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Research Article

Nutritional Composition and Fungi Isolates Associated with *Moringa Oleifera* Leaves and Seeds

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Abstract:

Moringa oleifera (miracle tree) as it is popularly called, has been found useful both medicinally and economically. *Moringa oleifera* is the most widely cultivated species of the monogeneric family, the Moringaceae, which is indigenous to South Asia. This fast-growing plant is a highly valued and cultivated one in the tropics and sub-tropics. With its high nutritive values, every part of the tree is suitable for either nutritional or commercial purposes. The leaves of this tree are worthy of special attention. The leaves are rich in minerals, vitamins, and other essential phytochemicals. This research is aimed at determining the nutritional composition and fungi isolates associated with *Moringa oleifera*. The Sabaraud Dextrose Agar was used to culture for pure growth and three microorganisms were isolated which consisted of *Aspergillus terreus*, *Aspergillus niger*, and *Aspergillus flavus.* The nutritional analysis of *Moringa oleifera* was carried out and its composition includes; crude lipids, crude fiber, moisture, ash, protein, and carbohydrates. They aid in treating digestive problems, reduce cholesterol and immunomodulation benefits, proper growth of the sperm cells, synthesis of DNA and RNA and they help to lower blood pressure. The mineral composition analyzed in *Moringa oleifera* leaves and seeds constituted zinc, copper, silver, and manganese. The microorganisms isolated produce toxins that cause harm and damage to humans. These toxins can be controlled if post-harvest interventions that reduce aflatoxin include rapid and proper drying, proper transportation and packaging, sorting, cleaning, drying, smoking, post-harvest insect control, and the use of botanicals or synthetic pesticides as storage protectants. The health benefits of *Moringa oleifera* include, they are anti-inflammatory in nature due to the presence of isothiocyanates, having anti-oxidative properties and protecting against the damaging effects of free radicals present in the body, stabilizing blood sugar levels due to the presence of isothiocyanates. They support brain health and work as neuro-enhancers, they are natural cleansers and help to detoxify the system. They are used as supplements in food to improve the nutritional composition that is lacking in the body.

Keywords: Nutrition, Fungi, *Moringa oleifera*, Leaf, Seed

Introduction

Plants have always been vital for mankind irrespective of the era and area all over the globe since the beginning of life. *Moringa oleifera* L. well-known as the "drumstick" or "horseradish" tree, is native to Northwest India, its main producer, but can also be found in South Africa, Northeast Africa, Madagascar, Tropical Asia, Southwest Asia, and Latin America. The *Moringa* genus comprises 14 species: *M. arborea*; *M. longituba*; *M. borziana*, *M. pygmaea*; *M. hildebrandtii*; *M. drouhardii*; *M. longituba*; *M. peregrina*; *M. stenopetala*; *M. rivae*; *M. ruspoliana*; *M. Ovalifolia*; *M. Concanensis* and *M. oleífera* (Rani *et al*., 2018). From the Moringaceae family, *M. oleifera* is the most known, studied, and used species (Olson, 2011) with human and animal applications. The various resources obtained from this plant's leaves, flowers, seeds, pods, bark, and roots can be used for cooking or in traditional medicine to treat several pathologies. *M. oleifera* can survive in humid or dry hot climates and poor soils (Anwar *et al.*, 2007 Mainenti 2018). *M. oleifera* is a highly nutritious plant, that is ideal for treating malnutrition in developing countries (Zongo 2013, Valdez-Solana *et al.*, 2015 Debajyoti *et al.*, 2017). *M. oleifera* gained the title of "Miracle Tree" and commercial attention supported several properties such as nutritional values, amino acids, and flavonols content which can be used in food supplements and the cosmetic industry. Moringa has been used in the traditional medicine passed down for centuries in many cultures around the world, for skin infections, anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhea, eye and ear infections, fever, glandular, swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation, diabetes, and pregnancy. The healing properties of Moringa oil, have been documented by ancient cultures. Moringa oil

has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner. Moringa oil has been used in skin preparations and ointments since Egyptian times. (Becker *et al.,* 2001). It is grown chiefly as an ornamental and in fencerows and hedges and has become naturalized along roadsides on the coastal plains and lower foothills. However, According to Agu *et al.* [46-49] and Okigbo et al. [50], between a quarter and two-fifths of agricultural produce is wasted yearly in the tropics due to poor storage conditions at the farm and village level. In its native habitat, annual temperature fluctuations tend to be very large, with minimum and maximum shade temperatures ranging from -1 to 3℃ and from 38 to 48℃ during the coldest and warmest months, respectively. In this region annual rainfall ranges from 750 to 2200 mm. Reseda is highly drought tolerant and is cultivated in semiarid and arid regions of India, Pakistan, Afghanistan, Saudi Arabia and east Africa, receiving an annual rainfall as low as 300 mm, although such sites are probably irrigated or are characterized by a high water table. In Puerto Rico, Reseda has become naturalized to a limited extent on sites with an annual rainfall between 1000 and 1800 mm. (Gopalakrishnan *et al.,* 1980). *Moringa oleifera* is cultivated in the Sind province of Pakistan. (Anwar K *et al.,* 2007).

The taxon name moringa comes from murunggi or muringa from Tamil and Malayalam. Moringa is available and known by more than 50 common names in Asia Africa, Europe South and Central America, Caribbean. Some are Drumstick tree, Horseradish tree, Mother's best friend, and West Indian ben are in English, Ben, Árbol del ben, Morango, and Moringa in Spanish. Moringa is a very impressive and amazing plant due to its tested, trusted, and potential benefits from nutritional as well as therapeutical points of view. This friendly plant is of great significance as shown to be useful in water purification, cosmetics, livestock fodder, plant enhancers, and biogas. In the last ten years, hundreds of research articles, theses, reports, and patents have been published on Moringa. Moringa which is also known as the miracle tree.

Materials and methods

Study Area:

This study will be carried out at Nnamdi Azikiwe University Awka, Anambra State.

Sample Collection:

The leaves were collected in the morning hours and were plucked from different trees in Nnamdi University, Awka.

Equipment Sterilization:

All rubber and glassware materials to be used were washed with a mixture of water, detergent, and hypochlorite. The glass wares were kept in an autoclave to be sterilized at 121^{o}C, 15psi for 15 minutes and allowed to cool before they were removed from the autoclave.

Production of ground *Moringa oleifera* leaves and seeds:

The fresh leaves were washed severally with clean water to remove all the undesirable materials and allowed to dry. The seeds were allowed to dry, peel, and ground, and put in an airtight container.

Microbial Isolation of Microorganisms:

The samples were diluted in a ten-fold serial dilution as 1ml from the 10^{-1} dilution tube was dispensed into several sterile petri dishes with the aid of a sterile pipette, and sabaraud dextrose agar was poured into the plates. The plates were rotated for easy mix-up of the sample and the media. All plates were allowed to solidify on the table. All the incubated plates were examined for colony growth.

Subculture and Purification:

After the incubation period, discrete colonies from the fungi plates were picked with a sterilized wire loop and subcultured onto a freshly prepared Sabaraud Dextrose Agar. The plates were incubated for 48 hours.

Microbial identification of fungi isolates Isolation and Characterization of the Fungi

This was done based on the description of the gross morphological appearance of fungal colonies on the modified slide culture method as described by Agu and Chidozie [45]. Microscopic evaluation was done under X10 and X40 magnification of the microscope; and referencing using the Manual of Fungal Atlases (Frey *et al.,* 1979; Barnett and Hunter, 2000. Watanabe, 2002. Ellis *et al.,* 2007).

Proximate Composition of *Moringa oleifera* Determination of moisture content:

This method is based on moisture evaporation. Here the aluminum dishes were washed dried in oven and in desiccators for cooling. The weight of each dish was taken 5.0 g of ground samples were weighed into a sterile aluminium dish, weight of the dish and weight of un-dried sample (in duplicate) were taken. This was transferred into an oven set at 80°C for 2 h and at 105°C for 3 hours respectively. This was removed and cooled in desiccators. Then the weight was measured using a measuring scale balance. It was transferred back into the oven for another one hour and then reweighed. The process continued until a constant weight was obtained.

The difference in weight between the initial weight and the constant weight gained represents the moisture content.
Calculation: The loss in weight multiplied by 100 over the original weight is the percentage moisture content.
Moisture content (g/100 g)
= loss in weight ((W2-W3)/ (W2-W1)) x 100
Where W1= initial weight of empty crucible, W2= weight of crucible + food before drying, W3 = final weight of crucible + food after drying.
% Total solid (Dry matter) (%) = 100- moisture (%) (AOAC 2005)

Determination of ash content:

The ash represents the inorganic component (minerals) of the sample after all moisture has been removed as well as the organic material. The method is a destructive approach based on the decomposition of all organic matter such that the mineral elements may be lost in the process. Twenty grams (20 g) of each of the samples were weighed into a clean dried and cooled platinum crucible. It was put into a furnace set at 550 °C and allowed to blast for 3 h. It was then brought out and allowed to cool in desiccators and weighed again.
Calculation: Percentage weight is calculated as the weight of ash multiplied by 100 over the original weight of the samples used.
Ash content = (weight of ash/ weight of original sample used) x100.
Loss in weight ((W3-W1)/(W2-W1)) x 100
Where W1 = weight of empty crucible, W2 = weight of crucible + food before drying and or ashing, W3 = weight of crucible + ash. (AOAC 2005)

Determination of lipid content:

The method employed was the soxhlet extraction technique described by (AOAC 2005). 15 g of the samples were weighed and carefully placed inside a fat-free thimble. This was covered with cotton wool to avoid the loss of sample. A loaded thimble was put in the Soxhlet extractor, about 200 ml of petroleum ether was poured into a weighed fat-free soxhlet flask, and the flask was attached to the extractor. The flask was placed on a heating mantle so the petroleum ether in the flask refluxed. Cooling was achieved by a running tap connected to the extractor for at least 6hrs after which the solvent was completely siphoned into the flask. A rotary vacuum evaporator was used to evaporate the solvent leaving behind the extracted lipids in the soxhlet. The flask was removed from the evaporator and dried to a constant weight in the oven at 60°C. The flask was then cooled in a desiccator and weighed. Each determination was done in triplicate. The amount of fat extracted was calculated by difference.
Ether extracts (100g) dry matter = (weight of extracted lipids/ weight of dry sample) x100 (AOAC 2005)

Determination of protein

Total protein was determined by the Kjeldahl method (AOAC, 2005). The analysis of a compound of its protein content by the Kjeldahl method is based upon the determination of the amount of reduced nitrogen present. About 20 g of the samples were weighed into a filter paper and put into a Kjedahl flask, 10 tablets of Na_2SO_4 were added with 1 g of $CuSO_4$ respectively. Twenty milliliters (20 mL) of conc. H_2SO_4 was added and then digested in a fume cupboard until the solution became colorless. It was cooled overnight and transferred into a 500 mL flat bottom flask with 200 mL of water. This was then cooled with the aid of packs of ice blocks. About 60 to 70 mL of 40% of NaOH were poured into the conical flask which was used as the receiver with 50 mL of 4% boric acid using 3 days of screened methyl red indicator. The ammonia gas was then distilled into the receiver until the whole gas evaporated. Titration was done in the receiver with 0.01M HCl until the solution became colourless.
Calculation: The percentage protein is calculated as follows:
Vs – Vb x 0. 01401x N acid (6.25) x 100 Original wt of sample used
Where Vs = Vol (ml) of acid required to titrate sample, Vb = Vol (ml) of acid required to titrate blank, N acid = normality of acid. (AOAC 2005)

Crude fiber

The bulk of roughage in food is referred to as fiber and is estimated as crude fiber. Twenty grams (20 g) of the different samples were defatted with diethyl ether for 8 hours and boiled under reflux for exactly 30 min with 200 mL of 1.25% H_2SO_4. It was then filtered through cheesecloth on a flutter funnel. This was later washed with boiling water to completely remove the acid. The residue was then boiled in a round-bottomed flask with 200 mL of 1.25% sodium hydroxide (NaOH) for another 30 min and filtered through a previously weighed couch crucible. The crucible was then dried with samples in an oven at 100°C, left to cool in a desiccator, and later weighed. This was later incinerated in a muffle furnace at 600°C for 2 to 3 hours and later allowed to cool in a desiccator and weighed. (AOAC, 2005).
Calculation = Weight of fiber = (C2-C3) y% fiber = C2-C3 x 100 /Wt. of the original sample

Carbohydrate determination

Available carbohydrate (%) = 100- (protein (%) + Moisture (%) + Ash (%) + Fibre (%) + Fat (%)).

Energy or Caloric Value (KJ/100g) = (Protein X 16.7) + (Lipids X 37.7) + (Carbohydrate X 16.7)

Result

In this study, moringa samples were used which consisted of seeds and leaves collected from Nnamdi Azikiwe University, Awka.

Microscopic and identification of fungi isolates

The SDA agar was used to culture the fungi and three isolates were seen with black, yellow, and green pigmentation. It was viewed under the microscope using lacto phenol cotton blue and ×40 magnification lens. The probable isolates include *Aspergillus niger*, *Aspergillus terreus*, and *Aspergillus flavus*. The fungi isolates and their pigmentation. *Aspergillus terreus* has a yellow pigmentation, *Aspergillus flavus* has a green pigmentation and *Aspergillus niger* has a black pigmentation as represented in Table 1. Colony Count of the fungi isolates on the Sabaraud Dextrose Agar, the count on the *Moringa oleifera* seed sample was forty and the *Moringa oleifera* leaves sample was twenty- five as represented in Table 2. Colonial and Microscopic Identification of the fungi isolates is indicated in Table 3. In the Proximate Analysis, the following nutritional components were found: crude lipids, crude fiber, moisture ash, protein, carbohydrate and calorific value as shown in Table 4. The mineral Composition of *Moringa oleifera* samples includes iron, copper, zinc, augmentin, manganese, and silver as represented in Table 5.

Table 1: FUNGI PROBABLE ISOLATES

Fungi Probable Isolates	Colour of Colony
Aspergillus niger	Black
Aspergillus terreus	Yellow
Aspergillus flavus	Green

Table 2: COLONY COUNT ON FUNGI ISOLATES

Sample	Agar	Total Colony Count(CFU/ML)
M L	SDA	25
M S	SDA	40

M L= *Moringa oleifera* leaves
M S= *Moringa oleifera* seeds
SDA= Sabaraud dextrose Agar

Table 3: COLONIAL AND MICROSCOPIC IDENTIFICATION OF FUNGI ISOLATES

Black centre, white fluffy Regular margin and pale yellowish on reverse on SDA	Smooth coloured conidiophores and conidia. The conidiophores are protrusions from a septate and hyaline hyphae. The phialides produce conidia that have a rough texture, are dark brown coloured.	*Aspergillus niger*
Yellow centre, white fluffy Regular margin and pale yellowish on reverse on SDA	Smooth coloured conidiophores and conidia. The conidiophores are protrusions from a septate and hyaline hyphae. The phialides produce conidia that have a rough texture, are dark brown coloured.	*Aspergillus terreus*
Green center produces white velvety colonies that turn yellowish-green, a pigment of the conidial spores.	Conidophore intermediate with aerial hyphae bearing conidiospores, conidial head typically columnar	*Aspergillus flavus*

Table 4: PROXIMATE COMPOSITION OF *Moringa oleifera* SAMPLES

Sample	Crude lipids%	Crude Fibre%	Moisture%	Ash %	Protein%	Carbohydrate%	Calorific value kj/100g
M L	9.2043	6.0496	7.4852	4.982	26.3495	45.9294	1248.29
	8.9204	6.0002	6.98326	5.02837	25.9194	47.14837	1220.232
M S	19.9584	4.58537	5.8832	3.5531	26.9437	39.84623	1115.392
	19.9584	4.0128	4.9937	3.9328	7.3646	22.9741	1120.608

ML = *Moringa oleifera* leaves
MS = *Moringa oleifera* seeds

Table 5: MINERAL COMPOSITION USING XRAY FLUORESCENCE SPECTROMETERS %.

Sample	Mineral Element	Value %
M L	Iron	6.969
	Zinc	0.972
	Augmentin	0.63
	Silver	8.56
	Palladium	7.445
	Copper	1.954
	Manganese	4.619

ML = *Moringa oleifera* leaves

Sample	Mineral Element	Value %
M S	Iron	3.562
	Zinc	1.138
	Augmentin	0.551
	Silver	8.705
	Copper	1.394
	Manganese	2.327

MS = *Moringa oleifera* seeds

Discussion and Conclusion

Moringa oleifera has always been vital to mankind, irrespective of the *era. Moringa oleifera* well known as "drumstick" has so many nutritional properties used in human and animal application. *Moringa oleifera* is used to treat malnutrition in some developing countries. The healing properties of *Moringa* oil have been tremendous for health use.

The study was carried out to discover the nutritional composition and fungi isolates associated with *Moringa oleifera*.

The fungi isolated from the *moringa oleifera* samples in the laboratory are *Aspergillus niger, Aspergillus flavus, and Aspergillus terreus.* These fungi produce potent mycotoxins called ochratoxins,highly carcinogenic toxins called aflatoxins which are a health hazard to humans and animals. Large doses of aflatoxins lead to acute poisoning (aflatoxicosis) that can be life-threatening, usually through damage to the liver. Outbreaks of acute liver failure (jaundice, lethargy, nausea, death), identified as aflatoxicosis, have been observed in human populations since the 1960s. Various studies have linked ochratoxin exposure with the human diseases Balkan endemic nephropathy (BEN) and chronic interstitial nephropathy (CIN) as well as other renal diseases. Ochratoxin A is a common food-contaminating mycotoxin. Contamination of food commodities, such as cereals and cereal products, coffee beans, dry vine fruits, wine, and grape juice and to avoid aflatoxin contamination from *Aspergillus flavus,* The toxins produced by these organisms damage plants by killing cells and causing plant stress. Sources of fungal infections are infected seeds, soil, crop debris, nearby crops, and weeds. Fungi are spread by wind and water splash, and through the movement of contaminated soil, animals, workers, machinery, tools, seedlings, and other plant material. These toxins can be controlled if post-harvest interventions that reduce aflatoxin include rapid and proper drying, proper transportation and packaging, sorting, cleaning, drying, smoking, post-harvest insect control, and the use of botanicals or synthetic pesticides as storage protectants. Delivering such powerful nutrition, these leaves could prevent the scourge of malnutrition and related diseases.

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