



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 oleifera 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 a cost 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 Ecuador to 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°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°C to evaporate any trace of the solvent to obtain a greenish brown residue. The extracts obtained were stored in the refrigerator at 4°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 $22.5^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ and relative humidity $55\% \pm 15\%$ 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 AOAC except 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 reduction & immune support.	+	+	Antioxidant, anticancer, cholesterol
Alkaloids cancer, cardiac dysfunction & pain.	+++	+	Treatment of malaria, diabetes,
Flavonoids	+	+	Antitumoral, anti-inflammatory, antibacterial & antioxidants.
Glycosides failure & cardiac arrhythmia.	++	+	Treatment of congestive heart
Reducing sugars function.	+	-	Provide energy for proper body

*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*

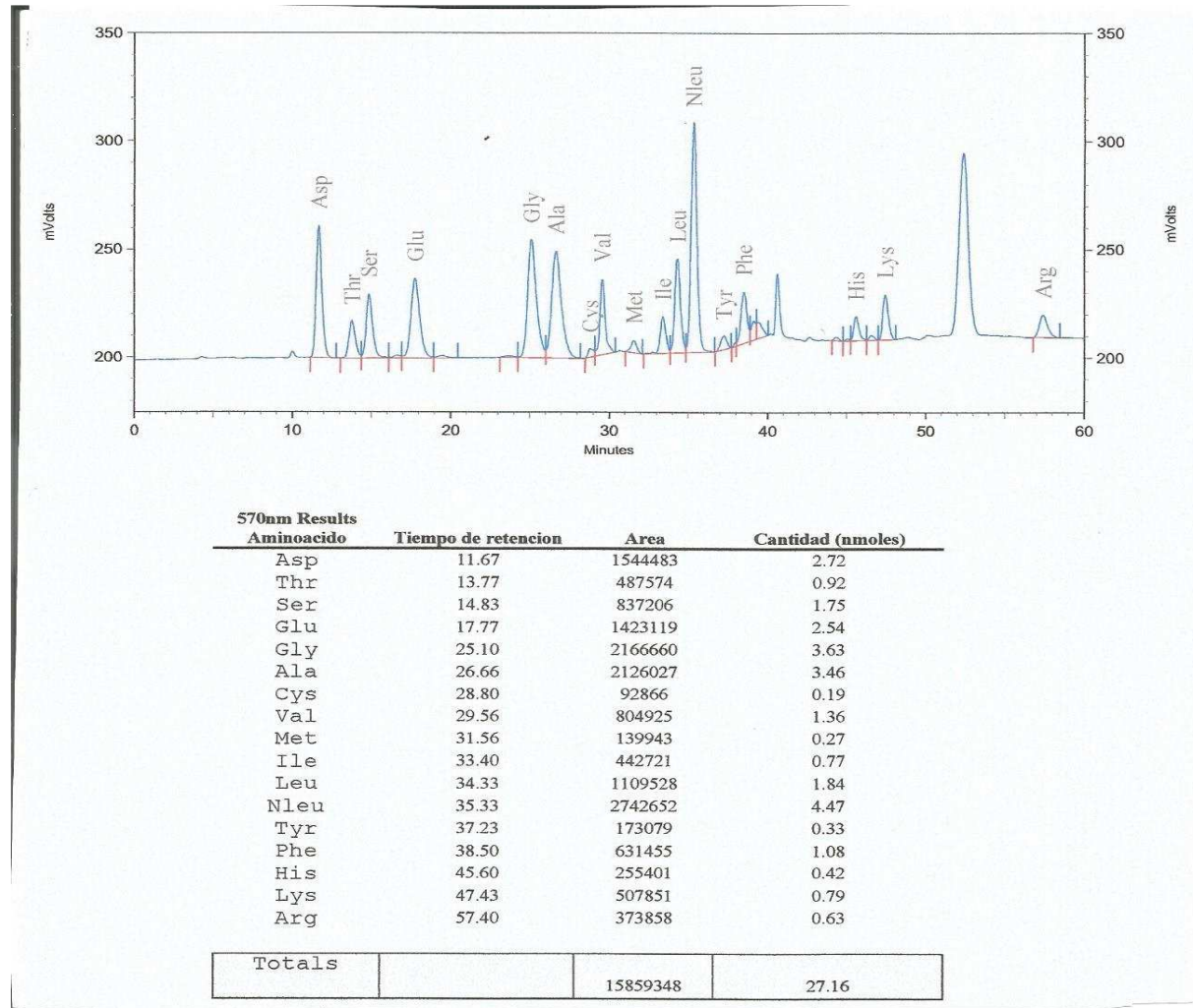


Figure 1: Ion-exchange chromatogram and composition of amino acids in *M. oleifera* leaf powder at 570 nm

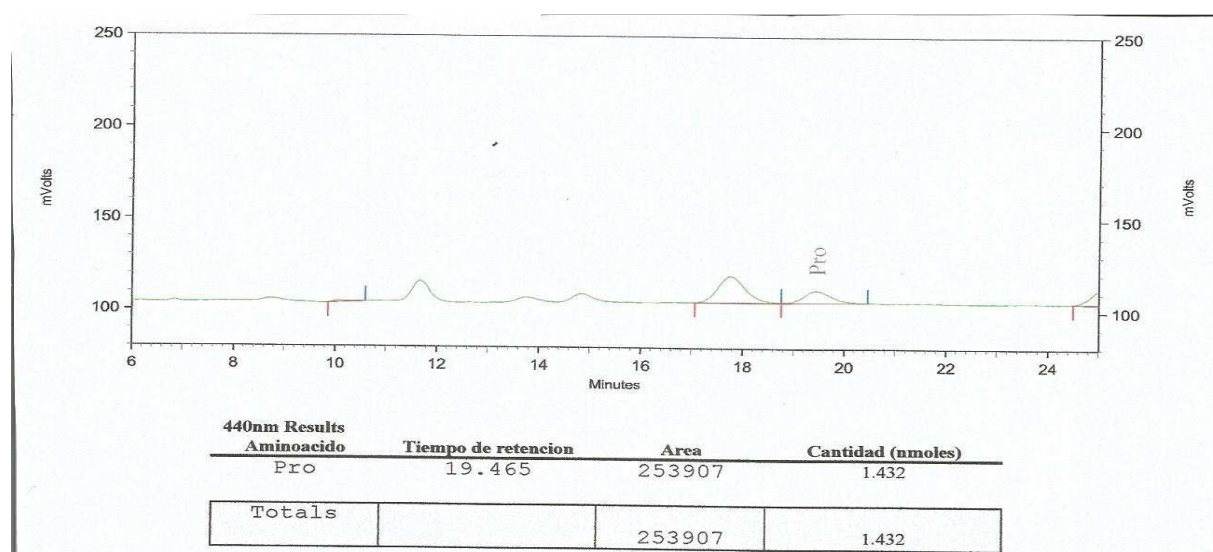


Figure 2: Ion-exchange chromatogram and composition of amino acids in *M. oleifera* leaf powder at 440 nm

The study showed that Moringa leaves contain nutritious compounds. Noteworthy is the crude protein (CP) content of 24.31 % observed in this study, although lower than sunflower seed cake's CP of 35.88% which is mostly used as protein concentrate [33]. This makes the Moringa leaves to be a good potential source of supplementary protein in animal diets. Other studies have reported variable protein contents ranging between 16, 22.42, 23.27, 27.4 and 40% [34], [35], [36], [37], [27], [38], and [26]. This level of crude protein content is of particular nutritional significance as it may meet animal's protein and energy requirements and boost the immune system against diseases [39], [40]. General growing ruminants like goats require 16% CP [41]. The CP supplied by Moringa is above the protein of goats making it ideal for use as a protein supplement. Moringa is reported to have high quality protein which is easily digested and that is influenced by the quality of its amino acids [42]. In this study, the dried Moringa leaves contained 17 amino acids, which slightly differ from the findings of Foidlet *et al.* [42], Sanchez-Machado *et al.* [38] and Moyo *et al.* [31] who reported 18, 16 and 19 amino acids respectively. Out of the 17 amino acids observed, 9 were classified as essential amino acids, namely; threonine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, histidine and lysine. Glycine had the highest value of 3.63 %, which differed with Moyo *et al.* [31] who reported the value of 1.53 %. In their work, Moyo *et al.* [31] reported alanine having the highest value of 3.033 %, which is lower than that of our findings (3.46 %). Cysteine had the least values followed by methionine, which is commonly deficient in green leaves. Methionine and cysteine are powerful antioxidants that help in the detoxification of harmful compounds and protect the body from radiation [40]. The variations in the amino acid composition could be influenced by protein quality and the origin of the plant (cultivated or wild). This may indicate that the Moringa was grown in fertile soils. Amino acids are organic compounds that combine to form proteins; as such, they influence the quantity and quality of protein. Amino acids are classified as essential and non-essential, which vary according to animal species and their production system [43]. Rumen microbes synthesize the essential amino acids from other amino acids or from nitrogen-containing substances. The efficiency of rumen microbial growth and activity in the rumen is enhanced by the presence of adequate amino acids, peptides and most macro and micro minerals [43]. Each amino acid has a specific function in the animal's body. In general, amino acids are required for the production of enzymes, immunoglobulins, hormones, growth and repair of body tissues and form the structure of red blood cells [40]. In addition, they contribute to the formation of glucose, acting as a buffer when other precursors are in short supply [43]. Amino acids also affect the function of other nutrients in the animal's body such as presence of lysine, which ensures adequate calcium absorption and aids in the antibody production.

The dry leaves could serve as a protein supplementary source in animal and human diets. This protein content is of particular nutritional significance since it has been suggested that amino acid supplementation is important in meeting a substantial proportion of an animal's protein and energy requirements [40]. Diets rich in amino acids help to boost the immune system against gastro-intestinal parasites infestations [39]. Proteins are also essential for continuous replenishment of the endogenous protein that is lost due to infections with gastro-intestinal helminths [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.

As observed in this study, Moringa contains more dietary polyunsaturated fatty acids (PUFA) than the saturated fatty acids (SFA). A higher content of PUFA and lower amount of SFA is desirable [47], as such, its inclusion in the diet is recommended as it prevents the occurrence of diseases thereby promoting good health. PUFA are important for human and animal health. They are of interest because they are precursors of long chain *n*-3 PUFA in the eicosanoid biosynthesis, which are viewed as important bioregulators of many cellular processes [48]. They are linked to the development and functionality of the immune system. Consumers have preference of food low in saturated fatty acids (SFA) because they are associated with an increased risk of cardio-vascular diseases and some cancers [49], [50]. Human nutritionists urge consumers to increase intake of polyunsaturated fatty acids (PUFA), particularly the *n*-3 PUFA at the expense of *n*-6 PUFA [47], [50]. The quantity and composition of fatty acids in the animals' body are related to the presence of some of their precursors in the diet, since some of the fatty acids are absorbed 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 °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 oleifera* 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 properties of the Moringa leaves, this probably explains the traditional use of the plant as an herbal tonic in India, because of its high levels of readily available essential nutrients and mineral resources, which may be required for the maintenance of electrical potential of nervous tissues and cell membranes. It can as well be used for the treatment of blood related disorders that is necessary for the improvement of the overall well-being of the body [53]. The nutritional variations observed among the studies could be attributed to the genetic background of the plant, in terms of ecotype and cultivar, environmental factors that include the soil and climate [38]. In addition, the cultivation method used encompasses the frequency of harvesting and age of the plant or leaves. Mode of conservation between collection and analysis (drying, refrigeration, freezing) might influence the leaves' nutritional composition [54], [55]. The data derived from phytochemical and nutrient characterization of Moringa are clear indications that the plant leaves are rich in healthy bioactive compounds and nutrients; and has potential to be used as medicine, feed and or food additive with multiple purposes. These include serving as a protein, fatty acid, mineral and vitamin resource for animal and human feed formulations. High nutritional content found in the dried leaves are important nutritional indicators of the usefulness of the plant as a likely feed resource. Drying the leaves assist to concentrate the nutrients, facilitate conservation and consumption, as such, it can be used during the time when feed is scarce or can be transported to areas where it is not cultivated. It is suggested that Moringa should be consumed in the powder form. Moringa has been reported to possess some medicinal properties [6]; its inclusion in the diets could function as curative and therapeutic therapy. As such, it can be used to improve 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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