About JMPR

The Journal of Medicinal Plants Research (JMPR) provides researchers, students and academicians an avenue to present their findings on the value of medicinal plants, indigenous medications, ethnobotany and ethnomedicine, herbal medicines and the cultivation of aromatic and medicinal plants.

The journal will consider for publication original research, reviews and meta-reviews, and short communication on areas covering nutraceuticals, drug discovery and development, pharmacopoeia, traditional medicine, monographs, and natural products research.

The Journal of Medicinal Plants Research is indexed in:

- CAB Abstracts
- CABI’s Global Health Database
- Chemical Abstracts (CAS Source Index)
- China National Knowledge Infrastructure (CNKI)
- Google Scholar
- Matrix of Information for The Analysis of Journals (MIAR)
- ResearchGate

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journals of Biotechnology is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

All articles published by Journal of Medicinal Plants Research are licensed under the Creative Commons Attribution 4.0 International License. This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited. Citation should include the article DOI. The article license is displayed on the abstract page the following statement:

This article is published under the terms of the Creative Commons Attribution License 4.0 Please refer to https://creativecommons.org/licenses/by/4.0/legalcode for details about Creative Commons Attribution License 4.0
**Article Copyright**

When an article is published by in the Journal of Medicinal Plants Research, the author(s) of the article retain the copyright of article. Author(s) may republish the article as part of a book or other materials. When reusing a published article, author(s) should;

Cite the original source of the publication when reusing the article. i.e. cite that the article was originally published in the Journal of Medicinal Plants Research. Include the article DOI

Accept that the article remains published by the Journal of Medicinal Plants Research (except in occasion of a retraction of the article)

The article is licensed under the Creative Commons Attribution 4.0 International License.

A copyright statement is stated in the abstract page of each article. The following statement is an example of a copyright statement on an abstract page.

Copyright ©2016 Author(s) retains the copyright of this article.

**Self-Archiving Policy**

The Journal of Medicinal Plants Research is a RoMEO green journal. This permits authors to archive any version of their article they find most suitable, including the published version on their institutional repository and any other suitable website.

Please see [http://www.sherpa.ac.uk/romeo/search.php?id=213&fIDnum=&mode=simple&la=en](http://www.sherpa.ac.uk/romeo/search.php?id=213&fIDnum=&mode=simple&la=en)

**Digital Archiving Policy**

The Journal of Medicinal Plants Research is committed to the long-term preservation of its content. All articles published by the journal are preserved by Portico. In addition, the journal encourages authors to archive the published version of their articles on their institutional repositories and as well as other appropriate websites..

[https://www.portico.org/publishers/ajournals/](https://www.portico.org/publishers/ajournals/)

**Metadata Harvesting**

The Journal of Medicinal Plants Research encourages metadata harvesting of all its content. The journal fully supports and implements the OAI version 2.0, which comes in a standard XML format. See [Harvesting Parameter](#)

**Memberships and Standards**
Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

Academic Journals is a member of the International Digital Publishing Forum (IDPF). The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

COUNTER (Counting Online Usage of Networked Electronic Resources) is an international initiative
serving librarians, publishers and intermediaries by setting standards that facilitate the recording and reporting of online usage statistics in a consistent, credible and compatible way. Academic Journals is a member of COUNTER

Portico is a digital preservation service provided by ITHAKA, a not-for-profit organization with a mission to help the academic community use digital technologies to preserve the scholarly record and to advance research and teaching in sustainable ways.

Academic Journals is committed to the long-term preservation of its content and uses Portico

Academic Journals provides an OAI-PMH (Open Archives Initiatives Protocol for Metadata Harvesting) interface for metadata harvesting.
Contact

Editorial Office: jmpr@academicjournals.org
Help Desk: helpdesk@academicjournals.org
Website: http://www.academicjournals.org/journal/JMPR
Submit manuscript online http://ms.academicjournals.org

Academic Journals
73023 Victoria Island, Lagos, Nigeria
ICEA Building, 17th Floor, Kenyatta Avenue, Nairobi, Kenya

Editor-in-chief

Prof. Akah Peter Achunike
Department of Pharmacology & Toxicology
University of Nigeria
Nsukka,
Associate Editors

Dr. Luís Cláudio Nascimento da Silva
Post-graduation program of Microbial Biology.
CEUMA University
Rua Josué Montello, nº 1, Renascença II
São Luís - MA, CEP 65.075-120

Dr. Isiaka A. Ogunwande
Department of Chemistry
Lagos State University
Ojo,
Nigeria.

Dr. Bachir Raho Ghalem
Biology Department
University of Mascara
Algeria.

Dr. Pramod V Pattar
Department of Botany
Davangere University
Karnataka,
India.

Dr. Parichat Phumkhachorn
Department of Biological Science,
Faculty of Science,
Ubon Ratchathani University,
Ubon Ratchathani 34190,
Thailand.

Dr. Anthoney Swamy
Department of Chemistry
School of Science and Technology
University of Eastern Africa
Baraton,
Kenya.

Dr. Arvind K Tomer
Department of Chemistry
University of Phagwara
Punjab
India

Dr. Foluso Oluwagbemiga Osunsanmi
Department of Agricultural Science,
University of Zululand,
South Africa.

Associate Editors

Dr. Shikha Thakur
Department of Microbiology,
Sai Institute of Paramedical and Allied Sciences,
India.

Dr. Naira Pelógia
Institute of Basic Sciences,
Taubaté University,
Brazil
Dr. Ravichandran Veerasamy
Faculty of Pharmacy
AIMST University
Semeling, Malaysia.

Dr. Bellamkonda Ramesh
Department of Food Technology,
Vikrama Simhapuri University,
India
Table of Content

Essential oil composition, antifungal activity and leaf anatomy of Lippia alba (Verbenaceae) from Brazilian Chaco
Rosani do Carmo de Oliveira Arruda, Cristiane Pimentel Victório, Amanda Galdi Boaretto, Carlos Alexandre Carollo, Cariolando da Silva Farias, Clarice Rossato Marchetti, Ronaldo José dos Santos, Giovana Cristina Giannesli and Denise Brentan Silva 79

Determination of concentration of some essential and heavy metals in roots of Moringa stenopetala using flame atomic absorption spectroscopy
Tsegaye Mega Kaba and Kusse Gudishe Goroya 89
Essential oil composition, antifungal activity and leaf anatomy of *Lippia alba* (Verbenaceae) from Brazilian Chaco

Rosani do Carmo de Oliveira Arruda¹, Cristiane Pimentel Victório², Amanda Galdi Boaretto³, Carlos Alexandre Carollo³, Cariolando da Silva Farias⁴, Clarice Rossato Marchetti⁵, Ronaldo José dos Santos¹, Giovana Cristina Giannesi⁵ and Denise Brentan Silva³

¹Laboratório de Anatomia Vegetal, Instituto de Biociências (INBIO), Universidade Federal de Mato Grosso do Sul (UFMS), 79070-900, Campo Grande, MS, Brazil.  
²Laboratório de Pesquisa em Biotecnologia Ambiental, Fundação Universidade Estadual da Zona Oeste (UEZO), Rio de Janeiro, RJ, 23070-200, RJ, Brazil.  
³Laboratório de Produtos Naturais e Espectrometria de Massas (LaPNEM), Faculdade de Farmácia, Alimentos e Nutrição (FACFAN), Universidade Federal de Mato Grosso do Sul (UFMS), 79070-900, Campo Grande, MS, Brazil.  
⁴Graduação em Alimentos – Tecnológico.  
⁵Laboratório de Bioquímica Geral e de Microrganismos, Universidade Federal de Mato Grosso do Sul, Instituto de Biociências (INBIO), Campo Grande, MS, 79070-900, Brazil.

Received 8 November, 2018; Accepted 10 January, 2019

This study aims to determine the essential oil chemical composition of *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson collected in the Brazilian Chaco where plants grow in conditions of high temperatures in the summer, periodic flood, low temperatures and air humidity in the winter. We also evaluate the oil antifungal activity against the animal and plant pathogenic fungi *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Fusarium* sp., *Penicillium funiculosum* and *Sclerotinia sclerotiorum*. Leaf essential oils were extracted by Clevenger hydrodistillation and characterized by GC-MS. The major essential oil components were linalool (38.26%), *trans*-ocimenone (6.57%) and caryophyllene oxide (6.48%). At first time *L. alba* from Brazilian Chaco was identified as a chemotype producing linalool. The essential oils showed antifungal activity, mainly against *S. sclerotiorum*, a fungi species related with diseases in soybean plants, with 100% of growth inhibition. These results suggest the potential alternative of this species to synthetic fungicides and confirm its popular uses as an important medicinal plant in South America.

Key words: Brazilian Chaco, ervança, essential oil, pathogenic fungi, glandular trichome, terpenes.

INTRODUCTION

*Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson (Verbenaceae), popularly known as bushy matgrass, bushy lippia, hierba negra, pitiona and ervança, is an aromatic subshrub, with chamaephyte life form, widely

*Corresponding author. E-mail: rosani.arruda@ufms.br.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
distributed throughout the Americas and found in different environments, such as forests, fields, and roadsides (Salimená and Múlgura, 2015). It is commonly used in South American folk medicine as an analgesic, anti-inflammatory, cold remedy, as well as a treatment for hepatic afflictions (Oliveira et al., 2006).

Essential oils from the leaves of *L. alba* have been categorized into different chemotypes, depending on their major constituents, such as linalool, citral, and carvone (Pandelo et al., 2012). Several biological properties of this plant, such as cytotoxicity, antioxidant, antibiofilm, anesthetic, antitumor, antibacterial, antifungal, anti-inflammatory, antispasmodic activities and anxiolytic-like effects differ according to essential oil chemotype (Glamočlija et al., 2011; Trevisan et al., 2016; Tofino-Rivera et al., 2016; Pandey et al., 2016, García et al., 2017).

*Aspergillus* species are associated with a wide range of diseases, including allergic bronchopulmonary aspergillosis (ABPA) and various forms of invasive Aspergillosis (Uniyal et al. 2012). Over many decades, the percentage of fungal infection by *Aspergillus* species has risen dramatically (Kocié-Tanackov and Dimié, 2013). Furthermore, *Aspergillus* species produce aflatoxins, a mycotoxin contaminant in several foods. Aflatoxins are genotoxic, hepatocarcinogenic and immunotoxic, harming both human and animal health (Passone et al., 2013).

Several essential oils have been described as inhibitors of aflatoxin and mycelial growth in *Aspergillus* species; thus, they are an attractive alternative method to avoid food contamination (Pandey et al., 2016; Passone et al., 2013). *Sclerotinia* is one of the most devastating and cosmopolitan of plant pathogens (Dalili et al., 2015). This pathogen infects more than 500 species of plants worldwide, including important field crops and numerous weeds (Saharan and Mehta, 2008). Chemical fungicides have been used against plant pathogenic fungi, and even though several of them are available, they are usually expensive, ineffective, and hazardous (Lu, 2003).

Plant essential oils are also good candidates for the control of fungi, as they consist of many bioactive chemicals with antifungal activity (Kocié-Tanackov and Dimié, 2013; Mahilirajan et al., 2014).

Therefore, this study aimed to determine the chemical composition of *L. alba* essential oils in specimens collected in the humid Brazilian Chaco and then evaluate their antifungal activity against *Penicillium funiculosum*, *Sclerotinia sclerotiorum* and some *Aspergillus* and *Fusarium* species.

In addition, histochemical, morphological and micromorphological analyses of glandular trichomes can provide support for studies useful to taxonomy, phylogeny and exploitation of substances produced by *Lippia* species. In this context, a histological analysis of glandular trichomes associated with oil accumulation in leaves of *L. alba* is presented.

**MATERIALS AND METHODS**

**Plant materials**

Plants were collected in the Brazilian Chaco region (21°41’56”W, 57°52’57”S) (Figure 1A). All experiments were conducted with leaves obtained from 20-30 individuals of *L. alba* (Mill.) N.E.Br. ex Britton & P. Wilson (Verbenaceae) (Figure 1B and C), and specimens were collected in June, 2015 and April, 2016. For anatomical, histochemical and micromorphological analyses, about 20 leaves were processed from five individuals. *Lippia alba* was identified by comparison with descriptions found in literature and with samples growing in the botanical garden at INBIO/UFMS. Representative dried specimens of the studied plants are preserved in the herbarium CGMS/UFMS under number 66619.

**Extraction of leaf essential oils**

Fresh leaves (30-40 g) of *L. alba* were collected in June 2015 and April 2016. They were cut and submitted to hydrodistillation using a Clevenger-type apparatus for 3 h. Essential oils were pooled and refrigerated until GC-MS analyses.

**Gas chromatography-mass spectrometry (GC-MS) analysis**

Samples of leaf essential oils were analyzed in a Shimadzu GCMS-QP2010 using a DB-5MS column (30 m x 0.25 mm; 0.25 mm in thickness). The initial oven temperature was 60°C, raised to 240°C at 3°C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min and at a pressure of 79.7 kPa. The injector temperature was 250°C, applying the split ratio of 1:5. Mass spectra were obtained using electron ionization source at 70 eV. The essential oil was solubilized in dichloromethane at concentration 2 mg/mL and 1 μL was injected on the chromatographic system. To identify constituents, the mass spectra were compared to data from NIST 08, FFNSC 1.3 and WILEY 7 libraries. A comparison of retention indices reported in the literature was also performed (Adams and Sparkman, 2007). Values were calculated using alkanes from C9 to C22.

**Characterization of isolated fungal strains**

The fungal species used in the experiments were *Aspergillus* CPV34.2A, *Aspergillus flavus* SM3, *A. turgidum*, *A. niger*, *A. terreus* MP 31, *Fusarium* sp., *P. funiculosum*, *Aspergillus* sp., *Aspergillus* sp. SM8 and *S. sclerotiorum*. Filamentous fungi were identified by Clarice Rossato Marchetti, in accordance with their morphological characteristics and deposited in the fungi collection of the Laboratory of Biochemistry and Microorganisms, UFMS, Campo Grande, Brazil. Stock cultures were propagated at 30°C on slats of solid potato dextrose agar (PDA) media and stored at 4°C.

**Antifungal activity assays**

For mycelial growth inhibition, 20 μL of essential oil were incorporated into PDA, giving a final concentration of 1 μg/mL. The medium with essential oil was poured into a Petri dish 9 cm in diameter. Culture medium without the essential oil served as control. After the incorporation of embedded discs (5 mm in diameter) with spores (6.10⁷/mL) on PDA medium, the plates were incubated for six days at 28°C. The diameters of the inhibition zones were measured in millimeters, and their means were calculated. Each treatment was tested on four plates as replications. The percentage of fungal growth inhibition was...
Results

The chemical analysis of *L. alba* leaf essential oils collected in Chaco, Mato Grosso do Sul, showed the monoterpene linalool (38.26%) (Figure 2) as the main component, followed by caryophyllene oxide (6.48%), *trans*-ocimenone (6.57%), *p*-mentha-1,8-dien-3-one (4.61%), and humulene epoxide II (4.03%) (Table 1). Based on these major chemical constituents, Brazilian Chaco *L. alba* could be classified as a linalool chemotype.

Accordingly, the effects *L. alba* essential oils against pathogenic fungi were evaluated in the present study. Specifically, the linalool chemotype from Chaquean *L. alba* showed inhibition of mycelial growth at 1 µg/mL (Table 2). The extension diameter (mm) of hyphae from the center to the sides of each dish, including replicates, was measured every 24 h for 6 days.

The fungal species tested did not grow after 3 days of incubation, except for *A. niger* (8 mm) and *Aspergillus* sp SM8 (10 mm). When compared with the control (55-90 mm of mycelial growth), the essential oil showed moderate antifungal activity against the growth of all fungal species tested (0-35 mm of mycelial growth) after 6 days of incubation.

Results showed variable inhibition between 77 to 100% and 50 to 100%, for 3 and 6 days, respectively (Table 3). *L. alba* essential oil was effective against *S. sclerotiorum*, *Aspergillus* sp5, and *Aspergillus* sp1 CPV34.2A with a
Table 1. Constituents identified of the leaf essential oil from Chaquean Lippia alba by GC-MS, June (2015).

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Compounds</th>
<th>Area (%)</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.60</td>
<td>Tiglicacid</td>
<td>1.67</td>
<td>901</td>
</tr>
<tr>
<td>2</td>
<td>6.93</td>
<td>β-Myrcene</td>
<td>0.94</td>
<td>991</td>
</tr>
<tr>
<td>3</td>
<td>9.67</td>
<td>cis-Linalool oxide</td>
<td>0.78</td>
<td>1073</td>
</tr>
<tr>
<td>4</td>
<td>10.25</td>
<td>trans-Linalool oxide</td>
<td>0.71</td>
<td>1087</td>
</tr>
<tr>
<td>5</td>
<td>10.78</td>
<td>Linalool</td>
<td>38.26</td>
<td>1099</td>
</tr>
<tr>
<td>6</td>
<td>12.52</td>
<td>n.i.</td>
<td>5.43</td>
<td>1146</td>
</tr>
<tr>
<td>7</td>
<td>14.42</td>
<td>cis-3-Hexenyl butyrate</td>
<td>1.77</td>
<td>1190</td>
</tr>
<tr>
<td>8</td>
<td>14.64</td>
<td>α-Terpineol</td>
<td>1.64</td>
<td>1194</td>
</tr>
<tr>
<td>9</td>
<td>16.13</td>
<td>trans-Ocimenone</td>
<td>6.57</td>
<td>1231</td>
</tr>
<tr>
<td>10</td>
<td>16.96</td>
<td>n.i.</td>
<td>1.73</td>
<td>1251</td>
</tr>
<tr>
<td>11</td>
<td>17.72</td>
<td>p-Mentha-1,8-dien-3-one</td>
<td>4.61</td>
<td>1269</td>
</tr>
<tr>
<td>12</td>
<td>26.94</td>
<td>β-Selinene</td>
<td>2.25</td>
<td>1488</td>
</tr>
<tr>
<td>13</td>
<td>30.24</td>
<td>Ledol</td>
<td>1.67</td>
<td>1572</td>
</tr>
<tr>
<td>14</td>
<td>30.58</td>
<td>Caryophyllene oxide</td>
<td>6.48</td>
<td>1581</td>
</tr>
<tr>
<td>15</td>
<td>30.77</td>
<td>Spathulenol</td>
<td>2.11</td>
<td>1585</td>
</tr>
<tr>
<td>16</td>
<td>31.63</td>
<td>Humuleneepoxide II</td>
<td>4.03</td>
<td>1607</td>
</tr>
<tr>
<td>17</td>
<td>31.97</td>
<td>n.i.</td>
<td>1.63</td>
<td>1617</td>
</tr>
<tr>
<td>18</td>
<td>35.10</td>
<td>n.i.</td>
<td>1.32</td>
<td>1701</td>
</tr>
<tr>
<td>19</td>
<td>46.77</td>
<td>n.i.</td>
<td>1.85</td>
<td>2069</td>
</tr>
<tr>
<td>20</td>
<td>49.21</td>
<td>n.i.</td>
<td>0.80</td>
<td>2151</td>
</tr>
<tr>
<td>21</td>
<td>50.81</td>
<td>n.i.</td>
<td>13.75</td>
<td>2205</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Total identified (%)</td>
<td>73.49</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Monoterpenes</td>
<td>55.28</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Sesquiterpenes</td>
<td>16.54</td>
<td>-</td>
</tr>
</tbody>
</table>

n.i., not identified; RT, Retention time; RI, retention indices on DB-5 capillary column.

growth inhibition average of 100, 78.3 and 72.2%, respectively.

Samples of L. alba herein investigated presented grayish color owing to a large number of trichomes covering the leaf surface (Figure 3A, B and C). The epidermal cells present right anticlinal wall and a very thick periclinal wall covered by a smooth wax layer and thin cuticle (Figure 3G, 3J). The L. alba leaf is amphystomatic with scarce stomata randomly distributed on the adaxial surface, whereas on the opposite side,
Table 2. Effect of essential oils on mycelial growth after 6 days (144 h) of incubation.

<table>
<thead>
<tr>
<th>Days</th>
<th>Aspergillus sp1 CPV34.2A</th>
<th>S. sclerotiorum</th>
<th>Penicillium funiculosum</th>
<th>A. flavus SM3</th>
<th>Aspergillus sp5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>ZI</td>
<td>C</td>
<td>ZI</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>5</td>
<td>55</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>18</td>
<td>70</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>25</td>
<td>90</td>
<td>-</td>
<td>62</td>
</tr>
</tbody>
</table>

C, PDA medium without the essential oil served as control; ZI, the diameters of the inhibition zones were measured in millimeters and their means were calculated. Mean growth measurements were calculated from four replicates for each of the fungal species.

Table 3. Percentage of growth inhibition* of fungal species in agar diffusion plate assay by *Lippia alba* essential oils.

<table>
<thead>
<tr>
<th>Days</th>
<th>Aspergillus sp1 CPV34.2A</th>
<th>S. sclerotiorum</th>
<th>Penicillium funiculosum</th>
<th>A. flavus SM3</th>
<th>Aspergillus sp5</th>
<th>A. terreus31</th>
<th>A. niger</th>
<th>Aspergillus sp SM8</th>
<th>A. fumigatus</th>
<th>Fusarium sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>91.6</td>
<td>100</td>
<td>82.3</td>
<td>64.5</td>
<td>83.8</td>
<td>79.1</td>
<td>63.3</td>
<td>54.5</td>
<td>77.0</td>
<td>77.0</td>
</tr>
<tr>
<td>5</td>
<td>76.0</td>
<td>100</td>
<td>70.0</td>
<td>61.5</td>
<td>72.5</td>
<td>61.0</td>
<td>53.4</td>
<td>50.0</td>
<td>6.0</td>
<td>66.0</td>
</tr>
<tr>
<td>6</td>
<td>72.2</td>
<td>100</td>
<td>59.6</td>
<td>60.0</td>
<td>78.3</td>
<td>60.0</td>
<td>53.0</td>
<td>50.0</td>
<td>5.5</td>
<td>54.5</td>
</tr>
</tbody>
</table>

*Growth inhibition (MIC) of treatment against control was calculated by percentage (%), using the formula \([C – T/ C] x 100\), where C is an average of four replicates of hyphal extension (mm) of control and T is an average of four replicates of hyphal extension (mm) of plates treated with essential oil.
substitute for synthetic fungicides based on their antibacterial, insecticidal and antifungal properties (Feng and Zheng, 2007). These natural compounds interact with microbial membranes and disrupt the permeability barrier leading to the leakage of cell content and impairing energy production (Tian et al., 2012).

The essential oil of *L. alba* showed 100% growth inhibition against *S. sclerotiorum*. Similar stomata are numerous and organized in patches protected by numerous non-glandular trichomes (Figure 3D and E).

Growth inhibition (MIC) of treatment against control was calculated by percentage (%), using the formula 

\[
\text{MIC} = \frac{C - T}{C} \times 100
\]

where Chaquean vegetation occupies an area of approximately 70,000 km². Chaco climate is marked by strong seasonality with hot summers and maximum temperatures reaching 49°C, but dry, cold winters with occasional frost. During and after the rainy season, flooding may occur owing to the poor drainage of compact soil (Pennington et al., 2000). In the collection area, the rainy season occurs from November to February (rainfall ≥ 100 mm) with soil flooding in subsequent months. The dry season starts in April; in September, water deficit occurs (Freitas et al., 2013). The soil is whitish in color and saline.

Conditions in particular environments can be determinants of both quantity and quality of essential oils. Studies done by Tavares et al. (2005) showed variation of leaf essential oils of *L. alba* which indicated three chemotypes from different regions in Brazil. These were characterized by the production of citral (Rio de Janeiro), carvone (Ceará) and linalool (São Paulo), as major constituents. Leaf essential oils of *L. alba* from Paraná (Brazil) presented geranial (50.94%) and neral (33.32%) as the main components, representing 97.69% of total essential oil identified (Glamočlija et al., 2011). Production of essential oils in plants is highly dependent on climatic conditions, especially day length, irradiance, temperature, and water supply (Gobbo-Neto and Lopes, 2007). Linalool is a common monoterpen in leaf essential oils of Lamiaceae and Verbenaceae plants, and it presents several biological activities against bacteria and fungi (Lang and Buchbauer, 2012).

A revision of 52 *Lippia* species, as reported by Pascual et al. (2001), showed p-cime, camphor, linalool, α-pinene, β-caryophyllene and thymol as the compounds most frequently detected in leaf essential oils. Ketone with unsaturated carbonyl and trans-ocimmenone, a natural organic compound classified as a monoterpene and used in the perfume industry, were also revealed. In fumigant and contact assays with plant essential oils, ketone-rich plants were verified as having insecticidal activity (Germinara et al., 2012; Herrera et al., 2014).

The presence of carbonyl groups has also been reported to increase toxicity. Herrera et al. (2014) verified the high toxicity of ocmone against *Sitophilus zeamais*, a beetle that attacks maize. Assays against fungus using essential oils from *Tagetes mexicana* (Asteraceae), which is rich in ocimene, showed activity in vitro against several yeasts, filamentous fungi, dermatophytes, Gram-positive and Gram-negative bacteria, and protozoa (Lima et al., 2009). *L. alba* cultivated in the Chaco ecosystem is subjected to dry and rainy seasons with temperatures ranging from warm to cold (Freitas et al., 2013), as well as periodic flooding. This chemical characterization of leaf essential oil is important in order to identify the components that are effective against fungi.

The antifungal activity of different chemotypes of *L. alba* essential oil against pathogenic fungi was also
Figure 3. *Lippia alba* (Verbenaceae): leaf micromorphological and anatomical details. **A and B:** Scanning electron microscopy of leaf epidermis. **A.** Glandular trichomes (white asterisc in type I and black asterisc in type II) and non-glandular trichomes (arrow). **B.** Non-glandular trichomes. **C-J:** Light microscopy. **C, D and E:** Adaxial and abaxial surface in front view showing trichomes (fine arrows) and stomata (horizontal arrow, ST). **E.** Stomata are organized in patches. **F-J:** In situ histochemical reactions. **F:** Nile Blue for acidic lipids; **G, Nadi** for essential oil and terpenoids; **H:** Ellran test for alkaloids: black content in type I glandular trichomes; **I and J:** Sudan VI evidencing lipophilic drops in type II glandular trichomes (I, arrows) and mesophyll cells (J, arrows) and in collar cells of type I glandular trichome.
recently investigated (Rao et al., 2000; Mesa-Arango et al., 2009; Glamočlija et al., 2011; Geromini et al., 2015; Pandey et al., 2016). Results similar to those of the present study were obtained from leaf essential oils at a concentration of 1 µg/mL against Aspergillus sp. CPV34.2A, A. flavus SM3, A. fumigatus, A. niger, A. terreus MP 31, Fusarium sp. P. funcicolus, Aspergillus sp., Aspergillus sp. SM8 and S. sclerotiorum.

Therefore, essential oils, as borne out by the results of the present study, show promise as an effective results were obtained using vinclozolin fungicide at 1 µg/mL against S. sclerotiorum (Mueller et al., 2002). Benjilali et al. (1984) examined the antifungal effects of essential oils obtained from three chemotypes of wild wormwood, thyme, eucalyptus and rosemary against Aspergillus and Penicillium species and other fungi.

The antifungal activity of essential oil from L. alba was investigated against Aspergillus ochraceus, A. versicolor, A. niger, A. fumigatus, Penicillium ochrochloron, P. funcicolus and Trichoderma viride. With a MIC of 0.300-1.250 mg/mL, the present study showed this essential oil to be a potential alternative to synthetic fungicides for green molds (Glamočlija et al., 2011).

The essential oil from L. alba also showed activity against S. cerevisiae, A. flavus, A. niger and C. albicans at a concentration of 500 µg/disc (Ara et al., 2009). In another work, the ranges of reduction in the growth of 91 isolates of S. sclerotiorum on potato dextrose agar (PDA) amended with thiophanate methyl and vinclozolin were 18 to 93% and 93 to 99%, respectively (Mueller et al., 2002). The essential oil of L. gracilis showed MIC of 5.0 µg/mL against Cladosporium sphaerospermum and C. cladosporioides (Franco et al., 2014).

Kumar et al. (2010) suggest that eugenol at a concentration of 0.2 µL/mL acts as a fungicide against Alternaria alternate, Aspergillus candidus, A. fumigates, A. niger, A. paradoxis, A. terreus, A. versicolor, Cladosporium cladosporioides, Culvularia lunata, Fusarium nivale, F. oxyporum and Penicillium species, while essential oil could achieve fungidal effect on the tested fungi at a concentration of 0.3 µL/mL. The essential oils of S. aromaticum, C. limon, C. aurantium and M. piperita showed antifungal activity against A. niger and C. candidum (Verma et al., 2011).

According to Metcalfe and Chalk (1979), species of Verbenaceae show a diverse type of glandular trichomes, describing up to 16 types. In glandular trichomes, the head is a storage compartment located on the tip of the hair, and it is part of the glandular cell, or cells, which are metabolically active (Glas et al., 2012).

Argyropoulou et al. (2010) pointed out the presence of alkaloids in a variation of type I trichome in L. citridora, termed by them as type D. Glandular trichomes are considered as an important front in the chemical defense of plants, therefore, they can accumulate and synthesize chemicals that can be released by the touch of herbivorous insects, for example. In Lippia alba, our chemical analyzes did not indicate the presence of alkaloids, although histochemical tests revealed the presence of these substances.

Further investigation on the content of isolated trichomes will confirm these results as it is notably the glandular trichomes that are on the surface of the leaf, more exposed, and must have an important defensive function for the plant. Sunflower leaves (Helianthus annuus L., Asteraceae) present different trichomes: non-glandular, capitulate glandular and linear glandular trichomes, each one with distinctive chemical composition of and distribution among epidermal cells being this pattern useful to infer the ecophysiological roles of metabolites (Brentan Silva et al., 2017).

Similarly, other species of Lippia can present different types of glandular trichomes, e.g., L. origanoides and L. stachyoids, with five and four types of glandular trichomes, respectively (Tozin et al., 2015). In L. citridora, five types of glandular trichomes were also described, four of them having unicellular head (Argyropoulou et al., 2010). Most studies on glandular trichomes use histochemical methods in order to identify chemical class and sites of accumulated synthesized substances (Nikolakaki and Christodoulakis, 2004, 2007). Although chemical analysis did not evidence alkaloids in leaves, our histochemical results did reveal that head cell vacuoles of type I glandular trichomes store substances that have the same reaction to this compound.

Lipophilic drops were detected in chlorenchyma cells. Leaves of L. alba found in Brazilian Chaco present isolateral mesophyll, that is, palisade parenchyma on both leaf sides in a compact organization. Lipophilic drops were detected in chlorenchyma cells. In contrast, morphotypes of L. alba growing under laboratory conditions presented dorsiventral leaves with loose parenchyma cells (Jezler et al., 2013).

L. alba growing in the Chaquean region exhibits leaf features typically observed in plants living in dry environments with saline soils, that is, thick external cell walls, numerous non-glandular trichomes, forming a barrier protecting stomata from evapotranspiration, the development of translucent parenchyma cells, most likely to improve water storage, and abundance of palisade parenchyma (Dickison 2000).

In sum, terpenoids play important roles in primary plant metabolism by their relationship to the production of chlorophylls, quinones, phytohormones and other signaling molecules. However, most terpenoids are functionally related to plant defense or attraction of pollinators (Gleason and Chollet, 2012). In L. alba analyzed here, we detected a high production of pinonalool as the major oil component in plants from Brazilian Chaco, possibly playing a role in direct defense against pests, as they have a deterrent or repellent, often toxic, effect (Glas et al., 2012).

This study demonstrated the antifungal activity of L. alba essential oils against Aspergillus species, such as A.
flavus, A. fumigatus, A. niger, A. terreus, and against Fusarium sp, P. funiculosum and S. sclerotiorum, but mainly S. sclerotiorum. Importantly, these results strongly indicate the potential of such essential oils as an alternative to synthetic fungicides, with the concomitant advantages of avoiding the development of resistance by pathogenic microorganisms and reduced environmental impact, especially if plants are growing in proper conditions. In addition, our study also points to the medicinal potential of L. alba plants already highlighted by the population in several locations in Brazil and South America.

**ACKNOWLEDGMENTS**

We thank the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) for financial support. RCOA thanks CNPq by a fellowship Proc. 311267/2012-2. We thank to Prof. Dr. Norberto Peporine Lopes of FCRPR-São Paulo University for the CG-MS analyses and Laboratory of Electron Microscopy (UFMS) for allowing the use of their equipment.

**CONFLICT OF INTERESTS**

The authors declare that they have no conflict of interest.

**REFERENCES**


Gleason FK, Chollet R (2012). Plant biochemistry. Jones and Bartlett Learning, Sudbury, Massachusetts. LLC 446.


Determination of concentration of some essential and heavy metals in roots of *Moringa stenopetala* using flame atomic absorption spectroscopy

Tsegaye Mega Kaba and Kusse Gudishe Goroya*

Department of Physics, College of Natural and Computational Sciences, Wolaita Sodo University, Ethiopia.

Received 10 December, 2018; Accepted 22 January, 2019

This study is aimed at determination of concentration of essential (Ca, K, Na and Mg) and heavy (Cd, Cr, Pb, Hg, Cu and Zn) metals using Flame Atomic Absorption Spectroscopy (FAAS) of samples taken from fresh roots of *Moringa stenopetala*. A wet digestion method involving use of mixture of (HCl and HNO₃, ratio 3:1) and 10 ml of 30% H₂O₂ at an optimum temperature and time duration was deployed during sample preparations. Results show that concentration of essential elements Mg, Ca, Na, and K range from 10.12 to 10.99 mg/kg, 2.6 to 5.64 mg/kg, 4.3 to 5.26 mg/kg, and 1.26 to 1.77 mg/kg, respectively. Among the heavy metals, concentration of Cu fall in the range of 0.81–1.44 mg/kg while that of zinc fall in the range of 0.37–2.34 mg/kg, both lying below toxic level. The levels of toxic metals (Cd, Cr, Pb and, and Hg) were not detected. The results of the study indicate that the concentration of the entire essential and heavy metals are below the range of WHO/FAO limits.

**Key words:** *Moringa stenopetala*, essential metals, heavy metals, roots, flame atomic absorption spectroscopy (FAAS)

**INTRODUCTION**

*Moringa* tree is a multi-purpose miracle tree with tremendous potential for food and medical uses (Agena, 2009). It belongs to the genus of family *Moringaceae*. *Moringa* requires an annual rainfall of 250-3000 mm to grow. It is drought resistant tree that grows best at altitude up to 600 m but still grows at altitude of 1000 m. Worldwide, some 14 species of the *Moringa* tree have been reported (Diriba et al., 2017). Among these, the best studied species with regard to potential nutritive, medicinal uses and the identification of compounds of potential therapeutic importance is *Moringa oleifera* which is native to the Indian subcontinent. *Moringa stenopetala* species is endemic to East Africa (Bosch, 2004) and grows widely in southern parts of Ethiopia.

Almost all parts of *Moringa* tree can be used as a source of nutrition for human and livestock as well as for other purposes (Bosch, 2004). *Moringa* tree is a deciduous plant with tuber-like root at the earlier stage. *Moringa oleifera* roots are important agents of healing and nourishment. The roots are used to make medicines, perfumes, natural pesticides, fertilizers, cleaning agents, animal feeding and many other important products. When

*Corresponding author. E-mail: anaxorma@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](http://creativecommons.org/licenses/by/4.0/)
Moringa oleifera seedlings are 60 cm tall or shorter, their roots can be used to make special sauce that can serve medicinal purposes. Individuals suffering from malnutrition are encouraged to consume sauce made from the Moringa oleifera roots sauce as it contains high levels of fiber, protein, vitamins and minerals, which are known to bring about a quick recovery (Fahey, 2005).

Moringa roots are popular in medicine both traditionally and in modern life. Traditionally, the restorative and healthful benefits of the Moringa root have been used by Ayurvedic practitioners in India for centuries to treat a wide variety of ailments and in controlling disorders of the circulatory system including minor cardiovascular complaints. In small doses, Moringa tree roots can be used to stimulate appetite and improve function of digestive tract, making it useful for individuals with gastric upset and irritable bowel syndrome. Additionally, roots of this tree have been used in controlled doses to treat impotence, sexual dysfunction, female reproductive tract issues and to bring on menstruation. In poultice form, Moringa roots are used for cramps and arthritis pains (Karuna and Rajni, 2014).

In modern medical uses, Moringa tree roots play great role. As stated in Bosch (2004) and Karuna and Rajni (2014), Moringa tree roots contain elements that can combat epithelial ovarian cancer and provide new hope for cancer sufferers. Roots extracts of Moringa tree can help to reduce or eliminate kidney stones by allowing body to flush calcium and phosphates from the kidneys more efficiently. This tree root is also used as anti-inflammatory agents with solid results in laboratory rats showing reduced swelling and improved healing in edema and other artificially induced inflammations. The analgesic and soporific effects of Moringa root compounds have undergone rigorous scientific testing and have been found to be useful in supplementing pharmaceutical remedies, allowing patients to experience longer, less interrupted sleep when taking pain medications.

Metal concentration in human diets are classified into essential like Na, K, Cu, Zn, Ca, Mg, Fe and Mn and non-essential or toxic such as Hg, Cd, Pb and Cr metals (Longhurst, 2010). Low intake of essential metals produces deficiencies, while higher consumption may cause toxicity. However, non-essential metals are lethal and toxic even at low concentrations to human and environment. Non-essential metals are ranked among the most hazardous toxic substances owing to their persistence in the environment and absorption in food chain (Khan et al., 2013; Muhammad et al., 2013). Toxic effects of metals include vomiting, diarrhea, headache, irritability, hypertension, heart, lung, kidney, liver and intellectual problems and cancer (Shah, 2012).

The term heavy metal refers to any metallic element that has a relatively high density and is toxic or poisonous at low concentration. Heavy metals include Cadmium (Cd), Copper (Cu), Lead (Pb), Zinc (Zn), Mercury (Hg), Arsenic (As), Silver (Ag), Chromium (Cr), Iron (Fe) and Platinum. Metal elements like copper and zinc are essential trace elements for living organisms at low concentration (< 10 mg/L). The characteristics of heavy metals are described by Loannidou et al. (2005) and Wang et al. (2005). Toxicity that can last for long time in nature cannot be degraded including bio-treatment and are very toxic even at low concentration (1.0 - 10.0 mg/L). With respect to their toxicity, heavy metals can be divided into two groups: micronutrients like Fe, Mn, Mo, Cu, Cr, Ni and Zn that are essential in small amounts and the only toxic ones are As, Cd, Hg and Pb without any known biological importance. Toxic metals are extremely persistence in the environment even at low concentrations and have been reported to produce damaging effects on human and animals because there is no good mechanism for their elimination from the body (Loannidou et al., 2005; Adah et al., 2013).

As deduced from different studies, Moringa tree have got different essential and non-essential, heavy as well as trace metals at different levels. According to Fagbohun et al. (2014), the following minerals were analyzed from Moringa oleifera tree roots in Nigeria using spectroscopic techniques FTIR and XRD. The research found zinc (Zn), iron (Fe), chromium (Cr) and copper (Cu) with concentration values (in ppm) 10.00, 21,510.00 and 20.00, 2.755, respectively.

Karuna and Rajni (2014) have analyzed M. oleifera tree roots using spectroscopic techniques FTIR and XRD for its elemental composition in India and found zinc (Zn), iron (Fe), calcium (Ca), potassium (K), sodium (Na) and magnesium (Mg) with concentration values (in ppm) 47.84, 5.04, 286.07, 860.59, 17.17 and 43.79, respectively. In addition to the nutritionally important minerals abovementioned, the study conducted by Karuna and Rajni (2014) had analyzed heavy metals such as Pb, Se, Hg and As, however, only Pb was detected (0.19 ppm) from the root part of Moringa oleifera using inductively coupled plasma atomic emission spectroscopy. Studies conducted in Nigeria have assessed concentration of heavy metals in Moringa oleifera and found lead, iron, copper and zinc with mean (mg/kg) of 0.9471±0.0173, 55.60±0.012, 0.1762±0.0230 and 3.225±0.022, respectively though atomic absorption spectrometer analysis (Abdulkadir et al., 2016).

Most of the data reported in research account for M. oleifera. However, all the plant parts of M. stenopetala, which is highly cultivated in southern part of Ethiopia, have not been analyzed for their metal concentration and nutritive values. The present study showcases a comprehensive investigation on the elemental constitutes of M. stenopetala tree roots. People in the study areas, especially Konso people, use M. stenopetala tree roots for abdomen pain relief just by digesting it and swallowing water component only. The usage is without any scientific dosage and presence of toxic and heavy metals could result in cause of health problems. Thus, the purpose of
this study was to determine the concentrations level of six heavy and four essential metals in *M. stenopetala* roots that are cultivated in urban and rural gardens in Southern part of Ethiopia.

**MATERIALS AND METHODS**

**Description of the study area**

The study was conducted in South Nation Nationalities and Peoples Region of Ethiopia. Three Woredas were selected: Gamo-Gofa Zuria, Konso and Derashe. Specifically, two places were taken: Karat and Dara from Konso, Gidole and Gato from Dirashe and Shara and Lante from Gamo-Gofa. Figure 1 displays study areas covered in the survey of this work.

**Sampling protocol**

Matured healthy and fresh *M. stenopetala* tree roots were collected from the farmlands of three areas mentioned in the section of study area; namely Gamo Gofa, Konso and Dirashe. The study areas were selected purposefully based on the productivity and traditional use of roots parts of *M. stenopetala* for medicinal purpose. To collect the representative sample from each sampling sites, two subsamples were taken from each site. In total, six samples were collected and put in clean polyethylene plastic bags, labeled and brought to laboratory for further pre-treatment.

**Sample preparations**

Roots samples collected from farmlands were washed with a running tap water to remove absorbed soil particulates and then rinsed with deionized water. The samples were chopped into pieces by stainless steel axe Teflon knife and Chopping board to facilitate drying and subsequently dried in drying oven at 70°C for constant weight. The dried samples were powdered using electronic blender and the powder was sieved through 2 mm sieve to prepare fine powder for digestion.

**Acid digestion procedure**

A gram of sieved powder of the samples was weighed out into acid washed glass beaker. Thereafter, the powder was digested with addition of 20 cm³ of aqua regia (mixture of HCl and HNO₃, ratio 3:1). 10 ml of 30% H₂O₂ was added to avoid any possible overflow leading to loss of material from the 100-ml conical flask. Hydrogen peroxide is also used to enhance the reaction speed and to ensure complete digestion. The analyte was digested for 2 h in 100-ml conical flask covered with watch glass and reflex over a hot plate at 90°C for 2 h. The volume was adjusted to 100-ml with distilled water. Blank solution was handled as detailed for the samples. Working standard solutions of all metals were prepared from stock.

![Figure 1. Map of study area (retrieved at: www.rippleethiopia.org/page/snnpr).](image-url)
ANOVA) shows no significant difference in the order; Derashe Gato > Derashe Gidole, except the two Derashe areas were manipulated with ASA and VGP, USA was used for absorbance recordings of the elements. Signal of each radiation for specific element was detected and were converted into concentration information for the analytes from calibration curves of each element. Results and discussion

Data entry management and preliminary summaries were done on Microsoft Office Excel spreadsheet. Means and standard deviations of data collected were determined. All analyses were carried out in triplicates and data presented as means ± standard deviations. One-way analysis of variance (ANOVA) at p < 0.05 was used to determine statistically significant differences in the mean concentrations of metals among groups of root samples from different study areas. Data were further manipulated with ASA and SPSS 20.

RESULTS AND DISCUSSION

Concentration of four essential and two heavy metals were obtained from absorption intensity. Average results of the elemental analysis of the metals observed in this work are displayed in Tables 1 and 2. It is to be noted that each result is an average of three independent triplicate measurements (n = 3). Results show that essential (Ca, K, Na, Mg) and heavy (Cu, Zn) metals were detected and the heavy metals Pb, Cd, Cr and Hg were not found to be to the level of detection limit of the technique used in this experiment. Results indicate that samples have variable composition of each analyte metals with different concentration ranges among different sample sites. Table 1 presents concentration of essential elements while Table 2 presents mean concentration of heavy metals.

**Table 1. Mean concentration of essential metals calculated in this work.**

<table>
<thead>
<tr>
<th>SN</th>
<th>Sample sites</th>
<th>Mean concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>1</td>
<td>Dirashe Gato</td>
<td>5.64±0.25</td>
</tr>
<tr>
<td>2</td>
<td>Dirashe Gidole</td>
<td>4.67±0.26</td>
</tr>
<tr>
<td>3</td>
<td>Konso Karat</td>
<td>3.64±1.59</td>
</tr>
<tr>
<td>4</td>
<td>Konso Dara</td>
<td>2.60±0.13</td>
</tr>
<tr>
<td>5</td>
<td>Gamo Gofa-Shara</td>
<td>3.36±0.01</td>
</tr>
<tr>
<td>6</td>
<td>Gamo Gofa-Lante</td>
<td>3.47±1.25</td>
</tr>
</tbody>
</table>

*Means with the same letter in a given column are not statistically significantly different.

**Table 2. Mean concentration of heavy metals calculated in this work.**

<table>
<thead>
<tr>
<th>SN</th>
<th>Sample Sites</th>
<th>Mean concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cu</td>
</tr>
<tr>
<td>1</td>
<td>Derashe Gato</td>
<td>0.81±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Derashe Gidole</td>
<td>1.42±0.74</td>
</tr>
<tr>
<td>3</td>
<td>Konso Karat</td>
<td>1.44±0.35</td>
</tr>
<tr>
<td>4</td>
<td>Konso Dara</td>
<td>1.43±0.01</td>
</tr>
<tr>
<td>5</td>
<td>Gamo Gofa shara</td>
<td>1.34±0.02</td>
</tr>
<tr>
<td>6</td>
<td>Gamo Gofa lante</td>
<td>1.35±0.28</td>
</tr>
</tbody>
</table>

*ND - Not Detected, *means with the same letters in a column are not significantly different.

Experimental setup

Flame atomic absorption spectrophotometer (FAAS) (Model: 210-VGP, USA) was used for absorbance recordings of the elements. Signal of each radiation for specific element was detected and were converted into concentration information for the analytics from calibration curves of each element.

**Statistical analysis**

Data entry management and preliminary summaries were done on Microsoft Office Excel spreadsheet. Means and standard deviations of data collected were determined. All analyses were carried out in triplicates and data presented as means ± standard deviations. One-way analysis of variance (ANOVA) at p < 0.05 was used to determine statistically significant differences in the mean concentrations of metals among groups of root samples from different study areas. Data were further manipulated with ASA and SPSS 20.

Essential metals

**Calcium (Ca)**

One-way analysis of variance (ANOVA) shows that mean concentration of calcium is statistically insignificant in all the areas of investigation, except the two Dirashe areas. The average concentration of calcium in the studied sites ranges from 2.60±0.13 to 5.64±0.25 mg/kg. Relatively high concentration was observed in Dirashe areas (Table 1). The pattern of concentration of calcium in the studied sample sites is in the order: Derashe Gato > Derashe Gidole > Konso Karat > Gamo Gofa Lante > Gamo Gofa Shara > Konso Dara.

**Potassium (K)**

As can be seen from Figure 2, mean concentration of standard solution (1000 ppm).
potassium in all areas is found to be lower. When sites are taken into consideration, there is statistical significant difference in the content of potassium concentration. The highest concentration of potassium is observed in Derashe-Gato (1.77±0.26 mg/kg). While relatively the lowest concentration is obtained in Gamo Gofa-Shara (1.26±0.00 mg/kg). Depending on the potassium levels in the studied samples, the order of the studied areas is Derashe-Gato > Derashe-Gidole > Konso-Karat > Gamo Gofa-Lante > Konso-Dara, Gamo Gofa-Shara.

Sodium (Na)
The concentration of sodium found in this work range from 4.33±0.80 to 5.31±0.28 mg/kg in studied root samples. As can be observed from Table 1, the mean concentration of sodium is next to that of magnesium, except, in Dirashe. The pattern of sodium level in the samples is in the order of; Gamo Gofa-Shara > Derashe-Gato > Konso-Karat > Gamo Gofa-Lante > Derashe-Gidole > Konso-Dara. One-way analysis of variance shows that the mean concentration of sodium does not show statistically significant difference among the six sample sites.

Magnesium (Mg)
As can be observed from Figure 1, in comparison to other elements, magnesium concentration is observed to be high in all areas covered in this work. Moreover, as can be seen from Table 1, its average is greater or equal to 10 mg/kg, which is far higher in content than all the metals determined in this work, in all the three study Woredas. Relatively, the concentration of magnesium is found to be higher in Derashe Gato (10.99±0.01 mg/kg) and followed by other sample sites in the order of 10.78±0.00 mg/kg, 10.53±0.05 mg/kg, 10.43±0.13 mg/kg, 10.43±0.01 and 10.12±0.22 mg/kg for Konso-Karat, Konso-Dara, Derashe-Gidole, Gamo Gofa-Shara and Gamo Gofa-Lante, respectively. Analysis of variance reveals that the mean concentration of magnesium is significantly different among the six sample sites.

Heavy metals
Copper (Cu) and Zinc (Zn)
Of the heavy metals considered in this work, copper and zinc are to the limit of detection level of the spectroscopic technique deployed in this experiment. The level of the heavy metals copper and zinc in this study samples are in the range of 0.81±0.01 – 1.44±0.35 mg/kg and 0.37±0.90 – 2.34±0.01 mg/kg, respectively. One-way analysis of variance shows that the concentration of copper has shown significantly no difference among sampled sites, except Dirashe-Gato. The Derashe Gato (0.81±0.01 mg/kg) copper concentration is significantly lower than other sample sites while Konso-Karat relatively records more (1.44±0.35 mg/kg) mean concentration. One-way analysis of variance shows that the concentration of zinc is significantly different among sample sites. Zinc concentration is observed to be higher than copper in Derashe and Konso-Karat. On the other
hand, it shows upper hand in Konso-Dara and Gamo Gofa areas, even with statistically significance figures. The concentration of zinc is in the range of 0.37-2.34 mg/kg. As can be observed from Figure 3, the lowest zinc content is obtained from Gamo Gofa Lante (0.37±0.90 mg/kg) and the highest one is from Konso-Karat (2.34±0.01 mg/kg) areas.

**DISCUSSION**

Average concentration of calcium in this study ranges from 2.6±0.13 to 5.64±0.25 mg/kg for *Moringa stenopetala* root. As cited in Kokou et al. (2001), concentration of calcium in *M. oleifera* root in Togo is found to be significantly higher than the concentration of calcium in *Moringa stenopetala* in this work. According to the studies conducted in Nigeria, the level of calcium concentration is reported (Fagbohun et al., 2014) to be 286.07 ppm in *M. oleifera* and 24.86 mg/g in medicinal plants (Oloyede, 2005). Research conducted in India for the determination of calcium in *M. oleifera* roots is 286.07 ppm (Karuna and Rajni, 2014). Result of this study show that the concentration of calcium in *M. stenopetala* root is below the aforementioned literature values. High concentration of calcium is important because of its role in bones, teeth, muscle system and heart functions (WHO, 2004).

The concentration of sodium and potassium in *M. stenopetala* root in this work is found to be 1.26-1.77 and 4.33-5.26 mg/kg, respectively. As reported by Karuna and Rajni (2014) in India, concentration (ppm) of sodium and potassium in *M. oleifera* roots were found to be 17.17 and 860.59, respectively. According to the study in Pakistan, the level of sodium ranges from 113.49 to 2174.38 mg/kg in different traditional medicinal plants. This indicates that the sodium and potassium concentration levels in *Moringa stenopetala* roots in this work is less than the concentration observed in the medicinal plants in Pakistan (Shazia et al., 2010). The highest concentration of potassium measured in this work is 1.77±0.26 mg/kg. Potassium is necessary for proper functioning of our body system. It maintains the electrolyte balance, manages blood pressure, keeps heart functioning properly and enhances muscle control, growth and health of the body cells. Its deficiencies can lead to varieties of mental and physical problems. The obtained data in this study indicated that the *M. stenopetala* roots are not deficient in potassium. Therefore, it is useful to be used as a food source, rich in K, for humans as it might help in the case of potassium deficiency. Results indicate that the concentration of potassium in all sample sites is found in the recommended daily intake level and also found below values obtained by Shazia et al. (2010).

The concentration of magnesium found in this work is observed to be 10.22-10.99 mg/kg. As studied by (Karuna and Rajni 2014), Mg is found to be 43.79 ppm in *M. oleifera* roots. Study conducted in Pakistan showed that content of Mg ranged from 2241.88 to 6350.63 mg/kg in different medicinal plants. As can be seen from the reports, the level of Mg in this study is below literature values and as a macronutrient it is below recommended level for plants. The level of the heavy metals Cu and Zn in this study are in the range of 0.811±0.01–1.44±0.35 mg/kg and 0.37±0.9–2.34±0.01 mg/kg, respectively. Reports in Nigeria indicate that the concentration of Cu and Zn in *M. oleifera* roots are found to be 2.755 mg/kg (Fagbohun et al., 2014) and 0.176±0.023 mg/kg (Abdulkadir et al., 2016) for copper and 10.00

![Figure 3. Concentration of heavy metals.](image-url)
mg/kg (Fagbohun et al., 2014) and 3.225±0.022 (Abdulkadir et al., 2016) for zinc.

Results indicate that the concentration of copper in all sample sites is below the permissible limit set by World Health Organization (WHO). Highest mean concentration of zinc in this study is 1.44 mg/kg which is much lower than the standard limit set by WHO (WHO, 2004; Kabata-Pendias, 2011; WHO, 2002) which is 10 to 160 mg/kg. The permissible limit of zinc set by WHO in edible plants is 27.4 mg/kg. On the other hand, the finding of this research differs from literature value of (Karuna and Rajni 2014) which is 47.84 mg/kg. The recommended limit of Zinc in medicinal plants by WHO is 50 mg/kg and its intake in food is 11 mg/day. In general, results of the heavy metals analyzed in this work show that the concentration level are below the standard guide lines for maximum limit proposed for medicinal plants by WHO.

Conclusions

Concentration of four essential (K, Ca, Na and Mg) metals and two heavy metals (Cu and Zn) in M. stenopetala tree roots are detected using FAAS with acid digestive method. Results indicate that M. stenopetala root contains low concentration of K, Ca, Na and Mg metals as compared to cited literatures, but optimum amount for consumption, and the concentration level of the toxic heavy elements are very low and could not cause any health threat to the consuming population. All essential elements analyzed are at the optimum required level and consumption of the M. stenopetala root could supplement essential metals required for human health. On the contrary, non-essential toxic metals analyzed in this study are below the permissible ranges presented by WHO standards revealing that the M. stenopetala root in the study area is safe for dietary as well as medicinal uses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES


http://www.ttljournal.org/article.php/20051201124931586


Related Journals:

- Clinical Reviews and Opinions
- Journal of Medicinal Plant Research
- African Journal of Pharmacy and Pharmacology
- Journal of Dentistry and Oral Hygiene
- Journal of Parasitology and Vector Biology
- Journal of Pharmacognosy and Phytotherapy
- Journal of Medical Laboratory and Diagnosis
- Journal of Diabetes and Endocrinology
- Medical Practice and Reviews

www.academicjournals.org