exhibit antimicrobial activity.

The bacterial strains viz., *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus and streptococcus pyogenes.* Dimethyl sulphoxide (DMSO) is used as a control while ciprofloxacin is used as a reference for bactrial study. The demonstration of antimicrobial activity against both gram-positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds as also reported by Masola et al 2009 (16). Phytochemical constituents such as tannins, saponins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores (17, 18).

Table 1: Antibacterial activity of Water extract of *Centella asiatica*

Bacteria	Zone of Inhibition (mm) (µg/ml)					MIC (µg/ml)	Ciprofloxacin (10µg/ml)
	62.5	125	250	500	1000		
Escherichia coli	00	00	00	12	15	500	20
Klebsiella pneumonia	07	10	13	14	17	62.5	26
Streptococcus aureus	00	00	06	11	13	250	22
Streptococcus pyogenes	00	08	13	15	16	125	24

MIC (minimum inhibitory concentration)

The antibacterial study revealed that 62.5,125, 250, 500 and 1000µg/ml of CA possess significant antimicrobial activity against *E.coli, K.pneumonia, S.pyogenes and S.aureus* which shown in table 1. Though CA having significant antimicrobial activity, the CA extract at the concentration of 500 µg/ml shows more potent antimicrobial activity against *E.coli,* 62.5µg/ml for *K. pneumonia,* 250 µg/ml for *S aureus* and 125 µg/ml for *S. pyogenes* which are the major pathogens causing infectious diseases to the human beings.

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