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Phytochemical and nutritional properties of dried leaf powder of *Moringa oleifera* Lam. from machala el oro province of ecuador

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ABSTRACT

Moringa oleífera commonly called Moringa, is a valuable tree whose fruits, roots and leaves have been advocated for traditional, medicinal and industrial uses. The phytochemical and nutritional properties of the dried leaf powder of M. oleifera used as nutraceuticals, dietary supplements, functional foods or a source of vegetable in meal preparation were investigated in this study to scientifically provide an empirical evidence for its use and benefits. Using standard phytochemical screening procedures the phytochemicals identified were tannins, saponins, alkaloids, flavonoids, cardiac glycosides and reducing sugars. The physico-chemical analysis using standard official methods and gas chromatography revealed the following nutrients; proteins (24.31%), carbohydrate (55.97%), ashes (11.50%), crude fiber (10.28%), total fat (9.22%), moisture (6.12 %), caloric value (404.10 Kcal/100g) and saturated fatty acids (3.77 %), unsaturated fatty acids (5.45 %), monounsaturated fatty acids (0.87 %), polyunsaturated fatty acids (4.58 %) and Trans fatty acid (0.00 %) for fatty acid profile. Using acid hydrolysis and ion-exchange chromatography, the amino acid analysis report showed the presence of essential and semi essential amino acids in varying amounts with a total of 27.16 nmol at 570nm and proline was 1.432 nmol at 440nm. These findings have far reaching nutritional importance in the healthcare system of this country and will help to address undernutrition in acost effective manner. Thus, the use of M. oleifera leaves as nutrients should be encouraged and sustained in this and other countries.

Keywords: *Moringa oleifera* Lam., Phytochemical, Nutritional, Leaf powder, Ecuador.

INTRODUCTION

The use of plants in traditional medical practice has a long drawn history, and remains the mainstay of primary health care in most of the third world. Traditional medicines are used by about 60% of the world population in both developing and developed countries where modern medicines are predominantly used [1]. An estimated 60-80% Africa's and Latin America's population depends solely on herbal remedies for its primary health care needs. In diversity, plants are thought to be between 250,000 to 400,000 species spread across all continents from the Antarctic to the Arctic. They thrive in all environments from the flooded planes to the deserts, and from those who

live on the seas and oceans to others that thrive on fresh water and ponds [2]. For classification and easy identification, plants were divided into different taxonomical groups known as kingdoms; these were further streamlined into phylum, class, order, family, genus and species. Within the family of the Moringaceae is found a miracle plant called *Moringa oleifera*.

M. oleifera in English is known as Drumstick tree, Horseradish tree and Ben tree; in Spanish it is called Moringa, Ben and Ángela, in Hindi it is known as Saguna and Sainjna and its common name is Moringa [3]. It is a small, fast growing, evergreen or deciduous tree with a soft and light wood indigenous to South Asia, mainly Himalaya's foothills, India [4]. It has been grown and naturalized in other countries like Pakistan, Afghanistan, Sri Lanka, Bangladesh, Arabian Peninsula, East and West Africa, throughout the West Indies and Southern Florida, in Central and South America from Mexico to Peru as well as in Paraguay and Brazil [5]; cultivated for human food, medicine, dye, textiles, fodder, water purification or clarification, etc. All its parts (leaves, roots, seeds, flowers, bark, stem-bark, green pods) have much impressive range of medicinal uses with higher nutritional value. The leaves mainly contain various glycosides of thiocarbamate and isocyanide class; pterygospermin, moringyne, niaziridin, 4 – (α – L – rhamnopyranosyloxy) benzyl isothiocyanate, 4 – (α – L – rhamnopyranosyloxy) benzyl glucosinolate, etc. are few of them which are isolated and therapeutically proved by scientific studies [6].

Traditionally, it is used to treat many diseases throughout the world (mainly in Thai) and many of them are scientifically proved, which mainly include; antihypertensive, antiasthmatic, diuretic, anticancer, antibiotic, antiulcer, analgesic, CNS- depressant, antiepileptic, anti-inflammatory, anthelmintic, antiurolithiatic and many more[7]. Recently, workshops are going on highlighting the importance of *M. oleifera* leaves in Africa and other developing nations, as it is used to overcome malnutrition especially in infants and nursing mothers. Three non-governmental organizations in particular – Tree for Life, Church World Service and Educational Concerns for Hunger Organization have advocated Moringa as natural nutrition for the tropics [4].

Proximate and nutrient analyses of edible plants and vegetables play a crucial role in assessing their nutritional significance [8]. As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species [8]. For as herbal drug's standardization is concerned, WHO also emphasize on the need and importance of determining proximate and micronutrients composition of the herbal plants. Such herbal formulations must pass through standardization processes [9]. Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world [10], [11], [12]. Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemical like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes [13]. Fortunately, chemical composition diversity in plants also includes many compounds that are beneficial to humans: vitamins, nutrients, antioxidants, anticarcinogens, and many other compounds with medicinal value [13].

In the ancient days of human existence, by instinct, intuition, or trial and error, man was able to identify various plants used to combat various ailments. In fact, it is the knowledge derived from the active components of these plant extracts that guided man to synthesize and use modern drugs in health care delivery. Presently, there has been a renewed interest in the study of medicinal plants such that much percentage of pharmaceutical preparations is based on natural products from plants. Over the years, our people have passed down knowledge of the types and applications of medicinal plants from generation to generation, often orally. The compilation of useful drugs derived from medicinal plant is impressive; these include; heart drugs, analgesics, anesthetics, antibiotics, anti-cancer and anti-parasitic compounds, anti-inflammatory drugs, oral contraceptive hormones, as well as laxative diuretics [14].
Plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides) known as secondary metabolites, which are biologically active [15]. Secondary metabolites may be applied in nutrition and as pharmacologically-active agents [15]. Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fiber[16]. In Ecuador, as in many Latin American countries, malnutrition is a serious health problem and although infant mortality has decreased, the survivors manifest lasting repercussions throughout life time [17]. The cultivation and awareness of the numerous benefits of *Moringa oleifera* is springing up in the Republic of Ecuador and there arise the need to produce herbal formulations from Moringa cultivated in Ecuadorto scale up nutrition –addressing undernutrition in a cost effective manner.Based on this, there is little or no report on the phytochemical and nutritional composition of Moringa cultivated in

Ecuador. Hence the aim of this work was to determine the nutrient composition of the dried leaf powder of this plant in order to scientifically provide an empirical evidence for its use and benefits to man.

MATERIALS AND METHODS

Source of plant material

The leaves of *Moringa oleifera* used for this study were collected from the matured tree in an orchard within the plantation of Faculty of Agricultural Sciences, Universidad Tecnica de Machala, Ecuador.

After collection, the leaves were removed from the branches, sorted out to separate the bad ones from the good ones, washed properly with sterile water and spread on clean mesh trays for drying. The leaves were shade dried for a period of 7 – 10 days for proper drying in the Pharmacy Pilot Plant Laboratory, Faculty of Chemical Sciences and Health, Universidad Tecnica de Machala, Ecuador. Upon drying, the leaves were pulverized under aseptic conditions using a grinder (Lab. Mill serial No. 56969, Type AR 400 Erweka® Apparatebau GmbH Heusenstamm Germany) into fine powdery form, sieved and stored in dry airtight glass jar for phytochemical and nutritional analyses.

Extraction of plant material

The powdered plant material (100g) was macerated in 98 % methanol (500 ml) in a glass jar for 72 hours and was shaken intermittently throughout the period. Another portion of the powdered plant material (100 g) was macerated in distilled water (500 ml) for 24 hours and was shaken intermittently. The aqueous filtrate was evaporated to dryness in a hot air oven set at 40^{o}C to obtain a brownish residue. The methanol extract was concentrated using a water bath to evaporate the extracting solvent (methanol) and later transferred to hot air oven set at 40^{o}C to evaporate any trace of the solvent to obtain a greenish brown residue. The extracts obtained were stored in the refrigerator at 4^{o}C until when required for phytochemical analysis.

Phytochemical screening

The aqueous and methanol extracts were separately screened for the presence of bioactive constituents using standard phytochemical techniques as described by [18] and [19].

Nutritional property analyses

The Moringa leaf powder sample was conserved in a fresh and dry atmosphere (climatic zone IV) with climatic test conditions of temperature 22.5^{o}C ± 2.5^{o}C and relative humidity 55 % ± 15% prior to analysis in the laboratory.

a) Proximate analysis

Determination of proximate composition was carried out in accordance with Association of Official Analytical Chemists (AOAC) methods [20]. The proximate analysis is a conventional system of analysis that gives the quantitative as well as qualitative idea of nutrients present in a particular food sample. Individual nutrients such as amino acids, fatty acids, monosaccharide etc. are not considered. The gross components considered are moisture content, ash content, protein, fat, crude fiber, carbohydrate, as well as caloric value calculated from values of carbohydrate, fat and protein [21], [22]. All the methods used in estimating the chemical composition of the plant samples were standard methods of AOACexcept where otherwise stated.

Carbohydrate content was estimated by subtracting the values obtained for fat and protein from organic matter. The percentage of organic matter was calculated by subtracting the percentage of ash from one hundred (100) [23]. The caloric value of the sample was calculated using “Atwater factor” by multiplying the value of the crude protein, lipid and carbohydrate by 4, 9, 4 respectively and taking the sum of the product [23].

b) Estimation of fatty acids

Fatty acid profile of the Moringa leaf powder was determined using gas chromatography in accordance with the methods outlined by AOAC [20]. The investigated fatty acids include: saturated, unsaturated, monosaturated, polysaturated and Trans fatty acids.

c) Amino acid analysis

Quantitative amino acid analysis of protein in *Moringa oleifera* leaf powder was determined using acid hydrolysis and ion exchange chromatography (Biochrom 30) technique.Sample hydrolysates were prepared in accordance with

the standard procedures of USP/ EP/ JP [24].Briefly, appropriate acid hydrolysis of the protein in the leaf powder sample (1.5 mg) was done and 3% injected into the apparatus for amino acid analysis. The amino acid standard used was norleucine. Free amino acids were separated by ion-exchange chromatography followed by postcolumn derivatization with ninhydrin. The postcolumn reaction between ninhydrin and amino acid eluted from the column was monitored at a wavelengths of 570nm and 440 nm, and the chromatogram obtained was used for the determination of the amino acid composition.

RESULTS AND DISCUSSION

The data obtained in the course of the experiments are shown in the tables and figures below and the discussion of these result are also given.

Pharmacologically active principles identified in *M. oleifera* leaf powder from Ecuador include; alkaloids, flavonoids, saponins, reducing sugars, tannins and glycosides with water showing a better extraction spectrum than methanol (Table 1). This finding is on a par with results of other researchers [25], [26]. These phytochemicals contribute significantly to protection against infection and degenerative diseases.

The macro nutrients and amino acids with their various compositions identified in this study are shown in Tables 2 and 3 and Figures 1 and 2 respectively. The moisture content (6.12 %), crude protein content (24.31 %), ash content (11.50 %), crude fiber (10.28), total carbohydrate (55.97 %), fat content (9.22 %) and caloric value (404.10 kcal/100g) of the present study were in good agreement with those obtained from Ghana [27], India [28], China and Rwanda [29] and Burkina Faso [30], but, was at variance with the values obtained from Nigeria [26]. The 17 amino acids and their compositions identified in this study, is in agreement with the results of several studies conducted in this regard on *Moringa oleifera* leaves, but with little variations [31], [32], [26]. These variations in the nutritional composition of Moringa leaf powder could be as a result of differences in agro-climatic conditions, age of the trees and possibly due to different stages of maturity of leaves. The highest amount of amino acids was glycine, which had a value of 3.63 % and the least content was cysteine with 0.19 %. The Moringa leaf powder were found to contain 5.45 % unsaturated fatty acids and 3.77 % of saturated fatty acids with no Trans fatty acids.

Table 1: Phytochemical constituents in extracts of *M. oleifera* leaf and their health benefits

Constituents	Aqueous extract	Methanol extract	Health Benefits
Tannins	++	+	Bactericidal, anti-inflammatory and anti-parasitic.
Saponins	+	+	Antioxidant, anticancer, cholesterol reduction & immune support.
Alkaloids	+++	+	Treatment of malaria, diabetes, cancer, cardiac dysfunction & pain.
Flavonoids	+	+	Antitumoral, anti-inflammatory, antibacterial & antioxidants.
Glycosides	++	+	Treatment of congestive heart failure & cardiac arrhythmia.
Reducing sugars	+	-	Provide energy for proper body function.

**Key: +++, Highly present; ++, Moderately present; +, Slightly present; -, Not present*

Table 2: Nutritional composition of *M. oleifera* leaf powder

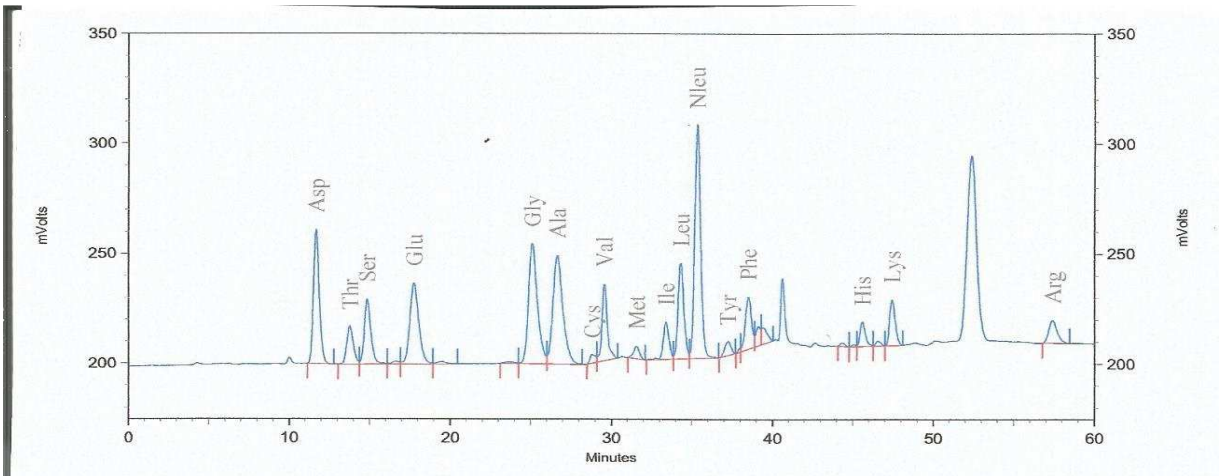
Parameters	**Values (Per 100g powder)**
Moisture (g)	6.12
Protein (g)	24.31
Crude fiber (g)	10.28
Ash (g)	11.50
Fat (g)	9.22
Total Carbohydrate (g)	55.97
Calories (Kcal)	404.10

**Values are mean of duplicate determinations*

Table 3: Fatty acid profile of *M. oleifera* leaf powder

Parameters	Values(Per 100g powder)
Saturated fatty acids	3.77
Unsaturated fatty acids	5.45
Monounsaturated fatty acids	0.87
Polyunsaturated fatty acids	4.58
Trans fatty acids	0.00

**Values are mean of duplicate determinations*



570nm Results

Aminoacido	Tiempo de retencion	Area	Cantidad (nmoles)
Asp	11.67	1544483	2.72
Thr	13.77	487574	0.92
Ser	14.83	837206	1.75
Glu	17.77	1423119	2.54
Gly	25.10	2166660	3.63
Ala	26.66	2126027	3.46
Cys	28.80	92866	0.19
Val	29.56	804925	1.36
Met	31.56	139943	0.27
Ile	33.40	442721	0.77
Leu	34.33	1109528	1.84
Nleu	35.33	2742652	4.47
Tyr	37.23	173079	0.33
Phe	38.50	631455	1.08
His	45.60	255401	0.42
Lys	47.43	507851	0.79
Arg	57.40	373858	0.63
Totals		15859348	27.16

Figure 1: Ion-exchange chromatogram and composition of amino acids in *M. oleifera* leaf powder at 570 nm

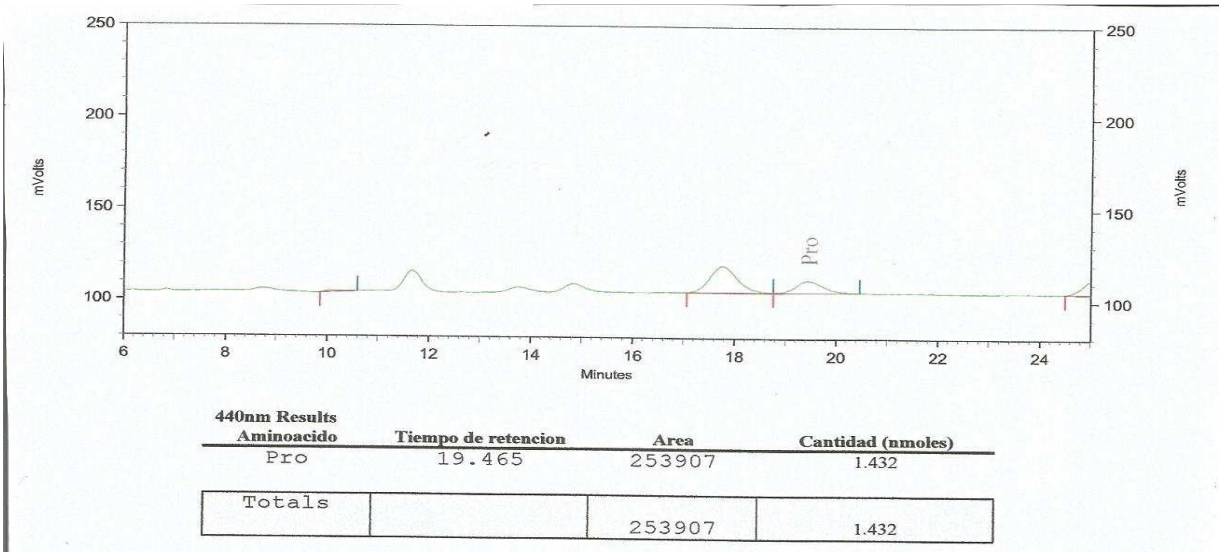


Figure 2: Ion-exchange chromatogram and composition of amino acids in *M. oleifera* leaf powder at 440 nm

The study showed that Moringa leaves containnutritious compounds. Noteworthy is the crude protein (CP)content of 24.31 % observed in this study, although lowerthan sunflower seed cake's CP of 35.88% which is mostlyused as protein concentrate [33]. Thismakes the Moringa leaves to be a good potential sourceof supplementary protein in animal diets. Other studieshave reported variable protein contents ranging between16, 22.42, 23.27, 27.4 and 40% [34], [35], [36],[37], [27],[38],and [26]. This level of crude protein content is of particularnutritional significance as it may meet animal's proteinand energy requirements and boost the immune systemagainst diseases [39], [40]. General growing ruminants like goatsrequire 16% CP [41]. The CPsupplied by Moringa is above the protein of goats makingit ideal for use as a protein supplement.Moringa is reported to have high quality protein whichis easily digested and that is influenced by the quality ofits amino acids [42]. In this study, the driedMoringa leaves contained 17 amino acids, which slightlydiffer from the findings ofFoidl*et al*. [42], Sanchez-Machado *et al.* [38] and Moyo*et al*. [31] who reported 18, 16 and 19 aminoacids respectively. Out of the 17amino acids observed, 09 were classified as essentialamino acids, namely; threonine, tyrosine, methionine,valine, phenylalanine, isoleucine, leucine, histidine andlysine. Glycine had the highest value of3.63 %, which differed with Moyo*et al*.[31] who reported the value of 1.53 %. In their work,Moyo*et al*. [31] reported alanine havingthe highest value of 3.033 %, which is lower than that ofour findings (3.46 %). Cysteine had the least values followed bymethionine, which is commonly deficient in green leaves.Methionine and cysteine are powerful antioxidants thathelp in the detoxification of harmful compounds andprotect the body from radiation [40]. The variations in theamino acid composition could be influenced by proteinquality and the origin of the plant (cultivated or wild). Thismay indicate that the Moringa was grown in fertile soils.Amino acids are organic compounds that combine toform proteins; as such, they influence the quantity andquality of protein. Amino acids are classified as essentialand non-essential, which vary according to animalspecies and their production system [43]. Rumen microbes synthesize the essential aminoacids from other amino acids or from nitrogen containingsubstances. The efficiency of rumen microbial growth and activity in the rumen is enhanced by the presence ofadequate amino acids, peptides and most macro andmicro minerals [43]. Each aminoacid has a specific function in the animal's body. Ingeneral, amino acids are required for the production ofenzymes, immunoglobulins, hormones, growthand repair ofbody tissues and form the structure of red blood cells[40]. In addition, they contribute to theformation of glucose, acting as a buffer when otherprecursors are in short supply [43].Amino acids also affect the function of other nutrients inthe animal's body such as presence of lysine, whichensures adequate calcium absorption and aids in theantibody production.

The dry leaves could serve as a protein supplementarysource in animal and human diets. This protein content isof particular nutritional significance since it has been suggested that amino acids supplementation is importantin meeting a substantial proportion of an animal's proteinand energy requirements [40]. Diets richin amino acids help to boost the immune system againstgastro intestinal parasites infestations [39]. Proteins are also essential for continuousreplenishment of the endogenous protein that is lost dueto infections with gastro-intestinal helminthes [44].Fat provides very good sources of energy and aids in transport of fat soluble vitamins, insulates and protects

internal tissues and contributes to important cell processes [45], [46]. Moreso, it is good to add fat to most of our diets, because many body functions depend on lipids.

Asobserved in this study, Moringa contains more dietarypolyunsaturated fatty acids (PUFA) than the saturated fatty acids (SFA).A higher content of PUFA and lower amount of SFA isdesirable [47], as such, itsinclusion in the diet is recommended as it prevents theoccurrence of diseases thereby promoting good health. PUFA are important for humanand animal health. They are of interest because they areprecursors of long chain *n*-3 PUFA in the eicosanoidsbiosynthesis, which are viewed as importantbioregulators of many cellular processes [48]. They are linked to the development andfunctionality of the immune system. Consumers havepreference of food low in saturated fatty acids (SFA)because they are associated with an increased risk ofcardio-vascular diseases and some cancers [49], [50]. Human nutritionists urge consumers to increase intake of polyunsaturated fattyacids (PUFA), particularly the *n*-3 PUFA at the expenseof *n*-6 PUFA [47], [50]. The quantity and composition of fatty acids in theanimals' body are related to the presence of some of theirprecursors in the diet, since some of the fatty acids areabsorbed in the body unchanged [51].

The low moisture content of the leaf powder is an attribute to a very high shelf life. Hence, long storage of the leaf powder would not lead to spoilage due to microbial attack and this supports the practice of storage in dry form by users. Also, moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food [23]. Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic materials (fat, protein, carbohydrates, vitamins, organic acid etc.) have been incinerated at a temperature of about 500 ^{o}C [23]. Thus, the ash content of the dried leaf powder is taken to be a measure of the mineral content of the original food. The crude fiber level (10.28%) obtained in this study is considered appropriate, because it aids absorption of glucose and fat. Crude fiber is made up largely of cellulose together with a little lignin which is indigestible in human. Although crude fibre enhances digestibility, its presence in high level can cause intestinal irritation, lower digestibility and decreased nutrient usage [52].

The dried *Moringa oleífera* leaf powder is a rich source of carbohydrate with great caloric value that can contribute to the caloric requirement of the body. Carbohydrates provide the body with a source of fuel and energy that is required to carry out daily activities and exercise. Our bodies need a constant supply of energy to function properly and a lack of carbohydrates in the diet can cause tiredness or fatigue, poor mental function and lack of endurance and stamina. Carbohydrates are also important for the correct working of our brain, heart and nervous, digestive and immune systems. They are an essential part of a healthy diet and should make up 50% of our daily calorie intake. Thus, majority should come from complex carbohydrates, preferably the wholemeal varieties, as well as a large intake of fruits and vegetables.

Looking at all the propertiesof the Moringa leaves, this probably explains the traditionaluse of the plant as an herbal tonic in India, because of itshigh levels of readily available essential nutrients andmineral resources, which may be required for themaintenance of electrical potential of nervous tissues andcell membranes. It can as well be used for the treatmentof blood related disorders that is necessary for theimprovement of the overall well-being of the body[53].The nutritional variations observed among the studiescould be attributed to the genetic background of the plant,in terms of ecotype and cultivar, environmental factorsthat include the soil and climate [38]. In addition, the cultivation method usedencompasses the frequency of harvesting and age of theplant or leaves. Mode of conservation between collectionand analysis (drying, refrigeration, freezing) mightinfluence the leaves' nutritional composition [54], [55]. The data derived from phytochemical and nutrientcharacterization of Moringa are clear indications that theplant leaves are rich in healthy bioactive compounds and nutrients; and has potential to beused as medicine, feed and or food additive with multiple purposes. Theseinclude serving as a protein, fatty acid, mineral andvitamin resource for animal and human feed formulations.High nutritional content found in the dried leaves areimportant nutritional indicators of the usefulness of theplant as a likely feed resource. Drying the leaves assiststo concentrate the nutrients, facilitate conservation andconsumption, as such, it can be used during the timewhen feed is scarce or can be transported to areas whereit is not cultivated. It is suggested that Moringa should beconsumed in the powder form. Moringa has beenreported to possess some medicinal properties [6]; its inclusion in the diets could function as curativeand therapeutic therapy. As such, it can be used toimprove health and nutrition.

CONCLUSION

This study can be considered as the first information on the nutritional and phytochemical composition of *Moringa oleifera* leaves from Ecuador. It indicates that the studied dry leaf powder is an excellent source of nutrients required for human existence in combating nutritional deficiencies like kwashiorkor, cardiovascular diseases amongst others. Its fiber content provides bulk in the diet and can help to enhance gastrointestinal function, prevents constipation and may reduce cholesterol content. The phytochemical content makes the leaf pharmacologically active and may serve as supplements for food, as they have potentials to improve the health status of its users. Therefore, *M. oleifera* dry leaf powder can be a cost effective, suitable functional ingredients for improving nutraceuticals, nutritional and organoleptic properties of food; its use in diets should be encouraged and sustained in Ecuador and other countries.

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