A Comparison of Biomechanical Properties and Antimicrobial Activities of Commonly Used Antibiotics with *Maclura pomifera* Extract Impregnated Bone Cements, An Experimental Study

Mehmet Nuri Konya¹*, Petek Konya², Neşe Demirtürk² and Beytullah Kenar³

¹Department of Orthopaedics and Traumatology, Afyon Kocatepe University, Afyon, Turkey
²Department of Infectious Disease, Afyon Kocatepe University, Afyon, Turkey
³Department of Veterinary Faculty, Afyon Kocatepe University, Afyon, Turkey

*Corresponding Author: *Mehmet Nuri Konya, Department of Orthopaedics and Traumatology, Afyon Kocatepe University, Afyon, Turkey.

Received: April 03, 2017; Published: April 29, 2017

Abstract

**Purpose:** *Maclura pomifera* also known as Osage orange is a small deciduous tree. *Maclura* species extracts have different activities. Antitumoral, Anti-inflammatory, Anti-microbial, Anti-malarial activities were studied previously by various authors. Aim of this study is to evaluate and compare the chemical composition, biomechanical and microbiological effect of *Maclura pomifera* extract augmented bone cements with different antibiotics; teicoplanin, daptomycin, rifampicin, gentamicin.

**Methods:** Approximately 100 cc milky juice extract was harvested and divided into 4 bottle for Gas Chromatograph Mass Spectrum (GC-MS) analysis, Total Antioxidant Activity and Total Phenolic Activity analysis, cytotoxity test on osteoblast cells. Microbiological tests were evaluated against *Staph. aureus, P. aeruginosa, C. albicans* and *E. coli* and also compared with Daptomycin, Rifampicin, Gentamicin, Teicoplanin impregnated bone cement.

**Results:** According to our Gas Chromatography (GC) and Mass Spectrum (MS) analysis test we identified the ϒ-palmitolakton (23.73% relative percent (RL)), lanosterol (22.47%) and Palmitic acid (9.47%). Total antioxidant analysis calculated with DPPH Method for *M. pomifera* 319.86 ± 2.58 mg Ascorbic acid/100 gr. Total phenol activity was 469.32 ± 10.77 Gallic acid equivalent. *M. pomifera* extract had no microbiological activities on *Staph. aureus, P. aeruginosa, C. albicans* and *E. coli*. In scanning electron microscopy, *M. pomifera* had Calcium more than four times other antibiotic impregnated bone cements.

**Conclusions:** As a results of this study, *Maclura pomifera* extract have anti phenolic and antioxidant effect. In previous studies, reported in text, have antimicrobial effect we couldn’t find any antimicrobial and anti-mitotic effect. In Biomechanical tests Teicoplanin and Gentamicin have found stronger than Rifampicin and MP extract embedded Bone Cements.

**Keywords:** Microscopy; Biomechanic, *Maclura pomifera*, Antimicrobial, Scanning Electron Microscopy, Antioxidant

Abbreviations

*M. pomifera*: *Maclura Pomifera*; PMMA: Polymethylmethacrylate; MMA: Methyl Methacrylate; GC-MS: Gas Chromatography- Mass Spectrophotometry; °C: Centigrade; DPPH: 2,2-diphenyl-1-picrylhydrazyl; *E. coli*: *Escherichia coli*; *C. albicans*: *Candida albicans*; SEM: Scanning Electron Microscopy; XRD: X-ray Diffraction; Cu: Copper; Mo: Molybdenum; ASTM: American Society for Testing and Materials Interna-


Introduction

*Maclura pomifera* also known as Osage orange [1] is a small deciduous tree, may be able to reach 8 - 15 metres. *M. pomifera* from a multiple fruit family and it's shape generally spherical and generally reach 10 to 13 centimetres (4 - 5 inch) in diameter, with a roughened and tuberculated surface. The fruit compounds of numerous small drupes in which the ovaries have grown together. Each small seeds are oblong, compressed and rounded. Fruit contains a milky juice, oozes when the is damaged or cut (Figure 1).

Figure 1: *Maclura Pomifera* Fruit.

Maclura species extracts have different activities. Antitumoral [2], Anti-inflammatory [3], Anti-microbial [4] and Anti-malarial [5] activities were studied previously by various authors. The ingredients of *Maclura pomifera* generally contains lectins, Fatty acid methylesters, Triacyl glycerol and tocopherol compositions [6].

Total joint arthroplasty is a well known operation methods in Orthopedics and the number of total hip and knee arthroplasty operations in 2030 is estimated to be around 2 million [7]. Failure rates are increasing in arthroplasty and approximately %1 percent in year. Bone cement binding prosthesis and subchondral bone, is a very important component in arthroplasties. Both one stage and two stage revision, bone cement is an essential component of the operations.

Bone Cement Properties

Bone cement consist of two major component Polymethylmethacrylate (PMMA) is a copolymer and methyl methacrylate (MMA) monomer. Polymerization consist of four different stage; mixing the component, waiting, working and hardening. Cementing technique is important and may cause aseptic loosening and one of the major cause of the implant failure. Hailer, et al. [8] reported 170.000 Total Hip Arthroplasty in a Swedish study, comparing uncemented and cemented arthroplasty in which increased risk in acetabular component in

uncemented cups and more revision rates were seen. Li [9] reported in meta-analysis, cemented fixation technique gets better functional results in hemiarthroplasty.

In total joint replacement surgery, deep periprosthetic infection one of the major cause of the revision nearly 15% in total hip arthroplasty [10] and 17% in total knee arthroplasties [11]. Beside the definitive treatment, using low dose antibiotic in bone cement can be a popular method for decreasing infection rates without decreasing compressing strength of bone cement [7].

In this study, our aim is to evaluate and compare of chemical component, biomechanical and microbiological effect of *Maclura pomifera* extract augmented bone cements with different antibiotics; teicoplanin, daptomycin, rifampicin, gentamicin.

**Materials and Methods**

**Material preparations**

*M. pomifera* fruits were collected from one tree. Each fruit was 0.5 kg and 8 mm diameter. Authenticated voucher specimen encoded GUE2234.

**Preparation of plant extracts**

All the fruits were washed in water, rinsed and dried at room temperature. Green shells of fruit was peeled and cut into small slices and meanwhile the milky juice was squeezed. Approximately 100 cc milky juice extract was harvested and divided into 4 bottle. One bottle was sent to Anadolu University Medicinal Plants, Drugs and Scientific Research Center for Gas Chromatograph Mass Spectrum(GC-MS) analysis. Another bottle was sent to Tubitak Marmara Research Center Food Institute for Total Antioxidant Activity and Total Phenolic Activity analysis. The other bottle was sterilized under Gama Radiation and used for microbiological tests and augmented with bone cement.

**GC-MS Analysis**

**Methods:** Weighing 5 mg extracted plant was stirred with 10 ml of hexane for 10 minutes in a seperating funnel then taken into Erlenmeyer, mouth closed, extraction continued for 15 minutes in an ultrasonic water bath. Erlenmeyer waited one night under +4°C and successive day haksan phase was seperated and concentrated under Nitrogen gas. GC-MS analysis was made using following conditions.

The System was HP 6890 Series Gas Chromatograph 5973 Mass Selective Deductor System, The Column was Agilent HP Innowax (60 mm Length, 0.25 mm internal diameter, 0.25μ film thickness. Injection Heat: 250°C, Ion Source Heat: 250°C, Ionisation modulus: Hand, Mass Spectrum 35 - 675 m/z, Electron energy: 70 ev, Supported gas: Helium

**Total Antioxidant Activity Analysis**

Total Antioxidant Activity Analysis was calculated with DPPH assay methods [12].

**Total Phenol Analysis**

Total phenol Analysis calculated with Folin Ciocalteu Methods [13].

**Microbiological examination of Maclura Pomifera**

To investigate the microbiological effect of *M. pomifera* extract was sterilized under Gamma radiation exposure. After sterilization 2 cc of (Tryptic Soy Broth -TSB) bouyon was mixed with 2 cc MP extract and diluted the ratio of 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64 in Microbiology Department Laboratories.

Media prepared in the 0.5 Mc Farland blurring with standard Staph. aureus ATCC-25923, P. aurogines ATCC-254992, E. coli ATCC-254986, C. albicans ATCC-90028 strains were cultivated 0.05 cc. and checked after 2 days.

Same microbiological agents were cultivated into bloody agar. 160 mg Gentamicin (Gentamed 80 mg sol Kocak Farma İlaç, Istanbul, Turkey), 800 (2X400) mg Daptomycin (Cubicin 400 mg IV flc, Novartis İlaç, Istanbul, Turkey) and 500 mg Rifampicin (Rifetem 250 mg IV sol. I.E Ulagay İlaç, Istanbul, Turkey) ve 2 cc maclura pomifera extract was mixed with 80 cc Bone cement (Cement Oliga 1 Bone Cement G21 srL SanPossidonio Italy) respectively in sterile condition. After 5 and 10 minute 2 cm³ ovoid shape antibiotic embedded bone cement were cultivated into Mueller-Hinton and bloody agar for eliminating heat effect on results. The results were checked after one and two day later.

XRD and Electron Microscopy Examination

Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD) techniques were used for investigating the effect of different antibiotics to bone cement in atomic level. The samples were examined in Application and Research Center with LEO1430VP Scanning Electron Microscopy which includes secondary electron - EDX Energy Dispersive X-ray, backscattered electron Spectroscopy detector. Samples were covered for examining.

XRD device (BRUKER D8 Advance) has Copper (Cu) and Molybdenum (Mo) X Ray tubes. Phase transformations can be performed at high temperatures up to 1500 degrees because of the oven.

Biomechanical Analysis

All of the cement specimens were prepared by hand mixing techniques and molded. The liquid and powder components were mixed using a sterile spatula and bowl mixing time of less than 3 min. Mixing and molding was made under 220C and 30% relative humidity. According to American Society for Testing and Materials International (ASTM) F451-99a Antibiotic impregnated cement was put into 6 mm diameter and 12 mm Height glass tube. Testing machine was (Microcomputer Control Electronic Universal testing Machine Mitech, MDW-20, Co, Ltd, China) was used for Compression forces (Figure 5).

Results and Discussion

GC-MS Analysis

Gas Chromatography(GC) and Mass Spectrum(MS) analysis , an effective combination for chemical analysis GC is a well described confirmation test [15]. GC analysis separates samples' components. GC devices vaporizes the sample ,separate and analysis different components. All components produces specific spectral peaks.The length of this peaks are measured. According to our test result we identified the Yi-palmitolakton (23.73% relative percent (RL)), lanosterol(22.47%) and palmititik asit (9.47%). The other components and peak levels shown in Table 1 and Figure 2.

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Component</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.23</td>
<td>Unidentified</td>
<td>1.94</td>
</tr>
<tr>
<td>7.69</td>
<td>Karyofilen</td>
<td>1.28</td>
</tr>
<tr>
<td>16.56</td>
<td>Unidentified</td>
<td>2.42</td>
</tr>
<tr>
<td>22.71</td>
<td>Etil stalat</td>
<td>3.01</td>
</tr>
<tr>
<td>26.25</td>
<td>Etil linoleat</td>
<td>1.55</td>
</tr>
<tr>
<td>30.77</td>
<td>Dibutil flalat</td>
<td>4.99</td>
</tr>
<tr>
<td>35.00</td>
<td>Yi-palmitolaktan</td>
<td>23.73</td>
</tr>
<tr>
<td>36.36</td>
<td>Palmitic acid</td>
<td>9.47</td>
</tr>
<tr>
<td>42.65</td>
<td>Skualen</td>
<td>2.21</td>
</tr>
<tr>
<td>46.28</td>
<td>Oleic acid</td>
<td>2.63</td>
</tr>
<tr>
<td>49.29</td>
<td>Linoleic acid</td>
<td>6.69</td>
</tr>
<tr>
<td>156.09</td>
<td>Lanosterol</td>
<td>22.47</td>
</tr>
<tr>
<td>180.78</td>
<td>Lanosterol acetat</td>
<td>8.84</td>
</tr>
</tbody>
</table>

Table 1: Components of Maclura Pomifera Extract and Retention Times.
Total Antioxidant Activity and Phenol Analysis:

The antioxidant activity analysis was calculated with DPPH analysis method.

DPPH [12] (1,1-diphenyl-2-picrylhydrazyl) is an obtainable commercially stable organic nitrogen radical, have maximum absorbance in 515 nm. Displacing a free electron in the molecule causes the formation of a violet color. DPPH solution when mixed with a substance which able to give the a hydrogen atom (Antioxidant), a dark violet color loss and reduced form can be occur. DPPH assay proposed the antioxidant capacity measured with amperometric reduction with carbon electrode. This method widely used by researchers to determine the antioxidant activity of the food extracts. It is a simple and fast technique. In our study, we calculated total antioxidant analysis for M. pomifera 319.86 ± 2.58 mg Ascorbic acid/100 gr.

Folin-Ciocalteu (FC) method [15] was first described for protein analyzing. FC method is based on the electron transfer molybdenum phenolic compounds and other reducing compounds. Blue colored complex formation is determined spectrophotometrically at 750 - 765 nm. Standard compound gallic acid as often used and results gallic acid equivalent (mg/L) are given. In our study, M. pomifera total phenol activity was 469.32 ± 10.77.

This result show us M. pomifera extract has strong antioxidant and phenolic activities.

Microbiological Results of Maclura Pomifera

In our study; we studied 3 different bacterial agent (Staph. aureus ATCC-25923, P. aeruginosa ATCC-254992, E. coli-254986) and C. albicans-90028 strains. Microdilution method was employed for antimicrobial and antimicotik activity test. 2 cc of (Trptic Soy Broth-TSB) buyyon was mixed with 2 cc MP extract and diluated the ratio of 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64. After one day in all tubes we couldn’t find microbial effect of M. pomifera.

Antibiotic embedded bone cements, ovoid 2 cm³, were inoculated into all bloody agar wells. Microplates were incubated at 35oC for 24 hour and 48 hour. These results show that, M. pomifera extract has no antimicrobial effect. Teicoplanin, Daptomycin Rifampicin and Gentamycin had (+++) antimicrobial effect only on Staph. aureus. Gentamicin affected E. coli and P. aeruginosa (Figure 3). These results shows us M. pomifera had no effect on microbiological agents but daptomycin embedded bone cement is a well treatment alternative for
the patient, infected with *Staph. aureus*. Clinical uses of Daptomycin Impregnated Bone cement reported in few studies about prosthesis infection and osteomyelitis [16].

Results of XRD and Scanning Electron Microscopy Examination:

XRD system; [14] Qualitative and semi-quantitative phase of inorganic solid materials (the mineralogical) content semiquantitative amorphous phase, the amount of the crystal size, mineralogical changes occurring in the structure of the material due to temperature, mineralogical content of the thin film coating on the substrate, the thickness of the generated or spontaneous knowing during production texture, remains located during use, which could cause distortion stresses in the structure of the material can be detected.

X-Ray diffraction methods commonly used for recognizing unknown crystalline materials. This method is commonly used in geology, material science and engineering and also it can be used for. To assess the structure of material, measuring the sample purity, to determine the crystal and amorphous structures.

Scanning Electron Microscopy and XRD gave us information how antibiotics change cement microstructure and atomic composition of the bone cement. Each antibiotic cement combination was investigated with SEM imaging (Figure 4). Only cements’ atomic composition mostly consist of Carbon (C) 55.41%, Oxygen (O) 31.29%, Sulfur (S) 1.16%, Chlorine (Cl) 4.49%, and Barium (Ba) was 7.20%. Targocid cement combination mostly consist of Carbon (C) 45.37%, Oxygen (O) 47.58%, Sulfur (S) 1.03%, Chlorine (Cl) 0.23%, Calcium 0.20% and Barium (Ba) was 5.59%. In Gentamicin -cement combination C rate was 47.58, O rate 46.82%, S 0.87%, Ca 0.34% and Ba was 4.35%. *M. pomifera*- Cement combination C rate 43.67%, O rate was 45.33%, S 1.03%, Ca 4.79% and Ba 5.17%. In Rifampicin- Cement Combination C rate was calculated as 49.99%, O 43.28%, S 0.94%, Cl 0.16%, Ca 1.02 %, Ba rate was 4.61%. Daptomycin- Cement combination atomic distribution was C 43.75, O 49.18%, S 0.98%, Cl 0.19%, Ca 1.98%, and Ba was 4.26 (Table 2). According to these results antibiotics can be change the atomic composition of bone cement. Ca rates of the *M. pomifera* extract- Cement group was higher than the other groups (Figure 4).

Results of Biomechanical Analysis

All of the cements were testing until 2000 N forces successively. For 6 different cement with/without antibiotic combination 6 specimens were tested. Mean stress/strain ratio were reported in Table 3 and Figure 5-7.

<table>
<thead>
<tr>
<th>Bone Cement Formulation</th>
<th>Compressive Modulus (MPa)</th>
<th>2% Offset Yield Strength (MPa)</th>
<th>Compressive Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cement</td>
<td>22 ± 1.5</td>
<td>102.51 ± 2.6</td>
<td>103.36 ± 1.8</td>
</tr>
<tr>
<td>DAPTO</td>
<td>17.4 ± 0.9</td>
<td>85.49 ± 1.5</td>
<td>86.94 ± 1.5</td>
</tr>
<tr>
<td>GENTA</td>
<td>19 ± 1.6</td>
<td>95.85 ± 8.4</td>
<td>97.72 ± 9.3</td>
</tr>
<tr>
<td>MP</td>
<td>10.6 ± 2.3</td>
<td>44.39 ± 12.2</td>
<td>44.92 ± 11.8</td>
</tr>
<tr>
<td>RIF</td>
<td>4.3 ± 1.2</td>
<td>16.85 ± 2</td>
<td>17.21 ± 1.9</td>
</tr>
<tr>
<td>T</td>
<td>26.6 ± 0.6</td>
<td>112.05 ± 1.5</td>
<td>113.46 ± 1.4</td>
</tr>
</tbody>
</table>

Table 3: Comparative Biomechanical Results of Antibiotic Embedded Bone Cements.
Discussion

Saloua, et al. [17] reported the containing material composition divided into 4 four groups. Fatty acid composition calculated on the basis of the peak area in sample. In this study, most common composition was linoleic acid (%76.2), palmitic acid (6.8%) and stearic acid...
In Orhan., et al. study [6] same compositions were reported. In our study, the most common components were Υ-palmitolakton and lanosterol.

The microbial effect of *M. pomifera* first described by Mahmoud Z. in 1981 [18]. In Mahmood’s study, a prenylated isoflavones showed strong antibacterial and antifungal activities against *E. coli* (2 μg/ml), *K. pneumoniae* (4 μg/ml), *E. faecalis* (0.5 μg/ml) and *C. albicans* (1 μg/ml). In another study, Salua reported [4] five different strains, and found the highest activities were against *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter faecalis* and *C. albicans*. In this study, we aimed to analyse how antibiotics impact on bone cement microstructure.

Bone cement consist of two different components powder component and liquid component. Powder component includes poly (methacrylic acid methyl ester), Barium sulphate and Dibenzoyl peroxide, Liquid components includes Methacrylic acid methyl ester stabilized with 1.4- Dihydroxybenzene and benzenamine, N,N,4-trimethyl. One package cement consist of 35.5 ± 1.8g PMMA, 0.5 ± 0.1g BPO and 4.0 ± 0.2 gr Barium Sulphate. Usually Powder/liquid rate is 2/1. Methyl methacrylate a monomer is the most important component. Another addictive is dimethyl-para-toluidine (DMPt). Hydroquinone can be added for lengthening storage time and prevent premature polymerization due to light and heat [20]. Benzoil Peroksit (BPO) is a starter co-polymer in powder component and Zirconium dioxide or barium sulphate radio opacifying acts. Neither antibiotics nor radio-opacifying substance has no role in chemical reactions. After the interactions between BPO and DMPt are chemical reactions starts and free radicals occur. Oliga-1 40 mg Bone Cement© was used for this study. We evaluate powder form, mixed powder and liquid component, Daptomycin, Rifampicin, *M. pomifera*, vancomycin, gentamycin impregnated bone cement in XRD imaging. Amorphous and crystalline structure and their interactions were examined.

The X-ray diffractograms are shown in Figure 4. All the materials exhibited typical peaks for BaSO₄. According to 2 Theta (Coupled TwoTheta/Theta) analysis Major peak of Powder PMMA was nearly 1000 and this shows amorph morphology. After mixing liquid part amorph structure change to crystallin structure and peaks were diminished. Each antibiotics showed different peaks but they are unable to change major differences on bone cement.

According to biomechanical results; the strongest combination of bone cement with/without antibiotic is Targocid- Bone Cement and the weakest combination is Rifampicin Bone cement combination was found. Several studies about bone cement biomechanical tests were evaluated. Antibiotic Loaded Bone Cement generally used in Arthroplasty operations and osteomyelitis treatment. Infectious organisms such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* have been developing resistance generally used Antibiotic Loading Bone Cements (ALBC) such as gentamycin, tobramycin and vancomycin [21]. Daptomycin improved for an alternative antibiotics to common antibiotics such as tobramycin, vancomycin and gentamicin [22]. Dretler, et al. [23] reported successful MRSA Knee Infection Treated Successfully with Daptomycin After Two Failed Prolonged High-Dose Courses of Vancomycin. When comparing Vancomycin and Daptomycin elusion from PMMA, similar rate were found [24].

Kaplan., et al. [25] reported antimicrobial effect and elusion of daptomycin and tobramycin Loaded Bone cement and found 2 g of daptomycin and 3.6 g of tobramycin per 40-g packet of cement should be used to promote daptomycin elution without effecting PMMA mechanical properties. As a biomechanical testing of ALBC we couldn’t find any study to compare Teikoplanin, Rifampicin, Daptomycin, Gentamycin and *Maclura pomifera* extract and PMMA. Teikoplanin and Gentamycin found the strongest ALBC group.

**Limitation of this study**

Limitation of this study was only compressive test were applied fatigue test. Elusion test of ALBC couldn’t applied because of using different type of antibiotics and Gr (+) and Gr (-) microorganism.

Conclusion

As a result of this study, *Maclura pomifera* extract have anti phenolic and antioxidant effect. In previous studies, reported in text, have antimicrobial effect we couldn’t find any antimicrobial and anti-mitotic effect. In this study, we want to evaluate and compare the properties of *M. pomifera* extract impregnated Bone Cement with commonly used Antibiotics. Further studies should be necessary for improving new additive for bone cements.

Acknowledgements

Author thanks to Mr Ozgur Verim for biomechanical tests.

Conflict of Interest

This project was supported by Afyon Kocatepe University, Scientific Research Project Coordination Unit project number was 15HIZ-DES97.

Bibliography


