

## **Antimicrobial Properties of *Euphorbia hyssopifolia* and *Euphorbia hirta* against Pathogens Complicit in Wound, Typhoid and Urinary Tract Infections**

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### **ABSTRACT**

Dehydrogenase activity (DHA) in Gram-positive *Staphylococcus aureus* isolated from degenerated wound, Gram-negative *Salmonella typhi* isolated from stool, and Gram-negative *Escherichia coli* from a high vaginal swab were assayed. Inhibition of dehydrogenase activity of the test organisms by ethanol extract of *Euphorbia hyssopifolia*, and *Euphorbia hirta*, were determined and compared to standard antibiotics (Ciprofloxacin and Gentamycin). The total dehydrogenase assay was done using 2, 3, 5-triphenyl tetrazolium chloride (TTC) as the artificial electron acceptor which was reduced to the red-coloured triphenyl-formazan (TPF). Response of the bacterial isolates varied with extract concentration. Dehydrogenase activity was progressively inhibited in a logistic dose-response fashion in the test organism by the extracts and standard drugs. All extract and standards achieved at least 70% inhibition within the tested doses (0-2000 $\mu$ g/ml), except for *Euphorbia hirta* against *Staphylococcus aureus*. Threshold inhibitory concentrations ( $IC_{50}$ ) for *Euphorbia hyssopifolia* against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* were 59.92 $\mu$ g/ml, 234.90 $\mu$ g/ml, and 492.46 $\mu$ g/ml respectively, while for *Euphorbia hirta*  $IC_{50}$  against *Salmonella typhi* and *Escherichia coli* was 99.67 $\mu$ g/ml, and 165.90 $\mu$ g/ml with no significant inhibition against *Staphylococcus aureus*. Inhibition of dehydrogenase activity in the test organism by the extract compared well with the standard antibiotics. *Euphorbia hyssopifolia* was effective against Gram-positive *Staphylococcus aureus* implicated in delayed wound healing than Gram-negative *Salmonella typhi* and *Escherichia coli* implicated in typhoid fever and urinary tract

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infections respectively, while *Euphorbia hirta* was effective against Gram-negative organisms implicated in typhoid fever and urinary tract infections, but not effective against Gram-positive *Staphylococcus aureus*. Secondary plant metabolites found in the extracts may be acting in synergy to bring about their pharmacologic functions and may explain reasons for ethno-medical usage.

**Keywords:** *Euphorbia hyssopifolia*; *Euphorbia hirta*; dehydrogenase enzymes; wound healing; typhoid fever; urinary tract infections.

## 1. INTRODUCTION

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). There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicines especially in developing countries (Murray, 1998). The use of plants and their natural products in Nigeria as either extract or infusion is a widespread practice in the treatment and management of diseases (Iwu, 1982). Kumar et al. (2007) and Biswas and Mukherjee (2003) reported that about 163 species of plants were used as wound healing plants in Indian systems of medicine. *Euphorbia* species have been used in the treatment of wounds in India ethnomedicine (Ayyanar and Ignacimuthu, 2009).

In Nigeria extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Igoli et al., 2005). *Euphorbia hirta* extract have been shown to have antidiarrhoeic activity (Galvez et al., 1993), analgesic, antipyretic and anti-inflammatory property Lanhers et al. (1991), Antibacterial effect against dysentery-causing *Shigella sp.*, (Vijaya et al., 1995; Sudhakar et al., 2006; Ogbulie et al., 2007) and showed activity against intestinal motility (Hore et al., 2006). *Euphorbia hirta* extract have also been shown to increase urine output and electrolytes in rats (Johnson et al., 1999). *Euphorbia hyssopifolia* produces latex which constitutes a health hazard to humans and livestock (Abo, 1994). It is known to contain substances which are inhibitory to seed germination and seedling growth as well as bacteria. Direct contact of the irritant latex with the eye can cause blindness. The toxic latex of *Euphorbia hyssopifolia* has a diuretic effect and purgative action. It has medicinal effect for inflammation of the respiratory tract and also is said to induce bronchial relaxation in asthma. The juice is said to remove warts and the leaves can be boiled with *Phyllanthus niruri* to make tea for the treatment of gonorrhoea (Adedapo et al., 2004). Hepatotoxicity effect of *Euphorbia hyssopifolia* has also been reported (Igwenyi et al., 2011).

The use of the total dehydrogenase assay has been used as a tool in probing response of microorganisms to antibacterial agents (Alisi et al., 2008) and is recognized as a useful indicator of the overall measure of the intensity of microbial, metabolism (Tabatabai, 1982; von Mersi and Schinner, 1991). This method is preferred over culture method for enumeration of microorganisms which can underestimate number of viable cells due to lack of homogeneity in distribution or difficulty in being readily desorbed from the substrate matrix (Oberbremer and Muller- Hurtig, 1989; Torstensson, 1997). Dehydrogenase assay is also an effective primary test for assessing the potential toxicity of metals to planktonic (Nweke, et al., 2006), and heterotrophic (Nweke, et al., 2007) bacteria. We have previously assessed toxicity of antimicrobial agents to pathogenic bacteria using the total dehydrogenase assay

(Nwaogu et al., 2007; Nwaogu et al., 2008 and Alisi et al., 2008, Alisi and Onyeze, 2009, Alisi, et al., 2011a).

Infections to wounds may be cross or auto as in the case of post-operative sepsis. Pathogenic bacteria find their way into wound` complicating healing process and bringing about delay in wound healing by contributing oxygen free radicals in the site of injury. Antimicrobial agents are used locally to aid in wound healing complicated by pathogenic organisms. Wound healing has been found to be accelerated in the presence of non-viable *Staphylococcus aureus* as a result of the contribution by its cell wall and cell wall Peptidoglycan components (Levenson et al., 2002). Bacteria have been rendered nonviable by physical and chemical means. Toxicity of antimicrobial agents to pathogenic bacteria has been accessed using the total dehydrogenase assay (Nwaogu et al., 2007; Nwaogu et al., 2008 and Alisi et al., 2008, Alisi and Onyeze 2009; Alisi et al., 2011a).

Infection of wounds and complications arising from such infections has become a source of worry to many local health providers. Scientists have shown concerns and research has been on-going on alternative therapies that could help alleviate problems associated with/causes of delayed wound healing (Alisi and Onyeze 2009). Infection with *Salmonella typhi* which is the causative agent of typhoid fever has become a public health concern in our communities where adequate treatment of water supplies including water carriage system of sewage disposal is lacking. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Davies, 1994). Urinary tract infections are now on the increase due to sexual recklessness. Multiple drug resistance caused by abuse of antibiotics among the teeming youth population is a public health concern. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio 1996; Iwu et al., 1999). Although many antibiotics are sensitive to *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli in-vitro*, satisfactory therapies remain scarce.

The antimicrobial actions of *Euphorbia hyssopifolia* and *Euphorbia hirta* against pathogens complicit in delayed wound healing, typhoid fever and urinary tract infection have not been previously studied. It was our intention to study the inhibition of total dehydrogenase activity in these pathogens implicated in these infections by ethanol extracts of *Euphorbia hyssopifolia* and *Euphorbia hirta*.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Preparation of Plant Samples

Fresh aerial part of *Euphorbia hyssopifolia* and *Euphorbia hirta* were collected from Ihiagwa and the forests in the Federal University of Technology Owerri, in owerri west local government area of Imo State. The plants were authenticated by a plant taxonomist at the School of Agriculture and Agricultural Technology, Federal University of Technology Owerri. Voucher specimen has been retained at the authors' laboratory. The sample was dried at 30°C and then reduced to a coarse powder in a mill (Kenwood BL357, Japan). A 200g portion was extracted with 800ml of ethanol by shaking for 48hrs. Soluble extract from filtration in a whatman No1 filter paper was concentrated by distillation under reduced

pressure at 49°C in a Buchi rotavapour (Switzerland). The extract was then dried to solid form in a vacuum desiccator (CNS Simax), and stored in a freezer ( $\leq 4.0^{\circ}\text{C}$ ) until needed.

## 2.2 Preliminary Phytochemical Analysis of Extract

This was carried out according to the methods described by Trease and Evans (1989).

## 2.3 Isolation of Test Organisms and Culture Conditions

Pathogenic bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*) were obtained from degenerated wound, patient stool and high vaginal swab respectively. 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 Marrienfeld rotary incubator (150 rpm) at room temperature ( $28 \pm 2^{\circ}\text{C}$ ). The cells were harvested by centrifugation at 4000 rpm for 10 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 0.85 at 420 nm.

## 2.4 Screen Test for TTC Reduction (Dehydrogenase Activity)

On a colony of each bacterial isolate growing on nutrient agar, one drop of 1:1 mixture of aqueous solution of TTC (0.4% w/v) and glucose (2% w/v) was placed. The plates were incubated at room temperature for 10 minutes. Production of red coloured formazan was suggestive of TTC reduction.

## 2.5 Determination of Antimicrobial Potentials of *Euphorbia hyssopifolia* and *Euphorbia hirta* Ethanol Extracts by Total Dehydrogenase Activity (DHA) Assay

Total dehydrogenase assay method as described by Alisi et al. (2008) was employed to determine the antimicrobial activity of the extract. Briefly, total dehydrogenase activity was assayed using 2, 3, 5-triphenyltetrazolium chloride (TTC) (BDH England) as the artificial electron acceptor, which was reduced to the red-colored triphenyl-formazan (TPF). The assay was carried out in a 4 ml volumes of nutrient broth-glucose-TTC medium supplemented with varying concentrations (0 – 2000  $\mu\text{g/ml}$ ) 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 any of *Euphorbia hyssopifolia*, *Euphorbia hirta*, Ciprofloxacin, or Gentamycin and pre-incubated on a rotary incubator (150 rpm) at room temperature ( $28 \pm 2^{\circ}\text{C}$ ) 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- 2000  $\mu\text{g/ml}$  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 *Euphorbia hyssopifolia*, *Euphorbia hirta* extract, Ciprofloxacin or Gentamycin. The reaction mixtures were further incubated statically at room temperature ( $28 \pm 2^{\circ}\text{C}$ ) for 8.0h. The TPF produced were extracted in 4 ml of amyl alcohol and determined colorimetrically at 500 nm.

$$\begin{aligned} \% \text{ Inhibition of DHA activity} &= 100 - (\text{Absorbance of test} / \text{Absorbance of control}) \times 100 \\ &= 100 - \text{Percent DHA of control} \dots\dots\dots(1) \end{aligned}$$

## 2.6 Data Analysis

Percentage Inhibition of dehydrogenase activity in pathogens by *Euphorbia hyssopifolia*, *Euphorbia hirta* ethanol extract, standard antibiotics (Ciprofloxacin and Gentamycin) was calculated relative to their controls as shown in equation (1). The percentage inhibition data calculated were fitted into logistic dose response model (see equations 2 and 3) by plotting inhibition (y) against extract or standard concentration (x).

$$Y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d} \dots\dots\dots (2) \qquad y = \frac{a}{1 + \exp\left(-\frac{x-b}{c}\right)} \dots\dots\dots (3)$$

Equations 2 and 3 are logistic dose response equations (abcd and abc respectively). The parameters were estimated by iterative minimization of least squares using Levenberg-marquardt algorithm (Table curve 2D systat USA) Marquardt (1964). The data of %inhibition fitted into equation (2 or 3) were used to evaluate the toxicity thresholds IC<sub>5</sub>, IC<sub>10</sub>, IC<sub>20</sub>, IC<sub>50</sub>, IC<sub>80</sub>, IC<sub>90</sub>, and IC<sub>100</sub> which are the concentrations of the extracts that inhibited 5%, 10%, 20%, 50%, 80%, 90% and 100%.

## 3. RESULTS

### 3.1 Phytochemical Constituents of *Euphorbia hyssopifolia* and *Euphorbia hirta*

Our result (Table 1) showed that *Euphorbia hyssopifolia* and *Euphorbia hirta* gave positive reactions for alkaloids, saponins, tannins, flavonoids, cardiac glycosides and steroidal aglycones. Cyanogenic glycosides were present in *Euphorbia hyssopifolia*, but not detected in *Euphorbia hirta*.

**Table 1. Phytochemical constituents of *Euphorbia hyssopifolia* and *Euphorbia hirta* ethanol extracts**

	Alkaloids	Tannins	Flavonoids	Saponins	Cyanogenic glycosides	Cardiac glycosides	Steroidal Aglycone
<i>E. hirta</i>	+	+	+	+	-	+	+
<i>E. hyssopifolia</i>	+	+	+	+	+	+	+

Key: + .....Presence, - .....Absence

### 3.2 Inhibition of Dehydrogenase Activity in the Wound Isolates (*Staphylococcus aureus*)

Our result (fig.1) showed that exposure of *Staphylococcus aureus* (isolated from degenerate wound with delayed healing) to *Euphorbia hyssopifolia* resulted in a dose-dependent inhibition of dehydrogenase activity in the organism. *Euphorbia hyssopifolia* at 59.92µg/ml resulted in the inhibition of 50% of the test population. At an extract concentration of 1972.04µg/ml, *Euphorbia hyssopifolia* exerted a complete inhibition of DHA in *Staphylococcus aureus*. Ethanol extract of *Euphorbia hirta*, however failed to significantly inhibit the organism at the tested concentrations (0-2000µg/ml). *Euphorbia hirta* at 2000µg/ml could not cause up to 9% inhibition in the test organism. The standard antibiotics (Ciprofloxacin and Gentamycin) inhibited dehydrogenase activity in *Staphylococcus aureus* in a dose dependent fashion that followed a logistic dose response model. Threshold

inhibitory concentrations ( $IC_{50}$ ) of *Euphorbia hyssopifolia*, Ciprofloxacin and Gentamycin against the wound isolate were 59.92 $\mu$ g/ml, 63.75 $\mu$ g/ml, and 74.25 $\mu$ g/ml respectively (see table 2), while *Euphorbia hirta* could not exert significant inhibition of DHA in the organism.

**Table 2. Comparative threshold inhibitory concentrations of extract and standard drugs in pathogenic bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*)**

Pathogenic bacteria	Threshold inhibitory concentration $IC_{50}$ ( $\mu$ g/ml)			
	<i>E. hyssopifolia</i>	<i>E. hirta</i>	Ciprofloxacin	Gentamycin
<i>Staphylococcus aureus</i>	59.92	ND	4.85	10.36
<i>Salmonella typhi</i>	234.90	99.67	4.89	10.80
<i>Escherichia coli</i>	492.92	165.90	187.93	238.76

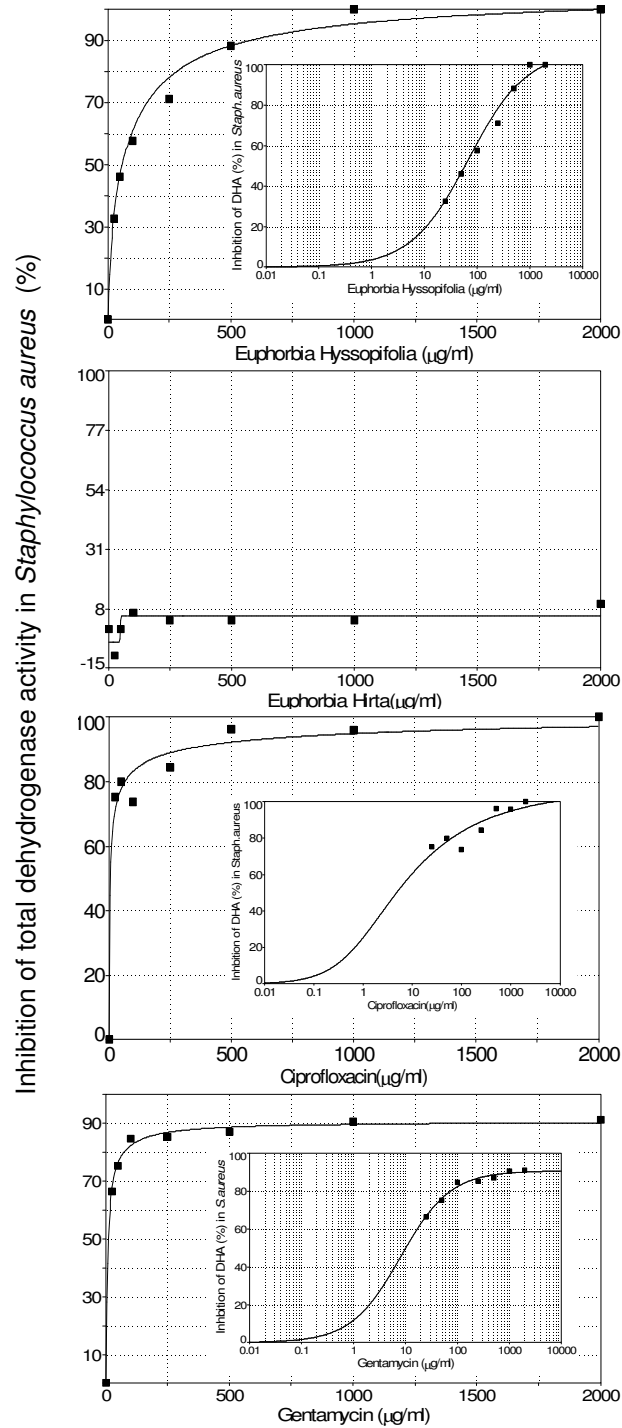
ND-Non determinable

### 3.3 Inhibition of Dehydrogenase Activity in the Stool Isolates (*Salmonella typhi*)

Our result (fig. 2) showed that exposure of *Salmonella typhi* (isolated from stool) to *Euphorbia hyssopifolia*, *Euphorbia hirta*, Ciprofloxacin and Gentamycin resulted in a dose-dependent inhibition of dehydrogenase activity in *Salmonella typhi*. Threshold inhibitory concentrations ( $IC_{50}$ ) of *Euphorbia hyssopifolia*, *Euphorbia hirta*, Ciprofloxacin and Gentamycin against the stool isolate were 234.90 $\mu$ g/ml, 99.67 $\mu$ g/ml, 4.89  $\mu$ g/ml and 10.80 $\mu$ g/ml respectively (see table 2), while their  $IC_{80}$  evaluated from the logistic dose response model were 2480.24 $\mu$ g/ml, 443.93 $\mu$ g/ml, 17.35  $\mu$ g/ml and 427.94 $\mu$ g/ml respectively. The plant extracts exerted inhibitory activities that followed a logistic dose response model like the standard antibiotics.

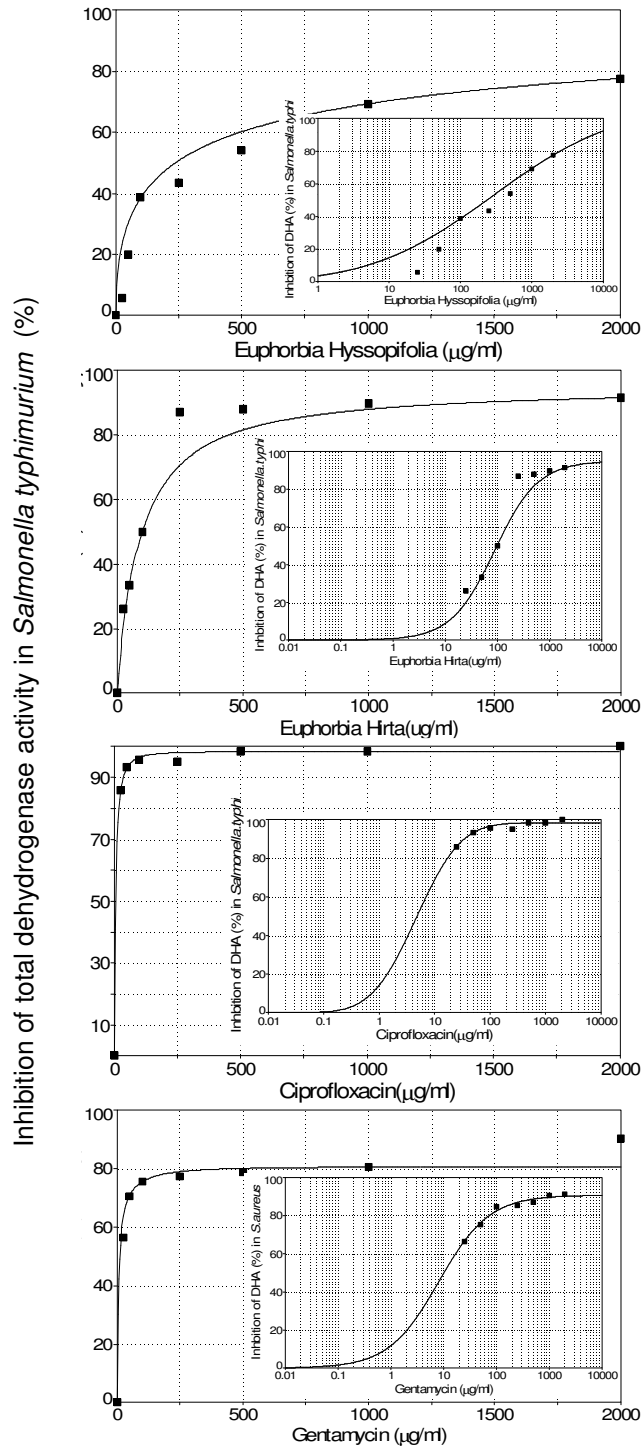
### 3.4 Inhibition of Dehydrogenase Activity in Higher Vaginal Isolate (*Escherichia coli*)

Our result (fig. 3) showed that exposure of *Escherichia coli* (From high vaginal swab) to *Euphorbia hyssopifolia*, *Euphorbia hirta*, Ciprofloxacin and Gentamycin each resulted in a dose-dependent inhibition of dehydrogenase activity in *Escherichia coli*. Threshold inhibitory concentrations ( $IC_{50}$ ) of *Euphorbia hyssopifolia*, *Euphorbia hirta*, Ciprofloxacin and Gentamycin against the stool isolate were 492.46 $\mu$ g/ml, 165.90 $\mu$ g/ml, 187.93  $\mu$ g/ml and 238.76 $\mu$ g/ml respectively (see table 2). The plant extracts exerted inhibitory activities that followed a logistic dose response model like the standard antibiotics. The extracts were however unable to completely (100%) inhibit dehydrogenase activity in *Escherichia coli* (see table 3).



**Figure 1. Inhibition of total dehydrogenase activity in pathogenic wound isolate (*Staphylococcus aureus*) by *Euphorbia hyssopifolia*, *Euphorbia hirta*, and standard antibiotics (*Gentamycin* and *Ciprofloxacin*).**

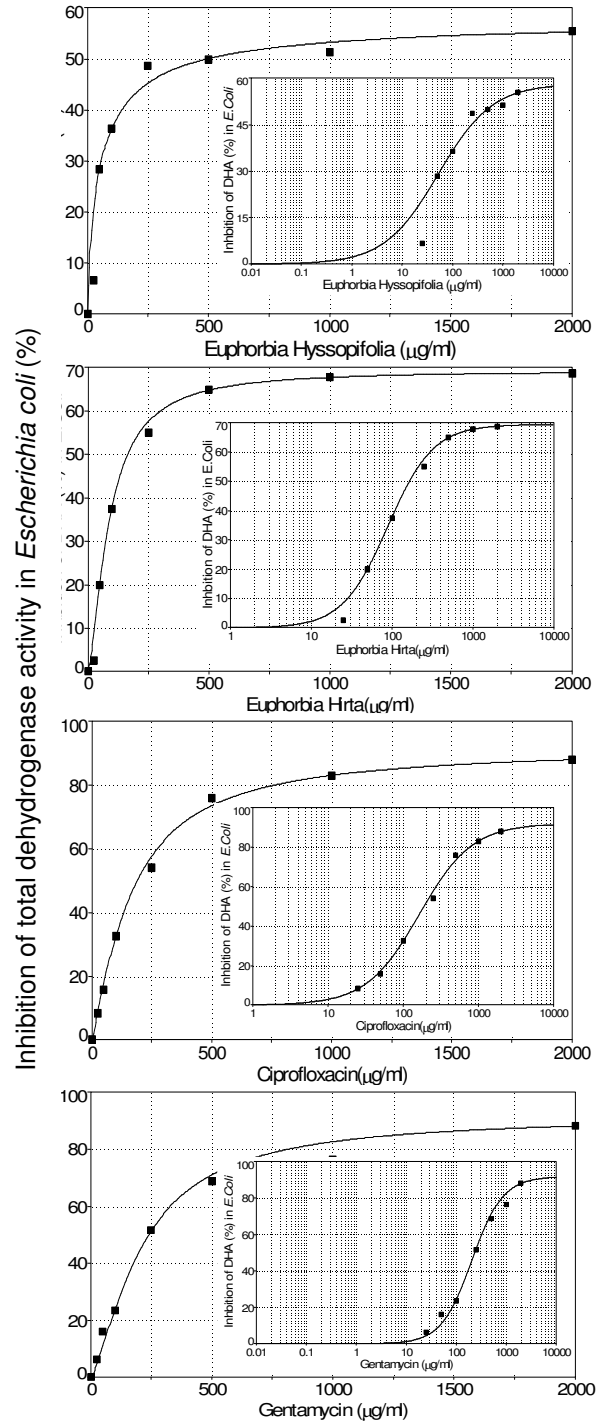
*Insets are same graph on a logarithmic x-axis that shows sigmoid association*



**Figure 2. Inhibition of total dehydrogenase activity in pathogenic stool isolate (*Salmonella typhi*) by *Euphorbia hyssopifolia*, *Euphorbia hirta*, and standard antibiotics (*Gentamycin* and *Ciprofloxacin*).**

*Insets are same graph on a logarithmic x-axis that shows sigmoid association*





**Figure 3. Inhibition of total dehydrogenase activity in pathogenic HVS isolate (*Escherichia coli*) by *Euphorbia hyssopifolia*, *Euphorbia hirta*, and standard antibiotics (*Gentamycin* and *Ciprofloxacin*).**

*Insets are same graph on a logarithmic x-axis that shows sigmoid association*

**Table 3. Threshold inhibitory concentrations of antimicrobial agents against DHA in pathogens**

	Threshold Inhibitory Concentration ( $\mu\text{g/ml}$ )						
	IC <sub>5</sub>	IC <sub>10</sub>	IC <sub>20</sub>	IC <sub>50</sub>	IC <sub>80</sub>	IC <sub>90</sub>	IC <sub>100</sub>
<i>Staphylococcus aureus</i>							
<i>Euphorbia hyssopifolia</i>	1.57	4.02	11.06	59.92	277.34	574.12	1972.04
<i>Euphorbia hirta</i>	ND	ND	ND	ND	ND	ND	ND
Ciprofloxacin	0.12	0.27	0.69	4.85	63.75	389	7192.3
Gentamycin	0.37	0.84	2.07	10.36	74.25	9354.12	ND
<i>Salmonella typhi</i>							
<i>Euphorbia hyssopifolia</i>	0.77	3.39	16.68	234.9	2480.24	7332.50	41047.00
<i>Euphorbia hirta</i>	5.68	11.65	25.48	99.67	443.93	1393.20	ND
Ciprofloxacin	0.68	1.18	2.17	6.14	17.85	34.75	2000.00
Gentamycin	0.37	0.74	1.77	10.80	427.94	ND	ND
<i>Escherichia coli</i>							
<i>Euphorbia hyssopifolia</i>	2.86	7.6	23.84	492.46	ND	ND	ND
<i>Euphorbia hirta</i>	17.23	28.43	50.13	165.9	ND	ND	ND
Ciprofloxacin	16.1	29.63	57.75	187.93	759.82	3582.66	ND
Gentamycin	27.9	47.52	85.11	238.76	815.31	3359.14	ND

ND – Non determinable (not able to inhibit up to that percentage of pathogen as evaluated from the logistic dose response equations)

#### 4. DISCUSSION

The ethanol extract of *Euphorbia hirta* was found to contain Alkaloids, Saponins, Glycosides, Cardiac glycosides, Steroidal aglycones, and Tannins. Plants are known to contain wide spectra of chemicals (Okwu, 2004). Some of these chemicals came into being by natural selection; through defunct metabolic pathways (Street and Cockburn, 1972). These phytochemicals have been found to have medicinal properties (Raza and John, 2007; Salah et al., 1995;) and antimicrobial effect (Alisi et al., 2011a).

We had earlier employed the use of the total dehydrogenase assay as a tool in probing response of microorganisms to antibacterial agents (Nwaogu et al., 2007; Nwaogu et al., 2008 and Alisi et al., 2008, Alisi and Onyeze 2009; Alisi et al., 2011a) and is recognized as a useful indicator of the overall measure of the intensity of microbial metabolism (Tabataba 1982; von Marsi and Schinner, 1991), and microbial activity (Gong, 1997; Mathew and Obbard, 2001).

Inhibition of total dehydrogenase enzymes by ethanol extracts of *Euphorbia hyssopifolia* (Fig 1) showed that *Euphorbia hyssopifolia* inhibited microbial metabolism, overall activity, and rendered *Staphylococcus aureus* non-viable. Wound healing has been found to be accelerated in the presence of non-viable *Staphylococcus aureus* as a result of the

contribution by its cell wall and cell wall Peptidoglycan components (Levenson et al., 2002), which increases number and alteration in location of macrophages, increased influx (or proliferation) of mesenchymal cells (notably fibroblasts), and increased angiogenesis and reparative collagen accumulation, as well as increasing the overall acute inflammatory response to wounding. (Killcullen et al.,1998).

Pathogenic bacteria find their way into wound complicating healing process and bringing about delay in wound healing by contributing oxygen free radicals in the site of injury. The antimicrobial action of *Euphorbia hyssopifolia* will halt the contribution of oxygen free radical to site of injury and hence facilitate wound healing. *Euphorbia hirta* failed to inhibit *Staphylococcus aureus* in this study. This result was however at variance with the report of Ogbulie et al. (2007) in which *Euphorbia hirta* was inhibitory to *Staphylococcus aureus*. Though the assay method was different, the variation may be due to the potentials for phenotypic variation with growth conditions in Gram positive bacteria. Both *Euphorbia hyssopifolia* and *Euphorbia hirta* significantly inhibited total dehydrogenase activity in Gram negative *Salmonella typhi*. This is at variance with the report of Ogueke et al. (2007) that ethanol extract of *Euphorbia hirta* was ineffective in inhibiting Gram negative *Salmonella typhi*. Both Ogbulie et al. (2007) and Ogueke et al. (2007), however seemed to be two sides of the same coin.

When tannic acid inhibited the growth of *Photobacterium phosphoreum* (Gram negative), one of the inhibitory sites was assumed to be the respiratory chain, another terminal oxidase (Konishi et al., 1987). Both plant had positive phytochemical reactions for tannins and saponins. Tannins, which are large phenolic molecules, and the more simple phenols and phenolic compounds like flavonoids are antimicrobial (Cowan 1999). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidised compounds, possibly through reaction with sulphhydryl groups or through more non-specific interactions with the proteins (Mason and Wasserman 1987). The extract of *Euphorbia hyssopifolia* was more active against the Gram-positive *Staphylococcus aureus* than against the Gram-negative microorganisms. This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than against Gram-negative bacteria (Vlietinck et al., 1995, Rabe and Van staden, 1997). *Euphorbia hirta* which could not exert total dehydrogenase inhibition in Gram positive *Staphylococcus aureus* was strongly inhibitory against *Salmonella typhi* and *Escherichia coli*. Antimicrobial action of both extract against *Escherichia coli* is welcome since emerging evidence shows increased resistance in organisms implicated in urinary tract infection.

The resistance mechanism of *Staphylococcus aureus* to *Euphorbia hirta* in this study could involve the secretion of tannin-binding polymers, tannin oxidation or siderophores. This suggestion needs further investigations. The co-existence of phenolic compounds with saponins which behave like the detergents 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. When compared with the very sensitive antibiotics used as standard/controls, the extracts could be said to be very promising as antimicrobial chemotherapeutic agents.

The observed antibacterial properties of *Euphorbia hirta* corroborate its use in traditional medicine. Traditionally, extracts of the plant are used in sore and wound healing, as ear drop for boils in the ear and treatment of boils in the control of diarrhea and dysentery (Kokwaro, 1993; Igoli et al., 2005). Since *Euphorbia hirta* could not inhibit the wound isolate, we hypothesize that its wound healing ability may be by extra-antimicrobial means including

antioxidant mechanism. Flavonoids are known to scavenge free radicals (Alisi et al. 2011b) and exert anti-inflammatory activity. Tissue oxidation and indeed lipid Peroxidation process is also quenched by phenolic compounds of plant origin (Alisi, et al., 2011c; Alisi, et al., 2011d).

The total phenolic compounds, flavonoids, Tannins, Saponins and other secondary plant metabolites found in the extract may be acting in synergy to bring about these observed antimicrobial actions and may explain reasons for ethno-medical usage as antimicrobial agents.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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