Antibacterial Activities of Ethanol and Aqueous Extracts of Five Nigerian Medicinal Plants on Some Wound Pathogens

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Abstract: The present study was undertaken to investigate the antibacterial activity of five medicinal plants used by traditional healers in Nigeria against wound pathogens. The antibacterial activity of aqueous and ethanolic extracts of *A. conyzoides* (Goat Weed); *A. indica* (Nee tree); *C. aurantifolia* (Lime fruit), *V. amygdalina* (Bitter leaf) and *F. exasperate* (Sandpaper tree) were determined against wound pathogens isolated from our study using disc diffusion method. The prevalence of the isolated wound pathogens were *Escherichia coli* (4%), *Proteus mirabilis* (9%), *Pseudomonas aeruginosa* (11%), *Klebsiella pneumoniae (*13%) and *Staphylococcus aureus (*63%). All the extracts (both aqueous and ethanol extracts) showed marked antibacterial activity but to varied zones of inhibition. The antibacterial activity of the extracts of *C. aurantifolia* (Lime fruit) was found to be apparently higher than other plant extracts ($p<0.05$).When the antibacterial activity of each of the plant extracts were compared for both ethanol and aqueous, no significant difference was noticed to exist in their activity ($p>0.05$).Our study therefore showed that crude extracts of the selected plant species could serve as a possible candidate for drug development.
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1. Introduction

Microorganisms are the causative agent of wound infections, which is an important cause of morbidity in surgical patients (Orrett, 2002).The widespread use of antibiotics has resulted in increased bacterial resistance to existing drugs, a phenomenon which threatens public health (Kavase *et al*., 2001). The emergence and dissemination of antimicrobial resistance in bacteria has been well documented as a serious problem world-wide (Cohen, 2000; Tenover, 2001; Orrett, 2002; Hsueh *et al*., 2002 and Akinyemi *et al*., 2005). Antimicrobial resistance results in increased illnesses, high cost of health maintenance and deaths (Cheley, 1998). As a result, there is need for the discovery of new antimicrobial compounds, probably acting through mechanisms different from those of existing drugs (Neu, 1989; Niccoli *et al*., 2001). Hence, the need to search for new antimicrobial agents from natural products of plants to combat the problems associated with drug resistant strains of microorganisms (Nickel, 1995). Tenover,(2001) described the new epidemic of multi-drug resistance as an emergent pathogen resulting from our own mismanagement of antibiotics. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius *et al*., 2003; Moreillion *et al*., 2005).

Herbal medicine is readily available in our diverse vegetation, cheap, and carries the potential of introducing new templates into modern medicine (Okwori *et al*., 2007). Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen substituted derivatives such as tannins. In many cases, these substances (particularly the alkaloids) serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Many of the herbs used by humans to season food are spices with useful medicinal compounds (Lai, 2004; Tapsell, 2006). As part of the search for new chemotherapeutics from natural products, this study investigated the antibacterial activities of five medicinal plants against isolates of wound infections.

2. Materials and Methods

2.1.Isolation of wound Pathogens

Twenty (20) wound samples each were collected from five (5) hospitals (Two teaching hospitals, two general hospitals and one private hospital) located in Ijebu – North Local Government Area of Ogun State, Nigeria. The samples were collected with the help of experienced Senior Nursing

Staff using sterile swab sticks. They were collected early in the morning from different parts of the wound of the patients. The samples were transported to the medical microbiology laboratory of the Department of microbiology, Olabisi Onabanjo University, Ago–Iwoye and were analyzed immediately in order to prevent drying of the swabs and subsequent dying of the organisms (Onche and Adedeji,2004).The samples were processed using standard microbiological techniques as described by Girish and Satish(2008).

2.2.Collection and Preparation of Plant Materials

The leaves of *A. conyzoides*, *V. amygdalina*, *A. indica*, *F. exasperata* and the fruits of *C. aurantifolia* were collected from Ago-Iwoye, in Ogun State, Nigeria. The samples were identified and authenticated at the herbarium Department, Forestry Research Institute of Nigeria (FRIN) Ibadan Oyo State where the voucher specimens were deposited. The following numbers, FHI 108200; FHI 108111; FHI 108199; FHI 108196; and FHI 108198 were given for *A. conyzoides*, *V. amygdalina*, *A. indica*, *F. exasperata*, and *C. aurantifolia* respectively.

2.3.Preparation of Extracts

2.3.1.Grinding of the Selected Plant Materials

After drying at 37°C for 24 h the plant material was ground in a grinding machine (Philips, Bolmixer Melangeur HR, 2846, Brazil) bought for the laboratory. Exposure to direct sunlight was avoided to prevent the loss of active components (Girish and Satish, 2008).

2.3.2.Preparation of Leaf Aqueous Extract

Fifty grams of selected fresh leaf materials was macerated with 50 ml of sterile distilled water in a grinding machine (Philips, Bolmixer Melangeur HR, 2846, Brazil)) for about 10-15 min. The macerate was first filtered through double layer muslin cloth then centrifuged at 3500 rpm for 30 min. The supernatant was filtered through. Whatman No. 1 filter paper and sterilized at 120°C for 30 minutes. The extracts were preserved aseptically at 5°C for further use (Gupta *et al.*,1996).

2.4.Antibacterial activity assay

Antibacterial activity of aqueous and solvent extracts of all the selected plant extracts was determined by the cup diffusion method on nutrient agar medium (Satish *et al.*,1999). Both the aqueous and solvent extracts of plants were screened for the antibacterial assay.

2.4.1.Aqueous Extract

The organism to be tested was inoculated into sterile nutrient agar. After incubation period of 24 h at 37°C, a loop of inoculum was transferred into 5 ml of nutrient broth and incubated for 2 h at 37°C which served as fresh suspension inoculum. Five wells (5 mm diameter) were made in sterile nutrient agar plate using cork borer (one in the center and four wells at the corner) and inoculum containing 10^6 CFU/ml of test bacteria were spread on solid plates with the help of sterile swab moistened with the bacterial suspension. Then 50 µl of aqueous extract of all the leaves were placed in the wells made in inoculated plates. The treatment also includes 50 µl of sterilized distilled water as control. All the plates were incubated for 24 h at 37°C and zone of inhibition if any around the well were measured in millimeter (mm). For each treatment six replicates were maintained.

2.4.2.Methanol Extract

One gram of all the selected plant leaf extract were dissolved in 9ml of methanol. The sterile nutrient agar medium in Petridishes was uniformly smeared with test culture. Well (5 mm) were made in each petridish to which 50 µl of solvent extracts dissolved in methanol were added. For each treatment six replicates were maintained. Methanol served as control.

3.Statistical Analysis:

Data were subjected to one way analysis of variance and student t-test using statistical package for social sciences (SPSS) to determine the significant differences between means

4.Results

The antibacterial activity of aqueous and methanol extracts of selected plants against human wound pathogenic bacteria both Gram-positive and Gram-negative bacteria are presented in Table 1. Activity was analyzed at 50 µl of aqueous and ethanolic extracts. All the plant species viz, *A. conyzoides* (Goat Weed); *A. indica* (Nee tree); *C. aurantifolia* (Lime fruit), *V. amygdalina* (Bitter leaf) and *F. exasperate* (Sandpaper tree) showed marked antibacterial but to varied zones of inhibition. The activity of these plant extracts were also compared as *C. aurantifolia* (Lime fruit) was found to be statistically more significant than other plants extract for both ethanol and aqueous extraction ($P<0.05$).The antibacterial activity of both ethanol and aqueous extract of all the plants were also compared(Table 4).In all, there was no statistical significant difference between the ethanolic and aqueous extract of all the plant species($p>0.05$).

Table 1: Prevalence of bacterial species isolated from wound samples

Bacterial isolates	Number of isolates	Percentage number (%)
S. aureus	86	63
P. aeruginosa	15	11
E. coli	5	04
P. mirabilis	12	09
K. pneumonia	18	13
Total	**136**	**100**

Table 1: Antibacterial activity of selected Nigeria Plants at 50µl

		Zone of inhibition (mm) (Mean ± SEM)	
Plant designate	**Test organisms**	**Ethanol Extract**	**Aqueous Extract**
FE	PA	17 ± 1.2	15 ± 0.3
	EC	21 ± 0.8	14 ± 0.1
	PM	19 ± 0.6	14 ± 0.1
	SA	20 ± 1.3	14 ± 1.2
	KP	1.5 ± 1.4	16 ± 0.6
AI	PA	16 ± 0.1	15 ± 0.3
	EC	19 ± 0.1	18 ± 1.2
	PM	12 ± 0.3	16 ± 2.1
	SA	23 ± 0.9	16 ± 2.4
	KP	18 ± 1.2	17 ± 1.6
VA	PA	16 ± 0.3	15 ± 2.1
	EC	19 ± 1.3	17 ± 1.4
	PM	20 ± 1.4	17 ± 1.6
	SA	19 ± 1.8	20 ± 2.3
	KP	16 ± 0.7	16 ± 2.5
AC	PA	19 ± 1.2	15 ± 1.9
	EC	16 ± 0.3	15 ± 1.8
	PM	10 ± 0.4	15 ± 0.3
	SA	14 ± 1.3	16 ± 0.1
	KP	15 ± 2.3	16 ± 2.3
CA	PA	22 ± 0.3	18 ± 1.3
	EC	24 ± 0.9	18 ± 2.6
	PM	20 ± 1.3	21 ± 2.1
	SA	18 ± 0.2	22 ± 3.2
	KP	08 ± 0.2	19 ± 0.7

FE = *Ficus exasperate,* AC = *Ageratum conyzoides,* VA = *Vernonia amygdalina,* AI = *Azadirachta indica,* CA = *Citrus auranti folic,* PA = *Pseudomonas aeruginosa,* EC = *Escherichia coli,* PM = *Proteus vulgaris,* SA = *Staphylococcus aureus,* KP = *Klebsiella pneumoriae.*

Table 2: Antibacterial activities of five selected ethanol extracts – a comparative analysis

Plant Extract		**Zone of inhibition (mm)**
	No of organisms	**Meant ± SEM**
CA	5	18.4 ±1.08
FE	5	17.6 ± 1.80
AI	5	1.80 ± 0.84
VA	5	18.0 ± 0.84
AC	5	14.8 ± 1.46
Control	5	11.2 ± 2.27

F value = 3.676, $P < 0.05$

Table 3: A comparative antibacterial activities of five selected aqueous extract

Plant Extract	No of organisms	Zone of Inhibition (mm) Meant ± SEM
CA	5	19.6 ± 0.81
FE	5	12.8 ± 3.23
AI	5	16.4 ± 0.51
VA	5	17.0 ± 0.84
AC	5	15.4 ± 0.55
Control	5	11.2 ± 5.07

F = 3.170, P < 0.05

Table 4: Antibacterial activities of Ethanol and Aqueous extract of five selected Nigeria plants

Plant Extract	N	Ethanol	Aqueous	t value	P value
CA	5	18.4 ± 1.08	12.8 ± 3.22	1.64	>0.05
FE	5	17.6 ± 1.81	16.4 ± 0.51	0.64	>0.05
AI	5	18.0 ± 0.84	17.0 ± 0.84	0.85	>0.05
VA	5	14.8 ± 1.46	15.4 ± 0.25	0.41	>0.05
AC	5	18.4 ± 1.08	19.6 ± 0.81	0.61	>0.05

N = Number of bacterial isolates

5. Discussion:

The antibacterial activity of five ethanol and aqueous plant extracts against some bacterial organisms isolated from wounds were investigated. The result of this study indicated that the Gram negative bacilli were more common in infected wounds than the Gram positive bacteria, although the prevalence rate of *S. aureus* (63%) was higher when compared with that of Gram-negative (37%). This finding is in line with the ones earlier reported in this environment (Sule *et al*., 2001; Thanni *et al*., 2003). *Staphylococcus aureus* is a normal flora of the skin and the leading cause of both surgical and accidental wound infections (Nester *et al.,* 2004). *Klebsiella pneumonia* was the most prevalent Gram-negative bacilli, followed by *P. aeruginosa*, *P. mirabilis* and *E. coli* in that order. This observation disagrees with that of Giacometti *et al*. (2000); and Adedeji *et al*. (2007) who reported *P. aeruginosa* (25.2%) and 54.2% respectively as the most predominant Gram-negative bacilli in wound infections. This thus suggest the fact that the distribution of pathogens causing nosocomial infections changes with time and varies among hospital and among different locations in the same hospital (Hsueh *et al*., 2002). The presence of *K. pneumoniae and P. aeruginosa* as the most and second most predominant isolates observed could be attributed to the fact that these organisms are frequently present in small number as normal flora of the intestine and skin of humans (Brooks *et al*., 2001). These bacteria are widely distributed in nature and is commonly present in moist environment in hospital and is pathogenic only when introduced into areas devoid of normal defences such as when mucous membrane and skin are disrupted by direct tissue damage (Brooks *et al*., 2001). *Pseudomonas aeruginosa* has also been described as one of the most important nosocomial pathogens and an important cause of death, particularly among patients with immunosuppression, malignancy, cystic fibrosis and burns or traumatic wound (Karakoc and Gerceker, 2001). In the case of the antibacterial study,there exist no statistically significant difference between ethanolic extracts and aqueous extracts of these plants (p>0.05). This could mean that the active ingredients of these plant extracts are equally soluble in ethanol and water. This result disagreee with the findings of Obi and Onuolia (2000) who reported ethanol as the best solvent for the extraction of plant active substances of medical importance. Other researchers reported hexane as the best solvent for extracting plants phytochemicals (Ijeh *et al*. 2005, Junaid *et al*., 2006). The high zone of inhibition exhbited by *C. aurantifolia (*P<0.05) compared to other plant extracts could mean that this extract contain more active ingredients which qualifies its use in the treatment of wound infections. The efficacy of *C. aurantifolia* on bacterial isolates has been reported by Aibinu *et al*. (2007); Fagade *et al*. (2007). The antimicrobial activities of these extracts, both ethanol and aqueous appeared to be broad spectrum since both the Gram-positive and Gram-negative bacteria responded to their inhibitory effects. However, these bacterial isolates, apart from being isolated from wound infections, have also been implicated in diseases such as respiratory tract infections, urinary tract infections, diarrhoea, abscesses etc. The sensitivity of ciprofloxacin

(positive control) at 50ul to these bacterial isolates as compared with the extracts could mean that the organisms are resistant to the antibiotic except *P. mirabilis* and *E. coli*. The antimicrobial resistance expressed by *K. pneumoniae*, *S. aureus* and *P. aeruginosa* in this study is similar to the findings of Todar, (2002); Deji-Agboola, (2007). The extensive use of antibiotics especially ciprofloxacin has been described to be responsible for the high prevalence of multi-drug resistant plasmids and transposons found in nosocomial strains of various bacterial genera (Vatopoulos and Kalapothaki, 1999). The strains harbouring these plasmids have been shown to survive in the hospital environment and can become the best candidates for the selection of resistant mutants (Vatopoulos and Kalapothaki, 1999). The resistance of these isolates to ciprofloxacin could be attributed to the indiscriminate use of antibiotics by the general public and also to the quality and potency of antibiotics in the market especially developing countries like Nigeria.In conclusion, the ethanolic and aqueous leaves extracts of the four plants and also the juice of *C. aurantifolia* significantly inhibited the growth of the tested bacterial organisms. The level of inhibition was found to be organism dependant and the particular extract. These finding may lead support to the traditional use of these plants in the treatment of microbial infections. Current researches and technology can be developed which will help to optimally extract all the bioactive molecules in the plant and formulated into appropriate dosage.

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REFERENCES

1. Adedeji GB, Fagade OE and Oyelade AA. Prevalence of *Pseudomonas aeruginosa* in Clinical Samples and its Sensitivity to Citrus Extract. *Afri. J. of Biomedical Res2007*;**10**: 183 – 187.
2. Ahmed SI. Potential of Using The Neem Tree (*Azadirachta indica*) for Pest Control and Rural Development. *Neem Nesl.*1998; (**5**): 49 – 55.
3. Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T and Odugbemi T. Evaluation of The Antimicrobial Properties of Different Parts of *Citrus aurantifolia* (lime fruit) as Used Locally. *African Journal of traditional complementary and alternative medicines2007;* 4(**2**): 185-195.
4. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, and Fasure KA. Screening of Crude Extracts of Six Medicinal Platns Used in South-West Nigeria Unorthodox Medicine Antimethicilin Resistant *Staphlococcus aureus* Activity. *BMC Complementary and Alternative Medicine* 2005;5(**6**): 1-7.
5. Atata R, Sani A, and Ajewole SM.Effect of Stem Back Extracts of *Enantia chloranta* on Some Clinical Isolates. *Biokemistri* 2003;15(**2**): 84-92.
6. Brooks GF, Butel JS, and Morse SA.Cell Structure: In Jawets, Melnick and Adelberg's Medical Microbiology 22nd Ed. Lange Medical Books/McGraw-Hill USA 2003:7- 37.
7. Cheley A. The Antibiotic Crisis. *The Nigerian Journal of Pharmacy* 1998;29(**4**): 183 – 188.
8. Cohen ML.Changing Patterns of Infectious Disease. *Nature 2000;* **406**: 762 – 767.
9. Deji-Agboola AM. In-Vitro Antimicrobial Activities of Some Nigerian Medicinal Plants Against Wound Pathogens. Ph.D thesis. Yet Unpublished. Olabisi Onabanjo University, Ago – Iwoye, Nigeria 2007:105 – 108.
10. Fagade OE, Adedeji GB, and Oyelade AA.Prevalence of *Pseudomonas aeruginosa* in clinical samples and its sensitivity to citrus extract. *Afr. J. Biomed. Res.2007;* **10**: 183 – 187
11. Giacometti AO, Cirioni A, Schimizzi M, Delprete M, and Barchiesi B. Epidemiology and Microbiology of Surgical Wound Infections. *J. of Clin. Microbial*. 2000;38(**2**): 218 – 922.
12. Girish HV.and Satish S.Antibacterial Activity of Important Medicinal Plants on Phytopathogenic *Xanthomonas compestris* Pathovars. *Letters in Microbiology*, 2000;**28:**145-147.
13. Gupta S,Raveesha KA, and Datta GR.Plant Extract: a Non Chemical Approach to Control Fusarium Diseases of Midberry. *Current Sci.* 1996;**71:**406-709.
14. Hsueh PR, Chen ML, Sun CC, Chen WH, Pan HJ, Yang LS, Chang SC, Lee SW, Hsieh CY and Luh KT. Antimicrobial Drug Resistance in Pathogens Causing Infections At a University in Taiwan, 1981 – 1999. *Emg. Infect. Dis. 2002;* 8(**1**): 63-68.
15. Ijeh II, Omodamiro OD, and Nwanna IJ. Antimicrobial Effect of Aqueous and Ethanolic Fraction of Two Species: *Ocimum gratissimum* and *Xylopia gethiopica*. *Afr. J. Biotechnol. 2005;* 4(**9**): 953 – 956.
16. Junaid SA, Olabode OA, Onwuuri FC, Okwori AEJ, and Agina SE.The Antimicrobial Properties of *Ocimum gratissum* Extracts on Some Selected

Bacterial Gastrointenstinal Isolates. *Afr. J. of Biotechnology*. 2006; 5(**22**): 2315-2321.
17. Karakoc B and Gerceker AA.In-Vitrio Activities of Various Antibiotics Alone and in Combination With Amikacin Against *Pseudomonas aeruginosa*. *Int. J. Antimicrob Agents. 2001* **18**: 567 – 570.
18. Kavase M, Motohashi N, Sakagami H, Kanamoto T, Nakashima T, Ferneczy L, Wolfard K, Miskoci C, and Molnar J.Antimicrobial Activity of Trifluoromethyl Ketones and their Synergism with Promethazine. *Int. J. Antimicrob Agents*. 2001; 18 (**2**): 161-165.
19. Lai PK. Antimicrobial and Chemopreventive Properties of Herbs and Spices. *Curr med. chem. 2004;*1451-1460
20. Moreillion P, Que YA, and Glauser MP. *Staphylococcus aureus* (including Staphylococcal Toxic Shock). In Principles and Practice of Infectious diseases (Ed) Mandell, G. L., Benneth, J. E., Dolin, R. Churchill Living Stone Pennsylvania 6th ed. (2). 2005:2333-2339.
21. Moura D. Anti-inflammatory and Chronic Toxicity Study of the Leaves of *Ageratum conyzoides*. L. in Rats. *Phytomedicine 2005:* 12 (**2**): 138-142.
22. National Committee for Clinical Laboratory Standards.Dilution Anti-microbial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard. 2nd Ed., NCCLS document M7 – A3. (ISBN 1-56238-469). Pennsylvania, USA. 2000.
23. Nester EW, Anderson DG, Roberts CE, Pearsall NN, and Nester MT.Microscopy and Cell structure, Application of Immune Responses, Wound Infections. In: Microbiology: A Human Perspective. 4th Edition, New York: McGraw-Hill. 2004:46-698.
24. Neu HC. The Crisis in Antibiotic Resistance. *Science* 1989;**257**: 1064 – 1073.
25. Niccoli DL, Tarsi L, and Thomas RJ. The Renewed Challenges of Antibacterial Chemotherapy. C*hem. Commun***. 2001;42**: 2333 – 2342.
26. Nickel LG 1995. Cited in Khan MR, Nkunya MHH. Antimicrobial Activity of Tanzanian Traditional Medicinal Plants. The United Republic of Tanzania. Dar ES Salam University Press. Ministry of Health. Tanzania.1991:391.
27. Obi VI. and Onuoha C. Extraction and characterization methods of plants and plant products. In Biological and Agricultural technique. Ogbuile JN and Ojiako OJ. Ed. Websmedia Publications, Owerri. 2000:271 – 286.
28. Okoegwale E, and Omefezi E. Some Herbal Preparations Among the People of Isoko Clan of Delta State, Nigeria. *J. Appl. Sci.2001;* (**4**): 2350-2371.
29. Okwori AE, Dina GO, Junaid S, Okeke IO, Adetunji JA. Antibacterial Activities of *Ageratum Conyzoides* Extracts on Selected Bacterial Pathogens. *The Internet Journal of Microbiology* 2007;4(**1**) 1-16.
30. Oladejo OW, Imosemi IO, Osuagwu FC, Oluwadara DO, and Aiku A. A comparative Study of The Wound Healing Properties of Honey and *Ageratum conyzoides*. *Afri. J. Med. Sci. 2003;*32(2): 193 – 196.
31. Onche I and Adedeji O .Microbiology of Post-Operative Wound infection in Implant Surgery. *Nigerian Journal of Surgical Research 2004;* 6(**1-2**): 37-40.
32. Orrett FA. Nosocomial Infections in an Intensive Care and in a Private Hospital. *West Indian Medical Journal* 2002;**51**: 21 – 24.
33. Pretorius JC, Magama S, and Zietsman DC. Growth Inhibition of Plant Pathogenic Bacteria and Fungi by Extracts from Selected South African Plant species. *South African Journal of Botany2003;* **20**: 188 – 192.
34. Satish S, Raveesha KA and Janardhana GR.Antibacterial Activity of Selected Plants on Phytopathogenic Xanthomonas compestris Pathovars. *Letters in Applied Microbiology* 1999;**28:**145-147.
35. Shahidi –Bonjar GH.Evaluation of Antimicrobial Properties of Iranian Medicinal plants against *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumonia* and *Bordetella bronchoseptica*. *Asian J. Plant Sci*. 2004; 3(**1**): 82 – 86.
36. Sule AM, Thanni LOA, Sule-odu OA, and Olusanya O.Bacterial Pathogens Associated With Infected Wounds in Ogun State University Teaching Hospital, Sagamu Nigeria. *Afri. J. Clin. Exp. Microbial 2002;* **3**: 13-16.
37. Tapsell LC.Health Benefits of Herbs and Spices: The Past, the Present, the Future. *Med. J. Aust 2006;*.**2:**144-1147
38. Taylor L. The Healing Power of Rainforest Herbs: http://www.rain-tree.com/prepmethod.htm. Date cited: 06/03/2002.
39. Tenover FC. Development and Spread of Bacterial Resistance to Antimicrobial Agents. An Overview. *Clinical infect Dis* 2001; 33 (suppl **3**) 5108 – 5115.
40. Thanni LOA., Osinupebi OA, and Deji-Agboola M. Prevalence of Bacterial Pathogens In Infected Wounds in a Tertiary Hospital, 1995 – 2001:

Any change in trend? *J. NaH Med. Assoc*. **2003; 95**: 1189-1195.
41. Todar K. Todar's Online Textbook of Bacteriology. www. pseudomonas. Intm. 2002:1 – 8.
42. Umerie SC, Ogbuagu AS, and Ogbuagu JO. Stabilization of Palm Oils By Using *Ficus exasperate* Leaves in Local Processing Methods. *Bioresour Technol. 2004;* 94(**3**): 307 – 310.
43. Valtopoulos AC and Kalapothaki V. Bacterial Resistance to Ciprofloxacin in Greece: Results from the National Electronic Surveillance System. *Emerg. Infect. Dis 1999;* 5(**3**): 471 – 476.
44. World Health Organization. The World Health Report 1998: life in the 21st century – a Vision for all. Geneva: WHO. 1998.

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