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BIOLOGICAL EFFICACY OF CENTELLA ASIATICA (L) urban AGAINST OPPORTUNISTIC PATHOGENS

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ABSTRACT

An assay was carried out to study the antimicrobial activity of ethyl acetate, ethanol, acetone, chloroform and petroleum ether extracts of Centella asiatica (L) urban herb by disc diffusion assay. The tested bacterial strains were Staphylococcus haemolyticus, Staphylococcus lentus, Staphylococcus aureus, Bacillus cereus, Eschericia coli, Serratia marcescens, Enterobacter amnigenous, Klebsiella pneumoniae, Klebsiella oxytoca and Brevibacterium paucivorans. Zone of inhibition produced by different extracts against the selected strains was measured and compared with standard antibiotic chloramphenicol (30µg). The present study demonstrated that the ethyl acetate, ethanol and acetone extract of Centella asiatica have higher antimicrobial activities (average 7-12 mm zone of inhibition) than n-hexpetroleum ether and chloroform extracts. All the extracts showed better results against the tested bacterial strains comparing with chloramphenicol (30µg). The results obtained in the present study suggest that the different extracts of Centella asiatica revealed a significant scope to develop a novel broad spectrum of antibacterial effect.

KEY WORDS

Centella asiatica, ethyl acetate extract, opportunistic pathogens, inhibitory effect, antibacterial activity

INTRODUCTION

Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries. [1] A wide range of medicinal plants parts is used to extract as raw drugs and they possess varied medicinal properties. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw materials for many herbal industries [2]. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics. [3] The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds. [4] The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. [5] Hence the sensitivity study of bacterial strains to the plant Centella asiatica was evaluated.

Centella asiatica (L) urban belonging to the family Umbeliferae is a common perennial herbaceous creeper flourishing abundantly in moist areas and distributing widely in tropical and subtropical countries including Bangladesh. Various chemical constituents are reported in Centella asiatica like asiaticoside, madecassoide, madecassicacid, asiatic acid, glucose, rhamnose, terpenoids, sitosterol, stigmasterol, fatty oils consist of glycerides of palmitic acid, stearic acid, linoleic acid, linolenic acid vitamins like ascorbic acid. It also contains

calcium, iron, and phosphate. [6,7] C. asiatica is claimed to possess a wide range of pharmacological effects.

Traditional knowledge suggests that "Centella asiatica" is used as anti-bacterial, anti-inflammatory, anti-diabetic, anti-oxidant, antifungal, for wound healing, can improve blood circulation, strengthens veins, helps to decrease anxiety, stress, fatigue and can be good remedy for skin conditions such as eczema, chronic ulcers, sore, & scleroderma, for cough expectoration and also helps to increase lactation. It is one of the most valuable herbs in Ayurvedic medicine, because as per Ayurveda knowledge it is used as Medhya Rasayana, ie. brain tonic, it can revitalize nerve & brain cells, increases memory & concentration & has an overall rejuvenating effects on our body. As per Chinese traditional medicinal knowledge, it has anti-ageing properties for skin & tightens older skin, helping to prevent wrinkling and can be used in cream to get rid of cane blemishes. Most of the tribes from India use it for wound healing, for bringing down the fever & treating dysentery in children & for diarrhea. The present study is aimed to make aware the people about the importance and role of natural products for our health care as compared to synthetic drugs.

MATERIALS AND METHODS

PLANT MATERIAL

The aerial part of plant of Centella asiatica was collected from Sirumalai hills of Dindigul district. It was then botanically identified.

PREPARATION OF PLANT POWDER

The plant parts were carefully examined and old insect damaged, fungus infected leaves, stems and roots were removed. The selected healthy plant parts were spread out and shade dried in the laboratory at room temperature for 5-8 days or until they broke easily by hand. The dried plant parts were ground to a fine powder by using an electronic blender and the powders were stored in a closed container at room temperature for further uses.

PLANT EXTRACTION

SOLVENT EXTRACTS

Fifty grams of the powdered leaf material (leaves and roots) was boiled separately with 300 ml of each of the solvents viz. ethyl acetate, ethanol, acetone, chloroform and petroleum ether in a soxhlet apparatus for 48 h at different temperatures (depends on the boiling point of the respective solvents). At the end of 48 h each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature. The paste like extracts were stored in pre-weighed screw cap bottles and the yield of extracts was calculated based on initial and final weight of the container. These screw cap bottles with the extracts were kept in refrigerator at 4℃. Each of the extract was individually reconstituted by using minimal amount of the extracting solvent prior to use.

ANTIBACTERIAL ACTIVITY TEST (Disc diffusion method)

DISC PREPARATION

The filter paper discs of uniform size are impregnated with the compound (plant extract) usually consisting of absorbent paper. It is most convenient to use Whatman No.1 filter paper for preparing the discs. Dried discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These dried discs were used for the test.

TESTED MICROORGANISMS

Antibacterial activity of Centella asiatica powder extracts was investigated against ten bacterial species such as Staphylococcus haemolyticus, Staphylococcus lentus, Staphylococcus aureus, Bacillus cereus, Eschericia coli, Serratia marcescens, Enterobacter amnigenous, Klebsiella pneumoniae, Klebsiella oxytoca and Brevibacterium paucivorans. The species that were not purchased were originally opportunistic pathogens isolated from clinical cases of suspected symptomatic HIV/AIDS patients of Perambalur district of Tamil Nadu. All the strains were confirmed by bio chemical characteristics and maintained in slants for further use.

PROCEDURE

Sterile liquid Muller Hinton Agar medium (pH 7.4 ± 2) was poured (10-15 ml) into each sterile petriplates. The growth media also seem to play an important role in the determination of the antibacterial activity. Based on the report by Lin et al. Muller- Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study. [8] After solidification, 100 µl of suspension containing 108 CFU/ml of each test bacteria was spread over Muller Hinton Agar plates. The sterile filter paper discs (6 mm in diameter) were impregnated with 10µl of the 3 mg/ml extracts (30µg/disc) placed on the inoculated agar. Negative controls were prepared in using the same solvents employed to dissolve the plant extract. Chloramphenicol (30µg/disc) was used as positive reference control to determine the sensitivity of the

plant extract on each bacterial species. The inoculated plates were incubated at 37° C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones. Each assay was conducted in triplicate.

RESULTS AND DISCUSSION

The antibacterial activity of the different organic solvents extracts of Centella asiatica leaf was studied against opportunistic bacterial pathogens isolated from HIV/AIDS patients of Perambalur district. The in vitro results were observed in terms of inhibition zone around each disc caused by diffusion of antibacterial properties from the plant extract impregnated disc into the surrounding medium. The results of antibacterial screening of ethyl acetate, ethanol, acetone, chloroform and petroleum leaf extracts of C. asiatica (30 mg/disc) are depicted in Table 1. All these extracts showed antibacterial activity to at least two of the tested bacterial pathogens. Among the various solvent extracts tested, ethyl acetate showed maximum activity against Brevibacterium paucivorans (12.33mm), Staphylococcus haemolyticus (11mm) and Bacillus cereus (10.33mm) respectively. The same extract showed moderate inhibition against S. aureus (9 mm), Enterobacter amnigenous and Klebsiella pneumoniae (8mm). Similarly, ethanol extract showed second highest inhibition zone against Staphylococcus haemolyticus (10mm), Klebsiella oxytoca (9.5mm) and Serratia marcescens (9mm). This confirmed the report of [Okwu and Josiah, 2006; Duangkamol et al., 2008 [9, 10] who reported ethanol to be the best solvent for extraction. The present study result is similar with the results of Udoh et al., 2012 [11] who reported ethanol extract of Centella asiatica showed significant activity against Staphylococcus aureus. Acetone extract also exhibit better activity against Bacillus cereus (10.33), Staphylococcus aureus (9.66mm) and Eschericia coli (9.33mm) Ethyl acetate extract was appeared to be the most effective extract than other extracts. All other extracts viz ethanol, acetone and chloroform extracts showed better inhibitory effect on the tested organisms. Petroleum extract showed complete absence of inhibition zones (chloroform and petroleum ether) against most of the tested bacteria. The leaf extract of Centella asiatica showed significant antibacterial activity against all tested bacteria. It was reported that *Centella asiatica* leaves are rich in asiaticoside and Asiatic acid which are considered as the active ingredients in the herb itself that proves its efficacy towards microorganisms such as S. aureus, E. coli, S. pneumonia.s [10, 12, 13] The diameter of inhibition zones for each of the samples were compared with standard antibiotics. It was noted that the inhibition zones of the tested plant extracts to be either less than or greater than or equal to the inhibition zones of standard antibiotics.

Table 1. Antibacterial screening of leaf extracts of *Centella asiatica* (L.) Urb.on pathogenic bacteria

Test bacteria	Ethyl acetate		Ethanol		Acetone		Chloroform		Petroleum ether	
	Experimental (30µg/disc)	Negative control	Experimental (30µg/disc)	Negative control	Experimental (30µg/disc)	Negative control	Experimental (30µg/disc)	Negative control	Experimental (30µg/disc)	Negative control
Staphylococcus haemolyticus	11±3.60	-	10±2	-	7.66±0.57	-	7.33±0.57	-	-	-
Staphylococcus lentus	8±1	-	8.5±2.12	-	8.55±2.12	-	7±0	-	-	-
Staphylococcus aureus	9±1	-	8±1	-	9.66±1.52	-	9±2.64	-	-	-
Bacillus cereus	10.33±0.57	-	8.33±1.52	-	10.33±1.52	-	9.33±0.57	-	7.5±0.70	-
Eschericia coli	7.33±0.57	-	7.66±0.57	-	9.33±2.08	-	7±0	-	8.5±2.72	-
Serratia marcescens	7.33±0.57	-	9±1	-	8.66±1.52	-	7.33±0.57	-	-	-
Enterobacter amnigenous	8±1	-	7±1	-	-	-	-	-	-	-
Klebsiella pneumoniae	8±1	-	8.66±1.15	-	8.66±1.52	-	8.33±1.52	-	-	-
Klebsiella oxytoca	7±0	-	9.5±2.12	-	8.33±1.15	-	9.33±0.57	-	-	-
Brevibacterium paucivorans	12.33±2.68	-	8.33±2.30	-	7.33±0.57	-	9±2	-	-	-

Note: –: No inhibition

CONCLUSION

The present study explores the scope for developing better cost effective and indigenous alternatives which can substitute conventional antibiotics or antimicrobial agents which suffer reduced efficiency due to the development of single or multidrug resistance by most of the pathogenic microorganisms.

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