International Research of Pharmacy and Pharmacology (ISSN 2251-0176) Vol. 1(9) pp. 228-236, December 2011
Available online http://www.interesjournals.org/IRJPP

Full Length Research Paper

Antimicrobial activity of lemongrass (*Cymbopogon citratus*) oil against microbes of environmental, clinical and food origin

Bhoj Raj Singh[1*], Vidya Singh[2], Raj Karan Singh[2] and N. Ebibeni[1]

[1]ICAR Research Complex for NEH Region, Jharnapani, Nagaland, India
[2]NRC on Mithun, Jharnapani, Nagaland, India

Accepted 30 November, 2011

Out of the 1114 strains belonging to 29 genera and 105 species of microbes (molds, yeasts and bacteria) isolated from different sources [clinical cases, environment (water, air, soil, droppings of lizards and birds), food and healthy animals], 38.2% were sensitive to lemongrass oil discs containing 50 µg oil/disc. All molds, yeasts, *Lactobacillus acidophilus, Morganella morganii*, most of the *Bacillus* spp. strains (84.3%), aeromonads (78%), *Edwardsiella* spp. (73.9%), 53.6% pseudomonads, 53.1% streptococci and 50% of *Budvicia aquatica* and *Leminorella ghirmontii* strains were sensitive to lemongrass oil (LGO). On the other hand, all *Hafnea alvei, Laclercia adecarboxylata, Xenorhabdus luminescens* and majority of *Salmonella enterica* (98.3%), *Citrobacter* spp. (93.7%), *Providencia* spp. and *Kluyvera cryocrescens* (83.3%), *Enterobacter* spp. (78.2%), *Proteus* spp. (78%), *Escherichia* spp. (77.7%), enterococci (73.7%), *Serratia* spp. (75%) and *Erwinia ananas* (75%), *Pragia fontium* (70.6%), staphylococci (69.8%) and *Klebsiella* spp. (62.7%) strains were resistant to LGO. MIC of LGO for sensitive strains (tested against discs containing 50 µg LGO) varied from 1 µg to 32 µg /ml while none of the resistant strains had MIC <64 µg LGO/ ml. MIC for yeast strains was the least i.e., 1 µg LGO/ ml. LGO had microbicidal activity on *E. coli, S. aureus* and *Candida albicans*. LGO instantly killed *C. albicans* and *E. coli,* and *S. aureus* in 10 min at 1 mg/ ml concentration, indicating of its wide spectrum antimicrobial activity at easily achievable concentrations. Study also indicated that LGO is more effective on enterococci in aerobic instead of microaerophilic growth conditions, it is indicative that *in-vivo* sensitivity results may differ from *in-vitro* tests.

Keywords: Lemongrass oil, Antimicrobial activity, Microbes

INTRODUCTION

Of more than 400,000 spp. of tropical flowering plants, varieties of several thousands species have been used for their medicinal properties in traditional medicine (Ali-Shtayeh and Abu Ghdeib, 1999; Odugbemi, 2006). 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 immunostimulant in India (Pearson, 2010; 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, emmenagogue, diuretic and antiseptic (Ghani et al., 1997). 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

*Corresponding authoremail: brs1762@gmail.com

Table 1. Antimicrobial effect of lemongrass oil on strains of different genera of microbes

Microbial strains tested (Number of species)	Strains tested	Strain resistant	Strains sensitive	% sensitive strains	% resistant strains
Aspergillus spp. *(2)*	11	0	11	100.0	0.0
Candida spp.	7	0	7	100.0	0.0
Lactobacillus acidophilus	1	0	1	100.0	0.0
Morganella morganii	3	0	3	100.0	0.0
Penicillium spp. *(1)*	3	0	3	100.0	0.0
Bacillus spp. *(15)*	115	18	97	84.3	15.7
Aeromonas spp. *(8)*	91	20	71	78.0	22.0
Edwardsiella spp. *(2)*	23	6	17	73.9	26.1
Micrococcus agilis	3	1	2	66.7	33.3
Pseudomonas spp. *(3)*	28	13	15	53.6	46.4
Streptococcus spp. *(8)*	32	15	17	53.1	46.9
Budvicia aquatica	8	4	4	50.0	50.0
Leminorella ghirmontii	2	1	1	50.0	50.0
Klebsiella spp. *(3)*	110	69	41	37.3	62.7
Staphylococcus spp. *(5)*	43	30	13	30.2	69.8
Pragia fontium	17	12	5	29.4	70.6
Ervinia ananas	12	9	3	25.0	75.0
Serratia spp. *(5)*	12	9	3	25.0	75.0
Enterococcus spp. *(15)*	213	157	56	26.3	73.7
Escherichia spp. *(4)*	112	87	25	22.3	77.7
Proteus spp. *(4)*	41	32	9	22.0	78.0
Enterobacter spp. *(9)*	55	43	12	21.8	78.2
Kluyvera cryocrescens	6	5	1	16.7	83.3
Providencia spp. *(2)*	6	5	1	16.7	83.3
Citrobacter spp. *(3)*	95	89	6	6.3	93.7
Salmonella enterica spp. *(3)*	59	58	1	1.7	98.3
Hafnea alvei	4	4	0	0.0	100.0
Leclercia adecarboxylata	1	1	0	0.0	100.0
Xenorhabdus luminescens	1	1	0	0.0	100.0

lemon grass has antibacterial and antifungal properties (Ushimaru et al., 2007).

LGO's antimicrobial properties make it an effective drug for bacterial and fungal infections. It can be used in cleaning wounds and treatment of skin diseases such as ringworm. It can also be used in food poisoning, staphylococcal infections, and other common infections of the colon, stomach, and urinary tract. Besides, bacteria, molds and yeasts, LGO has been reported to effectively control growth of agent of American foulbrood disease (AFD), the *Paenibacillus larvae* (Alippi et al., 1996) and malaria, the *Plasmodium* spp (Pearson, 2010). Although many studies have proved antimicrobial effect of LGO using reference strains of variety of bacteria (Chao and Young, 2000; Onawunmi, 1989; Syed et al., 1995; Alam et al., 1994; Sharma et al., 2003; Saikia et al., 1999) and fungi (Pratt and Hudson, 1991; Nieto et al., 1993; Abu-Seif, et al., 2009), only little is known about its action on field strains of clinical, environmental and food origin. Therefore, the present study was undertaken to elucidate the antimicrobial spectrum of LGO through testing it against 21 isolates of three genera of fungi and 1085 isolates of common bacteria belonging to 26 genera.

MATERIALS AND METHODS

Lemongrass oil (LGO)

Light yellow colored pure lemongrass oil was obtained as free gift from Naga Fragrance Pvt. Ltd. Dimapur, Nagaland, India.

Fungal and bacterial strains

Five *Aspergillus niger*, six *A. flavus*, three *Penicillium* spp., seven *Candida albicans* strains and 1093 bacterial strains of 26 genera (Tables 1, 2, 3) isolated (from clinical cases, water, fish, ponds, air, soil, cattle, pig, lizards, birds) and maintained at Microbiology Laboratory, ICAR Research Complex for NEH Region, Nagaland Centre, Jharnapani, Nagaland, India, were revived and checked for purity. Bacterial, yeast and

Table 2. Antimicrobial effect of lemongrass oil on strains of Gram negative bacteria

Microbial strains tested (Number of species)	Strains tested	Strain resistant	Strains sensitive	% sensitive strains	% resistant strains
Aeromonas caviae	12	2	10	83.3	16.7
A. eucranophila	18	10	8	44.4	55.6
A. hydrophila	18	3	15	83.3	16.7
A. media	9	0	9	100.0	0.0
A. salmonicida ssp. *achromogenes*	3	2	1	33.3	66.7
A. salmonicida ssp. *salmonicida*	5	2	3	60.0	40.0
A. salmonicida ssp. *smithia*	1	0	1	100.0	0.0
A. schubertii	8	0	8	100.0	0.0
A. sobria	3	0	3	100.0	0.0
A. veronii	14	1	13	92.9	7.1
Budvicia aquatica	8	4	4	50.0	50.0
Citrobacter amalonaticus	11	11	0	0.0	100.0
C. diversus	6	6	0	0.0	100.0
C. freundii	78	72	6	7.7	92.3
Edwardsiella hoshiniae	1	1	0	0.0	100.0
Edwardsiella. tarda	22	5	17	77.3	22.7
Enterobacter agglomerans	23	14	9	39.1	60.9
Enterobacter. amnigenus I	9	9	0	0.0	100.0
Enterobacter amnigenus II	3	1	2	66.7	33.3
Enterobacter cancerogenus	1	1	0	0.0	100.0
Enterobacter cloacae	5	5	0	0.0	100.0
Enterobacter gregoviae	11	11	0	0.0	100.0
Enterobacter hormaechei	1	1	0	0.0	100.0
Enterobacter sakazaki	1	1	0	0.0	100.0
Enterobacter spp.	1	0	1	100.0	0.0
Erwinia ananas	12	9	3	25.0	75.0
Escherichia blattae	6	4	2	33.3	66.7
Escherichia coli	96	77	19	19.8	80.2
Escherichia furgusonii	8	4	4	50.0	50.0
Escherichia vulneris	2	2	0	0.0	100.0
Hafnea alvei	4	4	0	0.0	100.0
Klebsiella oxytoca	9	7	2	22.2	77.8
K. pnumoniae ssp. *pneumoniae*	95	57	38	40.0	60.0
Klebsiella terrigena	6	5	1	16.7	83.3
Kluyvera cryocrescens	6	5	1	16.7	83.3
Leclercia adecarboxylata	1	1	0	0.0	100.0
Leminorella ghirmontii	2	1	1	50.0	50.0
Morganella morganii	3	0	3	100.0	0.0
Proteus mirabilis	12	8	4	33.3	66.7
Proteus myxofaciens	1	0	1	100.0	0.0
Proteus penneri	19	17	2	10.5	89.5
Proteus vulgaris	9	7	2	22.2	77.8
Pragia fontium	17	12	5	29.4	70.6
Providencia heimbachae	1	0	1	100.0	0.0
Providencia rettgeri	5	5	0	0.0	100.0
Pseudomonas aeruginosa	2	2	0	0.0	100.0
Pseudomonas fluorescens	1	1	0	0.0	100.0
Pseudomonas spp	25	10	15	60.0	40.0
Salmonella enterica ssp. *houtenae*	3	3	0	0.0	100.0
Salmonella enterica ssp. *indica*	45	44	1	2.2	97.8
Salmonella enterica ssp. *salamae*	11	11	0	0.0	100.0
Serratia fonticola	1	1	0	0.0	100.0
Serratia marcescens	2	2	0	0.0	100.0
Serratia odorifera	5	5	0	0.0	100.0
Serratia plymuthica	1	1	0	0.0	100.0
Serratia rubidiae	3	0	3	100.0	0.0
Xenorhabdus luminescens	1	1	0	0.0	100.0

Table 3. Antimicrobial effect of lemongrass oil on strains of Gram positive bacteria and fungi

Microbial strains tested (Number of species)	Strains tested	Strain resistant	Strains sensitive	% sensitive strains	% resistant strains
Aspergillus flavus	6	0	6	100.0	0.0
Aspergillus niger	5	0	5	100.0	0.0
Bacillus anthracoides	3	0	3	100.0	0.0
Bacillus badius	7	0	7	100.0	0.0
Bacillus brevis	4	1	3	75.0	25.0
Bacillus circulans	4	0	4	100.0	0.0
Bacillus coaggulans	51	10	41	80.4	19.6
Bacillus laterosporus	1	0	1	100.0	0.0
Bacillus lentus	8	0	8	100.0	0.0
Bacillus licheniformis	6	6	0	0.0	100.0
Bacillus marcerans	4	0	4	100.0	0.0
Bacillus mycoides	2	0	2	100.0	0.0
Bacillus pentothenticus	16	1	15	93.8	6.3
Bacillus stearothermophilus I	1	0	1	100.0	0.0
Bacillus stearothermophilus II	4	0	4	100.0	0.0
Bacillus subtilis	3	0	3	100.0	0.0
Bacillus spp.	1	0	1	100.0	0.0
Candida albicans	7	0	7	100.0	0.0
Eenterococcus asacchrolyticus	1	0	1	100.0	0.0
Eenterococcus avium	13	6	7	53.8	46.2
Eenterococcus caecorum	32	21	11	34.4	65.6
Eenterococcus casseliflavus	32	26	6	18.8	81.3
Eenterococcus dispar	29	26	3	10.3	89.7
Eenterococcus durans	2	1	1	50.0	50.0
Eenterococcus faecalis	13	8	5	38.5	61.5
Eenterococcus faecium	11	11	0	0.0	100.0
Eenterococcus gallinarum	16	13	3	18.8	81.3
Eenterococcus hirae	42	33	9	21.4	78.6
Eenterococcus malodoratus	3	3	0	0.0	100.0
Eenterococcus mundatii	7	4	3	42.9	57.1
Eenterococcus raffinosus	5	5	0	0.0	100.0
Eenterococcus solitarius	1	0	1	100.0	0.0
Enterococcus spp.	6	0	6	100.0	0.0
Lactobacillus acidophilus	1	0	1	100.0	0.0
Micrococcus agilis	3	1	2	66.7	33.3
Penicillium spp.	3	0	3	100.0	0.0
Staphylococcus aureus	13	8	5	38.5	61.5
Staphylococcus epidermidis	2	0	2	100.0	0.0
Staphylococcus sciuri	23	19	4	17.4	82.6
Staphylococcus xylosus	2	2	0	0.0	100.0
Staphylococcus spp.	3	1	2	66.7	33.3
Streptococcus gallinarum	2	1	1	50.0	50.0
Streptococcus milleri	3	3	0	0.0	100.0
Streptococcus agalactiae	1	0	1	100.0	0.0
Streptococcus alactolyticus	1	1	0	0.0	100.0
Streptococcus caseolyticus	1	1	0	0.0	100.0
Streptococcus mobilis	21	8	13	61.9	38.1
Streptococcus spp.	3	1	2	66.7	33.3

Table 4. Minimum inhibitory concentration of lemongrass oil for different microbes

Type of strain	Strain number	Results with disc diffusion method	Minimum inhibitory concentration of LGO in µg/ ml
Candida albicans	CV1PD	Sensitive	1
	ABY42	Sensitive	1
Enterococcus faecalis	SV7	Sensitive	16
	SV20	Sensitive	32
	E31	Resistant	64
	CV14NC	Resistant	128
Streptococcus mobilis	SV11	Sensitive	16
	SV27NC	Sensitive	32
	SV12	Resistant	64
	SV36NC	Resistant	64
Staphylococcus aureus	SK10S2	Sensitive	1
	SK5S1	Sensitive	8
	SK6S1	Resistant	64
	SKE111	Resistant	64
Bacillus coagulans	CB1	Sensitive	1
	CB6	Sensitive	4
	A12	Resistant	64
	B17	Resistant	64
Klebsiella pneumoniae	CP62	Sensitive	16
	M10	Sensitive	32
	LT81	Resistant	64
	LT121	Resistant	124
Edwardsiella tarda	26P	Sensitive	4
	1BCY	Sensitive	32
	56LT1	Resistant	128
	59LT3	Resistant	64
Escherichia coli	E382 (Control)	Sensitive	1
	C91	Sensitive	8
	P82	Resistant	128
	P86	Resistant	128

mold strains were confirmed according to Holt et al. (1986), Barnett et al. (2000) and Raper and Fennell (1977), respectively. A reference strain of *E. coli* (E382), received from National *Salmonella* Centre, IVRI, Izatnagar, Bareilly, India, was sensitive to all antimicrobial drugs and was used as control to determine the MIC of LGO.

Determination of Antimicrobial activity of LGO

The antibacterial activity was determined by disk diffusion method and minimum inhibitory concentration (MIC) determination assays methods of National Committee for Clinical Laboratory Standards (NCCLS) and Clinical and Laboratory Standards Institute (CLSI). For disk diffusion test, sterile disks of five mm diameter were soaked in methanolic solution of LGO and dried at room temperature to contain 50µg of the oil. Mueller Hinton agar (MHA; Hi-Media, Mumbai) plates were swabbed with 6-8 hour growth of test bacteria in tryptic soy broth (TSB, Hi-Media) medium or with overnight Sabrauds' broth (Hi-Media Mumbai) growth of yeast and mold strains, plates were allowed to dry. LGO discs with standard positive control disc (50µg mercuric chloride) and negative control disc (disc soaked in methanol and dried) was placed on the MHA plate. Plates were incubated overnight at 37°C for bacteria and for 48-72 hours at 22°C for yeast/fungi, the inhibition zone around discs was measured in mm.

To determine the effect of growth condition on disc diffusion assay, 8 strains of *Enterococcus avium* were tested under aerobic and microaerobic growth conditions simultaneously. For microaerophilic condition, plates were incubated in an anaerobic culture jar (Merck, Germany) using gas generating kit, Anaeocult® C (Merck) Cat No. 1.16275.0001. Plates were incubated for 24 h and zone of inhibition was recorded as for the aerobic plates.

For determination of MIC of selected LGO disc sensitive and resistant strains (Table 4) of *Klebsiella pneumoniae* (CP62, M10, LT 81, LT121), *Escherichia coli* (E382, C91, P82, P86), *Edwardsiella tarda* (26P, 1BCY, 56LT1, 59LT3), *Bacillus coagulans* (CB1, CB6,

A12, B17), *Staphylococcus aureus* (SK10S2, SK5S1, SK6S1, SKE111), *Streptococcus mobilis* (SV11, SV27NC, SV12, SV36NC), *Enterococcus faecalis* (SV7, SV20, E31, CV14NC) and *Candida albicans* (CV1PD, ABY42), agar dilution susceptibility test was performed based on modified method of NCCLS and CLSI. Briefly, LGO dissolved in sterilized dimethyl-sulphoxide (DMSO; 1024 µg /ml) was taken as standard and two fold dilutions were made to achieve 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg /ml concentration of essential oil in molten (at 45^0C) MHA. Plates were poured and after solidification, the plates were spot inoculated with loop-full (2 µl) of overnight grown bacterial/ yeast cultures. The test was carried out in triplicates and plates were incubated overnight at 37°C for bacteria and 22°C for yeast. After 18 to 24 hours, the MIC was determined.

To determine that LGO is either microbiostatic or microbicidal, LGO dissolved in sterilized dimethyl-sulphoxide (DMSO; 100 mg /ml) was mixed with sterilized normal saline solution (NSS) or with brain hear infusion (BHI) medium (Hi-Media) to the final concentration of 1 mg/ ml and 0.01 mg/ ml. In LGO containing BHI medium or NSS, washed (with NSS) cells of overnight grown bacteria (*S. aureus* SKE111, *E. coli* 382) and yeast (*C. albicans*, ABY42) were added at concentration of 42000 colony forming units per ml. Aliquots were drawn at an interval of 1 min for first 10 min and then at an hour interval for 30 h. Aliquots were plated in triplicate for counting the cfu/ ml after serial dilution in NSS. All tests were repeated thrice for conformity.

RESULTS

Results of antimicrobial activity of LGO using disc diffusion method revealed that 38.2% of 1114 strains of different microbes were sensitive. All molds (*Apergillus* spp., 11; *Penicillium* spp., 3), yeasts (*Candida albicans*, 7), *Lactobacillus acidophilus* (1) and *Morganella morganii* (3) strains tested were sensitive to LGO (Table. 1) while for other bacteria results varied with species of the microbes (Table 2, 3). The effect of reduced oxygen and enhanced carbon-di-oxide in incubating chamber was also evident, of the 8 *Enterococcus avium* strains tested simultaneously under aerobic and microaerobic conditions. Only three stains were resistant under aerobic incubation while six turned resistant under microaerobic incubation. Zone of inhibition also reduced significantly under microaerobic growth conditions.

Among the Gram negative bacteria there was a wide variation in sensitivity of bacterial strains to LGO discs among different genera and different species of a genus (Table 2). Although 78% aeromonads were sensitive to LGO, species wise analysis (Table 2) revealed that all strains of *A. media* (9), *A. schubertii* (8), *A. sobria* (3), *A. salmonicida* ssp. *smithia* (1), majority of the strains of *A. caviae* (10 of 12), *A. hydrophila* (15 of 18), *A. veronii* (13 of 14), *A. salmonicida* ssp. *salmonicida* (3 of 5) were sensitive to LGO discs. However, majority of the strains of *A. salmonicida* ssp. *achromogenes* (2 of 3) and *A. eucranophila* (10 of 18) were resistant to LGO. Many of the pseudomonads (46.4%) were sensitive but all strains of *P. aeruginosa* and *P. fluorescens* were resistant to LGO.

Among the members of Enterobacteriaceae majority of *Edwardsiella* (73.9%), and 50% of *Budvicia aquatica* and *Leminorella ghirmontii* strains were sensitive to LGO (Table 3). On the other hand, all *Hafnea alvei* (4), *Laclercia adecarboxylata* (1), *Xenorhabdus luminescens* (1) and majority of *Salmonella enterica* (98.3% of 59), *Citrobacter* spp. (93.7% of 95), *Providencia* spp. and *Kluyvera cryocrescens* (83.3% of 6 each), *Enterobacter* spp. (78.2% of 55), *Proteus* spp. (78% of 41), *Escherichia* spp. (77.7% of 112), *Serratia* spp., and *Erwinia ananas* (75% of 12 each), *Pragia fontium* (70.6% of 17), and *Klebsiella* spp. (62.7% of 110) strains were resistant to lemongrass oil (Table 1).

The only strains of *Edwardsiella hoshiniae* and 77.3% of 22 *E. tarda* were sensitive to LGO however, excepting a few strains of *Enterobacter agglomerans* (39.1% of 23) and two of the three strains of *E. amnigenus* group II along with one unidentified *Enterobacter* strain all *Enterobacter* strains belonging to other six species (Table 2) were resistant to LGO.

Out of 112 strains of *Escherichia*, 87 were resistant to LGO but 50% of *E. fergusonii* strains were sensitive. In contrast, 19.8% of *E. coli*, 33.3% of *E. blattae* and one of the two strains of *E. vulneris* were resistant to LGO. Among *Klebsiella* species strains, strains of *K. pneumoniae* were the most sensitive (40% of 95) while majority of *K. terrigena* (5 of 6) and *K. oxytoca* (7 of 9) were resistant to LGO. Similarly, of the 41 strains of four species of *Proteus,* 32 were resistant to LGO without much variation among different species except the only strain tested of *P. myxofaciens* (Table 2). On the same lines, out of 59 salmonellae 58 were resistant to LGO, the only one sensitive strain belonged to *S. enterica* ssp. *indica*, none of the *S. enterica* ssp. *houtenae* and *S. enterica* ssp. *salamae* strain was sensitive to LGO. All five strains of *Providencia rettgeri* but no strains of *P. haembachii* were resistant to LGO. All *Serratia* including *S. fonticola*, *S. marcescens*, *S. odorifera* and *S. plymuthica* strains were resistant to LGO but all the three strains belonging to *S. rubidiae* were sensitive to LGO. Similarly, only 92.3% strains of *Citrobacter freundii* and all strains of *C. diversus* and *C. amalonaticus* were resistant to LGO discs (Table 2).

Similar to Gram negative strains, variation in LGO sensitivity pattern was evident in Gram positive bacteria too (Table 3). Most of the *Bacillus* species strains (84.3%) and many of the streptococci (53.1%) were sensitive to LGO while majority of enterococci (73.7%) and staphylococci (69.8%) were resistant. None of the strains belonging to 11 *Bacillus* spp. (Table 3) was resistant to LGO; however, a few strains of *B. brevis* (25%), *B. coagulans* (19.6%), *B. pentothenticus* (6.3%) and all six *B. licheniformis* strains were resistant to LGO discs. Out of 213 strains of enterococci, 157 were resistant to LGO including all strains of *E. raffinosus* (5), *E. faecium* (11), and *E. malodoratus* (3) and majority of

the strains of *E. caseslliflavus* (26 of 32), *E. dispar* (26 of 29), *E. gallinarum* (13 of 16) and *E. hirae* (33 of 42) were resistant but all the unidentified enterococci and sole strains of *E. asacchrolyticus* and *E. solitarus* were sensitive to LGO. Both the strains of *Staphylococcus xylosus* were resistant but both the strains of *S. epidermidis* were sensitive; however, most of the strains of *S. sciuri* (82.6% of 23) and *S. aureus* (61.5% of 13) were resistant to LGO. Majority of the strains of streptococci were sensitive to LGO including all strains of *S. agalactiae*, most of the *S. mobilis* (61.9%) and 66.7 % of non-classified streptococcal strains; however, no strains of *S. milleri*, *S. alactolyticus* and *S. caseolyticus* was sensitive to LGO.

Determination of MIC through agar dilution method against resistant and sensitive strains of *Klebsiella pneumoniae* (CP62, M10, LT 81, LTLT121), *Escherichia coli* (E382, C91, P82, P86), *Edwardsiella tarda* (26P, 1BCY, 56LT1, 59LT3), *Bacillus coagulans* (CB1, CB6, A12, B17), *Staphylococcus aureus* (SK10S2, SK5S1, SK6S1, SKE111), *Streptococcus mobilis* (SV11, SV27NC, SV12, SV36NC), *Enterococcus faecalis* (SV7, SV20, E31, CV14NC) and *Candida albicans* (CV1PD, ABY42) revealed (Table 4) that all the strains tested sensitive to LGO (with disc diffusion method) had MIC ≤32 µg/ ml while those resistant had MIC ≥64 µg/ ml. Both the *C. albicans* strain had MIC 1 µg/ ml while for bacterial strains sensitive to LGO discs it ranged from 1 µg/ ml to 32 µg/ ml.

Studies to determine that the action of LGO on microbes is either microbiostatic or microbicidal, on cultures of LGO resistant *S. aureus* (SKE111) and LGO sensitive *E. coli* (E382) and *C. albicans* (ABY42), revealed that LGO was more active while bacteria were in NSS than they were in BHI. Both the sensitive cultures were killed within a minute while resistant *S. aureus* (SKE111) was detected even after 5 minutes but not at 10 minute of exposure of microbes to 1mg / ml in NSS, indicating the microbicidal action of LGO. On the other hand in BHI, LGO sensitive bacteria could be detected for 6 h and resistant strains was present up to 18 h of exposure. However, when cultures were suspended in NSS containing 0.01 mg/ ml of LGO it took 18 h to kill *C. albicans* and 24 h for killing *E. coli* strains but had no bactericidal effect on *S. aureus*. In BHI, LGO at 0.01 mg/ ml level was only bacteriostatic for *E. coli* (E382) and *C. albicans* (ABY42) while number of *S. aureus* (SKE111) started to increase after a bacteriostatic period of 3 h.

DISCUSSION

Of the 1114 strains of microbes tested for sensitivity to LGO discs (50µg LGO/ disc), 38.2% were sensitive and clear zone of growth inhibition (≥8 mm) was evident. Our observations revealed that all 14 fungal (*Aspergillus* spp., *Penicillium* species) and 7 yeast (*C. albicans*) strains were sensitive to LGO, and our findings are in concurrence to earlier reports (Abd-El Fattah et al., 2010; Abu-Seif et al., 2009; da Silva et al., 2008). Antifungal activity of LGO is proved to be due to its flavonoids (Pratt and Hudson, 1991; Nieto et al., 1993; Abu-Seif, et al., 2009) and phenolic compounds (Abu-seif et al., 2009). Due to LGO's antifungal activity it has been claimed as an effective fungi control agent suitable for protection of food (Patker et al., 1993). Although in earlier studies antimicrobial activity of LGO has been reported higher against bacteria than fungi and yeast (Helal et al., 2006) with a MIC for yeasts ~2 µl/ ml, in our study with *C. albicans* MIC was determined to be 1µg/ ml, it might be due to use of different strains in earlier studies (*Botrytis cinerea)* which might be more resistant than the strains of *C. albicans*. However we have not tested fungal strains for MIC of LGO but by analogy (that all strains tested sensitive with disc diffusion assay had MIC not more than 32 µg/ ml) we may predict that the isolates of *Penicillium*, *A. flavus* and *A. niger* also had MIC ≤32 µg/ ml which is much lower than that reported earlier 1.5 µl/ ml (Helal et al., 2006), it may be explained either on the basis of strain variation or the differences in LGO extracted from lemongrass in Nagaland and elsewhere.

LGO was effective against several Gram positive and Gram negative bacteria but its effectiveness cannot be generalized beyond certain levels of concentration. Observations revealed that all bacterial strains of a genus may not be equally sensitive as 115 *Bacillus* species strains (84.3%) and many of the streptococci (53.1%) were sensitive to LGO while majority of enterococci (73.7%) and staphylococci (69.8%) were resistant. Similarly among Gram negative bacteria 78% aeromonads, 73.9% *Edwardsiella* (73.9%) and 50% of *Budvicia aquatica* and *Leminorella ghirmontii* strains were sensitive to LGO while majority of *Salmonella enterica* (98.3% of 59), *Citrobacter* spp. (93.7% of 95), *Providencia* spp. and *Kluyvera cryocrescens* (83.3% of 6 each), *Enterobacter* spp. (78.2% of 55), *Proteus* spp. (78% of 41), *Escherichia* spp. (77.7% of 112), *Serratia* spp., and *Erwinia ananas* (75% of 12 each), *Pragia fontium* (70.6% of 17), and *Klebsiella* spp. (62.7% of 110) strains were resistant. But this resistance or sensitiveness is comparative and results vary with the concentration of LGO used. At higher concentration one may not find any strain resistant but at lower levels of LGO even the most sensitive may appear as resistant. Therefore we need to standardize the cut off limit for the concentration according to the tolerance of LGO. In earlier studies LGO is reported to possess potent bactericidal activity against Gram positive and Gram negative bacteria (Chao and Young, 2000; Onawunmi, 1989; Syed et al., 1995; Alam et al., 1994; Sharma et al., 2003; Saikia et al., 1999) but in most cases the concentration used to kill the bacteria was too high (1 to 100mg/ml) varying for different organisms (Ferdinand et al., 2009; Sue et al., 2008; Ohno et al., 2003). In our study, at 1mg/ ml concentration all the microbes tested including the resistant *S. aureus* were killed within 5 minutes while only those which were sensitive with disc diffusion method could be eliminated at 10 µg/ ml concentration. Similar results have been reported earlier

for *Hemophillus influenzae, S. pneumoniae, S. pyogenes* and *S. aureus,* inhibited at <12.5 µg/ ml, and *E. coli,* inhibited at at 100 µg/ ml concentration (Inouye et al., 2001).

Further, testing medium might also lead to variation in interpretation of sensitivity (Lalitha, 2004), in this study almost 100 fold higher concentration of LGO was needed to induce the same antibacterial effect when BHI was used as the medium instead of NSS. Therefore, the confusion regarding antimicrobial activity among different study in relation to MIC might be due to difference in the medium and the method used. Moreover, variation in disc concentration of herbal oil in different studies might be source of confusion while interpreting the results. Therefore, for uniformity a standard feasible (biologically achievable) concentration should be used in discs to determine sensitivity of different herbs, and 50 µg / disc concentration is quite feasible option to explore the affectivity of probable antimicrobial herbs.

The effect of oxygen deficient and CO_2 rich environment as expected under *in vivo* conditions was highly significant in reduction of the sensitivity of eight *E. avium* strains tested, indicating that the results of antimicrobial drug sensitivity particularly for LGO results obtained by general method of disc diffusion might lead to wrong perception of affectivity of the drug. The observations are in concurrence to reduction in antimicrobial activity of tobramycin, amikacin, and aztreonam under anaerobic conditions (King et al., 2010). However, more studies are required to understand effect of microaerophilic growth conditions on sensitivity of microbes and to have a broad idea about utility of general diffusion assay for facultative anaerobes and microaerophilic microbes.

It can be concluded from the observations that LGO is bactericidal and fungicidal at higher concentration (1mg/ ml) while bacteriostatic at lower concentrations (<10µg/ ml). Variation in LGO activity (MIC) on different strains of bacteria is inevitable as for most of the antimicrobials. All microbes are not equally susceptible to LGO as *Bacillus* spp. and streptococci among Gram positive and aeromonads and *E. tarda* among Gram negative bacteria are comparatively more susceptible to LGO than most of the other potentially pathogenic bacteria. Although number of yeast and mold strains was less (21) in the study, their uniform sensitivity was indicative of wide spectrum of LGO's antimicrobial action.

ACKNOWLEDGEMENTS

Authors are thankful to Naga Fragrance Pvt. Ltd. Dimapur, for providing LGO free of cost as and when required. Authors also thankful to Joint Director, Director of the ICAR Research Centre for NEH Region and Director NRC on Mithun, Jharnapani, Nagaland, for kindly providing the funds and facilities to conduct this study.

REFERENCES

Abd-El Fattah SM, Hassan ASY, Bayoum HM, Eissa HA (2010). The use of lemongrass extracts as antimicrobial and food additive potential in yoghurt. J. Am. Sci. 6: 582-594.

Abu-Seif FA, Abdel-Fattah ShM, Abo Sreia YH, Shaaban HA, Ramadan MM (2009). Antifungal properties of some medicinal plants against undesirable and mycotoxin-producing fungi. J. Agric. Mansuora Univ. 34: 1745-1756.

Aibinu I, Adenipekun T, Adelowowtan T, Ogunsanya T, 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.

Alam K, Agua T, Maren H, Taies R, Rao KS, Burrows I, Hubber ME, Rali T (1994). Preliminary screening of seaweeds, seagrass and lemongrass oil from Papua New Guinea for antimicrobial and antifungal activity. International J. Pharmacogn. 32: 396-399.

Alippi MA, Ringuelet JA, Cerimele EL, Re MS, Henning CP (1996). Antimicrobial activity of some essential oil against *Paenibacillus* larvae, the causal organism of Americal foulbrood disease. J Herbs Spices Med. Plants. 4: 9-16.

Ali-Shtayeh MS, Abu Ghdeib SI (1999). Antimycotic activity of twenty-two plants used in folkloric medicine in the Palestinian area for the treatment of skin diseases suggestive of dermatophyte infection. Mycoses. 42: 665-672.

Barnett JA, Payne RW, Yarrow D (2000). Yeasts: characteristics and identification. 3rd ed. Cambridge University Press. Cambridge, U.K.

Brian TS, Ikhlas AK (2002). Comparison of extraction methods for marker compounds in the essential oil of lemongrass by GC. J. Agric. Food Chem. 50: 1345-1349.

Chao SC, Young DG (2000). Screening of antibacterial activity of essential oils on selected bacteria, fungi and viruses. J. Essent. Oil Res. 12: 630-649.

Clinical and Laboratory Standards Institute (2008). Performance standards for antimicrobial susceptibility testing. Eighteenth informational supplement CLSI document M100-S18, Wayne.

Clinical and Laboratory Standards Institute (2009). Method for dilution antimicrobial susceptibility tests for bacterial that grow aerobically; approved standard - Eighth Edition. CLSI document M07-A8, Wayne.

da Silva CdB, Guterres SS, Weisheimer V, Schapoval EES (2008). Antifungal activity of the lemongrass oil and citral against *Candida* spp. Braz. J. Infect. Dis. 12: 1-14.

Depken KL (2011). Properties of Lemon Grass. http://www.ehow.com/about_5382246_properties-lemon-grass.html.

Ferdinand FJ, Esther U, Tayo A, Omotoyin A (2009). Evaluation of the antimicrobial properties of unripe banana (*Musa sapientum* L.), lemon grass (*Cymbopogon citratus* S.) and turmeric (*Curcuma longa* L.) on pathogens. Afr. J. Biotechnol. 8: 1176-1182.

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

Helal GA, Sarhan MM, Abu Shahla ANK, El-Khai EKA (2006). Antimicrobial activity of some essential oils against microorganisms deteriorating fruit juices. Mycobiol. 34: 219-229.

Holt JG, Sneath PHA, Mair MS, Sharpee ME (1986). Bergey,s manual of systematic bacteriology, vol. 2. Williams & Wilkins, 428 east Preston Street, Baltemore, MD 211202, U.S.A.

Huynh KP, Maridable J, Gaspillo P, Hasika M, Malaluan R, Kawasaki J (2008). Essential oil from lemongrass extracted by supercritical carbon dioxide and steam distillation. The Phillippine Agric. Sci. 91: 36-41.

Inouye S, Takizava T, Yamaguchi H (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J. Antimicrob. Chemother. 47: 565-573.

Cheel J, Theoduloz C, Rodríguez J, Schmeda-Hirschmann G (2005). Free radical scavengers and antioxidants from Lemongrass (*Cymbopogon citratus* (DC.) Stapf.). J. Agric. Food Chem. 53: 2511-2517.

King P, Citron DM, Griffith DC, Lomovskaya O, Dudley MN (2010). Effect of oxygen limitation on the in vitro activity of levofloxacin and other antibiotics administered by the aerosol route against *Pseudomonas aeruginosa* from cystic fibrosis patients. Diagnostic

Microbiol. Infect. Dis. 66: 181-186.
Lalitha MK (2004). Manual on Antimicrobial Susceptibility Testing. Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India. (www.ijmm.org/documents/Antimicrobial.doc)
Lawless J (1995). Illustrated Encyclopedia of Essential Oil : The Complete Guide to use of Oil in Aromatheraphy and Herbalism. Element Books: Rockport, MA, pp 56-67.
National Committee for Clinical Laboratory Standards (2003). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard - NCCLS document M7-A6, Wayne.
Nieto S, Gorrido A, Ssanhaeza J, Loyala LA, Marale G, Leighton FG, Valenzula A (1993). Flavonoid as stabilizers fish oil: an alternative to synthetic antioxidants. J. Am. Oil Chem. Soc. 70: 773-778.
Odugbemi T (2006). Medicinal plants as antimicrobials In: Outline and pictures of Medicinal plants from Nigeria. University of Lagos press. pp. 53-64.
Ohno T, Kita M, Yamaoka Y, Imamura S, Yamamoto T, Mitsufuji S, Kodama T, Kashima K, Imanishi I (2003) Antimicrobial activity of essential oils against *Helicobacter pylori*. J. Helicobacter. 8: 207-215.
Onawunmi GO (1989). Evaluation of the antimicrobial activity of citral. Lett. Appl. Microbiol. 9: 105-108
Patker KI, Usha CM, Shetty SH, Paster N, Lacey J (1993). Effect of spice oils on growth and aflatoxin B1 production by *Aspergillus flavus*. Lett. Appl. Microbiol. 17: 49-51.
Pearson O (2010). The Antibacterial Properties of Essential Oils. http://www.livestrong.com/article/168697-the-antibacterial-properties-of-essential-oils/# ixzz1W7u9hn6K.
Pratt DE, Hudson BJF (1991). Antioxidant activity of phenolic compounds in meat model systems. In phenol compounds in food and their effects on health. AC Symposium Serieus 506; American Chemical Society, 1990. Washington, DC, 1991; pp 214-222.
Raper K B, Fennell DI (1977). The genus *Aspergillus* Robert E. Krieger Publishing Company. Huntington, New York.
Saify ZS, Mustaq N, Noor F, Naqvi SBS, Mardi SA (2000). Antimicrobial activity of some commonly used herbs. Pakistan J. Pharm. Sci. 13: 1-3.
Saikia D, Shantha Kumar TR, Kaliol AP, Khanuja SPS (1999). Comparative bioevaluation of essential oil of three species of *Cymbopogon* for their antimicrobial activities. J. Med. Arom. Plant Sci. 21:24.
Sharma A, Tayung K, Baruah AKS, Sharma TC (2003). Antibacterial activity of lemongrass [*Cymbopogon fleuosus* (Steud) Wats] inflorescence oil. Indian Perfum. 47:389-393.
Sue C, Gary Y, Craig O, Karen N (2008). Inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) by essential oils. Flavour Frag. J. 23: 444–449.
Syed M, Qumar S, Riaz M, Choudhary FM (1995). Essential oil of the family *Gramineae* with antimicrobial activity part II. The antimicrobial activity of a local variety of *Cymbopogon citratus* and its dependence of storage. Pakistan J. Sci. Ind. Res. 38:146-148.
Ushimaru PI, Mariama TN, Luiz C, Di Luciano B, Ary FJ (2007). Antibacterial activity of medicinal plant extract. Braz. J. Microbial. 38:717-719.