www.ajbrui.net

Afr. J. Biomed. Res. Vol.17 (May, 2014); 93- 99

Full Length Research Paper

In vitro Anti-mycobacteria Sensitivity and Kill-kinetics of *Allium ascalonicum* L. (whole plant) on Nontuberculous Mycobacteria Species

Igbokwe, C.O, Lawal T.O and Adeniyi B.A*

Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria

ABSTRACT

Allium ascalonicum L. (Shallot) was one of the herbs repeatedly identified from the result of our ethnobotanical survey for the treatment of tuberculosis and nontuberculous mycobacteria diseases. It has been reported to show inhibitory potentials against several pathogens. This plant is also known to form part of the diet of many people across the world. In the quest for a more active and body-friendly therapeutic agents, extracts of *Allium ascalonicum* (whole plant) were screened against four nontuberculous mycobacteria species namely, *Mycobacterium fortuitum* ATCC 684, *Mycobacterium smegmatis* ATCC 19420, *Mycobacterium abscessus* and *Mycobacterium phlei* ATCC 19240. *In vitro* susceptibilities testing was done using agar diffusion method with the concentrations of extracts ranging between 25 to 200 mg/mL. The minimum inhibitory concentration (MIC) was determined by agar dilution methods while the kill or bactericidal kinetics was measured by viable counting technique. Methanolic extract demonstated significant inhibitory potentials against three of the organisms at the test concentrations with a minimum inhibitory concentration (MIC) of 100 mg/mL, and 72-98 % kill of susceptible organisms in 24 hours. This significant activity may be due to the presence and right combination of the secondary metabolites in the plant such as alkaloids, flavonoids saponins, cardiac glycosides and essential oil, as revealed from our phytochemical screening. This study therefore confirms the scientific bases and justifies the use of *Allium ascalonicum* L. in traditional medicine practice in Nigeria and other parts of the world; and encourages its consumption as a natural prophylaxis against tuberculosis and nontuberculous mycobacteria diseases.

Key words: *Allium ascalonicum*, nontuberculous mycobacteria, kill-kinetics, *in vitro*

INTRODUCTION

Allium ascalonicum L. commonly called shallot, is a member of the *Allium cepa* aggregatum group (Fritsch and Friesen, 2002). It is believed to have originated from Southeast Asia and reportedly used in the treatment of wide range of diseases, because the bulbs are considered stomachic, tonic, anthelmintic, antispasmodic, expectorant etc. (Hamid *et al*., 2011). Its antimicrobial principles are thought to differ from the antimicrobial compounds of common onion and garlic though members of the same family-Liliaceae. This is believed to be as a result of the relative heat-stability of the compounds of *A. ascalonicum*, when compared with the others (Amin and Kapadins, 2005). And such

*Corresponding author:
E-mail: baadeniyi@yahoo.co.uk

Date Received: December, 2013
Date Accepted:, April, 2014

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

antimicrobial activity of *A. ascalonicum* was reported to cut across both Gram-positive cocci and Gram-negative rods (Rahbar *et al.,* 2005). In the result of an ethnobotanical survey we carried out in an area of Ibadan, Southwest of Nigeria, *A. ascalonicum* was consistently mentioned as one of the plants used in the treatment of tuberculosis and nontuberculous mycobacteria diseases. The traditional healers prepare a decoction of the plant in combination with other plants, using water, locally made alcoholic drink or lemon-juice and give such decoctions to their patients.

This study was aimed at approaching indirectly and cautiously, this dangerous pathogen- *M. tuberculosis* by using other less dangerous (but often pathogenic) members of the same genus *Mycobacteria.* These non-tuberculous and rapidly growing mycobacteria species (especially the *M. smegmatis*), are known to share over 2000 gene homologues with *M. tuberculosis*, and they also share the same unusual cell wall structure of *M. tuberculosis* (McMurray, 1996). *N*-hexane and methanolic extracts of *A. ascalonicum* were used to test for *in vitro* susceptibilities of four nontuberculous mycobacteria species; viz; *M. fortuitum* ATCC 684, *M. smegmatis* ATCC 19420, *M. abscessus*, and *M. phlei* ATCC 19420. The minimum inhibitory concentrations (MIC) of the susceptible species were determined and the kill or bactericidal kinetics was measured.

MATERIALS AND METHODS

*Ethnobotanical Survey***:** As a preliminary investigation, to find out the various plants used by the local folks in the treatment of tuberculosis and non-tuberculous mycobacteria disease, ethnobotanical survey was carried out in Bodija, Oje and Bode markets, all within Ibadan, the Southwest of Nigeria. The survey was done using a semi-structured questionnaire, which was administered to about 50 respondents who were herb sellers.

Plant collection, extraction and preparation of extracts
The plant-*Allium ascalonicum:* (whole plant) was collected in Ogbomoso area of Oyo State, Nigeria in April 2011 and authenticated by Botanists at the Department of Botany (Herbarium) University of Ibadan, Ibadan, Nigeria. Voucher specimen was deposited at the Herbarium with voucher number UIH-22337. The plant was thoroughly washed, air-dried and subsequently pulverized for extraction. Soxhlet extraction was employed to allow for combination of percolation and immersion techniques. A known weight (543 g) of the plant sample was successfully extracted using pure *n*-hexane and methanol as solvents. Each fraction of the extracts (*n*-hexane and methanolic), was filtered and concentrated *in vacuo* and stored at 4ºC for further use. Extracts were prepared for the study by reconstituting in 20% dimethlsulphoxide (DMSO) to different final concentrations as required.

Microorganisms: The microorganisms employed in this study were the rapidly growing mycobacteria (RGM): *Mycobacterium fortuitum* ATCC 684, *Mycobacterium smegmatis* ATCC 19420, *Mycobacterium abscessus*, and *Mycobacterium phlei* ATCC 19420.These were grown over-night in Tryptic Soy broth (TSB) before use.

Media and antimicrobial agent: Mueller-Hinton agar (MHA) and Tryptic Soy broth (TSB), both from Oxoid, were employed for the growth of the organisms. Rifampicin was the positive control while 20% dimethyl sulfoxide (DMSO) was the negative controls.

Phytochemical screening: The plant extracts were subjected to quantitative chemical analysis for the presence of secondary metabolites such as anthraquinones, cardiac glycosides, flavonoids, alkaloids and saponins using methods previously described (Harborne, 1998; Abo and Adeyemi, 1999).

Antimycobacteria susceptibility testing: The susceptibility testing was investigated by the agar diffusion method (Adeniyi *et al*., 2006). A 0.2 mL of 1:100 dilutions (equivalent to 10^7 cfu/mL) of fresh overnight culture of the nontuberculous mycobacteria species grown in Tryptic Soy broth (TSB), was seeded into 20 mL of molten Mueller-Hinton agar supplemented with 5% sterile horse blood at 45ºC, and properly mixed in universal bottles. The mixture was aseptically poured into sterile Petri dishes and allowed to set. Using a sterile cork borer of 8 mm diameter, equidistant wells were made in the agar. A hundred microlitre (100 µL) of the resuspended extracts with concentrations between 25 to 200 mg/mL was introduced into the wells. The positive control (Rifampicin- 40 µg/mL) and negative control (20% DMSO) were also introduced into separate wells. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation. The plates were incubated at 37ºC for 24 to 48 hours. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. All experiments were performed in duplicates.

Determination of minimum inhibitory concentration (MIC): To determine the minimum inhibitory concentration (MIC) of the extract that inhibited the

growth of the organism, the agar-dilution method (Adeniyi *et al.*, 2009) was used. To 19 mL of molten Mueller-Hinton agar at 45ºC, was added 1 mL of the reconstituted extracts which were prepared to final concentrations of 400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL. The agar/extract mixture was poured into sterile Petri dishes and allowed to set. The surface of the agar was dried in an incubator for 30 mins before streaking with an inoculating loopful of the overnight culture of the organism. After incubation for 24-48 hours at 37ºC, the plates were examined for presence or absence of growth. The lowest concentration that prevented the growth of each organism was noted as the minimum inhibitory concentration. The experiment was performed in two replicates to ensure accuracy.

Determination of minimum bactericidal concentration (MIC): Minimum bactericidal concentration (MBC) of active plant extracts was determined by a modification of the method of Aibinu *et al.*, (2007). To a 0.5 mL extract at different concentrations as used in the MIC assay that showed no visible growth on the agar plates, was added 0.5 mL of test organism in a tube. These were incubated at 37ºC for about 24-48 hr. Samples were streaked out from the tubes on the surface of sterile extract-free Mueller-Hinton agar (Oxoid) medium supplemented with 5% sterile horse blood in Petri dishes to determine the minimum concentration of the extract required to kill the organisms. These concentrations were indicated by the inability of the organisms to grow on transfer to the extract-free agar. The lowest concentration that prevented bacterial growth after 48 hr of incubation was recorded as the minimum bactericidal concentration (MBC). The entire tests were carried out in duplicates to ensure accuracy. Agar plates without extracts and another agar-extract plate without any inoculated organism were also incubated to serve as organism and extract control plates respectively.

Table 1:
List of Nigerian plants used for the treatment of tuberculosis and nontuberculous mycobacteria diseases. (A result of ethnobotanical survey in three markets of Ibadan, Nigeria)

S/N	Botanical Names	Common Names	Local Names (Yoruba)	Family Names	Part used
1	*Parquetina nigrescens*	Silk vine	Ogbo ogbo	Periplocaceae	Root
2	*Crinium jagus*	Forest cranum	Ogede odo	Amaryllidaceae	Root & stem
3	*Chasmanthera dependens*	-	Egbo atoo	Menispermaceae	Stem
4	*Gladiolus psittacinus*	-	Alubosa Baka	Iridaceae	Corm
5	*Tetrapleura tetraptera*	Ese dae se a grandes fenilles (French)	A'ldan	Leguminosae (Mimosoideae)	Fruit (pod)
6	*Securideca logepedunculata*	Violet tree	Ipeta	Polygalaceae	Stem
7	*Euphobia laterifolia*	-	Eni-opiri	Euphorbiaceae	Stem
8	*Olax subscorpiodea*	-	Egbo ifon	Olacaceae	Stem
9	*Strophantus hispidus*	Arrow poison	Sagere	Apocynaceae	Stem
10	*Calliandra portoricens*	Powder puff	Tude	Mimosaceae	Root
11	*Allium ascalonicum*	Shallot	Ewe alubosa	Liliaceae	Whole plant
12	*Aframomum melegueta*	Alligator pepper	Ata are	Zingiberaceae	Seeds
13.	*Gossypium haisutum*	Upland cotton	Koro owu	Malvaceae	Seeds
14	*Garcinia kola*	Bitter kola	Orogbo gbigbe	Guttiferae	Fruit
15	*Xylopia aethopica*	Ethiopian pepper	Iru	Annonaceae	Fruit

The Kill-Kinetics

Concentrations of active extracts that correspond with the MIC value, 2 x MIC, 4 x MIC and 8 x MIC were used for the bactericidal studies by a modification of the viable counting methods described by Aibinu *et al*., (2007). A 0.5 mL of each culture was subcultured into a warm (37°C) 4.5 mL Tryptic Soy broth and incubated for 90 mins using a Gallenkamp orbital incubator to give a logarithmic phase culture. A 0.1 mL of the logarithmic phase culture was then inoculated into a warm 4.9 mL of Tryptic Soy broth containing the tested compound to give 1 in 50 dilution of the culture (equivalent to approximately 1×10^7 cfu/mL) and the required concentration of the extract. From the stock, 0.1mL was withdrawn aseptically for serial dilution. A 0.1ml of the final dilution (10^{-8}) was inoculated on to a solidified extract-free Mueller-Hinton agar plates. Sterile glass spreader was used to ensure even distribution of the inoculums over the agar surface. The withdrawal from the stock was strictly done at time intervals of 0 mins, 30 mins, 60 mins, 120 mins, 240 mins 360 mins, and 24 hours. This process was repeated for the various concentrations of extract used. The drug-positive and organism-negative controls also ran concurrently. The plates were incubated appropriately at 37ºC for 24-48 hours and the number of colonies on each plate was counted with the Stuart scientific colony-counter. The Colony Forming Unit (CFU) were calculated while taking into account, the final volume of inoculums plated out and the dilution factor. The results are averages of duplicate experiments, and the percentage survivals of the organisms were plotted against contact time on a semi-logarithmic graph.

RESULTS

The result of the ethnobotanical survey is presented in Table 1. With 1.98% and 17.7% respectively, the percentage yield of *n*-hexane extract to the methanol extract is approximately in the ratio 1:9. The phytochemical analysis revealed the presence of alkaloids, saponins, cardiac glycosides, flavonoids and essential oil, while tannins, anthraquinones and terpernoids were not detected (Table 2). The *n*-hexane fraction did not demonstrate any inhibitory potential against any of the screened organisms. Three out of the four tested organisms were susceptible to the methanolic extract (Table 3). The minimum inhibitory concentration for the susceptible species was 100 mg/mL (Table 4). The kill-kinetics revealed a drastic reduction in the population (72 – 98% kill) of the test organisms within 24 hrs of exposure to concentrations equivalent to and above the MIC of the extract (Figs. 1, 2 & 3).

Table 2:
Phytochemical screening of methanol extract of *Allium ascalonicum* (whole plant)

Phytochemicals	Observations
Tannins	-
Cardiac glycosides	++
Anthraquinones	-
Saponins	+
Flavonoids	++
Terpenoids	-
Carbohydrates	-
Essential oil	++
Alkaloids	++

Note: ++ = Present; + = present in low concentration; - = Not Present

Table 3:
Antimicrobial screening of lyophilized methanol extract of *A. ascalonicum* (whole plant) against nontuberculous mycobacteria species

	Methanol extract (mg/mL)				Rifampicin 40µg/mL	DMSO 20%
Organisms	**200**	**100**	**50**	**25**		
M. fortuitum ATCC 684	20±0.00	18±0.50	11±0.50	-	22±0.20	-
M. smegmatis ATCC 19420	20±0.20	17±0.00	11±0.50	-	21±0.00	-
M. abscessus	-	-	-	-	21±0.50	-
M. phlei ATCC 19240	19±0.50	17 ±0.60	10±0.40	-	21±0.00	-

Note: Diameter of cock borer = 8mm; Results are averages of duplicate experiments.

Table 4:
The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extract of *A. ascalonicum* (whole plant) on nontuberculous mycobacteria species

Organism	Methanol extract (mg/mL)		Rifampicin (µg/mL)	
	MIC	MBC	MIC	MBC
M. fortuitum ATCC 684	100	200	40	40
M. smegmatis ATCC 19420	100	200	40	40
M. abscessus	ND	ND	ND	ND
M. phlei ATCC 19240	100	250	40	60

Note: ND = Not determined

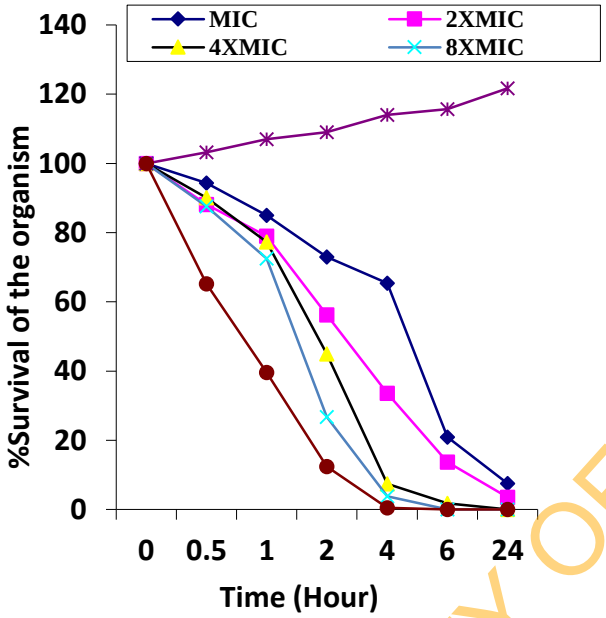

Fig. 1:
Kinetics of bactericidal activity of methanol extract of *A. ascalonicum* on *M. fortuitum* ATCC 684 showing the rate of kill of the organism by the extract at different exposure times.

DISCUSSION

Ethnobotanical studies remain a veritable source of information about the medicinal uses and efficacy of plants (Slikkerveer, 2006). For instance where indigenous people from geographically dispersed areas use closely related herbs for the same purpose, it is taken as supporting evidence for its efficacy. Many of the herbs and spices used by humans as food and food seasoning yield useful medicinal compounds (Lai and Roy, 2004; Tapsell *et al*., 2006). The result of the ethnobotanical survey in this study showed that *A. ascalonicum* L. (whole plant) is used in combination with other plants to form a decoction which is taken for the treatment of tuberculosis and nontuberculous mycobacteria diseases. This use of many plants and plant parts for the treatment of a disease may be justified by the principles of synergism, in which many bioactive substances in plants tend to combine in some order yet to be clearly understood, and potentiate the bioactivity of one another for maximum effect. The low yield and non activity of the *n*-hexane fraction relative to the methanolic fraction of the extract may be supported by the fact that most phenolic compounds dissolve readily in polar solvents than non-polar solvents. And thus, the polarity of solvents is known to affect the quantity and composition of the secondary metabolites of an extract (Parekh *et al.*, 2006).

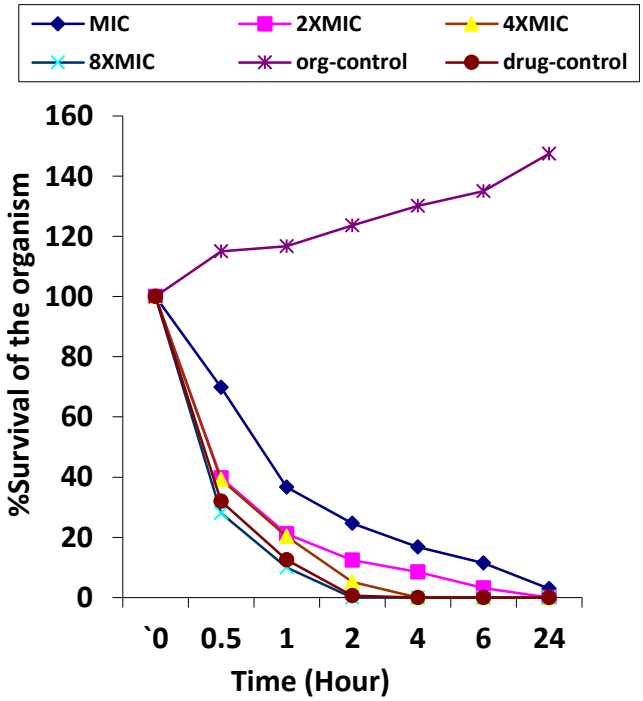

Fig. 2:
Kinetics of bactericidal activity of methanol extract of *A. ascalonicum* on *M. smegmatis* ATCC19420 showing the rate of kill of the organism by the extract at different exposure times.

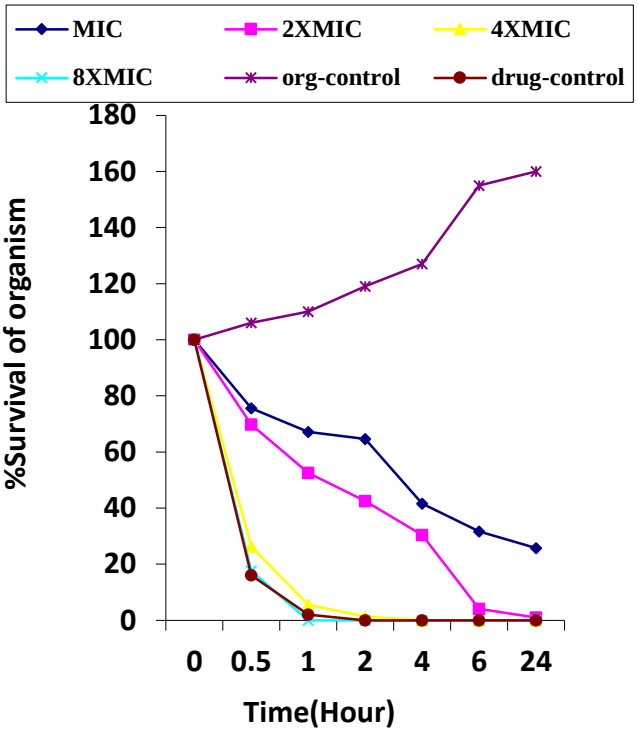

Fig. 3:
Bactericidal activity of methanol extract of *A. ascalonicum* on *M. phlei* ATCC 19240 showing the rate of kill of the organism by the extract at different exposure times

The result of the phytochemical screening revealed the presence of secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, saponins and essential oil, which could be responsible for the inhibitory potentials shown by the extract of this plant since these metabolites are known to possess antimicrobial activities. *Mycobacterium fortuitum* ATCC 684, *M. smegmatis* ATCC 19420, and *M. phlei* ATCC 19240, were susceptible to the extract of *A. ascalonicum*; while *M. abscessus* showed no susceptibility. Though the mechanisms of antimicrobial actions of the secondary metabolites are not fully understood, many investigations have been conducted. It is believed that single compounds may not be responsible for the bioactivity, but rather a combination of compounds interacting in an additive or synergistic manner (Javed *et al.*, 2012). The mechanism of flavonoids might be through cytoplasmic membrane, DNA-gyrase inhibition and β-hydroxyacyl-acyl carrier protein dehydratase activities (Cushnie and Lamb, 2005; Zhang *et al*., 2008). Ulanowska *et al.*, (2006) reported that cell morphology can be changed by isoflavone genistein through formation of filamentous cells and by inhibiting the synthesis of DNA and RNA of *Vibrio harvey*. Terpene present in essential oil promotes membrane disruption; coumarins cause cell respiration reduction and tannins bind to polysaccharides or enzymes, promoting inactivation and affecting the membrane of microorganisms (Ya *et al.*, 1988; Cowan, 1999).

The resistance shown by *M. abscessus* could be either intrinsic, attributed to a combination of the permeability barrier of the complex multilayer cell envelope, drug export system, antibiotic targets with low affinity and enzymes that neutralize antibiotics in the cytoplasm, or acquired resistance through mutation (Nessar *et al.*, 2012). However, the susceptibility of the other rapidly growing Mycobacteria (RGM) in this study offers the chance for possible chemotherapeutic alternatives, since some of these rapidly growing mycobacteria (e.g. *M. fortuitum*), are known to show resistance to some anti-RGM drugs, such as Telithromycin, meropenem and fluoroquinolones (Yang *et al.*, 2003).

With the MIC of the susceptible species at 100 mg/mL and the bactericidal kinetics of the extract showing a 72-98% kill rate of the organisms within 24 hrs of exposure to concentrations equivalent to and above the MIC of the extract. These suggest that the extracts contain compounds that could be developed to elicit better antimycobacteria activity that will compare favourably with the drug positive control which killed the organisms within the same contact time though at a lower concentration. This preliminary investigation agreed with the reports of Adeleye *et al*., (2008) that the ethanolic and aqueous extracts of *A. ascalonicum* inhibited the growth of *Mycobacterium tuberculosis*. Also, Mansour *et al.*, (2009) reported that partially purified extract of *A. ascalonicum* showed significant antimycobacteria activity against *M. tuberculosis*.

Conclusion

Methanolic extract of *Allium ascalonicum* L. (whole plant) demontrated *in vitro* activity against non-tuberculous mycobacteria species, thus justifying its use in traditional medicine in Nigeria and some other African countries, for the treatment of tuberculosis and nontuberculous mycobacteria diseases. Further research will focus on isolation and characterization of the bioactive components of this plant as a possible therapeutic alternative to existing anti-RGM drugs. More importantly, the prospect of formulating it into food supplement as a likely prophylaxis against the relevant diseases would be considered.

REFERENCES

Abo K. A., Adeyemi A. A. (1999). Introductory practical phytochemistry. Pharmacognosy Practical. Schedule II, Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Nigeria.

Adeleye I. A., Onubogu C. C., Ayolabi C. I., Isawumi A. O., Nshiogu M. E. (2008). Screening of Crude extracts of twelve medicinal plants and 'wonder – cure' concoction used in Nigeria unorthodox medicine for activity against *Mycobacterium tuberculosis* isolated from tuberculosis patients sputum. *Afr. J. of Biotechnol.,* 7 (18), 3182 – 3187.

Adeniyi B. A., Lawal T. O., Olaleye S. B. (2006): Antimicrobial and gastroprotective activities of *Eucalyptus camaldulensis* crude extracts. *J. Biol Sci* 6, 1141 – 1145.

Adeniyi B.A., Onwubuche B.C., Anyiam F.M., Ekundayo O., Mahady G.B. (2009). Anti-*Helicobacter pylori* activities of *Eucalyptus grandis*: Effects on susceptibility, urease activity and cell surface hydrophobicity. *Pharm. Biol.*, 47, 13-17.

Aibinu I., Adenipekun T., Adelowotan T., Ogunsanya T. Odugbami T. (2007). Evaluation of the Antimicrobial Property of different parts of *Citrus aurantifolia* (Lime fruit) as used locally. *Afr. J. Microbial. Res*., 4, 185-195.

Amin M., Kapadnis B. P. (2005). Heat stable antimicrobial activity of *Allium ascalonicum* against bacteria and fungi. *Indian J. Exp. Biol*. 43 (8): 751 – 4.

Cowan M. M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol Rev.*, 12, 564-582.

Cushnie T. P., Lamb A. J. (2005). Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 26, 343-356.

Hamid R., Mohammadi M., Ali M., Kamran M. (2011). Anticancer and anti-inflammatory activities of shallot (*Allium ascalonicum*) extract. *Arch. Med Sci* 7 (1), 38-44.

Harborne J. B. (1998). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. P 41-48 Chapman and Hall, London:

Fritsch R. M., Friesen N. (2002). Evolution, Domestication, and Taxonomy. In Rabinowitch H. D. Gurrah L. Allium Crop Science: Recent Advances. p. 21, CABI Publishing, UK.

Javed S., Ahmed A.S., Mohammed S.H., Aysha U., Rauf A., Sobia, M. (2012). Pharmacological Evaluation of *Nigella Sativa* (Kalonji) and *Trachyspermum Ammi Ajwain. J Med. Plants Res.*, 6 (5), 768-775.

Lai P. K., Roy J. (2004). Antimicrobial and chemopreventive properties of herbs and spices. *Curr. Med. Chem* 11 (11), 1451 – 60.

Mansour A., Sepideh S., Mohammad, H. (2009). Antimycobacteria activity of partial purified extract of *Allium ascalomicum*. *Jundishapur Journal of Microbiology*, 2 (4), 144 – 147.

McMurray D. N. (1996) Mycobacteria and Nocardia. Medical Microbiology 4th Edn. Pp. 423-443, University of Texas Medical Branch at Galuston, USA.

Nessar, R., Cambau E, Reyrat J. M., Murray A., Gicque B. (2012). *Mycobacterium abscessus*: a new antibiotic Nightmare. *J. Antimicrob. Chemother.,* 65(5): 1-9 doi:10.1093/jac/dkr578

Parekh J, Karathia N, Chanda S. (2006). Screening of some traditionally used medicinal plants for potential antibacterial activity. *Indian J. Pharm. Sci*., 68, (6), 832-834.

Rahbar M., Hoseini Tagavi S. A., Diba K., Haidari A. (2005). *In vitro* Antibacterial Activity of Shallot (*Allium ascalonicum*) Crude juice *Journal of Medicinal Plants* 4 (13), 45 – 49.

Yang, S.C, Hsueh P. R., Lai H.C., Teng L. J., Huang L. M., Chen J. M., Wang S. K., Shie D. C., Ho S. W., Luh K.T. (2003). High prevalence of Antimicrobial Resistance in Rapidly Growing Mycobacteria in Taiwan. Antimicrob Agents Chemother. 47(6), 1958-1962.

Slikkerveer L.J. (2006). The Challenge of Non-experimental Validation of Mac Plants. Springer. http://library.wur.nl/ojs/index.php/frontis/article

Tapsell, L. C., Hemphill I., Cobaic L., Patch C. S., Sullivan D. R.,Fenech M., Roodenrys S., Keogh J., Clifton P. M., Williams P. G., Fazio V. A., Inge K. E. (2006). Health benefits of herbs and spices: the past, the present, the future. *Med. J. Aust*., 185 (4 suppl), 54 – 24.

Ulanowska, K., Tkaczyk A.,Konopa G., Wegrzyn G. (2006). Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. *Arch. Microbiol*. 184, 271-278.

Ya, C., Gaffney S. H., Lilley T. H., Haslam E. (1988). Carbohydrate – polyphenol complexation. In: Hemingway R. W, Karchesy J. J, eds. Chemistry and Significance of condensed tannins. P 553, Plenum Press, New York NT.

Zhang, L., Kong Y., Wu D., Zhang H., Wu J. Chen J., Ding J., Hu L.,, Jiang H, Shen X (2008). Three flavonoids targeting the β-hydroxyacyl-acyl carrier protein dehydratase from *Helicobacter pylori*: crystal structure characterization with enzymatic inhibition assay. *Protein Sci*., 17: 1971-1978