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In vitro Antibacterial, Cytotoxic and Antioxidant Activities of Plant *Nephelium longan*

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Abstract: The petroleum ether, chloroform and ethyl acetate fractions of ethanol extract of leaf and stem from the plant *Nephelium longan* (Fam-Sapindaceae) was subjected to antioxidant, antibacterial and cytotoxic activity. All the fractions showed potent antioxidant activity, of which the ethyl acetate and chloroform fraction of leaf demonstrated the strongest antioxidant activity with the IC_{50} value of 44.28 and 44.31 μg mL^{-1}, respectively. The petroleum ether extracts (500 μg $disc^{-1}$) of leaf and stem of *N. longan*almost showed no activity against the tested pathogenic organisms except *Escherichia coli*. On the other hand, chloroform crude extracts of leaf and stem (500 μg $disc^{-1}$) showed excellent antibacterial activity with the average zone of inhibition of 13-21 mm among the tested bacteria. Besides this, ethyl acetate crude extracts showed good activity against the growth of *Sarcina lutea* (20 mm), *Vibrio mimicus* (18 mm), *Salmonella typhi* (18 mm), *E. coli* (17 mm) and *Staphylococcus aureus* (14 mm). However, in the brine shrimp lethality bioassay, all the crude extracts of leaf and stem possessed considerable cytotoxic activity. It was evident that, the chloroform and ethyl acetate extracts of leaf and stem have significant cytotoxic potentials with the LC_{50} value of 8.802, 9.587, 9.248 and 10.45 μg mL^{-1}, respectively. Both the stem and leaf of the experimental plant have considerable antibacterial, cytotoxic and antioxidant properties which indicates that the plant have potent bioactive principles.

Key words: *Nephelium longan*, Sapindaceae, antibacterial, antioxidant, brine shrimp

INTRODUCTION

Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. The first step towards this goal is the screening of plants used in popular medicine. Thus, antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented (Dubey *et al*., 2004). The demand for plant based medicines, health products, pharmaceuticals, food supplement and cosmetics etc., are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices (Kalia, 2005). In the continuation of this strategy of new drug discovery we have studied *N. longan* for their antibacterial, cytotoxic and antioxidant activities.

N. longan (Fam.-Sapindaceae; Bengali name *Kathlichu*) is a tree of 30 or 40 feet in height and 45 feet in width, with rough-barked trunk to 2 1/2 feet thick and long, spreading, slightly drooping, heavily foliaged branches. The longan is native to China and India and is cultivated in Bangladesh, Thailand, Cambodia, Laos, Vietnam and Taiwan. The longan is a handsome, symmetrical, evergreen tree with dense, dark green foliage. It may grow to approximately 35 feet high and 45 feet wide in favorable soil conditions, making an excellent specimen tree. The leaves are pinnately compound. The small greenish yellow flowers are borne on large panicles, usually at the tips of previous growth. The fruits are spherical to ovoid, vary from ¾ to 1½ inches in diameter and have a thin, leathery, light brown pericarp. The edible part is a whitish, somewhat translucent, gelatinous aril which surrounds a shiny, dark brown seed. The flavor is sweet and pleasant but different from that of the lychee. The fruits mature later than the lychee, mostly in July and August (Phillips *et al*., 1994).

Botanical synonyms for this species include *Dimocarpus longan* Lour. *Euphoria longan* Steud, *Euphoria longana* Lam. and *Nephelium longana* Cambess., closely allied to the glamorous lychee, in the family Sapindaceae, the longan, or lungan, also known as dragon's eye or eyeball and as *Mamoncillo chino* in Cuba, has been referred to as the little brother of the lychee (Morton, 1987). Longan has medicinal attributes and mineral salt properties that the body needs such as a

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slight amount copper, zinc etc. As a result, dried longan is a popular export for countries which have a Chinese population (Montatip *et al*., 2008). The extract of the plant is anxiolytic (Okuyama *et al*., 1999) and anti-mutagenic (Minakata *et al*., 1985). No extensive work has been recorded previously on this plant. It has been reported to contain gallic acid, corilagin (an ellagitannin), ellagic acid (Rangkadilok *et al*., 2005), soyacerebrosides I and II, 1-O-β-D-glucopyranosyl-(2S,3R,4E,8E)-2-(2'-lignoceroyl amino)-4,8-octadecadiene-1,3-diol (longan cerebroside I) and its 8 Zisomer (longan cerebroside II), momor-cerebroside I and phytolacca cerebroside (Ryu *et al*., 2003). Extensive chromatographic separation and purification of the organic solvent extracts of *N. longan* (Sapindaceae) stem bark afforded two compounds; scopoletin and stigmasterol (Khondaker *et al*., 2007). Hence, the study was aimed at screening of *N. longan* stem and leaf for their antibacterial, cytotoxic and antioxidant activities, evaluating their potential uses as safer drug.

MATERIALS AND METHODS

Plant material: The fresh leaf and stem of *N. longan* were collected from Tangail in the month of March, 2009 and identified by Dr. M.A. Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Dhaka, Bangladesh.

Plant materials extraction and fractionation: The fresh leaf and stem were collected, sun dried for seven days and ground. The dried powder of *N. longan*leaf (200 g) and stem (200 g) were soaked in 600 mL of methanol for 7 days and filtered through a cotton plug followed by Whatman filter paper number 1. The concentrated methanol extract of leaf (16 g) and stem (14 g) were fractionated by the modified Kupchan partitioning method (Van Wagenen *et al*., 1993) into petroleum ether, chloroform and ethyl acetate. The subsequent evaporation of solvents afforded petroleum ether (450 mg), chloroform (700 mg) and ethyl acetate (350 mg) from leaf extract and petroleum ether (400 mg), chloroform (650 mg) and ethyl acetate (750 mg) from stem extract, respectively.

Antibacterial assay: The disc diffusion method (Bauer *et al*., 1966) was used to test antimicrobial activity against sixteen bacteria. Solutions of known concentration (mg mL^{-1}) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the pathogenic test microorganisms. Standard antibiotic discs (Kanamycin 30 μg $disc^{-1}$) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4°C) for 24 h to allow maximum diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37°C for 24 h to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the mean of the reading is required (Bauer *et al*., 1966). The antibacterial activity of petroleum ether, chloroform and ethyl acetate of leaf and stem extract of *N. longan* were determined at a concentration of 500 μg $disc^{-1}$.

Cytotoxicity screening: Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Meyer *et al*., 1982; Zhao *et al*., 1992). Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method (Meyer *et al*., 1982). The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 μL in 5 mL solution) plus sea water (3.8% NaCl in water) to attain concentrations of 5, 10, 20, 40 and 80 μg mL^{-1}. A vial containing 50 μL DMSO diluted to 5 mL was used as a control. Standard Vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

Screening for antioxidant activity: Antioxidant activity of petroleum ether, chloroform and ethyl acetate of leaf and stem extracts of *N. longan* was determined on the basis of their scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

Qualitative assay: A suitable diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 min and the color changes (yellow on purple background) were noted (Sadhu *et al*., 2003).

Quantitative assay: The antioxidant activity of leaf and stem extract of *N. longan* was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay by the method of Blois (1958). The DPPH offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic anti-oxidants (Cao *et al*., 1997). The DPPH solution was prepared in 95% methanol. The crude extracts of *N. longan* were mixed with 95% methanol to prepare the stock solution (5 mg 50 mL^{-1}). The concentration of the sample solutions was 100 μg mL^{-1}. The test samples were prepared from stock solution by dilution with methanol to attain a concentration of 20, 40, 60, 80 and 100 μg mL^{-1}, respectively. Freshly prepared DPPH solution was added in each of these test tubes containing leaf and stem extracts of *N. longan*and after 20 min, the absorbance was taken at 517 nm. Ascorbic acid was used as a positive control. The DPPH solution without sample solution was used as control. Ninty five percent methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{1-As}}{\text{Ac}} \times 100$$

where, Ac is Absorbance of control, As is Absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated.

RESULTS

The result of antibacterial assay: The petroleum ether, chloroform and ethyl acetate crude extracts (500 μg $disc^{-1}$) of the leaf and stem of *N. longan* were screened against sixteen human pathogenic bacteria to check antibacterial activities by disc diffusion method. The petroleum ether extracts (500 μg $disc^{-1}$) of leaf and stem of *N. longan* almost showed no activity against the tested pathogenic orgamisms except *Escherichia coli*. On the other hand, chloroform crude extracts of leaf and stem (500 μg $disc^{-1}$) showed excellent antibacterial activity with the average zone of inhibition of 13-21 mm by disc diffusion method (Table 1, 2) among the tested bacteria, the growth of *Shigella dysenteriae* (21 mm) was extremely inhibited by the ethyl acetate extract of leaf. Besides this, ethyl acetate crude extract of leaf showed good activity against the growth of *Sarcina lutea* (20 mm), *Vibrio mimicus* (18 mm), *Salmonella typhi*

Table 1: *In vitro* antibacterial activity of leaf of *Nephelium longan* and standard Kanamycin discs

	Diameter of zone of inhibition			
Test organisms	Petroleum ether extract (500 μg $disc^{-1}$)	Chloroform extract (500 μg $disc^{-1}$)	Ethyl acetate extract (500 μg $disc^{-1}$)	Kanamycin (30 μg $disc^{-1}$)
Gram positive bacteria				
Bacillus megaterium	-	12	13	30
Bacillus subtilis	-	-	8	23
Bacillus cereus	-	-	10	22
Staphylococcus aureus	-	9	9	26
Sarcina lutea	-	9	14	24
Gram negative bacteria				
Escherichia coli	-	11	17	22
Pseudomonas aeruginosa	-	7	13	25
Salmonella paratyphi	-	10	12	25
Salmonella typhi	-	12	18	25
Shigella boydii	-	10	8	25
Shigella sonnei	-	11	10	24
Shigella shiga	-	12	9	23
Shigella flexneri	-	9	8	22
Shigella dysenteriae	-	8	21	25
Vibrio mimicus	-	8	18	28
Vibrio parahaemolyticus	-	9	17	26

-: Indicates no activity

Table 2: *In vitro* antibacterial activity of stem of *Nephelium lougan* and standard Kanamycin discs

	Diameter of zone of inhibition			
Test organisms	Petroleum ether extract (500 μg $disc^{-1}$)	Chloroform extract (500 μg $disc^{-1}$)	Ethyl acetate extract (500 μg $disc^{-1}$)	Kanamycin (30 μg $disc^{-1}$)
Gram positive bacteria				
Bacillus megaterium	-	14	-	30
Bacillus subtilis	-	20	14	23
Bacillus cereus	-	12	15	22
Staphylococcus aureus	-	13	-	26
Sarcina lutea	-	-	-	24
Gram negative bacteria				
Escherichia coli	16	13	10	22
Pseudomonas aeruginosa	-	8	-	25
Salmonella paratyphi	-	15	11	25
Salmonella typhi	-	18	20	25
Shigella boydii	-	10	-	25
Shigella sonnei	-	11	-	24
Shigella shiga	-	12	-	23
Shigella flexneri	-	9	-	22
Shigella dysenteriae	-	14	-	25
Vibrio mimicus	-	18	-	28
Vibrio parahaemolyticus	-	13	-	26

-: Indicates no activity

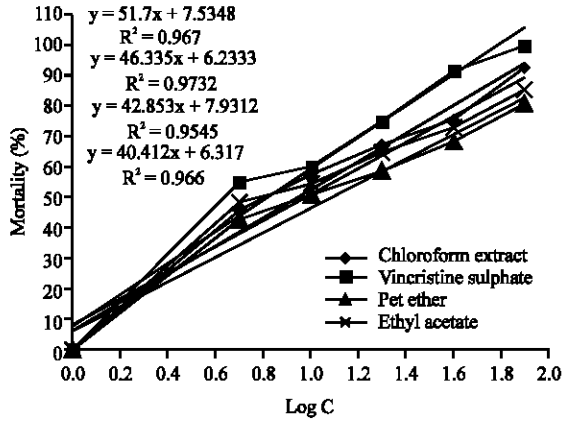

Fig. 1: Determination of LC_{50} values for standard crude petroleum ether, chloroform and ethyl acetate extract of leaf of *N. longan* from linear correlation between logarithms of concentration versus percentage of mortality

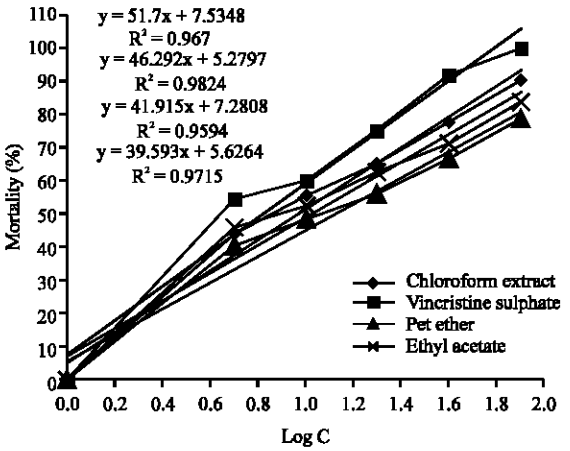

Fig. 2: Determination of LC_{50} values for standard crude petroleum ether, chloroform and ethyl acetate extracts of stem of *N. longan* from linear correlation between logarithms of concentration versus percentage of mortality

(18 mm), *E. coli* (17 mm) and *Staphylococcus aureus* (14 mm) where as the ethyl acetate and chloroform extracts of stem showed excellent activity against *Salmonella typhi* (20 mm) and *Vibrio mimicus* and *Salmonella typhi* (18 mm).

The results of brine shrimp lethality bioassay: Following the procedure of Meyer, the lethality of the crude petroleum ether, chloroform and ethyl acetate extracts of leaf and stem of *N. longan* to brine shrimp was determined on *A. salina* after 24 h of exposure the samples, the positive control and vincristine sulphate. This technique was applied for the determination of general toxic property of the plant extractives. From the Fig. 1and 2 we have observed that the LC_{50} values of petroleum ether extract of both leaf (LC_{50}-12.48 μg mL^{-1}) and stem (LC_{50}-13.205 μg mL^{-1}) of *N. longan* were outstanding in comparison to standard vincristine sulphate and the chloroform and ethyl acetate extracts of stem and leaf of the investigated plant were also found excellent and the results are shown in Table 3 and 4. No mortality was found in the control group, using DMSO and seawater. An approximate linear correlation was observed between logarithm of concentration and percentage of mortality.

The results of antioxidant activity: The DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract.

Qualitative assay: The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands.

Table 3: LC_{50} data of test samples of leaf of *Nephelium longan* and vincristine sulphate

Samples	LC_{50} (μg mL^{-1})
Vincristine sulphate	6.628
Petroleum ether extract	12.48
Chloroform extract	8.802
Ethyl acetate extract	9.587

Table 4: LC_{50} data of test samples of stem of *Nephelium longan* and vincristine sulphate

Samples	LC_{50} (μg mL^{-1})
Vincristine sulphate	6.628
Petroleum ether extract	13.205
Chloroform extract	9.248
Ethyl acetate extract	10.45

Table 5: IC_{50} data of test samples of leaf of *Nephelium longan* and ascorbic acid

Samples	LC_{50} (μg mL^{-1})
Ascorbic acid	43.11
Petroleum ether extract	50.95
Chloroform extract	44.28
Ethyl acetate extract	44.31

Quantitative assay: The antioxidant activity of the extracts was assessed by the DPPH free radical scavenging assay as shown in Table 5 and 6. All the six extracts exhibited potential antioxidant activity. The leaf extract showed comparatively more antioxidant activity than stem extract. The ethyl acetate and chloroform extract of leaf scavenged 50% DPPH free radical at the lowest inhibitory concentration (IC_{50}: 44.28 and 44.31 μg mL^{-1}) in Fig. 3. The petroleum ether extract of leaf and stem also revealed strong antioxidant activity (IC_{50}: 51.99 and 50.95 μg mL^{-1}) in Fig. 3 and 4. On the other hand, ethyl acetate and chloroform extracts of stem showed

Table 6: IC_{50} data of test samples of stem of *Nephelium longan* and ascorbic acid

Samples	LC_{50} (μg mL^{-1})
Ascorbic acid	43.11
Petroleum ether extract	51.99
Chloroform extract	48.09
Ethyl acetate extract	45.90

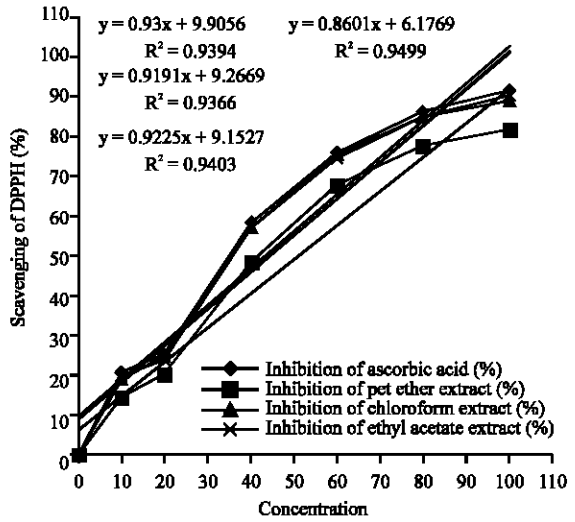

Fig. 3: Determination of IC_{50} values for standard crude petroleum ether, chloroform and ethyl acetate extract of leaf of *N. longan* from linear correlation between concentrations versus percentage of scavenging of DPPH

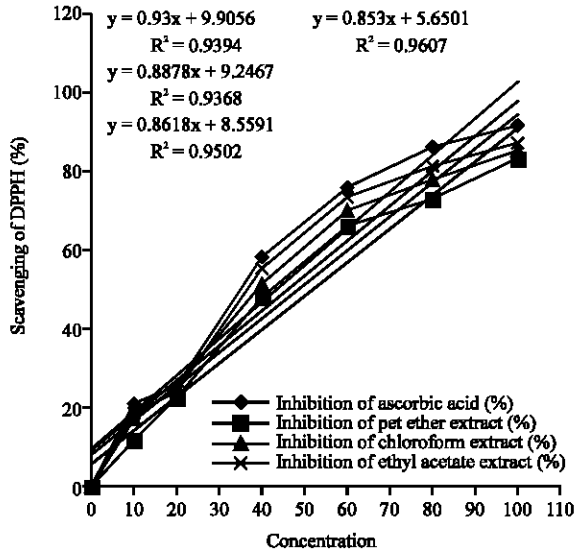

Fig. 4: Determination of IC_{50} values for standard crude petroleum ether, chloroform and ethyl acetate extract of stem of *N. longan* from linear correlation between concentrations versus percentage of scavenging of DPPH

antioxidant activity with IC_{50} of 45.90 and 48.09 μg mL^{-1}, respectively. These results denote the presence of antioxidant principles in the extractives.

DISCUSSION

The present study revealed that the plant *N. longan* has got antibacterial, cytotoxic and antioxidant effects and may have bioactive principles. The ethyl acetate extract of leaf and chloroform extract of stem showed the excellent antibacterial activity against both Gram positive and Gram negative bacteria.

The crude pet ether, chloroform and ethyl acetate extracts of *N. longan* showed good cytotoxic activities. The leaf extracts showed more cytotoxic effects than stem. This study is a general agreement with the results of previous investigations (Khondaker *et al*., 2007). Some chemical compounds have been isolated from *N. longan* (Rangkadilok *et al*., 2005; Ryu *et al*., 2003; Khondaker *et al*., 2007) in spite this, results should be encouraging other researchers to more work including phytochemical specially chloroform extract of leaf and ethyl acetate extract of stem and also biological investigation. The earlier reports of antimicrobial activities (Khondaker *et al*., 2007) support the findings of present studies. Good cytotoxic effects of crude extracts indicate that it can be selected for further cell line assay, because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts.

There is previous report on antioxidant activity. But the leaf and stem extract of *N. longan* showed profound antioxidant activity. Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy. The plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to free radical mechanisms.

CONCLUSION

In the present investigation we can conclude that the plant *N. longan* may have antibiotic, anticancer and antiaging agent as it showed good antibacterial, cytotoxic and antioxidant effect. Though, the crude extracts of stem and leaf of the investigated plant exhibited potent antibacterial, cytotoxic and antioxidant activities but we still don't know which of the components have the above properties and some principles may have toxic effects so now our study will be directed to explore the lead compound responsible for aforementioned activity from this plant.

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