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**ORIGINAL ARTICLE**

# Antimicrobial Effects of Spices on Spoilage Organisms of Moin-Moin

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**ABSTRACT**
*There is a renewed interest in the antimicrobial properties of spices. This study investigates the antimicrobial effects of spices on spoilage organisms of moin-moin - a traditional West African dish based on black-eyed peas or other beans. Serially diluted (up to $10^{-8}$) samples of spoilt moin-moin were plated on Mueller-Hinton Agar (MHA). Antimicrobial activities at the concentration of 0.4 g/ml each of methanolic and water extracts of Garlic (Allium sativum), Cinnamon (Cinnamommum verum), Nutmeg (Myristica fragrams) and Ginger (Zingiber offinale) on the isolated organisms were assessed using the cup plate diffusion method. These were compared with the activities of a standard, Ciprofloxacin. A total of seven (7) genera of bacteria - Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Enterobacter aerogenes and Proteus mirabilis - were implicated in the spoilt samples of the Moin-moin. Results showed that was a better extraction solvent. Phytochemical screening carried out on these spices revealed the presence of different biologically active ingredients such as tannin, saponin, alkaloid, phenol and phytate. Resultant inhibition zones displayed in plates were significantly different ($P< 0.05$) based on types of extraction solvent and extracts' concentration. This study confirms the efficacy of these spices as natural antimicrobials and suggests the possibility of employing them in the preservation of food products where spoilage is caused mainly by microbial activity.*
***Keywords:*** *Spoilage, spices, antimicrobial, foods, antibiotics.*

## INTRODUCTION

Spoilage is a metabolic process that causes food to be undesirable or unacceptable for human consumption due to changes in sensory and nutritional characteristics [1]. Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods [2]. Spices are often used in perfumes and cosmetics and many have been used in medicine and religious ritual as well. Spices are distinguished from other plant products used for similar purposes, such as herbs which are green leafy parts of plants aromatic vegetables and dried fruits [3]. Pungent spices can cause sweating, which may cause a cooling sensation in tropical climates, on the other hand they can add a sense of inner warmth when present in cooked foods used in cold climates. Spices also fitted into philosophic concepts of improving health, since it was understood that they could affect the four humours (blood, phlegm, yellow blue and black bile) and influence the corresponding moods sanguine, phlegmatic, choleric and melancholic [4].

Garlic (*Allium sativum*) has been used in all part of the world not only as spice or food but also for treatment of diseases. It is a vegetable member of the onion family which consist of a member of segment of cloves, each surrounded by a paper skin, joined together to form a spherical bulb [3]. Garlic contains several active substances, including sulphur compounds which are responsible for the pungent, aromatic odour. It has antiviral and antibacterial properties and recent congestion, bronchitis and cold symptoms and protect against re-infection. Garlic has traditional and medicinal applications as an anti-infective agent [5]. Evidence of the antimicrobial activity of fresh and freeze dried garlic extracts against many bacteria was established by Rees *et al*. [6]. Cinnamon is native to India and Sri Lanka (Ceylon). It is now cultivated in many tropical countries including Nigeria. This plant has been used in Ayurvedic (Indian traditional medicine) and other medicinal traditions in Africa. In the American continent, most of the original uses are still prevalent; mainly as a treatment for diarrhea, stomach upset, against respiratory ailments and externally as a skin antiseptic and rubefacient. Nutmeg (*Myristica fragrams*) seed is enclosed in a mottled yellow, edible fruit, the approximate size and shape of a small peach. Nutmeg is usually associated with sweet, spicy dishes-pies, puddings, custards, cookies and spice cakes. It compliments egg dishes and vegetables like cabbage, spinach, broccoli, beans onions and egg plant [7]. Ginger (*Zingiber*

*offinale*) is a strong antioxidant with antimicrobial properties used to treat sores and wounds. It was one of the earlier spices known in Western Europe. It is used in cooking either as silvers of fresh root or dried and ground as a reddish-coloured spices. It contains several active substances and volatile aromatic oil [3]. Food safety depends on their adequate manipulation, transportation and storage. Foods are not sterile, in the sense that they normally contain germs (bacteria, viruses, yeast and molds), some of which can lead to food intoxication and infections when present above the acceptable levels [8]. Hygiene conditions are poor when foods are produced in non-industrial establishments, mainly due to lack of required equipment for adequate processing. Those susceptible to food infections and or intoxications are children, elderly and immunesupressed individuals [9]. While food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with street-vended foods [10]. Moin Moin, also called and/or written as *moimoi*, *moyin moyin*, is a traditional West African dish based on black-eyed peas or other beans. It is usually conical or cylindrical in shape, based on the shape of the mould used to make it. Scientific studies have documented the antimicrobial properties of some spices, herbs and their components [11-13]. Other studies have reported that spices and herbs themselves may be highly exposed to bacterial contamination, based on conditions in which they were prepared. Moreso, contaminated spices have been reported to cause foodborne illness and spoilage [14]. In the global food industry today, 'natural' is a powerful force as there is increasing resistance at regulatory and consumer levels against chemical food preservatives [15]. Numerous naturally occurring antimicrobials are present in animal and plant tissues and many studies have evaluated the antimicrobial activities of several plant extracts, including *Sesamum radiatum* [16], *Allium cepa* [15], olives [15]*;* Chardonnay grapes and black raspberries [17] and orange essential oils [16]. The purpose of this study was to investigate the antimicrobial effects of some spices on bacteria associated with the spoilage of street-vended moin-moin.

## MATERIALS AND METHODS

### Collection of samples and sampling procedure

Samples of the street-vended food (moin-moin) were collected from six different locations in Ago-Iwoye, South-western Nigeria (Nigeria is located in the tropical zone of West Africa between latitudes 4°N and 14°N). From each of the sites, moin-moin samples were purchased and transported to the laboratory in sterile bags packed in insulated containers. These were left for five days to allow spoilage microorganisms to act on the food samples. The ginger, garlic, cinnamon and nutmeg were collected from local markets in Ago-Iwoye, Ogun State, South-western Nigeria. These were peeled, sliced and blended into an airtight container.

### Microbiological analyses

Ten grams of each sample for microbiological evaluation were aseptically transferred into 90 ml of 0.1% sterile peptone water, shaken thoroughly and appropriate dilutions (up to $10^{-8}$) prepared for microbiological studies (18Harrigan and McCance, 1976). 1 mL of this dilution was inoculated onto sterile Mueller-Hinton Agar (MHA). The plates were then incubated for 23 h at 37$^{o}$ C. Pure cultures were obtained by re-streaking isolates into fresh medium using standard methods. Total viable counts (aerobic mesophiles) were made on Plate Count Agar (PCA, Oxoid, U.K.) and plates incubated at 37$^{o}$C for 24 hrs. Pure strains were preserved at -20$^{o}$ C. The isolates were screened based on the size, colony aspect ratio, color, Gram's staining reactions, oxidase and catalase reactions. Identification and characterization were carried out using the API 20 NE Micromethods (Bio-Merieux, Lyon, France).

### Preparation of spice's extracts

The ginger, garlic, cinnamon and nutmeg were collected from local market in Ago-Iwoye, Ogun State, South-Western Nigeria. 300g each of the spices was blended into fine powder and soaked in 150 mls of distilled water and 75% methanol (methanolic extract) for 24hrs. The slurry obtained was left in clean, sterile glass container and shaken vigorously to allow for proper extraction. The slurry was filtered using a sterile muslin cloth after which the extract obtained was air dried and stored at 4$^{o}$C until required.

### Antimicrobial screening tests

The antimicrobial activity was carried out using the agar-well diffusion method of Lyudmila et al. [19]. Inocula of the bacteria culture were prepared from 18 h broth culture. A loopful of these

young actively growing culture was diluted in 0.9% NaCl to obtain a density comparable to 0.5 of McFarland standard turbidity scale corresponding to about 1.5 x $10^8$ colony forming unit (CFU) per ml. The bacteria suspensions were further adjusted and made to standard by adjusting the optical density to 0.1at 600 nm (Jenway 6105 UV/Vis spectrophotometer, 50 Hz/60 Hz) corresponding to about $10^6$ CFU/ml of the bacteria isolate. One milliliter of the bacteria suspension was spread over the surface of the agar medium [Mueller Hinton Agar (MHA)] Seeding was allowed to take place for 10 minutes. A sterile 6 mm cork borer was used to make wells in the MHA medium, the base of each well was sealed with 10 µl of the moulting MHA medium using sterile syringe. These wells were then loaded with 0.4 g/ml of the crude plant extract with three replicates. 0.5ml of each concentration was introduced into each hole on the medium and allowed to stand on the bench for one hour for proper diffusion, and thereafter incubated at $37^0$C for 24 hrs. The resulting inhibition zones were measured in millimeters and recorded against the corresponding concentration.

**Phytochemical analysis of samples extracts**

The preliminary phytochemical analysis was carried out employing the methods of Culer [20], Sofowora [21], Odebiyi and Sofowora [22] and Trease and Evans [23]. The extracts were screened for the presence of biologically active constituents such as tannins, saponins, alkaloids, betacyanins, phytate and total phenol.

**Statistical analysis**

Data obtained in the study were statistically analyzed using Analysis of Variance (ANOVA). The means were separated using Fisher's Least Significant Difference (LSD).

## RESULTS AND DISCUSSION

Figure 1 shows the percentage frequency of bacterial isolates in the spoilt moin-moin samples. *Staphylococcus aureus* had the highest frequency of 21.8% while *Escherichia coli* had the least frequency of 7.2%. *E. coli* and *Proteus mirabilis* exhibited the highest level of resistance as indicated by their lack of susceptibility to Ciprofloxacin while *Klebsiella pneumonia*, *Pseudomonas aeruginosa , Bacillu cereus, Enterobacter* aerogenes and *Proteus mirabilis* were all susceptible to Ciprofloxacin that was used as positive control.

**Table 1:** Antimicrobial effects of water extracts of spices on bacterial isolates from spoilt moin-moin.

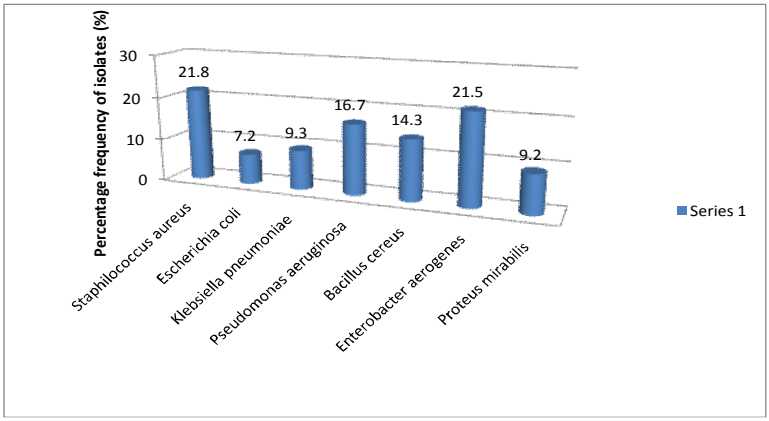
| Organisms | [Diameter of zones of inhibitions of different concentrations of spices (mg/ml) ± SD] in millimeters | | | | | |
|---|---|---|---|---|---|---|
| | **Garlic *(Allium sativum)*** | **Cinnamon *(Cinnamommum verum)*** | **Nutmeg *(Myristica fragrams)*** | **Ginger *(Zingiber officinale)*** | **Water** | **Ciprofloxacin** |
| *Staphylococcus aureus* | 22.0 ± 0.6[a] | 21.5 ± 1.0[a] | 17.0 ± 0.6[a] | 21.5 ± 0.8[a] | 6.0 ± 0.0[a] | 28.5 ± 0.8[a] |
| *Escherichia coli,* | 23.5 ± 0.8[a] | 16.8 ± 0.7[b] | 15.9 ± 1.0[b] | 21.0 ± 0.8[a] | 6.0 ± 0.0[a] | - |
| *Klebsiella pneumoniae* | 18.0 ± 0.9[b] | 18.0 ± 0.7[b] | 17.0 ± 0.6[a] | 21.5 ± 0.9[a] | 6.0 ± 0.0[a] | 19.0 ± 0.6[c] |
| *Pseudomonas aeruginosa*. | 21.0 ± 0.8[a] | 17.0 ± 0.8[b] | 19.0 ± 1.0[a] | 14.0 ± 0.7[c] | 6.0 ± 0.0[a] | 24.0 ± 0.5[b] |
| *Bacillus cereus* | 15.5 ± 0.8[c] | 13.5 ± 0.7[c] | 15.9 ± 0.8[b] | 23.5 ± 0.9[a] | 6.0 ± 0.0[a] | 22.0 ± 0.6[b] |
| *Enterobacter aerogenes* | 17.0 ± 0.9[b] | 14.0 ± 0.7[c] | 9.5 ± 0.7[c] | 18.0 ±0.9[b] | 6.0 ± 0.0[a] | 15.0 ± 0.4[d] |
| *Proteus mirabilis* | 17.0 ± 0.8[b] | 16.7 ± 0.9[b] | 14.8 ± 0.4[b] | 13.5 ± 0.9[c] | 6.0 ± 0.0[a] | - |

No Antimicrobial activity (NA)

Values followed by the same letter under the same line are not significantly different (Duncam's multiple range test at P > 0.05). Values represent mean ± standard deviation of experiment in duplicate

This study implicated a total of seven (7) genera of bacteria - *Staphylococcus aureus, Escherichia coli, Klebsiella* sp, *Pseudomonas aeruginosa, Bacillus* creus,, *Enterobacter* aerogenes, and *Proteus* mirabilis - in the spoilt samples of the food product (Figure 1). These organisms were found to occur in varying degrees and it, thus, became necessary to determine the percentage occurrence of each isolate. The most prevalent organism was *Staphylococcus aureus* with percentage frequency of

21.8%. This was followed by *Entobacter aerogenes* (21.5%), *Pseudomonas aeroginosa* (16.7%), *Bacillus cereus* (14.3%), klebsiella pneumonia (9.3%), *Proteus* mirabilis (9.2%) and Escherichia coli (7.2%). All organisms showed reasonable susceptibilities to the water extracts of the spices at the different levels of concentrations. The methanolic extracts of these spices showed stronger antimicrobial effects than water extract as indicated by the exhibition of larger zones of inhibition at virtually all concentrations of the spices (Table 2).




Bacteria isolates

**Figure 1:** Prevalence of bacteria genera in spices moin-moin

**Table 2:** Antimicrobial effects of methanolic extracts of spices on bacterial isolates from spoilt moin-moin.

| Organisms | [Diameter of zones of inhibitions of different concentrations of spices (mg/ml) ± SD] in millimeters | | | | | |
|---|---|---|---|---|---|---|
| | **Garlic *(Allium sativum)*** | **Cinnamon *(Cinnamommum verum)*** | **Nutmeg *(Myristica fragrams)*** | **Ginger *(Zingiber officinale)*** | **Methanol** | **Ciprofloxacin** |
| *Staphylococcus aureus* | 26.7± 1.0[a] | 23.0 ± 1.0[a] | 23.9 ± 1.0[a] | 22.0 ± 1.0[b] | 6.0 ± 0.0[a] | 27.5 ± 0.6[a] |
| *Escherichia coli,* | 20.5± 0.9[b] | 17.8 ± 1.0[c] | 22.5 ± 1.0[b] | 21.0 ± 1.0[b] | 6.0 ± 0.0[a] | - |
| *Klebsiella pneumoniae* | 21.5±0.8[b] | 22.0 ± 0.7[b] | 17.5 ± 0.4[d] | 24.5 ± 0.7[a] | 6.0 ± 0.0[a] | 20.0 ± 0.3[b] |
| *Pseudomonas aeruginosa*. | 22.5±1.0[b] | 21.0 ± 1.0[b] | 23.6 ± 1.0[a] | 27.0 ± 1.0[a] | 6.0 ± 0.0[a] | 25.0 ± 0.8[a] |
| *Bacillus cereus* | 25.5± 0.9[a] | 24.5 ± 1.0[a] | 25.9 ± 1.0[a] | 17.5 ± 0.7[c] | 6.0 ± 0.0[a] | 21.0 ± 0.4[b] |
| *Enterobacter aerogenes* | 22.0±1.0[b] | 22.0 ± 1.0[b] | 23.3 ± 1.1[a] | 26.0 ± 1.0[a] | 6.0 ± 0.0[a] | 17.0 ± 0.6[c] |
| *Proteus mirabilis* | 21.0±1.2[b] | 25.7 ± 1.2[a] | 20.5 ± 1.3[c] | 27.5 ± 1.0[a] | 6.0 ± 0.0[a] | - |

No Antimicrobial activity (NA)

Values followed by the same letter under the same line are not significantly different (Duncam's multiple range test at P > 0.05). Values represent mean ± standard deviation of experiment in duplicate

Results suggest methanolic extract as a better extracting solvent. The susceptibility or resistance patterns of these isolates were compared with a selected broad-spectrum conventional antibiotic, Ciprofloxacin, the results obtained were interesting. Both water and methanolic extract of the spices were found to be active against *E. coli* and *Proteus mirabilis* which proved be resistant to the antibiotic which served as the positive control.

**Table 3:** Phytochemical screening of spices

| Spices | Biologically Active Ingredients (%) | | | | |
|---|---|---|---|---|---|
| | **Tannin** | **Saponin** | **Alkaloid** | **Total phenol** | **Phytate** |
| Garlic | 40.00 | $4.87 \times 10^{-5}$ | 26.60 | 0.68 | 124.95 |
| Cinnamon | 38.40 | $4.62 \times 10^{-5}$ | 27.27 | 0.75 | 107.10 |
| Nut-Meg | 18.46 | $1.76 \times 10^{-5}$ | 23.28 | 0.50 | 47.60 |
| Ginger | 42.31 | $5.04 \times 10^{-5}$ | 28.60 | 0.78 | 148.75 |

It can be deduced in this study that the activity of both water and methanolic extracts of the spices were comparable to the activities of the standard antibiotic used in this study. Many factors could be responsible for this. For instance, it could be attributed to the difference in the concentrations of the antibiotics from that of the crude extracts of spices and also the efficiency of the extracting solvent in the total extraction of the active ingredients from the spices can be in doubt. Resultant inhibition zones displayed in plates were significantly different ($P< 0.05$) based on types of extracting solvent and extracts' concentration. Manandhar [24] observed the enhanced effect of garlic powder compared with other spices against *Listeria moncytogens*. The results obtained in this study further proved that the gram-positive bacteria are more susceptible to the methanolic extracts of the spices than the gram-negative bacteria (table 2), this is in agreement with the assertion of Burt [25] who stated that plant extracts are generally more active against gram-positive bacteria than gram-negative ones. It has been variously suggested by many authors that the outer membrane of surrounding the cell wall of gram-negative bacteria may hinder the diffusion of hydrophobic compounds through its lipopolysaccharide covering [26]. As could be observed from table 2, *Bacillus cereus* and *Staphylococcus aureus*, the only two Gram-negative bacteria in this study were more succeptible to the methanolic extract than the remaining bacteria used, which are gram-negative, in this study.

Nolan *et al.* [27] confirmed the sensitivity of certain food-borne pathogens to Allicin, which is a major component of garlic extracts. He used a chemically-synthesized and purified Allicin and confirmed the inhibitory property of Allicin against *Pseudomonas aeruginosa, Staphylococcus aureus, Clostridium perfringes, Escherichia coli, Salmonella typhimurim* and *Shigella dysenteriae.*

Phytochemical screening carried out on these spices revealed the presence of different biologically active ingredients such as tannin, saponin, alkaloid, phenol and phytate. Garlic contains 40% tanin, $4.87 \times 10^{-5}$ saponin, 26.60% alkaloid, 0.68% total phenol and 124.95% phytate. Cinnamon contains 38.405 tannin, $4.62 \times 10^{-5}$ saponin, 27.27% alkaloid, 0.75% total phenol and 107.1% phenol and 107% phytate. Nutmeg contains 18.40% tannin, $1.76 \times 10^{-5}$ saponin, alkaloid 23.28%, total phenol 0.50% and phytate 47.6%. Ginger contains 42.31% tannin $5.04 \times 10^{-5}$0% saponin 28.60% alkaloid, 0.78 total phenols and 148.75% phytate (Table 3). Plant antimicrobial containing phenolic compounds such as eugenol (clove, cinnamon leaf), cinnamic acid (chilli, cinnamon bark), carvacrol (oregano) or thymol (thyme) has been described to show higher antibacterial properties than some that has none [25, 28].

Generally, these active ingredients must have contributed to the inhibitory properties observed. Wiston (28001) further stressed that onions and cinnamons are rich sources of saponin and flavonoid substances which are known to provide protection against cardiovascular diseases. He also reported their uses in the treatment of poor appetite, coughs, colds, asthma, bronchitis and prevention of antherosclerosis. Benkeblia [10] described garlic as a "novel" means of extending the safety and quality of food. The sensitivity of the bacterial isolates to the standard antibiotic was different from that obtained for the spices. However, results obtained from the spices are encouraging when compared to those obtained of the standard used. In conclusion, the use of these spices in moin-moin preparation is highly desirable not only for their flavouring attributes and enhancement of its general organoleptic acceptability, but also for their possible health benefits and preservation stance in the keeping quality of the Moin-moin. However the addition of some of these spices has to be studied as it might bring in additional taste which might not be acceptable to consumers. Also there might be need to carry out studies on the color, solidity and firmness of the moin moin when the spices are added as preservative. A particular colour and firmness is

attributable to moin moin and consumers may reject this if the firmness is not maintained. It is expected that the next line of research will be able to address this.

This study confirms the efficacy of these spices as natural antimicrobials and suggests the possibility of employing them in the preservation of moin moin especially where spoilage is caused by the observed microbial activity.

## REFERENCES


1. Doyle EM (2007) Microbial Food spoilage- Losses and Control Strategies. Food Research Institute, University of Wisconsin – Madison, WI 53706.
2. Geneva (1982) Spices - A Survey of the World Market, vol.1: 68 – 712
3. Katherine W (2002). Healing Foods. Geddes and Cross Scotland.
4. Jennifer B and Paul NS (1998) Antimicrobial functions of Spices. Why some like it hot "*Quarterly review of Biology*" Vol.73, No. 1.
5. Lawson LD (1998) *Garlic:* a review of its medicinal effects and indicated active compounds.
6. Rees LP, Minney SF, Plummer NT, Slater JH, Skyrme DA (1993) A quantitative assessment of the antimicrobial activity of garlic. (*Allium sativum). World Journal. Microbial Biotechnol*, 303-307.
7. John L (1987) The Herb Book (Bantam Books, New York, U.S.A).
8. Frazier WC and Westhoff DC (1978) Food Infections And Intoxications From Microbiological Origin. In: Food Microbiology, 3rd Edn., Zaragoza Editorial Acribia S.A .(In Spanish).
9. Stagnitta PV, Micalizzi V, Stefanini de Guzman AM (2006) Prevalence of some bacteria, yeast, and molds in meat foods in san luis, Argentina. Cent. *European Journal Public Health*, 14(3): 141-144
10. Benkeblia N (2004) Antimicrobial activityof essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensm.- Wiss. U.-Technol.,* 37: 263–268.
11. Zaika LL (1980) Spices and herbs: their antimicrobial activity and its determination. *Journal of Food Safety*, 9: 97-118.
12. Mei-Chin Y, Wen-Shen C (2002) Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. *Meat* Science, 63: 23-28.
13. Sara B (2004) Essential oils: Their antibacterial properties and potential applications in foods- A Review. *International Journal of Food Microbiology*, 94: 223-253.
14. Perfumi J (1986) Assessment of microbiological quality of spices and herbs. *Journal of Food Protection*. 49: 958-963.
15. Agatemor C (2008) Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *Internet Journal of Food Safety*. 10: 77 – 80.
16. Singh HS, Malti M (2009) Postmortal changes in *Clarias gariepinus. Asian Journal of Experimental Science, 23, 1: 199-205.*
17. Harrigan WF and McCance, M.E. (1976). Laboratory methods in food and diary microbiology. Academic Press, London, U.K. p.
18. Lyudmila B, Sirigan D, Radka K, Nikolai K, Galina G, Ivan M, Rossen N, Zacharii K, (2003) Inhibition of *Helicobacter pylori* growth in vitro by Bulgarian propolis. Preliminary report. *Journal of Medical Microbiology* 52, 417–419.
19. Culer I (1982) Methodology for the analysis of vegetable drugs. Practical Manual on the Industrial Utilisation of Medicinal and Aromatic Plants Center Building, Romania.
20. Sofowora A (1993) Medicinal Plants and Traditional Medicine in Africa. 2nd Edn. Spectrum Books Limited, Ibadan, Nigeria. Pp. 1-153.
21. Odebiyi OO, Sofowora EA (1998) Antimicrobial alkaloids from Nigerian chewing stick (*Fagara zanthoxyloides). Plant medica.* 36: 204-210.
22. Trease GE, Evans WC (2002) Pharmacognosy. 15th Edn. Saunders. Pp 214-393.
23. Manandhar NP (2002) Plants and People of Nepal Timber Press. Oregon. ISBN 0-88192-527-6 004 *Asian Journal of Science and Technology,* Vol. 2, Issue, 3, pp.001-005, March, 2011.
24. Burt S (2004) Essential oils: their antibacterial properties and potential applica-tions in foodsda review. *International . Journal of Food Microbiol.* 94 (3), 223–253
25. Davidson PM Branen AL 2005 Food antimicrobials – an introduction. In:Davidson, P.M., Sofos, J.N., Branen, A.L. (Eds.), Antimicrobial in Food. CRC Press, pp. Taylor and Francis Group ed
26. Nolan LJ, Chore LJ, Christopher MCJ, Labbe DI, Ronald G (1996) Acta, hortic. (International Symposium on medicinal and aromatic Plant); 277-285.
27. Holley RA, Patel D 2005 Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials*. Food Microbiology*. 22, 273–292.
28. Cowan ST and Steel KJ (1985) Manual for the identification of bacteria. Cambridge University Press, Verlage, New York.
29. Giese J (1994) Spices and Seasoning blends. A taste for all seasons. *Food Technology*. 48 (4): 87-98.
30. Holt JG Krieg NR Smeath, P.H.A., Staley, J.T. and Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology, (9th edn). Williams and Williams Company, Baltimore, p. 783.
31. Okeke IN, Fayinka ST, Lamikanra A (2000) Antibiotic resistance in *Escherichia coli* from Nigerian students, 1986-98. *J. Em. Infect. Dis*., 6: 4.
32. Sneath PHA, Mair NS, Sharpe ME, Holt JG (1986) Bergey's Manual of Systematic Bacteriology Vol. 2. Williams and Wilkins Co. Baltimore.
33. Wiston FC (2001) Onions a global health benefit. A Professor of Nutrition at Andrews University, Berrien Springs, Michigan.