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Full Length Research Paper

Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extracts of *Musa paradisiaca* (var Sapientum)

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Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extract from the unripe fruit peels (called the bark) and leaves of *Musa paradisiaca* var sapientum were investigated via dehydrogenase assay using 2,3,5-triphenyl tetrazolium chloride (TTC) as the electron acceptor. Pure cultures of *Staphylococcus* and *Pseudomonas* species were exposed to varied concentrations of the extract [0 – 2000 µg/ml]. The extracts exhibited concentration dependent response against the tested organisms. Dehydrogenase activities (mg Formazan/mg cell dry weight/h) in the Gram-positive *Staphylococcus* sp. and Gram-negative *Pseudomonas* sp. were 1.125 ± 0.056 and 0.740 ± 0.040, respectively, and were progressively inhibited in the pure cultures. Threshold inhibitory concentrations (IC_{50}) of *M. paradisiaca* bark extract were 143.5 and 183.1 µg/ml against *Staphylococcus* and *Pseudomonas* species, respectively, while the threshold inhibitory concentrations (IC_{50}) of *M. paradisiaca* leaf extract were 401.2 and 594.6 µg/ml, respectively. The IC_{100} of the leaf extract against *Staphylococcus* and *Pseudomonas* species were 1850 and 2000 µg/ml respectively, while the bark could not completely inhibit the organisms at the tested concentrations. The bark and leaves of *M. paradisiaca* may be an available source of raw material for the production of chemotherapeutic agents against pathogenic bacteria.

Key words: *Musa paradisiaca*, dehydrogenase activity, pathogenic bacteria.

INTRODUCTION

Enzyme activity assay has been developed for accurate biochemical method to determine microbiological activity. The preference of dehydrogenase activity over cultural methods in enumeration of microorganisms and monitoring of their activities is due to underestimation of numbers of viable cells as a result of difficulty in being readily desorbed from the substrate matrix or lack of homogeneity in distribution common to culture methods (Mathew and Obbard, 2001).

Measurement of microbial enzyme activity has been used in the assessment of ecotoxicological impacts of environmental substrates. In this regard, dehydrogenase activity has been widely used. The dehydrogenase assay is an effective primary test for assessing the potential toxicity of metals to soil microbial activities (Aoyama and Nagumo, 1997; Chander and Brookes, 1995; Kelly and Tate, 1998; Rogers and Li, 1985), toxicity of metals to planktonic (Nweke et al., 2006) and heterotrophic (Nweke et al., 2007) bacteria from tropical river sediments. Toxicity of antimicrobial agents to pathogenic bacteria has been accessed using the dehydrogenase assay (Nwogu et al., 2007; Nwogu et al., 2008).

In southeastern Nigeria, many fruits, spices, herbs and leafy vegetables used as food and for medicinal purposes are obtained from wild tropical forest where they may be as many as a thousand species (Ibe and Nwufor, 2005). To date, plants continue to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plant products (Sofowara, 1982). The continued emergence or persistence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial

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agents currently in use. Therefore, the search for new drugs from novel sources, such as plant, is necessary (Fransworth and Morris, 1976).

Banana is a tropical tree-like herb, with large leaves of which the overlapping bases form the so-called false trunk. Fully grown, the stem reaches a height of 10 to 30 feet. The unripe fruit of banana, rich in starch, is cooked as food or dried and ground into flour. On ripening of the fruit, the starch turns into sugar. Although the banana family is of more interest for its nutrient than its medical properties, it has some value in traditional medicine. Herbalists use the leaves and the peel of the unripe fruit as antimicrobial agents (unpublished reports).

This study is aimed at investigating the antimicrobial properties of the aqueous extract on two bacterial isolates so as to justify or otherwise its traditional use as an antimicrobial remedy. The study will also expose new frontiers or improve on the current applications of the plant extract.

MATERIALS AND METHOD

Plant materials

Bark part of the fruit and Leaves of the plant *Musa paradisiaca var sapientum* was collected from Amaimo in Ikeduru LGA of Imo State Nigeria. Identity of the plant was confirmed by Mr. J. M. C Ekekwe (a plant kingdom scientific analyst). A voucher specimen (Voucher No.: 0033) is deposited in the authors' laboratory.

Extraction

Aqueous extracts were prepared according to the traditional methods. Briefly, 450 g of plant specimen dried at room temperature was mixed with 2.5 L of already boiling distilled water and boiled for 30 min, decanted and filtered. Extract was concentrated under *vacou* and freeze-dried (Yield 10.3%). The dry extract was stored in a desiccator protected from light and moisture.

Chemicals

All chemicals were analytical grade and chemicals (2,3,5-triphenyl tetrazolium chloride), nutrient broth, nutrient agar, amyl alcohol and triphenyl formazan required for all biochemical assays were obtained from BDH England, Lab M, Fluka, Kemie Lab and sigma Chemicals Co., USA respectively.

Phytochemical screening

Chemical tests were carried out on the powdered specimen using standard procedures to identify the constituents as described by Trease and Evans (1989).

Isolation of bacterial strains and culture conditions

Pathogenic bacteria (*Staphylococcus* sp. from higher vaginal swab (HVS) and *Pseudomonas* sp. from wound were obtained from the Federal Medical Centre Owerri. Isolates were purified on nutrient agar (Fluka) plates and characterizations were done using standard microbiological methods. Identifications to the generic level followed the schemes of Holt et al. (1994). The bacterial strains were grown to mid exponential phase in nutrient broth (Lab M) on a rotary incubator (150 rpm) at room temperature (28 ± 2°C). The cells were harvested by centrifugation at 6000 rpm for 8 min. Harvested cells were washed twice in deionised distilled water and re-suspended in water. The re-suspended cells were standardized in a spectrophotometer to an optical density of 0.70 at 420 nm. The dry weights of the standardized cells were determined by drying 15 ml of cell suspension to constant weight in an oven at 110°C. These standardized cell suspensions were used as inoculum in the dehydrogenase activity assay.

Dehydrogenase activity assay

Dehydrogenase assay method as described by Nweke et al. (2007) was employed with little modification. Briefly, dehydrogenase activity was determined using TTC (BDH England) as the artificial electron acceptor, which was reduced to the red-coloured triphenyl formazan (TPF). The assay was done in 4 ml volumes of nutrient broth-glucose-TTC medium supplemented with varying concentrations (0 – 2000 µg/ml) of extract in separate 20 ml screw-capped test tubes. Portions (0.3 ml) of the bacterial suspensions were inoculated into triplicate glass tubes containing 2.5 ml of phosphate-buffered (pH 6.8) nutrient broth-glucose medium amended with *M. paradisiaca* extract and preincubated on a rotary incubator (150 rpm) at room temperature (28 ± 2°C) for 30 min. Thereafter, 0.1 ml of 1 %(w/v) TTC in deionised distilled water was added to each tube to obtain final extract concentrations of 0, 20, 40, 80, 100, 200, 400, 800, 1000, 1400, 1800, and 2000 µg/ml in different test tubes. The final concentrations of nutrient broth, glucose and TTC in the medium were 2, 2 and 0.25 mg/ml, respectively. The controls consisted of the isolates and the media without *M. paradisiaca* extract. The reaction mixtures were further incubated statically at room temperature (28 ± 2°C) for 4.5 h. The TPF produced were extracted in 4 ml of amyl alcohol and determined spectrophotometrically at 445 nm (λmax). The amount of formazan produced was determined from a standard dose-response curve [0 - 20 µg/ml TPF (Sigma) in amyl alcohol; $y = 0.0487x$; $R^2 = 0.9977$]. Dehydrogenase activity was expressed as milligrams of TPF formed per mg dry weight of cell biomass per hour. Inhibition of dehydrogenase activity in the isolates by *M. paradisiaca* extract was calculated relative to the control. The percentage inhibitions for *Staphylococcus* sp. and *Pseudomonas* sp. were linearized against the concentrations of the extracts using gamma parameter (Γ) [Γ = % Inhibition / (100 - %Inhibition)] (Kim et al., 1994). The toxicity threshold concentrations (IC_{50}) were then determined from the linear regression plots. The total inhibitory concentrations (IC_{100}) were estimated from the linear regressions of log transformation plots of the dose-response data.

Statistical analysis

Data obtained from the study were analysed by the use of two-way analysis of variance (ANOVA) and values for $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the dried leaves and peels of fruit bark revealed the presence of some glycosides, anthocyanine, tannins, flavonoids and carbohydrate. The presence of tannins, flavonoids and saponins suggests possible antimicrobial activity by a plant as

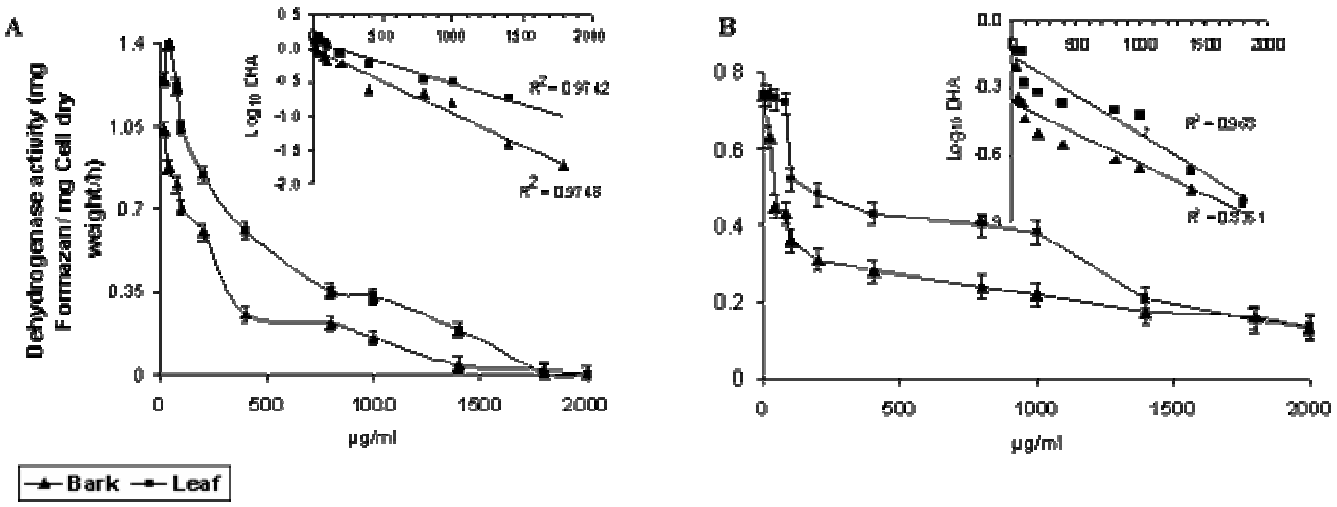

Figure 1. Dehydrogenase activity of *Staphylococcus* sp. [A] and *Pseudomonas* sp. [B] in response to graded concentrations of aqueous extracts of *M. paradisiacal*. Insets represent plot of Log dehydrogenase activity against the various concentrations.

Table 1. Uninhibited dehydrogenase activities in the isolates.

Strain	Dehydrogenase activity (mg Formazan/mg cell dry wt/h)
Staphylococcus sp.	1.125 ± 0.056
Pseudomonas sp.	0.740 ± 0.040

proposed by earlier workers (Levan et al., 1979; Ibrahim et al., 1997). The two organisms *Staphylococcus* sp. and *Pseudomonas* sp. were able to reduce TTC to its formazan and so were used to assess toxicity (antimicrobial effect) of the extract through the dehydrogenase assay.

The dehydrogenase activity (DHA) varied among the bacterial strains (Table 1). The Gram-positive *Staphylococcus* sp. had higher rate of dehydrogenase activity than the Gram-negative *Pseudomonas* sp. This is in agreement with Nweke et al. (2007), Nwogu et al. (2007) and Nwogu et al. (2008) in which the Gram-positive organisms had higher dehydrogenase activity than the Gram-negative organisms. Earlier report (Nweke et al., 2006) is however at variance with this observation. These variations may be due to differences in bacterial physiology, including cell wall components or dehydrogenase systems, since different microorganisms have been reported to have different dehydrogenase systems (Praveen-Kumar, 2003).

The effect of the extract concentration on the DHA and its percentage inhibition of the bacterial strains are shown in Figure 1 and Table 3 respectively. In both *Staphylococcus* sp. and *Pseudomonas* sp., dehydrogenase activity decreased with increase concentration of bark extract (20 - 2000 µg/ml). The leaf extract followed a similar pattern of inhibition of dehydrogenase activity in *Pseudomonas* sp. There was a stimulation of DHA in *Staphylococcus* sp. at low extracts concentration of the leaf (20 - 80 µg/ml), while at higher concentrations, it progressively inhibited dehydrogenase activity in a dose-dependent manner. Some substances that caused toxicity in certain organisms at higher concentrations have been shown to exert a stimulatory effect at micro concentrations (Nweke et al., 2007). The leaf may contain substances that support growth at lower concentration.

The Gram-negative *Pseudomonas* sp. seems to tolerate the extract more than the Gram-positive *Staphylococcus* sp. The evidence is seen from the threshold inhibitory concentration data (Table 2). Dehydrogenase activity correlates with extract concentration as shown in Figure 1. The higher R^2 values ($0.8361 \leq 0.9680 \leq 0.9742 \leq R2 \leq 0.9748$) indicate that the concentration was a strong determinant of dehydrogenase activities in the isolates. Thus, the extract at high concentration exerted serious stress on the organisms.

The log transformation plot of the dehydrogenase activity (Figure 1) and the gamma parameter model (Figure 2) gave good linearization of the dose response data for both bacterial isolates. The higher R^2 value for the linear regression plots of Log DHA and gamma parameter (Table 4) lays credence to the linear relationship. Gamma parameter models had higher R^2 values than the % inhibition plots (Table 4) and hence the linear regression models were used to assess the threshold inhibitory concentration of the extracts on the organism.

The 2-way analysis of variance shows that the dehydrogenase activity and its percentage inhibition varied significantly ($P < 0.05$) with bacteria strain and extract concentration. The result of this *in vitro* study indicated that both extracts were effective against the tested organisms. The bark was more effective than the leaf as an antimicrobial agent, while the Gram-positive *Staphylo-*

Table 2. Threshold inhibitory concentrations of *M. paradisiaca* extract against the pathogenic bacteria isolates.

Bacteria	Inhibitory concentrations		
Staphylococcus sp.	Extract	IC_{50}	IC_{100}
	Bark	143.5 ± 7.1	1600 ± 20.0
	Leaf	401.2 ± 10.2	1850 ± 32.3
Pseudomonas sp.	Bark	183.1 ± 9.7	ND
	Leaf	594.6 ± 15.4	ND

ND = Not determined (above 2000 µg/ml).

Table 3. *M. paradisiaca* inhibition (%) of dehydrogenase activity in pathogenic bacteria isolates.

Bacteria	Extract	*Musa paradisiaca* (µg/ml)										
		20	40	80	100	200	400	800	1000	1400	1800	2000
Staphylococcus sp.	Bark	8.11	22.16	28.65	37.30	46.49	77.30	80.81	86.22	96.49	98.38	100
	Leaf	-10	-24.32	-8.11	7.03	24.86	45.95	68.65	70.81	83.24	99.46	100
Pseudomonas sp.	Bark	15.48	38.71	41.93	50.97	58.71	62.19	67.74	70.32	76.77	78.08	82.01
	Leaf	0.64	1.93	3.22	29.67	35.48	42.58	45.80	48.39	71.61	79.35	83.6

Data are absolute values of mean of triplicate determinations.

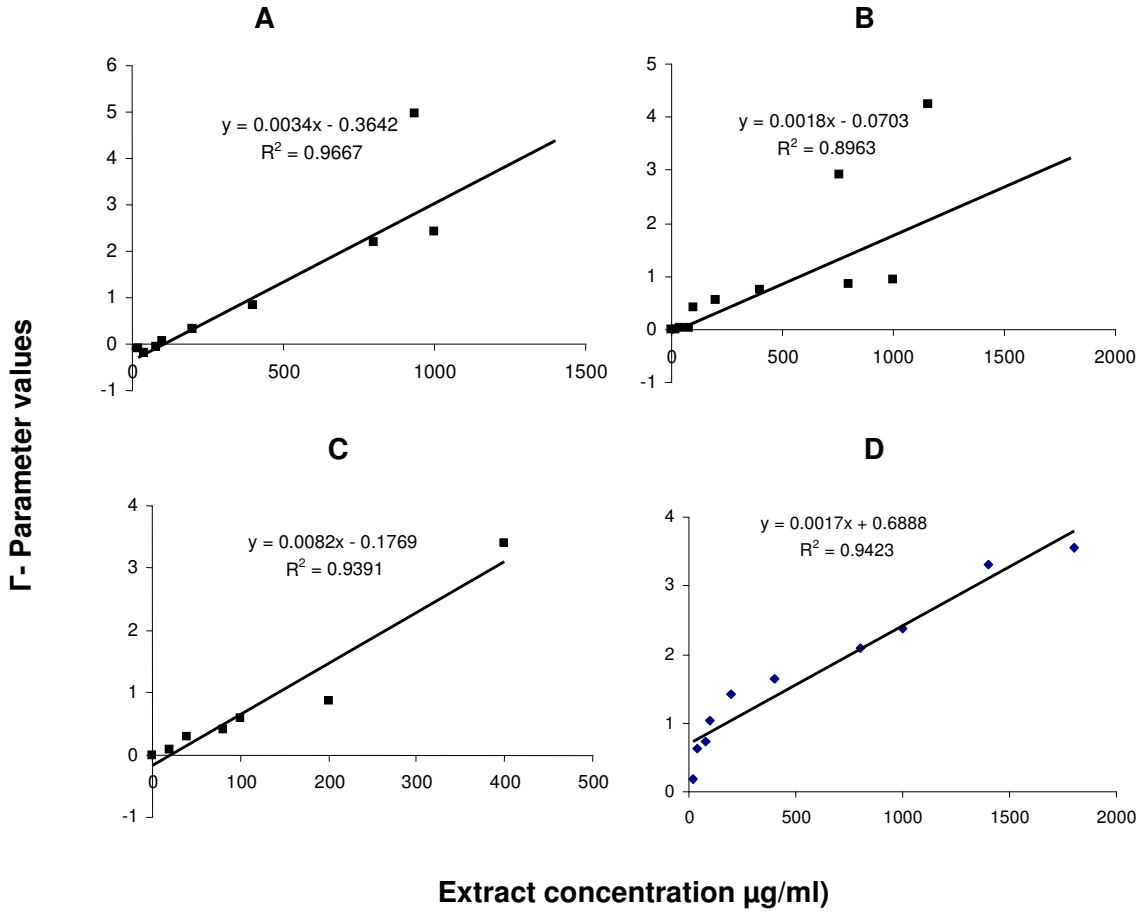

Figure 2. Gamma (Γ) parameter values of organisms (A, C = *Staphylococcus* sp. B, D = *Pseudomonas* sp.) in response to aqueous extract of *M. paradisiaca* (0 - 2000µg/ml), (A, B = Leaf; C, D = Bark).

Table 4. R^2 values of linear regression plots of transformation data of dehydrogenase activity of *Staphylococcus* sp. and *Pseudomonas* sp.

Regression plot	Extract	R^2-Values		
		Log Dehydrogenase	Inhibition (%)	Γ-Parameter
Staphylococcus sp.	Bark	0.9748	0.7819	0.9391
	Leaf	0.9742	0.8836	0.9667
Pseudomonas sp.	Bark	0.8361	0.6955	0.9423
	Leaf	0.968	0.8927	0.8963

coccus sp. was more responsive than the Gram-negative *Pseudomonas* sp.

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