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# Evaluation of antibacterial and antioxidant activity of three plant species from *Morus* genus

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## Abstract

Emergence and prevalence of antibiotic-resistant pathogenic bacteria and undesirable side effects of certain antibiotics have triggered immense interest in searching for new antimicrobial drugs of plant origin. Besides, the development of safer antioxidant, especially from plant source is also important to reduce oxidative stress related pathophysiological conditions. However, in this study, we aimed to evaluate antibacterial and antioxidant activity of the aqueous extracts of leaves of three different *Morus* species including *M. indica* (MI), *M. latifolia* (MLF), and *M. laevigata* (MLG). Antibacterial activity was investigated against two pathogenic bacteria (*E. coli* and *Pseudomonas*) and three non-pathogenic bacteria (*Acetobacter,* RCA, and RVM). The extracts were prepared at three different concentrations (0.25, 0.50, and 0.75 mg/disc) and the potency of the extracts was quantitatively assessed by the presence or absence of inhibition zone. Antioxidant potential of the three *Morus* species was evaluated using DPPH scavenging assay. All the three test plant possessed potent antibacterial and antioxidant capacity in a dose-dependent manner. The $IC_{50}$ (dose at which 50% DPPH was scavenged) of MLF, MI, and MLG extracts were 67.21, 69.23, and 92.74 µg/mL, respectively. Among the three species, MI and MLF showed the highest antibacterial and antioxidant activity respectively. Overall, findings suggest that the three studied plant species of *Morus* genera are the important sources of pharmaceutical value.

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## Introduction

The incidence of life-threatening infectious diseases caused by pathogenic microorganisms is an important cause of mortality and morbidity all over the world. Additionally, the emergence and prevalence of multidrug-resistant (MDR) bacterial pathogens in the last decades is a remarkable threat for the effectiveness of current antimicrobial therapy and significantly causing treatment failure of infections (Cohen, 1992; Giamarellou, 2010). Thus, antimicrobial drug resistance has long term effects on patients, doctors, health-care administrators, pharmaceutical companies and even the global economy. So, the discovery of substances from other sources with proven antimicrobial activity is a crucial need. Consequently, this condition has led to exploring more potential antimicrobial agents from the active ingredients of the plant that can be considered as a safe source for the synthesis of new antimicrobial drug (Pretorius *et al.,* 2003).

On the other hand, free radicals and other reactive oxygen species (ROS) are derived either from normal cellular metabolism in the human body or from the external sources such as exposure to X-rays, ozone, cigarette smoke, air pollutants, and industrial chemicals (Birben *et al.,* 2012). Those free radicals trigger various chain reactions and cause damage to cell components. Antioxidants are naturally abundant in plants (fruits, vegetables, medicinal herbs) and are able to neutralize free radicals by donating an electron and convert them into harmless molecules (Abdollahi *et al.,* 2005). Natural antioxidants have a wide range of bio-activities, including inhibition of ROS generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential (Leonard *et al.,* 2002).

The imbalance between the free radicals and antioxidants is termed as oxidative stress (Hasan *et al.,* 2018) which is responsible for a variety of pathological conditions including cardiovascular dysfunction, atherosclerosis, aging, inflammation, carcinogenesis, drug toxicity, reperfusion injury and neurodegenerative diseases (Aruoma, 1998).

Since the ancient time in Chinese medicine, Ayurveda, Arabic, and Unani medicine, the usefulness of plant extracts for antimicrobial therapy as well as other diseases have been proved to be outstanding remedies but the vast majority has not yet been adequately evaluated (Balandrin *et al.,* 1985). World health organization (WHO) has suggested adding the traditionally used medicines including phytomedicine to national health care system if they are ensured safe and effective through experiment (Eloff, 1998).

The genus *Morus* is widely distributed in Asia, Europe, North America, South America, and Africa, and is grown at considerably higher in the eastern, central, and southern Asia for silk production. Several *Morus* species have great nutritional value, medicinal uses and antioxidant potential (Elmacı and Altuğ, 2002; Imran *et al.,* 2010).

Keeping all of the mentioned information above in consideration, this study was aimed to investigate the antibacterial and antioxidant potency of the three selected *Morus* species namely *M. indica* (MI), *M. latifolia* (MLF), and *M. laevigata* (MLG).

## Materials and methods

### *Chemicals and reagents*

Mueller Hinton media and Ampicillin antibiotic were bought from Hi-Media (India). Butylated hydroxytoluene (BHT) was collected from Sigma-Aldrich (USA). All other chemicals used in this study were of analytical grade.

### *Plant sample collection*

*M. indica* (MI), *M. latifolia* (MLF), and *M. laevigata* (MLG) were identified and collected from Bangladesh Sericulture Research and Training Institute, Rajshahi, Bangladesh. Fresh leaves of those plants were collected maintaining standard botanical field collection methodology (Leonard *et al.,* 2002).

### *Preparation of crude extract*

Plant extracts were prepared by following the method as described previously with a little modification (Alade and Irobi, 1998). Fresh matured leaves were

washed with distilled water and dried at normal temperature. Then the dried leaves were kept in an air incubator at 37°Ctemperaturesuntil dried. The dried samples were then powdered by using a grinder machine and stored in an air tight container. The fine powder (25 g) was mixed with sterilized distilled water (250 mL) and was stirred for 24h.At the end of extraction, the extracts were passed through Whatman No. 1 filter paper and concentrated by using a rotary evaporator at 40°C. Finally, the extracts were stored at 4°C.

*Bacterial strains and media*

Among the bacterial strains used in this study, two were pathogenic strains (*E. coli* and *Pseudomonas*) collected from Rajshahi Medical College Hospital, Rajshahi, Bangladesh and three were non-pathogenic strains (*Acetobacter*, RCA and RVM) collected as stock from the Microbiology Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi, Bangladesh. All of the bacterial strains were cultured in Mueller Hinton Broth (MHB, Hi-Media) media for 18-24 h, followed by checking suspension turbidity equivalent to 0.5 McFarland solution (1-2 $\times 10^8$ CFU/mL) with the addition of sterile saline solution.

*Antimicrobial activity test*

Antibacterial activity of the plant extracts was screened by the disc-diffusion method with minor modifications (Wilkins *et al.,* 1972). 200 µL of overnight LB broth culture was added to 15 mL of molten Mueller Hinton Agar, mixed well, poured into a sterile Petri dish and allowed to set. Sterilized filter paper discs (5 mm in diameter) containing different concentrations (0.25, 0.50, and 0.75 mg/disc) of the sample were placed on the plate. In this study, Ampicillin (30 µg/disc) was used as a standard. Zone of inhibition (ZI) was measured after incubation at 37°C for overnight. The antimicrobial activity was determined by measuring the diameter of ZI. The experiment was carried out in triplicate and the mean of the diameter of the inhibition zones was calculated. Antibacterial activity was recorded as a zone of growth inhibition of greater than 5 mm around the disc.

*Antioxidant activity test*

Antioxidant activity of the extracts was checked by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity as described previously with little modification (Rahman *et al.,* 2018). The DPPH solution (0.004% w/v) was prepared in methanol. Then, the extracts and BHT (standard control) at different concentrations (25, 50, 100, 150, and 200 µg/mL) in methanol were mixed with the prepared DPPH solution. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of each extract and control was measured at 517 nm by using GENESYS 10S UV-VIS spectrophotometer (Thermo SCIENTIFIC, USA). Finally, the radical scavenging activity (RSA) was calculated as percentage of DPPH discoloration using following equation-

$$\% \text{ of RSA} = [(A_{DPPH} - A_E)/A_{DPPH}] \times 100$$

Where $A_{DPPH}$ is the absorbance of the DPPH solution and $A_E$ is the absorbance of the solution when extracts have been added at a particular concentration. The concentration of RVE giving 50% RSA ($IC_{50}$) was calculated from the graph of RSA percentage against extract concentration.

**Results and discussion**

As we investigated in this current study, we found that the aqueous extracts of MLF, MI, and MLG showed potent antibacterial activity against both of pathogenic (*E. coli,* and *Pseudomonas*) and non-pathogenic (*Acetobacter,* RCA, and RVM) (Fig. 1). The antibacterial activity was in a dose-dependent manner. On the basis of antibiotic sensitivity test, ZI in diameter ranging 5-10 mm was considered for resistance, 11-15 mm for intermediate resistance, and >15 mm for sensitive to antimicrobial agents. In case of the two pathogenic bacteria, the range of ZI for the standard Ampicillin was 17-21 mm which indicates these two clinical isolates were sensitive to Ampicillin. On the other hand, in case of *E. coli* and *Pseudomonas*, we found the highest ZI (16 mm) by MI extract at 0.75 mg/disc (Fig. 1A and 1B). At the

same concentration, MLG showed 16 mm and 12 mm inhibition zone against *E. coli* and *Pseudomonas* respectively (Fig. 1A and 1B). MLF also inhibited the growth of the two pathogenic isolates and the ZI was 10 mm for both strains (Fig. 1A and 1B). However, though 0.5 mg/disc dose showed a notable effect on growth inhibition, 0.25 mg/disc dose application of all three extracts exhibited a minute antimicrobial activity against the two pathogenic strains (Fig. 1A and 1B).

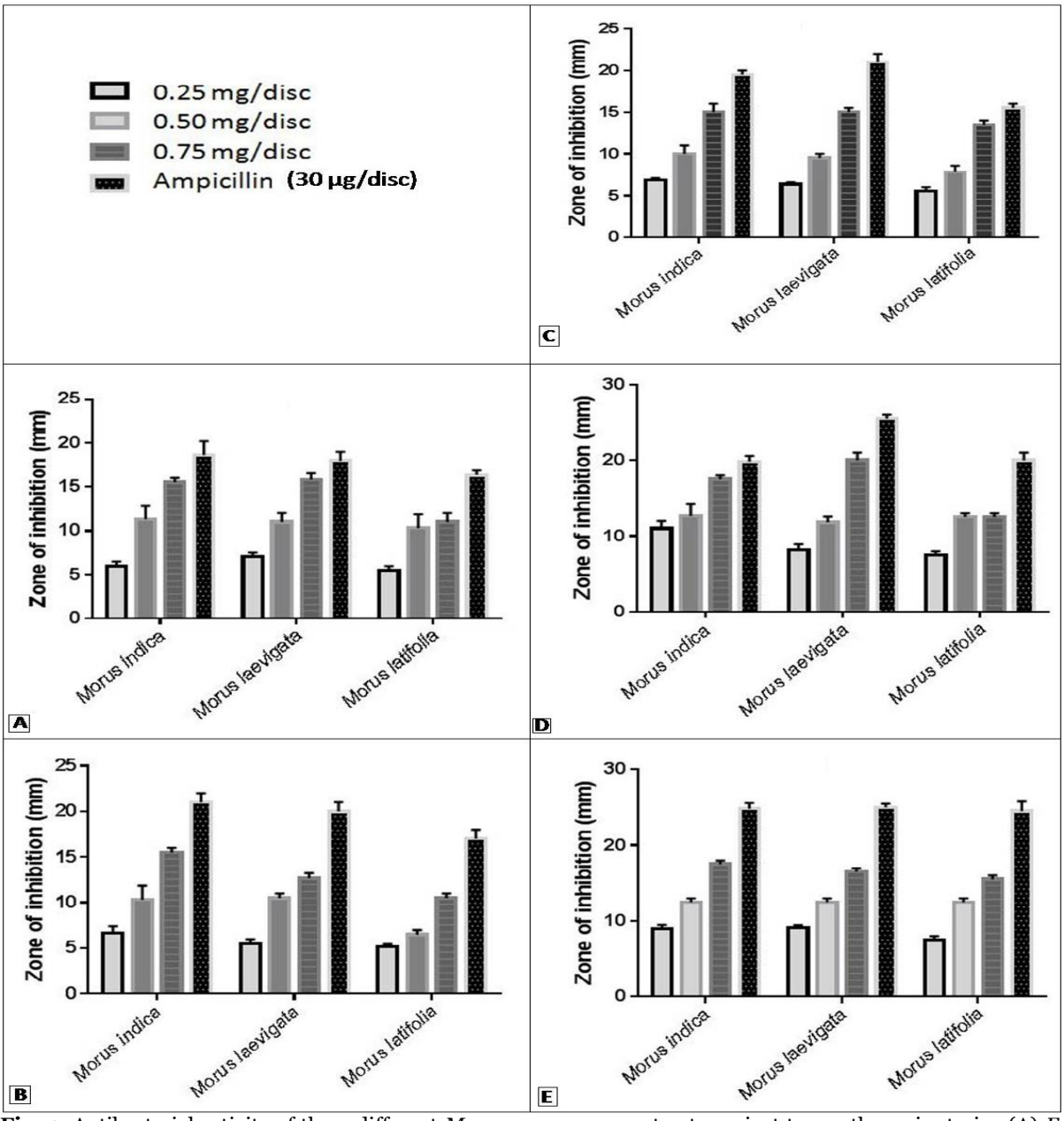


**Fig. 1.** Antibacterial activity of three different *Morus* sp. aqueous extracts against two pathogenic strains (A) *E. coli* and (B) *Pseudomonas* and three non-pathogenic strains (C) *Acetobacter*, (D) RCA, and (E) RVM compared to a standard Ampicillin (30µg/disc).

In case of the non-pathogenic strains, ZI for standard Ampicillin (30 µg/disc) was ranged from 15 to 26 mm. MI and MLG extracts showed outstanding activity with 18 mm and 20 mm ZI respectively against RCA bacteria at 0.75 mg/disc, while MLF showed only 12 mm ZI at the similar dose (Fig. 1D). Again, in case of RVM, at the highest dose, MI showed better activity than MLG and MLF and the ZI were 18, 17, and 15 mm respectively (Fig. 1E). And, the observed ZI against *Azotobacter* at 0.75 mg/disc

were 15, 15, and 13 mm by MI, MLG, and MLF respectively (Fig. 1C). So, it can be concluded that all the three non-pathogenic strains were susceptible to the extracts at the highest dose except MLF.Moreover, the DPPH radical has been widely used to assess the free radical scavenging aptitude of a variety of natural products and has been established as a model free radical (Imran *et al.,* 2010). However, the higher the DPPH scavenging activity, the higher is the antioxidant activity of the sample.

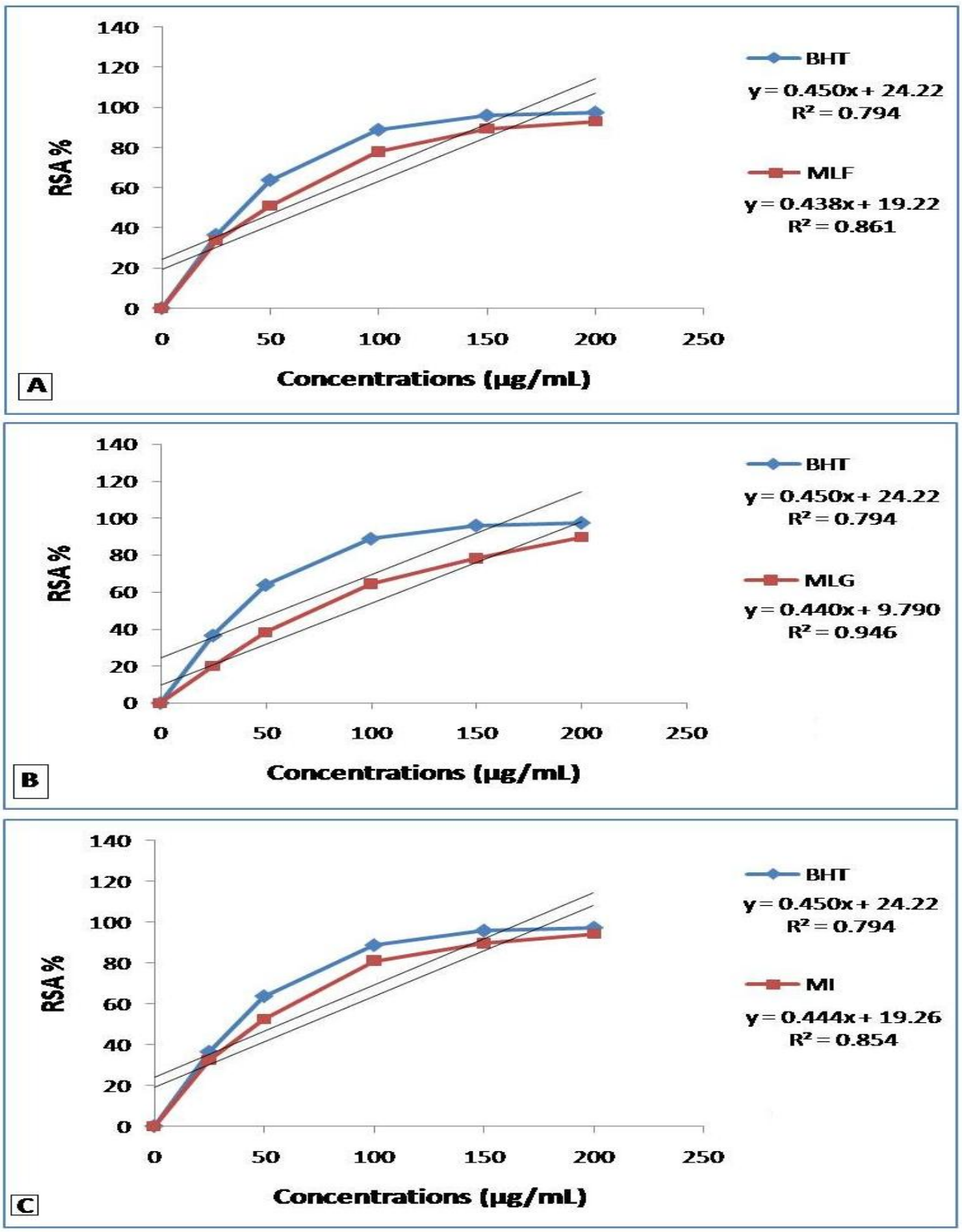


**Fig. 2.** Antioxidant activity of three different *Morus* sp. (MI, MLF, and MLG) aqueous extracts. The RSA % of the extracts was compared with standard BHT.

The leaves of Indian MI species was reported with strong free radical scavenging activity (Arabshahi-Delouee and Urooj, 2007). However, we found that the studied plant extracts possessed moderate antioxidant activity. At 200 µg/mL concentration, the RSA of the plant extracts were 93.07% by MLF, 89.48% by MLG, and 93.97% MI; whereas at the same concentration, the standard (BHT) showed 97.31% RSA (Fig. 2).

The $IC_{50}$ of MLF, MI, and MLG extracts were found 67.21, 69.23, and 92.74 µg/mL, respectively; in contrast, the $IC_{50}$ of BHT (standard) was 57.28 µg/mL. Higher the $IC_{50}$ means lower the radical scavenging activity. DPPH scavenging ability depends on the amount of antioxidants including flavonoids and phenolic compound in the extract.

However, the *Morus* genus is well-known for its prosperity in secondary metabolites including prenylated flavonoids, sterols, terpenes, anthocyanins, polyphenolic and isoprenoid-substituted phenolic compounds, phenyl bromides, arylbenzofurans, and stilbene derivatives which exhibited diverse biological activities like antibacterial, antifungal, antiviral, antinematodal, anticancer, antiplatelet, antioxidant, antiinflammatory, antiproliferative, cytotoxic, hypoglycemic, analgesic, cardio protective, and immune regulating activities (Lakshmi *et al.,* 2003). Subsequently, this study was supported by Lakshmi *et al.* (Lakshmi *et al.,* 2003) in term of antibacterial and antioxidant activity of the studied plant species from *Morus* genus.

**Conclusion**

Overall, all of the plant species have showed considerable antibacterial and antioxidant activity among which *M. indica* (MI) and *M. latifolia* (MLF) showed the highest activity respectively. The potent antibacterial and antioxidant properties may reflect to investigate phytochemicals in the three plant species.

**Conflict of interest**

The authors have no conflict of interest.

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