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In vitro Antimicrobial and Cytotoxic Activities of Leaves and Flowers Extracts from *Lippia alba*

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Abstract: The research was conducted to investigate the *in vitro* antimicrobial and cytotoxic activities of leaves and flowers extract extracted from *Lippia alba*. Disc diffusion technique was used for *in vitro* antibacterial and antifungal screening. Zones of inhibition were observed in disc diffusion for antibacterial screening against 4 Gram-positive pathogenic and 6 Gram-negative pathogenic bacteria. Among crude extracts chloroform extract showed good activity against all test organisms. A Large zone of inhibition was observed (18 mm) against *Vibrio parahaemolyticus*. In antifungal screening, the compound showed mild to moderate zones of inhibition against four tested organisms. A Large zone of inhibition was observed against *Aspergillus niger* (13 mm). Cytotoxic activities of crude extracts were determined using Brine shrimp lethality Bioassay and LC_{50} values of standard Vincristin sulphate as positive control, n-hexane and crude ethanol extracts were found to be 5, 15 and 20 μg mL^{-1}, respectively.

Key words: Gram-negative, gram-positive, antimicrobial, *Lippia alba*, cytotoxicity

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 immuno compromised 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 in order to help developing safer antimicrobial drugs (Rahman *et al*., 2001).

The plant *Lippia alba* (aromatic shrub) locally known as Motmotya belongs to the family Vebenaceae. The plant is extensively grown in all over the Bangladesh, is widely used all over South and Central America for different purposes. Different parts of this plant are used in the indigenous systems of medicine for the treatment of a variety of human aliments (Abad *et al*., 1999; Barbosa *et al*., 2005; Nikkon *et al*., 2003; Rahman *et al*., 2001; Gazola *et al*., 2004; Hennebelle *et al*., 2007).

The targeted plant has many uses in traditional medicine. The majority of *Lippia* species have been used traditionally for the treatment of stomach ailments, Cardiovascular troubles, coughs, colds and asthma, tranquilising remedy, prevention of gastritis etc. (Hennebelle *et al*., 2006; Barbosa *et al*., 2005; Gazola *et al*., 2004; Day and Mc Andrew, 2003). The aim of present study is to investigate the antimicrobial and cytotoxic activities of leaves and flowers extracts of *Lippia alba*.

MATERIALS AND METHODS

Plant materials: The fresh leaves of *Lippia alba* were collected from Natore City of Rajshahi District of Bangladesh in March 2008 and identified by Dr. M.A Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Dhaka, Bangladesh.

Plant materials extraction and fractionation: The collected leaves with flowers of *Lippia alba* were cut, sun dried for seven days and ground. The ground plant materials (400 g) were extracted with ethanol in cold condition (Nikkon *et al*., 2003) followed by solvent-solvent partitioning with n-hexane, Chloroform and ethylacetate (Haque *et al*., 2008).

Media: Nutrient agar media (Difco laboratories) pH 7.2, nutrient broth media (Difco laboratories) pH 6.8 and artificial seawater (3.8% NaCl solution) pH 8.4 were used for antibacterial screening, antifungal screening and cytotoxicity determination, respectively (Khan *et al*., 2008).

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Antimicrobial activity: *In vitro* antimicrobial 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 Department of Microbiology, University of Dhaka, Bangladesh. Antibacterial activities of n-hexane and chloroform leaves and flowers extracts of *Lippia alba* were determined at a concentration of 500 (μg $disc^{-1}$) against ten pathogenic bacteria (4 g positive and 6 g negative). Kanamycin 30 (μg $disc^{-1}$) was used as standard.

Antifungal activities: An antifungal activity of n-hexane and chloroform extracts of leaves and flowers of *Lippia alba* were determined at a concentration of 500 (μg $disc^{-1}$) against four pathogenic fungi. Nystatin 20 (μg $disc^{-1}$) was used as standard.

Cytotoxicity studies: Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Zhao *et al*., 1992). Natural products (extracts and pure compound) can be tested for their bioactivity by this method. Here the simple zoological organism (brine shrimp nauplii) is used as a convenient monitor for screening and fractionation in discovers of new bioactive natural products.

Cytotoxicity activities were determined against brine shrimp nauplii that were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial seawater (3.8% NaCl solution) for 48 h. The cytotoxicity assay was performed on brine shrimp nauplii using Mayer method. Dissolution of compound was performed in artificial seawater using DMSO. Each 5 mL solution of different concentrations (2.5, 5, 10,15, 20, 40, 60, 80 and 100 μg mL^{-1}) of the compound was taken in different vials where brine shrimp nauplii were placed and observed for mortality for 24 h. The resulting data were transformed to probit analysis for the determination of LC_{50} values of the compound. Artificial seawater medium containing DMSO was used as control. Standard vincristine sulphate was used as positive control in this assay (Hossain *et al*., 2006; Khan *et al*., 2008; Nikkon *et al*., 2003).

RESULTS

The result of antibacterial screening: Antibacterial activity were determined against 4 Gram positive bacteria (*Bacillus subtilis*, *Bacillus megaterium*, Staphylococcus aureus and *Sarcina lutea*) and 6 Gram-negative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella para typhi*, *Vibrio mimicus and Vibrio parahaemolyticus*) (Table 1).

Table 1: *In vitro* antibacterial activity of crude leaves and flower extracts (n-hexane and chloroform) of *Lippia alba* with standard Kanamycin

	Zone of inhibition (mm) of *Lippia alba* leaves and flowers extracts		
Tested organisms	n-hexane extract	Chloroform extract	Kanamycin (30 μg $disc^{-1}$)
Gram positive Bacteria			
Bacillus subtilis	12	10	30
Bacillus megaterium	11	15	32
Staphylococcus aureus	10	11	30
Sarcina lutea	10	10	25
Gram negative Bacteria			
Shigella dysentriae	12	17	28
Vibrio mimicus	8	10	30
Vibrio parahemolyticus	16	18	32
Salmonella paratyphy	9	10	32
Salmonella typhi	7	14	28
Escherichia coli	11	7	33

Among crude extracts chloroform leaves and flowers extracts of *Lippia alba* showed highest activity in comparison to others. Highest Zone of inhibition with chloroform extract of leaves and flower of *Lippia alba* was found to be 18 mm $disc^{-1}$ against *Vibrio parahemolyticus* and lowest zone of inhibition was found to be 8 mm $disc^{-1}$ against Gram negative *Escherichia coli*. The n-hexane extract of leaves and flowers of *Lippia alba* showed highest activity against Gram negative *Vibrio parahemolyticus* 16 mm $disc^{-1}$ and lowest zone of inhibition was found to be 7 mm $disc^{-1}$ against Gram negative *Salmonella typhi*. Except *Escherichia coli* chloroform extracts showed significant activity against all Gram-negative bacteria. Chloroform extract of leaves and flowers of *Lippia alba* also showed moderate antibacterial activity against all Gram positive bacteria whose zone of inhibition was ranging from (10-15 mm $disc^{-1}$). The n-hexane extract of leaves and flowers of *Lippia alba* showed mild antibacterial activity against all Gram positive bacteria whose zone of inhibition was ranging from (10-12 mm $disc^{-1}$).

The result of antifungal screening: Antifungal screening was carried out against four fungi (*Saccharromyces cerevaceae, Aspergillus flavus, Aspergillus niger* and *Candida albicans).* Both n-hexane and chloroform extract of leaves and flowers showed mild to moderate antifungal activity against a number of tested fungi. Chloroform leaves and flower extracts was found to be better antifungal activity (10-13 mm $disc^{-1}$) than n-hexane leaves and flower extracts (8-10 mm $disc^{-1}$) (Table 2).

The result of brine lethality bioassay: In cytotoxicity assay, the LC_{50} value for standard vincristine sulphate,

Table 2: *In vitro* antifungal activity of crude leaves and flower extracts (n-hexane and chloroform) of *Lippia alba* with standard nystatin

Name of fungi	Zone of inhibition (mm) of *Lippia alba* leaves and flower extracts		
	n-hexane extract	chloroform extract	Nystatin (20 μg disc^{-1})
Saccharromyces cerevaceae	8	10	20
Candida albicans	9	12	22
Aspergillus flavus	10	11	
Aspergillus niger	8	13	24

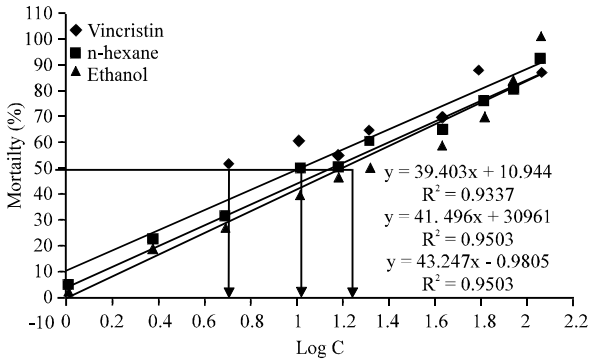

Fig. 1: Determination of LC_{50} values for standard, crude n-hexane, crude ethanol extracts of leaves and flowers of *Lippia alba* from linear correlation between logarithm of concentration versus percentage of mortality

n-hexane and crude ethanol extracts of leaves and flowers of *Lippia alba* were found to be 5, 15 and 20 μg mL^{-1}, respectively (Fig. 1). 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.

DISCUSSION

The present study revealed that the plant *Lippia alba* has got profound antibacterial, antifungal and cytotoxic effects and may have potential use in medicine. The crude chloroform extracts of leaves and flowers of *Lippia alba* possesses very remarkable antibacterial effects against gram negative bacteria such as *Vibrio parahemolyticus* (18 mm disc^{-1}) *Sygella dysentriae* (17 mm disc^{-1}). The plant *Lippia alba* also showed mild to moderate antifungal activities, especially chloroform extract showed good activity against *Aspergillus niger* (13 mm^{-1}disc). This study is in a general agreement with the results of previous investigations (Abad *et al*., 1999; Barbosa *et al*., 2005; Nikkon *et al*., 2003; Gazola *et al*., 2004). This results should be encouraging other researcher to more work on *Lippia alba* including phytochemical and biological investigation because different investigators have already been reported their popular use as medicinal plant as digestive, respiratory, cardiovascular, infectious problems and anxiety (Day and Andrew., 2003; Sena Filho *et al*., 2006; Singh *et al*., 2000).

Earlier reports of antibacterial, antifungal and cytotoxic activities (Khan *et al*., 2008) and traditional uses of the plant support the findings of present studies. Moderate cytotoxic effects of crude extracts (n-hexane and ethanol) indicate that it can be selected for further cell line assay, because many scientists have shown a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts (Martin *et al*., 1995).

In conclusion, this investigation reports that the plant possess antibacterial and cytotoxicity activity. This study has revealed further potentials of this plant in the area of pharmacology as anticancer and antibacterial agent. As a result of good LC_{50} value in Brine shrimp lethality and antibacterial activity the extract of *Lippia alba* would be a safer antibiotic and also a anticancer agent.

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