



**International Journal of Biology, Pharmacy  
and Allied Sciences (IJBPAS)**

*'A Bridge Between Laboratory and Reader'*

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**IN-VITRO ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT FROM *MWLIA AZEDARACH* FRUIT**

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**ABSTRACT**

This study was carried out to evaluate the *in vitro* antibacterial activities of the ethanolic extract of *Melia azedarach* fruit against 4 bacterial (2 gram-negative and 2 gram-positive bacteria) species. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were quantified by micro-dilution method. The lowest minimum inhibitory concentration (MIC) was observed 40 mg/ml and MBC=80 (against *Salmunella typhimurium*). The sensitivity of these bacteria was more than other bacteria. Results show significant antibacterial activity for this extract which suggest its capacity as a natural food preservative against bacteria in the food industry.

**Key words:** *Melia azedarach*, *Antibacterial activities*, *ethanolic extract*.

**INTRODUCTION**

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf-life [1]. Food conservation is based on an intermittent

search for foods that have high nutritional quality and microbial stability, and it involves controlling the growth/survival of spoiling and pathogen foodborne microorganisms. The improvement of the shelf-life of foods has an

important economic impact by reducing losses attributed to spoilage and allowing the products to reach distant and new markets [2]. Nowadays, the excessive use of synthetic antimicrobial compounds in food manufacture as additive agents is well known, many of which are suspected for their residual toxicity [3]. Because of increasing pressure of consumers and legal authorities, the food industry has tended to reduce the use of chemical preservatives in their products to either completely nil or to adopt more natural alternatives for the maintenance or extension of product shelf life [4]. Several Eos [4] and plant extracts [5] offer potential applications in food preservation, and the use of EOs [4] and plant extracts [5] in the food industry can help reduce the addition of chemical preservatives as well as the intensity of heat treatments, resulting in foods which are more naturally preserved and richer in organic properties [3]. Several compounds found in plants, which have long been used as natural agents for food preservation, are generally well accepted. Amongst these naturally occurring compounds, essential oils and extracts of various species of edible and medicinal plants, herbs and spices are considered by the food industry because of their antimicrobial potential. The aptitude of essential oils to inhibit the growth of certain

microorganisms is of paramount importance, particularly, when it is expressed against food-borne pathogens [6] and the antimicrobial activities of plant oils and extracts have formed the foundation of many applications such as in raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [7]. *Melia azedarach* L. (*M. azedarach*) belongs to the family *Meliaceae*, also placed in *Celtidaceae*, *Ulmaceae* and it is a small tree. There are about 50 shrubs or trees in the genus *Melia*, widely distributed in tropical and subtropical regions of the world, of which most of them are traditionally used in different therapeutic purposes [8]. It was introduced and naturalized in Philippines, United States of America, Brazil, Argentine, many African and Arab countries [9]. The photochemistry and pharmacological studies conducted on this medicinal herb have successfully isolated several natural products including diterpenes, monoterpenes, triterpenes, saponins, flavonoids hexoses, organic acids, rosmarinic acid, chromene and myoinositol [10]. Therefore the objective of the present study was to assess the biological activities of ethanolic extract as an antibacterial agent against standard microorganisms such as *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella*

*typhimurium*, *Escherichia coli* for the first time.

## MATERIALS AND METHODS

### Chemicals and Plant materials

Muller Hinton agar (Merck, Germany), BHI (Brain-heart infusion broth) (Merck, Germany), ethanol and Dimethyl sulfoxide (DMSO) (Merck, Germany) were purchased. Fruits of the *Melia azedarach* plant were collected in 2015 from Khorasan-Razavi Province (the northeast of Iran). The plant confirmed by Medicinal Plants Institute, Ferdowsi University, Mashhad, Iran.

### Preparation of plant extract

Fruit of *Melia azedarach* were air-dried at room temperature [11]. Extract was prepared by stirring the plant material (50 g) overnight at room temperature with 250 ml amount of ethanol. The suspension was filtered through a Whatman filter paper No. 1 [12, 13]. The filtered extracts were combined and organic solvent removed under reduced pressure at 40 °C by means of rotary evaporator [14]. concentrated to half of its original volume at room temperature, filter sterilized with 0.45 µm Millipore filters and stored in screw capped vials at [13] 4 °C [14]. The dry extract was dissolved in DMSO to obtain a concentration of 320 mg/ml [13].

### Organisms and Inoculation Conditions

The ethanolic extract was screened for antimicrobial activity against the gram-positive bacteria *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923); and the gram-negative bacterium *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922). The reference strains (ATCC) were obtained from American Type Culture Collection [15]. The bacteria strains were first grown on [16] BHI broth (Brain Heart Infusion broth, Merck) [17, 18, 19] medium [16] at 35 °C [15] for 18 to 24 hrs. 0.1 mL of each culture was added to 10 mL of BHIB (Brain Heart Infusion Broth, Merck) [20].

### Antibacterial activity assay

Minimum inhibitory concentration (MIC) was determined using the micro-broth dilution technique [21]. Micro-dilution broth test was performed in BHI broth (Brain Heart Infusion broth) [17, 18, 19] medium, in 96-well micro plates, as follows: 95 µl [22] of BHI broth (Brain Heart Infusion broth) [17, 18, 19] was added into each well of the micro plate and 100 µl of ethanolic extract was added to the first row of the micro plate and then serially twofold diluted in a final volume of 100 µl, respectively, with concentrations ranging from 320 to 0.039 mg ml<sup>-1</sup>. The wells were then inoculated with 5 µl of each bacterial suspension, adjusted to 0.5 McFarland [22]

(standardized at  $1.5 \times 10^6$  CFU/ml by adjusting the optical density to 0.1 at 600 nm by Shimadzu Uv-1601-pc spectrophotometer) [23]. The last row containing the bacterium in Mueller-Hinton broth without the test sample was used as a control for strain viability. The micro plates were covered and incubated for 24 hrs at 37 °C. Each experiment was performed in triplicate. By the end of the experiment, the MIC value of each well was measured spectrophotometrically at 620 nm with an ELIZA (anthos2020, Switzerland) micro plate reader [24]. The MIC was determined as the lowest concentration of the product inhibiting the growth of the microorganism [25]. The lowest concentrations of extract with no visible microbial growth on agar plates (after incubation for 18–24 h at 37°C) were recorded as MBC [22, 26].

## RESULTS AND DISCUSSION

### Results of MIC and MBC

The MIC values are summarized in Table 1, which show that the ethanolic extract was able to prevent the growth of all the tested bacteria. Generally, all the microorganisms were sensitive to the extract. The MIC values of the extract ranged from 40 to 80 mg ml<sup>-1</sup>, with *S. typhimurium* being the most sensitive while other bacteria were the least sensitive. MIC for *S. typhimurium* was 40 and for other

bacteria obtained 80 mg/ml<sup>-1</sup>, so this in study the gram-negative (*S. typhimurium*) bacteria were more sensitive than the gram-positive bacteria. All data on antimicrobial activity were average of triplicate.

Out of four bacterial species, all bacteria were found to be sensitive to plant extract. The mean MIC values of inhibition for the extract against Gram positive and Gram negative bacteria increased with the increasing concentration of the extract.

Some studies show that gram-negative bacteria are more resistant to essential oil others claim the same for gram-positive bacteria. In our study the gram-negative bacteria were in both categories [27]. Gram positive and Gram negative bacteria both were found to be sensitive indicating the broad spectrum activity of the extract [28].

The traditional use of plants provides the basis for indicating the type of essential oils and plant extracts useful for specific food purposes. Historically, many plant oils and extracts have been reported to have antimicrobial properties [5] and used in the food industry and are considered generally recognized as safe [29].

In contrast to the present study, Ramya et al 2009 reported the antibacterial activities of Dichloromethane, ethyl acetate, methanolic and ethanol leaf extract of *M. azedarach* [30].

Also Meziane et al 2014 obtained antibacterial activity of extract and essential oil from seeds, leaves and flowers this plants [9].

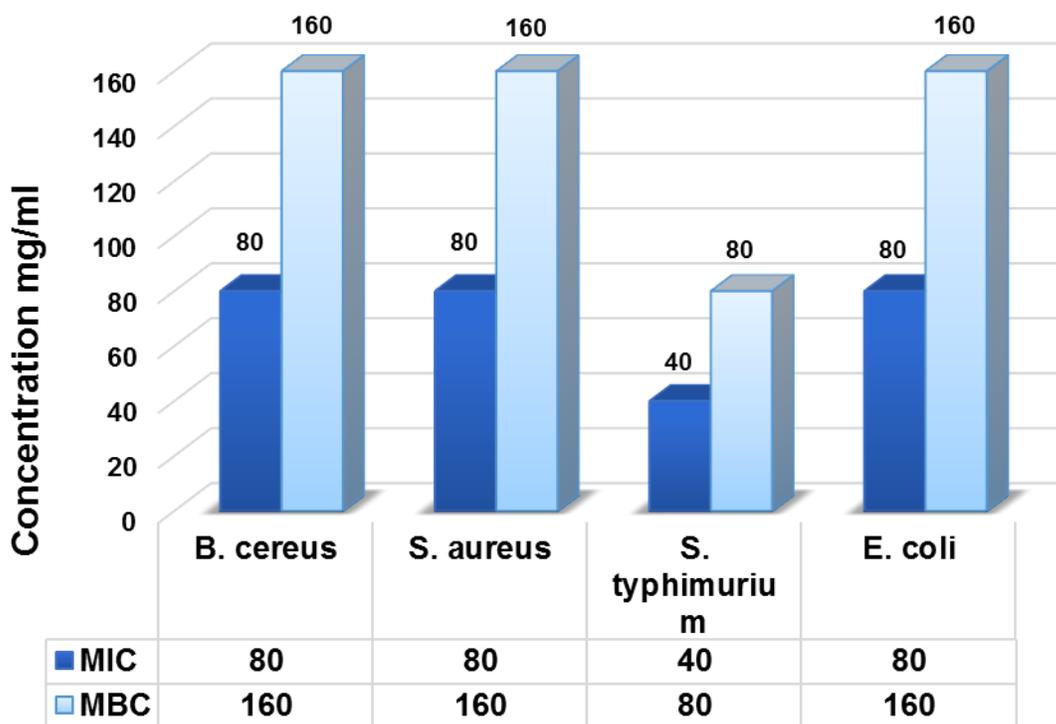
Although, some researches have carried out the test with different solvents and different strains for antimicrobial test, same activities

have been documented for the plant species. This plant has antioxidant, analgesic and antibacterial activities. The phytochemical screening also confirms alkaloids, flavonoids, glycosides, saponins and tannins like compounds [8].

**Table 1: MIC and MBC (mg ml<sup>-1</sup>) of ethanolic extract from *Melia azedarach* fruit**

MO	<i>B. cereus</i>	<i>S. aureus</i>	<i>S. typhimurium</i>	<i>E. coli</i>
MIC	80	80	40	80
MBC	160	160	80	160

Values are the mean of three replicate



**Figure 1: MIC and MBC (mg ml<sup>-1</sup>) of ethanolic extract from *Melia azedarach* fruit**

**CONCLUSION**

This is the first study to provide data on the ethanolic extract of *M. azedarach* fruit evaluated against four pathogenic bacteria. The results of our study suggest the possibility of using the extract of *M.*

*azedarach* as natural antimicrobials in food industry to control food-borne pathogens and to be used as a natural preservative in food systems against the well-known causal agents of foodborne diseases and food spoilage such

as *B. cereus*, *S. aureus*, *S. typhimurium* and *E. coli*.

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