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## Antimicrobial and Cytotoxic Activity of the Alkaloids of Amlaki (*Emblica officinalis*)

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**Abstract:** Alkaloids are important sources of drug that's why we have conducted our research to find out the biological activity of the alkaloids of a plant that is the Amlaki. Alkaloids were extracted from the methanolic extract of the fresh ripe fruits of Amlaki (*Emblica officinalis*) through solvent-solvent partitioning method with n-hexane and chloroform. The chloroform soluble fraction of the crude methanolic extract of the ripe fruits of Amlaki containing alkaloids was subjected to antimicrobial activity and brine shrimp lethality bioassay for observing cytotoxic activity. The chloroform soluble fraction of the methanolic extract exhibited significant antimicrobial activity against some Gram positive and Gram negative pathogenic bacteria and strong cytotoxicity having a  $LC_{50}$  of  $10.257 \pm 0.770 \mu\text{g mL}^{-1}$ . It is concluded that the chloroform soluble fraction of the ripe fruits of Amlaki containing alkaloids are biologically active.

**Key words:** Amlaki, alkaloids extraction, antimicrobial activity, cytotoxicity

### INTRODUCTION

Medicinal plants are natural resources yielding valuable herbal products which are often used in the treatment of various ailments (Dulger and Gonuz, 2004). In recent years attempt have been taken to investigate the indigenous drug against infectious diseases to help developing safer antimicrobial drugs (Rahman *et al.*, 2001). In the continuation of this strategy of new drug discovery we have studied the alkaloids of the ripe fruits of the plant, Amlaki (*Emblica officinalis*) to know their biological activity specially antimicrobial and cytotoxic activity.

The Amlaki also called Indian gooseberry (*Emblica officinalis*) is a deciduous tree of the Euphorbiaceae family. Amlaki has undergone preliminary research, demonstrating *in vitro* antiviral and antimicrobial properties (Saeed and Tariq, 2007). Another *in vitro* study shows that Amlaki extracts induce apoptosis and modify gene expression in osteoclasts involved in rheumatoid arthritis and osteoporosis (Letizia *et al.*, 2008).

Experimental preparations of leaves, bark or fruit have shown potential efficacy against laboratory models of disease, such as for inflammation, cancer, age-related renal disease and diabetes (Ganju *et al.*, 2003; Yokozawa *et al.*, 2007; Rao *et al.*, 2005).

A human pilot study demonstrated reduction of blood cholesterol levels in both normal and hypercholesterolemic men (Jacob *et al.*, 1988). Another very recent study with alloxan-induced diabetic rats given an aqueous Amlaki fruit extract has shown significant decrease of the blood glucose as well as triglyceridemic levels and an improvement of the liver function caused by a normalization of the liver-specific enzyme alanine transaminase (ALT) activity (Qureshi *et al.*, 2009).

Although, fruits are reputed to contain high amounts of ascorbic acid (vitamin C), 445 mg/100 g, (Tarwadi and Agte, 2007) the specific contents are disputed and the overall antioxidant strength of Amlaki may derive instead from its high density of tannins and other polyphenols. The fruit also contains flavonoids, kaempferol, ellagic acid and gallic acid.

Amlaki is one of the three ingredients of the famous ayurvedic preparation, triphala, which is given to treat chronic dysentery, bilousness and other disorders. Amlaki has antioxidant, cytoprotective (Bandyopadhyay *et al.*, 2000) hepatoprotective (Gulati and Agrawal, 1995) anti-hepatitis, anti-cancer, anti-tumor activity (Jeena *et al.*, 2001) and also have antimutagenic activity (Kaur *et al.*, 2002). It is used for constipation, peptic ulcer and scurvy (Joshi, 2000),

immunomodulator (Xia *et al.*, 1997). It is also useful for burning sensation in abdominal and cardiac regions and epigastric pain (Singh and Sharma, 1971).

Herbal drugs could be scientifically modified for better pharmacological activity and to establish safe and effective drugs. Among various types of metabolites alkaloids are very important source of drugs. Here we have emphasized to extract alkaloids from the plant and to evaluate their biological activity especially antimicrobial and cytotoxic activity.

## MATERIALS AND METHODS

Extraction of alkaloids from the ripe fresh fruits of Amlaki and experiments of the antimicrobial activity and cytotoxicity was conducted in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka from February to November, 2007.

**Plant material:** The Plant sample (ripe fresh fruit) of Amlaki was collected from Dhaka in February 2007. A voucher specimen had been deposited in Bangladesh National Herbarium, Dhaka for proper identification.

**Extraction of alkaloids from plant material:** Fruits of the plant (1 kg) were cut into small pieces and then soaked into methanol for two weeks at room temperature with occasional shaking and stirring. It was then filtered through a fresh cotton plug and finally with a Whatman filter paper. The volume of the filtrate was then reduced using a Buchii Rotavapor at low temperature and pressure. Through solvent-solvent partitioning method alkaloids were extracted (Ortiz and Mukherjee, 1982). Initially the extract was shaken with n-hexane to remove non polar compounds from the extract and the aqueous portion was collected which actually contained the alkaloids as salt form. Then the aqueous portion was basified with 10 N NaOH to get free alkaloids. This fraction was partitioned with  $\text{CHCl}_3$ . So the alkaloids came into the  $\text{CHCl}_3$  portion. This fraction was tested to know the presence of alkaloids with Dragendorff's reagent (Waldi, 1962) which gave positive test of alkaloids.

**Antimicrobial activity:** The crude chloroform extract was subjected to antimicrobial screening by disc diffusion method (Bauer *et al.*, 1966). The sample solution of the materials to be tested was prepared by dissolving a definite amount of material in appropriate solvent to attain a concentration of 40 mg mL<sup>-1</sup>. The 10  $\mu\text{L}^{-1}$  of such solution was applied on the sterile disc (6 mm diameter filter paper) and allowed the solvent to dry off in an

aseptic hood. Thus such discs contain 400  $\mu\text{g}$  of crude alkaloid extract. To compare the activity with standard antibiotics, kanamycin (30  $\mu\text{g}$  disc<sup>-1</sup>) was used.

The alkaloids extract was tested against some gram positive bacteria: *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina luteasome* some gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhi*, *Shigella boydii*, *Shigella dysenteriae*, *Vibrio mimicus*, *Vibrio parahemolyticus* and some fungi: *Candida albicans*, *Aspergillus niger* and *Sacharomyces cerevaca*.

The average zone of inhibition was 07-11 mm for chloroform extract at 400  $\mu\text{g}$  disc<sup>-1</sup>. Standard antibiotic disc of kanamycin at 30  $\mu\text{g}$  disc<sup>-1</sup> was used for comparison purpose. The chloroform extract showed strongest inhibitory activity against *Bacillus subtilis*. The growth of *S. typhi*, *B. cereus*, *P. aeruginosa*, *S. boydii*, *S. dysenteriae*, *S. aureus*, *S. lutea* were moderately inhibited.

**Cytotoxic activity:** The lethality of the chloroform extract (CLF) of the fruits of Amlaki to brine shrimp was determined following the procedure of Meyer *et al.* (1982). It gave the results of the brine shrimp lethality after 24 h exposure to the sample and the positive control, vincristine sulfate. The positive control compared with the negative control (sea water) was lethal, giving significant mortality to the shrimp. The lethal concentration LC<sub>50</sub> of the test sample after 24 h was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis.

The LC<sub>50</sub> value of chloroform fraction of the ripe fruits of Amalaki was found to be 10.257±0.770  $\mu\text{g}$  mL<sup>-1</sup>.

## RESULTS AND DISCUSSION

**The result of antimicrobial activity:** The chloroform fraction of Amlaki containing alkaloids was tested for antimicrobial activity against some Gram positive and Gram negative bacteria and some fungi. This fraction was used in a concentration of 400  $\mu\text{g}$  disc<sup>-1</sup>. This fraction showed significant antimicrobial activity against some microorganisms. The chloroform extract showed strongest inhibitory activity against *Bacillus subtilis*. This extract showed moderate inhibitory activity against *S. typhi*, *B. cereus*, *P. aeruginosa*, *S. boydii*, *S. dysenteriae*, *S. aureus*, *S. lutea*, *E. coli*, *S. paratyphi*, *V. parahemolyticus* and *V. mimicus* (Table 1). The test microorganisms were again tested with kanamycin, a

Table 1: Antimicrobial activity of the crude extract of alkaloids of the ripe fruits of Amlaki

Test microorganisms	Diameter of the zone of inhibition (mm)	
	Chloroform extract 400 ( $\mu\text{g disc}^{-1}$ )	Kanamycin 30 ( $\mu\text{g disc}^{-1}$ )
<b>Gram positive bacteria</b>		
<i>Bacillus cereus</i>	8.33±0.33	39.00±1
<i>Bacillus megaterium</i>	8.66±0.57	41.66±0.57
<i>Bacillus subtilis</i>	10.33±0.57	44.00±1
<i>Staphylococcus aureus</i>	8.33±1.15	40.66±1.52
<i>Sarcina lutea</i>	8.33±0.57	31.00±1
<b>Gram negative bacteria</b>		
<i>Escherichia coli</i>	--	33.33±1.52
<i>Pseudomonas aeruginosa</i>	--	29.66±1.52
<i>Salmonella paratyphi</i>	--	31.66±0.57
<i>Salmonella typhi</i>	--	39.33±1.52
<i>Shigella boydii</i>	9±1	39.00±1
<i>Shigella dysenteriae</i>	--	41.33±2.08
<i>Vibrio mimicus</i>	--	31.00±1
<i>Vibrio parahaemolyticus</i>	--	41.66±1.52
<b>Fungi</b>		
<i>Candida albicans</i>	--	43.00±1
<i>Aspergillus niger</i>	--	30.33±1.52
<i>Sacharomyces cerevacae</i>	--	30.00±1

Diameter less than 8 mm was considered as inactive. The zone of inhibition has been shown here as mean±SD (n=3). The test was done in triplicate

standard antibiotic to be ensured about the validity of the method. There exist a correlation between chloroform extract group and kanamycin group in this way that where the zones of inhibition by the chloroform extract are higher a higher zone of inhibition has also been found in case of kanamycin without a few cases.

**The result of brine shrimp lethality bioassay:** The cytotoxic activity of the crude extract of alkaloids of the ripe fruits of Amlaki was studied by brine shrimp lethality bioassay (Meyer *et al.*, 1982) and have been shown in the Fig. 1 and 2. The extract exhibited cytotoxic activity having a  $LC_{50}$  of  $10.257 \pm 0.770 \mu\text{g mL}^{-1}$ . The standard vincristine sulfate showed a  $LC_{50}$  of  $0.378 \pm 0.017 \mu\text{g mL}^{-1}$ .  $LC_{50}$  was obtained from the best-fit line slope of the graph. The test was done in triplicate (n = 3) and the  $LC_{50}$  has been shown here as Mean±SD.  $LC_{50}$  has been calculated from the regression equation by putting the value of  $y = 50$  and getting the value of  $x$ .

An approximate linear correlation was observed between logarithm of concentration and percentage of mortality.

The present study revealed that the plant Amlaki has got antimicrobial and cytotoxic effects due to the presence of some bioactive principles. The crude chloroform extract of the fruit showed antibacterial activity against both Gram positive and Gram negative bacteria.

Our objective in this research was to investigate the biological activity of the alkaloids of the plant. So there exist a correlation between the findings and the objective of our study.

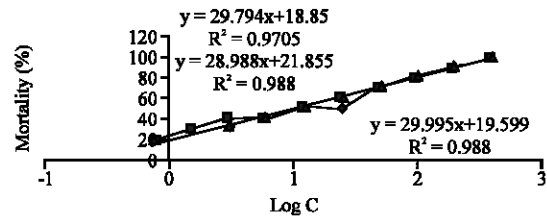


Fig. 1: Determination of  $LC_{50}$  value for chloroform soluble fraction of the ripe fruits of Amlaki containing alkaloids from linear correlation between logarithms of concentration versus percentage of mortality. The study was done in triplicate

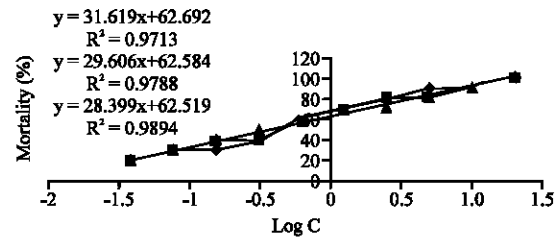


Fig. 2: Determination of  $LC_{50}$  value for standard (vincristine sulfate) from linear correlation between logarithms of concentration versus percentage of mortality. The study was done in triplicate

This study is a general agreement with the results of earlier investigators (Jasril *et al.*, 1999) these investigators showed the activity of crude n-hexane extract or crude methanolic extract of the fruit of this plant but our investigation has been focused on the bioactivity of the crude chloroform extract of alkaloids of the plant.

Our findings also support the previous results (Joshi, 2000) where it is declared that alkaloids are present in this plant but there is no indication of the biological activity of the alkaloids of this plant but this is shown here in our study. This study will encourage other researchers to study more including phytochemical and biological investigation.

It may be concluded from this study that the extract of alkaloids of the fruits of Amlaki are active against the tested microorganisms and also have cytotoxic effects. In addition the results confirm the use of the plant in traditional medicine. Now present study directed to explore the lead compounds responsible for aforementioned activity from this plant.

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REFERENCES

- Bandyopadhyay, S.K., S.C. Pakrashi and A. Pakrashi, 2000. The role of antioxidant activity of *Phyllanthus emblica* fruits on prevention from indomethacin induced gastric ulcer. J. Ethnopharmacol., 70: 171-176.
- Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Tenckhoff, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
- Dulger, B. and A. Gonuz, 2004. Antimicrobial activity of some Turkish medicinal plants. Pak. J. Biol. Sci., 7: 1559-1562.
- Ganju, L., W.D. Karan, S. Chanda, K.K. Srivastava, R.C. Sawhney and W. Selvamurthy, 2003. Immunomodulatory effects of agents of plant origin. Biomed. Pharmacother., 57: 296-300.
- Gulati, R.K. and S. Agrawal, 1995. Hepatoprotective studies on *Phyllanthus emblica*. Linn. and quercetin. Indian J. Exp. Biol., 33: 261-268.
- Jacob, A., M. Pandey, S. Kapoor and R. Saroja, 1988. Effect of the Indian gooseberry (Amla) on serum cholesterol levels in men aged 35-55 years. Eur. J. Clin. Nutr., 42: 939-944.
- Jasril, J., S.M. Mohamed, M.M. Mackeen, N.H. Lajis, A.A. Rahman and A.M. Ali, 1999. Antimicrobial and cytotoxic activities of some Malaysian flowering plants. Nat. Prod. Sci., 5: 172-176.
- Jeena, K.J., K. Girija and K. Ramadasan, 2001. Antitumor activity of *Emblica officinalis*. J. Ethnopharmacol., 75: 65-69.
- Joshi, S.G., 2000. Medicinal Plants. Oxford and IBH Publishing Co Pvt Ltd, New Delhi, pp: 281-282.
- Kaur, S., S. Arora, K. Kaur and S. Kumar, 2002. The *in vitro* antimutagenic activity of Triphala, an Indian herbal drug. Food. Chem. Toxicol., 40: 527-534.
- Letizia, P., I. Lampronti, M. Borgatti, M. Khan, M. Zennaro, R. Piva and R. Gambari, 2008. Induction of apoptosis of human primary osteoclasts treated with extracts from the medicinal plant *Emblica officinalis*. BioMed. Central, 8: 59-59.
- Meyer, B.N., N.R. Ferrigni, J.E. Putnam, J.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp; A convenient general bioassay for active plant constituents. Planta. Med., 45: 31-34.
- Ortiz, J.G.F. and K.D.C. Mukherjee, 1982. Extraction of alkaloids and oil from bitter lupin seed. J. Am. Oil Chem. Soc., 59: 241-244.
- Qureshi, S.A., W. Asad and V. Sultana, 2009. The Effect of *Phyllanthus emblica* Linn on Type-II diabetes, triglycerides and liver-specific enzyme. Pak. J. Nutr., 8: 125-128.
- Rahman, M.M., M.I.I. Wahed, M.H. Biswas, G.M. Sadik and M.E. Haque, 2001. *In vitro* antibacterial activity of the compounds of *Trapa bispinosa* Roxb. Science, 1: 214-216.
- Rao, T.P., N. Sakaguchi, L.R. Juneja, E. Wada and T. Yokozawa, 2005. Amla (*Emblica officinalis* Gaertn.) extracts reduce oxidative stress in streptozotocin-induced diabetic rats. J. Med. Food, 8: 362-368.
- Saeed, S. and P. Tariq, 2007. Antibacterial activities of *Emblica officinalis* and *Coriandrum sativum* against gram negative urinary pathogens. Pak. J. Pharm. Sci., 20: 32-35.
- Singh, B.N. and P.V. Sharma, 1971. Effect of amalaki on amalapitta. J. Res. Ind. Med., 5: 223-223.
- Tarwadi, K. and V. Agte, 2007. Antioxidant and micronutrient potential of common fruits available in the Indian subcontinent. Int. J. Food Sci. Nutr., 58: 341-349.
- Waldi, D., 1962. Thin-Layer Chromatography: A Laboratory Handbook. In: Thin Layer Chromatography, Stahl, E. (Ed.). Springer-Verlag, Berlin, pp: 483-502.
- Xia, Q., P. Xiao, L. Wan and J. Kong, 1997. Ethnopharmacology of *Phyllanthus emblica* L. Zhongguo Zhong Yao Za Zhi, 22: 511-518, 525, 574.
- Yokozawa, T., H.Y. Kim, H.Y., H.J. Kim, T. Takashi and S. Hidetoshi *et al.*, 2007. Amla (*Emblica officinalis* Gaertn.) attenuates age-related renal dysfunction by oxidative stress. J. Agric. Food Chem., 55: 7744-7752.