ABSTRACT

The research work was conducted to investigate the phytochemical analysis and antibacterial activity of ethanolic seeds extract of *Tamarindus indica*. The ethanolic seeds extract of this plant was obtained using cold extraction method. The antibacterial activity of ethanolic seeds extract of *Tamarindus indica* was carried out against four gram positive bacteria; *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus* and *Sarcina lutea* and four gram negative bacteria; *Shigella dysentriae*, *Escherichia coli*, *Salmonella typhi* and *Salmonella paratyphi* by disc diffusion method. Large zone of inhibition was observed (18 mm and 17.25 mm) against *Staphylococcus aureus* and *Shigella dysentriae* respectively. Phytochemical constituents present in the extract were found to include Flavonoids, saponins, alkaloids and glycosides and cardiac glycosides.

Keywords: Gram-negative, Gram-positive, Antibacterial, *Tamarindus indica*

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries. Although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to these agents (Al-Bari et al., 2006). In recent years, attempts have been made to investigate the indigenous drugs against infectious diseases. This may help to develop safer antimicrobial drugs (Rahman et al., 2001).

The plant *Tamarindus indica* locally known as Tetul belongs to the family Fabaceae. The plant is extensively grown in all over the Bangladesh, is widely used all over Tropical Africa, Sudan, India, Pakistan for different purposes. Different parts of this plant are used in the indigenous systems of medicine for the treatment of a variety of human ailments (Nikkon et al., 2003; Rahman et al., 2001). The targeted plant has many uses in traditional medicine. *Tamarindus indica* is widely used in traditional medicine in Africa for the treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Ferrara 2005). Pharmacological investigations on *Tamarindus indica* extracts reported them to have antibacterial, antifungal hypoglycaemic, cholesterolemic cytotoxic effects etc (Khanzada et al., 2008).

Because of the side effects, resistance and pathogenic microorganism build against antibiotics, much recent attention has been paid to extracts of biologically active component isolated from plant species used in herbal medicine. Medicinal plants may offer a new source of antimicrobial activities. In the present study therefore, ethanolic seeds extract
Phytochemical screening and in vitro antibacterial activity of Tamarindus indica, were screened for phytochemical constituents and antibacterial activity.

MATERIAL AND METHODS

Plant materials
The fresh seeds of Tamarindus indica were collected from Noakhali district of Bangladesh in January 2009 and identified by Dr. M.A Razzaque Shah, Tissu Culture Specialist, BRAC Plant Biotechnology Laboratory, Dhaka, Bangladesh.

Plant material extraction
The seeds of Tamarindus indica were collected, sun dried for seven days and ground. The ground plant materials (600gm) were extracted with ethanol in cold condition. The water extractions were carried on dried seeds with the use of mortar and pestle (Nikkon et al., 2003). Media: Nutrient agar media (Difco laboratories) pH 7.2 was used for antibacterial screening (Khan et al., 2008).

Phytochemical screening
Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by Sofowora (1993) method. The test for tannins was carried out by subjecting 3g of plant extract in 6 ml of distilled water, filtered and ferric chloride reagents added to the filtrate. For glycosides, Liebermann-Burvhard reaction test was adopted by subjecting 0.5 g extract was dissolved in chloroform and a few drops of concentrated sulfuric acid was added to it, followed by 2-3 drops of acetic anhydride. The test of alkaloid was carried out by subjecting 0.5 g plant extract in 5ml 1% HCl, boiled, filtered and Mayers reagent added.

The plant extract was subjected to frothing test for the identification of saponin. The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium turnins and potassium hydroxide solution (Awoyinka et al., 2007).

Antibacterial activity
In vitro antibacterial screening was generally performed by disc diffusion method for the primary selection of the compound as therapeutic agent. Disc diffusion method is highly effective for rapidly growing microorganisms and the activities of test drugs are expressed by measuring the diameter of the zone of inhibition. The standard test microorganisms were available in the microbiological laboratory of Pharmacy Dept., University of Southeast, Dhaka, Bangladesh. Pure cultures of these were collected from the Microbiological Laboratory of the institution of Nutrition and Food Science and Dept. of Microbiology, University of Dhaka, Bangladesh. Antibacterial activity of ethanolic seed extracts of Tamarindus indica was determined at a concentration of 500 (µg/disc) against eight pathogenic bacteria (four gram positive and four gram negative). Kanamycin 30 (µg/disc) was used as standard (Nikkon et al., 2003).

Table-1
Phytochemical screening of Tamarindus indica seeds extract

<table>
<thead>
<tr>
<th>Bioactive agent</th>
<th>Ethanolic seeds extract of Tamarindus indica</th>
<th>Dry seeds water extract of Tamarindus indica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: (+) sign indicates Presence (-) sign indicates Absence
RESULTS

The results of the phytochemical screening and antibacterial activity of Tamarindus indica ethanolic seeds extract are shown in Table-1 and 2.

1. Phytochemical screening of ethanolic seed extracts and dry seeds water extract of Tamarindus indica is presented in Table-1. The phytochemical analysis was carried out on ethanolic seed extract of Tamarindus indica showed the presence of some bioactive compounds in the plant. In ethanolic seeds extract six bioactive constituents were tested for. All the bioactive agents were shown positive result except tanins (Table-1).

2. In vitro antibacterial activity of ethanolic seed extracts Tamarindus indica with standard Kanamycin is presented in Table-2. Antibacterial activity of ethanolic seed extracts of Tamarindus indica with standard Kanamycin against four gram positive (Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus and Sarcina lutea) and four gram negative (Shigella dysenteriae Escherichia coli, Salmonella typhi, Salmonella para typhi) bacteria is presented in Table-2. Determination of the inhibition zones by means of disc diffusion method (Table-2) shows that seeds extract of Tamarindus indica exhibited an antibacterial effect against all the tested bacteria. Highest Zone of inhibition of seeds extracts of Tamarindus indica was found to be 18 mm/disc against gram negative Shigella dysenteriae and lowest zone of inhibition was found to be 12 mm/disc against gram positive Bacillus megaterium.

DISCUSSION

In literature it has been indicated that medicinal plants are the backbone of traditional medicine (Fransworth, 1994) and the antibacterial activity of plant extract is due to different chemical agent in the extract, which were classified as active antimicrobial compounds (Rojas et al., 1992). Plants have the capacity to synthesize a diverse array of chemicals and understanding how phytochemicals function in plants may further our understanding of the mechanisms by which they benefit humans. In plants, these compounds function to attract beneficial and repel harmful organisms, serve as photoprotectants and respond to environmental changes. In humans, they can have complementary and overlapping actions, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of steroid metabolism and antibacterial and antiviral effects (Johanna,

<table>
<thead>
<tr>
<th>Tested organisms</th>
<th>Zone of inhibition (mm) of Tamarindus indica seed extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol extract 500µg/disc</td>
</tr>
<tr>
<td></td>
<td>Gram positive Bacteria</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>13</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>12</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>17.25</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td>13</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td></td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>18</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>14</td>
</tr>
<tr>
<td>Salmonella paratyphi</td>
<td>13.5</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>13</td>
</tr>
</tbody>
</table>
Phytochemical screening and in vitro antibacterial activity of tamarindus

2003).

In the present study, ethanolic seeds extract and seeds water extract of Tamarindus indica tested positive for the presence of alkaloids, glycosides, cardiac glycosides, flavonoids and saponins. It was found that Alkaloids were only present in ethanolic seeds extract while absent in dried seed water extract. The main reason that can be aducted for this is the mode of extraction. Alkaloid is a plant-derived compound that is toxic or physiologically active, contains nitrogen in a heterocyclic ring with complex structure and is of limited distribution in the plant kingdom. Alkaloids are formed as metabolic by-products and have been reported to be responsible for the antibacterial activity (Doughari, 2006). Many phytochemicals are present in plants as glycosides (with a sugar moiety attached). Generally, glycosides are nonvolatile and lack fragrance. Cleaving the glycosidic bond yields the aglycon, which itself may be volatile and fragrant. Glycosides serve as defense mechanisms against predation by many microorganisms, insects and herbivores (De et al., 1999). This may therefore explain the demonstration of antimicrobial activity by the stem bark and leaf extracts of Tamarindus indica (Doughari, 2006). Saponins through positive for both extracts, persistent frosting was intense in the ethanolic extract than dry seed water extract (Awoyinka et al., 2007). This compound has been shown to have immense significance hypocholesterol, hypotensive and cardiac depressant properties (Trease and Evans, 1986).

From the present study we revealed that the plant Tamarindus indica has got profound antibacterial effects and could be used in medicine. From above results we observed that crude ethanolic seeds extract of Tamarindus indica possess very remarkable antibacterial effects against gram positive bacteria such as Staphylococcus aureus (17.25 mm/disc) and gram negative bacteria such as Shigella dysentriae (18 mm/disc). Other gram positive gram negative bacteria showed moderate to good antibacterial activities ranges from (12-13 mm/disc) and (13-14 mm/disc) respectively. This study has similarity with previous investigation (Bai, 1990; Nikkon et al., 2003).

The success of the ethnobotanical approach to drugs discovery can no longer be questioned. Historical and current discoveries demonstrate its power (Cox, 1994). A complete study conducted with the purpose of finding these chemicals is worthwhile. The optimal effectiveness of a medicinal plant may not be due to one main active constituent, but may be due to the combine action of different compounds originally in the plant (Bai, 1990).

From this study we can conclude that this is a promising plant and the result confirms the use of this plant in traditional medicine for the treatment of infections.

REFERENCES


