ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Inhibition Of Dehydeogenase Activity Of Escherichia Coli And Staphylococcus Aureusby Extracts Of Vernoniaamygdalina And Moringa Oleifera.

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ABSTRACT

The ability of extracts of Vernoniaamygdalina and Maringa oleifera to inhibit the Dehydrogenase activities (DHA) of Escherichia coli and Staphylococcus Aureus, used as a measure of its antimicrobial activity, was investigated. DHA of 1.418±0.046 and 2.213± 0.038 mg Formosan/mg cell dry weight/h was recorded for Escherichia coli and Staphylococcus Aureus. The Dehydrogenase activity correlated with extracts concentrations with R^2 values greater than 0.823 (0.823 ≤ R2≤ 0.9858) in all the bacterial isolates having IC_{50} values of 304.42µg/ml and 433.8µg/ml for Maringa oleifera plant extract and 820.69 µg/ml and 895.23 µg/ml for Vernoniaamygdalina plant extract on Escherichia coli and Staphylococcus Aureus respectively.
Keywords: Antimicrobial, Dehydrogenase, Formosan, Inhibitory concentrations.

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ISSN: 0975-8585

INTRODUCTION

Interest in the use of plants as sources of remedy is constantly on the increase owing to the increased resistance to most of the available antibiotics [1].As the increased demand for life expectancy is on the line, Access to potent antimicrobials is limiting and can be enhanced by exploring naturally endowed capacity domiciled in plants [1, 2, and 3]. Plants have been proven to be effective against a wide range of infections and diseases [4, 5, and 6]. Plants' antimicrobial capability is conferred by their possession of active variables called phytochemical which have been proven to be linked with chemotherapeutic bioactivities [1, 6, 7, and 8]. Therefore, quantitative and qualitative methods are needed to explore plants for their therapeutic capacities against infections and diseases. The Kirby bauer method of antimicrobial assay has been explored for its capacity to depict antimicrobial activities of plants against bacteria based on the formation of a "clear zone" also known as "zones of inhibition "which can be measured and reported in millimeters [6]. Due to the poor sensitivity of this assay method to low concentration of antimicrobials, other rapid techniques are constantly developing. Among others, inhibition of microbial Dehydrogenase activity can be used to test the toxicity of a plant extract or an antimicrobial agent against a microorganism [9, 10, 11, and 12). This research exploits this assay technique to investigate the activity of extracts of Maringa oleifera and Vernoniaamygdalina against Escherichia coli and Staphylococcus Aureus.

MATERIALS AND METHODOLOGY

Sample collection and preparation

The leaves of Vernoniaamygdalina and Maringa Oleifera were collected from Nekede, Owerri-west LGA of Imo State, Nigeria. Dr F.N. Bagwig, a plant taxonomist of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria, identified the plants. The fresh leaves of each plant were dried for four days. The dried leaves were ground into powder form using mechanical grinder. To 100 g of the leave powder in a conical flask were added 200 ml of 95% ethanol. This was covered, shaken every after 6 h and then allowed to stand for five days. The solution was subsequently shaken and filtered using Whatmann number 1 filter paper. The filtrate was evaporated to dryness using a rotary evaporator. The extract was stored at 4 ^{o}C.

Preparation of inoculums

The bacterial isolates were Escherichia coli and Staphylococcus Aureus were collected from the Anthony van Leuwenhoek's research centreumuoma, Nekede. The isolates were confirmed using relevant biochemical tests. The bacterial isolates were grown to mid exponential phase in nutrient broth (Lab M) on a rotary incubator (150 rpm) at room temperature (28 ± 2^{o}C). The cells were harvested by centrifugation at 6000 rpm for 8 min and washed thrice in distilled water. The washed cells were re-suspended in distilled water and the turbidity adjusted to an optical density of 0.85 at 500 nm. An aliquot of 0.3 ml of the cell suspension was used as inoculums in the Dehydrogenase activity assay. The dry weight of the cells was determined by drying a 10-ml aliquot of cell suspension in a pre-weighed crucible to constant weight in an oven at 110^{o}C.

Antimicrobial activity evaluation

The Dehydrogenase assay method as described by Alisiet al. [9] and newest al. [12] was adopted for this study. The Dehydrogenase activity (DHA) was determined using 2, 3, 5-triphenyltetrazolium chloride (TTC) as the artificial electron acceptor, which was reduced to the red colored triphenylformazan (TPF). The assay was done in 4 ml volumes of nutrient broth glucose–TTC medium supplemented with varying concentrations (0-2500 µg/ml) of the leaf extract in separate screw-capped test tubes. About 0.3 ml volume of the bacterial suspension was inoculated into triplicate glass tubes containing 2.5 ml of phosphate-buffered (pH 6.8) nutrient broth-glucose medium supplemented with varying concentrations of the plant extract solution. They were incubated in a rotary incubator (150 rpm) at room temperature (28 ± 2 ^{o}C) for 30 min. Thereafter, 1 ml of 0.4% (w/v) TTC in demonized water was added to each tube to obtain final extract concentrations of 0, 20, 50, 100, 200, 400, 800, 1400, 2000 and 2500 µg/ml in different test tubes. The control consisted of the isolates and the medium without Vernoniaamygdalina extract. The reaction mixture was further incubated statically at room temperature (28 ± 2 °C) for 16 h. The triphenylformazan produced was extracted in 4 ml amyl alcohol and

determined spectrophotometrically at 500 nm. The amount of Formosan produced was determined from a dose-response curve [0-200 µg/ml TPF (Sigma) in amyl alcohol].

Determination of inhibition of Dehydrogenase activity

Dehydrogenase activity was expressed as mg of TPF formed per mg dry weight of cell biomass per hour. Inhibition of Dehydrogenase activity in the isolates by Vernoniaamygdalina extract was calculated relative to the control. The percentages of inhibition of each of the test organisms were linear zed against the concentrations of the extracts using gamma parameter described by Kim et al. [13].

$$\% \text{ inhibition} = \left(1 - \frac{OD_{test}}{OD_{control}}\right) \times 100$$

$$\gamma = \frac{\% \text{ Inhibition}}{100 - \% \text{ Inhibition}}$$

The toxicity threshold concentrations (IC_{50}) were then determined from the linear regression plots.

RESULTS AND DISCUSSION

Dehydrogenase activity of isolates

The results of this work revealed that the test isolates can be used as a measure of Dehydrogenase activity (Table 1). Escherichia coli had a DHA of 1.418±0.046 mg Formosan/mg cell dry weight/h while Staphylococcus Aureus had a DHA of 2.213± 0.038 mg Formosan/mg cell dry weight/h. the results obtained from this work agrees with previous work published by Njokuet al. [14] where Staphylococcus sp had a DHA of 2.132 ± 0.12mg Formosan/mg cell dry weight/h. and E. coli had a DHA of 1.127 ± 0.032 mg Formosan/mg cell dry weight/h. In another research, Alisiet al. [9] published that the DHA of Staphylococcus sp. was 1.125 ± 0.056 mg Formosan/mg cell dry weight/h. These results also conform to the results from previous researchers [9, 11, 15, and 16). Praveen- Kumar [17] reported that characteristics such as differences in bacterial physiology, cell wall components or Dehydrogenase systems, causes different microorganisms to have different Dehydrogenase systems. This explains the similarities and the variations in the DHA obtained in this work.

Table 1: The Dehydrogenase activity of the test isolates.

Isolates	DHA (mg Formosan/mg cell dry weight/h)
Escherichia coli	1.418± 0.046
Staphylococcus Aureus	2.213± 0.038

Antimicrobial activity of the plant material using DHA.

The effect of the different concentrations of the extracts on the bacterial isolates with respect to the Dehydrogenase activity is shown in Figure 1a and b. The response of the bacterial Dehydrogenase activities to the extracts is concentration-dependent and varies among the organisms. Dehydrogenase activities were inhibited progressively with increase in concentrations of the extracts in all the bacterial species. However, Vernoniaamygdalina had a lower activity than Maringa oleifera leaf extracts on both isolates. Staphylococcus Aureus was more tolerant to the activity of the plant extracts than Escherichia coli. Previous research reported that Staphylococcus Aureus was more tolerant than Escherichia coli (Njokuet al. [14]. The Dehydrogenase activity correlated with extracts concentrations with R^2 values greater than 0.823 ($0.823 \leq R^2 \leq 0.9858$) in all the bacterial isolates (Figure 2 and 3). The IC_{50} of the Maringa oleifera plant extract 304.42µg/ml and 433.8µg/ml on Escherichia coli and Staphylococcus Aureus while Vernoniaamygdalinaplant extract had IC_{50} values of 820.69 µg/ml and 895.23 µg/ml on Escherichia coli and Staphylococcus Aureus **(Table 2).**

ISSN: 0975-8585

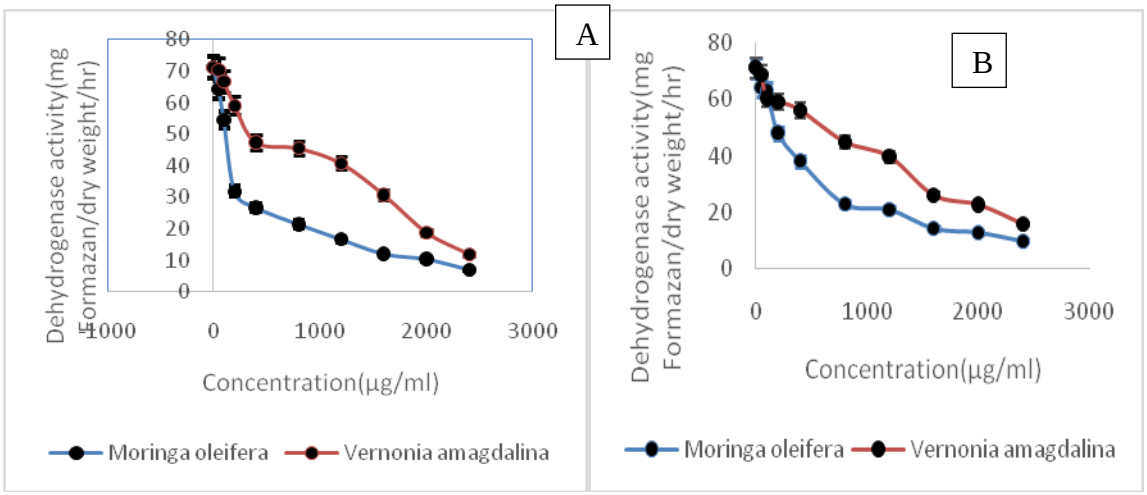

Figure 1: Dehydrogenase activity of extracts of Maringa oleifera and Vernoniaamygdalina against (a) Escherichia coli and (b) Staphylococcus Aureus

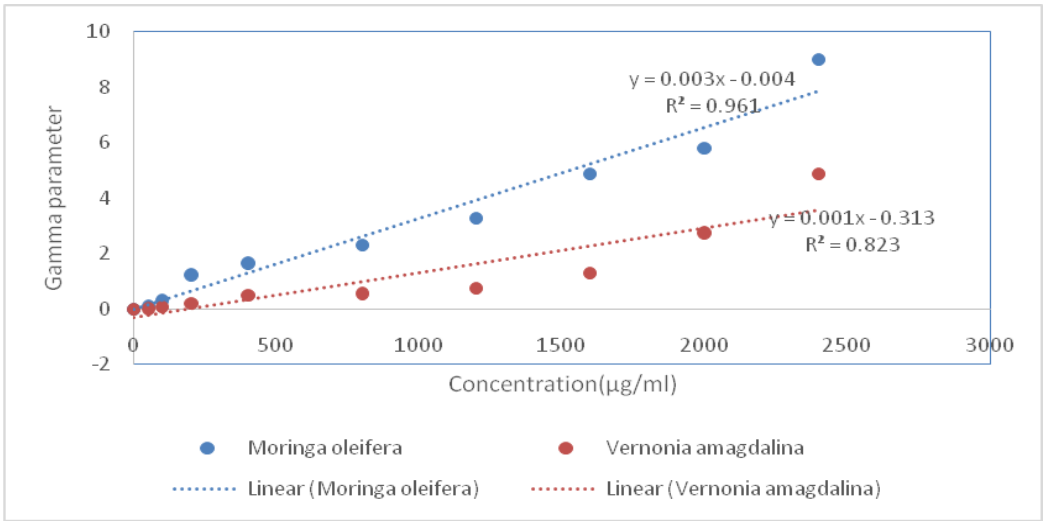

Figure 2: Gamma parameter of extracts of Maringa oleifera and Vernoniaamygdalina against Escherichia coli

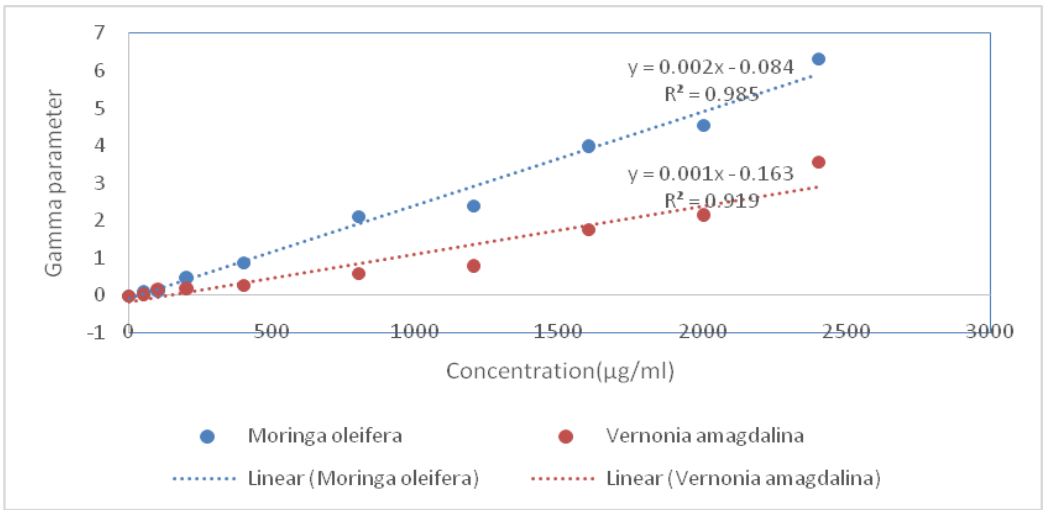

Figure 3: Gamma parameter of extracts of Maringa oleifera and Vernoniaamygdalina against Staphylococcus Aureus

ISSN: 0975-8585

Table 2: IC_{50} of Maringa oleifera and Vernoniaamygdalina on Escherichia coli and Staphylococcus Aureus

PLANT EXTRACT	ISOLATES	ϒ-parameter	R^2 VALUES	IC_{50}(µg/ml)
Maringa oleifera	Escherichia coli	y = 0.0033x - 0.0046	R^2 = 0.9619	304.42
Vernoniaamygdalina	Escherichia coli	y = 0.0016x - 0.3131	R^2 = 0.823	820.69
Maringa oleifera	Staphylococcus Aureus	y = 0.0025x - 0.0845	R^2 = 0.9858	433.8
Vernoniaamygdalina	Staphylococcus Aureus	y = 0.0013x - 0.1638	R^2 = 0.9192	895.23

CONCLUSION AND RECOMMENDATION

Vernoniaamygdalina and Maringa oleifera possess inhibitory activity on the tested isolates. The use of these plants as food condiments may aid in delivering the medicinal variables in the plant to target cells. Also, the use of the plant in inhibiting the activity of the tested isolates is a possibility. The plant should therefore be explored using several other protocols and phototherapeutic principles so as to get the best from the plants.

The IC_{50} of the Maringa oleifera plant extract 304.42µg/ml and 433.8µg/ml on Escherichia coli and Staphylococcus Aureus. Vernoniaamygdalinaplant extract 820.69 µg/ml and 895.23 µg/ml on Escherichia coli and Staphylococcus Aureus.

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