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# Antimicrobial drug resistance against *Eucalyptus citriodora* gum in strains of common microbes of public health concern isolated from food, animals and environment

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## ABSTRACT

Of the 812 strains of microbes belonging to 27 genera (isolated from food, fish-pond-water, lizards, cow, pigs and mithun) tested against *Eucalyptus citriodora* gum (EG), 157 (19.3%) strains were detected sensitive. All the strains detected sensitive through disc diffusion method had an MIC 0.25 mg to 5 mg/ ml while those resistant had an MIC of 10 mg to 25 mg/ ml. Significantly ($p < 0.01$) more number of microbial strains isolated from lizards (91.6%) and and mithuns (88.4%) were resistant to EG than strains of cattle (66.7%), pig (65.1%), water (75%) and food origin (65.8%). However, there was no significant difference in sensitivity pattern of strains of lizard and mithun origin (p, 0.33) also among strains of pig, cattle, water and food origin (p, 0.96). Resistant and sensitive strains were distributed among majority of them genera. All the strains of *Aeromonas salmonicida* ssp. *achromogenes* (2), *A. sobria* (2), *Citrobacter amalonaticus* (11), *Edwardsiella hoshiniae* (1), *Escherichia blattae* (3), *Hafnea alvei* (3), *Klebsiella oxytoca* (8), *Kluyvera cryocrescens* (6), *Lactobacillus acidophillus* (1), *Leclercia adecarboxylata*, *Proteus myxofacience* (1), *Raoultella terrigena* (6), *Salmonella enterica* ssp. *houtenae* (3), *Salmonella enterica* ssp. *salamae* (11), *Serratia fonticola* (1), *Se. Marcescens* (2), *Se. odorifera* (5), *Se. plymuthica* (1), *Streptococcus milleri* (3), *Str. alactolyticus* (1) and *Xenorhhabdus luminiscens* (1) were resistant to EG. On the other hand, all strains of *Candida albicans* (1), *Leminorella ghrimontii* (1), *Micrococcus* spp. (2), *Providencia heimbachae* (1), *Staphylococcus aureus* (4), *Staph. xylosus* (2) and *Streptococococcus caseolyticus* (1) were sensitive to EG. It may be an important question to ponder upon why majority of strains of certain bacteria were resistant to EG and a few strains were sensitive viz., *Citrobacter freundii* (3 of 74), *Erwinia ananas* (2 of 12), *Escherichia coli* (6 of 73), *Klebsiella pneumoniae* ssp. *pneumoniae* (4 of 63), *Proteus penneri* (3 of 16), *Pragia fontium* (1 of 14), *Providencia rettgeri* (1 of 5), *Salmonella enterica* ssp. *indica* (1 of 45). Similarly, resistance in a few strains of some species comprising mostly sensitive EG strains was also puzzling, such strains were detected among *Staph. sciuri* (3 of 17), *Proteus mirabilis* (3 of 8) and *Aeromonas caviae* (4 of 11). These exceptions may help in future in understanding the factors responsible for resistance or sensitivity to EG. The study has indicated that drug resistance against herbal products is common in strains of some species of microbes while in others it is rare.



## KEYWORDS

*Eucalyptus citrodora* gum;
*Salmonella enterica;*
*Escherichia coli;*
*Klebsiella pneumonia;*
*Raoultella terrigenae*;
*Staphylococcus aureus;*
*Bos frontalis;*
Axone.

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## INTRODUCTION

Indiscriminate therapeutic use of antimicrobials in medical, veterinary, agriculture and aquaculture, and much more in factory farming has lead to emergence of multiple drug resistant (MDR) and total drug resistant (TDR) super-bugs causing infections almost impossible to treat. Indiscriminate use of antibiotics daily is encouraging the development of drug resistance in bacteria. Such bacteria are able to transfer their resistance to other related bacterial strains. Stephen H Buhner[1] states "In a way that no researcher understands, bacteria learn resistance to multiple antibiotics from encountering only one antibiotic".

The use of herbs to treat diseases is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The WHO[2] estimated that 80 percent of the population of some Asian and African countries presently use herbal medicine. Much of the world population has only limited access to prescription drugs and is dependent on plant derived alternative therapies. Such alternative therapies are also perceived to be as effective as prescription drugs but with fewer side effects[3].

It is hypothesized that most of the bacteria are sensitive to herbal drugs! and there is no resistance development.

The top 15 antibiotic herbs used since ages includes acacia, aloe, cryptolepsis, echinacea, eucalyptus, garlic, ginger, golden seal, grapefruit seed extract, honey, juniper, licorice, sage, usnea and wormwood[1]. *Eucalyptus*, the gum tree, is an invasive worldwide plant which attracted attention from researchers and environmentalist because of its fast growth, multi-utility aromatic oil and its ability to drain swamps. More than 170 species, varieties and provenances of eucalypt have been tried in India[4] however only few have been grown at plantation scale including *E. hybrid, E. grandis, E. citriodora, E. globulus* and *E. camaldulensis*[5]. In North eastern India eucalyptus plantation is dominated by *E.citriodora*. This plantation helped not only in economic upliftment of the villagers but also in keeping the affected ecology in balance due to felling of perennial trees and practice of jhumming.

The eucalyptus oil has been used as component in pharmaceutical preparations to relieve the symptoms cold and flu, in products to soothe the bronchitis irritation as cough sweets, lozenges, ointments, inhalants due to its decongestant and antimicrobial activity[6,7]. Cineole in the oil controls secretion of inflammatory cytokines thus the mucus secretion and asthmatic attacks[8]. Eucalyptus oil not only acts against infectious agents but also modulate immune system[9]. Eucalyptus oil (EO) is a valuable topical anti-inflammatory and analgesic to become an ingredient of most of the liniments[10,11]. On the other hand gum of eucalyptus, the dried gummy exudate from injured bark of eucalyptus, which is mostly composed of kinotannic acid, kino red, glucoside, catechol, and pyrocatechol, is used in medicine as a strong astringent useful as antidiarrhoeal and haemostatic agent on injuries, since more than 100 years[12,13]. Although eucalyptus is famous as gum tree and its gum has been used in medicine, information is scant on antimicrobial activity of eucalytus gum (EG). Therefore, in the present investigation eucalyptus gum collected from *E. citriodora* in Jharnapani, Nagaland was evaluated for its antimicrobial effect on some important pathogens of zoonotic importance isolated from environment, food, water, pigs, cow and mithun (*Bos frontalis*).

## MATERIALS AND METHODS

### Eucalyptus gum

Reddish-brown resin was collected from 6-7 year old *Eucalyptus citriodora* trees in foot hills of Jharnapani, Nagaland. It was dried at 50°C for three days, cleaned and crushed to powder. Solution of powder was made (25%) in pure (99%) ethanol at 25°C on a rotary shaker for 18-24 hr. Each ml of alcoholic solution was adjusted to contain 250 mg of gum. The solution was stored at 20°C till used to make its dilutions in buffered peptone water or adsorbed on 6 mm sterile filter-paper discs (Hi-Media Mumbai). Each disc was dried after soaking in gum solution at 50°C for 18 hr. Each disc contained 5 mg of the gum.

### Microbial strains

A total of 812 strains (TABLE 1) of microbes belonging to 27 different genera and 85 species of public health concern, isolated earlier from fish-pond water (12), food (218, Axone/ Akhuni, a fermented local food

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of Nagaland), clinical samples of mithun (112), pig (83) and cows (12) and also from lizards inhabiting animal sheds (368) were revived from the glycerol stocks kept at Microbiology Laboratory at ICAR Research Complex for NEH Region, Jharnapani, Nagaland. All the strains were tested for purity and identity as described earlier[14]. A reference strain of *E. coli* (E-382), received from National *Salmonella* Centre, IVRI, Izatnagar, India, sensitive to all common antimicrobial drugs, was used as control sensitive strain in all experiments.

**TABLE 1 : Sensitivity of microbes (isolated from different sources) tested against 5 mg *Eucalyptus citriodora* gum discs in disc diffusion test.**

| Bacteria tested | Wall lizards | | Mithun | | Pig | | Cow | | Water | | Food (Axone) | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T | S | T | S | T | S | T | S | T | S | T | S | T | Sensitive (%) |
| *Aeromonas* spp. | 3 | 3 | 16 | 2 | 30 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 49 | 15(30.6) |
| *Bacillus* spp. | 0 | 0 | 0 | 0 | 7 | 2 | 0 | 0 | 0 | 0 | 87 | 34 | 94 | 36(38.3) |
| *Budvicia aquatica* | 0 | 0 | 6 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 2(25.0) |
| *Citrobacter* | 76 | 2 | 8 | 0 | 3 | 3 | 0 | 0 | 3 | 0 | 1 | 0 | 91 | 5(5.5) |
| *Candida albicans* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1(100.0) |
| *Edwardsiella* | 5 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 2 | 2 | 0 | 0 | 9 | 3(33.3) |
| *Enterobacter* | 25 | 4 | 9 | 1 | 0 | 0 | 1 | 0 | 3 | 1 | 10 | 1 | 48 | 7(14.6) |
| *Enterococcus* spp. | 79 | 8 | 12 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 68 | 20 | 159 | 29(18.2) |
| *Erwinia ananas* | 2 | 0 | 0 | 0 | 9 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 12 | 2(16.7) |
| *Escherichia* | 40 | 0 | 24 | 2 | 6 | 4 | 7 | 3 | 0 | 0 | 8 | 0 | 85 | 9(10.6) |
| *Hafnea alvei* | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0(0.0) |
| *Klebsiella* | 35 | 0 | 13 | 0 | 13 | 2 | 0 | 0 | 3 | 7 | 0 | 0 | 71 | 4(5.2) |
| *Kluyvera cryocrescens* | 0 | 0 | 5 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0(0.0) |
| *Lactobacillus acidophilus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0(0.0) |
| *Leclercia adecarboxylata* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0(0.0) |
| *Leminorella ghirmontii* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1(100.0) |
| *Micrococcus* spp. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2(100.2) |
| *Proteus* | 7 | 1 | 10 | 4 | 3 | 2 | 0 | 0 | 0 | 0 | 13 | 4 | 33 | 11(33.3) |
| *Pragia fontium* | 9 | 0 | 0 | 0 | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 14 | 1(7.1) |
| *Providencia* | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 5 | 1 | 6 | 2(33.3) |
| *Pseudomonas* | 2 | 1 | 2 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 9 | 3 | 15 | 6(40.0) |
| *Raoultella terrigena* | 5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0(0.0) |
| *Salmonella* | 59 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 59 | 1(1.7) |
| *Serratia* spp. | 2 | 0 | 6 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 9 | 0(0.0) |
| *Staphylococcus* spp. | 9 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 10 | 23 | 19(82.6) |
| *Streptococcus* | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1(20.0) |
| *Xenorhabdus luminescens* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0(0.0) |
| Total strains tested | 368 | 31(8.4) | 112 | 13(11.6) | 83 | 29(34.9) | 12 | 4(33.3) | 12 | 10(83.3) | 218 | 75(34.4) | 812 | 157(19.3) |

## Eucalyptus gum sensitivity assay

Disc diffusion assay as described earlier for herbal preparations[15-17] was used to determine antimicrobial activity of 5 mg EG discs. Discs were applied on to agar plates within 15 min of seeding with test strain grown for 8 h in brain heart infusion (BHI, Hi-Media) broth at 37°C. For all microbial strains sensitivity assays were performed on Mueller Hinton agar (MHA, Hi-Media) except the streptococci, enterococci and micrococci which were tested on brain heart infusion agar (BHIA, Hi-Media) to support the sufficient growth to observe the inhibition zone. Reference control strain of *E. coli* was tested on both BHIA and MHA. Antimicrobial activity was indicated by appearance of zone of inhibition was measured in mm after 24 h of aerobic incubation at 37°C. The strains showing no inhibition of growth

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around disc were considered as resistant to EG.

MIC of reference and selected five strains each of *E. coli*, *A. caviae* and *B. coagulans* and four strains of *Staph. aureus* was determined through broth dilution method[16], 17 using buffered peptone water (BPW, Hi-Media, Mumbai) as growth medium and EG dilutions used were 100 mg, 50 mg, 25 mg, 10 mg, 5 mg, 4 mg, 3 mg, 2 mg, 1 mg, 0.5 mg, 250 µg and 100 µg /ml). All diluations were made in medium before inoculation of the test strain (~1000 cfu/ ml). Tubes were incubated overnight at 37°C and then observed for turbidity an indicator for bacterial growth.

Comparison between strains of different origin and of different bacteria was statistically evaluated using $X^2$ test.

## RESULTS

Of the 812 strains of microbes belonging to 27 genera tested against 5 mg *Eucalyptus citriodora* gum (EG) discs, 157 (19.3%) strains showed zone of inhibition of growth indicating their sensitivity to EG (TABLE 1). Zone of inhibition around discs of EG varied from 8 mm to 20 mm ( ure 1). The zone of inhibition around discs of EG for reference sensitive strain was 15-16 mm under repeated tests both on BHIA and MHA indicating that growth medium has no or little effect on sensitivity assay against EG discs. Of the 157 sensitive strains only 19% strains had inhibition zone of e"15 mm. Although most of the staphylococci were sensitive to EG (82.6), zone of inhibition for most of the strains (95%) was below 15 mm. Similarly many of the aeromonads (30.6%) were sensitive to EG but inhibition zone of >15 mm was evident only in 34% EG sensitive strains. On the other hand majority (89.4%) of *E. coli* were resistant to EG, zone of inhibition for 56% of the sensitive strains was >15 mm. Although sizable number of *Bacillus* strains (38.3%) was sensitive to EG, zone of inhibition remained below 15 mm for 91% strains.

The difference between extents of sensitivity of different bacterial strains was further revealed by minimum inhibitory concentration (MIC) results (TABLE 2). From the results it was evident that MIC by broth dilution method and zone of inhibition around EG disc correlated well (r, -0.81). All the strains showing sensitivity against EG discs had an MIC 0.25 mg to 5 mg/ ml while those resistant to

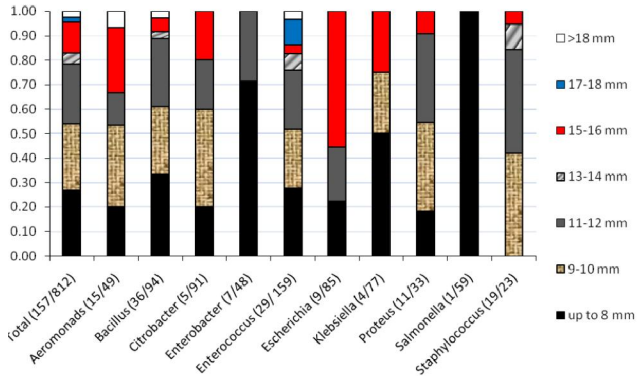


**Figure 1 : Proportions of eucalyptus gum sensitive strains of different genera showing inhibition zone (in mm) around discs containing 5 mg *Eucalyptus citriodora* gum.**

**TABLE 2 : Zone of growth inhibition around *Eucalyptus citriodora* gum (EG) discs (5 mg) and minimum inhibitory concentration (MIC) of selected strains of some bacteria isolated from different sources.**

| Bacteria | Source | Zone of inhibition in mm | MIC of EG /ml |
|---|---|---|---|
| *Aeromonas caviae* | Lizard | 10 | 3 mg |
| *Aeromonas caviae* | Mithun | 11 | 3 mg |
| *Aeromonas caviae* | Mithun | 0 | 25 mg |
| *Aeromonas caviae* | Pig | 8 | 5 mg |
| *Aeromonas caviae* | Pig | 0 | 10 mg |
| *Bacillus coagulans* | Axone | 22 | 250 µg |
| *Bacillus coagulans* | Axone | 11 | 3 mg |
| *Bacillus coagulans* | Axone | 0 | 25 mg |
| *Bacillus coagulans* | Pig | 15 | 500 µg |
| *Bacillus coagulans* | Pig | 0 | 10 mg |
| *Escherichia coli* | Reference | 16 | 500 µg |
| *Escherichia coli* | Lizard | 0 | 25 mg |
| *Escherichia coli* | Cow | 15 | 500 µg |
| *Escherichia coli* | Cow | 7 | 5 mg |
| *Escherichia coli* | Cow | 0 | 25 mg |
| *Escherichia coli* | Mithun | 12 | 2 mg |
| *Staphylococcus aureus* | Lizard | 11 | 2 mg |
| *Staphylococcus aureus* | Lizard | 11 | 2 mg |
| *Staphylococcus aureus* | Lizard | 10 | 3 mg |
| *Staphylococcus aureus* | Lizard | 10 | 3 mg |

EG had an MIC of 10 mg to 25 mg/ ml.

Most of the strains isolated from lizards (91.6%) and mithun (88.4%) samples were resistant to EG. The ratio of resistant strains isolated from lizards and and mithuns was significantly ($p < 0.01$) higher than strains of cattle (66.7%), pig (65.1%), water (75%) and food origin (65.8%). However, there was no significant dif-

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ference in sensitivity pattern of strains of lizard and mithun origin (p, 0.33) and, also among strains of pig, cattle, water and food origin (p, 0.96).

Of the 812 strains of 27 genera, resistant and sensitive strains were distributed among majority of them (TABLE 1). However, a few genera contained resistant strains only viz., *Hafnea* (3), *Kluyvera* (1), *Lactobacillus* (1), *Leclercia* (1), *Raoultella* (6), *Serratia* (9) and *Xenorhabdus* (1), while all strains of a few genera including *Candia* (1), *Leminorella* (1) and *Micrococcus* (2) were sensitive to EG. But both the exceptions were there in genera where numbers of representative strains were less.

Comparison of results among strains of different species indicated that that it was not only genus which largely decided the resistance but among strains of different species difference in sensitivity was marked. All the strains of *Aeromonas salmonicida* ssp. *achromogenes* (2), *A. sobria* (2), *Citrobacter amalonaticus* (11), *Edwardsiella hoshiniae* (1), *Escherichia blattae* (3), *Hafnea alvei* (3), *Klebsiella oxytoca* (8), *Kluyvera cryocrescens* (6), *Lactobacillus acidophillus* (1), *Leclercia adecarboxylata*, *Proteus myxofacience* (1), *Raoultella terrigena* (6), *Salmonella enterica* ssp. *houtenae* (3), *Salmonella enterica* ssp. *salamae* (11), *Serratia fonticola* (1), *Se. Marcescens* (2), *Se. odorifera* (5), *Se. plymuthica* (1), *Streptococcus milleri* (3), *Str. alactolyticus* (1) and *Xenorhhabdus luminiscens* (1) were resistant to EG. Although rare, all strains of a few species of microbes were always sensitive to EG, these included *Candida albicans* (1), *Leminorella ghrimontii* (1), *Micrococcus* spp. (2), *Providencia heimbachae* (1), *Staphylococcus aureus* (4), *Staph. xylosus* (2) and *Streptococococcus caseolyticus* (1).

It is important question to ponder upon why majority of strains of certain bacteria were resistant to EG and only a few strains were sensitive viz., *Citrobacter freundii* (3 of 74), *Erwinia ananas* (2 of 12), *Escherichia coli* (6 of 73), *Klebsiella pneumoniae* ssp. *pneumoniae* (4 of 63), *Proteus penneri* (3 of 16), *Pragia fontium* (1 of 14), *Providencia rettgeri* (1 of 5), *Salmonella enterica* ssp. *indica* (1 of 45). Similarly, resistance only in a few strains of some species comprising mostly EG sensitive strains was also puzzling; such strains were detected among *Staph. sciuri* (3 of 17), *Proteus mirabilis* (3 of 8) and *Aeromonas caviae* (4 of 11). These exceptions may help in understanding the factors responsible for resistance or sensitivity to EG.

Sensitivity to EG among 49 aeromonads of 8 different species was evident in 30.6% strains (TABLE 3). Though in general bacterial isolates from lizards were significantly more resistant to EG, all three aeromonads (*A. caviae*) from lizards were sensitive to EG and skewed the distribution significantly (p, 0.04). On the other hand the difference in sensitivity of aeromonads to EG among strains of pig or mithun origin was of little significance (p, 0.19). All species of aeromonads contained a few strains sensitive to EG except *A. samlonicida* ssp. *achromogenes* (mithun) and A. *sobria* (pig) but due to less number of strains under study the difference was statistically insignificant (p, 0.47).

**TABLE 3 : Sensitivity pattern of aeromonads from different sources tested against 5 mg *Eucalyptus citriodora* gum discs.**

| *Aeromonas* spp. | Wall lizards | | Mithun | | Pig | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | Sensitive | No. | Sensitive | No. | Sensitive | No. | Sensitive |
| *A.caviae* | 3 | 3 | 3 | 1 | 5 | 3 | 11 | 7(63.6) |
| *A.eucranophila* | 0 | 0 | 7 | 0 | 6 | 2 | 13 | 2(15.4) |
| *A.hydrophila* | 0 | 0 | 3 | 1 | 0 | 0 | 3 | 1(33.3) |
| *A.salmoicida ssp. achromogenes* | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0(0.0) |
| *A.salmonicida ssp. salmonicida* | 0 | 0 | 0 | 0 | 2 | 1 | 2 | 1(50.0) |
| *A.schubertii* | 0 | 0 | 1 | 0 | 2 | 1 | 3 | 1(33.3) |
| *A.sobria* | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0(0.0) |
| *A.veronii* | 0 | 0 | 0 | 0 | 13 | 3 | 13 | 3(23.1) |
| Total | 3 | 3(100.0) | 16 | 2(12.5) | 30 | 10(33.3) | 49 | 15(30.6) |

Testing of 94 strains of 14 species of *Bacillus* (TABLE 4) isolated from Axone (87) and pigs (7) revealed that resistance to EG was independent of origin

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of strains (p, 0.73) and species of bacteria (0.79) except a few deviations viz., all strains of B. *licheniformis* and *B. stearothermophillus* were resistant to EG while only one third number of strains of *B. brevis* and *B. marcerans* were resistant to EG.

Similarly among *Enterococcus* and *Enterobacter* strains (TABLE 5) origin effect on EG resistance was insignificant (p>0.29) and no difference was evident among strains of different species (p>0.8) of both group of potential opportunistic pathogens.

## DISCUSSION

Eucalyptus being one of the 15 most common antimicrobial herbs has been studied a lot and its essential oil has been found to be very effective on several pathogenic bacteria isolated from clinical samples particularly those associated with respiratory tract infections[6,7] and dental problems[13]. Eucalyptus gum has mostly

**TABLE 4 : Resistance pattern of *Bacillus* strains isolated from food (Axone) samples to 5 mg *Eucalyptus citriodora* gum discs.**

| *Bacillus* spp. | No. tested | Sensitive | Resistant | % Resistnat |
|---|---|---|---|---|
| *B. anthracoides* | 3 | 0 | 3 | 100.0 |
| *B. badius* | 5 | 2 | 3 | 60.0 |
| *B. brevis* | 3 | 2 | 1 | 33.3 |
| *B. circulans* | 4 | 2 | 2 | 50.0 |
| *B. coagulans** | 30 | 12 | 18 | 60.0 |
| *B. laterosporus* | 1 | 0 | 1 | 100.0 |
| *B. lentus* | 7 | 6 | 1 | 14.3 |
| *B. licheniformis* | 6 | 0 | 6 | 100.0 |
| *B. marcerans* | 3 | 2 | 1 | 33.3 |
| *B. mycoides* | 2 | 0 | 2 | 100.0 |
| *B. pentothenticus* | 15 | 8 | 7 | 46.7 |
| *B. stearothermophilus I* | 1 | 0 | 1 | 100.0 |
| *B. stearothermophilus II* | 4 | 0 | 4 | 100.0 |
| *B. subtilis* | 3 | 0 | 3 | 100.0 |
| Total | 87 | 34 | 53 | 60.9 |

**TABLE 5 : Resistance pattern of *enterococci and Enterobacter* spp. strains isolated from foods, Mithun and lizards to 5 mg *Eucalyptus citriodora* gum discs.**

| Bacteria tested | Food (Axone) | | Mithun | | Wall lizards | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | Resistant | No. | Resistant | No. | Resistant | No. | Resistant (%) |
| *E. asacchrolyticus* | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 (0.0) |
| *E. avium* | 4 | 3 | 0 | 0 | 1 | 1 | 5 | 4 (80.0) |
| *E. caecorum* | 28 | 21 | 0 | 0 | 0 | 0 | 28 | 21 (75.0) |
| *E. casseliflavus* | 3 | 2 | 10 | 9 | 15 | 15 | 28 | 26 (92.9) |
| *E. dispar* | 5 | 2 | 0 | 0 | 22 | 20 | 27 | 22 (81.5) |
| *E. faecalis* | 3 | 2 | 0 | 0 | 1 | 1 | 4 | 3 (75.0) |
| *E. faecium* | 4 | 2 | 0 | 0 | 1 | 1 | 5 | 3 (60.0) |
| *E. gallinarum* | 6 | 6 | 2 | 2 | 0 | 0 | 8 | 8 (100.0) |
| *E. hirae* | 7 | 5 | 0 | 0 | 35 | 31 | 42 | 36 (85.7) |
| *E. malodoratus* | 3 | 2 | 0 | 0 | 0 | 0 | 3 | 2 (66.7) |
| *E. mundatii* | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 1 (33.3) |
| *E. raffinosus* | 5 | 4 | 0 | 0 | 0 | 0 | 5 | 4 (80.0) |
| *En. agglomerans* | 6 | 5 | 4 | 3 | 5 | 5 | 15 | 13 (86.7) |
| *En. amnigenus I* | 1 | 1 | 3 | 3 | 5 | 5 | 9 | 9 (100.0) |
| *En. amnigenus II* | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 (0.0) |
| *En. cancerogenus* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 (100.0) |
| *En. cloacae* | 0 | 0 | 0 | 0 | 3 | 2 | 3 | 2 (66.7) |
| *En. gregoviae* | 3 | 3 | 1 | 1 | 7 | 7 | 11 | 11 (100.0) |
| *En. hormaechei* | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 (100.0) |
| *En. sakazaki* | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 (100.0) |
| *All Enterococci* | 72 | 50 (69.4) | 12 | 11 (91.7) | 75 | 69 (92.0) | 159 | 130 (81.8) |
| *All Enterobacter* spp. | 10 | 9 (90.0) | 9 | 8 (88.9) | 25 | 21 (84.0) | 44 | 38 (86.4) |

**Besides, strain of *En. aminigenus* I of water origin and *En. cloacae* strains, each of water and cow origin, were also resistant to 5 mg *Eucalyptus citriodora* gum discs.**

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been used as an astringent in medicine since more than a century[12], little is known about its antimicrobial activity. Antimicrobial activity of eucalyptus gum observed in the present investigation against more than 19% of bacterial strains having potential association either with throat infections, diarrhoeal infections and wound infections is very important. Although isolated from environment, water, food and animals, strains of the microbes in the study has frequently been reported to be associated with systemic as well as local infections of throat and upper respiratory tract gastrointestinal tract and wounds[18-23]. Thus antimicrobial activity of EG against the potentially pathogenic bacteria is of therapeutic value because EG has been used since centuries without knowledge of its true value as gurgle in throat infections, liniment in injuries and wounds and decoctum or powder in diarrhoea[12]. For wounds, injuries, gurgles and as haemostatis EG is used as 1:16 to 1:40 dilutions, i.e., 2.5% to 6.25% solution[12] the concentration sufficient to arrest the growth of even the strains showing resistance with disc diffusion assay (TABLE 2). In the study all *Staphylococcus aureus* strains, the major cause of wound infection and contamination[20], were sensitive to EG and MIC was not more than 3 mg/ ml i.e., 0.3% solution of EG can inhibit the growth of *Staph. aureus* while in practice EG has been safely used up 6.25% solution[13]. From the MIC values (TABLE 2) for different microbes it is evident that in therapeutic concentrations EG may effectively restrict the growth of most of the common pathogens as MIC was never more than 25 mg/ ml (2.5% solution).

MIC for aeromonads and *E. coli,* the two most common causes of acute, chronic and travellers' diarrhoea[23] varied from 250 µg to 25 mg/ ml while the recommended dose for diarrhoeic patient in only 100-300 mg[13], *i.e.*, even if the strain is sensitive to EG and MIC is 250 to 500 µg then to achieve the bacteriostatic conditions in the intestine dose will be much more than the recommended dosages *i.e.*, to have the bacteriostatic or bactericidal dose EG have little value in gastrointestinal disorders. However, to comment on its antidiarrhoeal value one must remember that even the use of most potent antimicrobials in management of diarrhoeal disorders is highly disputed[24]. Therefore, the use of EG for control of infectious diarrhoea may be very import due to its potent astringent and a limited antimicrobial action against common bacteria associated with diarrhoea.

Plant medicines are considered dent proof against the evolution of antimicrobial drugs resistance probably due to their much complex chemistry than antibiotics. Antibiotics are made of one pure chemical against which bacteria can easily mount an action to survive. Unlike antibiotics, plant extracts such as eucalyptus gum contain many antibacterial substances. It would be much harder for bacteria to develop resistance against an extract with multiple antibacterial substances[1, 25]. A limited work using thyme (on methicillin resistant *Staph. aureus*, MRSA), it is shown that bacteria can not develop resistance to herbal medicines[26-28]. Herbal antimicrobials therefore, may have a significant clinical value in treatment of infections caused by resistant microbial strains[29]. In contrast, observation of this study indicated that resistant to herbal drug, i.e., eucalyptus gum is not uncommon in common bacteria often associated with infections in human and animals. In the study only about 19% bacterial strains are sensitive to EG i.e, majority (>80%) were resistant to EG, the observations can not be compared in paucity of earlier observations on EG. However, the observations corroborate with studies on other herbs viz., report of herbal drugs as source of MDR strains[30] and resistance in >75% bacterial strains against essential oils of *Artemesia vulgaris*[17], >20% to *Selinum wallichianum* essential oil[15] and in >62% strains against lemon grass (*Cymbopogon citrates*) oil[16]. Thus it may be suggested that alternative therapies and herbal drugs though promising, may not be the final antimicrobial shot to infections.

## CONCLUSION

The study concludes that antimicrobial activity of EG, though feeble, was important against bacteria often associated with superficial infections. None of the strain tested had MIC more than >25 mg/ ml, which quite in range of recommended dilutions of this valuable gum. The study revealed that *Staph. Aureus* strains, mostly associated with wound infection were sensitive to EG while most of the *E. coli* strains were resistant. Detection of a few EG resistant *Staph. aureus* and few EG sensitive *E. coli* may be useful in further studies to un-

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derstand the mechanism of action of EG as antimicrobial and evolution of resistance against herbal drugs specifically to eucalyptus gum.

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