International Journal of Pharmacology, Phytochemistry and Ethnomedicine
ISSN: 2297-6922, Vol. 8, pp 27-40
doi:10.18052/www.scipress.com/IJPPE.8.27

Submitted: 2016-12-24
Revised: 2017-06-14
Accepted: 2017-07-06
Online: 2017-09-27

Determination of Antimicrobial Potentials of Ethanol Extract of *Combretum dolichopentalum* Leaves by Total Dehydrogenase Activity Assay

Ujowundu Favour Ntite

Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria

Corresponding Author E-mail: fnujowundu@yahoo.com

Keywords: Dehydrogenase activity, enzyme activity, inhibition, antioxidants, Phytochemicals, *E. Coli, S. typhi, S. aureaus, S. pneumonia, Combretum dolichopentalum*

Abstract. The viability of microorganisms can be determined by the total dehydrogenase activity (DHA). Thus, a reduction in total dehydrogenase activity is an indication of the bactericidal effect of plant extract. The antimicrobial potentials of ethanol extract of *Combretum dolichopentalum* (EECD) leaves on microbial isolates from stool, degenerated wound, and high vaginal swab were determined by the total dehydrogenase activity. The microbial cells were standardized in a spectrophotometer to an optical density of 0.70 at 420 nm and used as standardized cell suspension (inoculum) in the dehydrogenase assay. The results obtained indicated that EECD leaves were effective antimicrobial agents against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Streptococcus pneumonia* isolates. Threshold inhibitory concentrations of the extracts showed that EECD leaves inhibited dehydrogenase activity in all the organisms in a dose dependent manner. At 355.78 µg/ml, EECD leaves achieved an IC_{50} against *E. coli,* and at 349.42 µg/ml and 843.80 µg/ml EECD obtained an IC_{50} against *Streptococcus pneumonia* and *Staphylococcus aureus* respectively. Also, at 2270.68 µg/ml EECD leaves eliminated 100 % *S. typhi* to achieve 100 % inhibiting concentration. *C. dolichopentalum* makes a promising drug with bactericidal effect especially against *Escherichia coli* and *Salmonella typhi.*

Introduction

Researchers and pharmaceutical industries have increasing interest in traditional health practices used around the world, due to the growing drug discovery from natural products. This interest has been rekindled for years owing to systemic demonstration that plants are the richest source of drugs for traditional system of medicine, modern medicines, naturaceuticals, folk medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Most synthetic drugs have their origin from natural plant products [2]. Reports indicate that about 163 species of plants were used in wound healing in Indian systems of medicine [3-5]. Several plant based drugs are also used to treat diseases caused by pathogenic bacteria [6-7].

Pathogenic bacteria cause infections and disease by their entry into the body, rapid multiplication and crowding out healthy bacteria, or to grow in tissues that are normally sterile. Pathogenic bacteria may also emit toxins that damage the body [8]. Common pathogenic bacteria include: *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Streptococcus pneumoniae*, etc.

Staphylococcus aureus is the causative agent of boils, cellulitis, abscesses, wound infections, toxic shock syndrome, pneumonia and food poisoning [6]. A study by Kirker et al. [9] showed that wound healing can be accelerated in the presence of non-viable *S. aureus* as a result of the contribution by its cell wall and cell wall peptidoglycan components, which enhances number and alteration in location of phagocytes, increased proliferation of mesenchymal cells (notably fibroblasts), and increased angiogenesis and reparative collagen accumulation, as well as increasing the overall acute inflammatory response to injury [9].

Salmonella typhi is the causative agent of typhoid fever [9], a public health concern in communities where adequate treatment of water supplies including proper sewage disposal system

is lacking [10]. Risk factors include poor sanitation and poor hygiene [9]. *Escherichia coli* are a gram-negative, facultative anaerobe [11], with most strain harmless, but some serotypes can cause serious food poisoning in their hosts. *E. coli is* occasionally responsible for product recalls due to food contamination [12-13]. *Streptococcus pneumoniae*, is a gram-positive, facultative anaerobe [14] that causes many types of pneumococcal infections such as bronchitis, rhinitis, acute sinusitis, otitis media, conjunctivitis, meningitis, bacteremia, sepsis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, and brain abscess [15].

Medicinal plants and plant products serve as alternative to orthodox medicines especially in developing countries [16], either as extract or infusion in the treatment and management of diseases [17]. *Combretum dolichopentalum* leaves are used in the African and Indian traditional medicines and is commonly called Mmanya n'zeza by Igbos (Umuahia), 'Okoso' in Edo, all Nigerians. It is called Ohwirem by the Akan-Twi people in Ghana and Hakpa-nomu by the Mende people in Sierra Leone. *C. dolichopentalum* is used for treating disease conditions of the alimentary tract in Ibo ethnomedicine, which includes stomach ache, dysentery, gastrointestinal disorders, passage of bloody stool, diarrhea, and stomach ulcer in Imo State of Nigeria [18-19]. Several subclasses of flavonoids such as apigenin, kaempferol, luteolin, isorhamnetin etc have been isolated from *C. dolichopentalum* [19]. Furthermore, subclasses of alkaloid which includes; akuamidine, buphanidrine, crinamidine, 9-octadecinamide amongs others have also been isolated from *C. dolichopentalum* leaves, as well as saponins and tannins [19]. *Combretum dolichopentalum* have also been reported to possess free radical scavenging activities [20], and hepatoprotective potentials [18], thus it is expedient to carry out a cytotoxicity test on *C. dolichopentalum* to ascertain its antimicrobial potentials.

Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals and xenobiotics. A number of compounds used for cell viability detection rely on various cell functions [21, 7, 22, 23]. However, some of these methods cannot distinguish between the healthy cells and metabolically inactive cells, and can underestimate number of viable cells due to lack of homogeneity in distribution or difficulty in being readily desorbed from the substrate matrix [24]. Dehydrogenase-based assays reflect cell conditions with more sensitivity than other assays because they depend on several elements including dehydrogenase, NAD(H), NADP(H), and mitochondrial activity. This method is far superior to the previously mentioned methods because it is safe, has a high reproducibility, easy-to-use, and is widely used in both cell viability and cytotoxicity tests. Measurement of microbial enzyme activity has been used in the assessment of ecotoxicological impacts of environmental substrates. The inhibition of microbial enzyme (total dehydrogenase) activity is an important assay for potency of antimicrobial agents against pathogenic bacteria infection [25-27]. Dehydrogenase activity assay can be employed for the determination of bacterial growth and metabolism. The activity of the enzymes is directly proportional to the number of living cells during the growth phase and their activities during the production phase [28]. Recently, there has been a rekindled interest to rediscover medicinal plants as a source of potential drug candidate, this work determined the antimicrobial potentials of ethanol extract of *C. dolichopentalum* against *E. coli, S. aureaus, S. pneumonia,* and *S. typhi* using inhibition of total dehydrogenase activity.

Materials and Methods

Collection of plant sample and extraction

Fresh leaves of matured *C. dolichopentalum* plant were harvested from a farm in Obinze in Owerri West Local Government Area of Imo state of Nigeria with GPS coordinates N5^{o} 23'41.1' and E 6^{o} 57' 14.0'. The plant was identified by Mr. A. Ozioko, of the Bioresource Development and Conservation Program (BDCP), Research Centre at Nsukka, Enugu State with the assistance of Dr. F.N. Mbagwu, of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The sample specimen was deposited with voucher IMSUH12 at the University

Herbarium. The fresh leaves were removed from their stems, washed with distilled water and allowed to dry at room temperature (25± 2°C) for three weeks. The dried samples were pulverized in a grinding mill (Kenwood BL357) and stored at room temperature in an airtight container placed in a dark cupboard for 3 days.

Three hundred grams of the pulverized sample was extracted with 1.75 L ethanol/water in the ratio of 4:1 by soaking for 48 hours in three glass bottles. Exhaustive extraction was carried out in three separate glass bottles and finally pooled together. The sediment was removed by coarse filtration using a sieve followed by a Whatman No. 1 filter paper. The extract was concentrated using a rotary evaporator under mild temperature and reduced pressure and labeled ethanol extract of *C. dolichopentalum* (EECD).

Isolation of test organisms and culture conditions

The following pathogenic bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli and Streptococcus pneumonia*) were recovered from degenerated wound, high vaginal swab and patient stool. Isolated bacteria were purified on nutrient agar (Fluka) plates and characterized using standard microbiological methods. Following the scheme of Holt et al, [29] identification of the isolates was done to the generic level. The strains of bacteria were grown to mid exponential phase (14hrs) in nutrient broth (Lab M) on a Marrienfeld rotary incubator (150 rpm) at 28 ± 2°C. The cells were harvested by centrifugation at 4000 rpm for 10 min. Harvested cells were re-suspended in sterile water after washing twice in sterile distilled water. The re-suspended cells were standardized in a spectrophotometer to an optical density of 0.7 at 420 nm.

Total dehydrogenase activity (DHA) assay

The method as described by Alisi et al, [27] with minor modifications was employed in total dehydrogenase assay. Briefly; The substrate 2,3,5- Triphenyl tetrazolium chloride (TTC), an artificial electron acceptor is reduced to the red – coloured triphenyl- formazan (TPF) by electrons from viable cells such as *E. coli, S. aureaus, S. typhi* and *S. pneumonia.* The assay was done in 4 ml volumes of nutrient broth-glucose- TTC medium supplemented in varying concentrations of extract.

Inoculation: The microbial cells were standardized in a spectrophotometer to an optical density of 0.70 at 420 nm. These standardized cell suspension were used as inocula in the dehydrogenase assay. Portions (0.3 ml) of these bacterial suspensions were inoculated into triplicate glass tubes containing 2.5 ml phosphate buffered (pH 6.8) nutrient broth-glucose medium amended with *C. dolichopentalum* extract (0, 20, 50, 100, 150, 250, 500, 800, 1000, and 2000 µg/ml) and pre-incubated on a rotary incubator (150 rpm) at room temperature (28 ± 2^0C) for 30 minutes. Thereafter 0.1 ml of 1 % (w/v) TTC in de-ionized distilled water was added to each test tube. The final concentrations of nutrient broth, glucose and TTC in the medium were 2, 2, and 0.25 mg/ml, respectively.

Controls: The controls consisted of the isolates and the media without *C. dolichopentalum* extract. The reaction mixtures were further incubated at room temperature (28 ± 2^0C) for 8.0 hours. The TPF formed were extracted in 4 ml amyl alcohol and determined spectrophotometrically at 500 nm (λmax). The amount of formazan produced was determined from a standard dose– response curve (0-20 µg/ml TPF in amyl alcohol, $y = 0.487x$, $R^2= 0.997$). Dehydrogenase activity was expressed as milligrams of TPF formed per mg dry weight of cell biomass per hour.

***Calculation*:** Inhibition of dehydrogenase activity of the isolates by ethanol extract of *C. dolichopentalum* (EECD) was calculated relative to the control using equation 1.

$$\% \text{ Inhibition of DHA activity} = 100 - (\text{Absorbance of test} / \text{Absorbance of control}) \times 100$$
$$= 100 - \text{Percent DHA of control} \quad (1)$$

The percentage inhibitions for organisms were plotted against the concentrations of the extracts using the Table 2D curve V 5.01 system software. The toxicity threshold concentrations (IC_{20}, $1C_{50}$,

$1C_{70}$, $1C_{80}$ and $1C_{100}$) were then evaluated from the dose response plots. The total inhibitory concentrations ($1C_{100}$) values which were non-determinable from the simple inhibition plots were subjected to evaluation using a log transformation of % inhibition plots. Note that: Log % Inhibition = 2 = $1C_{100}$

Table 1. The inhibitory concentration of ethanol extract of *C. dolichopentalum* against the total dehydrogenase activity (DHA) of some bacteria.

	Inhibitory Concentration against some bacteria						
	EECD (µg/ml)						
	IC_5	IC_{10}	IC_{20}	IC_{50}	IC_{70}	IC_{80}	IC_{100}
Escherichia coli	1.8	9.06	42.95	355.78	822.58	1167.79	2167.1
Samonella Typhi	5.74	19.34	67.74	413.19	886.14	1234.23	2270.68
Streptococus pneumonia	2.59	9.24	36.74	349.42	1159.25	2195.66	11523
Staphylococcus aureus	705.38	716.82	741.69	843.8	975.47	1138.3	ND

ND = Non-determinable

Table 2. Inhibitory concentration of ciprofloxacin against total dehydrogenase activity (DHA) of some bacteria.

	Inhibitory concentration against some bacteria						
	Ciprofloxacin (µg/ml)						
	IC_5	IC_{10}	IC_{20}	IC_{50}	IC_{70}	IC_{80}	IC_{100}
Escherichia coli	0.13	0.56	2.66	29.21	92.58	161.57	530.7
Samonella typhi	0.97	3.27	11.73	79.25	187.78	278.61	605.45
Streptococcus pneumonia	7.15	0.00	0.01	4.03	42.7	118.94	781.96
Staphylococcus auraeus	0.00	0.03	0.5	25.4	120.28	229.76	714.81

Data analysis

The plant extract total dehydrogenase inhibition data (mean values from triplicate determinations) were fitted into kinetic equation- logistic-dose-response model and sigmoid abcd model. Percentage inhibition of dehydrogenase activity in pathogens by ethanol extract of *C. dolichopentalum*, standard antibiotics (Ciprofloxacin) was calculated relative to their controls as shown in equation (1). Furthermore, calculated percentage inhibition data were fitted into the logistic dose response model (equations 2, 3 and 4) by plotting inhibition (y) against extract or standard concentration (x).

$$y=a+b/(1+(x/c)^d) \quad (2)$$

$$y=a+4bn/(1+n)^2 \; n=\exp(-(x-c)/d) \quad (3)$$

$$y=a/(1+(x/b)^c) \quad (4)$$

where

a, b, c, d = Empirical values

$a = Y_{(predicted)}$ at $X_{(max)}$

$b = Y_{(predicted)}$

c = slope parameter defining inhibition rate

d = empirical value

x = extract concentrations (µg/ml)
y = % inhibition of DHAs

Equations 2, 3 and 4 are logistic dose response equations (abcd). The parameters were estimated by iterative minimization of least squares using Levenberg Marquardt algorithm (Table curve 2D systat USA) Marquardt [27]. The data of %inhibition fitted into equation (2, 3 or 4) were used to evaluate the toxicity thresholds IC5, IC10, IC20, IC50, IC80, IC90, and IC100 which are the concentrations of the extracts that inhibited 5%, 10%, 20%, 50%, 80%, 90% and 100%**.** Equation 2 was used for *E. coli, S. typhi* and *S. pneumonia*, while equation 3 and 4 were used for *S. aureus* and ciprofloxacin respectively.

Results

The inhibition of total dehydrogenase activity against *E. coli* by EECD is shown in Fig. 1. The extract dose dependently inhibited total dehydrogenase activity in *E. coli* following the logistic dose response curve although this was less than the inhibition caused by the standard drug ciprofloxacin. The threshold inhibitory concentrations are as shown in Table 1

Fig. 2 shows the inhibition of total dehydrogenase activity against *S. aureus* by EECD. At a higher concentration the extract dose dependently inhibited total dehydrogenase activity in *S. aureus* following the logistic dose response curve. But at lower concentrations, the extract stimulated the initial growth of *S. aureus* showing hormesis. Threshold inhibitory concentrations are as shown in Table 1

Fig. 3 shows the inhibition of total dehydrogenase activity against *S. pneumonia* by EECD. Following the logistic dose response curve (equation 2) as shown in Fig. 3, the extract dose dependently inhibited total dehydrogenase activity in *S. pneumonia*. Threshold inhibitory concentrations are as shown in Table 1.

Fig. 4 shows that EECD dose dependently inhibited total dehydrogenase activity in *S. typhi* following the logistic dose response curve in a manner less than that of the standard drug ciprofloxacin. At 2270.68 µg/ml EECD was able to eliminate 100 % *S. typhi*. The threshold inhibitory concentrations are as shown in Table 1.

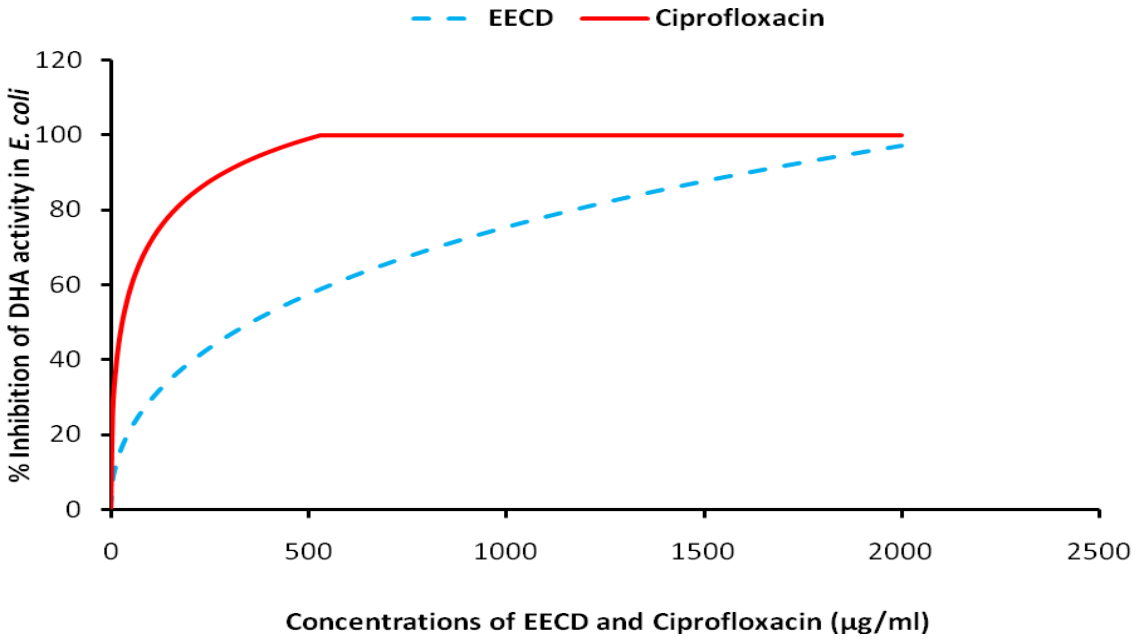


Figure 1. Percentage inhibition of dehydrogenase activity in *E. coli* by graded concentrations of standard drug (Ciprofloxacin) and ethanol extract of *C. dolichopentalum*. See appendices 1a and 1b for individual plot using the Table curve 2D SYSTAT.

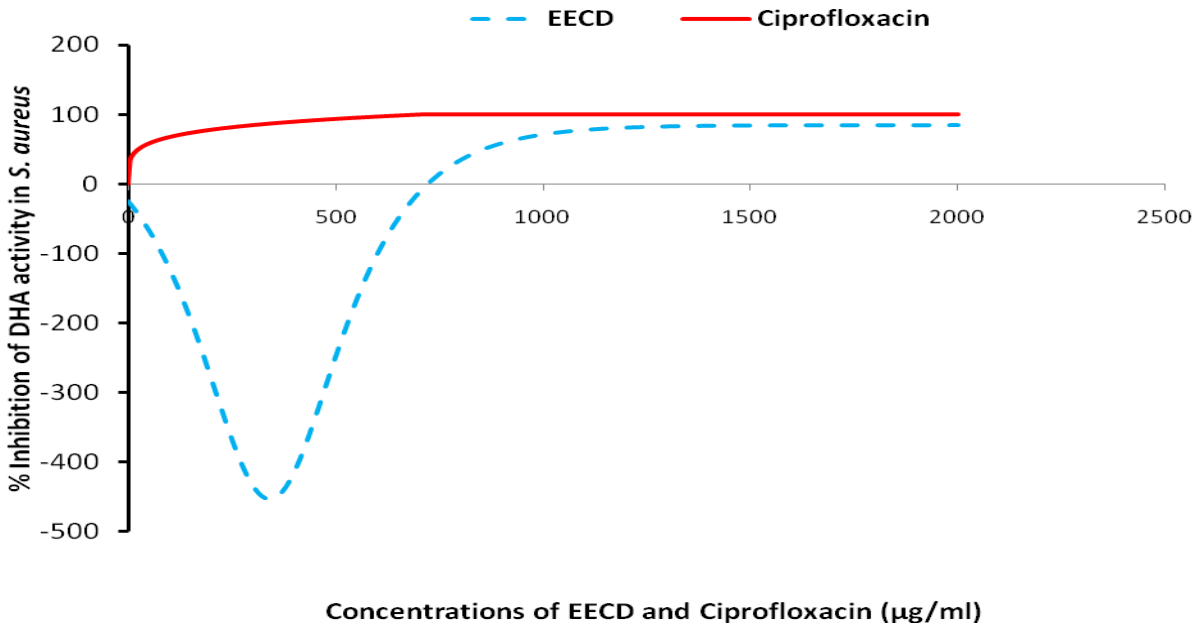


Figure 2. Percentage inhibition of dehydrogenase activity in *S. aureus* by graded concentrations of standard drug (Ciprofloxacin) and ethanol extract of *C. dolichopentalum*. See appendices 2a and 2b for individual plot using the Table curve 2D SYSTAT.

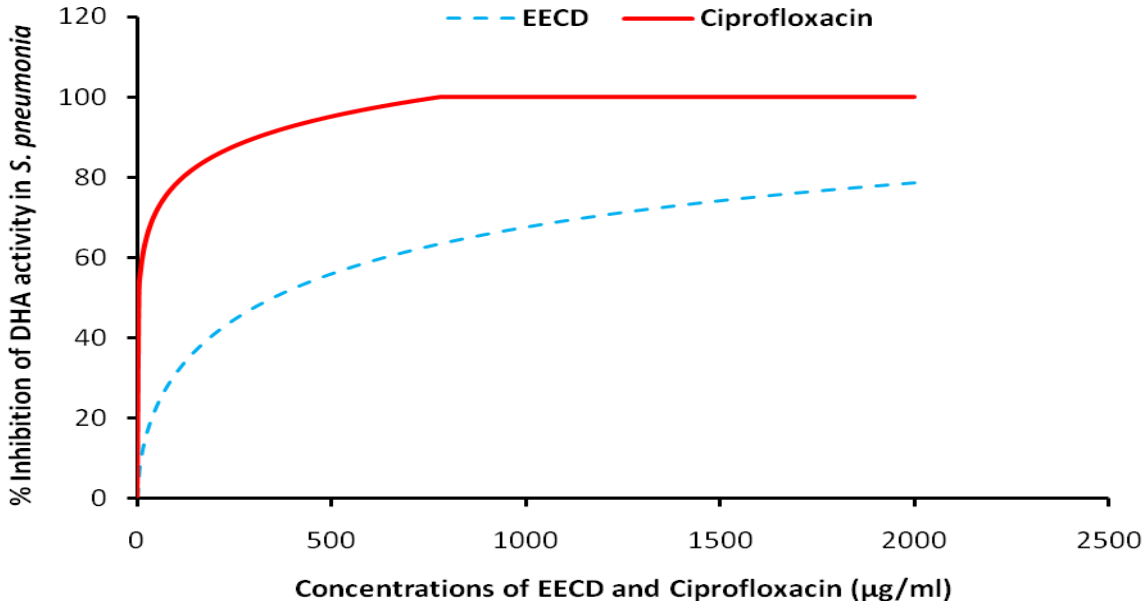


Figure 3. Percentage inhibition of dehydrogenase activity in *S. pneumonia* by graded concentrations of standard drug (Ciprofloxacin) and ethanol extract of *C. dolichopentalum*. See appendices 3a and 3b for individual plot using the Table curve 2D SYSTAT.

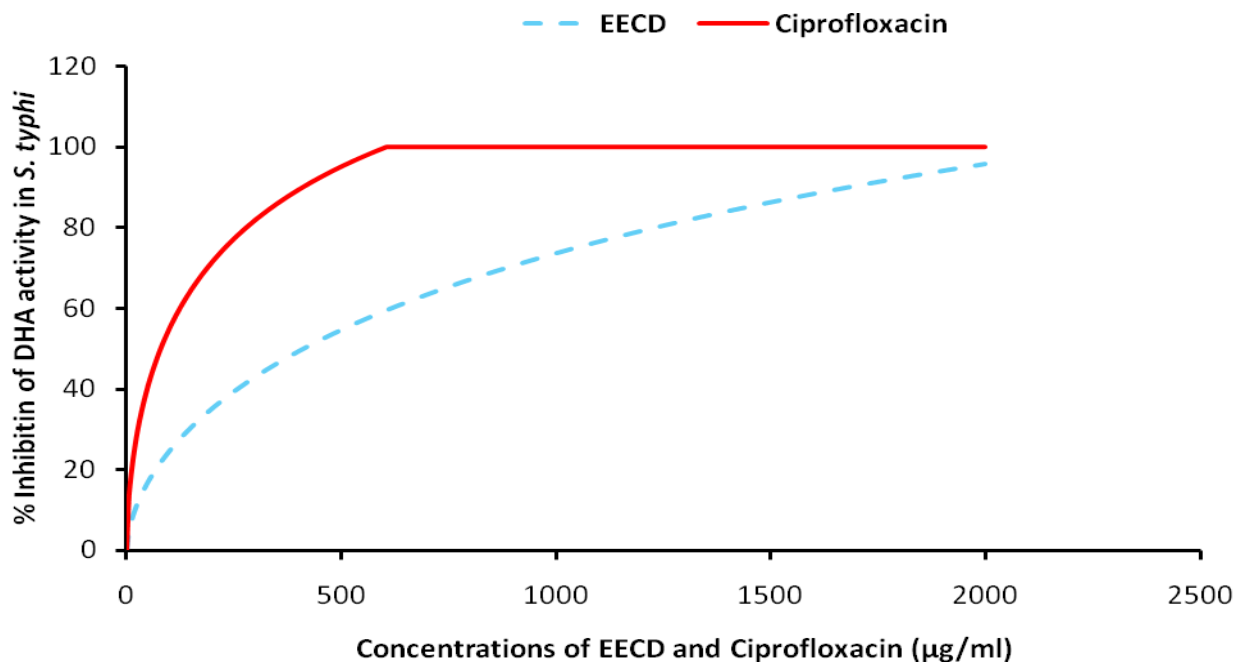


Figure 4. Percentage inhibition of dehydrogenase activity in *S. typhi* by graded concentrations of standard drug (Ciprofloxacin) and ethanol extract of *C. dolichopentalum*. See appendices 4a and 4b for individual plot using the Table curve 2D SYSTAT.

Discussion

Dehydrogenase is a wide group of endocellular enzyme, which is present in all living cells and are required for catalyzing the oxidation of organic compounds. They transfer hydrogen and electrons through a chain of intermediate electron carriers to oxygen as a final electron acceptor, which subsequently combine with them to form water [30]. Dehydrogenase activity is largely measured using the triphenyl tetrazolium chloride (TTC) salt as a hydrogen acceptor.

Triphenyl tetrazolium chloride (TTC) is a stable, water soluble heterocyclic organic salt that can be easily reduced to form a highly coloured insoluble product (red formazan), which can be quantified colorimetrically by visible light absorption. Formazan produced from the test is used as a measure of the amount of the living cells [28]. Dehydrogenase activity (DHA) is an effective primary test for the assessment of ecotoxicological impacts of environmental substrates such as toxicity of metals to planktonic [31], heterotrophic bacteria [32], and pathogenic organisms in wounds [29, 33, 6]]. The viability of microorganisms is represented by the total dehydrogenase activity, thus a reduction in total dehydrogenase activity is an indication of the bactericidal effect of the plant extract on the microorganisms. The toxicity of ethanol extract of *C. dolichopentalum* to microbial isolates from degenerated wound, isolated stool and high vaginal swab were determined. Our result (Figs. 1- 4) shows that EECD leaves were effective antimicrobial agents against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Salmonella typhi* isolates. Dehydrogenases are produced by viable cells only. TTC was used to measure the viability and growth of the pathogenic microorganisms [34]. Formazan produced from the reduction of TTC by dehydrogenases is used as a measure of the amount of the living cells [28]. A reduction in the amount of formazan formed from TTC in the presence of the crude extract indicates a drop in the activity of total dehydrogenase. This in turn is due to a reduction in the amount of viable cells. This evidently shows that the crude extract was bactericidal to the microorganisms. This corroborates with the work done by [7] using dehydrogenase.

Threshold inhibitory concentrations of the extracts showed that EECD inhibited dehydrogenase activity in all the organisms in a dose dependent manner. Although a standard drug-ciprofloxacin was more bactericidal. However, at 355.78 µg/ml, EECD achieved an IC_{50} against

E. coli when compared to *Euphobia hyssopifolia* at 492.46 µg/ml. While at 2270.68 µg/ml EECD eliminated 100 % *S. typhi* compared to *E. hyssopifolia* which needed a concentration as high as 41047.0 µg/ml, to achieve 100 % inhibiting concentration [7].

There was a stimulation of DHA in *Staphylococcus aureus* at low concentration of *C. dolichopentalum* leaf extracts (Fig. 2), while at higher concentrations it progressively inhibited dehydrogenase activity in a dose-dependent manner. Some bio-substances that are bactericidal in certain organisms at higher concentrations have been demonstrated to exert a stimulatory effect at micro-concentrations [32]. The leaf may contain substances that support growth at lower concentration. Pathogenic bacteria penetrate wound making healing process complicated and delaying wound healing by contributing oxygen free radicals in the site of injury [10]. The antimicrobial action of *C. dolichopentalum* will prevent the contribution (or could inhibit the generation) of reactive oxygen radical at the site of injury and thereby facilitate wound healing as it possesses antioxidant activity [19]. The rate of inhibition of total dehydrogenase activity in *Staphylococcus aureus* was not sustained making IC100 in *Staphylococcus aureus* non-determinable. This observation is in line with work of Alisi et al, [6] who also reported that *S. aureus* was not determinable at IC100 with *C. odorata*. However, at 1138.30 µg/ml ethanol extract of *C. dolichopentalum* eliminated 80 % of the pathogen. *Combretum dolichopentalum* has earlier been reported to possess secondary metabolites such as flavonoid, saponins, alkaloids and tannins [20, 19], which might inhibit bacteria by different mechanisms. Tannin content of *C. dolichopentalum* may exhibit antimicrobial activity [35], while flavonoids and saponins are known to exert antimicrobial activities as well [36-37]. The phytochemical and other antioxidant content of extracts of *C. dolichopentalum* may be acting synergistically to stimulate the observed inhibition of dehydrogenase activity. These extracts may actually be exerting their antimicrobial activity via inhibition of dehydrogenase activity in the test organisms.

Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria [38, 7]. These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall has a multilayered structure [39]. This restrains the penetration of active drug fraction. The inhibition of the growth of both *S. aureus* and *E. coli* in the presence of the ethanol extract of *C. dolichopentalum* indicates its effectiveness in penetrating the multilayered membrane structure of gram negative bacteria. Although the management and treatment of infections caused by bacterial, have remarkably been effective since the discovery of the various antibacterial drugs. Nevertheless some of these bacterial have rapidly become resistant to a number of the first discovered effective drugs. The incidence of drug resistance as well as development of untoward effects of certain antibiotics has led to the investigation of new bactericidal agents specifically from medicinal plants. Results obtained showed that *C. dolichopentalum* totally eliminated *E. coli*, *S. typhi* and *S. pneumonia* at relatively lower concentrations as shown in Table 1 above. The secondary plant metabolites identified in the extract [19, 20] may be acting synergistically to bring about the observed inhibition of dehydrogenase activity.

Recommendation

Numerous compounds within the crude extracts may have antagonizing interference with the actions of one another. Further separation and purification of the crude extracts into bioactive ingredient might increase its bioactivity relative to the crude extracts.

Conclusion

The observed antimicrobial properties of *C. dolichopentalum* supports its (aqueous and ethanol extract) use in traditional medicine in control of diarrhoea, dysentery and as an anti-ulcer agent. Thus, *C. dolichopentalum* can be investigated further as a therapy for its bactericidal effect against both gram negative and gram positive bacteria.

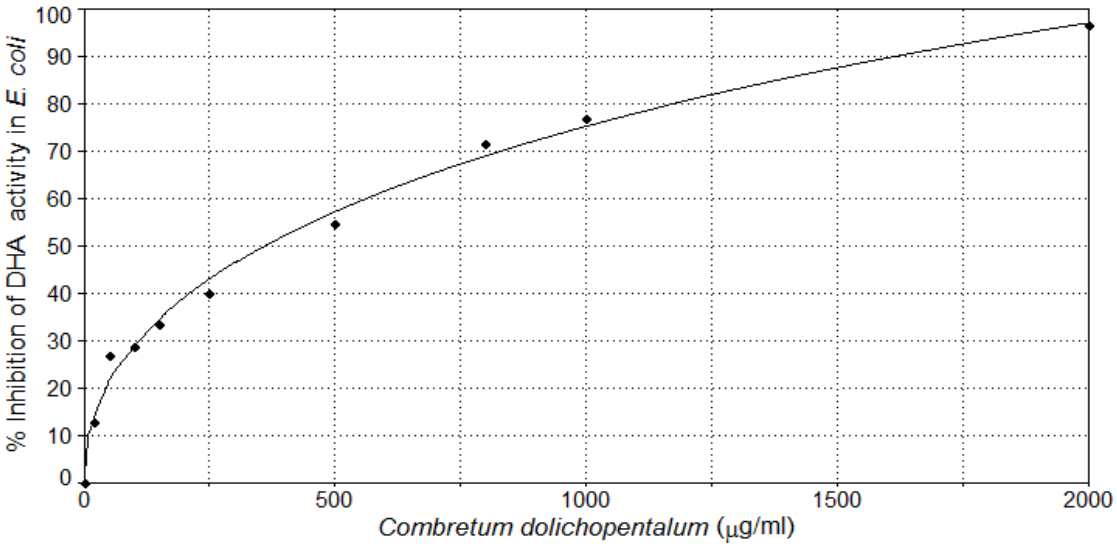
Competing interests

I declare no competing interest

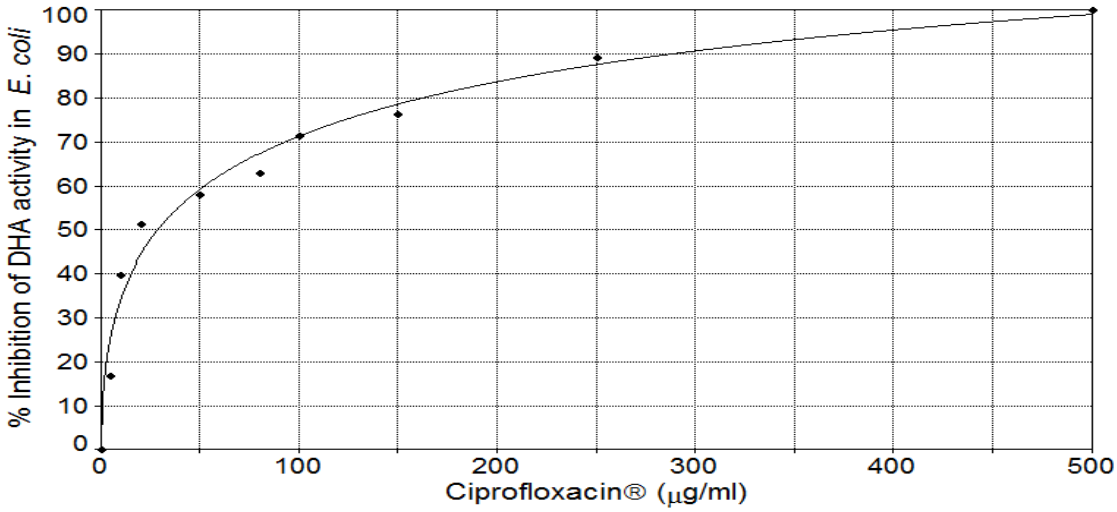
Acknowledgments

I acknowledge Dr. C.O. Ujowundu, Prof. A.I. Ukoha, Dr. R.N. Nwaoguikpe and Prof. A.O. Ojiako for their technical assistance and advice.

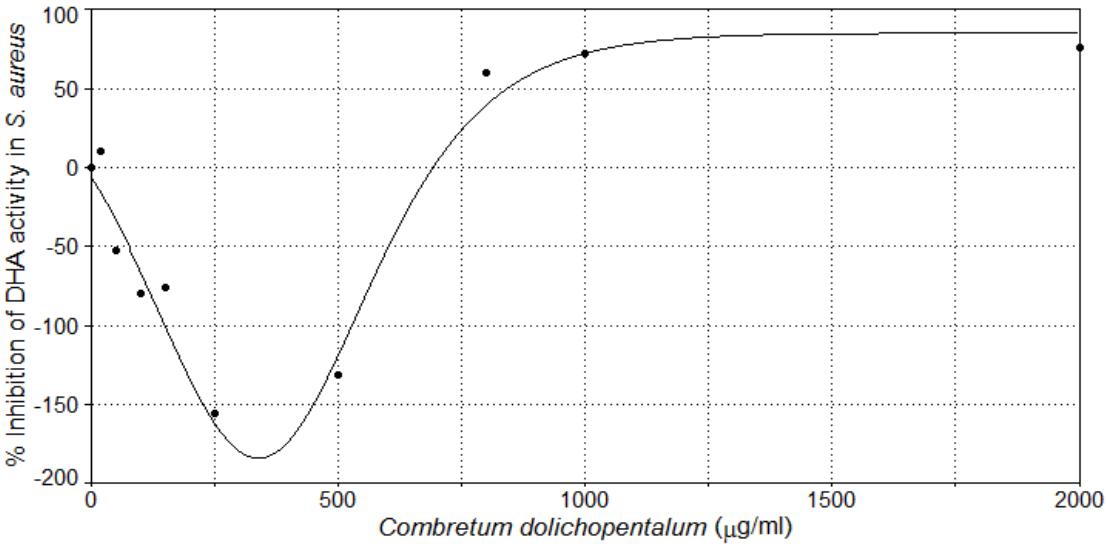
Appendix



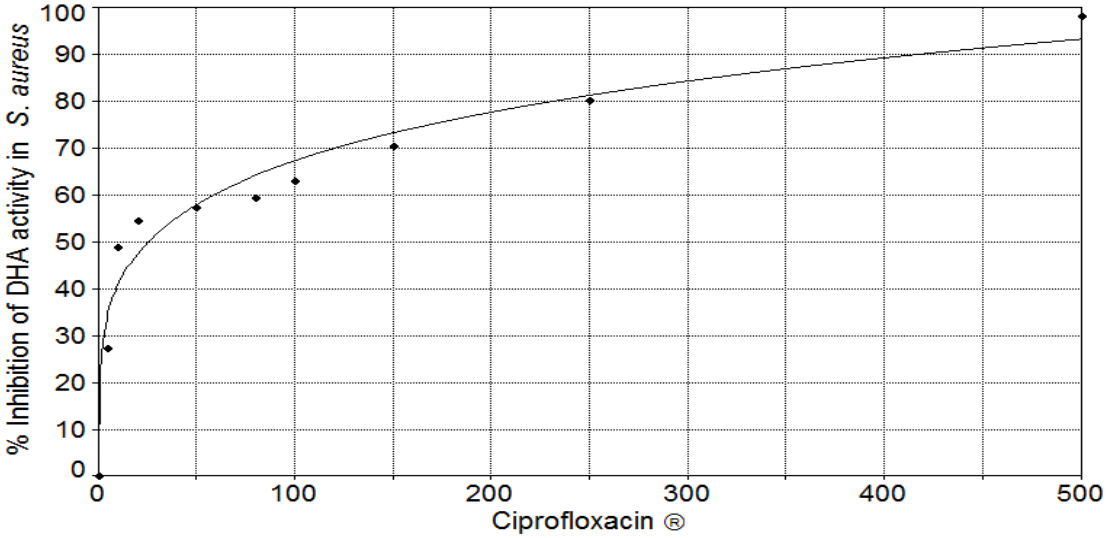
Appendix 1a. Inhibition of total dehydrogenase activity in *E. coli* by ethanol extract of *C. dolichopentalum.*



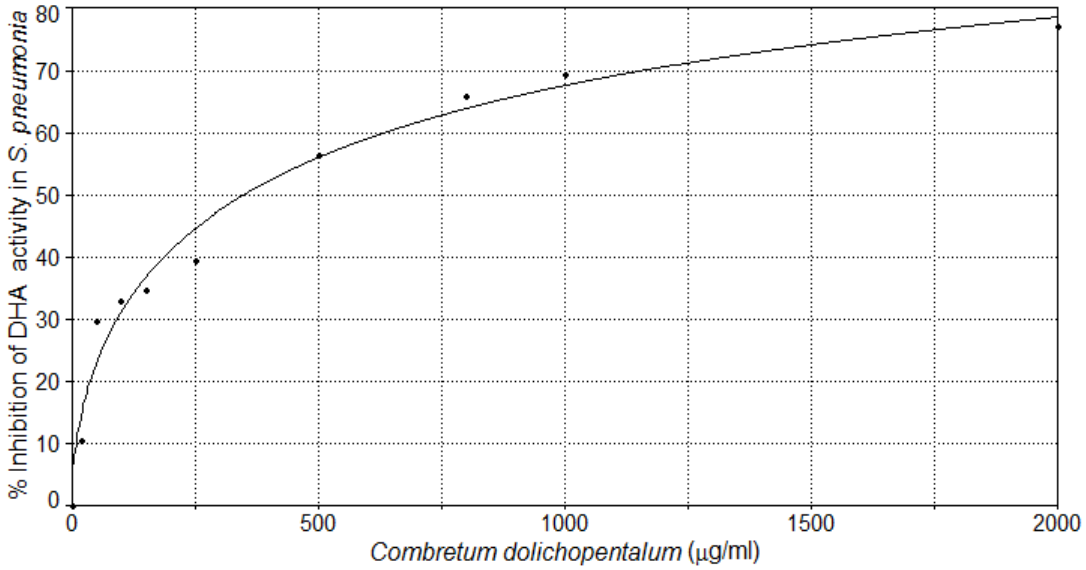
Appendix 1b. Inhibition of total dehydrogenase activity in *E. coli* by Ciprofloxacin.



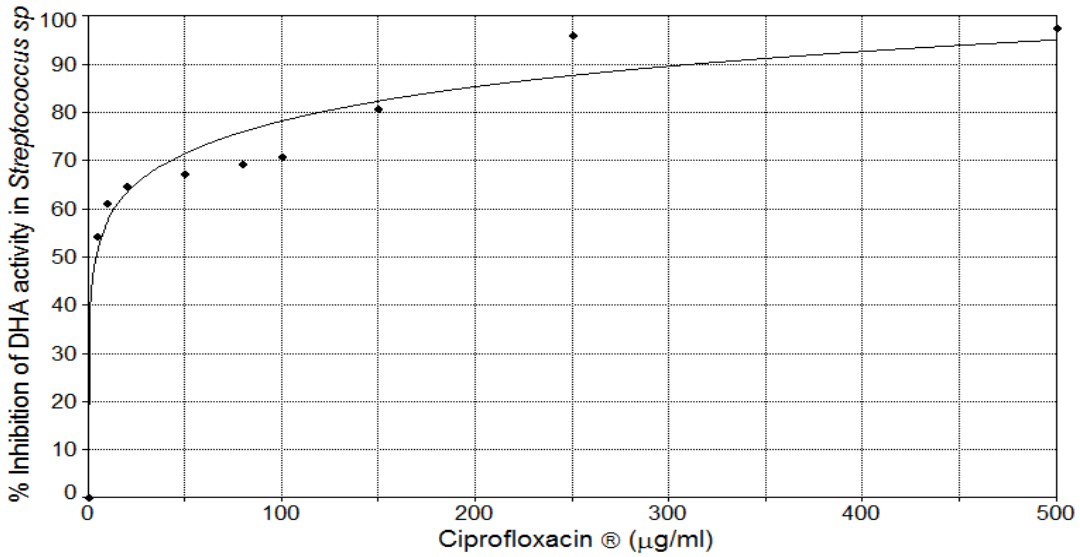
Appendix 2a. Inhibition of total dehydrogenase activity in *S. aureus* by ethanol extract of *C. dolichopentalum.*



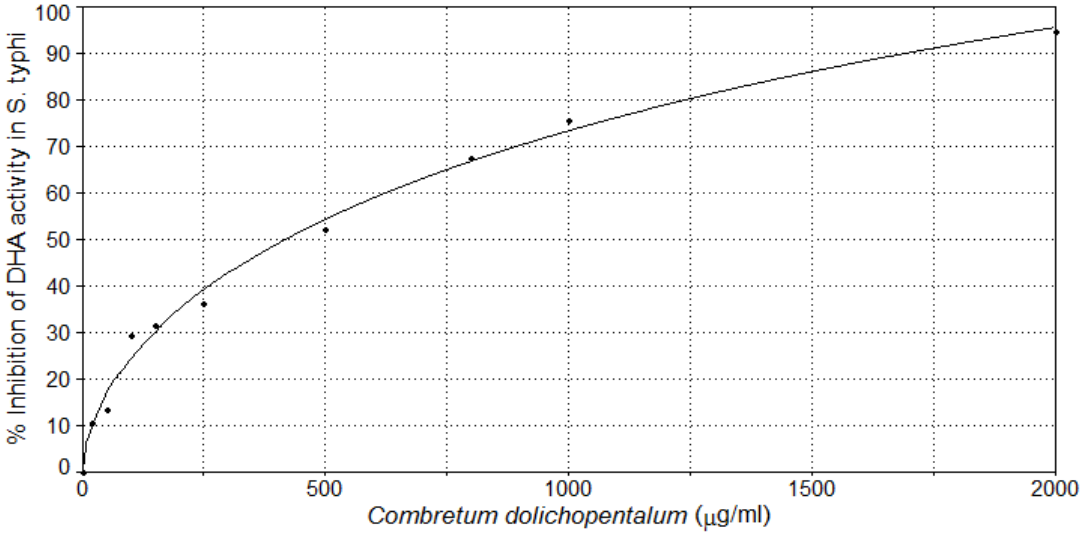
Appendix 2b. Inhibition of total dehydrogenase activity in *S. aureus* by Ciprofloxacin.



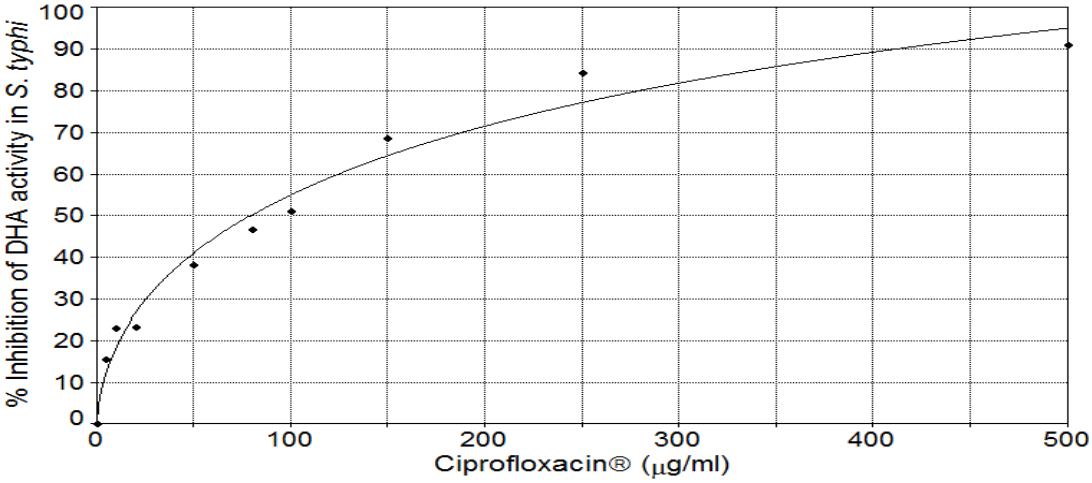
Appendix 3a. Inhibition of total dehydrogenase activity in *S. pneumonia* by ethanol extract of *C. dolichopentalum.*



Appendix 3b. Inhibition of total dehydrogenase activity in *S. pneumonia* by Ciprofloxacin.



Appendix 4a. Inhibition of total dehydrogenase activity in *S. typhi* by ethanol extract of *C. dolichopentalum*



Appendix 4b. Inhibition of total dehydrogenase activity in *S. typhi* by Ciproxacin

References

[1] A.K. Shakya, Medicinal plants: future source of new drugs, International Journal of Herbal Medicine. 4(4) (2016) 59-64.

[2] D.J. Newman, G.M. Cragg, D.G.I. Kingston, In the practice of medicinal chemistry, 4th ed. C.G. Wermuth et al. (Eds.), Elsevier: Amsterdam, 2015, pp. 101–139.

[3] B. Kumar et al., Ethnopharmacological approaches to wound healing – Exploring medicinal plants of India, J. Ethnopharmacol. 114 (2007) 103-113.

[4] M. Ayyanar, S. Ignacimuthu, Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences, Inter. J. Appl. Res. Nat. Prod. 2(3) (2009) 29-42.

[5] S.D. Sarker, L. Nahar, Chemistry for pharmacy students general, organic and natural product chemistry, John Wiley and Sons, 2007, pp. 283-359.

[6] C.S. Alisi et al., Antimicrobial action of methanol extract of C*hromolaena odorata* – Linn is Logistic and exerted by inhibition of dehydrogenase enzymes, J. Res. Biol. 3 (2011) 209-216.

[7] C.S. Alisi, S.E. Abanobi, Antimicrobial properties of *Euphorbia hyssopifolia* and *Euphobia hirta* against pathogens complicit in wound, typhoid and urinary tract infections, Inter. J. Trop. Disease Health. 2(2) (2012) 72-82.

[8] Centers for Disease Control and Prevention, 1600 Clifton Road Atlanta, GA USA, 2015.

[9] K.R. Kirker et al., Differential effects of planktonic and biofilm MRSA on human fibroblasts, Wound Repair Regen. 20(2) (2012) 253–261.

[10] M.C. Regan, A. Barbul. The cellular biology of wound healing. http://www.neuro.ki.se/neuro/KK2/Jimmy_GoogleCACHE1.html. Accessed on 30th June, 2017.

[11] J. Wain et al., Typhoid fever, Lancet. 385(9973) (2015) 1136–1145. Doi: 10.1016/s0140-6736(13)62708-7.

[12] K.C. Huang et al., Cell shape and cell-wall organization in Gram-negative bacteria, Proceedings of the National Academy of Sciences. 105(49) (2008) 19282-19287.

[13] CDC, Escherichia coli. National Center for Emerging and Zoonotic Infectious Diseases. Retrieved 2012-10-02.

[14] CDC, Notes from the field: Escherichia coli O157:H7 outbreak associated with seasonal consumption of raw ground beef - Wisconsin, December 2012-January 2013, MMWR. Morbidity and mortality weekly report. 62(48) (2013) 987.

[15] CDC, *Streptococcus pneumonia,* 2015. Available at https://www.cdc.gov/pneumococcal/clinicians/streptococcus-pneumoniae.html.

[16] R.A.C. Siemieniuk, D.B. Gregson, M.J. Gill, The persisting burden of invasive pneumococcal disease in HIV patients: an observational cohort study, BMC Infectious Diseases. 11 (2011) 314.

[17] D. Hada, K. Sharma, A review: plant extracts a new source of antimicrobial potential, Research Journal of Pharmaceutical, Biological and Chemical Sciences. 5(3) (2014) 597-628.

[18] F.N. Kalu et al., Aqueous extract of *Combretum dolichopentalum* leaf - a potent inhibitor of carbon tetrachloride induced hepatotoxicity in rats, Journal of Applied Pharmaceutical Science. 01(10) (2011) 114-117.

[19] F.N. Ujowundu et al., Isolation of bioactive phytochemicals in leaves of *Combretum dolichopentalum* and their hydrogen peroxide scavenging potentials, Pharm. Anal. Acta. 6 (2015) 444.

[20] F.N. Ujowundu et al., Gas chromatographic characterization of the flavonoids, alkaloids, saponins, and tannins isolated from *C. dolichopentalum* leaves, J. Chem. Pharmaceutical Res. 7(12) (2015) 1094-1103.

[21] D.S. Auld et al, A basis for reduced chemical library inhibition of firefly luciferase obtained from directed evolution, J. Med. Chem. 52 (2009) 1450-1458.

[22] S.J. Duellman et al., Bioluminescent, nonlytic, real-time cell viability assay and use in inhibitor screening, Assay Drug Dev. Tech. 13(8) (2015) 456-465.

[23] T.L. Riss et al., Cell Viability Assays, in: G.S. Sittampalam et al. (Eds.), Assay Guidance Manual, 2016.

[24] V. Quintas, I. Prada-López, I. Tomás, Analyzing the oral biofilm using fluorescence-based microscopy: what's in a dye? In: A. Mendez Vilas (Ed.), Microscopy: Advances in Scientific Research and Education, 2014, pp. 226-238.

[25] L.A. Nwogu et al., Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf, Afr. J. Biotechnol. 6(7) (2007) 890-893.

[26] L.A. Nwogu et al., A comparative study of the antimicrobial properties of the ethanolic extracts of *Landolphia owariensis* leaf and root, Afr. J. Biotechnol. 7(4) (2008) 368-372.

[27] C.S. Alisi et al., Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extracts of *Musa paradisiacal* (Van sapientum), Afr. J. Biotech. 7(12) (2008) 1821-1825.

[28] J. Xie et al., Detection of amount and activity of living algae in fresh water by dehydrogenase activity (DHA), Environmental Moni-toring and Assessment. 146 (2008) 473-478.

[29] C.S. Alisi, G.O.C. Onyeze, Biochemical mechanisms of wound healing using extracts of *Chromolaena odorata* Linn, Nig. J. Bioch. Mol. Biol. 24(1) (2009) 22-29.

[30] A.E. Ghaly, N.S. Mahmoud, Optimum conditions for measuring dehydrogenase activity of *Aspergillus niger* using TTC, American Journal of Biochemistry and Biotechnology. 2(4) (2006) 186-194

[31] C.O. Nweke et al., Response of planktonic bacteria of new Calabar river to zinc stress, Afr. J. Biotechnol. 5(8) (2006) 653-658.

[32] C.O. Nweke et al., Toxicity of Zinc to heterotrophic Bacteria from a tropical river sediment, Applied Ecology and Environmental Research. 5(1) (2007) 123-132.

[33] C.O. Ibegbulem, Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extracts of *Musa paradisiaca* (Var Sapientum), Afr. J. Biotech. 7(12) (2008) 1821-1825.

[34] T. Burdock et al., Effect of assay conditions on the measurement of dehydrogenase activity of *Streptomyces venezuelae,* using triphenyl tetrazolium chloride, Advances in Bioscience and Biotechnology. 2(4) (2011) 214-225.

[35] C.J. Dillard, J.B. German, Phytochemicals: nutraceuticals and human health, J. Sci. Food Agric. 80(12) (2000) 1744-1756.

[36] Y.L. Chew, J.K. Goh, Y.Y. Lim, Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia, Food Chemistry. 116(1) (2009) 13-18.

[37] M.B. Maatalah et al., Antimicrobial activity of the alkaloids and saponin extracts of *Anabasis articulata*, Journal of Biotechnology and Pharmaceutical Research. 3(3) (2012) 54-57.

[38] B. Biswas et al., Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria, International Journal of Microbiology. 2013 (2013) Article ID746165.

[39] T.J. Silhavy, D. Kahne, S. Walker, The bacterial cell envelope, Cold Spring Harb. Perspect. Biol. 2(5) (2010) a000414.