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Antimicrobial and Thrombolytic Activities of Decoction of *Azadirachta indica*

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Abstract

The decoction of *Azadirachta indica* leaves were evaluated for their antimicrobial and Thrombolytic activities respectively. The antimicrobial activity was done by disk diffusion method where kanamycin (30µg/disc) was used as positive control (standard). The experiment showed highest activity against gram negative bacteria especially against *E.coli and Vibrio parahaemolyticus* and fungi particularly against *Aspergillus niger*. However, it was moderately active against gram positive bacteria. For thrombolytic activity, we used streptokinase as standard and normal saline and distilled water as negative control. In the test it has been observed that the decoction was moderately active for lysis of the blood clot (27.53 ± 0.56)%. Our current studies for the first time, justified the use of decoctions of the leaves of *Azadirachta indica* for treating bacterial and fungal infection as well as use as thrombylitic agent in the indigenous system of medicine.

Key words: *Azadirachta indica,* Antimicrobial, Thrombolytic, streptokinase

Introduction

Drug resistance has become a major health related threat; globally challenging the clinicians, scientists as well as the pharmaceutical industries. Herbal medicines are targeted to be an important source of novel drugs (De N et al 2002 and Ncube N S et al 2008). Screening medicinal plants to get biologically active compounds becomes a platform to search newer agents like antimicrobial, thrombolytic, antidiabetic drugs etc. These naturally occurring compounds after some chemical modifications offers improved agents that may be used to treat different diseases (Natarajan et al 2003, Shah et al 2006). *Azadirachta Indica (A. Indica)* found in the family Meliaceae, is known as neem in Bangladesh. Different parts of neem (leaf, bark and seed oil) had been tested previously and exhibited various types of pharmacological activities like antioxidant, anticarcinogenic, anti-inflammatory, antihyperglycaemic, antimalarial, antimutagenic, antiulcer and antidiabetic properties (Talwar et al 1997). Antimicrobial and thrombolytic activities of *A. Indica* have been examined in previous studies. However, no studies on the decoction of leaves have been conducted yet. That's why; this study was designed to examine the antimicrobial and thrombolytic activities of decoction of *A. Indica*

Materials and Methods

Plant material and decoction preparation: The leaves of *A. Indica* were obtained from Satkhira and Gazipur and it is then authenticated by Bangladesh National Herbarium. The leaves were allowed to dry under shade. Hundred grams (100 g) each of *A. Indica* powdered leaves were boiled in one liter water for 10 minutes and were allowed to stand for 30 min. The contents were filtered through clean cloth and then Whatman no. 1 filter paper. The filtrates were further treated under vacuum to 100 ml resulted in a decoction as per the procedure described in our previous studies (Islam et al, 2014) in which contained 1 g equivalent powder per ml.

The antimicrobial study was conducted as per the method described by De Zoysa et al, 2019 where kanamycin (30µg/disc) was used as positive control (standard). The thrombolytic activity was done with a little modification of the method described by Ramjan et al., 2014 and Hussain et al., 2014 where streptomycin was used as positive control and normal saline and distilled water were used as negative control.

Results

The following gram posive bacterial were tested for the study: *Bacillus megaterium, Bacillus subtili, Sarcina lutea, Staphylococcus aureus, Bacills sereus.* In the test, it has been

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observed that all the bacteria were moderately sensitive with *A. indica* where *Bacills sereus* (Zone of inhibition = 15, Zone of inhibition of Kanamycin which used used as standard = 29) showed comparatively better susceptibility compared to other gram positive bacteria with this decoction (Table 01). Among the gram negative bacteria *E.coli, Pseudomonas aeruginos, Salmonella paratyphi, Shigella boydii, Shigella dysentriae, Vibrio mimicus, Vibrio parahaemolyticus, Salmonella typhi* were used to assay the activity of *A. indica.* Most of the tested gram negative bacteria showed higher activity with this plant extract where *E.coli* exibited highest activity (Zone of inhibition = 17) (Table 01). Activity of A. indica was also tested against some fungi like *Aspergillus niger, Saccharromyces cerevaceae, Candida albican.* Among the fungi *Aspergillus niger* showed highest (Zone of inhibition = 18) susceptibility against this decoction (Table 01).

Table 01: Antimicrobial activity of decoction of *Azadirachta indica*

Zone of inhibition in mm		
Name of the organism	Decoctions (400µg/disc)	Kanamycin (30µg/disc)
Gram (+) ve bacteria		
Bacillus megaterium	13	29
Bacillus subtilis	13	30
Sarcina lutea	13	29
Staphylococcus aureus	14	30
Bacills sereus	15	29
Gram (-) ve bacteria		
E.coli	17	29
Pseudomonas aeruginosa	15	29
Salmonella paratyphi	16	30
Shigella boydii	12	29
Shigella dysentriae	15	28
Vibrio mimicus	12	28
Vibrio parahaemolyticus	17	29
Salmonella typhi	13	29
Fungus		
Aspergillus niger	18	29
Saccharromyces cerevaceae	13	29
Candida albicans	12	30

For thrombolytic activity we used streptokinase [SK 30,000 IU (SK 30K) and SK 15,000 IU SK 15K)] as standard and normal saline and distilled water as negative control. The decoction exhibited moderate activity in lysing of the blood clot (27.53 ± 0.56) % in compared with positive control [SK 30K: (77.45 ± 0.89) %; SK 15K: (58. 21 ± 0.71) %] and negative control [0.9% NS: (8.25 ± 0.23) %; DW :(11.78 ± 0.47) % (Table 02).

Table 02: Thrombolytic activity of decoction of *Azadirachta indica*

Concentration of plant extract, control and standard	**Percentage of blood clot lysis (mean ± SEM)**
NC 0.9% NS	8.25 ± 0.23
NC DW	11.78 ± 0.47
PC SK 30,000 IU (SK 30K)	77.45 ± 0.89
PC SK 15,000 IU (SK 15K)	58. 21 ± 0.71
Decoction of *A.indica*	27.53 ± 0.56

NC= Negative control, PC= Positive control, NS= Normal Saline, DW= Distilled water

Discussion

From the dawn of human civilization, plants have been involved as an important source for the treatment of many diseases. Phytopharmacological investigations have opened a new window for the search of plant originated compounds which may be an effective alternative for treating certain diseases. It had been estimated that plant sources contributed about 30% of the current pharmaceuticals (Leta et al., 2002_; Gillman et al., 1995).

The antimicrobial compounds obtained from plants have better therapeutic benefit against microorganisms as they may treat the disease with few side effects as compared with synthetic antimicrobial agents (Sukanya et al., 2009). Large quantities of medicinal plants have been reported to have antimicrobial or antifungal activity (Cowan, 1999; Uniyal et al., 2006) [20,21]. Therefore, it has become a challenge for the scientist to find out the *in vitro* antimicrobial activity of naturally occurring compounds against the pathogenic bacteria.

A good number of studies have been conducted previously which confirmed the antimicrobial and antifungal activities of *A. indica* (Del Serrone & Nicoletti, 2013; Chaturvedi et al., 2011; Sharma et al., 2009; Okemo et al., 2001; Mahmoud et al., 2011).

However, no conclusive study was conducted yet with the decoction of *A. indica* which allure me to conduct this study. In the current study, most of the microorganisms showed activity in different grades and of some of the tested organisms like *E.coli, Vibrio parahaemolyticus*, *Aspergillus niger* showed higher susceptibility against this decoction of *A. indica* (Table 01).

Now days, for blood circulation, blood clot formation has become a major obstacle. Tissues get deprived of normal blood flow and oxygen due to the formation of thrombus or embolus which have been found to block the blood vessel leading to necrosis of the tissue. Blood clot is formed by thrombin from fibrinogen. It is then lysed by plasmin that is activated from plasminogen with the help of tissue plasminogen activator (tPA). The target of a fibrinolytic drug is to dissolve thrombin contributing to blockage of coronary arteries leading to restore normal blood supply to myocardium suffering from ischemia (Laurence et al., 1992_).

Many thrombolytic agents are being used for treating ischemic disorders of heart muscle. Streptokinase has been widely applied in myocardial infarction. All the available thrombolytic agents still have lots of demerits (Baruah et al., 2006; Gallus et al., 1998_; Wardlaw et al,. 2004; Capstick et al,. 2005_). Therefore, different steps have been taken to develop novel compounds to minimize deficiencies of thrombolytic agents (Adams et al., 1991_; Nicolini et al., 1992_; Lijnen et al., 1991_; Marder 1993_; Wu et al., 2006_).

A variety of studies have been carried out to find out suitable antithrombotic effect from the plants and natural food sources resulting in prevention of coronary events and stroke (Ratnasooriyaet al., 2008_; Joshipura et al., 1999_; Liu et al., 2000_; Bazzano et al., 2002_).

The present study was designed to investigate thrombolytic activity of *A. indica*. with the help of Streptokinase (SK), as a positive control (Prasad et al., 2007_). On the other hand, Normal saline and distilled water, was used as a negative control. It can be assumed that an enhanced thrombolytic activity was contributed by the decoction of *A.indica* in compared to the positive and negative control.

Our results exhibited an important antimicrobial activity of the *A. indica decoction* with visible zones of inhibition against some bacteria and fungi tested. It is safely assumed that the results obtained from the present study justify use of the plant in traditional medicine for the treatment of different infections. In third world countries like Bangladesh where contagious diseases are common, it is necessary to implement plant-derived medicines for treating infections. Our thrombolytic study got important findings indicating important implications in cardiovascular health. However, as it is a preliminary study it deserves extensive further study with the identification and isolation of compounds responsible for thrombolytic activity along with their mechanism of action.

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