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Inhibition of Dehydrogenase Activity in Pathogenic Bacteria Isolates by Aqueous Extract of *Curcuma Longa* (Turmeric) Rhizome

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Abstract

Inhibition of dehydrogenase activity in pathogenic Gram – positive and Gram – negative micro-organism exposed to ethanol extract of curcuma longa was used as an index for assessment of its antibacterial activity. Assay of dehydrogenase activity was done in the test organisms (*Escherichia Coli*, *Staphlococcus aureus* and *Salmonella* typhi) using 2, 3, 5-triphenyltetrazolium chloride (TTC) as an artificial electron acceptor which was reduced to the red-coloured triphenyl-formazan. Response of the bacterial isolates varied with extract concentration. Dehydrogenase activity was progressively inhibited in a logistic dose-response fashion. The Gram positive *staphylococcus aureus* responded more markedly than Gram negative *Escherichia Coli* and *Salmonella typhi* inhibitory concentrations (IC50) of ethanol extracts against *Escherichia Coli*, *Staphylococcus aureus* and *Salmonella typhi* were 250.51ug/ml, 55.80ug/ml, and 570.48ug/ml respectively. Preliminary phytochemical screening of the extract gave positive reactions for alkaloids, flavonoids, tannins, 4-hydroxybenzoic acid (phenolic compound) and saponins. These phytochemicals may be responsible for the observed inhibition of total dehydrogenase enzyme activity that translates to anti-bacterial action in these pathogenic organisms.

Keywords: Inhibition, Dehydrogenase, Anti-bacterial, Tumeric, Extracts.

Introduction

Medicinal plants are important source of the verification of pharmacological products and can be natural composite sources that act as new anti-infectious agents.[1] Different plant parts are used for medicinal purposes bulb, leaves, roots, barks, peels etc. The use of plants to treat illness is found throughout human culture.[2] The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine.[3] *Curcuma longa* is a medicinal plant that botanically is related to Zingiberaceae family.[4] *C. longa* the continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds.

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial

compounds in plants. *C. longa,* commonly known as 'turmeric', is widely used as a spice and colouring agent, and is well known for its medicinal properties. Curcumin a potent antioxidant believed to be the most bioactive and soothing portion of the herb turmeric and posses the properties like antioxidant, anti-inflammatory, anti-platelet, cholesterol 456 lowering antibacterial and anti-fungal effects. It contains a mixture of powerful antioxidant phytonutrients known as curcuminoids and inhibits cancer at initiation, promotion and progression stages of tumor development. It is a strong anti-oxidant, which supports colon health, exerts neuroprotective activity and helps to maintain a healthy cardiovascular system.[4]

C. longa (Turmeric) contains a wide variety of phytochemicals, including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols.[5]

The Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants. Keeping in view the important role of *curcuma longa* (turmeric) in inhibition of different cultures of bacteria and its role as antioxidant and antibacterial, the present research was conducted to compare the antibacterial activity of the extracts of *C. longa* potency on some bacteria.

Hydrogenases are a class of enzymes that remove a hydrogen atom from a substance and transfer it to an acceptor. They are essential in cellular respiration and are used by microorganisms in the soil to break down organic matter, metabolic processes that occur in abundance in healthy microorganisms. Dehydrogenase occurs only within soil bacteria. They do not act on their own without a bacterial host. Biological oxidation of organic components is generally a hydrogenation process, and there are many dehydrogenases (Enzymes catalyzing dehydrogenation).

The dehydrogenase enzyme systems apparently fulfill a significant role in the oxidation of orgaic matter as they transfer hydrogen (electron) from substrate to acceptors. Several studies have demonstrated that dehydrogenase enzyme activity (DHA) of microorganisms is among most sensitive parameter for evaluation of toxicity.[6, 7]

The use of dehydrogenase assay has been estabilished as a tool in probing response of microorganisms to antibacterial agents and waste water contaminated with both organic and inorganic contaminants and is recognized as a useful indicator of the overall measure of the intensity of microbial metabolism. This is preferred over culture method for enumeration of microogranisms which can underestimate number of viable cells due to lack of homogeneity in distribution or difficulty in being ready desorbed from the substrate matrix.[7]

Materials and Methods

Preparation of Extract:

The Preparation of extract was done according to Allisi et al.[7] The rhizome part of *Curcuma longa* (Turmeric) was washed with salt and water to reduce the microbial load or micro-organism. It was cut into pieces for easy drying and was air dried at room temperature and then further dried to constant weight in an oven at 600C and the dried rhizome were reduced to a coarse powder in a mill (Kenwood BL357). 500g powder was soaked in 2.0 litres of ethanol and left to stand for 4 days at room temperature. The extract was filtered, and the residue was re-extracted under the same conditions. The combined filtrate was concentrated in a rotary evaporator at <500C to obtain the ethanol extracts of curcuma longa (ETECL).

Isolation Of Bacterial Strains And Culture Conditions

Pathogenic bacteria (Salmonella sp, staphylococcus sp and Escherichia) were obtained from high vagina swab (HVS) and stool. Isolates were purified on nutrient agar (Fluka) plates and characterization was done using standard microbiological methods. Identification to the generic level followed the schemes of Holts et al. The bacterial strains were grown to mid exponential phase Chemical tests were carried out on the plant material (ETECL) and powdered dried *curcuma longa* rhizome) using standard procedures to identify their constituents. This was to ascertain their bioactive principles. The result would serve as a template for elucidating their possible mode of action and the biochemical/pharmacological effects that were observed.

Antimicrobial Potential of Ethanol Extract of *Curcuma Longa*

Inhibition of dehydrogenase assay method as described by Alisi et al.[7] was employed to determine the antimicrobial activity of the extract. Inhibition of dehydrogenase activity was assayed using 2, 3, 5-triphenylterazolium chloride (TTC) (BDH England) as the artificial electron acceptor, which was reduced to the red-coloured triphenyl-formazan

(TPF). The assay was carried out in 4ml volumes of nutrient broth-glucose-TTC medium supplemented with varying concentrations (0-2000ml) of extract in separate 20ml screw-capped test tubes. Portions (0.3ml) of the bacterial suspensions were inoculated into triplicate glass tubes containing 2.5ml of phosphate-buttered (pH 6.8) nutrient broth-glucose medium amended with curcuma longa extract and pre-incubated on a rotary incubator (150 rpm) at room temperature (28 + 20C) for 30 min. Thereafter, 0.1ml of 1% (w/v) TTC in deionised distilled water was added to each tube to obtain the final extract concentrations of 0-2000ug/ml in different test tubes. The final concentrations of nutrient broth, glucose and TTC in the medium were 2, 2 and 0.25mg/ml, respectively. The control consisted of the isolates and the media without curcuma longa extract. The reaction mixtures were further incubated at room temperature (28 + 20C) for 8 hrs. The TPF produced were extracted in 4ml of amyl alcohol and determined colorimetrically at 500nm. The amount of formazan produced was determined from a standard dose response curve [0-20ug/ml TPF (sigma) in amyl alcohol, Y=0.0487 x; R2 = 0.9977]. Dehydrogenase activity DHA was expressed as milligram of TPF formed per mg dry weight of cell biomass per hour. Percentage inhibition of dehydrogenase activity in the isolates by *curcuma longa* was calculated relative to the control. The percentage inhibitions for organisms were plotted against the concentrations of the extracts using the table 2, curve. The toxicity threshold concentrations (IC5, IC10, IC20, IC50, IC80 IC90 and IC100) values which were non-determinable from the simple inhibition plots were subjected to evaluation using a log transformation of % inhibition plots.

Results

Phytochemical Composition of *C. Longa* Extract

The phytochemical composition analysis of the ethanolic *Curcuma longa* rhizome extract revealed the presence of Alkaloid, flavonoids, glycosides, tannis, spaonins, hydrobenzoic acid, with the absence of steroidal aglycon (Table 1). The presence of flavonids, tannis, alkaloids & saponins have been associated with antimicrobial effects in various studies using plant extracts as shown in (Table 1). Many plants containing alkaloids and flavoniods have been shown to have dluretic, antisepamodic, anti-inflammatory and analgestic actions. The result obtained from the control samples showed that the three bacterial strains comprising gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*) and Gram-Positive (*Salnonella typhi*) were able to reduce 2, 3, 5-Triphenyltetrazolium Chloride (TTC) to its triphenyl formazan (TPF) at variable rates and extents (Table 2 and 3). Table 2 shows the threshold inhibitory concentrations of ethanol extracts of *C. longa* against the inhibition dehydrogenase activity of *Escherichia coli, Staphylococcus aureus* isolated from High vaginal swab (HVA) and salmonella typhi from stool.

Table 1: Phytochemcial profile of the rhizome extract of *curcuma longa*

Plant	Alkaloids	Flavonoids	Glycosides	Steroidal aglycone	Hydrobenozic Acid	Saponnis	Tennis
C.longa Rhizome extract	+	+	+	-	+	+	+

Key: + presence, - = absence

Table 2: Threshold inhibitory concentrations of ethanol extracts of *C. longa* against the inhibition dehydrogenase activity of *Escherichia coli* and *Staphylococcus aureus*

Orgs	IC_5	IC_{10}	IC_{20}	IC_{50}	IC_{80}	IC_{90}	IC_{100}
Escherichia coli	23.32	41.92	79.72	250.51	997.48	5890.57	ND
Staphylococcus aureus	7.16	12.05	21.21	55.80	147.50	262.47	ND
Salmonella typhi	1.48	8.02	45.57	570.48	2645.27	4102	6251

ND = Non-Determinable; Not Determined

Table 3: Equations of Logistic dose response model;

$$Y = \frac{a}{1 + (x/b)^c}$$ ……. equation (1),

Where Y = % inhibition of DHA

X= Concentration of C. Longa (ug/ml)

	A	B	C	R^2	Fit std Error
Escherichia Coli	91.32	215.77	-1.277	0.965	8.02
Staphylococcus aureus	99.68	55.56	-1.43	0.998	1.909
Salmonella Typhi	231.37	11893.54	-0.424	0.979	7.07

Table 4: Threshold inhibitory concentrations of standard antibiotic drug (Gentamycin) against the total dehydroganase activity *of E. coli, S. aureus* and *S. typhi*

Orgs	IC_5	IC_{10}	IC_{20}	IC_{50}	IC_{80}	IC_{90}	IC_{100}
E. coli	27.89	47.51	85.11	238.76	815.31	3359.14	ND
S. aureus	0.36	0.83	2.07	10.36	74.24	1354.12	ND
Salmonella typhi	0.36	0.73	1.76	10.80	427.93	ND	ND

ND = Non – Determined

Effect of Ethanol Extract of *C. longa* on Inhibition of Total Dehydrogenaseactivity on Pathogenic Hvs and Stool Isolates

The result (Figure 1, 2 and 3) shows the effect of ethanol extracts of *C. Longa* on the inhibition of dehydrogenase activity of *E. coli, S. aureus* isolated from high vaginal swab (HVs) and *S. typhi* from stool. The extract dose dependently inhibited dehydrogenase activity in these organisms following the logistic dose response model. Response of organism *(E. coli)* to extract followed a logistic dose response with equation (1) a = 91.32, b = 215.77, c = -1.277, R^2 – value = 0.965. Threshold inhibitory concentrations were; IC_5, =23.32, IC_{10} = 41.92, IC_{20} = 79.72, IC_{50}= 250.51, IC_{80} = 997.48, IC_{90}= 5890.57, IC_{100}= ND.

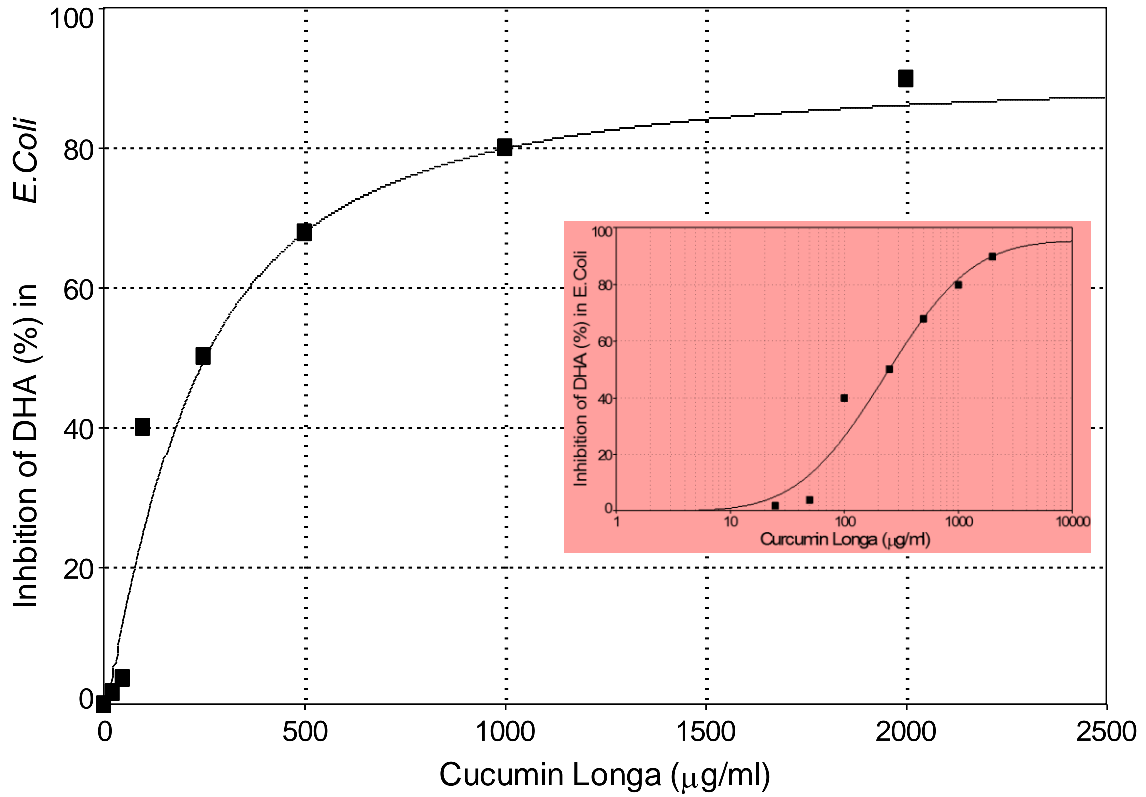

Figure 1: Plot of percentage inhibition of dehydrogenase activity in *Escherishia coli* by graded concentrations of ethanol extract of *Curcuma longa*. Inset shows the same plot on a logarithmic scale showing a sigmoid relationship.

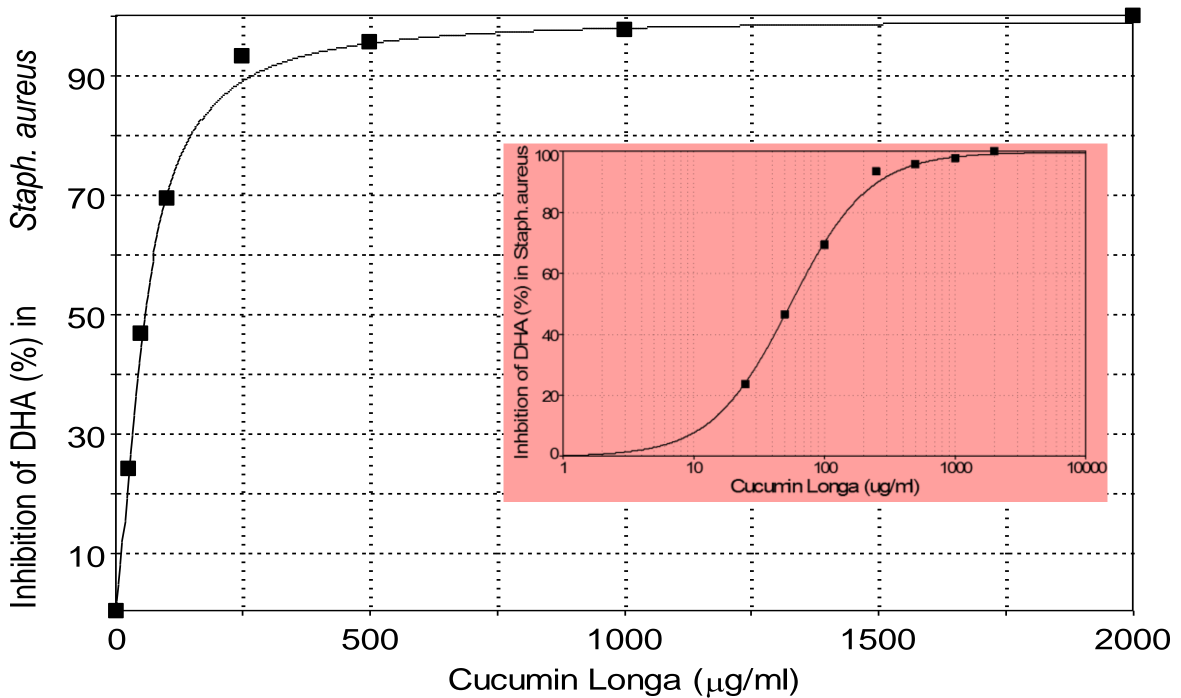

Figure 2: Plot of percentage inhibition of dehydrogenase activity in *staphylococcus aureus* by graded concentrations of ethanol extract of *Curcuma longa*. Inset shows the same plot on a logarithmic scale showing a sigmoid relationship.

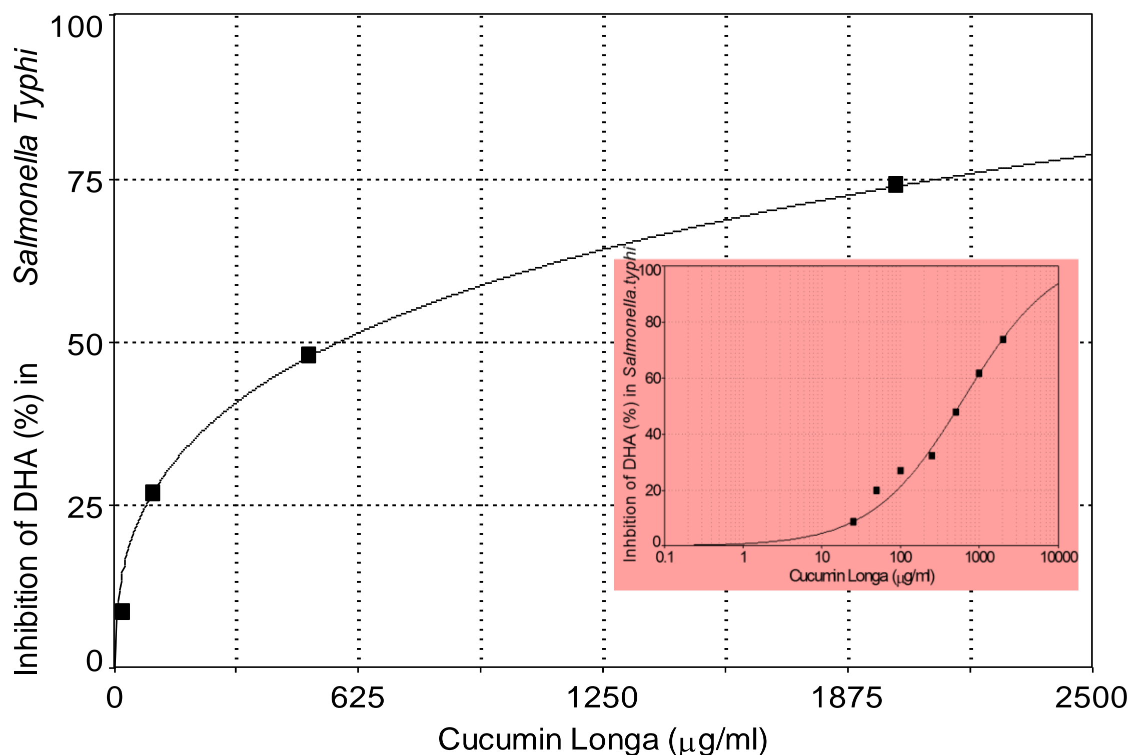

Figure 3: Plot of percentage inhibition of dehydrogenase activity in *Salmonella typhi* by graded concentrations of ethanol extract of *Curcuma longa*. Inset shows the same plot on a logarithmic scale showing a sigmoid relationship.

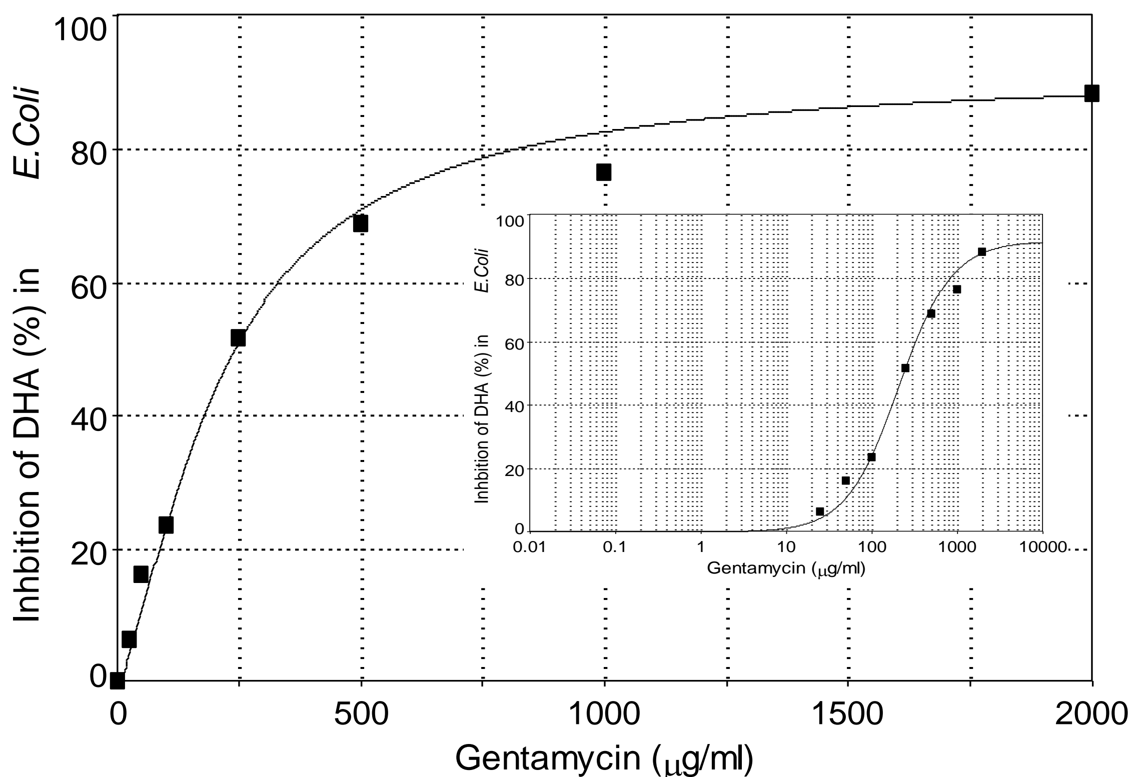

Figure 4: Plot of Percentage inhibition of dehydrogenase activity in *Escherishia coli* by graded concentrations of standard drug (Gentamycin). Inset shows the same plot on a logarithmic scale showing a sigmoid relationship.

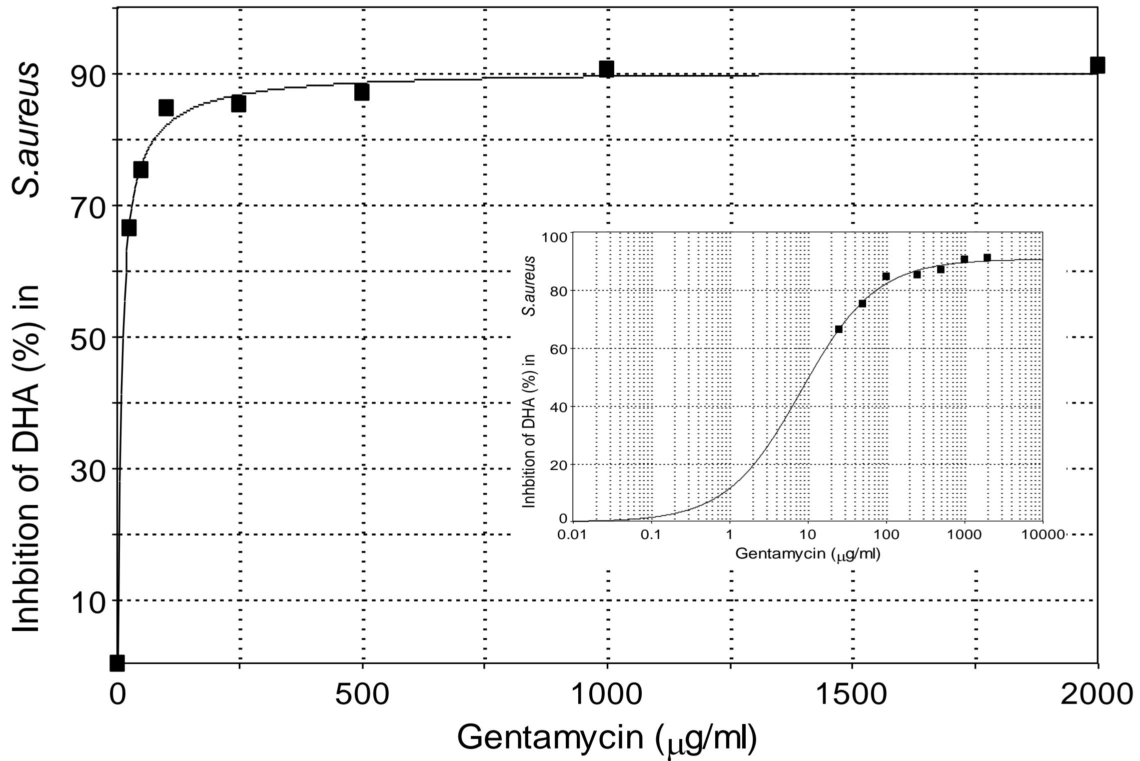

Figure 5: Plot of Percentage inhibition of dehydrogenase activity in *Staphylococcus aureus* by graded concentrations of standard drug (Gentamycin). Inset shows the same plot on a logarithmic scale showing a sigmoid relationship.

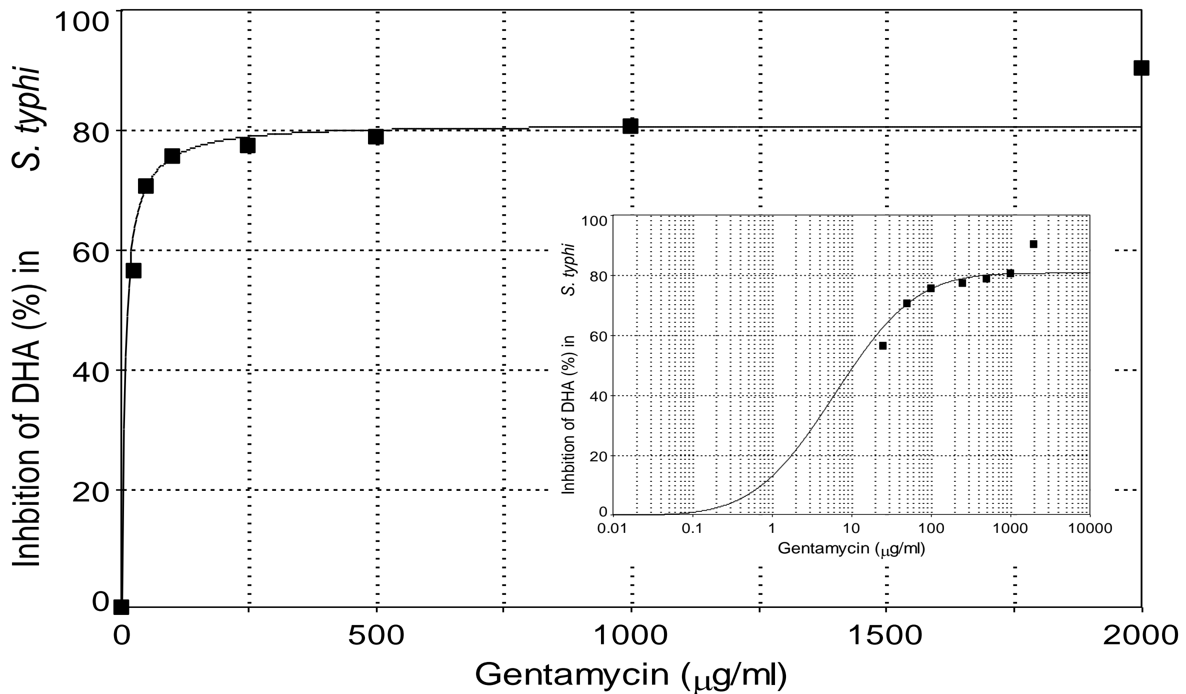

Figure 6: Plot of Percentage inhibition of dehydrogenase activity in *Salmonella typhi* by graded concentrations of standard drug (Gentamycin). Inset shows the same plot on a logarithmic scale showing a sigmoid relationship.

Response of organism (*S. aureus*) to extract followed a logistic does response as shown in equation (1) a = 99.68, b = 55.56, c = - 1.43, R^2 – value = 0.998, Threshold inhibitory concentration were; IC_5 = 7.16, IC_{10}= 12.05, IC_{20} = 21.21, IC_{50} = 55.80, IC_{80}147.50, IC_{90}= 262.47, IC_{100}= ND

Response of organism (*S. typhi*) to the extract followed a logistic dose response as shown in equation (1) a = 231.37, b = 11893.54, C= -0.424, R^2 – value = 0.979. Threshold

inhibitory concentrations were; IC_5 = 1.48, IC_{10} = 8.02, IC_{20} = 45.87, IC_{50} = 570.48, IC_{90} = 4102, IC_{100} = 6251.
Ethanol extracts of *C. longa* against *Escherishia Coli, Staphilococcus aureus* and *Salmonella typhi,* inhibited DHA in a logistic dose response fashion. Threshold inhibitory concentration of the extracts (Table 2) shows that *staphylococcus aureus* responded gradually but steadily. At lower concentrations, the extracts exerted strong inhibitory effect on DHA of *Escherichia coli* and *Salmonella typhi* than *staphylococcus aureus.* The equation used in the modeling of this result gave high correlation coefficient ($R^2 \geq$ 0.90) showing very strong relationship with very low fit standard errors.

Discussion

Measurement of microbial enzyme activity has been used in the assessment of ecotoxicity logical impacts of environmental pollutants. In this regard, dehydrogenase activity has been widely used. Dehydrogenase assay is also an effective primary test for assessing the potential toxicity of metals to planktonic bacteria.[6, 8]

Also, dehydrogenase assay had earlier been used to assess the toxicity of antimicrobial agent to pathogenic bacteria pathogenic organism in vagina and stool has been found to cause diseases and infections. Result (figure 1,2 and 3) showed that ethanol extracts of *C. longa* inhibited dehydrogenase enzyme activity in pathogenic HVS and stool isolate (urinary tract infections (UTI) and typhoid disease); *Escherishia coli, staphylococcus aureus,* and *salmonella typhi).*[6-8]

Inhibition of dehydrogenase activity in pathogenic HVS & stool isolates is indicative of a strong antimicrobial activity since inhibition of oxido-reductases like dehydrognases, affects respiration of the microbe.
Dehydrogenase assay involving the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) and 2-(P-iodophenyl)-5-phenyltetrazolium chloride (INT) to their formazans has been used to measure microbial activity.
The equation used in the modeling of this result gave high correlation coefficient ($R^2 \geq$ 0.90), showing very strong relationship with very low fit standard errors. Inhibition of dehydrogenase activity in vaginal & stool isolate could be one of the many mechanism employed by the plant extract for UTI and typhoid healing as well as antimicrobial activity.
The secondary plant metabolites identified in these extracts may be acting synergistically to bring about the observed inhibition of dehydrogenase activity. Extracts of the plant C.*longa* have earlier been shown to contain phenolic compounds like hydroxybenzoic acid. This is in line tandem with observation (table 1). Hydroxybenzoic acid is known to possess antimicrobial activity. These extracts may actually be exerting their antimicrobial activity via inhibition of dehydrognase activity in the test organisms.

The presence of surface active compounds is known to potentate the biological effect of an antimicrobial agent. The co-existence of phenolic compounds with saponins which behave like detergents may extend the activity of p-hydroxybenzoic acid and may explain the strong antimicrobial activity of the extract. Other phytochemicals found in the extract may also exert their own antimicrobial activity through different mechanisms.

The plant extract *C. longa* showed a logistic dose dependent inhibition of dehydrogenase activity in the test organisms. It was more effective against *staphylococcus aureus* and *salmonella typhis* than *E. coli.* It therefore can be useful as a promising antimicrobial agent in the treatment of infections caused by the test organisms.

More can be done with invasive plant *C longa,* which was seen as a non-used plant. Further work should be carried out on other organisms in order to include this plant as a reasonable alternative to chemotherapeutic drugs.
Research should be geared in the direction of understanding at molecular levels the genetic interactions that take place to produce these pharmacologic actions.

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