www.sospublication.co.in Journal of Advanced Laboratory Research in Biology

We- together to save yourself society e-ISSN 0976-7614

Volume 2, Issue 3, July 2011 Research Article

Syzygium cumini Leaf Extract Showed Vibriocidal Activity on Selected Diarrhea Causing Bacteria

Nilanjana Paul[1], Anwarul A. Akhand[1], Sahab Uddin Babu[1], Nazrul Islam[2] and Nazmul Ahsan[1*]

[1*]*Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh.*
[2]*Laboratory Science Division, ICDDR, B, Mohakhali, Dhaka-1212, Bangladesh.*

Abstract: The objective of the study was to investigate the effect of ethanolic leaf extract (ELE) of *Syzygium cumini* against *Vibrio cholerae* particularly two serogroups *Ogawa* and *Inaba*. The phenolic content of the ELE was found high which is comparable to ascorbic acid. Brine shrimp lethality bioassay was then performed to check the cytotoxic effects of ELE. The lower LC_{50} value of ELE obtained indicated its less cytotoxic properties. The antimicrobial activity of the extract was then evaluated by the disc diffusion method against multi-drug resistant *Vibrio* serogroups *Ogawa* and *Inaba*. The extract effectively inhibited the growth of both serogroups. Altogether, the results demonstrated that the ELE of *S. cumini* has a significant vibriocidal activity that might be useful as a drug for the treatment of cholera.

Keywords: Plant extract, *Vibrio*, Cholera, Vibriocidal activity.

1. Introduction

Cholera, an enteric diarrheal disease categorized as one of the "emerging and reemerging infections" has been threatening many developing countries including Bangladesh, where outbreaks occur in a regular seasonal pattern. According to World Health Organization (WHO), cases of cholera outbreak increased by 24% during 2004 to 2008 compared with that of 2000 to 2004. The true burden of the disease is estimated to be 3–5 million cases and 100,000–120,000 deaths annually [1]. A recent outbreak of cholera occurred in Haiti on late 2010, sickening more than 91,000 people and killing more than 2,000 of them [2]. In Bangladesh diarrheal disease is estimated to be the fourth biggest killer of children. *Vibrio cholerae* particularly two serogroups *Ogawa* and *Inaba* is considered the major causative agent of epidemic cholera, which causes explosive epidemic throughout Bangladesh, India and in other developing countries [3]. For the treatment of cholera, oral rehydration therapy, antibiotics, vaccines and good hygiene practice are recommended. However, the regular incidence of *Vibrio* at a considerable high percentage and frequent use/ misuse of antibiotics create an alarming situation for public health. During the last 10 years, the pace of development of new antimicrobial drugs has been slowed down while the prevalence of resistance has been increased astronomically [4]. Therefore, actions must be taken to reduce this problem through developing new drugs, either synthetic or natural. In this regard, herbal medicines are promising choice over modern synthetic drugs. Bangladesh is having an ancient culture of practicing herbal medicines due to availability and possession of a large variety of plant kingdoms. Among them, *Syzygium cumini* (L.), a member of the Myrtaceae family, is a common fruit plant in Bangladesh. It has also been attributed to possess several medicinal properties [5]. The extracts of *S. cumini* leaf showed inhibitory activity against various clinical isolates of the gram-negative bacteria such as *Salmonella enteritidis*, *S. typhi*, *S. paratyphi A, B*, *Pseudomonas aeruginosa and Escherichia coli*, and of gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* [6]. However, the effect of the plant extract on *V. cholerae* has still remained elucidated. In the present study, we have been exploring the possible effect of *S. cumini* leaf extracts of gram-

*Corresponding author:
E-mail: shishir_ahsan@yahoo.co.in; 8802-9661920/ 7822 (office), 88-02-01718340750.

negative *V. cholerae*. The research mainly focused on to find the vibriocidal activity of *S. cumini* plant leading to the development of phytomedicines that could be used to combat devastating cholera in our country with less expense.

2. Materials and Methods

2.1 Plant materials

The leaves of *Syzygium cumini* were collected from the orchards at Curzon Hall, University of Dhaka, Bangladesh. The plant was identified and authenticated by the National Herbarium of Bangladesh. A Voucher specimen (Accession no. 34742) of the plant was deposited in the National Herbarium.

2.2 Preparation of ethanolic leaf extract (ELE)

The leaves of *S. cumini* was clean, air-dried at room temperature on a cool dry place with keeping away from direct sunlight for 7 days and ground into a fine powder. Dried leaf powder (250gm) was soaked with 1L ethanol (95%) on a flask. The flask was covered and then kept at room temperature for around 1 week. After that, the solution was filtered through the Whitman filter paper and the filtrate was collected. The residue was soaked with 700ml ethanol (95%) and then was covered and kept again for 1 week and was filtered. All the filtrate was collected on a round bottom flask. Solvent evaporator was used to evaporate ethanol in the crude preparation under reduced pressure until a gummy substance was obtained. Then the substance was dried at room temperature for 10 days to prepare the powdered form. The powdered extract was weighed and stored at 4^0C for further work.

2.3 Determination of phenolic content of ELE

Total phenolic content of the extract was determined using Folin-Ciocalteu reagent in triplicate following the method of the Singleton and Rossi (1965) with slight modification using ascorbic acid as a standard [7].

2.4 Brine shrimp lethality bioassay

Brine shrimp (*Artemia salina*) lethality bioassay was carried out to the cytotoxic effect of the ELE. The assay was done according to Meyer's process (1992) with some modification [8].

2.5 Vibriocidal activity assay

Vibriocidal assay was done by agar diffusion method [9] using different antibiotic resistant *V. Cholerae* serogroups *Ogawa* and *Inaba*. Antibiogram was done to check the resistivity of the strains against different antibiotics by measuring the diameter of clear zone. The significance of zone of inhibition was determined using Kirby-Bauer method (1996) [10]. To find out the vibriocidal activity of the extract, different concentration (50, 75 and 100mg/ml) was applied to each disc and incubated overnight at 37°C. After incubation, the vibriocidal activity was determined by measuring the diameter of the zone of inhibition in millimeter using a scale.

2.6 Determination of Minimum Inhibitory Concentration (MIC) of ELE

The young cultures of desired serotypes of *Vibrio* were grown in nutrient broth at a density adjusted to 0.5 McFarland Standard and 50µl of bacterial suspension were added to susceptibility test broth. Different concentrations of plant extract ranging from 100 to 1000µg/ml were added to the broth medium containing *Vibrio*. The contents of each tube were mixed and shaken at 150 rpm during incubation at 37°C for 6-8 hrs. The growth of bacteria was determined by taking OD at 600nm. The lowest concentration of the extract that gave near zero absorbance reading was considered as the MIC of the plant extract.

3. Results

3.1 Physical properties of *S. cumini* ELE

An average of 34.10g (13.64%) of *S. cumini* extract was obtained from 250g of dried leaf powder in triplicate experiments. General physical properties of the extract were observed and it was found that the obtained extract was amorphous powder. The extract gave the bitter feeling when tested.

3.2 ELE showed high phenolics content

Phenols are very important plant components because of their radical scavenging properties due to the presence of hydroxyl groups [11]. The phenolic content may contribute directly to the anti-oxidative action [12]. So the antioxidant activities of plant extracts are often well correlated by their total phenolic contents. The total phenolic content in the ethanolic extract of *S. cumini* was calculated and found 510.40-± 7.08mg/g. This high phenolic content demonstrated the antioxidant potential *S. cumini* ELE.

3.3 ELE showed less cytotoxic properties

For demonstrating the safety and efficacy of such extract, generally, the cytotoxicity of the extract is. The brine shrimp bioactivity assay is a simple experimental process to the check the cytotoxicity of a chemical, drug or even plant extracts *in vivo* on simple zoologic organism *Artemia salina*. So, the lethal concentration LC_{50} of a drug or extract can be determined to estimate the cytotoxicity of that extract. The LC_{50} obtained for *S. cumini* extract was high (639.74µg/ml) (Fig. 1 & Table 1) comparing with positive control vincristine sulphate (13.90µg/ml) (data not shown). This result demonstrated that the extract would not be toxic in animal indicating the safety of the extract in the case of *in vivo* application.

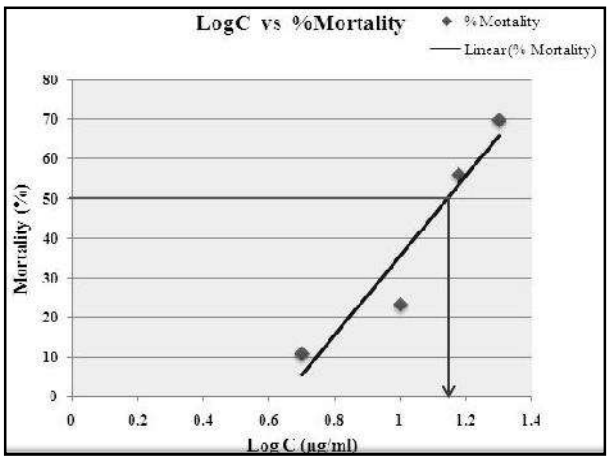

Fig. 1. Determination of LC_{50} of *S. cumini* ELE. A straight line obtained by plotting the percentage of mortality of Brine shrimps nauplii against the logarithm of the concentration of plant extract (from Table 1). From the graph, Log LC_{50} was obtained at 50% mortality. LC_{50} value was obtained by inversing the Log LC_{50} value. LC_{50} value obtained for *S. cumini* extract was 639.74µg/ml.

Table 1. Effect of *S. cumini* ELE on brine shrimp nauplii. Brine shrimp nauplii were treated with different concentrations of *S. cumini* extract as indicated and after 24 hrs number of live and dead nauplii was counted and percentage of mortality was calculated.

Concentration (mg/ml)	% Mortality
Blank	0.00
Solvent	0.00
300	30.00
400	33.33
500	47.50
600	53.33
1000	61.90

3.4 ELE showed Vibriocidal activity

To assure whether the *Vibrio* serogroup *Ogawa* and *Inaba* were antibiotic resistant or not, antibiogram of these strains was determined using some common antibiotics such as erythromycin, gentamicin, tetracycline, ampicillin, penicillin G, trimethoprim and ceftazidime. Both of the organisms showed resistance against all the above antibiotics tested (data not shown). Next, we investigated whether the extract of *S. cumini* could effectively inhibit the growth and activity of multi-drug resistant *Ogawa* and *Inaba*.

Different concentrations (50, 75 and 100mg/ml) 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 inhibition zone. 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 an almost similar effect against both *Inaba* and *Ogawa* (Fig. 2 and Table 2).

Table 2. Antibacterial activity of *S. cumini* ELE against *V. Ogawa and Inaba*.

Name of Serogroups	Diameter of Zone of Inhibition (mm)			
	Only solvent	Plant extract (mg)		
		50	75	100
V. ogawa	-	8	10	11
V. inaba	-	9	10	12

3.5 The MIC value of ELE against *Vibrio*

After getting significant inhibitory effects of the plant extract against *Ogawa and Inaba*, MIC of crude extract was determined. The maximal inhibition for these strains was in the range of 600-1000µg/ml (data not shown). The lowest concentration at which the extract of *S. cumini* effectively inhibited the bacterial growth was found to be 600µg/ml.

4. Discussion

Pharmacological industries have produced a number of new drugs in the last three decades; resistance to these drugs by microorganisms has increased due to the frequent use and misuse of drugs. As a result, scientists are increasingly recognizing plant remedies as a very important low cost alternative to industrially produced antibiotics.

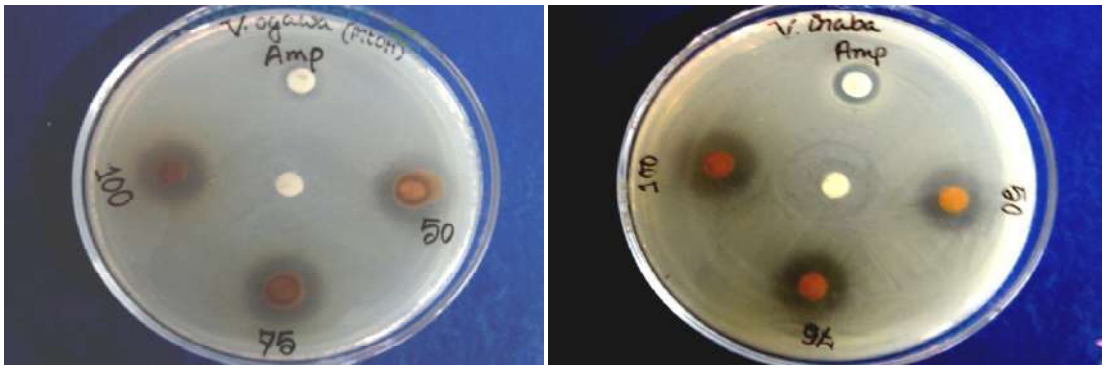

Fig. 2. Antibacterial activity of *S. cumini* ELE against *V. ogawa* (left) and *inaba* (right) at different concentrations (a. 50, b. 75 and c. 100µg/ml). Amp (ampicillin) and solvent (disc in the middle) used as negative control.

As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of drugs from plant origin should be emphasized. In this study, ethanolic extract of *S. cumini* leaf was investigated for its potential bioactivity against *V. ogawa* and *Inaba* that are known as multidrug resistant.

In this study, the phenolic content of *S. cumini* leaves were determined and it was found that phenolic content was high which is comparable with ascorbic acid. Reducing the power of the ethanolic extract was significantly related to its total phenolic content. The presence of polyphenolic compounds in ethanolic leaf extract of *S. cumini* might be responsible for this high antioxidant activity [13, 14].

In this study, *S. cumini* extract effectively inhibited the growth of *V. Ogawa* and *Inaba* almost equally. Brine shrimp bioactivity assay demonstrated the safety and efficacy of the extract by showing a relatively higher value of LC_{50} (639.74µg/ml for the extract and 13.90µg/ml for the control). This result argued that the extract would not be cytotoxic indicating its safety for *in vivo* application. Therefore, *S. cumini* extract seemed to be promising because for its effective inhibition or killing of multi-drug resistant *Vibrios* in one hand and its less cytotoxic properties on the other.

The extract was active with the concentrations ranging from 200-1000µg/ml, however, the MIC was found to be 600µg/ml for both *V. ogawa* and *inaba*. The high value of MIC may indicate a lower efficacy or the potential of the organisms for developing resistance against the bioactive compounds [15]. However, the predicted low MIC value observed for *Vibrio* in this study is a good indication of high efficacy against this bacterium. This outcome is remarkable considering that the plant extract could be a good candidate to be used as a potent drug against cholera.

Therefore, the components of this extract could be identified and characterized for future therapeutic applications.

References

[1]. World Health Organization Media Center. 2010.

[2]. Cholera in Haiti has spread to every part of the country, CDC reports. Booster Shots: Oddities, musings and news from the health world.

[3]. Albert, M.J., Ansaruzzaman, M., Bardhan, P.K., Faruque, A.F.M., Faruque, S.M., Islam, M.S., Mahalanabis, D., Sack, R.B., Salam, M.A., Siddique, A.K., Yunus, M.D. & Zaman, K. (1993). Large epidemics of cholera like disease in Bangladesh caused by *Vibrio cholerae* O139 synonym Bengal. Cholera Working Group, International Centre for Diarrhoeal Diseases Research, Bangladesh. *Lancet*, 342: 387-390.

[4]. Ramachandran, D., Bhanumathi, R. & Singh, D.V. (2007). Multiplex PCR for detection of antibiotic resistance genes and the SXT element: application in the characterization of *Vibrio cholerae*. *J. Med. Microbiol*., 56: 346-351.

[5]. Moresco, R.N., Sperotto, R.L., Bernardi, A.S., Cardoso, R.F. & Gomes, P. (2007). Effect of the Aqueous Extract of *Syzygium cumini* on Carbon Tetrachloride-induced Hepatotoxicity in Rats. *Phytother. Res.*, 21: 793–795.

[6]. Gowri, S.S. & Vasantha, K. (2010). Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts. *In. J. Pharm. Tech. Res.,* 2 (2): 1569-1573.

[7]. Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.*, 16: 144-158.

[8]. Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, J.B., Nichols, D.E. & Mclaughlin, J.L. (1982). Brine shrimp; a convenient general bioassay for active plant constituents. *Planta Medica*, 45: 31-34.

[9]. Perez, C., Paul, M. & Bazerque, P. (1990). An antibiotic assay by agar-well diffusion method. *Acta. Biol. Med. Exp.*, 15: 113-115.

[10]. Bauer, A.W., Kirby, W.M., Sherris, J.C. & Turck, M. (1996). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., 45:493–496.

[11]. Hatano, T., Edamatsu, R., Hiramatsu, M., Mori, A., Fujita, Y., Yasuhara, T., Yoshida, T. & Okuda, T. (1989). Effect of interaction of tannins with co-existing substances VI. Effect of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chem. Pharm. Bull.*, 37: 2016-2021.

[12]. Duh, P.D., Tu, Y.Y. & Yen, G.C. (1999). The antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). *LWT - Food Science and Technology*, 32, 269–277.

[13]. Benherlal, P.S. & Arumughan, C. (2007). Chemical composition and in vitro antioxidant studies on *Syzygium cumini* fruit. *J. Sci. Food Agric.*, 87: 2560-2569.

[14]. Zhi Ping Ruan, Liang Liang Zhang & Yi Ming Lin (2008). Evaluation of the Antioxidant Activity of *Syzygium cumini* Leaves. *Molecules*, 13: 2545-2556.

[15]. Anibijuwon, I.I. & Udeze, A.O. (2009). Antimicrobial activity of *Carica papaya* (Pawpaw Leaf) on some pathogenic organisms of clinical origin from South-Western Nigeria. *Ethnobotanical Leaflets*, 13: 850-864.