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Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Phytochemical composition and *in vitro* biological activities of *Morinda citrifolia* fruit juiceHaziz Sina^{a,*}, Gado Dramane^b, Philippe Tchekounou^a, Mahoudo Fidèle Assogba^d, Kamirou Chabi-Sika^{a,c}, Bawa Boya^a, Akim Socohou^a, Adolphe Adjanohoun^e, Lamine Baba-Moussa^a^aLaboratoire de Biologie et de Typage Moléculaire en Microbiologie, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, 05 BP 1604 Cotonou, Benin^bÉcole Normale Supérieure de Natitingou, Département des Sciences de la vie et de la Terre, Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques, Benin^cUniversité Nationale des Sciences, Technologies, Ingénierie et Mathématiques, Benin^dLaboratoire de Pharmacognosie et des Huiles essentielles sise à l'Institut des Sciences Biomédicales Appliquées, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Champ de Foire, Cotonou, Benin^eInstitut National de la Recherche Agronomique du Benin, Cotonou, Benin

ARTICLE INFO

Article history:

Received 20 September 2020

Revised 15 November 2020

Accepted 15 November 2020

Available online 23 November 2020

Keywords:

Phytochemical screening

Antibacterial activities

Fermentation

M. citrifolia

ABSTRACT

Morinda citrifolia is a plant with broad nutraceutical and therapeutic effects and used in the traditional treatment of several ailments. The objective of this work is to investigate the phytochemistry of the fruit juice of *M. citrifolia* on one hand and on other hand to evaluate its antiradical and antibacterial activity. The phytochemical investigation was carried out by tube staining tests of the extract of two types of fruit juice of *M. citrifolia*. The antioxidant activity of these juices was evaluated by reducing the DPPH radical method. Concerning the antibacterial activity, it was tested on the *in vitro* growth of 10 reference bacterial strains using the well diffusion method. Qualitative phytochemistry of *M. citrifolia* fruit juices revealed the presence of large groups of secondary metabolites including polyphenols, reducing compounds, mucilage and terpenoids. The antioxidant activity of *M. citrifolia* fruit juices is dose-dependent and higher than that of ascorbic acid. Antimicrobial activity on other hand revealed that fruit juices inhibit growth inhibitory activity of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *S. epidermidis*, *Proteus vulgaris*, *Streptococcus oralis*, *Enterococcus faecalis* and *Escherichia coli*. This observed difference is significant for each juices on the strains ($p < 0.001$). These results support the use of *M. citrifolia* in traditional medicine and are the starting points for the development of a new drug to combat both dietary conditions and chronic conditions associated with oxidative stress.

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1. Introduction

In developing countries, the socio-economic context makes it more difficult to manage the health status of the people living. However, infectious diseases cause more than 17 million deaths a year worldwide, more than half of them on the African continent (Traoré et al., 2012). Populations commonly use of medicinal plants to control diseases (Bessong et al., 2006). These medicinal plants produce various types of bioactive molecules, making them

sources of different types of potential drugs (Walton and Brown, 1999; Ghasemzadeh et al., 2010). Indeed, medicinal plants are a valuable heritage for humanity and in particular, for the majority of poor communities in developing countries who depend on them for their primary health care and livelihoods (Salhi et al., 2010). This is not only due to the low economic resources of the populations in these countries that limit the purchase of pharmaceuticals, but also the ineffectiveness of some synthetic drugs (Conlon et al., 2003). Thus, several plants are good sources of therapeutic agents and are traditionally used for different purposes, including treatments against bacteria, fungi and viruses (Bessong et al., 2006).

Morinda citrifolia is one of these plants with wide nutraceutical and therapeutic effects, known for its medicinal values since 2000 years in Asia and Australia (Whistler, 1985). It is a tropical and subtropical plant grown on the Pacific Islands and has been used to treat about 2000 diseases (Mc Clatchey, 2002). It is used as antifungal, antibacterial, anti-inflammatory, anticancer, antipar-

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Peer review under responsibility of King Saud University.



asitic, analgesic (Calzuola et al., 2006; Wang et al., 2002; Jasril et al., 2003; Pawlus et al., 2005; Potterat and Hamburger, 2007; Ruksilp et al., 2011). The juice extracted from the fruit is marketed under the name “Noni” as a dietary supplement in the USA (Phakhodee, 2012).

This plant, recently introduced in Benin, is recommended in the treatment of several ailments such as cough, skin infections, urinary tract infections, tuberculosis, etc. Natural pure juices (fresh or fermented), without water addition, are often used. Nevertheless, there is very little scientific evidence supporting the therapeutic values of *M. citrifolia* juice. Thus, this work aimed to do the phytochemical screening and evaluate the antioxidant and antibacterial activities of the fruits of *Morinda citrifolia*.

2. Material and methods

2.1. Collecting and obtaining of *Morinda citrifolia* juices

The fruits of *M. citrifolia* were harvested two times (May and July) in the Mono Department (Benin). The juices obtained in May 2019 were considered as fermented juice, whereas the fresh juice was in July 2019. Commercial noni juice is traditionally produced by fermentation of noni fruits in a sealed container for 2 months at room temperature (Nelson, 2006). Direct pressing of noni fruit produces Noni fresh juice. Thus, fresh noni juice is obtained by direct pressing of 500 g *M. citrifolia* fruits in tightly closed jars. The fermented fruit juice was obtained by introducing 500 g of fruit into a sealed container for at least two months at room temperature. In both cases, the resulting juice was filtered on cotton and then on wattman paper.

2.2. Phytochemical profiling

Tube staining tests described by Houghton et al. (1998) identified the chemical groups contained in the aqueous raw extracts of leaves and fruit juice extracts of *Morinda citrifolia*.

2.3. Evaluation of anti-radical activity

The anti-radical activity of our extracts was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method using the procedure previously described by Lamien-Meda et al. (2008). For this test, the samples were prepared by dissolution in distilled water (Panichayupakaranant and Kaewsuan, 2004). Each stock solution is diluted in reason 2 geometric series to have a concentration range from 0.8 to 0.003125 mg / ml. In dry and sterile test tubes, 1 ml of the extract solution to be tested is introduced, added to 1 ml of the DPPH solution (4%). After stirring in the vortex, the tubes are then placed away from the light at room temperature for 15 min. Absorbance was read at 517 nm at the spectrophotometer (UV/VIS). For each dilution, a blank was prepared under the same conditions. The anti-radical activity of the extracts was determined by determining for each extract, the effective concentration of the substrates reducing by 50% the activity of DPPH (EC₅₀) then compared to that of ascorbic acid (0.8–0.003125 mg/ml).

2.4. Evaluation of the antibacterial activity of *M. citrifolia* juices

2.4.1. Tested microorganisms

The tested microorganisms are part of the collection of the Laboratory of Biology and Molecular typing in Microbiology. The ten tested strains include five (05) gram positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *Micrococcus luteus* ATCC 10240, *Streptococcus oralis* and *Enterococcus*

faecalis ATCC 29212) and five (05) gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* 24974, *Proteus vulgaris* 25015, *Escherichia coli* ATCC 25922, *Escherichia coli* O157 and *Salmonella typhi* R 30951401).

2.4.2. Susceptibility of microorganisms to *Morinda citrifolia* juices

It was made according to the well diffusion method described by Bauer et al. (1966). From young reference strains (18–24 h), an isolated colony was collected and homogenized in 1 ml of Muller Hinton before being incubated for 18–24 h at 37 °C. From this culture, 10⁶ CFU/ml inoculum was obtained by dilution. One ml of the inoculum was used to flood on Muller Hinton petri dishes. After seeding, the wells were thoroughly impregnated with 30 µl of *M. citrifolia* juices (fresh and fermented). The impregnated dishes were left for 15–30 min at room temperature (25 °C ± 2 °C) for pre-diffusion of the substances before being incubated at 37 °C (Adesokan et al., 2007). The diameters of the possible inhibition zones were measured using a graduated ruler after an incubation time of 24–48 h. The experiment was performed in duplicate.

2.4.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by the tube dilution method used by Dah-Nouvlessounon et al. (Dah-Nouvlessounon et al., 2015) with a visual assessment of the microorganisms growth after 24 h of incubation. For this, bacterial inoculum (10⁶ CFU/ml) was brought into contact with a dilution range of plant juices. Thus, a series of 10 test tubes numbered T1 to T10, 1 ml of sterile distilled water was introduced in the tubes T2-T10. Into all tubes, 1 ml inoculum added to 10⁶ CFU / ml of nutrient broth MH. All tubes containing a final volume of 2 ml were incubated at 37 °C. After 24 h of incubation, the turbidity of the tubes was examined in relation to the control tube T10.

2.4.4. Determination of minimum bactericidal concentration (MBC)

It was made in conjunction with the MIC determination. The MIC tubes to high concentrations were seeded using a platinum loop on petri dishes containing MH agar medium. These boxes were incubated at 37 °C for 24 h. The concentration of the juices that does not show any microbial growth represents the minimum bactericidal concentration (MBC).

2.5. Statistical analysis

Data was entered and processed in MS Office Excel 2016 spreadsheet. These data were then subjected to an analysis of variance (ANOVA) using the SPSS software with a significance rate $p < 0.05$.

3. Results

3.1. Phytochemical screening

The phytochemical investigation revealed mainly the presence of some large chemical groups both in the fermented and fresh fruit. As summarized in Table 1, the main chemical group detected are glycosides, polyphenols, and alkaloids. In addition, it was observed that the composition is not influenced by the fermentation stat of the juice. Thus, all the chemical compounds observed in the fresh juice were in the fermented one. To end, compounds such as anthocyanin, coumarines, anthraquinones, triterpenoids, steroids, cardenolides, cyanogenic derivatives, free anthracene, C-heterosides, mucilage.

Table 1
Phytochemical composition of *M. citrifolia* fruits juice.

Chemical compounds	Fresh juice	Fermented juice
Alkaloids	+	+
Catechic tannins	+	+
Gallic tannins	+	+
Flavonoids	+	+
Anthocyanin	-	-
Leuco-anthocyanin	+	+
Coumarin	-	-
Anthra-quinone	-	-
Triterpenoids	-	-
Steroids	-	-
Cardenolids	-	-
Cyanogenic derivatives	-	-
Saponosids	+	+
Reducing compounds	+	+
Free anthracene	-	-
O-heterosids	+	+
C-heterosids	-	-
Mucilage	-	-

--: absence; +: presence.

3.2. Anti-radical activity of fresh and fermented *M. citrifolia* fruit juice

The results of the anti-radical activity of fresh and fermented fruit juice is shown in Table 2. Analysis of this table reveals that fresh fruit juice is more active ($IC_{50} = 0.024$ mg/ml) than ascorbic acid ($IC_{50} = 0.027$ mg/ml) because it has the lowest IC_{50} . In addition, the fermented fruit juice is less active ($IC_{50} = 0.047$ mg/ml) than ascorbic acid. The activity of the fresh juice is about twice higher than fermented one (Table 2). The same observation was made for the effective juice concentration reducing 50% of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Thus, the fermentation could be responsible for this difference in activity observed between the two fruit juices in spite of their identical phytochemical composition.

3.3. Antibacterial activity of *M. citrifolia* juice

The mean inhibition diameters induced by our two *M. citrifolia* fruit juices on the tested strains are shown in Fig. 1. Diameters vary significantly depending on strain and type of *M. citrifolia* juice ($p < 0.001$). Thus, at 20 mg/ml, both juices have no effect on *Micrococcus luteus* and *Salmonella typhi*. However, both fruit juices inhibited the growth of strains such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Streptococcus oralis*, *Enterococcus faecalis* and *Escherichia coli* with average inhibition diameters ranging from 10.0 cm to 14.5 cm for *M. citrifolia* fresh juice and from 9.5 cm to 12.0 cm for fermented juice. In addition, for each of these strains, the average inhibition diameter of fresh juice is greater than or equal to that of fermented juice. Thus, fresh juice is more active than three-month juice.

Table 2
Values of the anti-radical activity of *M. citrifolia* juices and reference molecule.

	IC_{50} (mg/ml)	EC_{50} (mg/ml)	Anti-radical Activity
Ascorbic acid	0.027	1.35	37
Fresh <i>M. citrifolia</i> fruit juice	0.024	1.2	41.67
Fermented <i>M. citrifolia</i> fruit juice	0.047	2.35	21.28

IC_{50} : Concentration of juice reducing 50% of DPPH; EC_{50} : Effective juice concentration reducing 50% of the DPPH activity.

3.4. Minimum inhibitory (MIC) and bactericidal (MBC) concentration of *M. Citrifolia* juices

Table 3 shows the minimum inhibitory (MIC) and bactericidal (MBC) Concentrations of the two *M. citrifolia* fruit juices. MIC values range from 2.5 to 5 mg / ml, while CMB values range from 10 to 20 mg/ml. The reports (MBC/MIC) show that both juices have bacteriostatic effects on strains. Fresh juice has a bacteriostatic effect on *Staphylococcus epidermidis* and *Escherichia coli*, while fermented juice induce bacteriostatic effect on *Staphylococcus epidermidis*, *Streptococcus oralis* and *Enterococcus faecalis*.

4. Discussion

The present study informed on the phytochemical composition, antioxidant and antibacterial activities of the fruit juice of *Morinda citrifolia*. Thus, the major groups of secondary metabolites observed on that juice are polyphenols, alkaloids and glycosides. These results are consistent with those of several authors who have worked on the fruits and leaves of this plant (Phakhodee, 2012; Wang and Su, 2001). These authors revealed in their study the presence of alkaloids, phenolic compounds, and glycosylated irideous. We note the presence of glycosylated irideous not found in our study. This relative difference could be related to climate and soil variation in both study areas (Houghton et al., 1998; Cybulski et al., 2000; Shen et al., 2008) or to the plant organ used. The total absence of anthocyanins significantly reduces the toxicological risk associated with the use of juice (Bruneton, 2009; Ortuno et al., 2006). Indeed, these compounds are responsible for toxicity due to the production of cyanide ions after ingestion and expressed by acceleration and then amplification of heart rate, respiratory depression, dizziness, impaired consciousness and even coma (Bruneton, 2009). The composition of secondary metabolites thus clearly explains the traditional use of *Morinda citrifolia* given the biological activities of the polyphenols and alkaloids contained therein (Mesia et al., 2005).

Evaluation of anti-radical activity according proves that fresh fruit juice is more active than ascorbic acid. Indeed, the EC_{50} of ascorbic acid is lower than that of fresh fruit juice. Ascorbic acid is then less active than the fresh juice of *M. citrifolia*. These results are consistent with those of Wang and Su (2001) who showed that the antioxidant activity of *M. citrifolia* fruits is 2.8 times greater than that of ascorbic acid. For fermented fruit juice, our results suggest that fermented fruit juice from *M. citrifolia* is less active than fresh fruit juice. Indeed, the EC_{50} of ascorbic acid is lower than that of fermented fruit juice and higher than that of fresh juice. Fresh fruit juice is then more active than fermented fruit juice. Fermentation therefore significantly reduces the anti-radical activity of *M. citrifolia* fruit juice. These results are consistent with those of Yang et al. (2007), which demonstrated that fermentation could reduce the anti-radical activity of *M. citrifolia* fruits by up to more than 90%. In fact, after two weeks, the initial antioxidant activity of fresh fruit juice dropped significantly and more gradually from two weeks to about three months. Thus, on day 4, this decrease in activity was estimated at 40% for conservation at 24 °C and then at 70% when conservation is made between 28 °C and 31 °C. In addition, the juice lost more than 90% of its antioxidant activity when conservation is done for three months under these two different conditions.

The antibacterial activity of the two fruit juices of *M. citrifolia* on the ten (10) reference strains indicates that the two fruit juices are active not only on Gram-positive strains (*Staphylococcus aureus*, *S. epidermidis*, *Streptococcus oralis* and *Enterococcus faecalis*) but also on gram-negative strains (*Pseudomonas aeruginosa*, *Proteus mirabilis*, *P. vulgaris* and *Escherichia coli*). These results are consistent

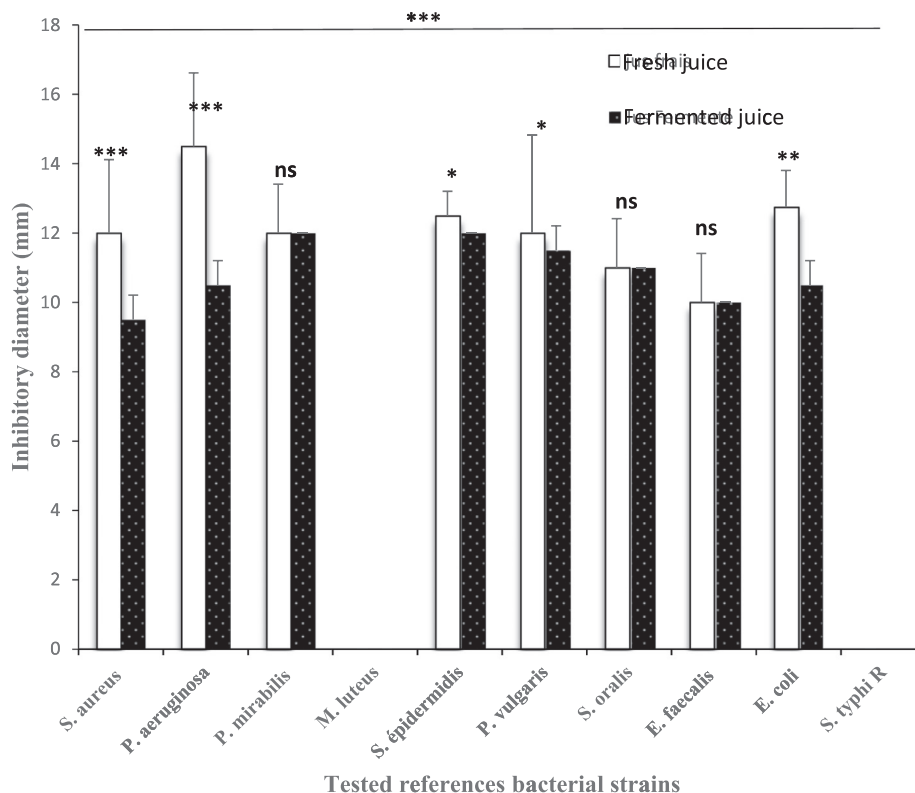


Fig. 1. Average inhibitory diameters of *M. citrifolia* juices ten bacterial strains. ns: not significant, *: p < 0.05, **: p < 0.01,***: p < 0.001.

Table 3

Minimum inhibitory and bactericidal concentrations (mg/ml) of *M. citrifolia* juices.

	Fresh juice			Fermented juice		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
<i>Staphylococcus aureus</i>	2.5	>20	ND	5	>20	ND
<i>Pseudomonas aeruginosa</i>	2.5	>20	ND	2.5	>20	ND
<i>Proteus mirabilis</i>	2.5	>20	ND	5	>20	ND
<i>Micrococcus luteus</i>	>20	>20	ND	>20	>20	ND
<i>Staphylococcus epidermidis</i>	5	20	4	5	20	4
<i>Proteus vulgaris</i>	5	>20	ND	5	>20	ND
<i>Streptococcus oralis</i>	2.5	>20	ND	2.5	20	8
<i>Enterococcus faecalis</i>	2.5	>20	ND	2.5	10	4
<i>Escherichia coli</i>	2.5	20	8	5	>20	ND
<i>Salmonella typhi</i>	>20	>20	ND	>20	>20	ND

ND: Not Determine.

with those of Srinivasahan and Durairaj (2014) who demonstrated the inhibitory effect of *M. citrifolia* fruit extracts on *E. coli*; *Pseudomonas aeruginosa*, *B. subtilis* and *S. aureus*. However, they are not consistent with those obtained by Jai Sunder et al. (2011) who noticed a lack of activity of *Morinda citrifolia* juice on *S. aureus*. This difference in activity would certainly be due to the phenology of the plant species (Cybulski et al., 2000; Shen et al., 2008; Houghton and Amala, 1998).

The greatest antibacterial activities were obtained with fresh juice on *Pseudomonas aeruginosa* (14.5 ± 2.12 mm) followed by *Escherichia coli* (12.75 ± 1.06 mm). The lowest activities were obtained with fermented juice on *Enterococcus faecalis* 10 mm and *Staphylococcus aureus* (9.5 ± 2.12 mm). These results are consistent with those of Esath et al. (2012) who demonstrated that *M. citrifolia* fruit juice has more antibacterial activity on Gram negative than on Grams positive bacteria. Since Gram-negative strains are much more sensitive than Gram-positive strains, it is inferred

that the accumulation of peptidoglycan layers by Gram-positive strains would be responsible for this bacterial resistance observed in Gram + (Basri and Fan, 2005; Vital and Rivera, 2009). In addition to the fact that the greatest antibacterial activities were observed with fresh fruit juice, we also noticed that for each of the eight strains for which both juices had activity, the inhibition diameter of fresh juice is either greater than or equal to that of fermented juice. It clears that fresh fruit juice is more active than fermented.

Thus, the present study shows that fermentation could also reduce not only the antioxidant activity but also the antibacterial activity of the fruit juice of *Morinda citrifolia*. The different minimum inhibitory concentration values guarantee the use of *M. citrifolia* fruit juice in the treatment of infectious diseases (Jayaraman et al., 2008). The results of our study are consistent with those of previous studies that suggest that tannins and flavonoids may allow the extract to overcome the bacterial cell wall barrier (Anyasor et al., 2011).

5. Conclusion

The fruits of *M. citrifolia* are excellent sources of antioxidants and polyphenols. The anti-radical activity of fresh fruit juice is more important than that of fermented juice. Storing noni fruit juice at room temperature can significantly reduce its anti-radical activity. Thus, for the maximum anti-radical potential of fruits of *M. citrifolia*, refrigeration and freezing of noni juice is strongly recommended.

6. Data availability statement

The data are available from the corresponding author upon request.

7. Funding statement

This was a non-funded project; the principal investigators used their own funds to support the data collection and logistics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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