Available online www.jsaer.com

***Journal of Scientific and Engineering Research*, 2016, 3(5):112-120**

ISSN: 2394-2630
CODEN(USA): JSERBR

Research Article

Inhibitory Potential Capacity of *Lantana camara* L. Extracts on Pathogenic Micro-organism using Dehydrogenase assay

Ukaoma AA[1], Okechukwu IR[1], Ukaoma VO[2], Ajuruchi VC[1]

[1]Department of Biological Science, Federal University Of Technology, Owerri, Nigeria
[2]Faculty of Medicine, Imo State University, Owerri, Nigeria

Abstract Inhibition of dehydrogenase activity in pathogenic micro-organism exposed to ethanol extract of *Lantana camara* was used as an index for assessment of its antibacterial activity. Assay of dehydrogenase activity was done in the test organisms (*Escherichia Coli*, *Staphylococcus aureus* and *Streptococcus spp*) using 2, 3, 5-triphenyltetrazolium chloride (TTC) as an artificial electron acceptor which was reduced to red-coloured triphenyl-formazan. Results obtained show that 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 and streptococcus spp* responded more markedly than Gram negative *Escherichia Coli* to the extract. Inhibitory concentrations (IC_{50}) of ethanol extract on *Escherichia Coli*, *Staphylococcus aureus* and *Streptococcus spp* were 652.06ug/ml, 369.23ug/ml, and 801.15ug/ml respectively. Preliminary phytochemical screening of the extract gave positive reactions for alkaloids, flavonoids, tannins, 4-hydroxybenzoic acid (phenolic compound) and saponins. These phytochemical may be responsible for the observed inhibition of dehydrogenase enzyme activity that translates to anti-bacterial action in these pathogenic organisms.

Keywords Dehydrogenase, Inhibition, Pathogenic, Logistic Dose, Response

Introduction

Medicinal plants are important elements of traditional medicine in virtually all cultures. Medicinal plants are important sources of pharmacological products. They 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 seeds, flowers. 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].

Plant-based products had primarily served as most important and indispensable sources of food and medicinal products [4] as such plant-based materials are used for medicinal purposes [5]. The World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs [6], as plants constitute a large reservoir of chemical substances that possess antimicrobial activity. Some of these substances are glycosides, phenolic compounds, steroids, saponin and terpenoids. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [7].

With the emergence of antibiotic-resistant pathogenic microorganisms, due to indiscriminate use of antibiotics, most especially in the developing world, there is continuous and urgent need to discover new antimicrobial

compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [8]. The increasing failure of chemotherapeutics, and antibiotics resistance exhibited by pathogenic microbial infectious agents, has led to the screening of several medicinal plants for their potential antimicrobial activity [5].

Lantana camara has several uses mainly as a herbal medicine and in some area as firewood and mulch [9]. The leaves are used in the treatment of tumours, tetanus, rheumatism and malaria. Raju, (2000) reported the diaphretic, and antiseptic properties of *Lantana camara*. The leaves have also been shown to have antifungal, insecticidal and wound- healing properties, hence there is need to evaluate the phytochemical compounds present in the leaf extract of *Lantana camara* and assess the antibacterial potential of the extract against selected bacteria isolate using dehydrogenase assay [10].

Figure 1: Leaves and flower of Lantana camara

Lantana camara is listed as one of the important medicinal plants of the world. Many studies have revealed the presence of terpenoids, steroids, and alkaloids as major chemical constituents. *Lantana. camara* oil and extracts are used in herbal medicine for the treatment of various human diseases such as skin itches, leprosy, cancers, chicken pox, measles, asthma, ulcers, tumors, high blood pressure, tetanus, rheumatism. Extracts from the leaves have been reported to have antimicrobial, fungicidal, insecticidal and nematicidal activity [11].

Use of Dehydrogenase Assay in Toxicity Assessment: Chemotherapeutic agents inhibit microorganisms by interfering with some important metabolic processes. Typical examples of this group of agent are the sulphonamides that inhibit the biosynthesis of folic acid as structural analogue. These anti-metabolites block metabolic pathways by completely inhibiting the use of metabolites by key enzymes [12]. Biological oxidation of organic compounds is generally a dehydrogenation process, and there are many dehydrogenases (enzymes catalyzing dehydrogenation), which are highly specific. The overall process for dehydrogenation may be represented as follows

$XH_2 + A \rightarrow AH_2$

Where XH_2 is an organic compound (hydrogen down) and A is a hydrogen acceptor. The dehydrogenase enzyme systems apparently fulfill a significant role in the oxidation of organic matter as they transfer hydrogen (electron) from substrate to acceptors.

Many different specific dehydrogenase systems are integral part of the micro-organisms. Thus, the result of the assay of dehydrogenase activity would show the average activity of the active component [5]. The most widely used substrate is 2, 3, 5-triphenyltrazolium chloride (TTC) which produces red coloured water insoluble triphenyltrazoliun chloride (INTF). The apparent redox potential of TTC is about -0.08V, which makes this compound act as an acceptor for many dehydrogenases [13].

Dehydrogenase assay is an effective primary test for accessing the potential toxicity of metals in soil microbial activities toxicity of metal to plankton [14] and heterotrophic bacteria from tropical river sediments. Toxicity of antimicrobial agents to pathogenic bacteria has been assessed using the dehydrogenase assay [5].

Materials and Methods

Fresh aerial parts of *Lantana camara* were collected from Federal University of Technology Owerri, in Owerri West local government area of Imo State. The method of Akujobi *et. al.* 2004 was adopted [15]. The aerial part of *Lantana camara* was dried at room temperature and reduced to a coarse powder in a mill (Kenwood BL357). 133g portion was extracted with 100mls of ethanol by shaking for 4 days. Soluble extract from filtration in a whatman No1 filter paper was recovered by distillation under reduced pressure at 49^{o}C in a rotary evaporator-Buchi rotavapour (Switzerland).

Phytochemical screening of the extract of leave of *Lantana*. *camara* was carried out by using the standard protocols. Plants were screened for phenolic compounds, saponins, alkaloids, flavonoids, glycosides, phytosterols and tannins (according to the methods described by [16-17].

Test for Tannins: 5g of the powdered plant sample was mixed with 5 ml of water. The mixture was boiled in a water bath and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate. Brownish green or a blue-black coloration indicates the presence of tannins.

Test for Flavonoids: The method of Kokate (2004) was adopted. 10 ml of ethyl acetate was added and heated in water bath and filtered. 1 ml of dilute ammonia solution was added to the filtrate and shaken well. A yellow coloration indicates the presence of flavonoids [17].

Test for Saponin: 5g of the dried powdered plant was added to 2-3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicates the presence of saponin.

Test for Phenolic Compounds: Phenolic presence was determined using the modified method of Trease and Evans (2002). 5mls of leaf extract was added to 5 ml of distilled water, few drops of 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds [16].

Test for Alkaloids: The method of Trease and Evans (2002) was adopted. 20 ml of distilled water was added to 2g of the powdered plant sample, was boiled in a water bath and filtered. 10 ml of this filtrate was mixed with 5 ml of Wagner's reagent and shaken vigorously for a stable persistent froth. The Reddish Brown precipitate indicates the presence of alkaloids [16].

Glycosides (Brontrager's Test): The method of Kokate (2004) was used. 50 mg of extract was added to concentrated HCl for two hours on water bath and filtered. 3ml of chloroform was added to the 1 ml of the filtrate. The mixture was shaken and the chloroform layer was separated out. 10% ammonium solution was added to the chloroform layer, pink colour indicates the presence of glycosides [17].

Phytosterols: The modified method of Kokate (2004) was adopted. 50 mg of extract was dissolved in 2 ml of acetic anhydride, few drops of concentrated sulphuric acid was added slowly along the sides of the test tubes. An array of colour changes showed the presence of phytosterols [17].

Isolation of Bacterial Strains and Culture Conditions

Pathogenic bacteria (*Staphlococcus sp., Escherichia sp., and Streptococcus sp.*), isolates were purified on nutrient agar (Fluka) plates and characterization was done using standard microbiological methods. Identification to the generic level followed the schemes. The bacteria strains were grown to mid exponential phase in nutrient broth (Lab M) on a Marrienfield rotary incubator (150 rpm) at room temperature (28 ± 2°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. The dry weights of the standardized cells were determined by drying volumes of cell suspension to constant weight in an oven at 110^{o}C. These standardized cell suspensions were used as inoculums in the dehydrogenase assay.

Determination of Antimicrobial Potentials of *Lantana Camara* Extracts By Total Dehydrogenase Activity (Dha) Assay

Screening 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 added. The plates were incubated at room temperature for 10 minutes. Production of red coloured formazan was suggestive of TTC reduction.

Total dehydrogenase assay method as described by Alisi *et al.* (2008) was employed to determine the antimicrobial activity of the extract. Total dehydrogenase activity was determined 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 done in 4 ml volumes of nutrient broth-glucose- TTC medium supplemented with varying concentrations (0 – 2000 µg/ml) of extract in separate 20 ml screw-capped test tubes was mixed. 0.3 ml of the bacterial suspensions were inoculated into triplicate glass tubes containing 2.5 ml of phosphate-buffer (pH 6.8) nutrient broth-glucose medium. *Lantana camara* leaf extract was pre-incubated on a rotary incubator (150 rpm) at room temperature (28 ± 2^{o}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 µg/ml in different test tubes. The final concentrations of nutrient broth, glucose and TTC in the medium were 2, 0 and 0.25 mg/ml, respectively. The controls consisted of the isolates and the media without *Lantana camara* extract.

The reaction mixtures were further incubated at room temperature (28 ± 2^{o}C for 8.0 h). The TPF produced were extracted in 4 ml of 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 (Sigma) in amyl alcohol; y = 0.0487x; R^2 = 0.9977]. Dehydrogenase activity (DHA) was expressed as milligrams of TPF formed per mg dry weight of cell biomass per hour.

% Inhibition of DHA activity = 100 – (Absorbance of test / Absrobance of control) x 100

= 100 – Percent DHA of control ……..……(1)

Percentage Inhibition of dehydrogenase activity in the isolates by *Lantana camara* was calculated relative to the control as shown in equation (1). The percentage inhibition data calculated were fitted into logistic dose response model (equations 2 and 3) by plotting inhibition (y) against extract or standard concentration (x).

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

Equations 2 and 3 are logistic dose response equations. The parameters were estimated by minimization of least squares using Levenberg-marquardt algorithm (Table curve 2D systat USA) Marquardt (1964). The toxicity thresholds concentrations (IC_5, IC_{10}, IC_{20}, IC_{40}, IC_{50}, IC_{70}, IC_{80} and IC_{100}) were then evaluated from the dose response plots. The total inhibitory concentrations (IC_{100}) 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 *Lantana camara*: Preliminary phytochemical screening of *Lantana camara* leaves revealed the presence of phenolic compounds hydrobenzoic acid, flavonoids, alkaloids, saponins, tannin, phytosterols.

Table 1: Phytochemical contents of *Lantana camara* leaf extract.

Phytochemicals	Results
Phenolic compounds	++++
Flavonoids	+++
Alkaloids	+++
Saponins	++
Tannins	++
Phytosterols	++
Glycosides	-

+ = present; = not present

Inhibition of Dehydrogenase Activity in *Staphlococcus aureus* by Graded Concentrations of Etnanol Extract of *Lantana camara* and Ciprofloxacin

Fig. 1 a & b showed that exposure of *Staphylococcus aureus* to *Lantana camara* leaf extract and Ciprofloxacin each resulted in a dose-dependent inhibition of dehydrogenase activity in *Staphylococcus aureus.* Threshold inhibitory concentrations (IC_{50}) of *Lantana camara* and Ciprofloxacin against the pathogenic isolate were

369.23ug/ml and 25.40ug/ml respectively. *Lantana camara* at 369.23ug/mg resulted in the inhibition of 50% of the test population but could not completely inhibit total dehydrogenase activity in *Staphylococcus aureus* at a threshold concentration of IC_{100}. The plant extract exerted inhibitory activities that followed a logistic dose response model like the standard antibiotic.

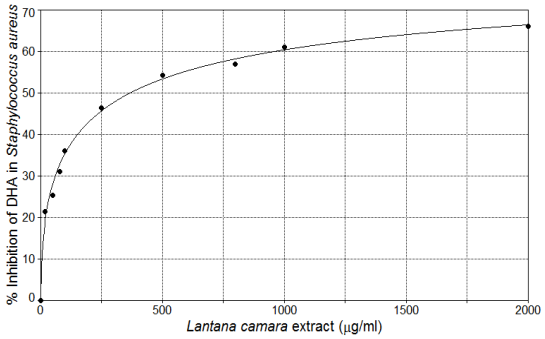

Figure 1a: Inhibition of dehydrogenase activity in staphylococcus aureus by graded concentration of ethanol extract of Lantana camara

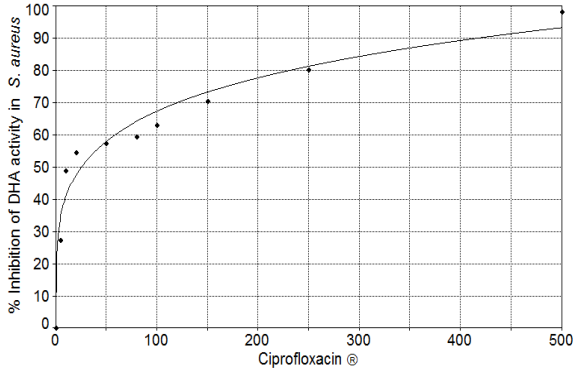

Figure 1b: Inhibition of dehydrogenase activity in Staphylococcus aureus by graded concentration of standard drug (Ciprofloxacin)

Inhibition of Dehydrogenase Activity in *Escherichia coli* by Graded Concentrations of Etnanol Extract of *Lantana camara* and Ciprofloxacin

Fig. 2a & b showed that exposure of *Escherichia coli* to *Lantana camara* and Ciprofloxacin each resulted in a dose-dependent inhibition of dehydrogenase activity in *Escherichia coli.* Threshold inhibitory concentrations (IC_{50}) of *Lantana camara* and Ciprofloxacin against the pathogenic isolate were 625.06 ug/ml and 29.21 ug/ml respectively. The plant extracts exerted inhibitory activities that followed a logistic dose response model like the standard antibiotic. Leaf extract were however able to completely (100%) inhibit total dehydrogenase activity in *Escherichia coli*.

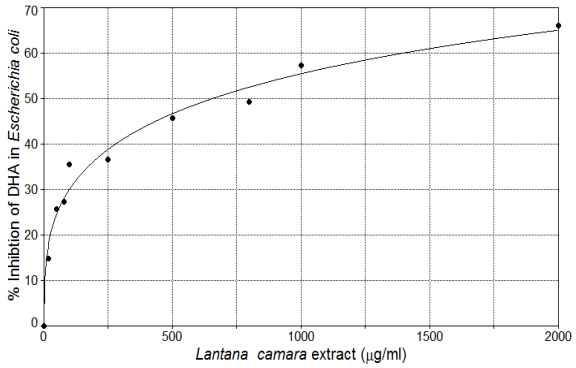

Figure 2a: Inhibition of dehydrogenase activity in Escherichia coli by graded concentration of ethanol extract of Lantana camara.

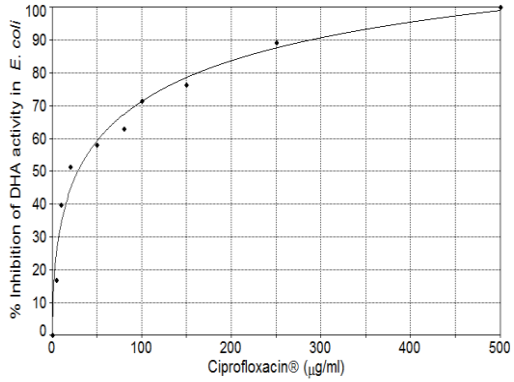

Figure2b: Inhibition of dehydrogenase activity in Escherichia coli by graded concentration of standard drug (Ciprofloxacin).

Inhibition of Dehydrogenase Activity in *Streptococcus spp* by Graded Concentrations of Etnanol Extract of *Lantana camara* and Ciprofloxacin

Fig. 3 a & b showed that exposure of *Streptococcus spp* to *Lantana camara* and Ciprofloxacin each resulted in a dose-dependent inhibition of dehydrogenase activity in the test organism. Threshold inhibitory concentrations (IC_{50}) of *Lantana camara* and Ciprofloxacin against the pathogenic isolate were 801.15 ug/ml and 4.03 ug/ml respectively. The plant extracts exerted inhibitory activities that followed a logistic dose response model like the standard antibiotic.

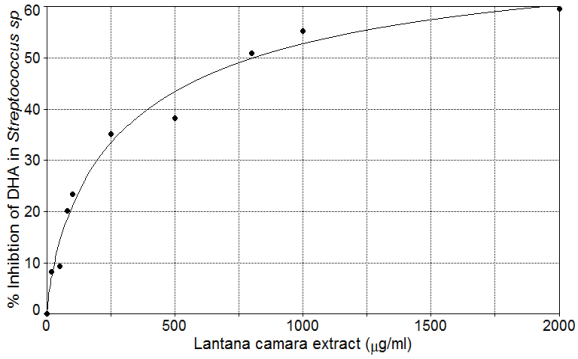

Figure 3a: Inhibition of dehydrogenase activity in Streptococcus spp by graded concentration of ethanol extract of Lantana camara.

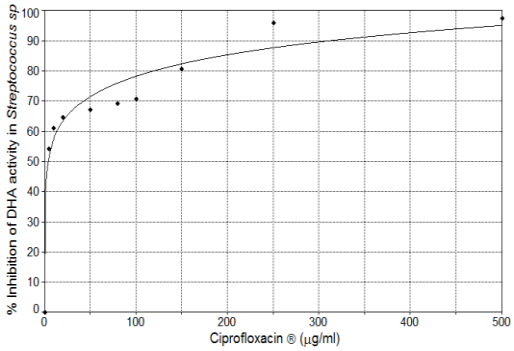

Figure 3b: Inhibition of dehydrogenase activity in Streptococcus spp by graded concentration of standard drug (Ciprofloxacin)

Discussion

Ethanol extract of *Lantana camara* against *Escherishia Coli*, *Staphylococcus aureus* and *Streptococcus spp*, inhibited dehydrogenase activity in a logistic dose response fashion. The dehydrogenase activity varied among the bacteria strains. This is in agreement with Nweke *et al.* (2007) and Nwogu *et al.* (2008) Alisi *et al.*, 2008)

[14, 18-19]] in which the Gram-positive organisms had higher dehydrogenase activity than the Gram-negative organisms. Earlier report [14] is however at variance with this observation. These variations may be due to differences in bacterial physiology, including cell wall components or dehydrogenase systems, since different microorganisms have been reported to have different dehydrogenase systems [13].

The response of the dehydrogenase activity to the extracts is concentration-dependent and varies among the organisms. Dehydrogenase activity was inhibited progressively with increase in concentration of the extracts in all the bacteria species. However, *Escherichia coli* was most sensitive to the extracts followed by *Streptococcus spp,* while *Staphylococcus aureus* was more resistant than the other isolates as obtained in the threshold inhibitory concentration of the extracts against the test organisms. The equation used in the modeling of this result gave high correlation coefficient (R^2> 0.90), showing very strong relationship with very low fit standard errors.

The high R^2 values (> 0.90) observed with all the bacteria isolates indicated that extract concentration was a strong determinant of the dehydrogenase activity. This indicated that increase in the concentration of the extract would significantly affect the carbon metabolism and respiratory activities in these bacterial isolates. This finding corroborates the reports of Osadebe and Ukwueze (2004) who found that various plant extracts inhibit the growth of some bacteria isolates [20].

Table 2: Comparative threshold Inhibitory concentration of extract and standard drug in pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli)*

Pathogenic bacteria	**Threshold inhibitory concentration IC_{50} (ug/ml)**	
	Lantana camara	***Ciprofloxacin***
Staphylococcus aureus	369.25	25.40
Escherichia coli	652.06	29.21
Streptococcus spp	801.15	4.03

Preliminary phytochemical screening of *Lantana camara* leaf revealed the presence of Phenolic compounds (hydrobenzoic acid), flavonoids, alkaloids, saponins, tannins, phytosterols. The presence of flavonoids, tannis, alkaloids & saponins suggest possible antimicrobial activity of plant extracts. Okwu (2004) also noted that plants are known to contain wide spectra of chemical, some of these chemicals came into being by natural selection; through defunct metabolic pathways [21-22]. These phytochemicals obtained in this work have been found to have medicinal properties. This finding agrees with the previous work [5, 23-24].

Measurement of microbial enzyme activity has been used in the assessment of ecotoxicity impacts of environmental pollutants. Dehydrogenase assay is also effective primary test for assessing the potential toxicity of metals to planktonic bacteria [14, 18].

Dehydrogenase assay had earlier been used to assess the toxicity of antimicrobial agent to pathogenic organisms that are found to cause diseases and infections [14]. Ethanol extract of *L. camara* inhibited dehydrogenase enzyme activity in pathogenic isolates (*Staphylococcus aureus* and *Escherishia coli)*. This finding agrees with the previous work [5, 14, 18-19]

Inhibition of dehydrogenase activity in pathogenic isolate is indicative of a strong antimicrobial activity since inhibition of oxido-reductases like dehydrognases, affect respiration of the microbe and fore stall infection as obtained in the result of this work. This agrees with the earlier reported work [5, 23].

Dehydrogenase assay involving the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) and 2-(P-iodophenyl)-5-phenyltetrazolium chloride (INT) to their formazans used in this work has been used to measure microbial activity. This method has once been used by Alisi *et al.* (2008) and Ukaoma *et al*. (2013) [5, 19].

The equation used in the modeling of this result gave high correlation coefficient (R^2> 0.90), showing very strong relationship with very low fit standard errors. Inhibition of dehydrogenase activity in pathogen could be one of the many mechanisms employed by the plant extract for 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 *L. camara* have earlier been shown to contain phenolic compounds like hydroxybenzoic acid as obtained in the works of Alisi *et al.* (2011) [23].

Hydroxybenzoic acid is known to possess antimicrobial activity [19]. These extracts may actually be exerting antimicrobial activity through inhibition of dehydrognase activity in the test organisms [5].

The presence of surface active compound 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 phytochemical found in the extract may also exert their own antimicrobial activity through different mechanisms [11].

The plant extract *L. camara* showed a logistic dose dependent inhibition of dehydrogenase activity in the test organisms. It was more effective against *Staphylococcus aureus* and *Escherichia coli.* It therefore can be useful as a promising antimicrobial agent in the treatment of infections caused by the test organisms [1].

The demonstration of activity against both gram-negative and gram-positive bacteria and fungi is an indication that the extracts of *Lantana camara* can be a source of bioactive substances that are anti pathogenic. The fact that the leaf extract inhibited dehydrogenase activity in the test organism is an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms. The search for new drugs to counter the challenges posed by resistant strains of bacteria and some fungi might have started yielding results as the investigation of this plant has demonstrated enormous therapeutic potential. It can serve the desired purpose with lesser side effects that are often associated with synthetic antimicrobial agents.

Multiple drug resistant microorganisms is one of the recent and major concern in public health. Hence, intensive studies on this plant might provide possible solutions to this problem as it was found to have antimicrobial properties. More research on the toxicology of *Lantana camara* would give us direction on how clinically utilizable the extracts are, in order to enhance proper health delivery. Research should be geared in the direction of understanding at molecular levels the genetic interactions that take place during inhibitory activity of the extract

References

[1]. Ushimaru, P.I., Mariama, C., Luiz, B., Luciano, D.I. and Ary, F.J. (2007). Anti- bacterial activity of medicinal plant extract. *Brazilian Journal of Microbiology.* 3(8):717-719.

[2]. Anne-Catherine, F. (2007). Medical plants. *A Botanic Garden for the Nation*. The United States Botanical Garden. Pp.121.

[3]. Odugbulemi, T. (2006). Medical plants and antimicrobial in: Outline and pictures of medicinal plants from Nigeria University of Lagos press. Pp. 53-64.

[4]. Enwuru, N.V., Ogbonna, S.O., Nkemehule, F., Enwuru C.A., and Tolani, O. (2008). Evaluation of antibacterial activity and acute toxicity of the hydroethanolic extract of *Stachytarpheta angustifolia* (Mill). *African Journal of Biotechnology.* 7(11): 1740-1744.

[5]. Ukaoma A. A., Ukaoma V. O., Opara, F. N. and Osuala, F. O. U. (2013). Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extract of *Curcuma Longa* (Turmeric) Rhizome. *The Journal of Phytopharmacology*. 2(3): 9-17.

[6]. Nascimento, G.G.F., Lacatelli, J., Freitas, P.C. and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistance bacteria. 31(4): 886-891.

[7]. Sukanya, S.L., Sudisha, J., Hariprasad, P., Niranjana, S.R., Prakash, H.S. and Fathima, S.K. (2009). Antimicrobial activity of of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. 8(23): 6677-6682.

[8]. Mgbemena, I. C., Opara, F.N., Ukaoma, A., Ofodu, C., Njoku, I. and Ogbuagu, D.H. (2010). Prophylatic potential of lemon grass and neem as antimalarial agents. *Journal of medical science.* 6(8): 503-507.

[9]. Day, M.D., Wiley, C.J., Playford, J. and Zalucki, M.P. (2003). *Lantana*: Current Management Status and Future Prospects. Australian Centre for International Agriculture Research: Canberra. Pp. 50-54.

[10]. Raju, A. (2000). Wild plants of Indian Sub-continental and their economic uses. CBS Pub & Distribution New Delhi. Pp. 65.

[11]. Sonibare, O.O. and Effiong, I. (2008). Antibacterial activity and Cytotoxicity of essential oil of *Lantana camara* L. leaves from Nigeria. *African Journal of Biotechnology*. 7(15): 2618-2620.

[12]. Prescott, M.L., Harlett, P.J. and Klein, A.D. (2004). Microbiology. 6th Edition. MacGraw Hill. Pp. 253-258.
[13]. Praveen-Kumar, J.C. (2003). 2,3,5-Triphenyltetrazolium chloride (TTC) and electron acceptor of culturable cell bacteria, fungi and actinomycetes. *Biological Fertilization of Soil*. 28: 186-189.
[14]. Nweke, C.O., Okolo J.C., Nwanyanwu, C.E. and Alisi, C.S. (2006). Response of planktonic bacteria of New Calabar River to Zinc Stress. *African Journal of Biotechnology.* 5(8): 653-650.
[15]. Akujobi, C., Anyanwu, B.N., Onyeze, C. and Ibekwe, V.I. (2004). Antibacterial activities and preliminary phytochemical screening of four medicinal plants. *Journal of applied sciences*. 7(3):4328 – 4338.
[16]. Trease, G.E. and Evans, W.C. (2002). *Pharmacognosy.* 13th edition. Bacilliere Tinall Ltd. London. Pp. 5-9.
[17]. Kokate, G.L., Skirsagar, K.A. and Walke, W.N. (2004). *Phytochemistry of secondary meatabolites*. 3:167-169.
[18]. Nwaogu, L.A., Alisi, C.S., Ibegbulem, C.O. and Igwe, C.U. (2007). Phytochemical and antimicrobial activity of ethanolic extract of *landolphia Owariensis* leaf. *African Journal of Biotechnology*. 6(7): 890-893.
[19]. Alisi, C.S., Nwanyanwu, C.E., Akujobi, C.O. and Ibegbulem, C.O. (2008). Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extract of *Musa paradisiacal* (*var sapie-entum*). *African Journal of Biotechnology.* 7(12): 1821-1825.
[20]. Osadebe, P.O. and Ukwueze, S.E. (2004). A comparative study of the phytochemical and antimicrobial properties of the Eastern Nigerian species of African mistletoe (*Loranthus micronthus*) sourced from different host trees. *Journal of Biologically Biotechnology.* 2(1): 18-23.
[21]. Okwu, D.E. (2004). Phytochemical and vitamin content of indigenous spices of southeastern Nigeria. *Journal of Sustainable Agricultural Environment*. 6: 30-34.
[22]. Street, H.E. and Cockburn, W. (2002). *Plant metabolism*. 2nd edition. Oxford Pergamon press. Pp. 70-90.
[23]. Alisi, C.S., Nwaogu, L.A., Ibegbulem, C.O. and Ujowundu, C.U. (2011). Antimicrobial action of methanol extract of *Chromolaena Odorata* Linn is logistic and exerted by inhibition of dehydrogenase enzymes. *Journal of Research in Biology*. 3: 209-216.
[24]. Raza, H. and John, A. (2007). In-vitro protection of reactive oxygen species-induced degradation of lipis, proteins and 2-deoxyribose by tea catechins. *Food and Chemical Toxicology.* 4: 1814-1820.