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Inhibition of dehydrogenase activity in *S. typhimurium* by ethanolic and methanolic extracts of *Carica papaya* and *Ocimum gratissimum*

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Abstract

Dehydrogenase and inhibitions of dehydrogenase activities in *Salmonella typhimurium* by ethanolic and methanolic leaf extracts of *Ocimum gratissimum* and *Carica papaya* were investigated. Dehydrogenase activity assay was carried out using 2, 3, 5-triphenyl tetrazolium chloride (TTC) as the electron acceptor. Pure culture of *S. typhimurium* was exposed to varied concentrations of ethanolic and methanolic extracts of *Ocimum gratissimum* and *Carica papaya* [0-4000 µg/ml]. The ethanolic and methanolic extracts exhibited a concentration dependent response against the tested organism. Results obtained revealed that the ethanolic extracts showed a higher bactericidal effect on the test organism than the methanolic extracts at the threshold and total inhibitory concentrations. The IC50 were 45.349 and 15.697; IC100 were 43.732 and 35.526 for ethanolic extracts of *Carica papaya* and *Ocimum gratissimum* respectively while the IC50 were 7.108 and 13.696; IC100 were 40.815 and 31.104 for methanolic extracts of *Carica papaya* and *Ocimum gratissimum* respectively. This in-vitro study further revealed that the leaf of *Carica papaya* was more potent on *Salmonella typhimurium* than the leaf of *Ocimum gratissimum*. The findings from this study seem to provide the in-vitro evidence that justifies *Carica papaya* and *Ocimum gratissimum* as good candidate medicinal plants for the potential treatment of Salmonella typhimurium infections

Key words: *Carica papaya*, *Ocimum gratissimum, S. typhimurium*, dehydrogenase activity, leaf extract

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INTRODUCTION

Ocimum gratissimum and *Carica papaya* are valuable multi-purpose medicinal plants which belong to the family *Lamiaceae* and *Caricaceae* respectively and are distributed in tropical and warm regions. They are commonly used in the treatment of various diseases such as upper

respiratory tract infections, diarrhea, headache, fever, ophthalmic and skin diseases and pneumonia (Gopi *et al*., 2006). Extracts of the plants contain antimicrobial, antibacterial, antifungal (Lemos *et al*., 2005), antimalarial (Ezekwesili *et al*., 2004) and antiprotozoal (Holetz *et al*., 2003) activities. The active compounds present as volatile oil from the leaves consist mainly of thymol (32-65%) and eugenol (Adeola *et al*., 2014). They also contain xanthones, terpenes and lactones together with cardiac glycosides, saponins, tannins and alkaloids (Akujobi *et al*., 2004).

Measurement of microbial enzyme activity has been used in the assessment of ecotoxicological impacts of environmental substrates. In this regard, dehydrogenase activity has been widely used. The dehydrogenase assay is an effective primary test for assessing the potential toxicity of metals to soil microbial activities (Anyanwu *et al.* 2016; Nwuche and Ugoji, 2008; Nwachukwu *et al.* 2011), toxicity of metals to planktonic (Nweke *et al*., 2006) and heterotrophic (Nweke *et al*., 2007) bacteria from tropical river sediments. Toxicity of plant extracts to pathogenic bacteria has been assessed using the dehydrogenase assay (Nwaogu *et al*., 2007; Nwaogu *et al*., 2008; Alisi *et al*., 2008).

Salmonella typhimurium has posed a problem in causing regular infections in hospitals and public health centers and has become a persistent pathogen in the environment able to easily survive and proliferate to cause serious infections in animals and humans thereby posing a major risk to public health (Martelli and Davies, 2012). Human infections with *S. typhimurium* originate mainly from livestock products such as meats, eggs and other products when consumed raw or undercooked as well as environmental contaminations from household pets or contaminated birds (De Knegt *et al*., 2015).

Thus this study carried out in Anthony van Leeuwenhoek Research Laboratory, Nekede in Imo State, Nigeria isolated *Salmonella typhimurium* from feces of different categories of livestock using selective/differential media and determined the effects of ethanolic and methanolic extracts of *O. gratissimum* and *C. papaya* on the dehydrogenase activity of the recovered *Salmonella typhimurium*. Several research questions were put forward to help us understand the efficacy of *O. gratissimum* and *C. papaya* against *Salmonella typhimurium in order* to validate or disprove the claim of herbalists who use the leaf extracts of these plants as cheaper antimicrobial herbal remedies against the expensive conventional antibiotics that are currently in use for these ailments.

The study will make for more economic and optimal use of O*. gratissimum* and *C. papaya* in alternative medicine. This is because the outcome of this study will contribute to current knowledge on the variations in the effects of ethanolic and methanolic extracts of *O. gratissimum* and *C. papaya* on the dehydrogenase activity of *Salmonella typhimurium.* This will be potentially useful to the relevant public health authorities, abattoir workers, livestock farmers, for the prevention, control and management of health problems caused by *Salmonella typhimurium*.

MATERIALS AND METHODS

Collection and Identification of *Ocimum gratissimum* and *Carica papaya*

Fresh leaves of *O. gratissimum* and *C. papaya* 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 identified by a plant taxonomist, Dr. S. E. Okeke in the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The freshly collected leaves were air-dried completely. The air-dried leaves were macerated and ground into powdery form using washed, air-dried and oven sterilized electric blender to avoid microbial contamination and stored in a clean airtight container until further use.

Preparation of the test bacterial isolate

Pure culture of *Salmonella typhimurium* was recovered by direct plating on selective/enrichment media of samples obtained from one-year surveillance in an integrated pig, poultry and cattle production farms. The recovered isolate of *Salmonella typhimurium* is pale on deoxycholate citrate agar (DCA). Microscopic identification and biochemical confirmation tests were performed to re-identify and confirm the identity of the test organism. Biochemically identified and confirmed *Salmonella typhimurium* was then grown to mid exponential phase (20 minutes) in nutrient broth on a rotary incubator (150 rpm) at room

temperature (28 ± 2°C). The cells were harvested by centrifugation at 6000rpm for 8 min. Harvested cells were washed three times in deionized distilled water and re-suspended in water. The re-suspended cells were adjusted in a spectrophotometer to an optical density comparable to 0.5 McFarland turbidity standards that is equal to 1.5 x 10^8 (One hundred and fifty million colony forming units/ml (CFU/ml) of bacterial suspension. The standardized cell suspension was used as the inoculum in the dehydrogenase activity assay as described by Alisi *et al*. (2008).

Extraction of Plant Materials

A 20g portion of *O. gratissimum* and *C. papaya* powder was weighed into 100ml of ethanol and methanol and kept in a conical flask for 72hrs. Soluble extracts from filtration (filtrate) in a Whatman number 42-filter paper was concentrated under vacuum and air-dried. A 0.4g portion of dried extracts was dissolved in 40mls of DMSO for further extraction in a conical flask. The extracts were then stored in a freezer at 4°C.

Dehydrogenase Assay

The dehydrogenase assay method as described by Alisi *et al*. (2008) was adopted for the study. The dehydrogenase activity (DHA) was determined using 2, 3, 5-triphenyltetrazolium chloride (TTC) as the artificial electron acceptor, which was reduced to the red colored triphenylformazan (TPF). The assay was done in 4 ml volumes of nutrient broth-glucose-TTC medium supplemented with varying concentrations (0- 4000 µg/ml) of the ethanolic and methanolic leaf extracts in separate screw-capped test tubes. About 0.3 ml volume of the standardized bacterial suspension was inoculated into triplicate glass tubes containing 0.4 ml of phosphate-buffered (pH 6.8) nutrient broth-glucose medium supplemented with varying concentrations of the extract solution 0, 50, 100, 200, 400, 800, 1600, 3200 and 4000 µg/ml in different test tubes. The different test tubes were incubated in a rotary incubator (150 rpm) at room temperature (28±2°C) for 30 min. Thereafter, 0.1 ml of 0.1% (w/v) TTC in deionized water was added to each tube to obtain final extract concentrations of 0, 50, 100, 200, 400, 800, 1600, 3200 and 4000 µg/ml in different test tubes. The control consisted of *Salmonella typhimurium* and the media without ethanolic or methanolic extracts of *C. papaya* or *O. gratissimum*. The reaction mixtures were further incubated at room temperature (28 ± 2°C) for 16 hours. The triphenylformazan produced was extracted in 4 ml of amyl alcohol and the absorbance determined using spectrophotometer at 500 nm. The amount of formazan produced was determined from a standard dose-response curve [0-4000 µg/ml TPF (Sigma) in amyl alcohol]. Dehydrogenase activity was expressed as mg of triphenylformazan (TPF) formed per mg dry weight of cell biomass per hour. Inhibition of dehydrogenase activity in the test organism by ethanolic and methanolic *C. papaya* and *O. gratissimum* extracts was calculated relative to the control. The percentage inhibition for the test organism was linearized against the concentrations of the extracts using gamma parameter (r-) [r- = % inhibition/ (100- % inhibition)] (Alisi *et al*., 2008). The toxicity threshold concentrations (IC50) were determined from the linear regression plots. The total inhibitory concentrations (IC100) were extrapolated from the plot of the inhibition data.

Statistical analysis

Data was analyzed using a two-way analysis of variance (ANOVA) and values for $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The results of this study on the effects of different concentrations of ethanolic and methanolic extracts of *C. papaya* and *O. gratissimum* on *Salmonella typhimurium* with respect to the dehydrogenase activity and its inhibition are shown in Figures 1, 3, 5 and 7. Dehydrogenase activities observed in the control samples (0 µg/ml i.e. no plant extract) of ethanolic and methanolic extracts of *C. papaya* and *O. gratissimum* indicated that the test organism was able to reduce TTC to the red formazan (Figures 1, 3, 5 and 7). The organism's dehydrogenase activity decreased with increase in concentration of ethanolic and methanolic extracts of *C. papaya* and *O. gratissimum* (0 - 4000 µg/ml) (Figures 1, 3, 5 and 7). The ethanolic extracts of *C. papaya* and *O. gratissimum* seem to have a higher rate of inhibition of dehydrogenase activity than the

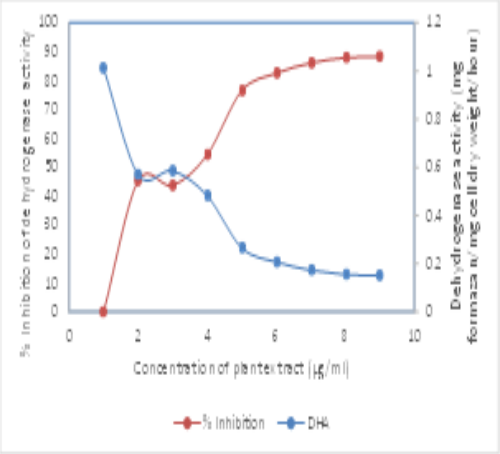

Figure 1. Dehydrogenase activity and %Inhibition of dehydrogenase activity in response to various concentrations of ethanol leaf extract of *Carica papaya* in *Salmonella species*.

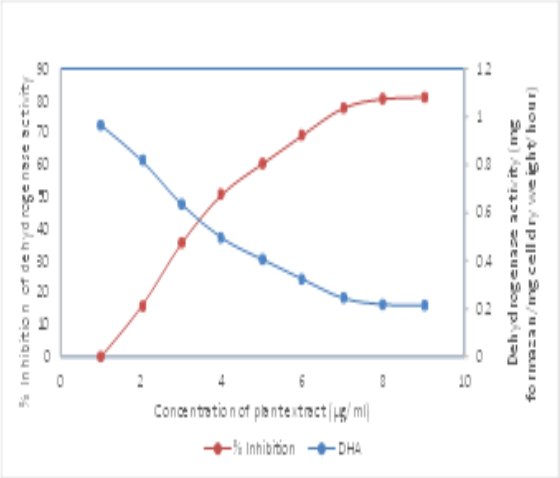

Figure 3. Dehydrogenase activity and %Inhibition of dehydrogenase activity in response to various concentrations of ethanol leaf extract of *Ocimum gratissimum* in *Salmonella species*.

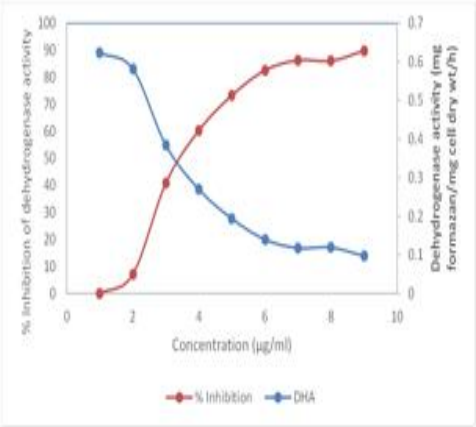

Figure 5: Dehydrogenase activity and %Inhibition of dehydrogenase activity in response to various concentrations of methanol leaf extract of *carica papaya* in *Salmonella* species.

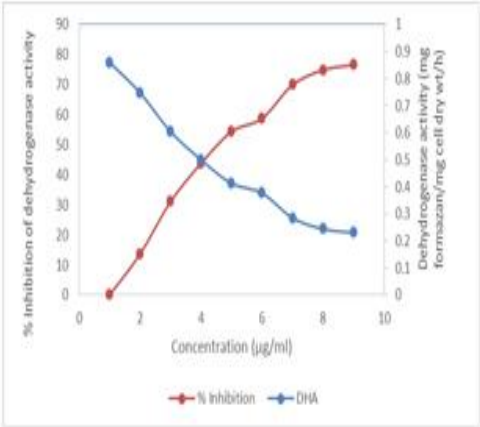

Figure 7: Dehydrogenase activity and %Inhibition of dehydrogenase activity in response to various concentrations of methanol leaf extract of *Ocimum gratissimum* in *Salmonella* species.

methanolic extracts of *O. gratissimum* and *C. papaya*. The evidence is seen from the threshold and total inhibitory concentrations data in Figures 1 and 3. According to Justina *et al.* (2017) ethanol could improve the extraction of essential oils and this might have been responsible for the observed enhanced activity of ethanolic extracts of C. *papaya* and *O. gratissimum* against *Salmonella typhimurium* compared to methanolic extracts. These results provide evidence suggesting that ethanol might be a better solvent in the extraction of phytochemicals from plant materials. However, the results of this *in vitro* study further justify *O*

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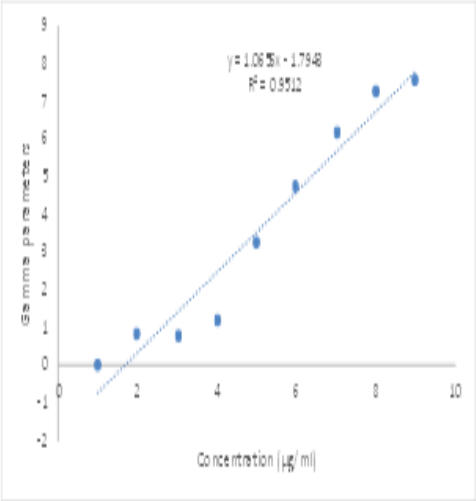

Figure 2. Linear regression of the gamma parameter (r-) values of ethanol leaf extract of *Carica papaya* against the *Salmonella species*.

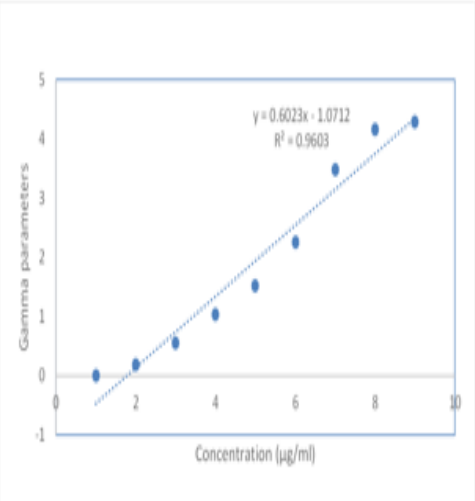

Figure 4. Linear regression of the gamma parameter (r-) values of ethanol leaf extract of *Ocimum gratissimum* against *Salmonella species*.

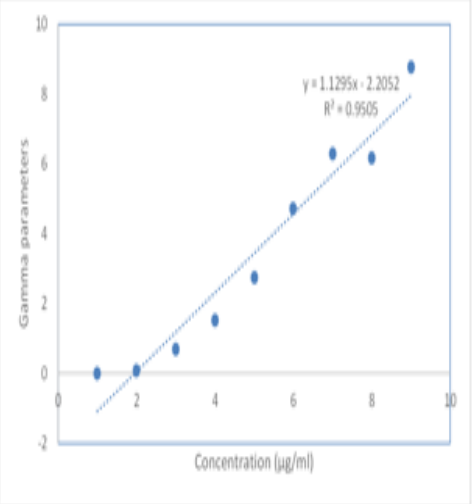

Figure 6: Linear regression of the gamma parameter (r-) values of methanol leaf extract of *carica papaya* against *Salmonella* species.

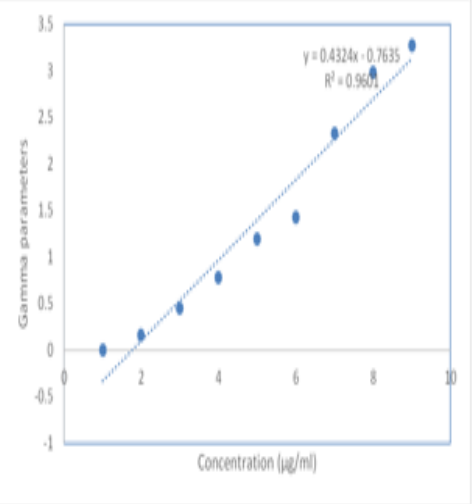

Figure 8: Linear regression of the gamma parameter (r-) values of methanol leaf extract of *Ocimum gratissimum* against *Salmonella* species.

gratissimum and *C. papaya* as good candidate medicinal plants for *Salmonella typhimurium* infections and further support the use of leaf extracts of *O. gratissimum* and *C. papaya* as antimicrobial herbal remedies as demonstrated by Akujobi *et al*. (2004). The observed high R^2 values greater than 0.90 ($0.9505 < R^2 < 0.9601 > 0.8000$) as shown in Figures 2, 4, 6 and 8 indicate that the concentration of ethanolic and methanolic extracts of *C. papaya* and *O. gratissimum* are both strong determinants of dehydrogenase activity in *Salmonella typhimurium*. It therefore implies that increase in the extract's concentration would have serious deleterious effect on carbon metabolism and respiratory activity of this bacterial isolate. The 2-way analysis of variance showed that the dehydrogenase activity and its percentage

inhibition varied significantly ($P < 0.05$) with extract concentration.

In conclusion, results obtained from this *in vitro* study show that the ethanolic and methanolic extracts of *O. gratissimum* and *C. papaya* inhibited the dehydrogenase activity of *S. typhimurium*. The inhibitory action may be due to the presence of different phytochemicals contained in these plants. The result of this *in vitro* study indicated that extracts of *O. gratissimum* and *C. papaya* were significantly effective against the tested organism and may serve *as* a cheaper antimicrobial herbal remedy in the management of salmonellosis.

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