ABOUT AJMR

The African Journal of Microbiology Research (AJMR) (ISSN 1996-0808) is published Weekly (one volume per year) by Academic Journals.

African Journal of Microbiology Research (AJMR) provides rapid publication (weekly) of articles in all areas of Microbiology such as: Environmental Microbiology, Clinical Microbiology, Immunology, Virology, Bacteriology, Phycology, Mycology and Parasitology, Protozoology, Microbial Ecology, Probiotics and Prebiotics, Molecular Microbiology, Biotechnology, Food Microbiology, Industrial Microbiology, Cell Physiology, Environmental Biotechnology, Genetics, Enzymology, Molecular and Cellular Biology, Plant Pathology, Entomology, Biomedical Sciences, Botany and Plant Sciences, Soil and Environmental Sciences, Zoology, Endocrinology, Toxicology. 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 are peer-reviewed.

Submission of Manuscript

Please read the Instructions for Authors before submitting your manuscript. The manuscript files should be given the last name of the first author.

Click here to Submit manuscripts online

If you have any difficulty using the online submission system, kindly submit via this email ajmr@academicjournals.org.

With questions or concerns, please contact the Editorial Office at ajmr@academicjournals.org.
Editors

Prof. Dr. Stefan Schmidt,
Applied and Environmental Microbiology
School of Biochemistry, Genetics and Microbiology
University of KwaZulu-Natal
Private Bag X01
Scottsville, Pietermaritzburg 3209
South Africa.

Prof. Fukai Bao
Department of Microbiology and Immunology
Kunming Medical University
Kunming 650031,
China

Dr. Jianfeng Wu
Dept. of Environmental Health Sciences,
School of Public Health,
University of Michigan
USA

Dr. Ahmet Yilmaz Coban
OMU Medical School,
Department of Medical Microbiology,
Samsun,
Turkey

Dr. Seyed Davar Siadat
Pasteur Institute of Iran,
Pasteur Square, Pasteur Avenue,
Tehran,
Iran.

Dr. J. Stefan Rokem
The Hebrew University of Jerusalem
Department of Microbiology and Molecular Genetics,
P.O.B. 12272, IL-91120 Jerusalem,
Israel

Prof. Long-Liu Lin
National Chiayi University
300 Syuefu Road,
Chiayi,
Taiwan

N. John Tonukari, Ph.D
Department of Biochemistry
Delta State University
PMB 1
Abraka, Nigeria

Dr. Thaddeus Ezeji
Assistant Professor
Fermentation and Biotechnology Unit
Department of Animal Sciences
The Ohio State University
1680 Madison Avenue
USA.

Associate Editors

Dr. Mamadou Gueye
MIRCEN/ Laboratoire commun de microbiologie
IRD-ISRA-UCAD, BP 1386,
DAKAR, Senegal.

Dr. Caroline Mary Knox
Department of Biochemistry, Microbiology and
Biotechnology
Rhodes University
Grahamstown 6140
South Africa.

Dr. Hesham Elsayed Mostafa
Genetic Engineering and Biotechnology Research
Institute (GEBRI)
Mubarak City For Scientific Research,
Research Area, New Borg El-Arab City,
Post Code 21934, Alexandria, Egypt.

Dr. Wael Abbas El-Naggar
Head of Microbiology Department,
Faculty of Pharmacy,
Mansoura University,
Mansoura 35516, Egypt.

Dr. Abdel Nasser A. El-Moghazy
Microbiology, Molecular Biology, Genetics Engineering
and Biotechnology
Dept of Microbiology and Immunology
Faculty of Pharmacy
Al-Azhar University
Nasr City,
Cairo, Egypt
Dr. Barakat S.M. Mahmoud  
*Food Safety/Microbiology*  
Experimental Seafood Processing Laboratory  
Costal Research and Extension Center  
Mississippi State University  
3411 Frederic Street  
Pascagoula, MS 39567  
USA  

Prof. Mohamed Mahrous Amer  
Poultry Disease (Viral Diseases of poultry)  
Faculty of Veterinary Medicine,  
Department of Poultry Diseases  
Cairo university  
Giza, Egypt  

Dr. Xiaohui Zhou  
Molecular Microbiology, Industrial Microbiology,  
Environmental Microbiology, Pathogenesis, Antibiotic resistance, Microbial Ecology  
Washington State University  
Bustad Hall 402 Department of Veterinary Microbiology and Pathology, Pullman,  
USA  

Dr. R. Balaji Raja  
Department of Biotechnology,  
School of Bioengineering,  
SRM University,  
Chennai  
India  

Dr. Aly E Abo-Amer  
Division of Microbiology, Botany Department, Faculty of Science, Sohag University.  
Egypt.  

---  

**Editorial Board**  

Dr. Haoyu Mao  
*Department of Molecular Genetics and Microbiology*  
College of Medicine  
University of Florida  
Florida, Gainesville  
USA.  

Dr. Rachna Chandra  
*Environmental Impact Assessment Division*  
Environmental Sciences  
Sálim Ali Center for Ornithology and Natural History (SACON),  
Anaikatty (PO), Coimbatore-641108, India  

Dr. Yongxu Sun  
*Department of Medicinal Chemistry and Biomacromolecules*  
Qiqihar Medical University, Qiqihar 161006  
Heilongjiang Province  
P.R. China  

Dr. Ramesh Chand Kasana  
*Institute of Himalayan Bioresource Technology*  
Palampur, Distt. Kangra (HP), India  

Dr. S. Meena Kumari  
*Department of Biosciences*  
Faculty of Science  
University of Mauritius  
Roadit  

Dr. T. Ramesh  
*Assistant Professor*  
Marine Microbiology  
CAS in Marine Biology  
Faculty of Marine Sciences  
Annamalai University  
Parangipettai - 608 502  
Cuddalore Dist. Tamilnadu, India  

Dr. Pagano Marcela Claudia  
Post doctoral fellowship at *Department of Biology,*  
*Federal University of Ceará - UFC,*  
Brazil.
Dr. EL-Sayed E. Habib
Associate Professor,
Dept. of Microbiology,
Faculty of Pharmacy,
Mansoura University,
Egypt.

Dr. Pongsak Rattanachaikunsopon
Department of Biological Science,
Faculty of Science,
Ubon Ratchathani University,
Warin Chamrap, Ubon Ratchathani 34190,
Thailand

Dr. Gokul Shankar Sabesan
Microbiology Unit, Faculty of Medicine,
AIMST University
Jalan Bedong, Semeling 08100,
Kedah,
Malaysia

Dr. Kwang Young Song
Department of Biological Engineering,
School of Biological and Chemical Engineering,
Yanbian University of Science and Technology,
Yanji,
China.

Dr. Kamel Belhamel
Faculty of Technology,
University of Bejaia
Algeria

Dr. Sladjana Jevremovic
Institute for Biological Research
Sinisa Stankovic,
Belgrade,
Serbia

Dr. Tamer Edirne
Dept. of Family Medicine, Univ. of Pamukkale
Turkey

Dr. R. Balaji Raja M.Tech (Ph.D)
Assistant Professor,
Department of Biotechnology,
School of Bioengineering,
SRM University,
Chennai.
India

Dr. Minglei Wang
University of Illinois at Urbana-Champaign, USA

Dr. Mohd Fuat ABD Razak
Institute for Medical Research
Malaysia

Dr. Davide Pacifico
Istituto di Virologia Vegetale – CNR
Italy

Prof. Dr. Akrum Hamdy
Faculty of Agriculture, Minia University, Egypt
Egypt

Dr. Ntobeko A. B. Ntusi
Cardiac Clinic, Department of Medicine,
University of Cape Town and
Department of Cardiovascular Medicine,
University of Oxford
South Africa and
United Kingdom

Prof. N. S. Alzoreky
Food Science & Nutrition Department,
College of Agricultural Sciences & Food,
King Faisal University,
Saudi Arabia

Dr. Chen Ding
College of Material Science and Engineering,
Hunan University,
China

Dr. Svetlana Nikolić
Faculty of Technology and Metallurgy,
University of Belgrade,
Serbia

Dr. Sivakumar Swaminathan
Department of Agronomy,
College of Agriculture and Life Sciences,
Iowa State University,
Ames, Iowa 50011
USA

Dr. Alfredo J. Anceno
School of Environment, Resources and Development (SERD),
Asian Institute of Technology,
Thailand

Dr. Iqbal Ahmad
Aligarh Muslim University,
Aligrah
India
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Josephine Nketsia-Tabiri</td>
<td>Ghana Atomic Energy Commission</td>
</tr>
<tr>
<td></td>
<td>Ghana</td>
</tr>
<tr>
<td>Dr. Juliane Elisa Welke</td>
<td>UFRGS – Universidade Federal do Rio Grande do Sul</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
</tr>
<tr>
<td>Dr. Mohammad Nazrul Islam</td>
<td>NIMR; IPH-Bangalore &amp; NIUM</td>
</tr>
<tr>
<td></td>
<td>Bangladesh</td>
</tr>
<tr>
<td>Dr. Okonko, Iheanyi Omezuruike</td>
<td>Department of Virology,</td>
</tr>
<tr>
<td></td>
<td>Faculty of Basic Medical Sciences,</td>
</tr>
<tr>
<td></td>
<td>College of Medicine,</td>
</tr>
<tr>
<td></td>
<td>University of Ibadan,</td>
</tr>
<tr>
<td></td>
<td>University College Hospital,</td>
</tr>
<tr>
<td></td>
<td>Ibadan,</td>
</tr>
<tr>
<td></td>
<td>Nigeria</td>
</tr>
<tr>
<td>Dr. Giuliana Noratto</td>
<td>Texas A&amp;M University</td>
</tr>
<tr>
<td></td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Phanikanth Venkata Turlapati</td>
<td>Washington State University</td>
</tr>
<tr>
<td></td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Khaleel I. Z. Jawasreh</td>
<td>National Centre for Agricultural Research and Extension, NCARE</td>
</tr>
<tr>
<td></td>
<td>Jordan</td>
</tr>
<tr>
<td>Dr. Babak Mostafazadeh, MD</td>
<td>Shaheed Beheshty University of Medical Sciences</td>
</tr>
<tr>
<td></td>
<td>Iran</td>
</tr>
<tr>
<td>Dr. S. Meena Kumari</td>
<td>Department of Biosciences</td>
</tr>
<tr>
<td></td>
<td>Faculty of Science</td>
</tr>
<tr>
<td></td>
<td>University of Mauritius</td>
</tr>
<tr>
<td></td>
<td>Reduit</td>
</tr>
<tr>
<td></td>
<td>Mauritius</td>
</tr>
<tr>
<td>Dr. Anju</td>
<td>Department of Biotechnology,</td>
</tr>
<tr>
<td></td>
<td>SRM University, Chennai-603203</td>
</tr>
<tr>
<td></td>
<td>India</td>
</tr>
<tr>
<td>Dr. Mustafa Maroufpor</td>
<td>Iran</td>
</tr>
<tr>
<td>Prof. Dong Zhichun</td>
<td>Professor, Department of Animal Sciences and Veterinary Medicine,</td>
</tr>
<tr>
<td></td>
<td>Yunnan Agriculture University,</td>
</tr>
<tr>
<td></td>
<td>China</td>
</tr>
<tr>
<td>Dr. Mehdi Azami</td>
<td>Parasitology &amp; Mycology Dept,</td>
</tr>
<tr>
<td></td>
<td>Baghaeei Lab.,</td>
</tr>
<tr>
<td></td>
<td>Shams Abadi St.</td>
</tr>
<tr>
<td></td>
<td>Isfahan</td>
</tr>
<tr>
<td></td>
<td>Iran</td>
</tr>
<tr>
<td>Dr. Anderson de Souza Sant’Ana</td>
<td>University of São Paulo.</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
</tr>
<tr>
<td>Dr. Juliane Elisa Welke</td>
<td>UFRGS – Universidade Federal do Rio Grande do Sul</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
</tr>
<tr>
<td>Dr. Paul Shapshak</td>
<td>USF Health,</td>
</tr>
<tr>
<td></td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Jorge Reinheimer</td>
<td>Universidad Nacional del Litoral (Santa Fe)</td>
</tr>
<tr>
<td></td>
<td>Argentina</td>
</tr>
<tr>
<td>Dr. Qin Liu</td>
<td>East China University of Science and Technology</td>
</tr>
<tr>
<td></td>
<td>China</td>
</tr>
<tr>
<td>Dr. Xiao-Qing Hu</td>
<td>State Key Lab of Food Science and Technology</td>
</tr>
<tr>
<td></td>
<td>Jiangnan University</td>
</tr>
<tr>
<td></td>
<td>P. R. China</td>
</tr>
<tr>
<td>Prof. Branislava Kocic</td>
<td>Specialist of Microbiology and Parasitology</td>
</tr>
<tr>
<td></td>
<td>University of Nis, School of Medicine Institute</td>
</tr>
<tr>
<td></td>
<td>for Public Health Nis, Bul. Z. Djindjica 50, 18000 Nis</td>
</tr>
<tr>
<td></td>
<td>Serbia</td>
</tr>
<tr>
<td>Dr. Rafel Socias</td>
<td>CITA de Aragón,</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
</tr>
</tbody>
</table>
Prof. Kamal I. Mohamed  
*State University of New York at Oswego*  
USA

Dr. Adriano Cruz  
*Faculty of Food Engineering-FEA*  
*University of Campinas (UNICAMP)*  
Brazil

Dr. Mike Agenbag (Michael Hermanus Albertus)  
*Manager Municipal Health Services,*  
*Joe Gqabi District Municipality*  
*South Africa*

Dr. D. V. L. Sarada  
*Department of Biotechnology,*  
*SRM University, Chennai-603203*  
*India.*

Dr. Samuel K Ameyaw  
*Civista Medical Center*  
*United States of America*

Prof. Huaizhi Wang  
*Institute of Hepatopancreatobilary Surgery of PLA Southwest Hospital,*  
*Third Military Medical University*  
*Chongqing 400038*  
*P. R. China*

Prof. Bakhiet AO  
*College of Veterinary Medicine, Sudan*  
*University of Science and Technology*  
*Sudan*

Dr. Saba F. Hussain  
*Community, Orthodontics and Pediatric Dentistry Department*  
*Faculty of Dentistry*  
*Universiti Teknologi MARA*  
*40450 Shah Alam, Selangor*  
*Malaysia*

Prof. Dr. Zohair I.F.Rahemo  
*State Key Lab of Food Science and Technology*  
*Jiangnan University*  
*P. R. China*

Dr. Afework Kassu  
*University of Gondar*  
*Ethiopia*

Prof. Isidro A. T. Savillo  
*ISCOF*  
*Philippines*

Dr. How-Yee Lai  
*Taylor’s University College*  
*Malaysia*

Dr. Nidheesh Dadheech  
*MS. University of Baroda, Vadodara, Gujarat, India.*  
*India*

Dr. Omitoyin Siyanbola  
*Bowen University,*  
*Iwo*  
*Nigeria*

Dr. Franco Mutinelli  
*Istituto Zooprofilattico Sperimentale delle Venezie*  
*Italy*

Dr. Chanpen Chanchao  
*Department of Biology,*  
*Faculty of Science,*  
*Chulalongkorn University*  
*Thailand*

Dr. Tsuyoshi Kasama  
*Division of Rheumatology,*  
*Showa University*  
*Japan*

Dr. Kuender D. Yang, MD.  
*Chang Gung Memorial Hospital*  
*Taiwan*

Dr. Liane Raluca Stan  
*University Politehnica of Bucharest,*  
*Department of Organic Chemistry “C.Nenitzescu”*  
*Romania*

Dr. Muhamed Osman  
*Senior Lecturer of Pathology & Consultant Immunopathologist*  
*Department of Pathology,*  
*Faculty of Medicine,*  
*Universiti Teknologi MARA,*  
*40450 Shah Alam, Selangor*  
*Malaysia*

Dr. Mohammad Feizabadi  
*Tehran University of medical Sciences*  
*Iran*
<table>
<thead>
<tr>
<th>Name</th>
<th>Position and Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Ahmed H Mitwalli</td>
<td>State Key Lab of Food Science and Technology, Jiangnan University, P. R. China</td>
</tr>
<tr>
<td>Dr. Mazyar Yazdani</td>
<td>Department of Biology, University of Oslo, Blindern, Oslo, Norway</td>
</tr>
<tr>
<td>Dr. Ms. Jemimah Gesare Onsare</td>
<td>Ministry of Higher, Education Science and Technology, Kenya</td>
</tr>
<tr>
<td>Dr. Babak Khalili Hadad</td>
<td>Department of Biological Sciences, Roudehen Branch, Islamic Azad University, Roudehen, Iran</td>
</tr>
<tr>
<td>Dr. Ehsan Sari</td>
<td>Department of Plan Pathology, Iranian Research Institute of Plant Protection, Tehran, Iran</td>
</tr>
<tr>
<td>Dr. Snjezana Zidovec Lepej</td>
<td>University Hospital for Infectious Diseases, Zagreb, Croatia</td>
</tr>
<tr>
<td>Dr. Dilshad Ahmad</td>
<td>King Saud University, Saudi Arabia</td>
</tr>
<tr>
<td>Dr. Adriano Gomes da Cruz</td>
<td>University of Campinas (UNICAMP), Brazil</td>
</tr>
<tr>
<td>Dr. Hsin-Mei Ku</td>
<td>Agronomy Dept. NCHU 250 Kuo, Kuang Rd, Taichung, Taiwan</td>
</tr>
<tr>
<td>Dr. Fereshteh Naderi</td>
<td>Physical chemist, Islamic Azad University, Shahre Ghods Branch, Iran</td>
</tr>
<tr>
<td>Dr. Adibe Maxwell Ogochukwu</td>
<td>Department of Clinical Pharmacy and Pharmacy Management, University of Nigeria, Nsukka, Nigeria</td>
</tr>
<tr>
<td>Dr. William M. Shafer</td>
<td>Emory University School of Medicine, USA</td>
</tr>
<tr>
<td>Dr. Michelle Bull</td>
<td>CSIRO Food and Nutritional Sciences, Australia</td>
</tr>
<tr>
<td>Prof. Dr. Márcio Garcia Ribeiro (DVM, PhD)</td>
<td>School of Veterinary Medicine and Animal Science-UNESP, Dept. Veterinary Hygiene and Public Health, State of Sao Paulo, Brazil</td>
</tr>
<tr>
<td>Prof. Dr. Sheila Nathan</td>
<td>National University of Malaysia (UKM), Malaysia</td>
</tr>
<tr>
<td>Prof. Ebiamadon Andi Brisibe</td>
<td>University of Calabar, Calabar, Nigeria</td>
</tr>
<tr>
<td>Dr. Julie Wang</td>
<td>Burnet Institute, Australia</td>
</tr>
<tr>
<td>Dr. Jean-Marc Chobert</td>
<td>INRA- BIA, FIPL, France</td>
</tr>
<tr>
<td>Dr. Zhihong Yang, PhD</td>
<td>Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health</td>
</tr>
<tr>
<td>Dr. Dele Raheem</td>
<td>University of Helsinki, Finland</td>
</tr>
<tr>
<td>Dr. Li Sun</td>
<td>PLA Centre for the treatment of infectious diseases, Tangdu Hospital, Fourth Military Medical University, China</td>
</tr>
<tr>
<td>Name</td>
<td>Institution and Address</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Dr. Biljana Miljkovic-Selimovic</td>
<td>School of Medicine, University in Nis, Serbia; Referent laboratory for Campylobacter and Helicobacter, Center for Microbiology, Institute for Public Health, Nis Serbia</td>
</tr>
<tr>
<td>Dr. Xinan Jiao</td>
<td>Yangzhou University, China</td>
</tr>
<tr>
<td>Dr. Endang Sri Lestari, MD.</td>
<td>Department of Clinical Microbiology, Medical Faculty, Diponegoro University/Dr. Kariadi Teaching Hospital, Semarang, Indonesia</td>
</tr>
<tr>
<td>Dr. Shojin Shin</td>
<td>Pusan National University Hospital, South Korea</td>
</tr>
<tr>
<td>Dr. Yi Wang</td>
<td>Center for Vector Biology, 180 Jones Avenue, Rutgers University, New Brunswick, NJ 08901-8536, USA</td>
</tr>
<tr>
<td>Dr. Heping Zhang</td>
<td>The Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural University, China</td>
</tr>
<tr>
<td>Prof. Natasha Potgieter</td>
<td>University of Venda, South Africa</td>
</tr>
<tr>
<td>Dr. Alemzadeh</td>
<td>Sharif University, Iran</td>
</tr>
<tr>
<td>Dr. Sonia Arriaga</td>
<td>Instituto Potosino de Investigación Científica y Tecnológica/División de Ciencias Ambientales, Mexico</td>
</tr>
<tr>
<td>Dr. Armando Gonzalez-Sanchez</td>
<td>Universidad Autonoma Metropolitana Cuajimalpa, Mexico</td>
</tr>
<tr>
<td>Dr. Pradeep Parihar</td>
<td>Lovely Professional University, Phagwara, Punjab, India</td>
</tr>
<tr>
<td>Dr. William H Roldán</td>
<td>Department of Medical Microbiology, Faculty of Medicine, Peru</td>
</tr>
<tr>
<td>Dr. Kanzaki, L I B</td>
<td>Laboratory of Bioprospection. University of Brasilia, Brazil</td>
</tr>
<tr>
<td>Prof. Philippe Dorchies</td>
<td>Laboratory of Bioprospection. University of Brasilia, Brazil</td>
</tr>
<tr>
<td>Dr. C. Ganesh Kumar</td>
<td>Indian Institute of Chemical Technology, Hyderabad, India</td>
</tr>
<tr>
<td>Dr. Farid Che Ghazali</td>
<td>Universiti Sains Malaysia (USM), Malaysia</td>
</tr>
<tr>
<td>Dr. Samira Bouhdid</td>
<td>Abdelmalek Essaadi University, Tetouan, Morocco, Morocco</td>
</tr>
<tr>
<td>Dr. Zainab Z. Ismail</td>
<td>Department of Environmental Engineering, University of Baghdad, Iraq</td>
</tr>
<tr>
<td>Dr. Ary Fernandes Junior</td>
<td>Universidade Estadual Paulista (UNESP), Brasil</td>
</tr>
<tr>
<td>Dr. Papaevangelou Vassiliki</td>
<td>Athens University Medical School, Greece</td>
</tr>
<tr>
<td>Dr. Fangyou Yu</td>
<td>The first Affiliated Hospital of Wenzhou Medical College, China</td>
</tr>
<tr>
<td>Dr. Galba Maria de Campos Takaki</td>
<td>Catholic University of Pernambuco, Brazil</td>
</tr>
</tbody>
</table>
Dr. Kwabena Ofori-Kwakye  
Department of Pharmaceutics,  
Kwame Nkrumah University of Science & Technology,  
KUMASI  
Ghana

Prof. Dr. Liesel Brenda Gende  
Arthropods Laboratory, School of Natural and Exact Sciences, National University of Mar del Plata  
Buenos Aires,  
Argentina.

Dr. Adeshina Gbonjubola  
Ahmadu Bello University,  
Zaria.  
Nigeria

Prof. Dr. Stylianos Chatzipanagiotou  
University of Athens – Medical School  
Greece

Dr. Dongqing BAI  
Department of Fishery Science,  
Tianjin Agricultural College,  
Tianjin 300384  
P. R. China

Dr. Dingqiang Lu  
Nanjing University of Technology  
P.R. China

Dr. L. B. Sukla  
Scientist – G & Head, Biominerals Department,  
IMMT, Bhubaneswar  
India

Dr. Hakan Parlakpinar  
MD. Inonu University, Medical Faculty, Department of Pharmacology, Malatya  
Turkey

Dr. Pak-Lam Yu  
Massey University  
New Zealand

Dr Percy Chimwamurombe  
University of Namibia  
Namibia

Dr. Eucléso Simionatto  
State University of Mato Grosso do Sul-UEMS  
Brazil

Dr. Hans-Jürg Monstein  
Clinical Microbiology, Molecular Biology Laboratory,  
University Hospital, Faculty of Health Sciences, S-581 85 Linköping  
Sweden

Dr. Ajith, T. A  
Associate Professor Biochemistry, Amala Institute of Medical Sciences, Amala Nagar, Thrissur, Kerala-680 555  
India

Dr. Feng-Chia Hsieh  
Biopesticides Division, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture  
Taiwan

Prof. Dra. Suzan Pantaroto de Vasconcellos  
Universidade Federal de São Paulo  
Rua Prof. Artur Riedel, 275 Jd. Eldorado, Diadema, SP  
CEP 09972-270  
Brasil

Dr. Maria Leonor Ribeiro Casimiro Lopes Assad  
Universidade Federal de São Carlos - Centro de Ciências Agrárias - CCA/UFSCar  
Departamento de Recursos Naturais e Proteção Ambiental  
Rodovia Anhanguera, km 174 - SP-330  
Araras - São Paulo  
Brasil

Dr. Pierangeli G. Vital  
Institute of Biology, College of Science, University of the Philippines  
Philippines

Prof. Roland Ndip  
University of Fort Hare, Alice  
South Africa

Dr. Shawn Carraher  
University of Fort Hare, Alice  
South Africa

Dr. José Eduardo Marques Pessanha  
Observatório de Saúde Urbana de Belo Horizonte/Faculdade de Medicina da Universidade Federal de Minas Gerais  
Brasil
Dr. Yuanshu Qian  
Department of Pharmacology, Shantou University Medical College  
China

Dr. Helen Treichel  
URI-Campus de Erechim  
Brazil

Dr. Xiao-Qing Hu  
State Key Lab of Food Science and Technology  
Jiangnan University  
P. R. China

Dr. Olli H. Tuovinen  
Ohio State University, Columbus, Ohio  
USA

Prof. Stoyan Groudev  
University of Mining and Geology “Saint Ivan Rilski”  
Sofia  
Bulgaria

Dr. G. Thirumurugan  
Research lab, GIET School of Pharmacy, NH-5, Chaitanya nagar, Rajahmundry-533294.  
India

Dr. Charu Gomber  
Thapar University  
India

Dr. Jan Kuever  
Bremen Institute for Materials Testing, Department of Microbiology, Paul-Feller-Str. 1, 28199 Bremen  
Germany

Dr. Nicola S. Flanagan  
Universidad Javeriana, Cali  
Colombia

Dr. André Luiz C. M. de A. Santiago  
Universidade Federal Rural de Pernambuco  
Brazil

Dr. Dhruva Kumar Jha  
Microbial Ecology Laboratory, Department of Botany, Gauhati University, Guwahati 781 014, Assam  
India

Dr. N Saleem Basha  
M. Pharm (Pharmaceutical Biotechnology)  
Eritrea (North East Africa)

Prof. Dr. João Lúcio de Azevedo  
Dept. Genetics-University of São Paulo-Faculty of Agriculture- Piracicaba, 13400-970  
Brasil

Dr. Julia Inés Fariña  
PROIMI-CONICET  
Argentina

Dr. Yutaka Ito  
Kyoto University  
Japan

Dr. Cheruiyot K. Ronald  
Biomedical Laboratory Technologist  
Kenya

Prof. Dr. Ata Akcil  
S. D. University  
Turkey

Dr. Adhar Manna  
The University of South Dakota  
USA

Dr. Cícero Flávio Soares Aragão  
Federal University of Rio Grande do Norte  
Brazil

Dr. Gunnar Dahlen  
Institute of odontology, Sahlgrenska Academy at University of Gothenburg  
Sweden

Dr. Pankaj Kumar Mishra  
Vivekananda Institute of Hill Agriculture, (I.C.A.R.), ALMORA-263601, Uttarakhand  
India

Dr. Benjamas W. Thanomsub  
Srinakharinwirot University  
Thailand

Dr. Maria José Borrego  
National Institute of Health – Department of Infectious Diseases  
Portugal
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Catherine Carrillo</td>
<td>Health Canada, Bureau of Microbial Hazards, Canada</td>
</tr>
<tr>
<td>Dr. Marcotty Tanguy</td>
<td>Institute of Tropical Medicine, Belgium</td>
</tr>
<tr>
<td>Dr. Han-Bo Zhang</td>
<td>Laboratory of Conservation and Utilization for Bio-resources, Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming 650091. School of Life Science, Yunnan University, Kunming, Yunnan Province 650091, China</td>
</tr>
<tr>
<td>Dr. Ali Mohammed Somily</td>
<td>King Saud University, Saudi Arabia</td>
</tr>
<tr>
<td>Dr. Nicole Wolter</td>
<td>National Institute for Communicable Diseases and University of the Witwatersrand, Johannesburg, South Africa</td>
</tr>
<tr>
<td>Dr. Marco Antonio Nogueira</td>
<td>Universidade Estadual de Londrina, CCB/Depto. De microbiologia, Laboratório de Microbiologia Ambiental, Caixa Postal 6001, 86051-980 Londrina, Brazil</td>
</tr>
<tr>
<td>Dr. Bruno Pavoni</td>
<td>Department of Environmental Sciences University of Venice, Italy</td>
</tr>
<tr>
<td>Dr. Shih-Chieh Lee</td>
<td>Da-Yeh University, Taiwan</td>
</tr>
<tr>
<td>Dr. Satoru Shimizu</td>
<td>Horonobe Research Institute for the Subsurface Environment, Northern Advancement Center for Science &amp; Technology, Japan</td>
</tr>
<tr>
<td>Dr. Tang Ming</td>
<td>College of Forestry, Northwest A&amp;F University, Yangling, China</td>
</tr>
<tr>
<td>Dr. Olga Gortzi</td>
<td>Department of Food Technology, T.E.I. of Larissa, Greece</td>
</tr>
<tr>
<td>Dr. Mark Tarnopolsky</td>
<td>McMaster University, Canada</td>
</tr>
<tr>
<td>Dr. Sami A. Zabin</td>
<td>Al Baha University, Saudi Arabia</td>
</tr>
<tr>
<td>Dr. Julia W. Pridgeon</td>
<td>Aquatic Animal Health Research Unit, USDA, ARS, USA</td>
</tr>
<tr>
<td>Dr. Lim Yau Yan</td>
<td>Monash University Sunway Campus, Malaysia</td>
</tr>
<tr>
<td>Prof. Rosemeire C. L. R. Pietro</td>
<td>Faculdade de Ciências Farmacêuticas de Araraquara, Univ Estadual Paulista, UNESP, Brazil</td>
</tr>
<tr>
<td>Dr. Nazime Mercan Dogan</td>
<td>PAU Faculty of Arts and Science, Denizli, Turkey</td>
</tr>
<tr>
<td>Dr Ian Edwin Cock</td>
<td>Biomolecular and Physical Sciences, Griffith University, Australia</td>
</tr>
<tr>
<td>Prof. N K Dubey</td>
<td>Banaras Hindu University, India</td>
</tr>
<tr>
<td>Dr. S. Hemalatha</td>
<td>Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi, 221005, India</td>
</tr>
<tr>
<td>Dr. J. Santos Garcia A.</td>
<td>Universidad A. de Nuevo Leon, Mexico, India</td>
</tr>
</tbody>
</table>
Dr. Somboon Tanasupawat
Department of Biochemistry and Microbiology,
Faculty of Pharmaceutical Sciences,
Chulalongkorn University,
Bangkok 10330
Thailand

Dr. Vivekananda Mandal
Post Graduate Department of Botany,
Darjeeling Government College,
Darjeeling – 734101.
India

Dr. Shihua Wang
College of Life Sciences,
Fujian Agriculture and Forestry University
China

Dr. Victor Manuel Fernandes Galhano
CITAB-Centre for Research and Technology of Agro-
Environment and Biological Sciences, Integrative
Biology and Quality Research Group,
University of Trás-os-Montes and Alto Douro,
Apartado 1013, 5001-801 Vila Real
Portugal

Dr. Maria Cristina Maldonado
Instituto de Biotecnología. Universidad Nacional de
Tucuman
Argentina

Dr. Alex Soltermann
Institute for Surgical Pathology,
University Hospital Zürich
Switzerland

Dr. Dagmara Sirova
Department of Ecosystem Biology, Faculty Of Science,
University of South Bohemia,
Branisovska 37, Ceske Budejovice, 37001
Czech Republic

Dr. E. O Igbinosa
Department of Microbiology,
Ambrose Alli University,
Ekpoma, Edo State,
Nigeria.

Dr. Hodaka Suzuki
National Institute of Health Sciences
Japan

Dr. Mick Bosilevac
US Meat Animal Research Center
USA

Dr. Nora Lía Padola
Imunoquímica y Biotecnología- Fac Cs Vet-UNCPBA
Argentina

Dr. Maria Madalena Vieira-Pinto
Universidade de Trás-os-Montes e Alto Douro
Portugal

Dr. Stefano Morandi
CNR-Istituto di Scienze delle Produzioni Alimentari
(ISPA), Sez. Milano
Italy

Dr. Line Thorsen
Copenhagen University, Faculty of Life Sciences
Denmark

Dr. Ana Lucia Falavigna-Guilherme
Universidade Estadual de Maringá
Brazil

Dr. Baoqiang Liao
Dept. of Chem. Eng., Lakehead University, 955 Oliver
Road, Thunder Bay, Ontario
Canada

Dr. Quanming Zou
Department of Clinical Microbiology and Immunology,
College of Medical Laboratory,
Third Military Medical University
China
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Ashok Kumar</td>
<td>School of Biotechnology, Banaras Hindu University, Varanasi, India</td>
</tr>
<tr>
<td>Dr. Chung-Ming Chen</td>
<td>Department of Pediatrics, Taipei Medical University Hospital, Taipei, Taiwan</td>
</tr>
<tr>
<td>Dr. Jennifer Furin</td>
<td>Harvard Medical School, USA</td>
</tr>
<tr>
<td>Dr. Julia W. Pridgeon</td>
<td>Aquatic Animal Health Research Unit, USDA, ARS, USA</td>
</tr>
<tr>
<td>Dr. Alireza Seidavi</td>
<td>Islamic Azad University, Rasht Branch, Iran</td>
</tr>
<tr>
<td>Dr. Thore Rohwerder</td>
<td>Helmholtz Centre for Environmental Research UFZ, Germany</td>
</tr>
<tr>
<td>Dr. Daniela Billi</td>
<td>University of Rome Tor Vergat, Italy</td>
</tr>
<tr>
<td>Dr. Ivana Karabegovic</td>
<td>Faculty of Technology, Leskovac, University of Nis, Serbia</td>
</tr>
<tr>
<td>Dr. Flaviana Andrade Faria</td>
<td>IBILCE/UNESP, Brazil</td>
</tr>
<tr>
<td>Prof. Margareth Linde Athayde</td>
<td>Federal University of Santa Maria, Brazil</td>
</tr>
<tr>
<td>Dr. Guadalupe Virginia Nevarez Moorillon</td>
<td>Universidad Autonoma de Chihuahua, Mexico</td>
</tr>
<tr>
<td>Dr. Tatiana de Sousa Fiuza</td>
<td>Federal University of Goias, Brazil</td>
</tr>
<tr>
<td>Dr. Indrani B. Das Sarma</td>
<td>Jhulelal Institute of Technology, Nagpur, India</td>
</tr>
<tr>
<td>Dr. Guanghua Wang</td>
<td>Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, China</td>
</tr>
<tr>
<td>Dr. Renata Vadkertiova</td>
<td>Institute of Chemistry, Slovak Academy of Science, Slovakia</td>
</tr>
<tr>
<td>Dr. Charles Hocart</td>
<td>The Australian National University, Australia</td>
</tr>
<tr>
<td>Dr. Guoqiang Zhu</td>
<td>University of Yangzhou College of Veterinary Medicine, China</td>
</tr>
<tr>
<td>Dr. Guilherme Augusto Marietto Gonçalves</td>
<td>São Paulo State University, Brazil</td>
</tr>
<tr>
<td>Dr. Mohammad Ali Faramarzi</td>
<td>Tehran University of Medical Sciences, Iran</td>
</tr>
<tr>
<td>Dr. Suppasil Maneerat</td>
<td>Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai 90112, Thailand</td>
</tr>
<tr>
<td>Dr. Francisco Javier Las heras Vazquez</td>
<td>Almeria University, Spain</td>
</tr>
<tr>
<td>Dr. Cheng-Hsun Chiu</td>
<td>Chang Gung memorial Hospital, Chang Gung University, Taiwan</td>
</tr>
<tr>
<td>Dr. Ajay Singh</td>
<td>DDU Gorakhpur University, Gorakhpur-273009 (U.P.), India</td>
</tr>
<tr>
<td>Dr. Karabo Shale</td>
<td>Central University of Technology, Free State, South Africa</td>
</tr>
<tr>
<td>Dr. Lourdes Zélia Zanoni</td>
<td>Department of Pediatrics, School of Medicine, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil</td>
</tr>
<tr>
<td>Name</td>
<td>Institution and Location</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------</td>
</tr>
<tr>
<td>Dr. Tulin Askun</td>
<td>Balikesir University, Turkey</td>
</tr>
<tr>
<td>Dr. Marija Stankovic</td>
<td>Institute of Molecular Genetics and Genetic Engineering, Serbia</td>
</tr>
<tr>
<td>Dr. Scott Weese</td>
<td>University of Guelph, Dept. of Pathobiology, Canada</td>
</tr>
<tr>
<td>Dr. Sabiha Essack</td>
<td>School of Health Sciences, University of KwaZulu-Natal, South Africa</td>
</tr>
<tr>
<td>Dr. Hare Krishna</td>
<td>Central Institute for Arid Horticulture, Bikaner-334 006, India</td>
</tr>
<tr>
<td>Dr. Anna Mensuali</td>
<td>Dept. of Life Science, Scuola Superiore, Sant’Anna</td>
</tr>
<tr>
<td>Dr. Ghada Sameh Hafez Hassan</td>
<td>Pharmaceutical Chemistry Department, Faculty of Pharmacy, Mansoura University, Egypt</td>
</tr>
<tr>
<td>Dr. Kátia Flávia Fernandes</td>
<td>Biochemistry and Molecular Biology, Universidade Federal de Goiás, Brasil</td>
</tr>
<tr>
<td>Dr. Abdel-Hady El-Gilany</td>
<td>Public Health &amp; Community Medicine, Mansoura University, Egypt</td>
</tr>
<tr>
<td>Dr. Hongxiong Guo</td>
<td>STD and HIV/AIDS Control and Prevention, Jiangsu provincial CDC, China</td>
</tr>
<tr>
<td>Dr. Konstantina Tsaousi</td>
<td>Life and Health Sciences, School of Biomedical Sciences, Ulster</td>
</tr>
<tr>
<td>Dr. Bhavnaben Gowan Gordhan</td>
<td>DST/NRF Centre of Excellence for Biomedical TB Research, Witwatersand, South Africa</td>
</tr>
<tr>
<td>Dr. Ernest Kuchar</td>
<td>Pediatric Infectious Diseases, Wroclaw Medical University, Poland</td>
</tr>
<tr>
<td>Dr. Mar Rodriguez Jovita</td>
<td>Food Hygiene and Safety, Faculty of Veterinary Science, Extremadura, Spain</td>
</tr>
<tr>
<td>Dr. Jes Gitz Holler</td>
<td>Hospital Pharmacy, Aalesund, Central Norway Pharmaceutical Trust, Trondheim, Norway</td>
</tr>
<tr>
<td>Prof. Chengxiang FANG</td>
<td>College of Life Sciences, Wuhan University, Wuhan 430072, China</td>
</tr>
<tr>
<td>Dr. Anchalee Tungtrongchitr</td>
<td>Siriraj Dust Mite Center for Services and Research, Parasitology, Siriraj Hospital, Mahidol University, Bangkok, Thailand</td>
</tr>
</tbody>
</table>
Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The cover letter should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process
All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review. Decisions will be made as rapidly as possible, and the Journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJMR to publish manuscripts within weeks after submission.

Regular articles
All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited. Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.
**Results** should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The **Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The **Acknowledgments** of people, grants, funds, etc should be brief.

**Tables** should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

**Figure legends** should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

**References:** In the text, a reference identified by means of an author’s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author’s name should be mentioned, followed by ‘et al’. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ‘a’ and ‘b’ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:


**Short Communications**

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.
Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the African Journal of Microbiology Research is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Copyright: © 2015, Academic Journals.
All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the AJMR, whether or not advised of the possibility of damage, and on any theory of liability.
This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.
Fermented milk enriched with passion fruit peel (passiflora edulis): Physicochemical and sensory aspects and lactic acid bacteria viability
Juliana Aparecida Célia, Marco Antônio Pereira da Silva, Kênia Borges de Oliveira, Jéssica Leal Freitas e Souza, Diene Gonçalves Souza, Ligia Campos de Moura, Richard Marins da Silva, Caroline Cagnin, Bheatriz Silva Morais de Freitas, Geovana Rocha Plácido and Márcio Caliari

Seroprevalence of Ehrlichia canis in dogs from Monterrey, Mexico

Detection of Mycobacterium bovis in bovine carcasses by multiplex-PCR
Ricardo César Tavares Carvalho, Vinicius Silva Castro, Flávia Galindo Silvestre Silva, Carlos Adam Conte Junior, Walter Lilienbaum, Vânia Margaret Flosi Paschoalin and Eduardo Eustáquio de Souza Figueiredo

Antioxidant and in-vitro anthelminthic potentials of methanol extracts of barks and leaves of Voacanga africana and Rauwolfia vomitoria
Francis Adu, John Antwi Apenteng, William Gariba Akanwariwiak, George Henry Sam, David Ntinagyei Mintah and Edna Beyeman Bortsie
Fermented milk enriched with passion fruit peel flour (*passiflora edulis*): Physicochemical and sensory aspects and lactic acid bacteria viability

Juliana Aparecida Célia¹, Marco Antônio Pereira da Silva²*, Kênia Borges de Oliveira¹, Jéssica Leal Freitas e Souza¹, Diene Gonçalves Souza¹, Ligia Campos de Moura¹, Richard Marins da Silva², Caroline Cagnin², Bheatriz Silva Morais de Freitas², Geovana Rocha Plácido² and Márcio Caliari³

¹Food Engineer, Master's students in Animal Science Post-Graduate Program at Instituto Federal Goiano - Rio Verde Campus, GO, Brazil.

²Instituto Federal Goiano - Rio Verde Campus, GO, Brazil.

³Universidade Federal de Goiás, Goiânia, GO, Brazil.

Received 4 April, 2015; Accepted 8 June, 2015

This study aimed to evaluate the physicochemical parameters, total and thermotolerant coliforms, lactic acid bacteria viability, instrumental color, and sensory analysis of fermented milks added to passion fruit peel flour (PFPF), throughout 29 days of storage, except composition. Four fermented milk treatments were prepared as follows: 1, fermented milk without addition of PFPF; 2, fermented milk added with 1% PFPF; 3, fermented milk added with 2% PFPF; 4, fermented milk added with 3% PFPF. According to the results obtained, acidity and pH values were inversely proportional, and microbiological analyses of coliforms showed no contamination, lactic bacteria were viable up to the 15th day of storage, treatment 3 showed the highest water holding capacity and syneresis decreased by raising the levels of PFPF. Fermented milk with the lowest level of addition of PFPF showed better scores and was the most preferred among panelists.

Key words: Whey, milk, pH, acidity, viable lactic acid bacteria.

INTRODUCTION

Passion fruit (*Passiflora edulis*) belongs to the family *Passifloraceae* (Sebrae, 2005) and is a fruit of tropical and subtropical climates; the fruit consists of approximately 52% peel, 34% pulp and 14% seeds and cultivation is aimed at the juice and pulp industry (Zeraik et al., 2010). Environmental care and waste reduction are increasing concerns of the food industry; therefore, viable alternatives to the use of food waste in the development of new products for human consumption must be proposed (Garmus et al., 2009).

*Corresponding author. E-mail: marcotonyrv@yahoo.com.br. Tel: +55 (64) 8122-1172. Fax: +55 (64) 3620-5640.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
Passion fruit by-products (peel and seeds) may have technological and biological characteristics of interest to the food industry (Martinez et al., 2012). According to Ambrosio-Ugri and Ramos (2012), after drying, passion fruit peel is ground to obtain passion fruit flour or passion fruit fibre. The passion fruit peel is composed of flavedo, which corresponds to the outer layer of yellow-green color, rich in insoluble fibre and albedo, corresponding to the white inner layer rich in soluble fibre, in particular pectin, with small amounts of mucilage (Janebro et al., 2008).

Dietary fibres helps the bowel function and are considered prebiotic; soluble fibres retard intestinal passage, gastric emptying and glucose uptake, helping to reduce blood cholesterol, insoluble fibres accelerate intestinal transit, increasing the fecal volume, slowing down glucose hydrolysis, contributing to the reduction of some colon diseases (Pereira, 2002) and can serve as a substrate for beneficial microorganisms such as probiotics (Gallina, 2009).

According to the World Health Organization (FAO, 2002), probiotics are defined as live microorganisms that when administered in adequate amounts, confer a health benefit to the host. Awaishheh et al. (2005) reports that bacteria belonging to the genus Lactobacillus, which colonize the small intestine of humans and combat pathogens such as Salmonella spp., and those of the genus Bifidobacterium, which colonize the large intestine of human and inhibits the growth of Escherichia coli and Candida spp., are major microbial species with probiotic properties. Lee et al. (1999) claims that products containing L. acidophilus and B. bifidum have the capacity of improving the peristaltic movements of the intestine, increasing absorption of nutrients, controlling or preventing intestinal infections by blocking the receptors of pathogens, inactivating the effects of enterotoxin and favoring the development of microorganisms resistant to pathogens, especially against Escherichia coli.

Gallina et al. (2012) have reported that the main technological challenge for the processing industry is the viability and stability of probiotic cultures and that probiotic foods should contain specific strains of probiotic microorganisms and maintain adequate levels of viable cells during product storage without interfering with flavor and texture.

Since the functional foods take an important place in the daily meal of the consumers, new studies must be carried out to: test ingredients, explore more options of food matrix that have not yet been industrially utilized, reengineer products and processes (Coman et al., 2012).

The aim of this work was to evaluate physical, chemical and microbiological parameters, sensory analysis and morphological structure of each fermented milk enriched of passion fruit peel flour (PFPF).

### MATERIALS AND METHODS

#### Development of Fermented milks

Refrigerated milk and milk whey were obtained from enzymatic coagulation of Mozzarella cheese in a dairy industry located in the city Rio Verde (GO, Brasil).

The processing of fermented milks was conducted at the Laboratory of Products of Animal Origin - Instituto Federal Goiano, Rio Verde Campus, GO, Brazil (IF Goiano). Milk and whey were filtered to eliminate physical contamination. Four treatments consisting of 4 L were prepared, with proportion of 60% milk and 40% milk whey added with 10% sucrose; subsequently, the mixture was submitted to heat treatment at 90°C for 3 min. After pasteurization, fermented milk was cooled to 42°C for the addition of the Bio Rich® lyophilized culture (Lactobacillus bulgaricus, Lactobacillus. acidophilus, Streptococcus termophilus and Bifidobacterium) and incubated at 42°C up to pH 4.5.

After coagulation, fermented milks were removed from the oven and cooled to reach 20°C, then clot breaking was performed using glass rod in circular movements for 1 min. After clot breaking, passion fruit peel flour was added in the following proportions: 1, fermented milk without addition with passion fruit peel flour (0%); 2, fermented milk added with 1% passion fruit peel flour; 3, fermented milk added with 2% passion fruit peel flour and 4, fermented milk added with 3% passion fruit peel flour. The formulations for each drink are presented in Table 1. After the addition of PFPF, fermented milks were packaged in aseptic polypropylene packages, identified and stored at 5°C for sensory evaluation, scanning electron microscopy (SEM), pH, acidity, syneresis, water holding capacity, viable lactic acid bacteria, and instrumental color during the 29 days of storage.

#### Physicochemical analyses

All analyses were performed in triplicate, except for the sensory analysis that was performed only once on the eighth day of storage.

<table>
<thead>
<tr>
<th>Table 1. Formulations of fermented milk drinks enriched with passion fruit peel flour (PFPF).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredients</strong></td>
</tr>
<tr>
<td>Whole milk (%)</td>
</tr>
<tr>
<td>Milk whey (%)</td>
</tr>
<tr>
<td>Sucrose (%)</td>
</tr>
<tr>
<td>PFPF (%)</td>
</tr>
<tr>
<td>Starter culture (mg/L)</td>
</tr>
</tbody>
</table>
Milk

Milk samples were collected for chemical composition evaluation at the Laboratory of Milk Quality, Research Center, School of Veterinary, Food and Animal Science - Federal University of Goiás, using MilkoScan 4000 equipment (Foss Electric A/S, Hillerod, Denmark) to obtain fat, protein, lactose and non-fat solid (NFS) results, expressed in percentage (%).

Milk whey

Analyses of pH, acidity, fat and protein were performed in Ecomilk equipment (Cap Lab) milk analysis.

Fermented milks

Fat

Fat was analyzed using the Gerber method as IAL (2005). About 10 ml of sulfuric acid were transferred to Gerber butyrometer and 11 ml of sample and 1 ml of isoamyl alcohol were added, sealed and centrifuged at 1200 ± 100 rpm for 15 min.

Protein determination

For crude protein analysis, total nitrogen was determined by the micro-Kjeldahl method according to official method No.960.52 of AOAC International (1997). Total nitrogen was converted into crude protein using factor 5.95 (Alencar; Alvarenga, 1991). The equipment used was digester block (Tecnal, TE-0363).

Titratable acidity determination

For titration of samples, 10 ml of diluted fermented milk were added to 10 ml of distilled water with five drops of 1% phenolphthalein solution, followed by titration with 0.1 N sodium hydroxide solution up to the appearance of a persistent pink color for approximately 30 seconds (Brazil, 2006). Acidity was determined according to the following equation: lactic acid (%) = (V x I x 0.9) /m (IAL, 2005).

pH

For pH assessment, bench Bel Engineer digital potentiometer was used. The electrode was inserted into the sample after homogenization without touching the bottom or the sides of the package, so the reading was carried out (Brazil, 2006).

Microbiological analyses

Analyses of total coliforms, Escherichia coli and estimation of viable lactic acid bacteria were performed at the Laboratory of Food Microbiology (IF Goiano) at storage times of 1, 8, 15, 22 and 29 days.

About 25 g of fermented milk were weighed and added to 225 ml of sterile peptone water and after homogenization, the solution was diluted to concentrations of 10⁻², 10⁻¹, 10⁻⁴, 10⁻⁵ and 10⁻⁶. The enrichment step for total coliform count with difference for Escherichia coli used 1 ml aliquots of concentrations of 10⁻¹, 10⁻² and 10⁻³, which were transferred into test tubes containing 10 ml of lauryl sulfate broth (LST) and incubated at 35°C for 24 h. Then, the presence of coliforms was confirmed using Brilliant Green Bile Broth (BGB) incubated at 35°C for 24 h and in E. coli broth (EC) incubated for 24 h in water bath at 45°C.

For viable cell count, the method used was “pour plate” in-depth plating, using MRS agar (Kasvi). Serial dilutions were made from 10⁰ to 10⁶ and plating performed in triplicate and incubated at 35°C for 48 h (Macedo, 1997). The results were presented as Log⁰ CFU/ml.

Syneresis determination

To determine syneresis, 30 g of yogurt were filtered in funnel and distributed on filter paper and after 5 h of draining, the supernatant was removed and weighed, and the syneresis rate was expressed in percentage (%), which was obtained by the ratio between supernatant weight and the total sample weight multiplied by 100 (Riener et al., 2010).

Water holding capacity

The water holding capacity (WHC) was determined in triplicate according to the modified method of Parnell-Clunies et al. (1986), being expressed as percentage (%), according to the following Equation:

WHC (%) = 100 x (initial sample weight - supernatant weight) / sample weight

Color parameters

Instrumental color parameters (L*, a* and b*) of fermented milk samples were analyzed in triplicate in colorimeter (HunterLab, 1998) at the Post-Harvest Laboratory of Plant Products (IF Goiano).

Sensory evaluation

This study has been submitted and approved by the ethics research committee of Instituto Federal Goiano, with number 20/2013.

Analyses were performed at the Laboratory of Sensory Analysis (IF Goiano). Fifty-one untrained panelists aged 18-56 years, 64.3% females and 35.7% males, participated at the sensory analysis. Fermented milk samples were coded with three-digit numbers and presented under white light in 50 ml white cups (± 20 g fermented milk) to each of the panelists.

The sensory analysis used acceptance and ordination tests: for acceptance, evaluation was based on scores awarded by panelists through a nine-point hedonic scale, where value one (1) represented "dislike extremely" and nine (9) "liked extremely", assessing flavour, aroma, texture and colour. The ordination test was analyzed, in which panelists put on an increasing order the samples they liked the most and those they liked less (IAL, 2005).

Scanning electron microscopy

Fermented milk samples were lyophilized in lyophilizer equipment (Enterprise II / Terroni®). Then, samples were defatted and analyzed in scanning electron microscope (JSM - 6610 / Jeol®) for the acquisition of images.

Statistical analyses

The experimental design adopted in the analysis was a completely randomized design (CRD) and syneresis, water holding capacity and were presented by means of regression while pH, acidity, viable lactic acid bacteria values, color parameters results were analyzed by comparison among treatment means using the Tukey's test. Analyses were performed using SISVAR and Sigma Plot 11.0 software.
RESULTS AND DISCUSSION

The composition of the milk used in the manufacture of fermented milks (fat 3.6% ± 0.04; acidity 0.16 ± 0.02; density 1.030 g/100 ml; cryoscopy -0.530ºH; NFS 8.4% ±0.03; protein 3.12% ± 0.15 and pH 6.70) shows that milk was in accordance with Normative Instruction N° 62/2011, which establishes the following minimum physicochemical parameters: fat, 3.0 g/100 g; acidity from 0.14 to 0.18 g of lactic acid/100 ml; relative density from 1.028 to 1.034 g/ml; Cryoscopic index from -0.512 ºC at 0.531 ºC; NFS at least 8.4%; protein 2.9%; whey showed fat 0.31% ± 0.16; acidity 0.10 ± 0.07; density 1.026 g/100 ml; cryoscopy of -0.500 ⁰H; NFS 0% ± 6.25; protein 1.10% ± 0.08 and pH 6.54.

The inclusion of increasing levels of PFPF in the preparation of fermented milks did not affect the fat content (Table 2); however, there was an increase of the protein content of fermented milks with the addition of PFPF, which was due to its average protein content of 15.4%, considered high.

The fat and protein values of the present study are lower than those observed by Gallina et al. (2011) in fermented milks with and without addition of probiotics and prebiotics (fat 2.8% and protein from 4.03 to 4.28%) by Toledo et al. (2013) in yogurts added of pulp and passion fruit flour (Passiflora edulis) (fat from 2.42 to 2.88% and protein from 2.98 to 4.20%) and Gerhardt et al. (2013) in fermented milks added of ricotta whey and collagen hydrolysate (fat from 2.90 to 3.10% and protein from 2.99% to 4.44%).

The titratable acidity and pH results of fermented milks throughout the storage period were inversely proportional. Fermented milk with 0% PFPF showed lower acidity while pH was the highest on the fifteenth day of storage. In this period, fermented milk with 3% PFPF showed high acidity and the lowest pH; at the end of the twenty-ninth day of storage, titratable acidity decreased and pH increased, and fermented milk with 0% PFPF showed the lowest acidity and fermented milk with 3% PFPF showed the highest pH (Table 3).

The titratable acidity results of this study were higher than those reported by Gallina et al. (2012), who worked with fermented milk produced from symbiotic fermented milk added of guava pulp and found variations from 0.41 to 0.42%, but the pH values were lower to those reported by the author who observed values from 4.40 to 4.42. Results similar to those of this study were observed by Gerhardt et al. (2013) in fermented milks using whey ricotta and collagen hydrolysate, which ranged from 0.72 to 0.91%; pH results corroborate those found by Toledo et al. (2013) in yogurts added of passion fruit pulp and flour (Passiflora edulis), ranging from 4.49 to 3.63.

Similar values were observed by Costa et al. (2013) in a study with fermented milk made with different stabilizers / thickeners (titratable acidity from 0.55 to 0.61% and pH from 3.95 to 4.07), and those reported by Gonçalves and Leão (2013) in yogurts added of mixed flours from apple, passion fruit and grape waste (titratable acidity from 0.84 to 0.89% and pH from 4.20 to 4.60).

The 29th day of storage the samples of fermented milk showed a decrease in acidity. This can be explained by the fact that the 29th day of storage the samples fermented milk showed incidence fungus samples, which caused decrease in acidity. Coelho et al. (2009), when evaluating shelf life in yogurt for 60 days there were decreased acidity for the high count of yeasts and molds. Franco and Langraf. (2003) reported that some species of yeasts and molds using lactic acid, leading consequently to an increase in the pH.

Regarding the count of coliforms, none of the samples showed typical colony formation, which results are similar to those reported by Tebaldi et al. (2007) in 20 samples of fermented milks commercialized in southern Minas Gerais and by Araújo et al. (2012) in passion fruit-flavored sundae-type yogurt, where microbiological results for analysis of coliform bacteria showed no turbidity with acidification (turning) and gas production, indicating absence of this microorganism in the samples analyzed. Paula et al. (2012) observed presence of <10 CFU ml⁻¹ of coliforms at 30°C and 45°C in fermented milk.

The viable cell count results of Figure 2 shows that 3% PFPF concentration up to the fifteenth day of storage showed the highest number of CFU/ml, followed by concentrations of 2 and 1%, which is in accordance with the current legislation that establishes minimum number of viable bacteria per milliliter (10⁶ CFU) during the validity period (Penna, 2002). On the fifteenth day of storage, it was observed that the 0% PFPF showed

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PFPF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.43 ±0.15a</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>2.19 ±0.03c</td>
</tr>
</tbody>
</table>

*Small letters in the line do not differ from each other according to Tukey’s test at 5% significance level.
Table 3. Acidity (%) and pH fermented milk with increasing levels of flour passion fruit peel during storage.

<table>
<thead>
<tr>
<th>Treatment (%)</th>
<th>Storage period (Days)</th>
<th>Acidity</th>
<th>pH</th>
<th>Acidity</th>
<th>pH</th>
<th>Acidity</th>
<th>pH</th>
<th>Acidity</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>15</td>
<td>22</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.63±0.01Ab</td>
<td>4.36±0.09Aa</td>
<td>0.68±0.28Bb</td>
<td>4.23±0.01Ab</td>
<td>1.12±0.16Aa</td>
<td>4.20±0.01Ab</td>
<td>1.32±0.04Aa</td>
<td>4.10±0.01Bb</td>
<td>0.56±0.06Ab</td>
</tr>
<tr>
<td>1</td>
<td>0.74±0.03Abc</td>
<td>4.31±0.05ABa</td>
<td>0.87±0.07ABbc</td>
<td>4.20±0.05Aa</td>
<td>1.05±0.01Aab</td>
<td>4.14±0.05ABb</td>
<td>1.27±0.03Aa</td>
<td>4.00±0.01Bb</td>
<td>0.70±0.06Ac</td>
</tr>
<tr>
<td>2</td>
<td>0.73±0.02Ab</td>
<td>4.26±0.09Ba</td>
<td>1.09±0.3Aa</td>
<td>4.25±0.01Aa</td>
<td>1.16±0.07Aa</td>
<td>4.06±0.05ABb</td>
<td>1.36±0.04Aa</td>
<td>4.02±0.01Bb</td>
<td>0.70±0.06Ac</td>
</tr>
<tr>
<td>3</td>
<td>0.72±0.02Ab</td>
<td>4.18±0.02Bb</td>
<td>1.19±0.1Aa</td>
<td>4.12±0.05Ab</td>
<td>1.19±0.03Aa</td>
<td>3.98±0.05Bb</td>
<td>1.34±0.03Aa</td>
<td>3.97±0.01Bb</td>
<td>0.80±0.09Ab</td>
</tr>
</tbody>
</table>

* Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey’s test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1% = 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

Table 4. Viable lactic acid bacteria (CFU/mL) in fermented milks with increasing levels of passion fruit peel flour during storage.

<table>
<thead>
<tr>
<th>Treatment (%)</th>
<th>Storage period (Days)</th>
<th>1</th>
<th>8</th>
<th>15</th>
<th>22</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>15</td>
<td>22</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.62x10^6±0.07Aa</td>
<td>9.05 x10^5±0.11Aa</td>
<td>5.05 x10^5±0.10Bb</td>
<td>4.67 x10^5±0.8Ab</td>
<td>0±0Ac</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.56 x10^5±0.30Aa</td>
<td>9.30 x10^5±0.12Aa</td>
<td>8.46 x10^5±0.01Aa</td>
<td>1.00 x10^5±0.1Bb</td>
<td>0±0Ab</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7.26 x10^5±0.20Aa</td>
<td>9.27 x10^5±0.15Aa</td>
<td>8.50 x10^5±0.5Aa</td>
<td>1.00 x10^5±0.1Bb</td>
<td>0±0Ab</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.27 x10^5±0.12Aa</td>
<td>9.96 x10^5±0.03Aa</td>
<td>8.41 x10^5±0.6Aa</td>
<td>0±0Bb</td>
<td>0±0Ab</td>
<td></td>
</tr>
</tbody>
</table>

* Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey’s test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1% = 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

Values lower than 10^6 CFU/ml. On the twenty second day of storage 3%, 2% and 1% PFPF drastically decreases while the 0% PFPF concentration remained constant, showing a decrease in CFU/ml only on the twenty-ninth day of storage (Table 4).

The lactic acid bacteria results corroborate those found by Gallina et al. (2011) assessing the viability of lactic acid and probiotic bacteria during shelf-life, which remained within adequate levels (10^6 CFU/ml) up to 15 days. Coman et al. (2012), while working with fermented milk added with different percentages of wheat flour and oat bran in concentrations (control, 2, 4 and 6%) with 2 types of sepas (L. rhamnosus IMC 501®, L paracasei IMC 502®, SYNBIO®) for each treatment; up to 28 days of storage all treatments showed lacticas viable with levels above 10^5 CFU/ml. Higher values were found by Matta et al. (2012) in symbiotic rice-based drink after 22 days of storage which showed from 10^9 to10^10 CFU/ml and those reported by Ribeiro et al. (2014) with fermented milk made with Camellia sinensis that after 30 days of storage showed 10^7 CFU/ml.

The increase in PFPF levels led to a decrease in syneresis values over a period of twenty nine days of storage, and fermented milk with the highest concentration (3% PFPF) resulted in the lowest syneresis value, while fermented milk with 0% PFPF showed the highest syneresis value,
indicating that there was a better whey release. WHC showed increased values in fermented milk with 3% PFPF and decreased values for 0% PFPF, these results show that syneresis and WHC have an inverse relationship (Figure 1).

Similar results were observed by Toledo et al. (2013) in a study with yogurt added of passion fruit pulp and flour (Passiflora edulis), who found that samples with lower PFPF content showed higher whey release and consequently higher syneresis values. The same behavior was observed by Antunes et al. (2007), who studied nonfat probiotic yogurt in combination with starter.
Table 5. Mean L* coordinate values of milk drinks with increasing levels of passion fruit peel flour during storage.

<table>
<thead>
<tr>
<th>Treatment (%)</th>
<th>Storage period (days)</th>
<th>1</th>
<th>8</th>
<th>15</th>
<th>22</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>89.86±0.02Abc</td>
<td>89.04±0.06Ad</td>
<td>89.42±0.06Ac</td>
<td>90.87±0.04Aa</td>
<td>90.50±0.02Aab</td>
</tr>
<tr>
<td>1</td>
<td>72.22±0.50 Bc</td>
<td>72.21±0.19Bc</td>
<td>73.11±0.11Bab</td>
<td>73.45±0.16Ba</td>
<td>72.46±0.17Bbc</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>65.34±0.77Cb</td>
<td>65.80±0.72Cabc</td>
<td>66.13±0.37Ca</td>
<td>66.19±0.16Ca</td>
<td>65.36±0.80Cb</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>62.67±1.13Db</td>
<td>62.67±0.26Db</td>
<td>64.95±0.37Da</td>
<td>62.51±0.14Db</td>
<td>62.38±0.13Db</td>
<td></td>
</tr>
</tbody>
</table>

*Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey’s test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1% = 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

Table 6. Mean a* coordinate values of milk drinks with increasing levels of passion fruit peel flour during storage.

<table>
<thead>
<tr>
<th>Treatment (%)</th>
<th>Storage period (Days)</th>
<th>1</th>
<th>8</th>
<th>15</th>
<th>22</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>1.99±0.04Dc</td>
<td>1.87±0.04Dab</td>
<td>1.99±0.02Da</td>
<td>1.71±0.02Cb</td>
<td>2.06±0.01Da</td>
</tr>
<tr>
<td>1</td>
<td>4.40±0.16Cb</td>
<td>4.01±0.04Cb</td>
<td>4.31±0.14Cb</td>
<td>4.33±0.10Cb</td>
<td>4.51±0.14Cb</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.39±0.14Ba</td>
<td>5.72±0.19Bb</td>
<td>5.89±0.14Ba</td>
<td>6.26±0.08Ba</td>
<td>6.11±0.25Bb</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.01±0.21Aa</td>
<td>6.69±0.13Aa</td>
<td>6.44±0.13Aa</td>
<td>6.93±0.03Aa</td>
<td>7.03±0.04Aa</td>
<td></td>
</tr>
</tbody>
</table>

*Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey’s test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1% = 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

Table 7. Average b* coordinate values of milk drinks with increasing levels of passion fruit peel flour during storage.

<table>
<thead>
<tr>
<th>Treatment (%)</th>
<th>Storage period (Days)</th>
<th>1</th>
<th>8</th>
<th>15</th>
<th>22</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>12.45±0.08Db</td>
<td>11.60±0.06Dc</td>
<td>13.25±0.06Ca</td>
<td>13.34±0.01Da</td>
<td>13.32±0.02Ca</td>
</tr>
<tr>
<td>1</td>
<td>19.64±0.29Cbc</td>
<td>19.27±0.12Cbc</td>
<td>19.26±0.04Bc</td>
<td>20.04±0.07Cabc</td>
<td>20.79±0.16Ba</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>22.93±0.18Bbc</td>
<td>22.26±0.12Bcd</td>
<td>21.81±0.19Ad</td>
<td>23.05±0.06Bb</td>
<td>24.27±0.29Aa</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>24.08±0.43Aa</td>
<td>24.24±0.08Aa</td>
<td>24.49±1.29Ab</td>
<td>24.24±0.04Aa</td>
<td>24.72±0.08Aa</td>
<td></td>
</tr>
</tbody>
</table>

*Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey’s test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1% = 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

culture and milk whey protein concentrate and found lower syneresis levels and increased whey retention capacity in formulations added of protein concentrate, and by Gerhardt et al. (2013), who prepared 11 treatments of fermented milks varying formulations with ricotta whey and collagen hydrolysate and observed that samples containing less collagen (0.65 and 0.5%) showed higher syneresis values. The results indicate that the addition of solids to fermented milks had an effect on syneresis.

The L* parameter (Table 5) indicates brightness and can determine values between zero (0) and one hundred (100), called black and white, respectively. The inclusion of increasing levels of PFPF in the preparation of fermented milks influence the brightness parameter (L*), and with increasing proportion of flour in treatments, there was a decrease in L*, corroborating results by Toledo et al. (2013) in the characterization of yogurts added of passion fruit pulp and flour (Passiflora edulis). Parameter a* (Table 6) showed significant difference (P<0.05) among treatments, and increasing flour levels led to an increase in a* values. Treatment with 0% was negative (-a*) towards green while treatments with 1, 2 and 3% flour were positive (+a*) towards red.

Parameter b* (Table 7) showed the addition of increasing levels of PFPF in fermented milks increased the b* chromaticity coordinate values. According to Caldeira et al. (2010), b* values greater than zero go
towards yellow and $b^*$ values less than zero go towards blue. Yellow coloration is related to the use of milk whey.

The test results are presented in Table 8: four types of fermented milks presented significant differences (P<0.05) in the aroma, flavour, acidity, viscosity, appearance and colour, presented notes between 4.4 to 7.4 (disliked slightly to liked moderately), from 3.20 to 7.85 (disliked moderately to liked moderately), from 4.22 to 7.31 (disliked slightly to liked moderately), from 4.38 to 5.04 (disliked slightly to indifferent), from 4.96 to 7.35 (disliked slightly to liked moderately), from 5.28 to 7.22 (indifferent to liked moderately).

However, fermented milk without addition of PFPF obtained higher score in relation to parameters of fermented milks added of PFPF.

Fermented milks added with PFPF showed sand-like texture; description corroborated by Espírito Santo et al. (2013) when studying probiotic yogurt enriched with passion fruit fibre. According to Gonçalves and Leão (2013), yogurt added with mixed flour containing passion fruit peel and apple bagasse showed acceptability between 5 (not liked nor disliked) and 6 (liked slightly).

Figure 2 shows the preference of consumers regarding the addition of PFPF to fermented milks, in which panelists evaluated from the most preferred to the least preferred fermented milk, and it was observed that among the 50 panelists, the control drink (without PFPF) was the most preferred, followed by fermented milks added of 1% PFPF and 2% PFPF and the least preferred fermented milk was that added of 3% PFPF.

According to Figure 2, it was observed that fermented milks added with passion fruit peel fibre were rejected, and according to Espírito Santo et al. (2013), this rejection is explained by the fact that panelists were unfamiliar with the consumption of yogurt added of fibre, which is corroborated by Ribeiro et al. (2010), when reporting that the Brazilian yogurt market is dominated by yogurt with fruit flavor (about 95% of the market), and colorful and sweet yogurts are preferred by consumers.

However, some sensory properties of fermented milks such as aroma, flavor, acidity, viscosity, appearance and color may have been underestimated by panelists because they are not used to consume fermented milks added of fibre. When the 51 panelists were asked if they would buy the fermented milk they liked the most, 90.2% responded yes and only 9.8% responded they would not buy.

Fermented milks added with passion fruit peel flour were intended for fast freezing in Ultra Freezer (Terroni®) at -50°C and then freeze-dried in lyophilizer equipment (Enterprise II/Terroni®). After lyophilization, samples were degreased by soxhlet method, stored in plastic bags and placed in desiccator with silica gel and then transported to the Laboratory of Electron Microscopy (LabMic) at the Federal University of Goiás. Samples were mounted on stubs and covered with gold plating. At the end of this procedure, stubs were examined in scanning electron microscopy (JSM - 6610 / Jeol®). Figure 3 shows the scanning electron microscopy images of fermented milk drinks added with passion fruit peel flour at 500x magnification.

The images at Figure 3 show the microstructure of a protein matrix and with the addition of flour passion fruit peel showed incidence of surface holes, which are called pits (Martins et al., 2009) and are more present the fermented milk added with 3% PFPF (Figure D), with higher porosity compared to control treatment. It was observed that fibres do not show a smooth and homogeneous surface, but rather quite irregular surface covered by recesses and protrusions.

**Table 8. Average acceptance of milk drinks with increasing levels of passion fruit peel flour after 8 days of storage.**

<table>
<thead>
<tr>
<th>Treatment (%)</th>
<th>Aroma</th>
<th>Flavour</th>
<th>Acidity</th>
<th>Viscosity</th>
<th>Appearance</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.42±1.31a</td>
<td>7.85±1.41a</td>
<td>7.31±1.28a</td>
<td>5.04±1.50c</td>
<td>7.35±1.30 a</td>
<td>7.22±1.49a</td>
</tr>
<tr>
<td>1</td>
<td>5.60±1.93b</td>
<td>5.10±2.22b</td>
<td>5.54±2.04b</td>
<td>5.80±2.40 a</td>
<td>6.08±1.96b</td>
<td>6.22±1.61b</td>
</tr>
<tr>
<td>2</td>
<td>4.96±2.02cb</td>
<td>3.86±2.17c</td>
<td>4.58±1.78cb</td>
<td>4.52±2.29b</td>
<td>5.22±2.27cb</td>
<td>5.76±1.78cb</td>
</tr>
<tr>
<td>3</td>
<td>4.36±1.99c</td>
<td>3.20±2.21c</td>
<td>4.22±2.16c</td>
<td>4.38±2.51b</td>
<td>4.96±2.15c</td>
<td>5.28±1.99c</td>
</tr>
</tbody>
</table>

*Means with same letters in the column do not differ by the Tukey’s test at 5% significance level.

**Conclusion**

The results allowed concluding that the use of passion fruit peel for the production of flour is an alternative for the reuse of this product, as it is rich in nutrients. Fermented milks produced achieved physical and chemical parameters established by brazilian law, with no contamination by total and thermotolerant microorganisms throughout the 29 days of storage.

The viability of fermented milks showed efficiency up to the fifteenth day of storage; and in relation to acceptance, the results demonstrated that fermented milks added of PFPF reached satisfactory sensory acceptance.

**Conflict of interest**

Authors declare there is no conflict of interest for this research.
REFERENCES


Araújo TF, Ferreira EG, Souza JRM, Bastos LR, Ferreira CLLF (2012). Desenvolvimento de iogurte tipo suíoa de morango adicionado de polpa de goiaba e avaliação de viabilidade simbiótica adicionado de polpa de goiaba e avaliação de viabilidade simbiótica. Ind Latic 67:65-76.


Figure 3. Scanning electron microscopy. (A) fermented milk drink with no addition of PFPF, (B) fermented milk drink with 1% PFPF, (C) fermented milk drink with 2% PFPF, (D) fermented milk drink with 3% PFPF.


Macedo REF (1997). Desenvolvimento de bebida láctea fermentada a base de extrato hidrossolúvel de soja e soro de leite de búfala por cultura mista de Lactobacillus casei Shirota e Bifidobacterium adolescentis. 112f. Dissertação (Mestrado em Tecnologia Química) - Universidade Federal do Paraná, Curitiba.


Pereira J (2002). Tecnologia e qualidade de cereais: arroz, trigo, milho e aveia. Lavras: UFLA/FAEPE.
Seroprevalence of *Ehrlichia canis* in dogs from Monterrey, Mexico


Universidad Autónoma de Nuevo León, Facultad de Medicina Veterinaria y Zootecnia. Av. Universidad S/N, Ciudad universitaria, San Nicolás de los Garza, Nuevo León. C.P. 66451, México.

Received 17 June, 2015; Accepted 17 August, 2015

Infection by *Ehrlichia canis* in dogs causes the worldwide tick-borne disease called canine monocytic ehrlichiosis (CME), and the presence of *E. canis* has been serologically demonstrated in all continents, with prevalence ranging from 0.2 to 80%. In southern Mexico, a prevalence of 44% was found, whereas in the northwest part of the country it varies from 21 to 49%. In the present study, a commercial kit for the detection of antibodies against *E. canis* was used in 391 dogs from the city of Monterrey, which is located at northeast of Mexico. A total of 54 samples were positive, giving a prevalence of 13%. According to sex, prevalence was 14% for males and 13% for females. Positive animals varied in age from 21 to 132 months old and only 10 of them presented ticks. As in the southern and northwest parts of Mexico, CME is present in northeast region, although with a lower prevalence.

**Key words:** *Ehrlichia canis*, dogs, serology, Mexico.

**INTRODUCTION**

The dog can be infected by different species of *Ehrlichia*, and *Ehrlichia canis* is the most important species; it is transmitted by *Rhipicephalus sanguineus* (Pusterla et al., 1998), although the American dog tick, *Dermacentor variabilis*, has also been shown to be a vector transmitter of this disease (Johnson et al., 1998).

*E. canis* is the primary causal agent of Canine Monocytic Ehrlichiosis (CME), a worldwide tick-borne disease (Kamani et al., 2013; Stich et al., 2008); it is an obligate intracellular gram-negative bacterium that multiply in eukaryotic cells, like monocytes and macrophages, developing leucopenia and thrombocytopenia (Stich et al. 2008).

CME can be divided in an acute phase, beginning from 8 to 20 days after infection, involving anemia, anorexia, ataxia, conjunctivitis, depression, fever, leucopenia, ocular discharge, thrombocytopenia and vomiting that end with a partial recovery of the dog, followed by an months-to-years subclinical phase. The chronic phase can be mild or severe with recurrent clinical and hematologic signs like pancytopenia, hemorrhage, monocytosis, lymphocytosis and weight loss (Stich et al., 2008). It is thought that *E. canis* is the only agent responsible for the development of CME. It has been

*Corresponding author. E-mail: vriojas@hotmail.com. Tel: (81) 1340-4390, ext. 3616.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
suggested that this bacteria could be the causal agent in the human granulocytic ehrlichiosis and it has even been successfully isolated from human patients with symptoms similar to those caused by other infections by \textit{E. chafeensis} and \textit{E. ewingii} (Perez et al., 2006; Nicholson et al., 2010).

Serologic evidence of previous studies around the world indicated that \textit{E. canis} is present among dogs throughout all continents, where serologic studies have found a prevalence ranging from 30 to 80\% in some countries of Africa (Azzag et al., 2015; Inokuma et al., 2006; Eoghain and Raoult, 2004; Ndip et al., 2005; Davoust et al., 2006), whereas in some Asian countries it was of 0.2 to 30\% (Inokuma et al., 1999; Rajamanickam et al., 1985; Stich et al., 2008).

In Europe, a prevalence ranging from 2 to 50\% have been found (Solano-Gallego et al., 2006; Cocco et al., 2003; Pusterla et al., 1998; Sainz et al., 1995). A study realized in the USA detected most often \textit{Ehrlichia} antibodies in dogs in the Southeast, with 1.3\% of samples testing positive, whereas other regions showed lower numbers ranging from 0.3 to 0.6\%. (Bowman et al., 2009). Different results were found in Oklahoma, where the prevalence of \textit{E. canis} was 10.8\% by serology and 3.1\% by the polymerase chain reaction (PCR) method (Murphy et al., 1998). Among dogs belonging to the U.S.A. military forces, seropositivity to \textit{E. canis} ranged from 8\% in cold zones (above 45° latitude) to 24\% in temperate places (between 40 and 45° latitude); a 13\% prevalence was found in tropical zones (below 40° latitude) (Keefe et al., 1982).

Several studies on \textit{E. canis} prevalence have been realized in Brazil. Melo et al. (2011) reported a prevalence of 74.4\% in urban and of 67.5\% in rural dogs (overall frequency of 70.9\%), whereas Witter et al. (2013) informed a seroprevalence of 70.1\%; in this last study the frequency of \textit{E. canis} infection was of 23.3\% by PCR. On the other hand, also in Brazil a prevalence of \textit{E. canis} of 41.5\% by IFA and of 9.4\% was found in cats (Braga et al., 2014).

In Grenade, 43.8\% of dogs tested were positive for \textit{E. canis} (Yabsley et al., 2008). In Mexico, studies performed in the southern area found 44\% of seropositive dogs to \textit{E. canis} with ELISA testing (Rodriguez-Vivaz et al., 2005), 36\% prevalence by PCR and 45\% in dogs located at animal shelters (Pat-Nah et al., 2015), whereas at the northwest region a prevalence of 49\% was found (Tinoco-Gracia et al., 2007). In another study (Haro-Alvarez et al., 2007), a 21.6\% prevalence, with 40\% of the dog population in contact with \textit{E. canis}, have been reported.

Although much have been said about the presence of this disease in Mexico, currently there are no reports of it in the northeast region; therefore, the goal of the present study was to estimate the seroprevalence of ehrlichiosis in dogs from the city of Monterrey, located in this part of Mexico.

**RESULTS AND DISCUSSION**

For the present work, 391 blood samples were taken from dogs located in the city of Monterrey, Mexico; antibodies against \textit{E. canis} were found in 54 samples, resulting in a prevalence of 13.8\%. Regarding to sex, animal’s samples comprised 173 males and 218 females of which 25 males and 29 females were positive, giving a prevalence of 14.5 and 13.3\% respectively (Table 1).

Positive animals varied in age from 21 to 132 months old; and according to size, 19 were small, 27 medium and eight large. Only 10 positive animals presented ticks (\textit{Rhipicephalus sanguineus}). The distribution of positive animals by breed is presented in Table 2; the biggest percentage of positive dogs was for mixed-breed.

Comparing the frequencies found in the present work to other studies on the subject can be difficult due to the wide range of prevalence reported according to the continent in which such studies were performed (from 0.2 to 80\% in Africa, Asia and Europe), as can be seen in the

**MATERIALS AND METHODS**

Blood samples were obtained from 391 dogs of different breeds in the city of Monterrey, using as inclusion factor only animals with fixed address, age over 6 months. It was decided to sample only one animal per house in case of having more than one dog. The examination of the dogs started with physical evaluation followed by blood sampling. All dogs showed no symptoms of any disease.

This study was carried out during 2014 in the city of Monterrey, Nuevo Leon, located in the northeast of Mexico, with a territorial extension of 451.30 square kilometers. Location coordinates are 25°40’17” N, 100° 18’31” W. Altitude is 530 m above sea level. The climate of the region has an average of 21°C, but because of annual thermal oscillation of 18°C, with important contrast among seasons. In summertime, temperatures above 30°C are common with an average in July and August of 34°C. In Winter, cold air arrive constantly to the region, often accompanied of humidity from the coast. making the temperature descend drastically, and every year at least two to three days are recorded with 0°C or less. The average annual precipitation is of 600 ml spread mainly in summer, with September as the rainiest month. The city was divided in quadrants in accordance with its cartographic plan. From this map, the 15 most urbanized quadrants were chosen, since the others belonged to non-well developed neighborhoods and little human population. Sampling was performed according to the dog population density and owner cooperation, and only one animal per city block and only one animal per house. To determine the sample size, calculations were made in basis of the population’s representative sample (infinite), with precision level of 5\%, confidence level of 95\% and a power of statistical test of 80\% in order to ensure reliability of the results and that they could be translated to the population under study. Sample size was determined using Epidat 3.1. For the \textit{in vitro} diagnosis for detection of antibodies against \textit{E. canis} in the samples, a commercial kit canine SNAP®4Dx (IDEXX labs, Inc. USA) was used. Before starting the procedure, samples must be at room temperature. The sera, either fresh or refrigerated, were utilized after no more than a week from the sampling. Sensitivity and specificity of the kit for the disease are reported with a minimum of 98.8\% and 100\%, respectively, and detects antibodies generated against peptides from the proteins p30 and p30-1 of \textit{Ehrlichia}. (O’Connor et al., 2004, 2006).
Introduction section. However, when we compare our work with studies done in the U.S.A., we find that a very similar prevalence (10.8%) was found in Oklahoma by serology, although in this same paper the prevalence was 3.1% by PCR (Murphy et al., 1998). This low prevalence of *E. canis* by PCR in the U.S.A. is confirmed by other work that informed 1.3% in the Southwest and 0.3 to 0.6% in other areas of that country (Bowman et al., 2009). On the other hand, a very large prevalence of *E. canis* in dogs has been informed in both Grenade (Yabsley et al., 2008) and south Mexico (Rodriguez-Vivaz et al., 2005); in the first, the prevalence was 43.8% and in the second of 44%. Other studies in Mexico concluded that the prevalence of *E. canis* is high, ranging from 40 to 49% in both the northwest and south part of the country (Haro-Álvarez et al., 2007; Pat-Nah et al., 2015; Tinoco-Coello et al., 2009). These results are in disagreement with the ones presented in our work. Therefore, a wide range of results regarding the prevalence of *E. canis* in dogs exist in the literature. One possible explanation to this disagreement could be the diagnostic method. Work in this subject indicate that the IFA method may be better than ELISA (Jimenez-Coello et al., 2009); using the IFA method these authors found a 8.7% prevalence, which is closer to the results informed in the present work; the previously mentioned work also indicates that the sampling method can also have an influence in the results. We think that both the sampling method and the technique used in the present study give an accurate view of the actual prevalence of *E. canis* infection in dogs located in the northeast region of Mexico; the prevalence we found is close to the ones reported in the U.S.A. and in the work done by Jimenez-Coello et al. (2009) in Mexico, but much lower to the prevalence informed in both the south and northwest areas of Mexico, as well as in other parts of the world as mentioned above.

### Conflict of interests

The authors did not declare any conflict of interest.

### ACKNOWLEDGEMENTS

We wish to thank the Programa Integral para el Fortalecimiento Institucional (PIFI) program of the Universidad Autónoma de Nuevo León for financial support to this work.

### REFERENCES


Eoghain GN, Raoul D (2004). Antibodies reactive with *Bartonella henselae* and *Ehrlichia canis* in dogs from the communal lands of

### Table 1. Distribution of positive animals for *Ehrlichia canis* by sex.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of dogs sampled</th>
<th>Number of positive dogs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>218</td>
<td>29</td>
<td>13.3</td>
</tr>
<tr>
<td>Male</td>
<td>173</td>
<td>25</td>
<td>14.5</td>
</tr>
<tr>
<td>Total</td>
<td>391</td>
<td>54</td>
<td>13.8</td>
</tr>
</tbody>
</table>

### Table 2. Number of positive animals to *Ehrlichia canis* according to breed.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number of positive animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basset Hound</td>
<td>1</td>
</tr>
<tr>
<td>Boxer</td>
<td>3</td>
</tr>
<tr>
<td>Bull Terrier</td>
<td>3</td>
</tr>
<tr>
<td>Chihuahua</td>
<td>2</td>
</tr>
<tr>
<td>Cocker Spaniel</td>
<td>2</td>
</tr>
<tr>
<td>Collie</td>
<td>1</td>
</tr>
<tr>
<td>Mixed breed</td>
<td>16</td>
</tr>
<tr>
<td>Doberman</td>
<td>1</td>
</tr>
<tr>
<td>French poodle</td>
<td>5</td>
</tr>
<tr>
<td>Great Dane</td>
<td>2</td>
</tr>
<tr>
<td>Maltese</td>
<td>4</td>
</tr>
<tr>
<td>Labrador</td>
<td>4</td>
</tr>
<tr>
<td>Schnauzer</td>
<td>2</td>
</tr>
<tr>
<td>German shepherd</td>
<td>1</td>
</tr>
<tr>
<td>Shar Pei</td>
<td>2</td>
</tr>
<tr>
<td>Shih tzu</td>
<td>4</td>
</tr>
<tr>
<td>Westhighland</td>
<td>1</td>
</tr>
</tbody>
</table>
Full Length Research Paper

Detection of *Mycobacterium bovis* in bovine carcasses by multiplex-PCR

Ricardo César Tavares Carvalho¹, Vinicius Silva Castro³, Flávia Galindo Silvestre Silva⁴, Carlos Adam Conte Junior⁵, Walter Lilienbaum⁶, Vânia Margaret Flosi Paschoalín¹ and Eduardo Eustáquio de Souza Figueiredo²,³*  

¹Programa de Pós Graduação em Ciência de Alimentos - Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro/RJ, Brasil.  
²Laboratório de Microbiologia Molecular de Alimentos, Faculdade de Nutrição, Universidade Federal de Mato Grosso, Cuiabá/MT, Brasil.  
³Programa de Pós Graduação em Ciência Animal, Universidade Federal de Mato Grosso, Cuiabá/MT, Brasil.  
⁴Laboratório de Imunogenética e Biologia Molecular, Hospital Geral Universitário - Universidade de Cuiabá, Cuiabá/MT, Brasil.  
⁵Faculdade de Veterinária, Universidade Federal Fluminense, Niterói/RJ, Brasil.  
⁶Instituto Biomédico, Universidade Federal Fluminense, Niterói/RJ, Brasil.

Received 20 May, 2015; Accepted 20 July, 2015

The causative agent of bovine tuberculosis (BTB) is *Mycobacterium bovis*, a bacterium belonging to the *M. tuberculosis* complex (MTC). The definitive diagnosis is achieved through isolation and identification of *M. bovis* from clinical samples, using a combination of traditional culture and biochemical methods, which is considered the “gold standard”. This procedure is cumbersome and time-consuming. We evaluated a multiplex-PCR (m-PCR) assay for the direct detection of *M. bovis* DNA from tissue with BTB-suspected lesions. A dairy herd consisting of 270 adult cattle where 34 animals were positive to the tuberculin skin test has been reported. At 30 days after the tuberculin test, all 34 reactive animals were slaughtered and subjected to a necropsy procedure. A pool of tissue samples representative of each animal (lung and mediastinal, scapular and retropharyngeal lymph nodes) were collected and subjected to bacteriological culture and m-PCR. *Mycobacterium* spp. was isolated in 50% (17/34) of the collected samples. When using m-PCR directly from tissue fragments, it was possible to detect *M. bovis* in 67.6% (23/34) of the collected samples including 15 samples isolated by bacteriological culture. High performance liquid chromatography (HPLC) was used to differentiate the 17 isolated strains of *Mycobacterium* spp., from the *Mycobacterium tuberculosis* complex (MTC) or other *Mycobacterium* sp. not belonging to the MTC. The use of m-PCR assays directly from tissue samples may be a valid supplementary tool for the *post mortem* diagnosis of BTB, since this is a faster and more specific technique than bacterial culturing, reducing the diagnosis time for diagnosis of the disease from three months to two days.

**Key words:** Bovine tuberculosis, high performance liquid chromatography (HPLC), *Mycobacterium tuberculosis* complex, multiplex polymerase chain reaction (PCR), tissues with macroscopic lesions.
INTRODUCTION

Bovine tuberculosis (BTB) is a major infectious disease among cattle in many countries. Although cattle are the main host and reservoir of this chronic infection, other mammals, including humans, are also susceptible to *Mycobacterium bovis* (Medeiros et al., 2010). Zoonotic TB can also be considered a socio-economic disease, as it causes direct economic losses in the agribusiness and hampers commercial exchange of animals and products (Zumárraga et al., 1999). Many countries around the world perform the control or eradication of BTB by their official control of infectious diseases, based on test-and-slaughter policy. Brazilian policies regarding the control and eradication of BTB include the National Plan for Control and Eradication of Bovine Brucellosis and Tuberculosis (PNCEBT), established in 2001 and reviewed in 2004, which is based on the slaughtering of all reactive animals to the tuberculin test (Brazil, 2006). According to Pollock et al. (2005) new tools, such as additional diagnostic tests, are needed to make a quick diagnosis of the disease and develop vaccines in order to prevent bovine tuberculosis.

There is a lack of official data regarding the current prevalence of BTB in Brazil. Based on official reports, there was a national average prevalence of 1.3% of cows infected from 1989 to 1998 (Brazil, 2006). Since the implementation of the PNCEBT in Brazil, the prevalence of the disease was reported to range from 0.7 to 3.3% (Furlanetto et al., 2012). According to the epidemiology of the disease, there is a higher incidence of BTB in dairy herds when compared to beef herds, due to the difference between the breeding systems of these animals.

BTB is usually diagnosed “in vivo”, based on delayed hypersensitivity reactions (intradermal tuberculin tests), which may lack high sensitivity and specificity. However, a definitive diagnosis is still established by the isolation and identification of the etiological agent (*M. bovis*) from lymph nodes or lungs, obtained during necropsy or at slaughter, using a combination of traditional culture and biochemical methods, which is considered the “gold standard method”. These methods are laborious, unreliable and time-consuming; it may take more than 90 days to grow the microorganism, and an additional 2 weeks for biochemical identification (OIE, 2009). Several alternative approaches have been attempted for the rapid and specific diagnosis of BTB, but molecular methods, especially the polymerase chain reaction (PCR) assay, are the most promising (Carvalho et al., 2015).

BTB lesions in cattle are most often found in organs rich in reticuloendothelial tissue, particularly the lungs and associated lymph nodes (Corner et al., 1990). Other studies conducted on naturally infected cattle experimentally infected with *M. bovis*, demonstrated that lesions are most commonly present in the lower respiratory tract, however the upper respiratory tract and its associated tissues also displays disease in many cases (Neill et al., 1994; Rodgers et al., 2007). Although tubercles are not pathognomonic of BTB, identifying *M. bovis* or its DNA confirms the disease.

PCR has been successfully applied by our group and other researchers in the detection of members from the *M. tuberculosis* complex (MTC), and DNA amplification of specific sequences is especially useful for this (Cardoso et al., 2009; 2015). However, the success of the PCR assay depends on the availability of intact and impunity-free DNA. Thus the presence of contaminants can interfere with the PCR technique, becoming an obstacle for its implementation (Cardoso et al., 2009). Vitale et al. (1998) showed that the QIAamp Blood and Tissue Kit (Qiagen®) was able to circumvent these problems, supplying DNA templates suitable to be amplified by PCR in most biological samples. We adopted this procedure to evaluate the efficiency of an m-PCR targeting for the *RvD1Rv2031c* and *IS6110* sequences, specific for *M. bovis* and MTC, respectively, to identify *M. bovis* DNA from tissues of slaughtered, skin-test positive, animals. The results were compared with those obtained from the skin test and conventional culture for *M. bovis*.

MATERIALS AND METHODS

Study design

This study was conducted on a dairy herd comprised of 270 adult crossbred Holstein and Gir cows, located in Macaé city, Rio de Janeiro State, in Southeastern of Brazil. Prior to the study, 34 adult cows had positive reactions to a single intradermal tuberculin test (SITT) and were kept in quarantine for 90 days, waiting for confirmatory tests to be conducted, in order to avoid bacillus transmission. After 90 days, a comparative intradermal tuberculin test (CITT) was performed in these same 34 cows (Group A - reagents), plus 16 randomly selected cows that were negative to the first SITT test (Group B - control), totaling 50 animals. After 30 days of the PPD injection, all CITT-reactive cattle (Group A) were slaughtered and subjected to a necropsy procedure (OIE, 2009). Mediastinal, scapular and retropharyngeal lymph nodes, as well as lung samples of lungs, independently of the macroscopic tuberculous lesions, were collected and analyzed by bacteriological culturing and PCR.

Intradermal tuberculin test

Intradermal tuberculin tests (both SITT and CITT) for BTB diagnosis were performed on all 50 cows, in accordance with the regulations of the Ministry of Agriculture, Livestock and Supply (Brasil, 2006). For the SITT, 0.1 mL of bovine PPD (bovPPD–*M. bovis* strain AN5, 

*Corresponding author. E-mail: figueiredoeduardo@hotmail.com / figueiredoeduardo@ufmt.br. Tel: +55 65 3615-8675.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
Isolate culturing and identification

All CITT-reactive cows were killed 30 days after the PPD injection, and a thorough necropsy was conducted. Mediastinal, scapular and retropharyngeal lymph nodes, as well as lung samples, independent of macroscopic necropsy lesions, were collected. A total of four tissue fragments were collected per animal. A pooled sample from each animal was packed in the same package and kept at -20°C. Tissues were collected after 30 days and were used to prepare DNA templates. DNA was extracted from colonies by a microprocessor system (Kromasil) with a suspension of acid bacteriophage grown in LJ medium. Mycolic acids were then separated from lipids by acidification with HCl at 2 M and 0.2 M of each primer JB21 (5'-TCGTCCGCTATGCAAGTCG-3') and JB22 (5'-CGTGAACGTAGTCGCCTGC-3'), targeting the RvD1-Rv2031c sequence, specific for M. bovis and INS1 (5'-CGTGAAGGACATCGAGTGGC-3') and INS2 (5'-GGTGAGCGTCGCGTAACAA3'), targeting the IS6110 region, specific for MTC. Amplification was carried out in a GeneAmp PCR System 9600 (Applied Biosystems) with the following cycling parameters: 94°C for 5 min, followed by 30 cycles of 1 min at 94°C, 1 min at 68°C and 1 min at 72°C, with a final extension at 72°C for 7 min. PCR products were checked by electrophoresis on 1.5% agarose gels stained with ethidium bromide (10 μg/mL).

Clinical samples were considered positive when double bands of 500 (M. bovis) and 245 bp (MTC) DNA were observed. DNA templates from reference strains M. avium (ATCC 13981), M. fortuitum (ATCC 6841), M. terrae (ATCC 15755), M. vaccae (ATCC 15483), M. xenopi (ATCC 33501), M. flavescens (ATCC 14474) and M. scrofulaceum (ATCC 19981) were used as negative controls to control cross-contamination and confirm the specificity of the m-PCR assays.

RESULTS AND DISCUSSION

From the 34 cows considered CITT-reactive, only nine animals presented macroscopic lesions compatible with granuloma in the lungs, were considered suggestive lesions. However, tissues from all 34 animals were collected, pooled and submitted to the culture and m-PCR assays. Seventeen (50%) of the samples were culture positive for Mycobacterium sp, where the presence of M. bovis was confirmed in 15/17 (88.2%) isolates, by m-PCR assays (Figueiredo et al., 2009) and HPLC analyses (Figure 1B). The others two isolated mycobacterium (m-PCR negative assay) were identified as M. fortuitum by HPLC analysis (Figure 1D). The totality of the remaining samples, 17, failed to grow in culture.

Decontamination with 0.75% HPC yielded M. bovis recovery from 10 samples, whereas 4% sodium hydroxide or 6% sulphuric acid yielded only recovered, M. bovis from six and five samples, respectively. The proportion of positive samples was higher for HPC than for each of the other two methods. When using both 0.75% HPC and 6% sulphuric acid methods for decontamination, it was possible to identify 13 of 15 (86, 6%) infected cows.
It was possible to identify all isolates (17) by HPLC, while the m-PCR technique identified only *M. bovis* (15). HPLC was more efficient than m-PCR adopted here because the mycolic acids from the cell wall generate characteristic chromatograms of each species or group. On the other hand, this is a technique that requires more expensive equipment and expertise for deployment as a BTB routine testing (Figure 1).

Multiplex PCR tests of tissue samples from CITT-reactive cows were able to amplify the target DNA in 23/34 (67.6%) of the assayed samples (Figure 2). *M. bovis* by m-PCR assays were identified in 10 samples where no culture growth was observed, which means that 59% of negative-culturing samples came from infected cows.

PCR assays have been successfully applied to detect MTC and *M. bovis* from clinical cattle samples (Cardoso et al., 2009; Figueiredo et al., 2009). In the present study, the PCR test was sensitive enough to detect *M. bovis* in a large proportion (59%) of the samples that failed to grow in culture. This was also emphasized by Liébana et al. (1995) and Zanini et al. (2001). For Miller et al. (2002) and Araujo et al. (2005), the efficiency of the culture method used as a first criterion for *M. bovis* identification is low because of the small number and live bacilli presence in some tissues, because of a short delay in delivering the tissues to the laboratory or because of the sensitivity of the mycobacteria to sodium hydroxide used in the Petroff method.

For the remaining 11 CITT-reactive cows, where both culturing and m-PCR assays failed to identify *M. bovis*, it is possible that there was an inhibitory effect during the PCR assay (Al-Soud and Radstrom, 2001; Cardoso et al., 2009). Some authors (Zanini et al., 2001; Cardoso et al., 2009) also observed less than 100% sensitivity. PCR assays are not able to detect samples that contain a small numbers of pathogens, mainly in paucibacillary tissue samples. The 11 samples from CITT-reactive cows, not confirmed by culturing and m-PCR tests, probably presenting paucibacillary lesions (low amount of *M. bovis* bacillus), fit the characteristics of a recent intra-herd infection. It is generally accepted that the CITT is related to *M. bovis* infections and not necessarily to disease (Neill et al., 1994).

**Conclusions**

Our results indicate that m-PCR is able to detect *M. bovis* DNA directly in tissue samples and represents a valid additional tool for the post mortem diagnosis of BTB. Multiplex PCR assay is faster and more specific than culture-based diagnosis in *M. bovis* detection and can reduce the diagnosis time from 90 days to approximately two days. Moreover, the m-PCR test is useful when the bacilli are non-viable and cannot be detected by culture methods, being a valuable aid during the sanitary inspection of slaughterhouses for the condemnation of carcasses that show suspected lesions of the bovine tuberculosis.

**Conflict of interests**

The authors did not declare any conflict of interest.
Figure 2. Direct detection of \textit{M. bovis} DNA in tissue samples from CITT-reactive animals. DNA templates obtained from tissue samples were tested by m-PCR. Amplicons were resolved on 1.5% agarose gels stained with ethidium bromide; lane M: 100 bp DNA ladder (Promega®); lane P, positive control \textit{M. bovis} IP; lane N, negative control (without mycobacterial DNA); lane 2-25, 500 bp (\textit{M. bovis}) and 245 bp (MTC) amplified fragments from tissue samples from CITT-reactive cattle; lane 15, negative control with DNA pool of \textit{M. avium} (ATCC 13950), \textit{M. fortuitum} (ATCC 6841), \textit{M. terrae} (ATCC 15755), \textit{M. vaccae} (ATCC 15483), \textit{M. xenopi} (ATCC 33501), \textit{M. flavescens} (ATCC 14474) and \textit{M. scrofulaceum} (ATCC 19981). Three independent PCR tests were performed for each cow, using the same template DNA.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support from FAPERJ, FAPEMAT, CAPES and CNPq.

REFERENCES


Antioxidant and *in-vitro* anthelminthic potentials of methanol extracts of barks and leaves of *Voacanga africana* and *Rauwolfia vomitoria*

Francis Adu¹*, John Antwi Apenteng², William Gariba Akanwariwiak⁴, George Henry Sam³, David Ntinagyei Mintah² and Edna Beyeman Bortsie⁴

¹Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
²Department of Pharmaceutical Sciences, Central University College, Accra, Ghana.
³Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
⁴Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Received 30 June, 2015; Accepted 17 August, 2015

*Voacanga africana* (Stapf) and *Rauwolfia vomitoria* (Apocynaceae) are traditional plants widely used in folkloric medicine. Methanol extracts of *V. africana* bark (VAB) and leaves (VAL), and *R. vomitoria* bark (RVB) and leaves (RVL) were evaluated for antioxidant and anthelmintic potentials. The antioxidant properties of the extracts were determined by the DPPH free radical scavenging method using ascorbic acid as reference antioxidant. The IC₅₀ values were then determined. Four concentrations (20, 30, 40 and 50 mg/mL) of extracts were evaluated for *in-vitro* anthelmintic activity by determining the effects of the extracts on the paralytic and death time of *Pheretima posthuma* using albendazole (ABZ) (10 mg/mL) as reference standard. Results reveal that, all the extracts exhibited some level of antioxidant activity with IC₅₀ values of 187, 43, 610 and 967 µg/mL for VAL, RVB, VAB and RVL, respectively. VAB and RVB demonstrated significant anthelmintic activity. RVB at a concentration of 50 mg/mL had a paralytic time of 11.17 ± 0.088 min (*p < 0.001*) with reference to ABZ. It also demonstrated a concentration dependent reduction in death time of the worms at all concentrations tested. VAB demonstrated a concentration dependent effect on the worms with decreasing paralytic and death times upon an increase in extract concentration. It also showed significant paralytic and death times (*p < 0.001*) at concentrations of 30, 40 and 50 mg/mL with reference to albendazole.

**Key words:** Paralytic time, free radical, death time, anthelmintic activity, *Pheretima posthuma*, *Voacanga africana*, *Rauwolfia vomitoria*.

INTRODUCTION

Medicinal plants have been used for decades in the management of various ailments in ethno-medicine.

*Corresponding author. E-mail: franceadu@yahoo.com, fadu.pharm@knust.edu.gh. Tel.: +233208168429.*

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
Research has it that about 80% of the world’s population especially in developing countries use plant materials as their source of primary health care (Farnsworth et al., 1985). Despite all the advancement in medical science, mankind still depends on medicinal plants as remedies to a number of ailments.

Anthelmintic resistance is now a serious problem particularly among farm animals and complete deworming of farm animals is currently very difficult (Prichard, 1994). High levels of drug resistance in human helminth infections such as soil-transmitted helminths (STH), (Ascaris lumbricoides, hookworms (Necator americanus and Ancylostoma duodenale) and Trichuris trichiura) have resulted from periodic mass administration of anthelmintic drugs to school age children and other at-risk groups (Vercruysse et al., 2011). Thus in the face of drug resistance it is imperative that new molecules be sought to curb the menace.

Many medicinal plants have been used in the management of helminth infections and these include: Carica papaya (Levecke et al., 2014), Annona senegalensis, (Alawa et al., 2003) and essential oil of Ocimum gratissimum (Linn.) and eugenol, (Pessoa et al., 2002).

The seeds of Voacanga africana, (Stapl.), are known to contain up to 10% indole alkaloids including voacamine and voacangine as well as many related compounds. Similar alkaloids are also found in the bark but in limited quantities (Bisset, 1985). Studies conducted have revealed that V. africana is a plant with a reservoir of alkaloids from which numerous alkaloid-based chemical compounds can be synthesized. Ibogaine, an alkaloid from V. africana has demonstrated numerous CNS effects (Kombian et al., 1997). V. africana has also been extensively studied for its alkaloids as well as its CNS and gastro-protective effects.

Rauwolfia vomitoria, (Aztel), (Apocynaceae) is a plant with numerous therapeutic uses (Irvine, 1961). Also known as serpent wood, (Kutalek and Prinz, 2007), the plant is traditionally used as treatment for snake bites, fever and nervous disorders (Kutalek and Prinz, 2007). The root, according to Prajapati et al. (2003) is a good anthelmintic and an antidote to snake venom. The root extracts are also known to possess good antioxidant effects (Okolie et al., 2011). Methanol extracts of the bark has demonstrated anti-ulcer activity in different models (Tan et al., 2000).

It is in this view that the leaves and barks of V. africana and R. vomitoria were evaluated to determine their effects on helminths and also explore their antioxidant potentials.

**MATERIALS AND METHODS**

**Collection and preparation of plant material**

Leaves and bark of R. vomitoria and V. africana were obtained from the forecourt of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. They were authenticated by Dr. G. H. Sam, a Botanist in charge of the physique garden and herbarium of the Department of Herbal Medicine, KNUST, where voucher specimens (KNUST/HM/2015L029 and KNUST/HM/2015L030 respectively) have been kept. The samples were thoroughly washed under running water to get rid of debris. The barks were sun dried for 5 days, while the leaves were dried at room temperature (25 to 28°C) for 5 days. The dried leaves and barks of V. africana and R. vomitoria were milled into powder using a laboratory mill machine (Type 8, Christy & Norris, UK). The powdered plant materials (50 g each) were each extracted by cold maceration for 72 husing methanol (70%v/v) and concentrated under reduced pressure (Type 8, Christy & Norris, UK). The powdered plant materials (50 g each) were each extracted by cold maceration for 72 h using methanol (70%v/v) and concentrated under reduced pressure using rotary vapour (Buchi, Germany). They were finally evaporated to dryness at 40°C in a hot-air oven and the weights of the extracts obtained recorded. The extracts were stored in a desiccator until needed. All chemicals used in the study were obtained from BDH, England, unless otherwise stated.

**Experimental organism**

Adult Indian earthworms (Pheretima posthuma; class Annelida; subclass Megascolecidiae) which have anatomical and physiological resemblance to human intestinal roundworms (Vidyarthi, 1967), were collected from the soil close to the Wiwi River in the Botanical Garden of KNUST. The earthworms were washed with 0.9% saline solution to remove all debris.

**In-vitro anthelmintic bio-assay**

An in-vitro anthelmintic bio-assay was performed according to the method described by Bhawar et al. (2009). P. posthuma samples, 4.0 to 5.0 cm in length and 0.10 to 0.20 cm in width were used. Extract solutions of concentrations of 20, 30, 40 and 50 mg/mL were prepared using a mixture of DMSO and distilled water in the ratio 2:8. Albendazole at a concentration of 10 mg/mL was used as the reference standard. A solution of 0.9% saline was used as the negative control.

**Experimental procedure**

The earthworms were placed in Petri dishes (five worms per Petri dish) into which the various extract solutions and reference standard were added. Observations were made for the time taken for the various extracts to cause paralysis and death of the individual worms. Paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was noted when the worms lost motility followed by a fading away of their body colour.

**Determination of antioxidant activity**

The antioxidant activity of the extracts were determined according to the method described in a previous study by Agyare et al. (2015) using the free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma- Aldrich, Damstadt, Germany). Solutions of the extracts and standard antioxidant (ascorbic acid) (Sigma-Aldrich, Damstadt, Germany) of concentrations 1.0, 3.0, 10.0, 30.0, 100.0, 300.0 and 1000.0 µg/mL were prepared in methanol. DPPH solution of concentration 5.0 x 10^{-6} M was prepared in methanol in a dark room. Three millilitres of this solution was added to 1.0 mL of the methanol test extracts and standard antioxidant. The tubes were kept in the dark for 30 min after which absorbance (A1) of excess DPPH in the extracts and standard solutions were measured at a wavelength of 517 nm using a UV spectrophotometer. The absorbance (A2) reading for a blank solution containing equivalent volumes of methanol and DPPH was used as control. The
Table 1. Paralysis time of RVB and RVL extracts against *P. posthuma*.

<table>
<thead>
<tr>
<th>Extract conc. (mg/mL)</th>
<th>RVB Time (min)</th>
<th>RVL Time (min)</th>
<th>0.9% saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>19.38 ± 0.409</td>
<td>31.08 ± 0.260</td>
<td>Na</td>
</tr>
<tr>
<td>30</td>
<td>16.50 ± 0.500</td>
<td>26.64 ± 0.049</td>
<td>Na</td>
</tr>
<tr>
<td>40</td>
<td>15.68 ± 0.266</td>
<td>24.02 ± 0.044</td>
<td>Na</td>
</tr>
<tr>
<td>50</td>
<td>11.17 ± 0.088***</td>
<td>21.68 ± 0.095</td>
<td>Na</td>
</tr>
</tbody>
</table>

Paralysis time ABZ 10 mg/mL 15.48 ± 0.180

RVB, *R. vomitoria* bark; RVL, *R. vomitoria* leaves; ABZ, Albendazole; Na, No activity; conc., concentration; ***p < 0.001.

Table 2. Paralysis time of VAB and VAL extracts against *P. posthuma*.

<table>
<thead>
<tr>
<th>Extract conc. (mg/mL)</th>
<th>VAB Time (min)</th>
<th>VAL Time (min)</th>
<th>0.9% saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>16.62 ± 0.347</td>
<td>34.01 ± 0.720</td>
<td>Na</td>
</tr>
<tr>
<td>30</td>
<td>12.51 ± 0.289***</td>
<td>31.46 ± 0.395</td>
<td>Na</td>
</tr>
<tr>
<td>40</td>
<td>9.43 ± 0.536***</td>
<td>27.62 ± 0.968</td>
<td>Na</td>
</tr>
<tr>
<td>50</td>
<td>7.03 ± 0.491***</td>
<td>22.55 ± 0.569</td>
<td>Na</td>
</tr>
</tbody>
</table>

Paralysis time of ABZ (10 mg/mL) 15.48 ± 0.180

VAB, *V. africana* bark; VAL, *V. africana* leaves; ABZ, Albendazole; Na, No activity; conc., concentration; ***p < 0.001.

Table 3. Death time of VAB and leaves extracts against *P. posthuma*.

<table>
<thead>
<tr>
<th>Extract conc. (mg/mL)</th>
<th>VAB Time (min)</th>
<th>VAL Time (min)</th>
<th>0.9% saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>24.59 ± 0.356</td>
<td>141.71 ± 0.918</td>
<td>Na</td>
</tr>
<tr>
<td>30</td>
<td>19.07 ± 0.261</td>
<td>138.72 ± 1.254</td>
<td>Na</td>
</tr>
<tr>
<td>40</td>
<td>16.43 ± 0.780***</td>
<td>125.65 ± 0.872</td>
<td>Na</td>
</tr>
<tr>
<td>50</td>
<td>14.77 ± 0.117***</td>
<td>113.99 ± 1.014</td>
<td>Na</td>
</tr>
</tbody>
</table>

Death time ABZ (10 mg/mL) 21.03 ± 0.258

VAB, *V. africana* bark; VAL, *V. africana* leaves; ABZ, Albendazole; Na, No activity; conc., concentration; ***p < 0.001.

The percentage of free radical scavenged was calculated from the equation \[% scavenging = ((Aₒ - A₁)/ Aₒ x 100)\]. The IC₅₀ was determined as the concentration of samples which scavenged 50% of free DPPH radicals. The experiment was performed in replicates.

Statistical analysis

All results were plotted and analysed with GraphPad Prism 5.0 for Windows (GraphPad software, San Diego, CA, USA) and analysed by two-way ANOVA followed by Bonferroni post-test analysis which recognises *p < 0.05, **p < 0.01, ***p < 0.001 as statistically significant.

RESULTS AND DISCUSSION

Anthelmintic activity

The extracts of *R. vomitoria* and *V. africana* demonstrated a concentration dependent paralytic and death times on *P. posthuma* (Tables 1 to 4).

Antioxidant activity

VAL and RVB demonstrated relatively high antioxidant activity with reference to their IC₅₀ as compared to ascorbic acid (Table 5 and Figure 1).

Studies conducted on the leaves and barks of *V. africana* and *R. vomitoria* revealed some pharmacological activity of the two plants. The methanol leaves and bark extracts of both plants demonstrated both anthelmintic and antioxidant activity. VAB demonstrated very potent anthelmintic activity with significant ($p <0.001$) paralytic and death times with reference to albendazole. VAB demonstrated a concentration dependent activity with
significant paralytic and death times ($p < 0.001$) at concentrations of 40 and 50 mg/mL (Tables 1 to 4). RVB also demonstrated anthelmintic activity at all concentrations with a significant ($p < 0.001$) paralytic time at 50
mg/mL. The anthelmintic activity of plants have been attributed to the presence of some phytochemicals in the plants particularly tannins which are polyphenolic compounds (Olusegun-Joseph et al., 2012). Research has shown that some synthetic phenolic anthelmintics such as niclosamide, interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or by binding to the free protein of the gastrointestinal tract of the worms. This eventually leads to death (Olusegun-Joseph et al., 2012). Tannins are presumed to exert the same effects on the worms. *V. africana* is known to possess tannins as some of its phyto-constituents (Ayoola et al., 2008). The anthelmintic effects demonstrated by the extracts could possibly be attributed to the presence of tannins in the extracts. Studies conducted on both plants have also revealed a wide array of alkaloidal content. The alkaloids can also cause paralysis of the worms by acting on its central nervous system (Mute, 2009), which could have also accounted for the anthelmintic effects of the extracts. The study revealed that the anthelmintic activities of the leaves of both plants are weaker (giving long paralysis and death times) than the barks that showed significant anthelmintic activities at concentrations of 30, 40 and 50 mg/mL ($p < 0.001$).

The study also revealed that the extracts possess good antioxidant activities. The IC$_{50}$ values clearly depict the extent of antioxidant activity of the various extracts (Table 5). It was evident that the bark extracts of *R. vomitoria* demonstrated the highest free radical scavenging activity (IC$_{50} = 43$ µg/mL) with the lowest being RVL (IC$_{50} = 967$ µg/mL). The situation was however the opposite with *V. africana*, in which the leaves rather demonstrated much scavenging activity (IC$_{50} = 187$ µg/mL) than the bark (IC$_{50} = 610$ µg/mL). The antioxidant properties could be due to the presence of flavanoids and tannins which are known to exert antioxidant activity (Agyare et al., 2015; Marja et al., 1999). These two plants are already known to contain phytochemicals which include tannins and flavonoids (Okolie et al., 2011; Korochi et al., 2009) and might therefore account for the antioxidant activity. The two plants; *V. africana* and *R. vomitoria* could therefore, be potential sources of antioxidant compounds.

**Conclusion**

The methanol extracts of *R. vomitoria* and *V. africana* demonstrated anthelmintic activity with the bark extracts demonstrating significant anthelmintic activity. The extracts also demonstrated antioxidant activity at concentrations tested.

**Conflict of interests**

The authors did not declare any conflict of interest.

**REFERENCES**


African Journal of Microbiology Research

Related Journals Published by Academic Journals

- African Journal of Biotechnology
- African Journal of Biochemistry Research
- Journal of Bacteriology Research
- Journal of Evolutionary Biology Research
- Journal of Yeast and Fungal Research
- Journal of Brewing and Distilling