Antibacterial and cytotoxic activities of *Areca catechu* L. (betel nut)

Md Shyfuzzaman Khan, Md. Shamim Akhter*

Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh

*Orcid id: 0000-0001-5630-3906, shamim11akhter@bge.ku.ac.bd

Abstract

The aim of the study was to evaluate the antibacterial and cytotoxic activities of acetone and ethanol extracts of *A. catechu* L. Antibacterial activity was tested against selected gram positive bacteria of *Staphylococcus aureus*, *Micrococcus* species and gram negative *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli*, *Vibrio cholera* using well diffusion assay. Acetonic extract showed better antibacterial activity against *E. coli* (20.83 mm for 100 µl/well), *S. typhi* (20.17 mm for 100 µl/well), *S. aureus* (20.17 mm for 100 µl/well). The ethanolic extract showed better antibacterial activity against *P. aeruginosa* (18.17 mm for 100 µl/well), *S. paratyphi* (17.67 mm for 100 µl/well), *Micrococcus* species (19 mm for 100 µl/well). Minimal inhibitory concentration (MIC) and minimal bacterial concentration (MBC) values were determined by using broth macro dilution assay which supports antibacterial potency of the extracts. Cytotoxicity was determined by brine shrimp lethality assay: both extracts showed moderate cytotoxicity compared to vincristine sulphate (with LC_{50} of 0.99 µg/ml). The acetonic extract showed more cytotoxicity than the ethanolic extract with LC_{50} values of 17.021 (µg/ml) and 20.136 (µg/ml). These findings are correlated with traditional medicinal uses of *A. catechu* and showed rationale for further investigation for screening out the possible bioactive constituents.

Keywords: *Areca Catechu* L., Betel Nut, Antibacterial Assay, Cytotoxicity, Brine Shrimp Lethality Bioassay.

Introduction

Areca catechu Linn is commonly known as betel nut and belongs to the family Arecaceae. It is commonly found in tropical and subtropical countries [1]. Many parts of the betel nut tree are used in traditional medicine as an herbal medicine with good diuretic, digestion-promoting, and anti-parasitic properties [2], anti-aging functions [3], stimulatory effect [4], antiovulatory and abortifacient effects [5], analgesic and anti-inflammatory effect [6-8], anti-allergic effects [9], blood glucose and lipids regulatory effects [10], antiplatelet effect [11], antimalarial and antimicrobial activites [12-13], antihyperglycemic activity [10, 14], burn wound healing capacity [15], and silver nanoparticle synthesis capacity[16]. The raw areca and charred betel nuts are the most common clinically used forms. The nut is used extensively as a narcotic in the Asia-Pacific region and in Asian communities elsewhere [17-22]. It is chewed with the leaf or in Xorescence of Piper betel (Piperaceae), lime and sometimes tobacco and spices, principally as a mild stimulant [4]. Its geographical origin is unknown, but it is widely cultivated and is used by an estimated 200–400 million people worldwide [23]. Traditionally, *A. catechu* has commonly been used to kill parasites and promote digestion. Currently, the areca nut is commonly used as the main traditional Chinese medicine (TCM) for the treatment of various gastrointestinal diseases (including abdominal distension, dyspepsia, dysentery, and constipation), parasitic diseases, and edematous disease, in the forms of powders, decoctions or infusions [2, 24].

To determine antibacterial activity of a substance there are a number of methods available. Majority of the researchers use one of the following in vitro assays: disc diffusion, broth dilution, and agar dilution method to determine antibacterial activity [25]. The test systems should ideally be simple, rapid, reproducible, and inexpensive and maximize high sample throughput in order to cope with a varied number of extracts and fractions [26-27]. To maximize high sample throughput and varied number of extracts and fractions, agar well diffusion is the reliable technique. And to determine cytotoxic activity, 'brine shrimp lethality assay' can be used [28] which has a correlation with cytotoxic activity on tumours in the human body.

The objective of this study was to investigate out the antibacterial potentiality and cytotoxic activity of crude

extracts of *A. catechu to* establish scientific relevance of the uses of *A. catechu* in traditional medicine.

Materials and Methods

Collection of seeds

Dry *Areca catechu* were purchased from the local market of Dumuria, Khulna, Bangladesh.

Preparation of sample/extract

The collected seeds were washed thoroughly two to three times with distilled water. Washed seeds were dried under sunlight naturally for 3 to 4 days. Hot water extraction methodologies were followed to obtain crude extracts from *A. catechu*. Five hundred gm fresh *Areca catechu* nut was collected and turned into powder through grinding. Fifty gm of sample powder was taken and soaked into 100 ml of pure ethanol and also in 100 ml of acetone. They were kept into water bath for 2 hours at (50-55) ° C. Then the contents were filtrated through Whatman filter paper. The filtrates were evaporated by rotary evaporator, then air dried. The air dried extracts were weighted and 13.36 gm of extracts were obtained from acetonic and 4.48gm from ethanolic extraction.

Antibacterial screening

Antibacterial screening of crude extracts was tested by the agar disc diffusion method. Seven pathogenic bacterial strain including five gram- negative and two gram-positive bacteria were chosen for antibacterial screening test (Table 1). They were maintained on nutrient agar media by streak plate method [29]. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [30-32]. Nutrient agar plate was prepared by pouring 25 ml nutrient agar media into Petri plates (100mm x 15 mm). The agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface (1.11 to 2.17 x 10^8 cfu/ml). Then, holes/wells with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer. Sample wells were filled with extracts of different volumes (50 µl and 100 µl), standard wells (positive control) were filled with antibiotic ciprofloxacin (15 µl) and the negative control wells were kept empty. The plates were then normally (not inverted) kept in refrigeration for about 24 hours at 4°C. This was sufficient for the materials to diffuse into a considerable area of the medium. Finally the plates were incubated normally as before at 37°C for 18-24 hours. After incubation, the antibacterial activity of the test samples were determined by measuring the diameter of zone of inhibition in term of millimeter with a transparent scale.

Table 1. List of bacteria

Gram positive(+ve)	**Gram negative(-ve)**
Staphylococcus aureus	*Vibrio cholera*
Micrococcus sp.	*Salmonella paratyphi*
	Escherichia coli
	Salmonella typhi
	Pseudomonas aeruginosa

Cytotoxic Screening

Brine shrimp lethality bioassay is an extensively used development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g, anticancer, antiviral, pesticidal etc. [33]. The larvae (nauplii, singular nauplius), about 22 mm long, are large enough to observe without high magnification and small enough for hatching in enormous amount without extensive workspace in a laboratory. The hatched shrimps were stored in a suitable environment in the dam attached to a lamp and then they were taken for the bioasaay. One hundred clean test tubes were taken, 14 of which were for the samples in seven different concentrations (two test tubes for each concentration), 14 for positive control (two test tubes for each concentration) and 2 for negative control. Then 4ml of seawater was given to the test tubes.

With the help of the micropipette specific volumes (5, 10, 20, 40, 80, 160 and 320 µl) of samples were transferred from the stock solutions to the test tubes. Different concentration of choloram phenicol (5, 10, 20, 40, 80, 160 and 320 µl) were taken in the rest of the 14 test tubes which were used as positive control and finally the volume was maintained at 10ml in each test tube (sample, positive control and negative control) using sea water. So, the concentration of samples became 2.5, 5, 10, 20, 40, 80 and 160 µg/ml respectively. Finally with the help of a Pasteur pipette, 10 live shrimp's nauplii were taken into each of the test tubes [34]. After 24 hours the test tubes were observed to count mortality and a graph of percentage of mortality and log concentration was plotted and median lethal concentration (LC_{50}) were calculated by using statistical analysis. Tests of acetonic and ethanolic extracts were done in triplicates to get a reliable result.

Results

Antibacterial assay

Acetonic extract of *A. catechu*

Acetonic extract showed the maximum zone of inhibition against three bacteria, *E. coli*, *S. aureus* and *S. typhi* respectively. Among these three, maximum zone was observed for *E. coli* (20.83 mm avg.). And maximum zone for these three were around 20 mm. On the other hand, minimum zone of inhibition was observed for *P. aeruginosa* (15.50 mm avg.). Positive control wells showed zone of inhibition around 30 mm for all those tested bacteria (Table 2, Fig. 1 and 2)

Table 2: Antibacterial activity of acetonic extract of *A. catechu*.

S/L No	Name of Bacteria	Zone of inhibition of test sample (mm+standard deviation)		Zone of Inhibition of positive and negative control (mm ± standard deviation)	
		Absolute acetone			
		50µl/well	100µl /well	Positive control(15µl /well)	Negative control
01	*P. aeruginosa*	**12.00±0.50**	**15.50±0.50**	**30.00±1.00**	**n.d**
02	*S. paratyphi*	13.17±0.76	18.17±0.76	29.00±1.00	n.d
03	*Micrococcus sp.*	13.50±0.50	17.33±1.53	30.17±1.04	n.d
04	*S. Aureus*	17.17±1.26	20.17±1.04	30.67±1.53	n.d
05	*S. Typhi*	16.17±0.76	20.17±0.76	31.50±0.50	n.d
06	*E. Coli*	17.50±0.50	20.83±1.26	29.50±0.50	n.d
07	*V. cholerae*	10.17±1.04	18.00±1.00	32.00±2.00	n.d

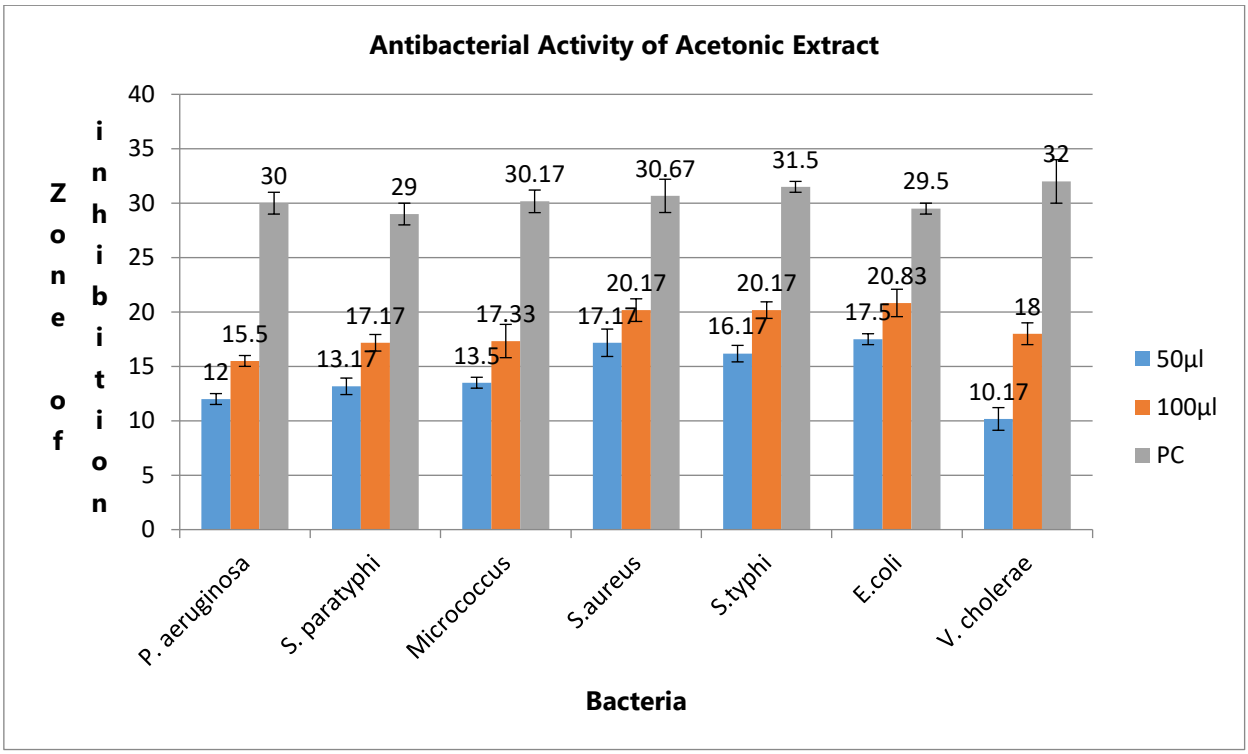

Figure 1. Evaluation of antibacterial activity of acetonic extract of *A. catechu* (Bacteria vs Zone of inhibition in millimeter).

Figure 2. Antibacterial activity of acetonic extract of *A. catechu* against *P. aeruginosa* and *S. aureus*.

Ethanolic extract of *A. catechu*

Ethanolic extract showed the maximum zone of inhibition against *Micrococcus sp.* (19 mm avg.). On the other hand, minimum zone of inhibition was observed for *P. aeruginosa* (15.50 mm avg.). Positive control wells showed zone of inhibition around 30 mm for all those tested bacteria. Maximum for *V. cholera* (31.17 mm avg.) and minimum for *E. coli* (28.17 mm avg.) (Table 3, Fig. 3 and 4). The statistical p-value showed the significance for tested organisms (Table 4). The extracts showed good zone of inhibition ($p<0.05$) against the tested microbes. The variation of the result may be due to the potentiality to extract out molecules by acetone and ethanol, respectively.

Table 3. Antibacterial activity of ethanolic extract of *A. catechu*.

S/L No	Name of Bacteria	Zone of inhibition of test sample (mm ± standard deviation)		Zone of Inhibition of positive and negative control (n=2) (mm ± standard deviation)	
		Absolute Ethanol			
		50µl/well	100µl/well	Positive control (15µl /well)	Negative control
01	*P. aeruginosa*	13.5±0.50	18.17±0.76	29.83±0.76	n.d
02	*S. paratyphi*	13±1.00	17.67±0.76	31±1.00	n.d
03	*Micrococcus sp.*	12.83±0.76	19±1.00	31.5±1.50	n.d
04	*S. Aureus*	12.5±0.50	15.50±0.76	30.5±0.50	n.d
05	*S. Typhi*	13.67±0.76	16.83±0.29	30.5±0.50	n.d
06	*E. Coli*	12±1.00	16.17±0.76	28.17±0.76	n.d
07	*V. cholerae*	13.5±0.50	16.67±0.58	31.17±0.76	n.d

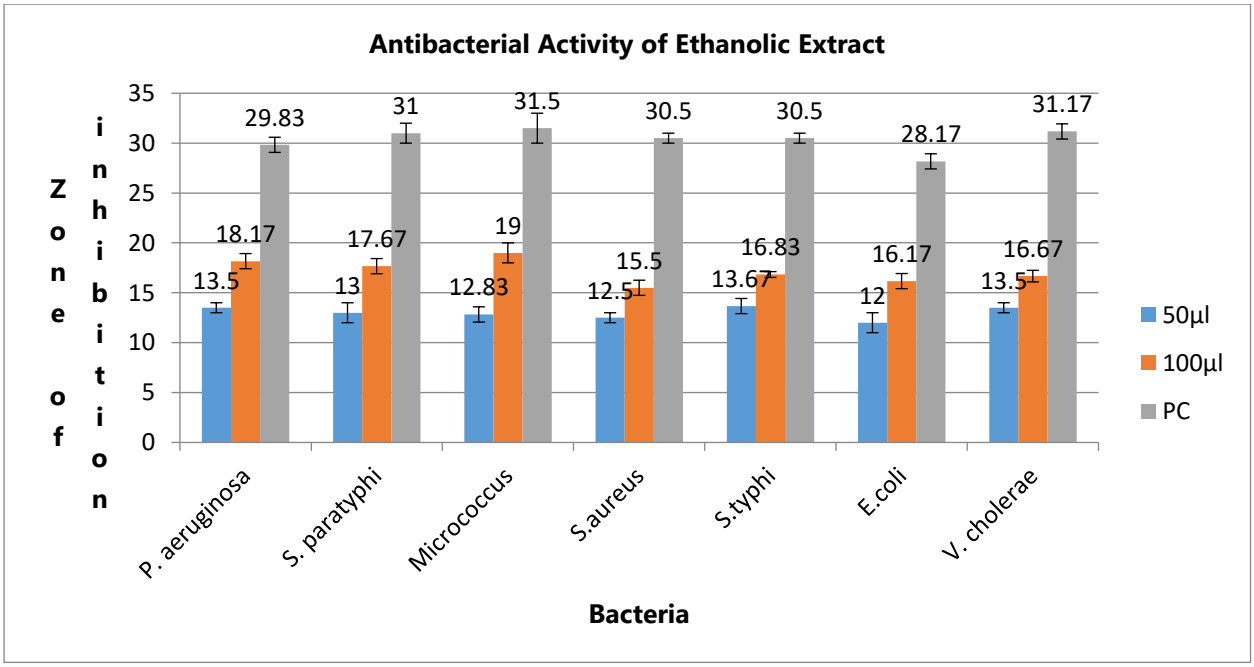

Figure 3. Evaluation of antibacterial activity of ethanolic extract of *A. catechu* (Bacteria vs Zone of inhibition in millimeter).

Figure 4. Antibacterial activity of ethanolic extract of *A. catechu* against *P. aeruginosa* and *S. paratyphi.*

Table 4. Result of significance test ($p < 0.05$) for mean of zone of inhibition at two different volumes.

Bacteria	Statistical analysis					
	Absolute acetone			Absolute ethanol		
	t-value	p-value	Significant (yes/no)	t-value	p-value	Significant (yes/no)
P. aeruginosa	8.5732	0.0010	yes	8.8544	0.0017	yes
S. paratyphi	8.0178	0.0013	yes	6.4236	0.0038	yes
Micrococcus sp.	4.1309	0.0384	yes	8.4884	0.0014	yes
S. Aureus	3.1820	0.0351	yes	4.4272	0.0159	yes
S. Typhi	6.4143	0.0030	yes	7.7782	0.0076	yes
E. Coli	4.2640	0.0310	yes	5.7354	0.0056	yes
V. cholerae	9.4000	0.0007	yes	7.1813	0.0022	yes

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assay

The tubes were allowed to incubate overnight. Broth tubes that appear turbid are indicative of bacterial growth [35] while tubes that remain clear indicated no growth. The MIC of the antibiotic is the lowest concentration that does not show growth. MIC is the lowest concentration of extract that prevent bacterial visible growth.

The resultant MIC test tubes are used for MBC test and showed the concentration of the antibacterial where it killed around 99% of the bacteria in the growth tube (Table 5).

Table 5. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of ethanolic and acetonic extracts of *A. catechu*.

Bacteria	Acetonic extract		Ethanolic extract	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
P. aeruginosa	2.5	5.0	1.25	2.5
Micrococcus sp.	1.25	2.5	0.625	1.25
S. Aureus	0.625	1.25	2.5	5.0
E. Coli	0.625	1.25	1.25	2.5

Cytotoxic activity related assay

Brine shrimp lethality bioassay

Based on the lethality of brine shrimp the cytototoxicity effect of the crude extract of *A. catechu* showed moderate lethality indicating the biological activity of the compound present in the extract. Test samples are showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample and plot of percent mortality versus different concentrations of extracts on the graph paper produced an approximate linear correlations at which 50% mortality (LC_{50}) of brine shrimp naupli occurred were obtained by extrapolation. The ethanolic extract of *A. catechu* seeds has the highest value LC_{50} of 20.1358 µg/ml. On the other hand acetonic extract of *A. catechu* seed has the lowest value LC_{50} of 17.0208 µg/ml. The lower the LC_{50} value higher the activity. So acetonic extract of seed has higher cytotoxicity effect than the ethanolic extract (Table 6, 7 and 8; Fig. 5, 6). In Fig. 7 hatching of brine shrimp is shown.

Table 6. Cytotoxic effect of the acetonic extract and ethanolic extract.

Sample Conc. (µg/ml)	Log C	Mortality (%) For Acetonic Extract		LC_{50} (µg/ml)	Mortality (%) For Ethanolic Extract		LC_{50} (µg/ml)
2.5	0.39	5	y = 55.765x-13.843	17.0208	5	y = 56.361x-13.189	20.1358
5	0.70	15			20		
10	1.00	55			45		
20	1.30	60			70		
40	1.70	80			85		
80	1.90	95			95		
160	2.20	100			100		

Table 7. Cytotoxic activity of vincristine sulphate.

Concentration (µg/ml)	LogC	% Mortality	LC_{50} (µg/ml)
0.06	-1.22	15	0.997
0.12	-0.903	25	
0.25	-0.602	35	
0.50	-0.30	45	
1.00	0	55	
5.00	0.7	60	
10.00	1.0	80	

Table 8. Regression equation and value of R^2 of ethanolic, acetonic extract *A. catechu* and vincristine sulphate.

Sample	Regression equation	R^2
Acetone	y = 55.765x-13.843	0.9526
Ethanol	y = 56.361x-13.189	0.9598
Vincristine sulphate	y= 26.575x+50.03	0.9565

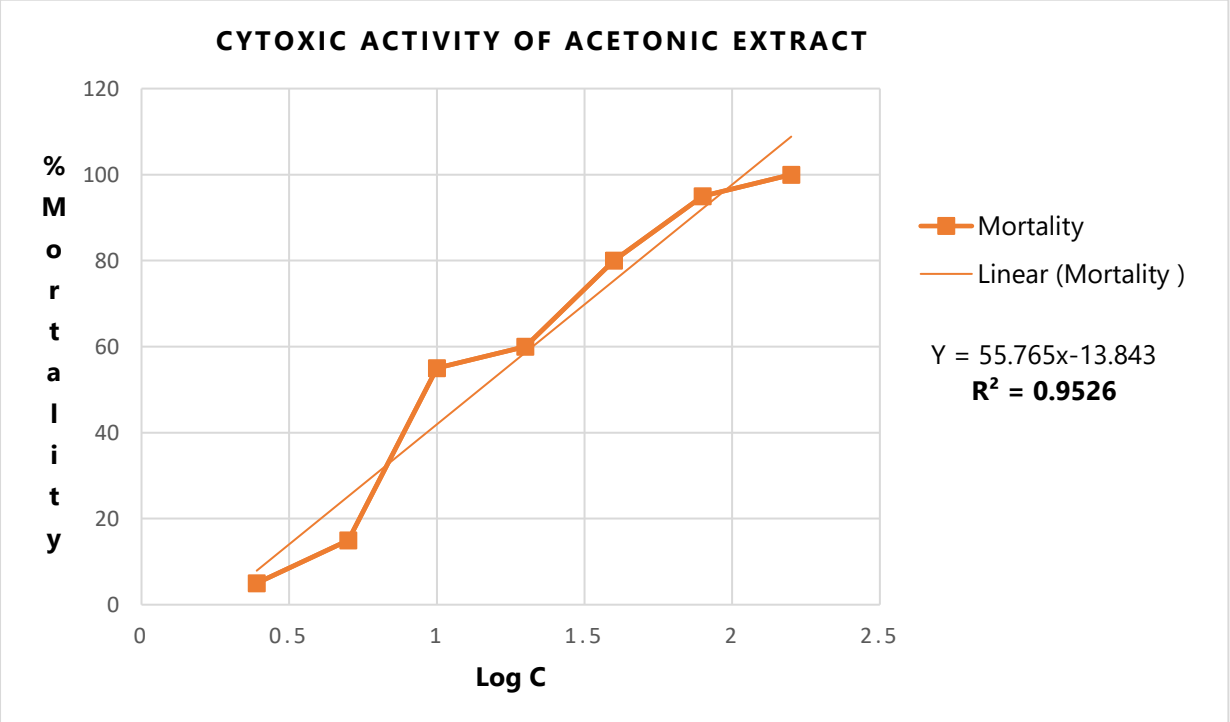

Figure 5. Log concentration of acetonic extract of *A. catechu* (extract vs percent shrimp mortality).

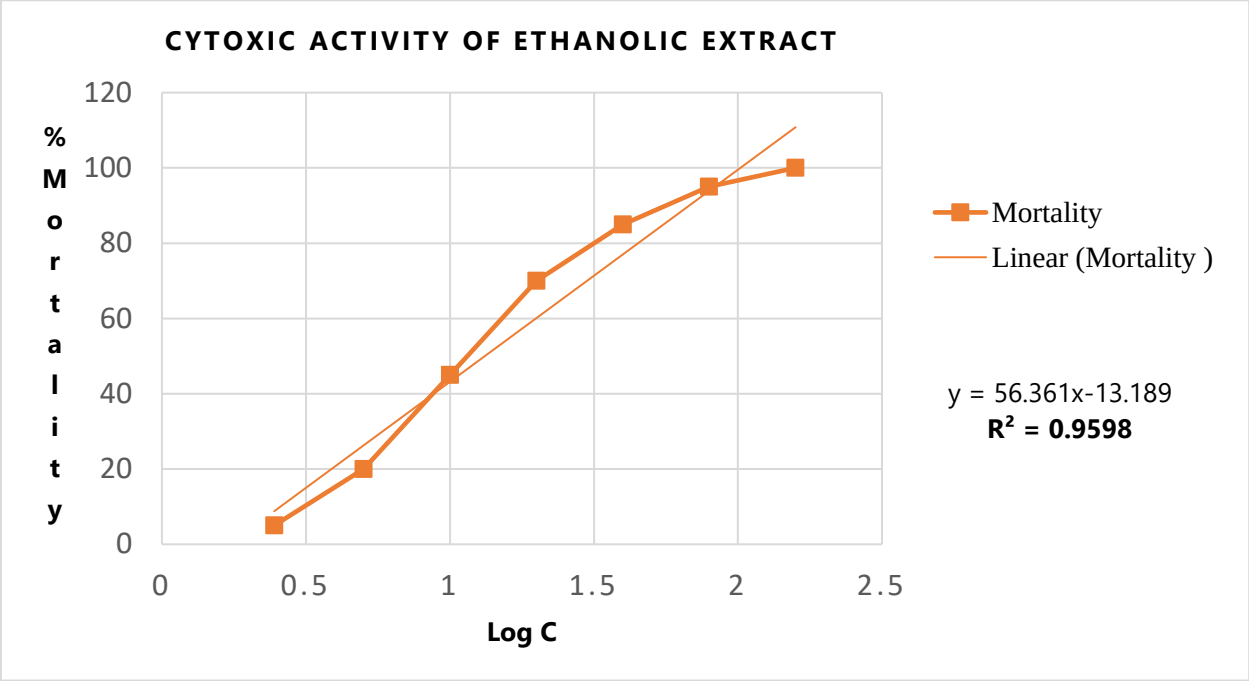

Figure 6. Log concentration of ethanolic extract of *A. catechu* (extract vs percent shrimp mortality).

Figure 7. Hatching of *Artemia salina* leach

Discussions

The antiseptic qualities of aromatic and medicative plants and their extracts are recognized since antiquity, whereas the attempts to characterize these properties within the laboratory were initiated to the first decenary [36-37]. Pharmaceutical and scientific communities have proved therapeutic worth of natural compounds and validated their biological activity [38-39]. Profuse use of economic antibiotic and artificial pesticides for human and crop protection is harmful to human health, system and atmosphere. This has enabled exploitation of medicinal plants for the treatment of microbial infections of both plants and humans by developing new antimicrobial agents [13]. To prevent or cure infectious conditions, traditional healers have long used plants. Western medicine is trying to duplicate their successes. Plants have been found to have antimicrobial properties *in vitro* because they are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids [40]. The compounds having phenolic structures, such as carvacrol, eugenol and thymol, were highly active against the test microorganisms. Members of this class are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used [41]. Aqueous extracts of the *A. catechu* contains

major alkaloid arecoline and the components tannic acid and catechin of its tannin fraction. The antibacterial properties of the above were tested on *Streptococcus mutans*, *Streptococcus salivarius*, *Candida albicans* and *Fusobacterium nucleatum* and, as a control, *Staphylococcus aureus*. After chewing boiled *A. catechu*, its effect was examined on salivary organisms cultured from the saliva. Extracts inhibited the growth of the selected organisms in a concentration dependent manner [42]. This study supports that providing scientific proof of using *A. catechu* as antibacterial agents.

The acetonic extract showed maximum zone of inhibition against gram negative bacteria *E. coli*, *S. typhi* and gram positive bacteria of *S. aureus*. The diameter of zone of inhibition found against these three can be evaluated as the extract showed succeptible result against the growth of this three organisms. The extract also showed good zone of inhibition ($p < 0.05$) against other tested microbes including gram positive bacteria. The possible causes of excellent potentiality showed against gram positive bacteria due to the presence of outer membrane of gram negative bacteria which act as barrier against numerous antibiotic molecules and the enzymes of the periplasmic spaces which have the ability to breakdown foreign molecules [43]. Compared to ethanol, acetonic extract showed more response to inhibit growth of all tested microorganisms. Variation of this result may be due to the different potentiality of extraction using acetone and ethanol respectively. Result analysis indicates that the acetonic and ethanolic extracts have antibacterial properties. A. catechu extracts may be used to develop drugs against bacteriological diseases like infections caused by *S. aureus* [44], drugs against many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveller's diarrhoea, and other clinical infections such as neonatal meningitis and pneumonia caused by *E. coli* [45], drugs against inflammation and suppuration caused by *Micrococcus* species [46]. Ethanolic extract showed significant results against all tested organisms and maximum for *Micrococcus* species; acetonic extract showed maximum for *E. coli* with significant results against all tested microbes [47].

Another part of this research was to find out the potential cytotoxic activity of *A. catechu*. The brine shrimp lethality bioassay is considered as a useful tool for the preliminary assessment toxicity [48] and normally conducted to draw inferences on the safety of the plant extracts and to further depict trends of their biological activities [49]. The dried extracts showed cytotoxicity at such a level that it could be termed as moderate cytotoxicity compare to positive control according to Meyer *et al*., 1982 [34]. This can be caused due to the presence of toxic ingredients in the active fraction that possess ovicidal properties [50]. The toxicity may be due to the presence of cyanogenic glucosides present in the dried seeds [51]. Considerable interest arose regarding the use of a synthetic cyanogenic glucoside as an alternative anticancer compound [52]. *In vitro* cytotoxic effect showed by *A. catechu* extract can be an initial indicator of *in vivo* antitumor and anticancer activity. Therefore isolation of active compounds and further cell line assay is required to eliminate cytotoxic compounds and to develop potential anticancer agent [53]. Cytotoxicity can be caused due to wide range of phytochemicals which have nonspecific cytotoxicity [53-55].

Conclusions

Here *in vitro* antibacterial and cytotoxic activity of the *A. catechu* extracts provide scientific footing to enhance confidence in the traditional claims of its use, suggesting the isolation of bioactive components and the elimination of toxic compounds, as well, through further bioassays. *In-vivo* trials would help to sort out active compounds of the seed as pharmaceutical and therapeutic agents.

Acknowledgements

The authors express thanks to Khulna University Research Cell (KURC), Khulna University, Bangladesh for providing the grant to conduct this fundamental research. The authors also express thanks to their respective institute: Khulna University, Bangladesh.

Conflict of interest: There is no conflict of interest anywhere.

References

1. Xing ZHANG, Jiao WU, Zhuang HAN, Wen-Li M, Hao-Fu D (2010) Antioxidant and cytotoxic phenolic compounds of areca nut (Areca catechu), Journal Chem Res Chin Univ, 26(1), 161-64.

2. Yan D, Peng W, Liu Y, Xie D, Hu M, Tao X, & Wu C (2016) Spectrum-effect relationships between high performance liquid chromatography fingerprints and bioactivities of charred areca nut, Trop J Pharm Res, 15(5), 1017-1024.

3. Amudhan MS, Begum VH, Hebbar KB (2012) A review on phytochemical and pharmacological potential of Areca catechu L. seed, Inter. J of Pharm Sc and Res, 3(11), 4151.

4. Heatubun CD, Dransfield J, Flynn T, Tjitrosoedirdjo SS, Mogea, JP, Baker WJ (2012) A monograph of the betel nut palms (Areca: Arecaceae) of East Malesia, Bot. J of the Lin Soc., 168(2), 147-173.

5. Shrestha J, Shanbhag T, Shenoy S, Amuthan A, Prabhu K, Sharma S, Kafle S (2010) Antiovulatory and abortifacient effects of Areca catechu (betel nut) in female rats, Indian J of pharma., 42(5), 306.

6. Khan S, Mehmood MH, Ali ANA, Ahmed FS, Dar A, Gilani AH (2011) Studies on anti-inflammatory and analgesic activities of betel nut in rodents. J of Ethnopharma, 135(3), 654-661.

7. Bhandare AM, Kshirsagar AD, Vyawahare NS, Hadambar AA, & Thorve VS (2010) Potential analgesic, anti-inflammatory and antioxidant activities of hydroalcoholic extract of Areca catechu L. nut. Food Chem. Toxicol., 48(12), 3412-3417.

8. Bhandare A, Kshirsagar A, Vyawahare N, Sharma P, Mohite R (2011) Evaluation of anti-migraine potential of Areca catechu to prevent nitroglycerin-induced delayed inflammation in rat meninges: Possible involvement of NOS inhibition, J of ethnopharma, 136(1), 267-270.

9. Wang CC, Lin YR, Liao MH, Jan TR (2013) Oral supplementation with areca-derived polyphenols attenuates food allergic responses in ovalbumin-sensitized mice, BMC com and alt med, 13(1), 154.

10. Ghate R, Patil VP, Hugar S, Matha NH, Kalyane NV (2014) Antihyperglycemic activity of Areca catechu flowers. Asian Pac J of Trop Dis, 4, S148-S152.

11. Ghayur MN, Kazim SF, Rasheed H, Khalid A, Juman, MI, Choudhary MI, Gilani AH (2011) Identification of antiplatelet and acetylcholinesterase inhibitory constituents in betel nut, J of Chinese integra med, 9(6), 619-625.

12. Boniface P, Verma SK, Cheema HS, Darokar MP, Pal A (2014) Evaluation of antimalarial and antimicrobial activites of extract and fractions from Areca catechu, Inter J of Infec Dis, 21, 228-229.

13. Das K, Tiwari RKS, Shrivastava DK (2010) Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends, J of med plants res, 4(2), 104-111.

14. Huang PL, Chi CW, Liu TY (2013) Areca nut procyanidins ameliorate streptozocin-induced hyperglycemia by regulating gluconeogenesis, Food Chem. Toxicol.,, 55, 137-143.

15. Bharat M, Verma DK, Shanbhag V, Rajput RS, Nayak D, Amuthan A (2014) Ethanolic extract of oral Areca catechu promotes burn wound healing in rats, Int J Pharm Sci Rev Res, 25(2), 145-148.

16. Bhat R, Ganachari S, Deshpande R, Ravindra G, Venkataraman A (2012) Rapid Biosynthesis of Silver Nanoparticles Using Areca Nut (Areca catechu) Extract Under Microwave-Assistance. J of Cluster Sc., 24, 107–114. doi:10.1007/s10876-012-0519-2.

17. Mehrtash H, Duncan K, Parascandola M, David A, Gritz ER, Gupta PC, Mehrotra R, Amer Nordin AS, Pearlman PC, Warnakulasuriya S, Wen CP, Zain RB, Trimble EL (December 2017) Defining a global research and policy agenda for betel quid and areca nut, Lancet Oncol. 18 (12), e767–e775. doi:10.1016/S1470-2045(17)30460-6. PMID 29208442.

18. Garg A, Chaturvedi P, Gupta PC (2014) A review of the systemic adverse effects of areca nut or betel nut, Indian J of Med and Paedi Onco., 35 (1): 3–9. doi:10.4103/0971-5851.133702. PMC 4080659. PMID 25006276.

19. Auluck, A, Hislop G, Poh C, Zhang L, Rosin MP (2009) Areca nut and betel quid chewing among South Asian immigrants to Western countries and its implications for oral cancer screening, Rural and Rem Health. 9 (2). PMID 19445556.

20. Ahuja SC, Ahuja U (2011) Betel Leaf and Betel Nut in India: History and Uses. Asian Agri-History, 15, 13-35.

21. Peter GO, Tung-Shan C, Tsu-Wang S (2011) Characterization of the Psychological, Physiological and EEG Profile of Acute Betel Quid Intoxication in Naïve Subjects, PLoS One, 6(8), e23874. doi: 10.1371/journal.pone.0023874

22. Padmavathamma V (2011) Areca nut in Indian economy-present status and future strategies. E-Journal of Dentistry, 1 (3): 46-49.

23. Gupta PC, Warnakulasuriya S, (2002) Global epidemiology of areca nut usage, Addic biol, 7(1), 77-83.

24. Peng W, Liu YJ, Wu N, Sun T, He XY, Gao YX, Wu CJ (2015) Areca catechu L.(Arecaceae): a review of its traditional uses, botany, phytochemistry, pharmacology and toxicology, J of ethnopharma, 164, 340-356.

25. Bauer AW, Kirby WMM., Sherris JC, Turck M, (1966) Antibiotic susceptibility testing by a standardized single disk method, American j of clinic patho, 45(4), 493-496.

26. Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: A review, J of Pharma Anal., 6(2), 71-79.

27. Hadacek, F, Greger H (2000) Testing of antifungal natural products: methodologies, comparability of results and assay choice. Phytochemical Analysis, an Inter J of Pl Chem and Biochem Tech, 11(3), 137-147.

28. Michael AS, Thompson CG, Abramovitz, M (1956) Artemia salina as a test organism for bioassay, Science, 123(3194), 464-464.

29. Bauman RW (2013) Microbiology with diseases by taxonomy. Pearson Higher Ed.

30. Dahiya P, Purkayastha S (2012) Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. Indian j of pharma sc, 74(5), 443.

31. Magaldi S, Mata-Essayag S, De Capriles CH, Perez C, Colella MT, Olaizola C, Ontiveros Y (2004) Well diffusion for antifungal susceptibility testing. Inter j of infec dis, 8(1), 39-45.

32. Valgas C, Souza SMD, Smânia EF, Smânia Jr, A (2007) Screening methods to determine antibacterial activity of natural products, Brazilian J of Microb., 38(2), 369-380.

33. Syahmi ARM, Vijayarathna S, Sasidharan S, Latha LY, Kwan YP, Lau YL, Shin LN, Chen Y (2010) Acute oral toxicity and brine shrimp lethality of Elaeis guineensis Jacq., (oil palm leaf) methanol extract, Molecules, 15(11), 8111-8121.

34. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents, Planta medica, 45(05), 31-34.

35. Goldman E, Green LH (2015) Practical handbook of microbiology, CRC press.

36. Martindale WH (1910) Essential oil in relation to their antiseptic powers, Purfum. Essent. Oil Rec., 1, 266-274.

37. Hoffmann C, Evans AC (1911) The Use of Spices as Preservatives. Indus & Engin Chem, 3(11), 835-838.

38. Newman DJ, Cragg GM (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. J of nat. prod, 75(3), 311-335.

39. Shen B (2015) A new golden age of natural products drug discovery, Cell, 163(6), 1297-1300.

40. Cowan MM (1999) Plant products as antimicrobial agents. Clinical microbiol rev., 12(4), 564-582.

41. Pelczar MJ, Chan ECS, Krieg NR (1988) Control of microorganisms, the control of microorganisms by physical agents, Microbiol., 469, 509.

42. Basson NJ (1996) The effect of areca nut on salivary and selected oral microorganisms. Inter dental j, 46(4), 350-356.

43. Neidhardt FC, Ingraham JL, Schaechter M (1990) Physiology of the bacterial cell: a molecular approach, Sunderland, MA: Sinauer Associates.

44. Lowy FD (1998) Staphylococcus aureus infections. New England j of med, 339(8), 520-532.

45. Kaper JB, Nataro JP, Mobley HL (2004).Pathogenic escherichia coli, Nat rev microbiol, 2(2), 123.

46. Ogston A (1882) Micrococcus poisoning, J of anat and physiol, 16(4), 526.

47. Shapiro S, Meier A, Guggenheim B (1994) The antimicrobial activity of essential oils and essential oil components towards oral bacteria, Oral microbiol and immunol, 9(4), 202-208.

48. Manila A, Sujith S, Kiran GS, Selvin J, Shakir C (2009) Cytotoxic potentials of red alga, Laurencia brandenii collected from the Indian coast, Global J Pharmacol, 3(2), 90-94.

49. Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos, MD (2002) A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC biotech, 2(1),17.

50. Ullah MO, Haque M, Urmi KF, Zulfiker AHM, Anita ES, Begum M, Hamid K (2013) Anti–bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh, Asian Pac j of trop biomed, 3(1),1-7.

51. Al-Snafi AE (2017) The pharmacology and medical importance of Dolichos lablab (Lablab purpureus)-A review, IOSR J of Pharm, 7(2), 22-30.

52. Poulton JE (1983) Cyanogenic compounds in plants and their toxic effects, Handbook of natural toxins, 1, 117-157.

53. Hasan MM, Rahman SA, Akhter MS (2019) Antibacterial and Cytotoxic Activity of Tamarindus Indica (Tamarind) Seeds, J. Bio-Sci., 27, 83-88. doi: https://doi.org/10.3329/jbs.v27i0.44673.

54. Rahman SA, Akhter MS (2018) Antibacterial and cytotoxic activity of seeds of white hyacinth bean (Lablab purpureus L. sweet 'white'), J of Adv Biotech and Experimental Therap., 1(2), 49-54.

55. Rahman SA, Akhter MS (2018) Antibacterial and cytotoxic activities of Carica papaya L. (Papaya) seeds, Khulna Univ. Studies, 15 (1), 371-48.