EFFECT OF ESSENTIAL OILS FROM THE LEAVES OF *Ocimum gratissimum*, *Callistemon rigidus*, peels of *Citrus paradisi* and extract of *C. paradisi* seeds on *Staphylococcus* SPECIES FROM CLINICAL SPECIMENS.

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Abstract:Essential oils from grapefruit *(Citrus paradisi)*, scent leaf *(Ocimum gratissimum)* and bottle brush *(Callistemon rigidus)* were obtained by steam distillation. Hot water extract of the grape seed was also obtained. They were screened for antimicrobial activity against 115 clinical isolates of *Staphylococcus* spp and a locally isolated antibiotic sensitive strain of *S. aureus* using the agar-well diffusion method. *Ocimum gratissimum* oil showed the largest inhibitory zone sizes (14-4lmm), followed by that of *Callistemon rigidus* (10-33mm). Grape seed extract gave the least inhibitory zone sizes of 6 - 17 mm. O. *gratissimum* oil was the most effective against the staphylococcal isolates as 38 (80.85%) of the *S. aureus* and 56 (82.35%) of the coagulase - negative staphylococci were susceptible. They were least susceptible to the grape seed extract to which only 11 (23.40%) of the *S. aureus* and 23 (33.82%) of the coagulase - negative staphylococci screened were susceptible. The development of essential oils from these plants into effective antibacterial herbal preparations should be aggressively pursued to augment the available antibiotics for treating multi-drug resistant staphylococci.

Keywords: Antimicrobial activity, *Callistemon rigidus* , *Citrus paradisi*, Essential oils, *Ocimum gratissimum*, *Staphylococcus* spp.

Introduction.

Staphylococcus species were traditionally divided into pathogenic and relatively non pathogenic strains based on the synthesis of the enzyme coagulase (Prescott *et al.*, 2008). The coagulase-positive staphylococci (*S. aureus*) is the most important human pathogen in this genus. *S. aureus* infections are many, and include: bacteremia, septicaemia, osteomyelitis, wound infections, boils, carbuncles, scalded skin syndrome, etc (Emmerson, 1994; Brooks *et al.*, 1998; Mins *et al.*, 2004). The coagulase-negative staphylococci (CONS) are known to comprise over 30 species including *S. epidermidis*, *S. saprophyticus*, *S. lugdunensis*, etc (Pantucek *et al.*, 2005; Longauerova, 2006); and are considered to be basically opportunistic microorganisms that prevail in numerous organic conditions, producing serious infections (Lark *et al.*, 2000) such as nosocomial infections in immunocompromised individuals, neonates and those subjected to invasive medical devices (Von Eiff *et al.*, 2001; Bjorkqvist *et al.*, 2002; Caierao *et al.*, 2006). Most staphylococcal infections have become increasingly resistant to

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antibiotic treatment. Nosocomial infections caused by methicillin resistant strains often pose therapeutic dilemma to clinicians because of their multi-antibiotic resistant nature (Taiwo *et al.*, 2004). Like *Staphylococcus aureus*, about 90% of coagulase-negative strains (CONS) isolated from human specimens produce an inducible beta-lactamase (Diekema *et al.*, 2001) Methicillin resistance/multiple drug resistance has been documented more often in disease-causing strains of *Staphylococcus epidermidis* (Archer, 1991). With the increase in staphylococcal resistance to methicillin, vancomycin (or teicoplanin) is often the antibiotic of choice in infections with methicillin resistant *S. aureus* (MRSA) (Hiramatsu *et al.*, 1997; Rasheed and Awole. 2007). The emergence of vancomycin resistant enterococci which gave rise to the possibility of horizontal transfer of the genetic elements of resistance to *S.aureus* has generated a lot of concern, as this development limits treatment options for antibiotic resistant staphylococcal infections. Alternative therapies are being sought for MRSA infections, and one area of interest is the use of essential oils (Edwards-Jones *et al.*, 2004). Many common essential oils have medicinal properties that have been applied in folk medicine since ancient times and are still being used today. *Ocimum gratissimum* (scent leaf) is used in the treatment of different diseases including upper respiratory tract infections, diarrhea, headache, fever, ophthalmic, skin diseases and pneumonia (Onajobi, 1986; Ilori *et al.*, 1996s). It has also been demonstrated to have antimicrobial properties (Sartoratto *et al.*, 2004; Adebolu and Oladimeji, 2005; Lopez *et al.*, 2005; Junaid *et al.*, 2006 and Nwinyi *et al.*,2009). *Citrus paradisi* (grape fruit) seed extract has been claimed to be a strong antimicrobial agent, but information on its proven efficacy is scarce. Extracts and volatile oils of *Callistemon rigidus* have also been reported to have some antimicrobial and insect-repelling properties respectively (Gomber and Saxena, 2007; Aisien *et al.*, 2004). Although essential oils are known for their antimicrobial properties, medical teams rarely use them. This is primarily due to lack of scientific evidence of their efficacy, toxicity issues and availability of conventional therapy (Edwards-Jones *et al.*, 2004). This research survey documents the antibacterial effects of essential oils of *O. gratssimum*, *C. paradisi* and *C. rigidus* on clinical isolates of *S. aureus* and coagulase negative staphylococci hn.

Materials and methods

Source of Samples.

Samples of *O. gratissimum* leaves and fruits of *C. paradisi* were bought from a market in Benin City, while leaves of *C. rigidus* were collected, growing wild within the campus of the University of Benin, Benin City.

Bacterial strains

One hundred and fifteen strains of staphylococci, consisting of 47 *S. aureus* and 68 coagulase negative staphylococci (CONS) were used. They were isolated from clinical specimens obtained from public hospitals in Benin City, Edo State, Nigeria.

Essential oils

Essential oils from the leaves of *O. gratissimum, C. rigidus* and *C. paradisi* peels were obtained by subjecting samples to steam distillation using modified clavenger-type distilling apparatus. Briefly, two litres flat-bottomed flask was filled up to ¾ of its volume with the test sample, 500ml of sterile distilled water was added, and plugged with quickfits. The flask and contents were mounted on the electro-thermal heating mantle, regulated to a temperature of 90-95°C for 2-3hrs. The essential oils were collected via a burette (half-filled with water) connected to a condenser that was attached to the flask. The oil floats on the water which is subsequently separated.

Extract from *C. paradisi* seeds was obtained by boiling 880 g of fresh seed with 500ml of distilled water in a conical flask mounted on an electro-thermal heating mantle for 1hr. The extract obtained was sieved and concentrated by evaporation in a water bath. One gram of the paste extract was dissolved in 9ml of sterile distilled water as stock solution for the experiment

Antimicrobial susceptibility testing of essential oils and extract

Aliquots (20 µL) of each of the test oils and 1 in 10 dilution of the grape seed extract in combination with an equal volume of dimethylsulfoxide as carrier, were placed separately in 6mm diameter wells bored in nutrient agar plates seeded with test staphylococcal suspensions adjusted to 0.5 McFarland's turbidity standard. Fifty percent DMSO and water (40 µL) was used as a negative control. Cultures were incubated at 37°C for 24hrs and zones of inhibition measured. A locally isolated strain of *S. aureus* that was sensitive to the following antibiotics: erythromycin, pefloxacine, gentamicin, cefuroxime, cefriaxone, ciprofloxacine, streptomycin, cotrimoxazole and vancomycin was also screened for sensitivity to the test essential oils in each batch of tests for the purpose of interpreting observed zones of inhibition. The sensitivity of the test isolates was determined by comparing the inhibitory zone sizes with that of the sensitive strain.

Results

All the isolates of staphylococci showed some sensitivity to each of the essential oils screened, but the inhibitory zone sizes varied with oil and strain. Oils from *O. gratissimum* produced the largest zones of inhibition (range 14-41mm), followed by *C. rigidus* (zone range 10-33mm), while *C. paradisi* seed extract produced the least zone sizes ranging from 6 to 17 mm (Table 1) . Eleven out of the 115 staphylococcal isolates subjected to *O. gratissimum* oil produced inhibition zone sizes of 38-41mm (Table 1). Forty-four isolates subjected to *C. rigidus* oil had inhibitory zone sizes of 22mm and above, while none of those subjected to grape seed extract had zone sizes above 17mm. Eleven (23.4%)of the *S. aureus* isolates were sensitive to *C. paradisi* seed extract, 32 (68.09%) to the essential oil, 38 (80.85%) to *O. gratissimum* oil and 18 (38.30%) to *C. rigidus* oil. Similarly, 23 (33.82%) of the 68 CONS isolates were sensitive to grape seed extract, 45 (66.18%) to grape peel oil, 56 (82.35%) to scent leaf oil and 26 (38.24%) to bottle brush oil (Tables 2 and 3). Isolates were therefore most sensitive to scent leaf oil, followed by grape peel oil, and least susceptible to grape seed extract.

TABLE 1

Effect of some essential oils and grape seed extract on staphylococci-zones of inhibition.

	Grape Seed (extract)			Grape Peels (volatile oil)			Scent Leaf (volatile oil)			Bottle brush (volatile oil)		
Zone Size (mm)	NO of Isolates with zone size	COPS with zone size	CONS with zone size	NO of Isolates with zone size	COPS with zone size	CONS with zone size	NO of Isolates with zone size	COPS with zone size	CONS with zone size	NO of Isolates with zone size	COPS with zone size	CONS with zone size
6-9	81	36	45	9	6	3	0	0	0	1	0	1
1o-13	28	9	19	25	9	16	0	0	0	15	7	8
14-17	6	2	4	36	19	17	3	2	1	20	7	13
18-21	0	0	0	25	7	18	7	3	4	35	15	20
22-25	0	0	0	18	6	12	17	6	11	27	12	15
26-29	0	0	0	1	0	1	28	12	16	13	5	8
30-33	0	0	0	0	0	0	30	10	20	4	1	3
34-37	0	0	0	1	0	1	19	10	9	0	0	0
38-41	0	0	0	0	0	0	11	4	7	0	0	0
Total	115	47	68	115	47	68	115	47	68	115	47	68

[a] COPS = Coagulase Positive staphylococci, [b] CONS = Coagulase Negative Staphylococci

TABLE 2

Antimicrobial susceptibility of clinical isolates of *Staphylococcus aureus* (coagulase positive)

Source	Total number of isolates	Number of isolates sensitive (percent) to:			
		Grape seed extract	Grape peel oil	Scent leaf Oil	Bottle brush oil
Urethral	9	3(33.33)	6(66.67)	7(77.78)	3(33.33)
Wound	9	1(11.11)	5(55.56)	7(77.78)	2(22.22)
Vaginal	6	2(33.33)	5(83.33)	5(83.33)	1(16.67)
Urine	1	0(0.0)	1(100.0)	1(100.0)	1(100.0)
Blood culture	7	3(42.86)	4(57.14)	5(71.43)	4(57.14)
Semen	2	2(100.0)	2(100.0)	2(100.0)	2(100.0)
Endocervix	4	0(0.0)	3(75.0)	4(100.0)	2(50.0)
Ear	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Eye	3	0(0.0)	3(100.0)	3(100.0)	2(66.67)
Others	4	0(0.0)	3(75.0)	4(100.0)	1(25.0)
Total	**47**	**11(23.40)**	**32(68.09)**	**38(80.85)**	**18(38.30)**

TABLE 3

Antimicrobial susceptibility of clinical isolates of coagulase negative staphylococci

Source	Total number of isolates	Number of isolates sensitive (percent) to:			
		Grape seed extract	Grape peel oil	Scent leaf oil	Bottle brush oil
Urethral	11	1(9.09)	8(72.73)	9(81.82)	3(27.27)
Wound	8	4(50.0)	6(75.0)	4(50.0)	3(37.5)
Vaginal	9	5(55.56)	7(77.78)	9(100.0)	7(77.78)
Urine	12	6(50.0)	8(66.67)	12(100.0)	7(58.33)
Blood culture	5	1(20.0)	3(60.0)	5(100.0)	2(40.0)
Semen	7	1(14.29)	2(28.57)	5(71.43)	1(14.29)
Endocervix	4	2(50.0)	3(75.0)	4(100.0)	2(50.0)
Ear	5	0(0.0)	4(80.0)	4(80.0)	0(0.0)
Eye	3	2(66.67)	3(100.0)	2(66.67)	1(33.33)
Others	4	1(25.0)	1(25.0)	2(50.0)	0(0.0)
Total	**68**	**23(33.82)**	**45(66.18)**	**56(82.35)**	**26(38.24)**

Discussion

All test essential oils and grape seed extract showed some activity against the test microbial isolates as in earlier reports (Edwards-Jones *et al.*,2004; Adebolu and Oladimeji, 2005; Nwinyi *et al.*, 2009). Sensitivity to patented antimicrobial agents is normally based on results of comparisons of zones of inhibition of test isolates with that of a standard known sensitive strain (Bigos *et al.*, 2012). It is therefore imperative that tests of activity of antimicrobial herbal extracts to be similarly standardized.

Zones of inhibition produced by all preparations used varied with different strains of CONS and *S. aureus*. Four out of the 47 *S. aureus* strains had zones of inhibition above the 37mm earlier reported for *O. gratissimum* (Adebolu and Oladimeji, 2005) (Table 1). However, when zones of inhibition for test *S. aureus* and CONS were compared with a locally isolated antibiotic sensitive *S. aureus* sensitive strain, over 80% of test isolates showed sensitivity (Tables 2& 3). The essential oils of *O. gratissimum* has been reported to have components such as eugenol, thymol, citral and linalool, some of which have antimicrobial properties (Sartoratto *et al.*, 2004; Janine *et al.*, 2005). The antimicrobial activity observed in this and other reports may be the scientific basis of the observed effectiveness by alternative medicine practitioners in the treatment of various infectious diseases (Ilori *et al.*, 1996; Afolabi *et al.*, 2007). Over 60.1% of *S. aureus* and CONS were susceptible to *C. paradisi* peel while *C. rigidus* oil was effective against 38.3% of *S. aureus* and 38.2% of CONS respectively.

Data on the antimicrobial activity of *Callistemon rigidus* and *Citrus paradisi* are scarce. There are however reports of some antimicrobial activity by these herbs (Gomber and Saxena, 2007; Sawer *et al.*, 2005). Lysis occurs when susceptible *S. aureus* isolates are exposed to the alkaloid, cryptolepine in *Callistemon* sp (Sawer *et al.*, 2005). *C. paradisi* seed extract was the least active as 23.4% of *S. aureus* and 33.8% of CONS were susceptible. Previous reports showed that Citricidal™ (a commercially available antibacterial agent) prepared from *C. paradisi* seed extract showed some activity against *S. aureus* strains (Edwards-Jones *et al.*,2004). Components of *C. paradisi* juice have also been shown to enhance the susceptibility of methicillin resistant *S. aureus* to agents such as ethidium bromide and norfloxacin, to which they are normally resistant (Abulrob *et al.*, 2004).

In conclusion, additional research needs to be conducted on these essential oils and extracts, with a view to documenting their active components and precise mechanisms of action. This will aid their development into potent antimicrobials for therapeutic uses.

REFERENCES

Abulrob, A-N., Suller, M.T.E., Gumbleton, M., Simons, C. and Russell, A.D. (2004). Identification and biological evaluation of grapefruit oil components as potential novel efflux pump modulators in methicillin-resistant *Staphylococcus aureus* bacterial strains. *Phytochem.* 65 (22):3021-3027.

Adebolu, T.T. and Oladimeji, S.A. (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarroea causing bacteria in South-Western in Nigeria. *Afri. J. Biotech.* 4 (7): 682-684.

Afolabi, C.A., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum. Sci. Res. Essay* 2(5):163-166.

Aisien, M.S.O., Imasuen, A.A., Wagbatsoma,V.A. and Ayinde, B.A. (2004). Preliminary evaluation of the repellant activity of some plant essential oils against *Simulium damnosum* S.I., the vector of human onchocerciasis. *Int'al J. Trop. Insect Sci.* **24** (2): 196-199.

Archer, G.L. (1991). Alteration of cutaneous staphylococcal flora as a consequence of antimicrobial prophylaxis. *Rev. Infect Dis.* **13** (suppl 10): 805-809.

Bigos, M., Wasiela, M., Kalemba, D. and Sienkiewicz, M. (2012). Antimicrobial activity of geranium oil against clinical strains of *Staphylococcus aureus* . *Molecules* 17(9):10276-10291.

Bjorkqvist, M., Soderquist, B., Tornqvist, E., Sjorberg, L., Fredlund, H., Kuhn, I., Colque-Navarro, P. and Schollin, J. (2002). Phenotypic and genotypic characterization of blood isolates of coagulase-negative staphylococci in the newborn. *APMIS* **110**: 332-339.

Brooks, G.F., Butel, J.S. and Morse, S.A. (1998). *Medical Microbiology.* **21st** edn. Appleton and Lange, NY 740pp.

Caierao, J., Superti, S., Dias, C.A.G. and d'Azevedo, P.A. (2006). Automated systems in the identification and determination of methicillin-resistance among coagulase-negative staphylococci. *Mem. Inst. Oswaldo Cruz.* **101** (3):277-279.

Diekema, D.J., Pfaller, M.A.. and Schmitz, F.J. (2001). Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Programme. *Clin. Infect. Dis.*, **32** (Suppl 2): 5114-5132.

Edwards-Jones, V., Buck, R., Shawcross, S.G., Dawson, M.M. and Dunn, K. (2004). The effect of essential oils on methicillin-resistant *S. aureus* using a dressing model. *Burns* **30**: 772-777.

Emmerson.,M. (1994). Nosocomial Staphylococcal Outbreak. *Scand J. Infect. Dis. Suppl.* **93**: 47-54.

Gomber, C. and Saxena, S. (2007). Anti-staphylococcal potential of *Callistemon rigidus. Cen. Euro. J. Med.* 2 (1): 79-88.

Hiramatsu, K., Hanaki, H., Ino,T.,Yabuta, K., Oguri, T. and Tenover, F.C. (1997). Methicillin-resistant *Staphylococcus aureus* strain with reduced vancomycin susceptibility. *J. Antimicrob. Chem.* **40**: 135-136.

Ilori, M., Sheteolu, A.O., Omonighehin, E.A. and Adeneye, A.A. (1996). Antibacterial activity of *Ocimum gratissimum* (Lamiaceae) *J.Diarr. Dis. Res.* **14**:283-285.

Janine, A.L., Passos, X.S., Fernande, O.F.L., Paula, J.R., Ferri, P.H., Souza, L.K.H., Lemos, A.A. and Silva, M.R.R. (2005). Antifungal activity from *Ocimum gratissimum* L. towards *Cryptococcus neoformans. Mem. Inst. Oswaldo Cruz.* **100** (1):55-**58**.

Junaid, S.A., Olabode, A.O., Onwuliri, F.C., Okwori, A.E.J. and Agina, S.E. (2006). The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial in South-Western in Nigeria. *Afri. J. Biotech.* **4** (7): 682-684.

Afolabi, C.A., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum. Sci. Res. Essay* **2**(5):163-166.

Aisien, M.S.O., Imasuen, A.A., Wagbatsoma,V.A. and Ayinde, B.A. (2004). Preliminary evaluation of the repellant activity of some plant essential oils against *Simulium damnosum* S.I., the vector of human onchocerciasis. *Int'al J. Trop. Insect Sci.* **24** (2): 196-199.

Archer, G.L. (1991). Alteration of cutaneous staphylococcal flora as a consequence of antimicrobial prophylaxis. *Rev. Infect Dis.* **13** (suppl 10): 805-809.

Bigos, M., Wasiela, M., Kalemba, D. and Sienkiewicz, M. (2012). Antimicrobial activity of geranium oil against clinical strains of *Staphylococcus aureus . Molecules* 17(9):10276-10291.

Bjorkqvist, M., Soderquist, B., Tornqvist, E., Sjorberg, L., Fredlund, H., Kuhn, I., Colque-Navarro, P. and Schollin, J. (2002). Phenotypic and genotypic characterization of blood isolates of coagulase-negative staphylococci in the newborn. *APMIS* **110**: 332-339.

Brooks, G.F., Butel, J.S. and Morse, S.A. (1998). *Medical Microbiology.* 21st edn. Appleton and Lange, NY 740pp.

Caierao, J., Superti, S., Dias, C.A.G. and d'Azevedo, P.A. (2006). Automated systems in the identification and determination of methicillin-resistance among coagulase-negative staphylococci. *Mem. Inst. Oswaldo Cruz.* **101** (3):277-279.

Diekema, D.J., Pfaller, M.A.. and Schmitz, F.J. (2001). Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Programme. *Clin. Infect. Dis.,* **32** (Suppl 2): 5114-5132.

Edwards-Jones, V., Buck, R., Shawcross, S.G., Dawson, M.M. and Dunn, K. (2004). The effect of essential oils on methicillin-resistant *S. aureus* using a dressing model. *Burns* **30**: 772-777.

Emmerson.,M. (1994). Nosocomial Staphylococcal Outbreak. *Scand J. Infect. Dis. Suppl.* **93**: 47-54.

Gomber, C. and Saxena, S. (2007). Anti-staphylococcal potential of *Callistemon rigidus. Cen. Euro. J. Med.* **2** (1): 79-88.

Hiramatsu, K., Hanaki, H., Ino,T.,Yabuta, K., Oguri, T. and Tenover, F.C. (1997). Methicillin-resistant *Staphylococcus aureus* strain with reduced vancomycin susceptibility. *J. Antimicrob. Chem.* **40**: 135-136.

Ilori, M., Sheteolu, A.O., Omonighehin, E.A. and Adeneye, A.A. (1996). Antibacterial activity of *Ocimum gratissimum* (Lamiaceae) *J.Diarr. Dis. Res.* **14**:283-285.

Janine, A.L., Passos, X.S., Fernande, O.F.L., Paula, J.R., Ferri, P.H., Souza, L.K.H., Lemos, A.A. and Silva, M.R.R. (2005). Antifungal activity from *Ocimum gratissimum* L. towards *Cryptococcus neoformans. Mem. Inst. Oswaldo Cruz.* **100** (1):55-58.

Junaid, S.A., Olabode, A.O., Onwuliri, F.C., Okwori, A.E.J. and Agina, S.E. (2006). The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *Afr. J. Biotech.* **5** (22): 2315-2321.

Lark, R.L., Chenoweth, C., Saint, S., Zemeneuk, J.K., Lipsky, B.A. and Plorde, J.J. (2000). Four-year prospective evaluation of nosocomial bacteremia: Epidemiology, microbiology and patient outcome. *Diagn. Microbiol. Infect. Dis.*, **38**: 131-140.

Longauerova, A. (2006). Review: Coagulase-negative staphylococci and their participation in pathogenesis of human infections. *Bratisl Lek Listy* **107** (11-12):448-452.

Lopez, P., Sanchez, K., Batlle, R. and Nerin, C. (2005). Solid and vapor phase antimicrobial activities of six essential oils susceptibility of selected food borne bacterial and fungal strains. *J. Agric. Fd. Chem.* **53** (17): 6939-6946.

Mims, C., Dockrell, H., Georing, R., Raitt, I., Wakelin, D. and Zuckerman, M. (2004). *Medical Microbiology.* 3rd edn. Elsevier ltd, 660pp.

Nwinyi, O.C., Chinedu, N.S., Ajani, O.O., Ikpo, C.O. and Ogunniran, K.O. (2009). Antibacterial effects of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *Afri. J. Fd. Sc.* **3** (3):077-081.

Onajobi, F.D. (1986). Smooth muscle contracting soluble principles in chromatographic fractions of *Ocimum gratissimum*. *Ethnopharmacol.* **18**: 3-11.

Pantucek, R., Sedlazek, I., Petras, P., Koukalova, D., Svec, P., Stetina, V., Vancanneyt, M., Chrastinova, L., Vokurkova, J., Ruzickova, V., Doskar, J., Swings, J. and Hajek, V. (2005). *Stapylococcus simiae* Nov., isolated from South American squirrel monkeys. *Intern. J. Syst Evol. Microbiol.* **55**:1953-1958.

Prescott, L.M., Harley, J.P. and Klein, D.A. (2002). *Microbiology.* 4th edn. McGraw-Hill, New York, 962pp.

Rasheed, M.U. and Awole, M. (2007). *Staphylococcus epidermidis*: A commensal emerging as a pathogen with increasing clinical significance especially in nosocomial infections. *Int. J. Microbiol.* **3** (2): 1-17.

Sartoratto, A., Machado, A.L.M., Delarmelina, C., Figueira, G.M., Duarte, M.C.T. and Rehder, V.L. (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Braz. J. Microbiol.* **35**: 275-280.

Sawer, I.K., Berry, M.I. and Ford, J.L. (2005). The killing effect of Cryptolepine on *Staphylococcus aureus*. *Let. Appl. Microbiol.* **40**(1):24-29.

Taiwo, S.S., Orile, B.A. and Akanbi, A.A. (2004), Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Ilorin, Nigeria. *Afri. J. Cl and Exp. Microbiol.* **5** (2): 1-4.

Von Eiff, C., Proctor, R.A. and Peters, G. (2001). Coagulase-negative staphylococci: pathogens have major role in nosocomial infections. *Postgrad Med.* **110**(4):63-76.