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Original Article

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

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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

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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

Table 1
Diameters of zones of inhibition (mm) of *A. lebbeck* bark extracts against different gram positive and gram negative bacteria.

Test Organism	Petroleum ether extract	Ethyl acetate extract	Methanol extract	Ciprofloxacin at 10 µg/ml
Gram positive bacteria				
Bacillus polymyxa	10	–	–	32
Bacillus subtilis	11	14	–	40
Bacillus megaterium	–	11		36
Sarcina lutea	–	–	–	38
Staphylococcus aureus	13	11	–	36
Gram negative bacteria				
Vibrio mimicus	11	12	–	40
Vibrio cholera	8	8	–	32
Salmonella typhi	13	14	–	38
Shigella boydii	10	9	–	42
Shigella flexneri type-1	8	10	–	40
Shigella dysenteriae	14	13	–	40
Pseudomonas aeruginosa	–	9	–	38
Klebsiella pneumoniae	12	–	–	36
Escherchia coli	–	–	–	30

(–) = No significant antibacterial activity.

Table 2
Diameters of zones of inhibition (mm) of *A. lebbeck* bark extracts against different fungal strains.

Test Organism	Petroleum ether extract	Ethyl acetate extract	Methanol extract	Griseofulvin at 25 µg/ml
Candida arrizae	7	10	–	26
Aspergillus fumigatus	–	–	–	20
Aspergillus niger	8	9	–	24
Rhizopus oryzae	–	–	–	16
Candida albicans	6	8	–	24
Saccharomyces cerevisiae	7	7	8	20
Candida krusei	–	–	–	16

(–) = No significant antifungal activity.

26.04–81.13% for *A. lebbeck* and 20.68–68.10% for *A. Nilotica* within the concentrations of 20–100 µg/ml. The petroleum ether extract showed lowest free radical scavenging activity as compared to the ethyl acetate and methanol extracts.

The IC_{50} values obtained by the hydroxyl free radical scavenging assay were 70.93, 64.69, and 68.99 µg/ml for the petroleum ether, ethyl acetate and methanol extract of *A. lebbeck*, respectively. On the other hand, the IC_{50} value of 83.73, 74.29, and 78.88 µg/ml was found for the petroleum ether, ethyl acetate and methanol extract of *A. Nilotica*, respectively. The pattern of free radical scavenging activity was found to be kind of similar in both the DPPH and hydroxyls scavenging assays. The ethyl acetate barks extracts of *A. lebbeck* and *A. nilotica* showed most significant IC_{50} values in hydroxyl free radical scavenging assays. The standard, ascorbic acid was found to have the IC_{50} of 43.80 µg/ml. Like DPPH scavenging study, all the tested extracts showed higher scavenging activity than the standard compound in hydroxyl scavenging assay too. The complete result of hydrogen peroxide scavenging study is presented in Table 6.

Table 3
Diameters of zones of inhibition (mm) of *A. Nilotica* bark extracts against different gram positive and gram negative bacteria.

Test Organism	Petroleum ether extract	Ethyl acetate extract	Methanol extract	Ciprofloxacin at 10 µg/ml
Gram positive bacteria				
Bacillus polymyxa	12.0	10.0	–	30
Bacillus subtilis	13.0	11.0	–	36
Bacillus megaterium	11.0	9.0	–	32
Sarcina lutea	–	–	–	40
Staphylococcus aureus	10.0	11.0	–	38
Gram negative bacteria				
Vibrio mimicus	11.0	10.0	–	38
Vibrio cholera	10.0	–	–	42
Salmonella typhi	12.0	10.0	–	40
Shigella boydii	–	–	–	44
Shigella flexneri type-1	10.0	9.0	–	38
Shigella dysenteriae	–	9.0	–	36
Pseudomonas aeruginosa	12.0	10.0	–	34
Klebsiella pneumoniae	9.0	–	–	30
Escherchia coli	–	–	–	36

(–) = No significant antibacterial activity.

Table 4
Diameters of zones of inhibition (mm) of *A. Nilotica* bark extracts against different fungal strains.

Test Organism	Petroleum ether extract	Ethyl acetate extract	Methanol extract	Griseofulvin at 25 µg/ml
Candida arrizae	7	8	–	24
Aspergillus fumigate	7	–	–	22
Aspergillus niger	8	8	–	24
Rhizopus oryzae	–	–	–	16
Candida albicans	–	8	–	22
Saccharomyces cerevisiae	–	7	–	22
Candida krusei	–	–	–	14

(–) = No significant antifungal activity.

3.3. Cytotoxicity text

In the cytotoxicity assay, LC_{50} were observed for all the extracts within the concentration range tested (Table 7). In all cases, a concentration dependent increase in cytotoxicity was observed. Among the extracts, ethyl acetate extracts of *A. lebbeck* and *A. Nilotica* bark showed the highest LC_{50} which were 42.36 and 37.32 µg/ml, respectively. These results were much comparable with the results of positive control, Vincristine sulfate for which the LC_{50} was 33.42 µg/ml. In our study no brine shrimp nauplii was found for the 1% DMSO solution which was used as negative control. This indicates that the only solvent was not involved for the death of nauplii.

4. Discussion

In this study, the zone of inhibition observed for the petroleum ether, ethyl acetate, and methanol extracts of *A. lebbeck* and *A. Nilotica* bark are found in the range of 8–14 mm against all tested Gram positive and Gram negative bacteria. Among the three extracts, petroleum ether extracts showed highest inhibitory activity against all the tested bacteria which was followed by ethyl acetate extract. Further isolation,

Table 5
The DPPH free radical scavenging activity of barks extracts of *A. lebbeck* and *A. Nilotica*.

Concentration (μg/ml)	Percent inhibition						
	Ascorbic acid	*A. lebbeck* extract			*A. Nilotica* extract		
		Petroleum ether	Ethyl acetate	Methanol	Petroleum ether	Ethyl acetate	Methanol
20	27.16	21.36	26.04	23.45	15.74	20.68	18.14
40	56.13	28.36	35.63	34.26	22.12	33.12	23.15
60	79.00	40.75	49.79	48.21	32.26	45.79	37.21
80	106.95	61.48	67.13	65.12	53.14	58.21	55.26
100	134.43	74.82	81.13	78.23	64.12	68.10	66.12

purification and elucidation of the exact structure of the active compounds in these extracts are required to establish the exact mechanism for this antimicrobial activity [24].

The petroleum ether extracts of *A. lebbeck* showed maximum zone of inhibition of 14 and 13 mm against the *S. aureus* and *S. dysenteriae*. This extract also gave zone of inhibition of around 7–8 mm against all the tested fungus. These results are comparable to the reports of Ferdous et al. and Srinivasan et al. where they found a moderate to strong broad spectral activity of petroleum ether extract of *A. lebbeck* bark against these same microorganisms [9,25]. In this study, we didn't found any activity of methanol extract of A. lebbeck bark against any tested microorganisms. In this study, *A. Nilotica* was also assessed for antibacterial activity. The petroleum ether extract of this plant showed significant activity against the almost all the tested bacteria. The results supported the previous reports of Banso et al. [26]. Here, we observed that the overall antimicrobial activity of the *A. lebbeck* extract is higher than that of the *A. Nilotica*. The methanol extract of both plants didn't show any antimicrobial activity.

The reactivity of the organic compounds with the stable free radicals is determined by using DPPH and hydroxyl scavenging potential which in turn gives the antioxidant potential of any organic compounds [20]. In DPPH quantitative antioxidant assay, ethyl acetate extract of *A. lebbeck* gave the most promising DPPH scavenging activity and it was 81.13% (IC_{50} = 57.25 at 100 μg/ml concentration). On the other hand, the highest DPPH free radical scavenging activity observed for the *A. Nilotica* was for ethyl acetate extract with an IC_{50} of 68.03 μg/ml. Hydrogen peroxide is not very reactive but it may cause hydroxyl free radical generation in cell. In normal cellular condition, hydroxyl free radical might be originated due to the reaction of hydrogen peroxide (H_2O_2) with different natural iron complexes which lead to many toxic effects [27]. Thus removal of this radical is very important to normal physiologic condition. In this study, the ethyl acetate extract of A. lebbeck and A. nilotica demonstrated very significant hydroxyl free radical scavenging potential which was IC_{50} 64.69 and 74.29 μg/ml, respectively. In both cases, the ethyl acetate extract gave the highest activity which may be the results of the presence of polyphenolic compounds in this extract [5,28,29]. The obtained results support the previously reported in vitro antioxidant activity for the bark of *A. lebbeck* and *A. nilotica* [30–32]. In developing new drugs which can cause the protection of cellular DNA, lipids and proteins from the free radical damage, the ethyl acetate extract could be further analyzed for identifying the exact compounds which is responsible for the observed activity.

Different plant extracts are now widely implemented to treat various cancerous cell lines. Brine shrimp cytotoxicity assay is a widely used laboratory test for the determination of preliminary cytotoxicity profile of a plant extract. It's a rapid, simple, inexpensive bioassay which is mostly allies with the pesticidal, antiplasmodial, antitumor, antimalarial properties [22,23]. Previously, several works reported the cytotoxic effects of different parts of *A. Nilotica* and *A. lebbeck* on different cancer cell lines [11,33,34]. In 2012 Hussain et al. reported the methanol, ethyl acetate and chloroform root extracts [35]. We obtained a LC_{50} value of 37.32 μg/ml for the ethyl acetate bark extract which is significantly lower than that of the methanol and petroleum ether extracts. In this study, petroleum ether, ethyl acetate and methanol bark extract of *A. lebbeck* was also investigated for the cytotoxicity assay. The LC_{50} values of this plant extracts were 64.71, 42.36 and 88.1 μg/ml, respectively. The presence of saponins in the ethyl acetate extract may be responsible for the most potential cytotoxic effect showed by this extract [36].

5. Conclusions

The ethyl acetate extracts of *A. lebbeck* and *A. Nilotica* bark have the most potential antimicrobial, antioxidant and cytotoxic potentials as compared to the petroleum ether and methanol extracts. Further isolation and identification of specific phytochemicals from the ethyl acetate extracts may pave the way to develop some natural derived novel compounds which can be used as potential antimicrobial, antioxidant and cytotoxic agent.

Conflict of interest

The authors declare that there is no conflict of interests.

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Table 6
The hydrogen peroxide free radical scavenging activity of berks extracts of *A. lebbeck* and *A. Nilotica*.

Concentration (μg/ml)	Percent inhibition						
	Ascorbic acid	*A. lebbeck* extract			*A. Nilotica* extract		
		Petroleum ether	Ethyl acetate	Methanol	Petroleum ether	Ethyl acetate	Methanol
20	32.10	24.25	28.47	26.59	19.56	23.92	22.31
40	49.56	32.12	39.71	35.12	26.12	31.49	29.54
60	62.30	41.27	47.22	44.31	36.21	43.67	40.58
80	74.54	54.21	56.12	55.26	49.42	52.21	49.23
100	82.15	68.12	67.41	66.33	63.87	63.23	61.77

Table 7
Effect of plant extracts and Vincristine sulfate on shrimp nauplii.

Conc. (µg/ml)	$Log_{10}C$	A. lebbeck extract			A. Nilotica extract			Vincristine sulfate		
		Petroleum ether	Ethyl acetate	Methanol	Petroleum ether	Ethyl acetate	Methanol	Conc. (µg/ml)	$Log_{10}C$	Percent mortality
		Percent mortality								
10.0	1.000	10	20	0	0	10	0	5.0	0.699	10
20.0	1.301	20	30	10	20	30	10	10	1.0	20
40.0	1.602	30	40	30	40	50	30	25	1.398	30
60.0	1.778	50	60	40	50	60	40	35	1.544	50
80.0	1.903	60	70	50	70	80	60	50	1.699	70
	LC_{50} (µg/ml)									
		64.71	42.36	88.1	51.16	37.32	73.28	33.42		

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