

Leaf Extract of *Syzygium cumini* Shows Anti-*Vibrio* Activity Involving DNA Damage

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ABSTRACT: The aim of the study was to investigate the effect of an ethanol extract of leaf (EEL) of *Syzygium cumini* against *Vibrio cholerae* serogroups *Ogawa* and *Inaba*. The antimicrobial activity of EEL was evaluated by the disc diffusion method against multi-drug resistant *Ogawa* and *Inaba*. The EEL effectively inhibited the growth of both serogroups. This growth inhibition was accompanied by fragmentation of genomic DNA as revealed by agarose gel electrophoresis. This result suggested that the EEL might inhibit bacterial growth involving DNA damage either through activation of signal transduction pathways or through direct interaction of the metabolites present in the EEL with DNA. Therefore, EEL of *S. cumini* has potential growth inhibitory activity against multi drug resistant *Vibrios*. This inhibitory effect of EEL might be explored to develop effective candidate(s) to combat cholera.

Key words: *Syzygium cumini*, anti *Vibrio* activity, DNA fragmentation

INTRODUCTION

Cholera is an infectious disease that has been threatening many developing countries including Bangladesh. According to World Health Organization (WHO), during 2004 to 2008 cholera outbreak increased by 24% compared to that of 2000 to 2004. The incidence of the disease is estimated to be 3–5 million cases and 100,000–120,000 deaths annually.¹ In Haiti, after tsunami cholera became epidemic sickening more than 91,000 people and killing more than 2,000 of them in late 2010.² *Vibrio cholerae* particularly two serogroups *Ogawa* and *Inaba* are considered the major causative agents of epidemic cholera throughout Bangladesh, India and in other developing countries.³ For the treatment of cholera, oral rehydration therapy, antibiotics, good hygiene practice and sometimes vaccines are recommended. However, the high percentage of regular incidence infection with of *Vibrio* and frequent use and misuse of antibiotics create a very

alarming situation for public health. During the last 10 years the development of new antimicrobial drugs has been slowed down but the prevalence of resistance has been increased considerably.⁴ Therefore, preventive actions must be taken to address this problem through developing new drugs, which may be either synthetic or natural. In this regard herbal medicines would be an alternate promising choice over synthetic drugs. Bangladesh is having ancient culture of using herbal medicines due to availability and possession of a large variety of plant kingdoms. *Syzygium cumini* (L.), a member of Myrtaceae family, is a common fruit plant of Bangladesh. It has several medicinal properties.⁵ The phenolic content of *S. cumini* leaves and antioxidant activity were found high and the latter was comparable to ascorbic acid.⁶ The presence of polyphenolic compounds in ethanolic leaf extract of *S. cumini* might be responsible for its high antioxidant activity.⁶⁻⁸ The extracts of *S. cumini* leaf showed inhibitory activity against various clinical isolates of the Gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *S. typhi*, *S. paratyphi* A and B,

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and of Gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*.⁹ However, the effect of *S. cumini* extract on *V. cholerae* has not been studied until today.

In the present study we explored the possible effect of *S. cumini* leaf extract on Gram negative *V. cholerae*. The research mainly focused to find out the anti *Vibrio* activity of *S. cumini* plant leading to the development of a phytomedicine that could be used to combat cholera in our country with less expense.

MATERIALS AND METHODS

Plant materials: The leaves of *Syzygium cumini* were collected from orchards at Curzon Hall campus, University of Dhaka, Bangladesh. The plant was identified and authenticated and a voucher specimen (Accession no. 34742) of the plant was deposited in Bangladesh National Herbarium.

Preparation of ethanolic extract of leaf (EEL): The leaves of *S. cumini* were cleaned, air-dried at room temperature on a cool dry place keeping away from direct sunlight for 7 to 10 days and finally ground to a coarse powder. Dried leaf powder (300 g) was soaked in 1 L ethanol (95%) in a flask. The flask was covered and then kept at room temperature for around 1 week. After that, the solution was filtered by Whatman filter paper (no. 1) and the filtrate was collected. The residue was again soaked with 700 ml ethanol (95%) to get additional extractive. All the filtrates were collected in a round bottom flask. Gummy substance was obtained by using vacuum rotary evaporator. Then the substance was dried at room temperature. The powdered extract was weighed and stored at 4°C for further work. From 300 g dried leaf powder, 40 g (13.33%) of extract was finally obtained. The extract was dissolve in 40% ethanol before using.

Anti *Vibrio* activity assay: This assay was done by agar diffusion method¹⁰ using two different antibiotic resistant *V. cholerae* serogroups *Ogawa* and *Inaba*. To find out the anti *Vibrio* activity of the extract, different extract dose (3, 4.5 and 6 mg) were applied to each disc and incubated overnight at 37°C. After incubation, the activity was determined by

measuring the diameter of the zone of inhibition in millimeter using a scale. The diameter of zone of inhibition was determined by using Kirby-Bauer method.¹¹

Genomic DNA isolation and analysis by gel electrophoresis. EEL (600 µg/ml) was added in the liquid culture medium containing *V. cholerae* serogroup *Ogawa* (~10⁶ cells/ml) and kept at 37°C in shaking incubator for different time interval. After 0.5, 1, 1.5, 2 and 3 hours, cells were harvested through centrifugation for 5 min at 5000 rpm. Genomic DNA was isolated according to manufacturer instruction (G-spin Genomic DNA extraction Kit for Bacteria, iNtRON Biotechnology INC, Korea). For control, genomic DNA was isolated from *V. cholerae* serogroup *Ogawa* that was cultured without EEL. Genomic DNA sample (10 µl) mixed with 1 µl of dye was loaded on a 1% agarose gel (SIGMA) with 0.1µg/ml ethidium bromide. The sample was run for 1 hour at 50 mV. Gel was observed under UV light and photographs were taken using gel documentation system (Gel DiWise Doc, Korea).

RESULTS

EEL showed anti *Vibrio* activity. Both of the *V. cholerae* serotypes *Ogawa* and *Inaba* showed resistance against many antibiotics like erythromycin, gentamicin, tetracycline, ampicillin, penicillin G, trimethoprim, ceftazidim.^{4,6} We investigated whether the extract of *S. cumini* could effectively inhibit the growth and activity of multi-drugs resistant *Ogawa* and *Inaba*. Different doses (3, 4.5 and 6 mg) of the extract were applied on each disc, where ampicillin and ethanol (40%) were applied as controls. The antibacterial activity of the extract and its potency was assessed by the presence or absence of zone of inhibition. From our observation, it was clear that *S. cumini* extract was active in inhibiting the growth of *Ogawa* and *Inaba*. It was also observed that the extract showed almost similar effect against both *Ogawa* and *Inaba* (Figure 1 and Table 1).

Genomic DNA degradation was observed in EEL-treated bacterial cells. Many drugs or

chemicals have been reported to cause cellular apoptosis accompanied by DNA fragmentation.¹²⁻¹⁴ As we observed in the study that *S. cumini* extract inhibited bacterial growth, we next tried to examine whether this inhibition was caused by fragmentation of bacterial genomic DNA. We previously determined the minimum inhibitory concentration (MIC) of *S. cumini* EEL against *Vibrio* strains and

that was 600 µg/ml.⁶ Here, *V. cholerae* serogroup *Ogawa* was treated with or without 600 µg/ml of EEL for different time intervals followed by isolation and analysis of genomic DNA by gel electrophoresis. Interestingly, compared to control, multiple bands for fragmented DNA were detected at the lower portion of the gel in EEL-treated sample (Figure 2).

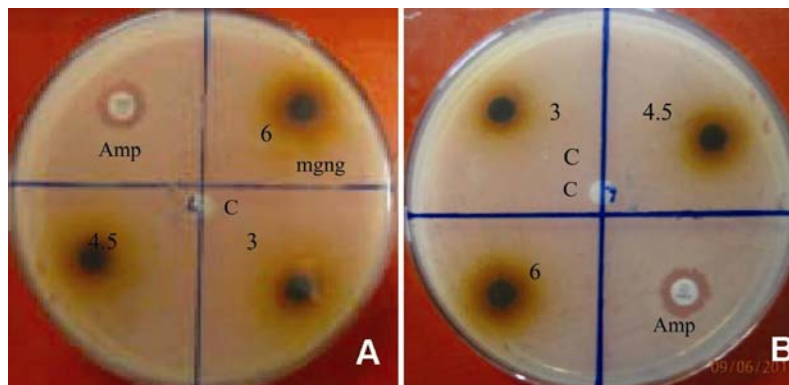


Figure 1. Antibacterial activity of *S. cumini* EEL against *V. cholerae* serogroups *Ogawa* (A) and *Inaba* (B) at different doses (3, 4.5 and 6 mg). Amp (ampicillin) and solvent only (40% ethanol) (C) used as negative control.

Table 1. Antibacterial activity of *S. cumini* EEL against *V. cholerae* serogroups *Ogawa* and *Inaba*.

EEL dose (mg)	Diameter of zone of inhibition (mm)	
	<i>Ogawa</i>	<i>Inaba</i>
3	7	8
4.5	9	9
6	11	12
Solvent only	-	-



Figure 2. Fragmentation of genomic DNA of *V. cholerae* serogroup *Ogawa* treated with *S. cumini* extracts (600 µg/ml) *in vivo*. Here, Lane 1 and 2 represent for genomic DNA from *V. Ogawa* incubated without plant extract and with plant extract respectively for 3 hours.

DISCUSSION

Resistance of microorganisms against drugs has increased due to the frequent use and misuse of drugs. Consequently, scientists are increasingly recognizing plant remedies as very important low cost alternatives to industrially produced antibiotics.

In this study, *S. cumini* extract effectively inhibited the growth of *Ogawa* and *Inaba* almost equally. Brine shrimp bioactivity assay of *S. cumini* extract demonstrated the safety and efficacy of the extract.⁶ This result suggested that the extract is safe for *in vivo* application. Therefore, the use of *S. cumini* extract seemed to be promising because of its potential for inhibiting multi-drug resistant *Vibrios* in one hand and its less cytotoxic properties on the other.

Many plant extracts and cytotoxic chemicals have been shown to induce death of cells involving chromosomal DNA fragmentation.¹²⁻¹⁶ In this study, it was also investigated whether or not EEL-induced *Vibrio* cell death was accompanied by DNA fragmentation. Interestingly, fragmentation of

genomic DNA was observed in case of EEL-treated bacterial cells (Figure 2). This genomic DNA fragmentation was an early event and initiated within 3 hrs after exposure (Figure 2). EEL-mediated intracellular signaling possibly activated endonucleases that might be responsible for bacterial DNA damages.

Further investigation is needed to explain the detailed mechanism of EEL-mediated anti *Vibrio* activity and to explore its use as a potential candidate for the treatment of cholera.

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