Effect of betel and lemon aqueous extracts on clinical isolates of *Escherichia coli*

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ABSTRACT

Escherichia coli causes a plethora of human infections and can be difficult to treat due to biofilm formation and high degree of antibiotic resistance. Hence, natural inhibitory compounds are being studied against this pathogen. Our study evaluated the inhibitory role of paan leaf and lemon on this pathogen. Both paan leaf and lemon juice showed inhibition of growth and virulence factors at 4 g% concentration. This can show the road for further studies to characterize these inhibitory natural moieties.

Key words: *Betel, Biofilms, Escherichia coli, Lemon*

Escherichia coli is a frequent cause of urinary tract infection, diarrhea, and dysentery and is often multidrug resistant [1]. It is normal gut commensal and diarrheagenic *E. coli* may be enteropathogenic, enterotoxigenic, enteroinvasive, and enterohemorrhagic among other types [2]. Gut *E. coli* is often resistant to multiple antibiotics, for example, ampicillin, chloramphenicol, and cotrimoxazole [3]. Herbal and natural compounds can be an excellent source of non-toxic natural antibiotic molecules against this pathogen [4]. For example, products such as apple, beans, fenugreek, and black tea have been documented to kill enterotoxigenic *E. coli* (ETEC) [4]. Pea and liquorice have also been documented to produce inhibition of disease production by ETEC by inhibiting binding of Labile toxin with gut GM1 ganglioside [4]. Extracts of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) are also very potent inhibitors of the growth of *Pseudomonas aeruginosa* and *E. coli* as per recent scientific data [5]. This is important and such green pharmacy may, in the long run, become the base for the development of medicines by providing a compound which can be used for developing of new drugs with novel mechanisms of action [5]. Keeping these things in mind, our study was planned to assess and evaluate the antibacterial effect (effect on growth, lecithinase, lipase, protease, and biofilm) of aqueous extracts of betel leaf and lemon juice against clinical isolates of *E. coli*.

MATERIALS AND METHODS

This was a laboratory-based observational study carried out in the department of microbiology of the institute from June 2017 to August 2017 (3 months) as a part of short-term training. Twenty-nine randomly selected isolates of *E. coli* from various samples such as urine, pus, and stool were taken for the study. Betel leaf (sweet variant) and lemon (*Citrus×limon*) were sourced and bought from local markets. Betel leaf was dried. Lemon juice was extracted and weighed. *E. coli* isolates were inoculated on 3 sets of media: (a) Peptone water, (b) peptone water with betel leaves, and (c) peptone water with lemon juice. Media (b) and (c) were prepared in 3 different concentrations: 1%, 2%, and 4%, all weight/volume. All were autoclaved at 10 lbs/in^2 and 110°C and at 15 lbs/in^2 and 121°C (2 sets). When pH was measured, betel extract showed pH of 7 and lemon extract showed pH of 5.5 on all occasions. *E. coli* isolates were purified by subculture and inoculated in these media by adjusting turbidity to 0.5 McFarland turbidity standard. After incubation for 24 h at 37°C, 10 µl of liquid from each tube was subcultured on Mueller-Hinton agar and egg yolk agar (prepared in-house) and these plates incubated similarly overnight. Liquid contents of tubes were decanted and tubes were washed thrice with sterile normal saline. Then, 0.5% aqueous Safranine was added to them and kept for 1 min. After that, tubes were again washed thrice with normal saline and dried in inverted position and observed for visible biofilm formation by naked eye. In egg yolk agar, lecithinase and protease activities were defined as zones of opalescence and clearing, respectively, around colonies seen in transmitted light, and lipase by pearly sheen seen in reflected light. Simultaneously, extracts were checked for host toxicity by making amount of buffy coat from leftover human plasma samples (received for other purposes), mixing it with extracts and observing for cell lysis for 20 min under ×40 microscope objective. By a commercial assay, amino acid content of betel and lemon extract was studied. Commercial kit (SD Urocolor, Korea) was used to estimate the amino acid and sugar content of the extracts.

RESULTS

E. coli isolates did not show lecithinase and protease activities on egg yolk agar. Lipase was present, and it was not well inhibited

by betel extract. However, lipase was inhibited very well by 4% (w/v) lemon extract. Both betel and lemon at concentrations of 4% inhibited biofilm formation of *E. coli* quite appreciably, as observed by test tube method. Results have been summarized in Tables 1-3.

None of the extracts showed any lysis of host white blood cells and red blood cells. Thus, they were safe and non-toxic to host cells.

By the commercial assay, amino acid content of betel extract was not high, but the lemon extract showed very high level of leucine. Exact quantity was not measurable.

DISCUSSION

Herbal compounds have since long been used in our oriental food habits and have been claimed to possess numerous putative health benefits. Betel leaf, also called paan in India, is often used as a mouth freshener. Betel leaf (*Piper betle*) has been shown by researchers to effectively inhibit *E. coli* and *Staphylococcus aureus in vitro* [6]. Both methanolic and ethanolic extracts of betel leaf are good in this regard [7]. Similarly, lemon juice has been documented to effectively reduce the population of *E. coli* and *Salmonella* enteritidis in meat [8]. This property of lemon has been attributed to the citric acid content. In a study from Iraq, lemon juice mixed with green tea showed remarkable inhibitory activity on uropathogenic *E. coli* isolates [9]. It is commonplace in our country to incorporate paan as a confectionery and lemon as juice or as an accompaniment of diet. Given the high burden of antibiotic resistance shown by *E. coli*, the results shown with lemon juice and paan leaf are indeed very encouraging and should be the topic of further research. As far as we know, this type of study showing the *in vitro* effect of aqueous paan extract and lemon juice on clinical isolates of *E. coli* has not been carried out or published in our country. This can serve as a initial study to further characterize the inhibitory compounds in paan and lemon juice extracts to synthesize new natural antibiotics which are badly needed. That the inhibition was seen more with extracts autoclaved at 10 lbs/in^2 and 110°C than at 15 lbs/in^2 and 121°C, the inhibitory compounds

Table 1. Isolation of *E. coli* from urine, pus, and stool samples

	A total number of experiments	Normal peptone water	1% betel extract with peptone water		1% lemon extract with peptone water	
			Positive	Negative	Positive	Negative
Biofilm	29	29	15	14	17	12
Growth	29	29	27	00	21	06
Lipase	29	29	27	00	21	06
Protease	29	00	00	00	00	00
Lecithinase	29	00	00	00	00	00

Positive: Organisms growth has seen in different % of betel and lemon extract, Negative: Organisms growth have inhibited in different % of betel and lemon extract. *E. coli: Escherichia coli*

Table 2: Isolation of *E. coli* from urine, pus, and stool samples

	Total number of experiments	Normal peptone water	2% betel extract with peptone water		2% lemon extract with peptone water	
			Positive	Negative	Positive	Negative
Biofilm	13	10	13	00	03	10
Growth	13	13	13	00	11	02
Lipase	13	13	13	00	11	02
Protease	13	00	00	00	00	00
Lecithinase	13	00	00	00	00	00

Positive: Organisms growth has seen in different % of paan and lemon extract, Negative: Organisms growth have inhibited in different % of paan and lemon extract. *E. coli: Escherichia coli*

Table 3: Isolation of *E. coli* from urine, pus, and stool samples

	A total number of experiments	Normal peptone water	4% betel extract with peptone water		4% lemon extract with peptone water	
			Positive	Negative	Positive	Negative
Bio film	17	08	05	12	00	17
Growth	17	17	14	03	03	14
Lipase	17	17	14	03	03	14
Protease	17	00	00	00	00	00
Lecithinase	17	00	00	00	00	00

Positive: Organisms growth has seen in different % of paan and lemon extract, Negative: Organisms growth have inhibited in different % of paan and lemon extract. *E. coli: Escherichia coli*

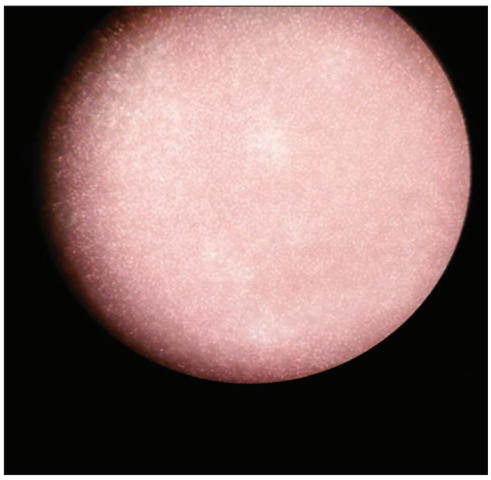

Figure 1: No lysis of RBC and WBC with paan extract

might be mildly thermolabile. The excellent inhibitory effect of lemon extract on *E. coli* can be partly due to the high leucine content as found out in our study since some reports suggest that leucine-containing peptides effectively inhibit *E. coli in vitro* [10]. Some studies highlight this inhibitory effect of lemon extract on *E. coli* on *S. aureus* and *Aeromonas veronii* [11]. As far as we now, such studies have not been carried out earlier in this part of the country. More such studies are waited in this context (Fig. 1).

CONCLUSION

Thus lemon extract and to a lesser extent, betel leaf extract were lethal to *E. coli*, and were non-toxic to host cells also. These findings can be used to further study and assess the inhibitory compounds present in these extracts.

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