JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2013. Vol. **7**(2), p. 1055-1063

Antibacterial Potentials of the Crude Dichloromethane Extract of *Garcinia kola* (Heckle) Seeds against some *Listeria* species Isolated from Wastewater Effluents

Penduka Dambudzo and Okoh I. Anthony*

Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, P. Bag X1314 Alice, 5700, South Africa.

(Received: 30 September 2012; accepted: 10 November 2012)

The anti-*Listerial* activities of the dichloromethane extract of *Garcinia kola* seeds were assessed against a panel of 42 *Listeria* bacteria. The extract was active against 19 of the isolates with the minimum inhibitory concentrations (MICs) ranging between 0.079 mg/ml and 0.313 mg/ml whilst the minimum bactericidal concentrations (MBCs) ranged between 0.625 mg/ml and 10 mg/ml. The extract's rate of kill against four representative *Listeria* isolates showed a concentration and time dependent profile, being more lethal to the bacteria at the highest concentration (4× MIC value) at the maximum exposure time of 2 h. The extract was bacteriostatic against *Listeria grayi* (LAL 15) giving a less than $3Log_{10}$ decrease in the viable cell counts after 2 h exposure time at all four MICs. However the extract was bactericidal against *Listeria ivanovii* (LEL 18) and *Listeria monocytogenes* (LAL 8) after 105 min and 120 min respectively at 4× MIC value. The extract was also bactericidal against *Listeria ivanovii* (LEL 30) achieving complete elimination of all the viable cells at 3× MIC and 4× MIC values after 90 min and 45 min exposure times respectively. These results therefore show the possible presence of therapeutic compounds in *Garcinia kola* seeds that have potential in listeriosis treatment.

Key words: *Listeria* species; Rate of kill; *Garcinia kola* seeds; MIC; Dichloromethane.

Antibiotics are naturally-occurring, semi-synthetic and/or chemically synthesised antimicrobial compounds used mainly in the treatment and prevention of diseases in both humans and animals and also as growth promoters in animal intensive industries [1]. The therapeutic use of an antibiotic, in either human or animal population, creates a selective pressure that favours survival of bacterial strains resistant to the antibiotic. The result is that many bacteria strains to which the antibiotic is used against become resistant to it, rendering the antibiotic ineffective as treatment of choice against that respective bacterial strain [2; 3]. Some of the mechanisms of resistance include: alteration of permeability barriers across bacterial outer membranes, prevention of antibiotic uptake through inhibiting its corresponding transport carrier, modification of the antibiotic' s target binding sites to prevent recognition of the antibiotic, and the ability by the bacteria to chemically and/or enzymatically degrade the antibiotic [1].

Unused and or unmetabolised antibiotic substances such as those from hospital effluents are sometimes disposed off into the sewage system [4]. The biological treatment process in a conventional wastewater treatment plant may result in a selective increase of the antibiotic resistant bacteria population and the increased occurrence of multi antibiotic resistant bacteria [5]. Wastewater treatment plants may facilitate the spread of antibiotics, antibiotic resistance genes and antibiotic resistance bacteria in the aquatic

* To whom all correspondence should be addressed.
Tel: +27-40-6022365; Fax: +27-086-6286824;
E-mail: aokoh@ufh.ac.za

environment as they link different aquatic environments including municipal sewage and surface waters [6]. The occurrence and spread of antibiotic resistant bacteria species is a major threat to public health as it is limiting treatment options thereby causing an increase in morbidity and mortality [5]. Most medicinal plants and their purified constituents have been proven to possess beneficial therapeutic potentials [7], such that they can be a useful and effective alternative in mitigating the spread of antibiotic resistance.

Garcinia kola is one such traditional medicinal plant that is evergreen and can be found in the equatorial forest of Sub-Saharan Africa where it grows wild and can also be domesticated due to its numerous medicinal values [8]. The plant is also known as "bitter kola" because of its bitter taste or "male kola" because of its claimed aphrodisiac activity [9]. *Garcinia kola* seeds form a major part of the herbal preparation used for the treatment of various respiratory tract diseases including asthma [10]. Studies by Olaleye and Farombi [11] showed that treatments with kolaviron extracted from the powdered seeds of *Garcinia kola* significantly inhibited gastric lesions produced by indomethacin and acidified ethanol in rats.

There are some studies that have proven the antibacterial activities of *Garcinia kola* seeds extracts *in-vitro* [12; 13; 14; 15], however information on the *anti-Listerial* activities of the seeds is very rare. *Listeria* species are Gram positive, facultatively anaerobic, psychrotrophic and catalase positive rod shaped bacterium [16]. The genus *Listeria* is composed of six species namely *Listeria grayi*, *Listeria innocua*, *Listeria ivanovii*, *Listeria welshimeri*, *Listeria seeligeri* and *Listeria monocytogenes*, however only *L. monocytogenes* and *L. ivanovii* are considered pathogenic [16; 17]. Human listeriosis is a food borne disease normally caused by *L. monocytogenes* [18], which because of its ubiquitous nature, commonly contaminates raw produce and, through cross-contamination infects other food items such that humans are routinely exposed to the organism [19], but the defined high risk groups to listeriosis are the pregnant, neonates, aged and immunocompromised persons [20].

Most reported cases of listeriosis present as life-threatening illness in one of three clinical syndromes: maternofetal listeriosis or neonatal listeriosis, blood stream infection, and meningoencephalitis [19]. Despite efficient antibiotic therapy, listeriosis is fatal in up to 30% of the cases making it a major public health threat [21]. A number of authors have reported the resistance of *Listeria* species to antibiotics [22; 23; 24; 25]. The need to provide alternative listeriosis treatment options becomes a necessity and in this paper, we report on the anti-*Listerial* activities of the dichloromethane extract of *Garcinia kola* seeds.

MATERIALS AND METHODS

Plant Material

The ground seed powder of *Garcinia kola* was obtained from the plant material collection of the Applied and Environmental Microbiology Research Group (AEMREG) laboratory, University of Fort Hare, Alice, South Africa.

Preparation of extracts

The method of Basri and Fan [26] was used to prepare the dichloromethane solvent extracts. A 100 grams measurement of the seed powder was steeped in 500 ml of the solvent for 48 h with shaking on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The resultant extract was centrifuged at 3000 rpm for 5 min at 4°C (Beckman Model TJ-6RS Centrifuge, Great Britain) and the supernatant filtered through Whatman No.1 filter paper while the residue was used in the second extraction process involving 300 ml of the solvent. The combined extracts were concentrated using a rotary evaporator at 50°C (Steroglass S.R.L, Italy), after which they were dried to a constant weight under a stream of air in a laminar flow cabinet at room temperature. Dimethyl sulphoxide (DMSO) at a concentration of 5% (v/v) was used to aid the reconstitution of the dried extract when making different test concentrations.

Test *Listeria* strains

The 42 test *Listeria* isolates used in this study were obtained from the culture collection of the Applied and Environmental Microbiology Research Group (AEMREG) laboratory at the University of Fort Hare, Alice, South Africa. The bacteria were previously isolated from wastewater effluents in the Eastern Cape Province of South Africa and belonged to three species groups which are *L. ivanovii*, *L. grayi* and *L. monocytogenes* [23].

Preparation of the Inoculum

Colonies were picked from 24 h old

cultures grown on nutrient agar and suspended in saline solution (0.85% NaCl) to give an optical density of approximately 0.1 at 600 nm for each organism. The suspension was then diluted a hundred-fold before use [27].

Antibacterial susceptibility test

The susceptibility of the *Listeria* bacteria to the extract was determined using the agar well diffusion method described by Irobi *et al*. [28] with modifications. A 100 µl volume of the prepared bacterial suspension (100 µl) was inoculated into sterile molten Mueller-Hinton agar medium at 50ºC in a MacCarthney bottle, mixed and poured into a sterile petri dish. A sterile 6 mm diameter cork borer was used to bore wells into the solidified agar medium after which approximately 100 µl of 10 mg/ml extract solution was put in the wells. The plates were then left to stand on the laboratory bench for 1 h to allow proper diffusion of the extract into the medium before incubation at 37ºC for 24 h, and thereafter the zones of inhibition were observed and measured. Ciprofloxacin (2 µg/ml) was used as a positive control, and sterile distilled water was used as the negative control while 5% DMSO was also tested to determine its effect on each organism.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MICs of the susceptible *Listeria* bacteria were determined using the broth microdilution assay method of EUCAST [27] and carried out in sterile disposable flat-bottomed 96-well microtiter plates. Two-fold serial dilutions using sterile distilled water were carried out from 10 mg/ml of the stock plant extract to make 9 test concentrations ranging from 0.039 to 10 mg/ml. The assay procedures follow after our recent report [15]. Double strength Mueller-Hinton broth (100 µl) was introduced into all the 96 wells. Column 1 was used as the sterility wells containing 100 µl of sterile distilled water in addition to the 100 µl of Mueller-Hinton broth, column 2 was used as the positive

Table 1. The anti *Listerial* activities of ciprofloxacin and the crude dichloromethane extract of *Garcinia kola* seeds

Organism	Zones of inhibition (mm)		Organism	Zones of inhibition (mm)	
	DCM	Cipro		DCM	Cipro
L. grayi (LAL 13)	0	20±3.055	*L. ivanovii* (LEL 18)	11±0.577	20±3.215
L. ivanovii (LEL 17)	8±0	19±1.528	*L. ivanovii* (LEL 29)	0	8±0.577
L. ivanovii (LEL 30)	10±1.528	30±0.577	*L. ivanovii* (LEL 15)	0	13±2.082
L. ivanovii (LDB 11)	9±0.577	20±1	*L. ivanovii* (LDB 9)	8±0.577	25±2.082
L. ivanovii (LEL9)	10±1.155	16±2.082	*L. ivanovii* (LDB 10)	13±1.155	25±0.577
L. ivanovii (LEL 1)	13±2.646	17±0.577	*L. ivanovii* (LEL 2)	0	28±1.528
L. ivanovii (LEL 5)	0	11±0.577	*L. ivanovii* (LEL 6)	0	11±1.732
L. ivanovii (LEL 3)	0	35±3.055	*L. ivanovii* (LEL 4)	0	14±1
L. ivanovii (LEL 19)	0	25±4.041	*L. ivanovii* (LEL 10)	0	20±2.082
L. ivanovii (LAL 9)	11±0.577	25±1.732	*L. ivanovii* (LAL 11)	10±0.577	17±2.646
L. grayi (LAL 12)	9±1.155	17±1.155	*L. ivanovii* (LAL 10)	11±0	15±2.082
L. grayi (LAL 15)	11±1.732	18±2.082	*L. ivanovii* (LAL 14)	0	30±2.517
L. ivanovii (LDB 1)	0	15±2.082	*L. ivanovii* (LDB 2)	0	14±0
L. ivanovii (LAL 6)	0	19±1.155	*L. ivanovii* (LAL5)	0	20±1.528
L. ivanovii (LAL 7)	0	20±1.528	*L. monocytogenes* (LAL 8)	13±0.577	12±1
L. ivanovii (LDB 7)	16±0.577	27±0.577	*L. ivanovii* (LDB 12)	14±1	25±1.528
L. ivanovii (LDB 3)	11±1	15±1	*L. ivanovii* (LDB 8)	0	20±1.732
L. ivanovii (LEL 7)	0	9±1	*L. ivanovii* (LEL 8)	0	30±1.528
L. ivanovii (LEL 14)	0	35±2	*L. ivanovii* (LEL 16)	12±1	15±1.528
L. grayi (LAL 3)	0	13±3.055	*L. ivanovii* (LAL 4)	0	20±2
L. ivanovii (LAL 2)	13±2.082	16±1	*L. ivanovii* (LAL 1)	0	20±2

Keynotes: DCM denotes dichloromethane, Cipro denotes ciprofloxacin, number±number denotes mean zone of inhibition±standard deviation whereby each observation is a mean±SD of 3 replicate experiments (n=3), mm denotes millimeters

control wells containing 100 µl of the broth, 50 µl of ciprofloxacin and 50 µl of the test organism whilst column 3 was used as the negative control wells containing 100 µl of the broth, 50 µl sterile distilled water and 50 µl of the test organism whilst columns 4 to 12 were used as test wells containing 100 µl of the broth, 50 µl of the test extract concentration and 50 µl of the test organism. The plates were then incubated at 37°C for 18-24 h. Results were read visually by adding 40 µl of 0.2 mg/ml of ρ-iodonitrotetrazolium violet (INT) dissolved in sterile distilled water into each well [29]. A pinkish coloration is indicative of microbial growth because of their ability to convert INT to red formazan [30]. The MIC was recorded as the lowest concentration of the extract that prevented the appearance of visible growth of the organism after 24 h of incubation [27].

The method of Sudjana *et al.* [31] was used to determine the MBC from the MIC broth microdilution assays through subculturing 10 µl volumes from each well that did not exhibit growth after 24 h of incubation and spot inoculating it onto Mueller-Hinton agar plates. The plates were incubated for 48 h after which the numbers of viable colonies were counted. The MBC was defined as the lowest concentration killing more than or equal to 99.9% of the inoculum compared with initial viable counts [31].

Rate of kill assay

The time kill assay was done according to the method of Odenholt *et al.* [32] as described by Akinpelu *et al.* [33]. The selected test *Listeria* isolates namely *L. ivanovii* (LEL 18), *L. grayi* (LAL 15), *L. monocytogenes* (LAL 8) and *L. ivanovii* (LEL 30) were used for the rate of kill studies as representatives of the *Listeria* species used in the study. The turbidity of the 18 h old test *Listeria* was first standardized to 10^8 cfu/ml. Four different concentrations of the plant extract were made starting from the MIC to 4×MIC value for each test organism. A 0.5 ml volume of each organism suspension was added to 4.5 ml of the extract's different concentrations, held at room temperature and the rate of kill determined over a period of 2 h. After 15 min intervals a 0.5 ml volume of each suspension was withdrawn and transferred to 4.5 ml of nutrient broth recovery medium containing 3% "Tween 80" to neutralize the effects of the antimicrobial compound carryovers on the test

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration values of dichloromethane extract of *Garcinia kola* seeds against *Listeria* species

Organism	Dichloromethane extract MIC (mg/ml)	MBC (mg/ml)
L. ivanovii (LEL9)	0.157	10
L. ivanovii (LEL 18)	0.079	10
L. ivanovii (LAL 10)	0.079	10
L. ivanovii (LEL 30)	0.157	0.625
L. ivanovii (LEL 16)	0.157	10
L. monocytogenes (LAL 8)	0.079	5
L. ivanovii (LDB 12)	0.157	10
L. ivanovii (LDB 10)	0.079	10
L. ivanovii (LEL 1)	0.079	10
L. ivanovii (LAL 11)	0.079	10
L. ivanovii (LDB 3)	0.079	5
L. grayi (LAL 15)	0.079	10
L. grayi (LAL 12)	0.313	10
L. ivanovii (LDB 11)	0.079	10
L. ivanovii (LAL 2)	0.313	10
L. ivanovii (LEL 17)	0.079	10
L. ivanovii (LDB 7)	0.079	5
L. ivanovii (LDB 9)	0.079	5
L. ivanovii (LAL 9)	0.079	10

organisms [33]. The suspension was then serially diluted and 0.5 ml was plated out for viable counts and incubated at 37 °C for 48 h. The control plates contained the test organism without the plant extract. The emergent colonies were counted and compared with the counts of the culture control.

Statistical Analysis

The SPSS 19.0 version for windows program was used to determine the means and standard deviations of the zones of inhibitions results, with the one way analysis of variance (ANOVA) of the same program being used to determine the means and standard deviations of the rate of kill results. All experiments were carried out in triplicates.

RESULTS

Antibacterial susceptibility test

The results of the antibacterial susceptibility test are as shown in Table 1. The zones of inhibition ranged from 8-16 mm and 19 out of the 42 isolates were susceptible to the extract. The highest zone of inhibition was observed against *L. ivanovii* (LDB 7), whilst the lowest zones of inhibitions were observed against *L. ivanovii* (LEL 17) and *L. ivanovii* (LDB 9). The 5% DMSO and the sterile distilled water negative controls had no antibacterial activity on all the tested *Listeria* isolates.

MIC and MBC

The results of the MIC and MBC of the extract are as shown in Table 2. The MICs ranged between 0.079 mg/ml and 0.313 mg/ml, of which the extract had MIC values of 0.079 mg/ml against 13 *Listeria* isolates, of 0.157 mg/ml against 4 isolates and of 0.313 mg/ml against 2 isolates. The MBC values ranged from 0.625 mg/ml to 10 mg/ml with the lowest MBC value of 0.625 mg/ml being recorded against *L. ivanovii* (LEL 30) isolate only. The extract had an MBC value of 5 mg/ml against four isolates, whilst against the remaining 14 isolates it had an MBC value of 10 mg/ml.

Rate of kill

The highest number of viable cells killed was noted at the maximum exposure time of 2 h at all the concentrations tested with the highest concentration (4× MIC) being most lethal for all

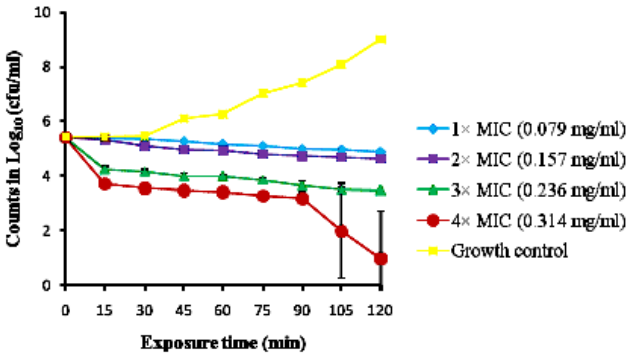

Fig. 1. Rate of kill for the dichloromethane extract of *Garcinia kola* seeds against *L. ivanovii* (LEL 18)

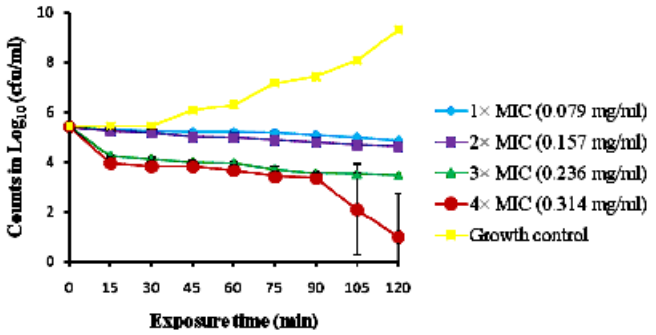

Fig. 2. Rate of kill for the dichloromethane extract of *Garcinia kola* seeds against *L. monocytogenes* (LAL 8)

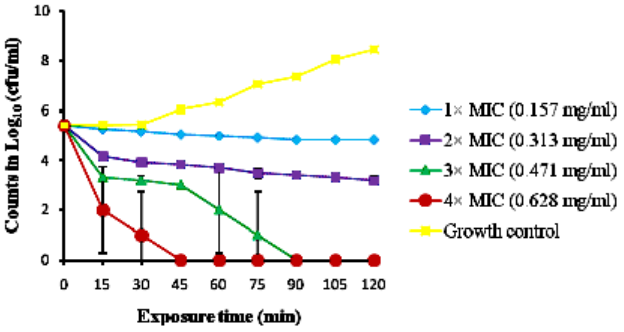

Fig. 3. Rate of kill for the dichloromethane extract of *Garcinia kola* seeds against *L. ivanovii* (LEL 30)

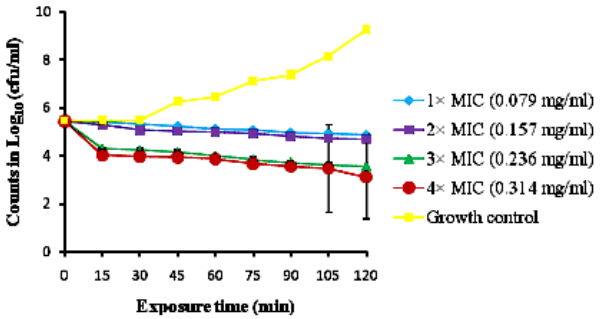

Fig. 4. Rate of kill for the dichloromethane extract of *Garcinia kola* seeds against *L. grayi* (LAL 15)

the four test isolates. The extract was bactericidal against *L. ivanovii* (LEL 18) (Fig. 1) and *L. monocytogenes* (LAL 8) (Fig. 2) at 105 min and 120 min at 4×MIC values only. The extract was also bactericidal against *L. ivanovii* (LEL 30) (Fig. 3) at 15 min at 4× MIC value and after 60 min at 3× MIC value and also went on to achieve a complete elimination of all viable cells of the organism after 45 min at 4× MIC value and after 90 min at 3× MIC value of the extract. However for *L. grayi* (LAL 15) (Fig. 4) the extract was bacteriostatic at all MICs even after 2 h exposure time achieving a maximum of 2.347Log_{10} decrease in viable cell count after 2 h at 4×MIC value concentration.

DISCUSSION

The dichloromethane extract of *Garcinia kola* seeds was active against 45% of the test bacteria including the pathogenic species *L. ivanovii* and *L. monocytogenes*. The MIC values ranged from 0.079 mg/ml to 0.313 mg/ml whilst the MBC values ranged between 0.625 mg/ml and 10 mg/ml. In a similar study [34] involving the dichloromethane extract of *Garcinia kola* seeds against *Vibrio* species which are Gram negative bacteria we reported higher MICs and MBCs ranging between 0.313 mg/ml – 0.625 mg/ml and 5 mg/ml – 10 mg/ml respectively. Similary results from studies by Sibanda and Okoh [35] also showed the Gram positive bacteria tested in that particular study to be more susceptible than the Gram negative bacteria to the aqueous and acetone extracts of *Garcinia kola* seeds. These findings suggest that the active compounds in *Garcinia kola* seeds have broad spectrum activity and they are more antagonistic towards Gram positive bacteria than Gram negative ones.

Rate of kill curves are used to determine the kinetics of bacterial killing *in- vitro* and can be used in distinguishing whether bacterial killing is concentration and/or time dependent [36]. In this particular study the rate of kill studies showed a concentration and time dependent characteristic for all the four test *Listeria* isolates as shown in Fig.1 to Fig 4 since an increase in the concentration of the extract from MIC value to 4×MIC value resulted in more bacteria cells being killed in shorter exposure times and also for each MIC value the highest

bacterial cells were killed at the maximum exposure time of 2 h. For an antibacterial agent to be termed bactericidal it should be able to kill bacteria by achieving a ≥99.9% or ≥3log$_{10}$ reduction in viable bacterial density, whilst a bacteriostatic agent does not reach the above required killing activity points [36]. The extract proved to bactericidal against three of the tested *Listeria* isolates namely *L. ivanovii* (LEL 18), *L. monocytogenes* (LAL 8) at 4×MIC value only and *L. ivanovii* (LEL 30) at 4×MIC and at 3×MIC values only and this was within the 2 h exposure time whilst it was bacteriostatic against *L. grayi* (LAL 15) at all test concentrations even at the maximum exposure time of 2 h used in the study. The results suggest that the extract can be either bactericidal or bacteriostatic against *Listeria* species, which is not an unexpected result in any antibacterial agent [36], although in this instance the extract appears to be more of a bactericidal nature than of a bacteriostatic one since it was bactericidal against three of the four tested isolates.

Phytochemical analysis of the crude methanolic extract of *Garcinia kola* seeds showed the presence of flavonoids, tannins, cardiac glycoside, steroids, saponins and reducing sugars which are known to play vital roles in the bioactivity of medicinal plants [33]. Dichloromethane solvent as shown by some studies on plants can also extract some of these bioactive compounds from plant material such as saponins and tannins [37] and steroids [38]. Besides these phytochemicals, dichloromethane solvent is also known to extract essential oils from plant material [39], of which most plant species are known to exhibit antimicrobial activity due to their essential oils content. anti-*Listerial* activities of different plants' essential oils have also been reported by several authors[40-43]. The mode of action of essential oils and their components is based on their lipophilic nature, which enables them to partition the lipids of the bacterial cell membrane. This disrupts the membrane's intergrity causing a loss of chemiosmotic control which leads to bacterial cell death [44; 45; 46.] Studies by Aniche and Uwakwe [47] have shown the presence of essential oils in *Garcinia kola* seeds which also may have attributed to the observed antibacterial activities in this study.

CONCLUSION

The dichloromethane extract of *Garcinia kola* seeds has been shown in this study to exhibit *anti-Listerial* activities which could be bacteriostatic or bactericidal in nature. Isolation and characterization of the active compounds in the extract remain the vital follow up steps and these are subjects of ongoing research in our group.

ACKNOWLEDGEMENTS

This study has been made possible by a grant from the National Research Foundation (NRF) of South Africa.

REFERENCES

1. Jury, K.L., Vancov, T., Stuetz R.M., Khan, S.J. Antibiotic resistance dissemination and sewage treatment plants. *Curr. Res. Tech. Edu. Topics. Appl.Microbiol.Microb.Biotechnol*, A. Mendez-Vilas (ed)., 2010; pp 509-519.
2. Altekruse, S.F., Cohen, M.L., Swerdlow, D.L. (1997). Emerging Foodborne diseases. *Emerg. Infect. Dis*., 1997; **3**(3): 285-293.
3. Tenover, F.C. Mechanisms of antimicrobial resistance in bacteria. *The. Amer. J. Med*., 2006; 119 (6A): S3-S10.
4. Kummerer, K., Significance of antibiotics in the environment. *J. Antimicrob. Chemother*., (2003) 2002; 52: 5-7.
5. Zhang, Y., Marrs, C.F., Simon, C., Xi, C. Wastewater treatment contributes to selective increase of antibiotic resistance among *Acinetobacter* spp. *Sci. Total. Env*., 2009; **407**: 3702–3706.
6. Schluter, A., Szczepanowski, R., Puhler, A., Top, E.M. Genomics of IncP-1antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. *FEMS. Microbiol. Rev*., 2007; **31**: 449-477.
7. Venkatachallam, S.K.T., Pattekhan, H., Divakar, S., Kadimi, U.S. Chemical composition of *Nigella sativa* L. seed extracts obtained by supercritical carbon dioxide. *J. Food. Sci. Technol*., 2010; **47**(6): 598–605.
8. Oze, G., Okoro, I., Obi, A., Nwoha, P. Hepatoprotective role of *Garcinia kola* (Heckel) nut extract on methamphetamine: Induced neurotoxicity in mice. *Afr. J. Biochem. Res*., 2010; **4**(3): 81-87.

9. Uko, O.J., Usman, A., Ataja, A.M. Some biological activities of *Garcinia kola* in growing rats. *Vet. Arhiv*., 2001; **71**(5): 287-297.
10. Okojie, A.K., Ebomoyi, M.I., Ekhator, C.N., Emeri, C.O., Okosun, J., Onyesu, G., Uhuonrenren, O., Atima J. Review of physiological mechanisms underlying the use of *Garcinia kola* in the treatment of Asthma. *Intern. J. Pulmon. Med*., 2009; 11 (1).
11. Olaleye, S.B., Farombi, E.O. Attenuation of Indomethacin- and HCl/ Ethanol-Induced Oxidative Gastric Mucosa Damage in Rats by Kolaviron, A Natural Biflavonoid of *Garcinia kola* Seed. *Phytother. Res*., 2006; **20**: 14-20.
12. Ofokansi, K.C., Mbanefo, A.N., Ofokansi, M.N., Esimone, C.O. Antibacterial Interaction of Crude Methanol Extract of *Garcinia kola* Seed with Gatifloxacin. *Trop. J. Pharm. Res*., 2008; **7**(4): 1159-1165.
13. Okigbo, R.N., Mmeka, E.C. Antimicrobial effects of three tropical plant extracts on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *Afr. J. Trad. CAM*., 2008; **5**(3): 226-229.
14. Sibanda, T., Olaniran, A.O., Okoh, A.I. In-vitro antibacterial activities of crude extracts of *Garcinia kola* seeds against wound sepsis associated *Staphylococcus* strains. *J. Med. Plants. Res*., 2010; **4**(8): 710-716.
15. Penduka, D., Okoh, O.O., Okoh, A.I. In-vitro antagonistic characteristics of crude aqueous and methanolic extracts of *Garcinia kola* (Heckel) seeds against some *Vibrio* bacteria. *Mol*., 2011; **16**(4): 2754-2765.
16. Warriner K., Namvar A. What is the hysteria with *Listeria*? *Trends. Food. Sci. Technol*., 2009; **20**: 245-254.
17. Rocourt J., Buchrieser C.: The genus *Listeria* and *Listeria monocytogenes*: phylogenic position. In: *Listeria, listeriosis and food safety* (Ryser ET, Marth EH, eds). CRC Press, 2007; pp 1-20.
18. Singh, J., Batish, V.K., Grover, S. Simultaneous detection of *Listeria monocytogenes* and *Salmonella* spp. in dairy products using real time PCR-melt curve analysis. *J. Food. Sci. Technol*., 2012; **49**(2): 234–239.
19. Swaminathan, B., Gerner-Smidt, P. The epidemiology of human listeriosis. *Microb. Infect*., 2007; **9**: 1236-1243.
20. Zunabovic, M., Domig, K.J., Kneifel, W. Practical relevance of methodologies for detecting and tracing of *Listeria monocytogenes* in ready-to-eat foods and manufacture environments - A review. *LWT. Food. Sci. Technol*., 2011; **44**: 351-362.
21. Yucel, N., Citak, S., Onder, M. Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. *Food. Microbiol*., 2005; **22**: 241-245.
22. Rahimi, E., Ameri, M., Momtaz, H. Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. *Food. Cont*., 2010; **21**: 1448-1452.
23. Odjadjare, E.E.O., Obi, L.C., Okoh, A.I. Municipal wastewater effluents as a source of *Listerial* pathogens in the aquatic milieu of the Eastern Cape Province of South Africa: A concern of public health importance. *Int. J. Environ. Res. Public. Health*., 2010; **7**: 2376-2394.
24. Acciari, V.A., Torresi, M., Migliorati, G., Di Giannatale, E., Semprini, P., Prencipe, V. Characterisation of *Listeria monocytogenes* strains isolated from soft and semi-soft cheeses sampled in a region of Italy. *Vet. Ital*., 2011; **47**(1): 15-23.
25. Adetunji V.O., Isola T.O. Antibiotic resistance of *Escherichia coli*, *Listeria* and *Salmonella* isolates from retail meat tables in Ibadan municipal abattoir, Nigeria. *Afr. J. Biotechnol*., 2011; **10** (30): 5795- 5799.
26. Basri D.F., Fan S.H. The potential of aqueous and acetone extracts of galls of *Queercus infectoria* as antibacterial agents. *Indian. J. Pharmacol*., 2005; **37**: 26-29.
27. EUCAST (European Committee for Antimicrobial Susceptibility Testing). Determination of Minimum lnhibitory Concentration (MICs) of antimicrobial agents by broth dilution. *Clin. Microbiol. Infect*., 2003; **9**: 1-7.
28. Irobi O.N., Young M., Anderson W.A. Antimicrobial activity of Annato *(Bixaorella)* extract. *Int. J. Pharmacog*., 1996; **34**: 87-90.
29. Eloff, J.N. A sensitive and quick microplate method to determine the minimum inhibitory concentration of plants extracts for bacteria. *Planta. Med*., 1998; **64**: 711-713.
30. Iwalewa, E.O., Suleiman, M.M., Mdee, L.K., Eloff, J.N. Antifungal and antibacterial activities of different extracts of *Harungana madagascariensis* stem bark. *Pharm. Biol*., 2009; **47**(9): 878-885.
31. Sudjana, A.N., D'Orazio, C., Ryan, V., Rasool, N., Ng, J., Islam, N., Riley, T.V., Hammer, K.A. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. *Int. J. Antimicrob. Agents*., 2009; **33**: 461-463.
32. Odenholt, I., Lowdin, E., Cars, O. Pharmacodynamics of telithromycin in vitro against respiratory tract pathogens. *Antimicrob. Agents. Chemother*., 2001; **45**(1): 23-29.

33. Akinpelu, D.A., Adegboye, M.F., Adeloye, O.A., Okoh, A.I. Biocidal activity of partially purified fractions from methanolic extract of *Garcinia kola* (Heckel) seeds on bacterial isolates. *Biol. Res*., 2008; **41**: 277-287.
34. Penduka, D., Okoh, A.I. In-vitro antagonistic activities of crude dichloromethane extracts of *Garcinia kola* (Heckel) seeds against potentially pathogenic *Vibrio* species. *J. Med. Plants. Res*., 2011; **5**(10): 2071-2077.
35. Sibanda, T., Okoh, A.I. *In-vitro* antibacterial regimes of crude aqueous and acetone extracts of *Garcinia kola* seeds. *J. Biol. Sci*., 2008; **8**(1): 149-154.
36. Pankey, G.A., Sabath, L.D. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram positive bacterial infections. *Clin. Infect. Dis*., 2004; **38**: 864-870.
37. Doughari, J.H., El-mahmood, A.M., Tyoyina, I. Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). *Afr. J. Pharm. Pharmacol*., 2008; **2**(1): 007-013.
38. Nazifi, E., Delazar, A., Movafeghi, A., Hemmati, S., Nazemiyeh, H., Nahar, L., Sarker, S.D., GC-MS Analysis of the Dichloromethane Extract of the Bulbs of *Ornithogalum cuspidatum* Bert. (Family: Liliaceae) from Iran. *Rec. Nat. Prod*., 2008; **2**(3): 94-99.
39. Gamiz-Gracia, L., Luque de Castro, M.D. Continuous subcritical water extraction of medicinal plant essential oil: comparison with conventional techniques. *Talanta*., 2000; **51**: 1179-1185.
40. Smith-Palmer, A., Stewart, J., Fyfe, L. The potential application of plant essential oils as natural food preservatives in soft cheese. *Food. Microbiol*., 2001; **18**: 463-470.
41. Singh, A., Singh, R.K., Bhunia, A.K., Singh, N. Efûcacy of plant essential oils as antimicrobial agents against *Listeria monocytogenes* in hotdogs. *Lebensm-Wiss.u Technol*., 2003; **36**: 787-794.
42. Sokovic, M., Marin, P.D., Brkic, D., van Griensven, L.J.L.D. Chemical Composition and Antibacterial Activity of Essential Oils of Ten Aromatic Plants against Human Pathogenic Bacteria. *Food*., 2007; **1**(1).
43. Celikel, N., Kavas, G. Antimicrobial properties of some essential oils against some pathogenic microorganisms. *Czech. J .Food Sci*., 2008; **26**(3): 174-181.
44. Sikkema, J., de Bant, J.A.M., Poolman, B. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev*., 1995; **59**(2): 201-222.
45. Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R., Wyllie, S.G. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J. Appl. Microbiol*., 2000; **88**: 170-175.
46. Prabuseenivasan, S., Jayakumar, M., Ignacimuthu, S. In vitro antibacterial activity of some plant essential oils. BMC CAM., 2006; **6**: 39.
47. Aniche G.N., Uwakwe G.U. Potential use of *Garcinia kola* as hop substitute in lager beer brewing. *World. J. Microbiol. Biotechnol*., 1990; **6**: 323-327.