Bulletin of Faculty of Pharmacy, Cairo University xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bulletin of Faculty of Pharmacy, Cairo University

journal homepage: www.elsevier.com/locate/bfopcu

Original Article

A comparative study of in vitro antimicrobial, antioxidant and cytotoxic activity of *Albizia lebbeck* and *Acacia nilotica* stem bark

Mohammad Tuhin Ali[a], Sheikh Tanzina Haque[b], Md. Lutful Kabir[c], Sohel Rana[a], Md. Enamul Haque[a,*]

[a] Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh
[b] Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Dhaka 1000, Bangladesh
[c] Dept. of Biochemistry and Molecular Biology, Bangabandhu Sheikh Mujibur Rahman Science & Technology University, Gopalganj 8100, Bangladesh

ARTICLE INFO

Keywords:
Albizia lebbeck
Acacia nilotica
Antimicrobial assay
Antioxidant assay
Cytotoxicity test

ABSTRACT

Petroleum ether, ethyl acetate and methanol extracts of the stem bark of *Albizia lebbeck* and *Acacia nilotica* was investigated for antimicrobial, antioxidant and cytotoxicity assay. The powdered stem bark was extracted successively with petroleum ether, ethyl acetate and methanol solution. The crude extracts were subjected to antimicrobial, antioxidant and cytotoxicity assay by using disc diffusion method, DPPH and hydroxyl free radical scavenging assay, and brine shrimp lethality test, respectively. The petroleum ether and ethyl acetate extracts of both plants showed the most prominent activity in antimicrobial susceptibility test as compared to methanol extracts. These extracts showed moderate activity against the *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio mimicus*, *Salmonella typhi*, *Shigella dysenteriae*, *Candida arrizae* and *Aspergillus niger*. The zone of inhibition against the tested bacteria and fungi were 11–14 mm and 8–10 mm, respectively. The ethyl acetate extract of both plants showed the highest antioxidant and cytotoxic activity when compared with that of petroleum ether and methanol extracts. All the tested extracts showed higher free radical scavenging activity than that of the standard, ascorbic acid. In compared with *A. lebbeck*, *A. nilotica* was found to have the lower IC_{50} value which was 74.29 μg/ml and 68.03 μg/ml in DPPH and hydroxyl free radical scavenging assay, respectively. The LC_{50} value was 42.36 and 37.32 μg/ml for the ethyl acetate extract of *A. lebbeck* and *A. nilotica*, respectively. We suggest further study for the identification of active compounds from the bark extracts of *A. lebbeck* and *A. nilotica*.

1. Introduction

Herbal drugs are useful for the treatment of the diverse range of diseases. According to World Health Organization (WHO), almost 80% people of the developing countries depend on traditional and folk medicines. In spite of great advances in the modern medicines, plants still make an important role in developing new medicines [1]. In Bangladesh, a large portion of the people depends on different folk medicines for their primary health care. Therefore, medicinal plants are becoming the focus of interest to evaluate whether their traditional uses are supported by their actual pharmacologic effect [2–4].

Albizia lebbeck (*A. lebbeck*) (known as Shirish in Bangladesh) is a widely distributed plant in tropical and subtropical Asia and Africa. It is a leguminous plant belonging to the family Fabaceae. All parts of *A. lebbeck* are widely used as a general and universal antidote in traditional medicine [5,6]. The bark extract of *A. lebbeck* is useful for asthma, skin diseases, erysipelas, allergy, infectious diarrhea, and anxiety. The flower of this plant has anti-pulmonary activity [7–9]. The major phytochemical constituents of this plant are saponins. It also contains macrocyclic alkaloids, phenolic glycosides and flavonoids [10].

Acacia nilotica (*A. nilotica*) (known as Bablain Bangladesh) is an important ornamental and medicinal plant that belongs to the family Fabaceae. The major classes of phytochemical constituents of this plant include tannins, flavonoids, alkaloids, fatty acids and polysaccharides (gums) [11]. The leaves of this plant are used as antibacterial, chemo preventive, astringent, anti-inflammatory and as anti-Alzheimer's [11–13]. The root is used against tuberculosis and tumors of ear, eye, and testicles [11,14]. Bark is reported to have antibacterial, antioxidant, antimutagenic, and cytotoxic activity. Flowers are used in gastrointestinal disorders [15,16].

These two plants are the sources of many active secondary metabolites which may serve as potential candidates for drug development in future. The present works aimed at providing an up-to-date

Peer review under responsibility of Faculty of Pharmacy, Cairo University.
* Corresponding author.
E-mail addresses: m.tuhinali@gmail.com (M.T. Ali), kabir.bmb@bsmrstu.edu.bd (Md. L. Kabir), enamulmd@du.ac.bd (Md. E. Haque).

http://dx.doi.org/10.1016/j.bfopcu.2017.10.002
Received 16 July 2017; Received in revised form 22 October 2017; Accepted 24 October 2017

comparative study of anti-bacterial, anti-fungal, antioxidant, and cytotoxicity profiles of the stem bark extracts of *A. lebbeck* and *A. nilotica*.

2. Materials and methods

2.1. Collection of plant materials and preparation of plant extracts

The stem bark of *A. lebbeck*, and *A. nilotica* were collected from March 2015 to April 2015 with the authentication from Bangladesh National Herbarium, Dhaka, Bangladesh. After thoroughly washing, the bark was sun-dried for seven consecutive days and ground into fine powder. The extraction of plant material on the powdered stem bark had previously described and in this study it was employed with several modifications [17]. About 1.5 L of petroleum ether, ethyl acetate and methanol were used for the successive extraction of 300 g powdered plant material at room temperature for 10 days with regular shaking and stirring. The crude extracts were then filtered through Whatman filter paper No. 1 (Whatman Ltd., England) and concentrated through evaporation of solvents by using a rotary vacuum evaporator. The amounts of petroleum ether, ethyl acetate, and methanol extracts were 15 g, 20 g and 30 g, respectively. The organic soluble extractives were then collected and preserved in glass vials at 4 °C for future use.

2.2. Antimicrobial assay

Antimicrobial assay was carried out by using disc diffusion method [18,19]. In this study, the sterile filter paper discs (5 mm diameters) were impregnated with a known concentration of sample, so that each dried discs contains 300 µg of crude extract. Antibacterial and antifungal activities of the crude extracts were compared with the standards (ciprofloxacin 10 µg/disc and griseofulvin25 µg/disc, respectively). Sterile blank discs containing only respective solvents (methanol without extract) were used as negative controls to ensure that there is no zone of inhibition due to the residual solvents and the filter paper. Pure cultures of the microorganisms were supplied by the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

The tested microorganisms were -

Bacteria: Gram positive bacteria: *Bacillus polymyxa*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, *Staphylococcus aureus*. Gram negative bacteria: *Vibrio mimicus*, *Vibrio cholera*, *Salmonella typhi*, *Shigella boydii*, *Shigella flexneri* type-1, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherchia coli*.

Fungi: *Candida arrizae*, *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus oryzae*, *Candida albicans*, *Saccharomyces cerevisiae*, *Candida krusei*.

2.3. Antioxidant assay

2.3.1. DPPH free radical scavenging assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging assay was used for the determination of antioxidant activity of the plant extracts [20]. Different concentrations of the extracts were mixed with 3.0 ml of DPPH solution in methanol. Bleaching of the purple color of DPPH solution by plant extracts was recorded at 517 nm by UV spectrophotometer and compared with that of ascorbic acid. Free radical scavenging activity was calculated using following equation-

% DPPH radical scavenging = $[1 - (A_S/A_C)] \times 100$, where, A_C = absorbance of control and A_S = absorbance of sample solution. Finally, IC_{50} was calculated from the inhibition versus concentration graph (graph not shown).

2.3.2. Hydrogen peroxide free radical scavenging assay

Scavenging activity of extracts was also evaluated by hydrogen peroxide scavenging method [21]. 1 ml of each extract at different concentrations in ethanol was mixed with 2 ml of hydrogen peroxide solution in phosphate buffered saline (PBS, pH 7.4). After 10 min the absorbance was taken at 230 nm against a blank solution of phosphate buffer saline without any hydrogen peroxide. Ascorbic acid was used as control. The percentage of inhibition was determined using the following equation:

% inhibition of hydroxyl free radicals = $[1 - (A_S/A_C)] \times 100$, where, A_C = absorbance of control and A_S = absorbance of sample solution. Finally, IC_{50} was calculated from the inhibition versus concentration graph (graph not shown).

2.4. Cytotoxicity assay

For the determination of cytotoxic potential of the plant extracts, brine shrimp lethality bioassay was used [22,23]. At 37 °C temperature and constant oxygen supply, the eggs of brine shrimp (*Artemia salina* leach) were hatched for two days and were allowed to become the mature nauplii. Using Pasteur pipette, 10 nauplii were transferred to each of 6 test tubes containing 10, 20, 40, 60, 80 µg/ml concentration of plants extracts in artificial sea water with the final volume adjusted to 5 ml. Vincristine sulfate and 1% DMSO were used as positive control and negative control, respectively. After 24 h of incubation, the number of survived nauplii was counted and the percentage of death of brine shrimp nauplii for each concentration was determined. Percent of inhibition versus concentration graph was plotted to determine LC_{50}.

3. Results

3.1. Antimicrobial assay

In our study, there were no zones of inhibition were found for the blank discs containing only the solvent, methanol which were used as negative controls in the antibacterial and antifungal study. The petroleum ether and ethyl acetate extracts of *A. lebbeck* bark demonstrated moderate zone of inhibition against both Gram positive and Gram negative bacteria tested. These extracts showed moderate activity against *S. aureus*. In case of Gram negative bacteria, the petroleum ether and ethyl acetate extracts showed maximum activity against *S. typhi* (13 and 14 mm, respectively) and *S. dysentriae* (14 and 13 mm, respectively). Methanol extract of this plant didn't show any activity in antibacterial screening (Table 1). *C. arrizae*, *A. niger*, *C. albicans*, and *S. cerevisiae* showed moderate to strong sensitivity against the petroleum ether and ethyl acetate extract of this plant. Methanol extract of this plant showed strong activity only against *S. cerevisiae* culture (10 mm of zone of inhibition compared to 12 mm of that by the standard) (see Table 2).

Except *S. lutea*, *S. boydii*, *S. dysenteriae*, and *E. coli*, all the bacteria showed mild to moderate sensitivity towards the petroleum ether and ethyl acetate extracts of *A. Nilotica*. The petroleum extract of this plant showed highest activity against *B. polymyxa* with a zone of inhibition of 13 mm. On the other hand ethyl acetate extract of this plant showed zone of inhibition of 11 mm against *B. subtilis*, *S. aureus*, and *S. typhi* bacteria. Moderate activity by the petroleum ether and ethyl acetate extracts of this plant was also observed against the *C. arrizae*, *A. fumigatus*, *A. niger*, *C. albicans*, and *S. cerevisiae*. Methanol extract of this plant showed no activity in antimicrobial screening. Results are summarized in Table 3 and Table 4.

3.2. Antioxidant activity

In DPPH free radical scavenging assay, IC_{50} values of 66.63, 57.25 and 60.21 µg/ml was determined for the petroleum ether, ethyl acetate, and methanol extracts of *A. lebbeck*, respectively, whereas for *A. nilotica*, the IC_{50} values were 79.64, 68.03 and 75.64 µg/ml, respectively. The IC_{50} value obtained for the standard ascorbic acid was 36.83 µg/ml. Prior to the determination of IC_{50}, it was observed that the crude extracts showed concentration dependent free radical scavenging of DPPH as summarized in Table 5. For both plants, the ethyl acetate extracts demonstrated the highest free radical scavenging activity which was