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Phytochemical profile and associated microflora of *Morinda citrifolia* L. (Rubiaceae) juice used as source of traditional nutraceuticals

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Abstract
The aims of this study was to investigate the phytochemical profile and associated microflora of juice from *Morinda citrifolia* L. (Rubiaceae), commonly call ''*Noni*'' which is currently used as nutraceuticals in traditional medicine. The results of phytochemical analysis indicated that anthocyanins, anthraquinone, leucoanthocyans, coumarins, as well as flavonoids are presents in the juice extracted from Noni fruits. However cyanogenics derivatives compounds are not detected. Microbiological analysis revealed that Noni juice has a low level of microbial contamination with the absence of pathogens such as *Staphylococcus aureus* (<1 ufc/ml). The Noni juice, with the presence of these secondary metabolites, offers a novel approach to the management of therapeutic failures, encountered in the treatment of severe forms of certain pathologies. However, further research and clinical trials are required to fix appropriate doses and combinations to be consumed in specific individual cases.

Keywords: *Morinda citrifolia* L., *Noni*, nutraceuticals, phytochemical, microflora, Benin

Introduction
For a long time, plants are considered as the basis for nutrition and health preservation [1]. The use of complementary traditional medicine which include herbal medicines in the treatment of various diseases has expanded rapidly in both developed and developing countries, attributable to affordability, accessibility and efficacy. Then, faced with the repeated therapeutic failures encountered in the treatment of severe forms of certain pathologies such as arteriosclerosis, cardiovascular and neurological diseases, as well as cancer, nutraceutical products, nowadays, have become progressively popular to improve health, and to prevent or treat diseases. Nutraceutical offers medical or health benefits to the consumer by providing means for the maintenance of health and protection from disease [2]. On the other hand a functional plant food provides the body with the required amounts of vitamins, fats, protein, carbohydrates and many other compounds that are needed for its survival. However, botanicals are often used as synonyms for herbal products. Then, there is a need for a legal distinction of plant nutraceuticals from pharmacologically active herbals or botanicals [3]. Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world [4]. "Let food be your medicine and let medicine be your food" is world famous advice of father of medicine "Hippocrates" [5]. Then, the recommendations for consumption of nutraceuticals from plant origin have emerged as new concepts of health aid such as nutritional therapy and phyto-therapy. *Morinda citrifolia* L. (Rubiaceae) has a long history linked to medical uses in most countries of Southeast Asia and in India, where this tropical plant grown naturally. This plant produced fruit in all seasons. These fruits are thick and ovoid in shape with circular veins. They are green when unripe and yellowish-white when fully ripe. The fruits have a soft, watery flesh, and a cheese aroma that becomes more and more pronounced and pungent during the ripening process [6]. In Benin, based on the varied biodiversity [7], and the endogenous knowledge relating to the many therapeutic virtues linked to the fruit of *Morinda citrifolia* L. (Rubiaceae), the juice is therefore traditionally extracted, packaged and then marketed in other to be used in folk medicine, which recommend its periodical consumption for well-being and in the prevention of some kinds of diseases, especially those related to hormonal imbalance and oxidative stress. The present study aims to investigate the phytochemical profile and associated microflora of *Morinda citrifolia* L. (Rubiaceae) juice currently used in traditional medicine. This study also aims to deepen knowledge on ''*Noni*'' juice with a view to its industrial valorization as a source of nutraceuticals.

Material and Methods

Collection of fruits and extraction of juice

The fresh green colored of fruits of *Morinda citrifolia* L. (Rubiaceae) were obtained from the city of Lokossa (southern Benin). Fresh, fully matured and dark green colored fruits were selected and then allowed to ripen till green color changes to dark brown or amber color. The juice is extracted according to the method described by Joshi *et al*. [8] (Figure 1).

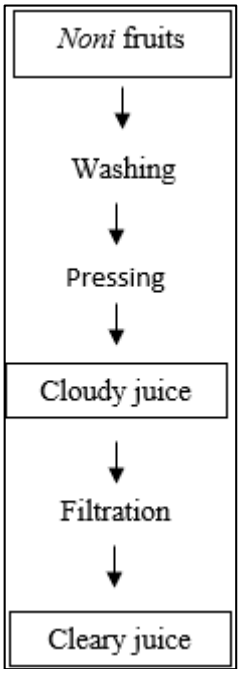

Fig 1: Technological flowsheet of *Morinda citrifolia* L. (Rubiaceae) juice production

Identification of the secondary metabolites

The procedure used for the research of every compound enumerate as follows:

- The gallic tannins has been characterized by an aqueous solution of ferric chloride (FeCl3) to 2% driving to the development of a coloration bruise-black or green black characterizing the presence of tannoïdes [9].;
- The catechic tannins has been put in evidence by the reagent of Stiasny (formalin 30% in HCl extract: 2/1 v/v) [9].
- The flavonoïdes as for them are revealed by the reaction to the cyanidine [9].
- The anthocyanes have been revealed by hydrochloric acid addition to 5% and some drops of ammonia water to infuse it. A red coloration that turns to the purplish or greenish bruise indicates the presence of anthocyanes [9];
- The leuco-anthocyanes is put in evidence by the hydrochloric alcohol (ethanol to distilled 95°alcool/water; HCl concentrated in proportion equivolumic) and heating to the bath gets married to 90°C. A coloration red cherry or purplish indicates the presence of leuco-anthocyanes;
- The alkaloids have been identified by the test of Meyer and have been confirmed by the test of Bouchardât [9];
- Reducers compounds have been identified by the test of Felhing Liqueur and Saponins were determined by the test of moss; degree of dilution of an aqueous décoction giving persistent moss after shaking [10];
- The Coumarineses were identified by fluorescence in UV at 365nm [11];
- Cyanogenic derivatives were detected by the picric acidic test 1% resulting in a brown coloration characteristic of the presence of cyanogenic derivatives;
- Mucilages were revealed by the obtaining a flaky precipitate of a décoction in ethylic ether indicating the presence of mucilages [12].

Microbiological analyses

To 25 g of each sample, 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total coliforms count and plates were incubated at 30°C for 24 h. *Staphylococcus aureus* count was performed as described by the standard NF. EN. ISO. 6888-1 [13; 14]. After incubation, the number of colonies was tracked using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, considering the factors of dilution. The method used for detection of yeast and fungi in samples was performed using dilution plating method. 10 g of each sample were separately added to 90 ml of sterile water containing 0.1% peptone water. This was thoroughly mixed to obtain the 10^{-1} dilution. Further tenfold serial dilutions up to 10^{-4} were made. One milliliter of each dilution was separately placed in Petri dishes, over which 15 ml of Potato Dextrose Agar with 60µg/ml of chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days [15]. All media used for microbiological analysis were prepared as indicated by the manufacturer.

Statistical Analyses

The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. [16].

Results and discussion

The juice extracted from ‘’Noni’’ fruits are brown in color with a smell characteristic of the fruit. The results obtained during phytochemical investigations on the juice (Table 1) revealed the presence in the juice of secondary metabolites with importance in the field of medicine, such as anthocyanins, anthraquinone, leucoanthocyans, coumarins, flavonoids as well as reducer compounds. Cyanogenic derivatives are however absent in analyzed ‘’*Noni*’’ juice. The presence of these secondary metabolites of therapeutic interest in ‘’*Noni*’’ juice is closely linked to the composition of ‘’*Noni*’’ fruit, despite its many nutritional characteristics. Indeed according to Singh [17], the fruit protein content representing 11.3% of the juice dry matter. The main amino acids are aspartic acid, glutamic acid and isoleucine [18]. Minerals account (8.4% of the dry matter), are mainly potassium, sulfur, calcium, phosphorus and traces of selenium [19]. The most abundant monosaccharides found were arabinose, galactose, galacturonic acid, and rhamnose [20]. The vitamins reported in the fruit are mainly ascorbic acid (24 to 158 mg/100 g dry matter) [21] and provitamin A [22]. These secondary metabolites present in ‘’*Noni*’’ juice could also justify the many uses of the juice in the field of traditional medicine. For example, Anthraquinones presents in the ‘’*Noni*’’ juice, are one of the major phenolic compounds that have also been identified and isolated from different parts of the plant [14]. Among the reported anthraquinones, damnacanthal appear quite unique with respect to their function for its anti-cancer and anti-HIV activities [23; 24].

Other studies have also reported therapeutic properties of the fruit and fruits extracts in several fields such as Antiviral activity [20], Antihelmintic activity [25], Antioxidant activity [26], Hepatoprotective activity [27], Analgesic activity [28], Anxiolytic activity [29], Anti-inflammatory activity [30], Wound healing activity [31], Hypotensive activity [32], Cardiovascular activity [33], Estrogenic activity [34] and Immunological activity [35]. However, according to Patil [36], the lack of quality control is a major area of concern for nutraceuticals. The quality of plant material and manufacturing processes used for nutraceuticals are regulated by food laws, which lack the specificity required for botanical drugs. Contamination, for instance, with toxins after fungal infection of raw plant material or with other ingredients has been repeatedly reported in juice [37], or plant product used as food [38]. and could have potential fatal consequences. Adulterations and numerous other types of impurity of nutraceuticals conceivably remain undetected simply because there is an almost total absence of specific quality control. Absence of quality control not only increases the risk to the consumer, it also results in a total lack of impetus to conduct adequate research that demonstrates the potential benefits of nutraceuticals or ensures their safety [39]. Then, in order to assess the microbiological risks associated with the consumption of this ‘’*Noni’’* juice from traditional production, the results obtained (Table 2) showed that the Noni juice analyzed does not present a direct microbiological risk for the consumer. This result could also highlight the antimicrobial properties of Noni fruit extracts. Indeed, Locher *et al*. [38] showed that extract of the dried fruit inhibited the growth of *P. aeruginosa*, *B. subtilis*, *E. coli*, and *Streptococcus pyrogene*. Lee *et al*. [39] reported the antibacterial property of the fruit extract against *E. coli*, *Streptococcus* species, *Vibrio alginolyticus*, *Vibrio harveyi*, *Mycobacterium tuberculosis* and *Salmonella typhi.* Based on this antimicrobial property, the juice from *Morinda citrifolia* L. (Rubiaceae) fruits could also be used in other to prevent many diseases caused by microorganisms and life span could also be prolonged with appropriate use of this nutraceutical from plant origin.

Table 1: Phytochemical composition of *Morinda citrifolia* L. (Rubiaceae) juice

Secondary metabolites	Results
Tanins gallics	-
Tanins catechics	-
Alkaloids	-
Anthocyanes	+
Leucoanthocyanes	+
Anthraquinones	+
Saponosides	-
Coumarineses	+
Cyanogenic derivatives	-
Flavonoids	+
Reducer compounds	+
Mucilages	-

Table 2: Microbiological characteristics of *Morinda citrifolia* L. (Rubiaceae) juice

Microbiological parameters	Load
Total bacteria count	<1ufc/ml
Total coliform count	<1 ufc/ml
Yeast and mold count	<10 ufc/ml
Staphylococcus aureus count	<1 ufc/ml

Conclusion

In health care, adequate nutritious food is required to combat nutritional deficiency and malnutrition. Disorders and malnutrition could be rectified with an adequate intake of balanced nutritious food. Then, the ‘’*Noni* ‘’ juice appears as functional food which could also aids in the prevention or treatment of diseases or disorders. The industrial valorization of this product could allow consumers to have enriched foods with good medicinal property.

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