

# cytotoxicity and antitubercular activity of allium sativum and lantana camara against mycobacterial isolates from people living with HIV/AIDS

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## Citation

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## Abstract

The Antitubercular activity of *Allium sativum* and *Lantana camara* on multiple-drug-resistant *Mycobacterium* was investigated among Nigerian HIV-infected-persons. Minimal inhibitory concentration (MIC) was estimated by the well-in-agar-diffusion method and potency of extracts compared with standard drugs. Cytotoxicity was determined using brine shrimps. MIC of drugs was 0.33mg/ml, 0.25mg/ml and 0.20mg/ml for streptomycin, isoniazide and rifampicin. *L. camara* had MIC of 0.63mg/ml for *M. tuberculosis* and Unidentified *M. avium* complex and 0.89mg/ml for *M. avium* Complex while garlic had 0.64mg/ml for *M. tuberculosis*, 0.97mg/ml for *M. avium* Complex and 1 mg/ml for Unidentified species.  $LC_{50}$  of garlic was 49.1 ppm for *M. tuberculosis*, 56.5ppm for *M. avium* complex and 63.0ppm for the Unidentified species, while *L. camara* had 32.6ppm for *M. tuberculosis*, 55.9ppm for *M. avium* complex and 51.3ppm for Unidentified species. The observed activity of the extracts is consistent with their use in traditional medicine for the treatment of *Mycobacterium* species.

## INTRODUCTION

Tuberculosis (TB), by members of the *Mycobacterium tuberculosis* complex, causes three million deaths a year worldwide<sup>1</sup>. The disease is associated with impoverished economic conditions. The resurgence of *M. tuberculosis* infection in the developing countries is due to immigration, the emergence of drug-resistant strains, inadequate treatment, continuing poverty, malnutrition, overcrowding, alcoholism and the AIDS epidemic<sup>2</sup>. In advanced stages of AIDS, where TB infections are commonly found, *Mycobacterium* infections due to members of the *M. avium-intracellulare* complex (MAC) are also on the increase. Thus the Morbidity and Mortality Weekly Report of the Centre for Disease Control (CDC), in 1998, declared tuberculosis a deadly disease, and the major cause of death of immune suppressed persons and especially those living with HIV/AIDS.<sup>3</sup> Of the 34.3 million persons living with HIV/AIDS, 24.5 million (71%) live in sub-Saharan Africa and approximately one-third of them are co-infected with tuberculosis.<sup>4</sup> Treatment of TB involves a long period of drug administration, usually lasting for 6-24 months for effective elimination of the bacilli from the lungs, organs and macrophages. However, the drug-resistance of the pathogen has necessitated combination therapy of at least

two drugs: isoniazide (INH), rifampicin, ethambutol, streptomycin, pyrazinamide and paraaminosalicylic acid (PAS). WHO on the other hand recommends the short course directly observed therapy (DOTS) using a combination of isoniazide, rifampicin, pyrazinamide and ethambutol on a daily dose for 2 months, followed by 4 months therapy of isoniazide and rifampicin given thrice weekly.<sup>5</sup>

Garlic (*Allium sativum*), is the most extensively studied herb for inhibition of infections. Its antimicrobial activity was first noted in 1944 by<sup>6</sup> and since then, garlic extract has been considered an effective alternative for the treatment of various infections including TB and MAC infections.<sup>7</sup> All parts of this plant have been used traditionally for several ailments throughout the world. The leaves are used as a bechic, antitumoural, antibacterial and antihypertensive agent. The root of this plant is used for the treatment of malaria, rheumatism and skin rashes: dermatitis, eczema, pruritis.<sup>8</sup> It is also indicated for influenza, cough, mumps, high fever and tuberculosis<sup>9</sup>; and has also been shown to be helpful in gum care.<sup>10</sup> The medicinal efficacy of *L. camara* has been widely reported. Its rich phytochemical components including cardiac glycosides, useful in

enhancing myocardial contraction as well as the present alkaloids, flavonoids and other essential oils are indicative of its antioxidant activity.<sup>11-12</sup> The root has established uses in malarial control, rheumatism, skin rashes as well as dermatitis, eczema, and related mycotic infections as well as in the management of respiratory tract infections including influenza, and tuberculosis.<sup>10</sup>

Thus, the burden of HIV/AIDS and TB co-infection has prompted this research aimed at screening the leaves of *L. camara* and the bulbs of *A. sativum* for antitubercular activity in order to establish a basis for their use in Nigerian herbal practice.

## **MATERIALS AND METHODS**

### **SUBJECT AND LOCATION**

The subjects used for the survey were volunteers of known HIV status (people living with HIV/AIDS) from the Local Chest Unit and Shanahan Hospital, Nsukka. Sputum samples were collected before mouthwash and analysed within 24h of collection macroscopically to determine parameters of medical importance: colour, whether blood tinged, presence of pus and parasites according to the methods of Cheesbrough<sup>13</sup>

### **CULTIVATION OF ORGANISM**

Preliminary identification of Mycobacterial isolates was based on the rate of growth, temperature of optimal growth, colony morphology, colony texture and pigment production. Purulent, muco-purulent or cheesy sputa and nasopharyngeal samples were first digested or hydrolyzed with two drops of 40% Potassium hydroxide solution and incubated for 1hr until fluid as described by<sup>14</sup> Hydrolyzed samples were then cultivated on duplicate plates of freshly prepared Lowenstein-Jensen medium (LJ-medium), consisting of 6-8 homogenized hen's eggs (275ml), 8ml hydrochloric acid, (IN), 153ml salt Glycerol solution, 2.75ml of 2% (w/v) malachite green, and 25000iu penicillin G (Benzyl penicillin), MacConkey, Blood, and Chocolate agar (5-10% CO<sub>2</sub>), and incubated at both room temperature and at 37C for 24 hours, observed for fast growers and subsequently re-incubated for 3 – 4 weeks. Acid-fast bacilli were then screened for using the Zeihl-Neelsen Staining method 1 for identifying AFB bacilli as described by Ellen et al<sup>15</sup>

The isolates were further characterized using various biochemical tests: catalase, urease production, nitrate reduction, lipase (Tween 80 hydrolysis), growth in 5% NaCl,

growth rate and pigment production as described by<sup>13</sup>

### **COLLECTION AND IDENTIFICATION OF PLANTS (AUTHENTICATION)**

The fresh leaf of the plant, *Lantana camara* (Verbenaceae) was collected from the botanical garden of the University of Nigeria, Nsukka, dried for one week under room temperature, pulverized using a clean mechanical grinder into fine powder, and then stored in airtight containers and kept in the refrigerator until use. Bulbs of *A. sativum* were bought at the Ogige Market, Nsukka and the back peeled. These were crushed into fine powder using a clean laboratory mortar and preserved for further use.

### **EXTRACTION OF ACTIVE COMPONENTS OF THE PLANTS**

The methanolic extraction of the leaves of *Lantana camara* was carried out using the percolation method. Approximately 50gm of each of the powdered plant extracts were weighed and macerated separately in 250ml each of methanol in a clean conical flask and plugged with cotton wool and foil. The mixture was allowed to stand for 72hrs and subsequently filtered using Whatman No. 1 filter paper. The filtrate was poured into clean Petri dishes and evaporated to dryness using vacuum evaporator.

### **PREPARATION OF STOCK SOLUTION**

A 500 mg quantity of the plant extracts was weighed and dissolved separately in 5ml each of the solvent (20% dimethylsulfoxide) to a concentration of 100 mg/ml. The stock solution was doubly diluted to a concentration of 100, 50, 25, 12.5, and 6.25mg/ml in each set of tubes.

### **PHYTOCHEMICAL TESTS**

The Phytochemical analysis described by<sup>16-17</sup> for the determination of the presence of secondary constituents of plants such as alkaloids, flavonoids, tannins, steroids, carbohydrates, reducing sugars, cardiac glycosides, terpenes, fats and oil and saponins were adopted in the study using methanolic extract of *L. camara* leaves and *Allium sativum* bulbs

### **THE DISC DIFFUSION METHOD USING INDUSTRIAL GRADE ANTIMYCOBACTERIAL AGENTS**

Industrial grade disc antimycobacterial agents (in mg/ml) were used for the antibiogram. These included streptomycin (0.33), rifampicin (0.2), ethambutol (0.2), isoniazide (0.25) and pyrazinamide (0.1). After seeding/streaking (state which

one you did!!) the test microorganisms on sterile Lowenstein-Jensen medium (LJ-medium, the discs were aseptically placed on the plates and the set-up incubated at 27C for 14 days. After the incubation period, the plates were observed for presence or absence of zones of inhibition around each antimicrobial disc. Zones were measured with a pair of calipers and a ruler calibrated in millimeters

### **THE WELL-IN-AGAR DILUTION METHOD**

Antitubercular activity of the plant extracts (500mg/ml) was assayed using the well-in-agar technique described by .<sup>18</sup> Sterile plates of the L-J medium were flooded with normal saline culture of the test organisms of turbidity comparable to one (1) McFarland standard. Excess bacterial suspension was drained and the plates allowed drying. Wells of diameter 8mm were bored on the agar plates with a cork borer, and about 0.2ml of each dissolved extracts was introduced into each well and allowed to stand for 2 hrs at room temperature to allow extracts to diffuse into the agar. The plates were then incubated at 37C for 18-21 days. Control experiments were done by dispensing 0.1ml of the test organism dissolved in 0.5ml each of DMSO and distilled water without adding the plant extracts. The Inhibition Zone Diameters (IZDs) were measured with a pair of calipers and a ruler calibrated in millimeters.

### **MINIMUM INHIBITORY CONCENTRATION (MIC) BY THE BROTH DILUTION METHODS**

The MIC and MCC of the standard antimycobacterial agents and the plant extracts were determined by making two series of doubling dilutions (ranging from 250-12.625 mg/ml) of each drug and plant extracts in sterile L-J medium plates. The test tube series were inoculated with overnight broth cultures of the organism and incubated at 37 for 24hr. Uninoculated media with and without the test agents were introduced as controls. Tubes with the highest dilution of each drug (chemotherapeutants) and extract showing no visible turbidity were considered as containing the MIC of the drugs and the test plant extracts. MCC was determined by streak-inoculating sterile L-J plates with loopfuls of the broth cultures from the inhibition zones in each dilution series and incubated at 27°C for 48-72hrs. Cultures with highest dilution of each plant extract and the used chemotherapeutants that yielded no growth in the LJ sub-culture were considered as containing the MCC

### **BRINE SHRIMP LETHALITY ASSAY**

About 100 liters of seawater was dispensed in 10-m amounts

into 10 Petri dishes. These were partitioned into 2; one side was slightly covered with polyethylene sheets and retained dark while the other was uncovered. Brine Shrimp eggs were placed on the darkened ends, covered and left in a secure position. Hatching was observed between 24-48hrs during which the hatched eggs or nauplii swarm to the uncovered lit end of the Petri dishes. These were used for the bioassay. The freshly collected seawater was dispensed in 2ml volumes into 10ml test –tubes and the this was added 20mg of each plant extract and slightly shaken to homogenize. Using graduated finne pipettes, 500, 50 and 5µl of the homogenate was dispensed into McCartney bottles and made up to 5ml and a final concentration of 1000, 100 and 10µ/ml respectively. Ten (10) of the nauplii was carefully placed into each of the McCartney bottles using sterile Pasteur pipettes. Control experiment was done with only the seawater and no added plant extract. The set up was then observed closely for significant behavioural responses of nauplii such as erratic and/or sluggish movement, mortality and survival rates. The lethal dose of the plant extracts that could kill 50% of the nauplii (LC<sub>50</sub>) was determined using the graphic methods of<sup>19</sup> for estimating the median effect dose (ED<sub>50</sub> or LD<sub>50</sub>, etc) and the dose percent effect curve. The interpolated value at 50% mortality ratio gave the LD<sub>50</sub> of each extract at the corresponding concentrations.

## **RESULT**

### **PHYTOCHEMISTRY OF AND**

The Phytochemistry of *A. sativum* and *L. camara* (Table 1) indicated the presence of secondary metabolites including alkaloids, cardiac glycosides, steroids, saponins, tannins, flavonoids, reducing sugars, terpenes, essential oils and carbohydrates. However, while terpenes is present in *L. camara*, it was not observed in *A. sativum* Nevertheless, protein was absent in both test plants.

### **Figure 1**

Table 1: The Phytochemical Analysis of Used Plant Extracts

Metabolites	<i>L. camara</i>	<i>Allium sativum</i>
Alkaloids	+	+
Cardiac Glycosides	+	+
Steroids	+	+
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Protein	-	-
Starch (Carbohydrates)	+	+
Reducing sugar	+	+
Oils	+	+
Terpenes	+	-

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Key: + = positive

- = negative

The antitubercular activity of test plants expressed in their inhibitory zone diameter (IZD) compared with standard antibiotics is presented in Table 2. *Allium sativum* exhibited maximal activity against all isolates even at reduced concentrations with IZD of 9.0mm for *M. tuberculosis*, 17.5mm for *M. avium* Complex and 10.0 mm for the Unidentified *M. avium* Complex at 6.25 mg/ml concentration. The activity of *L. camara* was expressed only on high concentrations of extracts: *M. avium* Complex was inhibited at 25mg/ml with IZD of 11.0mm (Table 2). Only two (2) of the standard antitubercular antibiotics used: Streptomycin and Rifampin showed significant activity against test isolates. The IZD of Streptomycin (0.33mg/ml) was 1.6cm on *M. tuberculosis*, 1.4cm on *M. avium* Complex and 1.1cm on the Unidentified *M. avium* Complex. Rifampin ((0.2mg/ml) on the other hand had IZD of 1.2cm on *M. tuberculosis*, 1.6cm on *M. avium* Complex and 1.5cm on the Unidentified isolate. The activity profile of the test plant extracts Mycobacterial isolates is summarized in their dose-response (Figures 1- 6).

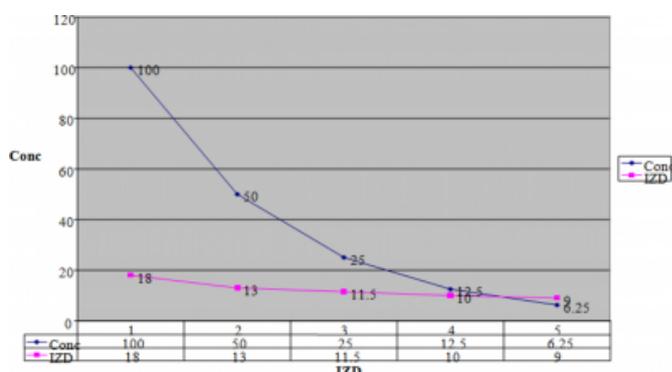
**Figure 2**

Table 2: INHIBITORY ZONE DIAMETER OF PLANT EXTRACTS AND CONTROL ANTIBIOTIC

Test Plant	Concentration (mg/ml)	IZD (mm)		
		<i>(M. tuberculosis)</i>	<i>(M. avium)</i>	Unidentified <i>Mycobacterium avium</i> complex
Streptomycin	(0.33)	1.6cm	1.4cm	1.1cm
Rifampicin	(0.2)	1.2cm	1.6cm	1.5cm
Ethambutol	(0.2)	Nil	Nil	Nil
Isoniazide	(0.25)	Nil	Nil	Nil
Pyrazinamide	(0.10)	Nil	Nil	Nil
<i>Allium sativum</i> (Garlic)	100	18.0	22.8	23.5
	50	13.0	22.0	19.0
	25	11.5	20.5	16.0
	12.5	10.0	20.0	12.5
	6.25	9.0	17.5	10.0
<i>L. camara</i>	100	12.0	17	11.5
	50	10.0	13.5	10.0
	25	-	11.0	-
	12.5	-	-	-
	6.25	-	-	-

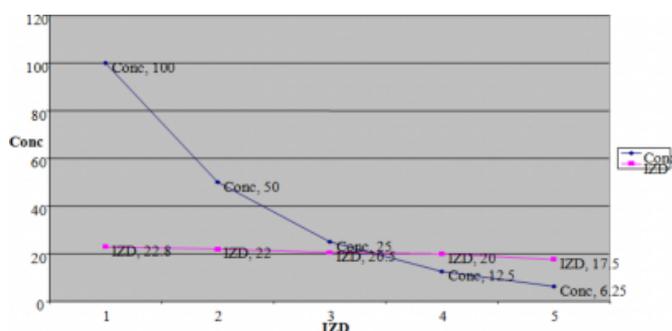
**Figure 3**

Figure 1: Dose-response effect of the Ethanolic extracts of on



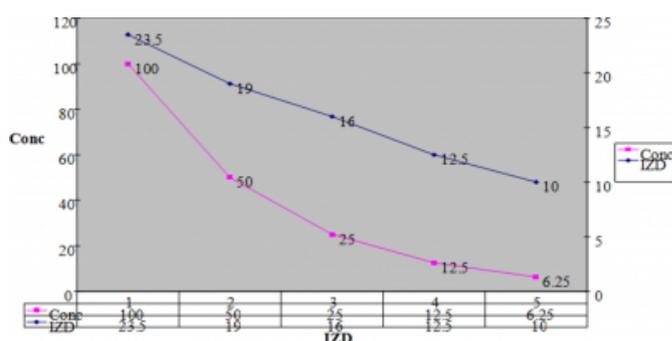
**Figure 4**

Figure 2: Dose-response effect of the Ethanolic extracts of on Complex



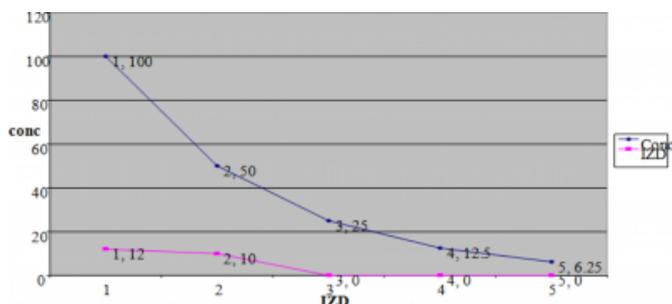
**Figure 5**

Figure 3: Dose-response effect of the Ethanolic extracts of on Unidentified Complex



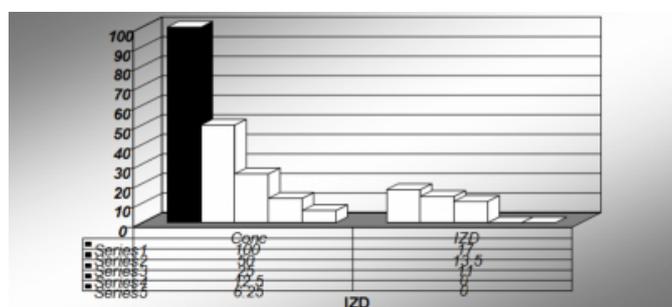
**Figure 6**

Figure 4: Dose-response effect of the Ethanolic extracts of *L. camara* on



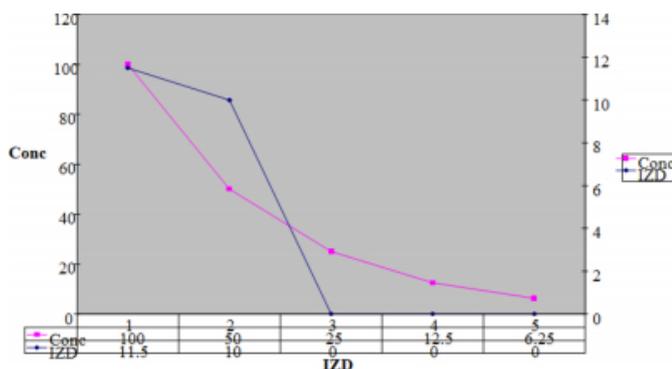
**Figure 7**

Figure 5: Dose-response effect of the Ethanolic extracts of on



**Figure 8**

Figure 6: Dose-response effect of the Ethanolic extracts of on Unidentified Complex



The activity profile of test plant extracts on isolated Mycobacterial species expressed in their minimal inhibitory concentration (MIC) is shown in the increasing order: *M. tuberculosis* (0.63mg/ml), *M. avium* Complex (0.97mg/ml) and Unidentified *A. avium* Complex (1mg/ml) for *A. sativum*; while for *L. camara*, activity increased as follows: *M. tuberculosis* (0.3mg/ml), *M. avium* Complex (0.89mg/ml) and Unidentified *A. avium* Complex (0.63mg/ml) (Table 3).

**Figure 9**

Table 3: MINIMAL INHIBITORY CONCENTRATION (MIC) OF TEST PLANT EXTRACTS ON TEST ISOLATES

Isolate	MIC (mg/ml)	
	<i>A. sativum</i>	<i>L. camara</i>
<i>M. tuberculosis</i>	0.643	0.63
<i>M. avium</i> Complex	0.97	0.89
Unidentified <i>M. avium</i> Complex	1	0.63

The cytotoxic effect of the test ethanolic plant extracts on brine shrimps (Table 4) indicated that garlic extracts had LC<sub>50</sub> at 49.1 ppm for *M. tuberculosis*, 56.5ppm for *M. avium* Complex and 63.0ppm for the Unidentified *M. avium* Complex, while *L. camara* had 32.6ppm for *M. tuberculosis*, 55.9ppm for *M. avium* Complex and 51.3ppm for Unidentified *M. avium* Complex.

**Figure 10**

Table 4: LD OF TEST PLANT EXTRACTS AT DIFFERENT CONCENTRATIONS

Test Plants	LD50 at different Concentrations		
	1000	100	10
	<i>M. tuberculosis</i>	<i>M. avium</i> Complex	Unidentified <i>M. avium</i> Complex
<i>A. sativum</i>	49.1	56.5	63
<i>L. camara</i>	32.6	44.9	51.3

**DISCUSSION**

Current strategies to overcome the global problem of antitubercular resistance include research in finding new and innovative alternative plant extracts of medicinal valued. The emergence of drug-resistant *M. tuberculosis* has created additional concern in the event of TB diagnosis. These strains are resistant to the primary antituberculosis drugs and require a specialized antibiotic treatment. The opportunism of *Mycobacterium tuberculosis* and *Mycobacterium avium* complex in HIV/AIDS infection was apparent in this study as previously observed by<sup>20-21</sup>. This is as expected because tuberculosis is often the first opportunistic infection and manifests in HIV-infected persons with relatively higher CD4+ counts (336/ml/ - 441/ml) than in other opportunistic infections such as *Pneumocystis carinii*; hence among infected patients the risk of disseminated MAC infection was observed to increase with progressive immune dysfunction and was greatest among those with a CD4 count less than 50cells per micro liter (<50/u).<sup>22</sup> The observed prevalence of *Mycobacterium* infection among the people living with HIV/AIDS correlates with the findings of<sup>23</sup>, who reported that persons infected with human immunodeficiency virus (HIV) are at risk for having active tuberculosis (TB) disease

because of either reactivation of latent infection with *Mycobacterium tuberculosis* or rapid progression of newly acquired infection. However,<sup>24</sup> suggested that MAC in patients with HIV disease could represent recent acquisition rather than latent infection reactivating (which is the case in many other opportunistic infections in immunocompromised patients), and that the risk of MAC is inversely related to the patient's CD4 count, and increases significantly when the CD4 count decreases below 50 cells/mm<sup>3</sup>

Susceptibility test was therefore carried out using some conventional antitubercular drugs, and the ethanolic extract of *Allium sativum* (garlic) and *L. camara*, in the estimation of minimal inhibitory concentration (MIC). Of all the several drugs tested, streptomycin (IZD of 1.6cm for *M. tuberculosis*; 1.4cm for *M. Avium* complex and 1.1cm for the Unidentified isolate); and rifampicin (IZD of 1.2cm for *M. tuberculosis*, 1.6cm for *M. avium* Complex and 1.5cm for the unidentified *M. avium* complex) were found most effective against test isolates. The susceptibility of the isolates to Streptomycin was generally observed among all the isolates, making it a possible drug of choice for combined therapy probably with any of those drugs to which the isolates were moderately sensitive, rifampicin and ethambutol respectively. However, combination therapy with a minimum of 2 drugs was recommended by the Centre for Disease Control and Prevention.<sup>3</sup> Nevertheless, the newer macrolides, azithromycin and clarithromycin were found more effective single dose chemotherapeutants for the treatment of this disease<sup>22</sup> although they were not tried in the research. Monotherapy with any other drug would not be recommended because multiple drug resistance observed among people with multiple infection of HIV and TB. Drug resistance potential of *Mycobacterial* species especially in immunocompromised persons could be attributed to some immunological and generic mechanisms: its ability to prevent phagosome maturation, survival in stress conditions, modulation of antigen processing and presentation as well as the depletion of CD4 and CD8 T-cells in the course of co-infection with HIV. Furthermore, the pathogen also secretes a cytotoxic factor known as nucleoside diphosphate kinase (Ndk), which facilitates ATP-dependent P2Z receptor-mediated macrophage death or apoptosis vital for initiation of infection, survival and escape of host immune surveillance, and consequently reactivation of latent tuberculosis.<sup>25</sup> Phytochemical analysis of the ethanolic plant extracts used showed the presence of vital chemical constituents cardiac glycosides, tannins, and flavonoids,

suggesting their potential for therapeutic purposes. Antitubercular activity (expressed as MIC) of garlic extract and *L. camara* were remarkably observed in this study in the order: 1mg/ml (Unidentified *M. avium* complex) > 0.97mg/ml (*M. avium* Complex) > 0.63mg/ml (*M. tuberculosis*). This is in consonance with previous report by<sup>26</sup> indicating the antitubercular activity of the plant extract at lower concentration. The recorded antitubercular activity of *L. camara* could be associated with its rich phytochemical components: cardiac glycosides present function to enhance myocardial contraction, and they exert their hypotensive effect by inhibiting Sodium and Potassium ion (Na<sup>+</sup> and K<sup>+</sup> ATPase). The presence of alkaloids, flavonoids saponins and oils in the plant may also account for its observed activity against the isolates: while alkaloids interferes with cell division, flavonoids exert potent antioxidant activity against the superoxide radical, hence the inhibition of low density lipid oxidation as reported by<sup>11-12</sup> respectively. The root of this plant has been found useful in the management of malaria, rheumatism and skin rashes: dermatitis, eczema, pruritis.<sup>8</sup> It is also indicated for influenza, cough mumps, high fever and tuberculosis and has also been shown to be helpful in gum care.<sup>10</sup>

The susceptibility of the isolates to garlic extract as shown by the MIC and dose-response effect was remarkable demonstrates its potential for therapeutic uses Garlic contains a stable, effective, and safe organosulfur compound, S-allyl cysteine (SAC major garlic constituent), which is noted for protection against oxidation, free radicals, pollution, cancer, and cardiovascular diseases. SAC has cardiovascular effect: lowers total serum cholesterol and LDL cholesterol hypercholesterolemia by inhibiting the activity of the key enzymes in cholesterol synthesis,  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA synthetase and reductase, in the liver<sup>27</sup> It's blood thinning effect was observed by<sup>28-29</sup> who showed that SAC lowered the levels of plasma thromboxane B2, and factor 4 (blood clotting factors) in those with hypercholesterolemia up to 30%; and also decreased platelet aggregation, or blood clotting, induced by the potent clotting agents, collagen and adenosine diphosphate. Inhibition of Vascular Smooth-Muscle Cell (SMC) and Umbilical Endothelial Cell Proliferation was reported by<sup>30</sup> SMC proliferation constitutes an essential aspect in the development of atherosclerosis and of restinosis (narrowing or constriction) of blood vessels subjected to angioplasty.<sup>31</sup> examined the potential role of Aged Garlic Extract as an antioxidant for sickle red blood cells.

Unanimously, the patient's count of Heinz bodies decreased from 58.9% to 29.8% during the 4 weeks of the study. These data suggest the significant antioxidant activity of AGE on sickle cell anemia, and may represent a potential therapy to combat complications of the disease. Antioxidative effects of SAC was further reported by <sup>32</sup> who found that SAC could prevent copper, a potent oxidant, from oxidizing LDL cholesterol. <sup>33-34</sup> found that SAC decreased the emission of low level chemiluminescence (LLC) initiated by t-butyl hydroperoxide. SAC inhibited LLC emissions 33% at 5 mmol/L and 45% at 10mmol/L. SAC also demonstrated radical and hydrogen peroxide scavenging activities in vivo. SAC demonstrated a scavenging effect on hydrogen peroxide and also inhibited the chain oxidation induced by a hydrophilic radical initiator in another study by <sup>33</sup>. SAC inhibited the emission of low level chemiluminescence and the early formation of TBA-RS (markers of oxidation), whereas water extracts of raw and heat-treated garlic enhanced such emissions<sup>35</sup> suggested that SAC has antioxidative efficacy. Attenuating Ischemic Brain Damage. <sup>36</sup> studied the efficacy of SAC as a free radical scavenger and reported that SAC improved (i) motor performance and (ii) memory impairment, and reduced (iii) water contents and (iv) the infarct size. Also, (i) the production of free radicals (alkoxyl radicals) as studied by electron paramagnetic resonance spectroscopy (EPR) was biphasic. Anticancer and Cancer-Preventive Effects of SAC were similarly reported: SAC inhibited the growth of carcinogen-induced tumors of the breast in the studies carried out by <sup>37</sup>:SAC dose-dependently reduced the carcinogen DMBA-DNA adduct formation in the mammary glands, and (0.5 or 2.5 mg/kg diet) supplementation markedly depressed the occurrence of DMBA-DNA adducts in mammary cells by 70 or 80%, respectively. On the other hand,<sup>38</sup> found that SAC is an effective inhibitor of N-methylnitrosourea mammary tumors.

The cytotoxic effect of the test ethanolic plant extracts on brine shrimps is apparent in the study: garlic extract was most highly effective, with LC<sub>50</sub> at 49.1 ppm for M. tuberculosis, 56.5ppm for M. avium Complex and 63.0ppm for the Unidentified M. avium Complex. However, moderate cytotoxic activity was exerted by L. camara on the brine shrimp: 32.6ppm for M. tuberculosis, 55.9ppm for M. avium Complex and 51.3ppm for Unidentified M. avium Complex respectively (less than 100 ppm: effective LD<sub>50</sub> value for cytotoxicities is about one-tenth LD<sub>50</sub> values for brine shrimp test. <sup>39</sup> Thus, the brine shrimp lethality index obtained in the study further provides evidence of the antitubercular activity

of garlic and relatively for L. camara, whose uses have been well documented for the treatment of a wide range of illnesses.

## CONCLUSION

The antitubercular activity of some plant extracts have been elucidated in this study, and their pharmacokinetics are clear evidence of their possible uses (as natural sources) in solving the global problem of antituberculosis resistant Mycobacterial species especially in this wake of HIV/AIDS epidemic. Due to demonstrated high LD<sub>50</sub> value in brine shrimps lethality and high antitubercular activity of L. camara and A. sativum (garlic) extracts in particular, their uses in TB chemotherapy is thus suggested. However, further studies on the antagonistic and/or synergistic effect of the active constituents of the used plants extract is to be furthered using thin layer chromatography (TLC), in order to effectively evaluate the antitubercular activity of individual constituents and determine the actual pharmacologically active constituents responsible for the antitubercular activity.

## References

1. Raviglione MC, Snider DE, Kochi A, Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. JAMA 1995; 273:220-226
2. Prescott LM, Harley JP, Klein DA, Human diseases caused by viruses. In: Microbiology 4th ed. Boston: WCB/McGraw Hill, 2005; 743-752.
3. CDC, Incidence of HIV-2, HIV/AIDS Surveillance Report, 1998; 10 (2): 5-26-28
4. WHO, Traditional Medicine Strategy 2002–2005 Geneva: World Health Organization 2003.
5. Adetokunbo OL, Herbert MG, Short Text book of Public Health Medicine for the Tropics. 4th Ed. Edward Arnold, London 2003.
6. Cavalitto CJ, Allium sativum; antimicrobial activity, J. Am. Chem. Soc 1944; 66: 1950.
7. Subhuti D, Garlic as the Central Herb Therapy For AIDS. Institute for traditional Medicine 1996.
8. Raghun C, Ashok G, Dhanaraj SA et al. In vitro cytotoxicity activity of Lantana camara (Linn.). Indian Journal of Pharmacology 2004; 36(2) 94-95.
9. Smith I, Mycobacterium tuberculosis Pathogenesis and Molecular Determinants of Virulence. Clin. Microbiol. Rev 1998; 16: 463-496
10. Idu M, Onyibe L, Antimicrobial activity of some plants in Africa. Research Journal of Medicinal plants 2007; 4(2): 32-41.
11. Knekt P, Jarvinen R, Reunanen A et al. Flavonoid intake and coronary mortality in Finland: a cohort study. Biomed. J, 1996; 312:478-481
12. Hertog MGL, Feskens EJM, Holman DCH et al. Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen Elderly Study. Lancet, 1993; 342:1007-1011.
13. Cheesbrough M, Medical Laboratory Manual for Tropical Countries Butterworth-Heinemann Ltd, Oxford, Cambridge, 1991; 11: 151-352.
14. Stokes EJ, Clinical Bacteriology. (3rd Edition). Edward

- Arnold Publishers, London.1970.
15. Ellen Jo B, Lance RP, Sydney MF, Bailey and Scott's Diagnostic Microbiology. (9th Edition). Mosby Publishers, St. Louis, U.S.A 1994.
  16. Trease GE, Evans WC, Textbook of Pharmacognosy. 14th Ed. W.B. Sanders, London 1996; 46-47
  17. Sofowor EA, Medicinal plants and Traditional Medicine in Africa. J.Wiley and Sons LTD New York 1993; 512-513.
  18. Cruickshank R, Handbook of Bacteriology. A guide to Laboratory Diagnosis and Control of Infection. 10th edn. Church Hill, Livingstone Ltd. Edinburgh 1960.
  19. Litchfield and Wilcoxon A simplified method of evaluating dose-effect experiment. J. Pharm 1949.
  20. Havlik JA, Horsburgh Jr CR, Metchock B, et al. Thompson III SE. Disseminated *M. avium* complex infection: clinical identification and epidemiologic trends. J Infect Dis 1992; 165: 577-580.
  21. Grange JM Collins CH, Bovine Tuberculosis and Disease in Animal and Man. Epidemiology and Infection 1990; 9: 221-234.
  22. Cohen PT, Merle AS, Volberding PA, The AIDS Knowledge Base. (2nd Edition). Little, Brown and Company, New York 1994.
  23. Castro KG, Tuberculosis as an opportunistic disease in persons infected with human immunodeficiency virus. Clin Infect Dis 1995; 21(suppl. 1):S66-S71.
  24. Teirstein, AS, Damsker, B, Kirschner, PA, et al. Pulmonary infection with MAI: Diagnosis, clinical patterns, treatment. Mt Sinai J Med 1990; 57:209.:220-226
  25. Puneet C, Anubha S, Anil K et al. Cytotoxic activity of nucleoside diphosphate kinase secreted from *Mycobacterium tuberculosis*. European Journal of Biochemistry 2003; 270:625-634.
  26. Adelina J, Mariana M, Raquel R, Activity against multidrug-resistance *Mycobacterium tuberculosis* in Mexican plants used to test respiratory diseases. Escuela Nacional de Ciencias Biologicas 2003; 5:1340-1346.
  27. Abuirmeileh N, Yu S, The FASEB J. Journal, 1991; 5(6):A1756, #8048.
  28. Qureshi N, Lin R, First World Congress on the Health Significance of Garlic and Garlic Constituents (FWC). Washington, D.C. August 28-30 1990; p. 17.
  29. Yu S, Qureshi N, National Conference on Cholesterol and high blood pressure. Sponsored by Cholesterol Education Program of the National Institutes of Health. April 8-10 1990; Washington, D.C.
  30. Takasu J.. Blood Disorders, BMC 2002; 2:3.
  31. Ide N, Lau B, Planta Med, 1997; 63: 263-264.
  32. Ide N , Matsuura H. Phytothe . Res 1996; 10: 340-341.
  33. Amagase H, Ch. Phytochemicals and Phytopharmaceuticals. AOCS Press, Champaign, Illinois, 1999; 6: 62-78.
  34. Imai J, Itakura Y, Planta Medica, 1994; 60:417-420.
  35. Numagami Y, Ohnishi S, Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998.
  36. Song K, Milner J, J. Nutr. 1999; 29(3): 657-661.
  37. Liu J, Milner J, Experimental Biology, 1995; 95.
  38. MacLaughlin JL, Chang CJ, Smith DL, "Bench-Top" Bioassays for the discovery of Bioactive Natural Products: An update (Atta Url-Rahman ed). Studies in Natural Product Chemistry. Elsevier Science Publishers B.V. Amsterdam, 1991; 9:101-103.

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