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The Pattern of Potassium and Protein Leakage from Microbial Cells by *Vernonia tenoreana* Leaf Extract

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Abstract: The mechanism of action of the leaf extracts of *V. tenoreana* on some pathogens was investigated. The pathogens included *Staphylococcus aureus* (NCIB 67), *Bacillus subtilis* (NCIB3610), *Pseudomonas aeruginosa* (NCIB 950) and *Escherichia coli* (NCIB 86). Crude extract of the leaf was obtained using methanol and distilled water (in ratio 2:3 v/v). The extract leaked potassium and protein from the cells of *S. aureus, B. subtilis, E .coli* and *Pseudomonas aeruginosa*. Protein was leaked increasingly from the bacterial cells during the first 60 mins of interaction, after which a continuous decrease in the protein leakage began. However, the leakage of potassium ions from the cells of the pathogen followed an increasing trend throughout. The leaf extract leaked 0.30 M of potassium from *S. aureus* at ten minutes interaction, while as much as 103.87 M was leaked at 60 min interaction from the same organism.

Key words: Extracts, leakage, potassium ion, protein, organisms

INTRODUCTION

Plants as members of the kingdom Plantae, play a vital role in the existence and survival of man; they provide food and also supply the much required oxygen for breathing, they provide fibres for diverse purposes; wood for housing and shelter, they are also utilized for medicines. Different plants produce different compounds, which vary in their antimicrobial action and microorganisms differ in their sensitivity to these compounds.

The Genus Vernonia includes sixty different species in areas covered by flora of West Tropical Africa[1]. About thirty-three species occur in Nigeria[2]. Vernonia species are generally known to contain chemicals with antimicrobial properties that are useful for producing therapeutic drugs. The leaves are used for human consumption as vegetables, which stimulate the digestive system. Dalziel, in 1956 reported that the active principles in most medicinal plants vary at different stages of plant growth under different climates and ecological conditions. He also reported that the active principles of medicinal plants are usually more concentrated in storage organs like roots, stem, bark and leaves. It is now estimated that, plant materials are present in, or have provided the models for 50% of western drugs[4]. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity[5]. The introduction of antibiotics for the chemotherapy of bacteria infections has been one of the most important medical achievements of the past 50 years.

The basic mechanisms of action of antibiotics are acting as inhibitors of peptidoglycan synthesis, protein synthesis and nucleic acid synthesis by interrupting nucleotide metabolism, inhibiting RNA polymerase, or inhibiting DNA gyrase[6]. In addition, some antibiotics interfere with membrane integrity. Antimicrobial agents exercise similar action on the bacteria, as well as, interacting with the bacterial cell wall or envelopes, produce changes in cytoplasmic membrane, inhibit membrane enzymes and act as alkylating agents[7]. In order for antibiotics to exert their bacteristatic or bactericidal actions on bacteria, they must access intracellular targets. This necessitates, in Gram–negative bacteria that they cross the outer membrane, a substantial permeability barrier[8]. In the last few decades, there has been more interest in drugs obtained from vegetable sources than at any in history. This is due to the success with antibiotics and other plant drugs such as Rauwolfia (for treating mental diseases), Podophyllum and Catharanthus alkaloids for cancerous tumours[9].

A record of medicinal plant practice in early period in Nigeria was virtually not available because there was no record for the isolation, selection and preparation[10]. Every fact about potent herbal plants was passed by words of mouth to generation. Another limiting factor to rapid development of herbal medicine in Nigeria apart from documentation is that most potent plants were in custody

of the medicine men and selected numbers of a particular sect or cult that has sworn under oath not to relay such facts. It has also been proposed that the knowledge of medicinal plants in Nigeria began by accident although some of the traditional medicine practitioners claimed that their ancestors in various ways communicated the information in such plants. However, early men could have gained some knowledge by watching the effects produced by various plants when eaten by domestic animals[9].

MATERIALS AND METHODS

Microorganisms used: The microorganisms used included: *Staphylococcus aureus* (NCIB 67), *Bacillus subtilis* (NCIB3610), *Pseudomonas aeruginosa* (NCIB 950), *Escherichia coli* (NCIB 86) and they were collected from the Department of Microbiology, Obafemi Awolowo University, Ile -Ife.

Collection of plant materials: *V. tenorreana* was collected on a mountain top behind the Agip Filing Station, Ajilosun, Ado-Ekiti, Ekiti State, Nigeria where it was found growing. The plant was authenticated by the curator of the herbarium at the Obafemi Awolowo University, Ile-Ife and confirmed by comparison with herbarium specimens. Voucher specimen was then deposited at the herbarium for future reference.

Fresh leaves of *V. tenoreana* collected between 10 a.m. and 12.00 noon were air-dried on the laboratory bench until well dried. The dried leaves were soaked in methanol/distilled water solvent mixture (2:3 v/v) and left on the laboratory bench for four days. On the fourth day, the solution was filtered and the filtrate dried, using the rotary evaporator, until all the methanol was removed while the aqueous part remaining was removed by freeze drying.

Studies on the potassium ions leakage from four bacterial isolates: The method of Gale[11] was adopted. Eighteen hour cultures of bacteria on nutrient broth namely: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were used. Four sterile test tubes labeled A, B, C and D, each containing 9 mL of the extract (5 mg mL^{-1}) were prepared. Into each test tube was added 1 mL of the bacterial suspension. The solutions were centrifuged at 7000 rpm after 10, 20, 40 and 60 min interaction of organisms with extracts. The supernatant solution obtained after centrifugation were analyzed for potassium ions using the flame photometer. The potassium ion concentration of a blank prepared with the extract solution alone was measured at zero minute and set up as control.

Studies on the protein leakage from selected organisms: The biuret reaction method as described by Olutiola[12] was employed to determine the quantity of protein leaked from the cells of the organisms. Known concentration of protein was prepared as standards using bovine serum albumin. One ml of an eighteen hour bacteria grown on nutrient agar namely: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* were treated with 9 mL of 5 mg mL^{-1} concentration of the extract. After 30, 60, 90 and 120 min of suspension in the extract, each suspension was centrifuged at 7000 rpm. To 2 mL of each supernatant obtained was 1 ml of 3 N of NaOH and 1 mL of a $CuSO_4$ solution the mixture was shaken and the absorbance was measured using a digital spectrophotometer. Triplicate readings were made for each supernatant. The absorbance of a blank prepared with the extract solution alone was measured. From the values obtained for the standard, a calibration curve was plotted and from this curve the protein concentration leaked from the microorganisms were calculated.

RESULTS AND DISCUSSION

Crude extract of the leaf of *V.tenoreana* leaked potassium ions and protein from *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* increasingly for sixty min. Fig. 1 and 2.The extract leaked 0.30M of potassium from *S. aureus* at ten minutes interaction, while as much as 103.87 M was leaked at 60 min interaction from the same organism Fig. 1. At thirty minutes 1.45 mg of protein was leaked from *E. coli* by the extract. Figures showed that 1.13 mg mL^{-1}, 1.6 mg mL^{-1}, 1.5 mg mL^{-1} and 1.18 mg mL^{-1} of protein were leaked by the extract from the same organism at 30, 60, 90 and 120 min interaction Fig. 2.

Gale[11], reported on the release of potassium ions from *Candida albicans* in the presence of polyene antibiotics. The behaviour of the extract is probably similar to that of polyene antibiotics.

Also, Oloke[13] recorded continuous increase for sixty min in the leakage of potassium from *Escherichia coli* by the volatile oil of *Aframamum melegueta.*The trend of protein leakage by the leaf extract from test organisms did not follow that of potassium. The trend is that there is a usual increase in protein leakage up to sixty minutes, after which it decreases and begins to increase again at about 120 min interaction. The same pattern was reported by Oloke[13], whose *Aframamum melegueta*, leaked 21.6, 26.7, 33.0, 20.7 and 25.1 (µg mL) of protein from *Staphylococcus aureus* at 30 min, 1, 2, 3 and 4 hrs of interaction, respectively. The release of potassium ion and protein from the test bacteria by the extract Fig. 1 and 2 showed that release of potassium ion and protein from

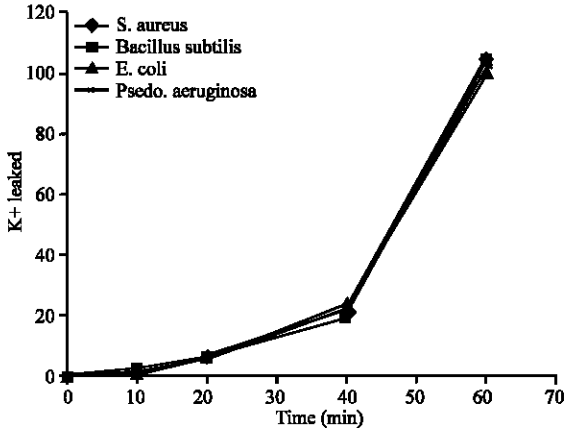

Fig 1: The effect of Vtl on the potassium ion leakage from test organisms Vtl= *V.tenoreana* leaf extract

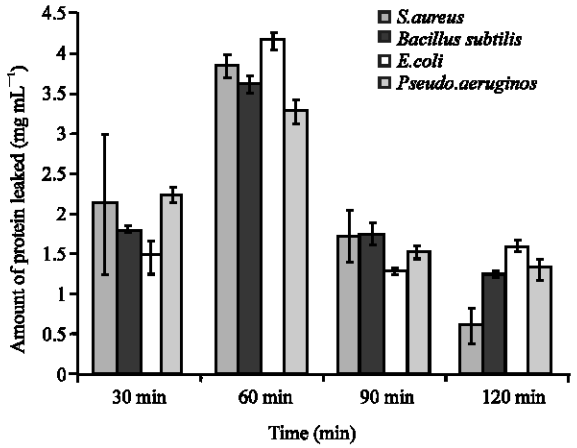

Fig 2: The effect of the Vtl on protein leakage of test isolates Vtl= *V.tenoreana* leaf extract

the cells of the microorganisms are part of the mechanisms of action of these extracts.

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