Original Research

ESTIMATION OF POTENTIAL ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *Averrhoa bilimbi* LEAVES

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ABSTRACT

The aim of this study was to examine the antimicrobial and antioxidant activities of *Averrhoa bilimbi* (*A. bilimbi*) leaves. For determination of antimicrobial activity disc diffusion method was used and antioxidant activity was determined by DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging activity. In this study *A. bilimbi* leaves were extracted with ethanol followed by fractionation with the help of n-Hexane, chloroform and petroleum ether respectively. In case of gram positive bacteria among three fraction n-Hexane fraction showed highest antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Sarcina luteae*. Among gram negative bacteria, n-Hexane fraction showed superior activity against *Shigella dysenteriae, Salmonella paratyphi, Pseudomonus aeruginosa* and Petroleum Ether fraction showed highest activity against *Escherichia coli, V. parahemolyticus* with respect to other fractions. In case of antioxidant test the sequence of DPPH radical scavenging activity was in the following order: Ascorbic acid > n-Hexane fraction > Petroleum Ether fraction > Chloroform fraction. The IC_{50} values of Ascorbic acid, n-Hexane fraction, Petroleum Ether fraction and Chloroform fraction were 48.27 µg/ml, 67.05 µg/ml, 49.99 µg/ml and 76.64 µg/ml. The results suggested that *A. bilimbi* leaves had strong antibacterial and antioxidant activity.

KEYWORD: *Averrhoa bilimbi*, Antibacterial activity, Antioxidant activity, DPPH.

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1. INTRODUCTION

Infectious diseases produced by pathogenic microorganism are deliberated as a major cause of mortality and morbidity in humans. Though numerous antibiotics have been established to manage these diseases with most favorable efficacy, their mismanagement and mal-administration, in addition to microbial mutation have directed to the emergence of drug-resistant strains and has now become a universal alarm [1]. The worldwide emergence of multi-drug resistant (MDR) bacteria is progressively warning the efficiency of existing drugs and remarkably triggering failure of treatment [2]. Bacterial resistance to chemically distinct antibiotics is one of the prime public health concerns [3] and may be initiated by over-expression of MDR efflux pumps [4]. An unselective use of antimicrobial agents by the wide-ranging population has steered to an intricate structure of resistance of microorganisms to conventional antimicrobial therapy, what may be a pertinent factor in the emergence of problematic control infectious diseases and has encouraged researchers to pursue different sources of antimicrobial agents, among them the medicinal or natural plants [5]. Among the potential sources of new agents, medicinal plants have long been considered because; they contain many bioactive agents that can be of interest in therapeutics. Consequently, the exploration of new antimicrobial drugs from natural sources is warranted.

The cells of living organisms always produce free-radicals on account of pathophysiological and biochemical processes in response to some factors such as chemicals, radiation, environmental pollutants and toxins. Free radicals are reactive oxygen species (ROS) that are uninterruptedly generated in the human body as a byproduct of cellular aerobic respiration. Excessive production of ROS lead a disclosure to external oxidant substances, consequent failure in the defense mechanism or impairment to biomolecules such as DNA, lipids or proteins [6]. Furthermore, the cellular damage subsequently initiates aging and several chronic diseases such as cancer, diabetes, and atherosclerosis in addition to cardiovascular, inflammatory, and other degenerative diseases in humans [7]. Antioxidants are the agents that are capable of successfully neutralizing these free radicals by meddlesome with oxidation process, chelating catalytic metals and also by acting as oxygen scavengers [8]. The capability of definite phytochemical extracts to constrain or delay the oxidation of other molecules by overwhelming the initiation or propagation of oxidizing chain reactions have made them active substitutions in complementary medicine. These naturally occurring antioxidant agents have been stated to be composed of phenolic (such as phenolic acids, flavonoids and tocopherols) and nitrogen compounds (alkaloids, amines, chlorophyll derivatives, and amino acids) along with carotenoids and ascorbic acid [9]. Actually, phytochemical extracts comprising ingredients such as plant-derivatives alkaloids carotenoids, flavonoids, terpenoids, vitamins, polyphenols, and phenolic compounds stated to display antioxidant and anticancer activities [10]. The present investigation has proofed that consumption of natural antioxidants has been concomitant with decreased risk of cancer and many chronic diseases [11].

Averrhoa bilimbi Linn. (Bengali name- Bilimbi) belongs to a family- Oxalidiaceae is an attractive, long-lasting tree, and ranges 5-10 m in height, has a short stem soon dividing into a number of upright divisions that grows in Indonesia, Moluccas and cultivated or found throughout Bangladesh, Indonesia, the Philippines, Sri Lanka, Myanmar, India, Malaysia and Zanzibar [12]. Leaves mainly bunched at the branch tips, are substitute, imparipinnate; 30-60 cm long, with 11-37 cm different or sub opposite leaflets, ovate or oblong, with rounded base and pointed tip; downy; medium-green on the upper surface, pale on the underside; 2-10 cm long, 1.2-1.25 cm wide. Fruit ellipsoid, obovoid or nearly cylindrical, faintly 5-sided, 4-10 cm long; covered by a thin, star shaped calyx at the stem-end. The outer skin is smooth, very thin, soft and tender, and the flesh green, jelly-like, juicy and extremely acid [12]. *A. bilimbi* is medicinally used as a folk therapy for many symptoms. It is used as hypertension, antibacterial, antiscorbutic, astringent; postpartum protective medicine. It is also recommended for the treatment of fever, pimples, mumps, inflammation of the rectum and diabetes,

itches, boils, syphilis, rheumatism, whooping cough, bilious colic, stomach ache and as a cooling drink [13]. *A. bilimbi* fruits have therapeutic properties for the effective controlling of several human diseases [14]. Syrup that is prepared from the fruit is administered as a treatment for fever and inflammation and to stop rectal bleeding and improve internal hemorrhoids. The leaves are used as a paste on itches, swellings of mumps and rheumatism, and on skin eruption. They are also applied to the bites of poisonous creatures. Malaysians take the fresh leaves or fermented as a treatment for venereal disease [15]. A leaf infusion is a medication for coughs and is administered after childbirth as a tonic. A leaf decoction is administered to relieve rectal inflammation. A flower infusion is said to be effective against coughs and thrush. A paste of soused bilimbi is smeared all over the body to accelerate recovery after a fever.

The present study was designed to assess the antibacterial and antioxidant activities of ethanolic, chloroform and petroleum ether fractions of *A. bilimbi* leaves.

2. METHODOLOOGY

2.1. Drugs and chemicals

DPPH (1,1-diphenyl-2-picryl hydrazyl) was obtained from Sigma Aldrich USA. Ascorbic acid was obtained from SD Fine Chem. Ltd, Biosar, India. DMSO (dimethylsulfoxide) was purchased from Merck, Germany. Kanamycin was collected from Square Pharmaceuticals Ltd., Bangladesh.

2.2. Collection and identification of the plant

The fresh leaves of *A. bilimbi* were collected in the month of April, 2013 from Mymensing, Bangladesh and were taxonomically recognized by Bangladesh National Herbarium. The Accession number of this plant is DACB-49675.

2.3. Drying and grinding of plant material

The fresh leaves of the plants were first thoroughly rinsed in running tap water and then in sterile water to remove adhering dirt. Then leaves were sun dried for 7 days and finally dried in an oven at temperature not more than 50°C for better grinding. The dried leaves were ground into course powder using an electric blender, then stored in sealed and labeled sterilized glass container for further use.

2.4. Extraction and fractionation of the plant material

Powdered sample having a weight of 250 g of *A. bilimbi* was extracted by cold extraction process using ethanol (1000 ml) with daily shaking and stirring for 15 days at room temperature. After 7 days the extract was filtered through cotton followed by filter paper (Double filter paper 102, 11.0 cm). Then the concentrated liquid extract was dried at 37°C to obtain a greenish mass. The weight of the crude extract obtained from leaves was 30 grams. The whole process was repeated with n-Hexane, chloroform and petroleum ether instead of ethanol using as solvent.

2.5. Antimicrobial activity

2.5.1. Test microorganism

Antimicrobial activity was carried out against five Gram positive (*S. aureus, B. cereus, B. megaterium, B. subtilis and S. luteae*) and five Gram negative (*E. coli, V. parahemolyticus, S. dysenteriae, S. paratyphi and P. aeruginosa*) bacteria. These bacteria were chosen to be studied as they are important pathogens and also due to rapidly developed antibiotic resistance. The microorganisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. For bacteria, the culture media was prepared by nutrient agar, reconstituting with distilled water according to specification (2.8% w/v).

2.5.2. Preparation of inoculum

Preparation of inoculum of the test organisms was done by using the colony suspension method [16]. The bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C. The stock cultures were maintained at 4°C. The bacterial strains were adjusted to a turbidity of 0.5 McFarland standards approximately 108 CFU/ml for bacteria with the addition of sterile saline (0.9 % NaCl) based on the optical density (OD) measurement at 530 nm.

2.5.2. Antibacterial activity

Antibacterial activity of A. bilimbi was carried out by the help of disc diffusion method [17]. Solution of known concentration (500 µg /disc) of the test sample was made by dissolving measured amount of the sample (50 mg) in 1 ml of methanol. Then sterile filter paper disc (5 mm diameters) was impregnated with known test substance and dried. The dried disc was placed on plates (Petri dishes, 120 mm diameter) containing a suitable medium (nutrient agar) seeded with the test organisms. Standard disc of kanamycin (30µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control. These plates were kept at low temperature (4ºC) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37ºC) for 24 hours to allow the growth of microorganisms. Antibacterial activity of the test sample was observed by growth inhibition of organisms forming clear, distinct zone surrounding the discs. The antibacterial activity was expressed in terms of millimeter by measuring the diameter of the zone of inhibition. The greater zone of inhibition indicates the greater activity of the test material against the test organism.

2.6. DPPH free radical scavenging activity

The free radical scavenging activity (antioxidant capacity) of the three leaves extracts on the stable radical 1,1-diphenyl-2- picrylhydrazyl (DPPH) was determined by the method of Brand-Williams [18-20]. The stock solution (24 mg DPPH/100 mL methanol) was diluted with methanol to get an absorbance of 1.1 at 515 nm using UV spectrophotometer (Model NO. 1501PC Shimadzu, Japan). 0.6 mL of the sample extracts at different concentrations, blank and ascorbic acid as standard were permitted to react with 3 mL of the DPPH working solution for 20 min under dark conditions. Then, the absorbance was taken at 515 nm. DPPH free radical scavenging capacity was calculated from the absorbance of sample, blank, and ascorbic acid as standard. Percent scavenging of the DPPH free radical was measured using the following equation-

$$\% \text{ DPPH radical scavenging} = [1 - (A_s/A_c)] \times 100$$

Here, A_c = absorbance of control,
A_s = absorbance of sample/standard solution.

2.7. Statistical analysis

Results were expressed as mean ± standard deviation (SD) from three separate observations. Student's t test was used to find the significance of standard and sample for IC50 values. Microsoft Excel 2010 (Roselle, IL, USA) was used for the statistical and graphical evaluations. A probability of $p < 0.05$ was considered as significant.

3. RESULTAND DISCU SSION

3.1. Antibacterial activity

The antibacterial activity of the plant extract against gram positive bacteria is given in Figure 1. The results show that n-Hexane fraction showed maximum 19 mm zone of inhibition (ZI) against *Sarcina luteae* and minimum ZI was 10 mm against *Bacillus cereus.* For Chloroform fraction maximum 10 mm ZI was reported against *Bacillus subtilis.* Petroleum Ether fraction showed highest 14 mm ZI against *Staphylococcus aureus. The standard* Kanamycin exerted maximum 26 mm ZI against *Bacillus subtilis* and minimum 22 mm ZI against *Bacillus megaterium.* In the study of Uddin MS. *et al.,* reported that crude methanol extract of *Litsea monopetala* leaves had 15 mm ZI against *Bacillus cereus* [21].

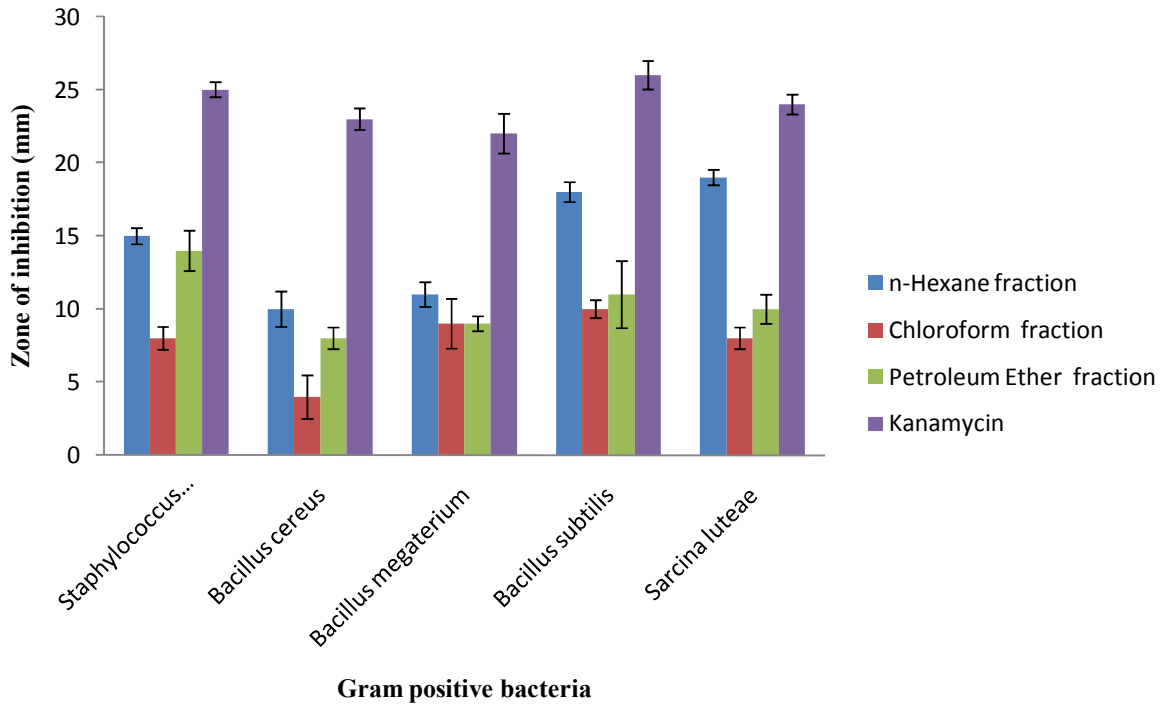

Fig 1: Antibacterial activity of *A. bilimbi* leaves extracts against gram positive bacteria. Values are expressed as mean ± SD (n = 3).

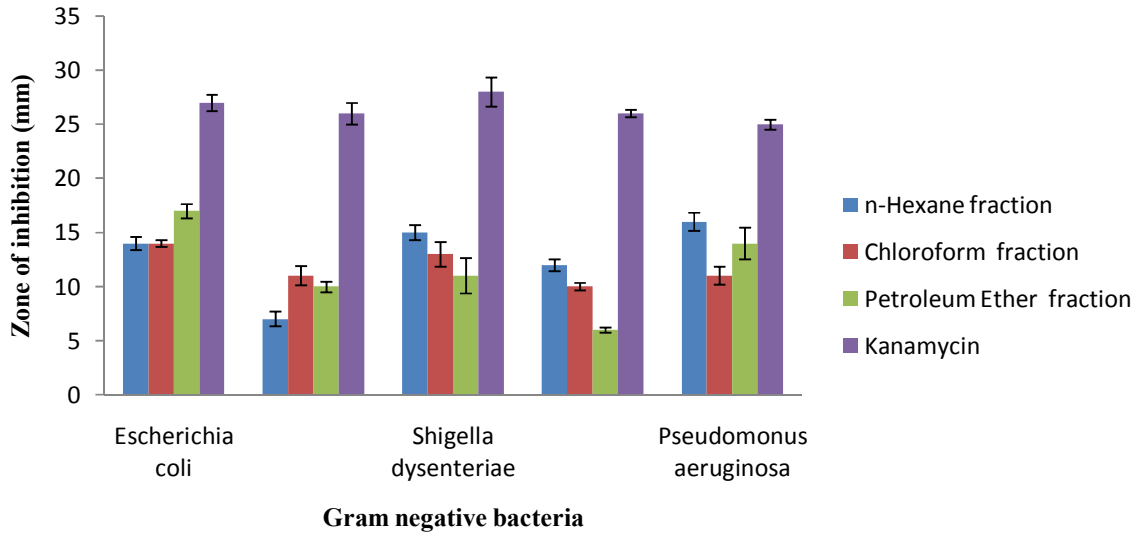

Fig 2: Antibacterial activity of *A. bilimbi* leaves against gram negative bacteria. Values are expressed as mean ± SD (n = 3).

Figure 2 represents the antibacterial activity of the plant extract against gram negative bacteria. In case of n-Hexane fraction maximum 15 mm ZI was stated against *Shigella dysenteriae* but lowest ZI was only 7 mm against *V. parahemolyticus.* Chloroform fraction showed highest 14 mm ZI against *Escherichia coli. For* Petroleum Ether fraction highest 17 mm ZI was testified against *Escherichia Coli.* On the other hand standard, Kanamycin displayed maximum 28 mm ZI against *Shigella dysenteriae* and minimum 25 mm ZI against *Pseudomonus aeruginosa.* Afolayan AJ. showed that ethanol extract of *Hydnora africana* had 20 mm ZI against *Pseudomonas aeruginosa* and *Escherichia coli* [22].

3.2. Antioxidant activity

The antioxidant activity of the plant extract was tested by using the DPPH radical scavenging test. This test is most widely used testing methods for searching antioxidant potential of unknown plant extract. As a standard ascorbic acid was used in this study. At a maximum concentration among 3 fractions maxium DPPH radical scavenging activity was reported by n-Hexane fraction followed by Petroleum Ether fraction and then Chloroform fraction. Hossain MS. *et al.,* in the study of antioxidant and cytotoxic of *Xanthosoma sagittifolium* leaf noticed almost similar results [23].

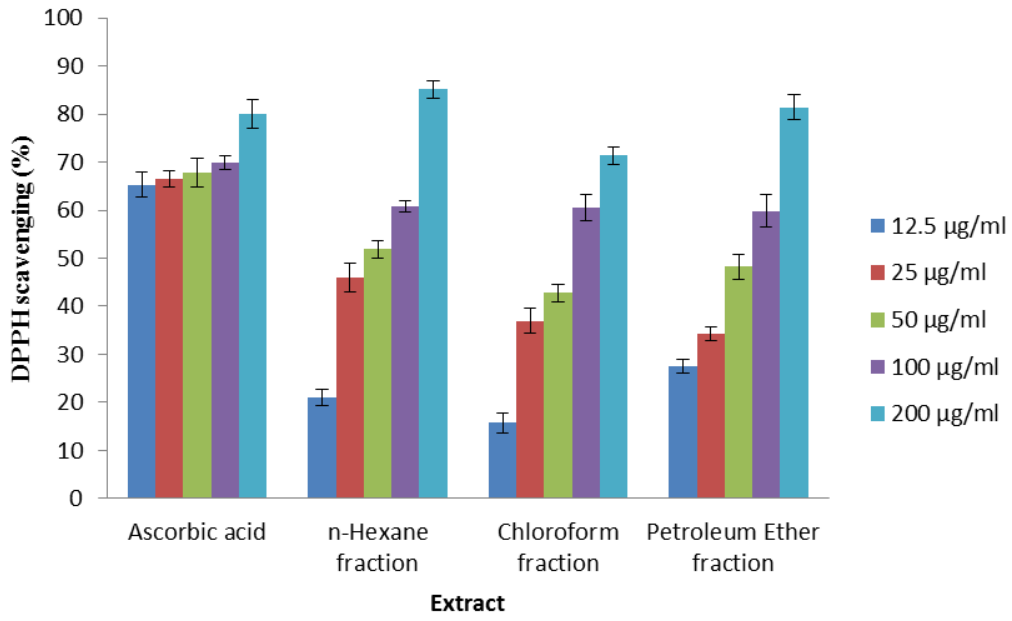

Fig 3: DPPH radical scavenging activity of standard and *A. bilimbi* leaves. Values were expressed as mean ± SD (n = 3).

The IC_{50} value of the extracts is given in Figure 4. Results showed that the IC_{50} value of the Chloroform fraction was 49.99 µg/ml. For n-Hexane fraction IC_{50} value was 67.05 µg/ml ($p < 0.01$) followed by Petroleum Ether fraction (76.64 µg/ml). The IC_{50} value of the ascorbic acid was 48.27 µg/ml. In the study of antioxidant activity of *Caryota urens* fruits Uddin MS. *et al*., reported almost similar finding [24]. Details information is given below:

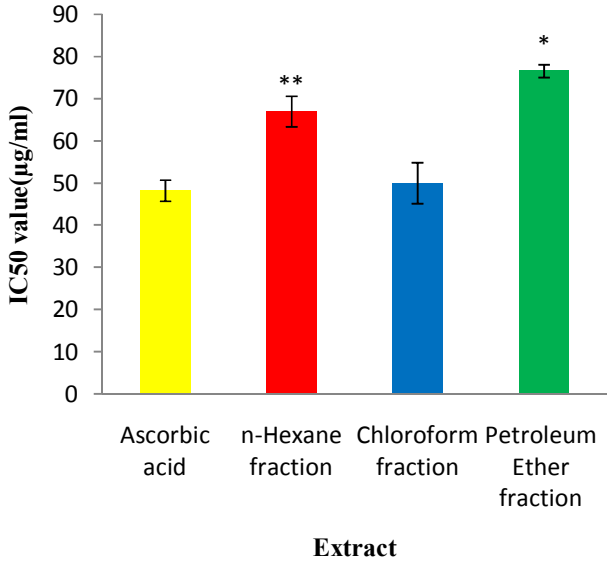

Fig 4: IC_{50} values of standard and *A. bilimbi* leaves. Values were expressed as mean ± SD (n = 3). *p < 0.05, **p < 0.01 significance difference from standard

4. CONCLUSION

This study showed that *A. bilimbi* leaves have strong antibacterial and antioxidant activity. However, further study will require to prove the active compounds for possible development of lead compound responsible for antibacterial and antioxidant potentiality.

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COMPETING INTERESTS

Author have declared that no competing interests exist.

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