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# In Vitro Antimicrobial Activity Screening of *Maclura pomifera* Fruits Against Wide Range of Microorganisms

Kerem Canli[1*],Mustafa Eray Bozyel[2],Ergin Murat Altuner[3]
[1]*Dokuz Eylül University, Faculty Of Science, Department Of Biology, Buca, Izmir, Turkey*
[2]*Department Of Biology, Faculty Of Arts And Science, Çanakkale Onsekiz Mart University, Çanakkale, Turkey*
[3]*Kastamonu University, Faculty Of Science And Arts, Department Of Biology, Kastamonu, Turkey*
**Corresponding Author:biyoloji@gmail.com*

***Abstract:****The use of plants as medicine has been practiced from ancient time in World. In developing country, traditional herbs have critical significance for disease treatment and they aid to detect novel antibiotics.Maclurapomifera(Raf.) C.K.Schneid, commonly known as the Osage orange, is a tree of the Moraceae or mulberry family. There are antimicrobial researches about M. pomifera in the literature and results are promising.Therefore fruits of this medical plant investigated against 21 microorganisms by using disk diffusion method according to show its antimicrobial potential more clearly. A wide range of Gram positive and Gram negative bacteria and yeast were selected to test the antimicrobial effect of M. pomifera. Most of these strains are standard, some of the strains are food isolated and the rest are clinic isolated. These microbial species are Bacillus subtilis DSMZ 1971, Enterecoccusfaecalis ATCC 29212, Listeria monocytogenes ATCC 7644, Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, Enterobacter aerogenes ATCC 13048, Escherichia coli ATCC 25922, Pseudomonas aeruginosa DSMZ 50071, Pseudomonas fluorescens P1, Salmonella enteritidis ATCC 13075, Salmonella typhimurium SL1344, Candida albicans DSMZ 1386, Enterococcus durans, Enterecoccusfaecium, Listeria innocua, Klebsiella pneumoniae, Salmonella infantis, Salmonella kentucky, Staphylococcus aureus (CI), Escherichia coli (CI) and Proteus vulgaris (CI). The results were presented that M. pomiferafruits ethanol extract has antimicrobial activity against all tested microbial strains.*

***Keywords:*** *Maclurapomifera, antimicrobial activity, disc diffusion method,ethanol extract.*

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## I. Introduction

Plants have many bioactive components which have beneficial health effects. Pharmacological features of them haven't been completely determined and the antimicrobial potential and biochemical compositions of many plant species weren't analysed yet [1].

World Health Organization (WHO) has predicted increasing antimicrobial resistance as a major threat for the public health for the 21st century. In order to prevent spreading of antibiotic resistant infections, scientists have been conducting intensive researches to determine new antimicrobial agents. One way to prevent antibiotic resistance of microorganisms is by using new compounds that are not based on existing antimicrobial agents. [2-3].

In the last three decades, antimicrobial activity related experiment have been applied by using plant extracts [4]. Although the antimicrobial activity of many natural plant species were determined until today, the broad range antimicrobial activity of *M. pomifera*fruit haven't been analysed by disk diffusion method yet.

The purpose of present research was to detect the antimicrobial activity of *M. pomifera*fruits ethanol extract against 21 microorganisms by diskdiffusion method.

## II. Materials And Methods

**Plant sample**

*M. pomifera* (Raf.) C.K.Schneid, commonly known as the Osage orange, is a tree of the Moraceae or mulberry family. This tree is planted in parks and on roadsides for landscaping purposes. Other than its uses as hedge trees and hardwood, the use of the fruit as an insect repellent is perhaps the most attractive traditional usage. The distinctive fruit, from a multiple fruit family, and turns a bright yellow-green in the fall. Fruits of *M. pomifera* were collected from central park, which was also known as the Public Gardens in Canakkale/Turkey.

**Extraction procedure**

All *M. pomifera*fruit samples were dried after collection and the samples were ground by a mortar and a pestle. In order to extract active substances, ethanol (Sigma-Aldrich) was chosen as an extraction solvent. Ground samples were shaken in ethanol at 140 rpm for 2 days at room temperature [5-7].

All the extracts were filtered through Whatman No. 1 filter paper into evaporation flasks. The filtrate was evaporated by a rotary evaporator (HeidolphHei-Vap Value HL/HB-G1) at 45°C [8]. After evaporation the residues were collected and used to prepare 4, 8 and 12 mg extracts.

**Microorganisms**

A wide range of Gram positive and Gram negative bacteria and yeast were selected to test the antimicrobial effect of *M. pomifera*. The pathogenic microorganisms were chosen for the analyses on the basis of their significance because of potential for contamination of food and human infection. Most of these strains are standard, some of the strains are food isolated and the rest are clinic isolated.

*Bacillus subtilis* DSMZ 1971, *Enterecoccusfaecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Enterobacter aerogenes* ATCC 13048, *Escherichia col*i ATCC 25922, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075,*Salmonella typhimurium* SL1344, *Candida albicans* DSMZ 1386, *Enterococcus durans*, *Enterecoccusfaecium*, *Listeria innocua*, *Klebsiella pneumoniae*, *Salmonella infantis*, *Salmonella kentucky*, *Staphylococcus aureus* (CI), *Escherichia coli* (CI) and*Proteus vulgaris* (CI)were used in the study.

**Preparation of inoculum**

All bacterial strains were incubated at 37°C for 24 hours. But since the requirements for *C. albicans* is different, *C. albicans* was inoculated at 27°C for 48 hours. Inoculum were prepared by transferring morphologically similar colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland, thus standard inoculum is adjusted to contain approximately $10^8$cfu/mL for bacteria and $10^7$cfu/mL for C. albicans [9-11].

**Disk diffusion method**

Disk diffusion test was performed as described previously by Andrews [12]. The culture medium was poured into 120 mm sterile petri dish to give a mean depth of 4.0 mm ± 0.5 mm [13].

30 µL, 100 µL and 150 µL aliquots of each extract was applied on sterile paper disks of 6 mm diameter end up with sample on each disk. To get rid of any residual solvent which might interfere with the results, disks were left to dry overnight at 30°C in sterile conditions. The surface of the plates was inoculated using previously prepared inoculum containing saline suspension of microorganisms. Inoculated plates were then left to dry for 5 min at room temperature before applying the disks [14].

Disks were firmly applied to the surface of the plate which had an even contact with the agar. Plates were incubated and inhibition zone diameters were expressed in millimetres.

**Controls**

Empty sterile disks and extraction solvent (ethanol) loaded on sterile disks which were dried at sterile conditions to remove solvent as done in the study were used as negative controls.

**Statistics**

The statistical analysis was executed using a non-parametric method Kruskal-Wallis which is one-way analysis of variance with $p< 0.05$.

## III. Results And Discussion

Antimicrobial activity of *M. pomifera*fruits ethanol extract was analysed. In order to load extracts, empty sterile disks were used. These disks were applied on a Mueller Hinton Agar, after they were inoculated with microorganism. Inhibition zone was observed, when the extracts had activity against these microorganisms. The diameters of these zones were measured in millimetres as Table 1.

**Table 1.** Disk diffusion test results for M. pomifera (Inhibition zones in mm).

| | 30µL | 100µL | 150µL |
|---|---|---|---|
| *B. subtilis* DSMZ 1971 | - | - | - |
| *C. albicans*DSMZ 1386 | - | - | - |
| *E. aerogenes* ATCC 13048 | - | - | - |
| *E. durans* | 7 | 8 | 9 |
| *E. faecalis*ATCC 29212 | - | - | 7 |
| *E.faecium* | 9 | 10 | 11 |
| *E. coli* ATCC 25922 | - | - | - |
| *E. coli* (CI) | - | - | - |
| *K. pneumoniae* | 7 | 8 | 9 |
| *L. innocula* | 8 | 9 | 9 |
| *L. monocytogenes* ATCC 7644 | - | - | - |
| *P. aeruginosa*DSMZ 50071 | - | - | - |
| *P. fluorescens*P1 | 7 | 9 | 9 |
| *P. vulgaris*(CI) | 9 | 11 | 13 |
| *S. enteritidis* ATCC 13075 | - | - | - |
| *S. infantis* | - | - | - |
| *S. kentucky* | - | - | - |
| *S. typhimurium* SL 1344 | 7 | 8 | 9 |
| *S. aureus* ATCC 25923 | 8 | 8 | 8 |
| *S. aureus* (CI) | 10 | 11 | 13 |
| *S. epidermidis*DSMZ20044 | 8 | 9 | 10 |
| "-": No inhibition | | | |

In our study, *M. pomifera*fruits ethanol extract antimicrobial activity was determined against 21 microorganisms with disc diffusion method at 4 mg 8 mg and 12 mg. According to our result, *M. pomifera* has antimicrobial activity against*E. durans*(9 mm), *E. faecalis* ATCC 29212 (7 mm), *E. faecium*(11 mm), *K. pneumoniae* (9 mm), *L. innocula*(9 mm), *P. fluorescens* P1 (9 mm), *P. vulgaris*(CI) (13 mm), *S. typhimurium* SL 1344 (9 mm), *S. aureus* ATCC 25923 (8 mm) , *S. aureus* (CI) (13 mm) and *S. epidermidis* DSMZ 20044 (10 mm) at 12 mg. However there is no activity determined against *B. subtilis* DSMZ 1971, *C. albicans*DSMZ 1386, *E. aerogenes* ATCC 13048, *E. coli* ATCC 25922, *E. coli* (CI), *L. monocytogenes* ATCC 7644, *P. aeruginosa* DSMZ 50071, *S. enteritidis* ATCC 13075, *S. infantis* and *S. kentucky*.

When compare to gram negative and gram positive bacteria, gram negative bacteria have more resistance than gram positive bacteria against bioactive component [15, 16]. Therefore, much greater activity was obtained against gram positive bacteria in related research. Our experiment has similar results and most of the activity was observed against gram positive bacteria.

Leven et al. [17] found that *M. pomifera*has antimicrobial activity against *S. aureus* and *P. vulgaris*on the other hand *M. pomifera* shows no activity against *E.coli*, *P. aeriginosa* and *C. albicans*, which is in parallel to our results.

*E.faecium* has long been thought of as a harmless commensal of the mammalian GI tract. However, *E. faecium*has become an important cause of nosocomial infections. These infections are often difficult to treat owing to the resistance of *E. faecium*to a large number of antibiotics [18]. Mojab et al. [19] identified that methanol extract of *Thymus daenensis* caused 8 mm of inhibition zone against *E. faecium* whereas Ilhan et al. [20] identified that methanol extract of *Palustriellacommutata* observed no activity. In our study we observed 11 mm zone for 12 mg of *M. pomifera* fruits.

*S. aureus* is known one of the common nosocomial infections in medical intensive care units [21]. Several researchers study antimicrobial activity of some plant extracts on *S. aureus* strains. In our study we observed 13 mm zone against clinic isolated *S. aureus*. *M. pomifera*fruits are highlyactive against *S. aureus* when compared to some other higher plants [22].

## IV. Conclusion

As a result, it can be concluded that there is clear antimicrobial activity of *M. pomifera* fruits against 11 of the strains tested. The results of our study clearly presents that *M. pomifera* fruits could have a possible medicinal uses especially against *P. vulgaris*, *S. aureus* and *E. faecium*.But further researches are needed to be conducted in order to analyse the active substances and their activity mechanisms in details.

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