Journal of Sciences and Multidisciplinary Research
Volume 7, No. 1, 2015
ISSN: 2277-0135

# ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS AND ESSENTIAL OIL OF SPICES (Lemon grass and Holy basil) ON SELECTED HUMAN PATHOGENS

Afuape A.O., Osifeso O.O., and Adewunmi A.R

Department of Science Laboratory Technology,
Moshood Abiola Polytechnic, Ojere Abeokuta.
E-mail: afuapeolajumoke@yahoo.com

**Abstract:** Aim is to ascertain the effectiveness of lemon grass and holy basil extracts with their essential oils against five human pathogens. The ethanol extracts and essential oils were screened against four gram-negative organisms viz: *E.coli, klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae*, and one gram- positive organism viz: *Staphylococcus aureus*. Essential oils were obtained through steam distillation. The MIC/MBC of extracts was tested using agar dilution method at 50/100mg/ml concentrations each, active component of the oils were tested by photochemical screening. Essential oils of the species showed antibacterial activity on all pathogens but significant activity shown similarly on both *Salmonella typhi* and *Staphylococcus aureus* followed by *E. coli* and *Klebsiella pneumonia* while *Shigella dysenteriae* showed least inhibitory activity. Although ethanolic extracts and oil of both spices showed promising antibacterial activity on all pathogens, the broadest activity was verified on their essential oils; being good preservative, can also be good sources of antibacterial agents.

**Keywords:** Lemon Grass, Holy Basil, Essential Oil, MIC: Minimum inhibitory Concentration and MBC: Minimum Bactericidal Concentration.

## INTRODUCTION

*Ocimum sanctum* (Holy Basil) is a shrub, belongs to family Labiatae (Mint). Different parts of *O. sanctum* are commonly employed for the treatment of fever, bronchitis, cough, arthritis, digestive complaints, and also this plant has anticancer, antidiabetic and antimicrobial properties. Though it is closely related to the sweet basil (Ocimum basilicum) frequently used in cooking, holy basil has a much richer history. The plant, which is native to tropical Asia, is now found in most tropical parts of the world. It has only recently gained popularity in the United States although it has been grown in India for more than 3,000 years (Hammer *et al.*, 1999).Holy Basil is a traditional plant considered sacred by the Hindus. It is a most common household plant in India and grows wild in tropics. Native to India, it is a short lived perennial herb or small shrub of Mint family Labiatae (Lamiaceae). It has small leaves with a strong smell and purple flowers. The foliage is green or purple, strongly scented. Oil extracted from leaves of this plant possesses significant insecticidal properties (Nanasombat and Lohasupthawee, 2005). *Ocimum sanctum* has been extensively studied for therapeutic potentials in various areas like immuno-stimulation, anticancer antioxidant, as adjuvant to radiotherapy, antiulcer, analgesic and antidiabetic (Hammer *et al.*, 1999). Lemongrass (*Cymbopogon citratus),* a tall perennial grass comprising of about 55 species, is native to warm region and grows in almost all tropical and subtropical countries (Cheel *et al.*, 2005). The biologically active constituent of lemon grass is citral constituting more than 75% (w/w) of its essential oil (Huynh *et al.*, 2008). Lemongrass is widely used as an essential ingredient in Asian cuisines because of its sharp lemon flavour. Herbal tea of lemongrass is used as sedatives, febrifuge and immune-stimulant in India (Brian and Ikhlas, 2002) while, lemongrass essential oil is applied for its medicinal value to cure acne, oily skin, scabies, flatulence, headaches, blood circulation problems (Pearson, 2010) and excessive perspiration due to its antimicrobial and antibacterial activities

(Lawless, 1995). It has also been used as carminative, stimulant, diuretic and antiseptic (Ghani *et al.*, 1997). Lemongrass is native to India and tropical Asia. It is widely used as an herb in Asian cuisine. It has a subtle citrus flavour and can be dried and powdered, or used fresh. *C. citratus*, lemon grass (Poaceae/Graminae) is a native to India but found growing naturally in tropical grass land. It used in Ayurvedic medicine to help bring down fevers and threat infectious diseases. Traditionally, it is believed to cleanse the body so used as an antioxidant (Cheel *et al.*, 2005). In Nigeria, lemon grass is used for stomach problem and it is also used in combination with few other plants for effective treatment of malaria (Aibinu *et al.*, 2007) and typhoid (Depken, 2011). Although, in a few preliminary antimicrobial screenings, LGO had shown no activity against four Gram positive (*Bacillus subtilis, Corynebacterium diphtheriae, Streptococcus pyogenes* and *Staphylococcus aureus*) and three Gram negative (*Salmonella paratyphi* A*, Escherichia coli* and *Pseudomonas aeruginosa*) bacterial cultures (Saify *et al.*, 2000), later on several studies have shown that the lemon grass has antibacterial and antifungal properties (Ushimaru *et al.*, 2007). Essential oils are volatile aromatic concentrated hydrophobic oily liquids which are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots. Essential oils are usually terpenoids responsible for the aroma and flavour associated with herbs, spices and perfumes, also called volatile oils because they easily diffuse into the air. The main constituents of essential oils are mono and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones responsible for the biological activity as well as for their fragrance. Phenolic compounds present in essential oils have also been recognized as antimicrobial bioactive components. Plant essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Burt, 2004; Kordali, *et al*, 2004).

Some oils have based in cancer treatment (Sylvestre, *et al,* 2006} some other oils has been used in food preservation (Fraid, *et al,* 1995), aromatheraphy and fragrance industries. (vander Braak and Leijten 1999). Essential oil are rich source of biologically active compounds, there has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. Therefore, it is reasonable to expect a variety of plant compounds in this oil with specific as well as general antimicrobial activity and antibiotic potential (Seenivasan, *et al,* 2006). Essential oil can be obtained by expression, fermentation or extract but the method of steam distillation is most commonly used for commercial production. An estimated 3000 essential oils are known of which 300 are commercially important in fragrance market (Vander and Leijten 1999).Essential oils are complex mixer comprising many single compounds. Chemically they are derived from tarpenes and their oxygenated compounds, each of these constituents contribute to the beneficial or adverse effect (Seenivasan, *et al,*2006).

## MATERIALS AND METHODS

### MATERIALS

The following materials were used during the practical aspects of this project, which are:
Holy basil leaves extract (essential oil), Lemon grass leaves extract (essential oil) ;Nutrient agar; Clinical bacterial isolates ;Filter paper; Autoclave; Incubator; Test tubes; Petri dishes; Refrigerator; Clinical flask; Micro titer plates ; Absorbent and non absorbent cotton wood; Spirit lamp; Rubber pipette; Forceps ; and Adhesive tape.

## COLLECTION OF SAMPLES

Fresh and matured leaves of *Occimum gratissimum* and *Cymbopogon citratus* were collected from the Botanical Garden of Moshood Abiola Polytechnic, Ojere. Each sample was identified according to various literatures and taxonomic texts. Holy basil was cut while lemon grass was uprooted from source.

## SAMPLE PREPARATION

The leaves were destalked; wash to remove stones and debris, the sample were drained, dried and ground into powdery form, this was kept for various extractions.

## PREPARATION OF EXTRACTS

Extractions of *Occimum gratissimum* leaf and *Cymbopogon citratus* rhizomes was carried out using modified method of Omala *et, al,*2010. 25g of the powdered leaf were weighed into 250ml sample bottle, 300ml of the ethanol and aqueous solvents were added. The mixture was allowed to stand for 72hours (3days) then filtered. The filtrates were concentrated using a rotary evaporator.

## PREPARATIONS OF ESSENTIAL OILS

300g of *Cymbopogon citrates* and *Occimum gratissimum* was weighed and placed in a 2 litre volume flask together with distilled water (1 litre) after steam distillation; the 100% pure essential oils were collected and dispensed into dark bottles. This was stored at 4°c until used.

## PHOTOCHEMICAL SCREENING

This was done on the ethanolic extracts and essential oils from holy basil leaves and lemon grass extracts to ascertain the presence of bio active components present in both samples. The presence of Alkaloids, Flavonoids, Saponins,. Tannis, Steroids, Glycoside and Anthraquine were determined as described by (Okwu 2005, Sofowora 1993, Trease and Evans 2002).

## TEST FOR ALKALOIDS

Few quantities of the sample was stirred with 5ml of 1% aqueous HCL on water both and then flittered, 1ml was placed into two (2) test tubes, to the first portion, a few drops of dragendorff's reagent were added, occurrence of orange-red precipitate was taken as positive. To the second 1ml, Mayer's re-agent was added and appearance of buff- color showed the presence of alkaloids (Sofowora, 1993).

## TEST FOR FLAVONOIDS

5ml of 10% dilute ammonia solution was added to a portion of the plant extracts (holy basil and lemon grass) followed by addition of concentrated $H_2SO_4$. A yellow coloration observed in the extracts indicated the presence of flavonoids (Okwu, 2005).

## TEST FOR SAPONIN

Two (2ml) of the sample was boiled with 20ml distilled water in the water bath, the mixture was filtered and about 5ml of distilled water was added to 10ml, of the filtrate, this was shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaken vigorously and then observed for the formation of emulsion (Okwu, 2005)

## TEST FOR TANNINS

About 5ml portion of different sample (lemon grass and holy basil) was stirred and shaken with 10ml distilled water and then filtered, few drops of 1% ferric chloride solution were added to

2ml of the filtrate, occurrence of a blue black or blue green precipitate indicated the presence tannins (Trease and Evans, 2002).

## TEST FOR STEROIDS

2ml of aceticanhydride was added to 0.5ml of each extract, this was followed by the addition of 2ml $H_2SO_4$. The colour change from violet to blue or green in some sample indicating the presence of steroids (Okwu, 2005).

## TEST FOR CARDIAC GLYCOSIDES

5ml of the extracts were treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%). This was underplayed with 1ml of concentrated $H_2SO_4$, a brown ring of the interface indicated a deoxysugar characteristics of cardenolides. A violet ring may appear below the ring, while in the acetic layer, a greenish ring may form just gradually throughout their layer (Okwu, 2005).

## TEST FOR ANTHRAQUINONE

About 2ml of some portion to be tested was taken with 10ml of benzene and then filtered, 5ml of the 10% ammonia solution was then added to the filtrate and thereafter was shaken appearance of a pink, red or violet colour in the ammonical (lower) phase showed as the presence of free anthraquinons (Sofowora, 1993).

## COLLECTION OF TEST ORGANISM AND PREPARATION OF STOCK CULTURE

The clinical isolates of *Staph. aureus*, *E. coli*, *klebsiclla, pneumonia, salmonella typhi* and *shigella dysenteriae* were obtained from the department of microbiology, Sacred Heart hospital, Lantoro, Abeokuta, Ogun State, Nigeria, and reconfirmed by gram staining and sub-sculturing in appropriate selective media at $37^0$C.

## PREPARATION OF DISK

Paper puncher was used to punch whit-man number one filter paper to produce 5mm disc. The discs were sterilized by hot air, the funded filter paper placed in an oven at 180°c for 10 minutes. They were stored in a sterile container until ready to use.

## AGAR DIFFUSION TEST

Nutrient agar was inoculated with the test organisms adjusted to $10^6$ using 0.5 Mc farmland standards. The discs were soaked in the extract and also the essential oil separately, soaked disc were applied over the seeded nutrient ager plates at equidistance, and the control placed on their middle. Petri dishes were labeled inside the laminar flow according to the name of bacteria strain. The plates were incubated at 37°C for 24hours. After the incubation period, the inhibition zone diameter around the disc was measured using a transparent ruler, this indicates a positive antibacterial activity of the respective extracts and essential oils.

## MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC was determined according to Ochei and Kolhatker (2008), using microtubes dilution method. Twelve sterile test tubes were set up the 0.5ml diluents was introduced into the tubes with a micropipette starting from tube 2 to tube 12, then 0.5ml of the extracts and essential oils of both sample was added to tube 2 to tube 11, after which one drop each of the test organisms were added to each tube to tube 11. The microtitre plate were incubated at $37^0$C for 24hours

and observed for visible growth. The lowest concentration at which no detectable bacterial growth occurred was considered as minimum inhibitory concentrations (MIC).

## MINIMUM BACTERICIDAL CONCENTRATION (MBC)

MBC was determined as described by Ochi and Kolhatar (2008). Subculture was made from the last tubes showing no growth durin MIC determination on the nutrient agar. The plate was incubated at 37$^0$C for 24 hours the highest dilution that showed no growth was observed and noted as MBC.

## RESULTS

**Table 1: Antibacterial Sensitivity Pattern of Essential Oil and Holy Basil extracts on some Pathogenic Bacteria.**

Zone diameter (mm) / Concentration (mg/m)

| Organisms | Essential Oil | | | Ethanol | | | Aqueous | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 100 | 50 | 25 | 100 | 50 | 25 | |
| *Escherichia coli* | 15 | 11 | 8 | 11 | 9 | 6 | 7 | nil | nil | 18 |
| *Staphylococcus aureus* | 16 | 12 | 8 | 9 | 7 | 6 | 7 | 4 | nil | 18 |
| *Klebsiella sp.* | 11 | 9 | 6 | 9 | 8 | nil | 7 | 5 | nil | 19 |
| *Salmonella sp.* | 14 | 10 | 7 | 11 | 9 | 6 | 9 | 6 | nil | 24 |
| *Shigella sp.* | 11 | 8 | 6 | 8 | 6 | nil | 7 | 5 | nil | 20 |

* CONTROL: Ciprofloxacin

**Table 2: Antibacterial Sensitivity Pattern of Lemon Grass extract on some Pathogenic Bacteria**

Zone diameter (mm) / Concentration (mg/m)

| Organisms | Essential Oil | | | Ethanol | | | Aqueous | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 100 | 50 | 25 | 100 | 50 | 25 | |
| *Escherichia coli* | 12 | 10 | 6 | 11 | 8 | 6 | 7 | nil | nil | 18 |
| *Staphylococcus aureus* | 16 | 8 | 6 | 9 | 7 | 6 | 7 | 4 | nil | 18 |
| *Klebsiella sp.* | 12 | 9 | 6 | 9 | 6 | nil | 7 | 5 | nil | 19 |
| *Salmonella sp.* | 14 | 11 | 8 | 11 | 9 | 6 | 9 | 6 | nil | 24 |
| *Shigella sp.* | 10 | 7 | 6 | 8 | 6 | nil | 7 | 5 | nil | 20 |

* CONTROL: Ciprofloxacin

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Essential oil and holy basil extracts against some pathogenic bacteria

(mg/ml of extract)

| Organisms | Essential Oil | | Ethanol | | Aqueous | | Control |
|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC/MBC |
| *Escherichia coli* | 50 | 100 | 50 | 100 | 100 | 100 | 12.5/12.5 |
| *Staphylococcus aureus* | 50 | 50 | 50 | 50 | 100 | 100 | 12.2/12.5 |
| *Klebsiella sp.* | 50 | 100 | 50 | 100 | 100 | 100 | 6.25/12.5 |
| *Salmonella sp.* | 50 | 100 | 100 | 100 | 100 | 100 | 3.13/6.25 |
| *Shigella sp* | 50 | 100 | 100 | 100 | 100 | 100 | 6.25/6.25 |

* CONTROL: Ciprofloxacin

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Lemon Grass extracts against some Pathogenic Bacteria

(mg/ml of extract)

| Organisms | Essentail | | Ethanol | | Aqeuous | | Control |
|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC/MBC |
| *Escherichia coli* | 50 | 100 | 100 | 50 | 10 0 | 100 | 12.5/12.5 |
| *Staphylococcus aureus* | 50 | 50 | 50 | 50 | 100 | 100 | 12.2/12.5 |
| *Klebsiella sp.* | 50 | 100 | 50 | 100 | 100 | 100 | 6.25/12.5 |
| *Salmonella sp.* | 50 | 100 | 100 | 100 | 100 | 100 | 3.13/6.25 |
| *Shigella sp* | 50 | 100 | 50 | 100 | 100 | 100 | 6.25/6.25 |

* CONTROL: Ciprofloxacin

Table 5: PHYTOCHEMICAL COMPOSITION OF HOLY BASIL AND LEMON GRASS

| | HOLY BASIL | | | LEMON GRASS | | |
|---|---|---|---|---|---|---|
| Parameters | Essential oil | Ethanol | Aqueous | Essential oil | Ethanol | Aqueous |
| Alkaloids | +++ | + | + | ++ | + | + |
| Flavonoids | ++ | + | – | ++ | + | + |
| Saponins | +++ | + | + | ++ | + | + |
| Tannins | + | + | - | + | + | + |
| Steroids | ++ | ++ | + | ++ | + | + |
| Glycoside | + | + | - | + | + | + |
| Anthraquinone | + | + | + | + | + | - |

Keys: - Absent, + Present

## DISCUSSION

In vitro studies in this work showed that the ethanolic extracts and essential oils of the spices (lemon grass and holy basil) inhibited growth of bacteria but their effectiveness varied. The antibacterial activity of many plants extracts has been previously reviewed and classified as

strong, medium or weak (Bishnu*, et al,* 2009). The medicinal plant like holy basil and lemon grass are being used traditionally for the treatment of inflammation fever, antiseptics expectorant and typhoid, and the antibacterial activity has been attributed to the presence of some active constituents in the extracts. In this study, spices (lemon grass and holy basil) extracts and oils exhibited strong activity against the selected bacterial strains. Several studies (Bishnu, *et al*, 2004; Seenivasan, *et al*, 2006; Suree and Pana, 2005) have shown that essential oils of holy basil and lemon grass had strong and highly consistent inhibitory effects against human pathogens. This study is in line with the past research that the essential oil possess antibacterial activity than the ethanolic extracts, which might result from the presence of some active parameters in the extracts. Holy basil oils contain high concentration of alkaloids and saponins to lemon grass oil, which was moderately present as revealed in the result (table 5). Even though earlier studies have reported strong antibacterial activity of essential oil of holy basil on *Salmonella typhi* (Suree and Pana, 2005). This study showeld less inhibitory activity on salmonella and strong effect on *Staphylococcus areus*. Similarly, this result is in line with the previous findings (Seenivasan *et al*; 2006), that lemon grass oil and holy basin oil have equal effect against both gram positive and gram negative organism. This study also support the previous findings (Burt, 2004; Kodali, *et al,* 2004), that holy basil oil and lemon grass oils have been tested to possesses antibacterial, antifungal, antiviral insecticidal and anti oxidant properties through this study based on only on five human pathogenic organism in which staphylococcus *aureus* exhibited the most significant susceptibility pattern to both spices which is in line with previous. (Staples and Michael,1999) that the aqeous extract of holy basil showed growth inhibition on *Klebsiella*, E.*coli* and better activity against the β-*Lactamase* producing methicililn resistant *Staphyloccus aureus* organism (Staples and Michael, 1999) in which these oils may be adopt preferable to synthesize modified medicine or drugs in treating or curing disease caused by these pathogens. The fact that holy basil oil and lemon grass oils inhibit the growth and kills some organism has shown in this study and these reveal the active component of holy basil and lemon grass has bactericidal and bacteriostatic efficiency, thereby supporting the folk medicinal usage.

## CONCLUSION

It was concluded that essential oils of spices holy basil and lemon grass possess significant antibacterial activity than ethanolic extracts though active against some pathogen, the oils has the potential bactericidal properties. It is believed that this findings compared to previous studies provide support to the antibacterial properties of spices (lemon grass and holy basil) which can be of use in treatment of microbial infections by the communities.

## RECOMMENDATION

It is suggested that essential oils of these spices may be adopt in modification of effectives medicinal drugs.

## REFERENCES

Adamson M. W. (2004). Food in Medieval Times. Westport, Conn: Greenwood Press. ISBN 0-313-32147-7.

Aibinu I., Adenipekun T., Adelowokan T., Ogunsanya T. and Odugbemi T. (2007). Evaluation of the Antimicrobial Properties of Different Parts of *Citrus aurantifolia* (Lime Fruit) as Used Locally. Afr. J. Tradit. CAM. 2: 185 – 190.

Behboud J., Amirreza E., Babak M. A. and Zarifeh H. (2012). Antibacterial Activities of Lemon Grass Methanol Extract and Essence on Pathogenic Bacteria. American-Eurasian J. Agric. & Environ. Sci., 12 (8): 1042 – 1046.

Blanco M. M., Costa C. A., Freire A. O., Santos J. G. and Costa M. (2009). Neurobehavioral effect of Essential Oil of *Cymbopogon citratus* in mice. Phytomedicine 16 (2–3): 265 – 70.

Bleasel N., Tate B. and Rademaker M. (2002). Allergic Contact Dermatitis following Exposure to Essential Oils. Australas. J. Dermatol. 43 (3): 211–3.

Brian T. S. and Ikhlas A. K. (2002). Comparison of Extraction Methods for Marker Compounds in the Essential Oil of Lemongrass by GC. *Journal of Agric. Food Chem.* 50: 1345 – 1349.

Chatterjee G. (2001). Sacred Hindu Symbols. Abhinav Publications. 93. ISBN 978-81-7017-397-7.

Cheel J., Theoduloz C., Rodríguez J. and Schmeda-Hirschmann G. (2005). Free Radical Scavengers and Antioxidants from Lemongrass (*Cymbopogon citratus (DC.) Stapf.*). *Journal of Agric. Food Chem.* 53: 2511 – 2517.

Civitello L. (2007). Cuisine and Culture: A History of Food and People. John Wiley and Sons. ISBN 0-471-74172-8.

Claus P. J., Sarah D. and Margaret A. M. (2003). South Asian Folklore: An Encyclopedia. Taylor & Francis. 619. ISBN 978-0-415-93919-5.

Cohen M. L. (1992). Epidemiology of Drug Resistance, Implications for a Post Antimicrobial Era. Sci., 257, 1050 – 1055.

Cragg G. M., Newman D. J. and Sander K. M. (1997). Natural Products in Drug Discovery and Development. *J. Nat. Prod.* 60: 52 – 60.

Dahiru D., William E. T. and Nadroi M. S. (2005). Protective effects of *Ziziphus mauritiana* Leaf extract on Carbon Tetrachloride-Induced Liver Injury. *African Journal of Biotechnology,* 4(10):1177 – 1179.

Depken K. L. (2011). Properties of Lemon Grass. http://www.ehow.com/about_5382246_properties-lemongrass. html.

Devi P. U. and Ganasoundari A. (1999). Modulation of Glutathione and Antioxidant Enzymes by *Ocimum Sanctum* and Its Role in Protection against Radiation Injury. *Indian Journal of Experimental Biology* 37 (3): 262 – 268.

Ghani K. U., Saeed A. and Alam A. T. (1997). Indusyonic Medicine; Traditional Medicine of Herbal, Animal and Mineral Origin in Pakistan. University of Karachi.

Golshahi H., Ghasemi E. and Mehranzade E. (2011). Antibacterial activity of *Ocimum Sanctum* extract against *E. coli, S. aureus and P. aeruginosa*. Clinical Biochemistry. Conference: 12th Iranian Congress of Biochemistry, ICB and 4th International Congress of Biochemistry and Molecular Biology 44 (13 SUPPL. 1): 352.

Gupta S. S. (1994). Prospects and Perspectives on Natural Plant Products in Medicine. Indian *Journal of Pharmacology*. 26:1 – 2.

Hammer K. A., Carson C. F. and Riley T. V. (1999). Antimicrobial activity of essential Oils and other Plant Extracts. J. Appl. Microbiol., 86: 985 – 990.

Huynh K. P., Maridable J., Gaspillo P., Hasika M., Malaluan R. and Kawasaki J. (2008). Essential oil from Lemongrass Extracted by Supercritical Carbon Dioxide and Steam Distillation. *The Phillippine Agric. Sci.* 91: 36 – 41.

Iwu M. M., Duncan A. R. and Okunji C. O. (1999). New Antimicrobials of Plant Origin. In: Janick J., ed. Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA, 457 – 462.

Jae-Young C., Dereje D., Seung-Jin L., Jong-Choon K. and Seung-Chun P. (2012). Antimicrobial activity of Lemongrass and Oregano Essential Oil against Standard Antibiotic Resistant Staphylococcus Aureus and Field Isolates from Chronic Mastitis Cow. *International Journal of Phytomedicine*. 4 (1): 12 – 17.

Lawless J. (1995). Illustrated Encyclopedia of Essential Oil: The Complete Guide to Use of Oil in Aromatheraphy and Herbalism. Element Books: Rockport, MA. 56 – 67.

Leite J. R., Seabra M. L., Maluf E. (1986). Pharmacology of lemongrass (*Cymbopogon citratus Stapf*). III. Assessment of Eventual Toxic, Hypnotic and anxiolytic effects on humans. *J Ethnopharmacol* 17 (1): 75 – 83.

Mondal S., Varma S., Bamola V. D., Naik S. N., Mirdha, B. R., Padhi M. M., Mehta N. and Mahapatra S. C. (2011). Double-Blinded Randomized Controlled Trial for Immunomodulatory effects of Tulsi (*Ocimum sanctum Linn.*) Leaf extract on Healthy Volunteers. *Journal of Ethnopharmacology* 136 (3): 452 – 456.

Murray M. (1995). The Healing Power of Herbs. Prima Publishing, Rocklin, CA. 162 – 171.

Nanasombat S. and Lohasupthawee P. (2005). Antibacterial Activity of Crude Ethanolic extracts and Essential Oils of Spices against *Salmonellae* and Other Enterobacteria. KMITL *Sci. Tech. J.,* 5(3): 527 – 538.

National Institute of Industrial Research (NIIR) Board (2004). Compendium of Medicinal Plants. 2004. National Institute of Industrial Research, India. 320. ISBN 978-81-86623-80-0.

Nostro A., Germano M. P., D'Angelo V., Marino A. and Cannattelli M. A. 2000. Extraction Methods and Bioautography for Evaluation of Medicinal Plants Antimicrobial Activity. Letters in Applied Microbiology, 30: 379 – 384.

Padalia R. C. and Verma R. S. (2011). Comparative Volatile Oil Composition of Four *Ocimum* Species from Northern INDIA. Natural Product Research 25 (6): 569 – 575.

Pearson O. (2010). The Antibacterial Properties of Essential Oils. http://www.livestrong.com/article/168697-the-antibacterialproperties- of-essential-oils/# ixzz1W7u9hn6K.

Peng X., Zhao Y., Liang X., Wu L., Cui S., Guo A. and Wang W. (2006). Assessing the Quality of RCTs on the effect of Beta-Elemene, One Ingredient of a Chinese herb, against Malignant Tumors. Contemporary clinical trials 27 (1): 70 – 82.

Prakash P. and Gupta N. (2005). Therapeutic Uses Of *Ocimum Sanctum Linn* (Tulasi) With A Note On Eugenol And Its Pharmacological Actions: A Short Review. *Indian Journal of Physiology and Pharmacology* 49 (2): 125 – 131.

Qingdi Q. L., Gangduo W., Manchao Z., Christopher F. C., Lan H. and Eddie R. (2009). β-Elemene, A Novel Plant-Derived Antineoplastic Agent, Increases Cisplatin Chemosensitivity of Lung Tumor Cells by Triggering Apoptosis. Oncology Reports 22: 161 – 170.

Rai V., Mani U. V. and Iyer U. M. (1997). Effect of *Ocimum sanctum* Leaf Powder on Blood Lipoproteins, Glycated Proteins and Total Amino Acids in Patients with Non-insulin-Dependent Diabetes Mellitus. *Journal of Nutritional and Environmental Medicine* 7 (2): 113 – 118.

RHS (2008) A-Z Encyclopedia of Garden Plants. United Kingdom: Dorling Kindersley. 1136. ISBN 1405332964.

Russell A. D. (2002). Antibiotic and Biocide Resistance in Bacteria: Introduction. *J. Appl. Microbiol. Symposium Supplement,* 92: 1S – 3S.

Saify Z. S., Mustaq N., Noor F., Naqvi S. B. S. and Mardi S. A. (2000). Antimicrobial activity of some Commonly Used Herbs. Pakistan J. Pharm. Sci. 13: 1 – 3.

Scully T. (1995). The Art of Cookery in the Middle Ages. Ipswich: Boydell Press. ISBN 0-85115-611-8.

Sethi J., Sood S., Seth S. and Talwar A. (2004). Evaluation of Hypoglycemic and Antioxidant effect of *Ocimum sanctum. Indian Journal of Clinical Biochemistry* 19 (2): 152 – 155.

Shadab Q., Hanif M. and Chaudhary F. M. (1992) Antifungal activity by Lemongrass Essential Oils. *Pak. J. Sci. Ind. Res.* 35: 246 – 249.

Sharma P., Kulshreshtha S. and Sharma A. L. (1998). Anti-cataract activity of *Ocimum sanctum* on experimental cataract. *Indian Journal of Pharmacology* 30 (1): 16 – 20.

Staples G. and Michael S. K. (1999). Ethnic Culinary Herbs. University of Hawaii Press. 73. ISBN 978-0-8248-2094-7.

Suanarunsawat T., Boonnak T., Na Ayutthaya W. D. and Thirawarapan S. (2010). Anti-hyperlipidemic and cardioprotective effects of *Ocimum sanctum L.* fixed oil in rats fed a High Fat Diet. *Journal of Basic and Clinical Physiology and Pharmacology* 21 (4): 387 – 400.

Takeguma M. (2013). Gowing Citronella. Retrieved 12 June 2013 from http://www.livestrong.com/article/168697-the-antibacterialproperties- of-essential-oils/# ixzz1W7u9hn6K.

Ushimaru P. I., Mariama T. N., Luiz C., Di Luciano B. and Ary F. J. (2007). Antibacterial Activity of Medicinal Plant extract. Braz. *J. Microbial.* 38: 717 – 719.

Warrier P. K. (1995). Indian Medicinal Plants. Orient Longman. 168. ISBN 0-86311-551-9.

Wells P. J. (2007). Chinese Herbal Medicine. Retrieved 2013-06-19.

World Health Organisation (WHO) (1993). Regional Office for Western Pacific, Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. Manila. 2.

**Reference** to this paper should be made as follows: Afuape A.O., Osifeso O.O., and Adewunmi A.R (2015), Antibacterial activity of Ethanolic extracts and Essential Oil of Spices (Lemon Grass and Holy Basil) on Selected Human Pathogens. *J. of Sciences and Multidisciplinary Research,* Vol. 7, No. 1, Pp. 62 – 72.