Musa paradisiaca – AN AUXILIARY AID

TAXONOMY

Musa or banana is an exotic plant with its origin in the tropics of south eastern Asia. They are large woodless flowering plants belonging to the musaceae family. Amongst 100 other species, *Musa paradisiaca* is the most widely cultivated and popular edible banana.

***Musa paradisiaca* in natural habitat**

MATERIALS & METHODS

The shredded banana leaves were collected from the edges of the aquatic bodies. The leaves were cleaned thoroughly with double distilled water in the laboratory and lyophilised. The dried leaves were processed preferably within 24hrs. Following the preparative steps, around 3.5 kg leaves were recovered and powered using electrical mixer to obtain a dry weight of approximately 1.2 kg. The procedure of extraction is as follows:

The powdered leaf sample was homogenized in methanol for 24hrs using mechanical stirrer at 700 rpm.

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The homogenized sample is concentrated in Rotary vacuum evaporator at 48°C -52°C with 72 rpm.

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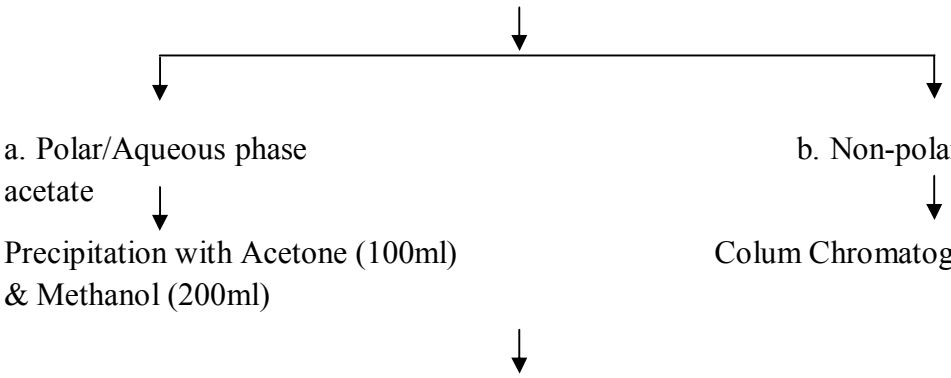
It is now subjected to liquid-liquid extraction (LLE) by partition into two polarity phases: a. Polar in Aqueous phase and b. Non-polar in Ethyl acetate phase.



Each fraction is subjected to thin layer chromatography in three different solvent systems:

a. Pet Ether : Chloroform :: 5:5; b. Hexane: Ethyl acetate:: 7:3 and c. Acetone: Methanol:: 9.9:0.1

The target fraction selected and subjected to biochemical analysis and bioactivity.

Fraction No.	Solvent/Solvent System	Ratio	Quantity (ml)
F1	Petroleum Ether	-	250
F2	Pet Ether:Chlo	7:3	500
F3	Chloroform	-	500
F4	***Ethyl acetate***	**-**	***300***
F5	Acetone : Water	7:3	250
F6	Ethanol : Water	7:3	300
F7	Ethanol : Water	3:7	250

F4 fraction taken for further work

Biochemical analysis [Phenols, Tannins & Antioxidants]

Microbiological screening [Antibacterial Assay]

The leaves of the plants have been subjected to antimicrobial assay by disc diffusion assay against *Edwardsiella tarda* and *Streptococcus aureus* which are one of the few major fish disease causing microorganisms.

The antibacterial assay was done by Disc diffusion method. The culture media was swabbed with the overnight grown bacterial culture using sterile cotton swabs. The cultures were maintained in Trypton Soya broth. 5µl of the respective dilutions were adsorbed in 10mm disc and kept in static incubation in the plates at room temperature for 15 mins allowing diffusion. The incubation of the plates was done for 36-48 hrs at 35°C. The diameter of the inhibition zone (mm) was measured.

↓

Cytotoxicity assay

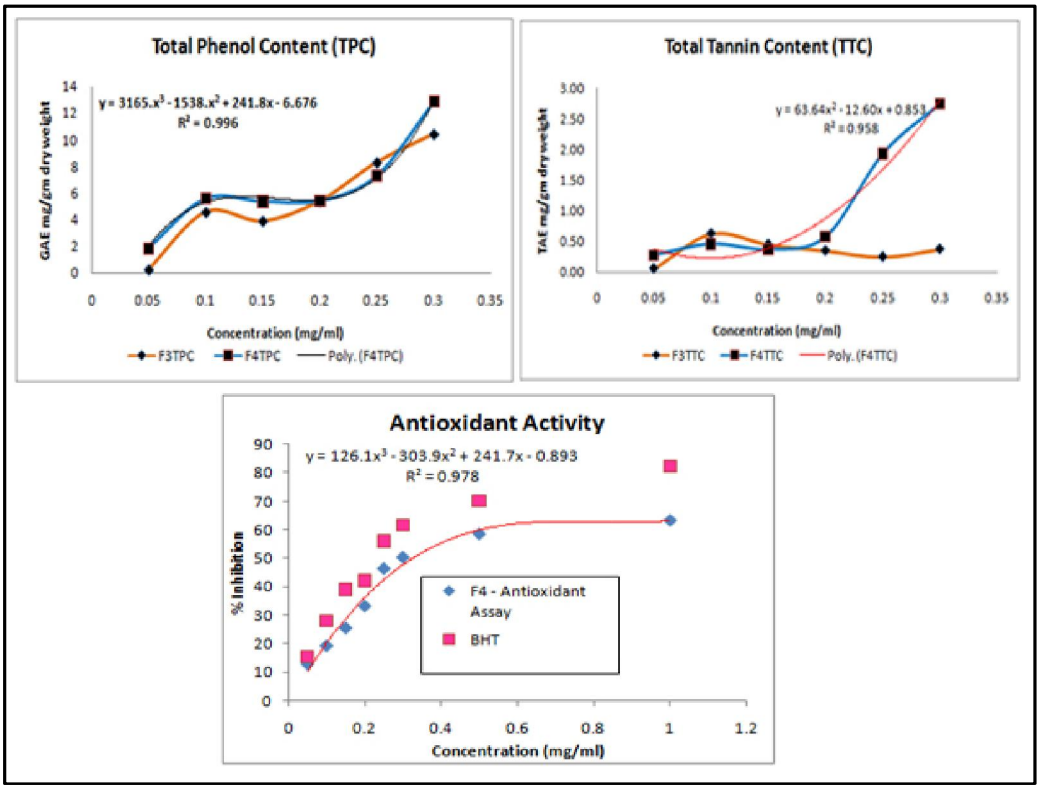
Brine Shrimp lethality Test

RESULTS

***Phenols*:** The phenol content was nonlinear with the concentration grade and recoded a fall in value at concentration 0.15 mg/ml with 5.44 GAE mg/g & 0.20 mg/ml with 5.41 GAE. The maximum content was recorded at 0.3 mg/ml with 12.85 GAE mg/g. The R^2 = 0.996 showed the polynomial equation as best fit regression model.

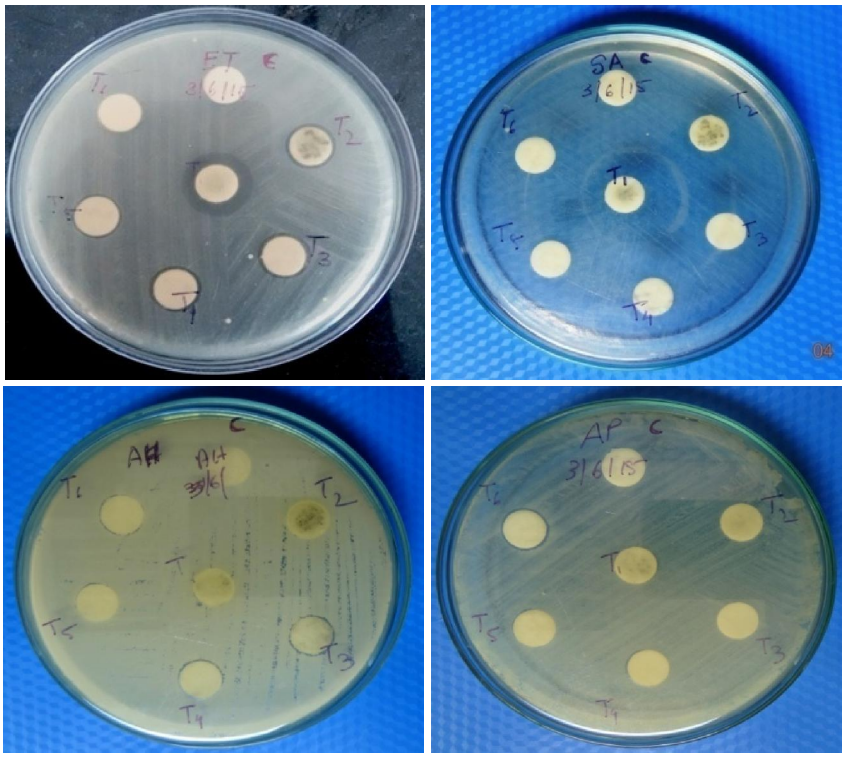
Tannins: Tannic acid equivalents (TAE) in mg/g dry weight is preferred as a yard stick for the progress of tannin The maximum tannin content was confirmed at 0.3 mg/ml of the leaf fraction with 2.74 mg/gm TAE with R^2 = 0.958.

***DPPH Radical Scavenging Assay*:** The highest antioxidant was recorded at 0.3 mg/ml with 63.14% as compared to commercially available antioxidant, BHT at 82.16%. The curve fit model followed a power equation for IC50 of 2.23mg/ml with R^2 = 0.978.



Biochemical activity of the F4 fraction

Microbiological screening: The inhibition zone diameter is tabulated below. The ethyl acetate fraction of *M. paradisiaca* showed appreciable inhibition on *Edwardsiella tarda* but showed very negligible impact on *Streptococcus aureus* and *Aeromonas hydrophila*. Hence MIC and MBC was proceeded only with *E. tarda*.1250 µg was found to be the MIC because turbidity appeared till 625µg. OD was measured at 600nm. Following which four concentrations 1250 µg, 2500 µg, 5000 µg, 10,000 µg were plated and incubated at 37°C for 24 hrs/48hrs. 5000µg was found to be the MBC as no growth appeared at 5000 µg and 10,000 µg.

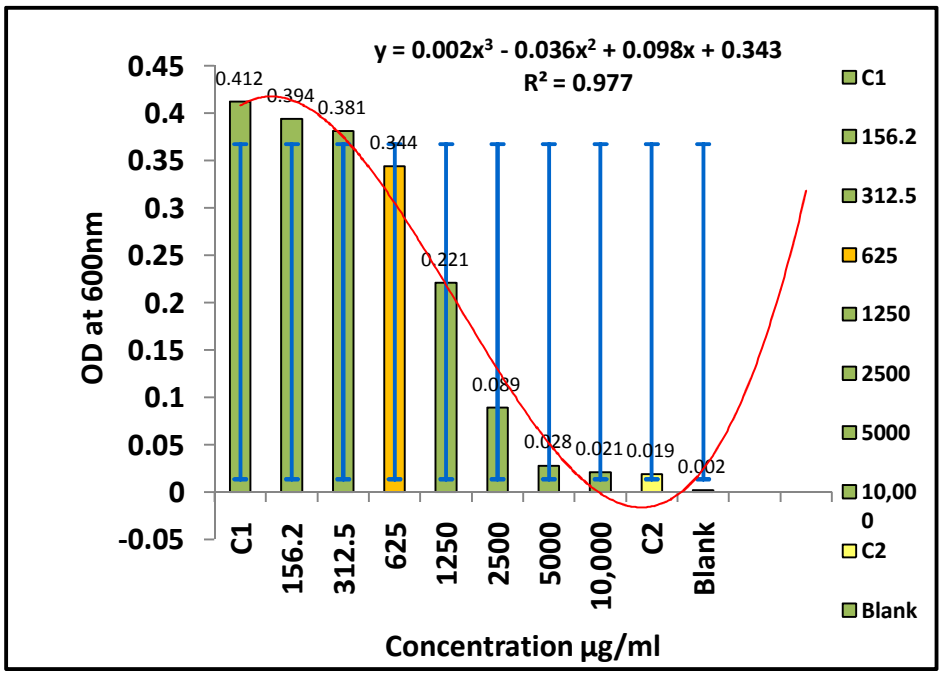


Antibacterial assay of Ethyl acetate fraction of banana leaves on (clockwise) *Edwarsiella tarda*, *Streptococcus aureus*, *Aeromonas hydrophila* and *A.popoffi.*

Bacteria	C	T1	T2	T3	T4	T5	T6
S. aureus	0 (15)	3(18)	2 (17)	1 (16)	0 (12)	0 (15)	0 (16)
E. tarda	0	10	6	0	0	0	0
A. hydrophila	0	0	0	1	1	0	0.5
A. popoffi	0	0	0	0	0.5	0	1

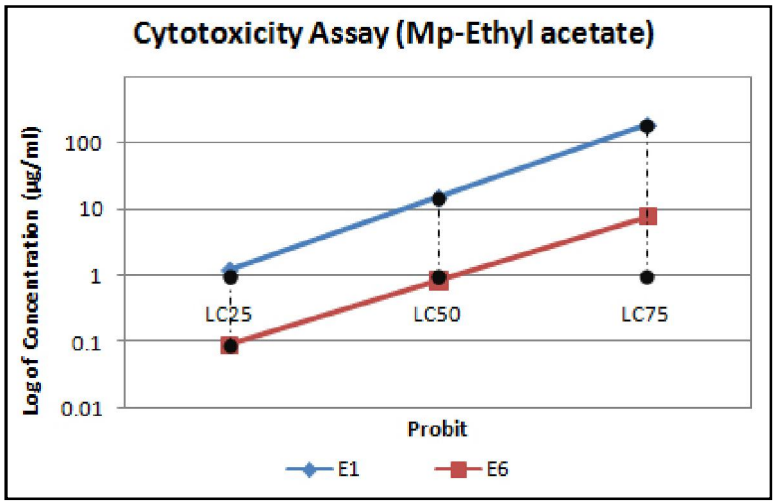
Zone of Inhibition of *Edwarsiella tarda (EA)*, *Streptococcus aureus (SA)*, *Aeromonas hydrophila (AH)* and *A. popoffi (AP)* as displayed on plates.

Concentration	OD
C1	0.412
156.2	0.394
312.5	0.381
625	0.344
1250	**0.221**
2500	0.089
5000	**0.028**
10,000	0.021
C2	0.019
Blank	0.002



Picto- graphical illustration of MIC and MBC of F4 fraction of *M. paradisiaca* against *E. tarda*

Cytotoxicity Test: *M. paradisiaca* showed uniformity with concentration dependent mortality. The 1 hr exposure time to be a better fit model than 6hrs time interval. The LC_{50} for 6hrs was 0.845µg/ml and that of 1 hr was 15.005µg/ml. Nevertheless the extract showed a toxicity effect beyond 6hrs. This suggested that the fraction could contain cytotoxicity compounds.



Graphical representation of LC25, LC50 and LC75 against log of concentration for *M. paradisiaca* at 1hr, 6hrs and 12hrs exposure time of Brine shrimp lethality assay of Ethyl Acetate fraction

Statistically, the Shapiro-Wilk significance test confirmed its normal distribution. However chi-square test showed the 1 hr exposure time had better curve fit than 6hrs interval.

Musa

Tests of Normality

Time	6hr-Banana	Kolmogorov-Smimov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Mortality	6	.223	5	.200*	.879	5	.304

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

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a. Lilliefors Significance Correction

Parameter Estimates

Time: 1hr	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	.620	.054	11.533	.000	.515	.726
	Intercept	-.730	.107	-6.809	.000	-.837	-.622

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

Parameter		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	9.336	3	.025[a]

a. Since the significance level is less than .150, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Parameter Estimates

Time: 6hrs	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	.699	.083	8.453	.000	.537	.861
	Intercept	.051	.111	.460	.646	-.060	.162

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

Parameter		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	3.518	3	.318[a]

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Test of normality (Shapiro Wilk) and Kolmogorov-Smirnov Test and Probit and logit analysis of Ethyl acetate fraction of *M. paradisiaca* on brine shrimp lethality at 1hr and 6hr exposure time.

DISCUSSION

The use of chemicals in aquaculture is a very common practise which not only devastates the aquatic health but also accounts for major diseases upon consumption. By virtue of plant based products this chemicals could be substituted (Direkbusarakom, 2000; Dung, 1990) in terms of antibacterial for fish and other disinfectants. The ethyl acetate fraction of *M. paradisiaca* contained high phenol and antioxidants. The fraction showed inhibitory effect against *E. tarda*, causative for emphysematous putrefactive

disease of catfish but could not provide satisfactory output on *S. aureus*. The graphs for biochemical and antimicrobial activity follow a non-monotonic cubic model of data. The cytotoxicity test reveals the compound may be toxic at its crude form at it shows 50% mortality at less than 50ppm which is quite a diminutive concentration and hence signifies the compound. However in terms of real time implication as an antimicrobial agent it has to be in encapsulated form with polar sheeting effortlessly soluble in water as ethyl acetate is not very safe solvent for nature release.