



EVALUATION OF ANTIOXIDANT ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF *MORINDA CITRIFOLIA* (NONI) LEAVES CULTIVATED IN KOUTONGUINÉ (WESTERN REGION CÔTE D'IVOIRE)

Mohamed Anderson YEO*¹, Gilles Léonce Niamketchi², Kalamourou Silue¹ and Lacina Coulibaly³

¹Department of Agronomy and Forestry, Training and Research Unit of Agronomic, Forestry and Environmental Engineering, University of Man, P.O. Box V 20 Man, Ivory Coast.

²National Centre for Agronomic Research (CNRA), 01 P.O. Box 1740 Abidjan, Côte d'Ivoire

³Training and research unit of Environmental Sciences and Management (SGE), Nangui Abrogoua University 02 B.P. 802 Abidjan, Ivory Coast.

***Corresponding Author: Mohamed Anderson YEO**

Department of Agronomy and Forestry, Training and Research Unit of Agronomic, Forestry and Environmental Engineering, University of Man, P.O. Box V 20 Man, Ivory Coast.

Article Received on 28/10/2021

Article Revised on 18/11/2021

Article Accepted on 08/12/2021

ABSTRACT

The study was designed to investigate the phytochemical screening and antioxidant properties of *Morinda citrifolia* leaves (Noni; Rubiaceae) cropped in Man, West region of Cote d'Ivoire. Aqueous and methanolic extract obtained from *M. citrifolia* were subjected to phytochemical analysis. It proved that *M. citrifolia* contain large amount of secondary metabolite like polyphenols, flavonoids, tannins, alkaloids, saponins, cardiotonic glycosides, polyterpenes and sterols whereas quinones and coumarins were absent. Spectrophotometric analysis confirmed the presence of total polyphenols and flavonoids contents in methanolic and aqueous extracts with value of 56 mg GAE/g and 5 mg GAE/g for polyphenols and 11.06 mg GAE/g and 1.7 mg GAE/g for flavonoids respectability. Antioxidant activity is measured by their ability to scavenge the free radicals of DPPH method. These assays confirmed the presence of antioxidants in the methanolic extract of *Morinda citrifolia*.

KEYWORDS: *Morinda citrifolia*, phytochemical screening, antioxidant activity, leaves, Man.

INTRODUCTION

In recent years, there has been an increase in interest in the biological effects of natural antioxidants included in the fight against oxidative stress generated by reactive oxygen species (ROS). These biological effects are involved in aging and in the onset and progression of several diseases such as cancer, atherosclerosis, cardiovascular accidents (stroke), osteoporosis, inflammatory diseases and neurodegenerative diseases, etc. Thus, a great deal of scientific research carried out on bioactive compounds, particularly on polyphenols (PP) which act against ROS, has fostered their use in food, cosmetic and pharmacological industries (Kebbab, 2014). These substances arouse a great deal of interest in several fields, namely that of nutrition because of their preventive or therapeutic nature to cure diseases (Manach et al., 2004). According to the World Health Organization (WHO), medicinal plants represent one of the sources of medicines used by about 80% of the african populations in primary care of their daily life (Ladoh et al., 2014).

Morinda citrifolia (Noni), belonging to the Rubiaceae family is a plant used in traditional medicine in the

Tonkpi region against certain diseases. This plant is widely used to treat digestive disorders, ulcers, burns, diarrhea (Udoamaka et al., 2014; Suroowan & Mahomoodally, 2016). The leaves of young Noni plant are eaten as vegetable and used as a wrapper to heat food in foil (Morton, 1992; Dixon et al., 1999). They are applied to the burns until wilting and are then replaced with fresh leaves. The treatment is repeated until the leaves no longer dry out and the fever stops (Nicolas, 2004). After childbirth, if the child is not breathing, the midwife wraps the umbilical cord with a pair of Noni leaves to initiate breathing. If breathing is still delayed, then the midwife begins stacking Noni leaves on the child until breathing begins (Nicolas, 2004). Moreover, Noni leaves contain most of the amino acids, namely alanine, arginine, aspartic acid, cysteine, glycine, glutamic acid, histidine, leucine, isoleucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. It is also admitted the presence of anthraquinones, glycosides, phenolic compounds, sitosterol resins and ursolic acid in Noni leaves (Chunideng, 2003). In southern Vietnam the roots are commonly used in local medicine. The roots and leaves of *Morinda citrifolia* L. are used in Mauritius, Tangatapu,

Vietnam, the Philippines and India as an analgesic or anti-rheumatic and also for the treatment of urinary problems (Adjanohoun *et al.*, 1983; Singh *et al.*, 1986). The leaves, crushed with spices, help stop diarrhea and dysentery suffered by adults. The main medicinal properties of Noni are known to be antibacterial, analgesic, anticongestive, antioxidant, anti-inflammatory, astringent, emollient, laxative, sedative and hypotensive. Thus, our choice to study the Noni plant was motivated by its frequent traditional use for culinary and medicinal purposes in the Tonkpi region in order to make a promotion of our national heritage.

Hence, this study is focused on the phytochemical sorting, the determination of phenolic compounds and the evaluation of the antioxidant activity of the aqueous and methanolic extracts of the leaves of *Morinda citrifolia*, being cropped in Man.

MATERIEL AND METHODS

1. Materiel

1.1. Plant material

The plant material studied consists of *Morinda citrifolia* leaves. These leaves were collected from the department of Agronomy and Forestry of Man University, region of Tonkpi, West of Côte d'Ivoire (7°19'60" N 7°25'0" W). Leaves sample were deposited at the Botany herbarium department with the voucher number of plant 1071/2021. The leaves were dried at 18°C during 5 days, crushed and packaged in sachets for analysis.

1.2. Chemical and Standard

All reagents and solvents were of analytical grade. Methanol and ethanol were purchased from Carlo Erba (Spain), Folin-Ciocalteu reagent from Panreac (USA), gallic acid and quercetin, Sigma-Aldrich (Germany), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) Carlo Erba (Spain), sodium carbonate and chloride aluminium, Merck (Germany), sodium hydroxide, Sharlau (Spain). Water was purified by a Milli-Q water purification system.

2. METHODS

2.1. Preparation of the aqueous and methanolic extract

Soluble dry matter content of the leaves was prepared according to the method described by Ano *et al.*, (2021). For the preparation, a mass of 50 g of leaves powder was macerated in 500 mL of distilled water and in 500 mL of methanol under magnetic stirring during 24 hours. The aqueous and methanolic macerates were filtered through cotton wool and once on Whatman filter paper. The filtrate obtained were first placed in petri dishes and then dried in an oven at 50°C for 3 days to obtain the dry extract leaves of *Morinda citrifolia*.

2.2. Phytochemical screening

Phytochemical screening of *Morinda citrifolia* leaves of secondary metabolites such as polyphenols, flavonoids, sterols and terpenes, alkaloids, tannins, saponins were

screened according to the method described by Bidié *et al* (2011), Koné (2009), Békro *et al* (2007) and Bagre *et al* (2007). The presence of a bioactive compound was indicated by a color change and a precipitate.

Test for polyphenols

A drop of 2% alcoholic ferric chloride solution was added to 2 mL of the extracts (aqueous and methanolic solution). The appearance of blackish-blue or more or less dark green coloring indicates a positive reaction.

Test for flavonoids

A volume of 2 mL of the extracts was dried on a sand bath. The dry residue obtained was cooled and taken up in 5 mL of hydrochloric alcohol (mixture of 10 mL of ethanol, 10 mL of distilled water and 10 mL of concentrated hydrochloric acid). Then two to three magnesium shavings were added to it. The appearance of a pink-orange or purple color after adding 3 drops of isoamyl alcohol indicates the presence of flavonoids.

TEST FOR TANNINS

Catechetal tannins

A volume of 15 mL of STIASNY reagent (10 mL of 40% formaldehyde added with 5 mL of concentrated HCl) was added to 1 g of dry extract. The mixture was kept in a water bath at 80 °C for 30 min and cooled under flowing water. The appearance of large precipitates in form of flakes indicates the presence of catechetal tannins.

Gallic tannins

The solution containing the flakes was filtered and the collected filtrate was then saturated with sodium acetate. To the mixture, 3 drops of ferric chloride were added to it. The appearance of an intense blue black color indicates the presence of gallic tannins.

Test for leucoanthocyanins

A volume of 2 mL of the extracts was dried on a sand bath. The dry residue obtained was cooled and taken up in 1 mL of hydrochloric alcohol and 1 mL of isoamyl alcohol. The mixtures were kept in a water bath at 80 °C for 15 min and cooled under flowing water. The appearance of a red-cherry or purplish color indicates the presence of leucoanthocyanins.

Test for sterols and polyterpenes

Liebermann's reagent was used for this demonstration. A mass of 0.1 g of dry extract of the leaves was dissolved at hot in 1 mL of acetic anhydride and collected in a test tube. Then, 0.5 mL of concentrated sulfuric acid was added. The appearance of a purple or violet ring at the interphase, turning blue and then green, indicates the presence of polyterpenes and sterols.

Test for saponins

A mass of 0.1 g of dry extract was dissolved in 10 mL of distilled water. The solutions obtained were stirred vigorously during 45 seconds. After stirring, the

solutions were allowed to stand for 15 minutes. The observation of persistent foam, greater than 1 cm in height, indicates the presence of saponins.

Test for cardiotonic glycosides

A volume of 10 mL of chloroform was added to 2 mL of the extracts. After decantation, the chloroform phases were extracted using a pipette and then divided into 2 test tubes and dried on a sand bath. The residues were taken up in 1 mL of isopropanol and 1 mL of 2% alcoholic potassium. The mixtures were heated on hot plate for 3 minutes. The appearance of a purplish red color indicates the presence of cardiotonic glycosides.

Test for quinones

A volume of 2 mL of the extracts was added to 5 mL of HCl, mixed and diluted to 1/5. The mixtures were kept in a water bath at 80 °C for 30 min and cooled under flowing water. The hydrolyzate solutions were extracted with 20 mL of chloroform. A volume of 1 mL of Borntraeger's reagent (ammonia diluted twice) was added to the chloroform phase. The appearance of an intense red or purple color indicates the presence of quinones.

Test for alkaloids

A mass of 1 g of dry extract was dissolved in 6 mL of 60° absolute ethanol. 2 drops of DRAGENDORFF reagent (aqueous solution of potassium iodobismuth) were added to the alcoholic solution thus obtained. The appearance of a precipitate or an orange color indicates the presence of alkaloids.

2.3. Polyphenols Levels

2.3.1. Total Polyphenols

Total polyphenols were determined using the method described by Singleton and Rossi (1965), as amended by Wood et al. (2002). A volume of 2.5 mL of Folin-Ciocalteu diluted (1/10) was added to 30 µL of extract. The mixture was held for 2 minutes in the dark at room temperature, and then 2 mL of calcium carbonate (75 g.L⁻¹) were added. The mixture was placed for 15 minutes in a water bath at 50°C, and then cooled quickly. The absorbance was measured at 760 nm with distilled water as blank. A calibration curve was performed with gallic acid at different concentrations (0, 0.2, 0.3, 0.6, 0.7, and 0.9 g.L⁻¹). Analyses were performed in triplicate and polyphenols level was expressed in mg gallic acid equivalents (GAE)/g dry matter.

2.3.2. Total flavonoids

Total flavonoids were determined by the method of Marinova et al. (2005). A volume of 0.75 mL of sodium nitrite (NaNO₂) to 5% (w/v) was added to 2.5 mL of extract in a 25 mL flask. The mixture was added 0.75 mL of aluminum chloride (AlCl₃) to 10% (m/v) and incubated for 6 minutes in the dark. After incubation, 5 mL of sodium hydroxide (NaOH 1N) were added and the volume made up to 25 mL. The mixture was stirred vigorously before being dosed with UV-Visible

spectrophotometer. The reading was taken at 510 nm with distilled water as a blank. A calibration curve was performed with quercetin (0-1.5 g.L⁻¹) at different concentrations (0, 0.15, 0.3, 0.9, 1.2, 1.5 g.L⁻¹). The tests were performed in triplicate and the flavonoids content was expressed in mg quercetin equivalents (QE)/g dry matter.

2.4. Antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was solubilized in methanol to obtain a solution with a concentration of 0.1 mg/mL. Different concentration ranges (6.25, 12.5, 25, 50, 100 and 200 µg/mL) of methanol plant extract were prepared in the same solvent. 1 mL of methanol plant extract and 1.5 mL of DPPH methanolic solution were introduced into test tubes. After shaking, the tubes were placed in a dark place for 30 min. The absorbance of the mixture was then read at 517 nm with a UV-visible spectrophotometer (Jasco V-530) against a blank consisting of 1 mL methanol and 1.5 mL DPPH. The positive reference control is quercetine. The percentage reduction (% I) of DPPH was calculated according to the following equation (Parejo et al., 2000):

$$I(\%) = \left(1 - \frac{As}{Aw}\right) \times 100$$

As: absorbance of the extract; Aw: absorbance of the white.

This parameter was used to determine the median effective reduction concentration (EC50) of DPPH, determined graphically using Graph Pad Prism.

RESULTS AND DISCUSSION

3. Results

3.1. Phytochemical screening

The results of Table 1 indicate the presence of secondary metabolites in the aqueous and methanolic extracts of *Morinda citrifolia* leaves. These are polyphenols, flavonoids, tannins (catechetical and gallic), leucoanthocyanins, sterols and terpenes, saponins, cardiotonic glycosides and alkaloids. A strong intensity of the coloring and precipitate was observed in the methanolic extracts. Phytochemicals such as coumarins and quinones were not detected in the extracts leaves of *Morinda citrifolia*.

3.2. Content of phenolic compounds

3.2.1. Total Phenolics Content

Total polyphenols distribution of aqueous and methanolic extracts of *M. citrifolia* leaves are show in Figure 1. The highest rate is obtained by extracting with solvent methanol with a value of 56 mg GAE/g DM while the lowest value is obtained by aqueous solvent (5 mg GAE/g DM).

Figure 3 shows the distribution of total flavonoids content in different extracts of *M. citrifolia* leaves studied. Just as the level of total polyphenols, methanolic extract contain the highest levels of flavonoids compared

to aqueous solvent with value of 11.06 mg GAE/g and 1.7 mg GAE/g DM.

Table 1: Phytochemicals in the aqueous and methanolic leaves extracts of *Morinda*.

Secondary metabolites		<i>Morinda citrifolia</i> leaves	
		Aqueous extract	Methanolic extract
Polyphenols		++	+++
Flavonoids		++	+++
Tannins	Catechetical	++	+++
	Gallic	++	+++
Leucoanthocyanins		+	++
Sterols and Polyterpenes		++	++
Saponins		++	+
Cardiotonic glycosides		++	++
Coumarins		-	-
Quinones		-	-
Alcaloids		+	++

+++ : strong presence, + : average presence, - : absence

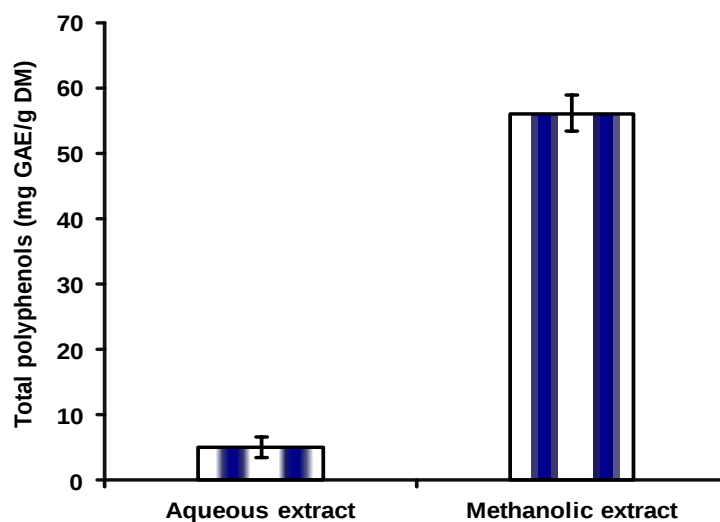


Figure 1: Polyphenolic contents of *M. citrifolia* extracts.

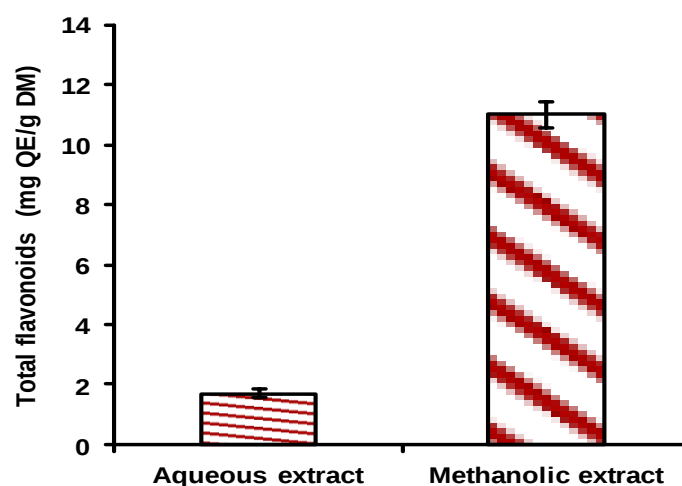


Figure 2: Flavonoids contents of *M. citrifolia* extracts.

4. Antioxidant Activity of *M. citrifolia*

The antioxidant activity of *M. citrifolia* leaves was estimated by considering the capacity of its aqueous and methanolic extracts to reduce the stable radical of DPPH,

compared to quercetin (reference antioxidant). The results were expressed in graphical form (Figure 3). In general, it appears that the leaves of *M. citrifolia* reduction activity are concentration-dependent. It

decreases progressively with decreasing concentration of the methanolic extract. As far as the aqueous extract is concerned, no activity was obtained. The EC_{50} inhibitory concentration, determined graphically from the reduction

percentage of DPPH, is the reliable median concentration that shows the antioxidant effectiveness of *M. citrifolia* leaves (Table 2).

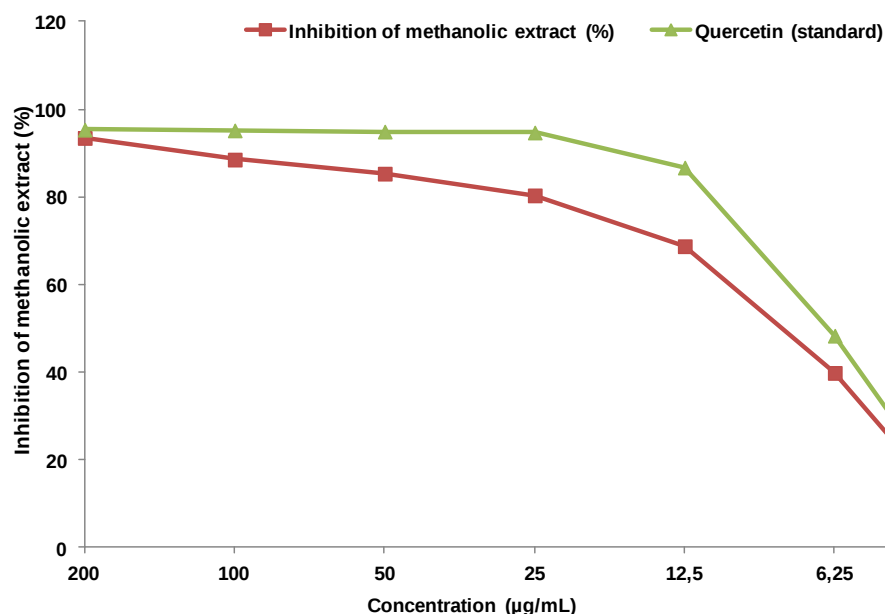


Figure 3: Inhibition of quercetin and methanolic extract.

Table 2: EC_{50} of the extracts of *M. citrifolia* leaves.

Extracts	EC_{50} (µg/mL)
Methanolic	2.30 ± 0.12
Aqueous	ND

*ND: not determined

DISCUSSION

This study was designed to investigate the phytochemical screening, phenolic compounds and antioxidant activity of two extract of *Morinda citrifolia* leaves.

Phytochemical screening revealed the presence of all major secondary metabolites such as polyphenols, flavonoids, tannins, alkaloids, saponins, cardiotonic glycosides, polyterpenes and sterols in *Morinda citrifolia* leaves whereas quinones and coumarins were absent. Results of this study are in agreement with those of Sajani and Maya, 2020; Sasikumar et al., (2012). The presence of these phytonutrients in *M. citrifolia* leaves could justify its therapeutic use. They are recognized as having antioxidant properties, which allows them to play a role in the inflammatory treatment, cardiovascular or neurodegenerative diseases (Mesia et al., 2005; Amarowicz, 2007; Phakhodee, 2012). Most of these diseases find part of their cause in the effects produced by oxidative stress (Wang and Su, 2001). For this, the leaves could intervene in the fight against this stress. As for saponins, they act like anti-hyperlipidemia, hypotensive and have cardiodepressive properties (Ogboru et al., 2015). In addition to the role of antimicrobial (Faizi et al., 2003), alkaloids play a detoxifying and antihypertensive role (Awoyinka et al, 2007). Steroids are known to be an important Cardio

tonic activities posse's antimicrobial property and also used in herbal medicines and cosmetics. The presences of cardiac glycosides are known to play a major role in heart muscles by inhibiting Na^+ and K^+ pump that increase the availability of sodium ions and calcium ions to heart muscles which improves cardiac output and reduce heart distension. Thus are used in the treatment of congestive heart failure and cardiac arrhythmia (Schneider et al., 2004). Flavonoids and tannin are the group of phenolic compounds that act as primary antioxidants and posse's also antimicrobial, anti-inflammatory, antiallergic, anticancer, antineoplastic activity, and for the treatment of intestinal disorders (Gulcin et al., 2005; Rievere et al., 2009).

The results of the determination of total phenols and flavonoids showed that the methanolic extract of the leaves represents the richest extract with respectively 56 mg GAE/g of extract and 11.06 mg QE/g of extract. The radical scavenging capacity of the Noni leaves extract reduce and decolorize the 2, 2-diphenyl-1- picrylhydrazyl (DPPH) into a yellow compound (Ardestani & Yazdanparast, 2007). Results are expressed as a percentage of anti-free radical activity using the EC_{50} parameter, which is defined as the concentration of the substrate that causes a 50% loss of DPPH activity (Markowicz et al., 2007). This study revealed that only the methanolic extract of the plant studied possessed anti-free radical activity. Thus, just 200 µg/mL is enough for the methanolic extract to inhibit the DPPH radical at 85.51%. DPPH scavenging activity of the extracts can be correlated to the presence of flavonoids (Turkmen et al., 2007; Bourgou et al., 2008; Miguel, 2010; Sajani and

Maya, 2020). Antioxidants are biologically synthesized as defensive mechanism having an important role in preventing or alleviating chronic diseases by reducing the oxidative damage to cellular components caused by free radicals. The present study shows that *Morinda citrifolia* leaves extract possess significant amount of phytochemicals and *in vitro* antioxidant activity. A significant linear relationship between antioxidant activity and phytochemicals, that are responsible for the *in vitro* antioxidant property of the leaves extracts.

Analyzing the results, it is clear that the methanolic extract of *Morinda citrifolia* account for the plethora of bioactive compounds. However isolation and characterization of the compounds and validating their therapeutic efficacy against different pathologies is required for clinical implementation.

CONCLUSION

This study has shown that in addition to the presence of bioactive compounds, the leaves of *M. citrifolia* have antioxidant activity. The results showed that the aqueous and methanolic extracts of *M. citrifolia* contains polyphenols, flavonoids, tannins, alkaloids, saponins, cardiotonic glycosides, polyterpenes and sterols. It also emerges from the analyzes that the methanolic extract was found to be greater antioxidant activity than the aqueous extract and even similar to that of quercetin. In combination and at useful doses, the *M. citrifolia* leaves extracts could provide an alternative to synthetic molecules and have their place in the prevention and treatment of neurodegenerative diseases.

ACKNOWLEDGEMENTS

We authors, express our gratitude to the the President of “Université Polytechnique de Man” for his help in the realisation of this study.

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