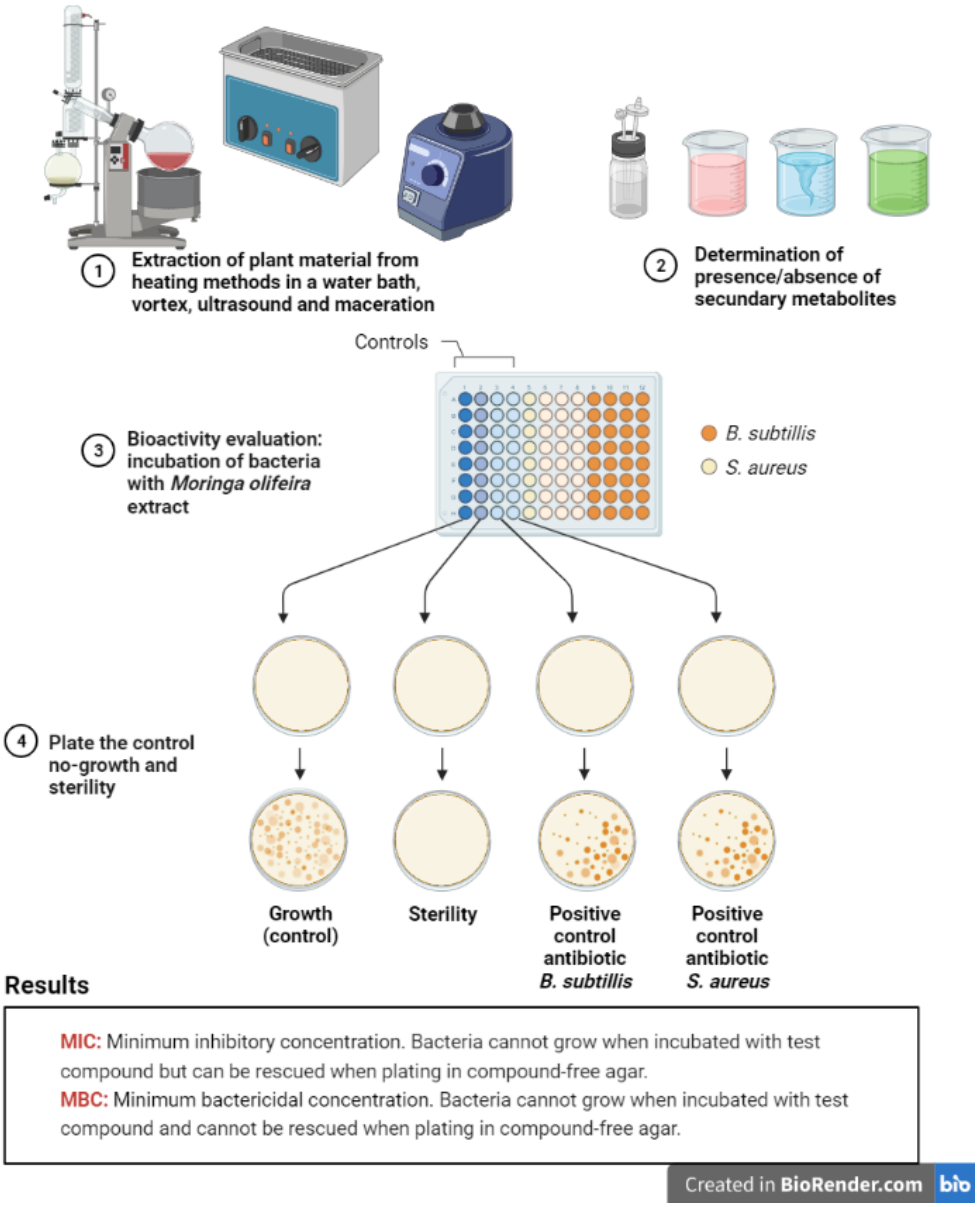
# Phytochemical characterization and evaluation of in vitro biological activity of *Moringa olifeira* leaf extracts in highly positive strains

Ramírez M. Valentina

ramirzm.valentina@uces.edu.co

Facultad de Ciencias y Biotecnología- Universidad CES

Medellín, Colombia




## Abstract

Moringa (*Moringa oleifeira*) is a tree native to the southern Himalayas rich in vitamins and different amino acids, for nutritional value it is used in human and animal nutrition. Moringa leaves contain many secondary metabolites such as: glucosinolates, isothiocyanates, flavonoids, anthocyanins, proanthocyanidins and cinnamates in vitro studies showed control of infections caused by pathogenic microorganisms. In this work, the evaluation of the antimicrobial activity is carried out in accordance with the Standard Reference Standard CLSI M07-A9 and implementing the method of microdilution in 96-well plates, to establish the effect of ethanol extracts obtained through different leaf extraction processes on strains of *Staphylococcus aureus* (ATCC: 6538) and *Bacillus subtillis* (ATCC: 6633). The measurement of bacterial growth at a wavelength of 625 nm showed an inhibition percentage of approximately 61% (*S. aureus*) and 19% (*B. Subtillis*); This result is representative, compared with the percentage obtained with the commercial antibiotic ciprofloxacin at a concentration of 2mg / mL. The antimicrobial efficacy moringas's extract provides a quantitative scientific basis for its possible use as an antimicrobial, however, it is required the isolation and purification of the different phytochemicals of this extract, to establish the compounds responsible for this activity and the evaluation of cytotoxicity and genotoxicity of the fractions. The above is a contribution to the constant search for new antimicrobial substances that allow therapies to be offered to multi-resistant strains of microorganisms from natural resources of plant origin.

**Keywords**

*Moringa olifeira*, Moringaceae, antimicrobial activity, phytochemical, secondary metabolites

## 1. Introduction

*Moringa oleifera*, a tree belonging to the Moringaceae family, is native to the southern foothills of the Himalayas and is currently cultivated in virtually all tropical, subtropical, and semi-arid regions of the world. It can grow under conditions of water scarcity, but its intensive cultivation, with irrigation and fertilization, increases biomass yields to exceed 100 tons per hectare [1]. The leaves, flowers, fruits and roots are appreciated for their nutritional value and can be used in human and animal nutrition. The leaves are exceptionally rich in vitamins and different amino acids [2]. Reports on chemical composition reveal the presence of different phytochemicals such as: glucosinolates, isothiocyanates, flavonoids, anthocyanins, proanthocyanidins and cinnamates [3]. The high content of vitamins, minerals and other phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, β-sitosterol, octacosanoic acid, moringin, moringinin and phytoestrogens is also an important factor in the therapeutic effects of *M. oleifera* [4 ]. Compounds isolated from the plant such as benzyl isothiocyanate and benzyl 4- (α-Lramnopyranosiloxy) -glucosinolate have anticancer, hypotensive and antibacterial activity [5]. The scientific evaluation of the processes of use of the plant, as well as the identification of active principles and mechanisms of action, have made it possible to explain many of the previously known beneficial effects, optimize their exploitation and propose new applications. The present work presents the

evaluation of the antimicrobial activity of moringa extract in order to provide a quantitative scientific basis for its eventual use as an antimicrobial. To obtain the extract, different methods were carried out, which were discussed about its performance and the qualitative recognition of secondary metabolites.

## 2. Results

Table 1 shows the yield percentage of the moringa extract obtained after performing different extraction methods.

Table 1. Plant material extraction performance

| Extraction method | Plant material obtained | Performance |
|---|---|---|
| Vórtex | 0.1349±0.001g | 13.49±0.001% |
| Water bath | 0.1336±0.001g | 13.36±0.001% |
| Ultrasound | 0.1861±0.001g | 18.61±0.001% |
| Maceration | 5.7885±0.1 g | 32.1583±0.001% |

Table 2 shows the secondary metabolites identified for the moringa extract after performing different extraction methods, in the table the presence is identified with a "+" sign and the absence with a "-" sign.

Table 2. Secondary metabolites in ethanolic extracts of moringa

| Secondary metabolite | Vórtex | Water bath | Ultrasound | Maceration |
|---|---|---|---|---|
| Alkaloids | + | - | + | + |
| Flavonoids | - | + | + | + |
| Saponins | - | - | + | + |
| Tannins | + | - | + | + |
| Anthocyanins | + | - | + | + |
| Coumarins | - | - | + | + |

Table 3 shows the results of biological activity against *S. aureus and B. subtillis* obtained with the moringa extract by the maceration method, evaluated at two concentrations, and the result obtained by the antibiotic ciprofloxacin at the concentration of a commercial presentation. 2mg / mL injectable.

Table 3. Activity of the moringa extract obtained by maceration and the result of the positive control

| Sample | %inhibición *S. aureus* | %inhibición *B. subtillis* |
|---|---|---|
| Extract 5 mg/mL | 57.57 | 10.40 |
| Extract 10 mg/mL | 61.39 | 19.31 |
| Ciprofloxacin 2 mg/mL | 87.63 | 83.12 |

With the maceration process, the best percentage of yield was obtained and the presence of all the secondary metabolites evaluated, it is for this reason that it was the extract obtained for the biological activity tests. With the ultrasound method, the suspension of plant tissue is enhanced, which is suitable for obtaining metabolites, however, the performance is still low. The votex method generates a gentle agitation of the material and the water bath a thermal process that can prevent the extraction of temperature-sensitive materials, therefore, these two methods do not generate high percentages of yield or the presence of the different phytochemicals.

The secondary metabolites obtained correspond to that established in the literature [3, 5].

The inhibition obtained with the ethanolic extract of moringa showed representative results, if compared with the percentage obtained with the commercial antibiotic ciprofloxacin at a concentration of 2mg / mL, the above represents a quantitative scientific basis that allows establishing the eventual use of the extract as an antimicrobial [9].

## 3. Conclusions

The efficiency of plant material extraction processes is determined by several factors that are directly related to the solubility of the components to be extracted, temperature, particle size, porosity, impurities, agitation, the dissolution capacity and the amount of solvent, that is why these procedures have been perfected to obtain high percentages of yield and the presence of different secondary metabolites, such as the maceration process.

The result of antimicrobial activity obtained with the ethanolic extract of moringa obtained with the maceration process represents a contribution to the constant search for new antimicrobial substances that allow offering therapies to multi-resistant strains of microorganisms from natural resources of plant origin, however, The isolation and purification of the different phytochemicals of this extract is required to establish the compounds responsible for this activity and for the evaluation of cytotoxicity and genotoxicity of these fractions.

## 4. Experimental

4.1 methods of extraction of plant material:

The plant material is dried for 48 hours at 40 ° C in a natural convection oven, crushed in a double helix mill and taken to the following extraction processes established in the literature [6]:

-Vortex extraction method: 1 g of plant material was stirred at 500 rpm at 25 ° C for 30 minutes.

-Extraction method by water bath: 1 g of plant material was subjected to a water bath at 40 ° C for 30 minutes.

- Ultrasound extraction method: 1 g of plant material was sonicated at 25 ° C for 30 minutes.

-Maceration extraction method: 25g of the material are added in 160mL of 96% ethanol and left to rest in a hermetic glass bottle protected from light for 8 days.

All the extracts are subjected to rotary evaporation to remove the solvent and are stored at -20 ° C in amber hermetic glass bottle for later use. The calculation of the extract yield for each method is given by the following equation:

$$\%\text{Performance} = \frac{Final\ weight\ extract}{Initial\ weight\ extract} * 100$$

The weight of the final extract corresponds to the grams of extract obtained after the rotary evaporation process and the weight of the initial extract is the grams of dry material used in each extraction method.

4.2. Recognition of secondary metabolites: it is carried out by means of phytochemical tests reported in literature [7], which are a chemical characterization test consisting of a chemical reaction that produces rapid alteration in the molecular structure of a compound, they are tests of qualitative type that give an indication of the presence or absence of a particular secondary metabolite.

4.3. Evaluation of biological activity: it was carried out by micro dilutions following the CLSI-M07A9 protocol (“Performance Standards for Antimicrobial Susceptibility Testing”, 2012) with some modifications [8]. For the test, the colonies of the bacteria started with an absorbance measured at 625nm of 0.08, which is equivalent to 0.5 on the McFarland scale.

In each microwell, 100 µL of inoculum, 50 µL of MH broth and 50 µL of the samples to be analyzed were placed, for the positive control the sample was replaced by the antibiotic, for the growth control 50 µL of sterile water was used and for the control of Sterility included 100 µL of Mueller Hinton broth and 100 µL of sterile water, all wells retained a final volume of 200 µL. Before measurement in the spectrophotometer at 625nm, all dishes were incubated at 37 ° C for 24 hours. All the extracts were previously filtered through a sterile 0.22 µm membrane, and the extracts were measured for absorbance correction. The result of antimicrobial activity was determined by the growth inhibition percentage using the following equation

$$\%\text{inhibition} = \frac{Control\ absorbance - extract\ absorbance}{Control\ absorbance} * 100$$

For the statistical design measurements were made in triplicate, with extract concentrations of 10 mg / mL and 5 mg / mL prepared with DMSO. The data were tabulated in Microsoft® Excel® where the means of the absorbances of each analysis were obtained, in addition, the behavior of each of the samples was plotted for each type of bacteria.

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