

Journal of Microbiology and Antimicrobials

Volume 8 Number 6, December 2016
ISSN 2141-2308



*Academic
Journals*

ABOUT JMA

The Journal of Microbiology and Antimicrobials (JMA) (ISSN 2141-2308) is published monthly (one volume per year) by Academic Journals.

Journal of Microbiology and Antimicrobials (JMA), is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as Disorders of the immune system, vaccines and antimicrobial drugs, Microbial Metabolism, Protozoology etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMA are peer-reviewed.

Contact Us

Editorial Office: jma@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: <http://academicjournals.org/JMA>

Submit manuscript online <http://ms.academicjournals.me/>

Editors

Ass. Prof. Aamer Ikram

*Department of Microbiology,
Armed Forces Institute of Pathology,
Pakistan*

Prof. Wang Jianhua

*Gene Engineering Lab
Feed Research Institute,
Chinese Academy of Agricultural Sciences
China*

Dr. Mohd. Shahid

*Antimicrobial Agents & Drug Section
Department of Medical Microbiology
Jawaharlal Nehru Medical College & Hospital
Aligarh Muslim University
India*

Dr. Anil Vyas

*Microbial Biotechnology & Biofertilizer Lab.
Department of Botany
J.N.V.University
India*

Dr. (Mrs.) Amita Jain

*Dept. of Microbiology
King George Medical University,
India*

Dr. Eduardo Mere

*Department of Biochemistry
University Federal of Rio de Janeiro,
Brazil*

Dr. Shwikar Mahmoud Abdel Salam

*Department of microbiology
Faculty of Medicine
Alexandria University
Egypt.*

Dr. Gideon Mutie Kikui

*Institute of Tropical Medicine and Infectious Diseases
Jomo Kentatta University of Agriculture and Technology
Kenya.*

Editorial Board Members

Dr. Manal El Said El Sayed

*Bilharz Research Institute (TBRI)
Ministry of Scientific Research
Egypt.*

Dr. Amber Farooqui

*Sardinian Research and Development (SARD)
Porto Conte Research Institute
Alghero,
Italy.*

Dr. Chang-Gu Hyun

*Laboratory of Bioresources
Jeju Biodiversity Research Institute (JBRI)
Jeju Hi-Tech Industry Development Institute (HiDI)
Korea.*

Dr. Vasant P. Baradkar

*Department of Microbiology
Government Medical College
Aurangabad,
India.*

Prof. Omar Abd El-Fattah Mohamed Fathalla

*Medicinal Chemistry Department
National Research Centre
Dokki,
Egypt.*

Dr. Amber Farooqui

*Dept. di Scienze Biomediche
Universita di Sassari
Italy.*

Dr. Kosta V. Kostov

*Military Medical Academy
Department of Pulmonology
Bulgaria.*

Dr. Antonio Rivera

*Benemérita Universidad Autónoma de Puebla
Puebla,
Mexico.*

Dr. Mohammad Rahbar

*Department of Microbiology
Iranian Reference Health Laboratory
Iran.*

Dr. Abd El-Latif Hesham

*Genetics Department
Faculty of Agriculture
Assiut University
Egypt.*

Dr. Samuel Sunday Taiwo

*Department of Medical Microbiology and Parasitology
College of Health Sciences
Nigeria.*

Dr. Anil Vyas

*J.N.V. University
Jodhpur
India.*

Dr. Najla Dar-Odeh

*University of Jordan
Jordan.*

Prof. Asiye Meric

*Anadolu University
Faculty of Pharmacy
Department of Pharmacy and Chemistry
Turkey.*

Prof. Salah M. Azwai

*AlFateh University
Libya.*

Prof. Abdel Salam Ahmed

*Department of Microbiology
Faculty of Medicine
Alexandria University
Egypt.*

Dr. Kuldeep Kumar Shivalya

*Indian Veterinary Research Institute
Izatnagar,
India.*

Prof. Viroj Wiwanitkit

*Hainan Medical University
China.*

Dr. Hafizah Chenia

*School of Biochemistry
University of KwaZulu-Natal
Durban,
South Africa.*

Dr. Gholamreza Salehi Jouzani

*Microbial Biotechnology and Biosafety Department
Agricultural Biotechnology Research Institute of Iran
(ABRII)
Iran.*

Dr. Wilson Parawira

*Institute of Food, Nutrition and Family Sciences
University of Zimbabwe
Zimbabwe.*

Dr. Subhash C. Mandal

*Division of Pharmacognosy
Department of Pharmaceutical Technology
Jadavpur University
India.*

Dr. Adesemoye A. O.

*Department of Plant Pathology
Centre for Integrated Plant Systems
Michigan State University
USA.*

Dr. Giselli Fernandes Asensi

*Universidade Federal do Rio de Janeiro
Brazil.*

Prof. Hongyue Dang

*Centre for Bioengineering and Biotechnology
China University of Petroleum
China.*

Dr. Babu Joseph

*Acharya''s Bangalore School
India.*

Dr. Aamer Ali Shah

*Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad,
Pakistan.*

Dr. Tadele Tolosa

*Jimma University
College of Agriculture and Veterinary Medicine
Ethiopia.*

Dr. Urveshkumar D. Patel

*Department of Pharmacology and Toxicology
Veterinary College
Anand Agricultural University
India.*

Dr. Saeed Zaker Bostanabad

*Islamic Azad University
Iran.*

Dr. Rakesh Kumar Singh

*Florida State University
College of Medicine
USA.*

Assoc. Prof. Vintila Iuliana

*Dunarea de Jos University
Romania.*

Dr. Saganuwan Alhaji Saganuwan

*University of Agriculture Makurdi
Dept. of Physiology, Pharmacology and Biochemistry
Makurdi,
Nigeria.*

Dr. Eskild Petersen

*Dept. of Infectious Diseases
Aarhus University Hospital
Norbrogade,
Denmark.*

Dr. Elpis Giantsou

*Cambridge University Hospitals
UK.*

Ass Prof. Emanu Getu Degaga

*Addis Ababa University
Ethiopia.*

Dr. Subramanian Kaviarasan

*Dept of Molecular Medicine
University Malaya
Kuala Lumpur,
Malaysia.*

Ass Prof. Nongyao Kasatpibal

*Faculty of Nursing,
Chiang Mai University
Thailand.*

Dr. Praveen Rishi

*Panjab University
India.*

Prof. Zeinab Nabil Ahmed Said

*Microbiology & Immunology Department
Faculty of Medicine
Al-Azhar University
Egypt.*

Ass. Prof. Abdulaziz Zorgani

*Medical School
Edinburgh University
Edinburgh,
UK.*

Dr. Adenike Adedayo Ogunshe

*University of Ibadan
Nigeria.*

Prof. Itzhak Brook

*Department of Pediatrics and Medicine
Georgetown University
Washington, DC
USA.*

Dr. Eduardo Mere Del Aguila

*Universidade Federal do Rio de Janeiro
Rio de Janeiro
Brazil.*

Dr. Md. Shah Alam Sarker

*School Agric and Rural Development
Bangladesh Open University
Bangladesh.*

Dr. Ramnik Singh

*Khalsa College of Pharmacy
Amritsar,
India.*

Prof. Amita Jain

*Chhatrapati Shahuji Maharaj (CSM) Medical University
Lucknow,
India.*

Prof. Yulong Yin

*Institute of Subtropical Agriculture
The Chinese Academy of Science
China.*

Prof. Mohan Karuppaiyl

*School of Life Sciences
Swami Ramanand Teerth Marathwada (SRTM) University
Maharashtra,
India.*

Dr. Sunil Gupta

*National Centre for Disease Control
India.*

Dr. Elpis Giantsou

*Cambridge University Hospitals
England.*

Dr. Mustafa Gul

*Kahramanmaras Sutcuimam University
Faculty of Medicine
Department of Microbiology and Clinical Microbiology
Turkey.*

Dr. Nese Karaaslan Biyikli

*Anadolu Medical Center
Turkey.*

Dr. Zafar Iqbal

*Dept Plant Pathology
University College of Agriculture
András Fodor
Pakistan.*

Ass Prof. Habil András Fodor

*Department of Plant Protection
Georgikon Faculty
Pannonia University
Hungary.*

Dr. Neelam Mewari

*Department of Botany
University of Rajasthan
Rajasthan,
India.*

Dr. Elpis Giantsou

*Cambridge University Hospitals
UK.*

Dr. Sanjib Bhattacharya

*Bengal School of Technology
India.*

Dr. Habibur Rahman

*PSG Colege of Pharmacy
India.*

Md. Elisa Bassi

*Department of Dermatology
Delmati Hospital
Italy.*

Iheanyi Omezuruike Okonko

*University of Ibadan
Nigeria.*

Ass. Prof. Weihua Chu

*Dept. of Microbiology
School of Life Science & Technology
China Pharmaceutical University
China.*

Dr. Mat Yamage

*World Organization for Animal Health (OIE)
Japan.*

Dr. Ali Abbas Qazilbash

*United Nations Industrial Development Organization
Pakistan.*

Dr. Kulachart Jangpatarapongsa

*Department of Clinical Microbiology
Mahidol University
Thailand.*

Dr. Nasrin Ghasemi

*Research and Clinical Centre for Infertility
Yazd Shahid Sadoughi University of Medical Sciences
Yazd,
Iran.*

Dr. Johnson Afonne

*Department of Pharmacology
College of Health Sciences
Nnamdi Azikiwe University
Nigeria.*

Dr. Branka Vasiljevic

*Institute of Molecular Genetics and Genetic Engineering
Serbia.*

Dr. Mehmet Ulug

*BSK Anadolu Hospital
Infectious Diseases and Clinic Microbiology
Turkey.*

Dr Ömür Baysal

*Turkish Ministry of Agriculture and Rural Affairs
West Mediterranean Agricultural Research Institute
(BATEM)
Plant Pathology and Molecular Biology Departments
Antalya,
Turkey.*

Dr. Pooja Jain

*University of California
Department of Pathology
Medical Sciences
Irvine, CA
USA.*

Dr. Chellaiah Edward Raja

*Cancer Biology Unit
School of Biological Sciences
M.K. University
India.*

Prof. Zeinab Nabil Ahmed Said

*Faculty of Medicine (for girls)
Al-Azhar University
Egypt.*

Prof. Manal Mohammad Baddour

*Alexandria University
Faculty of Medicine
Microbiology and Immunology Dept.
Azarita,
Egypt.*

Dr. Bechan Sharma

*Department of Biochemistry
Centre for Biotechnology
University of Allahabad
Allahabad,
India.*

Ass. Prof. Ravichandran Veerasamy

*Faculty of Pharmacy
AIMST University
Malaysia*

Dr. Mohammad Ibrahim

*Programa de Pós-Graduação em Bioquímica Toxicológica
Centro de Ciências Naturais e Exatas
Universidade Federal de Santa Maria
Brazil.*

Dr. Sudheer Bobba

*Department of Drug Metabolism and Pharmacokinetics
Covance Laboratories
USA.*

Dr. Kannan Alpadi

*Department of Molecular Biology and Biochemistry
Baylor College of Medicine
USA.*

Dr. Shaohua Chen

*Department of Plant Pathology
South China Agricultural University
Guangzhou,
China.*

Dr. Prasun Kumar

*Department of Microbial Biotechnology and Genomics
CSIR-Institute of Genomics and Integrative Biology
India.*

ARTICLE

- Antimicrobial analysis of copaiba oil extract from *Passiflora cincinnata* and endodontic substances** **34**
Lucinea Barbosa de Oliveira Santos, Caroline de Souza Leitao, Amaro de Mendonça Cavalcante, Marcos Aurelio Bomfim da Silva, Zenaldo Porfirio, Antonio Euzebio Goulart Santana

Full Length Research Paper

Antimicrobial analysis of copaiba oil extract from *Passiflora cincinnata* and endodontic substances

Lucinea Barbosa de Oliveira Santos¹, Caroline de Souza Leitao¹, Amaro de Mendonça Cavalcante¹, Marcos Aurelio Bomfim da Silva¹, Zenaldo Porfirio², Antonio Euzebio Goulart Santana³

Department of Restorative Dentistry, Faculty of Dentistry, UFAL -Federal University of Alagoas, Maceió, AL, Brazil.

Department of Microbiology, university of Health Science of Alagoas, Maceio, Alagoas, Brazil

Institute of Chemistry and Biotechnology, UFAL-Federal University of Alagoas, Maceio, Al, Brazil.

Received 22 August, 2016; Accepted 4 November, 2016

The aim of this study was to evaluate the antimicrobial activity and minimum inhibitory concentration (MIC) of copaiba oil, extracts of *Passiflora cincinnata* and substances commonly used against endodontic infections of bacterial strains certified by the American Type Culture Collection (ATCC) and against clinical isolates (CI). The methodology involved the preparation of crude extracts of the plants, the selection of copaiba oil and the standardization of samples. The antibacterial activity of these substances was tested against *Enterococcus faecalis* (ATCC), *Escherichia coli* (CI) and *Pseudomonas aeruginosa* (ATCC and CI). The MIC was determined by broth dilution and the nitroblue tetrazolium chloride dye reduction test. The data were statistically analyzed by the Kolmogorov-Smirnov normality test and by the Kruskal-Wallis test with a confidence level of 95%. The analysis of the antimicrobial activity showed that the ethanol extracts of *P. cincinnata* and the combination of calcium hydroxide with polyethylene glycol with and without camphorated monochlorophenol showed no antimicrobial activity. However, the copaiba oil and other substances evaluated showed some antimicrobial activity against the microorganisms used ($p < 0.01$), exhibiting a MIC ranging from < 0.3 to $> 400 \mu\text{L/mL}$ ($p < 0.05$). Copaiba oil showed antimicrobial activity and could represent a potential phytotherapeutic agent to be used against microorganisms causing endodontic infections.

Key words: Endodontics, *Enterococcus faecalis*, *Passiflora cincinnata*, *Pseudomonas aeruginosa*.

INTRODUCTION

The microflora of endodontic infections is polymicrobial, and there is generally a prevalence of anaerobic bacteria. Microorganisms such as *Enterococcus faecalis* and *Escherichia coli*, but *Pseudomonas aeruginosa*, an aerobic bacterium, can also be present (Dianat et al.,

2015). Mechanical cleaning of the root canal individually cannot disinfect the canal precisely, because the bacteria can hide in out-of-reach areas, isthmi and dentinal tubules. In addition to the mechanical action of the rotary or manual instrumentation, treatments frequently include

*Corresponding author. E-mail: marcos.silva@foufal.ufal.br.

Table 1. Substances used and their respective trademarks.

| Substances | Trademarks |
|---|--|
| Calcium hydroxide + polyethylene glycol | Calen - SSWhite [®] , Rio de Janeiro, RJ, Brazil |
| Calcium hydroxide + polyethylene glycol with CMCP | Calen with PMCC - SSWhite [®] Rio de Janeiro, RJ, Brazil |
| Formocresol | Biodinâmica [®] Ibiporã, PR, Brazil |
| Tricresol formalin | Biodinâmica [®] Ibiporã, PR, Brazil |
| Camphorated monochlorophenol (CMCP) | Biodinâmica [®] Ibiporã, PR, Brazil |
| Sodium hypochlorite 2.5% | Q-boa [®] , Osasco, SP, Brazil |
| Hydrocortisone + neomycin sulfate + polymyxin B sulfate | Otosporin - Farmoquímica [®] , Rio de Janeiro, RJ, Brazil |

the use of irrigating solutions of agents such as sodium hypochlorite and chlorhexidine (Dianat et al., 2015) and the intra-canal administration of calcium hydroxide, camphorated paramonochlorophenol-(CMCP), formocresol, tricresol formalin and/or a combination of corticosteroid and antibiotics. Given these limitations and based on the traditional use and relative ease of the isolation of active components, plants may provide potential sources of new drugs for the safe and effective treatment of diseases (Santos et al., 2008; Pimenta et al., 2015).

Among the plants that have been found to have pharmacological properties, *Copaifera* spp. are known to have anti-inflammatory effects on the airway and on the skin and to promote the healing of wounds and intrauterine ulcers, in addition to exhibiting antibacterial actions. Copaiba is an oil-resin produced by the exudation of the trunks of trees from the *Copaifera* genus. The material excreted is a transparent liquid, bright, with a coloration ranging from yellow to brown (Veiga et al., 2001). Among these plants, *Passiflora cincinnata* has been used to treat hypotension, as an anti-inflammatory agent, as an anti-anxiety agent and as an antitussive agent (Wolfman et al., 1994).

The aim of this study was to evaluate the antibacterial activity of preparations of copaiba oil and the extracts of *P. cincinnata* (passion flower) against *P. aeruginosa*, *E. faecalis* and *E. coli*. These effects were also compared with the antimicrobial effects of substances commonly used in endodontic practice.

MATERIALS AND METHODS

Plant and extraction procedure

Two fractions of ethanolic extracts of the flowers and leaves of *P. cincinnata* obtained from the Laboratory of Chemistry and Biochemistry, Federal University of Sergipe were used. The samples were placed in an oven (Model MA-037) at 37°C with continuous circulation of air for 48 h until they were completely dehydrated. The dried leaves and flowers were crushed and pulverized for 72 h to obtain a powder. The powder was subjected to extraction by maceration in 70% ethanol for 5 days. After concentration of the solvent in a rotary evaporator under reduced pressure, a crude ethanolic extract of the flowers and leaves were

obtained. The copaiba oil fractions were prepared and donated by the Laboratory of Natural Products ICB (Institute of Chemistry and Biotechnology – UFAL). Due to the difficulty in the synthesis of the products, the fractions were acquired in 2007, 2008 and 2009. These fractions were denominated as samples I, II and III, used in their pure form.

Microorganisms

E. faecalis (ATCC 4083) and *P. aeruginosa* (ATCC 9027) from the National Institute of Quality Control and Health - INCQS Oswaldo Cruz Foundation – FIOCRUZ – RJ, and also clinical isolates of *Pseudomonas aeruginosa* (204 C) and *Escherichia coli* (73 C) obtained from the Tropical Disease Hospital - HDT-AL were used.

Commercial substances

Commercial formulations of substances commonly used in conventional endodontic therapy were used, as shown in Table 1.

Antimicrobial activity

10 mg of the leaves and flowers were solubilized in 2 mL of distilled water to obtain a standard solution with a concentration of 5.0 mg/mL of each extract. 2.4 mg of calcium hydroxide paste (CALEN, SS WHITE, Rio de Janeiro, Brazil) and calcium hydroxide paste with camphorated paramonochlorophenol-CMCP (CALEN with CMCP, SS WHITE, Rio de Janeiro, Brazil) were used, which were each diluted in 2 mL of distilled water to yield a concentration of 1.2 mg/mL of each aliquot.

The following substances were used in the antimicrobial tests: Ciprofloxacin, formulations of the copaiba oil I, II and III, formocresol, tricresol formalin, camphorated paramonochlorophenol, 1% sodium hypochlorite and hydrocortisone + neomycin sulfate + polymyxin B sulfate. The bacteria from the American Type Culture Collection (ATCC), *E. faecalis* and *P. aeruginosa*, were received as lyophilized samples and were placed on maintenance medium, which was prepared with Miller-Hinton agar and Brain Heart Infusion (BHI) broth. The clinical isolates of *E. coli* and *P. aeruginosa* were derived from pre-cultures of the bacteria grown for 18 h at 37°C on Miller-Hinton agar.

The inoculum was diluted in saline to achieve an optical density (OD) equivalent to 0.5 on the McFarland scale, which corresponds to 10⁸ colony forming units (CFU/mL). The antimicrobial activity was assessed according to the method reported by Bauer et al. (1966) (disk agar dilution method). Previously sterilized paper discs were soaked in the solutions of standardized extracts and medications and were placed at pre-dish

Table 2. Distribution of the halo of inhibition of antibacterial action (in mm) on the microorganisms tested ($p < 0.01$).

| Samples | Microorganisms | | | |
|------------------------|--------------------|-----------------------------|---------------------------|----------------|
| | <i>E. faecalis</i> | <i>P. aeruginosa</i> (ATCC) | <i>P. aeruginosa</i> (CI) | <i>E. coli</i> |
| Copaiba oil I | 18.5 | – | 16.5 | – |
| Copaiba oil II | 27 | – | 23 | – |
| Copaiba oil III | – | – | 15 | – |
| Leaf extract | – | – | – | – |
| Flower extract | – | – | – | – |
| Calen | – | – | – | – |
| Calen with CMCP | – | – | – | – |
| Formocresol | – | 25.5 | 17 | 10 |
| Tricresol formalin | – | 14 | 8.5 | – |
| CMCP | – | 18 | – | – |
| NaOCl 2.5 % | – | 9 | 8 | – |
| Otosporin [®] | 15 | 13.5 | 22 | 15 |
| Ciprofloxacin | 30 | 32 | 21 | – |

Table 3. Minimum inhibitory concentration (MIC) of the tested substances in $\mu\text{L}/\text{mL}$ ($p < 0.05$).

| Samples | Microorganisms | | | |
|--------------------|--------------------|-----------------------------|---------------------------|----------------|
| | <i>E. faecalis</i> | <i>P. aeruginosa</i> (ATCC) | <i>P. aeruginosa</i> (CI) | <i>E. coli</i> |
| Copaiba oil I | < 3.12 | – | > 400 | – |
| Copaiba oil II | < 3.12 | – | > 400 | – |
| Copaiba oil III | – | – | > 400 | – |
| Formocresol | – | 50 | 50 | 200 |
| Tricresol formalin | – | 50 | 100 | – |
| CMCP | – | 200 | – | – |
| NaOCl 2.5% | – | > 400 | > 400 | – |
| Otosporin | < 3.12 | > 400 | > 400 | > 400 |
| Ciprofloxacin | < 0.3 | 25 | > 50 | – |

and incubated at 37°C for 24 h. The readings were performed by measuring the inhibition halo around the disc in millimeters, using a calibrated caliper.

Minimum inhibitory concentration (MIC)

The plants and substances used for the determination of the minimum inhibitory concentration were copaiba oils, formocresol, tricresol formalin, camphorated paramonochlorophenol, sodium hypochlorite 2.5%, hydrocortisone + neomycin sulfate + polymyxin B sulfate and ciprofloxacin. A total of 15 μg of ciprofloxacin was diluted in 300 μL of saline to a concentration of 5 $\mu\text{g}/100 \mu\text{L}$. The other substances used were applied as commercial formulations.

To determine the minimum inhibitory concentration, 96-well microdilution plates (Andrews, 2001) were used. Ciprofloxacin was used at concentrations ranging from 0.3 to 50 $\mu\text{g}/\text{mL}$, and the other substances were used in concentrations ranging from 3.12 to 400 $\mu\text{L}/\text{mL}$. For the negative controls, the wells with saline and brain heart infusion (BHI) broth were treated. Each well containing culture medium (BHI) and the substance (100 μL) was inoculated with 10 μL of a microbial strain. On the other hand, Calen was diluted in the same manner as in antimicrobial activity test and used in the

evaluation of MIC. Then, the plates were sealed and incubated at 37°C for 24 h. After this incubation period, 10 μL of p-lodinitrotetrazolium was added to reveal whether microbial growth was present, and the minimum inhibitory concentration of each substance was determined in triplicate.

The data were analyzed regarding the normal distribution by the Kolmogorov-Smirnov test. Since the normal distribution was not detected, the nonparametric Kruskal-Wallis test was used and the data were analyzed. The software ASSISTAT 7.0 software (UFCG, Campina Grande, Brazil) was used to apply the statistical tests which were set at a confidence level of 95%.

RESULTS

The ethanolic extracts of *P. cincinnata* and the combination of calcium hydroxide and polyethylene glycol with and without camphorated paramonochlorophenol showed no antimicrobial activity. However, the opaiba oils and the other substances evaluated showed antimicrobial activity against the target microorganisms (p

<0.05) (Table 2).

The minimum inhibitory concentrations for the copaiba oil, formocresol, tricresol formalin, camphorated paramonochlorophenol, sodium hypochlorite, hydrocortisone + neomycin sulfate + polymyxin B sulfate and ciprofloxacin are shown in Table 3. The Copaiba II oil, the hydrocortisone + neomycin sulfate + polymyxin B sulfate (MIC < 3.12 $\mu\text{L/mL}$ to > 400 $\mu\text{L/mL}$) and ciprofloxacin (<0,03 a >50 $\mu\text{L/mL}$) were more effective agents against *E. faecalis* and *P. aeruginosa* as compared to the other substances ($p < 0.01$).

DISCUSSION

To test the antimicrobial properties of plants and chemicals, testing was performed using agar diffusion, as advocated by Bauer et al. (1966) which is a reliable, inexpensive, reproducible and simple method that is widely used in microbiological research (Ohara et al., 1993; Estrela et al., 2000; Gomes et al., 2002; Drumond et al., 2004; Silva et al., 2006; Costa et al., 2008).

It was shown that the copaiba oil preparations (copaiba oil I and II) were effective against *E. faecalis* (ATCC) and *P. aeruginosa* (CI), with MIC values < 3.12 and > 400 $\mu\text{L/mL}$, respectively, while *P. aeruginosa* (ATCC) and *E. coli* (CI) were resistant to all the three oil preparations tested. However, in a study by Mendonça et al. (2009), the copaiba oil was effective against *P. aeruginosa* and *E. coli*, with MIC values of 12.5 and 1.56%, respectively, which may be explained by differences in bacterial resistance, such as the removal of proteins by an efflux pump, the differences in the expression of outer membrane proteins and differences in the protein production of DNA gyrase.

In another study, Lima et al. (2006) stated that the presence of the terpenoid in copaiba oil was responsible for its activity against Gram-positive bacteria, while it had no effects against *E. coli*, which is a Gram-negative bacterium. The present study and previous work by Packer and Luz (2007) showed similar results regarding the effects of copaiba oil on *E. coli*. However, Pieri et al. (2012) suggested that copaiba oil exerts an antimicrobial action that is only bacteriostatic in nature, because no concentration led to 99.9% destruction of bacteria in his studies performed to determine the bacteriological concentration maximum. In addition to these microorganisms, copaiba oil has shown antimicrobial activity against cariogenic agents such as *Streptococcus mutans* and some biofilm-forming agents, such as *Streptococcus salivarius*, *Streptococcus pyogenes* and *E. faecalis* (Pieri et al., 2012).

In the present work, the analysis of antimicrobial activity showed that the extracts of the leaves and flowers of *P. cincinnata* demonstrated no activity against any of the bacteria tested. However, Nicolls et al. (1973) assessed the antimicrobial activity of the bark extract of the fruit of

P. mollissima and observed antimicrobial activity against *E. coli*, which may be attributed to the presence of polyacetylenes occurring in these antimicrobial plants (Birner et al., 1973; Nicolls et al., 1973), which were absent from the ethanol extracts of *P. cincinnata* tested in the present study.

Similar results were found for calcium hydroxide + polyethylene glycol with and without CMCP, which showed no zone of inhibition for any of the test organisms. However, the analysis was performed after 24 h, while Estrela et al. (1998) found that calcium hydroxide exerted antibacterial effects against bacteria after 48 h; moreover, in the work by Gomes et al. (2002), antimicrobial effects were observed after 7 days. Notably, in another study by Estrela et al. (2003a), complete inhibition of microorganisms occurred after 60 days, showing that the timing of studies is fundamental and that it may take a long time for the calcium hydroxide to exert its full activity, likely because the antimicrobial activity is directly proportional to the rate of diffusion of hydroxyl ions.

On the other hand, formocresol was effective against most of the bacteria tested in the present study, except *E. faecalis*, with MIC values of 50 $\mu\text{L/mL}$ for *P. aeruginosa* (ATCC and CI) and 200 $\mu\text{L/mL}$ for *E. coli*. However, Ferreira et al. (2007) also found that formocresol had antibacterial effects against *E. faecalis*. Similarly, an antimicrobial action of formocresol against anaerobic bacteria was observed by (Ohara et al., 1993).

Tricresol formalin showed an antibacterial front for *P. aeruginosa* (ATCC and CI), with MIC values of 50 and 100 $\mu\text{L/mL}$, respectively. However, the other bacteria tested were resistant. This result was in agreement with a previous study by (Melo et al., 2004), in which tricresol formalin's bactericidal actions were observed only against *P. aeruginosa*, and with a study by Silva et al. (2006), in which tricresol formalin did not inhibit the growth of *E. faecalis*.

In the current study, camphorated paramonochlorophenol was only effective against *P. aeruginosa* (ATCC), with a MIC of 200 $\mu\text{L/mL}$. In contrast, Costa et al. (2008) showed that CMCP had antibacterial effects against *E. faecalis*. Moreover, the work of Orstavik and Haapasalo (1990) showed that CMCP eliminated *P. aeruginosa* within four hours; however, there was also an effective front for *E. faecalis* (24 h) and *E. coli* (20 min).

The sodium hypochlorite solution (2.5%) was effective against *P. aeruginosa* (ATCC and CI), with a MIC > 400 $\mu\text{L/mL}$ for both. In another study by Estrela et al. (2003b), a similar result was found for *P. aeruginosa* when they tested the effects of 2% alkaline hypochlorite by direct exposure and agar diffusion. Those authors observed antimicrobial effects against *E. faecalis* and *P. aeruginosa* for both methods, but better performance was observed with the direct exposure for sodium hypochlorite.

The combination of hydrocortisone + neomycin sulfate

+ polymyxin B sulfate was the only treatment tested that showed activity against all strains tested, with a MIC of < 3.12 µL/mL for *E. faecalis* and > 400 µL/mL for the other microorganisms. Similarly, Siqueira Jr. and Lopes (1999) tested the effectiveness of this combination treatment on the bacteria present in saliva under aerobic and anaerobic conditions, and found that this drug only exhibited inhibitory effects in the presence of oxygen. The authors suggested that this phenomenon could be explained by the fact that the antibiotics (neomycin sulfate and polymyxin B sulfate) are effective against both aerobic and facultative anaerobic bacteria.

Ciprofloxacin showed activity against *E. faecalis* and *P. aeruginosa* (ATCC and CI), with MIC values of 0.03, 2.5 and > 5 µg/mL, respectively, because the strain of *E. coli* was resistant. Likewise, the work of Sader et al. (2001) showed that ciprofloxacin eliminated 61.4% of *P. aeruginosa* strains isolated from clinical infections, with a MIC of up to 0.5 µg/mL. Similar results were obtained in a study by Gales et al. (1997), who observed inhibition of the growth of *P. aeruginosa* (ATCC) by ciprofloxacin.

Conclusion

Based on the results of this study, copaiba oil exerted antimicrobial activity against *E. faecalis* and *P. aeruginosa*; however, the combination of hydrocortisone + neomycin sulfate + polymyxin B sulfate was the best antimicrobial treatment. Our study also showed that *E. coli* is the most resistant to the various substances tested.

Conflicts of interests

The authors have not declared any conflict of interests.

REFERENCES

- Andrews JM (2001). Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.* 48:5-16.
- Bauer AW, Kirby WM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45:493-496.
- Birner J, Nicolls J.M (1973). Passicol, an antibacterial and antifungal agent produced by passiflora plant species: preparation and physicochemical characteristics. *Antimicrob. Agents Chemother.* 3:105-109.
- Costa EMMB, Esmeraldo MRA, Carvalho MGF, Daniel RLAP, Pastro MF, Silva Júnior FL (2008). Evaluation of the Antimicrobial Action of Propolis and Substances Used in Endodontics against *Enterococcus faecalis*. *Pesq. Bras. Odontoped. Clin. Integr.* 8:21-25.
- Dianat O, Saedi S, Kazem M, Alam M (2015). Antimicrobial Activity of Nanoparticle Calcium Hydroxide against *Enterococcus faecalis*: An *In Vitro* Study. *Iran Endod J.* 10:39-43.
- Drumond MRS, Castro RD, Almeida RVD, Pereira MSV, Padilha WVN (2004). Comparative Study in vitro of the Antibacterial Activity from Phytotherapeutic Products Against Cariogenic Bacterias. *Pesq. Bras. Odontoped. Clin. Integr.* 4:33-38.
- Estrela C, Bammann LL, Estrela CRA, Silva RS, Pécora JD (2000). Antimicrobial and Chemical Study of MTA, Portland Cement, Calcium Hydroxide Paste, Sealapex and Dycal. *Braz. Dent. J.* 11:3-9.
- Estrela C, Estrela CRA, Pecora JD (2003a). A study of the time necessary for calcium hydroxide to eliminate microorganisms in infected canals. *J. Appl. Oral Sci.* 11:133-137.
- Estrela C, Pimenta FC, Ito IY, Bammann LL (1998). In vitro determination of direct antimicrobial effect of calcium hydroxide. *J. Endod.* 24:15-17.
- Estrela C, Ribeiro RG, Estrela CRA, Pécora DJ, Sousa-Neto MD (2003b). Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. *Braz. Dent. J.* 14:58-62.
- Ferreira FBA, Torres SA, Rosa OPS, Ferreira CM, Garcia RB, Marcucci MC, Gomes BPFA (2007). Antimicrobial effect of propolis and other substances against selected endodontic pathogens. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 104:709-716.
- Gales AC, Pignatari AC, Jones RN, Baretta M, Sader HS (1997). Evaluation of in vitro activity of new fluoroquinolones, cephalosporins and carbapenems against 569 gram-negative bacteria. *Rev. Assoc. Med. Bras.* 43:137-44.
- Gomes BPFA, Ferraz CCR, Vianna ME, Rosalen PL, Zaia AA, Teixeira FB, Souza-Filho FJ (2002). In vitro antimicrobial activity of calcium hydroxide pastes and their vehicles against selected microorganisms. *Braz. Dent. J.* 13:155-161.
- Lima MRF, Luna JS, Santos AF, Andrade MCC, Sant'ana AEG, Genet JP, Marquez B, Neuville L, Moreau N (2006). Anti-bacterial activity of some Brazilian medicinal plants. *J. Ethnopharmacol.* 105:137-147.
- Melo ABP, Albuquerque DS, Castro CMMB (2004). Comparative study in vitro antimicrobial capacity of tricresol. *J. Bras. Endod.* 17:126-131.
- Mendonça DE, Onofre SB (2009). Antimicrobial activity of the oil-resin produced by copaiba copaiferamultijugaHayne (Leguminosae). *Rev. Bras. Farmac.* 19:577-581.
- Nicolls JM, Birner J, Forsell P (1973). Passicol an antibacterial and antifungal agent produced by *Passiflora* Plant Species: Qualitative and Quantitative Range of Activity. *Antimicrob. Agents Chemother.* 3:110-117.
- Ohara P, Torabinejad M, Kettering JD (1993). Antibacterial effects of various endodontic medicaments on selected anaerobic bacteria. *J. Endod.* 19:498-500.
- Orstavik D, Haapasalo M (1990). Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endod. Dent. Traumatol.* 6(4):142-9.
- Packer JF, Luz MMS (2007). Evaluation and research method for natural products inhibitory activity. *Rev. Bras. Farmacogn.* 17:102-107.
- Pieri FA, Mussi MCM, Fiorini JE, Moreira MAS, Schneedorf JM (2012). Bacteriostatic Effect of Copaiba Oil (*Copaifera officinalis*) against *Streptococcus mutans*. *Braz. Dent. J.* 23:36-38.
- Pimenta HC, Violante IM, Musis CR, Borges ÁH, Aranha AM (2015). In vitro effectiveness of Brazilian brown propolis against *Enterococcus faecalis*. *Bras. Oral Res.* 29:1-6.
- Sader HS, Mendes RE, Gales AC, Jones RN, Pfaller MA, Zoccoli C, Sampaio J (2001). Antimicrobial susceptibility of bacteria isolated from the lower respiratory tract of inpatients with pneumonia in Brazilian hospitals – Results from the SENTRY surveillance program, 1997 and 1998. *J. Pneumol.* 27:59-67.
- Santos AO, Ueda-Nakamura T, Dias Filho BP, Veiga Jr VF, Pinto AC, Nakamura CV (2008). Antimicrobial activity of Brazilian copaiba oils obtained from different species of the *Copaifera* genus. *Mem. Inst. Oswaldo Cruz.* 3:277-281.
- Silva KP, Irala LED, Salles AA, Limongi O, Soares RG (2006). Antimicrobial Activity Evaluation of Tricresol Formalin Opposite of *Enterococcus faecalis* and *Bacillus subtilis*. *Rev. Endod. Pesq. Ensi On Line* 4:1-11.
- Siqueira Jr JF, Lopes HP (1999). Comparative study of the antimicrobial activity of drugs intra-canals through direct contact and distance. *UFES Rev. Odontol.* 1:60-64.
- Veiga VF, Jr, Zunino L, Calixto JB, Patitucci ML, Pinto AC (2001). Phytochemical and antioedematogenic studies of commercial copaiba oils available in Brazil. *Phytother. Res.* 15:476-80.
- Wolfman C, Viola H, Paladini A, Dajas F, Medina JH (1994). Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. *Pharmacol. Biochem. Behav.* 2(47):1-4.

Journal of Microbiology and Antimicrobials

Related Journals Published by Academic Journals

- *Journal of General and Molecular Virology*
- *African Journal of Food Science*
- *Journal of Ecology and The Natural Environment*
- *African Journal of Environmental Science and Technology*
- *African Journal of Microbiology Research*

academicJournals