IJPSR (2011), Vol. 3, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 August, 2011; received in revised form 15 December, 2011; accepted 29 December, 2011

ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC EFFECTS OF THE BARK OF *TERMINALIA ARJUNA*

Md. Shafikur Rahman* and Salma Sultana

Department of Pharmacy, Southeast University, PO Box: 1213, Banani, Dhaka, Bangladesh

Keywords:
Antimicrobial,
Antioxidant,
Cytotoxicity,
Bark,
Terminalia arjuna

Correspondence to Author:

Md. Shafikur Rahman

Senior Lecturer, Department of Pharmacy, Southeast University, PO Box: 1213, Banani, Dhaka, Bangladesh

ABSTRACT

The aim of this study was to investigate the antimicrobial, antioxidant and cytotoxic effects of the bark of *Terminalia arjuna*. The plant part was extracted with methanol to yield the crude extract. The antimicrobial activity test of the methanol extract of the bark of *Terminalia arjuna* was done using disc diffusion method. Standard antibiotic discs of Kanamycin (30 µg/disc) were used as standard. The crude extract was used at a concentration of 500µg/disc. All the microorganisms were susceptible in various degrees to the extract. The methanol extract was found to be moderately active against *Staphylococus aereus, Bacillus megaterium, Bacillus subtilis Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa*. The zone of inhibition was found from 12 mm to 19 mm. The crude methanol extract was again studied for investigating free radical scavenging potentiality and was subjected to this study with 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). The methanol extract of the bark of the plant exhibited the potential free radical scavenging activity (antioxidant effect) having IC_{50} value of 7.05 µg/ml. The cytotoxic activity of the crude methanol extract was determined by Brine Shrimp Lethality Bioassay. In this bioassay methanol extract showed positive results and the LC_{50} was 6.163 µg/ml indicating that the some of the compounds of the extract are biologically active. From this experiment, it was reveled that the test sample showed different response at different concentrations. The mortality rate of brine shrimp was found to be increased with the increased concentrations of sample, and a plot of log of concentration versus percent mortality on the graph produced an approximate linear correlation.

INTRODUCTION: The study of disease and their treatment has existed since the beginning of human civilization. Norman R. Farnsworth of the University of Illinois declared that, for every disease that affect mankind there is a treatment and cure occurring naturally on the earth. Plant kingdom is one of the major search areas for effective works of recent days. The importance of plants in search of new drugs is increasing with the advancements of medical sciences. In fact, plants are the important sources of a diverse range of chemical compounds. Some of these compounds possessing a wide range of pharmacological activities are either impossible or to difficult to synthesize in the laboratory. A phytochemist uncovering these resources is producing useful materials for screening programs for drug discovery. Emergence of newer disease also leads the scientists to go back to nature for newer effective molecules. Thus, plants are considered as the most important and interesting subjects that should be

explored for the discovery and development of newer and safer drug candidates.

Terminalia arjuna is a medicinal plant of the genus Terminalia, widely used by ayurvedic physicians for its curative properties in organic/functional heart problems including angina, hypertension and deposits in arteries. According to Ayurvedic texts, it is also very useful in the treatment of any sort of pain due to falls, ecchymosis, spermatorrhoea and sexually transmitted diseases such as gonorrhea. It is thought to be a useful astringent, cooling, aphrodisiac, cardio tonic, and is used for ulcers, leucorrhoea, diabetes, cough, tumor, excessive perspiration, asthma, inflammation and skin disorders etc [1].

Arjuna bark (*Terminallia arjuna*) is thought to be beneficial for the heart. Powdered extract of the above drug provided very good results to the people suffering from Coronary heart diseases [2, 3].

Research suggests that Terminalia is useful in alleviating the pain of angina pectoris and in treating heart failure and coronary artery disease. Terminalia may also be useful in treating hypercholesterolemia [4]. The cardioprotective effects of Terminalia are thought to be caused by the antioxidant nature of several of the constituent flavonoids and oligomeric proanthocyanidins, while positive inotropic effects may be caused by the saponin glycosides. In addition to its cardiac effects, Terminalia may also be protective against gastric ulcers, such as those caused by NSAIDs [5].

Arjuna was introduced into the *materia medica* of Ayurveda as a treatment for heart disease by Vagbhata. It is used in the treatment and prevention of cardiovascular disease, traditionally prepared as a milk decoction, a process that renders the triterpenes more bioavailable. In the Ashtanga Hrdaya, Vagbhata mentions Arjuna in the treatment of wounds, hemorrhages and ulcers, applied topically as a powder. Typical dose is 3-5 grams of the powder twice daily [6].

The bark powder of *Terminalia arjuna*, an indigenous plant has been found to have antianginal, decongestive and hypolipidemic effect. *Terminalia arjuna* showed significant decrease in mitral regurgitation (IMR), improvement in E/A ratio and considerable reduction in anginal frequency [7].

Terminalia arjuna, a deciduous tree belonging to Combretaceae family, is of 20-30 m height and is found ubiquitously in Bangladesh and India. The bark powder from *Terminalia arjuna* tree has been used in Ayurvedic medicine for over 2,500 years, primarily as a cardiotonic. Improvement of cardiac muscle function and subsequent improved pumping activity of the heart seem to be the primary benefits of the bark powder.

It has been documented that bark extract from *Terminalia arjuna*, contains following compounds: acids such as arjundic acid, terrninic acid, glycosides-argentine arjunosides I-IV, strong antioxidants such as, flavones, tannins, oligomeric proanthocyanidins and minerals [8]. However, not much is known about the specific biological activity of individual constituents. The aim of this study was to determine the antimicrobial, antioxidant and cytotoxic activity of selected indigenous Bangladeshi medicinal plant like *Terminalia arjuna.*

MATERIALS AND METHODS:

Extraction of the Plant Material: The plant powdered materials (100gm) was extracted with 350ml methanol in a flat bottom glass container, through occasional shaking and stirring for 20 days. The extract was then filtered through filter paper. The filtrate was concentrated at 50°C under reduced pressure to afford a radish mass of extract.

Antioxidant Activity Study: The free radical scavenging activity (antioxidant capacity) of the plant extract on the stable radical DPPH was estimated by the method of Brand-Williams [9]. 2.0 ml of methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH in methanol solution (20µg/ml).

The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer (**Table 2**). DPPH is used to evaluate the free radical scavenging activity (antioxidant potentiality) of the medicinal plants [10, 11].

DPPH is stable free radical potentially reactive with substance able to donate a hydrogen atom and thus useful to asses' antioxidant activity of specific

compounds of extracts [12]. Because of its odd electron, DPPH has a strong absorption band at 517 nm.

Since this electron becomes paired in the presence of a free radical scavenger, the absorption decreases stoichiometrically with respect to the number of electrons taken up. This change in absorbance produced by this reaction has been widely used to test the ability of several molecules to act as free radical scavengers [12]. The absorbance was measured by UV-Spectro- photometer and methanol was taken for extraction and as a solvent. Ascorbic acid was used as a standard (Table 2).

2.0 ml of a methanol solution of the extract at different concentrations (500 to 0.977µg/ml) were mixed with 3.0 ml of a DPPH in methanol solution (20µg/ml). After 30 minutes reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. Inhibition of free radical (DPPH) in percent (I %) was calculated as follows:

$$I\% = (1 - A\ sample/A\ blank) \times 100,$$

Where, A blank is the absorbance of the control reaction (containing all reagents except the test material) and A sample is the absorbance of the mixture of test sample and DPPH. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as inhibition percentage against extract concentration. BHT was used as positive control.

Cytotoxicity Study: Brine shrimp lethality bioassay [13] is a rapid general bioassay method for the bioactive compound of the natural and synthetic origin. Bioactive compounds are almost always toxic at high dose. Pharmacology is simply toxicology at a higher dose or toxicology is simply pharmacology at a lower dose. Brine shrimp lethality bioassay is a bench top bioassay method for evaluating anticancer, antimicrobial and pharmacological activities of natural products and it is a recent development in the bioassay for the bioactive compounds. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their biosphere- activity.

Here, *in-vivo* lethality in a simple zoological organism (Brine shrimp nauplii) is used as a convenient monitor for screening and in the discovery of new bioactive natural products. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of the compounds [14]. Brine shrimp eggs are hatched in simulated sea water to get nauplii. Sample solutions are prepared by dissolving the test materials in pre-calculated amount of Dimethyl sulfoxide (DMSO). Ten nauplii are taken in vials containing 5 ml of simulated sea water. The samples of different concentrations are added to the pre-marked vials with a micropipette. The assay is performed using three replicates. Survivors are counted after 24 hours. These data are processed in a simple program to estimate LC_{50} values.

Preparation of Simulated Sea Water (Brine Water): Since the lethality test involves the culture of brine shrimp nauplii that is the nauplii should be grown in sea water. Sea water contains 3.8% of sodium chloride & hence 3.8% salt solution should be needed for this purpose. Accordingly 3.8% of sodium chloride solution was made by dissolving sodium chloride (38 gm) in distilled water (to make 1000 ml solution) & was filtered.

Hatching of Shrimps: Sea water was kept in a small tank & shrimps eggs were taken into the divided tank, constant oxygen supply was carried out & constant temperature (37°C) was maintained. Two days were allowed for the shrimp to hatch and mature as nauplii. These nauplii were taken for bioassay.

Preparation of Test Solutions: Measured amount (4.00 mg) of sample was dissolved in 100µl of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 50µl were added to pre-marked glass test tubes containing 5 ml of seawater and 10 shrimp nauplii. So, the final concentration of samples in the test tubes were 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.56 µg/ml, 0.78125 µg/ml for 10 dilutions.

Preparation of Controls: Vincristine sulphate served as the positive control. 0.2 mg of vincristine sulphate was dissolved in (DMSO) to get an initial concentration of 20 µg/ml from which serial dilutions were made using pure DMSO to get 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25

μg/ml, 0.625 μg/ml, 0.3125 μg/ml, 0.15625 μg/ml, 0.078125 μg/ml, 0.0390 μg/ml.

The control groups containing 10 living brine shrimp nauplii in 5 ml simulated sea water received the positive control solutions. For negative control, 30 μl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii. The test was considered valid as the negative control showed no rapid mortality rate.

Counting of nauplii and Analysis of Data: After 24 hours, the vials were inspected using a magnifying glass and the numbers of survivors were counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed statistically (**Table 4**). The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

Antimicrobial Activity Study: Any chemical substance or biological agent that destroys or suppresses the growth of microorganism is called antimicrobial agent. Antimicrobial screening of a crude extract or pure compound isolated from natural sources is essential to ascertain its activity against various types of pathogenic organisms.

The primary assay can be done in three ways as: Diffusion method, Dilution method and Bioautographic method.

Among these methods, the disc diffusion method is widely acceptable for the preliminary evaluation of antimicrobial activity. Disc Diffusion is essentially a qualitative or semi- qualitative test indicating the sensitivity or resistance of microorganisms to the test materials. However no distinction between bacteriostatic or bactericidal activity can be made by this method.

Disc Diffusion Method: Diffusion is based on the ability of a drug to diffuse from a confined source through the nutrient agar medium and creates concentration gradient. If agar is seeded with a sensitive organism, a zone of inhibition will result where the concentration exceeds the minimum concentration (MIC) for that particular organism.

In this method, measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentration (μg/ml). Then, sterile filter paper discs (5 mm diameters) are impregnated with known amounts of the test substances and dried. The dried discs are placed on plates (Petri dishes, 120mm diameters) containing a suitable medium (nutrient agar) seeded with the test organisms. These plates are kept at low temperature ($4^{o}C$) for 24 hours to allow maximum diffusion.

A number of events take place simultaneously which includes-the dried discs absorb water from the agar medium and the material under test is dissolved. The test material diffuses from the discs to the surrounding medium according to the physical law that controls the diffusion of molecules through agar gel. There is a gradual change of test material concentration on the agar surrounding each disc.

The plates are then kept in an incubator ($37^{o}C$) for 12-18 hours to allow the growth of microorganism. If the test material has antimicrobial activity, it will inhibit the growth of the microorganism, giving a clear, distinct zone called “Zone of Inhibition”. The antimicrobial activity of the test agent is determined in term of millimeter by measuring the diameter of the zone of inhibition. The greater the zone of inhibition, the greater will be the activity of the test material against the test organism.

Test Materials: Methanolic extract of the bark of *Terminalia arjuna.*

Test Organisms: Both Gram-positive and Gram-negative strains of bacteria we used as the test organism to observe the antibacterial activity of the compounds. The bacterial strains used for this investigation are listed in the Table 1. These organisms were collected from the Microbiology research laboratory, Department of Pharmacy, Southeast University, Dhaka.

Culture Medium: The main requirement for the growth of bacteria was as follows-source of energy such as (carbohydrate, protein and nucleic acid), essential trace elements (Mg, Mn, Fe, and Co).

Optimum pH of media and Optimum temperature for incubation

Preparation of the Medium: The instant nutrient agar media was accurately weighted and then reconstituted with distilled water in a conical flask according to specification (4% w/v). It was then heated with water bath to dissolve the agar and a transparent solution was obtained. The prepared media was then transferred in 9 ml and 5 ml in a number of clean test tubes, respectively to prepare plates and slants. The slants were used for making subculture of microorganism, which in turn use for sensitivity tests.

The test tubes were then plugged with cotton and sterilized in an autoclave at temperature of 126°C and pressure of Ib/sq inch for 15 minutes. In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on for one hour before working in the Laminar Hood. Petri dishes and other glasswares were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Preparation of Subculture: With the help of a inoculating loop, the test organisms were transferred from the pure culture to the agar slants in a laminar airflow unit. The incubated slants were then incubated at 37°C for 18-24 hours to ensure the growth of test organisms. This culture was used for sensitivity test.

Preparation of the Test Plate: The test organism was transferred from the subculture to the test tube containing 9ml autoclaved medium with the help of an incubating loop in aseptic area. The test tube was shaken by rotation to get a uniform suspension of the organism. The bacterial suspensions were immediately transferred to the sterile Petri dishes in an aseptic area and were rotated several times, first clockwise and anticlockwise to ensure homogeneous dispersion of the organism into the medium. The depth of media into each Petri dish was approximately 4mm. After plates were cooled to room temperature, they were stored in a refrigerator at 4°C.

Preparation of Discs: Three types of discs were used for antibacterial screening. These were-Sample discs, Standard discs and Blank/control discs.

- **Sample discs**: Sterilized filters discs (5 mm in diameter) were taken in a blank Petri dish. Sample solution of the desired concentration was applied on the discs with the help of a micropipette in an aseptic condition. These discs were left for a few minutes in aseptic condition for complete removal of solvent.

- **Standard discs**: These were used to compare to the antibacterial activity of test material. In our investigation Kanamycin (30 µg/disc) was used as a reference.

- **Blank discs**: Only solvent was applied to the disc to determine the antibacterial effects of the solvent used.

Application of the Test Samples: Standard Kanamycin (30 µg/disc) discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

Determination of Antimicrobial Activity: After 24 hour incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zone of inhibition in millimeter with transparent scale (**Table 1**).

RESULTS AND DISCUSSION:

Result of Antibacterial Activity: The methanolic extract of the bark of *Terminalia arjuna* was tested for the antibacterial activity against a number of Gram positive and Gram negative bacteria. Standard antibiotic discs of Kanamycin (30µg/disc) for bacterial species were used. In this antibacterial screening crude extract was used at a concentration of 500µg/disc. The results of antibacterial activity of crude extract against a number of Gram positive and Gram negative bacteria are given below (**Table 1**).

TABLE 1: DIAMETER OF THE ZONE OF INHIBITION (mm) OF THE PLANT EXTRACT

Name of Bacteria	Methanolic extract	Kanamycin (30µg/disc)
Gram positive		
Staphylococcus aereus	19±1	25±1
Bacillus megaterium	15.33 ± 0.58	30.33 ±0.58
Bacillus subtilis	15 ±1	32 ± 1
Sarcina lutea	13.33 ±1.53	28.33±0.58
Gram negative		
Salmonella paratyphi	14.67 ± 0.58	30± 1
Salmonella typhi	16.67± 0.58	32.33 ± 0.58
Escherichia coli	16.33 ± 0.58	31.67 ±1.53
Shigella dysenteriae	13 ±1	28±1
Sheigella boydii	13.33 ±.058	35.67 ± 1.53
Vibro parahemolytica	12 ± 1	26.33 ± 0.58
Vibro minius	14.67±0.58	30 ± 1
Pseudomonas auregonosa	15.33 ±1.53	30.67±0.58

The test was done in triplicate. Diameter of the zone of inhibitions is given here as mean ± standard deviation. The control disc containing the solvent had no zone of inhibition, so their data were omitted from the above data.

DISCUSSION: From the above data, it is evident that methanol extract has shown 13-19 mm zone of inhibition against mentioned gram positive bacteria and 12-17 mm zone of inhibition against mentioned gram negative bacteria. So we can say that some compounds are present in the methanolic extract of the bark of *Terminalia arjuna* that are responsible for this antimicrobial activity.

Result of Antioxidant Activity: The antioxidant activity (DPPH radical scavenging activity) of *Terminalia arjuna* is depicted in **Figure 1**. This activity was increased by increasing concentration of the sample. The IC_{50} value of the crude methanol extract of the bark of the plant was found to be 7.058 µg/ml (**Table 2**) while the IC_{50} value of the reference standard ascorbic acid was found to be 6.964 µg/ml (**Table 3**).

TABLE 2: LC_{50} VALUE CALCULATION OF THE CRUDE METHANOL EXTRACT OF THE BARK OF *TERMINALIA ARJUNA* AGAINST DPPH

Conc. of extract	Absorbance of extract	Absorbance of blank	% inhibition	IC_{50} (µg/ml)
200	0.061± 0.00058	0.405	84.93	
100	0.063± 0.001	0.405	84.44	
50	0.076± 0.001	0.405	81.23	
25	0.076± 0.001	0.405	80.99	
12.5	0.110± 0.002	0.405	72.84	7.058
6.25	0.122± 0.002	0.405	69.87	
3.125	0.185± 0.00252	0.405	54.32	
1.56	0.262±0.002	0.405	35.31	
0.78	0.295± 0.00351	0.405	27.16	
0.39	0.299± 0.001	0.405	26.17	

The test was done in triplicate. The absorbance of extract are given here as mean ± standard deviation

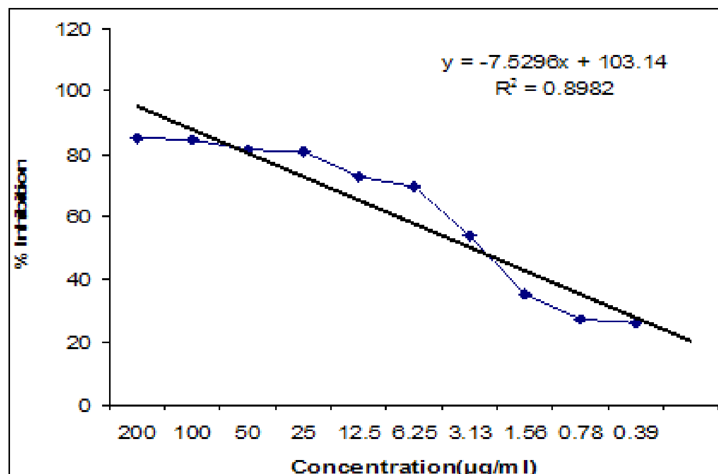

FIG. 1: EFFECT OF CRUDE METHANOL BARK EXTRACT OF *TERMINALIA ARJUNA* AGAINST DPPH

TABLE 3: CALCULATION OF THE LC_{50} VALUE OF ASCORBIC ACID AGAINST DPPH

Conc. of Ascorbic Acid	Absorbance of Ascorbic Acid	Absorbance of blank	% inhibition	IC_{50} (µg/ml)
500	0.053±0	0.42	86.75	
250	0.099±0.001	0.42	75.25	
125	0.0154±0.002	0.42	61.5	
62.5	0.166±0	0.42	58.5	
31.25	0.179±0.001	0.42	55.25	6.964
15.625	0.198±0.001	0.42	50.5	
7.812	0.207±0	0.42	48.25	
3.906	0.213±0.002	0.42	46.75	
1.953	0.227±0.002	0.42	43.25	
0.976	0.238±0.001	0.42	40.5	

The test was done in triplicate. The absorbances of Ascorbic acid are given here as mean ± standard deviation

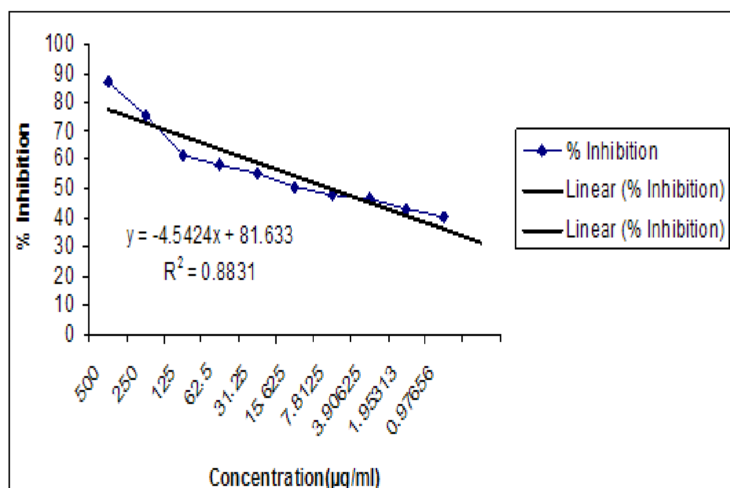

FIG. 2: EFFECT OF ASCORBIC ACID AGAINST DPPH

DISCUSSION: In antioxidant activity test, the methanol extract of the bark of *Terminalia arjuna* exhibited significant antioxidant activities with the IC_{50} value of 7.05 µg/ml. So we can say that this extract contains some active principles which are responsible for this free radical scavenging activity.

Result of Cytotoxicity: In this bioassay, methanol extract showed positive results indicating that the compounds are biologically active. From this experiment, it was revealed that each of the test samples showed different mortality rates at different concentrations (**Table 4**).

The mortality rate of brine shrimp was found to be decreased with the increase of concentration of samples, and a plot of log of concentration versus percent morality on the graph produced an approximate linear correlation between them (**Figure 3**). From the graph, the concentration at which 50% mortality (LC_{50}) of the brine shrimp nauplii occurred was determined and the LC_{50} for the crude methanol extract of the bark of *Terminalia arjuna* was found to be 6.163µg/ml.

TABLE 4: RESULT OF CYTOTOXICITY OF THE METHANOL EXTRACT OF THE BARK OF *TERMINALIA ARJUNA*

Conc.(C) (µg/ml)	LogC	% Mortality (Methanol extract)	LC_{50} (µg/ml)
400	2.602	100±0	
200	2.301	73.33±5.78	
100	2.000	66.67±5.78	
50	1.699	63.33±5.78	
25	1.398	60±0	
12.5	1.097	56.67±5.78	6.163
6.25	0.796	50±0	
3.125	0.495	46.67±5.78	
1.562	0.194	40±0	
0.781	-0.107	33.33±5.78	

The test was done in triplicate. The % Mortality is given here as mean ± standard deviation

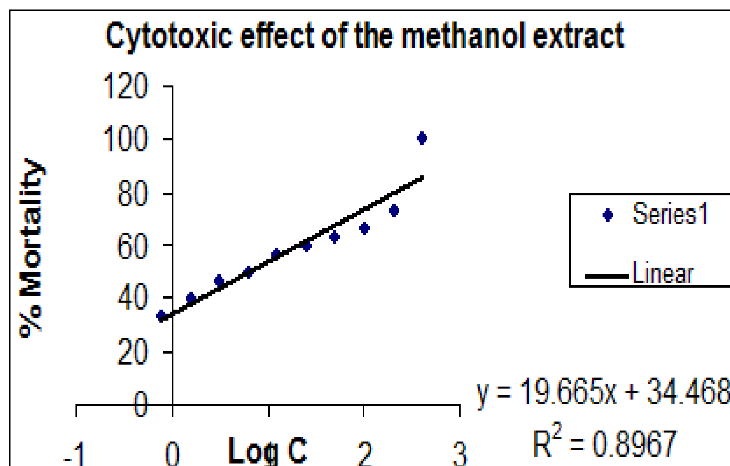

FIG. 3: EFFECT OF BARK OF *TERMINALIA ARJUNA* ON BRINE SHRIMP NAUPLII

TABLE 5: EFFECT OF VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII

Conc.(C) (µg/ml)	LogC	% Mortality (Vincristine Sulphate)	LC_{50} (µg/ml)
20	1.3010	100±0	
10	1	90±0	
5	0.6989	86.67±5.78	
2.5	0.3979	83.33±5.78	
1.25	0.0969	73.33±5.78	0.339
0.625	-0.2014	60±0	
0.312	- 0.5051	46.67±5.78	
0.156	- 0.8061	36.67±5.78	
0.078	- 1.1072	30±0	
0.039	-1.4089	20±0	

The test was done in triplicate. The % Mortality is given here as mean ± standard deviation

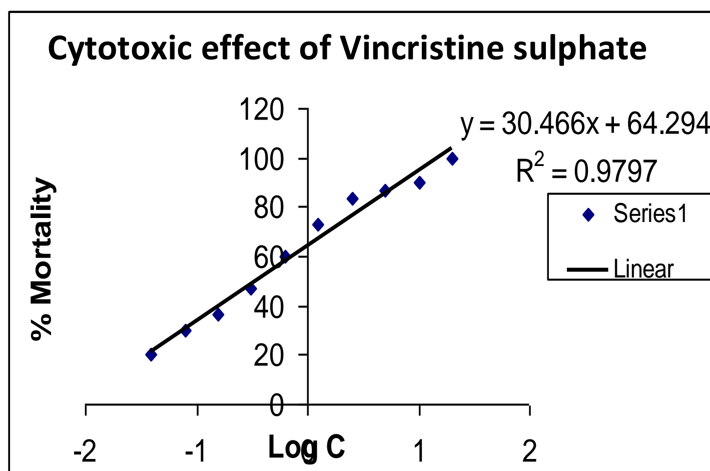

FIG. 4: EFFECT OF VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII

DISCUSSION: In brine shrimp lethality bioassay, the methanol extract of the bark of *Terminalia arjuna* exhibited significant cytotoxic activity with the LC_{50} value of 6.163µg/ml. So from this result, we can say that the methanol extract of the bark of *Terminalia arjuna* contains some compounds which are cytotoxic up to a certain level of concentration.

CONCLUSION: The present study indicates that the methanol extract of *Terminalia arjuna* has got intense antimicrobial, antioxidant and cytotoxic activity and may have potential use in medicine. From the previous studies and our current investigation it may be concluded that further study can be carried out to investigate the individual bioactive principles.

ACKNOWLEDGEMENTS: The authors thank the University of Dhaka, for helping on the microbiological works, and Southeast University, for providing required facilities to carry out this research work.

REFERENCES:

1. Paarakh PM: *Terminalia arjuna* (Roxb.) wt. and am.: A review. *International Journal of Pharmacology* 2010; 6(5): 515-534.
2. Maulik SK: Focused Conference Group: P16 - Natural products: Past and future? Role of *Terminalia arjuna* an Indian medicinal plant in cardiovascular diseases. *Basic and Clinical Pharmacology and Toxicology*, 2010; 107 (1): 445-446.
3. Shukla SK, Dwivedi S, Singh SB & Sharma UR: *Terminalia arjuna* as a therapeutic and preventive modulator in experimentally induced myocardial infarction. *Diabetes and Vascular Disease Research* 2011; 8(1): 80-81.
4. Miller AL: Botanical influences on cardiovascular disease. *Altern Med Rev* 1998; 3 (6): 422–31.
5. Devi RS, Narayan S, Vani G & Shyamala DCS: Gastroprotective effect of *Terminalia arjuna* bark on diclofenac sodium induced gastric ulcer. *Chem Biol Interact* 2007; 167 (1): 71–83. 6.
6. Dwivedi S: *Terminalia arjuna* Wight & Arn. A useful drug for cardiovascular disorders. *Journal of Ethnopharmacology* 2007; 114: 114–129.
7. Kalola J & Rajani M: Extraction and TLC desitometric determination of triterpenoid acids, (Arjungenin, Arjunolic Acid) from *Terminalia arjuna* stem arjuna, without interference of tannins. *Chromatographia* 2006; 63: 475-481.
8. Brand-Williams W, Cuvelier ME & Berset C: Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology* 1995; 28: 25-30.
9. Choi HY, Jhun EJ, Lim BO, Chung IM, Kyung SH & Park DK: Application of flow injection chemiluminescence to the study of radical scavenging activity in plants. *Phytother Res* 2000; 14: 250-253.
10. Desmarchelier C, Repetto M, Coussio J, Liesuy S & Ciccia G: Antioxidant and pro-oxidant activities in aqueous extracts of Argentine plants. *Int J Pharmacog* 1997; 35: 116-120.
11. Dinis TCP, Madeira VMC & Almeida LM: Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors membrane lipid peroxidation and peroxyl radical scavengers. *Arch Biochem Biophys* 1994; 315(1): 161-169.
12. Meyer BN, Ferringni NR, Puam JE, Lacobsen LB, Nichols DE & McLaughlin JL: Brine shrimp: a convenient general bioassay for active constituents. *Planta Med* 1982; 45: 31-32,
13. Persoone G, Sorgeloos P, Roels O & Jaspers E: The brine shrimp Artemia. Proceedings of the International symposium on the brine shrimp Artemia salina. Physiology, Biochemistry, Molecular Biology. 1980; 2: 636

****