Isolation of Phytochemical Components Present in Philippine *Morinda citrifolia* Variety (PhilNONI)

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Abstract

Various modern techniques currently determine phytochemicals, but conventional qualitative tests are still popular for preliminary phytochemical screening of plants. This study was patterned on a previous work of Nagalingam et al. from India, who extracted and did preliminary phytochemical screening of active components in their Indian noni fruit variety. We aimed to isolate the phytochemical components present in our local *Morinda citrifolia* variety (called PhilNONI by our company). It is a fact that different noni varieties from different countries may differ in phytochemical contents. Recent scientific studies have established a relationship between phytochemicals and health benefits, such as diabetes, cancer, and heart diseases, in boosting immunity and as powerful antioxidants. Phytochemicals, moreover, fulfill the action of cell regeneration in wound healing. There are about 65-200 phytochemicals that can fight diseases, boost energy, and revitalize health. They are powerful innovators, as discussed by Rita Elkins in her book on the Noni Revolution. Using three extracts from noni powder, ethanol, methanol, and water, we isolated qualitatively in appreciable amounts from the three extracts of PhilNONI the following groups of phytochemicals: steroids, cardiac glycosides, terpenoids, alkaloids, carbohydrates, flavonoids, and saponin. Gallic catechol tannin, acidic compounds, and reducing sugar are also in appreciable amounts but only in water extracts. Both ethanol and methanol PhilNONI extracts gave appreciable amounts of phenolic compounds. Protein came out in large amounts from methanol and water extracts and large amounts in ethanol extract, but only for asparagine amino acids. We isolated anthraquinone in medium amounts from methanol extract but in appreciable amounts from ethanol and water extracts. There was no lipid or fat isolated from the three extracts.

Keywords: Philippine Noni, extraction & isolation, phytochemicals

Introduction

Morinda citrifolia L., Rubiaceae (noni), is a small to medium evergreen shrub growing in tropical areas worldwide. Originally native to Southwestern Asia, the plant spread to Australia, Hawaii, Polynesia, and other tropical areas, including the Philippines, by dispersing buoyant seeds through water. There are many known components of *Morinda citrifolia* fruit categorized into different therapeutic values such as antioxidant to various organ functions, as well as growth and development of the body, which make noni a functional food (Kumar, et al., 2023). Many of these interventions are activated by phytochemicals.

Shaikh and Patil (2020) also studied medicinal plants, rich bio-reservoirs of various phytochemicals. These phytochemicals can be identified and isolated from our local *Morinda citrifolia* variety, which may or may not differ from other varieties from other countries. Due to the increase of commercial noni fruit products in the global market, an extensive phytochemical comparison of noni varieties is necessary to understand their internal quality. The variation of phytochemical content in noni may be attributed to the diversity of the geographical environment. There could be differences in the type of soil, sunlight, temperature, precipitation, and other post-growth factors like harvesting, storage, transportation, manufacturing processes, formulation, and others (Deng et al., 2011).

So far, about 200 broad spectrums of these secondary metabolites have been identified, grouped as steroids, glycosides, phenol, tannins, terpenoids, alkaloids, saponins, flavonoids, protein, anthraquinones, and many more major bioactive compounds in noni.

Methodology

Extraction was used to obtain the desired compounds from sourced material, which was the noni powder produced from 70% of its fruits and 30% from its leaves at Phil Morinda Citrifolia, Inc. (PMCI) company, Los Banos, Laguna, Philippines. The solvents used for the extraction in this study were ethyl alcohol (ethanol), methyl alcohol (methanol), and distilled water to produce the so-called green extracts. The three solvents were selected because they are non-toxic, biodegradable, recyclable, and renewable and have high flash points or lower fuel hazards to high temperatures (Kumar et al., 2023).

Water is the most used and universal solvent. It is non-selective and can separate all hydrophilic substances, such as saponins, phenolics,

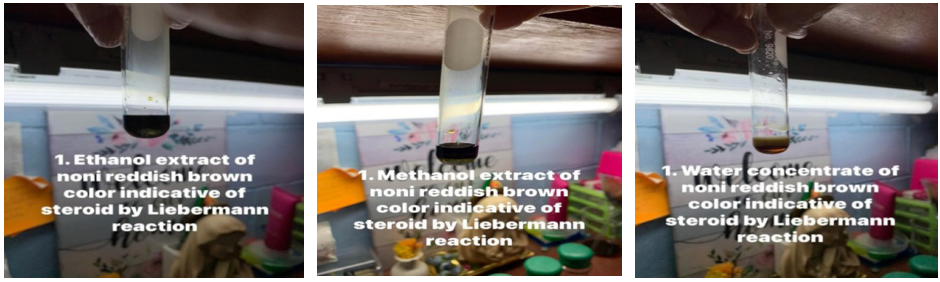
and polysaccharides. The extraction efficiency is enhanced by constant shaking and heating because it decreases the dielectric constant of water and provides better penetration. Ethanol is selective in action and used to extract polyphenols and triterpenes. Methanol has inherent toxicity. It is used for the extraction of saponins, alkaloids, and anthraquinones.

The extraction method used in this study was 20 grams of PhilNONI powder, which was added separately, along with about 50 ml of each solvent: ethanol, methanol, and distilled water. Each bottle with the solvent and the noni powder was shaken vigorously every 10-15 minutes during the first 8 hours, then left overnight at room temperature. Each bottle was filtered with Whatman cellulose filter paper the following day, pre-pleated to give an improved flow rate and increased capacity. Each filtrate was bottled separately and properly labeled. Extracts of ethanol and methanol were suspended in 0.25% dimethyl sulfoxide (DMSO). Water extract was condensed to half its volume before being used by heating.

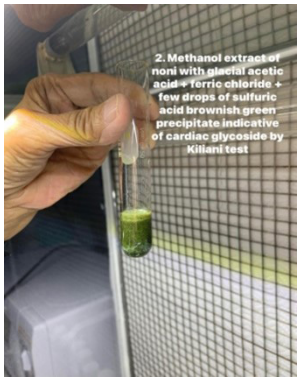
All three extracts were used in the isolation of each phytochemical compound as determined from selected standard tests compiled by Shaikh and Patil (18) and also used by Nagalingam and co-workers (2012), Auwal et al. (2014) and Ezeonu and Ejikeme (2016).

1. Determination of Steroids like cholesterol by Liebermann Burchard Reaction:

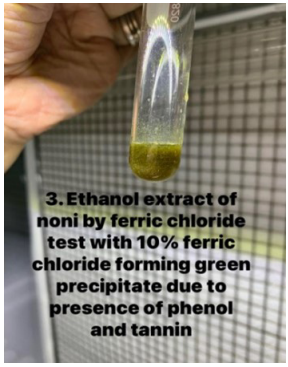
To 300 ul of each PhilNONI extract, add 1 ml of chloroform plus a few drops of concentrated sulfuric acid (conc. H_2SO_4) along the side of the test tube. A reddish-brown layer or precipitate was observed at the bottom of the test tube, indicating the presence of steroids.

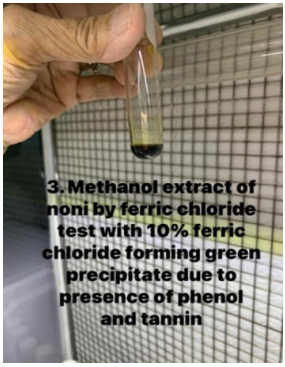


2. Glycoside by Keller Kiliani test:
300 ul of each extract + 1 ml glacial acetic acid + 300 ul of
10% ferric chloride ($FeCL_3$) + few drops of conc. H_2SO_4 along the sides of the test tube.
Brownish green precipitate at the bottom of the test tubes in ethanol and methanol and more brown in water extracts indicated the presence of glycosides, including cardiac glycoside or digitoxose moiety.

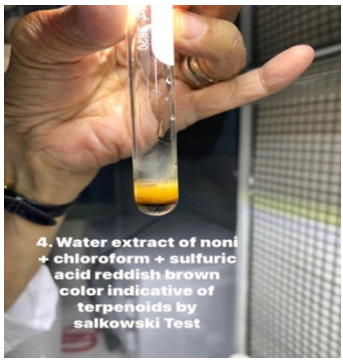


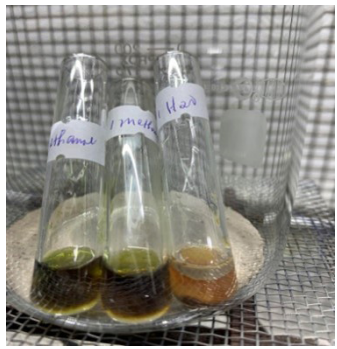
3. Phenol and Tannin by Ferric Chloride Test:
To 300 ul of each extract + a few drops of 10% $FeCL_3$.
Blue to green precipitate formation was due to phenol and tannin.
Blackish blue to greenish-black color in the water extract indicated the presence of gallic tannin and catechol tannin.





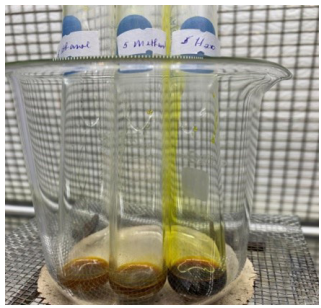
4.Terpenoids by Salkowski test:
To 300ul of each PhilNONI extract + 1 ml chloroform + few drops of conc. H_2SO_4 along the sides of the test tube.
A reddish-brown color was seen in the presence of terpenoids, especially in the water extract.



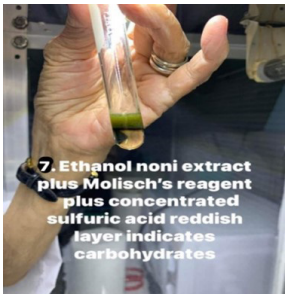


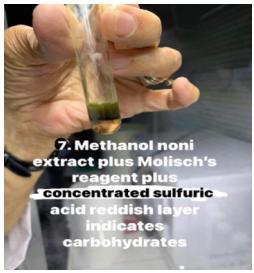
5. Alkaloids by Dragendorff's Reaction:
The formula of Dragendorff's reagent: Part A = 0.85 gm of bismuth nitrate in 40 ml H_2O + 10 ml glacial acetic acid, Part B = 8 g of bismuth iodide + 20 ml of H_2O.
Add equal parts before use. To each PhilNONI extract, add an equal part of Dragendorff's rgt.

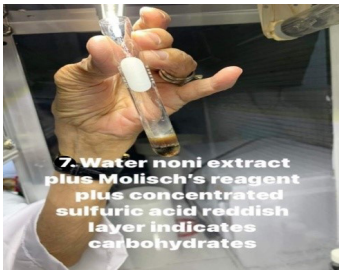
Orange to orange-red precipitate indicated the presence of alkaloids.



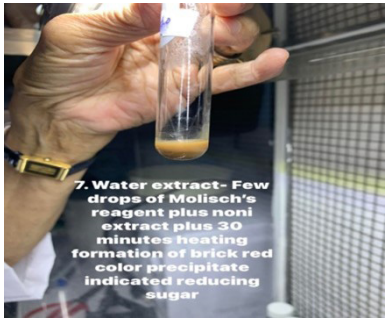
6. Carbohydrates by Molisch's Test
Formula of Molisch's rgt. 3.75 gm of α-naphtol in 25% ethanol
300 ul of PhilNONI extract + 300ul of Molisch's rgt + few drops of conc. H_2SO_4 .
The reddish layer color was indicative of the presence of mono, di, and polysaccharide.

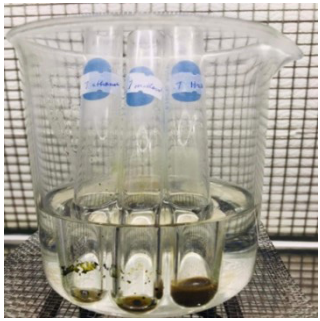




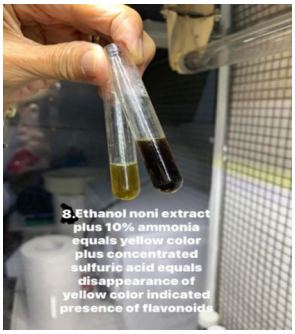


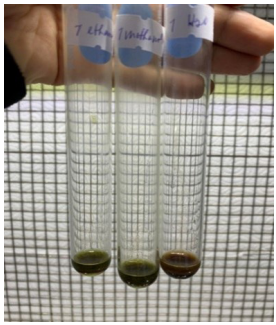
Alternate procedure for Reducing Sugar:
500ul of PhilNONI extract + a few drops of Molisch's rgt.
Heat for 30 minutes and observance of brick red colored precipitate indicated reducing sugar in water extract. The 2nd pic showed the brick color still forming.

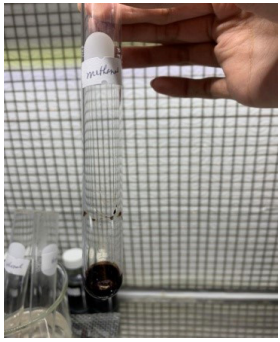




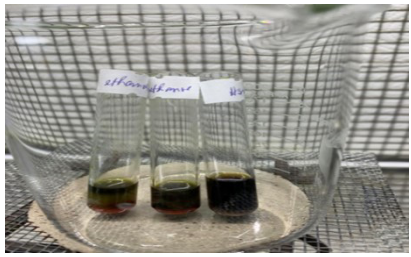
7. Flavonoids:
300 ul of PhilNONI extract + 1 ml of 10% ammonia gave yellow solution + 1 ml conc. H_2SO_4
Disappearance of yellow color indicated the presence of flavonoids.
Alternate procedure: (2nd & 3rd pics)
300 ul of PhilNONI extract + conc. hydrochloric (HCL) acid
The immediate red color in the water extract indicated the presence of flavonoids, and the Shinoda test gave a purple color.



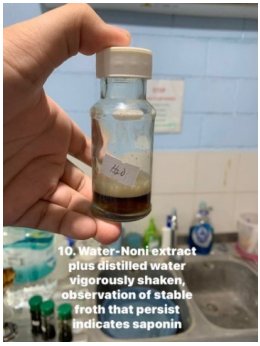




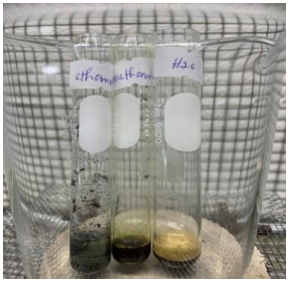
8. Anthraquinones:
300 ul of PhilNONI extract + $FeCl_3$ + 1 ml of 10% ammonium hydroxide.
The formation of pink to red to violet color showed the presence of anthraquinones.

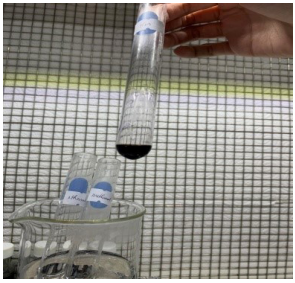


9. Saponins by Froth Test:
300 ul of PhilNONI extract + 2 ml H_2O in a test tube.
Tubes were vigorously shaken.
Observation of stable froth that persisted indicated the presence of saponins.



9. Protein by Ninhydrin test:
300 ul of PhilNONI extract + 1 ml of H^2O + 300 ul of ninhydrin rgt.
The dark purple color indicated the presence of protein and amino acids.
Black for asparagine.

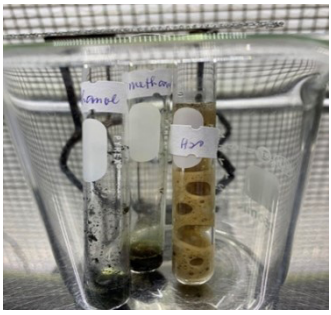




10. Lipids and Fats:
A small quantity of PhilNONI powder was rubbed on clean, neat filter

paper and observed for permanent translucent stain.
We used olive oil as control on one side of the rubbed powder. No translucent stain was seen.

Acidic compounds:
To 300 ul of PhilNONI extract + a pinch of bicarbonate.
Effervescence indicated the presence of acidic compounds, greatest in water extract.



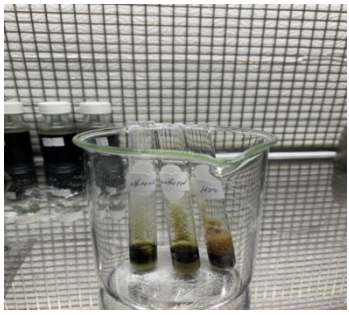


Table 1
Isolated Phytochemicals from Philippine Morinda citrifolia (PhilNONI)

Phytochemical	Ethanol Extract	Methanol Extract	Water Extract	Remarks/ endpoints
1. Steroid, Liebermann Rx	4+	4+	4+	Red/brown color
2. Glycoside, K. Kiliani Test	4+	4+	4+	Brownish green ppt
3. Phenol, $FeCl_3$ Test	4+	4+	0	Blue to green color
4. Tannin, Gallic/ catechol	0	0	4+	Black color
5. Terpenoids, Salkowski Rx	4+	4+	4+	Red/brown layer
6. Alkaloids, Dragendorff's	4+	4+	3+	Orange ppt.
7. Carbohydrates, Molisch's	4+	4+	4+	Reddish layer
8. Reducing sugar Test	+	+	4+	Brick red ppt.
9.Anthraquinone, $FeCl_3$ + Ammonia	4+	2+	4+	Reddish violet
10. Flavonoids, 10% Ammonia	4+	4+	4+	Yellow solution
11. Flavonoids, , +conc H_2SO_4	4+	4+	4+	Yellow color disappeared
12. Flavonoids, Shinoda test	0	0	4+	Red purple
13. Protein, Ninhydrin test	0	4+	4+	violet
14. Asparagine	4+	0	0	black
15. Lipids & Fat	0	0	0	
16. Acidic cpds	2+	2+	2+	effervescence
17. Saponin, Froth test	4+	4+	4+	Persistent foam

All qualitative tests were scored in plusses:
0 = absent
+ = present
2+ =present in medium amt.
3+- 4+ = present in appreciable amount
All tests were done in duplicate maintained endpoints.

Results and Discussions

Isolation of phytochemical compounds from Morinda citrifolia, Philippine variety (PhilNONI) revealed the presence of various bioactive compounds. Please see Table 1.

In the 3 PhilNONI extracts, steroids were found in considerable amounts by Liebermann Burchard reaction.

The presence of phytochemical steroids in noni can be in the form of anabolic steroids, which can increase muscle strength very quickly. This means that a person can endure any activity more often for a longer period of time with improved recovery and a rapid increase in lean muscle tissue. Since their identification nearly 80 years ago, steroids have played a prominent role in the treatment of many disease states. Many clinical roles of steroids are related to their potent anti-inflammatory and immune-modulating properties (William & Kaye, 2014). Examples of steroids are the costicosteroid which are anti-inflammatory. They treat rheumatologic diseases like rheumatoid arthritis, lupus, and vasculitis or inflammation of blood vessels.

Glycosides were found in the local noni variety in the Philippines from the three PhilNONI extracts: ethanol, methanol, and, most significantly, in water in appreciable amounts.

Glycosides, which are compounds formed from a simple sugar with another compound by replacement of the hydroxyl group in sugar molecule, exist in the form of anthraquinones, coumarin, cyanogens, flavonoids, thioglycoside, phenols, steroids, terpenoids, and saponins. Cardiac glycosides found in our local noni work in the body system by holding cell membranes from pumping out, increasing the sodium in the cells, and allowing calcium to build up in the cells, which causes stronger heart muscle contraction. It is also used in cancer remediation (Ayogu & Amaechi, 2020).

The phenolic compounds derived from our local noni variety were isolated from the ethanol and methanol extracts and tannin from the water extract, all in appreciable amounts.

These compounds have antioxidant properties to lower total cholesterol, keep the heart healthy, lower blood pressure, and stimulate the immune system. They also have antibacterial properties and fight tooth decay. Tannins in the phenolic group are used to treat varicose ulcers, hemorrhoids, minor burns, and gum inflammation. Internally, they are useful in diarrhea and as an antidote for heavy metals poisoning (Maslog, 2020).

Terpenoids in our PhilNONI variety were found to be 4+ (present in

appreciable amounts) in the three PhilNONI extracts by the Salkowski test. Terpenoids, also known as isoprenoids, are a class of chemical compounds produced from isoprene and terpenes. Terpene is a medicinal molecule with a mode of action affecting calmness. Its medicinal use includes anti-infection, anti-anxiety, and sedative or anxiolytic effects (Maslog, 2020).

Alkaloids isolated from the local noni variety were seen in appreciable amounts in all three PhilNONI extracts. According to Yangfang et al. (2021) and Zhang et al. (n.d.), alkaloids from noni have potential α-glucosidase inhibitory activity. These α-glucosidase are enzymes that are supposed to catalyze the hydrolysis of starch/carbohydrates to glucose for intestinal absorption, leading to increased blood glucose. Alkaloids, therefore, prevent diabetes mellitus by its α-glucosidase inhibiting action.

Bin Zhang and co-workers isolated four new alkaloids called noni alkaloid A-D,1-4, and six known analogues 5-10 from noni (Zhang et al., n.d.).

The presence of carbohydrates in this study showed four plusses results by Molisch's reaction in all the 3 PhilNONI extracts and 4+, as well as in reducing sugar in water extract.

All these isolated carbohydrates contain free aldehyde, a ketonic group called reducing sugars. Examples of reducing sugars are glucose, galactose, glyceraldehydes, fructose, ribose, and xylose. Our brains and organs need sugar to function. Glucose is the main source of nutrients for our body cells. So, low or no sugar entering the body's cells can be life-threatening. The polysaccharide contents and aroma characteristics in noni changed during fermentation scale and time conditions (Wang, 2021).

Flavonoids came out in our analyses with the highest score of 4 plusses in the three PhilNONI extracts in a couple of standard tests.

According to the Salu Genecists, Inc. (2009), flavanoids, once known as Vitamin P, are a large class of plant compounds found in deeply colored vegetables and fruits, including noni. They were discovered in 1938 by Hungarian scientist Dr. Albert Szent Gyorgyi. Flavonoids' phytochemicals include flavonols, dihydroflavonoids, flavones, isoflavones, anthocyanins, and anthocyanidin. Well-known flavonols include quercetin, rutin, apigenin and luteolin. Flavonoid content in noni gradually increased with fermentation (Wang et al., 2021).

Frequent colds and/or infections, reflective of generally weakened immune function, can be a symptom of flavonoid deficiency. Conditions like excessive bruising, swelling after injury, nose bleeds, and hemorrhoids are also signs of inadequate intake of flavonoids (Ulah et al., 2020).

Additional functions of flavonoids are enhancing the antioxidant

effect of Vitamin C, playing a role in preventing excessive inflammation, and involving alterations of the behavior of many types of immune system cells like T cells, B cells, natural killer cells (NK cells,) mast cells and the neutrophils (West & Deng, 2021). They also possess antibiotic activity from bacteria and anti-virus properties, especially HIV and herpes simplex (Bansal & Priyadarsini, 2021; Deng et al., 2011). A recent publication by Ghildiyal et al. (2020) showed that not only the flavonoid groups mentioned above are anti-viral, but they also found out that phenolics, alkaloids, terpenoids, tannins, and carotenoids are anti-viral agents as well.

Anthraquinones presented a high amount with PhilNONI ethanol and water extracts in our present study. Rainer W. Bussman et al. (2013) and Tiwari and Singh (1977) reported from a health perspective that noni contains anthraquinones, especially lucidin, alizarin, and rubiadin forms of anthraquinones glycosides. Tiwari and Singh (1976) studied anthraquinone from noni's flowers.

Anthraquinone derivatives have been used for centuries for medical applications as laxative, anti-microbial, and anti-inflammatory agents. Also, it is for constipation, arthritis, multiple sclerosis, and cancer. Anthraquinones in PhilNONI such as scopoletin, acubin, l-asperuloid and alizarin serve as bactericidal (Maslog, 2020)

Saponin is present in the 3 PhilNONI extracts in large amounts by froth test. Saponin is taken first as fruit, then as an antioxidant. Clinical studies have suggested that saponins affect the immune system in ways that help protect the human body against cancers and lower cholesterol levels. Saponins, moreover, decrease blood lipids.

Found in grains, legumes, and plant-like noni, it serves as a "natural antibiotic," Recently, scientists have been looking at it as a help from fungal and viral infections and boosting certain vaccine effectiveness. As a bonus, saponin is very sweet. It tastes about 200 times sweeter than table sugar, according to Dr. Mary Clarke, an extension specialist from Kansas State University (Clarke, n.d.)

Proteins, peptides, glycoprotein, and amino acids proved to be present in the methanol and water PhilNONI extracts in large amounts, but the amino acid, asparagines, was evident from the ethanol extract.

Proteins and amino acids in noni are important as immunomodulators or immune system boosters. They help prevent cancer, bacterial infections, viral infections, and inflammatory diseases like multiple sclerosis (Maslog, 2020; West & Deng, 2021).

Acidic compounds in our present study showed a strong four-plusses reaction in the PhilNONI water extract and only with a medium amount

in both the ethanol and methanol extracts. The most predominant acidic compounds in noni are acetic acid, aucubin, caprylic acid, and benzoic acid. Acidic compounds are the key components in noni against fungi infections, especially in candidiasis and aspergillosis (Maslog, 2020).

Conclusions

It is important to isolate and identify the phytochemicals in our local *Morinda citrifolia* variety (PhilNONI), the variety we use in our PMCI company. These phytochemicals determine the pharmacological activities of noni plants in treating various diseases. Following Food & Drug Administration (FDA) guidelines, we cannot simply claim from the literature the benefits derived from our products without proper research to isolate and identify such biochemicals in our local noni variety. We need to test whether these phytochemicals exist in our PhilNONI products.

Thus, this project, being the first time that such biochemicals were isolated from the native variety of Morinda citrifolia in the Philippines, we can claim and advertise its broad range of therapeutic effects with all honesty. The isolation of steroids, for example, proved Dr. Ray Sahelian's theory of its being a supplement as an energy enhancement and well-being as well as being antibacterial, antiviral, antifungal, antitumor, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune-enhancing effects. Ray Sahelian, MD, obtained a BS in nutrition from Drexel University and completed his doctoral training at Thomas Jefferson Medical School in Philadelphia; he is a well-known preacher/lecturer on noni health benefits (Sahelian, 2016).

Now, it is documented that PhilNONI juice contains a mixture of anthraquinones from the isolation of glycosides to which category it belongs, which helps build stronger heart muscle and can potentially reduce cancer risk. The presence of phenolic compounds in the local variety of noni made us realize for the first time the beneficial effect of PhilNONI juice on the anti-oxidant, hematological, and biochemical alterations it can do to ameliorate the effect of oxidative stress. Alkaloids containing inhibitory enzyme activity versus diabetes, now proven to be present in the PhilNONI variety of noni plants, can greatly help blood sugar control. Isolation of flavonoids phytochemical, acidic compounds, proteins, and saponins in local noni variety is now proven why it helps improve the immune system, ultimately healing dozens of conditions including simple colds and other infections, bacterial, fungal, viral, and even human parasites.

With these analyses using standard qualitative procedures and

publication of our results in a reputable journal, we will be able to advertise evidence-based health benefits from phytochemicals isolated from our PMCI products aside from the nutrition facts already analyzed by reputable labs in the Philippines containing Vitamins C, E, & B complex, and minerals like calcium, sodium, potassium, magnesium, and iron.

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