Journal of Applied Pharmaceutical Science Vol. 2 (10), pp. 142-147, October, 2012 Available online at http://www.japsonline.com DOI: 10.7324/JAPS.2012.21028 ISSN 2231-3354 CC) BY-NC-SA

Evaluation of antimicrobial, antioxidant and cytotoxic activities of methanolic extracts of *Lagerstroemia speciosa* leaves and barks

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ARTICLE INFO

Article history: Received on: 16/09/2012 Revised on: 08/10/2012 Accepted on: 16/10/2012 Available online: 29/10/2012

Key words: MLL, MBL, antimicrobial activity, antioxidant, free radical, Brine shrimp lethality bioassay.

ABSTRACT

Methanolic extracts of Lagerstroemia speciosa leaves (MLL) & barks (MBL) have been evaluated for their antimicrobial, antioxidant & cytotoxic activity. Antimicrobial activity of the extracts was evaluated against 11 Gram-positive, Gram-negative bacteria and 3 fungi using disk diffusion technique. Kanamycin ($30\mu g/disc$) was used as standard. Antioxidant potentiality of the extracts was investigated on DPPH scavenging activity, Total antioxidant capacity, Reducing ability as well as total phenolic contents. Cytotoxic study was done by brine shrimp lethality bioassay and vincristin sulphate was used as standard. In antimicrobial study, the average zone of inhibition exhibited by MLL & MBL (each $500\mu g/disc$) was 10-20 mm & 12-21mm respectively. In DPPH scavenging activity, IC₅₀ value was found $27.89 \pm 0.83\mu g/ml, 21\pm0.61 \mu g/ml and 16.76 \pm 1.11\mu g/ml for MLL, MBL and standard ascorbic acid, respectively. Total antioxidant capacity was found <math>0.22 \pm 398.37$ mg/g and 4.02 ± 346.37 mg/g equivalent of ascorbic acid for MLL & MBL, respectively. Reducing ability was found concentration dependent for both the extracts. The total phenolic content was found 71.06 \pm 2.01 and 60.65 \pm 2.16 mg/g equivalent of gallic acid for MLL & MBL, respectively. The cytotoxicity exhibited by MLL was promising with LC₅₀ value 9.602 $\mu g/m$], comparing with the LC₅₀ (6.25 $\mu g/m$]) values of standard vincristin sulphate as a positive control. The results suggest into the plant extracts could be used as a potential therapeutics in many pathological conditions.

INTRODUCTION

Natural products have been used for a wide variety of purposes for many thousands of years and for a long time, mineral, plant and animal products were the main sources of drugs (Hammer *et al.*, 1999).Developed organic chemistry & industrial revolution have been facilitated identification & isolation of pure compounds, structural modifications to produce potentially more active and safer drugs could be easily performed. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Yue-Zhong, 1998). The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants.

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Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger & Hostettmann, 1991) The present study was designed to search for newer, safer and more potent antimicrobials, antioxidants and cytotoxic components which may accomplish our present need.

Lagerstroemia speciosa (Lythraceae) or Banaba locally known as 'Jarul' in Bangladesh. It is a medium sized to large deciduous tree with a rounded crown, have been used in traditional medicine to treat diabetes mellitus in Southeast Asia for a many years. Banaba extracts are also known to have antiobesity (Suzuki *et al.*, 1999), anti-oxidant (Unno *et al.*, 1997) and anti-gout (Unno *et al.*, 2004) effects. Corosolic acid, an active ingredient in these extracts, displays a potential anti-diabetic activity (Shi *et al.*, 2008),

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as well as anti-oxidant, anti-inflammation and antihypertension properties (Yamaguchi *et al.*, 2006).

The leaves of this tropical plant have been used as a folk medicine for treatment of diabetes, kidney diseases and also the tribal people use it for heart diseases. It is also used for abdominal pain, mouth ulcers, stimulant and febrifuge (Kirtikar & Basu, 1987). Ethanol and water extracts of leaves showed prominent antimicrobial activity against all micro-organisms (Ambujakshi *et al.*, 2009). Free radical scavenging and anti-inflammatory properties have been demonstrated in leaf extracts of the plant (Priya *et al.*, 2008).

Ethyl acetate extract of leaves has been shown to ameliorate cisplatin-induced nephrotoxicity in BALB/c mice (Priya *et al.*, 2007). The preliminary phytochemical studies reveal the presence of tannins, triterpenoids, proteins and amino acid. Xanthine oxidase inhibitors (valoneic acid dilactone and ellagic acid) have been isolated from leaves of the plant (Unno *et al.*, 2004). The literature study revealed that the different extracts of the seeds have been shown to possess antimicrobial properties (Sinhababu *et al.*, 1999).

A phytochemical, orobol 7-O-D-glucoside has been isolated from the plant, reported to have inhibitory effects on human rhinoviruses replication (Choi *et al.*, 2010). Anti-fungal activity has been demonstrated with hot water as well as methanol extract of the plant against *Arthrinium sacchari* M001 and *Chaetomium funicola* M002 strains (Sato *et al.*, 2000). Despite the various pharmacological activities of this plant are reported, no studies on antimicrobial and cytotoxic action of this plant have so far been undertaken. Taking this in view, an attempt was made this time to investigate the antimicrobial, antioxidant and cytotoxic activity of the methanolic extract of leaves (MLL) & barks (MBL) of *Lagerstroemia speciosa*.

MATERIALS AND METHODS

Plant Materials

The leaves & bark of *Lagerstroemia speciosa* were collected from Gazipur in the month of October 2011 and identified by Bangladesh National Herbarium. A voucher specimen No- DACB 37502 has been maintained for it in the Bangladesh National Herbarium, Dhaka, Bangladesh.

Preparation of the extracts

The leaves and barks of *L. speciosa* were first washed with water to remove adhering dirt and then dried separately at 45°C for 36 hrs in an electric oven, then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container.

The dried powdered of each of the material (1kg) was taken in a clean, flat bottomed glass container and soaked in methanol for seven days. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. The total filtrate was concentrated to dryness, in vacuum at 40° C to render the methanolic extract (390 g) of brownish red color.

Antimicrobial assay

Microorganisms

Antimicrobial activity was tested against *B. megaterium*, *B. subtilis, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Salmonella paratyphi, S. typhi, Shigella boydii, S. dysenteriae, Vibrio mimicus & V. parahemolyticus, Saccharromyces cerevaceae, Candida albicans* and *Aspergillus niger.* These microbial strains were isolated from clinical samples and obtained as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Antimicrobial screening

Antimicrobial activity of the crude extracts of L. speciosa (MLL & MBL) were investigated against above mentioned bacterial strains by the paper disk diffusion technique (Bauer et al., 1966) using 100µl of suspension containing 108 CFU/ml of bacteria spread on nutrient agar medium. Sterile 6 mm diameter filter paper discs were impregnated with 500µg of each of the sterile test material and placed into nutrient agar medium. Kanamycin (30µg/disc) disc were used as positive control to ensure the activity of standard antibiotic against the test organisms. The sample discs and the standard antibiotic discs were placed gently on the previously marked zones in the agar plates preinoculated with the test bacteria and fungi. The plates were then kept in a refrigerator at 4^oC for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37[°]C for 12 hours. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (Jones et al., 1913). The experiment was carried out in triplicate and the mean value was taken.

In vitro antioxidant activity screening The amount of phenolic compounds

The total phenolic content of MBL & MLL was determined using Folin–Ciocalteu reagent (Yu *et al.*, 2002). The content of total phenol in the extracts was calculated from regression equation of the calibration curve (y = 0.0138x + 0.1275, $r^2 = 0.988$) and is expressed as Gallic acid equivalents (GAE).

Assay of total antioxidant capacity

The antioxidant activity of the extracts of *L. speciosa* was evaluated by the phosphomolybdenum method (Prieto *et al.*, 1999). The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The total antioxidant in the extract of was calculated from regression equation of the calibration curve (y = 0.0043x + 0.1503, $r^2 = 0.8874$) and is expressed as Ascorbic acid equivalents (AAE).

DPPH Free radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2picrylhydrazyl (DPPH) free radical was determined by the method described by Braca (Braca *et al.*, 2001). The percentage inhibition activity was calculated from the following equation.

Percentage of inhibition = $[(A_0-A_1)/A_0] \ge 100$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. IC₅₀ value was calculated from the equation of line obtained by plotting a graph of concentration (µg/ml) versus % inhibition.

Reducing power activity

The reducing power of was determined according to the method described by Oyaizu (Oyaizu, 1986). Increased absorbance of the reaction mixture indicated increased reducing power.

Cytotoxicity screening by Brine shrimp lethality bioassay

Brine shrimp lethality bioassay (Meyer et al., 1982; Rahman et al., 2008) technique was applied for the determination of general toxic property of MLL & MBL. Here, in vivo lethality test has been carried out using brine shrimp nauplii eggs (Artemia salina). Vincristin sulphate was used as a positive control in the bioassay. Eggs were placed in one side of a small tank divided by a net containing 3.8% NaCl solution for hatching. In the other side of the tank was placed a light source to attract the nauplii. After 2 days of hatching period the nauplii were ready for the experiment. Four milligrams of the complexes was accurately measured and dissolved in DMSO to get a concentration of varying concentrations100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781, 0.39, $0.19 \,\mu$ g/ml. ten brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 ml was used. After 24 hour of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. From these data, the percentage of mortality of the nauplii was calculated for each concentration and the LC50 values were determined.

RESULTS AND DISCUSSION

Invitro Antimicrobial activity

The antimicrobial efficacy of MLL & MBL against eleven pathogenic bacteria and three fungi were shown in Table 1 & table 2.

MLL showed moderate to very good antibacterial activity against gram positive bacteria with average zone of inhibition 13 ± 0.93 -16 ±0.76 mm and good to excellent antibacterial activity with 10 ±0.19 -20 ±1.65 mm zone of inhibition against gram negative bacteria. The maximum zone of inhibition (20 ±1.65 mm) showed against *V. mimicus* and the minimum zone of inhibition (10 ±0.19 mm) showed against *P. aeruginosa*. Again MBL showed moderate to very good antibacterial activity against gram positive bacteria with average zone of inhibition 14 ±1.55 -18 ±0.20 mm and good to excellent antibacterial activity with 12 ±0.13 - 21 ±0.15 mm zone of inhibition against gram negative bacteria. The found maximum

and minimum zone of inhibition was 21±0.15 mm & 120.13 mm against *V. mimicus* & *P. aeruginosa* respectively.

 Table . 1: In vitro antibacterial activity of the methanol extracts of Lagerstroemia speciosa (leaves & barks) and kanamycin discs .

Name of the Test	Diameter of zone of inhibition			
organisms	Mean (mm) ± SD			
	MBL	MLL	Kanamycin	
	(500µg/disc)	(500µg/disc)	(30µg/disc)	
Gram positive bacteria				
Bacillus subtilis	17 ± 0.87	14 ± 2.05	28 ± 0.67	
Bacillus megaterium	13 ±0.34	13 ±0.93	33 ±1.05	
Sarcina lutea	13 ±0.05	16 ±0.55	30 ± 0.03	
Staphylococcus aureus	18 ±0.20	13 ± 1.02	28 ±1.22	
Bacillus cereus	14 ±1.55	16 ±0.76	32 ±0.44	
	Gram negative	bacteria		
Salmonella paratyphi	19 ±1.97	14 ±0.32	30 ± 0.76	
Vibrio mimicus	21 ±0.15	20 ±1.65	35 ±.23	
Vibrio	14 ±0.39	12 ± 2.23	33 ±0.3	
parahemolyticus				
Pseudomonas	12 ±0.13	10 ±0.19	28 ±2.13	
aeruginosa				
Escherichia coli	18 ±0.44	16 ±00	30 ± 1.88	
Shigella dysenteriae	18 ±1.72	17 ±2.32	30 ±00	

The values are expressed as Mean ±standard deviations (SD). "-" Indicates no zone of inhibition

 Table. 2: In vitro antifungal activity of methanolic extracts of Lagerstroemia speciosa and kanamycin discs.

Test Organisms	MBL	MLL
Aspergillus niger	19 ±0.00	12 ±1.45
Sacharomyces cerevaceae	19 ±0.32	13 ±2.33
Candida albicans	17 ± 1.77	14 ± 1.06

Similar studies on fungi, MBL showed very good $(17\pm1.77 - 19\pm0.32 \text{ mm}, \text{ zone of inhibition})$ antifungal activity whereas MLL showed moderate $(12\pm1.45 - 14\pm1.06 \text{ mm}, \text{ zone of inhibition})$ antifungal activity.

Medicinal plants are now gaining popularity and considered as clinically effective and safer in the treatment of bacterial infections due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics. The result of antimicrobial activity of the present study revealed that both MBL & MLL showed significant antimicrobial activity towards both bacteria and fungi, suggested the presence of antimicrobial compounds. Previous phytochemical investigation of *L. speciosa* leaves found the presence of tannins, steroids/triterpinoids & flavonoids (Woratouch *et al.*, 2011), suggests the ability of this plant to play a major role in the treatment of infectious diseases (Asquith and Butler, 1986), as tannins have shown antioxidant and protein-precipitating properties (Ruch *et al.*, 1989). Again, flavones are known to

be synthesized by plants in response to microbial infection and causes metabolic perturbation (osmotic imbalance, denaturation of enzymes and modification of ion channels) of microorganisms (Havsteen, 2002).Result of huge amount of total phenolic compounds in both MLL & MBL are founded able to exert antimicrobial action (Critchfield *et al.*, 1996). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Cowan, 1999). Amongst the gram positive and gram negative bacteria, gram negative bacterial strains were more susceptible to both the extracts when compared to gram positive bacteria. This may be attributed to the fact that these two groups differ in their structure of the cell wall components. In case of antifungal activity both MBL & MLL showed significant potentiality but MBL was more potent than MLL.

Antioxidant activity

The total phenol content was found to be 71.06 ± 2.01 and 60.65 ± 2.16 mg/g plant extract (in GAE) for MLL & MBL respectively. Total antioxidant capacity of *L. speciosa* is expressed as the number of equivalents of ascorbic acid. Total antioxidant capacity was found to be 0.22 ± 398.37 mg/gm and 4.02 ± 346.37 mg/gm equivalent of ascorbic acid for MLL & MBL respectively. The percentage (%) scavenging of DPPH free radical was found to be concentration dependent i.e. concentration of the extract between 25-200 µg/ml greatly increasing the inhibitory activity (Figure 1) with the IC₅₀ value 27.89 ± 0.83 µg/ml and 21 \pm 0.61 µg/ml for MLL & MBL respectively. while IC₅₀ value of standard ascorbic acid was found to be 16.76 ± 1.11 µg/ml. Therefore, antioxidative abilities of MBL & MLL were investigated in terms

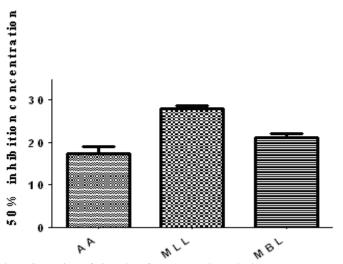


Fig. 1: Comparison of IC50 value of MLL, MBL & standard AA.

For the measurement of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of *L. speciosa* extracts compared with standard ascorbic acid and showed in Figure 2. The reducing power of extracts was found to be concentration dependent. Safer Antioxidants from plant origin are essential to prevent the progression of free radical mediated disorders. They can either scavenge ROS/RNS to stop radical chain reactions (primary antioxidants or free radical scavengers) or inhibit the reactive oxidants from being formed into ROS/RNS (secondary or preventive antioxidants) (Karadag, 2009). No single assay accurately reflects the mechanism of action of all antioxidants in a complex system (Prior, 2005) due to various oxidative processes.

of total phenol content, total antioxidant capacity, DPPH radical scavenging activity & reducing power.

Phenolic compounds are important plant constituents, known as powerful chain breaking antioxidants (Shahidi & Wanasundara, 1992), due to their hydroxyl groups scavenging ability(Suresh *et al*, 2008) and effective hydrogen donors (Michalak, 2006), which makes them good antioxidant. Our findings of the total phenolic contents were $60.65\pm$.216 mg /g plant extract (in GAE) and $71.06\pm$ 2.01 mg /g plant extract (in GAE) for MBL and MLL respectively. Such yield of total free phenolics in both the extracts is very important for their antioxidative activity as it is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1g daily with a diet rich in fruits and vegetables (Tanaka *et al.*, 1998).

The percentage (%) scavenging of DPPH free radical revealed by MBL & MLL was found to be concentration dependent i.e. concentration of the extract between 25-200 μ g/ml greatly increasing the inhibitory activity (Figure 1). This Potential DPPH radical scavenging activity *might* confirm its hydrogen donating capacity and also its proposed ability to protect the consumers' health from various free-radical related diseases.

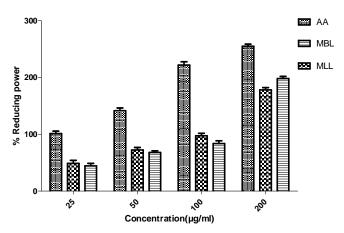


Fig. 2: Comparison of reducing power of MLL, MBL & standard AA.

Reducing capacity of a compound serves as a significant indicator of its potential antioxidant activity (Yang, 2002), which will be given by the amount of reductones present in them. The ability of the hydroxyl groups present in the flavonoids / phenolics to reduce the free radicals by donating their electrons will determine their activity. Dose dependent high yield Reducing ability of both MBL & MLL exert antioxidant action by breaking the free radical chain by donating hydrogen atom (Duh, 2002).

Cytotoxic activity

In cytotoxic test activity, % mortality increased gradually with the increase in concentration of the test samples. LC_{50} values obtained from the best-fit line slope (Fig 3) were 9.602 µg/ml and 72.06 µg/ml for MLL & MBL respectively in comparison with vincristine sulphate as standard whose LC_{50} value was 6.25µg/ml. From the above result, it can be well predicted that MLL have considerable cytotoxic potency. Previous phytochemical screening indicated the presence of triterpenoids and sterols in *L. speciosa* which are responsible for anti-inflammatory and anti-tumour activities (Lui, 1995). Huge amount of phenolics & flavonoids present in MLL might be responsible for its promising cytotoxic activity and the possible mechanism of cytotoxicity against brine shrimp nauplii due to poisonous effect on cell mitosis.

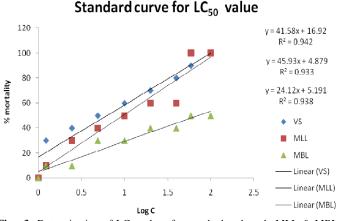


Fig. 3: Determination of LC $_{50}$ values for standard and crude MLL & MBL from linear correlation between logarithms of concentration versus percentage of mortality.

CONCLUSION

This work has demonstrated that the methanolic extracts of the leaves & barks of *L. speciosa* possesses significant antimicrobial, antioxidant and cytotoxic potentiality, thereby lends support to the traditional use of the plant in various disorders. However, further studies are needed to be conducted to understand the exact mechanisms of such actions and to isolate the active principles responsible for the observed activity.

ACKNOWLEDGEMENT

The authors are thankful to Department of Pharmacy, Southeast University, Banani, Dhaka-1213,Bangladesh, for providing lab facilities to carry out this research work

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How to cite this article:

Fatema Nasrin, Sabir Ahmad, Kamrunnahar. Evaluation of antimicrobial, antioxidant and cytotoxic activities of methanolic extracts of *Lagerstroemia speciosa* leaves and barks. J. App. Pharm. Sci. 2012; 2 (10): 142-147.