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Tinospora cordifolia- leaves aqueous extract biocidal activity on gram-negative bacteria

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Abstract

Tinospora cordifolia- leaves aqueous extract is having strong biocidal properties and used as a main home remedial plant since ancient times. Herbal extraction is having slow-acting properties, so a single dose is not effective as allopathic medicine, perhaps long-term exposure is required. Biocidal activity of leaves aqueous extract is tested using human gastro-intestinal gram-negative pathogenic bacterium Aeromonas hydrophila isolated from the wastewater treatment plant. Satisfactory results were obtained that confirm the safety of *Tinospora cordifolia* leaves aqueous extraction as an effective home remedial as well as pharmaceutical product due to having effective biocidal properties. To the best of our knowledge, aqueous extraction of *Tinospora cordifolia* leaves with its biocidal activity is first reported in this study.

Keywords: *Tinospora cordifolia*; Aqueous extract; Herbal extraction; Biocide

1. Introduction

Herbal extractions and their bioactive constituents are used as traditional medicine by 80% of the world population reported the World Health Organization [1] among this *Tinospora cordifolia* a member of the Menispermaceae family is a commonly used herb in general Ayurvedic practices. In ancient Ayurvedic practices, this is a commonly used shrub and also known as “Amrutha”, “Gudhuchi” (Sanskrit), “Gurcha” (Hindi). The plant body is a widely spread deciduous shrub with elongated branches. Leaves having long petiole, ex-stipulate, pulvinate, somewhat roundish, simple and alternate, apex and base twisted partially, one way long and halfway round [2]. Plant body bearing juicy stem is creamy or white-grey with thin bark. The flowering season extends over summer and winter bearing yellow florae with stalked racemes. In India, due to diversity rich soil ranging from alkaline to acid, plants grow easily everywhere and need normal humidity levels. Herbal extractions prepared by using the plant *Tinospora cordifolia* are extensively used for different ailments treatment due to having anti-microbial, anti-inflammatory, anti-periodic, anti-osteoporotic, anti-allergic antidiabetic and anti-spasmodic, anti-cancerous, cardioprotective properties [3]. Biocidal activity of leaf aqueous extract tested by using human suspected opportunistic pathogenic species associated with Gastrointestinal disease Aeromonas hydrophila, a member of genus Aeromonas, ubiquitous in the aquatic environment such as River, lake, raw and processed drinking water and domestic sewage [4,5]. Susruta an ancient physician explained various therapeutic and medicinal values of medicinal plants in “Susruta Samhita” [6]. These old traditional practices contain herbal compounds including metals and minerals with well-designed concentrations in a controlled manner for curative and protective effects [7].

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2. Material and methods

2.1. Plant collection

Tinospora cordifolia leaves were collected from the medicinal and aromatic plants section, Department of Genetics and Plant Breeding College of Agriculture, CCS Haryana Agricultural University, Hisar Haryana, India. Collected leaves were washed with running tap water then with autoclaved water for proper removal of sand and dust particles.

2.2. Aqueous extraction preparation

Collected leaves of *Tinospora cordifolia* dried in a shady area at room temperature (25_+ 2 °C) dried leaves were grounded into powdered form with the help of a grinder. Then leaf powder was stored in a sample container until extraction. Aqueous extraction of leaves was prepared by using Soxhlet (Jain Scientific Glass Works, 18782). Extraction is further subjected to a rotary evaporator/ vacuum oven (Metrex Scientific Instruments Ltd, New Delhi) till obtaining complete extract.

2.3. Biocidal activity

Biocidal activity of *Tinospora cordifolia* leaves extract was performed on Aeromonas hydrophila isolated from medical college Sewage Treatment Plant, Hisar (sequence id MN865804.1) with the help of agar well diffusion method [8]. Bacterial culture prepared at [10]6 CFU used for biocidal activity then plated using Mac Conkey media (Himedia). Different concentration like 10 µl, 50 µl, 100 µl of aqueous extract was used for biocidal activity and it was studied at different regular interval of time respectively 6, 12, 18, 24, 30, 36 hr. All activities are performed in triplicates.

2.4. Data analysis

The biocidal activity graph was plotted by using Origin Pro 8.5 win 10.

3. Results

3.1. Biocidal activity

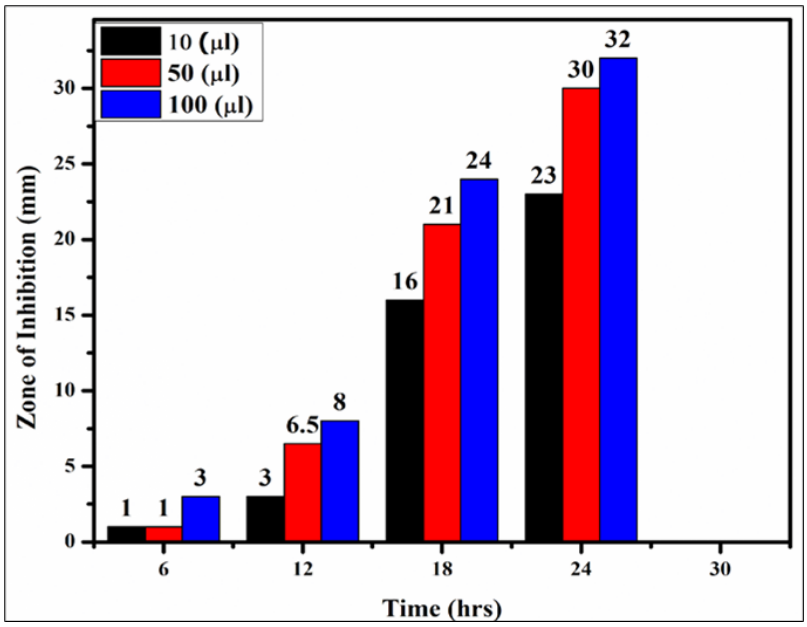

Figure 1 Biocidal activity of plant extract on *A. Hydrophila*

Agar well diffusion method showed biocide activity of *T. cordifolia* at different concentrations specifically 10, 50, 100µL. In 10µL Petri plate A. hydrophila showed a 23 mm zone of inhibition at 24 hrs while at 30 hr there is no bacterial growth obtained. In 50 µL Petri plate maximum zone of inhibition observed was 30mm at 24hr incubation. Similarly, there was bacterial growth observed at 30 hrs. In 100µL Petri plate at 24 hrs showed a 32 mm zone of inhibition. All three

concentrations provide no bacterial growth at 30 hrs and the colour of the Meuller Hinton media plate turned light brown. Fig 1 shows the biocidal activity of plant extract on *A. Hydrophila*.

4. Discussion

Excessive use of antibiotics in day-to-day life created a life-threatening situation that gives rise to superbug formation [9]. Extensive and without prescribed use of antibiotics helps bacteria in gaining antibiotic resistance through horizontal gene transfer. In such situations, we have to opt for alternative medicine or drug product. Biocide isolated from plants proved a better alternative approach to combat with superbug situation. In our study, we observed that *Aeromonas hydrophila* multiple drug-resistant bacteria that can be effectively treated by using plant extract isolated from *Tinospora codifolia* [10]. Plant biocides naturally show coevolution that helps to deal with such situations [11]. As the hypothesis clearly explains when pathogen or selective agents become specialized on the genotype of common host sexual reproduction/asexual favouring frequency-dependent selection in the host population increases the production of novel genotype and adaptation rate. Heavy metal impurities or toxicants can be easily removed with the help of biotechnological applications like chromatography, dilution, extraction, ultrafiltration and diafiltration. Further, we can move with biotechnological approaches like conjugates of antibodies and drugs, where elemental impurities can be controlled with small molecular components.

5. Conclusion

Biocides isolated from plants have slow-acting properties but showed effective results on gram-negative antibiotic resistance bacteria. This could be a possible solution to deal with multiple drug-resistant bacteria.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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