Study of trace metals concentration and antimicrobial properties of tropical Aloe vera plant from southern India

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Abstract
This study was carried out with an objective to investigate the antibacterial and antifungal potentials and trace metals concentrations in Aloe vera (Linn) plant leaves. Fresh leaves of Aloe vera were collected from Tiruchirappalli district of Tamil Nadu during the period of February - March 2014. The 100 g of shade dried A. vera leave power was used to collect the methanol extraction of the test plant by the soxhlet apparatus. The extracted solutions were dried by hot air oven at 60 ℃ for 48-72 h for further analysis. The antimicrobial activity of Aloe vera methanol extract was examined with six various pathogenic microorganisms such as gram positive, gram negative and fungal strains using the disk diffusion test. The two tested concentrations such as 0.60 and 1.20 mg/disc produce zone of inhibition on muller hinton agar (MHA) and potato dextrose agar (PDA) plates for bacteria and fungi, respectively. In this study, higher (1.20 mg) concentration get greater sensitivity than lower (0.60 mg) concentration against all strains. All the microbial strains depict higher sensitivity to the higher concentration (1.2 mg / disc) for the test sample when compared to the positive control except bacterial strains such as Aeromonas liquefaciens MTCC 2645 (B1). The trace metal analyses of the plants were also carried out. The mean concentration of trace metals such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) were 0.04, BDL, 0.06, 0.08, BDL, 0.02 and 0.22 mg kg⁻¹ respectively. Therefore, it is signified that Aloe vera plant extract is safe to be used as an antimicrobial agent. Hence, throughout impoundment is needed to verify the trace metal levels in plants.

Keywords: Aloe vera, Heavy metal, Antimicrobial activity, Ethanol extraction

1. Introduction
Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional medicines. Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms. Genus Aloe is a perennial succulent herb have grown in temperate and subtropical parts of the world. The genus includes 200 or more species. Some of them are cultivated for therapeutic purposes. The present study was emphasized the antibacterial activity and trace metals concentrations.
2. Materials and Methods

2.1 Collection and preservation of specimen

The herbal plant of Aloe vera was collected from the Tiruchirapalli district, Tamil Nadu. They were taken to the laboratory for further analysis and were preserved in 10% neutralized formalin. The Aloe vera was identified based on the taxonomical characteristic studies. The shade dried plant powders (100 g) were successively extracted with methanol by soxhlet apparatus and was used as test sample.

2.2 Extraction of plant leaves

Fresh leaves of Aloe vera were collected from Tiruchirapalli district of Tamil Nadu during the period of February - March 2014. The shade dried plant powder (100 g) was loaded in the thimble of Soxhlet apparatus. The methanol solvent was used in this study. After completion, the extracts were weighted and the percentage yield was calculated (initial weight of raw material / final weight of extract). Then, the extracted solution was dried by hot air oven at 60 °C for 48-72 h.

2.3 Testing of antimicrobial activity

The test strains were: Aeromonas liquefaciens MTCC 2645 (B1), Enterococcus faecalis MTCC 439, Salmonella typhimurium NCIM 2501 (B3), Candida albicans MTCC 1637 (F1), Cryptococcus sp. MTCC 7076 (F2), Trichophyton rubrum MTCC 3272 (F3). The cultures were obtained from MTCC (Microbial Type Culture Collection), Chandigarh and NCIM (National Collection of Industrial Microorganisms), Pune, India. Microbial strains were tested for antimicrobial sensitivity using the disc diffusion method. The antibacterial and antifungal activity of test samples was analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to inoculate the standardized microbial suspension on surface of agar plate. The 0.60 and 1.20 mg/disk of sample coated disks were placed in agar plates, separately. For negative control study, the methanol solvent was used. The plates were incubated at 37±1 °C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition was measured with ruler. The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control. All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

2.4 Testing of trace metals concentration in plants

The Aloe vera plant sample was collected from the Tiruchirapalli district, Tamil Nadu. The plant leaves (surface and jelly part) were carefully removed and washed with sterile distilled water, separately. The cleaned leaves were dried in shadow area and were grinded with agate mortar and pestle. The powdered plant samples were stored in sterile plastic container. The 1 g of powdered plant samples was treated with aqua regia mixture (hydrochloric acid + nitric acid) in Teflon bomb and was incubated at 140 °C for 3–4 days. After incubation, the reaction mixture was filtered with nitrocellulose (0.45 µm) filter paper by Millipore vacuum filtration unit. Then the extraction was test for trace metals (Fe, Cu, Zn, Pd, Cd, Cr and Ni) analysis. The extraction of the studied metals in the solutions was determined by the 797 VA Computrace voltammetry, Metrohm.

3. Results and Discussion

The antimicrobial activity of Aloe vera methanol solvent sample was examined with various pathogenic microorganisms using the disk diffusion test. The results of the antimicrobial activities are summarized in Figure 1. The two tested concentrations such as 0.60 and 1.20 mg/disc produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (1.20 mg) concentration of sample got greater sensitivity than (0.60 mg) lower concentration in all the tested microorganisms. Thuis and his co-workers (2013) stated that the methanol extraction of A. vera were affected the gram positive and gram negative strains in the higher concentration such as 2.5 and 5.0 mg/well. But in this study, we confirmed that the low concentrations (0.60 and 1.20 mg/disk) of the test sample was examined with various pathogenic microorganisms using the disk diffusion test. The results of the antimicrobial activities are summarized in Figure 1. The two tested concentrations such as 0.60 and 1.20 mg/disk produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. All the microbial strains depict higher sensitivity to the higher concentration (1.2 mg / disc) for the test sample when compared to the positive control except bacterial strains such as Aeromonas liquefaciens MTCC 2645 (B1) while smaller effect was noticed from Enterococcus faecalis MTCC 439 (B2). In fungi, this was effective against Candida albicans MTCC 1637 (F1) whereas smaller effect was observed in Cryptococcus sp. MTCC 7076 (F2). All the microbial strains depict higher sensitivity to the higher concentration (1.2 mg / disc) for the test sample when compared to the positive control except bacterial strains such as Aeromonas liquefaciens MTCC 2645 (B1). But, the fungal strains were highly sensitive to test samples than the positive control. There is no antimicrobial activity in negative control such as methanol.

Soil pH, anions and cations is very important because it influences the availability and plant uptake of micronutrients including heavy metals. Toxic chemicals and trace metals are very important pollutants which affects all ecosystems at large extent. The accumulation of trace metals in large quantities to the green plants causes physiological, biochemical, growth and functional changes to the large extent. The results of the trace metal concentrations are summarized in Figure 2. The mean concentration of trace metals such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) were 0.04, BDL, 0.06, 0.08, BDL, 0.02 and 0.22 mg kg⁻¹, respectively. Through the natural process of biomagnifications, minute quantities of metals become part of the various food chains and concentrations become elevated to levels which can prove to be toxic to human, animal, plant and other living organisms. In particularly, higher trace metal in the plants caused progressive reduction in the photosynthetic ability of leaves, closure of leaf stomata, productivity of plants, ascorbic acid content and chlorophyll content.
4. Conclusion

This study has revealed that Aloe vera gel extract had excellent antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in humans. The result of the antimicrobial report explains the use of this plant in folk medicine for the treatment of different diseases and it could be a source of new antibiotic compounds being nontoxic and less expensive than the modern drugs. The trace metal concentrations were not crossing the standard levels and it gives the alarming for bioaccumulation/bio magnifications studies.

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References