OPEN ACCESS

March 2022
ISSN 1684-5315
DOI: 10.5897/AJB
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

About AJB

The African Journal of Biotechnology (AJB) is a peer reviewed journal which commenced publication in 2002. AJB publishes articles from all areas of biotechnology including medical and pharmaceutical biotechnology, molecular diagnostics, applied biochemistry, industrial microbiology, molecular biology, bioinformatics, genomics and proteomics, transcriptomics and genome editing, food and agricultural technologies, and metabolic engineering. Manuscripts on economic and ethical issues relating to biotechnology research are also considered.

Indexing

CAB Abstracts, CABI's Global Health Database, Chemical Abstracts (CAS Source Index) Dimensions Database, Google Scholar, Matrix of Information for The Analysis of Journals (MIAR), Microsoft Academic, Research Gate

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 African Journal of Biotechnology 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

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.

Contact

Editorial Office: ajb@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJB

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

Editorial Board Members

Table of Content

Vol. 21(3), pp.95-105, March 2022
DOI: 10.5897/AJB2021.17414
Article Number: 5663E6668779
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Assessment of proximal, mineral composition and content of vitamin A and C of leaves and flowers from *Lippia multiflora* vegetable in Benin

Hihoto Wilfrid Djengue[1,2], Arlette Adjatin[1,2*], Rock Djehoue[3], Zaki Bonou-gbo[1], Codjo Tiburce Odjo[1], Zinsou Com Koukpo[4], Arsène Jacques Fassinou[4], Gérard Tiko[3], Hyacinthe Ahissou[5] and Alexandre Dansi[1]

[1]Laboratory of Biotechnology, Genetic Resources and Improvement of Animal and Plant Species (BIORAVE), Superior National Schooll of Applied Bioscience and Biotechnology (ENSBBA), National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM), BP 14, Dassa-Zoumè, Benin.
[2]Laboratory of Biochemistry, Food and Medicinal Formulations, Superior National Schooll of Applied Bioscience and Biotechnology (ENSBBA), National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM), BP 14, Dassa-Zoumè, Benin.
[3]Laboratory of Biochemistry and Bioactive Natural Substances, Faculty of Sciences and Technology (FAST), University of Abomey-Calavi (UAC), 04 BP 0320, Cotonou, Benin.
[4]Faculty of Sciences and Technology (FAST), University of Abomey-Calavi (UAC), P. O. Box 526 Cotonou, Benin.
[5]Laboratory of Protein Biochemistry and Enzymology, Faculty of Sciences and Technology (FAST), University of Abomey-Calavi (UAC), P. O. Box 526 Cotonou, Benin.

Received 29 September, 2021; Accepted 23 December, 2021

Lippia multiflora **is a plant whose leaves and flowers are used as a food in Benin, so it is important to determine the nutritional properties of leaves and flowers of** ***L. multiflora*****. Biochemical analyses for the determination of macronutrients, mineral salts and vitamins A and C on leaves and flowers of this leaf vegetable were done. The result indicates that the contents in leaves and flowers are, respectively: 5.44± 1.27 and 6.37± 1.61% for proteins, 13.17± 2.79 and 2.1 ± 0.54% for lipids, 0.46±0.09 and 12.53± 2.38% for carbohydrates, 19.65± 1.05 and 23.65± 0.46%, for fiber, 570±110.99 and 283.868± 26.64 mg/100 g for phosphor, 2872± 371.13 and 2755.83± 427.7 mg/100g for calcium, 683.5±113.35 and 2755.83± 427.7 mg/100 g for magnesium, 2009.83± 167.12 and 1722± 94.55 mg/100 g for potassium, 57.25± 14.97 and 46.75± 6.36 mg/100 g for iron, 64.47±10.73 and 40.52 ± 8.47 mg/100 g for vitamin A, 3511.5±200.03 and 2778.83±444.66 mg/100 g for vitamin C. In conclusion, leaves and flowers of** ***L. multiflora*** **are rich in nutrients and could constitute for this population an important food supplement.**

Key words: *Lippia multiflora*, nutritional properties, food supplement, Benin.

INTRODUCTION

Leaf vegetables constitute excellent contribution for diversification of human food (Agbankpé et al., 2014). Indeed, they play an important role in food regimes of all world population, especially in Africa, Asia and Oceania,

where they assure essential part of nutritional and medicinal needs (Batawila et al., 2005; Vodouhè and Dansi, 2012; Akakpo and Achigan-Dako, 2019). These leaf vegetables contain micronutrients (vitamins, minerals) that contribute to human well-being (Food and Agriculture Organization, 1988; Rubaihayo, 1992; Achikanu et al., 2021). They are foods with high nutritional value because they contain carotenes (provitamin A), various B vitamins (thiamin, riboflavin, niacin), folic and folates acid, vitamin C, minerals and proteins (Stevels, 1990; Maundu, 2005; Soro et al*.,* 2012, Melse-Boonstra, 2020). Recent nutritional studies carried out in Benin on some leafy vegetables showed that *Crassocephalum rubens* and *Crassocephalum crepidioides* are rich in nutrients and minerals and are very good sources of vitamin C (Adjatin et al., 2013). *Vernonia amygdalina*, *Crateva adansonii* and *Sesamum radiatum* are also rich in nutrients and mineral salts (Agbankpé et al., 2015). An ethnobotanical survey in Benin on LFTs revealed 187 plant species including *Lippia multiflora* which proved to be of major interest (Dansi et al., 2012; Djengue et al*.,* 2017). *L. multiflora* is used in Benin like a nutritious food in addition to its medicinal value and possessing antibiotic, antidiabetic, anti-malarial, anti-diarrheal, antidiuretic properties and treats indigestion problems indigestion, headache and toothache, chicken pox, ulcer, fever, stress, hemorrhoids, anemia, dysentery, and epilepsy. It is also used as an aphrodisiac and laxative and is without risk of toxicity for consumers (Djengue et al., 2017).

In spite of its medicinal importance, its nutritional value is not known in Benin. Therefore, the aim of this study was to determine the nutritional properties of *L. multiflora* in order to stimulate interest in its use by raising awareness among the populations on its nutritional importance as a food. Specifically, it was about:

(1) Assessing macronutrients content (water, lipids, carbohydrates, proteins, ash and fiber) of leaves and flowers;
(2) Determinate mineral salts content and vitamins A and C of leaves and flowers.

MATERIALS AND METHODS

Samples collect and preparation

The samples (leaves and flowers) of *L. multiflora* were collected from six districts (Bantè, Dassa Zoumè, Djidja, Glazoué, Savalou and Savè) of central Benin. To prepare the samples, leaves and flowers of *L. multiflora* were washed thoroughly under running tap water followed by sterile distilled water, cut into smaller pieces and dried under shade for 9 days. The dried plant parts were ground using electric blending machine and the powdery samples obtained were sieved using two sieves of 0.2 mm (mesh size) and stored in air tight sterile containers until needed. Chemical analyses were done on the powders of leaves and flowers for assessment of the following constituents: water, proteins, lipids, carbohydrates, fiber, ashes, mineral components (calcium, copper, iron, magnesium, manganese, phosphor, potassium, and sodium) and of vitamin A and C (Senga et al., 2013).

Determination of macronutrients content

Chemical analysis was carried out on powdered materials of leaves and flowers of *L. multiflora.* Analyses were made for: water, crude proteins, crude lipids, ashes, fibers and carbohydrates (Adjatin et al., 2013; Nair et al., 2012; Senga et al., 2013; Salma 2020). Crude protein was determined by using the Kjeldahl method (Nair et al., 2012; Salma, 2020). Water and crude lipids were determined according to the procedure of Association of Official Analytical Chemist (AOAC, 1990). The total crude fiber content of powders was determined using method described by Diallo et al. (2015). The percentage was calculated based on the dry weight. Ashes were determined after incineration in a muffle furnace following Bangash et al. (2011). Fehling's method was adopted to detect the presence of carbohydrates in leaves and flowers of leaf vegetable (EPSIC, 1999).

Determination of mineral composition

Mineral composition of samples was determined according to methods recommended by Association of Official Analytical Chemists (AOAC, 1990), Badau et al. (2013), and Kamal et al. (2021). The samples were incinerated in the oven at a temperature of 550°C for 3 h. The samples of *Lippia multifora* were each digested using a mixture of concentrated Nitric (HNO_3), perchloric ($HClO_4$) and sulphuric (H_2SO_4) acids in the ratio 9:2:1 (v/v), respectively (Nair et al., 2012). Copper (Cu), iron (Fe), zinc (Zn), sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) and manganese (Mn) were determined by Atomic Absorption Spectrophotometer (AAS) (PerkinElmer A Analyst 700, England). Phosphorus contents of the samples were determined using Flame photometer as specified in Alinnor and Oze (2011).

Dosage of vitamins

Dosage of provitamin A and Vitamin C

Provitamin A was assayed according to AOAC method (AOAC, 2005). The assay was performed with 1 mL of trifluoroacetic acid, using a Biomate 3 spectrophotometer at 620 nm. Vitamin C was assayed by the Camag TLC Scanner III densitometer according to the method of Nair et al. (2012).

Statistical analysis

ANOVA was performed to compare the 2 factors (zone and parts) and the interaction. Level of significance was set at 5% ($p< 0.05$). Quantitative data were processed using XSLTAT version 2015 and CATS 1.2softwares.

*Corresponding author. E-mail: aarlette2000@yahoo.fr. Tel: (00229) 97440798.

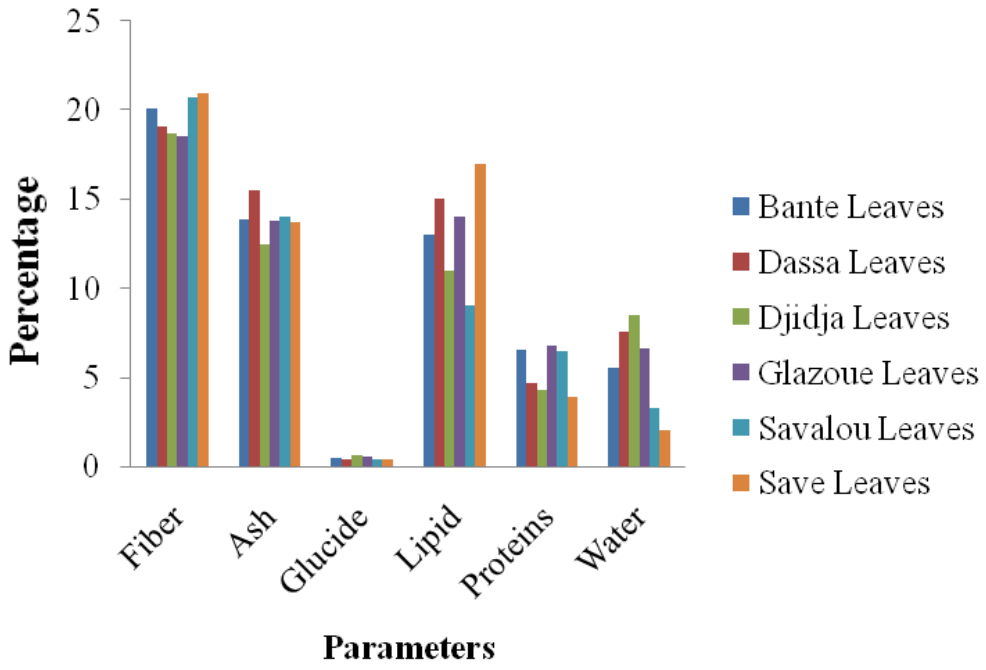


Figure 1. Proximal compositions of *L. multiflora* leaves from six areas.

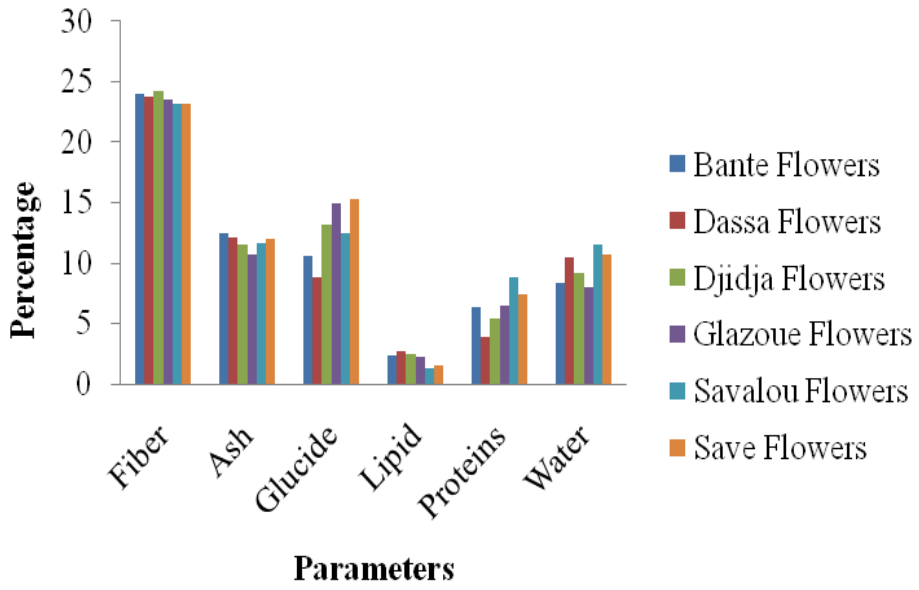


Figure 2. Proximal compositions of *L. multiflora* flowers from six areas.

RESULTS AND DISCUSSION

Proximal composition

Figures 1 and 2 show variations of proximal compositions of leaves and flowers of six areas (Bante, Dassa-Zoume, Djidja, Glazoue, Savalou and Save). Fiber content is between 18.525 and 20.93% for leaves and 23.15 and 24.22% for flowers. Ash content is between 12.46 and 15.49% for leaves and 10.65 and 12.51% for flowers. That of carbohydrates is between 0.4 and 0.62% for leaves and 8.8 and 15.25% for flowers. Lipids content is between 0.9 and 1.7% for leaves and 1.3 and 2.7% for flowers. That of proteins is between 3.92 and 6.75% for

Table 1. Analysis of variance proximal composition of leaves and flowers from *L. multiflora* according to area collection.

Source	DF	Model	Zones	Parts	Zone*Part
		11	1	5	5
Fiber	MS	14.213	0.696	95.760	1.888
	F	18.886	0.925	127.238	7.282
	Pr > F	< 0.0001	0.490	< 0.0001	0.004
Ash	MS	5.250	1.723	27.255	0.976
	F	4.599	1.509	23.874	0.744
	Pr > F	0.005	0.242	0.000	0.608
Glucide	MS	129.412	6.403	873.868	6.139
	F	57.178	2.829	386.103	11.128
	Pr > F	< 0.0001	0.051	< 0.0001	0.001
Lipid	MS	111.412	9.011	734.827	7.971
	F	34.181	2.764	225.443	6.482
	Pr > F	< 0.0001	0.055	< 0.0001	0.006
Proteins	MS	5.181	6.184	5.245	2.925
	F	4.217	5.033	4.270	7.299
	Pr > F	0.008	0.006	0.055	0.004
Water	MS	17.712	4.569	101.106	12.118
	F	4.242	1.094	24.215	23.165
	Pr > F	0.008	0.401	0.000	< 0.0001

leaves and 3.84 and 8.8% for flowers. Finally, the water content is between 2 and 8.5% for leaves and 7.96 and 11.54% for flowers. We note that there is a variation of these parameters in leaves and flowers of one area to another. This variation could be explained by phenology or plant development stage. These results are similar to those reported in Ivory Coast by Kane et al. (2010) which have demonstrated there is a variation of these parameters on different types of soil.

Table 1 describes the significance of these different values. From these results, no significant difference ($p>0.05$) was observed about water, lipid, carbohydrates, fiber, ash and sodium content by area. However significant difference ($p<0.05$) was shown with protein content. In leaves and flowers of the same area, a significant difference ($p<0.05$) was observed with fiber, ash, carbohydrates, protein, lipid and water. But the difference in protein content was not significant ($p<0.05$). This significant difference could be due to used organs. Furthermore, Dougnon et al. (2012) showed that there is a significant difference in terms of proximal composition between *Solanum macrocarpon* leaves and fruits. With regard to interaction zone-part, the difference is significant ($p<0.05$) for fibers, carbohydrates, proteins, lipids and water content but is not significant ($p>0.05$) for ash content.

Results on proximal composition revealed that leaves and fibers were most abundant followed by ash, lipids, water, proteins and carbohydrates (Table 2). In flowers, fibers are also most abundant followed by carbohydrates, ash, water, proteins and the least abundant elements are lipids. Thus, flower was richer in fiber, carbohydrates, protein and water than leaf while leaf was richer in ash and lipids.

The water contents of leaves and flowers of *L. multiflora* were 5.58 and 9.68%, respectively. These values were respectively less than 12 and 17% reported by Kane et al. (2010) and 20.36 and 36.82% according to Ekissi et al. (2011) in Ivory Coast. In addition, Ekissi et al. (2011) reported a water content of less than 19.86% in the flowers of this plant. This difference may be related to phenology. Moreover, these values were also lower than those reported by Adjatin et al. (2013) on *C. crepidioides* and *C. rubens*, Yameogo et al. (2011) on *Moringa oleifera* (73.90%). The differences observed between the results of this study and those of these authors could be due to the plant species used. The low content in water of leaves and flowers of *L. multiflora* indicates that this species would not be susceptible to microbial attack during storage and would not also be highly perishable

Table 2. Means of proximal composition of leaves and flowers from *Lippia multiflora*.

Parameter	*L. multiflora*	
	Leaves	Flowers
Water content	5.58 ± 2.35[b]	9.68 ± 1.39[a]
Total proteins (%)	5.44 ± 1.27[b]	6.37 ± 1.61[a]
Total lipids (%)	13.17 ± 2.79[a]	2.1 ± 0.54[b]
Carbohydrates (%)	0.46 ± 0.09[b]	12.53 ± 2.38[a]
Fibers (%)	19.65 ± 1.05[b]	23.65 ± 0.46[a]
Total ashes (%)	13.89 ± 1.25[a]	11.76 ± 0.87[b]

Number of repetitions n=3; significant difference $p \leq 0.01$.

(Pillai and Nair, 2013).

The average protein contents of leaves and flowers of *L. multiflora* are 5.44 and 6.37% g^{-1}, respectively of dry matter. According to the results reported by Kane et al. (2010) and Ekissi et al. (2011), the protein content of fresh leaves were lower than that of dry leaves with values of (8.05%; 8.75%) and (9.63%; 11.21%). In the same plant, the protein content in flowers was also lower (12.95%) according to Ekissi et al. (2011). These results are similar to those reported by Itoua et al. (2015) on *Phytolacca dodecandra* and different from those reported by Kouame et al. (2015) on *Myrianthus arboreus* with a protein content of 57.02%. Food plants whose protein content is more than 12% of caloric value are considered to be good sources of protein.

The total lipid contents in leaves and in dried flowers of *L. multiflora* were 1.32 and 2.1%, respectively. These values are lower than those reported by Adjatin et al. (2013) on *C. crepidioides* (3.45%) and *C. rubens* (2.75%), and Kouame et al. (2015) on leaves of *M. arboreus* (5.8%). A diet providing 1 to 2% of caloric energy as fat may be sufficient for humans because excess in fat consumption leads to cardiovascular disorders such as arteriosclerosis, cancer, and aging (Antia et al., 2006).

Fiber content in leaves and flowers were 19.65 and 23.65%, respectively. These values are higher than those reported by Adjatin et al. (2013) on *C. crepidioides* (8.18%) and *C. rubens* (7.95%), and Sodamade (2013) on *V. amygdalina* (10.46 mg/100 g). However, these values are lower than those reported by Ejoh et al. (2007) on *Gymnantheum amygdalinum* (25.47%) known as one of leafy vegetables particularly rich in fiber. *L. multiflora* is a very good source of dietary fiber and deserves more attention. Fiber is involved in digestion and reduces the risk of cardiovascular disease (Badau et al., 2013).

Studies have shown that increasing fiber intake may help reducing the incidence of some diseases such as diabetes, coronary heart disease, colon cancer, and various digestive disorders (Badau et al., 2013). Consuming fiber also softens the stool and lowers blood cholesterol levels in the body (Pillai and Nair, 2013). Maintaining internal distension for normal peristaltic movement of the gastrointestinal tract is a physiological function provided by crude fibers. However, very high fibers content can cause intestinal irritation and decreased nutrient bioavailability (Pillai and Nair, 2012).

The average ash content was 13.89% for the leaves and 11.76% for the flowers of *L. multiflora*. These results are similar to those reported by Ekissi et al. (2011). However, Adjatin et al. (2013) and Kouamé et al. (2015) reported higher values on *C. crepiodioides* (19.02%), *C. rubens* (19.76%) and *M. arboreus* (36%) while that Yaméogo et al. (2011) and Andzouana and Mombouli (2012) reported lower values on *M. oleifera* (11.10%) and *Ochthocharis dicellandroides* (4.19%). The high ash content is a reflection of the mineral salt content in the food. Further study would be needed to determine the types of mineral elements as they are essential for the functioning of tissues and necessary for daily needs.

The average carbohydrate content of *L. multiflora* was 0.46% for leaves and 12.53% for flowers. These results are similar to those reported in Ivory Coast by Kane et al. (2010). The leaves of *C. crepidiodes* (42.22%), *C. rubens* (43.11%), *Talinum triangulare* (10.87%) and *O. dicellandroides* (11.73%) are relatively richer in carbohydrates than those of *L. multiflora* (Adjatin et al., 2013; Aja et al., 2010; Emebu and Anyika, 2011). Most vegetables are not generally good sources of carbohydrates. Some of them are richer while others contain trace (Andzouana and Mombouli, 2012), which means that *L. multiflora* is not a good source of carbohydrate.

Mineral and vitamins compositions

Figures 3 and 4 show variations of mineral and vitamins compositions of leaves and flowers of studied areas. The mineral elements (mg/100 g) in the leaves and flowers from Bantè, Dassa-Zoumè, Djidja, Glazoué, Savalou and Savè was as follows: phosphorus: leaves (from 393.3 to 723.9 mg) and flowers (from 243.07 to 318.4 mg), calcium: leaves (from 2494 to 3270 mg) and flowers

(from 1923 to 3270 mg), magnesium: leaves (from 513 to 860 mg) and flowers (from 424 to 620 mg), potassium: leaves (from 1748 to 2246 mg) and flowers (from 1620 to 1910 mg), sodium: leaves (from 49 to 92 mg) and flowers (from 25 to 52 mg), iron: leaves (from 41.85 to 85.92 mg) and flowers (from 39.26 to 56.72 mg), copper: leaves (from 1.86 to 4.8 mg) and flowers (from 1.1 to 1.25 mg) and manganese: leaves (from 5.98 to 9.8 mg) and flowers (from 3.6 to 5.1 mg). (Figures 3 and 4).

The provitamin A content varied from 54 to 79.6 mg /100 g in leaves and from 32.6 to 56.6 mg/100 g in flowers. Vitamin C content ranges from 2442to 3521 mg/100 g in leaves and from 3248 mg/100 g to 3870 mg/100 g in flowers. This variation could be explained by phenology. These results are similar to those reported in Ivory Coast by Kouassi et al. (2013) on okra varieties. These authors have shown that there is a variation in magnesium, potassium, manganese and sodium contents depending on the growing areas.

The results in Table 3 show that the phosphorus, calcium, magnesium, potassium, iron, copper, manganese, provitamin A and vitamin C contents of leaves and flowers are significantly different between areas ($p<0.05$). The difference between sodium contents were not significant ($p> 0.05$). These results differ from those reported in Ivory Coast by Kouassi et al. (2013). These authors have shown that there is no significant difference in magnesium, potassium, and manganese content of okra varieties by growing area. A significant difference ($p<0.05$) between phosphorus, calcium, magnesium, potassium, sodium, iron, copper, manganese, provitamin A and vitamin C contents in leaves and flowers in the same area was observed. This significant difference could be due to the particular metabolism of these organs. In addition Dougnon et al. (2012) have shown that there is a significant difference in mineral and vitamin content between leaves and fruits of *S. macrocarpon*. In terms of zone-Organs interaction, the difference is significant ($p< 0.05$) for phosphorus, calcium, magnesium, potassium, sodium, iron, copper, manganese, provitamin A and vitamin C contents. This justifies that the contents of these minerals and vitamins were specific to the area in which the species were found.

The results presented in Table 4 show that *L. multiflora* was a leaf vegetable rich in provitamin A, vitamin C, and minerals. The main minerals found in leaves and flowers were calcium (Ca), potassium (K), magnesium (Mg), Iron (Fe), copper (Cu) and manganese (Mn). Sodium (Na) and phosphorus (P) were found in low quantity. Leaves were richer in provitamin A and minerals than the flowers. However, flowers were richer in vitamin C.

Calcium contents of leaves and flowers of *L. multiflora* were 2872 and 2755.83 mg/100 g, respectively. These values are higher than those reported by Agbankpé et al. (2015) on *V. amygdalina* (1180 mg/100 g), *C. adansonii* (2400 mg/100 g) and *S. radiatum* (1520 mg/100 g). These values are also higher than those reported on *C. crepidioides* (1012 mg/100 g) by Adjatin et al. (2013) and lower than that of *C. rubens* (3845.88 mg/100 g) reported by the same authors. This leafy vegetable could then be considered as a potential source of calcium. Calcium is the most abundant mineral in body and is involved in blood clotting, muscle contraction, neurological function, bone and tooth formation (Senga et al., 2013). It is also an important factor in enzymatic metabolic processes (Karau et al., 2012).

The average phosphorus contents were 570 and 283.868 mg/100 g in leaves and flowers of *L. multiflora*, respectively. According to results reported by Agbankpé et al. (2015) as well as those reported by Adjatin et al. (2013), these contents are lower than those of *V. amygdalina* (873 mg/100 g), *C. adansonii* (693 mg/100 g), *S. radiatum* (650 mg/100 g), *C. crepidiodes* (1039.2 mg/100 g) and *C. rubens* (1409 mg/100 g). The recommended daily intake of phosphorus for adults and children is 800 mg/day (Pillai and Nair, 2013). Phosphorus associated with calcium, helps strengthen bones and teeth, especially in children and nursing mothers (Andzouana and Mombouli, 2012). The phosphorus content obtained from leaves and flowers of *L. multiflora* is below the recommended norm. Therefore, *L. multiflora* is not a good source of phosphorus.

The average contents of potassium in leaves and flowers were 2009.83 and 1722 mg/100 g, respectively. These values are lower than those reported by Agbankpé et al. (2015) on *V. amygdalina* (4820 mg/100 g), *C. adansonii* (2810 mg/100 g) and Adjatin et al. (2012) on *C. crepidioides* (2291.86 mg/100 g) and *C. rubens* (4469.91 mg/100 g). Potassium is important in regulating heart rate, body water balance and neurotransmission (Alinnor and Oze, 2011). A high quantity of potassium in body increases iron use (Nair et al., 2012) and is beneficial for people taking diuretics to control high blood pressure (Nair et al., 2012).

The average magnesium content in leaves and flowers were 683.5 and 530.16 mg/100 g respectively. These values are higher than those reported by Agbankpé et al. (2015) on *C. adansonii* (670 mg/100 g) and *S. radiatum* (510 mg/100 g) as well as those reported by Adjatin et al. (2012) on *C. crepidioides* (336.46 mg/100 g) and *C. rubens* (434.13 mg/100 g). According to the results reported by Agbankpé et al. (2015), these values are lower than that of *V. amygdalina* (900 mg/100 g). The recommended dietary allowance for magnesium is 350 mg/100 g for adults and 170 mg/100 g for children. From these results, it emerges that *L. multiflora* is rich in magnesium and could meet the daily needs of adults and children. Magnesium is known to prevent cardiomyopathy, muscle degeneration, growth retardation, alopecia (premature hair loss), dermatitis, immune system dysfunction, gonad atrophy, impaired spermatogenesis, birth defects and coagulation disorders (Andzouana and Monbouli, 2012). According to Alinnor and Oze (2011),

Table 3. Analysis of variance of mineral and vitamins compositions of leaves and flowers from *L. multiflora* according to area collection.

Source	DF	Model	Zone	Part	Zone-Part
		11	1	5	5
Phosphor	MS	53796.780	15065.338	491227.984	15801.322
	F	295.261	82.685	2696.078	86.725
	Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Calcium	MS	321383.278	98962.167	80968.167	656005.367
	F	48.875	15.050	12.313	99.762
	Pr > F	< 0.0001	0.000	0.005	< 0.0001
Magnesium	MS	29510.500	13163.867	141066.667	29434.667
	F	169.306	75.523	809.318	168.871
	Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Potassium	MS	76032.042	38915.767	497088.167	44141.367
	F	30.828	15.779	201.552	17.898
	Pr > F	< 0.0001	0.000	< 0.0001	< 0.0001
Sodium	MS	860.389	61.500	6600.167	673.767
	F	15.938	1.139	122.260	12.481
	Pr > F	< 0.0001	0.396	< 0.0001	0.000
Iron	MS	319.610	505.527	661.343	129.246
	F	1487.064	2352.090	3077.058	601.347
	Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Copper	MS	3.221	1.520	22.885	1.635
	F	14719.773	6944.463	104571.224	7468.733
	Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Manganese	MS	8.249	2.690	73.395	2.429
	F	5732.348	1869.422	51001.511	1687.883
	Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Vitamin A	MS	471.755	304.974	3441.615	138.707
	F	226.229	146.249	1650.415	66.516
	Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Vitamin C	MS	506126.958	261735.067	3220802.667	308788.267
	F	34402.038	17790.437	218921.705	20988.698
	Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001

magnesium plays an essential role in calcium metabolism and in bone formation and is also involved in the prevention of diseases related to the circulatory system. It helps regulate blood pressure and insulin secretion.

Sodium is an important mineral that helps regulate blood flow and maintain electron potential in body tissues (Alinnor and Oze, 2011). The sodium content average in leaves and flowers were 68.83 and 35.66 mg/100 g, respectively. These contents are much lower than those reported by Adjatin et al. (2013) on *C. crepidioides* (2291.86 mg/100 g) and *C. rubens* (2921.04 mg/100 g). They are higher than those reported by Sanoussi et al. (2016) on *Ipomoea Batatas* (29 and 34 mg/100 g).

The average iron content was 57.25 mg/100 g in leaves and 46.75 mg/100 g in flowers of *L. multiflora* (Table 4). This content is much higher than that recommended by

Table 4. Average composition of mineral elements, provitamins A and vitamin C of leaves and flowers from *L. multiflora* in mg/100 g of dry matter.

Composition (mg)	*L. multiflora*		Norm/WHO
	Leaves	Flowers	
Phosphor (P)	570 ± 110.99[b]	283.868 ± 26.64[a]	800
Calcium (Ca)	2872 ± 371.13[b]	2755.83 ± 427.7[a]	800
Magnesium (Mg)	683.5 ± 113.35[b]	530.16 ± 71.17[a]	350
Potassium (K)	2009.83 ± 167.12[b]	1722 ± 94.55[a]	2000
Sodium (Na)	68.83 ± 16.09[b]	35.66 ± 10.04[a]	500
Iron (Fe)	57.25 ± 14,97[b]	46.75 ± 6.36[a]	10-15
Copper (Cu)	3.12 ± 1.16[b]	1.66 ± 0.05[a]	3
Manganese (Mn)	7.58 ± 1.37[b]	4.09 ± 0.5[a]	-
Ca/P	5.03[a]	9.7[b]	> 1
Na/K	0.034[b]	0.02[a]	< 1
Vitamin A	64.47 ± 10.73[b]	40.52 ± 8.47[a]	-
Vitamin C	2778.83 ± 444.66[a]	3511.5 ± 200.03[b]	-

Number of repetition n=3; significant difference p ≤ 0.01.

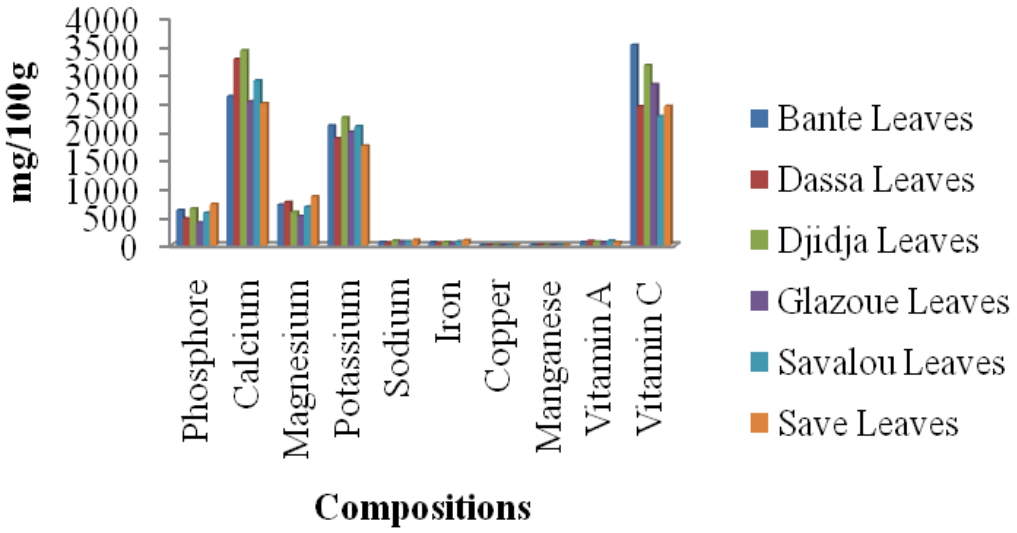


Figure 3. Mineral and vitamins composition of *L. multiflora* leaves from six areas.

WHO, which is 10 to 15 mg/day (Senga et al., 2013).

According to Andzouana and Monbouli (2012), iron as a trace element plays many biochemical roles and is a fundamental element in the metabolism of almost all living organisms. In humans, iron is an essential constituent of several types of proteins and enzymes (Andzouana and Monbouli, 2012). It is important for the normal functioning of the central nervous system (Alinnor and Oze, 2011) and facilitates the oxidation of carbohydrates, proteins and lipids. Iron is necessary for formation of hemoglobin contained in blood and is an important component in diet of pregnant women, nursing mothers, infants and the elderly who often suffer from anemia and other illnesses related to blood (Alinnor and Oze, 2011).

The average copper content was 3.12 mg/100 g in leaves and 1.66 mg/100 g in flowers. Copper content in leaves is higher than that reported by Adjatin et al. (2013) on *C. crepidioides* (1.4 mg/100 g) and *C. rubens* (2.6 mg/100 g). Copper is necessary for enzymes production and electrons transport in body (Alinnor and Oze, 2011).

The average manganese content was 7.58 mg/100 g in leaves and 4.09 mg/100 g in flowers. These values are lower than those reported by Adjatin et al. (2013) on *C. crepidioides* (7.7 mg/100 g) and *C. rubens* (8.22 mg/100 g). Manganese is a trace element that plays an important

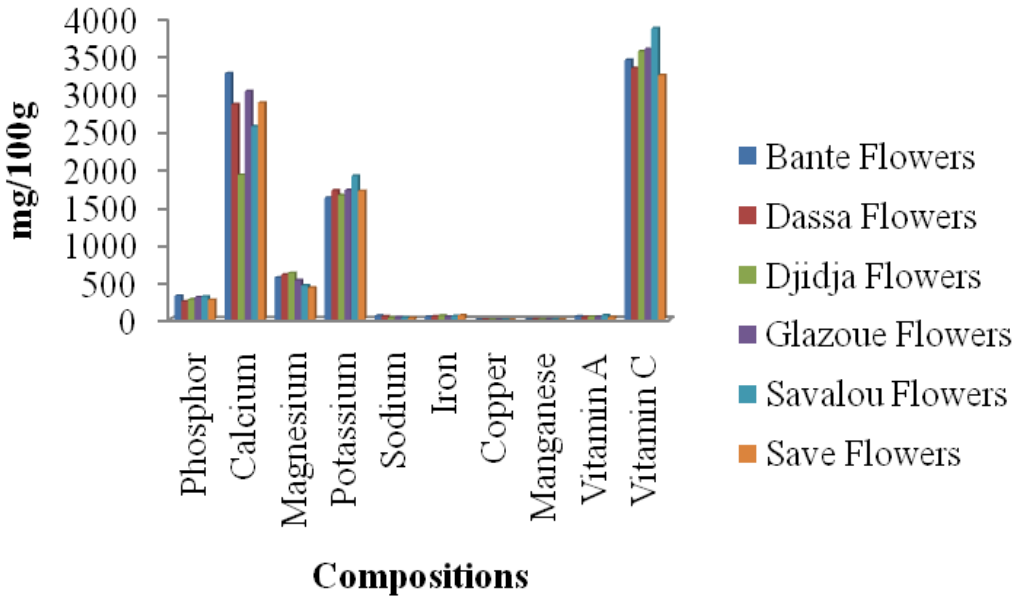


Figure 4. Mineral and vitamins composition of *L. multiflora* flowers from six areas.

role in synthesis of bones, amino acids and metabolism of carbohydrates. It contributes to the proper functioning of thyroid gland and serves to heal inflammation and sprains (Erikson et al., 2005). Manganese deficiency can lead to problems with infertility, diabetes, and joint pain. It is an antioxidant which protects body against damage caused by free radicals. Despite its essential character, the accumulation of manganese in blood is toxic to central nervous system (Sidoryk and Aschner, 2013).

The value of Ca/P ratio was, respectively 5.03 and 9.7 for leaves and flowers. According to Adeyeye and Aye (2005) and Alinnor and Oze (2011), a Ca/P ratio greater than 2 helps to increase the absorption of calcium in small intestine. Diet is considered good if Ca/P ratio is greater than 1 and poor if the ratio is less than 0.5. Therefore, *L. multiflora* appears to be a good source of food. Furthermore, Na/K ratio of leaves and flowers were, respectively 0.034 and 0.02. Alinnor and Oze (2011) reported that Na/K ratio promotes blood pressure control and a Na/K ratio less than 1 lowers blood pressure. So *L. multiflora* could be used as a food to treat or prevent blood pressure problems.

The results of Table 4 have shown that the average of provitamin A content was 64.47 mg/100 g in leaves and 40.52 mg/100 g in flowers. These values are higher than those reported by Millogo-Koné et al. (2010) on *M. oleifera* leaves (39 mg/100 g) and lower than those reported by Tchiégang and Aissatou (2004) on *Hibiscus cannabinus* (60 mg/100 g) and *Cerathotheca sesamoïdes* (90 mg/100 g). Vitamin A is involved in the synthesis of bones, teeth, hair and reproduction (Wardlaw and Kessel, 2002). It is mainly involved in the processes of vision maintenance, regulation of gene expression and cell differentiation (Herrero et al., 2012). According to Vanisha et al. (2008), the vitamin A requirements for an adult weighing 70 kg are 1000 µg/d which means that *L. multiflora* could be considered as a potential source of provitamin A.

The average vitamin C content was respectively 2778.83 and 3511.5 mg/100 g in leaves and flowers of *L. multiflora*. These values are much higher than those reported by Millogo-Koné et al. (2010) on *M. oleifera* (210 mg/100 g), *C. crepidioides* (9.17 mg/100 g) and Adjatin et al. (2013) on *C. rubens* (3.60 mg/100 g). According to Tchiégang and Aissatou (2004), these values are less than 4590 mg/100 g in *T. triangulare* and 4920 mg/100 g in *Vigna unguiculata*. Daily vitamin C requirements vary between 40 and 90 mg/day (Vanisha et al., 2008). So this leafy vegetable would be a very good source of vitamin C. Vitamin C plays an important role in maintaining good health and preventing disease. It has immuno-stimulating, anti-allergic and antioxidant effects and protects the cardiovascular system and eyes. Vitamin C is necessary for the synthesis of collagen, the intercellular substance that provides the structure of muscles, vascular tissues, bones, tendons and ligaments (Olayinka et al., 2012).

Conclusion

The results of this work provided data on the nutritional composition of leaves and flowers of *L. multiflora* in Benin. Leaves and flowers of *L. multiflora* are rich in fiber, ash, calcium, magnesium, potassium, copper and especially iron, provitamin A and vitamin C. So this plant

is a good source of nutrients. So it is important to use it in improving the health status of populations and in the fight against malnutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

REFERENCES

Achikanu CE, Akpata E, Uwa J (2021). Level of minerals in ten leafy vegetables eaten in Enugu State Nigeria. Special Journal of Public Health, Nutrition, and Dietetics 1(2):1-10.

Agbankpé AJ, Dougnon TVB, Bankole S, Yèhouénou B, Yedomonhan H, Legonou M et Dougnon TJ (2014). Etude ethnobotanique des légumes feuilles thérapeutiques utilisés dans le traitement des diarrhées au sud-Bénin (Afrique de l'Ouest). International Journal of Biological and Chemical Sciences 8(4):1784-1795.

Agbankpé AJ, Bankolé SH, Dougnon TJ, Yèhouénou B, Hounmanou YMG, Baba-Moussa LS (2015). Comparison of nutritional values of *Vernonia amygdalina*, *Crateva adansonii* and *Sesamum radiatum*: Three main vegetables used in traditional medicine for the treatment of bacterial diarrhoea in southern Benin (West Africa). Food and Public Health 5(4):144-149

Adjatin A, Dansi A, Badoussi E, Sanoussi AF, Dansi M, Azokpota P, Ahissou H, akouegninou A, Akpagana K, Sanni A (2013). Proximale, mineral and vitamine C composition of vegetable Gbolo (*Crassocephalum rubens* and *C. crepidioides*) in Benin. International Journal of Biological and Chemical Sciences 7(1):319-331.

Aja PM, Okaka ANC, Ibiam UA, Uraku AJ, Onu PN (2010). Proximate analysis of *Talinum triangulare* (water leaf) leaves and its softening principle. Pakistan Journal of Nutrition 9(6):524-526.

Akakpo Amandine DM, Achigan-Dako Enoch G (2019). Nutraceutical Uses of Traditional Leafy Vegetables and Transmission of Local Knowledge from Parents to Children in Southern Benin. Agronomy 9(12):805 doi:10.3390/agronomy 9120805.

Alinnor IJ, Oze R (2011). Chemical evaluation of the nutritive value of *Pentaclethra macrophylla* Benth (African Oil Bean) Seeds. Pakistan Journal of Nutrition 10(4):355-359.

Antia BS, Akpan EJ, Okon PA, Umoren IU (2006). Nutritive and Anti-Nutritive valuation of Sweet Potatoes (*Ipomoea batatas*) Leaves. Pakistan Journal of Nutrition 5:166-168.

Association of Official Analytical Chemists (AOAC) (1990). Official methods of Analysis (15th Edition): Helrich K. Ed.; Association of Official Analytical Chemists, Washington D.C.

Association of Official Analytical Chemists (AOAC) (2005). Official method of analysis of the Association of official Analytical Chemist. 5th ad. AOAC Press Arlington, Virginia, USA.

Andzouana M, Mombouli JB (2012). Assessment of the chemical and phytochemical constituents of the leaves of a wild vegetable *Ochthocharis dicellandroides* (Gilg). Pakistan Journal of Nutrition 11(1):94-99.

Badau MH, Abba HZ, Agbara GI, Yusuf AA (2013). Proximate composition, mineral content and acceptability of granulated maize dumpling (*Dambu Masara*) with varying proportions of ingredients. Global Advanced Research Journal of Agricultural Science 2(1):320-329.

Batawila K, Akpavi S, Wala K, Kanda M, Vodouhe R, Akpagana K (2005). Diversité et gestion des légumes de cueillette au Togo. African Journal of Food Agriculture Nutrition and Development 21 p.

Dansi A, Vodouhè R, Azokpota P, Yedomonhan H, Assogba P, Adjatin A, Loko YL, Dossou-Aminon I, Akpagana K (2012). Diversity of the Neglected and Underutilized Crop species of importance in Benin. The Scientific World Journal pp. 1-19.

Djengue HW, Dansi A, Adjatin A, Dossou-Aminon I, Dansi M and Sanni A (2017). Ethnobotanical Investigation of *Lippia multiflora* Moldenke, a Local Aromatic Leafy Vegetable under Domestication in Benin. International Journal of Current Research in Biosciences and Plant Biology 4(5):44-51.

Diallo KS, Koné KY, Soro D, Assidjo NE, Yao KB, Gnakry D (2015). Caractérisation biochimique et fonctionnelle des graines de sept cultivars de voandzu (*Vigna subterranea* L., Fabaceae), cultivés en Côte d'Ivoire. European Scientific Journal 11(27):288-304.

Dougnon TV, Bankolé HS, Johnson RS, Klotoé JR, Fernand GD, Assogba GF (2012). Phytochemical screening, nutritional and toxicological analyses of leaves and fruits of *Solanum macrocarpon* Linn (Solanaceae) in Cotonou (Benin). Food and Nutrition Sciences 3(11):1595-1603.

Ekissi Alice Christine, Konan Amoin Georgette, Yao-Kouamé Albert, Bassirou bonfoh and Kati-Coulibaly Séraphin (2011). Evaluation of the chemical constituents of savannah tea (*Lippia multiflora*) leaves. Journal of Applied Biosciences 42:2854-2858.

Ejoh RA, Nkonga DV, Innocent G, Moses MC (2007). Nutritional components of some non-conventional leafy vegetables consumed in Cameroon. Pakistan Journal of Nutrition 6(6):712-717.

Emebu PK, Anyika JU (2011). Proximate and mineral composition of kale (*Brassica oleracea*) grown in Delta State, Nigeria. Pakistan Journal of Nutrition 10(2):190-194.

EPSIC (1999). Nutriments et énergie. Menu de la chimie: démonstrations disponibles et Travaux Pratiques (TP), Chapitre 7 pp. 1-6.

Erikson KM, Syversen T, Aschner JL, Aschner M (2005). Interactions between excessive manganese exposures and dietary iron-deficiency in neuro-degeneration. Environmental Toxicology and Pharmacology 19(3):415-421.

Food and Agriculture Organization (FAO) (1988). Traditional food plants. Food and Nutrition Paper 42:1-3.

Herrero M, Cifuentes A, Banez E (2012). Comprehensive sampling and sample preparation, Elsevier, J. Pawliszyn Editor, Amsterdam3.

Itoua OYS, Elenga M, Moutsamboté JM, Mananga V, Mbemba F (2015). Évaluation de la consommation et de la composition nutritionnelle des légumes-feuilles de *Phytolacca dodecandra* consommés par les populations originaires des districts d'Owando et de Makoua. Journal of Animal and Plant Sciences 27(1):4207-4218.

Kamal B, Farid M, Abdessamad BM, Marianne S, Marie-Laure F, Mohamed B, Hana SC, Ahmed E (2021). Proximate Composition, Amino Acid Profile, and Mineral Content of Four Sheep Meats Reared Extensively in Morocco: A Comparative Study. The Scientific World Journal 6633774, 11 pages.

Kane F, Yao-kouame A, Konan AA, N'guessan AK (2010). Dosage de quelques composantes biochimiques des feuilles de *Lippia multiflora* (verbénacée) à deux stades de développement et qualité des infusions, en fonction de la dose d'urée. Agronomie Africaine 22(3):227-235.

Karau GM, Njagi NM, Machocho AK, Wangai LN (2012). Phytonutrient, mineral composition and *in vitro* antioxidant activity of leaf and stem bark powders of *Pappea capensis* (L.). Pakistan Journal of Nutrition 11(2):123-132.

Kouame NM, Soro K, Mangara A, Diarrassouba N, Coulibaly AV, Boraud N, Kama M (2015). Étude physico-chimique de sept (7) plantes spontanées alimentaires du centre-ouest de la Côte d'Ivoire. Journal of Applied Biosciences 90(1):8450-8463.

Kouassi JB, Massara CC, Daniel ES, Tiahou GG, Djohan FY (2013). Détermination des teneurs en Magnésium, Potassium, Manganèse et Sodium de deux variétés de gombo. Journal of Applied Biosciences 67:5219-5227.

Maundu P (2005). Les légumes-feuilles traditionnels. http://www.tilz.tearfun.org.

Melse-Boonstra Alida (2020). Bioavailability of Micronutrients From Nutrient-Dense Whole Foods: Zooming in on Dairy, Vegetables, and Fruits. Frontiers in Nutrition 7:101. doi: 10.3389/fnut.2020.00101

Millogo-Koné H, Kini BF, Yougbaré Z, Yaro MB, Sawadogo M (2014). Etudes de la phytochimie et de l'activité antimicrobienne *in vitro* des feuilles de *Moringa oleifera* (Moringaceae). Pharmacopée et Médecine Traditionnelle Africaine P 16.

Nair AG, Pradeesh S, Devi CM, Mini I, Swapna TS (2012). Diplazium esculentum: A Wild Nutrient-Rich Leafy Vegetable from Western Ghats. Prospects in Bioscience 1:293-301.

Olayinka OO, Kareem AM, Ariyo IB, Omotugba SK, Oyebanji AO (2012). Antioxidant contents (vitamin C) of raw and blanched different

fresh vegetable samples. Food and Nutrition Sciences 3:18-21.
Pillai LS, Nair BR (2013). Proximate composition, Mineral elements and Anti-nutritional factors in *Cleome viscosa* L. and *Cleome burmanni* W. & A. (Cleomaceae). International Journal of Pharmacy and Pharmaceutical Sciences 5(1):384-387.
Rubaihayo EB (1992). The diversity and potential use of local vegetables in Uganda. In: Guarino L (Ed.). The First National Plant Genetic Resources Workshop: Conservation and Utilization. IPGRI, Kenya, pp. 109-114.
Salma S (2020). Nutritional and functional properties of *Moringa oleifera***.** Metabolism Open 8 (100061).
Sanoussi AF, Adjatin A, Dansi A, Adebowale A, Sanni LO, Sanni A (2016). Mineral composition of tenelites sweet potato (*Ipomoea Batatas* L. Lam.) Landraces of Benin. International Journal of Current Microbiology and Applied Sciences 5(1):103-115.
Senga KP, Opota OD, Tamba VA, Tona LG, Kambu KO, Covaci A (2013). Chemical composition and nutritive value study of the seed oil of *Adenanthera pavonina* L. (Fabaceae) growing in Democratic Republic of Congo. International Journal of Pharmtech Research 5(1):205-216.
Sidoryk-Wegrzynowicz M, Aschner M (2013). Manganese toxicity in the central nervous system: the glutamine/glutamate-γ-aminobutyric acid cycle. Journal of Internal Medicine 273(5):466-477.
Soro LC, Ocho-Anin AAL, Kouadio KKA, Kouamé C (2012). Evaluation de la composition nutritionnelle des légumes feuilles. Journal of Applied Biosciences 51:3567-3573.
Sodamade A (2013). Proximale analysis, mineral content, amino acid composition and functional properties of *Vernonia amygdalina* vegetable leaf protein concentrates. Greener Journal of Agricultural Sciences 3(3):204-210.
Stevels JMC (1990). Legumes traditionnels du Cameroun, une etude agro-botanique. Agricultural University, Wageningen, the Netherlands Papers N° 90, 262 p.
Tchiégang C, Aissatou K (2004). Données ethnonutritionnelles et caractéristiques physico-chimiques des légumes-feuilles consommés dans la savane de l'Adamaoua (Cameroun). Tropicultura 22(1):11-18.
Vanisha S, Ambiar N, Shilpa P (2008). Standardization and organoleptic evaluation of Drumstick (*Moringa oleifera*) leaves incorporated into traditional Indian recipes. Trees for life Journal 3(2):1-7.
Vodouhè R, Dansi A (2012). The "Bringing into Cultivation" Phase of the plant domestication process and its contributions to *In Situ* conservation of genetic resources in Benin. The Scientific World Journal, pp. 1-13.
Wardlaw GM, Kessel M (2002). Perspective in nutrition (5^{th}ed). Mc graw-hill, Boston pp. 271-274.
Yaméogo CW, Bengaly MD, Savadogo A, Nikiema PA, Traore SA (2011). Determination of chemical composition and nutritional values of *Moringa oleifera* leaves. Pakistan Journal of Nutrition 10(3):264-268.

Vol. 21(3), pp.106-115, March 2022
DOI: 10.5897/AJB2021.17436
Article Number: 638B14C68810
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Liver enzyme evaluation and NAT2 polymorphism in patients on anti-tuberculosis and antiretroviral drugs at Jamot Hospital in Yaoundé-Cameroon

Ambassa Axel Cyriaque[1,2], Tiani Lionel Ulrich[1,2], Djuidje Ngounoue Marceline[1,3], Assam Assam Jean Paul[1,4], Andoseh Généviève[1,2], Kamdem Thiomo Diane[1,2], Tchinda Fossi Cédric[1,2], Nkah Numfor Leonard[5], Gonsu Kamga Hortense[11], kouanfack Charles[7,8], Pefura Yone Eric Walter[9,10], Ntoumi Francine[5,6] and Penlap Beng Véronique[1,2*]

[1]Department of Biochemistry, Faculty of Science, University of Yaoundé I, Cameroon.
[2]Laboratory for Tuberculosis Research and Pharmacology, Biotechnology Center of Nkolbisson (BTC), University of Yaoundé I, Yaoundé, Cameroon.
[3]Research and Health Ethics Committee in Central Africa (CERSAC), Cameroon.
[4]Department of Microbiology University of Yaoundé I, Cameroon.
[5]Fondation Congolaise pour la Recherche Médicale, Brazaville, Republic of Congo.
[6]Privatdozentin, Faculty of Medicine, Institute for Tropical Medicine, University of Tubingen, Germany.
[7]Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon.
[8]HIV-Day Care Service, Yaoundé Central Hospital, Yaoundé, Cameroon.
[9]Pneumology Service, Yaoundé Jamot Hospital, Cameroon.
[10]Department of Internal Medicine and Subspecialties, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon.
[11]Department of Microbiology and Infectious Diseases, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon.

Received 1 December, 2021; Accepted 21 February, 2022

Hepatotoxicity is reported frequently as an adverse reaction during tuberculosis (TB) and HIV treatment. This study aimed to investigate the incidence of antiretroviral and anti-tuberculosis drug-induced liver enzymes activities variation in TB and TB-HIV co-infected patients at Jamot Hospital in Yaoundé-Cameroon. From April 2018 to May 2019, 336 treatment-naïve TB patients were enrolled. Liver enzymes (AST, ALT, ALP) and total bilirubin were evaluated at baseline and 12 weeks after treatment initiation. Blood was spotted on filter paper for DNA extraction by the chelex method. Standard nested PCR followed by restriction enzyme analysis with *KpnI, TaqI,* and *BamHI* to detect NAT2 polymorphisms was performed. TB-HIV co-infection prevalence was 29.46%. There was a significant rise of transaminases ($p < 0.05$) at baseline in TB-HIV co-infected patients. At 12 weeks, there was a substantial rise of transaminases in TB patients, and total bilirubin in TB-HIV co-infected patients ($p < 0.05$). The prevalence of slow and fast acetylators was 85.71 and 14.29%, respectively. NAT2*5/5 and NAT2*5/6 genotypes were most represented. Slow acetylating NAT2 phenotype was significantly associated with drug hepatotoxicity ($p < 0.05$). The prevalence of TB-HIV co-infections remains high, and the rise in transaminases is linked to the slow acetylating NAT2 phenotype.

Key words: TB/HIV co-infection, hepatotoxicity, NAT2, Jamot Hospital, Cameroon.

INTRODUCTION

Tuberculosis is an infectious and contagious disease caused by the mycobacteria of the *Mycobacterium tuberculosis* complex and the leading cause of morbidity and mortality in sub-Saharan Africa and worldwide, in people with HIV/AIDS. In 2018, 10 million people contracted the disease, and 1.2 million died. In addition, 2.48 million new cases were recorded in Africa, with 397,000 deaths (WHO, 2019). Tuberculosis also represents one of Cameroon's major threats to public health and a significant cause of preventable mortality in the adult population (NTCP, 2019). In 2018, 47,000 cases of tuberculosis were registered, with 7,700 cases of death (WHO, 2019). In 2019, 82% of tuberculosis patients were tested for HIV, with a prevalence of TB-HIV co-infection of 27% (NTCP, 2019).

Anti-tuberculosis therapy has always been an inducing toxicity factor due to the combination of Isoniazid, Rifampicin, and Pyrazinamide (NTCP, 2012). Similarly, a 14-fold increased risk of anti-TB hepatotoxicity has been reported in patients co-infected with HIV (NTCP, 2012). That implies HIV infection increases the risk of antiretroviral (ARV)-induced hepatotoxicity (Younossian et al., 2005; Sadaphal et al., 2001; Pan et al., 2005). Efavirenz is the first antiretroviral drug usually given in combination with rifampicin-based anti-TB treatment therapy in case of TB/HIV co infection (WHO, 2012). Though effective, this antiretroviral has been associated with liver injury (Abrescia et al., 2002) and is metabolized in the liver by many liver enzymes (Yimer et al., 2011). Rifampicin, proved as a potent inducer of these enzymes, is known to reduce plasma efavirenz concentrations. Therefore overlapping toxicities can complicate multidrug therapy and cause treatment failure, relapse or drug-resistance (Tostmann et al., 2008; Saukkonen et al., 2006; Wares et al., 2003). Although rifampicin and efavirenz are the principal molecules used in the case of concomitant TB and HIV therapy in low- income countries, data are still limited in high endemic HIV/AIDS and TB areas (Desta et al., 2007).

Moreover, N-acetyltransferase2 enzyme (NAT2) initiates the metabolism of isoniazid in the liver by biochemical acetylation, with variable rates depending on the genetic component of each individual (Mc Donagh et al., 2014). Despite considerable evidence demonstrating that the relationship between hepatotoxicity induction and NAT2 gene distribution differs according to ethnicity, there are few studies involving TB/HIV co-infected individuals in sub-Saharan Africa countries, as well as in Cameroon (Lee et al., 2010; An et al., 2012; Ben Mahmoud et al., 2012). This suggests that careful monitoring should be carried out in all patients after initiation of antiretroviral therapy to identify risk factors for better management of drug-induced hepatotoxicity and design prevention measures. Hence this study was undertaken to study seroprevalence of TB-HIV co-infection and hepatotoxicity during anti-TB treatment in people living with HIV/AIDS at Jamot Hospital in Yaoundé-Cameroon.

MATERIALS AND METHODS

Study design and population

This prospective study with descriptive aims was carried out at Jamot Hospital in Yaoundé from April 2018 to March 2019. The study population consisted of patients in consultation and observation in the Pneumatology Unit. A total of 336 pulmonary tuberculosis patients were enrolled in this study. The serum, obtained from the collected blood, was directly analyzed or stored at -20°C.

Inclusion and exclusion criteria

According to the defined inclusion criteria, participants in this study were aged 15 and more, regardless of gender ethnicity. In addition, only volunteers who agreed to sign an informed consent form after being informed on the nature, study procedure, potential benefits, and foreseeable risks were recruited, excluding patients with forms of tuberculosis other than pulmonary TB.

Data collection procedure and laboratory analyses

Peripheral venous blood samples (10 mL) were obtained from all studied patients at baseline and after 12 weeks of anti-TB and antiretroviral treatment. For confidentiality management, an anonymous identification code was assigned to each patient for laboratory analyses, data entry, and data analyses. Sputum samples were collected successively over three days after microscopic confirmation of positive cases. In parallel, blood samples were collected in dry tubes and EDTA tubes, in strict compliance with the conditions of asepsis. The serum was separated by centrifugation at 3000 (g) for 5 min, aliquots done, and stored at -20°C for subsequent analyses. Anti-HIV antibodies were determined using the whole blood by immuno-chromatography using ALERE Determine, and all positive cases were confirmed using Oraquick. An aliquot of the serum was then used to determine the serum enzymes activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) by kinetic method, and the total bilirubin using colorimetric method. The kinetic method for determining ALT and AST was based on the recommended International Federation of Clinical Chemistry (IFCC). Hepatotoxicity was defined as elevated aminotransferase levels and identified as three times higher than normal before initiating TB treatment, with associated symptoms considered jaundice, nausea, vomiting, dyspepsia, and asthenia. The reference values adopted were AST 37 UI/mL and ALT 40 UI/mL, ALP 92 UI/mL and total bilirubin 1

*Corresponding author. E-mail: v.penlap@yahoo.fr. Tel: +237 77695459.

mg/dL) according to the manufacturer's instructions. The biological variables potentially related to hepatotoxicity were analyzed, and the use of antiretroviral drugs at the time of initiating TB treatment was confirmed through medical records. The results of all the 336 tests for HIV antibodies conducted on the 173 PLWHA patients included in this study were positive. All patients who had initiated TB treatment due to clinical diagnosis or after a bacteriological test confirmation were said to have TB infection.

NAT2 polymorphism evaluation

The most common alleles in the African population were investigated. They contained the following mutations: C481T (rs1799929, silent mutation, amino acid change L161L), G590A (rs1799930, amino acid change R197Q), A803G (rs1208, amino acid change K268R) and G857A (rs1799931, amino acid change G286E). The primers used to amplify the gene was: NAT2 (+) 5'-GCCTCAGGTGCCTTGCATTT-3' and NAT2 (-) 5'-CGTGAGGGTAGAGAGGATAT-3'. The PCR amplification was carried out using a T3 thermal cycler (Biometra, UK), and performed in a total volume of 25 µl containing: nuclease-free water, 10× thermopol buffer, 10 mM dNTPs (200 µM of each deoxyribonucleotide), 20 pmol primers, 5 U/µL Taq polymerase, and 3 ng of gDNA. After initial denaturation at 95°C for the 5 min, 30 cycles of amplification was carried out with denaturation at 95°C for the 50 s, annealing at 55°C for the 50 s and extension at 72°C for the 50 s, followed by a final extension at 72°C for the 5 min. To confirm the presence of NAT2 alleles, PCR products were electrophoresed on a 2% agarose gel and polymorphisms determined by restriction endonuclease digestion of amplified gene fragments as previously described (Chen et al., 2007). The amplicons were digested under conditions stipulated for the restriction enzymes *KpnI* and *BamHI* (New England Biolabs, USA), followed by inactivation at 80°C for 20 min, a 2% agarose gel electrophoresis with Ethidium Bromide, and the analysis of the migration by UV trans-illumination.

Acetylator genotype classification

NAT2 acetylator genotypes were produced according to previously published data (Yokogawa et al., 2001). In this procedure, homozygotes (NAT2*4/NAT2*4) or heterozygotes (NAT2*4/NAT2*5, NAT2*4/NAT2*6, and NAT2*4/NAT2*7 combinations) for the dominant NAT2*4 wild type allele were classified as fast acetylator genotypes, while homozygotes of the mutant alleles (NAT2*5, NAT2*6 ,and NAT2*7) were classified as slow acetylator genotypes.

Data preparation and analysis

The sample size was calculated by the formula

$n = z^2 \times p(1-p)/m^2$

With p = 0.5, z = 1.96, m **=** margin of error (5%).

The data obtained were entered, cleaned, and analyzed using the statistical software for social sciences (SPSS) version 22.1. The means, frequencies, and percentages were used to summarize the descriptive statistics of the data. The chi-square test ($\chi 2$) was used to assess the relationships between the qualitative variables, namely sex, marital status, level of education, occupation. Values of $P \leq 0.05$ were considered to be statistically significant.

Ethical considerations

Ethical approval for this study was obtained from the Regional Ethics Committee of the Centre Region, Cameroon N°: 2018/01/970/CE/SP. An authorization from the "Jamot Hospital in Yaoundé" collection site (N°:00001478/L/MINSANTE/SG/DHJY) was also obtained. All participants were fully informed about the objectives, procedures, potential risks, benefits, and purpose of the study. The included study population gave their signed informed consent form (provided in French and English languages).

RESULTS

Socio-demographic characteristics of the subjects of study

A total of 336 newly diagnosed pulmonary tuberculosis patients were recruited prospectively in this study, and they were followed up to 12 weeks. Baseline demographic and clinical data, plus laboratory results of the 336 patients at 12 weeks, were used for analyses. ALT, AST, PAL, and bilirubin levels were evaluated at baseline and 12 weeks. Of the total TB patients, 215 (63.98%) were men, while 121 (36.01%) were women, with a male to female ratio of 1.77:1. The average age of the patients was (35.16 ± 14.04 years), the minimum was 15 years, and the maximum was 94 years. Regarding marital status and level of education, 222 (66.07%) were single, 94 (27.97%) were married, 14 (4.16%) were widowed, and 6 (1.78%) were divorced, while 17 (5.05%) were out of school, 77 (22.91%) had a primary level, 194 (57.73%) had a secondary level, and 48 (14.28%) had a university level. Regarding the HIV status of the study patients, 99 (29.46%) tested positive for HIV. Table 1 presents the various socio-demographic characteristics in a grouped manner.

Biochemical parameters

Table 2 presents the values of liver enzymes activity in TB and TB-HIV patients. It appears that in TB-HIV co-infected patients, there is a significant rise in Alanine Aminotransferase (ALT: 28.97±2.98) (p = 0.01) and Aspartate Aminotransferase (AST: 67.98±5.75) (p= 0.008).

To assess the variation in liver enzymes and the impact of anti-tuberculosis treatment on hepatotoxicity, two samples were taken on Day 0 before the anti-TB treatment and three months after initiation. The results of Table 3 show that in TB mono-infected patients, there is a significant rise in ALT (32.2 ± 3.56) (p=0.01) and AST (64.67 ± 5.23) (p=0.008), three months after treatment initiation, compared to the value on Day 0. While in TB-HIV co-infected patients, there is a significant rise in Alkaline Phosphatase (ALP) (143.8±8.75; p= 0.001) and total bilirubin (1.44±0.27; p=0.018), but an insignificant increase in ALT.

Table 1. Socio-demographic characteristics of the study population.

Caracteristics	Effective	Percentage
Sex		
Female	121	36.01
Male	215	63.98
Total	366	100
Age in years		
<20	28	8.33
21-30	112	33.33
31-40	96	28.57
41-50	49	14.58
51-60	28	8.33
61-70	18	5.35
71 and above	5	1.48
Total	336	100
Marital status		
Singles	222	66.07
Married	94	27.97
Divorced	6	1.78
Widowers	14	4.16
Total	336	100
School level		
Out of school	17	5.05
Primary	77	22.91
Secondary	194	57.73
University	48	14.28
Total	336	100
Type of infection		
TB	237	70.53
TB-HIV	99	29.46
Total	336	100

Genetic polymorphism of NAT2 and association with hepatotoxicity

In this study, a total of 7 genotypes were obtained with variable proportions NAT2*5/5 (47.61%), NAT2*5/6 (19.04%), NAT2*5/7 (14.28%), NAT2*4/6 (4.76%), NAT2*4/7 (4.76%), NAT2*4/5 (4.76%), NAT2*6/7 (4.71%), with a predominance of four alleles: NAT2*5 (66.66%), NAT2*6 (14.28%), NAT2*7 (11.98%), and NAT2*4 (7.14%). And the results of phenotypes, genotypes and alleles are represented by Figures 1 to 3: NAT2*5/5 fast phenotype frequency 14.28%, and slow phenotype frequency 85.71%.

Impact of anti-tuberculosis treatment in tuberculosis patients and co-infected TB-HIV and variation in liver enzymes

In Table 4, liver enzyme values after three months of initiation of anti-tuberculosis treatment show variations in ALT (from 29.37 to 40.48 UI), AST (from 35.96 to 43.34 UI), and total bilirubin (from 0.83 to 1.38).

Association between hepatotoxicity and NAT2 polymorphism

In this study, 21 patients were genotyped. Among these, 5 were co-infection TB/HIV cases and 16 cases of mono-infection TB. Unfortunately, no statistically significant difference was found between acetylation phenotype and co-infection, as shown in Table 5.

The results of Table 6 have shown a significant association (p=.026) between the slow acetylators phenotype and the hepatotoxicity in mono-infected TB patients and TB/HIV co-infected patients.

DISCUSSION

Hepatotoxicity is reported frequently as an adverse

Table 2. Serum levels of liver enzymes according to the types of infection.

Infection	Total	Mean ± SD			
		ALT (U/L)	AST (U/L)	ALP (U/L)	TBR (mg/dl)
TB	237	15.98±1.32	34.89±3.84	141±8.65	1.48±0.28
TB-VIH	99	28.97±2.98	67.98±5.75	71.43±5.95	0.65± 0.19
p values		0.01	0.008	0.001	0.1984

ALT : Alanine Aminotransferase, AST : Aspartate Aminotransferase, ALP : Alkaline phosphatase, TBR : total Bilirubin.

Table 3. Evolution of serum levels of liver enzymes for a period of three months.

Type of infection	Sample	Mean ± SD			
		ALT U/l	AST U/l	ALP U/l	TBR mg/dl
TB	Day 0	15.98±1.32	34.89±3.84	141±8.65	1.48±0.28
TB	Day 90	32.2 ± 3.56	64.67 ± 5.23	98.4 ± 7.39	0.80 ± 0.23
P-Values		0.001	0.006	0.0021	0.19
TB-HIV	Day 0	28.97±2.98	67.98±5.75	71.43±5.95	0.65± 0.19
TB-HIV	Day 90	32.11±1.86	53.85±3.2	143.8±8.75	1.44±0.27
P-values		0.32	0.23	0.001	0.018

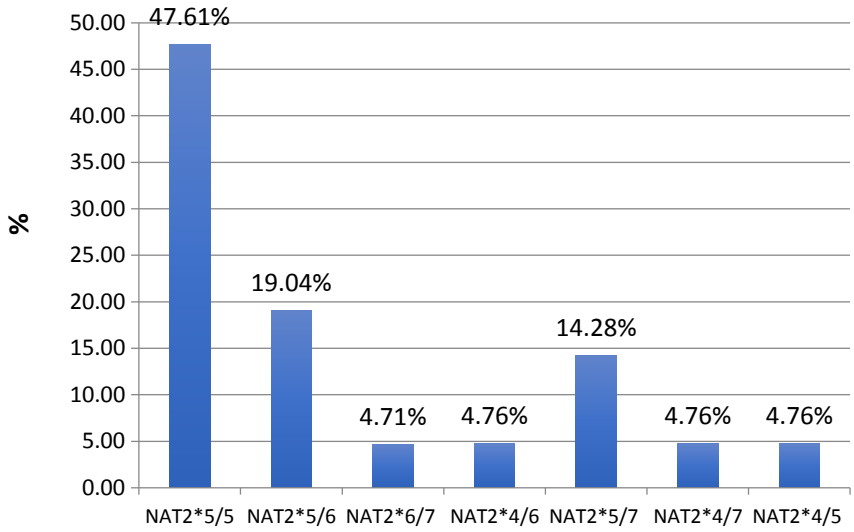


Figure 1. Genotypic frequency of NAT2.

reaction during the treatment of tuberculosis. This study aimed to study the seroprevalence of TB-HIV co-infection and hepatotoxicity during anti-TB treatment in people living with HIV/AIDS at Jamot Hospital in Yaoundé-Cameroon.

This study showed that TB/HIV co-infection prevalence was high (29.46%) (Table 1). This prevalence is higher than those reported by Assam-Assam et al. (2011) and Sidze et al. (2014) in Cameroon, which was 20.77 and 28.5%, respectively, but remains lower than that reported by Sama et al. (2017), which was 32.8%. This high prevalence was linked to Cameroon's being classified as a highly endemic area for TB, with the national prevalence of TB-HIV co-infection of 27% (NTCP, 2019). Previous studies have shown that HIV+ patients have an increased risk of TB infection (Havlir and Barnes, 1999; Colebunders and Lambert, 2002). The cellular immunosuppression induced by HIV infection prepares the entry of *Mycobacterium tuberculosis* and is a factor of TB recrudescence (Gollub et al., 1997). TB-HIV co-

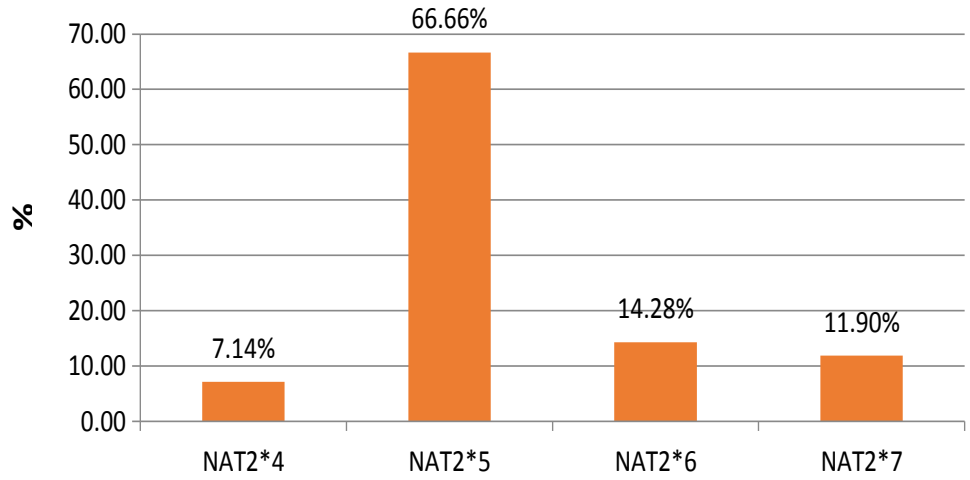


Figure 2. Allele frequency of NAT2.

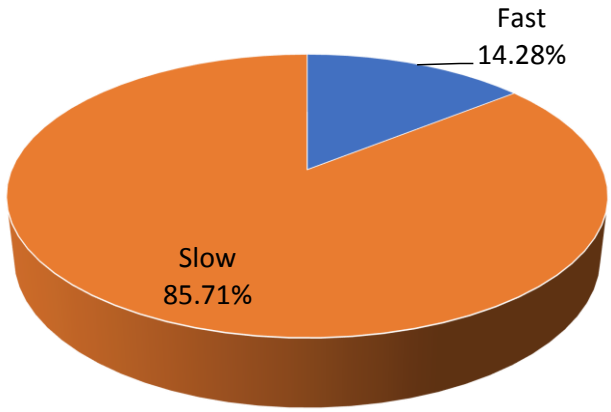


Figure 3. Global phenotypic frequency of NAT2.

Table 4. Impact of treatment on hepatotoxicity.

Biochemical parameters	Enzyme values	
ALT	Day 0	29.37
	Day 90	40.48
p-Value	0.05	
AST	Day 0	35.96
	Day 90	43.34
p-Value	0.31	
ALP	Day 0	154.5
	Day 90	153.27
p-Value	0.18	
Total bilirubin	Day 0	0.83
	Day 90	1.38
p-Value	0.018	

Table 5. Correlation between co-infection and N-acetyltransferase 2 phenotype.

Type of infection	Type of phenotype	
	Slow	Fast
Co-infected TB/HIV	4	1
Mono-infected	14	2
p-values	p=0.306	p=0.425

Table 6. Association between the slow acetylators phenotype and hepatotoxicity.

NAT2 Acetylators		Slow	Fast	Total
Hepatotoxicity	No	4	3	7
	Yes	14	0	14
Total		18	3	21
p-value		p=0.026		

infected subjects are 20 to 30 times more likely to develop active TB than HIV-negative subjects (WHO, 2018). Also, the obtained prevalence value of TB-HIV co-infection was slightly high among men (15.18%) compared to women (14.28%), more common in the 21 to 49 age group, representing 82.82% of co-infected cases, and also more prevalent among singles and married people as well, with 88.88% of co-infected cases. Similar results have been reported by other authors (Sama et al., 2017) and can be explained that TB is the main opportunistic disease in HIV-positive people. Moreover, in the Demographic and Health Survey reports and Multiple Indicators, Cameroon's most sexually active age group is between 15 and 49 years (Demographic and Health Survey and Multiple Indicators, 2011).

Analysis of the assay data showed a significant rise in serum transaminase activity (ALT/AST, p = 0.005) in TB-HIV coinfected patients. These results agree with the work of Sama et al. (2017) conducted in a TB-HIV coinfected population in the West region of Cameroon and show a rise in the levels of the transaminases. The high level of ALT and AST transaminases obtained in the present study could be explained by the percentage of HIV-positive patients in the study population (29.46%), already under ARV treatment for most of them at the start of the study. Indeed, HIV has been shown to directly attack liver cells (Oluwafemi et al., 2003) and cause cell death. The release in the environment of cell contents made of 20% of enzymes (Wild-Up et al., 1990) can be responsible for the significant increase in liver enzymes in infected patients confirmed by several studies in ARV-naive patients. In addition, tuberculosis, which accelerates morbidity and mortality in HIV-positive people, can lead to the degeneration of the liver connective tissue (Margulis et al., 1986) and result from hepatobiliary obstruction occurring in these patients. This high level of transaminase might also be due to the difficulties in accessing treatment in developing countries where most of the population barely visits hospitals for treatment and use drugs from unknown origin, which can lead to severe hepatotoxicity and even cirrhosis.

The results of the second sampling from patients infected by tuberculosis and co-infected with TB-HIV after three months showed a significant increase in ALP (143.8±8.75UI) and total bilirubin (1.44±0.27 mg/dl) in TB-HIV co-infected patients (Table 4). This high rise in ALP and total bilirubin could be due to cumulative hepatotoxicity arising from the combined anti-tuberculosis (RHEZ) and antiretroviral therapies the patients took during the three-month treatment period. In addition, cotrimoxazole (Yang et al., 2014) and Isoniazid (Raghu and Karthikeyan, 2016) have been shown to induce liver damages resulting in raised levels of these liver enzymes. On the other hand, in TB patients, a significant rise of ALT (32.2 ± 3.56 UI) and AST (67 ± 5.23 UI) transaminases was noted after three months of the start of Anti-TB treatment, confirmed in other published studies (Van Hest et al., 2004; Schechter et al., 2006). In addition, anti-tuberculosis therapy in treating tuberculosis has the problem of hepatotoxicity resulting from the combination of these anti-tuberculosis drugs (Isoniazid, Rifampicin, Pyrazinamide) (Schechter et al., 2006).

Indeed, the information gathered on the distribution of the genetic polymorphism of genes that encode N-acetyltransferase2 (NAT2) in populations is essential for understanding the inter-individual differences in drug metabolism and the risk of developing side effects. In this study, seven genotypes were identified, and among them, NAT2*5/5 (47.61%), NAT2*5/6 (19.04%), and NAT2*5/7 (14.28%) were predominant. These results do

not corroborate those published by Kengne et al. (2016) in a study in children under ten years of age, having malaria and receiving antimalarial treatment, in the North-West and South-West regions of Cameroon. In this previous study, the following genotypes were described: fast acetylators: NAT2*4/4; slow acetylators: NAT2*5/5, NAT2*6/6, NAT2*5/6, NAT2*5/7, NAT2*6/7, and intermediate acetylators: NAT2*4/5, NAT2*4/6, NAT2*4/7. The difference observed with the results of our study could be linked to the disease (malaria instead of TB) and the small size of the study population. Our findings indicated 85.71% of slow metabolizers and 14.28% of fast acetylators, as mentioned in the previous study (Kegne et al., 2016; Achonduh et al., 2013). Similarly, previous work done in Africa and around the world on tuberculosis patients or TB/HIV co-infected individuals has also reported high frequencies of slow metabolizers (Soukaina et al., 2014, 2016; Mariz et al., 2020). Indeed, the main side effects of anti-tuberculosis drugs are ocular toxicity, hypersensitivity reactions, and liver toxicity. These are the most severe side effect of the adverse effects of anti-TB medications due to isoniazid in particular (Tostmann et al., 2008). Factors that initiated this hepatotoxicity include metabolizer phenotype, anti-tuberculosis drug doses, patient's nutritional status, and disease severity. The relationship between the genetic factors and hepatotoxicity induced by anti-TB drugs has been published already (Soukaina et al., 2016; Hyun et al., 2007; Sun et al., 2008). The TB/HIV co-infected patients on anti-TB treatment and characterized by a slow acetylation phenotype were significantly more likely to develop hepatotoxicity ($p < 0.05$) in a previous study (Mariz et al., 2020). One of the reasons could be their capacity to slowly remove drugs from the body that can lead to drug persistence and liver toxicity. Likewise, some authors suggested the role of translational or post-translational defects in the NAT2 enzyme degradation acceleration (Blum et al., 1991), inducing an accumulation of toxic metabolites and free radicals generation. Our results showed that slow acetylators are the most frequent phenotype among TB patients who have developed high liver toxicity ($p = 0.026$). It provides an existing correlation between slow acetylation and anti-TB drugs induced hepatotoxicity among the study participants. These results agree with other researches carried out by several authors (Khalili et al., 2011; Leiro-Fernandez et al., 2011; Ben-Fredji et al., 2016). However, it should be noted that this study has some limitations on the size of the samples used for this NAT2 genetic polymorphism evaluation that should be extended in the future. In addition, assessing the CYP450 enzymes involved in the biotransformation of drugs might be essential to draw more valuable conclusions.

Conclusion

Finally, the present study has demonstrated that providing more significant support for TB patients living with HIV/AIDS undergoing anti-TB treatment and antiretrovirals may well contribute to more excellent management and control of the drugs used, especially those TB patients co-infected or not, with genotypes NAT2*5/5 (47.61%), NAT2*5/6 (19.04%) and NAT2*5/7 (14.28 %). Thus, we can say that NAT2*5/5 (47.61%) and NAT2*5/6 (19.04%) variant alleles are the main risk factors for developing hepatotoxicity. TB patients having these genotypes present a higher chance of developing liver toxicity during anti-TB treatment. These findings demonstrate that the NAT2 phenotype may also be a good indicator for assessing adverse events due to anti-TBs drugs or in combination with antiretrovirals. Further studies with different populations and larger sample sizes are required to verify the validity of these findings. Values from control individuals not having TB or HIV might bring interesting complementary information for a better understanding and use of liver enzymes activities variation as indicators of toxicity prevention in these populations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors greatly thank EDCTP (European and Developing Clinical Trials Partnership) for having financially supported the realization of this work through CANTAM (Central Africa Network for Tuberculosis, AIDS/HIV and Malaria) Network and its Coordinator Prof Francine Ntoumi. The authors also thank Prof. Assam Assam Jean-Paul, Associate Professor in Microbiology and research scientist at the LTR Laboratory (BTC/UYI), for all the scientific and technical support and fieldwork supervision; Dr. Cedric Tchinda Fossi (LTR, BTC, UYI) and Mrs. Augusta Tsasse for their technical assistance in the laboratory investigations. In addition, all the study participants, counselors, and health staff from the Jamot Hospital and Day Care of the Central Hospital in Yaoundé are appreciated for their technical support in carrying out this research work. Finally, sincere thanks go to the National Tuberculosis Control Program (NTCP).

REFERENCES

Abrescia N, D'Abbraccio M, Figoni M, Busto A, Butrico E, Marco MD, Viglietti R (2002). Fulminant hepatic failure after the start of an efavirenz-based HAART regimen in a treatment-naive female AIDS patient without hepatitis virus co-infection. Journal Antimicrobial Chemotherapy 50(5):763-765.

Achonduh OA, AtoghoTB, Mbulli IA, Chedjou JP, Achu M, Nji AM, Fokou E, Kamgue E, Veyee V, Karana O, Sahfe D, Mbacham WF (2013). Adverse events clustering with NAT2 slow metabolisers following deparasitization in children in Bangolan, NWR Cameroon.

Journal of Life Science 7(7):742-748
An HR, Wu XQ, Wang ZY, Zhang JX, Liang Y (2012). NAT2 and CYP2E1 polymorphisms associated with antituberculosis drug-induced hepatotoxicity in Chinese patients. Clinical and Experimental Pharmacology and Physiology 39(6):535-543.
Assam-Assam JP, Beng VP, Cho-Ngwa F, Tedom JC, Anyangwe AI, Titanji VPK (2011). Mycobacterium tuberculosis complex drug resistance pattern and identification of species causing tuberculosis in the West and Center regions of Cameroon. BMC Infectious Diseases 11(1):1-6.
Ben Mahmoud L, Ghozzi H, Kamoun A, Hakim A, Hachicha H, Hammami S, Sahoung Z, Zalila N, Makmih H, Zeghal K (2012). Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatotoxicity 364 in Tunisian patients with tuberculosis. Pathologie Biologie 60(5):324-330.
Ben-Fredj N, Gam R, Kerkni E, Chaabane A, Chadly Z, Boughattas N, Aouam K (2016). Risk factors of isoniazid-induced hepatotoxicity in Tunisian tuberculosis patients. Pharmacogenomics Journal 17(4):372-377. DOI: 10.1038/tpj.2016.26.
Blum M, Demierre A, Grant DM, Heim M, Meyer UA (1991). Molecular mechanism of slow acetylation of drugs and carcinogens in humans. Proceedings of the National Academy of Sciences of the United States of America 88(12):5237-5241
Colebunders R, Lambert ML (2002). Management of co-infection with HIV and TB: improving tuberculosis control programmes and access to highly active antiretroviral treatment is crucial. British Medical Journal 342(7341):802-803
Demographic and Health Survey and with Multiple Indicators (2011). http://dhsprogram.com
Desta Z, Saussele T, Ward B, Blievernicht J, Lang L, Klein K, Flockhart D, Zanger UM (2007). Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro. Pharmacogenomics 8(6):547-558.
Gollub EL, Trino R, Salmon M, Moore L, Dean JL, Davidson BL (1997). Co-occurrence of AIDS and tuberculosis: results of a database “match” and investigation. Journal of Acquired Immune Deficiency Syndromes 16(1):44-49.
Havlir DV, Barnes PF (1999). Tuberculosis in Patients with Human Immunodeficiency Virus Infection. New England Journal of Medicine 340:367-373.
Hyun JC, Won JK, Yon JR, Chang SK, Myung HN, Jong WK, Soo Y (2007). Genetic polymorphisms of NAT2 and CYP2E1 associated with antituberculosis drug-induced hepatotoxicity in Korean patients with pulmonary tuberculosis. Tuberculosis 87(6):551-556. Doi: 10.1016/j.tube.2007.05.012.
Kengne CJP, Akindeh MN, Innocent MA, Achonduh OA, Ekollo MA, Mbacham WF (2016). Predominance of N-acetyl transferase 2 slow acetylator alleles in children less than ten years experiencing adverse treatment events following artemisinin-based combination therapy in North and South West Regions of Cameroon. African Journal of Biotechnology 15(25):1285-1291.
Khalili H, Fouladdel S, Sistanizad M, Hajiabdolbaghi M, Azizi E (2011). Association of N-Acetyltransferase-2 Genotypes and Anti-Tuberculosis Induced Liver Injury; First Case-Controlled Study from Iran. Current Drug Safety 6(1):17-22.
Lee SW, Chung LS, Huang HH, Chuang TY, Liou YH, Wu LS (2010). NAT2 and CYP 2E1 polymorphisms and susceptibility to first-line anti-tuberculosis drug-induced hepatitis. International Journal of Tuberculosis and Lung Diseases 14(5):622-626.
Leiro-Fernandez V, Valverde DX, Vázquez-Gallardo K, Botana-Rial M, Constenla L, Agúndez JA, Fernández-Villar A (2011). N-acetyltransferase 2 polymorphisms and risk of anti-tuberculosis drug-induced hepatotoxicity in Caucasians. International Journal of Tuberculosis and Lung Diseases 15(10):1403-1408. Doi: 10.5588/ijtld.10.0648.
Margulis SJ, Honig CL, Soave R, Govoni AF, Mouradian JA (1986). Biliary Tract Obstruction in the Acquired Immunodeficiency Syndrome. Annals of Internal Medicine 105(2):207-210.
Mariz CA, Albuquerque MFPM, Lopes EP, Ximenes RAA, Lacerda HA, Filho DBM, Martins BBC, Pastor AFP, Santos BA (2020). Hepatotoxicity during TB treatment in people with HIV/AIDS related to NAT2 polymorphisms in Pernambuco, Northeast Brazil. Annals of Hepatology 19(2):153-160.
Mc Donagh EM, Boukouvala S, Aklillu E, Hein DW, Altman RB, Klein TE (2014). PharmGKB summary: very important pharmacogene information for N acetyltransferase 2. Pharmacogenetics and Genomics 24(8):409-425
NTCP (2012). Epidemiological situation of tuberculosis in Cameroon. http:onsp.minsante.cm
NTCP (2019). Epidemiological situation of tuberculosis in Cameroon. http:onsp.minsante.cm
Oluwafemi O, Oguntibeju B, Olatubosan B (2003). A Study on the Activities of Liver Enzymes in HIV/ AIDS Patients. Journal of Medicine and Science 3:106-109.
Pan L, Jia ZS, Chen L, Fu EQ, Li GY (2005). Effect of anti-tuberculosis therapy on liver function of pulmonary tuberculosis patients infected with hepatitis B virus. World Journal of Gastroenterology 11(16):2518-2521.
Raghu R, Karthikeyan S (2016). Zidovudine and isoniazid induced liver toxicity and oxidative stress: evaluation of mitigating properties of silibinin. Environmental Toxicology and Pharmacology 46:217-226.
Sadaphal JP, Astemborski NM, Graham, Sheely L, Bonds M, Madison A, Vlahov D, Thomas DL, Sterling TR (2001). Isoniazid preventive therapy, hepatitis C virus infection, and hepatotoxicity among injection drug users infected with Mycobacterium tuberculosis. Clinical Infectious Diseases 33(10):1687-1691.
Sama LF, Nganou-Djinou OI, Wam EC, Bamou R, Ali IM, Noubom M, Tume CB (2017). Sero-prevalence of Hepatitis B and C virus and High Risk of Hepatotoxicity among TB / HIV Positive and HIV Negative Population in Western Cameroon. Global Journal of Infectious Diseases 3(1):001-008. DOI: http://doi.org/10.17352/2455-5363.000011
Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, Nolan CM, Peloquin CA, Gordin PM, Nunes D, Strader DB, Bernardo J, Venkataramanan R, Sterling TR (2006). An official ATS statement: hepatotoxicity of antituberculosis therapy. American Journal of Respiratory and Critical Care Medicine 174(8):935-952.
Schechter M, Zajdenverg R, Falco G, Barnes GL, Faulhaber JC, Coberly JS, Moore RD, Chaisson RE (2006). Weekly rifapentine / isoniazid or daily rifampin / pyrazinamide for latent tuberculosis in household contacts. American Journal of Respiratory and Critical Care Medicine 173(8):922-926.
Sidze LK, Mouafo TE, Kuaban C, Assam Assam JP, Tedom JC, Eyangoh S, Fouda FX, Nolna D, Ntoumi F, Frank M, Beng VNP (2014). Strong Decrease in Streptomycin-Resistance and Absence of XDR 12 Years after the Reorganization of the National Tuberculosis Control Program in the Central Region of Cameroon. Plos One 9(6):e98374. Doi: 10.1371 / journal. Pone. 0098374
Soukaina G, Ilham R, Fatima ZL, Siham CE, Imane CJ, Amina B, Abdelaziz S (2014). Distribution of allelic and genotypic frequencies of NAT2and CYP2E1variants in Moroccan population. BMC Genetics 15(1):1-6.. DOI 10.1186/s12863-014-0156-x.
Soukaina G, Ilham R, Omaima El Bouazzi, Sanaa H, Amina T, Jamal EB, Rachida SB, and Abdelaziz S (2016). NAT2 Genotypes in Moroccan Patients with Hepatotoxicity Due to Antituberculosis Drugs. Genetic Testing and Molecular Biomarkers 20(11):680-684. DOI: 10.1089/gtmb.2016.0060.
Sun F, Chen Y, Xiang Y, Zhan S (2008). Drug-metabolising enzyme polymorphisms and predisposition to anti-tuberculosis drug-induced liver injury: a meta-analysis. International Journal of Tuberculosis and Lung Diseases. 12(9):994-1002.
Tostmann A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R (2008). Antituberculosis drug-induced hepatotoxicity: concise up -to-date review. Journal of Gastroenterology and Hepatology 23(2):192-202.
Van Hest R, Baars H, Kik S, van Gerven P, Trompenaars MC, Kalisvaard N, Keizer S, Borgdorff M, Mensen M, Cobelens F (2004). Hepatotoxicity of rifampin-pyrazinamide and isoniazid preventive therapy and tuberculosis treatment. Clinical Infectious Diseases 39(4):488-496.
Wares DF, Singh S, Acharya AK, Dangi R (2003). Non-adherence to tuberculosis treatment in the eastern Tarai of Nepal. International Journal of Tuberculosis and Lung Disease 7(4):327-335.
Wild-Up M, Fortuin F, Whittle DHC, Hall AJ, Wolf CR, Montesonai R

(1990). Liver and hepatitis B virus in Gambian children. Cancer Epidemiology, Biomarkers and Preventions 2:555-561.
World Health Organization (2012). Library Cataloguing-in-Publication Data: Treatment of tuberculosis: guidelines - 4th ed.WHO/HTM/TB/2009.420 http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf (accessed 28 May 2012).
World Health Organization (2018). Global tuberculosis report 2018. Geneva (Switzerland)
World Health Organization (2019). Global tuberculosis report 2019. Geneva (Switzerland).
Yang JJ, Huang CH, Liu CE, Tang HJ, Yang CJ, Lee YC, Lee KY, Tsai MS, Lin SW, Chen YH, Lu PL, Hung CC (2014). Multicenter study of trimethoprim/sulfamethoxazole related hepatotoxicity: incidence and associated factors among HIV-infected patients treated for Pneumocystis jirovecii pneumonia. PloS One 9(9):e106141.
Yimer G, Ueda N, Habtewold A, Amogne W, Suda A, Kiedel KD, Burhenne J, Aderaye G, Lindquist L, Mkonnen E, Aklillu E (2011). Pharmacogenetic & Pharmacokinetic Biomarker for Efavirenz Based ARV and Rifampicin Based Anti-TB Drug Induced Liver Injury in TB-HIV Infected Patients. PloS One 6(12):e27810. doi:10.1371/journal.pone.0027810.
Younossian AB, Rochat T, Ketterer JP, Wacker J, Janssens JP (2005). High hepatotoxicity of pyrazinamide and ethambutolfor treatment of latent tuberculosis. European Respiratory Journal 26(3):462-464.

Vol. 21(3), pp.116-124, March 2022
DOI: 10.5897/AJB2021.17411
Article Number: B30911A68828
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Leaf microscopy, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) analyses of *Croton zambesicus* Müll.-Arg. leaf (Family: Euphorbiaceae) in Nigeria

Ugbabe G. E.[1*] and Dalyop J. D.[2]

[1]Medicinal Plant Research and Traditional Medicine (MPR and TM) Department, National Institute for Pharmaceutical Research and Development (NIPRD), Idu Industrial Area, P. M. B. 21, Garki - Abuja, Nigeria.
[2]Plant Science and Biotechnology Department, Federal University Lafia, Nassarawa State, Nigeria.

Received 21 September, 2021; Accepted 9 February, 2022

This study was done to get information on the chemical composition and the internal structure of the plant which could aid in its further characterization. The study is aimed at determining the epidermal leaf anatomy, HPLC of the hot water extract, chemical constituents of the oil from the leaves by GC-MS and chemo-microscopic properties of *Croton zambesicus* for proper identification of the plant. The leaf epidermal microscopy of *C. zambesicus* showed abundant trichome on the abaxial surface arising from a multi-cellular base and radiating radially and paracytic stomata were observed on the lower surface of the leaf. Chemo-microscopic analysis of the leaves showed the presence of cellulose, lignin, starch, oxalate crystals, tannin, oils, proteins and absence of mucilage. The physicochemical parameters evaluated on the leaves had moisture content of 4.10% and total ash value of 11.91%. HPLC analysis of the hot water extract revealed 12 peaks with number 3 having the highest peak of 93 mAU at 3.5 min. The GC-MS analysis of the oil of the leaves had 57 components. Caryophyllene had the highest percentage composition (15.53%) followed by Copaene (11.38%), Phellandrene (8.65%), 1,6-Octadien-3-ol, 3,7-dimethyl (4.67%), Humulene (3.94%), Pinene (3.85%) and Ar-tumerone (3.41%).

Key words: *Croton zambesicus*, high performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), microscopy, Nigeria.

INTRODUCTION

Plants have been used by mankind for its medicinal value and the healing of different ailments. Quite a number of modern drugs are plant based and of natural sources. Many of these active agents isolated were based on their

*Corresponding author. E-mail: graceeyinehi@yahoo.com; graceeyinehi@gmail.com. Tel: +234(0)7038338313.

uses in traditional medicine (Boukhira et al., 2016). The material medica of these systems contains a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India (Manikandaselvi et al., 2016). The books on ayurvedic medicine such as *Charaka Samhita* and *Susruta Samhita* refer to the use of more than 700 herbs (Manikandaselvi et al., 2016).

Croton zambesicus Mull. Arg. has wide application in Africa for medicinal purposes (Kumar et al., 2011) and grown in many Nigerian communities for medicinal purposes (Ogundajo et al., 2014). The wood is pale yellow, fine-grained, hard, gives a good polish and the stems are used in parts of West Africa for hut-posts ((Irvine, 1961). The bark emits an aromatic smell; an infusion of bark is used in Nigeria in cases of malaria, and the leaves are considered strengthening (Bello et al., 2014). A soup made from the leaves is given to dysentery cases in Southern Nigeria, whereas in both Nigeria and Sierra Leone a leaf decoction is used as a wash and is taken internally for dysentery, fever, convulsions, headache, and as a vermifuge (Irvine, 1961). The fruits and bark are aromatic and are used in the Adamawa region of Nigeria to spice food and to prepare a sort of scent (Ogundajo et al., 2010). The seeds are said to have medicinal use in Togo. Examination of Nigerian material has shown the presence of a trace of alkaloid in the stem and leaf (Ogundajo et al., 2010).

About 80% of the people in developing countries rely mostly on traditional medicine for their health care needs, of which the use of plant extracts or their active principles are involved (Springfield et al., 2005). One of the criticisms of herbal medicine is lack of standardization and quality control profiles, as well as the correct identification of the species concerned, whether in the fresh, dried or powdered state (Springfield et al., 2005).

Phytochemistry deals with a variety of organic substances accumulated in plants (Ogundajo et al., 2014). Further, besides their chemical compounds like carbohydrates, protein, and lipids being used as food by man, other compounds like glycosides, alkaloids, and flavonoids are used as medicines by him in various ways. The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is an important step in medicinal plant research (Kokate, 1994). Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals (Banso and Adeyemo, 2007).

C. zambesicus has wide application in Africa for medicinal purposes and other economic uses. However, there is insufficient information on the chemical constituents and internal structure of the plant which could aid in its further characterization. Based on the presented information, this study was done with a view to characterize the epidermal leaf anatomy, chemical constituents by HPLC and GC-MS analyses, and chemo-microscopic properties of *C. zambesicus* for proper identification and standardization of the species.

MATERIALS AND METHODS

Plant collection and identification

The plant material was collected from NIPRD, Abuja on 13th October, 2020. Identification was done at the Herbarium and Ethnobotany Unit, Medicinal Plant Research and Traditional Medicine (MPR and TM) Department, NIPRD-Abuja.

Leaf epidermal microscopy

Epidermal preparations (abaxial and adaxial surfaces) followed the methods of Ayodele and Olowokudejo (1997, cited by Ugbabe and Ayodele, 2008). Slides were labeled appropriately and examined under the light microscope (ACCU-SCOPE 3025 Microscope Series) while photographs of the micro morphological features were taken using camera (Industrial Design Camera E31SPM12000KPA) with magnifications x100 and x400. Terminologies are based on Metcalfe and Chalk (1979).

Physicochemical determination and chemo-microscopy

Two physicochemical parameters, moisture content and ash values were determined following WHO guidelines (African Pharmacopoeia, 1986).

Chemo-microscopic studies of the comminuted dried leaf sample was carried out using the methods of Adamu et al. (2018) to test for the presence of different metabolites (African Pharmacopoeia, 1986; Evans, 2002).

High performance liquid chromatography (HPLC)

The method described by Adamu et al. (2018) was used with some modifications. The dried samples (0.2 g) were weighed into clean and well labeled sample bottles, 10 ml of 70% ethanol was added to each sample bottle, allowed to stand for 24 h and the mixture was filtered into clean bottle. An aliquot of each sample was taken with the aid of a 2-ml syringe, filtered through a 0.45-µm Millipore membrane filter and then transferred into HPLC vial before injecting into the HPLC machine. The HPLC operating conditions were programmed to give the following: solvent B: 20% at a flow rate of 0.6 ml/min; and column oven was set to 40°C temperature. The total run time was 20 min.

Gas chromatography–mass spectrometry (GC-MS) analysis

Extraction of oils by hydro distillation

The methods of Okhale et al. (2018) were used where fresh *C. zambesicus* leaf sample was chopped into pieces and subjected to hydro-distillation for 4 h using Clevenger-type apparatus. The essential oil obtained was dried over a hydrous sodium sulphate and used immediately for GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy =70Ev), scan range =45400 amu, and scan rate = 3.99 scan/s], and Shimadzu GCMS solution data system). The percentage of each component was reported as raw percentages based on the total ion current (Okhale et al., 2018).

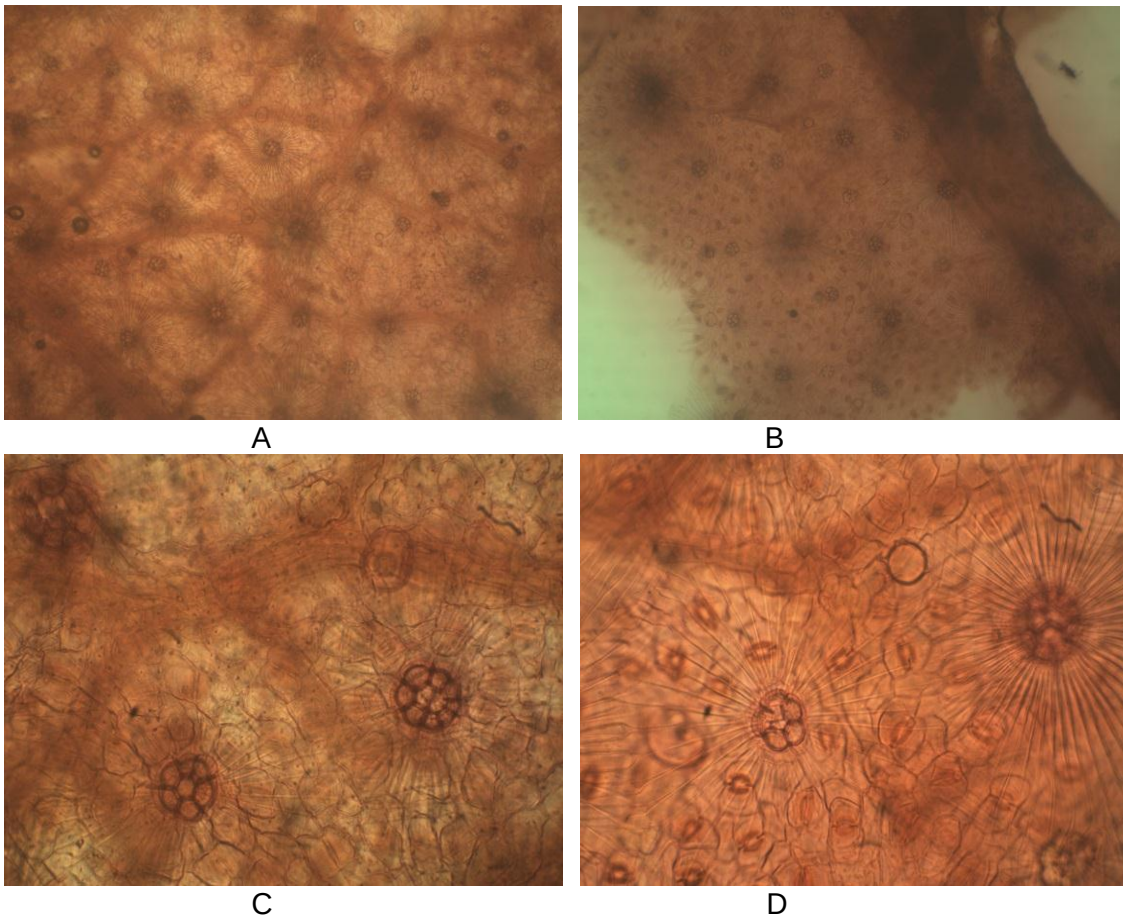


Figure 1. Leaf epidermal microscopy of *Croton zambesicus (*A) Abaxial surface X 100 showing the outline of the epidermal cells and reticulate venation on the leaf surface (B) Abaxial surface X 100 showing the outline of the epidermal cells (C) Abaxial surface X 400 showing trichomes radiating from multi-cellular base and glandular trichome with a rounded head, and D) Abaxial surface X 400 showing paracytic stomata.

RESULTS

Leaf epidermal microscopy

Figures 1 and 2 showed the *C. zambesicus* leaf epidermis qualitative characters. It revealed a paracytic stomata on the abaxial surface while the adaxial surface has no stomata. Trichomes are numerous radiating from a multicellular base radially. The cells are polygonal with nearly straight cell walls on the adaxial surface (Figure 1).

The powdered leaf microscopy revealed oil globules, calcium oxalate crystals and trichomes arising from a base and branching radially (Figure 3).

Chemo-microscopic evaluation of *C. zambesicus* leaf

The chemo-microscopic evaluation of the leaf of *C. zambesicus* revealed the presence of lignin, cellulose, tannins, starch, calcium, oxalate crystals, oils, protein and the absence of mucilage. The leaf has a moisture content of 4.10 and an ash value of 11.91 (Table 1).

HPLC analysis of *C. zambesicus* leaf extract

The HPLC analysis revealed 12 peaks; number 3 has the highest peak (Figure 4) and retention time of 3.214 min. (Table 2 and Figure 4).

GC-MS analysis of *C. zambesicus* leaf oil

The chromatogram of the GC-MS analysis of the leaf of *C. zambesicus* had 57 compounds (Figure 5 and Table 3). The major compounds in the analysis were Caryophyllene (15.53%); beta-copaene (11.38%); alpha-myrcene (8.65%); 1,6-octachen3-ol-1,7-dymethyl- (4.77%); alpha-pinene (3.85%) and Ar-turmerone (3.41%).

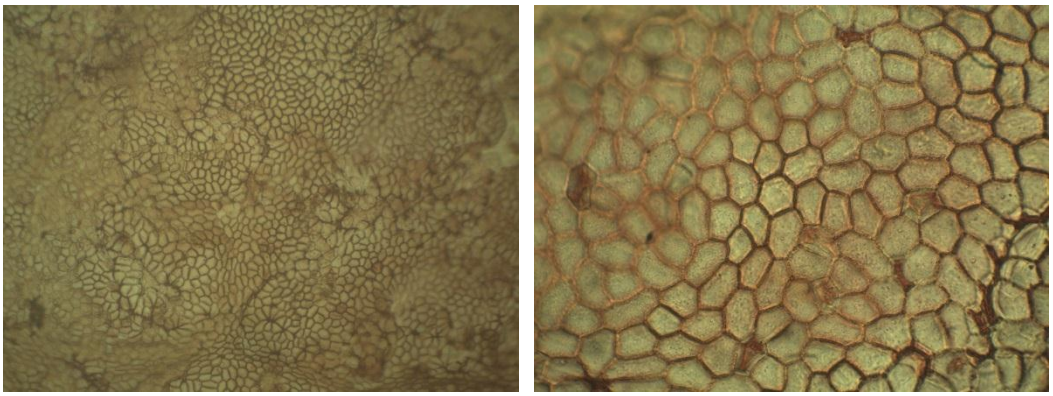


Figure 2. Leaf epidermal microscopy of *Croton zambesicus* A) Adaxial surface ×100 showing the outline of the epidermal cells B) Adaxial surface ×400 showing nearly straight cell walls and have no trichomes and no stomata.

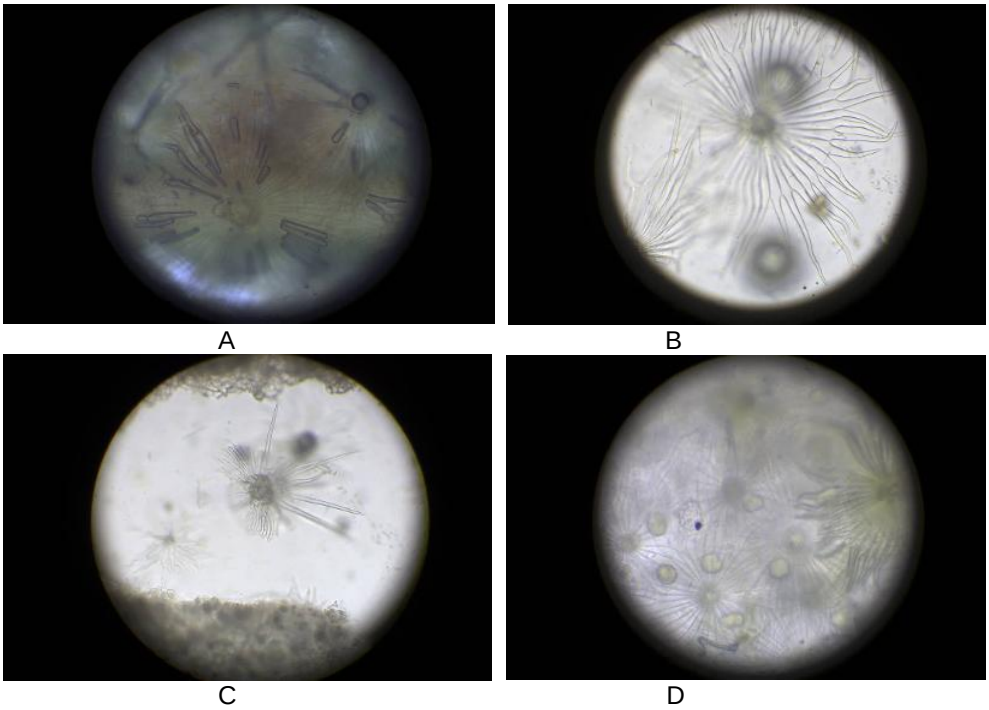


Figure 3. Powdered leaf microscopy of *Croton zambesicus.* A) Calcium oxalate crystals, (B) Trichomes radiating from multi-cellular base and branching radially, (C) Trichome and D) Showing oil globules and trichomes.

Chemical structures of some compounds in *C. zambesicus* leaf oil

Some compounds in *C. zambesicus* leaf oil and their chemical structures are as presented in Scheme 1. These include Caryophyllene, Ar-turmerone, alpha-Phellandrene, alpha-Myrcene, 1,6-octachen3-ol-1,7-dymethyl and beta-copaene.

DISCUSSION

The leaf epidermal microscopy of *C. zambesicus* showed abundant stellate trichomes arising from a multi-cellular base and branching radially on the abaxial surface with paracytic stomata also observed on the lower epidermis of the leaf. The comminuted dried leaf of *C. zambesicus* showed stellate trichome with paracytic stomata. Chemo-

Table 1. Chemo-microscopy and physico-chemical evaluation of *Croton zambesicus* leaf.

Parameter	Inference
Lignin	+
Mucilage	_
Cellulose	+
Tannins	+
Starch	+
Calcium Oxalate crystal	+
Oil	+
Proteins	+
Moisture content	4.10%
Total ash value	11.91%

+= present, - = absent.

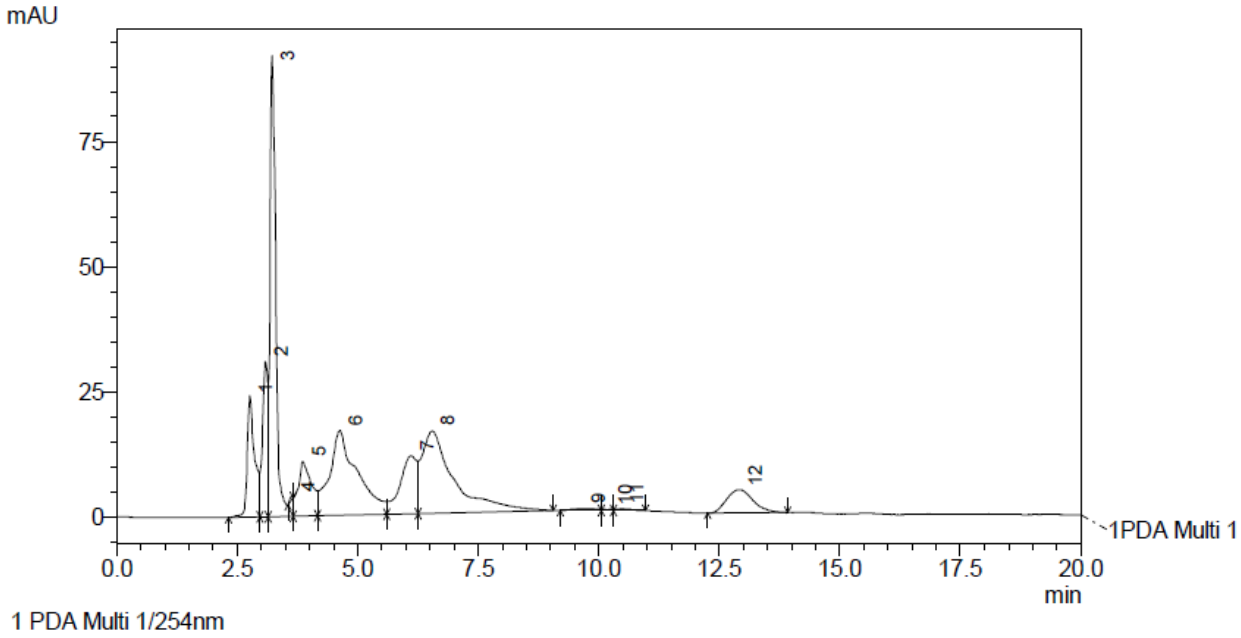


Figure 4. HPLC chromatogram of *Croton zambesicus* leaf extract.

microscopic analysis of leaves revealed the presence of cellulose, lignin, starch, oxalate crystals, tannin, oils, proteins and absence of mucilage in the leaves. The physicochemical parameters evaluated were: moisture content (4.10%) and total ash value (11.91%). The HPLC analysis had 12 peaks with number 3 having the highest peak. The chromatogram of the GC-MS analysis of the leaf of *C. zambesicus* had 57 compounds (Figure 5 and Table 3). The major compounds in the analysis were Caryophyllene (15.53%), beta-copaene (11.38%), alpha-myrcene (8.65%), 1,6-octachen3-ol-1,7-dymethyl- (4.77%), alpha-pinene (3.85%) and Ar-turmerone (3.41%).

Stomata are the microscopic pores on the leaf that facilitate gas and water exchange with the atmosphere and have a characteristics associated with photosynthesis and transpiration (Mansfield et al*.,* 1990). Stomata vary in size and density in different species and are greatly influenced by the environment. Both glandular and non-glandular trichomes were observed in *C. zambesicus*. Trichomes are tiny outgrowths from the plant epidermis that have ability to secret water, nectar, resins, mucilage and terpenes (Mansfield et al*.,* 1990). They serve as physical and chemical protection for the leaf against microbial organisms, aphids and insects ((Mansfield et al*.,* 1990). The cell wall provides mechanical strength and

Table 2. HPLC chromatogram of *Croton zambesicus* leaf extracts.

Retention time	Area
2.757	265244
3.082	245547
3.214	755307
3.616	3538
3.854	213504
4.623	680552
6.102	283292
6.536	766816
9.658	14719
10.219	3622
10.452	8637
12.912	179337

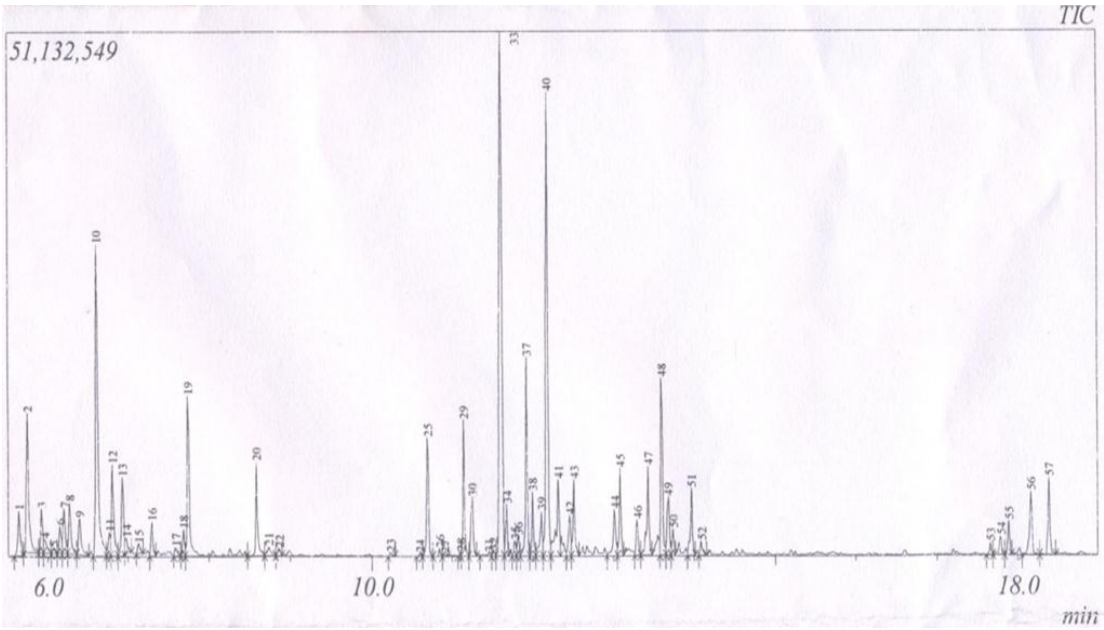


Figure 5. Chromatograph of GC-MS analysis of *Croton zambesicus* leaf oil.

support to the plant. Moisture content determination will lead to activation of enzymes and give the proliferation of living organism. Moisture content of crude plant is a function of its shelf life; the lower the moisture contents the longer the shelf life. Ash value is useful in determining authenticity and purity of samples and also these values are important qualitative standards. The ash value and moisture content for *C. zambesicus* is found to be 4.10 and 11.91% respectively. The ash value of *C. zambesicus* is slightly above the standard and the moisture content is within the acceptable value.

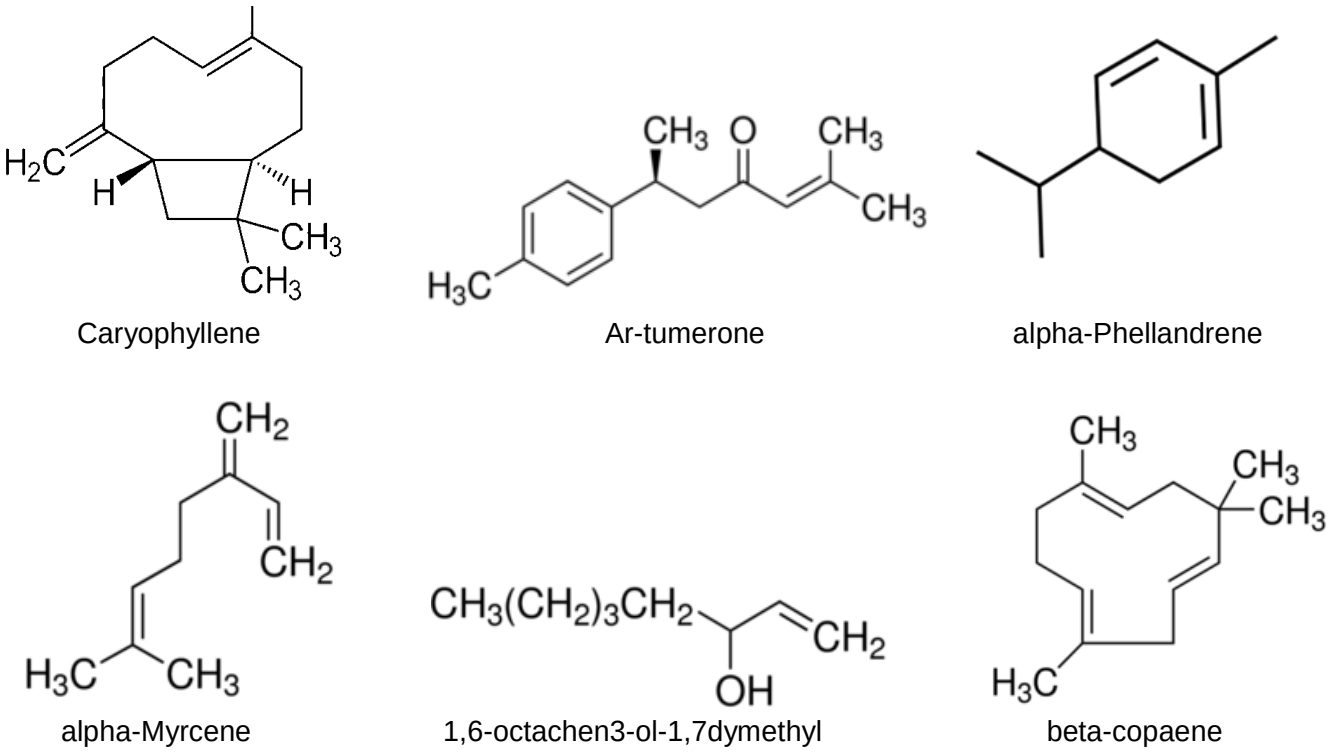
HPLC is a tool used in the standardization of complex drug which has the ability to estimate the presence of active marker quantitatively and qualitatively. GC-MS is a method that combines gas-chromatography and mass spectrometry to identify different substances within a test sample in drug detection and identification of unknown sample (Sparkman et al*.,* 2011). Caryophyllene is a terpene used for its pain-relieving and antimicrobial properties; beta-caryophyllene improves mood, and may also help prevent osteoporosis (Jürg et al., 2008). Terpenes are common compounds in plants responsible for distinct aromas and flavors in essential oils (Jürg et al., 2008). Due to the smell, taste, and ability to act as a dietary cannabinoid, beta-caryophyllene can be used as a flavoring agent or food additive (Jürg et al.,2008). It can also be used in cosmetics, creams, toothpaste, and other commercial products to enhance their therapeutic effects.

Table 3. Chromatographic profile of the oil of *Croton zambesicus* leaf.

Name of compound	Retention Time	Molecular formula	% Composition
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	5.633	$C_{10}H_{16}$	1.28
alpha.-Pinene	5.741	$C_{10}H_{16}$	3.85
Camphene	5.918	$C_{10}H_{17}$	1.03
Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl	5.956	$C_{11}H_{18}O$	0.44
Benzene, 1,2,4-trimethyl	6.067	C_9H_{12}	0.11
- 1-Octen-3-ol	6.150		0.66
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl	6.201	$C_{10}H_{16}$	1.16
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene	6.273	$C_{10}H_{16}$	1.35
beta.-Myrcene	6.398	$C_{10}H_{16}$	1.08
alpha.-Phellandrene	6.615	$C_{10}H_{16}$	8.65
Cyclohexene, 4-methyl-3-(1-methylethylidene	6.770	$C_{10}H_{16}$	0.61
o-Cymene	6.809	$C_{10}H_{14}$	2.24
Cyclohexene, 1-methyl-5-(1-methylethenyl)-,	6.933	$C_{10}H_{16}$	1.96
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	6.975	$C_{10}H_{16}$	0.35
beta.-Ocimene	7.129	$C_{10}H_{16}$	0.28
gamma.-Terpinene	7.294	$C_{10}H_{16}$	0.77
Benzene, 1-ethyl-3,5-dimethyl-	7.585	$C_{10}H_{14}$	0.26
Cyclohexene, 1-methyl-4-(1-methylethylide	7.683	$C_{10}H_{16}$	0.56
1,6-Octadien-3-ol, 3,7-dimethyl-	7.742	$C_{10}H_{18}O$	4.67
endo-Borneol	8.586	$C_{10}H_{18}O$	2.15
Terpinen-4-ol	8.734	$C_{10}H_{18}O$	0.36
alpha.-Terpin	8.861	$C_{15}H_{24}$	0.22
4,4-Dimethyl-cyclohex-2-en-1-ol	10.227	$C_{10}H_{16}O$	0.09
gamma.-Elemene	10.593	$C_{15}H_{24}$	0.07
Cyclohexene, 4-ethenyl-4 -methyl-3 -(1-methylethenyl	10.694	$C_{15}H_{24}$	2.37
alpha.-Cubebene	10.838	$C_{15}H_{24}$	0.17
alpha.-Guaiene	10.899	$C_{15}H_{24}$	0.06
alpha.-ylangene	11.099	$C_{15}H_{24}$	0.08
alfa.-Copaene	11.142	$C_{15}H_{24}$	2.51
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl	11.248	$C_{15}H_{24}$	1.67
Caryophyllene	11.453	$C_{15}H_{24}$	0.18
1H-Cycloprop [e]azulene, 1a,2,3,4,4a,5,6,7b- octahydro:	11.508	$C_{15}H_{24}$	0.14
Caryophyllene	11.616	$C_{15}H_{24}$	15.53
1H-Cyclopenta [1,3] cyclopropa[1,2] benzene,	11.673	$C_{15}H_{24}$	1.07
Isoledene	11.774	$C_{15}H_{24}$	0.33
gamma.-Muurolene	11.814	$C_{15}H_{24}$	0.42
Humulene	11.923	$C_{15}H_{24}$	0.42
Alloaromadendrene	11.993	$C_{15}H_{24}$	1.27
.gamma.-Muurolene	12.101	$C_{15}H_{24}$	1.32
beta.-copaene	12.182	$C_{15}H_{24}$	11.38
gamma.-Elemene	12.312	$C_{15}H_{24}$	2.46
Naphthalene, 1,2,3,4,4a,5,6,8a- octahydro-7-methyl-4-methylene	12.452	$C_{15}H_{24}$	1.11
Naphthalene,1,2,3,5,6,8a-hexahydro- 4,7-dimethyl	12.506	$C_{15}H_{24}$	1.68
(-)-Spathulenol	13.011	$C_{15}H_{24}O$	1.34
Caryophyllene oxide	13.083	$C_{15}H_{24}O$	1.59
Globulol	13.292	$C_{15}H_{26}O$	0.84
1H- Cycloprop[e]azulen-7-ol, decahydro-1,1	13.432	$C_{15}H_{24}O$	2.16
Ar-turmerone	13.601	$C_{15}H_{200}$	3.41
Turmerone	13.682		1.48
1- Naphthalenol, decahydro-1,4a- dimethyl-7-(1-methylethylidene	13.741	$C_{15}H_{26}O$	0.52
Curlone	13.970	$C_{15}H_{22}O$	1.55

Table 3. Contd.

Tetradecanal	14.088	$C_{14}H_{28}O$	0.35
Acetic acid, 10- hydroxy-12a-methyl- 7-oxo-1,2,3,3a,3b,4	17.657	$C_{20}H_{28}O_4$	0.23
Trachylobane	17.785	$C_{20}H_{32}$	0.46
Podocarp-7-en-3-one, 13.beta.-methyl-13-vinyl	17.888	$C_{20}H_{30}O$	0.68
Podocarp-7-en-3-one, 13 a.-methyl-13-vinyl	18.167	$C_{20}H_{30}O$	1.77
Podocarp-7-en-3.beta.-ol, 13.beta.-methyl-13	18.391	$C_{19}H_{28}O_2$	1.72



Scheme 1. Chemical structures of some compounds in *Croton zambesicus* leaf oil.

Beta-copaene is a sesquiterpenoids and was first reported by Türkez et al. (2013).

Conclusion

The analyses carried out in this study can be used for identification and standardization of the plant. *C. zambesicus* has wide application in Africa for medicinal purposes and other economic uses. However, there is insufficient information on the chemical constituents, and internal structure of the plant which could aid in its further characterization. Hence, this study is aimed at bridging this research gap by determining the epidermal leaf anatomy, chemical constituent of the oil from the leaves by GC-MS analysis, HPLC analysis from the leaf extract, chemo-microscopic properties of *C. zambesicus* for proper identification, standardization of the plant as well as drug manufacturing from isolation of the various beneficial compounds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adamu A, Esievo KB, Ugbabe G, Okhale SE, Egharevba HO (2018). High performance liquid chromatography-diode array detection (HPLC-DAD) profiling, antioxidant and anti-proliferative activities of ethanol leaf extract of Berlinia grandiflora (Vahl) Hutch. Dalziel. Journal of Pharmacognosy and Phytotherapy 10(11):187-194.

African Pharmacopoeia (1986). General Methods for Analysis. OAU/SRTC Scientific Publications Lagos pp. 137-149.

Ayodele AE, Olowokudejo JD (1997). Systematic Importance of leaf and epidermal characters in West African species of family Myrtaceae. Boletim da Sociedade Broteriana 68:35-72.
Banso A, Adeyemo SO (2007). Phytochemical and antimicrobial evaluation of ethanolic extract of *Dracaena manni* Bark. Nigerian Journal of Biotechnology 18(1-2):27-32.
Bello MO, Hammed MA, Adekunle AS, Fasogbon OT (2014). Nutrient Contents and Fatty Acids Profiles of Leaves and Seeds of *Croton zambesicus*. Advance Journal of Food Science and Technology 6(3):398-402.
Boukhira S, Balouiri M, Bousta F, Moularat S, Taleb MS, Bousta D (2016). Antimicrobial Activities of Essential Oil of Five Plant Species from Morocco against Some Microbial Strains. International Journal of Pharmacognosy and Phytochemical Research 8(11):1901-1906.
Charak Samhita, Charaka Chandrika (2007). Hindi commentary, by Dr. Brahmanand Tripathi and Dr. Ganga Sahay Pandey, Siddhi Sthana Chapter 12, Verse No. 39. Chaukhamba Surbharti Prakashan P 1342.
Evans MC (2002). Trease and Evans pharmacognosy. 15th Edition. WB Sanders. London, pp. 183-393.
Irvine FR (1961). Woody plant of Ghana. Oxford University Press. London.
Jürg G, Marco L, Stefan R, Ildiko R, Jian-Zhong C, Xiang-Qun X, Karl-Heinz A, Meliha K, Andreas Z (2008). Beta-caryophyllene is a dietary cannabinoid. Proceedings of the National Academy of Sciences of the United States of America 105(26):9099-9104.
Kokate CK (1994). Practical Pharmacognosy, 4th Edition. Vallabh Prakashan, New Delhi pp. 112-120.
Kumar RS, Rajkapoor B, Perumal P (2011). Antioxidat Potetial *of Digofera liaeialia* vitro study. Pharmacology online 1: 710-720.
Manikandaselvi S, Vadivel V, Brindha P (2016). Studies on Nutraceutical Properties of *Caesalpinia bonducella* L.: An Indian Traditional Medicinal Plant. Research Journal of Medicinal Plants 10:127-139.
Mansfield TA, Hetherington AM, Atkinson CJ (1990). Some current aspects of stomatal physiology. Annual Review: Plant Physiololgy. Annual Review of Plant Biology 41(1):55-75.
Metcalfe CR, Chalk L (1979). Anatomy of Dicotyledons. Vol. I. Second Edition. Oxford University Press, New York.
Ogundajo IA, Akintayo L, Ogunwande HG. Gbadamosi RG, Guido F (2014). Chemical Composition of the Leaf Essential Oils of Croton zambesicus Müll.-Arg. Grown in Lagos, South-West Nigeria. European Journal of Medicinal Plants 4(12):1524-1533.
Ogundajo LA, Adeniyi OO, Denloye, OAL (2010). Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry Lagoons, Lagos, Nigeria.
Okhale SE, Ugbabe GE, Oladosu PO, Ibrahim JA, Egharevba HO (2018). Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* L (Rubiaceae) collected from North Central Nigeria. International Journal of Bioassays 7:5630-5637.
Sparkman DO, Penton Z, Kitson FG (2011). GC-MS a practical guide. Academic press. ISBN 98-0-08-092015-3.
Springfield EP, Eagles PK, Scott G (2005). Quality assessment of South African herbal medicines by means of HPLC fingerprinting. Journal of Ethnopharmacology 101(1-3):75-83.
Türkez H, Celik K, Togar B (2013). Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro. Cytotechnology 66(4):597-603. DOI: 10.1007/s10616-013-9611-1.
Ugbabe GE, Ayodele AE (2008). Foliar epidermal studies in the family Bignoniaceae Juss. in Nigeria. African Journal of Agricultural Research 3(2):154-166.

Vol. 21(3), pp.125-132, March 2022
DOI: 10.5897/AJB2021.17430
Article Number: CD42B8D68831
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Agronomic performances of temporary immersion bioreactor-derived potato microtubers in a Peruvian low input cropping agriculture system

María de Lourdes Tapia y Figueroa[1*], José Faustino Beraún Tapia[1], Elliosha Hajari[2], Maritza Escalona[3], Hervé Etienne[4,5] and José Carlos Lorenzo[6]

[1]National Agricultural University La Molina, Lima, Peru.
[2]Plant Improvement, Agricultural Research Council-Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa.
[3]Laboratory for Cell and Tissue Culture, University of Ciego de Ávila, CP 69450, Cuba.
[4]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR DIADE, F-34398 Montpellier, France.
[5]UMR DIADE, Montpellier University, CIRAD, IRD, F-34398 Montpellier, France.
[6]Laboratory for Plant Breeding and Conservation of Genetic Resources, Bioplant Center, University of Ciego de Ávila, CP 69450, Cuba.

Received 10 November, 2021; Accepted 9 February, 2022

In Peru, potato cultivation represents 25% of the agricultural gross domestic product yet only 0.2% of the agamic seed used is from certified sources. The use of temporary immersion bioreactors (TIB) has improved the quality of microtubers micropropagated along with savings in costs of production. The current study investigated the agronomic performances of Peruvian Canchan potato microtubers derived from TIB (basic agamic seed 1 and 2) under the low-input agro-technology in the coastal zone of Peru. Following 75 days of growth, plants derived from microtubers produced in TIBs displayed slower vegetative growth than those from conventional tubers. However, at harvest, these differences were no longer apparent as the plants from TIB derived-basic agamic seed 1 and 2 produced the highest numbers of tubers per plant. Although plants raised from conventional tubers produced the highest fresh mass of tubers, significantly more propagules were produced by plants regenerated from basic agamic seed 1 and 2 derived from micropropagation in liquid media. These results demonstrate that much more planting material (seed tubers) can be obtained from microtubers in the field (basic agamic seed 1) than from the conventional commercial seed tubers.

Key words: Agronomic traits, micropropagation, potato microtuber, *Solanum tuberosum* L., temporary immersion bioreactors.

INTRODUCTION

Feeding an ever increasing global population is placing unprecedented pressure on agricultural systems and finite natural resources. This is exacerbated by the impacts of climate change, regional conflicts, migration,

the COVID-19 pandemic, etc. The aforementioned factors have highlighted the importance ensuring a stable, sustainable supply of food. Cultivation of potato (*Solanum tuberosum* L.) makes a valuable contribution to food security with production levels steadily increasing in the last 20 years (Devaux et al., 2020). The crop is cultivated in temperate regions of the northern hemisphere, highlands of the Andes and Africa, in the Rift valley, volcanic mountains of West Africa and South East Asia. In the sub-tropics, production occurs in the Mediterranean areas, North India and Southern China (Devaux et al., 2020). Currently, global production is estimated to be approximately of 370 million tons on 17 million hectares of land. Peru is regarded as the major center of origin of potato (Devaux et al., 2020) and farmers of this country cultivate four species of potatoes in the highlands and on the coast.

The cultivation of potato faces several challenges including pests, diseases, increasing soil salinity, incidences of drought and susceptibility to high temperatures (Gastelo et al., 2014). A major difficulty faced by producers worldwide is access to high quality planting material, that is, seed tubers (Sharma-Thomas et al., 2015). In many developing countries, seed tubers are not regularly renewed. This leads to the accumulation of endophytic pathogens which ultimately causes gradual degeneration in both quality and yield (Wasilewska-Nasciemento et al., 2020). In Peru, only a dramatically limited proportion of the agamic seeds used by farmers are certified (Corrêa et al., 2009).

Over the years alternative propagation technologies have been investigated in efforts to promote production efficiency. One of such avenue of investigation has been the use of micropropagation techniques. This, when combined with methods for disease detection, has allowed for the production of seedlings with significantly improved phytosanitary status and with efficient multiplication rates. In this regard, virus-free plants have been propagated through meristem cultures, successfully multiplied and minitubers produced *in vitro* (Al-Shareefi et al., 2020; Mamiya et al., 2020; Rojas et al., 2020; Yagiz et al., 2020; Belguendouz et al., 2021). *In vitro* plants can be planted in the field but they generally require a fairly technical acclimatization intermediate stage to outdoor conditions prior to field transfer and since these plants are delicate, they are also difficult to handle and transport (Wróbel, 2015). Microtubers are more robust than *in vitro* plants and provide an easier alternative to *in vitro* plants for potato propagation. Microtubers produced from in vitro plants provide ideal propagules for direct field planting. This strategy can be planned with consideration of planting seasons to ensure that sufficient in vitro plants are produced for subsequent generation of appropriate amounts of minitubers when required for planting (Igarza et al., 2011; Igarza et al., 2012, 2014; Rokka et al., 2014). An added advantage of microtubers is that they can be planted directly into the field without the need for acclimatization, provided that microtubers are large enough unlike *in vitro* plants (Jiménez et al., 1999).

There also exist the possibility of reducing production costs of microtubers through semi-automation in liquid media to produce basic potato agamic seed (Jiménez et al., 1999). A range of bioreactors have been developed over the years (e.g. temporary immersion systems) (Jiménez et al., 1999; Valdiani et al., 2019; Vidal and Sánchez, 2019). Temporary immersion systems have been successfully used to produce microtubers in potato (Jiménez et al., 1999; Higgins et al., 2017; Tapia et al., 2018) and yam (Jova et al., 2011; Balogun et al., 2014). However, as with any new technology, it is imperative that the agronomic performance of bioreactor-derived microtubers be investigated under local conditions before the micropropagation system can be scaled-up and applied on a large scale for roll out in potato agamic seed production schemes. Therefore, the current study investigated the agronomic characteristics of the Peruvian Canchan potato, propagated from microtubers regenerated in TIBs, in on farm field trials in the coastal zone of Peru and under the low-input agro-technology.

MATERIALS AND METHODS

Plant and culture conditions to obtain microtubers

Tubers were sourced from the germplasm bank of the International Potato Center (IPC, Lima, Peru) and *in vitro* plants were obtained via meristem culture. The elite plant tubers received were planted in greenhouses under controlled conditions allowing the material to keep free of pest and diseases. After 60 days of growth, apical cuttings were harvested and subsequently disinfected with 2.5% (v/v) sodium hypochlorite for 15 min. Meristems (0.1-0.3 mm) from apical and lateral buds were excised from plants using a dissecting microscope. The excised meristems were placed onto culture medium in a growth chamber at 25°C with a Photosynthetic Photon Flux Density (PPFD) of 50 µmol m^{-2} s^{-1} with a photoperiod of 16 h light/8 h dark. The meristems were transferred weekly onto fresh medium. After 6 to 8 weeks of growth, seedlings were obtained, which were micropropagated for indexing. The plants were evaluated at the IPC for any persistent virus infection. The following tests were carried out: nucleic acid spot hybridization (NASH) for the detection of potato spindle tuber viroid (PSTVd) and serological enzyme linked immunosorbent assay (ELISA) tests of indicator plants (Lizarraga et al., 1980). Pathogen-free plants generated from meristems with a height of approximately 15 cm and diameter of 1.5 mm were sectioned into nodal segments and placed in culture

*Corresponding author. E-mail: ltapia@lamolina.edu.pe.

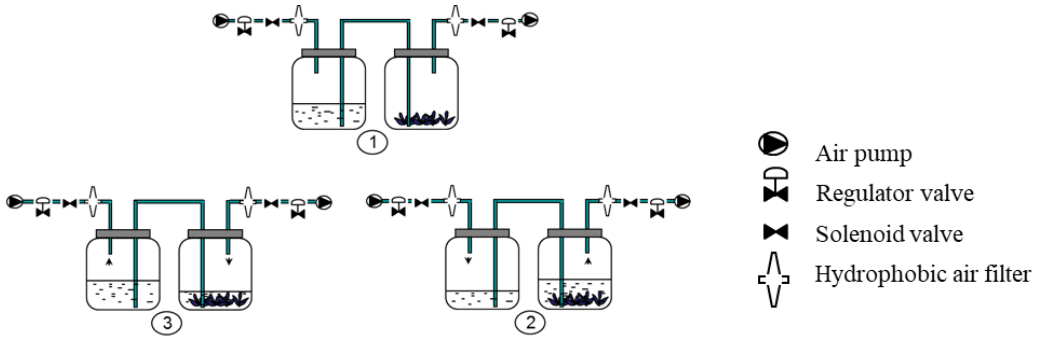


Figure 1. Operating cycle of a TIB. (1) Non-immersed stage, plant materials were free-standing on the bottom of the culture vessel. (2) Beginning of the immersed stage; an overpressure were applied and the medium was pushed up into the plant container immersing the plant material for 4 min. (3) End of the immersed stage, a second solenoid valve was opened and the culture medium was removed into the reservoir. These steps were performed every 3 h. The air pump and electric valves were under control of a timer.
Source: Lorenzo et al. (1998) and Escalona et al. (1999).

flasks with semi-solid medium for further multiplication.

The microtubers (pre-basic agamic seed) were obtained in 4 L-TIB (Lorenzo et al., 1998; Escalona et al., 1999) (Figure 1) in two stages, the first targeted for growth and multiplication of the nodal segments and the second was for microtuberization. The inoculation density was 50 explants/TIB (segments with 2 nodes) and 15 mL medium per segment in the bioreactors. Plants were immersed in liquid medium for a frequency of 4 min every 3 h over a period of 28 d under a photoperiod 16 h light/8 h dark. The microtuberization was induced in the same bioreactor by replacing the nutritive medium with the same medium enriched in 50 g/L sucrose to reach 80 g/L as final concentration. In such conditions, microtubers were induced after 60 day in the dark at 22°C.

All culture media were composed of Murashige and Skoog (1962) salts and vitamins modified as follows: 1750 mg/L ammonium nitrate, 2 g/L potassium nitrate, 450 mg/L calcium chloride, 175 mg/L phosphate, 0.4 mg/L thiamine, 2 mg/L glycine, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine, 1.0 mg/L calcium pantothenate, 1 mg/L folic acid and 4 mg/L arginine. The medium was supplemented with 100 mg/L myo-inositol. Sucrose was supplied at 30 g/L except for the microtuberization step which contained 80 g/L. The pH of all culture media was adjusted to 5.8 before autoclaving by steam sterilization at 121°C and 1.2 kg/cm^2.

In vitro culture procedure to obtain basic 1 and 2 agamic seeds

In order to obtain basic agamic seed 1, microtubers were rinsed and placed in trays exposed to ambient environmental conditions (natural light and 22 ± 2°C) for a month until they started budding. Before planting, they were disinfected with a 1 g/L (w/v) Benomyl solution for 10 min. Only microtubers with a fresh weight greater than 0.5 g (Kawakami and Iwama, 2012) were planted directly in sandy loam soil. Basic agamic seed 2 was obtained from the sowing of basic 1 agamic seed with 50 g of fresh weight. The procedure was the same as described earlier.

Assessment of agronomic performances

The performances of Peruvian Canchan potato plants derived from different propagation methods were compared in May to August, 2018. The following planting materials were used (about 50 g/seed tuber; planting density: 0.3 m × 1.0 m): (1) first generation tubers obtained in TIBs (basic agamic seed 1), (2) second vegetative generation (basic agamic seed 2), and (3) a commercial agamic seed control used by the Peruvian farmers. After 30, 75 and 150 days of field growth, agricultural traits of plants were evaluated (Egúsquiza, 2014). Plants grew on sandy soil, without any fertilizer or pesticide, at 200 m above sea level, with superficial irrigation, and at 15 to 27°C. Average rainfall was 0.12 mm and temperature 16.7°C.

Statistical analysis

The field experiment was composed of 4 independent blocks used as replicates. Each block was divided in three lines of 15 plants, each line representing one of the 3 planting/propagation material tested. In total, 60 plants for each type of propagation material were characterized agronomically. All data were statistically evaluated using SPSS (Version 8.0 for Windows, SPSS Inc., New York, NY) to perform one-way analysis of variance (ANOVA) and Tukey post-hoc tests (p=0.05).

RESULTS AND DISCUSSION

Potato agamic seed production remains a challenge in many developing countries. TIBs have been used in attempts to improve the efficiency of microtuber production for this purpose. However, it is necessary to first evaluate the field performance of propagules produced in this manner before this technology can be rolled out. This is particularly important when local cultivars are used under resource constrained conditions, as is the case in potato production in Peru. Hence, the current study investigated the field performance of Canchan potato microtubers produced in TIBs cultivated under typical low input systems characteristic of this country. The performances of this new planting material

Table 1. Field performance of TIB-derived potato microtubers in under a low input cropping system in Peru.

Agronomic trait observed after field planting		Types of propagated tubers compared as planting material		
		Commercial tubers used by marginal farmers	Basic agamic seed 1: TIB-derived microtubers	Basic agamic seed 2: Tubers harvested from basic seed 1-derived plants
After 30 days	Percentage of sprouting*	80.0 ± 7.3^{c}	90.0 ± 8.7^{a}	85.0 ± 7.6^{b}
After 75 days	Number of leaves per plant*	187.2 ± 15.4^{a}	86.7 ± 7.5^{c}	142.7 ± 11.5^{b}
	Total leaf fresh weight per plant (g)*	692.8 ± 55.3^{a}	188.1 ± 13.3^{c}	413.3 ± 39.4^{b}
	Total stem fresh weight per plant (g)*	523.7 ± 45.1^{a}	101.7 ± 9.5^{c}	380.5 ± 35.2^{b}
	Total root fresh weight per plant (g)*	67.7 ± 4.7^{a}	5.4 ± 0.4^{c}	23.2 ± 2.3^{b}
	Number of tubers per plant*	53.0 ± 4.3^{a}	14.2 ± 1.2^{b}	14.0 ± 1.2^{b}
	Total tuber fresh weight per plant (g)*	23.5 ± 1.9^{a}	16.5 ± 1.5^{a}	25.7 ± 1.9^{a}
	Tuber diameter (cm)*	28.9 ± 2.4^{a}	24.9 ± 2.1^{a}	33.1 ± 3.0^{a}
	Tuber length (cm)*	32.4 ± 2.8^{a}	26.1 ± 1.8^{a}	32.4 ± 2.8^{a}
After 150 days	Total number of tubers per plant*	8.0 ± 0.7^{b}	12.6 ± 1.1^{a}	10.9 ± 1.1^{a}
	Total tuber fresh weight per plant (g)*	143.9 ± 12.5^{a}	86.6 ± 7.6^{b}	93.0 ± 8.8^{b}
	Tuber diameter (cm)*	59.1 ± 4.8^{a}	51.6 ± 4.9^{b}	53.3 ± 4.5^{b}
	Tuber length (cm)*	63.3 ± 5.6^{a}	53.1 ± 4.1^{b}	53.9 ± 5.2^{b}

*Results with the same *letter* are not statistically different (One-Way ANOVA, Tukey, p>0.05). For the statistical analysis only, numbers of leaves and tubers were transformed according to $y' = y^{0.5}$, and the percentage variables as $y' = 2 \arcsin(y/100)^{0.5}$. Vertical bars represent ±SE of original data.

were compared to those of conventional tubers propagated by farmers themselves in non-controlled conditions.

The agronomic performances of microtubers derived from cultures in TIBs are summarized in Table 1 and Figure 2. Sprouting of tubers was recorded following 30 days of field growth and other agronomic parameters after 75 and 150 days. Good levels of sprouting were achieved from all propagation material with the highest values observed from bioreactor-derived microtubers (90%) followed by basic agamic seed 2 (85%) and commercial tubers (80%). At the mid-point of the trial (that is at 75 days), plants derived from commercial tubers were more vigorous showing the significantly highest number of leaves (187), leaf FW (692 g), stem FW (523 g) and root FW (67 g). For all the aforementioned parameters, the significantly lowest indicators were observed in plants directly derived from bioreactors (basic agamic seed 1). At this intermediate stage, plants from commercial agamic seeds also produced the highest number of tubers per plant (53) compared with basic agamic seed 1 and basic agamic seed 2 that regenerated similar numbers of tubers (14 tubers/plant). No significant difference was observed between the three planting materials for the tuber FW per plant and the diameter and length of tubers.

The field trial was harvested 150 days after planting and final agronomic performances of plants depending on the sowed tuber type were assessed. In contrast to the preliminary findings at 75 days, the plants derived from both types of microtubers derived from TIB-basic agamic seed 1 and 2 generated the significantly highest numbers of tubers per plant (12.6 and 10.9, respectively) while the plants grown from commercial tubers produced 8 tubers per plant (Table 1). However, these plants grown from commercial tubers still yielded the highest tuber FW/plant (143.9 g), compared with basic 1 and 2 agamic seeds (86.6 and 93.0 g, respectively). Furthermore, the tuber size as expressed by both diameter and length was larger in plants from commercial tubers (59.1 and 63.3 cm, respectively) than in the plants grown from the basic agamic seed sources. Plants propagated from commercially sourced tubers produced fewer but larger tubers than those regenerated by microtubers produced from bioreactors. It is noteworthy that tubers of a similar size were produced by plants grown from basic agamic seed 1 and 2 (Table 1).

As the objective of the study is to develop methods for the production of more efficient planting material in potato, tuber morphological characteristics were also measured to determine the ability of plants from each of the three sources to generate tubers suitable for subsequent tuber propagation. The results showed that the significantly higher numbers of agamic seed tubers per plant were produced by plants generated by microtubers basic agamic seeds 1 (8.5) and 2 (6.7) in comparison with plants from commercial tubers yielding very low number (3.0) (Figure 2A). Then, an elevated

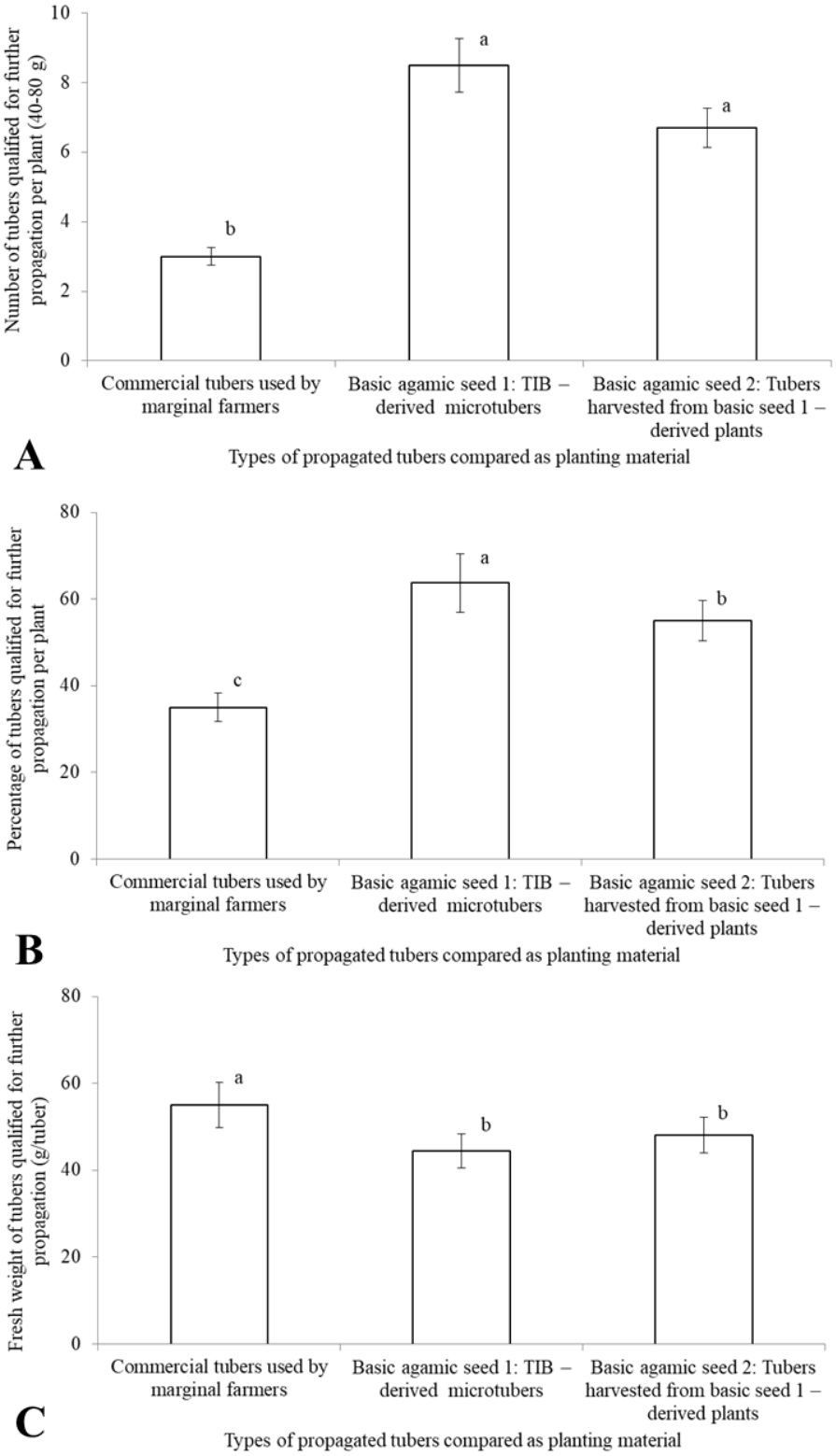


Figure 2. Quality of the materials obtained for further propagation after 150 days of planting. Results with the same letter are not statistically different (one-way ANOVA, Tukey, $p>0.05$). For the statistical analysis only, numbers of tubers were transformed according to $y" = y^{0.5}$, and the percentage as $y" = 2 \arcsin(y/100)^{0.5}$. Vertical bar represent ±SE of original data.

percentage of tubers harvested on plants generated from basic agamic seed 1 (63.7%) can be used for further new propagation cycle, higher than those obtained with plants from basic agamic seed 2 (55%) and much higher than the low percentage obtained with plants from commercial tubers (35%) (Figure 2B). In terms of the FW of tubers, selectable for subsequent propagation cycles, plants raised from commercial tubers produced bigger tubers (55 g) than plants from microtubers that produced similar tubers in a range of 44 to 48 g (Figure 2C). The highest emergence of sprouts observed in microtubers derived from bioreactors during the initial 30 days-vegetative growth stage must be related to the setting up of a series of particular biochemical, physiological and morphological processes leading to the induction of buds to ultimately give rise to new organs of the plant (Wattimena et al., 1983). This process is also influenced by environmental conditions and genotype (Dieme and Sy, 2013; Wróbel, 2015). It has been postulated that the size of tubers might influence the rate of emergence, however there are conflicting reports regarding this. Ranalli et al. (1994) reported that smaller tubers were prone to slower rates of emergence with reduced vigor of plants while Kawakami and Iwama (2012) found differences in emergence only with the smallest tubers tested (0.3 - 0.5 g) and not with other size classes. However, this observation was not consistent across years. Others authors argue that it is the nutrient status of tubers that determines sprouting. In this regard, Escalona et al. (2003) highlighted that the superiority of *in vitro* derived microtubers is as a consequence of the improved assimilation of nutrients that occurs under *in vitro* conditions leading to more vigorous tubers than from conventional sources. It has been shown that a substantial pool of reserves in tubers, particularly carbohydrates, allows for further more efficient plant development (Desire et al., 1995). In addition, the method of *in vitro* propagation has also been reported to affect nutrient accumulation in microtubers and subsequent plant emergence. For example, potato microtubers generated from *in vitro* culture on semi-solid nutritive media have been reported to display lower percentages of emergence (Lommen and Struik, 1994).

Despite the higher levels of sprouting observed in microtubers derived from bioreactors, these plants displayed the slowest relative growth rates within the first 75 days in the field as evidenced by the low levels of biomass generated. While there are studies on the field performance of plants derived from microtubers and commercial tubers, there is still a scarcity of information on the field behavior of the basic agamic seed 1 and 2 microtubers obtained from *in vitro* culture compared with the conventional sowing of commercial tubers. Nevertheless, this lower plant development did not ultimately affect the yield of tubers obtained as on the contrary plants grown from microtubers produced a higher number of tubers at harvest. Kawakami and Iwama (2012) also noted that the observation of initial low leaf area index in plants derived from microtubers was transient and disappeared after flowering.

Plants from basic agamic seed 2 microtubers also displayed consistently higher vegetative growth than those from basic agamic seed 1 microtubers. Although both basic agamic seed 1 and basic agamic seed 2 originated from the same source, that is, culture in TIBs, the latter was able to produce more vigorous plants. Ultimately, the faster vegetative development of these plants derived did not translate into higher tuber yields as both plant types produced similar numbers of tubers with comparable morphological characteristics.

Wattimena et al. (1983) and Leclerc and Donnelly (1990) also found no differences in the FW of tubers per plant between basic agamic seed 1 and 2. Similar results were obtained by Kawakami and Iwama (2012) who observed that the differences found in the vegetative development of plants from microtubers and commercial tubers disappeared as plants developed, with no significant differences in the number of tubers produced at harvest. However, tubers produced from commercial sources were larger (with higher diameters and lengths) and heavier than those produced from basic agamic seed 1 and 2. Similarly, Kawakami et al. (2003), Wróbel (2015) and Higgins et al. (2017) also reported a larger fresh mass of tubers generated from commercial tubers. Hence, in the present study, it was observed that fewer, larger tubers were produced by plants raised from commercial tubers while many smaller tubers were produced by basic seed 1 and 2. A similar finding was reported by Wattimena et al. (1983) in potato and by Jova et al. (2011) in yam. The reason for this observation is not clear but a few suggestions have been proposed. For example, it has been highlighted that the temporary immersion system permits contact between liquid culture medium and all parts of the plant for prescribed amounts of time. This allows for the induction of tubers in a more uniform manner among axillary buds leading to the formation of more tubers (Jiménez et al., 1999). It has also been suggested that microtubers might have a greater number of eyes than tubers from other sources leading to more stems which in turn could generate more tubers (Radouani and Lauer, 2015). Regardless of the exact mechanism involved, the results indicate that microtubers and basic agamic seed 2 provide appropriate and optimized sources of material for potato propagation schemes.

Furthermore, there are phytosanitary advantages in using microtubers as these are generated from virus-free *in vitro* meristem cultures, therefore the quality of these propagules are superior to commercial tubers in this regard. Indeed this might have contributed to the observation of reduced sprouting in commercial tubers, which are known to accumulate pathogens over time (Badoni and Chauhan, 2009b, a) leading to deterioration

(particularly in informal or non-certified schemes). These results suggest that the use of agamic seed from biotechnological methods (microtubers from temporary immersion: pre-basic) would be especially useful in countries where the production of quality agamic seed with phytosanitary conditions is challenging. In addition, the observation in the current study that plants generated from basic agamic seed 1 and 2 can produce high levels of tubers for subsequent propagation, makes this method feasible for potato agamic seed schemes.

The current work evidences the possible use/potential of TIBs to produce microtubers as a source of material for potato agamic seed schemes. The results showed that bioreactor-derived propagules initially displayed slower rates of vegetative growth than commercial tubers but ultimately produced a higher number of tubers per plant. Bioreactor-derived microtubers were smaller in mass but more abundant in number than conventional tubers with a higher percentage of tubers suitable for subsequent propagation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This research was supported by the National Agricultural University La Molina (Peru), the Bioplant Centre, University of Ciego de Avila (Cuba), and the Agricultural Research Council (South Africa).

REFERENCES

Al-Shareefi MJ, Abbass JA, Abdulhussein MAA (2020). Effect of light sources and culture systems on microtubers production of potato (*Solanum tuberosum* L.) *in vitro*. International Journal of Agricultural and Statistical Sciences 16(2):679-686

Badoni A, Chauhan J (2009a). A note on micro tuber seed production of potato: Necessitate steps for Uttarakhand Hills. Report and Opinion 1(5):9-11.

Badoni A, Chauhan J (2009b). Effect of growth regulators on meristem-tip development and *in vitro* multiplication of potato cultivar 'Kufri Himalini'. Nature and Science 7(9):31-34.

Balogun MO, Maroya N, Asiedu R (2014). Review: status and prospects for improving yam seed systems using temporary immersion bioreactors. African Journal of Biotechnology 13(15):1614-1622

Belguendouz A, Kaide Harche M, Benmahioul B (2021). Evaluation of different culture media and activated charcoal supply on yield and quality of potato microtubers grown *in vitro*. Journal of Plant Nutrition 44(14):2123-2137.

Corrêa RM, Pinto JB, Faquin V, Pinto CB, Reis ES (2009). The production of seed potatoes by hydroponic methods in Brazil. Fruit, Vegetable and Cereal Science and Biotechnology 3(1):133-139.

Desire S, Couillerot J, Vasseur J (1995). Dormancy and sprouting of *in vitro* potato (*Solanum tuberosum* L.) microtubers: effects of sucrose concentration from tuberization medium, storage period at 4 degrees C and gibberellic acid treatment. Acta Botanica Gallica 142(4):371-378.

Devaux A, Goffart J, Petsakos A, Kromann P, Gatto M, Okello J, Suarez V, Hareau G (2020). Global food security, contributions from sustainable potato agri-food systems. In: Campos H, Ortiz O (eds) The Potato Crop: Its Agricultural, Nutritional and Social Contribution to Humankind. Springer Nature, Switzerland, pp. 3-36

Dieme A, Sy MO (2013). Environmental, morphological and physiological factors analyzes for optimization of potato (*Solanum tuberosum* L.) microtuber *in vitro* germination. Advances in Bioscience and Biotechnology 4(11):986.

Egúsquiza B (2014). La papa en el Perú. Universidad Nacional Agraria La Molina, Perú

Escalona M, Lorenzo JC, González B, Daquinta M, Borroto C, González JL, Desjardines Y (1999). Pineapple micropropagation in temporary immersion systems. Plant Cell Reports 18:743-748

Escalona M, Samson G, Borroto C, Desjardins Y (2003). Physiology of effects of temporary immersion bioreactors on micropropagated pineapple plantlets. *In Vitro* Cellular & Developmental Biology-Plant 39(6):651-656

Gastelo M, Kleinwechter U, Bonierbale M (2014). Global potato research for a changing world. International Potato Center. https://cipotato.org/publications/global-potato-research-for-a-changing-world/

Higgins S, Francis R, Smith A (2017). Production of *Solanum tuberosum* L. microtubers using temporary immersion system. European Journal of Experimental Biology 7(6):38. doi: 10.21767/2248-9215.100038

Igarza J, Agramonte D, de Feria M, Jaime J, Pérez M, san Roman M (2011). Obtención de microtubérculos de papa cv "Andinita" en Sistemas de Inmersión Temporal. Biotecnología Vegetal 11(1):59-62.

Igarza J, Agramonte D, Alvarado Y, De feria M, Pugh T (2012). Empleo de métodos biotecnológicos en la producción de semilla de papa. Biotecnología Vegetal 12(1):3-24

Igarza J, De feria M, Alvarado Y, Pugh T, Pérez M, San Roman M, Agramonte D (2014). Caracterización morfo-agronómica de plantas de papa cv. "Andinita" a partir de la siembra en campo de microtubérculos obtenidos en Sistemas de Inmersión Temporal. Biotecnología Vegetal 14(2):81-89

Jiménez E, Pérez N, De Feria M, Barbón R, Capote A, Chávez M, Quiala E, Pérez JC (1999). Improved production of potato microtubers using temporary immersion system. Plant Cell, Tissue and Organ Culture 59(1):19-23.

Jova M, Kosky R, Cabrera A, De Feria M, Perez M, Vega V, López-Torres J (2011). Performance of yam microtubers from temporary immersion system in field conditions. African Journal of Biotechnology 10(46):9268-9271.

Kawakami J, Iwama K, Hasegawa T, Jitsuyama Y (2003). Growth and yield of potato plants grown from microtubers in fields. American Journal of Potato Research 80(6):371-378.

Kawakami J, Iwama K (2012). Effect of potato microtuber size on the growth and yield performance of field grown plants. Plant Production Science 15(2):144-148.

Leclerc Y, Donnelly DJ (1990). Seasonal differences in the field performance of micropropagated potato under a short growing season in Quebec. American Potato Journal 67(8):507-516.

Lizarraga R, Salazar L, Roca W, Schilde-Rentschler L (1980). Elimination of potato spindle tuber viroid by low temperature and meristem culture. Phytopathology 70(8):754-755.

Lommen W, Struik P (1994). Field performance of potato minitubers with different fresh weights and conventional seed tubers: crop establishment and yield formation. Potato Research 37(3):301-313.

Lorenzo JC, González B, Escalona M, Teisson C, Espinosa P, Borroto C (1998). Sugarcane shoot formation in an improved temporary immersion system. Plant cell, Tissue and Organ Culture 54(3):197-200.

Mamiya K, Tanabe K, Onishi N (2020). Production of potato (*Solanum tuberosum* L.) microtubers using plastic culture bags. Plant Biotechnology 37(2):233-238.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum

15(3):473-497. https://doiorg/101111/j1399-30541962tb08052x
Radouani A, Lauer F (2015). Field performance of cultivars Nicola and Russet Burbank micro and minitubers. American Journal of Potato Research 92(2):298-302.
Ranalli P, Bassi F, Ruaro G, Del Re P, Dicandilo M, Mandolino G (1994). Microtuber and minituber production and field performance compared with normal tubers. Potato Research 37(4):383-391.
Rojas GP, Sanchez H, Barahona IR, Ayme VA, Segura-Turkowsky M, Jimenez RE (2020). Alternative inputs for micropropagation of *Solanum tuberosum*, *Ullucus tuberosus* and *Oxalis tuberosa* in semi-solid and liquid medium, and temporary immersion system. Tropical and Subtropical Agroecosystems 23(2):1-15.
Rokka VM, Kamarainen-Karppinen T, Virtanen E, Pirttila AM (2014). Bioreactor technologies for mass propagation of potato: future prospects. In: Ramawat KG, Mérillon JM (eds) Bulbous Plant Biotechnology. CRC Press,
Sharma-Thomas S, Abdurahman A, Ali S, Andrade-Piedra JL, Bao S, Charkowski AO, Crook D, Kadian M, Kromann P, Struik PC, Torrance L, Garrett KA, Forbes GA (2015). Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. Plant Pathology 65(1):3-16.
Tapia MdL, Arbizu C, Beraún F, Lorenzo J, Escalona M (2018). Pre-basic seed potato (*Solanum tuberosum* L.) production using temporary immersion bioreactors. Peruvian Journal of Agronomy 2(1):9-14.
Valdiani A, Hansen O, Nielsen U, Johannsen V, Shariat M, Georgiev M, Omidvar V, Ebrahimi M, Dinanai E, Abiri R (2019). Bioreactor-based advances in plant tissue and cell culture: challenges and prospects. Critical Reviews in Biotechnology 39(1):20-34.
Vidal N, Sánchez C (2019). Review: Use of bioreactor systems in the propagation of forest trees. Engineering in Life Sciences 19(12):896-915.
Wasilewska-Nasciemento B, Boguszewska-Mańkowska D, Zarzyńska K (2020). Challenges in the production of high quality seed potatoes (*Solanum tuberosum* L.) in the tropics and subtropics. Agronomy 10(2):260. doi:10.3390/agronomy10020260.
Wattimena G, McCown B, Weis G (1983). Comparative field performance of potatoes from microculture. American Potato Journal 60(1):27-33.
Wróbel S (2015). Assessment of potato microtuber and *in vitro* plantlet seed multiplication in field conditions - growth, development and yield. Field Crops Research 178:26-33.
Yagiz AK, Yavuz C, Tarim C, Demirel U, Caliskan ME (2020). Effects of growth regulators, media and explant types on microtuberization of potato. American Journal of Potato Research 97(5):523-530.

Vol. 21(3), pp.133-138, March 2022
DOI: 10.5897/AJB2022.17454
Article Number: 348A6E068838
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

A study of the antimicrobial activity of *Psidium guajava* L. and *Lawsonia inermis* leaf extracts against some foodborne pathogens

Nagwa T. Elsharawy[1,2*] and Afra Mohammed Baghdadi[1]

[1]Department of Biology, College of Science, University of Jeddah, Jeddah, Saudi Arabia.
[2]Department of Food Hygiene, Faculty of Veterinary Medicine, New Valley University, Egypt.

Received 5 January 2022; Accepted 21 February 2022

The study aims to determine the antibacterial activity of *Psidium guajava* and *Lawsonia inermis* leaf extracts against some foodborne pathogens. The leaf samples were collected, rinsed, dried, ground, and then stored. The leaf powder was soaked in ethanol and boiled in distilled water. Then, it was filtered. The antibacterial activity of *P. guajava* and *L. inermis* leaf extracts against *Salmonella enteritidis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and multidrug resistant *S. aureus* (MRSA) was studied. Microbial enzymes were examined including Amylase, Protease and Lipase. The results showed that the most effective extract against all the tested microorganisms was *Psidium guajava* ethanol extract. Similar results were almost detected in *L. inermis* water extract. Although *P. guajava* water extract had wider inhibition zone, its effect was limited against *E. coli, S. aureus* and MRSA. In conclusion, the results obtained provide valuable knowledge for food preservation. There is need for further research to better extract high concentrations of *P. guajava* and *L. inermis* extracts.

Key words: Antibacterial agents, guava, Henna, *Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

The most recent research seeks to overcome microbial resistance to traditional drugs consisting of natural bioactive compounds and their derivatives obtained from plants which have antimicrobial effects against different pathogenic microorganisms including foodborne microorganisms (Vaou et al., 2021).

Guava leaf (*Psidium guajava* L.) is obtained from one of the most common tropic fruit trees with about 133 genera and 3,800 species worldwide. It belongs to Magnoliophyta phylum, Magnoliopsida class and Myrtaceae family. It is commonly used for traditional medicine to treat many digestive and respiratory disturbances due to its richness in vitamins and minerals such as phosphorus, calcium, pectin, tryptophan, lysine plus its antimicrobial polyphenolic compounds such as flavonoids, saponins, eugenol, tannins, and triterpenoids (Seo et al., 2014; Naseer et al., 2018).

Henna (*Lawsonia inermis*) is also one of the most common dying plants related to Lythraceae family grown in most tropical countries all over the world. This plant

*Corresponding author. E-mail: dr.nagwa2004@yahoo.com.

leaf contains amino acids and fixed oils. It also has high concentrations of many medicinal ingredients such as triterpenoids, alkaloids, lignins and flavonoids which have antimicrobial effect in accelerating wound healing. Furthermore, it has sedative, cardio-inhibitory, anti-hemorrhagic and hypotensive effects (Parimalam et al., 2021).

Salmonella species (*Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST)) and *Escherichia coli* are Gram negative common foodborne microorganisms mainly found in the intestines of animals and humans. They contaminate food and water through fecal matter. They are considered as the second common cause of gastrointestinal upsets. Over 90,000 infected cases are reported annually in Europe. The infection signs are abdominal cramps, fever and diarrhea within few hours of consumption of contaminated and undercooked meat, fish, chicken or their products. Recovery mostly occurs spontaneously within few days (The Standard of the National Committee for Clinical Laboratory Standards, 2012).

The most global Gram positive microorganisms are *Staphylococcus* species. Especially, *Staphylococcus aureus* is the bacterium that spreads most in the skin, mucus membranes, urogenital and upper respiratory systems of healthy animals and people. *S. aureus* (MRSA) strain has resistance to methicillin and several antibiotics. It is commonly transported via contaminated high salt tolerance meat, chicken, milk and fish or their products. Its high virulence makes it to secrete seven toxins leading to food poisoning. However, its methicillin resistance is not due to its ability to produce enterotoxins which cause food poisoning. The symptoms of illness caused by this strain appear within few minutes after eating food contaminated by the heat stable toxins like mild gastroenteritis. People mainly recover within three days after being infected (da Silva et al., 2019; Le et al., 2021).

Most foodborne infectious agent is diagnosed by stool and blood samples, although almost all of these microorganisms become resistance to almost all antibiotics especially in suppressed immune patients. The major problem caused when the infectious agents do not respond to traditional antimicrobials is high cost of treatment using orthodox medicines, including their side effect and the patients' inability to work. These motivated the researchers to look for new antimicrobials. This study aims to determine the antibacterial activity of ethanol and aqueous leaf extracts of guava and henna against some clinical Gram negative and Gram positive microorganisms including some foodborne pathogens.

MATERIALS AND METHODS

Plant extracts preparation

Random fresh guava (*P. guajava*) and henna (*L. inermis*) green leaf samples were collected from guava trees from different Jeddah fruit farms, Jeddah, Saudi Arabia. The samples collected were stored in labeled plastic bags and then transported to the laboratories of College of Science, University of Jeddah. The leaves of the samples were rinsed, dried, grinded by blender and then sieved by 1 mm aluminium sieve. It was stored inside labeled air tight bottles until used (Seo et al., 2014).

Methods of extractions

About 25 g of the leaf powder was soaked in 100 ml ethanol (>99.5%) and boiled in distilled water inside an aluminium flask to prevent light exposure and evaporation. It was stirred in a shaker incubator at 70 rpm for 3 days under sterile conditions to obtain 25% concentration. The solution obtained was centrifuged at 4,000 rpm/25°C for 10 min. The supernatant was filtered by Whatman No. 1 filter paper and then stored at 4°C until it was used (Seo et al., 2014).

Antibacterial activity

Fresh guava (*P. guajava*) and henna (*L. inermis*) green leaves were extracted for use against sex strains of microorganisms: *S.* Enteritidis (ESBL700613)*, S. aureus* (ATCC25923)*, S.* Typhimurium (ATCC14028)*, Escherichia coli* (NCTC9001)*, pathogenic E. coli* (MC-SC376609), and MRSA (ATCC43300) using the well diffusion technique. Muller Hinton was used to streak the microorganisms and then were punched by a sterile borer of 5 mm diameter to impede six antibiotics discs (50 mg/mL) as follows: Cefoxitin (FOX), Cephalothin (KF), Cotrimoxazole (TS), Gentamicin (GM), Augmentin (AUG) and Ampicillin (AP)" [Mast Group/ MASTRING-S] (Figure 1). They were compared with the discs of the different plants' leaf extracts. Three plates were prepared from the same extracts and then incubated at 37°C/24 h. Then the halo/clear zone of each plate was measured by a ruler to estimate the inhibition zone by millimeters. The average of the three readings (The Standard of the National Committee for Clinical Laboratory Standards, 2012) was calculated.

Microbial enzymes' examination

This was performed to know the effect of the plant extracts on the microbial enzymes.

Amylase assay: About 25 g of starch agar medium was suspended in 1000 ml distilled water. About 4 mm of bacterial culture was cut on the labeled plate and then incubated at 37°C/24 h with a drop of iodine solution for 30 s. The color of the medium changed because amylase is a starch hydrolyzing enzyme (Ross, 1976).

Protease assay: Skim milk agar plate was suspended in about 51.5 g of 1000 ml distilled water. The bacterial culture was inoculated separately. There were clear zones (zone of hydrolysis) around the bacterial colonies after being incubated at 25°C for 48 h (Ali, 1992).

Lipase assay: This was done by the addition of 2.5% agar, 2% Tween 20-80 and 0.01% Victoria Blue B (or other indicators). The different tested microorganisms were grown at 30°C in a circular well of about 1 cm diammeter). Lipolytic microorganisms were picked out from the culture plates (Samad et al., 1989).

Statistical analysis

The statistical program, SPSS version 16 for window, was used to

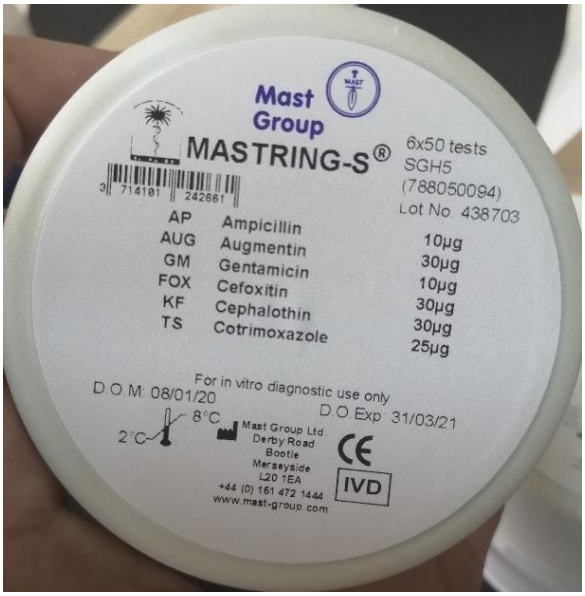


Figure 1. Antibiotic discs.

Table 1. Antimicrobial activity of *Psidium guajava* L. and *Lawsonia inermis* leaf extracts (cm).

Microorganisms	*Psidium guajava* L.		*Lawsonia inermis*		FOX	KF	TS	GM	AUG	AP
	Ethanolic extract	Water extract	Ethanolic extract	Water extract						
Salmonella enteritidis	4.5	00	1.8	4.0	3.0	2.5	2.6	2.5	2.5	2.0
S. aureus	00	6.5	1.5	2.0	3.0	00	3.5	3.5	3.5	4.5
Salmonella typhimurium	4.0	00	1.8	2.3	3.4	2.5	2.5	2.0	2.5	2.0
E. coli	4.0	7.3	1.6	2.0	2.5	1.5	2.5	2.5	2.5	1.5
Pathogenic E. coli	2.5	00	2.7	1.4	2.7	2.0	2.5	3.0	2.5	2.0
MRSA	2.5	3.0	1.2	1.2	2.5	00	2.3	2.5	2.5	00

determine the means (at the significance level of $P<0.05$), standard error, and analysis of variance (ANOVA) with one way. Statistical significance was tested at the 5% level of significance in this study (SPSS 16, 2007).

RESULTS

Comparison between the antimicrobial activity of *P. guajava* L. leaf extracts and *L. inermis* leaf extracts

Table 1 and Figure 2 show the *in vitro* inhibitory effects of the water and ethanol extracts in comparison with commercial antibiotics. The most effective extract against all the tested microorganisms was the ethanol extract of *P. guajava* (4.5-2.5 cm). Almost similar results were obtained from the water extract of *L. inermis* (4.0-1.2 cm). Although the water extract of *P. guajava* had wider inhibition zone, its effect was limited against *E. coli* (7.3 cm), *S. aureus* (6.5 cm) and MRSA (3.0 cm). While, the ethanol extract of *L. inermis* ranged from 2.7 to 1.2 cm. The study results showed there was higher inhibition effect against Gram negative microorganisms than Gram positive species.

Effects of *P. guajava* L. leaf extracts and *L. inermis* leaf extracts on microbial enzymes

Figure 3 shows the comparison of the *in vitro* inhibitory effect of the water and ethanol extract. The most effective extract against all the tested microbial enzymes was the water extract of *P. guajava*. Almost similar results were detected in the water extract of *L. inermis.* Although, the ethanol extracts of *P. guajava* and *L. inermis* had almost similar inhibition effect against the tested microbial enzymes. All the herbal extracts had higher inhibition effect against lipase followed by amylase and then protease enzymes.

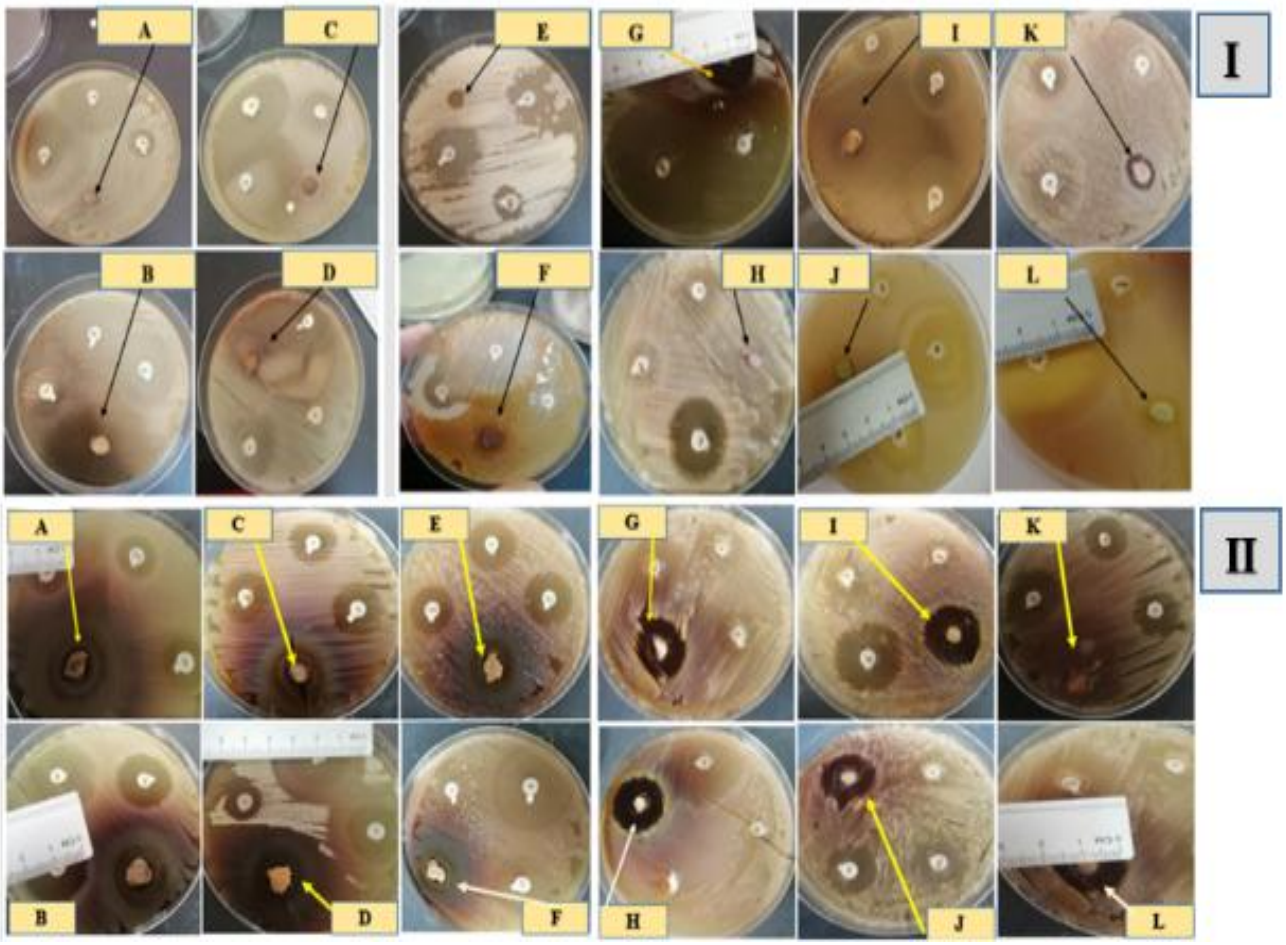


Figure 2. Antimicrobial effect of *psidium guaiava* (I) and *Lawsonia inermis* (II) Aqueous and Ethanol leaf extracts against different microorganisms compared with Antibiotics. (A,G) *Salmonella enteritis; (*B, H) *Salmonella typhimurium; (*C, I) Pathogenic *E. coli; (*D, J) *E. coli; (*E, K) *Staphylococcus aureus; (*F, L) MRSA.

DISCUSSION

This study examines the antibacterial effect of *P. guajava* and *L. inermis* in overcoming the side effects of commercial used antibiotics and the resistance of foodborne pathogens to antibiotics. The study estimates the aqueous and ethanol extracts of *P. guajava* and *L. inermis* against the most common foodborne pathogens which resist almost all commercial antibiotics. It also examines the effects of the different plant extracts on the bacterial enzymes. The results obtained revealed that the most effective extract against all the tested microorganisms was the ethanol extract of *P. guajava;* it has higher inhibition effect against the tested Gram negative microorganisms than Gram positive tested species. On the other hand, the most effective extract against all the tested microbial enzymes was the water extract of *P. guajava*. Almost similar results were obtained from the water extract of *L. inermis.* Although, the ethanol extracts of *P. guajava* and *L. inermis* showed almost similar inhibition effect against the tested microbial enzymes. All the herbal extracts had higher inhibition effect against lipase followed by amylase and then protease enzymes.

According to Santhoshkumar et al. (2014), the aqueous and ethanol extracts of *P. guajava* leaves have patent effect against *S. aureus.* Samiha et al. (2017) reported that *P. guajava* leaf extract has effective antimicrobial activity against *S. aureus* strains. Puntawong et al. (2012) detected the antibacterial effect of *P. guajava* leaf ethanol extract against Gram negative and positive microorganisms, especially against gastrointestinal pathogens. Naseer et al. (2018) reported the high effect of *P. guajava* leaf extract against *Salmonella* spp., *E. coli* and *S. aureus.* Antibacterial effect of *P. guajava* refers to the presence of quinone compounds which can adhere to bacterial cell polypeptides and attach to bacterial enzymes which inhibit the growth of microorganisms. Another antibacterial agent derived from *P. guajava* containing flavonoids, saponins, and tannins has great

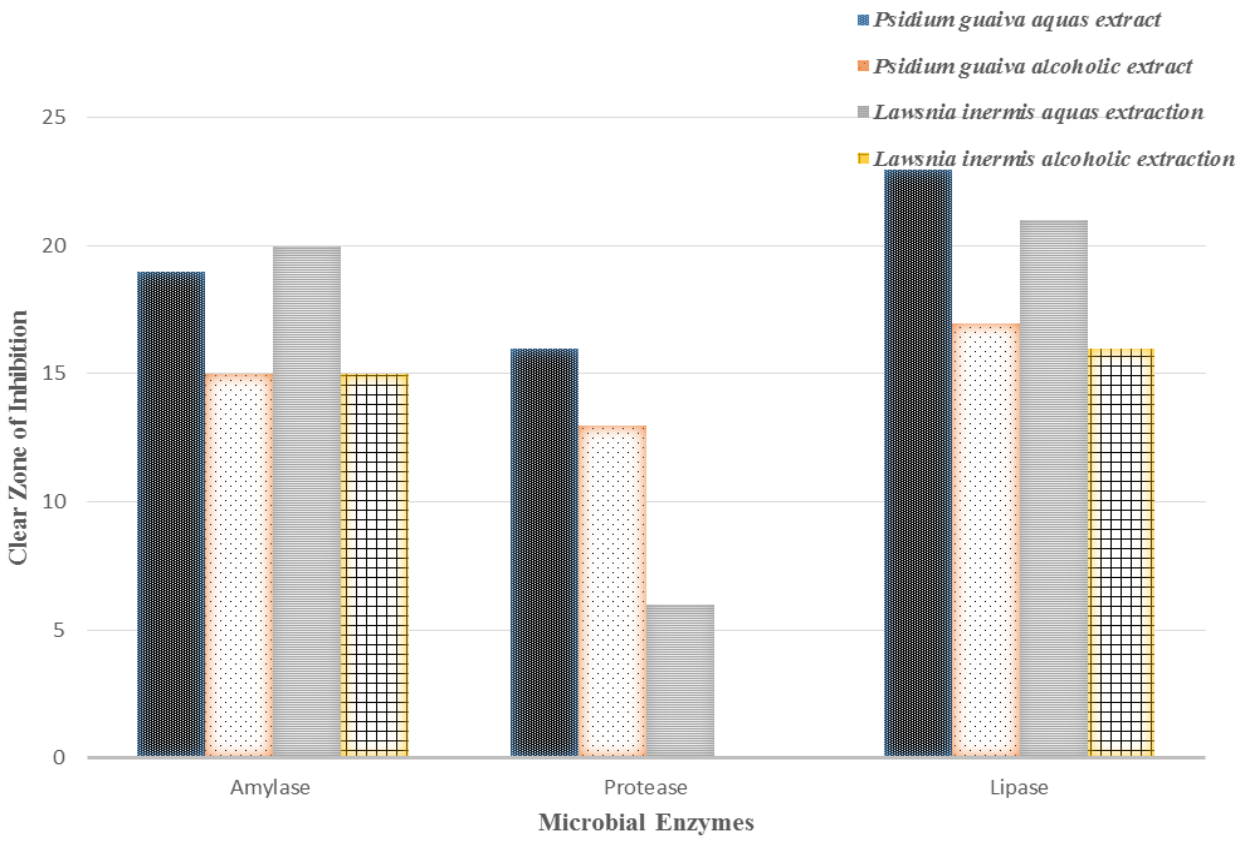


Figure 3. Comparison between the effect of *Psidium guaiava, Lawsonia inermis* extracts against different bacterial enzymes.

inhibitory effect against *S. aereus* and *E. coli* and other microorganisms' enzymes (Díaz-de-Cerio et al., 2017).

Nearly similar results were obtained by Elmanama et al. (2011) who recorded the antimicrobial activity of *L. inermis* that can combine with proteins and carbohydrates which are found in bacterial cell wall and inactivate bacterial enzymes. Santhamari et al. (2011), Borade et al. (2011) and Pereira et al. (2010) recorded the effectiveness of *L. inermis* against *S. aureus, E. coli*, *Bacillus* species, *Klebsiella pneumonia, Proteus* species, *Pseudomonas aeruginosa, Salmonella* spp. and MRSA. Arun et al. (2010) observed it has less effect on Gram negative microorganisms. On the other hand, Habbal et al. (2010) stated that the high potency of the ethanol extract of *L. inermis* is considered as a promising broad spectrum antibacterial agent. The effectiveness of *L. inermis* is because it contains flavonoids, alkaloids, cardenolides, saponins and other active substances (Santhamari et al., 2011).

Conclusions

This study has shown that the aqueous and ethanol extracts of *P. guajava* and *L. inermis* leaves possess good inhibitory antimicrobial effect. The results obtained provided valuable knowledge that natural antimicrobial agents have potential applications in food and pharmaceutical industries for controlling foodborne pathogens. More and further research needs to be done to better extract and use high concentrations of *P. guajava* and *L. inermis* leaf extracts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The article was fully funded by the authors.

REFERENCES

Ali OG (1992). Formation of proteases by *Aspergillus fumigatus* and *Pencillium* sp. King Saud University–Science 4(2):127-136.

Arun P, Purushotham K, Jayarani J, kumara V (2010). *In vitro* Antibacterial activity and Flavonoid contents of *Lawsonia inermis* (Henna). International Journal of PharmTech Research 2(2):1178-1181.

Borade A, Kale B, Shete R (2011). A phytopharmacological review on *Lawsonia inermis* (Linn.). International Journal of Pharmacy and Life Sciences 2(1):536-541.

da Silva A, Rodrigues M, Silva N (2019). Methicillin-resistant *Staphylococcus aureus* in food and the prevalence in Brazil: a review. Brazilian Journal of Microbiology 51(1):347-356.

Díaz-de-Cerio E, Verardo V, Gómez-Caravaca A, Fernández-Gutiérrez A, Segura-Carretero A (2017). Health Effects of *Psidium guajava* L. Leaves: An Overview of the Last Decade. International Journal of Molecular Sciences 18(4):897-905.

Elmanama A, Alyazji A, Gheneima A (2011). Antibacterial, Antifungal and Synergistic Effect of *Lawsonia inermis, Punica granatum* and *Hibiscus sabdariffa*. Annals of Alquds Medicine 7(3):33-41.

Habbal O, Hasson S, El-Hag A, AlMahrooqi Z, Al-Hashmi N, AlBimani Z, Al-Balushi M, Al-Jabri A (2010). Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*. Asian Pacific Journal of Tropical Biomedicine 1(3):173-176.

Le H, Dalsgaard A, Andersen P, Nguyen H, Ta Y, Nguyen T (2021). Large-Scale *Staphylococcus aureus* Foodborne Disease Poisoning Outbreak among Primary School Children. Microbiology Research 12(1):43-52.

Naseer S, Hussain S, Naeem N, Pervaiz M, Rahman M (2018). The phytochemistry and medicinal value of *Psidium guajava* (guava). Clinical Phytoscience 4(1):1-8.

Parimalam M, Deepak K, Bharath A, Pushpa V (2021). Pharmacological Investigation of *Lawsonia Inermis* L. Seed Extracts: An *In vitro* Study. Annals of the Romanian Society for Cell Biology 25(3):1723-1733.

Pereira M, Sandhu D, Samleti A (2010). Evaluation of Antibacterial Activity of *Punica granatum* Peels extract. International Journal of Current Trends in Science and Technology 1(4):232-236.

Puntawong S, Okonogi S, Pringproa K (2012). *In vitro* antibacterial activity of *Psidium guajava* Linn. Leaf extracts against pathogenic bacteria in pigs. Chiang Mai University Journal of Natural Sciences 11(2):127-134.

Samad A, Razak C, Salleh A, Yunus W, Ampon K, Basri M (1989). A plate assay for primary screening of lipase activity. Journal of Microbiological Methods 9(1):51-56.

Samiha S, Jahan M, Hossain H, Siddique R (2017). Apple, guava and pineapple fruit extracts as antimicrobial agents against pathogenic bacteria. American Journal of Microbiological Research 5(5):101-6.

Santhamari T, Meenakshi R, Velayutham S (2011). *In vitro* Antibacterial Activity of Extracts of *Lawsonia inermis* and *Punica grantum* Against Clinically Isolated Antibiotic Resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Asian Journal of Pharmaceutical and Clinical Research 4(1):0974-2441.

Santhoshkumar T, Rahuman A, Jayaseelan C, Rajakumar G, Marimuthu S, Kirthi V, Valayutham K, Thomas J, Venkatesan J, Kim S (2014). Green synthesis of titanium dioxide nanoparticles using *Psidium guajava* extract and its antibacterial and antioxidant properties Green synthesis of titanium dioxide nanoparticles using *Psidium guajava* extract and its antibacterial and antioxidant properties. Asian Pacific Journal of Tropical Medicine 7(12):968-976.

Seo J, Lee S, Elam L, Johnson A, Kang J, Arjmandi H (2014). Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. Food Science and Nutrition 2(2):174-180.

Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E, Tomasz M, Ożarowski M (2021). Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms 9(10):2041-2050.

Related Journals: